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Speciation of volatile forms of selenium and inorganic selenium in sediments by gas chromatography–mass spectrometry

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Abstract

Methods are presented for the speciation of both inorganic and organic compounds of selenium, including selenite, selenate, dimethylselenide (DMSe), dimethyldiselenide (DMDSe), diethyselenide (DESe) and diethyldiselenide (DEDSe). Parameters affecting the extraction efficiency from sediments are discussed and the performance of the methods is described. Inorganic selenium species are quantitatively leached from sediments using alkaline solutions. Separation of selenite and selenate is carried out using anion-exchange phases packed in cartridges. Selenite is analysed as piaselenol derivative using GC–MS. Selenate is determined after conversion into selenite. The detection limit achieved using the proposed method is 0.6 ng of Se g^{-1} (wet mass basis) for both selenite and selenate using sample portions of 1 g. Volatile selenium species are desorbed from sediments using a dynamic headspace desorption method with active carbon as sorptive trap. The species are eluted with carbon disulphide. The four organic selenium species are directly analysed by GC–MS but sensitivity of the method is improved for dialkyldiselenides after derivatization with 1-fluoro-2,4-dinitrobenzene followed by extraction into ethyl acetate and evaporation to dryness under a N_2 stream. Extraction recoveries obtained by this method are higher than 75% for all the species considered in the present work. Estimated detection limits for DMSe, DMDSe, DESe and DEDSe using portions of 20 g of wet sediments are 33, 1.0, 22 and 2.3 ng of Se g^{-1} (wet mass basis). The methods were applied to the determination of inorganic and volatile organic selenium species in several sediments collected from different areas in the Southwest of Spain. Finally, incubation experiments were performed at two temperatures for 15 days on sediments and a raw sewage sludge collected from Ayamonte city. Only from this latter were both DMDSe and DESe generated.

Keywords: Sediments; Environmental analysis; Selenium; Inorganic anions; Organoselenium compounds

1. Introduction

Selenium is widely distributed in the environment specially included in metal sulphide deposits [1]. Otherwise, selenium is obtained industrially as a by-product of the electrolytic refining of copper, and is used as an additive to improve the properties of alloys, in the manufacture of ceramics, plastics, paints and lubricants, in the rubber industry, in the production of photometers, rectifiers and semiconductors, xerography, etc. Organoselenium compounds are used as herbicides, fungicides and bactericides in agriculture. Selenium is present in aquatic systems in different oxidation state species: selenide (both in inorganic and organic compounds), selenite and selenate. Although the selenium concentration in most drinking and natural waters is less than 10 $\mu g\, l^{-1}$, pore water from seleniferous soil in

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semiarid areas may contain up to hundreds or thousands of micrograms of dissolved selenium per liter [2]. The mean level of selenium content for a variety of soils has been reported in the range of 0.5 $\mu g\ g^{-1}$ [3].

Selenium is one of the minor but biologically essential elements. Low levels of selenium are necessary for human metabolism, but higher concentrations of this element may cause damage to health [4]. Selenium has different functions, for example, protection of cell membranes from oxidative damage [5] or interaction with toxic heavy metals [6]. The nutritional requeriments for selenium have been stated to lie in the range 0.1–0.3 $mg\ kg^{-1}$, but higher concentrations (from 2 to 10 $mg\ kg^{-1}$) produce chronic toxicity symptoms [7]. Selenium compounds cause liver carcinoma, cirrhosis, teeth, hair and nail loss, irritation of eyes and paralysis [8]. Genotoxic effects of selenium on DNA, retrovirus gene expression, bacterial systems, mammalian chromosomes, yeast and plant mutagenesis and on *Drosophila melanogaster* have also been reported [9].

Organic species of selenium have a different toxicity than inorganic ones. For example, dimethylselenide has been considered to be 500 times less toxic than selenite [10], methylation being an effective detoxification mechanism. There is evidence of production of volatile organic species of selenium (mainly dimethylselenide and dimethyldiselenide) from inorganic selenide salts, as well as from selenocystine and selenomethionine by fungi, plants and animals in the environment and it has been well documented in the literature [11–17]. Dimethylselenide has been found in human breath at levels ranged from 0.08 to 0.98 $\mu g\ m^{-3}$ [18]. As a consequence analytical methods for the chemical speciation of selenium including both inorganic (selenite and selenate) and organic species (dialkylselenide and dialkyldiselenide) in environmental samples are necessary.

A variety of analytical methods have be applied for selenium estimation. Spectrophotometry [19,20], fluorescence analysis [21,22], neutron activation analysis [23,24], anodic [25,26] and cathodic [27,28] stripping voltametry, inductively coupled plasma (ICP) [29,30] or atomic absorption spectrometry (AAS) [31–34] have been used for this purpose. A comparison of instrumental methods for the determination of total selenium in environmental samples was performed concluding that fluorimetry (FL), hydride generation (HG)-AAS and HG-ICP-atomic emission spectrometry (AES) are usually employed for the routine analysis of selenium, although the fluorimetric method is becoming dated. FI-HG-AAS was superior to HG-AAS. The best absolute detection limits were reached by HG-ICP-MS (0.6 ng in 20 ml) and by RNAA (0.5 ng in solids), although both of them have a high capital cost [35]. Graphite furnace (GF)-AAS has a good sensitivity, but suffers from interferences which need to be overcome by the use of matrix modifiers and background corrections [36]. Gas chromatographic methods for the analysis of inorganic selenium require the conversion of these compounds to volatile species such as selenium hydride [37] or alkylselenium compounds [38]. The derivatized species are usually trapped at low temperature followed by heating to transfer the products to the analytical column. More frequently, gas chromatography–electron-capture detection (ECD) has been applied to determine Se(IV) in several matrices [39] using selenium derivatization with *o*-phenylenediamines to form piaselenol and the best results were obtained using 5-nitropiaselenol [40] with a detection limit of 1 pg. However, a source of interferences in the GC–ECD system is the appearance of spurious chromatographic peaks close to the piaselenol peak [39]. Moreover, ECD does not directly respond to selenium itself. The use of selenium specific detectors such as microwave emission detector, flame-photometric detector and mass spectrometer [41], can reduce the effects of these interferences.

Speciation of selenium has been reviewed by several authors [42–46]. Speciation of inorganic forms of selenium has been performed using capillary electrophoresis [47] and HPLC [42]. When an anion-exchange stationary phase was used, the coupling of HPLC–ICP-AES yields absolute detection limits of 140 ng and 91 ng for Se(IV) and Se(VI), respectively [48]. However, reversed-phase HPLC–ICP-AES yields values of 8 ng for Se(IV) and 14 ng for Se(VI) [49]. Better figures (5 ng for each species) were reported by Chakraborti et al. using an ion chromatography (IC)–GF-AA system [50] or by Measures and Burton (2.4 pg) using GC–ECD [51].

Volatile organic selenides (with Se(II): R_2Se,

R_2Se_2, where R=CH_3, C_2H_5) [52] have been analysed by gas chromatography, especially in samples of air, which are directly injected into the chromatograph or trapped on an adsorbent for subsequent desorption by both solvent elution or temperature programming of the trap. A number of coupled GC–AAS systems with a previous purge and trap isolation technique have also been proposed for dimethylselenide (DMSe), diethylselenide (DESe) and dimethyldiselenide (DMDSe) [17,53]. Jiang et al., using GC–GF-AAS, determined up to 0.1 ng of DMSe and DESe and 0.2 ng of DMDSe [54]. The same detection limit (0.1 ng) was reached by Chau et al., for both DMSe and DMDSe, using GC–QF-AAS [55]. Cutter could determine up to 0.5 ng of Se(IV), Se(VI), DMSe and DMDSe using HG-GC–QF-AAS with an air–hydrogen flame [56]. Lower detection limits are obtained using GC–AES for DMDSe (0.015 ng) and for DEDSe (0.012 ng) [57] and using GC–microwave-induced plasma (MIP)-AES for DESe (0.062 ng) [58] and for DMSe and DMDSe (0.01 ng) [59].

Extraction of inorganic selenium species from sediments has been performed by using alkaline leaching [60]. Alkylated selenium compounds have been detected in the atmosphere just above soils and sediments [16,61,62], but they have been scarcely analyzed directly in soils and sediments [63]. Volatile alkylselenium compounds are usually removed from air samples by gas stripping and swept into a liquid or solid cold trap [64–67], being thermally desorbed or extracted using a solvent prior to analysis [66,68,69].

The aims of this work are the optimization of methods for the extraction of selenite, selenate, dimethylselenide, diethylselenide, dimethyldiselenide and diethyldiselenide from sedimentary samples, using GC–MS for speciation, and their application to natural samples from the Southwest of Spain.

2. Experimental

2.1. Reagents and standards

The reagents used in the experiments were analytical grade and obtained from Merck (Zn powder, HF 40%, $HClO_4$ 70%, HCl 37%, HNO_3 65%, H_2SO_4 95–97%, $NaHCO_3$, NaOH, anhydrous sodium sulphate, 1-octanol glucose, elemental selenium and 4-chloro-*o*-phenylenediamine), Sigma (iodoethane, 2,4-dinitrofluorobenzene, selenourea) and Zeneca (propofol, 2,6-diisopropylphenol). Active carbon (100–400 mesh ASTM) was purchased from Sigma, active carbon strips were obtained from Pro-Tek Systems (Portland, CT, USA) and SAX cartridges from Alltech. Pesticide grade solvents were purchased from Merck.

Stock solution of Se(IV) and Se(VI) (1000 mg of Se l^{-1}) were prepared from analytical-reagent grade selenium dioxide and sodium selenate (Merck), respectively. Organoselenium stock solutions were prepared at a concentration of ca. 100 mg (as Se) l^{-1} in benzene from dimethyldiselenide (b.p. 155–157°C, Aldrich), diethylselenide (Pfaltz & Bauer), dimethylselenide (Pfaltz & Bauer) and diethyldiselenide (synthesised by the authors) and were kept in a refrigerator.

Intermediate solutions of 1 mg l^{-1} of Se(IV) and Se(VI) were prepared by dissolving appropriate volumes of stock solutions bringing the volume to 50 ml with water. Intermediate solutions of 1 mg (as Se) l^{-1} of DMSe, DESe, DMDSe and DEDSe were prepared by dissolving appropriate volumes of stock solutions in 1 ml of methanol and bringing the volume to 50 ml with water. Working solutions were prepared daily by appropriate dilutions of the intermediate solutions with water.

Water used in the experiments was double-distilled and deionized and gave blank readings in all the analyses. Plastic and glassware used for experiments were previously soaked in 0.08 *M* nitric acid for 24 h and rinsed carefully with doubly distilled water as recommended in the literature [70].

2.2. Apparatus

Selenium species analysis was carried out using an HP Model 5890 gas chromatograph and HP Model 5970 mass detector, controlled by 9836C Hewlett-Packard Data System (Hewlett-Packard, Avondale, PA, USA). The chromatograms and mass spectra obtained were printed as hard copies by Hewlett-Packard 7470A plotter and Hewlett-Packard 82906A printer. Selenium species were separated on a fused-silica capillary column, 25 m×0.20 mm I.D. and a

film thickness of 0.33 μm HP-1 crosslinked methylsilicone gum.

Sample aliquots of 1 μl were manually injected into the capillary column using splitless injection mode (purge time =0.5 min off). Helium was used as carrier gas at a head pressure of 100 kPa (14.5 p.s.i.) resulting in a flow-rate between 0.5 and 1 ml min^{-1}. The interface temperature was operated at 260°C. Electron impact (EI) mass spectrometry was used as detector and the filament remained off until the solvent was eluted. A scan time of 1.0 s was used over a mass range of 40 to 500 *m/z*. The electron multiplier was set at autotune value; the emission current at 0.4 mA and the electron energy at 70 eV. Calibration was performed with perfluorotributylamine. Data were stored as total ion chromatogram (TIC) and quantification was performed by using SIM (single ion monitoring) mode.

2.3. Diethyldiselenide synthesis

Diethyldiselenide was synthesized by a modification of the procedure proposed by Ganther and Kraus for dimethyldiselenide [71,72]. A 0.5 g portion of selenourea was placed in a round-bottomed flask and 25 ml of water was added giving a red color), then 3 ml of ethyl iodide was added and the mixture was boiled under reflux at 200°C with continuous stirring for 2 h. The excess of reagent was removed by rotatory evaporation (40–50°C), and then 25 ml of *n*-heptane and 40 ml of 5 *M* NaOH were added. The mixture was refluxed for 1 h and then allowed to cool. The upper yellow heptane layer was dried over anhydrous sodium sulphate (Na_2SO_4) and filtered. Finally, the heptane was removed by rotatory evaporation (30°C). The resulting extract was a brown–yellowish oily liquid, whose purity was studied by GC–MS and FAAS.

2.4. Purity of the organoselenium standards

The purity of the individual compounds was evaluated by gas chromatography–mass detection. The chromatographic determination of each compound showed the presence of only one peak, whose nature was confirmed by the fragmentation spectrum [52,55]. The characteristic fragments were as follows: for dimethylselenide, base peak and the molecular ion at *m/z* 110; for dimethyldiselenide, base peak and the molecular ion at *m/z* 188; for diethylselenide, molecular ion at *m/z* 138 and base peak at *m/z* 110 and for diethyldiselenide, molecular ion at *m/z*=216, *m/z* 158 (Se–Se) as base peak and *m/z* 187 (loss of CH_3–CH_2).

In addition, the total selenium content in each standard was checked by flame AAS after an acid digestion method and by using a 1000 $mg\,l^{-1}$ inorganic selenium Titrisol standard (Merck) for calibration. A suitable portion (10 μl) of each organoselenium compound was digested with 2 ml of concentrated nitric acid and diluted to 10 ml [55]. Purities of 97±3%, 98±2%, 98±3% and 97±3% were assessed for diethyldiselenide, diethylselenide, dimethyldiselenide and dimethylselenide, respectively.

2.5. Sample collection and preparation

Sediment samples were collected in Summer 1995 at low tide using a PTFE spatule. In all of the stations five aliquots of about 1 cm depth corresponding to four crossed sites 1 m apart from the same location were taken and then carefully mixed, homogenized and placed in polyethylene bottles. They were frozen for transporting and storage.

Separate samples of the sediments were dried in an oven at 110°C for 5 h to obtain mass loss data. Undried samples were analysed for inorganic and organic selenium species to avoid the effects of selenium loss. The results for the undried samples were multiplied by the undried sample mass ratio obtained from the separate drying experiments. All reported data are based on dry mass.

2.6. Analysis of total selenium in sediments

2.6.1. Sediment digestion

The digestion performed to evaluate the total selenium content in sediments was a wet method involving the use of HNO_3–$HClO_4$–HF, being a modification of that used by Kubota et al. [36]. In short, approximately 1.5 g of wet sample was accurately weighed into a PTFE beaker. Then 4 ml of nitric acid (65%, w/v), 3 ml of perchloric acid

(70%, w/v) and 5 ml of hydrofluoric acid (40%, w/v) were added. The solution was allowed to stand for 30 min to avoid any loss of volatile selenium compound. The beaker was capped with a PTFE lid, heated to 170°C on a hot plate and kept at this temperature until 2–3 ml of the solution remained in the beaker. The process was performed a second time adding the acid mixture and heating again. After the solution was cooled to room temperature, it was diluted to 25 ml in a standard flask with distilled water.

2.6.2. Reduction of Se(VI) to Se(IV)

The digest was transferred to a Pyrex tube (50 ml) and Se(VI) was quantitatively reduced to Se(IV) by adding 10 ml of 5 *M* HCl and boiling for 30 min. After allowing the residual solution to cool, pH was adjusted to 2.1 and selenium was derivatised.

2.6.3. Derivatization of Se(IV)

5 ml of 0.1% 4-chloro-*o*-phenylenediamine in 0.1 *M* HCl was added to the digestion solution and the mixture was heated at 75°C for 7 min to form the corresponding piaselenol. After allowing the solution to cool to room temperature, the selenium derivative was extracted twice with 1 ml of toluene by shaking for 1 min. The organic phase was separated from the aqueous phase and reduced to dryness under a N_2 stream. The residue was dissolved in 50 μl of hexane containing fluorodinitrobenzene (FDNB) as internal standard (230 ng l^{-1}). Aliquots of 1 μl were analysed by GC–MS.

2.6.4. Instrumental analysis of Se(IV) derivative

The chromatographic analysis of the piaselenols was performed by using the following oven temperature program: 40°C for 1 min after injection, followed by a 60°C min^{-1} ramp to 125°C and isothermal maintenance at this temperature for 1 min. Then a second heating ramp of 10°C min^{-1} up to 250°C and a final isotherm for 1 min. The injector block was heated at 240°C and the injection volume was 1 μl. Retention times: FDNB (internal standard), 9.8 min (*m*/*z* 186) and piaselenol, 10.5 min (*m*/*z* 218).

2.7. Leaching of inorganic selenium species from sediments

Inorganic selenium species were leached from the sediment using a method adapted from that of Cutter [60]. In short, 1 g of wet homogenized sediment sample was accurately weighed into a 50 ml polystyrene centrifuge tube, and 5 ml of 2 *M* sodium hydroxide solution was added. The centrifuge tube was then placed in an ultrasonic bath (Ultrasons, Selecta) for 4 h. The aqueous phase was separated by centrifugation (Sigma 4-10) at 10 000 rpm for 10 min. The supernatant was transferred into a 25-ml standard flask. 2 ml of fresh sodium hydroxide solution was added to the tube and centrifuged again at 10 000 rpm for 10 min. The supernatant was then decanted into the standard flask and sufficient distilled water was added to bring the volume to 25 ml. This solution was passed through a 0.45 μm membrane filter and Se(VI) and Se(IV) were separated by using SAX cartridges.

2.7.1. Separation of inorganic selenium

Selenite was separated from selenate by means of solid-phase extraction based on a method proposed in the literature [1]. Briefly, an anion-exchange resin (SAX cartridge) was conditioned by passing 10 ml of 3 *M* HCl followed by 10 ml of distilled water with a flow-rate of 5 ml min^{-1}. The pH of the 25 ml water extract containing selenite and selenate was adjusted to a value of 7–8 using HCl or NaOH and then it was passed through the cartridge at a flow-rate of 8 ml min^{-1} with the aid of a vacuum pump. Selenite and selenate were successively eluted with 25 ml of 1 *M* formic acid, which recovers Se(IV), and 25 ml of 3 *M* HCl, which recovers Se(VI).

In order to determine the concentration of Se(VI), it was quantitatively reduced to Se(IV) (see above). Se(IV) was derivatised using 4-chloro-*o*-phenylenediamine (see above). Finally, aliquots of 1 μl were analysed by GC–MS.

2.8. Extraction of volatile selenium species from sediments

The extraction of the dialkylselenide and the dialkyldiselenide from the sediments was performed

using two methods: passive headspace adsorption and dynamic headspace adsorption.

2.8.1. Passive headspace adsorption

20 g of wet sediment were accurately weighed in a polystyrene bottle. Then 1 cm^2 of active carbon strip was placed at the end of a nylon thread which was hanging from the lid of the bottle. The bottle was sealed with PTFE. The system was heated to 125°C for 2 h. Then the system was cooled down to room temperature. After 24 h, the active carbon was extracted with 1 ml of carbon disulphide for 10 min. 200 ng l^{-1} of internal standard (propofol) was added and the solution was analyzed by GC–MS.

2.8.2. Dynamic headspace adsorption

The apparatus used in the extraction of organic selenium species from sediments consists of a polystyrene bottle where the sample (20 g of wet sediment) is placed. The bottle was fitted with a polyethylene stopper and sealed with PTFE to avoid losses of the volatile selenium species. A N_2 stream (20 ml min^{-1}) was delivered through an orifice inserted into the lid of the bottle. An adsorption tube was inserted into another orifice in the lid. The adsorption tube was made by inserting a small plug of glass wool in the bottom of a PTFE (1/8 in. I.D.×3/16 in. O.D.; 1 in.=2.54 cm) then 2 g of active carbon was added and finally, the active carbon was held in place with an additional 1 cm of glass wool. The bottle was heated at 50°C for 1 h. Then the organic selenium species were eluted with 2 ml of carbon disulphide. 200 μl of a solution of 2000 ng l^{-1} of internal standard (propofol) in carbon disulphide was added and the solution was analyzed by GC–MS.

2.9. Instrumental analysis of volatile selenium species

The carbon disulphide extract was analyzed by GC–MS and the organic selenium species were separated with the following oven temperature program: 35°C for 2 min after injection, followed by a 10°C min^{-1} ramp to 180°C and isothermal maintenance at this temperature for 5 min. The injector block was heated at 240 °C and the injection volume was 1 μl. Retention times: 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 (internal standard), 17.1 min (m/z 178).

2.10. Derivatization of dialkyldiselenides

To improve the sensitivity of the chromatographic method, a derivatization step for the dialkyldiselenide was introduced. The apparatus used in the derivatization step is depicted in Fig. 1. A suitable aliquot of carbon disulphide extract containing the organoselenium compounds was placed in the volatilization polystyrene vial (internal diameter 16 mm and length 100 mm) and 350 mg of zinc powder and 3–4 drops of *n*-octanol were added. This vial was fitted with a polyethylene stopper and sealed with PTFE to avoid losses of the volatile selenols. The trapping vial contained 0.4 ml of double-distilled water, 0.6 ml of dimethylformamide (DMF), 1 ml of freshly prepared FDNB in DMF (1%, v/v) and 14 mg of $NaHCO_3$. A N_2 stream (as carrier gas) was passed through the volatilization manifold to remove the oxygen. Then 3 ml of 12 *M* HCl was injected into the volatilization vial through a septum with a syringe. A N_2 flow-rate of 100 ml min^{-1} over 10 min was used to complete the reaction in the trapping vial.

The trapping vial content was extracted with 4 ml of ethyl acetate (three times) by shaking for 15 min on a mechanical shaker. The ethyl acetate extract was separated from the aqueous phase and then concentrated using a rotatory evaporator to a volume of about 3 ml. The extract was subsequently evaporated to dryness under a N_2 stream. The residue was dissolved with 50 μl of toluene containing 200 ppb

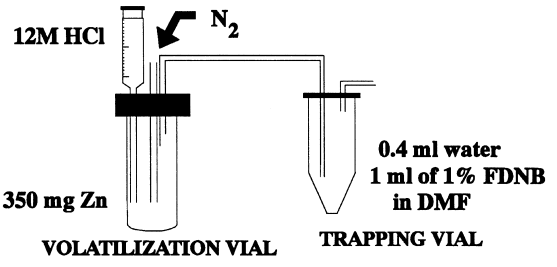

Fig. 1. Volatilization and trap device for the derivatization of dialkyldiselenides using dinitrofluorobenzene.

of propofol which was used as internal standard for chromatographic quantification.

2.11. Instrumental analysis of the dialkylselenide derivatives

The gas chromatographic oven temperature was programmed as follows: 40°C for 1 min after injection, followed by a 60°C min^{-1} ramp to 125°C and isothermal maintenance at this temperature for 1 min. Then a second heating ramp of 10°C min^{-1} up to 250°C and a final isotherm for 1 min. Injector block temperature, 250°C. Retention times: DMDSe derivative 16.3 min (m/z 262), DEDSe derivative 18.7 min (m/z 276) and propofol (internal standard) 9.1 min (m/z 178).

2.12. Sediments incubation experiments

Batches of five different sediments and a raw sewage sludge collected from Southwest Spain were incubated with the following procedure: 20 g of wet sediment was place in a polystyrene bottle and one of the following incubator reagents were added: 2 g of glucose, 0.3 g of elemental Se, 0.3 g of Se(IV) or 0.3 g of Se(VI). Sediments were incubated at 25 and 50°C for 15 days. Then the bottles were connected to a N_2 flow system at 20 ml min^{-1} and heated at 50°C for 1 h. The generated organselenium compounds were trapped in a PTFE tube containing active carbon and were subsequently eluted with 2 ml of carbon disulphide, being analysed by GC–MS.

3. Results and discussion

3.1. Reduction of Se(VI) and derivatization of Se(IV)

Methods for inorganic selenium analysis by GC are mostly based on the derivatization of selenite by formation of a piaselenol, using *o*-phenylenediamine in acid medium [39]. Several piaselenols have been proposed in the literature for this purpose which are stable and volatile. The piaselenol can be quantitatively separated from excess reagent by extraction with organic solvents. However, some of them (e.g. 4,5,6,7-tetracloropiaselenol and 4,6-dinitropiaselenol) have very long chromatographic retention times. We have chosen 4-chloropiaselenol because it is commercially available, has a short retention time, a high distribution ratio ($1.41 \cdot 10^{-3}$ in toluene) [73] and has been previously used in the analysis of inorganic selenium by GC–ECD [39]. In spite of others piaselenols (e.g. 5-nitropiaselenol or 4,6-dibromopiaselenol) have a higher sensitivity to ECD [74], they were not considered because of the relative sensitivity of the piaselenols may change by using a mass detector and they have also longer retention times than that corresponding to the 4-chloro-*o*-phenylenediamine used in the present work.

The reduction of the time necessary for the formation of the piaselenol makes the analytical method more attractive. This reduction is achieved when the derivatization is applied at higher temperatures [75]. Therefore, the influence of both the temperature and the reaction time on the derivatization of Se(IV) using 4-chloro-*o*-phenylenediamine was studied. Progress of the reaction under conditions of controlled temperature, ranged from 25 to 90°C, and reaction times, ranged from 1 to 150 min, was determined from the peak height of the piaselenol formed in the derivatization reaction. The experiments were carried out on a 25 ml solution containing 30 ng of Se(IV) in distilled water applying the procedure described in the Experimental. Optimum values giving good yields were obtained when the derivatization was performed at 75°C for 7 min. Higher temperatures or longer reaction times did not improve the derivatization yield.

The chromatographic approach based on the use of piaselenols is unable to directly determine selenate, which must be reduced to Se(IV) prior to the derivatization step. Therefore two chromatographic runs must be performed to analyze selenite and selenate species. A convenient method for reducing selenate to selenite is based on the use of hydrochloric acid, but the reaction must be carefully controlled to avoid losses of selenium as various volatile species. Therefore, the effect of increasing acidity, ranging from 3 to 12 *M*, and reaction times, ranging from 10 to 60 min, on the piaselenol chromatographic peak height was studied. For this purpose, a solution of 25 ml of water containing 30 ng of Se(VI) was analysed. Optimum conditions for the reduction of Se(VI) were obtained when the reaction

was performed using 10 ml of 5 *M* HCl for 30 min at boiling temperature. No differences were found between peak heights corresponding to reduced Se(VI) and Se(IV) derivatives, which indicated that the reduction of Se(VI) to Se(IV) was quantitative. However, lower acid concentrations or shorter reaction times did not quantitatively reduce the Se(VI) and higher acid concentrations or longer reaction times produced losses of up to 50% of selenium.

3.2. Analytical performance of the chromatographic determination method for the analysis of inorganic selenium species

Most of the chromatographic methods based on the use of piaselenols use ECD as detection method, which detects the complex rather than selenium itself. Therefore, we propose the mass detector for the final determination of piaselenol. This system has the major advantage that it is an element-specific detector, and it has already been used to determine Se(IV) as its 5-nitro-*o*-phenylendiamine derivative, using a quadrupole mass detector [41,76,77].

A calibration curve from aqueous selenium standard solutions was constructed. The stock solution of Se(IV) was diluted to obtain the working standard. Aliquots of this solution were taken to prepare a working range of selenium(IV). The reagent 4-chloro-*o*-phenylenediamine was added. The piaselenol was extracted and analyzed by GC–MS. The calibration curve was linear for selenium (as Se atoms) amounts less than 15 ng (correlation coefficient 0.9995). The determinations were carried out using FDNB as internal standard, which improved the precision. The minimum absolute detection limit was computed as [3×standard deviation of the mean]+ the value for the mean standard blank], for $n=10$ standard blank runs. It was estimated to be 12 pg of Se. The sensitivity (slope of the calibration curve) achieved by this method was $242\pm4.8\ \mu g^{-1}$ of Se. The solutions were analyzed at least five times with relative standard deviation lower than 5% when peak height was used. The response of the detector using peak area was usually greater than 5%; for this reason peak height was used throughout.

The absolute detection limit obtained by using several chromatographic techniques and detectors are summarized in Table 1, which shows that comparable detection limits are obtained by using mass detector, electron-capture detector or ICP-MS detector. In general, better figures are obtained using a gas chromatographic separation than HPLC, although the higher volumes generally used in HPLC can balance this differences.

Table 1
Absolute detection limit (pg of Se) achieved by using different chromatographic systems

Technique	Absolute detection limit (pg)	Ref.
GC–MS	12	This paper
GC–MS	<40	[76]
GC–ECD	4–86	[39]
GC–ECD	40	[86]
GC–MES	40	[87]
GC–ICP-ID-MS	3	[88]
GC–HG-MIP	25 000	[89]
HPLC–FAAS	100 000	[90]
HPLC–FAAS	31 000–51 000	[91]
IC–ET-AAS	1200–1700	[92]
IC–GF-AAS	20 000	[50]
HPLC–ET-AAS	1000	[93]
HPLC–ET-AAS	2500	[94]
HPLC–ET-AAS	1000–1200	[92]
IC–HG-AAS	1200–1500	[95]
HPLC–HG-AAS	680	[96]
IC–ICP-AES	91 000–140 000	[48]
HPLC–DIN-ICP-AES	8000–14 000	[49]
HPLC–ICP-AES	3000	[96]
IC–ICP-MS	1200–2000	[97]
HPLC–ICP-MS	120–160	[98]
HPLC–ICP-MS	1000	[90]
HPLC–ICP-MS	16	[96]
HPLC–USN-ICP-MS	40–70	[99]
IC–UV	10 000–25 000	[100]
HPLC–FD	100	[101]

MES=Microwave emission spectrometric detector; ID=isotopic dilution; DIN=direct injection nebulizer; USN=ultrasonic nebulizer; FD=fluorescence detector.

3.3. Total selenium content in natural samples: recovery studies

The method proposed for total selenium analysis was applied to five sediments and one raw sludge sample collected from areas of Southwest Spain. The results obtained in these experiments are listed in Table 2. Levels of total selenium were low in

Table 2
Total selenium content (ng of Se g^{-1}, dry mass basis) in natural sediments collected from Southwest Spain[a]

Sample	Se content (ng g^{-1})	Amount of each selenium specie added (ng)	Recovery (%)
Tinto river	13.4 (0.7)	10	99.1
Ayamonte city	98.1 (6)	100	95.5
Portil boatyard	26.2 (1.2)	30	104
Portil lake	33.4 (1.1)	30	96.3
Rompido estuary	53.8 (2.3)	50	97.4
Puntaumbria channel	110 (7.1)	100	102

[a] Standard deviations (three replicates) are given in brackets. Selenium recovery percentages when known amounts of selenite, selenate, DMSe, DMDSe, DESe, DEDSe and seleneurea are added to sediment samples.

sediments collected from all of the locations and ranged from 13 to 110 ng g^{-1} (dry mass basis). However, higher total selenium levels have been found in marine sediments collected around Singapore [78] and the USA [60], or in river and marine sediment certified reference materials [79], which indicated that selenium pollution was not severe in the sediments considered in this work.

Recovery studies were performed adding to each sample amounts of each inorganic (selenite and selenate) and organic selenium species (DMSe, DMDSe, DESe, DEDSe and seleneurea) comparable to the natural selenium content of the sediments. The spiked samples were homogeneized by magnetic stirring at 4°C for 24 h and then analyzed. The results obtained in these experiments are listed in Table 2, which shows quantitative recoveries. Therefore, no interferences occurred using the GC–MS method.

The evaluation of total selenium in sediments using GC–MS compares favourably with other usual techniques for the analysis of this element. Graphite furnace atomic absorption spectroscopy has been applied to the determination of total selenium in sediments after digestion with HNO_3–$HClO_4$–HF mixture [80]. However, atomic absorption of selenium is difficult to measure in some cases because of a large molecular absorption attributable to the presence of AlF_3 [36]. Other instrumental methods, including fluorimetry, HG-AAS, HG-ICP-AES, HG-ICP-MS, have also been used for this purpose, but moderate or serious interferences were found [40] in contrast with the results obtained in the present work using GC–MS method.

3.4. Speciation of inorganic selenium species in sediments

Using the alkaline leaching method proposed in the Experimental section, both selenite and selenate are released from sediments. However, the gas chromatographic method only determines selenite. Selenate must be reduced to selenite in order to be quantified, and both species have to be separated before derivatization and analysis. For this purpose a solid-phase extraction was optimized.

3.4.1. Separation of inorganic species of selenium with SAX cartridges

A selective elution of selenite and selenate is necessary to evaluate these compounds by GC–MS. For this purpose, a SAX cartridge was used. Retention of Se(IV) and Se(VI) in the cartridge may depend on the acidity and volume of the sample, while the subsequent sequential elution of both species is related to the concentration and volume of the solution used as eluent. Experiments for the optimization of these parameters were performed using 12 μg of both Se(IV) and Se(VI) in 25 ml of double distilled water, following the procedure described in the Experimental section.

3.4.1.1. Effect of acidity

Loading of inorganic selenium species in the cartridge has been studied using aqueous solutions of selenite or selenate at different pH values, ranging between 3 and 9. The pH was fixed using HCl and NaOH solutions. The presence of selenium species in the aqueous effluent was evaluated by the GC–MS

method. Quantitative retention of both species was only achieved when the pH of the sample was between 7 and 8. Therefore, a pH of 7 was used in further experiments.

3.4.1.2. Influence of the sample volume

It is interesting to evaluate the volume of aqueous sample that can be passed through the cartridge with quantitative retention of the selenium species. Inorganic selenium species were dissolved in 10, 20, 30, 50 and 75 ml of water and passed through the cartridge. The presence of selenium species in the aqueous effluent was evaluated by the GC–MS method. Concentration of selenium species in the effluent were below the detection limit which indicated their quantitative retention on the cartridge.

3.4.1.3. Influence of the concentration of HCOOH and HCl on the recovery of inorganic selenium species

Concentrations of formic acid ranging between 0.5 and 4 *M* were tested for the elution of inorganic selenium species. Quantitative recoveries were achieved for Se(IV) when 1 *M* HCOOH was used as eluent but selenate was not eluted. A higher concentration of this acid up to 4 *M* did not modify the results. A different stronger acid such as HCl was studied for quantitative elution of selenate, a concentration of at least 2 *M* of this acid being necessary.

3.4.1.4. Influence of the volume of HCOOH and HCl on the recovery of inorganic selenium species

Volumes of each eluent ranging between 10 and 40 ml were tested to study the elution of inorganic selenium species. At least 25 ml of each eluent must be used for quantitative elution of both selenite and selenate.

In summary, selenite and selenate were found to be retained on the cartridge and then eluted quantitatively. Moreover, the extraction method did not influence the identity of selenite and selenate, and speciation was possible.

3.4.2. Extraction of Se(IV) and Se(VI) from sediments

The alkaline leaching of inorganic selenium species from sediments has been proposed as a quantitative method to solubilize these species in different operationally defined phases of sediments: exchangeable, carbonate and iron and manganese oxide phases. Moreover, no detectable speciation change has been observed [60]. Therefore, this approach was selected from the literature to determine the selenite and selenate content in the sediments. In order to optimize the extraction of these species from river and marine sediments, the concentration and volume of sodium hydroxide and the leaching time were studied in two representative samples from the area considered in the present work (Southwest Spain): a raw sewage sludge collected from Ayamonte city and a sediment collected from Rompido boatyard.

3.4.2.1. Concentration of NaOH solution

5 ml of NaOH solution at different concentrations ranging from 0.5 to 4 *M* was tested to extract selenite and selenate from 1 g of wet sediment using an ultrasonic bath for 5 h. Results from this experiment are depicted in Fig. 2. For an optimum extraction of the species in both samples, at least a concentration of 2 *M* NaOH solution had to be used to extract selenite. Selenate could be extracted with 1 *M* NaOH solution. However, selenate was not found in the sewage sludge. Higher concentrations of leaching agent did not significantly increase the selenium amount extracted from the sediments (ANOVA, $P > 0.61$, at the 95% confidence level). Therefore, a concentration of 2 *M* was used in further experiments.

3.4.2.2. Volume of 2 M NaOH

Volumes of NaOH ranged from 1 to 10 ml were tested but no significative differences were found between the amounts of selenite and selenate leached from the sediments (ANOVA, $P > 0.15$, at the 95% confidence level). Relative standard deviations for selenium extraction with NaOH volumes up to 2 ml ranged from 11 to 16%. A better precision was obtained using a leaching volume higher than 2 ml (relative standard deviations ranging from 4 to 7%). Therefore, 5 ml of 2 *M* NaOH was used throughout.

3.4.2.3. Leaching time

Different leaching times ranged from 1 to 8 h were tested. Results are shown in Fig. 3. The amounts of

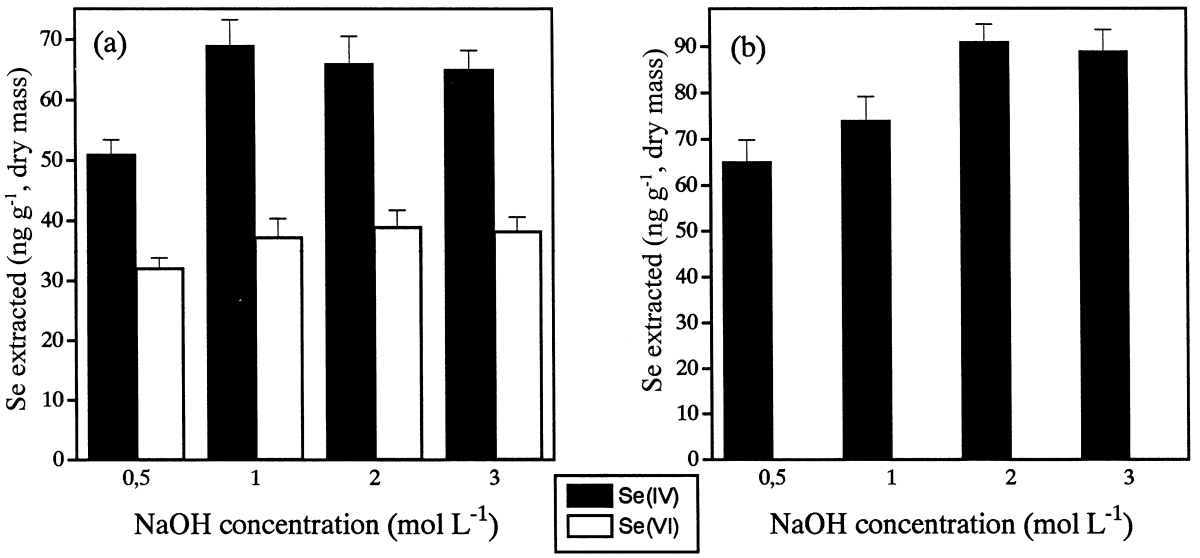

Fig. 2. Influence of the sodium hydroxide concentration (5 ml solution) on the 5 h extraction of selenite and selenate from 1 g (wet mass) portions of sediment collected from Rompido boatyard (a) and raw sewage sludge from Ayamonte city (b).

both selenite and selenate increased significantly (Student's *t*-test $P>0.056$ at the 95% confidence level) with leaching time up to 3 h. Longer leaching times did not improve the extraction (ANOVA $P>0.38$, at the 95% confidence level) but the precision was poorer by leaching the selenium species for only 3 h. Therefore, a leaching time of 4 h was taken as the optimum value.

3.4.2.4. Recovery experiments

The possibility of changes in species distribution during the leaching procedure was studied by spiking

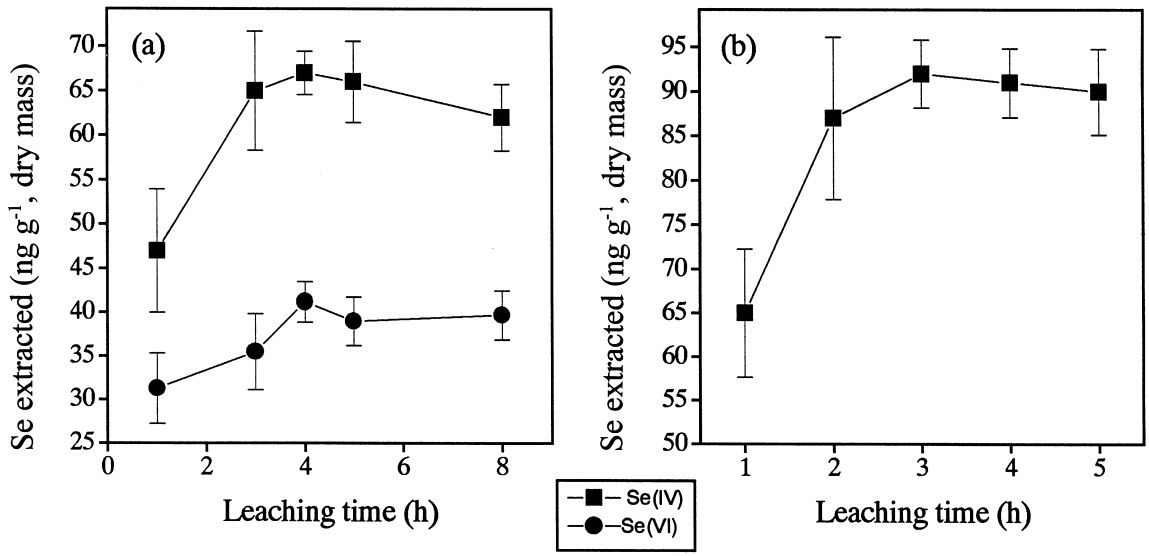

Fig. 3. Influence of the leaching time on the selenite and selenate extraction from 1 g (wet weight) portions of sediment collected from Rompido boatyard (a) and raw sewage sludge from Ayamonte (b), using 5 ml of 2 *M* NaOH as extractant.

each sediment with known amounts of selenite or selenate. Quantitative recoveries for both species were obtained which indicated negligible interconversion between Se(IV) and Se(VI) during the experiments.

3.5. Inorganic selenium speciation in natural samples

This method was applied to five sediments and a sewage sludge collected from areas of Southwest Spain. Results are summarized in Table 3. The given means with standard deviations are the result of three parallel analysis of subsamples of a given sediment. Selenite was always more abundant in the studied samples than selenate, being detected in all of the samples. Se(IV) levels (dry weight basis) ranged from 13.8 ng g^{-1} (in the Tinto river) to 92 ng g^{-1} (in the raw sludge collected in Ayamonte city). However, in two samples (collected from Tinto river and from Ayamonte city) no selenate was detected. In the remaining samples, Se(VI) was present in levels ranged from 3.9 to 41.2 ng g^{-1} (dry mass). Therefore, selenite was shown to have a greater affinity for mineral oxides and sediments than does selenate, as reported by several authors [81–83].

Quantitative recoveries were obtained by spiking experiments in all the samples. In addition, the sum of selenite and selenate agreed with the total selenium content quantified in Table 2, in all of the studied samples within the given uncertainties. This could indicate the absence of organic selenium compounds at ng g^{-1} levels in the samples considered in this study.

In summary, the proposed method allows the analysis of selenite and selenate in sediments. Estimated detection limit for both selenite and selenate using aliquots of 1 g of wet sediments is 0.6 ng of Se g^{-1} (wet mass basis). Relative standard deviation of the whole procedure was lower than 8%.

3.6. Analytical performance of the chromatographic system for the determination of the underivatised organic species of selenium

A calibration curve from organoselenium standard solutions in hexane was constructed. The stock solution of each organoselenium (DMSe, DMDSe, DESe and DEDSe) was diluted to obtain the working standard. Aliquots of this solution were taken to prepare a working range of the organoselenium compounds. Aliquots of 1 μl were injected into the chromatograph and analyzed by GC–MS. The calibration curves were linear for selenium (as Se atoms) amounts less than 17 ng (correlation coefficient 0.9991) for DMSe, 27 ng (correlation coefficient 0.995) for DESe, 60 ng (correlation coefficient 0.998) for DMDSe and 62 ng (correlation coefficient 0.998) for DEDSe. The determinations were carried out using propofol as internal standard, which improved the precision. The minimum absolute detection limits were computed as [3×standard deviation of the mean]+the value for the mean standard

Table 3
Levels (ng Se g^{-1}, dry mass) and recovery percentages of selenite and selenate in natural samples collected from Southwest Spain[a]

Sample	Se(IV) (ng g^{-1})	Se(VI) (ng g^{-1})	Recovery experiments			
			Se(IV) added (ng)	Se(VI) added (ng)	Recovery of Se(IV) (%)	Recovery of Se(VI) (%)
Tinto river	13.8 (0.8)	<DL	10	5	97	96
Ayamonte city	92 (3.8)	<DL	100	5	96	97
Portil boartyard	23.4 (1.6)	3.9 (0.3)	20	5	98	100
Portil lake	25.7 (1.5)	4.9 (0.3)	20	5	102	97
Rompido boatyard	41.9 (2.6)	10.1 (0.6)	40	10	100	104
Puntaumbria boatyard	67 (2.4)	41.2 (2.3)	70	40	97	97

[a] Standard deviations are given in brackets (three replicates).

blank], for $n=10$ standard blank runs. They were estimated to be 0.3 ng, 0.2 ng, 0.1 ng and 0.2 ng for DMSe, DESe, DMDSe and DEDSe, respectively. The sensitivities (slope of the calibration curve) achieved by this method were 87.7 ± 1.3, 101 ± 3.1, 268 ± 4.7 and 111 ± 2.0 μg^{-1} of Se for DMSe, DESe, DMDSe and DEDSe, respectively. The solutions were analyzed at least five times with relative standard deviation lower than 5% when peak height was used. The response of the detector using peak area was usually greater than 5%; for this reason peak height was used throughout.

3.7. Extraction of volatile selenium species from sediments

Parameters controlling the extraction of the alkylselenium species were studied using samples in which the absence of these species was previously checked. The sample selected for the optimization study was collected from Rompido boatyard, in which further experiments were performed three times. Portions of 20 g of sediment were spiked with 30 μg of each species dissolved in water and stirred overnight at 4°C.

3.7.1. Passive headspace adsorption

Optimal conditions for the volatilization and trapping of the organic selenium species were obtained by examining the effects of time and temperature on the recoveries. Sediment samples were heated for 2 h at temperatures ranged from 25 to 150°C. The organic selenium species adsorbed on the active carbon strip were dissolved using 2 ml of carbon disulphide and the recoveries were evaluated (Table 4). Maximum recoveries for all the species were obtained by heating the samples at 50°C. By using lower temperatures the extraction was poorer but the recovery did not improve by heating the sediment at higher temperatures. Consequently, heating at 50°C was used in further experiments. Several heating times were tested ranged from 15 min to 4 h. The organic selenium species adsorbed on the active carbon were dissolved using 2 ml of carbon disulphide. The results are shown in Table 4. At least 2 h must be used to yield maximum recoveries which were not improved using longer heating times.

The elution of the organic selenium species from the active carbon was studied by using different volumes of carbon disulphide ranged from 0.5 to 5 ml. Results are summarized in Table 4. The optimum

Table 4
Recovery percentages obtained using the passive headspace adsorption method for extraction of organic selenium species from 20 g of a sediment collected from Rompido boatyard (Spain), spiked with 30 μg (as Se) of DMSe, DESe, DMDSe and DEDSe[a]

Heating time (min)	Temperature (°C)	Elution volume (ml)	Recovery (%)			
			DMSe	DMSe	DESe	DEDSe
120	25	2	37 (5.9)	38 (6.4)	28 (7.8)	39 (3.7)
	50		56 (2.9)	59 (3.7)	51 (2.9)	57 (4.5)
	100		41 (7.3)	44 (6.5)	38 (3.7)	43 (6.5)
	125		44 (5.7)	48 (3.6)	43 (7.3)	47 (4.3)
	150		43 (5.6)	48 (3.3)	32 (8.0)	47 (3.6)
15	50	2	27 (5.4)	19 (4.3)	24 (5.4)	23 (4.9)
60			43 (2.8)	39 (4.5)	35 (3.6)	46 (2.9)
120			56 (2.8)	59 (2.4)	51 (2.2)	57 (2.9)
180			59 (3.6)	55 (4.5)	53 (3.3)	57 (5.0)
240			54 (3.3)	57 (4.5)	55 (3.7)	59 (3.6)
120	50	0.5	33 (3.7)	24 (3.6)	29 (3.3)	36 (5.0)
		1	57 (2.9)	54 (2.9)	58 (2.4)	55 (3.7)
		2	56 (2.4)	59 (2.2)	51 (2.9)	57 (3.3)
		3	54 (2.9)	55 (4.3)	51 (3.3)	57 (3.6)
		5	58 (3.7)	57 (2.9)	56 (4.5)	54 (2.4)

[a] Standard deviations (three replicates) are given in brackets.

volume was 1 ml and higher volumes did not improve the recoveries.

In summary, maximum recoveries were obtained by heating the sediment at 50°C for 2 h and eluting the compounds from the active carbon strips with 1 ml of carbon disulphide. However, the recoveries were lower than 60%. The extraction procedure was carried out a second time over the same sediment and no more organoselenium compounds were recovered. This can be explained by the loss of selenium species by volatilization, as well as the differential strength of linkage of selenium species to the active points of the sediments.

3.7.2. Dynamic headspace adsorption

Recoveries of organic selenium species were evaluated by heating the sediment at temperatures ranged from 25 to 150 °C for 2 h using a N_2 stream at 20 ml min^{-1}. Results are presented in Table 5. Higher recoveries were obtained by heating at 50°C, but they were not improved by using temperatures higher than 50°C. Different heating times were tested ranging from 15 min to 4 h (Table 5). At heating time of at least 1 h was necessary to obtain maximum recoveries for the extraction of the organoselenium species. Finally different volumes of carbon disulphide, ranged from 1 to 5 ml, were used for the elution of the organic selenium compounds from the active carbon trap. Results are summarized in Table 5. The optimum elution volume was 2 ml insofar as higher volumes did not improve the recoveries.

In summary, maximum recoveries were obtained by heating the sediment at 50°C for 1 h and eluting the compounds from the active carbon with 2 ml of carbon disulphide. The recoveries were 91%, 77%, 89% and 78% for DMSe, DMDSe, DESe and DEDSe, respectively, and were higher than using the passive headspace adsorption method.

Finally, a thermal desorption technique was tested, using the injector liner of the chromatograph filled with active carbon to trap the selenium species using the dynamic headspace adsorption method. After adsorption of the selenium species onto the active carbon, the injector liner was placed into the

Table 5
Recovery percentages obtained using the dynamic headspace adsorption method to extract the organic selenium species from 20 g of sediment collected from Rompido boatyard (Spain) spiked with 30 μg (as Se) of DMSe, DESe, DMDSe and DEDSe[a]

Heating time (min)	Temperature (°C)	Elution volume (ml)	Recovery (%)			
			DMSe	DMDSe	DESe	DEDSe
120	25	2	52 (3.7)	45 (5.3)	51 (5.9)	37 (6.2)
	50		87 (4.3)	83 (2.4)	86 (4.5)	81 (3.3)
	100		73 (4.5)	69 (5.7)	70 (5.7)	77 (4.9)
	125		54 (3.7)	51 (6.2)	63 (6.4)	55 (3.6)
	150		49 (6.2)	40 (5.7)	37 (5.4)	41 (4.5)
15	50	2	40 (3.3)	42 (5.7)	36 (4.5)	29 (4.5)
60			91 (3.7)	77 (3.3)	89 (4.5)	78 (3.7)
120			87 (3.7)	83 (5.0)	86 (3.6)	81 (3.7)
180			81 (3.6)	82 (4.2)	78 (5.4)	80 (4.3)
240			62 (5.4)	74 (5.7)	68 (3.7)	76 (5.1)
120	50	1	83 (6.5)	71 (9.1)	80 (5.7)	69 (7.0)
		2	91 (3.7)	77 (4.5)	89 (4.1)	78 (3.7)
		3	93 (5.9)	81 (4.1)	86 (3.7)	80 (3.3)
		4	90 (4.3)	79 (4.3)	84 (4.2)	82 (3.7)
		5	88 (7.0)	80 (3.3)	86 (3.7)	79 (5.7)

[a] Standard deviations (three replicates) are given in brackets.

chromatograph and the sample was run. However, recoveries lower than 60% were obtained.

3.8. Improvement of the detection of dialkyldiselenide species

Direct analysis of dialkylselenide and dialkyldiselenide by GC–MS, considered in the previous section, does not give good detection limits to evaluate the presence of volatile organic selenium species in natural sediments. However, these species are not able to be preconcentrated without losses due to their high volatility. Loss percentages of 80% for dialkylselenide and 100% for dialkyldiselenide were obtained when carbon disulphide solutions of 10 ml containing these compounds were concentrated to a final volume of 0.5 ml under a N_2 stream.

Therefore, an alternative method based on derivatization with dinitrofluorobenzene (FDNB) to form less volatile species with alkylselenium has been tested. Se–FDNB derivatives are non-volatile enough to be concentrated under a N_2 stream. However, dialkyl- selenides and diselenides do not directly react with FDNB and they were not found in the final organic extracts by using GC–MS. Therefore, a pretreatment of samples based on volatile selenol formation followed by the reaction of this compounds with FDNB was necessary for this purpose.

The volatilization of selenium compounds has been described by Diplock et al. [84,85] and modified by Ganther [71], who trapped ^{75}Se-labeled volatile species in FDNB and assayed them in a γ-counter. The volatilization–derivatization system is depicted in Fig. 1 and was made in polystyrene, polyethylene and PTFE materials because organic selenium is readily deposited as elemental selenium on glass surfaces [85]. The evaporation vessel must be sealed with PTFE to avoid losses of the highly volatile selenols. One or two drops of *n*-octanol must be added to this vessel to avoid frothing during selenol formation but it is easily swept out with the N_2 stream and it was found as a minor component in the TIC of the final extract. A mass of 14 mg of $NaHCO_3$ must be added to the trapping vial to provide the basic pH necessary in the reaction between the dissociated form of the volatile selenol and FDNB. In addition, it neutralized HCl fumes.

Several parameters controlling the derivatization step were optimized and included the choice of the acid medium, the nitrogen flow-rate, the purge and derivatization times, the extraction volume and the final evaporation of the solvent.

3.8.1. Choice of the acid medium

The optimization experiments were performed (five replicates) by using 25 ng and 50 ng (as selenium) of dimethyldiselenide and diethyldiselenide, respectively, in 2 ml of carbon disulphide, following the recommended procedure described under Experimental. Different volumes of 10 *M* HCl ranged between 1 and 5 ml were tested and higher derivatization yields were obtained by using 3 ml of HCl, but higher volumes did not cause any significant improvement.

3.8.2. Nitrogen flow-rate and purge and derivatization times

A N_2 stream (as carrier gas) must be passed through the volatilization manifold to remove the oxygen prior to the derivatization reaction and a purge time of 5 min was chosen for this purpose. During the derivatization step a flow-rate of 100 ml min^{-1} was passed for 10 min to give satisfactory results and was used in all subsequent experiments. Faster flow-rates may avoid the complete trapping of the selenols in the FDNB solution, but by using lower flow-rates there was a greater chance of the oxidation of the volatile material.

3.8.3. Extraction and concentration of the organselenium derivatives

Extraction of the organoselenium derivatives with a solvent is a prior step necessary for the GC–MS determination. The concentration of these species by removing the solvent under a N_2 stream will improve the detection limit. We have studied the influence of several variables on the extraction: type and volume of solvent, number and time of extractions and final volume to be concentrated. Several solvents such as carbon disulphide, hexane, benzene, ethyl acetate and cyclohexane were tested to extract the organoselenium derivatives in the trapping vial, using

single and multiple (up to four) extractions for 5 min. Two successive extractions were insufficient, but three and four extractions gave higher, although similar, results. Therefore, three successive extractions were chosen for further experiments, ethyl acetate being the best solvent. Several extraction times ranging from 1 to 10 min were tested and better results were obtained by using extraction times longer than 4 min. Loss of the organoselenium derivatives during the concentration step was studied by removing the solvent under a N_2 stream to different final volumes and then diluting to the initial volume prior to the analysis by GC–MS but no losses were observed when the volume was reduce to dryness.

Finally, when dimethyl- and diethylselenide were subjected to the volatilization procedure, they did not give detectable dinitrophenyl (DNP) derivatives, which could be due to both the higher volatility of these compounds which could cause their loss through the nitrogen flow [36] prior to the trapping reaction, or to the difficulty of dialkylselenide in forming the corresponding selenol.

3.9. Analytical performance of the chromatographic system for the determination of the organic selenium derivatives

The calibration curves for dimethyldiselenide and diethyldiselenide using peak heights were linear for selenium amounts less than 25 and 50 ng, respectively. The determinations were carried out using propofol as internal standard, which improved the precision. The minimum detection limits were computed as [3×standard deviation of the mean]+the value for the mean standard blank], for $n=10$ standard blank runs. They were estimated to be 0.3 ng of Se for DMDSe (correlation coefficient 0.998) and 0.8 ng of Se for DEDSe (correlation coefficient 0.996). The sensitivities (slope of the calibration curve) achieved by this method were 66.9 ± 1.8 and 26.8 ± 0.4 μg^{-1} of Se for DMDSe and DEDSe derivatives, respectively. The solutions were analysed at least five times with relative standard deviations lower than 5% when peak height was used. The standard deviations using peak area were usually greater than those evaluated using peak heights. For this reason peak height was used throughout.

The chromatographic sensitivity for the dialkylselenium derivatives did not improve that obtained for the corresponding underivatized species. However, the major advantage of the derivatization approach is that the species may be concentrated to dryness (preconcentration ratio=40) under a N_2 stream without evidence of losses of these compounds by volatilization, which improve the sensitivity of the analytical procedure.

3.10. Organic selenium content in natural sediments.

The method proposed for the extraction of volatile selenium species was applied to five sediments and a sewage sludge collected from areas of Southwest Spain. However, levels in all of the sediments were below the detection limit, even if derivatization of the dialkyldiselenide was performed to improve the sensitivity of the method. Two possible reasons supporting these results are the absence of bioalkylation microorganisms in the sediments and the normally high temperatures in the Southern Spain summer (higher than 35°C) which may cause volatilization of the alkylselenides. However, DMSe at a maximum concentration of 1 ng g^{-1} and DMDSe at a maximum concentration of 2.1 ng g^{-1} have been evaluated in sediments samples of different locations of a river in Germany [63].

Therefore, recovery experiments were carried out on these samples, which were spiked with 8 μg of DMSE and DESe and 0.8 μg of DMDSe and DEDSe. Results are summarized in Table 6. Recoveries were higher than 76% for all the organic selenium species. Estimated detection limits in these natural samples for DMSe, DMDSe, DESe and DEDSe using aliquots of 20 g of wet sediments are 33, 1.0, 22 and 2.3 ng of Se g^{-1} (wet mass basis). Relative standard deviation of the whole procedure was lower than 9%.

3.11. Samples incubation experiments

A variety of microorganisms are capable of producing volatile organic selenium compounds, and methylated selenium species have been observed emanating from soils, sediments and sewage sludges [42]. Organoselenium compounds have been gener-

Table 6
Recovery percentages obtained using the dynamic headspace adsorption method to extract the organic selenium species from 20 g of sediments collected from areas of Southwest Spain[a]

Sample	Recovery percentage			
	DMSe	DMDSe	DESe	DEDSe
Tinto river	86 (4.1)	79 (4.9)	79 (3.7)	80 (6.1)
Ayamonte city	92 (4.5)	83 (4.3)	85 (4.1)	79 (5.7)
Portil boatyard	87 (3.6)	85 (4.2)	82 (3.1)	79 (5.1)
Portil lake	86 (4.9)	80 (3.7)	84 (7.0)	83 (4.9)
Rompido boatyard	91 (3.7)	78 (5.1)	85 (5.1)	76 (3.7)
Puntaumbria boatyard	89 (4.1)	81 (3.7)	83 (5.0)	80 (5.4)

[a] Samples are spiked with 8 μg (as Se) of DMSe, DESe and 0.8 μg (as Se) of DMDSe and DEDSe. Standard deviations (three replicates) are given in brackets.

ated from incubated sediments collected from the River Avon [52]. These sediments were enriched with selenite, nutrient broth, glucose and yeast extract and incubated at room temperature. In 14 days total evolved selenium was found to be ranged between 20 to 572 ng g^{-1}, and the presence of DMSe and DMDSe was observed by GC–ion trap (IT)–MS.

Similar comparative incubation experiments were performed in the samples considered in this work. The detection of the organic selenium species evolved during the course of 15 days of sample incubation at 50°C is presented in Table 7. Organic selenium species (DMSe and DEDSe) were detected in the sample collected in Ayamonte city once it was enriched with glucose, selenite or selenate (but not elemental selenium). This sample was a sewage sludge and the presence of microorganisms may be guaranteed. Results showed that microorganisms may promote methylation and ethylation processes from selenite and selenate when raw sewage sludges are incubated at 50°C. Experiments performed at 25°C did not show the presence of any detectable organic selenium species.

4. Conclusions

The use of GC–MS provides a good alternative for speciation of inorganic and organic selenium against more common couplings based on atomic spectrometry detection with comparable detection limits. However, four chromatographic analysis are necessary to determine the presence of selenite, selenate, DMSe, DESe, DMDSe and DEDSe.

The number of methods proposed for selenium speciation in sediment samples are scarce, especially for volatile organoselenium compounds, due to the transfer of this compounds to the atmosphere which reduces their presence in natural solid samples and makes very sensitive speciation procedures with cumbersome preconcentration steps necessary. The procedure developed in this study based on the use of GC–MS allows the determination of both selenite and selenate at levels higher than 2 ng g^{-1}, usually found in natural sediments. Comparable levels may be determined for dialkyldiselenide, but the methodology must be improved to evaluate the usually low levels of dialkylselenide found in natural sediments. However, the GC–MS system constitutes an interesting approach to solve selenium environmental problems if we consider that it is a commercial coupling which does not require very skilled operators as traditional hyphenated systems do.

Selenium levels present in sediments from rivers and estuaries in southwest Spain indicate the absence

Table 7
Organic selenium species detected during sediment incubation at 50°C for 15 days after adding inorganic selenium species or glucose

Sample	Glucose (0.1 g g^{-1})	Elemental selenium (15 mg g^{-1})	Sodium selenite (15 mg g^{-1})	Sodium selenate (15 mg g^{-1})
Tinto River	ND	ND	ND	ND
Ayamonte city	DESe+DMDSe	ND	DESe+DMDSe	DESe+DMDSe
Portil boatyard	ND	ND	ND	ND
Portil lake	ND	ND	ND	ND
Rompido boatyard	ND	ND	ND	ND
Puntaumbria boatyard	ND	ND	ND	ND

ND: below detection limit.

of severe pollution problems in this area caused by the presence of selenium.

Finally, it has been shown that alkylation processes may only occur in these solid samples when the presence of both microorganisms and selenite or selenate is guaranteed in the sample.

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