## Appendix XIV: Detection of Aconitine, Hypaconitine and Mesaconitine

The Aconitum diester alkaloids mainly include aconitine, mesaconitine and hypaconitine. It is commonly present in herbs derived from plants belonging to the genera of Aconitum of the family Ranunculaceae. The major toxicity expresses in cardiotoxicity and neurotoxicity. To reduce the toxicity of the Chinese Materia Medica, an appropriate processing method should be adopted in order to reduce the total content of Aconitum diester alkaloids. In view of this, Chinese herbs containing aconitine, mesaconitine and hypaconitine should be examined and the limits of the above compounds should be established.

**Method**

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

**Standard solutions**

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

Weigh accurately 5.0 mg of aconitine CRS and dissolve in 5 mL of 0.01 M hydrochloric acid.

*Hypaconitine standard stock solution, Std-Stock (2000 mg/L)*

Weigh accurately 10.0 mg of hypaconitine CRS and dissolve in 5 mL of 0.01 M hydrochloric acid.

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

Weigh accurately 5.0 mg of mesaconitine CRS and dissolve in 5 mL of 0.01 M hydrochloric acid.

*Mixed aconitine, hypaconitine and mesaconitine standard solution for detection*

Measure accurately the volume of aconitine, hypaconitine and mesaconitine Std-Stock, mix and dilute with 0.01 M hydrochloric acid to produce a series of solutions of 0.5, 1, 2, 3, 4 mg/L for both aconitine and mesaconitine, and 5, 10, 20, 30, 40 mg/L for hypaconitine.

**Test solution**

Weigh accurately 0.5 g of the powdered sample and put into a 10-mL centrifugal tube, then add accurately 5 mL of methanol (50%). Sonicate (490 W) the mixture for 60 min. Centrifuge at about 1800 × *g* for 5 min. Filter through a 0.45-μm RC filter.

**Chromatographic system**

The liquid chromatograph is equipped with a detector (240 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size, pH: 1-12). The flow rate is about 1.0 mL/min. Programme the chromatographic system as follows –

| Time (min) | Ammonium bicarbonate* solution (%, v/v) | Acetonitrile (%, v/v) | Elution |
|---|---|---|---|
| 0 – 60 | 70 → 45 | 30 → 55 | linear gradient |

***Ammonium bicarbonate solution**

Dissolve 0.2 g of ammonium bicarbonate in 1 L of water and adjust the pH to 10 with 1 mL of ammonia solution.

**System suitability requirements**

Perform at least five replicate injections each with 20 µL of hypaconitine (standard solution for detection, 20 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of hypaconitine should not be more than 3.0%; the RSD of the retention time of hypaconitine peak should not be more than 2.0%; the column efficiency determined from hypaconitine peak should not be less than 30000 theoretical plates.

The *R* value between hypaconitine peak and the closest peak in the chromatogram of the test solution should not be less than 1.0.

**Calibration curve**

Inject a series of the mixed aconitine, hypaconitine and mesaconitine (standard solution for detection, 20 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 (standard solution for detection). Obtain the slopes, y-intercepts and the $r^2$ values from the corresponding 5-point calibration curves.

**Procedure**

Inject 20 µL of the test solution into the HPLC system and record the chromatogram. Identify aconitine peak, hypaconitine peak and mesaconitine peak in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed aconitine, hypaconitine and mesaconitine (standard solution for detection). The retention times of aconitine peaks, hypaconitine peaks and mesaconitine peaks from the two chromatograms should not differ from their counterparts by more than 2.0%. 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 indicated in Appendix IV(B). Calculate the sum of the content.

**Limit**

The total content of aconitine ($C_{34}H_{47}NO_{11}$), hypaconitine ($C_{33}H_{45}NO_{10}$) and mesaconitine ($C_{33}H_{45}NO_{11}$) in CMM samples should comply with the limit specified in the individual monograph.