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Speciation of selenite and selenoamino acids in biota samples by dual stir bar sorptive extraction-single desorption-capillary gas chromatography/ mass spectrometry

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ABSTRACT

A method of speciation of selenite (Se(IV)), selenomethionine (SeMet) and selenomethylselenocysteine (SeMeSeCys) has been developed in clams. It is based on dual stir bar sorptive extraction (dual-SBSE) coupled to thermal desorption (TD) and capillary gas chromatography mass spectrometry (GC–MS) operating in selected ion-monitoring mode (SIM). Samples are extracted by ultrasonic probe assisted enzymatic hydrolysis. Se(IV) is derivatized to piazselenol and selenoamino acids to the N-isobutoxycarbonyl methyl ester derivatives and diluted with water prior to SBSE. The optimised method consists of a dual SBSE extraction performed on both derivatized extracts. After extraction, the two stir bars are placed in a single glass thermal desorption liner and simultaneously desorbed. The method showed good linearity ($R > 0.999$), high sensitivity (limits of detection of 0.008, 0.070 and 0.180 ng as Se for Se(IV), SeMet, SeMeSeCys, respectively), reproducibility (better than 13%) and recoveries (higher than 85%). The accuracy of the analytical method applied to the determination of SeMet was studied in the certified reference material SELM-1 yeast, and no significant differences were found between the determined value (1419 ± 115 mg Se kg^{-1}) and the certified value (1364 ± 70 mg Se kg^{-1}). The method was applied to the speciation analysis of selenium in clams exposed to waterborne Se(IV) (750 μg Se L^{-1}), SeMeSeCys (10 μg Se L^{-1}) and SeMet (10 μg Se L^{-1}) for two months.

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1. Introduction

Selenium is an essential micronutrient that plays an important role in many biological processes through the action of seleno-proteins that have an important antioxidant and detoxification function in animals [1]. Selenium bioavailability and toxicity depend on its chemical form and concentration in foods. Generally, organic compounds of Se are more bioavailable than the inorganic analogs [2].

The chemical speciation of selenium is of great interest to study the presence of inorganic selenium and selenoamino acids. These works are carried out by high-performance liquid chromatography (HPLC) in combination with sensitive and selective detectors including inductively coupled plasma mass spectrometry (ICP-MS) [3,4], atomic fluorescence (AFS) [5] and mass spectrometry (MS) [6]. Gas chromatography technique was also applied for selenium speciation because of its high resolution. It has been coupled with ICP-MS [7,8], atomic emission detector (AED) [9], flame photometric detector (FPD) [9], microwave induced plasma atomic emission spectrometry (MIP-AES) [10] and MS [11–13]. On the other hand, selenoamino acids and inorganic selenium are nonvolatile species and a derivatization reaction is needed before gas chromatographic analysis. Determination of inorganic selenium is based on volatilisation of ionic Se(IV) by the use of suitable derivatization reagents. Sodium tetraethylborate and sodium tetrapropylborate [14–17] have been used to form diethylselenide. Another group of reagents which was reported for increased sensitivity included 4-chloro-o-phenylenediamine or 4,5-dichloro-1,2-phenylenediamine compounds [15,18]. They have been used to form the corresponding piazselenol. In the case of selenoamino acids, the alkyl chloroformates have been used to formation of derivatives in just one step [7–13].

In order to improve the sensitivity and selectivity for speciation of selenium in real samples, a sample pre-treatment step is usually required before the analysis. Several authors used solid phase microextraction (SPME) as a sample preparation strategy for selenite [15–18] and selenoamino acids [7]. More recently, stir bar sorptive extraction (SBSE) was introduced by Baltussen et al. [19], as another preconcentration technique and it has been employed to extract selenoamino acids from a variety of matrices [12,20].

The aim of this work was to develop an extraction and preconcentration procedure for selenite and selenoamino acid analysis in biota samples. The method involved conversion of the selenoamino acids of interest to their corresponding volatile alkyl chloroformate

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derivatives and selenite to piazselenol in two aliquots of sample. Then the derivatives were extracted using two stir bars. After sampling, both stir bars were introduced in one thermal desorption tube and analysed simultaneously by capillary GC–MS. The proposed method was applied to the speciation of selenium in clam soft tissues with satisfactory results.

2. Experimental

2.1. Reagents

All chemicals and solvents were of analytical-reagent grade and purchased from Sigma-Aldrich and Fluka. The metallic forms of ^{74}Se (99.60 ± 0.2% enriched) were purchased from Chemotrade, Düsseldorf, Germany. Ultra pure Milli-Q water (18 MΩ cm) (Millipore, Bedford, MA, USA) was used throughout.

Stock solutions of selenomethionine (purity > 99%), seleno-methyl-selenocysteine (>99.5%), selenomethionine-(methyl-^{13}C) (99 atom % ^{13}C) and Se(IV) (approximately 1000 mg Se L^{-1}) were prepared in 0.1 M HCl and stored at 4 °C. Working solutions were prepared daily by dilution. All dosages were controlled gravimetrically. Sodium selenite enriched with ^{74}Se was prepared by oxidation of the enriched metallic ^{74}Se in concentrated metal-free nitric acid and subsequent neutralization with 1 M NaOH.

A 1.0% (w/v) aqueous solution of sodium tetraethylborate was prepared in a 2% (w/v) sodium hydroxide medium. A 1.0% (w/v) aqueous solution of 4-chloro-1,2-phenylenediamine was prepared in a 0.1 M hydrochloric acid ethanol solution.

2.2. Apparatus

A SONOPULS ultrasonic homogenizer (Bandelin, GmbH & Co. KG) fitted with a HF generator 2200 and a Digiten 21 centrifuge were used for sample preparation. The homogenizer was equipped with a titanium microtip of 3 mm diameter. Different stir bars (glass-encapsulated magnetic stir bar coated externally with a PDMS layer) obtained from Gerstel (Mülheim an der Ruhr, Germany) were studied taking into account its PDMS thickness (0.5 or 1 mm) and length (10 or 20 mm). They were conditioned for 15 min at 300 °C with helium at a desorption flow of 50 mL min^{-1}, and kept in new 2 mL vials until immediately prior to use. For the extraction, a 20 mL vial from Supelco was used.

2.3. Derivatization and extraction of selenoamino acids

A 100 μL aliquot of standard solution or sample were placed in a 20 mL headspace vial, adding 200 μL of the water:methanol:pyridine (60:32:8) mixture and 40 μL of isobutyl chloroformate. The mixture was vigorously shaken for 60 s at room temperature. Gas evolution (carbon dioxide) usually occurs. After derivatization, the mixture was diluted to 1.5 mL of 100 g NaCl L^{-1} in water, a 63 μL PDMS stir bar was added and a teflon-coated silicone septum cap was placed on the vial without crimping. SBSE was performed at room temperature (23 ± 1 °C) for 30 min while stirring at 900 rpm.

2.4. Derivatization and extraction of selenite using 4-chloro-1,2-phenylenediamine

Portions of 3 mL of water containing 500 μL of standard solution or hydrolyzed sample were placed in a 40 mL headspace and 200 μL of the 1.0% (w/v) 4-chloro-1,2-phenylenediamine solution was added. Then a 126 μL PDMS stir bar was immersed in the sample and the vial was closed with a cap provided with PTFE silicone septum. SBSE was performed at room temperature (25 ± 1 °C) for 60 min while stirring at 600 rpm.

2.5. Derivatization and extraction of selenite using sodium tetraalkylborate

Portions of 5 mL of water containing 500 μL of standard solution were placed in a 40 mL vial and 0.5 mL of acetate/acetic buffer solution (1 M) to adjust the pH to 5 and then 150 μL of 1.0% (w/v) $NaBEt_4$ solution was added. SBSE was performed as in Section 2.4 but using an extraction time of 20 min.

Derivatization and extraction conditions are summarized in Table 1.

2.6. Instrumentation and TD-GC–MS conditions

Once the extraction step was finished, the stir bar was rinsed with Milli-Q water in order to eliminate the possible rests of salts and dried with a paper tissue before introduction into a glass thermodesorption tube (TD). The TD tube was then placed in the TD system where the stir bar was subjected to on-line thermal desorption coupled to gas chromatography–mass spectrometry (TD-GC–MS).

TD-GC–MS was performed using a TDS 2 thermodesorption system equipped with a TDS-A autosampler and a CIS 4 programmable temperature vaporization (PTV) inlet (Gerstel), and an Agilent 6890N gas chromatograph with a 5973 mass spectrometry detector (Agilent Technologies).

TDS 2 temperature was programmed to increase from 40 °C (held for 1 min) to 270 °C (held for 6 min) at 60 °C min^{-1}. The desorbed compounds were trapped at 5 °C on a quartz wool packed liner in the PTV system with liquid carbon dioxide. Finally, the PTV system was programmed to increase from 5 to 250 °C (held for 7 min) at 10 °C s^{-1} to inject the trapped compounds into the analytical column (splitless mode for 1 min). Separations were conducted on a HP-5MS fused silica column (30 m × 0.25 mm, 1 μm film thickness, J&W Scientific, Agilent Technologies). Oven temperature was programmed to increase from 120 °C (held for 1 min) to 200 °C at 5 °C min^{-1} and then to 260 °C at 30 °C min^{-1} (held during 10 min). Helium was used as the carrier gas at 0.8 mL min^{-1}. The mass spectrometer was operated in the selected-ion monitoring (SIM) mode with electron ionization (EI) (ionization voltage: 70 eV). A dwell time of 100 ms was selected. Each compound was identified using two or more characteristic ions (Table 2) one was used as quantifier and the others as qualifiers, and the relative intensity of qualifier to quantifier ion (±20%). This test together with the retention times (which should be within 3 s of the corresponding standard) were used to ensure the correct peak assignment in real samples.

Table 1
Derivatization and extraction conditions for selenium speciation.

	4-Chloro-1,2-phenylenediamine	$NaBEt_4$	Isobutyl chloroformate
Sample aliquot/selenium species	500 μL/Se(IV)	500 μL/Se(IV)	100 μL/selenoamino acids
Derivatization reagent	200 μL of 1% (w/v) 4-Chloro-1,2-phenylenediamine	150 μL of 1% (w/v) $NaBEt_4$	40 μL of isobutyl chloroformate and 200 μL of the water:methanol:pyridine (60:32:8)
PDMS volume	126 μL	126 μL	63 μL
Extraction time and temperature	60 min at 25 °C	20 min at 25 °C	30 min at 23 °C
Stirring rate	600 rpm	600 rpm	900 rpm
Ionic strength		Acetate/acetic solution 1 M	100 g NaCl L^{-1} in water
Final volume	3 mL	5 mL buffered at pH 5	1.5 mL

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