Use of solid phase extraction for speciation of selenium compounds in aqueous environmental samples

The Analyst

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The performance and optimization of an anion exchanger phase (SAX) in line with a hydrophobic phase (C_{18}) was studied for the preconcentration of selenite [Se(IV)], selenate [Se(VI)], dimethylselenide (DMSe), dimethyldiselenide (DMDSe), diethylselenide (DESe) and diethyldiselenide (DEDSe), using a GC-MS system to characterize the different selenium species. Optimum separation and preconcentration of the inorganic selenium species were based on their retention in an SAX cartridge, with Se(IV) eluted in 25 ml of 1 mol l^{-1} HCOOH and Se(VI) in 25 ml of 1 mol l^{-1} HCl. Organic selenium species were retained in a C_{18} cartridge and eluted in 2 ml of carbon disulfide. The detection limits of the method were 1.4, 1.6, 0.6, 6.0, 400 and 900 ng l^{-1} for Se(VI), Se(IV), DMDSe, DEDSe, DMSe and DESe, respectively, using a 1000 ml water sample, and the repeatability was less than 10%. The method was applied to natural water samples of different origins.

Introduction

Selenium is present in the environment from both natural and anthropogenic inputs, at concentrations ranging between 50 and 4000 μg l^{-1} in waters. This element has been recognized as an essential nutrient and amounts of 55–70 μg of Se have been recommended as the daily intake in humans. However, at concentrations higher than 130 μg l^{-1}, selenium becomes toxic; 10 μg l^{-1} Se is the maximum allowed concentration in drinking water as recommended by the WHO. The toxicity, bioavailability and essential nature of selenium depend on its chemical forms,[1] Se(IV) and Se(VI) being the predominant species in natural waters. Otherwise, biomethylation of inorganic selenium produces volatile dimethyl selenide (DMSe), dimethyl diselenide (DMDSe) and diethyl selenide (DESe), and this process constitutes the major mechanism for selenium detoxification. Therefore, the development of reliable techniques for selenium speciation is necessary to understand the biogeochemical cycle, mobility, uptake–depuration mechanisms and toxicity of this element.

Se(IV) and Se(VI) are separated using ion pair HPLC or IC and detected by AAS, AES or ICP-MS,[2,3] which are preferred to non-specific detection methods because they are less influenced by the matrix in environmental samples. Without previous separation of Se(VI), Se(IV) can be determined by GC-ECD, GC-MS, GC-IDMS or fluorimetry after the formation of volatile piazselenols or hydride generation (HG)-AAS.[4,5] Speciation of volatile organic selenium compounds has been successfully achieved by GC coupled with either quartz and graphite furnace AAS, MIP-AED or non-dispersive AFD.[6]

Despite the very sensitive analytical methods available for selenium, the low levels of this element found in water require preconcentration to improve the detectability and remove matrix interferences. Usually, organic and inorganic interferences are eliminated from water samples using column chromatography on XAD-8 resin[7] and on Dowex 50W-X8,[8] respectively. Preconcentration is performed by liquid–liquid extraction of piazselenols,[9] on-line coprecipitation of inorganic selenium species with $La(OH)_3$,[10] complexation and adsorption on activated carbon[11] and solid phase extraction (SPE) based on anionic sorbents.[3] On the other hand, the volatile selenides can be separated from water by helium stripping and swept into a cold trap with solid sorbents such as activated carbon, glass-wool and GC stationary phases, and then thermally desorbed or extracted with organic solvents.[6,12] However, no method for the simultaneous preconcentration of Se(IV), Se(VI), DMSe, DMDSe, DESe and diethyl diselenide (DEDSe) in water have been reported.

This work was focused on the determination of inorganic and volatile organic selenium species in water samples. An SPE based on the use of two different sorbent phases, octadecyl (C_{18}) and quaternary amoninum (SAX), in separate cartridges was optimized for the preconcentration of trace amounts of the selenium species. The SAX cartridge was stacked upon the C_{18} cartridge for the application of the method to different natural aqueous matrices. The final analytical determination uses a GC-MS approach, which presents a sensitivity comparable to that of other methods, with an additional confirmation of the nature of each species.

Experimental

Reagents, standards and apparatus

Analytical-reagent grade reagents and pesticide grade solvents were obtained from Merck (Darmstadt, Germany) and Sigma (St. Louis, MO, USA). SAX and C_{18} (600 mg of sorbent) cartridges were obtained from Alltech (Deerfield, IL, USA) and Waters (Milford, MA, USA), respectively.

Stock standard solutions of Se(IV) and Se(VI) (1000 mg l^{-1} of Se) were prepared from analytical-reagent grade selenium dioxide and sodium selenate (Merck), respectively. Organoselenium stock standard solutions were prepared at a concentration of approximately 100 mg l^{-1} (as Se) in benzene from DMDSe (Aldrich, Gillingham, Dorset, UK), DESe, DMSe (Pfaltz and Bauer, Waterbury, CT, USA) and DEDSe (synthesized by the authors[13]) and were kept in a refrigerator. Aqueous working solutions were prepared daily and water used in the

experiments was doubly distilled and de-ionized and gave blank readings in all the analyses. Plastic and glassware used for experiments were previously soaked in 0.08 mol l^{-1} HNO_3 for 24 h and rinsed carefully with water.

Selenium speciation was carried out using an HP Model 5890 gas chromatograph and HP Model 5970 mass detector, with a fused silica capillary column, 25 m × 0.20 mm id and a film thickness of 0.33 μm HP-1 cross-linked methylsilicone gum (Hewlett-Packard, Palo Alto, CA, USA). Sample aliquots of 1 μl were injected manually using the splitless injection mode. Helium was used as the carrier gas at a head pressure of 100 kPa. The interface was operated at 260 °C. Electron ionization (EI) mass spectrometry was used for detection. A scan time of 1.0 s was used over a mass range of *m/z* 40–500.

Preconcentration of selenium species using cartridges

Preconcentration of inorganic selenium was performed on a SAX cartridge conditioned with 10 ml of 3 mol l^{-1} HCl and 10 ml of water at 5 ml min^{-1}. For organic selenium species, a C_{18} cartridge was conditioned with 10 ml of CS_2, 10 ml of MeOH and 10 ml of water, using a flow rate of 5 ml min^{-1}. The SAX cartridge was stacked on top of a C_{18} cartridge and 1000 ml of water with the pH adjusted at 7–8 was passed through the cartridges at 8 ml min^{-1}. The cartridges were separated and the analytes were eluted separately, Se(IV) with 25 ml of 1 mol l^{-1} of HCOOH and Se(VI) with 25 ml of 3 mol l^{-1} of HCl, using a flow rate of 5 ml min^{-1}. The C_{18} cartridge was dried under a stream of nitrogen and the organic selenium species were eluted with 2 ml of CS_2 at 1 ml min^{-1}.

Determination of inorganic selenium

Selenium(IV) was derivatized with 5 ml of 0.1% 4-chloro-*o*-phenylendiamine in 0.1 mol l^{-1} HCl at 75 °C for 7 min. The solution was allowed to cool to room temperature and the piazselenol was extracted twice with 1 ml of toluene by shaking for 1 min. The organic phase was separated and evaporated just to dryness under a stream of nitrogen. The residue was dissolved in 50 μl of hexane containing fluorodinitrobenzene (FDNB) as internal standard (230 ng l^{-1}) and analysed by GC-MS. Selenium(VI) was quantitatively reduced to Se(IV) by adding 10 ml of 5 mol l^{-1} HCl and boiling for 30 min. After allowing the residual solution to cool, the pH was adjusted to 2.1 and selenium was derivatized. The retention times obtained were: FDNB 9.8 min (*m/z* 186) and piazselenol 10.5 min (*m/z* 218).

Determination of organic selenium

A 200 μl volume of a solution of 2.0 μg l^{-1} of 2,6-diisopropylphenol (propofol) as internal standard in CS_2 was added to the extract containing the organic selenium species and the solution was analysed by GC-MS. The retention times were DMSe 2.97 min (*m/z* 109), DESe 5.07 min (*m/z* 109), DMDSe 7.11 min (*m/z* 188), DEDSe 8.41 min (*m/z* 218) and propofol 17.1 min (*m/z* 178).

A derivatization step using FDNB was introduced to improve the sensitivity of the dialkyl diselenide determination.[14] The derivatives were extracted with 4 ml of ethyl acetate (three times), the extracts were evaporated to dryness, the residues were dissolved in 50 μl of toluene containing 200 μg l^{-1} of propofol and the solutions were analysed by GC-MS. The retention times were DMDSe derivative 16.3 min (*m/z* 262), DEDSe derivative 18.7 min (*m/z* 276) and propofol 9.1 min (*m/z* 178).

Statistical treatment

The data were analysed statistically for differences using factorial analysis of variance (ANOVA). Prior to analysis, all the data were tested for homogeneity of variance using the Barlett and Levene tests. A parametric statistical test (Student's *t*-test) was applied to different hypotheses. An α-value of 0.05 was adopted as the critical level for all statistical testing giving a 95% confidence level (CSS: STATISTICA).

Results and discussion

Preconcentration and separation of inorganic selenium species using strong anionic exchange cartridges

Two strong anion exchange phases, SAX and Acell plus QMA (Waters), were used for Se(IV) and Se(VI) preconcentration and a similar performance was found in both cases (ANOVA, $p > 0.61$). The parameters controlling the SPE were the pH and volume of sample, both affecting the retention of inorganic selenium species, and also the concentration and volume of the eluents, which affect the elution of these analytes. The experiments were performed (three replicates) by using 12 μg of both Se(IV) and Se(VI) in 250 ml of doubly distilled water, following the procedure described under Experimental.

Loading of inorganic selenium species into the cartridge was studied using aqueous solutions of pH 3–9. Fig. 1 shows that the best recoveries were obtained for pH values typically found in natural waters (7–8) (*t*-test, $p < 0.01$). Therefore, a pH of 7 was used in further experiments and no special pre-treatment of the samples was required.

Selenium preconcentration from different volumes of water ranging from 100 to 1000 ml was tested and no differences were found in the recoveries. Therefore, it is possible to use large volumes of water when selenium species levels are very low in order to load suitable amounts of this element on the cartridges. However, this point should be confirmed by the analysis of natural water, because other compounds from the matrix may overload the packing material.

Elution of inorganic species of selenium was tested using 0.5–4 mol l^{-1} aqueous HCOOH solutions. Selenium(IV) was quantitatively recovered with 1 mol l^{-1} HCOOH and higher concentrations did not improve the results (ANOVA, $p > 0.98$). Elution of Se(VI) required 2 mol l^{-1} HCl (*t*-test, $p < 0.01$). The volumes of each eluent between 10 and 40 ml were tested, and 20 ml were needed to obtain quantitative recoveries (*t*-test, $p < 0.01$). No improvement was obtained by using larger volumes of elutant (ANOVA, $p > 0.50$).

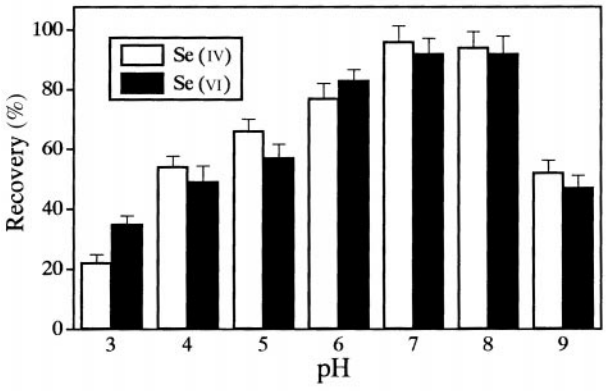

Fig. 1 Influence of pH on the retention of Se(IV) and Se(VI) on SAX cartridges. Results represent the percentage recovery ± standard deviation for three replicates, obtained by elution with an acidic medium [HCOOH and HCl solutions for Se(IV) and Se(VI), respectively].

Preconcentration and separation of organoselenium species using non-polar sorbents

Cartridges with non-polar (C_{18} and activated carbon) and polar (CN) sorbents were tested for the preconcentration of organoselenium compounds from water samples. As the extract obtained from the cartridge has subsequently to be injected into the GC system for analysis, a minimum elution volume must be used in order to avoid losses of the volatile organoselenium species during solvent removal. Moreover, the chromatographic determination establishes restrictions in the eluent choice, which must not be eluted from the chromatographic column at the same time as the most volatile organoselenium species (DMSe), because the filament used for the EI must remain off during the solvent elution.

The parameters controlling the SPE were the sample volume and the nature and volume of the solvent used as the eluent. The experiments were performed (three replicates) by using samples of 100 μg (as Se) of both DMSe and DESe and 1 μg (as Se) of DMDSe and DEDSe in 250 ml of doubly distilled water, following the analytical procedure described under Experimental.

Recoveries of the four organoselenium species using both polar and non-polar sorbents and CS_2 volumes of 1–6 ml are given in Table 1. Recoveries higher than 80% were obtained using both C_{18} and activated carbon, the non-polar character of the primary interaction between analytes and sorbents being obvious. Poorer recoveries were obtained using CN cartridges. This indicated the absence of polar interactions. Higher recoveries from C_{18} were obtained using at least 2 ml of solvent (*t*-test, $p < 0.01$), but 4 ml were needed for the elution of the organoselenium compounds from the activated carbon sorbent (*t*-test, $p < 0.015$), which indicated stronger retention of the organoselenium compounds in this sorbent. Higher volumes did not improve the recoveries (ANOVA, $p > 0.55$). Finally, lower recoveries ranging from 20 to 70% were obtained using 6 ml of more polar eluents such as MeOH and Et_2O, irrespective of the sorbent.

To evaluate the volume of aqueous sample that can be treated with the cartridge without overloading the packing material, recovery experiments were carried out on spiked samples ranging from 100 to 1000 ml, and no differences were found (ANOVA, $p > 0.81$). However, no experiments were developed to check the total adsorption capacity of the cartridge, which will depend on the presence of other substances in the sample such as hydrocarbons and fats. This point should be checked by the analysis of natural samples.

Calibration and analytical quality control

Calibration curves were constructed by the analysis of 1000 ml aliquots of water which were submitted to the whole preconcentration method. The calibration curves were linear up to selenium concentrations of 0.750 μg l^{-1} for Se(IV) and Se(VI) and 130, 110, 1.20 and 2.40 μg l^{-1} for DMSe, DESe, DMDSe and DEDSe, respectively, with correlation coefficients higher than 0.997. Detection limits (DLs), sensitivity, repeatability and reproducibility of the method for water analysis are given in Table 2. These results are comparable to those reported in the literature and obtained using HPLC-ICP-MS[3] [DL 160 and 80 ng l^{-1} for Se(IV) and Se(VI), respectively], GC-AAS[15] [DL 0.8 ng l^{-1} for methylated species and 1.6 ng l^{-1} for Se(IV)] and GC-MIP-AED[12] (DL 2 ng l^{-1} for methylated species), allowing the evaluation of these species in natural aquatic environments. However, lower detection limits, 0.0044 and 0.0069 ng l^{-1} for DMSe and DMDSe, respectively, have been reported using 1 l samples in a purge-and-trap, low temperature GC-AFD device, but no attempt to separate ethylated selenium species was made.[6]

Table 1 Recoveries ± standard deviations (%) of organoselenium compounds eluted from octadecylsiloxane (C_{18}), activated carbon (AC) and cyanopropylsiloxane (CN) sorbents using CS_2 as eluent

Sorbent	Volume/ml	DMSe	DESe	DMDSe	DEDSe
C_{18}	1	53 ± 3	47 ± 5	61 ± 5	53 ± 4
	2	84 ± 5	87 ± 5	88 ± 5	89 ± 5
	4	86 ± 4	88 ± 4	83 ± 4	91 ± 5
	6	83 ± 4	84 ± 4	86 ± 4	86 ± 4
AC	1	39 ± 5	42 ± 8	45 ± 6	53 ± 6
	2	51 ± 5	64 ± 6	65 ± 5	69 ± 5
	4	79 ± 5	82 ± 4	85 ± 5	87 ± 5
	6	81 ± 5	76 ± 5	74 ± 6	86 ± 5
CN	1	ND[a]	11 ± 4	8 ± 3	9 ± 4
	2	12 ± 3	9 ± 4	14 ± 5	17 ± 6
	4	33 ± 4	27 ± 6	34 ± 5	30 ± 7
	6	27 ± 7	33 ± 8	24 ± 6	26 ± 5

[a] Not detected.

Application to environmental samples

The method was validated for Se(IV) determination using a CRM (CASS-3 from Laboratory of the Government Chemist, Teddington, UK) consisting of nearshore sea-water with 0.5 mg l^{-1} total dissolved organic matter, acidified at pH 1.6 and with an Se(IV) content of 0.020 ± 0.005 μg l^{-1}, and applied to the speciation of selenium in sea-, river and tap water samples. The results are given in Table 3. No organic selenium species were found in any samples. Consequently, samples were spiked at two levels, using high concentrations of organoselenium compounds (50, 25, 1 and 1 μg l^{-1} for DESe, DMSe, DMDSe and DEDSe, respectively) to check the absence of sorbent overloading low concentrations (5, 2.5, 0.006 and 0.05 μg l^{-1}

Table 2 Detection limits (DL), correlation coefficient (r^2), sensitivity (*S*), repeatability (*r*) and reproducibility (*R*) of selenium determination in water samples. The repeatability and reproducibility were assessed in solutions containing 0.150 μg l^{-1} of Se(IV), 0.150 μg l^{-1} of Se(VI), 16.8 μg l^{-1} of DMSe, 33.6 μg l^{-1} of DESe, 0.240 μg l^{-1} of DMDSe and 0.490 μg l^{-1} of DEDSe (as Se)

Species	DL/ng l^{-1}	*S*/l μg^{-1}	r^2	*r* (%)	*R* (%)
Se(IV)	1.6	4.89	0.998	5.6	8.6
Se(VI)	1.4	4.35	0.997	6.6	8.5
DMSe	388	0.033	0.998	6.1	9.9
DESe	903	0.029	0.999	3.7	7.0
$DMDSe_d$[a]	0.6	2.30	0.998	7.1	9.2
$DEDSe_d$[a]	6.0	0.93	0.999	7.3	9.7

[a] Organselenium derivative.

Table 3 Concentration of inorganic species of selenium ± standard deviation (μg l^{-1}, as Sn) (three replicate samples) in water samples collected from the southwest of Spain and in a certified reference sample. Recovery studies

Location	Type pH	Species	Se concentration ± *s*/μg l^{-1} Initial	Added	Recovery (%)
Niebla	River 4.1	Se(IV)	0.033 ± 0.003	0.04	96
		Se(VI)	0.025 ± 0.003	0.025	97
Portil	Lake 7.8	Se(IV)	0.18 ± 0.01	0.2	94
		Se(VI)	0.21 ± 0.02	0.2	94
Portil	Sea 7.6	Se(IV)	0.34 ± 0.02	0.3	96
		Se(VI)	0.31 ± 0.02	0.3	97
Rompido	Sea 7.7	Se(IV)	10.5 ± 0.9	10	98
		Se(VI)	39 ± 3	40	96
Punta Umbria	Sea 7.8	Se(IV)	270 ± 20	300	97
		Se(VI)	81 ± 6	100	99
Ayamonte	Sea 6.8	Se(IV)	5.7 ± 0.3	5	94
		Se(VI)	3.4 ± 0.3	4	93
Sevilla	Tap 7.1	Se(IV)	0.98 ± 0.06	1	96
		Se(VI)	0.43 ± 0.02	0.5	91
CASS-3	CRM	Se(IV)	0.017 ± 0.002 (duplicate)		

for the same species) to simulate more realistic environmental levels. Recoveries of 80–100% were invariably found for the four organoselenium species in all the samples in Table 3, with no significant differences between the two concetration levels tested. High levels of both Se(IV) and Se(VI) were found in water from Punta Umbria, a fishing harbour located on the Huelva coast. Lower concentrations were found in water from Rompido and Ayamonte fishing harbours, located in estuarine areas in the Piedras and Guadiana Rivers. The lowest concentrations for both Se(IV) and Se(VI) were found in water from the Tinto River (Niebla station). These levels are in the usual range found in natural waters.[16]

In general, Se(IV) was present in higher concentrations than Se(VI), which is in contrast to the stability predictions, indicating slow oxidation kinetics of Se(IV) to Se(VI) in natural waters. Finally, selenium concentrations found in a tap water from Seville were also in the range usually found in drinking waters by several workers[16] and below the maximum selenium concentration in drinking water recommended by the WHO.

Conclusions

Both inorganic and volatile organic selenium species were recovered from water samples using stacked SAX and C_{18} cartridges, which allow 40- and 500-fold preconcentration for inorganic and organic species, respectively. Moreover, the separation of Se(IV), Se(VI) and organic selenium compounds can be achieved by fractional elution, which allows the independent determination of these species using different analytical methods. The procedure is robust enough for the analysis of tap, river and sea-water samples at environmentally representative levels of ng l^{-1} for Se(IV), Se(VI), DMDSe and DEDSe and low μg l^{-1} for DMSe and DESe, using a GC-MS system.

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