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NOTE

Inorganic and Organic Selenium Compound Speciation with Coupled HPLC–MW–HG–AFS

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Coupling between high-performance liquid chromatography, microwave-induced reduction, hydride generation and atomic fluorescence spectrometry (HPLC–MW–HG–AFS) has been used for selenium speciation. The concentrations of a redox mixture (HCl solution of KBr and $KBrO_3$ aqueous solutions) used for both destruction of organic matter and reduction of the selenium species have been optimized, as well as the concentration of $NaBH_4$ in the hydride generation step. Chromatographic separation of selenocysteine, selenite and selenate took place in 15 min, employing a polymer-based strong anion-exchange column. Calibrations with correlation coefficients $r > 0.998$ were obtained for the three compounds at concentrations between 0.5 and 10 $\mu g\,l^{-1}$. Detection limits for selenite, selenocysteine (SeCys) and selenate were 0.2, 0.3 and 0.5 $\mu g\,l^{-1}$, respectively. The coupling has been tested with spiked water samples (tapwater and seawater), obtaining recoveries in the range 94–104%. Copyright © 1999 John Wiley & Sons, Ltd.

Keywords: high-performance liquid chromatography; microwave radiation; hydride generation; atomic fluorescence spectrometry; selenium speciation; water samples

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INTRODUCTION

Selenium species are widely distributed in the environment and can be present as inorganic or organic species, exhibiting different chemical and biological properties. Inorganic compounds, such as selenite and selenate ions, are present in water and soils, whereas inorganic selenides and elemental selenium are insoluble compounds. Some volatile organoselenium compounds, such as dimethyl selenide and dimethyl diselenide, are the result of biomethylation of inorganic selenium by microorganisms.[1] More complex selenoamino acids (e.g. selenocysteine and selenomethionine) can be found in biological tissues as a consequence of biological pathways by which selenium is incorporated into proteins.[2]

Low levels of selenium are necessary for human metabolism, as it is a component of glutathione peroxidase, necessary for the removal of hydrogen peroxidase and lipid peroxidases from cells.[3] In addition, this element prevents tissue interactions with toxic heavy metals such as arsenic and mercury.[4] The nutritional requirements for selenium have been established to be in the range 0.1–0.3 mg kg^{-1}; higher concentrations (2–10 mg kg^{-1}) are toxic.[5,6]

Liquid chromatography (HPLC) has been widely used for the speciation of non-volatile selenium compounds. Different selenoamino acids (selenoethionine, selenocysteine or selenomethionine) have been separated employing a reverse-phase column.[7,8] However, inorganic compounds (selenite and selenate) are not retained on this type of column, and co-elute.[9] The use of a vesicle-mediated HPLC (with didodecylammonium bromide), allows separation of inorganic compounds.[10] Also, use of a mixed packing consisting of octadecyl and ion-exchange stationary phases

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(ODS/AMINO) has been proposed.[11] Finally, separation of selenoamino acids and inorganic selenium has been achieved by use of strong anion-exchange columns.[12,13]

Several couplings based on HPLC and atomic detectors have been proposed for selenium speciation. Direct coupling with flame atomic absorption spectrometry (HPLC–FAAS)[12] provides poor detection limits, in the milligram per litre range. Better results (micrograms per litre) are obtained with coupling between HPLC and electrothermal atomic absorption spectrometry (HPLC–ETAAS).[14] HPLC–ICP–MS provides high sensitivity, as has been reported,[7,11] with detection limtits in the microgram per litre range.

Another approach involves use of an intermediate step based on microwave radiation in order to destroy the organic part of the organoselenium molecules, followed by hydride generation[15] and atomic absorption detection (HPLC–MW–HG–AAS). This approach employs a redox reagent also (a HBr/$KBrO_3$ mixture) in order to reduce all the species to selenium(IV) before the hydride generation step, and it is suitable for complex molecules, especially selenoamino acids. Other authors[16,17] have also employed atomic fluorescence detection (HPLC–MW–HG–AFS) for the speciation of inorganic selenium.

In this paper a procedure based on the separation of selenium(IV), selenium(VI) and SeCys by HPLC followed by on-line microwave reduction oxidation of the selenium species to selenium(IV) with a redox mixture (HCl + KBr and $KBrO_3$), hydride generation and detection with atomic fluorescence spectrometry (AFS) has been developed and optimized (HPLC–MW–HG–AFS). This approach has been used for selenium speciation in spiked water samples.

EXPERIMENTAL

Reagents

Stock solutions (each 1000 mg l^{-1} as Se) of Na_2SeO_3, Na_2SeO_4, seleno-DL-cysteine (all purchased from Sigma, Gillingham, Dorset, UK) were prepared in deionized water obtained from a Milli-Q Gradient system (Millipore, Bedford, MA, USA) and stored in the dark at 4 °C. Dilute working solutions were prepared daily.

HBr (47%) (Merck, Darmstadt, Germany), $KBrO_3$, $NaBH_4$, KoAc and K_2SO_4 (all purchased from Panreac, Barcelona, Spain) were of analytical grade. All aqueous solutions were prepared using Milli-Q water. Analysed water samples were filtered through a 0.45 μm syringe filter (Whatman, Clifton, NJ, USA).

Instrumentation

The HPLC system consisted of a Varian 9012 ternary pump. The sample was introduced via a Rheodyne 7125 injector fitted with a 200 μl loop. Separation of the selenium compounds took place in a Hamilton PRP X-100 column. The HBr, $KBrO_3$ and $NaBH_4$ solutions were pumped using two Gilson Minipulse-3 peristaltic pumps. Microwave radiation was supplied by a Moulinex Y 55 domestic microwave. Separation of the volatile selenium hydrides was achieved in a type A gas–liquid separator (PS Analytical, Kent, UK). Argon was used as carrier gas and was dried before detection with a hygroscopic membrane drying tube (Perma Pure Products, Farmingdale, NJ, USA). Detection of the selenium hydrides was performed using a PSA Excalibur 10.33 AFS (PS Analytical, Kent, UK). Integration of the chromatographic peaks was performed by connecting the analogue output of the detector to a computer (Varian, San Fernando, CA, USA).

Procedure

A 200-μl sample solution was injected in the HPLC strong anion-exchange column. Separation of the selenium species was performed using a gradient program with two mobile phases (A, 2 g l^{-} KoAc aqueous solution; B, 2 g l^{-1} K_2SO_4 aqueous solution), both at a flow rate of 1 ml min^{-1} and pH 6.5: 100% A was pumped for 1 min, then changed in 1 min to 100% B and maintained until the end of the chromatogram. A redox reagent was added after the chromatographic separation, consisting of two solutions, 2% (w/v) KBr in 10 M HCl solution (flow rate 1.2 ml min^{-1}) and 15 mM $KBrO_3$ solution (flow rate 0.6 ml min^{-1}) added on-line. Destruction of the organic portion of the selenocysteine and reduction of the different selenium compounds was achieved with the microwave operated at 150 W. In order to stabilize the signal, an ice-bath-cooled loop was placed after the microwave. Hydride generation was carried out by adding 1 ml min^{-1} of 1.5% (w/v) $NaBH_4$ in 1% (w/v) NaOH. A flow of argon of 100 ml min^{-1} transported the selenium hydrides to a gas–liquid separator. A second argon flow of 200 ml min^{-1}

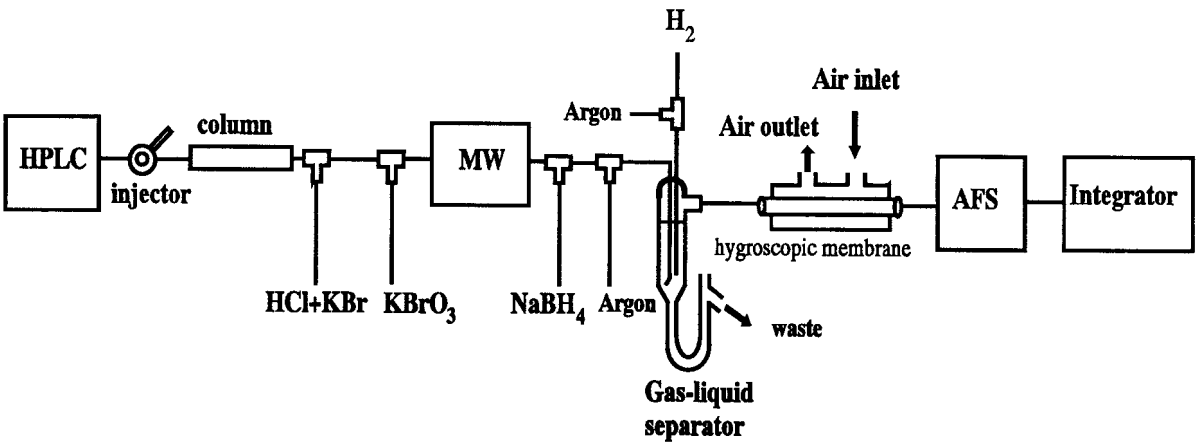

Figure 1 Scheme for HPLC–MW–HG–AFS coupling. AFS, atomic fluorescence spectrometer.

carried the hydrides to the AFS. A hydrogen flow of 60 ml min^{-1} was also used in order to maintain the argon–hydrogen diffusion flame of the detector. A boosted discharged hollow cathode lamp (BDHCL) was used as radiation source, with both the primary and boost currents at 25.0 mA. Peak areas were considered throughout the experiments. A diagram of the coupling is shown in Fig. 1.

RESULTS AND DISCUSSION

Chromatographic separation

Separation of the selenium species was carried out using the strong anion-exchange column, taking as the starting point the results obtained by Gilon *et al.*,[14] who used HPLC–ETAAS, with a gradient program employing $Ni(OAc)_2$ and $NiSO_4$ solutions as mobile phases, with a run time of 36 min. In our approach, some modifications were introduced. Nickel solutions were substituted by the corresponding potassium salts, maintaining the original concentrations of 2 g l^{-1} for each solution and the pH of 6.5. Gilon *et al.* used GFAAS for final detection of the selenium species; thus a matrix modifier was needed to obtain a suitable signal. In our case, mobile phases based on potassium salts were chosen. Following the conditions described by Gilon *et al.*, we separated the species in 25 min (Fig. 2a). The gradient program was then optimized, pumping initially 100% of mobile phase A (KOAc) for 1 min, then changing to 100% of mobile phase B (K_2SO_4) in 1 min and maintaining this phase until the end of the chromatogram. Under these conditions, the time of analysis was reduced to 15 min. A typical chromatogram obtained with

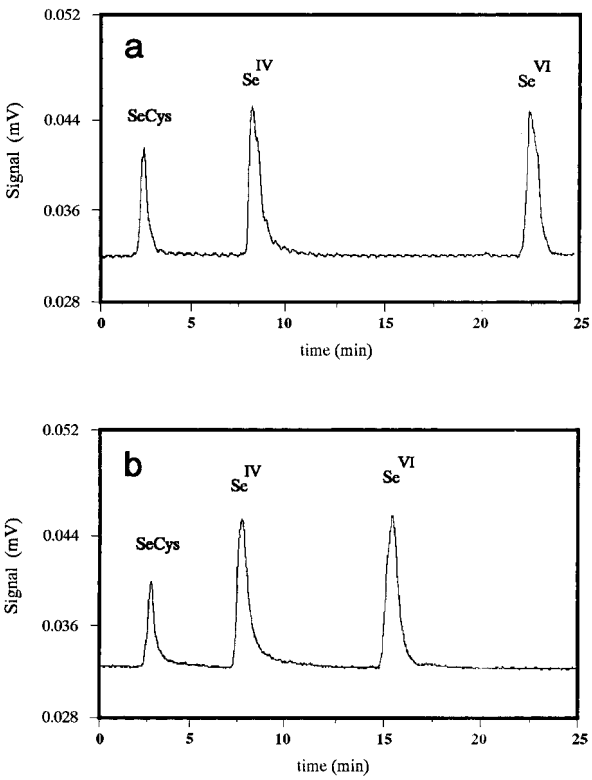

Figure 2 HPLC–MW–HG–AFS traces for selenium standards (each 10 $\mu g\, l^{-1}$ as Se) using the gradient program described in: (a) Ref. 14; (b) this paper.

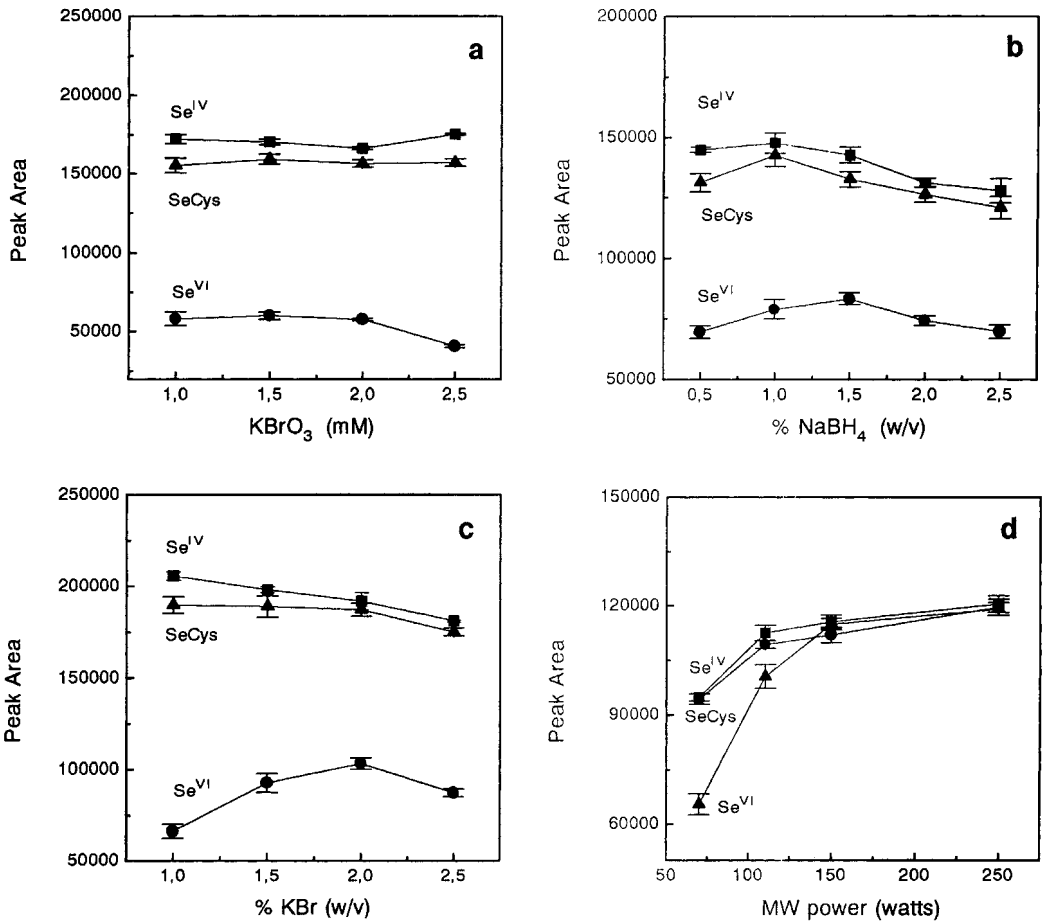

Figure 3 Influence of experimental variables on selenium hydride generation for a mixture of Selenium (IV) (Se^{IV}), Selenium (VI) (Se^{VI}) and SeCys on: (a) $KBrO_3$ concentration; (b) $NaBH_4$ concentration; (c) KBr concentration; (d) microwave power.

the HPLC–MW–HG–AFS coupling and the optimized gradient programme is depicted in Fig. 2(b).

Derivatization optimization

The concentrations of the different solutions pumped with the peristaltic pump were optimized. In Fig. 3 the peak areas obtained for selenocysteine, selenite and selenate are shown for several concentration ranges of $KBrO_3$, KBr and $NaBH_4$. The weakest signal was always obtained for selenium(IV). Hence, optimal concentrations were chosen in accordance with the maximum signal for this species. Both selenium(IV) and SeCys resulted in strong signals for all the conditions tested. On the other hand, an increase in the microwave power (Fig. 3d) resulted in a significant increase in the selenium(IV) signal, but produced an increase in baseline noise. A compromise value of 150 W was chosen.

Calibration and detection limits

Calibration curves were determined for the selenium species. Several standards containing the three compounds were injected into the chromatograph, covering concentrations between 0.5 and 10 $\mu g\,l^{-1}$ (as Se). Linear fits were obtained for the three compounds with correlation coefficients $r = 0.9994$, 0.9993 and 0.998 for selenium(IV), SeCys and selenium(VI), respectively. Detection limits (DL) were obtained as three times the

standard deviation calculated at the intercept divided by sensitivity (slope of the calibration curve). The DL obtained for selenium(IV), SeCys and selenium(VI) were 0.2, 0.4 and 0.3 $\mu g\,l^{-1}$ (as Se), respectively.

Analysis of spiked samples

Two types of water samples of low and high ionic strengths were studied (tapwater and seawater). Samples were filtered through a 0.45-μm syringe filter and analysed. Results revealed the absence of selenium species in both samples. Therefore, a spiking experiment was performed to evaluate the recovery and possible influence of the matrix. A 100 μl portion of a standard aqueous solution (1 $mg\,l^{-1}$) containing the three species were placed in a 10-ml volumetric flask which was filled to give a final concentration of 10 $\mu g\,l^{-1}$. Recovery values were between 94 and 104% with precision (standard deviation) better than 5%. Neither retention time shift nor peak shape deformities were observed, using seawater samples.

CONCLUSIONS

Coupled HPLC–MW–HG–AFS gave detection limits below the microgram per litre level. These results are comparable with those obtained by other sensitive atomic detectors, such as ICP–MS. AFS detection also presents the advantage of low costs and easy coupling to the HPLC system. Forthcoming work will be directed to the analysis of other selenoamino acid species and to studies with enumerated samples.

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