Aconiti Kusnezoffii Radix (unprocessed)

Figure 1 A photograph of Aconiti Kusnezoffii Radix (unprocessed)

A. Aconiti Kusnezoffii Radix (unprocessed)

B. Magnified image of transverse section of root tuber

1. NAMES

Official Name: Aconiti Kusnezoffii Radix (unprocessed)

Chinese Name: 草烏 (生)

Chinese Phonetic Name: Caowu (Sheng)

2. SOURCE

Aconiti Kusnezoffii Radix (unprocessed) is the unprocessed dried root tuber of *Aconitum kusnezoffii* Reichb. (Ranunculaceae). The root tuber is collected in autumn when the stem and leaves withered, rootlets and soil removed, washed clean, then dried under the sun to obtain Aconiti Kusnezoffii Radix (unprocessed).

3. DESCRIPTION

Irregularly long-conical or conical, slightly curved, 2-9 cm long, 7-25 mm in diameter. Apex usually with remnants of stem and a few remnant bases of adventitious roots, sometimes apex bearing a withered bud on one side and a circular or oblate remnant base of adventitious root on the other side. Externally blackish-brown, shrunken, with longitudinal wrinkles, dotted rootlet scars and several tubercular lateral roots. Texture hard. Fracture greyish-white or dark grey, sometimes with fissures; cambium ring subrounded, the pith relatively large or hollow. Odour slight (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Metaderm consists of 1-9 layers of brownish-yellow suberized cells. Cortex narrow. Stone cells subretangular, square or elliptical, lumen large, scattered singly or 2-5 in groups in cortex. Endodermis mostly indistinct. Phloem broad, with groups of sieve tubes and irregular clefts scattered throughout. Sclerenchyma occasionally present near the xylem vessels across the cambium layer. Cambium ring subrounded or subpolygonal. Xylem vessels several to dozens in groups, located inside each angle of the cambium, some containing brownish-yellow contents. Pith relatively large. Parenchymatous cells filled with starch granules (Fig. 2).

Powder

Colour greyish-brown. Single starch granules subrounded, 2-26 µm in diameter; black and cruciate-shaped under the polarized microscope; compound starch granules composed of 2-6 units. Metaderm cells brown, subsquared or elongated polygonal in surface view, walls unevenly thickened, some appeared tuberculate and projected into lumen. Sclerenchymatous cells long stripe-shaped or long fusiform, 21-37 µm in diameter; bright white under the polarized microscope. Stone cells colourless, brown when adhered to the metaderm cells, subsquare, subrectangular, subrounded, fusiform or long stripe-shaped, 22-155 µm in diameter, walls varied in thickness, the thick-walled stone cells distinctly striated and finely pitted, some contained brown contents; bright white under the polarized microscope. Vessels mainly reticulate, annular vessels occasionally found, 4-59 µm in diameter; white under the polarized microscope (Fig. 3).

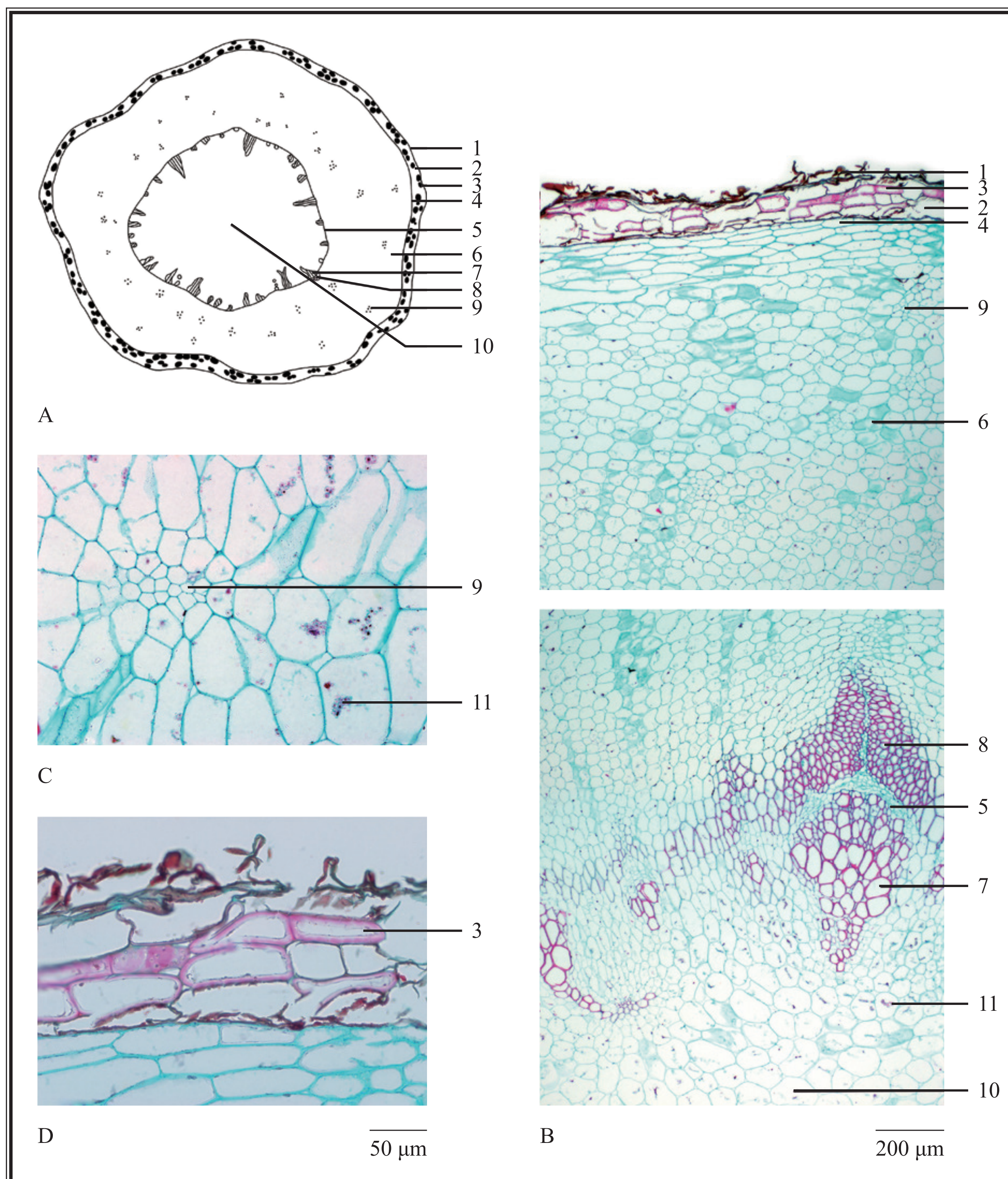


Figure 2 Microscopic features of transverse section of Aconiti Kusnezoffii Radix (unprocessed)

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

1. Metaderm 2.Cortex 3. Stone cells in cortex 4. Endodermis 5. Cambium 6. Phloem
7. Xylem vessels 8. Sclerenchyma 9. Sieve tube group 10. Pith 11. Starch granules

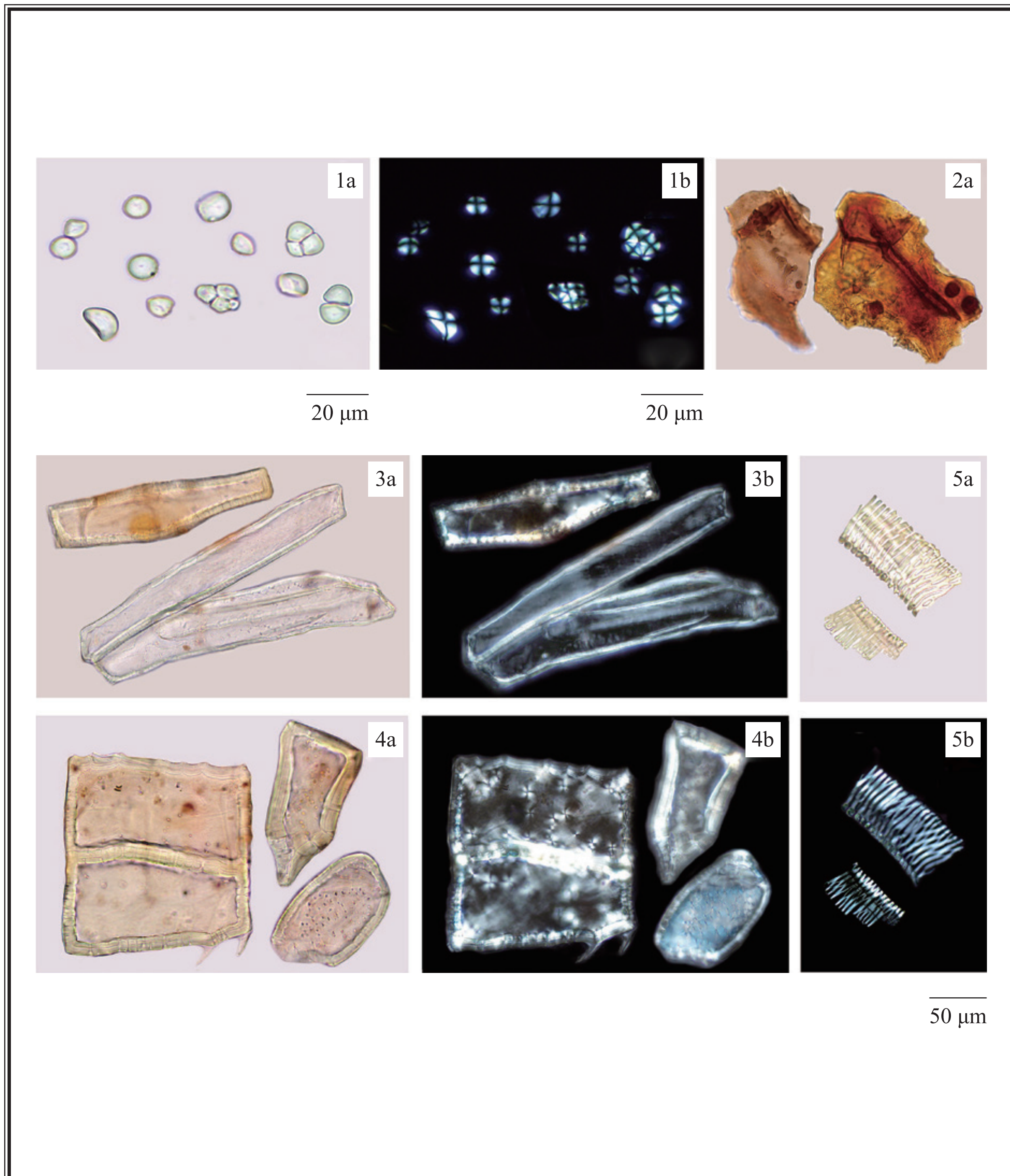


Figure 3 Microscopic features of powder of Aconiti Kusnezoffii Radix (unprocessed)

1. Starch granules 2. Metaderm cells 3. Sclerenchymatous cells 4. Stone cells 5. Vessels

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 3.5 mg of aconitine CRS (Fig. 4) and dissolve in 2 mL of a mixture of isopropanol and ethyl acetate (1:1, v/v). Keep at about 4ºC.

Hypaconitine standard solution

Weigh 2.5 mg of hypaconitine CRS (Fig. 4) and dissolve in 2 mL of a mixture of isopropanol and ethyl acetate (1:1, v/v). Keep at about 4ºC.

Mesaconitine standard solution

Weigh 2.5 mg of mesaconitine CRS (Fig. 4) and dissolve in 2 mL of a mixture of isopropanol and ethyl acetate (1:1, v/v). Keep at about 4ºC.

Developing solvent system

Prepare a mixture of ethyl acetate, *n*-hexane and methanol (6.8:6.4:1, 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

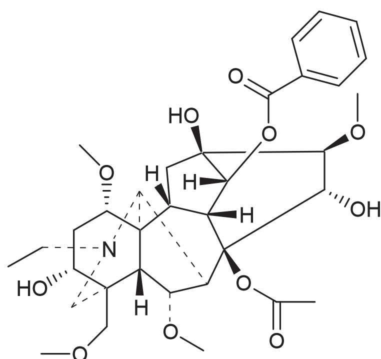
Weigh 2.0 g of the powdered sample and place it in a 50-mL conical flask, then add 2 mL of ammonium hydroxide solution (9.1%, w/v) and 20 mL of diethyl ether. Cap the flask. Sonicate (300 W) the mixture for 30 min in an ice water bath. Filter and transfer the filtrate to a 100-mL round-bottomed flask. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 1 mL of a mixture of isopropanol and ethyl acetate (1:1, v/v).

Procedure

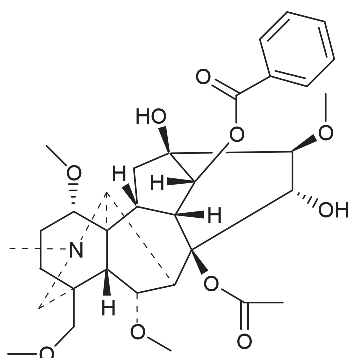
Carry out the method by using a HPTLC silica gel G60 plate, a twin trough chamber and a freshly prepared developing solvent system as described above. Apply separately aconitine standard solution (3 μL), hypaconitine standard solution (5 μL), mesaconitine standard solution (10 μL) and the test solution (10 μL) to the plate. Before the development, add

the developing solvent to one of the troughs of the chamber and 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 15 min. Carefully place the HPTLC plate in the trough containing the developing solvent for development. Develop over a path of about 7 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)

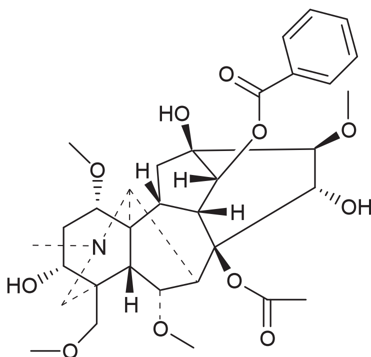


Figure 4 Chemical structures of (i) aconitine (ii) hypaconitine and (iii) mesaconitine

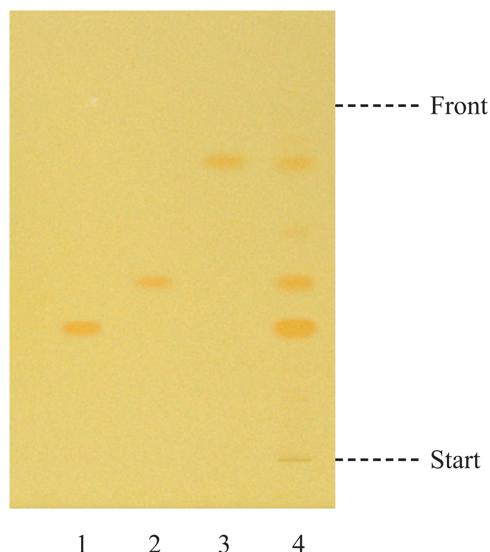


Figure 5 A reference HPTLC chromatogram of Aconiti Kusnezoffii Radix (unprocessed) extract observed under visible light after staining

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 (180 mg/L)

Weigh 0.9 mg of aconitine CRS and dissolve in 5 mL of 0.01% hydrochloric acid in methanol. Keep at about 4ºC.

Hypaconitine standard solution for fingerprinting, Std-FP (320 mg/L)

Weigh 1.6 mg of hypaconitine CRS and dissolve in 5 mL of 0.01% hydrochloric acid in methanol. Keep at about 4ºC.

Mesaconitine standard solution for fingerprinting, Std-FP (420 mg/L)

Weigh 2.1 mg of mesaconitine CRS and dissolve in 5 mL of 0.01% hydrochloric acid in methanol. Keep at about 4ºC.

Test solution

Weigh 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 2 mL of ammonium hydroxide solution (9.1%, w/v) and 20 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 10 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 four 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 four characteristic peaks of Aconiti Kusnezoffii Radix (unprocessed) extract are listed in Table 2.

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

Peak No.	RRT	Acceptable Range
1	0.31	± 0.03
2 (mesaconitine)	0.86	± 0.03
3 (marker, hypaconitine)	1.00	-
4 (aconitine)	1.04	± 0.03

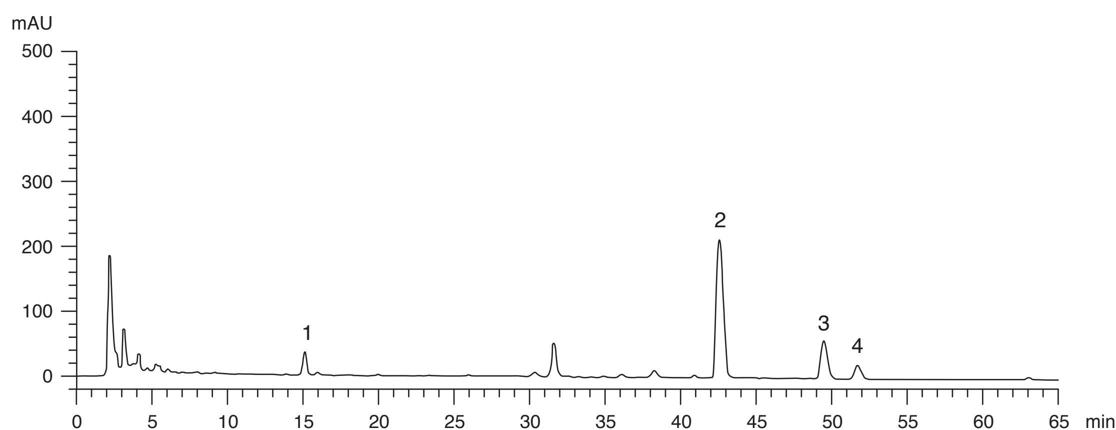


Figure 6 A reference fingerprint chromatogram of Aconiti Kusnezoffii Radix (unprocessed) extract

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. 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 XVII)*: meet the requirements.

5.5 Foreign Matter *(Appendix VIII)*: not more than 5.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 6.0%.
Acid-insoluble ash: not more than 1.5%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 10.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 23.0%.
Ethanol-soluble extractives (hot extraction method): not less than 20.0%.

7. ASSAY

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

Standard solution

Mixed aconitine, hypaconitine and mesaconitine standard stock solution, Std-Stock (900 mg/L for aconitine, 1600 mg/L for hypaconitine and 2100 mg/L for mesaconitine)
Weigh accurately 9.0 mg of aconitine CRS, 16.0 mg of hypaconitine CRS and 21.0 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 4.5, 9, 18, 36, 180 mg/L for aconitine, 16, 32, 64, 320, 640 mg/L for hypaconitine and 21, 84, 420, 840, 1050 mg/L for mesaconitine. Keep at about 4ºC.

Test solution

Weigh accurately 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 2 mL of ammonium hydroxide solution (9.1%, w/v) and 20 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 10 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 (18 mg/L for aconitine, 64 mg/L for hypaconitine and 420 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 0.39% to 0.77% 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.