Appendix XIII: Detection of Aristolochic Acid I

Aristolochic Acid I (AAI) is a known nephrotoxin and potential carcinogen that is commonly present in herbs derived from plants belonging to the genera of Aristolochia and Asarum of the family Aristolochiaceae. In view of this, the Department of Health announced that importation and sale of Chinese herbs containing AAI were prohibited starting from 1st June, 2004. There are unclear factors in local Chinese herbal market that may lead to inappropriate or misuse of the herbs containing Aristolochic Acid. It is suggested that the trader may apply the following method to detect AAI in the suspected Chinese herbs.

Method

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

Standard solution

Aristolochic acid I standard stock solution, Std-Stock (50 mg/L)

Weigh accurately 5.0 mg of aristolochic acid I CRS and dissolve in 100 mL of methanol.

Aristolochic acid I standard solution for detection

Measure accurately the volume of aristolochic acid I Std-Stock, dilute with methanol to produce solutions of 0.05 and 5 mg/L for aristolochic acid I.

Test solution

Weigh accurately 0.5 g of the powdered sample and put into a 50-mL centrifugal tube, and add 8 mL of methanol. Sonicate (490 W) the mixture for 30 min. Centrifuge at about 1800 × *g* for 10 min. Transfer the supernatant to a 25-mL volumetric flask. Repeat twice. Combine the extracts and make up to the mark with methanol. Mix and filter through a 0.45-μm RC filter.

Chromatographic system

The liquid chromatograph is equipped with a detector (396 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The flow rate is about 1.0 mL/min. The mobile phase is a mixture of acetonitrile and 1% acetic acid (52:48, v/v). The elution time is about 25 minutes.

System suitability requirements

Perform at least five replicate injections each with 10 μL of aristolochic acid I (standard solution for detection, 5 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of aristolochic acid I should not be more than 3.0%; the RSD of the retention time of aristolochic acid I peak should not be more than 2.0%; the column efficiency determined from aristolochic acid I peak should not be less than 10000 theoretical plates.

Procedure

Separately inject aristolochic acid I (standard solution for detection, 0.05 mg/L) and the test solution (10 μL each) into the HPLC system and record the chromatograms. Identify aristolochic acid I peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of aristolochic acid I (standard solution for detection). The retention times of aristolochic acid I peaks from the two chromatograms should not differ by more than 2.0%.

Limit

Aristolochic acid I ($C_{17}H_{11}NO_7$) should not be detected in CMM samples, unless otherwise specified.