

Methods for Determining Aristolochic Acid in Various Matrices

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Key Words

Aristolochic Acid, Chinese Herbal Remedies, Nephrotoxin

Goal

To develop an HPLC-coulometric electrode array method capable of resolving aristolochic acid I (AA1) and aristolochic acid II (AA2).

Introduction

Aristolochia species (e.g., Virginia snakeroot, guaco) are common ingredients in traditional Chinese herbal remedies and herbal dietary supplements. Unfortunately, over the last ten years consumption of herbals containing *Aristolochia* has resulted in numerous cases of late stage renal failure often associated with urothelial tract carcinoma. Initially termed “Chinese herbs neuropathy” the disease has now been renamed “aristolochic acid nephropathy” in recognition of the active toxin(s) present and the fact that *Aristolochia* species are used in many non-Chinese herbal supplements. In 2000, the FDA released a warning to health care professionals warning of the consequences of *Aristolochia* consumption.¹

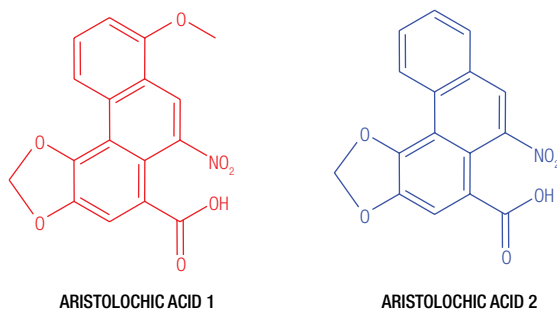


Figure 1. Chemical structures of Aristolochic Acid 1 and 2.



The principal nephrotoxin present is aristolochic acid (AA). AA is actually composed of a group of several nitrophenanthrene carboxylic acids – aristolochic acid I and aristolochic acid II being the most abundant (see Figure 1 for structures). Neither AA1 nor AA2 are toxic per se. However, upon metabolic activation they form reactive intermediates that are capable of damaging DNA. The first part of the mechanism involves partial reduction of AA1/AA2 by cytosolic reductases (e.g., DT-diaphorase, xanthine oxidase or prostaglandin H synthase, the latter being very abundant in the kidney).^{2,3} The resulting nitrenium ion preferentially forms an adduct with purines. The most persistent adduct is 7-(deoxyadenosin-N(6)-yl) aristolactam,¹ which leads to AT→TA transversion in vitro.⁴ As this lesion occurs with a high frequency in codon 61 of the H-ras oncogene, this may explain why AA is so carcinogenic.⁴ It is currently thought that such adduct formation is not only responsible for tumor development but also the destructive fibrotic process in the kidney.

Several methods are currently being used to measure AA in plant extracts including HPLC with photodiode array detection^{5,6,7} and LC-MS.⁸ Presented here in this application brief is a highly selective and sensitive method for measurement of AA. These preliminary data suggest that this method will be of use in the measurement of AA in both plant and animal tissues.

Materials and Methods

The isocratic analytical system consisted of a pump, an autosampler, a thermostatic chamber, a single channel UV detector placed before a twelve-channel Thermo Scientific™ Dionex™ CoulArray™ Electrochemical Detector (ECD).

Conditions

LC

Column:	C18, 5 μ m, 4.6 \times 150 mm
Mobile Phase A:	Acetonitrile – water, 70:10 (v/v) containing 100 mg/L sodium dodecyl sulfate; final pH 2.0 with phosphoric acid
Flow Rate:	1.0 mL/min
Temperature:	Ambient
Injection Volume:	50 μ L

Detector

Electrochemical Detector:	Model 5600A, CoulArray
Applied Potentials:	200 to 900 mV in 100 mV increments (vs. Pd).
Cell Cleaning:	All cell potentials were set to 1000 mV (vs. Pd) for 0.5 min at the end of each analysis to minimize electrode surface adsorption
Detector Wavelength:	252 nm

Standard Preparation

Commercially available standard (aristolochic acid sodium salt, Sigma – A4951) was dissolved in water at a concentration of 1 mg/mL. The percentage composition reported was 61% AA1 and 19% AA2.

Results and Discussion

The chromatographic and response behavior of the aqueous standard is presented in Figure 2. Comparison of EC (dominant channel) and UV (optimal wavelength) responses is presented in Figure 3. The sensitivity of EC detection is calculated to be more than 50 times that of UV detection based on maximal response only or more than 500 times that of UV based on detection limits (S/N of 3:1). The limit of sensitivity for EC detection is calculated to be \sim 100pg on column.

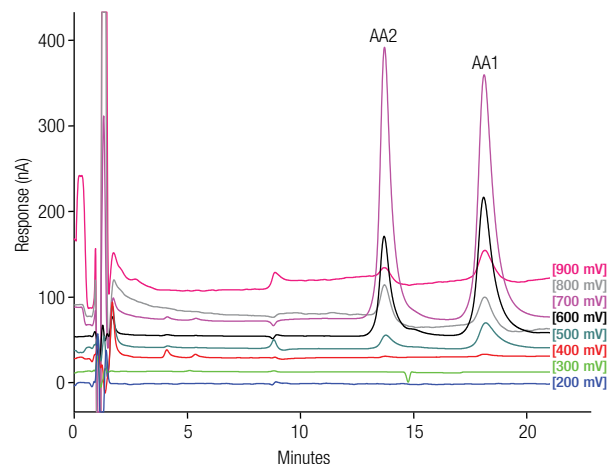


Figure 2. Separation and voltammetric behavior of AA1 and AA2 (50 ng each on column).

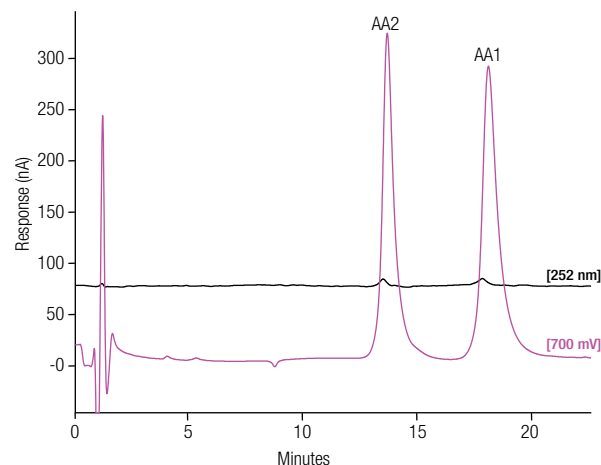


Figure 3. Comparison of EC (700 mV) and UV (252 nm) responses for AA1 and AA2 (50 ng each on column).

Conclusion

This preliminary method shows the measurement of AA1 and AA2. The method is much more sensitive than PDA-based methods and is less complex and versatile than expensive LC-MSn approaches. Using this method it should be possible to measure AA in both herbal extracts and animal tissues.

Ordering Information

Description	Part Number
CoulArray, Model 5600A - 12 Channels	70-4329
CoulArray Organizer with Temp. Control	70-4340T
Accessory Kit, CoulArray Detector to UltiMate 3000 System	70-9191

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