Determination of Dialkyldiselenides in Water by Gas Chromatography–Mass Spectrometry Using 1-Fluoro-2,4-dinitrobenzene as Derivatization Reagent

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Abstract

The feasibility of gas chromatography–mass spectrometry for the speciation of traces of dialkyldiselenides in the presence of dialkylselenides and inorganic selenium species is described, and the procedure is applied to environmental samples. The analysis is based on the reaction of dialkyldiselenide species with 1-fluoro-2,4-dinitrobenzene after volatilization of selenium species as alkylselenols using a volatilization and trap device. Parameters affecting the volatilization and derivatization of the selenium compounds are discussed, and the performance of the method is described. The approach reaches detection limits in the order of nanograms (after a preconcentration step) and has been applied to the analysis of dimethyldiselenium and diethyldiselenium in natural waters.

Introduction

Selenium is one of the minor but biologically essential elements in the biosphere. Low levels of selenium are necessary for human metabolism, but higher concentrations of this element may cause damage to human health (1,2). Selenium is mainly found in metal sulphide deposits but is also widely distributed in the environment. Although the selenium concentration of most drinking and natural waters is less than 10 µg/L, the pore water in seleniferous soil in semiarid areas may contain up to hundreds or thousands of micrograms of dissolved selenium per liter (3). Selenium is present in aquatic systems in different oxidation states: selenide (both in inorganic and organic compounds), selenite, and selenate. However, the organic species of selenium have a different toxicity than that of inorganic forms, with dimethylselenide (DMSe) considered 500 times less toxic than selenite. Therefore, methylation is an effective detoxification mechanism for selenium (4). There is evidence of volatile selenium organic species production, mainly DMSe and dimethyldiselenide (DMDSe), from inorganic selenide salts. Moreover, selenocysteine and selenomethionine have been found in fungi, plants, and animals in the environment (1,5). As a consequence, analytical methods for the chemical speciation of dialkylselenide and dialkyldiselenide in environmental samples are necessary, particularly in natural waters.

Volatile organic selenides have been analyzed by gas chromatography (GC), with most applications to air, soil, and water samples by direct gas injection or by trapping the volatile compounds in an adsorbent with desorption later by solvent elution or temperature programming of the trap (6). A number of detectors have been tested in different instrumental couplings. GC–atomic absorption spectrometry (AAS) systems with a previous purge-and-trap step have been proposed for DMSe, diethylselenide (DESe), and DMDSe speciation. Jiang et al. (7), using a GC–graphite furnace (GF) AAS system could determine up to 0.1 ng of DMSe and DESe and 0.2 ng of DMDSe. A similar detection limit (0.1 ng) was reached by Chau et al. (8) for both DMSe and DMDSe using GC-quartz furnace (QF) AAS, and Cutter (9) could determine up to 0.5 ng of Se(IV), Se(VI), DMSe, and DMDSe using hydride generation (HG)-GC-QFAAS with an air–hydrogen flame. Other detection techniques, including atomic fluorescence spectrometry (AFS) (10), chemiluminescence (11), microwave-induced plasma atomic emission spectrometry (MIP-AES) (12), inductively coupled plasma mass spectrometry (ICP-MS) (13), and mass spectrometry (MS) (14), have been successfully used. However, analytical methods sometimes include a derivatization step to improve the selectivity and the sensitivity of the determination. The use of 1-fluoro-2,4-dinitrobenzene (FDNB, the Sanger's reagent) as a trapping agent after the volatilization of selenium species as volatile selenols has been proposed for Se(IV) and methylselenol analysis by Ganther et al. (15). This treatment involved a two-step procedure: first, the DMDSe reacts with a reducing agent, such as $Zn^{+}HCl$, to form the corresponding volatile selenol, which is subsequently trapped and stabilized in an alkaline aqueous-DMF solution of FDNB for final selenium estimation in liver tissues by a gamma counter (15).

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In this paper, the possibilities of the separation by capillary GC coupled to a high sensitivity MS (GC–MS) has been studied for the analysis of dialkyldiselenides (DMDSe and DEDSe) in the presence of dialkylselenides (DMSe and DESe) using FDNB as a trapping agent after the volatilization of dialkyl compounds as volatile selenols from the matrix. FDNB is used to form stable, organic-soluble selenium derivatives that avoid the thermal decomposition of the compounds in the chromatographic system (7), because at low temperature, dialkyldiselenides tend to be adsorbed both on the injector and detector of the GC, whereas excessive high temperatures cause organoselenium degradation. Results confirm the potential use of FDNB in selenium speciation.

Experimental

Reagents and standards

The reagents used in the experiments were analytical and pesticide grade and obtained from Merck (Darmstadt, Germany) and Sigma (St. Louis, MO). Active carbon (100–400 mesh ASTM), Florisil (100–200 mesh ASTM), and Celite 545 were purchased from Sigma, Merck, and Fluka (Ronkonkoma, NY), respectively. Water used in the experiments was double-distilled and deionized, giving blank readings in all the analyses. Plastic and glassware used for experiments were previously soaked in 0.08M nitric acid for 24 h and rinsed carefully with doubly distilled water as recommended in the literature (16).

Stock solutions of selenite and selenate (1000 mg Se per liter) were prepared from analytical reagent-grade selenium dioxide and sodium selenate (Merck), respectively. Organoselenium stock solutions were prepared at a concentration of approximately 100 mg/L (as Se) in benzene from DMDSe (Aldrich, Gillingham, Dorset, U.K.), DESe, DMSe (Pfaltz and Bauer, Waterbury, CT), and DEDSe (synthesized by the authors) and were kept in a refrigerator. Intermediate solutions of 1 mg/L of Se(IV) were prepared by dissolving appropriate volumes of stock solution and bringing the volume to 50 mL with water. Intermediate solutions of 1 mg/L (as Se) 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 dilutions of the intermediate solutions with water.

Synthesis of Diethyldiselenide and purity of the organoselenium standards

DEDSe was synthesized by a modification of the procedure proposed by Ganther and Kraus (15) for DMDSe. Selenourea (0.5 g) was placed into a round-bottomed flask, and 25 mL of water was added (appearing red in colour). Then, 3 mL of ethyl iodide was added, and the mixture was heated at reflux at 100°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 5M NaOH was added. The mixture was heated at reflux for 1 h and then allowed to cool. The upper yellow heptane layer was dried over anhydrous Na_2SO_4 and filtered. Finally, the heptane was removed by rotatory evaporation (30°C). The resulting extract was a brown–yellow oily liquid, and purity was studied by FAAS. The chromatographic study of the different organoselenium compounds evaluated revealed the presence of only one peak for each compound, and structure was confirmed by the fragmentation spectrum (14,17). The characteristic fragments were as follows: DMSe, base peak and the molecular ion at *m/z* 110; DMDSe, base peak and the molecular ion at *m/z* 190; DESe, base peak at *m/z* 110 and molecular ion at *m/z* 138; and DEDSe, molecular ion at *m/z* 218, base peak at *m/z* 158 (Se–Se), and *m/z* 189 (loss of CH_3–CH_2). In addition, the total selenium content in each standard was checked by flame AAS after acid digestion using a 1000 mg/L inorganic selenium Titrisol standard (Merck) for calibration. A suitable aliquot (10 µL) of each organoselenium compound was digested with 2 mL of concentrated HNO_3 and diluted to 10 mL (8). Purities of 97 ± 3%, 98 ± 2%, 98 ± 3%, and 97 ± 3% were assessed for DEDSe, DESe, DMDSe, and DMSe, respectively.

Extraction procedure

Separation and preconcentration of volatile organic species of selenium from water were carried out using solid-phase extraction on active carbon. 1.5 g of active carbon was placed in a glass column (25 × 0.8-cm i.d.). The bottom side of the column was previously covered with a 0.5-cm layer of celite to avoid carbon trapping losses. The column was rinsed with 10 mL of double-distilled water, and the water sample (1 L) was immediately transferred into the extraction assembly to avoid drying of the active carbon. A vacuum pump was used and adjusted to keep the extraction flow rate at 10 mL/min. After selenium species retention, the column was dried under a nitrogen stream for 5 min. Elution was developed using 4 mL of carbon disulphide (CS_2) at a flow rate of 1 mL/min.

Derivatization

The apparatus used in the derivatization step is depicted in Figure 1. A suitable aliquot of CS_2 extract containing the organoselenium compounds was placed into a volatilization polystyrene vial (100 × 16-mm i.d.), and both 350 mg of zinc dust and 3–4 drops of *n*-octanol were added. This vial was fitted with a polyethylene stopper and sealed with teflon to avoid losses of the volatile selenols. The trapping vial contained 0.4 mL of double-distilled water, 0.6 mL of DMF, 1 mL of freshly prepared 1% (v/v) FDNB in DMF, and 14 mg of $NaHCO_3$. A stream of N_2 (as carrier gas) was passed through the volatilization manifold to remove the oxygen. Then, 3 mL of 12N HCl was injected into the volatilization vial through a poly(tetrafluoroethylene) tube by a

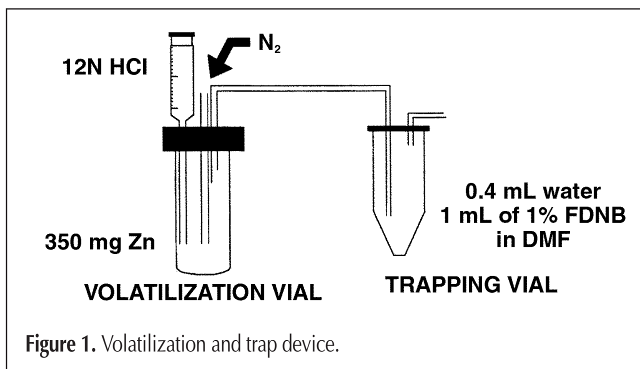


Figure 1. Volatilization and trap device.

syringe. An N_2 flow rate of 100 mL/min was passed for 10 min to assure the complete reaction in the trapping vial. Then, the aqueous solution was extracted with 4 mL of ethyl acetate (3 times) by mechanical shaking. The organic layer was concentrated using a rotatory evaporator to a volume of approximately 3 mL, and the extract was evaporated to dryness under an N_2 stream. The residue was dissolved with 50 µL of benzene containing 200 µg/L of 2,6-diisopropylphenol (propofol), which was used as internal standard for chromatographic quantitation.

Instrumental analysis

A 5890 Hewlett-Packard (Palo-Alto, CA) GC interfaced with an HP 5970 mass selective detector via a capillary direct inlet with a fused-silica crosslinked methyl silicone capillary column (25 m × 0.20-mm i.d., 0.33-µm film thickness) HP-1 was used. Sample aliquots of 1 µL were injected using a splitless injection mode (purge time, 0.5 min off). Helium was used as carrier gas at a head pressure of 100 KPa. The injector port temperature was set at 250°C, and the interface temperature was operated at 260°C. Electron ionization MS was used for detection, and the filament remained off until the solvent 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 was set at 0.4 mA, and the electron energy was set at 70 eV. Calibration was performed with perfluorotributylamine. Data were stored as total ion chromatograms (TIC), and quantitation was performed using single ion monitoring (SIM) mode. The GC oven temperature was programmed as follows: the initial column temperature was held at 30°C for 1 min, then programmed at 30°C/min to 125°C, and isothermally maintained at this temperature for 1 min, then ramped a second time at 10°C/min to 250°C, and finally isothermally maintained for 1 min.

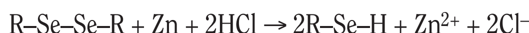
Statistical treatment

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

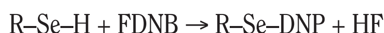
Results and Discussion

Optimization of the derivatization step

Before treatment with FDNB, the sample had to be pretreated for dialkyldiselenides reduction and volatilization via the corresponding selenols, which were trapped by FDNB prior to the analysis by GC–MS. The chemical reactions involved in these steps are as follows:



$$R–Se–Se–R + Zn + 2HCl \rightarrow 2R–Se–H + Zn^{2+} + 2Cl^-$$



$$R–Se–H + FDNB \rightarrow R–Se–DNP + HF$$

These reactions were performed in the manifold presented in Figure 1. Except for the trapping vial, which was made of pyrex glass, the other components of the volatilization–derivatization system were made in polystyrene, polyethylene, and teflon materials, because the underivatized organic selenium species were readily deposited as elemental selenium on glass surfaces. The volatilization vessel had to be sealed with teflon to avoid losses of the highly volatile selenols, and 1 or 2 drops of *n*-octanol were added to avoid frothing during the selenol formation. This alcohol was easily released into the N_2 stream and found as a minor component in the TIC of the final extract. A weight of 14 mg of $NaHCO_3$ was added to the trapping vial to provide the basic pH necessary for the reaction between the dissociated form of the volatile selenol and FDNB. In addition, this base neutralized the HCl fumes from the volatilization vial. Several parameters controlling the volatilization-derivatization step were studied: (*a*) the nature of the acid for selenol formation, (*b*) the nitrogen flow rate to purge out the selenols, (*c*) the stripping and derivatization times, and (*d*) the length and the material of the connecting tubes. The DMF-aqueous solution of organoselenium derivatives was extracted with a nonaqueous solvent for analysis by GC–MS, and the nature and volume of this solvent, as well as the final volume of this organic extract, were optimized. The optimization experiments were performed (5 replicates) by using 20 and 36 ng of DMDSe and DEDSe (as selenium), respectively, in 2 mL of CS_2 and following the proposed analytical procedure.

Choice of the acid medium

An acid medium was necessary to form the corresponding volatile selenols. Three different concentrated acids (HNO_3, H_2SO_4, and HCl) were tested. Chromatographic peak heights resulting from derivatization of the working solution using 3 mL of acid are collected in Table I. The best performance was obtained using 10N HCl (*t*-test, $p < 0.001$). Consequently, different volumes of this acid ranging between 1 and 5 mL were tested, obtaining a significant high-derivatization efficiency with at least 2 mL of this acid (*t*-test, $p < 0.001$). Higher volumes did not cause any significant improvement in the reaction (ANOVA, $p > 0.07$).

Table I. Influence of the Type and Volume of Acid on the Volatilization–Derivatization Step*

	Peak height of selenium derivative/peak height of propofol ± standard deviation	
Type of acid	DEDSe	DMDSe
H_2SO_4	1.09 ± 0.09	1.35 ± 0.07
HNO_3	1.13 ± 0.07	1.49 ± 0.12
HCl	1.33 ± 0.06	1.65 ± 0.08
Volume of HCl (mL)	**DEDSe**	**DMDSe**
1.0	0.543 ± 0.037	0.734 ± 0.048
2.0	1.27 ± 0.06	1.50 ± 0.07
3.0	1.33 ± 0.06	1.65 ± 0.08
4.0	1.27 ± 0.06	1.60 ± 0.07
5.0	1.31 ± 0.09	1.58 ± 0.08

* For 20 and 36 ng (as Se) of DMDSe and DEDSe, respectively, dissolved in 2 mL of CS_2.

Nitrogen flow rate, purge and derivatization times, and type of the connecting tubes in the derivatization manifold

Previous to the derivatization reaction, an N_2 stream had to be passed through the volatilization manifold to remove the oxygen. Purge times ranging from 0 to 15 min were tested (Table II). The best results were obtained when purging for 5–10 min (t-test, $p < 0.001$). Several derivatization reaction times and nitrogen flow rates were also tested, and the results are summarized in Table II. A flow rate of 100 mL/min for 10 min gave satisfactory results and was selected for all subsequent experiments. Faster flow rates caused nonquantitative trapping of the selenols in the FDNB solution, but at lower flow rates the oxidation of the volatile species also produced inferior results (t-test, $p < 0.001$). Several materials (teflon, polypropylene, and polyethylene) were checked for the interconnecting tubes using lengths ranging from 20 to 50 cm and internal diameters of ⅛ inch (Table III). No significant differences were found with tubes made of teflon or polyethylene (t-test, $p > 0.34$), but the results obtained with polypropylene were inferior (t-test, $p < 0.001$). Tube lengths longer than 40 cm also gave poor results (t-test, $p < 0.001$).

Extraction and concentration of the organoselenium derivatives

Extraction of the selenium derivatives with an organic solvent is required prior to GC–MS determination. Moreover, concentration of these species by removing the solvent under an N_2 stream improved the detection limit. Several variables related with this step, including type of solvent, number and time of extractions, and final volume of the extract, were studied. A preliminary experiment using 4 mL of different solvents such as CS_2, hexane, benzene, ethyl acetate, and cyclohexane was attempted in the extraction of the organoselenium derivatives from the trapping vial using single and multiple extractions for 5 min. Results from these experiments are depicted in Table IV, showing good recoveries with the use of 3 successive extractions. Benzene and ethyl acetate gave the best performance, but the latter was selected for further experiments because of its lower toxicity. Several extraction times ranging from 1 to 10 min were tested, with better results obtained for extractions longer than 4 min (t-test, $p < 0.008$). Losses of the organoselenium derivatives during the concentration step were studied by removing the solvent under an N_2 stream to different final volumes ranging from dryness to 4 mL and then diluting to the initial volume prior to the analysis by GC–MS. No significant losses were observed, even after evaporation to dryness (ANOVA, $p > 0.70$), and a preconcentration factor of 40 was obtained when the residue was dissolved with 50 µL of benzene containing the internal standard. These results compared favorably with methods based on the direct underivatized selenium analysis by GC–MS, because underivatized dialkylselenide had losses of approximately 80% when they suffered a similar preconcentration treatment. Detection limits using both the derivatization and preconcentration steps were 0.2 and 0.4 mg/L (as selenium) for DMDSe and DEDSe, respectively. However, these limits are not sufficiently sensitive to make the evaluation of these species in natural waters possible (12,19). Therefore, a preconcentration step using solid-phase extraction was optimized, as discussed later in this paper.

Speciation of DMSe, DESe, Selenite, and Selenate.

When DMSe and DESe were subjected to the volatilization and derivatization procedure, they did not yield any detectable derivatives. This could be attributed to both the high volatility of these

Table II. Influence of N_2 Flow Rate, Purge Time, and Reaction Time on the Volatilization–Derivatization Step*

	Peak height of selenium derivative/peak height of propofol ± standard deviation	
Purge time (min)	**DEDSe**	**DMDSe**
0		0.876 ± 0.05
1.37 ± 0.06		
5.0	1.28 ± 0.08	1.64 ± 0.11
10.0	1.33 ± 0.06	1.65 ± 0.08
15.0	1.17 ± 0.07	1.28 ± 0.06
N_2 flow rate (mL/min)	**DEDSe**	**DMDSe**
25		0.986 ± 0.09
1.11 ± 0.12		
50		1.25 ± 0.08
1.48 ± 0.09		
100	1.33 ± 0.06	1.65 ± 0.08
150	0.900 ± 0.08	1.06 ± 0.08
Reaction time (min)	**DEDSe**	**DMDSe**
1.0	0.297 ± 0.045	0.164 ± 0.022
5.0	0.841 ± 0.038	0.947 ± 0.072

Table III. Influence of Type and Length of the Connecting Tubes on the Volatilization–Derivatization Step*

	Peak height of selenium derivative/peak height of propofol ± standard deviation	
Polypropylene length (cm)	**DEDSe**	**DMDSe**
20	1.05 ± 0.09	1.52 ± 0.11
30	1.08 ± 0.09	1.54 ± 0.12
40	0.933 ± 0.050	1.44 ± 0.09
50	0.810 ± 0.046	0.931 ± 0.086
Teflon length (cm)	**DEDSe**	**DMDSe**
20	1.32 ± 0.05	1.65 ± 0.10
30	1.33 ± 0.06	1.65 ± 0.08
40	1.24 ± 0.06	1.54 ± 0.12
50	1.03 ± 0.10	1.11 ± 0.10
Polyethylene length (cm)	**DEDSe**	**DMDSe**
20	1.28 ± 0.09	1.55 ± 0.06
30	1.30 ± 0.05	1.66 ± 0.06
40	1.24 ± 0.06	1.49 ± 0.10
50	0.907 ± 0.079	1.01 ± 0.08

* For 20 and 36 ng (as Se) of DMDSe and DEDSe, respectively, dissolved in 2 mL of CS_2.

compounds and the difficulty of the alkylselenides to form the corresponding alkylselenols. Moreover, it has not been possible to characterize the presence of inorganic selenium using the GC–MS system in spite of the fact that the derivatization procedure was originally proposed for inorganic selenium species (15). However, the presence of red elemental selenium in the injector glass liner surface was observed, which indicated the thermal instability of the derivative formed in the reaction and explained the absence of signal. To confirm this assertion, the 2,4-dinitrophenylselenide was synthesized and isolated by using FDNB. Thermal decomposition of this compound in the injector was also observed, justifying the absence of the corresponding peaks in the chromatogram when the derivatization step was used.

Optimization of the instrumental response

The investigated parameters included the temperature program of the chromatographic oven, the temperatures of the injector block and the transfer line between the chromatograph and the mass detector, and the flow rate of the carrier gas. The experiments were performed (5 replicates) by using 20 and 36 µg (as Se) of the FDNB derivatives from DMDSe and DEDSe, respectively, in 2 mL of CS_2, following the recommended analytical procedure.

Temperature program

Retention times and peak heights of the DMDSe and DEDSe derivatives were evaluated using the following temperature programs: 30°C for 1 min, 30°C/min to 125°C, isotherm 1 min, 10°C/min to 250°C; 125°C for 1 min, 10°C/min to 250°C; isotherm at 250°C; 180°C for 1 min, 5°C/min to 250°C. Results indicated that the temperature program is not a decisive factor for a reliable separation when a few number of compounds are separated. The optimum conditions were obtained when using the first temperature program listed (30°C for 1 min, 30°C/min to 125°C, isotherm 1 min, 10°C/min to 250°C). The retention times were 9.22 ± 0.03, 16.27 ± 0.03, and 18.65 ± 0.02 min for propofol, DMDSe, and DEDSe, respectively. The reproducibility of the retention times was very good, with a relative standard deviation below 0.3%. With this temperature program, peak heights were 151 ± 13, 255 ± 20, and 206 ± 15 for propofol, DMDSe, and DEDSe, respectively, and were higher than those using other temperature programs. Moreover, lower standard deviations of the signal were obtained using this temperature program (t-test, $p < 0.03$).

Injector and transfer line temperatures

Temperatures ranging from 100 to 260°C were tested for the injector block. The minimum injector temperature to ensure a rapid volatilization was 240°C for both DEDSe and DMDSe. Higher temperatures did not significantly affect the organoselenium signals (ANOVA, $p > 0.98$), but the baseline noise increased. A range of temperatures from 250 to 280°C were tested for the transfer line, and the peak heights of the organoselenium derivatives were compared. Temperatures lower than 250°C altered the vacuum in the detector system. Otherwise, no significant differences were found between the temperature conditions tested in these experiments (ANOVA, $p > 0.96$), thus a temperature of 260°C was selected for further experiments.

Carrier gas and chromatographic column

Two columns were tested: HP-1 (25 m × 0.20-mm i.d., 0.33-µm film thickness) and SPB-1 (30 m × 0.32-mm i.d., 0.5-µm film thickness). Flow rates of helium (as carrier gas) ranging from 0.4 to 1.0 mL/min were tested using the HP-1 column. The best performance was obtained using flows between 0.6 and 0.8 mL/min, and no significant differences were found in this flow rate range (t-test, $p > 0.3$). Low and poor peak resolution resulted with flows lower than 0.6 mL/min. Otherwise, peak heights decreased significantly using a flow of 1.0 mL/min (t-test, $p < 0.001$). Therefore, a flow rate of 0.8 mL/min was selected for further experiments. The performance obtained with the SPB-1 column was lower.

Table IV. Influence of the Organic Solvent, Number of Extractions, and Extraction Time on the Extraction of DMDSe and DEDSe Derivatives*

	Peak height of selenium derivative/peak height of propofol							
	DEDSe				DMDSe			
	a	b	c	d	a	b	c	d
Carbon disulphide	0.095 ± 0.014	0.224 ± 0.021	0.299 ± 0.025	0.414 ± 0.037	0.140 ± 0.056	0.388 ± 0.069	0.555 ± 0.051	0.769 ± 0.051
Hexane	0.163 ± 0.013	0.265 ± 0.019	0.374 ± 0.034	0.652 ± 0.052	0.267 ± 0.044	0.408 ± 0.036	0.582 ± 0.043	0.829 ± 0.072
Benzene	0.965 ± 0.082	1.03 ± 0.06	1.32 ± 0.06	1.30 ± 0.06	1.24 ± 0.07	1.41 ± 0.06	1.68 ± 0.07	1.63 ± 0.07
Ethyl acetate	1.01 ± 0.07	1.18 ± 0.05	1.39 ± 0.06	1.37 ± 0.06	0.950 ± 0.085	1.16 ± 0.05	1.70 ± 0.08	1.68 ± 0.08
Cyclohexane	0.122 ± 0.022	0.374 ± 0.072	0.815 ± 0.066	0.686 ± 0.057	0.120 ± 0.026	0.368 ± 0.057	0.508 ± 0.063	0.675 ± 0.060

Extraction time (min)	Peak height of selenium derivative/peak height of propofol: DEDSe	DMDSe
1	1.13 ± 0.08	1.26 ± 0.08
3	1.25 ± 0.05	1.52 ± 0.06
5	1.39 ± 0.06	1.71 ± 0.08

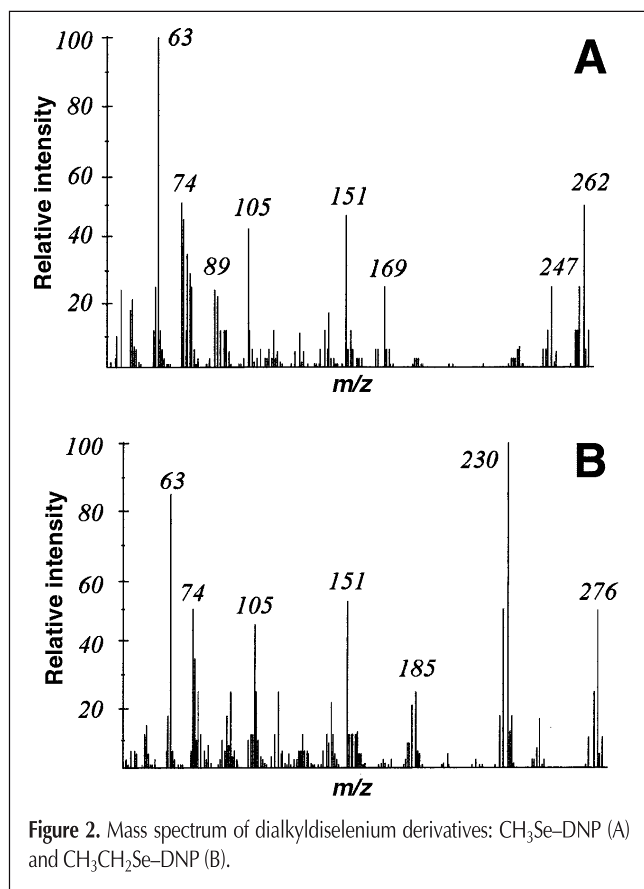


Figure 2. Mass spectrum of dialkyldiselenium derivatives: CH_3Se–DNP (A) and CH_3CH_2Se–DNP (B).

Detector performance

Contamination or significant memory effects were not observed in the chromatograms. Therefore, detector baking was not required to remove traces of the organometallic compounds from the detector. Mass spectra of the FDNB derivatives of DMDSe and DEDSe (CH_3–Se–DNP and CH_3–CH_2–Se-DNP, respectively) are shown in Figure 2. The CH_3–Se–DNP mass spectra (Figure 2A) shows the base peak coinciding with the molecular ion at *m/z* 262, which gives a higher sensitivity to the method with low interference in SIM mode. Other intense fragments are *m/z* 247 (loss of CH_3), *m/z* 169 (loss of a Se–CH moiety), *m/z* 151 (loss of H_2O from ion *m/z* 169), and *m/z* 63. This fragmentation indicates a high stability for the Se–DNP bond, because no ion corresponding to the loss of CH_3–Se was found. Mass spectra for CH_3–CH_2–Se–DNP (Figure 2B) shows the molecular ion at *m/z* 276 (with approximately 50% abundance, enough to characterize and detect this compound in complex mixtures), the base peak at *m/z* 230 (loss of NO_2 from molecular ion), and intense fragments at *m/z* 247 (loss of CH_3–CH_2), *m/z* 185, *m/z* 151 ($C_6H_3N_2O_3$), and *m/z* 63.

Calibration and detection limits

The quantitative analysis of the two compounds of interest was carried out in the SIM mode for the following fragments: *m/z* 178 (propofol), *m/z* 262 (CH_3–Se–DNP), and *m/z* 276 (CH_3–CH_2–Se–DNP). The dwell time of each mass was 100 ms. The solutions were analyzed at least 5 times, and the use of propofol as internal standard improved the precision. Quantitation was carried out using intensity, obtaining relative standard deviations lower than 5%, better than those corresponding to the use of peak area. The calibration curves for DMDSe and DEDSe established on the basis of their derivatives (CH_3–Se–DNP and CH_3–CH_2–Se–DNP) were linear for selenium amounts less than 25 and 50 ng, respectively, with correlation coefficients higher than 0.999. The absolute detection limits (evaluated as 3 times standard deviation of the mean plus the mean value for the blank, for 10 blanks) were 0.2 and 0.4 ng, and the sensitivities (slope of the calibration curve) were 0.180 and 0.0786 ng^{-1} for DMDSe and DEDSe, respectively. Comparative detection limits are obtained using GC–QFAAS (7) being lower than those for GC–flame-ionization detection (18) but higher than those for GC–SCD (11), GC–MIP (12), and purge-and-trap low-temperature (PT-LT) GC–AFS (10).

Determination of selenium species in water samples

Separation and preconcentration of dialkyldiselenides from sea water were carried out by adsorption on 1.5 g of active carbon placed in a glass column. A 1-L aliquot of sea water, filtered through a 0.45-µm glass fiber filter for the elimination of suspended matter, was used as a test sample. Elution was accomplished using CS_2. The efficiency of the extraction was assessed in samples spiked with DMDSe and DEDSe at two concentration

Table V. Dialkyldiselenide Recoveries (%) Using a Solid-Phase Extraction on Active Carbon and Several Volumes of CS_2 as Eluent at a Flow Rate of 2 mL/min and Several Flow Rates of 4 mL of CS_2

	Spiking level (ng/L)	CS_2 volume (mL)				
		0.5	1.0	2.0	4.0	10.0
DEDSe	100	29 ± 4	42 ± 5	63 ± 6	70 ± 5	76 ± 5
DEDSe	500	26 ± 6	34 ± 6	56 ± 5	73 ± 5	74 ± 6
DMDSe	100	19 ± 6	39 ± 6	52 ± 6	75 ± 6	78 ± 7
DMDSe	500	13 ± 4	41 ± 6	58 ± 7	80 ± 6	82 ± 6
	Spiking level (ng/L)	CS_2 flow rate (mL/min)				
		0.2	1.0	2.0	5.0	10.0
DEDSe	100	79 ± 5	83 ± 5	70 ± 5	61 ± 5	49 ± 6
DEDSe	500	76 ± 5	81 ± 5	73 ± 5	59 ± 5	55 ± 5
DMDSe	100	81 ± 6	79 ± 5	75 ± 6	54 ± 5	47 ± 5
DMDSe	500	80 ± 5	82 ± 5	80 ± 6	48 ± 6	52 ± 5

Table VI. Detection Limits (DL), Correlation Coefficient (r^2), Sensitivity (S), Repeatability (r), and Reproducibility (R) of Selenium Species in Water*

	DL (ng/L)	S (L/µg)	r^2 (%)	r (%)	R (%)
DMDSe	12	3.00 ± 0.098	0.996	6.83	10.8
DEDSe	23	1.41 ± 0.031	0.997	7.19	11.6

* The repeatability and the reproducibility were assessed in solutions containing 0.480 µg/L of DMDSe and 0.975 µg/L of DEDSe (as Se).

levels (100 and 500 ng/L). Several volumes of CS_2 ranging from 0.5 to 10 mL were tested as eluent using a flow rate of 2.0 mL/min. The better recoveries were obtained from a CS_2 volume higher than 4 mL (Table V). Flow rates of CS_2 ranging between 0.2 to 10 mL/min were tested for the elution (Table V) using 4 mL of solvent, giving optimum results for a flow rate of 1 mL/min.

The performance of the complete procedure, including the sample extraction, derivatization, and preconcentration, is summarized in Table VI. The repeatability of the procedure, evaluated for 5 replicates of aqueous standard solutions analyzed in a period of 8 h, was in the order of 7%, and the reproducibility over a month (5 different replicates) was less than 11%. Calibration curves were linear from the detection limit up to 1200 and 2700 ng/L for DMDSe and DEDSe, respectively. The detection limits (evaluated as 3 times standard deviation of the mean plus the mean value of 10 blanks) using 1 L of water were 12 ng/L for DMDSe and 23 ng/L for DEDSe. These results are comparable with those using GC–AAS (12) and GC–MIP (19), allowing the evaluation of this species in natural aquatic environments.

The procedure has been applied to the determination of DMDSe and DEDSe in 7 natural waters, including river and seawater, collected from southwest Spain. However, concentrations of both species were below the detection limit, and recoveries were evaluated by spiking the samples with 200 ng/L of both species (DMDSe and DEDSe), with recoveries higher than 87%, indicating the feasibility of the proposed method for high- and low-ionic-strength waters.

Conclusion

The conversion of dialkyldiselenides into the corresponding selenols and, in turn, their derivatization by trapping with Sanger's reagent for subsequent quantitation by GC–MS, offers a good approach for the identification of specific forms of selenium in the environment. The formation of derivatives with Sanger's reagent increases the resistance of selenium species to oxidation and makes their handling and preconcentration in the absence of an inert atmosphere possible, which represents a simplification in comparison with other procedures recommended for selenium speciation. In addition, the approach avoids the possible interference from alkylselenides. The procedure is suitable for the speciation of dimethyl- and diethyl-diselenide with detection limits in the nanogram range, making the method suitable for the analysis of natural waters. Finally, although this speciation procedure based on GC–MS does not allow for the determination of inorganic selenium (selenite and selenate) due to the thermal instability of their derivatives, the method could be extended to these inorganic species using HPLC.

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