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Arsenic speciation in tree moss by mass spectrometry based hyphenated techniques

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ABSTRACT

A method based on ion-pair reversed phase high performance liquid chromatography (HPLC) hyphenated with inductively coupled plasma mass spectrometry (ICP-MS) was developed for arsenic speciation in extract of tree moss. Under the optimal conditions, the limit of detection of eight arsenic species including arsenite (As^{III}), arsenate (As^{V}), monomethylarsonic acid (MMA), dimethylarsonic acid (DMA), trimethylarsinoxide (TMAO), tetramethylarsonium (Tetra), arsenocholine (AsC) and arsenobetaine (AsB) is between 0.04 and 0.07 ng/mL, with a linear range of 0.2 − 500 ng/mL. Three unknown arsenic species (Unk1, Unk2 and Unk3) and six specific arsenic species (As^{III}, As^{V}, DMA, TMAO, Tetra and AsB) were detected in the extract of tree moss. Unk3 was identified as a kind of arsenosugars (2,3-dihydroxypropyl-5-deoxy-5(dimethylarsenoso)furanoside, arsenosugar X) by electrospray ionization quadrupole time-of-flight mass spectrometry (ESI-qTOF-MS).

1. Introduction

Arsenic is a carcinogenic and mutagenic source with an average concentration of 5 μg/g in the earth's crust [1]. The emission of arsenic into the atmosphere are caused by natural phenomena such as weathering, biological activity, volcanic activity, and anthropogenic inputs. Then it is redistributed on the earth's surface by rain and dry fallout from atmosphere. The toxicity of arsenic species is related to its chemical forms, generally arsenite (As^{III}) > arsenate (As^{V}) > monomethylarsonic acid (MMA) > dimethylarsonic acid (DMA) > trimethylarsinoxide (TMAO), tetramethylarsonium (Tetra), arsenobetaine (AsB), and arsenocholine (AsC) [2].

Tree moss (*Ramalina fastigiata (Pers.) Ach.*) is a kind of lichen, which is a composite organism that arises from algae and/or cyanobacteria living among filaments of a fungus in a symbiotic relationship [3]. It spreads over the surface of stones and trees, and the extract of tree moss is applied to produce fragrant substances [4], and widely used in cigarette, food and perfume industry to get a fragrance of mixture of grass and tree. When the surrounding environment was polluted by arsenic, the tree moss would uptake arsenic and cause potential health risk. Nearing et al. [5] studied the uptake and transformation of arsenic during the vegetative life stage of terrestrial fungi. It was found that As^{V} could transform to TMAO in *Sparassis crispa*. It is also reported that arsenic could be accumulated in lichens [6], and the concentration of total arsenic is about ten micrograms per gram in dry lichen. Two kinds of arsenosugars were first identified by Edmonds *et al.* in brown kelp (*Ecklonia radiata*) [2], which may be the intermediates in the biological methylation of inorganic arsenic. In the extracts of terrestrial fungi and lichens from Yellowknife, Canada, Koch et al. [7] found various arsenic species including As^{III}, As^{V}, MMA, DMA, TMAO, Tetra, AsB and Arsenosugar X. The natural occurrence of arseno compounds in plants, lichens, fungi, algal species, and microorganisms was reviewed by Dembitsky et al. [8]. The above studies indicate that tree moss has the capability to accumulate arsenic and convert arsenic into organo-arsenic species. Due to the wide use of tree moss extracts as fragrant substances and possible toxicity caused by arsenic, arsenic speciation in the extracts of tree moss should be a vital step before it is used as an additive in products.

Hyphenated techniques are effective methods for elemental speciation. Various separation techniques, including gas chromatography (GC) [9,10], capillary electrophoresis (CE) [11,12] and high performance liquid chromatography (HPLC) [13,14], have been applied for the separation of arsenic species. Among them, HPLC is the most widely used method in arsenic speciation. Ultraviolet (UV) is the most commonly used detector for HPLC, but the limit of detection for arsenic species is relatively high. As element-specific detectors, atomic absorption spectrometry (AAS) [15], atomic fluorescence spectrometry (AFS) [9], inductively coupled plasma optical emission spectrometry (ICP-OES) [16] and inductively coupled plasma mass spectrometry (ICP-MS) can provide higher sensitivity for arsenic than UV detector. Compared with other elemental-specific techniques, ICP-MS exhibits high sensitivity, wide dynamic range, and good resistance to complex

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matrix. HPLC hyphenated with ICP-MS detection has become one of the most powerful techniques for arsenic speciation [17,18]. In HPLC analysis of arsenic species, ion exchange chromatography (IEC) [13,18] and ion pair reversed phase (IP-RP)-HPLC [14,19–21] are commonly used separation modes. In contrast to IEC, simultaneous separation of both charged and uncharged analytes can be achieved in RP-HPLC with the addition of ion pair reagents.

However, HPLC-ICP-MS only provides quantification information. For those unknown arsenic species observed in real samples, organic mass spectrometry is an essential tool to get the structure information. By using HPLC-ICP-MS together with electrospray ionization (ESI)-MS, Madsen et al. [22] identified four arsenosugars in an algal extract. Bluemlein et al. [23] analyzed arsenic peptides in an ornamental garden plant (*Thunbergia alata*) by LC-ES-MS/ICP-MS. Low-molecular-mass thio-organoarsenical compounds in the form of As^{III} -phytochelatins were found in root of *Thunbergia alata* exposed to arsenate. Therefore, hyphenated technique of HPLC-ICP-MS supplemented with ESI-MS would provide more useful information for arsenic speciation.

The aim of this work is to develop a new method by combining HPLC-ICP-MS with high resolution ESI-quadrupole time-of-flight (qTOF)-MS/MS for the identification and quantification of arsenic species in the extracts of tree moss. The extracts of tree moss sample were prepared by Soxhlet extraction prior to HPLC-ICP-MS analysis. For identification of unknown arsenic species by ESI-qTOF-MS/MS, the extracts of tree moss were further subjected to solid phase extraction and HPLC separation for the removal of complex matrix and purification, respectively.

2. Materials and methods

2.1. Materials and chemicals

Stock solutions (1.000 mg/mL as As) of eight arsenic standards (As^{III}, As^{V}, MMA, DMA, AsB, TMAO, Tetra and AsC) were prepared with $NaAsO_2$ (> 90%, Wako, Japan), $Na_2AsO_7{\cdot}H_2O$ (> 99%, Wako, Japan), $CH_3AsO_3Na_2$ (> 98.5%, J&K Chemical Ltd, China), $C_2H_6AsO_2Na{\cdot}H_2O$ (> 98.5%, Genebase Bioscience Co., Ltd , China), $C_5H_{14}AsBrO$ (> 95%, Wako, Japan), $(CH_3)_3AsO$ (> 95%, J&K Chemical Ltd, China), $(CH_3)_4AsI$ (> 97%, J&K Chemical Ltd, China) and $C_5H_{11}AsO_2$ (> 95%, Wako, Japan) in high purity water, respectively. The mixed standard solution was prepared by diluting the stock solution daily. All the concentration units stated in the whole manuscript are expressed as the concentration of As rather than that of As species. Malonic acid was obtained from Sinopharm Chemical Reagent Co. Ltd (Shanghai, China), and 1-Butanesulfonic acid sodium (BSAS) was obtained from Tianjin Aoran Fine Chemical Research Institute (Tianjin, China). Tetramethylammonium hydroxide (TMAH) (~25% in H_2O) was obtained from Aladdin Industrial Corporation (Shanghai, China). High purity water was obtained by a Milli-Q water purification system (18.25 MΩ cm, Millipore, Molsheim, France). Other reagents used in the experiment were analytical grade unless otherwise mentioned. The containers employed in experiment were stored in 10% (v/v) nitric acid over 24 h, and rinsed with tap water and high purity water prior to use. Tree moss samples were kindly provided by Mr. Weiping Yang (Zhengzhou Institute of Tobacco, Zhengzhou, Henan, China).

2.2. Instruments

A CAPCELL PAK C18 column (250 mm × 4.6 mm, 5 μm particle size) was used for the separation of target arsenic species. The HPLC system consisted of an LC-10AD high pressure pump and a DGU-12A degasser (Shimadzu, Japan). A quadrupole ICP-MS (Agilent 7500a, Japan) with a Babington nebulizer was interfaced to HPLC via a minimum length piece of Teflon tubing (i.d. 0.5 mm, length 30 cm) with a finger-tight PEEK fitting. Optimization of the ICP-MS instrument (i.e. lens settings, sampling depth and carrier gas flow rate) was performed with conventional pneumatic nebulization (PN)-ICP-MS prior to being connected with HPLC. A semi-preparative C18 chromatographic column (250 mm × 10 mm, 10 μm particle size) was purchased from Soochow High Tech Chromatography CO., LTD. (Soochow, China) to enrich and purify unknown arsenic species. A microTOF-Q III MS with an ESI ion source (Bruker, German) was used for identification of unknown arsenic species. The operating conditions for HPLC-ICP-MS are summarized in Table 1. An electrically-heated thermostatic water bath (DF-101S, Keer, Wuhan) was used for Soxhlet extraction of arsenic species in tree moss. A rotary evaporator (RE-52AA, Yarong, Shanghai) was employed to remove the extraction solvent and enrich target analytes at 70 °C. Ultracentrifugation (ThermoFisher scientific, German) was applied to separate insoluble matter in the extract of tree moss. C18 solid phase extraction (SPE) cartridge (Agilent) was used to eliminate the impurities in tree moss extract.

Table 1
Operation conditions for HPLC-ICP-MS.

Instrumental conditions	
HPLC	
Column	CAPCELL-PAK C18 MG-II (250 mm × 4.6 mm, 5 μm)
Mobile phase A	10 mmol/L sodium butanesulfonate, 4 mmol/L TMAH and 4 mmol/L malonic acid, methanol/water (0.1/99.9, v/v), pH 3.0
Mobile phase B	5 mmol/L ammonium acetate, methanol/water (1/99, v/v), pH 7.0
Mobile phase C	methanol/water (0.1/99.9, v/v), pH 3.0
Flow rate	1.0 mL/min
Column temperature	ambient temperature
Injection volume	60 μL
ICP-MS	
Rf power	1150 W
Rf matching	6.2 V
Sampling depth	6.8 mm
Carrier gas	1.1 L/min
Time-resolved data acquisition	
Scanning mode	Peak-hopping
Dwell time	100 ms
Integration mode	Peak area
Detected isotope	^{75}As

2.3. Analytical procedure

The analysis procedure for arsenic speciation in tree moss is presented in Fig. 1. It can be divided into two parts, one is the quantification of specific arsenic species, and the other is the identification of the unknown arsenic species.

2.3.1. Quantification of specific arsenic species

2.3.1.1. Soxhlet extraction. To prepare the extracts of tree moss, benzene, petroleum ether and ethanol are the usually employed extraction solvents [24]. Here, ethanol was chosen as the extract solvent. Briefly, 5.38 g of tree moss and 200 mL extraction solvent consisting of ethanol and water (3:1, v/v) were added into a round bottomed flask. Soxhlet extraction was carried out for 3 h in water bath at 95 °C. After extraction, the majority of extraction solvent in the crude extract of tree moss was removed with the rotary evaporation apparatus. Next, the condensed crude extract was centrifuged at 12,000 rpm for 10 min and the supernatant was collected. The sediment after centrifugation was washed twice by high purity water with the assistance of vortex and then centrifuged to get the supernatant. All supernatant was merged as the extraction fraction and diluted to 25 mL with high purity water for following HPLC-ICP-MS analysis.

2.3.1.2. HPLC-ICP-MS. The operation conditions of HPLC for the separation of As^{III}, As^{V}, MMA, DMA, AsB, TMAO, Tetra and AsC was