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Simultaneous characterization of selenium and arsenic analytes via ion-pairing reversed phase chromatography with inductively coupled plasma and electrospray ionization ion trap mass spectrometry for detection Applications to river water, plant extract and urine matrices

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ABSTRACT

With an increased awareness and concern for varying toxicities of the different chemical forms of environmental contaminants such as selenium and arsenic, effective methodologies for speciation are paramount. In general, chromatographic methodologies have been developed using a particular detection system and a unique matrix for single element speciation. In this study, a routine method to speciate selenium and arsenic in a variety of "real world" matrices with elemental and molecular mass spectrometric detection has been successfully accomplished. Specifically, four selenium species, selenite, selenate, selenomethionine and selenocystine, and four arsenic species, arsenite, arsenate, monomethylarsonate and dimethylarsinate, were simultaneously separated using ion-pairing reversed phase chromatography coupled with inductively coupled plasma and electrospray ionization ion trap mass spectrometry. Using tetrabutylammonium hydroxide as the ion-pairing reagent on a C_{18} column, the separation and re-equilibration time was attained within 18 min. To illustrate the wide range of possible applications, the method was then successfully applied for the detection of selenium and arsenic species found naturally and spiked in river water, plant extract and urine matrices.

1. Introduction

Selenium, an element essential for human survival, has been shown to have a narrow range between deficiency and toxicity [1]. Needed at only trace levels in the human body, selenium provides many pertinent biological properties including evidence for cancer chemoprevention [2–4] and protection against oxidative damage through enzymes such as glutathione peroxidase [5]. The common forms of selenium in the environment are selenite (Se^{IV}), selenocystine ($SeCys_2$), selenomethionine (SeMet) and selenate (Se^{VI}) [6], ordered in decreasing toxicity [7]. When considering the range of toxicities from the different chemical forms of selenium and the growing concern drawn from high levels found in various contaminated environments [8], selenium speciation is vital for proper analysis and assessment in the environment and biological systems.

While there is possible evidence for the essentiality of arsenic at trace levels [9], its toxic effects are well known. Anthropogenic activities, such as wood treatment [10] and mining/processing of ore [11], have increased arsenic levels beyond natural concentrations thereby causing worldwide concern for the environment. The most common arsenic species include arsenite (As^{III}), arsenate (As^{V}), monomethylarsonate (MMA) and dimethylarsinate (DMA), which are listed with decreasing toxicity [12]. Due to the significantly different levels in toxicity of arsenic forms, speciation of arsenic is paramount to effectively assess the risk of a given environmental or biological sample.

In general, speciation methods have been developed for varying forms of single elements. With the knowledge of accumulating environmental contamination from different sources, a need for routine multi-element speciation methods is apparent in order to meet the impending financial and time requirements of today. In the past, several separation techniques have been developed to speciate different selenium and arsenic forms in various matrices with some of the more recent given here [13,14]. In addition to the separation of arsenic species, which has been typically accomplished using anion exchange chromatography [15–17], simultaneous speciation of selenium and arsenic species has also been shown, but in far fewer publications [18–20]. With these methods, only the inorganic selenium species were resolved along with varying arsenic

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species. This study addresses this shortcoming by including both organic and inorganic species of these metalloids in one chromatographic run.

Ion-pairing reversed phase liquid chromatography (IPRP) has been utilized for organic selenium speciation in prior studies [21–23]. In addition, a few studies have incorporated IPRP for the simultaneous separation of selenium and arsenic species. Le et al. separated 13 arsenic and selenium species using hexanesulfonate as the ion-pairing reagent [5]; however, when the method was tested with a urine matrix, a different column and mobile phase were needed along with elevated temperatures to achieve the desired separation. Do et al. separated 10 arsenic and selenium species employing tetrabutylammonium phosphate as the ion-pairing reagent [24]; although, online compatibility with inductively coupled plasma mass spectrometry was not investigated. More recently, Pan et al. separated 12 selenium and arsenic species using tetrabutylammonium hydroxide as the ion-pairing reagent [25]; however, Se^{VI}, a major species in nature, had an elution time of approximately 40 min. Currently, there remains a need for the simultaneous separation of the eight predominant selenium and arsenic species: As^{III}, As^{V}, MMA, DMA, Se^{IV}, Se^{VI}, SeMet and $SeCys_2$, in a timely and sensitive manner with online detection.

Several instruments have been used for the detection of selenium and arsenic including atomic fluorescence spectrometry [26,27], atomic absorption spectrometry [28,29] and inductively coupled plasma mass spectrometry (ICPMS) [30,31]. Of the frequently used techniques listed, ICPMS has proven to be one of the most sensitive analytical techniques for fast multi-element determination of metals at ultra trace concentrations in different sample matrices, and is amenable to coupling with various chromatographic methodologies [32]. A key reason for the enhanced speciation capability with ICPMS was the advent of the collision/reaction cell, which gave rise to the removal of polyatomic interferences, through collision, reaction and energy discrimination, from the analyte of interest (i.e. $^{40}Ar^{40}Ar$ for ^{80}Se and $^{40}Ar^{35}Cl$ for ^{75}As). This allowed for a greater signal to noise ratio and improved detection capabilities.

Newer speciation methodologies should include molecular mass spectrometry techniques in order to provide specific molecular weight information. Electrospray ionization ion trap mass spectrometry (ESI-ITMS) has been used as an advantageous companion to the element specific detection of ICPMS [33,34]. While ESI-ITMS suffers from high detection limits when compared to ICPMS, ESI-ITMS can provide structural information to confirm unknown compounds by mass (MS^n) as a compliment to retention time matching and sample spiking assignments of ICPMS. To date, the chromatographic methods mentioned above have not investigated the feasibility of ESI-ITMS in conjunction with the ICPMS for species identification.

In past studies, chromatographic methodologies for the speciation of varying arsenic and selenium analytes generally have been limited to a single application in "real world" matrices. The variance in composition of different matrices often causes insurmountable challenges in developing a universal separation; however, the ability of a speciation method to successfully separate the analytes of interest within various sample matrices is imperative to the overall achievement of a robust and valuable separation. Comprehensive studies reviewing arsenic and selenium separations listed water, plant and urine samples as common environmental and biological matrices for method applications [13,14]. Currently, a single method for the simultaneous separation of common selenium and arsenic analytes within these matrices has not yet been shown.

In this study, a method was developed to accommodate the growing demands of multi-elemental speciation. Eight common environmentally and biologically observed arsenic and selenium standards were baseline separated including re-equilibration time within 18 min on a C_{18} chromatography column via ion-pairing reversed phase chromatography. Both ICPMS and ESI-ITMS were incorporated as detectors without modifying the separation methodology, which lead to retention time matching and confident structural identification. The chromatographic method was then successfully applied to three environmental and biological matrices: river water, plant extract and urine to illustrate the wide range of potential applications.

2. Experimental

2.1. Instrumentation

2.1.1. High-performance liquid chromatography

Chromatographic separations were accomplished with an Agilent 1100 liquid chromatograph by Agilent Technologies (Santa Clara, CA) equipped with a binary HPLC pump, an autosampler, a vacuum de-gasser system and a thermostated column compartment. Reversed phase chromatography was carried out with a ZORBAX Eclipse XDB-C_{18} column (5 μm × 4.6 mm id × 250 mm) from Agilent Technologies (Santa Clara, CA).

2.1.2. Inductively coupled plasma mass spectrometry

The ICPMS used for specific element detection was an Agilent 7500ce by Agilent Technologies (Santa Clara, CA). The instrument was equipped with a microconcentric nebulizer made by Glass Expansion (Pocasset, MA), a Scott double channel spray chamber (2 °C), a shielded torch, a CE lens stack, an octopole collision/reaction cell with hydrogen gas pressurization (purity of 99.999%), a quadrupole mass analyzer and an electron multiplier for detection.

2.1.3. Electrospray ionization ion trap mass spectrometry

The ESI-ITMS used for molecular identification was a 6300 Agilent LC/MSD Trap XCT Ultra from Agilent Technologies (Santa Clara, CA) used in the conventional LC introduction mode. The effluent from the column was sent directly, via a 40 cm length 0.25 mm ID PEEK tube from the column outlet, to the ESI interface with a high-flow spacer installed. The ESI-ITMS spectra were acquired in negative ion mode. For tuning purposes, individual arsenic and selenium standards diluted in buffer A were introduced via a syringe pump obtained from KD Scientific (Holliston, MA) at a rate of 10 μl min^{-1}.

For all instrumental conditions, see Table 1.

2.2. Reagents and standards

All the solutions were prepared in 18 MΩ cm^{-1} doubly deionized water (DDW) processed by Sybron/Barnstead (Boston, MA). Standards used for sample spiking and identification were the following: disodium methylarsonate hexahydrate (MMA) purchased from Chem Service (West Chester, PA); L(+)-selenomethionine (SeMet) was selected as it is the form found within biological samples such as plants [35] and obtained from Acros Organics (Morris Plains, NJ); sodium (meta)arsenite (As^{III}), Cacodylic acid (DMA) and seleno-L-cystine ($SeCys_2$) were acquired from Fluka (Milwaukee, WI); potassium arsenate (As^{V}), potassium selenate (Se^{VI}) and sodium selenite (Se^{IV}) were purchased from Sigma–Aldrich (St. Louis, MO).

Claritas PPT arsenic and selenium elemental standards used for quantification were acquired from SpexCertiPrep (Metuchen, NJ). Calibration standards of 1.0–500 μg l^{-1} were prepared through dilution from a stock solution with 5% (v/v) HNO_3.