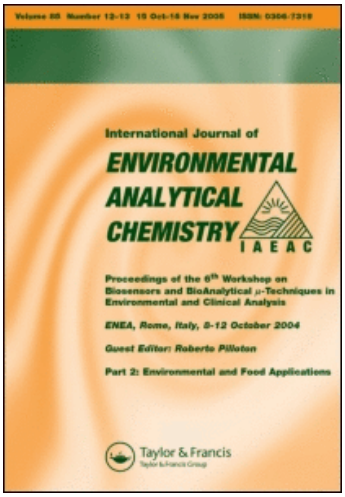

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Pervaporation as a Separation Technique for Direct Determination of Volatile Selenium Species by Atomic Fluorescence Spectrometry

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PERVAPORATION AS A SEPARATION TECHNIQUE FOR DIRECT DETERMINATION OF VOLATILE SELENIUM SPECIES BY ATOMIC FLUORESCENCE SPECTROMETRY

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This paper reports for the first time a suitable way to determine methylated selenium compounds using the new approach of pervaporation coupled to atomic fluorescence spectrometry (PV-AFS).

The method developed allows direct extraction, separation, preconcentration and determination of dimethylselenium (DMSe) and dimethyldiselenium (DMDSe) from slurry samples. Under the optimum conditions, the detection limits (LODs) were found to be 0.66 ng and 0.39 ng for DMSe and DMDSe, respectively, the precision being about 6–9 % for 10 ng mL^{-1}as selenium concentration. The linearity ranges were from the LOD to 0.7 $\mu g\ mL^{-1}$ for DMSe and from the LOD to 0.4 $\mu g\ mL^{-1}$ for DMDSe (as Se). The pervaporation efficiencies were 55 ± 1 % and 85 ± 5 % for DMSe and DMDSe, respectively. The proposed method was successfully applied to determine methylated selenium species in sewage sludge, garlic and oyster samples. The concentrations found were from 0.07 to 1.42 $\mu g\ g^{-1}$.

As no certified reference materials are available for these analytes, validation was carried out by recovery studies in these matrices, and the results showed that the proposed method performed satisfactorily.

Keywords: Organomethylselenium species; pervaporation; atomic fluorescence spectrometry; slurry; sewage sludge; garlic and oyster

INTRODUCTION

Bioalkylation processes have been well characterized as playing an important role in the biogeochemical cycle of selenium, as well as that of other elements like lead, mercury, tin or arsenic[1]. The increasing use of fossil fuels and indus-

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trial products with high quantities of selenium can lead to more transformation in the environment by bacteria and other micro-organisms. All these transformations generate volatile Se species (mainly methylated compounds) which are released into the atmosphere[2,3]. This has been suggested as a Se decontamination pathway in polluted areas. The formation of these compounds in the different compartments is represented in Figure 1. From this figure, it is seen that the volatile Se compounds are released from different sources: bacterial activity in soils; transformation of the inorganic selenium absorbed by plants; animal exhalation from seleno-protein degradation.[4,5]

The main volatile Se species identified in the environment are two methylated compounds: dimethylselenide (DMSe) and dimethyldiselenide (DMDSe). However, the occurrence of other volatile alkyl selenides have also been reported[2]. The occurrence of these Se compounds in the environment is still very low. Therefore the development of sensitive and rapid analytical methods which allow the determination and the speciation of these compounds is required. The determination of these species has been accomplished by using on-line cryogenic trapping coupled to gas chromatography. The detectors coupled to this purge system are atomic emission spectrometry (AES)[2], microwave induced plasma atomic emission spectrometry[6], non-dispersive atomic fluorescence spectrometry (NDAFS)[7,8],, or inductively coupled plama-mass spectrometry (ICP/MS)[9], all of them being highly powerful. The use of a technique such as pervaporation coupled to element-specific detectors has been proposed as an alternative to chromatographic separation. This new approach integrates extraction, clean-up, separation and preconcentration steps in the same unit.

Pervaporation is a membrane process that combines the evaporation of volatile analytes with their diffusion through a selective membrane. The mechanism of transport by pervaporation can be divided into the following steps: i) sorption of the volatile analytes from a liquid phase at the membrane surface; ii) diffusion through the polymeric surface; and iii) evaporation of the analytes from the membrane[10], The selectivity of this process is determined not only by differences in the vapour pressure of the components, but also by their permeation rate through the membrane. This last factor is crucial in the selection of an appropriate membrane to enhance selectivity and sensitivity[11,12].

Some pervaporation studies have been carried out for the separation of water/alcohol mixtures[13,14]. However, the application of this technique for analytical purposes is still uncommon. The first papers published described the determination of ethanol and diacetyl in biological liquids[15,16]. In the last ten years, Luque de Castro et al. have developed a wide number of applications of this technique to the determination of different analytes[17], such as sulphide[18], fluoride[19], acetaldehyde[20], trimethylamine[21], and mercury speciation.[22,23]

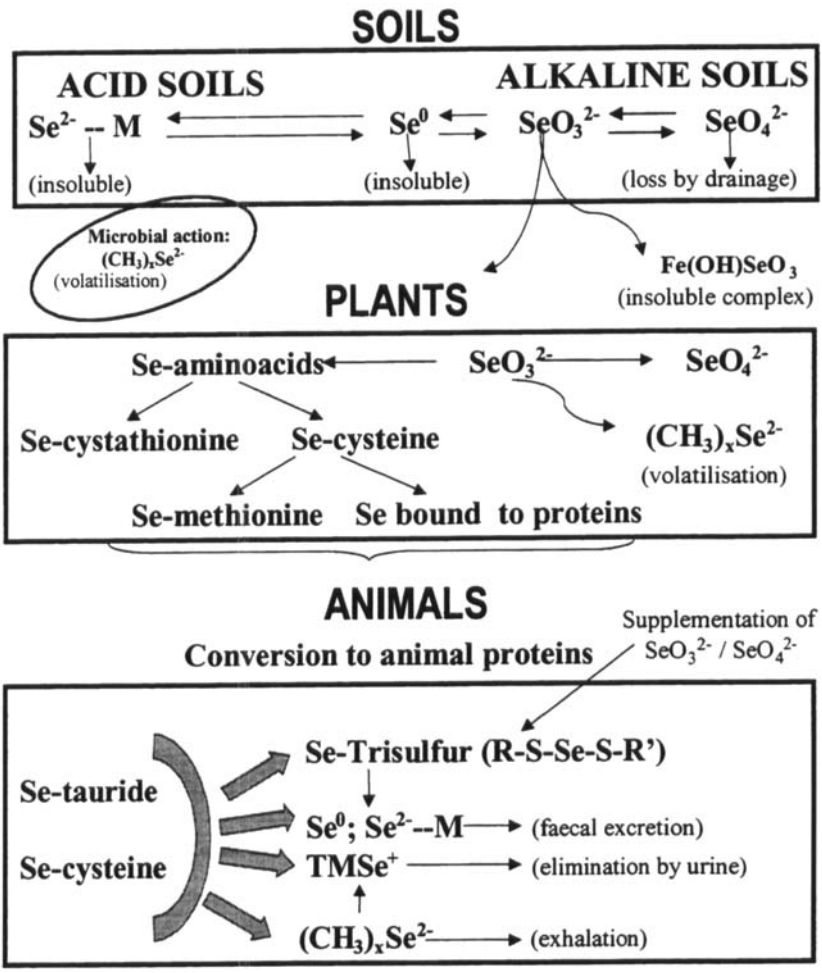


FIGURE 1 Biogeochemical cycle of selenium. Exchanges between soils, plants and animals (adapted from refs. 4 and 5)

This work focused on the determination of DMSe and DMDSe in solid samples by pervaporation coupled to AFS. Such a coupling combines the selectivity of pervaporation and the high sensitivity of AFS. Three kinds of solid samples were analysed. First, sewage sludges were taken from a water treatment plant after biological treatment, which can lead to biomethylation processes as mentioned above[2,3], The second group of samples studied were foodstuffs. It is well known that *Allium* species are rich sources of selenium in the diet. Garlic is

claimed to contain a high number of selenoproteins, and also volatile organoselenium compounds (methyl-, allyl-, propenyl species).[24,25] The third group of samples were oysters as a seafood, which can biomethylate selenium species. In all cases, the samples were freeze-dried and ground in order to obtain a homogenised solid. The slurry technique was then applied for introduction in the pervaporation chamber. After heating, the volatile selenium species were preconcentrated in the upper part of the chamber and then driven to the detector by the carrier gas.

The advantages of this method are: i) low manipulation of the sample thereby avoiding possible contamination problems derived from the pre-treatment procedures; ii) low consumption of sample and reagents due to the small dimensions of the pervaporation unit; iii) lower analysis time and cost than for chromatographic methods; iv) detection limits in the ng/L level.

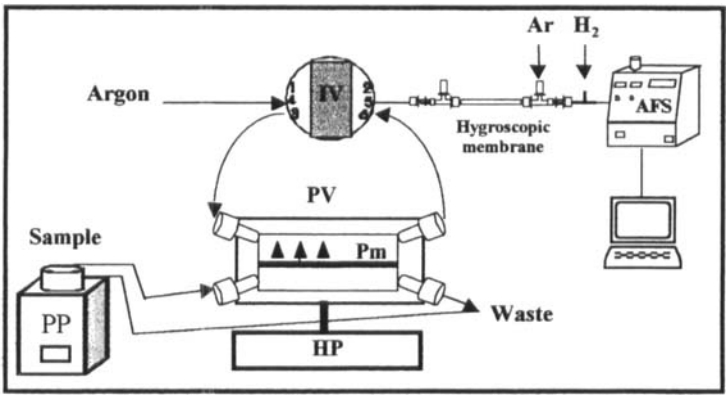
EXPERIMENTAL

Instrumentation

Pervaporation was carried out using a pervaporation chamber designed in our laboratory. This chamber was made of methacrylate and divided in two chambers, called the donor chamber and the acceptor chamber. There were two inner o-ring fittings, one made of Teflon and the other of methacrylate. The ability to change the size of the Teflon ring made it possible to adjust the volumes in the lower part (donor chamber). The other ring supported the membrane between the two chambers that was necessary to carry out the pervaporation procedure.

Hydrophobic Teflon membranes (Durapore GVHP) with a pore size of 0.22 μm were used in the system. A Peristaltic pump (4-channel Gilson), teflon tubes of 0.5 mm inner diameter, pumping tubes and connectors were used to introduce the samples into the pervaporation module. A heating plate (Bunsen MC-8, S.A.) was used to heat the pervaporation chamber. The pervaporation (PV) module was coupled as the same way as the coil placed in a six-channel injection valve (Omnifit) to achieve a FIA system (see Figure 2)

A hygroscopic membrane of Nafion was used to remove the moisture from the pervaporated analytes before they entered the atomic fluorescence spectrometer (P.S. Analytical Ltd., type 10.033). The atomization flame was a hydrogen/argon diffusion flame. A solar photomultiplier tube positioned at right angle to the incident light was used as detector. Signals were collected using an integrator (Waters 746) for subsequent processing.



PP: Peristaltic pump	**IV: Injection valve**
PV: Pervaporation Unit	**AFS: Atomic Fluorescence Spectrometer**
Pm: Pervaporation membrane	**HP: Heating Plate**

FIGURE 2 Schematic diagram of the PV-AFS system for determination of methylated selenium species

Reagents

Argon, purity ≥99,995% was used as carrier and dryer gas, and Hydrogen, purity ≥99,999%, was employed to fuel the atomization flame (both from Carburos Metálicos).

Dimethylselenide (b.p 58°C) and dimethyldiselenide (b.p. 154°C) (Alfa Aesar) standard stock solutions of 1000 mgl^{-1} as Se and 10 mg l^{-1}were obtained by diluting the appropriate amount in methanol. Working solutions from 5 ng Se mL^{-1} to 100 ng mL^{-1} were prepared daily in de-ionised water in sealed glass flasks and kept on ice until measurement in order to preserve their integrity.

Operating conditions

All tubes and transfer line lengths were minimised in the pervaporation-atomic fluorescence spectrometry (PV-AFS) system shown in Figure 2.

It is divided in three modules:

(1) Pervaporation module to achieve quantitative separation of pervaporated analytes (methyl selenium species).

(2) Transfer module to couple the PV system to AFS

(3) Detection module to quantify the selenium in samples

(1) The samples were driven to the donor chamber using a peristaltic pump until a volume of 700 μL (total chamber volume) was reached. Then the entrance and exit were closed, the pervaporation chamber was heated to 60°C using a heating plate with a Copper-plate system to enable full contact with the donor pervaporation part. After six minutes the injection valve was changed from the load position to the inject position allowing the argon carrier gas to drive the pervaporated analytes from the acceptor chamber through the transfer line to the AFS.

(2) The transfer line was a hygroscopic membrane (Perma Pure Products, Farmingdale, NJ, USA). It had two concentric tubes, the inner one of Nafion and the outer one of Teflon. The moisture was removed using an argon flow rate of 0.4 L min^{-1}.

(3) Before the AFS, a hydrogen flow is required to fuel a diffusion flame Ar/H_2 in the atomisation cell.

The non-dispersive AFS was equipped with a selenium boosted discharge hollow cathode lamp at 25 mA as primary and boosted intensities. The PMT was 600 V and the integration time used was 1/4 s.

Sample preparation

Three kinds of samples were chosen due to the interest in the determination of volatile selenium species containing methyl groups:

1- environmental samples-Sewage sludges, due to the microbiological activity on soils. They were obtained from a water treatment plant and from WRC, Aquachek UK.

2- biological and marine samples-Fresh garlic, which is known to have a high content of sulphur and selenium compounds, and oysters, which can methylate selenium compounds. These vegetal and marine materials were freeze-dried for subsequent analysis.

Fresh garlic samples were purchased at a supermarket and accurately weighed and freeze-dried with and without spiking with different amounts of DMSe and DMDSe. Then they were ground to achieve homogenisation. Recovery tests did not show losses from the enriched samples. (Recovery achieved was about 97%)

Sewage sludges, obtained from a water treatment plant, were ground in a centrifugal ball mill (agate balls).

Slurries of these samples were prepared by weighing accurately 0.15 g of sample, adding de-ionised water to 25 mL and treatment in an ultrasonic bath for 10 minutes, after which they were stirred until measurement.

RESULTS AND DISCUSSION

Ability of the selenium species to pervaporate

The coupled PV-AFS device was tested for several inorganic and organic selenium species to determine which of them can pervaporate and can thus be determined by the proposed method.

The results showed that selenites and selenates do not pervaporate, and neither do selenomethionine, selenocystine and trimetylselenonium. Dimethylselenium and dimethyldiselenium however do pervaporate and therefore they were chosen as the analytes to be determined by the proposed method in different samples.

Optimization of parameters

All the parameters, likely to affect the pervaporation efficiency (heating temperature, pervaporation time, carrier argon flow rate and sample volume) and detection of selenium compounds, were optimized.

A- Pervaporation module

Heating temperature and heating time

In order to obtain the maximum pervaporation efficiency, the effect of the donor chamber heating temperature on 5 $\mu g\ l^{-1}$ of DMSe and DMDSe was tested within the range 10–80°C using a carrier argon flow rate of 600 mL min^{-1} and a pervaporation time of 6 minutes.

It was observed that the pervaporation efficiency for DMDSe was higher than for DMSe and in both cases it increased with increasing temperature. Results on experiments carried out on the effect of temperature showed that the analytical signal increases with increasing heating temperature of the donor chamber until reaching a plateau at 60°C. Therefore this temperature was selected as optimum for further experiments.

The pervaporation time, needed to obtain a quantitative process before the injection of volatile compounds in the carrier argon, was evaluated over 1 to 15 minutes using 5 $\mu g\ l^{-1}$ of DMSe and DMDSe. The results obtained are reflected in Figure 3 A, which shows that maximum sensitivity is obtained after 6 minutes.

The use of higher heating temperatures and pervaporation times produced a significant decrease in precision without any improvement in analytical signal intensity. This was due to water condensation on the acceptor chamber, so a compromise of 60°C and 6 minutes was chosen as the optimum conditions.

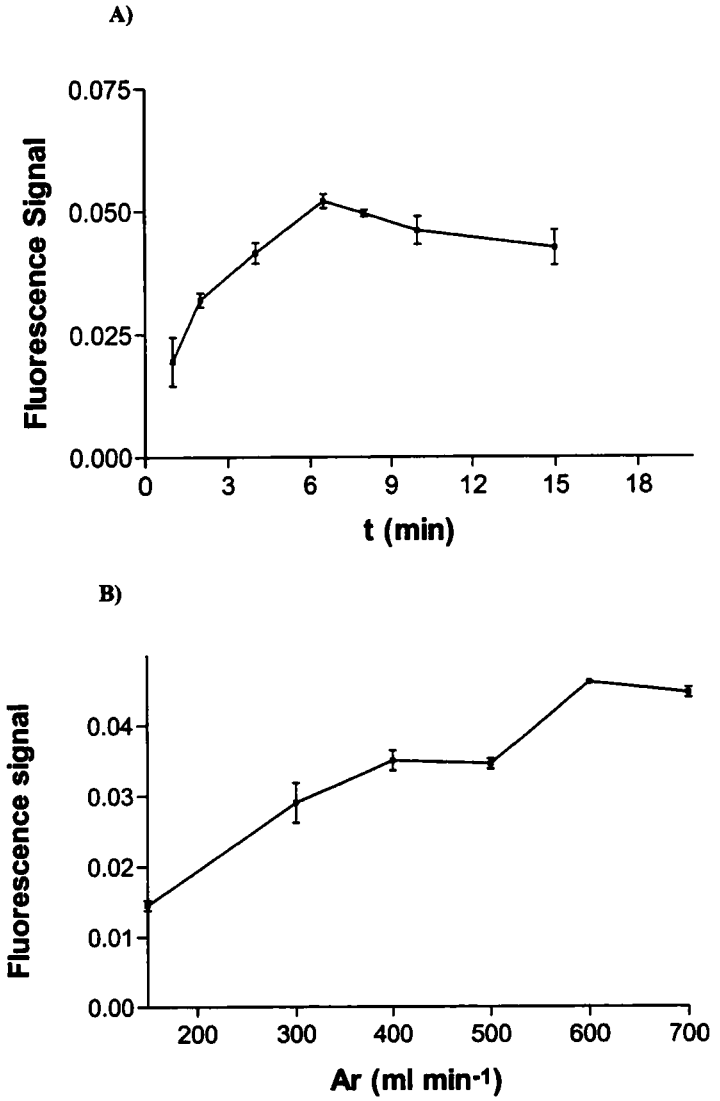


FIGURE 3 Effect of pervaporation time and carrier argon flow rate on Fluorescence signal

Argon flow rate

It was also important to optimise the argon flow rate because it plays an important role in volatile analyte transport from the acceptor chamber to the detector.

The flow rate was tested from 150 to 700 mL min^{-1}. As can be seen in Figure 3 B, the signal increased, due to transport of selenium species improved, with increasing flow up to 600 mL min^{-1} after which a steady signal was obtained. Therefore 600 mL min^{-1} was chosen as optimum flow rate for further experiments.

Sample volume

It was important to optimise the volume in the donor chamber. The effect of sample volume was tested from 30 µL to 700 µL (full chamber). Three experiments on different absolute amounts (0.5, 8, 32 ng) of DMSe and DMDSe were carried out.

Figure 4 shows that, independently of the standard, of whether DMDSe or DMSe was tested and of the absolute amount of each, higher signals were observed for higher volumes, the optimum sensitivity being achieved when a full donor chamber was used.

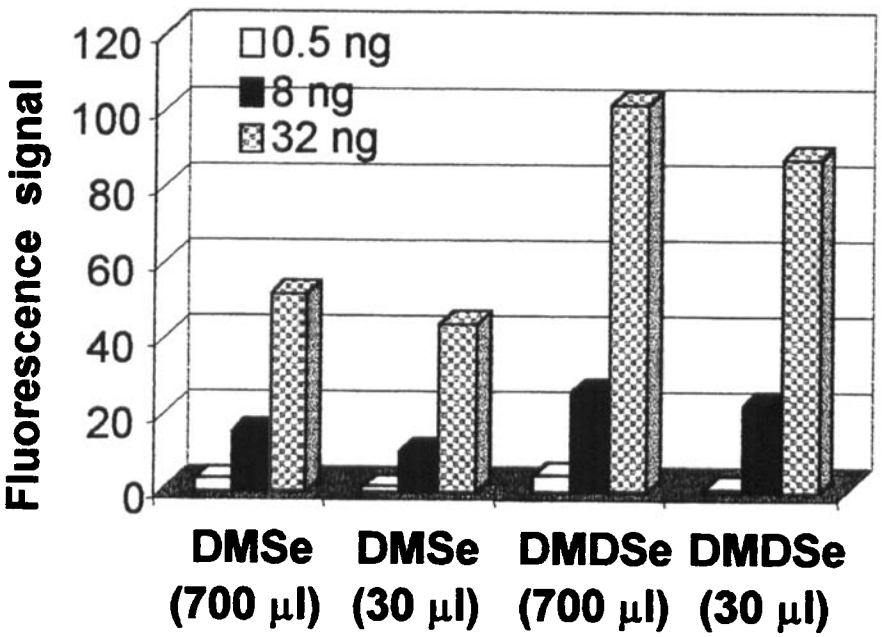


FIGURE 4 Effect of donor chamber volume of the pervaporation system, for different absolute amounts of DMSe and DMDSe

Therefore 700 µL was chosen as the optimum volume. This test demonstrated that diffusion also contributes to the pervaporation process because the solutions touch the membrane slightly when the donor chamber is full, but otherwise do not.

Pervaporation membranes

Several Teflon membranes with pore size between 0.22 and 5 μm were tested. The results obtained led us to choose hydrophobic teflon membranes Durapore GVHP 0.22 μm for the pervaporation module in further experiments.

B-Transfer line

In this module a hygroscopic Nafion membrane length of 24 cm with a 400 mL min^{-1} Argon drier gas flow was used to drive the pervaporated analytes to the detector and to remove the moisture originated during the pervaporation time. This provided better results that without the Nafion membrane.

C-Detection module

A hydrogen flow rate of 250 mL min^{-1} was necessary to fuel a flame in the atomization cell. It was incorporated by means of a T-connection before the atomic fluorescence spectrometer. This external auxiliary hydrogen with carrier argon made it possible to sustain an adequate diffusion flame, and also to improve the atomization mechanism for these analytes by relaxing and breaking specific bonds to varying extents.

Table I summarises the experimental conditions for the three modules used throughout the study.

TABLE I Experimental conditions for DMSe and DMDSe determination using PV-AFS

Pervaporation Module	
Membrane	Durapore GCHP 0.22 μm
Pervaporation time	6 min
Heating temperature	60°C
Acceptor chamber volume	300 μl
Donor Chamber volume	700 μl
Carrier gas (Ar)	600 ml min^{-1}
Transference Module	
Hygroscopic membrane	Nafion 24 cm
Drier gas (Ar)	400 ml min^{-1}
Atomic Fluorescence Spectrometer	
Auxiliary gas (H_2)	250 ml min^{-1}
Primary Current	25 mA
Boost Current	25 mA

Pervaporation efficiency

To determine the process efficiency, the following experiment was performed.

After pervaporating samples, air was purged through the donor chamber and the residue was collected and analysed by hydride generation-atomic fluorescence spectrometry technique (HG-AFS). Sample collection was carried out after three consecutive measurements to obtain a representative solution and this operation was repeated three times. We previously evaluated the ability of DMSe and DMDSe to form volatile selenium compounds when treated with sodium borohydride in a manner similar to that used in selenium hydride generation.

The pervaporation efficiency (PE) was evaluated as the difference between the initial selenium amount before (A_B) and after (A_A) pervaporation divided by the initial selenium amount and multiplied by 100:

$$PE = \frac{(A_B - A_A)}{A_B} \times 100$$

The experiment was carried out in three consecutive measurements. The efficiency achieved was 55 ± 1 % and 85 ± 5 % for DMSe and DMDSe, respectively, which indicates that DMDSe quantitatively pervaporates while DMSe does so with much lower efficiency (see Table II). This explains the different slopes of their calibration curves.

TABLE II Evaluation of pervaporation efficiency

Species	A_A[a]	A_B[b]	*Pervaporation efficiency*[c] *(%)*
DMSe	40.8	93.9	56
	42.2	93.9	55
DMDSe	13.9	109.6	87
	20.0	109.6	82

a. Amount after pervaporation determined by HG-AFS
b. Amount before pervaporation determined by HG-AFS
c. $\frac{(A_B - A_A)}{A_B} \times 100$

Analytical characteristics of the proposed method

For the purpose of this study, the system was calibrated from 0–100 ng mL^{-1} of DMSe and DMDSe. Typical calibration curve equations, correlation coefficients, linearity ranges and detection and quantification limits (LOD, LOQ) are given in Table III.

TABLE III Performance Characteristics of the proposed method

Species	*Concentration (ng ml^{-1})*	*Equation*	*Correlation Coefficient, r^2*	*Linearity (μg)*	*LOD[a] (ng)*	*LOQ[b] (ng)*	*Precision[c] (%)*
DMSe	0–100	y= 2.3391x –0.06	0.9981	LOD-0.7	0.66	2.10	9
DMDSe	0–100	y= 4.0074x +0.03	0.9986	LOD-0.4	0.39	1.30	6

a. LOD calculated as 3 s_{n-1} of the standard deviation of 2 ng ml^{-1} (n=10)
b. LOQ calculated as 3 s_{n-1} of the standard deviation of 2 ng ml^{-1} (n=10)
c. % Relative standard deviation n=5 [Se]=10 ng ml^{-1}

LOD and LOQ were calculated as three or ten times the standard deviation, respectively, of ten runs on standards of 2 ng mL^{-1}. The results showed that the sensitivity achieved for DMDSe is about 1.7 times higher than that for DMSe, which is in good agreement with the results of the pervaporation efficiency study.

The precision of the proposed method, determined for DMSe and DMDSe by carrying out repeated pervaporations (n= 5) of a 10 ng mL^{-1} standard, was 9 and 6 %, respectively.

Applications of the proposed method

The proposed method was applied to the determination of methylated selenium species in sewage sludges, fresh garlic and oysters.

Matrix effects were observed when the slurries were analysed and the standard additions method, making use of DMDSe, was used to determine the species. As an approximation way, it was necessary to express the total volatile selenium concentration as selenium from DMDSe, because the system used did not enable each selenium species to be distinguished.

Methylated species were found in most of the sewage sludges tested within the range 0.35–1.42 μg g^{-1} and in the analysed garlic and oysters (0.07–1.15 μg g^{-1}).

The concentrations of methylated selenium species found in each sample are shown in Table IV. Analysis of total selenium was carried out by a HG-AFS system described in a previous paper[26]. The total selenium concentrations are also given in Table IV.

As no certified reference material for methylated selenium species is currently available, the method had to be validated by spiking samples with known concentrations of each selenium species and evaluating the recovery in each matrix.

All samples were spiked with both 5 and 100 μg g^{-1} of each selenium species. The spike recoveries, shown in Table V, demonstrate quantitative recovery of both species in all matrices tested. Therefore good agreement was observed with the results using the proposed PV-AFS system.

TABLE IV Results for real samples

Sample	*Name*	*Total Selenium*[a] *($\mu g\ g^{-1}$)*	*Volatile selenium found*[b] *($\mu g\ g^{-1}$)*
	Sur	2.87 ± 0.08	1.24 ± 0.04
Sewage sludge	Butazqua	1.79 ± 0.05	--
	Aquacheck 1	10.35 ± 0.09	0.79 ± 0.08
	Aquacheck 2	2.57 ± 0.08	1.42 ± 0.08
Garlic	A	0.63 ± 0.06	0.10 ± 0.03
	B	6.03 ± 0.10	1.05 ± 0.07
Oyster	T-36	157.7 ± 3.30	0.98 ± 0.07
	T-37	57.8 ± 7.00	0.80 ± 0.04

a. Total selenium determined by HG-AFS.
b. Volatile Selenium determined using DMDSe for standard addition method.

TABLE V Results for spike recoveries

Sample	*Spike recoveries ($ng\ ml^{-1}$)*					
	DMSe			*DMDSe*		
	Added	*Found*	*Recovery (%)*	*Added*	*Found*	*Recovery (%)*
Sewage Sludge (Stir)	5	4.5 ± 0.4	90	5	4.3 ± 0.5	86
	100	99.0 ± 0.8	99	100	98.0 ± 1.0	98
Garlic (B)	5	4.6 ± 0.3	92	5	4.2 ± 0.9	84
	100	98.0 ± 1.0	98	100	99.0 ± 0.8	99

CONCLUSIONS

The ability and efficiency of DMSe and DMDSe to pervaporate has been reported for the first time in the literature.

The pervaporation system used acts as a combined pre-concentration and separation step and has been shown to be a good alternative way to introduce sample in the atomic fluorescence spectrometer, as it is easily coupled. This features together with the extremely sensitive detection system used, makes the coupling a good method for the determination of methylated selenium species in sewage sludges, garlic and oysters as demonstrated by the results reported.

In addition, the method needs little sample pre-treatment and manipulation, thus reducing contamination or losses, and has the advantage of low reagent consumption and short measurement time.

Future trends in experimentation will probably include the evaluation of other selective hydrophobic membranes, other hydrophobic polymers and modified membranes with active sites, and the use of other couplings for the speciation of selenium species.

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