Aconiti Radix (unprocessed)

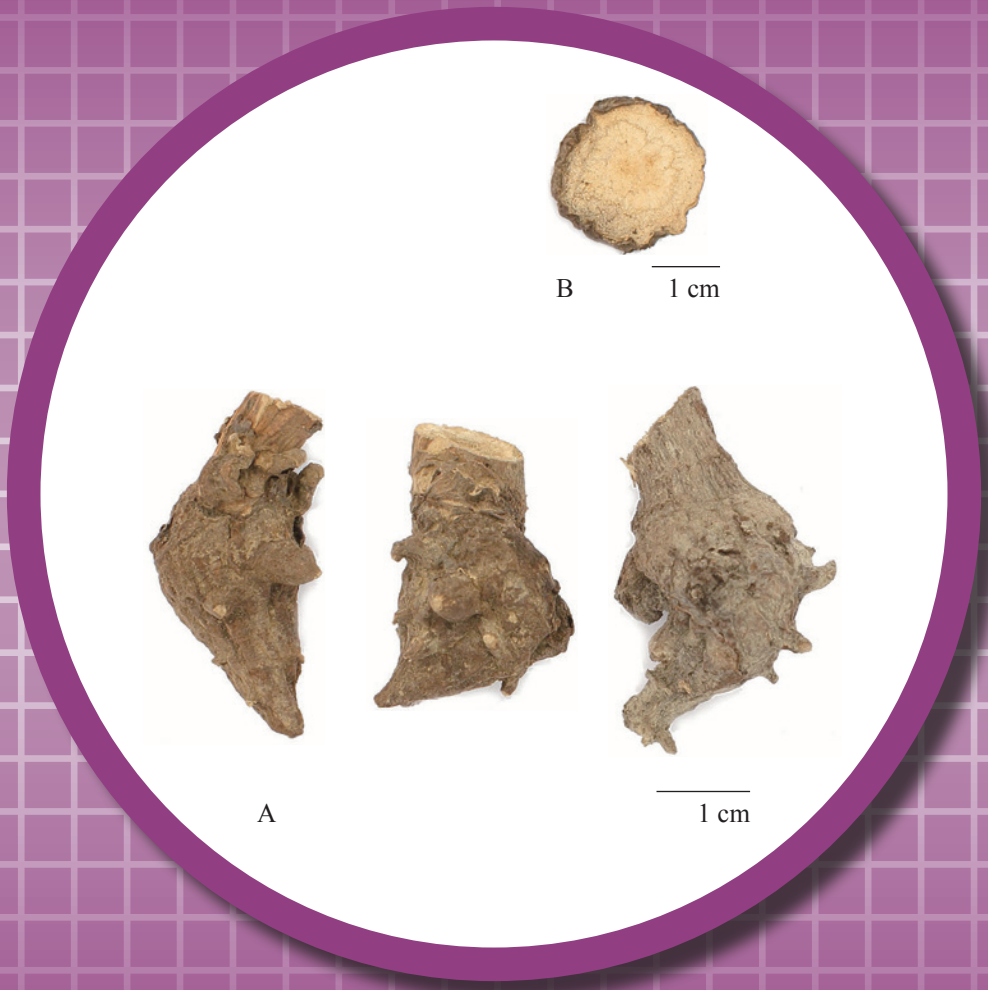


Figure 1 A photograph of Aconiti Radix (unprocessed)

A. Aconiti Radix (unprocessed)
B. Magnified image of transverse section of primary root tuber

1. NAMES

Official Name: Aconiti Radix (unprocessed)

Chinese Name: 川烏 (生)

Chinese Phonetic Name: Chuanwu (Sheng)

2. SOURCE

Aconiti Radix (unprocessed) is the unprocessed dried primary root tuber of *Aconitum carmichaelii* Debx. (Ranunculaceae). The root tuber is collected from late June to early August, branch roots, rootlets and soil removed, then dried under the sun to obtain Aconiti Radix (unprocessed).

3. DESCRIPTION

Irregularly conical, slightly curved, usually with remnants of stem at apex, mostly swollen towards one side in the middle, 2-7.5 cm long, 12-30 mm in diameter. Externally brown or greyish-brown, shrunken, with tuberculate lateral roots and scars of branch roots . Texture hard and compact. Fracture whitish to greyish-yellow, cambium ring polygonal. Odour slight (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Metaderm consists of brown suberized cells. Stone cells subrectangular, squared, elongated-elliptical or subtriangular, with a relatively large lumen, occurring scattered singly or in groups, occasionally found in cortical parenchyma. Endodermis indistinct. Sieve tube groups scattered in the phloem, with fibre bundles occasionally found on the inner side of phloem. Cambium in a ring, subpolygonal. 1 or several anomalous vascular bundles occasionally found on the inner side of the cambium. Xylem vessels consist of several rows of cells, arranged radially or in V-shaped. Pith distinct. Parenchymatous cells filled with starch granules (Fig. 2).

Powder

Colour pale brown to brown. Single starch granules spherical, oblong or reniform, 3-25 μm in diameter; black and cruciate-shaped under the polarized microscope; compound starch granules composed of 2-15 units. Stone cells nearly colourless or pale yellowish-green, subrectangular, subsquare, polygonal or tapering at one side, 62-280 μm long, 49-117 μm in diameter, walls 4-13 μm thick, the thick-walled stone cells distinctly striated, pits relatively sparse. Metaderm cells brown, some walls tuberculate thickened and projected into lumen. Vessels nearly colourless or pale yellow, mainly bordered-pitted, 12-70 μm in diameter, endings truncate or mucronate, perforated at the end walls or side walls, some vessels thick and short, tortuous or connected in crisscross pattern (Fig. 3).

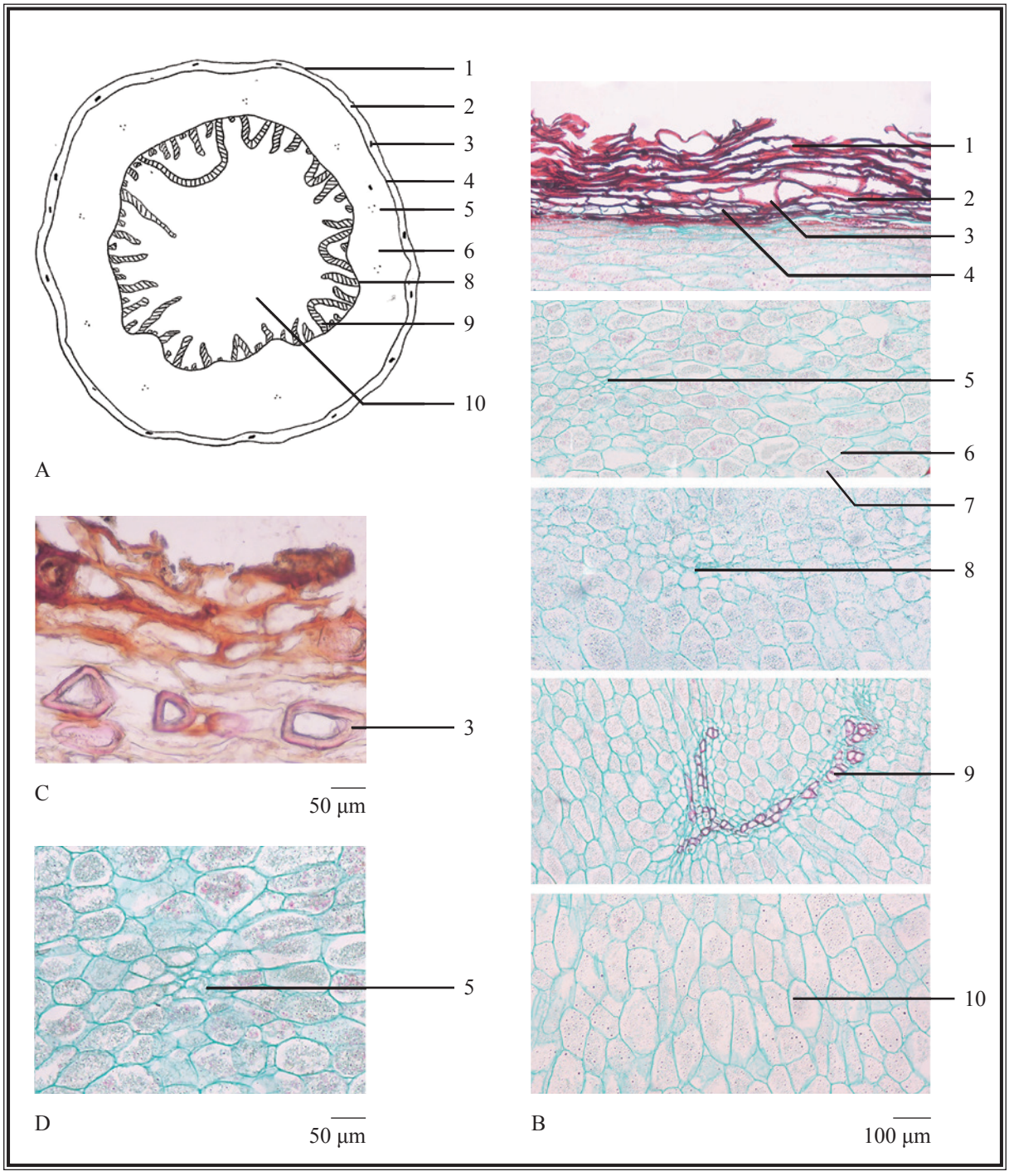


Figure 2 Microscopic features of transverse section of Aconiti Radix (unprocessed)

A. Sketch B. Section illustration C. Stone cells in cortex D. Sieve tube in phloem

1. Metaderm 2. Cortex 3. Stone cells in cortex 4. Endodermis 5. Sieve tube group 6. Phloem 7. Starch granules 8. Cambium 9. Xylem 10. Pith

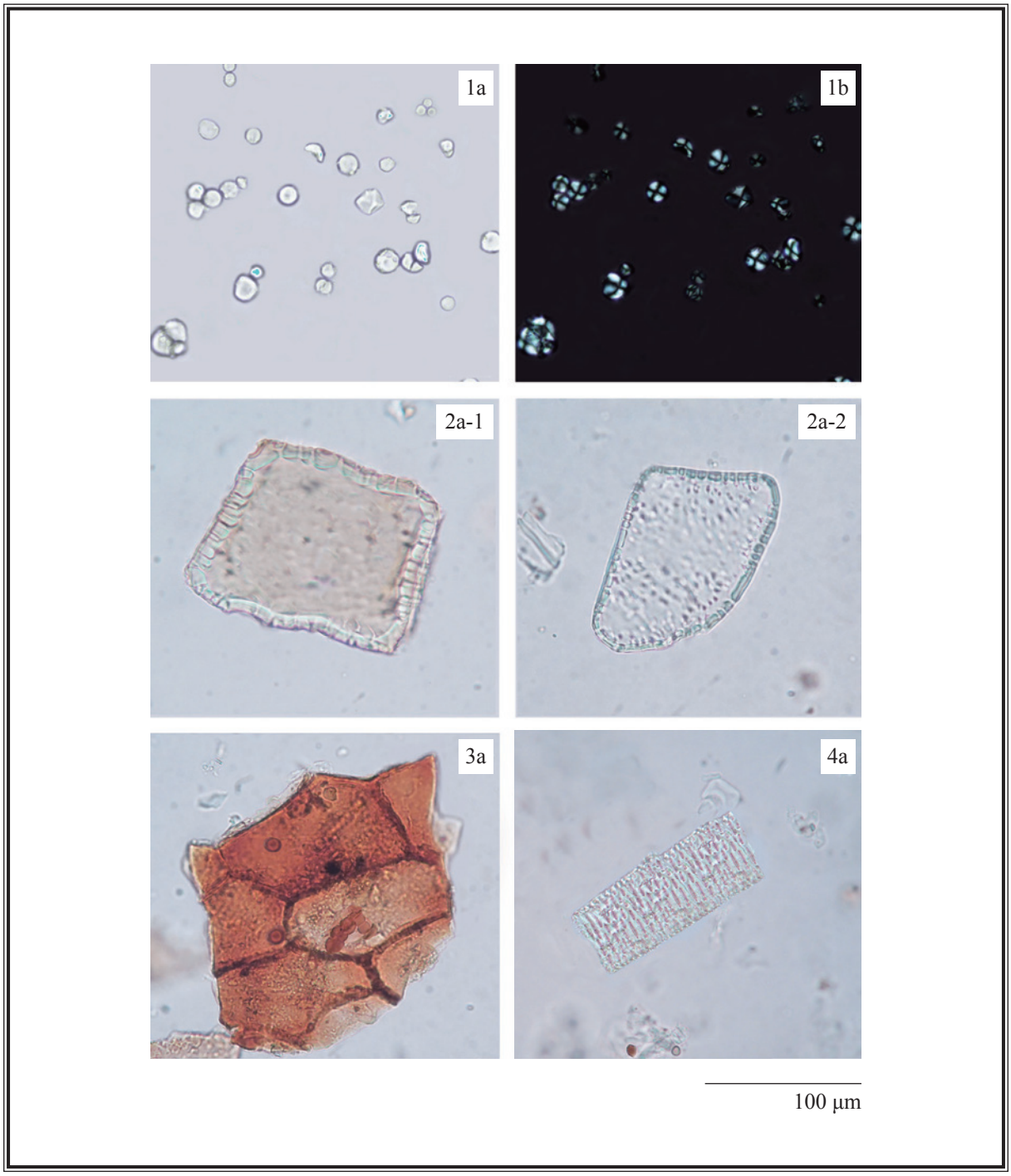


Figure 3 Microscopic features of powder of Aconiti Radix (unprocessed)

1. Starch granules 2. Stone cell (2-1 subsquare stone cell, 2-2 polygonal stone cell)
3. Metaderm cells 4. Vessel

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

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

Standard solutions

Aconitine standard solution

Weigh 1.0 mg of aconitine CRS (Fig. 4) and dissolve in 1 mL of a mixture of isopropanol and ethyl acetate (1:1, v/v). Keep at about 4ºC.

Hypaconitine standard solution

Weigh 1.0 mg of hypaconitine CRS (Fig. 4) and dissolve in 1 mL of a mixture of isopropanol and ethyl acetate (1:1, v/v). Keep at about 4ºC.

Mesaconitine standard solution

Weigh 1.0 mg of mesaconitine CRS (Fig. 4) and dissolve in 1 mL of a mixture of isopropanol and ethyl acetate (1:1, v/v). Keep at about 4ºC.

Developing solvent system

Prepare a mixture of *n*-hexane, ethyl acetate and methanol (6.4:5:1, v/v).

Spray reagent

Solution A

Weigh 0.85 g of bismuth oxynitrate 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

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

Test solution

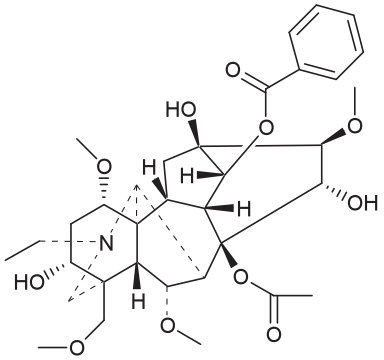
Weigh 2.0 g of the powdered sample and place it in a 50-mL conical flask, then add 3 mL of ammonium hydroxide solution (9.1%, w/v) and 25 mL of diethyl ether. Cap the flask. Sonicate (300 W) the mixture for 30 min in an ice water bath. Filter and transfer the filtrate to a 100-mL round-bottomed flask. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 0.5 mL of ethyl acetate.

Procedure

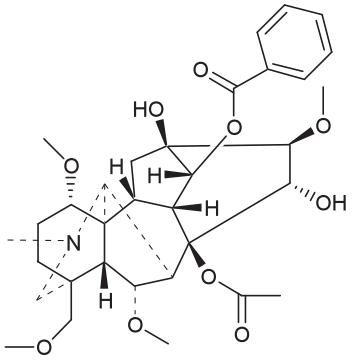
Carry out the method by using a HPTLC silica gel G60 plate, a twin trough chamber and a freshly prepared developing solvent system as described above. Apply separately aconitine standard solution (4 µL), hypaconitine standard solution (4 µL), mesaconitine standard solution (4 µL) and the test solution (8 µL) to the plate. Before the development, add the

developing solvent to one of the troughs of the chamber and add ammonium hydroxide solution (9.1%, w/v) to the other trough. Hang the HPTLC plate in the chamber, cover the chamber with a lid and let equilibrate for about 10 min. Carefully place the HPTLC plate in the trough containing the developing solvent 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 dry in air. Examine the plate under visible light. Calculate the R_f values by using the equation as indicated in Appendix IV (A).

(i)



(ii)



(iii)

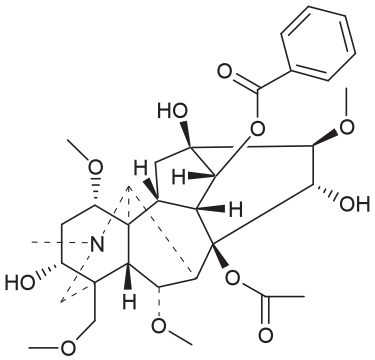


Figure 4 Chemical structures of (i) aconitine (ii) hypaconitine and (iii) mesaconitine

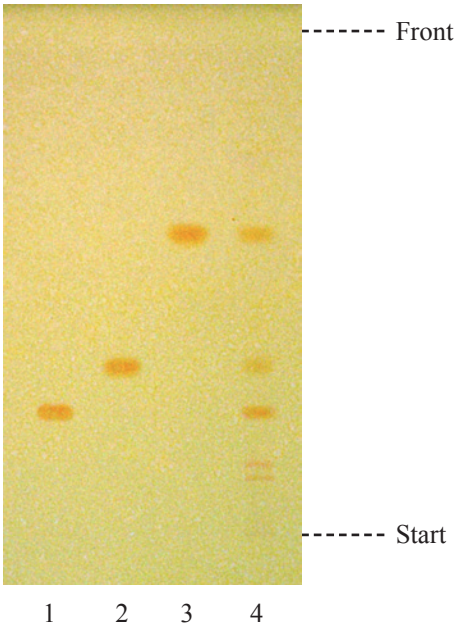


Figure 5 A reference HPTLC chromatogram of Aconiti Radix (unprocessed) extract observed under visible light after staining

1. Mesaconitine standard solution 2. Aconitine standard solution
3. Hypaconitine standard solution 4. 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 aconitine, hypaconitine and mesaconitine (Fig. 5).

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

Standard solutions

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

Weigh 0.5 mg of aconitine CRS and dissolve in 10 mL of 0.01% hydrochloric acid in methanol. Keep at about 4ºC.

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

Weigh 0.5 mg of hypaconitine CRS and dissolve in 10 mL of 0.01% hydrochloric acid in methanol. Keep at about 4ºC.

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

Weigh 0.5 mg of mesaconitine CRS and dissolve in 10 mL of 0.01% hydrochloric acid in methanol. Keep at about 4ºC.

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 (9.1%, w/v) and 30 mL of a mixture of isopropanol and ethyl acetate (1:1, v/v). Cap the tube. Sonicate (300 W) the mixture for 30 min in an ice water bath. Centrifuge at about 3000 × *g* for 5 min. Filter and transfer the filtrate to a 250-mL round-bottomed flask. Wash the residue for three times each with 10 mL of a mixture of isopropanol and ethyl acetate (1:1, v/v). Combine the solutions. Evaporate the solvent to dryness below 40ºC at reduced pressure in a rotary evaporator. Dissolve the residue in 3 mL of 0.01% hydrochloric acid in methanol. Transfer the solution to a 5-mL volumetric flask and make up to the mark with 0.01% hydrochloric acid in methanol. Filter through a 0.45-μm nylon filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (235 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The column temperature is maintained at 30ºC during the separation. The flow rate is about 1.0 mL/min. Programme the chromatographic system as follows (Table 1) –

Table 1 Chromatographic system conditions

Time (min)	0.2% Acetic acid* (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 44	79 → 69	21 → 31	linear gradient
44 – 65	69 → 65	31 → 35	linear gradient

*Adjust the pH to 6.2 with triethylamine

System suitability requirements

Perform at least five replicate injections, each using 10 μL of aconitine Std-FP, hypaconitine Std-FP and mesaconitine Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of aconitine, hypaconitine and mesaconitine should not be more than 5.0%; the RSD of the retention times of aconitine, hypaconitine and mesaconitine peaks should not be more than 2.0%; the column efficiencies determined from aconitine, hypaconitine and mesaconitine peaks should not be less than 20000 theoretical plates.

The *R* value between peak 2 and the closest peak; the *R* value between peak 3 and the closest peak; and 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. 6).

Procedure

Separately inject aconitine Std-FP, hypaconitine Std-FP, mesaconitine Std-FP and the test solution (10 μL each) into the HPLC system and record the chromatograms. Measure the retention times of aconitine, hypaconitine and mesaconitine peaks in the chromatograms of aconitine Std-FP, hypaconitine Std-FP, mesaconitine Std-FP and the retention times of the five characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify aconitine, hypaconitine and mesaconitine peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of aconitine Std-FP, hypaconitine Std-FP and mesaconitine Std-FP. The retention times of aconitine, hypaconitine and mesaconitine 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 Aconiti Radix (unprocessed) extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the five characteristic peaks of Aconiti Radix (unprocessed) extract

Peak No.	RRT	Acceptable Range
1	0.68	± 0.03
2 (mesaconitine)	0.89	± 0.05
3 (marker, hypaconitine)	1.00	-
4 (aconitine)	1.05	± 0.03
5	1.18	± 0.03

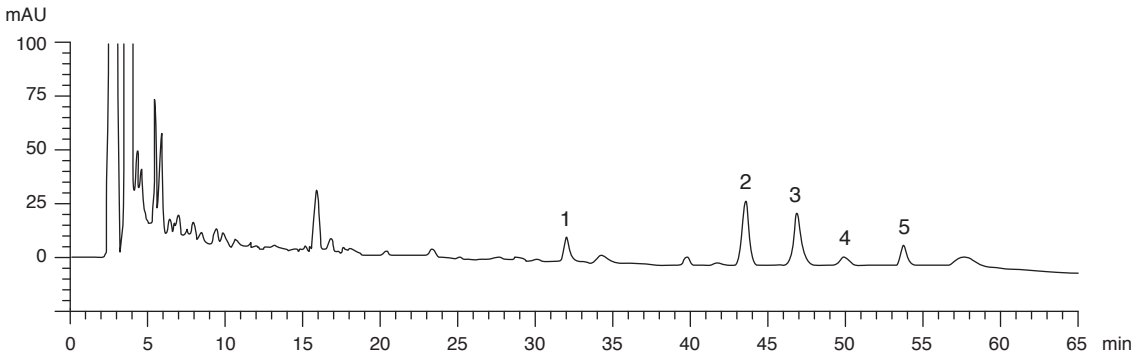


Figure 6 A reference fingerprint chromatogram of Aconiti Radix (unprocessed) extract

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

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

5.5 Foreign Matter *(Appendix VIII)*: not more than 2.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 9.0%.
Acid-insoluble ash: not more than 1.5%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 12.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (hot extraction method): not less than 37.0%.
Ethanol-soluble extractives (hot extraction method): not less than 19.0%.

7. ASSAY

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

Standard solution

Mixed aconitine, hypaconitine and mesaconitine standard stock solution, Std-Stock (1000 mg/L each)
Weigh accurately 10.0 mg of aconitine CRS, 10.0 mg of hypaconitine CRS and 10.0 mg of mesaconitine CRS, and dissolve in 10 mL of 0.01% hydrochloric acid in methanol. Keep at about 4ºC.

Mixed aconitine, hypaconitine and mesaconitine standard solution for assay, Std-AS

Measure accurately the volume of the mixed aconitine, hypaconitine and mesaconitine Std-Stock, dilute with 0.01% hydrochloric acid in methanol to produce a series of solutions of 5, 8, 10, 20, 50 mg/L for aconitine, 5, 10, 20, 50, 100 mg/L for hypaconitine and 10, 20, 50, 100, 200 mg/L for mesaconitine. Keep at about 4ºC.

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 (9.1%, w/v) and 30 mL of a mixture of isopropanol and ethyl acetate (1:1, v/v). Cap the tube. Sonicate (300 W) the mixture for 30 min in an ice water bath. Centrifuge at about 3000 × *g* for 5 min. Filter and transfer the filtrate to a 250-mL round-bottomed flask. Wash the residue for three times each with 10 mL of a mixture of isopropanol and ethyl acetate (1:1, v/v). Combine the solutions. Evaporate the solvent to dryness below 40ºC at reduced pressure in a rotary evaporator. Dissolve the residue in 3 mL of 0.01% hydrochloric acid in methanol. Transfer the solution to a 5-mL volumetric flask and make up to the mark with 0.01% hydrochloric acid in methanol. Filter through a 0.45-μm nylon filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (235 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The column temperature is maintained at 30ºC during the separation. The flow rate is about 1.0 mL/min. Programme the chromatographic system as follows (Table 3) –

Table 3 Chromatographic system conditions

Time (min)	0.2% Acetic acid* (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 44	79 → 69	21 → 31	linear gradient
44 – 65	69 → 65	31 → 35	linear gradient

*Adjust the pH to 6.2 with triethylamine

System suitability requirements

Perform at least five replicate injections, each using 10 μL of the mixed aconitine, hypaconitine and mesaconitine Std-AS (10 mg/L for aconitine, 20 mg/L for hypaconitine and 50 mg/L for mesaconitine). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of aconitine, hypaconitine and mesaconitine should not be more than 5.0%; the RSD of the retention times of aconitine, hypaconitine and mesaconitine peaks should not be more than 2.0%; the column efficiencies determined from aconitine, hypaconitine and mesaconitine peaks should not be less than 20000 theoretical plates.

The *R* value between aconitine peak and the closest peak; the *R* value between hypaconitine peak and the closest peak; and the *R* value between mesaconitine peak and the closest peak in the chromatogram of the test solution should not be less than 1.5.

Calibration curves

Inject a series of the mixed aconitine, hypaconitine and mesaconitine Std-AS (10 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of aconitine, hypaconitine and mesaconitine against the corresponding concentrations of the mixed aconitine, hypaconitine and mesaconitine Std-AS. Obtain the slopes, y-intercepts and the r^2 values from the corresponding 5-point calibration curves.

Procedure

Inject 10 µL of the test solution into the HPLC system and record the chromatogram. Identify aconitine, hypaconitine and mesaconitine peaks in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed aconitine, hypaconitine and mesaconitine Std-AS. The retention times of aconitine, hypaconitine and mesaconitine peaks in the chromatograms of the test solution and the Std-AS should not differ by more than 5.0%. Measure the peak areas and calculate the concentrations (in milligram per litre) of aconitine, hypaconitine and mesaconitine in the test solution, and calculate the percentage contents of aconitine, hypaconitine and mesaconitine in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains 0.056% to 0.12% of the total content of aconitine ($C_{34}H_{47}NO_{11}$), hypaconitine ($C_{33}H_{45}NO_{10}$) and mesaconitine ($C_{33}H_{45}NO_{11}$), calculated with reference to the dried substance.

8. CAUTION

This CMM is potent/toxic and should be prescribed by registered Chinese medicine practitioner.