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Comparison of two derivatizing agents for the simultaneous determination of selenite and organoselenium species by gas chromatography and atomic emission detection after preconcentration using solid-phase microextraction

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Abstract

Two methods for the simultaneous determination of selenite and two organoselenium compounds, dimethylselenide (DMSe) and dimethyldiselenide (DMDSe), are proposed. Both methods involve sample preconcentration by solid-phase microextraction (SPME) and capillary gas chromatography coupled to atomic emission detection (GC-AED). The main difference between the methods is the derivatizing agent used to complex the inorganic species: 4,5-dichloro-1,2-phenylenediamine. sodium tetraethylborate and The parameters affecting the derivatization and preconcentration steps, chromatographic separation as well as detection of the compounds were optimized. Direct immersion (DI) mode and a relatively long extraction time were selected for the method involving the formation of the piazselenol complex, better sensitivity being achieved for the three analytes under study. In this case, detection limits ranged between 3 to 25 ng L⁻¹, depending on the compound. Headspace mode (HS) and extraction times of 20 min were selected for the method involving tetraalkylborate, and detection limits of between 7.3 and 55 ng L⁻¹ were obtained. DMSe and Se(IV) were found in several of the water samples analyzed at concentrations of 0.07-1.0 ng mL⁻¹.

Keywords: Selenite; Dimethylselenide; Dimethyldiselenide; Sodium tetraethylborate; 4,5-dichloro-1,2-phenylenediamine; Solid-phase microextraction (SPME); Gas Chromatography – Atomic Emission Detection (GC-AED); Waters.

1. Introduction

Trace levels of selenium occur naturally in waters and is also released into the environment from anthropogenic sources, because this element is used in the microelectronic, semiconductor and optoelectronic industries [1]. Selenium concentration levels ranging between 0.03 and 6000 ng mL⁻¹ have been found in waters, depending on the origin of the sample [2,3]. Selenium is an essential trace element with only a small difference between essential and toxic levels, depending on the chemical form in which it is present [1]. The concentration permitted in drinking water is federally regulated in the USA and must not exceed $10 \,\mu$ g L⁻¹ [4].

The biomethylation process of inorganic selenium by micro-organisms in environmental matrices produces mainly dimethylselenide (DMSe) and dimethyldiselenide (DMDSe), and is well documented in the literature [5], methylation being an effective detoxification mechanism. Volatile methylated species are considered to be 500 times less toxic than selenite [1].

The importance of selenium speciation is evident as regards the great number of reviews related with this point [6-10]. The volatility of DMSe and DMDSe means that gas chromatography is frequently used for their determination, while application of this technique to determine selenite involves its volatilisation by suitable derivatization agents. Sodium tetraethylborate [11-13], sodium tetrapropylborate [11], 4,5-dichloro-1,2-phenylenediamine [14,15], and 4-chloro-o-phenylenediamine [2,16] have been used to transform selenite into less polar compounds which are amenable for gas chromatography to be carried out.

Microwave induced plasma coupled to atomic emission detection (MIP-AED) [1,3,11,13,17-19] has been used as a good alternative to the expensive detector based on inductively coupled plasma (ICP) [20-23]; other detection techniques used in selenium speciation are atomic fluorescence spectrometry (AFS) [24], electrothermal atomic absorption spectrometry (ETAAS) [25,26], mass spectrometry (MS) [12,14,15,27] and photoionization (PID) [28]. Selectivity and sensitivity are major advantages of the detection system used in the present work (MIP-AED), which is even more sensitive than inductively coupled plasma mass spectrometry (ICP-MS) and AFS for Se detection [18].

Despite the very sensitive analytical procedures available, the speciation of selenium in environmental samples requires preconcentration systems. The literature reports different preconcentration techniques, the most common involving cryogenic trapping (CT) followed by thermal desorption [11,22,24,26] or purge-and-trap (PT) without the cryogenic module [19]. Solid-phase extraction (SPE) has also been used [2]. Solid-phase microextraction (SPME) has proved to be very useful for preconcentrating both inorganic [12,13,15] and organoseleno compounds [1,17,18,20,21,23]. As far as we know no previous work describing the use of SPME for the simultaneous preconcentration of selenite, DMSe and DMDSe has been found. This environmental friendly technique brings together extraction, preconcentration and clean-up procedures in one step [29,30]. The described procedures for the simultaneous determination of inorganic and organic species represent additional ways to carry out selenium speciation.

2. Experimental

2.1. Chemicals

Dimethylselenide [(CH₃)₂Se, DMSe; 99% purity], dimethyldiselenide [(CH₃)₂Se₂, DMDSe; 98% purity] and stock standard solution of 1000 µg mL⁻¹ of Se(IV) were obtained from Fluka (Buchs, Switzerland), Aldrich (Steinheim, Germany) and Panreac (Barcelona, Spain), respectively. Standard solutions of 1000 µg mL⁻¹ of the volatile compounds were prepared by appropriate dilution in methanol and stored at 4 °C. Lower concentration stock solutions of the organo-compounds were prepared daily in methanol and were stored in the refrigerator. Working standard solutions of the three analytes were prepared immediately before use by diluting with water obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA).

The two derivatizing agents assayed were prepared as follows: a 1.0% (m/v) aqueous solution of sodium tetraethylborate (NaBEt₄, 98% purity, Strem Chemicals, Newburyport, MA, USA) was prepared in a 2% (m/v) sodium hydroxide medium. Fractions of this solution were stored in the dark at -20 °C. The solution remained stable for one month. A 1.0% (m/v) aqueous solution of 4,5-dichloro-1,2-phenylenediamine (97% purity, Aldrich) was prepared in a 0.1 M hydrochloric acid ethanol solution.

Sodium chloride, sodium acetate and potassium dihydrogen phosphate were obtained from Sigma (St. Louis, MO, USA). Acetic acid (99.8% v/v, Fluka) and phosphoric acid (85% v/v, Panreac) were used to prepare buffer solutions.

The plasma gas and carrier gas used for GC was helium. The reagent gases for the AED were oxygen and hydrogen. Nitrogen was used for purging the AED system. All the gases (99.999% purity) were supplied by Air Liquide (Madrid, Spain).

2.2. Instrumentation

The SPME device for manual sampling consisted of a holder assembly and several replaceable fibers, all obtained from Supelco (Bellefonte, PA, USA). SPME fibers coated with non-bonded polydimethylsiloxane (PDMS) of 100 µm thickness, bonded polydimethylsiloxane/divinylbenzene (PDMS/DVB) of 65 µm, bonded stableFlex divinylbenzene/Carboxen/polydimethylsiloxane (DVB/CAR/PDMS) of 50/30 µm, bonded Carbowax/divinylbenzene (CW/DVB), bonded Carboxen/polydimethylsiloxane (CAR/PDMS) of 75 µm and bonded polyacrylate (PA) of 85 µm, were obtained from Supelco. The fibers were conditioned prior to use by heating in the injection port of the chromatographic system under the conditions recommended by the manufacturer for each fiber coating. Whenever needed, the conditioning step was repeated for fiber cleanup. All analyses were performed in 15 mL clear glass sealed vials. An RH-KT/C magnetic stirrer (IKA, Staufen, Germany) and a home-made heating system consisting of a drilled block provided with an electronic temperature control system were used for stirring and heating, respectively, during the extraction step. PTFE-coated magnetic stir bars (10 mm x 6 mm O.D.) were used for stirring the samples.

An Agilent 6890 gas chromatograph was directly coupled by a transfer line to a G2350A microwave-induced plasma atomic emission detector (Agilent, Waldbronn, Germany). Updated G2070AA ChemStation application with the G2360AA GC-AED software was used to control and automate many features of the GC and AED systems, and for data acquisition and treatment. The chromatograph was fitted with a 30 m x 0.32 mm I.D. DB-624 capillary column from Agilent with a 1.8 µm film thickness. The GC programs finally selected for each derivatizing agents assayed appear in Table 1. Desorption of the loaded fibers into the injection port was carried out in the splitless mode. A SPME liner (Supelco) of 0.75 mm I.D. was used. Helium was used as the carrier gas and as AED make-up gas at 4 and 180 mL min⁻¹, respectively. Oxygen and hydrogen were used as the scavenger gases at 20 and 10 psi, respectively. The transfer line and the cavity temperatures were set at the same value as recommended by the manufacturer, 250 °C. Filter and backamount (base-line correction parameter) adjustment in the AED system were set according to Agilent default specifications. The spectrometer was purged with a nitrogen gas flow rate of 2.5 L min⁻¹. All compounds were quantified in the selenium 196.018 nm wavelength emission line, using peak area as the analytical parameter.

2.3. Samples. SPME and in situ derivatization

Seventeen water samples of different origins (five seawaters, two waters from a watercourse close to a mining area, one subterranean, three mineral and six tap) from SE Spain were analysed as obtained, with no filtration. Two

hundred-millilitre volumes of water were collected in polycarbonate flasks and care was taken to ensure that all the recipients were completely filled with the samples to avoid the presence of a gaseous phase. Samples were stored in the dark at 4 °C until analysis.

To carry out the extraction when using the tetraalkylborate reagent, 5 mL of water sample were placed in a 15 mL SPME glass vial and 0.5 mL of acetate/acetic buffer solution (1 M) were added to adjust the pH to 5. 150 µL of 1.0% (m/v) NaBEt₄ solution were added. The vial was immediately sealed with the cap after introducing the magnetic stir bar and the mixture submitted to homogenization and derivatization by inserting the vial in the home-made heating block programmed at 25 and maintaining the stirring speed at 1500 rpm for 3 min. Then a DVB/CAR/PDMS fiber was exposed to the headspace for 20 min over the aqueous mixture, which was continuously stirred at 650 rpm at ambient temperature. After this time the fiber was thermally desorbed at 215 °C for 1 min at the GC injector port.

When 4,5-dichloro-1,2-phenylenediamene was used, 280 µL of the 1.0% (m/v) derivatizing solution were added to 7 mL of water sample, placed in a 15 mL SPME glass vial, which was immediately sealed with the cap after introducing the magnetic stir bar. The chemical reaction was allowed to proceed for ten minutes, after which a DVB/CAR/PDMS fiber was immersed in the sample for 30 min, while the solution was continuously stirred at 650 rpm at 40 °C. The adsorbed compounds were desorbed by heating the fiber at 270 °C for 1 min in the GC injection port.

2.4. Recovery assays

Since no reference materials are available for the validation of the methods, spiked samples were prepared. The fortification procedure was applied to three different water samples at two concentration levels and three replicates were analyzed in each case. Twenty-mL volume sample were spiked with 0.1 mL of a working aqueous standard solution containing the analytes at between 20 and 300 ng mL⁻¹, depending on the compounds, corresponding to fortification levels of approximately 0.1 and 1.5 ng mL⁻¹, respectively. Aliquots of 5 or 7 mL were submitted to the above described procedures, using sodium tetraethylborate or 4,5-dichloro-1,2-phenylenediamine, respectively.

3. Results and discussion

3.1. Optimization of the procedure for ethylation derivatization reaction

Preliminary experiments were carried out to separate the derivatization products, after preconcentration on a carboxen/polydimethylsiloxane coated SPME fiber. The optimized program (Table 1) elutes DMSe, DMDSe and Se(IV) at retention times of 4.0, 6.5 and 8.7 min, respectively. While DMSe is not chemically transformed in the presence of the tetraalkylborate, DMDSe and Se(IV) are eluted as methylethylselenide (MEtSe) and diethylselenide (DEtSe), at approximately 95 and 115 °C, respectively. Because the stationary phase does not have to be submitted to high temperatures to elute the three analytes,

a post run phase at 200 °C was included in the oven program to prevent retention of the matrix components when real water samples were being analyzed. Separation was carried out using a constant helium flow rate of 4 mL min⁻¹, which reduced the analysis time necessary and avoided overlapping peaks. Higher helium flow-rate values are not recommended by the manufacturer to preserve the stationary phase.

A 5 mL standard solution volume, containing the three analytes at 10 ng mL⁻¹, was used to optimize the derivatization step, concentrating the derivatized compounds on a CAR/PDMS fiber in the headspace mode with the following SPME conditions: extraction for 20 min at ambient temperature and desorption for 0.2 min at 250 °C. The selection of the pH of the extraction medium was carried out adding 0.5 mL of acetate buffer solution (1 M) (for pH values up to 5) and 0.5 mL of phosphate buffer solution (1M) (for pH values ranging between 6 and 9.5). In all cases 80 µl of the tetraalkylborate solution were added and the reaction allowed to proceed for 10 min. Whereas the derivatization efficiency did not vary for any of the compounds at pH values lower than 4, at pHs higher than 5, DMSe and DMDSe showed the opposite behaviour to selenite, the sensitivity for the organic compounds increasing. Therefore, a pH value of 5 was selected so that all three analytes could be determined simultaneously (Fig. 1A).

The amount of sodium tetraethylborate necessary to carry out the derivatization step was studied, by adding different volumes ranging between 25 μ L and 1 mL of a 1% (m/v) solution of the reagent, to 5 mL of the sample previously buffered at pH 5, roughly corresponding to concentrations of 0.005

and 0.16% (m/v), respectively (Fig. 1B). As expected, reagent concentration did not affect the sensitivity for DMSe, while the highest signal was obtained for Se(IV) with 0.05% (m/v). The signal obtained for DMDSe hardly varied between 0 and 0.05% (m/v), increasing with higher concentration. The finally selected concentration was 0.05% (m/v).

When the reaction time was varied between 1 and 15 min, sensitivity did not vary for DMSe or Se(IV), probably because DMSe does not undergo derivatization and because the derivatization reaction for selenite is very fast. The highest signal was attained at 3 min for DMDSe, which was transformed to MEtSe. Therefore a reaction time of 3 min was selected (Fig. 1C).

Make-up gas flow-rate and reagent gas pressure were optimized for AED. The helium make-up flow was varied between 120 and 200 mL min⁻¹. No overlapping peaks were observed with any of the flow-rates assayed, and even though the greatest sensitivity was attained using the lowest flow-rate, 180 mL min⁻¹ was adopted in order to prolong the discharge tube life time, and to obtain more reproducible results. The effect of oxygen pressure on the sensitivity of the studied compounds was studied in the interval 5-30 psi, maintaining the hydrogen pressure at 10 psi. Since no significant differences were observed between pressures, a value of 20 psi was adopted to avoid accumulation of elemental carbon in the AED discharge tube. Similarly, when the hydrogen pressure was varied between 5 and 30 psi, with the oxygen at the previously selected value of 20 psi, no great differences in sensitivity were observed, although best results were achieved at 10 psi, which was the adopted value.

Six fiber coatings (CAR/PDMS, DVB/CAR/PDMS, CW/DVB, PA, PDMS and PDMS/DVB) of different polarities and retention powers were assayed to preconcentrate the analytes from a 5-mL volume solution containing DMSe, DMDSe and Se(IV) at 5, 2 an 2 ng mL⁻¹, respectively, after submission to the optimized derivatization step. In all cases 20 min and 25 °C were the conditions used in the extraction stage from the headspace solution. Temperatures 20 °C lower than those recommended by the manufacturer as the maximum for each particular fiber coating were applied as the desorption temperature for 0.5 min. The PA, PDMS and CW/DVB coatings were discarded because the first did not adsorb any of the compounds, PDMS absorbed the three analytes to the same extent but with very low extraction efficiency and the last coating only extracted DMDSe but with low sensitivity. As can be observed in Fig. 2A, the DVB/CAR/PDMS fiber was found to be optimal in terms of sensitivity and repeatability, and was therefore selected.

The most suitable extraction mode was checked for the selected fiber by extracting the analytes from a 7 mL sample volume in the direct immersion and the headspace modes. As shown in Fig. 2B, similar responses were obtained for DMSe and Se(IV) in both modes, while the signal for DMDSe was higher when extracting in the HS mode. HS was therefore selected because it would also prolong the useful life time of the fiber coating.

The influence of the extraction time was studied between 5 and 60 min. As can be observed in Fig. 3A, equilibrium between the gaseous phase and the fiber coating was reached at 15 min for DMSe and at 20 min for DMDSe and

Se(IV). Therefore, twenty minutes was selected for the extraction step. For adsorption times higher than 30 min sensitivity decreased for the two organic compounds, a decrease only observed after 45 min for selenite. The effect of extraction temperature was evaluated by applying temperatures ranging from room temperature to 75 °C. In the case of the two organic compounds signals were constant up to 40 °C, after which they decreased, while for selenite the signals decreased with only a slight increase in temperature. Consequently, further experiments were carried out at room temperature (Fig. 3B).

The effect of the salt concentration was studied between 0 and 20% (m/v) by adding different masses of sodium chloride to the extraction vial, the mixture being derivatized and then extracted for 20 min at 25 °C. Whereas the presence of the salt hardly affected sensitivity in the case of DMSe and DMDSe, sensitivity increased substantially for selenite in the presence of 2.5% (m/v) of sodium chloride, a concentration which was adopted (Fig. 4A).

The sample volume was studied between 2 and 7 mL for a standard mixture solution containing the analytes at concentrations between 2 and 5 ng mL⁻¹, depending on the compound. A 5-mL volume was selected because it provided a slight increase in sensitivity for the organoselenium compounds, although not for selenite in the volume range studied. Sample volumes higher than 7 mL were not assayed because of possible problems keeping the fiber in the headspace. Of the different stirring speeds assayed (300-2000 rpm) 650 rpm provided the lowest RSD for three consecutive analyses, and so was adopted.

When the desorption temperature was investigated between 180 and the maximum value permitted for the DVB/CAR/PDMS fiber coating selected (270 °C), each compound behaved differently. In the case of DMSe, no differences in sensitivity were observed, while sensitivity increased with temperature for DMDSe, and the highest signal was attained for selenite at 200 °C (Fig. 4B). A desorption temperature of 215 °C was adopted as a compromise value. As regards the desorption time, although 0.3 min was sufficient to desorb the trapped analytes, the fiber was maintained for 1 min in the injection port. Fig. 5A and C shows the chromatograms obtained for a standard mixture solution and an unfortified mining water sample, respectively, using the optimized procedure with sodium tetraethylborate as the derivatizing agent.

Two GC-inlet liners of 2 and 0.75 mm internal diameter were used, and, as expected, peak width decreased by approximately 10% and sensitivity increased by 10-25% for DMSe and selenite, respectively, when the narrower liner was used.

3.2. Optimization of the procedure using 4,5-dichloro-1,2-phenylenediame

Preliminary experiments were carried adsorbing the derivatized compounds on a DVB/CAR/PDMS fiber from a 7 mL solution, containing DMSe, DMDSe and selenite at 20, 5 and 5 ng mL⁻¹, respectively, to which 150 μ L of HCl 10% (v/v) and 200 μ L of a 0.4% (m/v) solution of the derivatizing agent were added. Extraction of the compounds was first carried out in the immersion

mode, since this was the extraction mode selected in the only previous work describing extraction of the piazselenol complex formed by selenite using SPME [15]. Because this complex elutes at higher temperature than the corresponding tetraethylderivative, the oven program was optimized (Table 1). The complex now eluted at 10 min, a retention time which corresponds to 310 °C. The two organoselenocompounds did not undergo derivatization with 4,5-dichloro-1,2-phenylenediamine, but eluted at 80 and 180 °C (4.9 and 7.5 min), respectively.

The hydrochloric acid and the reagent concentrations as well as the reaction time were studied for the derivatization reaction. When volumes of a 10% (v/v) hydrochloric acid solution ranging from 0 to 150 μ L were added to 7 mL of the extraction medium, (containing DMSe, DMDSe and Se(IV) at 20, 5 and 5 ng mL⁻¹,) corresponding to pH values from 5 to 1.5, no significant differences were observed. Therefore, no acid was added in subsequent experiments in order to preserve the fiber coating (Fig. 1D).

As can be observed from Fig. 1E, the presence of the derivatizing agent decreased sensitivity for the volatile organoselenocompounds. On the other hand, the best sensitivity was achieved for selenite at 0.04% (m/v) of the reagent, a concentration that was adopted.

The reaction time was studied between 1 and 60 min once the reagent had been added and before inserting the SPME fiber in the sample solution, maintaining the vial sealed and stirring at 650 rpm. The adsorption time for this

experiment was 10 min. As shown in Fig. 1F, the equilibrium between the aqueous solution and the surface fiber coating was reached at 10 min for the piazselenol complex.

The same AED values selected as when using sodium tetraethylborate also proved to be optimal. The adsorption capacity of the three fiber coatings which provided best results with NaBEt₄ was now tested: DVB/CAR/PDMS, CAR/PDMS and PDMS/DVB, in the immersion mode at 25 °C, for a sample volume of 7 mL. As can be observed in Fig. 2C, CAR/PDMS was discarded because it adsorbed the piazselenol complex to a very low extent. Best results were again obtained with DVB/CAR/PDMS, which provided better sensitivity and repeatability than PDMS/DVB. Fig. 2D shows the results obtained when direct immersion and headspace extraction modes were compared using the DVB/CAR/PDMS fiber. The three analytes showed higher sensitivity when the fiber was immersed in the solution, and so this extraction mode was adopted. When sample volumes between 7 and 15 mL were assayed, 7 mL provided the best sensitivity.

When the adsorption time was studied between 5 and 60 min, maintaining the sample vial at ambient temperature, DMSe and the piazselenol complex reached the equilibrium in 20 and 30 min, respectively; however, DMDSe did not reach equilibrium in the time range studied (Fig. 3C). An adsorption time of 30 min was selected, since this provided good sensitivity without excessively lengthening the total analysis time.

Although the extraction temperature is generally less important when using direct immersion than it is when working in headspace mode, the effect of this parameter was evaluated from room temperature to 75 °C. Fig. 3D shows the influence of this parameter on the three compounds studied. The maximum sensitivity was attained at 40 °C for both organoselenocompounds, while this effect was more pronounced for DMDSe. On the other hand, adsorption efficiency increased with temperature in the case of the piazselenol compound. As a compromise value 40 °C was selected.

The effect of salt concentration was studied between 0 and 20% (m/v). While no effect was observed for DMSe in the presence of salt, the addition of sodium chloride led to a decrease in the extraction efficiency for DMDSe and selenite (Fig. 4C). Consequently, no salting-out agent was included in the procedure. Nevertheless, this point should be taken into account when seawater samples are analyzed, as will be described in Section 3.3.

When investigating the desorption temperature between 180 and 270 °C, no effect was observed for DMSe, whereas sensitivity increased with temperature for DMDSe and Se(IV), as shown in Fig. 4D. Therefore, 270 °C was selected, which corresponds to the highest temperature recommended for the fiber material used. When the desorption time was varied between 0.2 and 1 min, the three analytes reached their maximum sensitivity at 0.5 min, after which the obtained signals remained constant. A desorption time of 1 min was selected, ensuring the absence of carryover effects.

Sample volumes ranging from 7 to 15 mL were submitted to the analytical procedure. Lower volumes would not have permitted immersion of the fiber in the sample solution, while 15 mL-volume corresponds to the maximum capacity of the SPME vial. For all three analytes under study sensitivity decreased with sample volume, this effect being particularly pronounced for selenite but less so for DMSe. Consequently, a sample volume of 7 mL was adopted. A stirring speed of 650 rpm, under the selected conditions, increased sensitivity and repeatability. Fig. 5B shows the chromatograms obtained for a standard mixture solution and for an unfortified seawater sample, respectively, when the piazselenol complex is obtained by derivatization with 4,5-dichloro-1,2-phenylenediamine.

3.3. Analytical characteristics and validation of the methods

For calibration, aqueous standard solutions of 5 and 7 mL were prepared containing 2.5 and 0% (m/v) sodium chloride concentration, respectively, and submitted to the corresponding optimized derivatization procedures with sodium tetraethylborate and 4,5-dichloro-1,2-phenylenediamine, respectively, as well as to the SPME preconcentration step. Six concentration levels were assayed in duplicate, using peak areas for calibration purposes. Table 2 shows the characteristics of the calibration graphs obtained for each compound for the two proposed procedures. As can be seen, the correlation coefficients showed a high degree of correlation between concentration and peak area. The detection limits were calculated using a signal-to-noise ratio of 3, being shown in Table 2.

Absolute detection limits were 275, 75 and 35 pg for DMSe, DMDSe and Se(IV) when sodium tetraethylborate was used as derivatizing agent and 190, 50 and 20 pg when 4,5-dichloro-1,2-phenylenediame was used. The quantification limits, calculated using a signal-to-noise ratio of 10, ranged between 25 and 180 ng L⁻¹ for Se(IV) and DMSe, respectively, when using sodium tetraethylborate, and between 10 and 85 ng L⁻¹ for the same compounds, when using 4,5-dichloro-1,2-phenylenediamine. The repeatability was calculated using the relative standard deviation for 10 successive injections of a tap water sample free of the analytes and fortified with a standard mixture prepared at twenty-fold the quantification limits: 4.9 and 7.2 for DMSe, 5.1 and 6.8 for DMDSe and 6.1 and 8.1% for Se(IV), for the procedures where sodium tetraethylborate and 4,5-dichloro-1,2-phenylenediamine, respectively, were used. For all three analytes 4,5-dichloro-1,2-phenylenediamine provided slightly higher RSD values than NaBEt₄, probably due to the immersion mode used for the extraction step, when the surface coating was in direct contact with the sample matrix.

When the slopes of the aqueous calibration graphs obtained were compared with those obtained when the standard additions method was applied to three water samples of different origin (tap, river and seawater), no significant differences were found in any case, except for the seawater when 4,5-dichloro-1,2-phenylenediamine was used. The absence of a matrix effect was corroborated by applying a statistical t-test (95% confidence level). On the other hand, when the quantification of seawaters is required, no standard addition method needs to be applied, by using 4,5-dichloro-1,2-phenylenediamine, if the aqueous calibration is carried out by preparing the standard solutions in the

presence of 3.5% (m/v) of sodium chloride, which roughly corresponds to the salt content of seawater.

Average recoveries \pm standard deviations (n=54) of 96.4 \pm 7.1 and 98.4 \pm 6.4% were obtained using the method optimized with sodium tetraethylborate and 4,5-dichloro-1,2-phenylenediamine, respectively.

3.4. Real samples

The two optimized methods for the simultaneous analysis of DMSe, DMDSe and Se(IV) were applied to 17 different water samples of different origins. The obtained results appear in Table 3. Three of the samples analyzed were free of the studied compounds. It is interesting that DMSe was found in only one of the samples analyzed, a seawater sample (Fig. 5D). Concentration levels of DMSe, DMDSe and Se(IV) in this seawater sample were 0.65, 0.09 and 0.06 ng mL⁻¹, respectively. The concentrations of DMDSe and selenite found in the water samples ranged between 0.07 and 1.0 ng mL⁻¹, as can be observed from Table 3.

4. Conclusion

Solid phase microextraction appears as an interesting preconcentration system for the analytes studied. The inherent advantages of extracting in the headspace mode and the fact that a relatively short derivatization and

preconcentration step is needed means that the sodium tetraethylborate procedure can be recommended. Nevertheless, if greater sensitivity is required the method using 4,5-dichloro-1,2-phenylenediamine is preferable. Both methods allow direct sample quantification against aqueous standards, although in the case of seawater samples when using 4,5-dichloro-1,2-phenylenediamine, the addition of 3.5% (m/v) sodium chloride to the aqueous standards is required to avoid the need of using the standard additions method. Furthermore, the excellent selectivity of the atomic emission detector provides nearly specific chromatograms. The analytical characteristics of the proposed methods make them useful tools for the routine monitoring of selenocompounds in water samples

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FIGURES



Fig. 1. Effect of pH (A, D), derivatizing agent concentration (B,E) and reaction time (C,F) on peak area when using sodium tetraethylborate (A,B,C) and 4,5-dichloro-1,2-phenylenediamine (D,E,F). (•) DMSe, (\circ) DMDSe and (\mathbf{V}) Se(IV).



Fig. 2. Extraction efficiencies for the different species with different fiber coatings (A,C) and comparing the two extraction modes (B,D), for derivatization with sodium tetraethylborate (A,B) and 4,5-dichloro-1,2-phenylenediamine (C,D).



Fig. 3. Influence of time (A,C) and temperature (B,D) of the adsorption stage on the peak area of the three analytes when using derivatization with: sodium tetraethylborate (A,B) and 4,5-dichloro-1,2-phenylenediamine (C,D). (•) DMSe, (\circ) DMDSe and (\mathbf{V}) Se(IV).



Fig. 4. Effect of sodium chloride concentration (A,C) and desorption temperature (B,D) on peak area when using NaBEt₄ (A,B) and 4,5-dichloro-1,2-phenylenediamine (C,D).
(●) DMSe, (○) DMDSe and (▼) Se(IV).



Fig. 5. SPME-GC-AED chromatograms obtained from (A,B) a standard mixture (0.9, 0.15 and 0.6 ng mL⁻¹ of DMSe, DMDSe and Se(IV), respectively), (C) mining water 1 and (D) a seawater sample (contaning 0.3, 0.05 and 0.07 ng mL⁻¹ of DMSe, DMDSe and Se(IV), respectively), when using sodium tetraethylborate (A,C) and 4,5-dichloro-1,2-phenylenediamine (B,D). 1, DMSe; 2, DMDSe and 3, Se(IV).

TABLES

	NaBEt ₄	4,5-Dichloro-1,2- phenylenediamine	
GC parameters			
Oven program	40 °C (1 min)	40 °C (4 min)	
	40–130 °C (10 °C min⁻¹)	40–140 °C (40 °C min⁻¹)	
	Post run: 200 °C (5 min)	140–310 °C (60 °C min ⁻¹)	
		310 °C (2 min)	
SPME parameters			
Extraction time and temperature	20 min at 25 °C	30 min at 40 °C	
Extraction mode	Headspace	Immersion	
Desorption time and temperature	1 min at 215 °C	1 min at 270 °C	
Fiber material	DVB/CAR/PDMS	DVB/CAR/PDMS	
Sample volume	5 mL buffered at pH 5	7 mL non-buffered	
Derivatizing agent concentration	0.05% (m/v)	0.04% (m/v)	
Sodium salt concentration	2.5% (m/v)	0	
Reaction time	3 min	10 min	

Table 1. Experimental conditions of the GC-SPME system with both derivatizing agents assayed

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Compound	NaBEt₄			4,5-Dichloro-1,2-phenylenediamine		
	Slope \pm SD ^a (mL ng ⁻¹)	Detection limit (ng L ⁻¹)	Correlation coefficient	Slope \pm SD ^a (mL ng ⁻¹)	Detection limit (ng L ⁻¹)	Correlation coefficient
DMSe	16.29 ± 0.25	55	0.9998	28.18 ± 0.22	25	0.9990
DMDSe	68.50 ± 0.29	15	0.9990	109.65 ± 1.19	7.0	0.9970
Se(IV)	123.21 ± 1.35	7.3	0.9997	132.47 ± 1.55	3.0	0.9996

^a Mean value \pm standard deviation (n = 3).

Sample	NaBEt₄		4,5-Dichloro-1,2-phenylenediamine		
	DMDSe	Se(IV)	DMDSe	Se(IV)	
Seawater 1	0.151 ± 0.007	1.02 ± 0.01	0.170 ± 0.015	1.01 ± 0.01	
Seawater 2	ND	0.270 ± 0.008	ND	0.280 ± 0.007	
Mining 1	0.073 ± 0.010	0.232 ± 0.010	0.074 ± 0.005	0.23 ± 0.01	
Mining 2	0.095 ± 0.007	0.245 ± 0.007	0.101 ± 0.009	0.25 ± 0.009	
Mineral 1	0.105 ± 0.007	0.34 ± 0.005	0.098 ± 0.01	0.36 ± 0.008	
Mineral 2	0.095 ± 0.02	0.38 ± 0.02	0.102 ± 0.02	0.37 ± 0.01	
Mineral 3	0.07 ± 0.005	0.33 ± 0.003	0.07 ± 0.008	0.34 ± 0.008	
Well water	ND	0.37 ± 0.07	ND	0.35 ± 0.01	
Tap 1	ND	0.25 ± 0.05	ND	0.23 ± 0.02	
Tap 2	ND	0.35 ± 0.06	ND	0.37 ± 0.02	
Tap 3	ND	0.23 ± 0.05	ND	0.21 ± 0.03	
Tap 4	ND	0.19 ± 0.03	ND	0.20 ± 0.02	
Tap 5	ND	0.25 ± 0.02	ND	0.26 ± 0.01	

Table 3. Concentrations (ng mL⁻¹) obtained for the analysis of the samples under the two optimized procedures

ND: not detected.

^a Mean value \pm standard deviation (n = 3).