Determination of volatile organochalcogens using liquid phase microextraction and gas chromatography with fluorine-induced chemiluminescence detection

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ABSTRACT

In this study, a simple and rapid liquid phase microextraction technique coupled with gas chromatography and fluorine-induced chemiluminescence detection has been developed for determination of volatile organochalcogens (dimethyl sulfide, dimethyl disulfide, dimethyl trisulfide and dimethyl diselenide and dimethyl telluride). The effects of extraction parameters such as extracting solvent and its volume, stirring time, extraction time and salt effect were optimized. Also, analytical figures of merit such as response linearity, repeatability and reproducibility, and limits of detection have been evaluated. The proposed method was found to be a simple and rapid analytical procedure for determination of volatile organochalcogens in real aqueous samples with detection limits of 5-620ng·l^{-1}. The relative recoveries of these analytes for spiked bacterial cultures, tap water and lake water ranged from 95 to 105% with the relative standard deviations of 0.3-11.0%.

INTRODUCTION

Biomethylation of the chalcogens sulfur, selenium and tellurium (Group 16 elements) in environmental matrices has been well-documented in the literature (Chasteen and Bentley 2003; Meija et al. 2002; Pinel-Raffaitin et al. 2008). Many plants and microorganisms can transform these elements from nonvolatile, oxyanionic form (for example, SeO_4^{2-}) into volatile organic compounds (for example, $CH_3SeSeCH_3$). This biological activity is important in the global cycling of many of these species and is an important step in changing the toxicity of inorganic species in the water and soil samples. Volatile species can escape from soil and water and enter the atmosphere (Frankenberger and Benson 1994; Haygarth 1994). Volatile organosulfur compounds (VSCs) are of interest due to their adverse organoleptic characteristics (stench) even at very low concentration. These compounds are commonly associated with anaerobic decomposition of organic matter. In waste water, VSCs can originate from the reduction and methylation of proteins and other biological compounds. In addition, VCSs are byproducts of some industries such as leather tanning, paper production, and wood treatment.

Organic forms of selenium and tellurium, though generally less toxic than their more water soluble oxyanions, probably interfere with redox balance in living cells (Nogueira et al. 2005). The epidemiology, biology and biochemistry of these species are subject of intense current interest, and therefore the development of inexpensive and accurate analytical methods for identification and quantification of these compounds is important.

Separation and sensitive determination of these volatile chalcogen-containing organic species can be achieved by using gas chromatography (GC) coupled with different detection systems such as flame ionization detection (FID) (Dilli and Sutikno 1984), electron capture detection (ECD) (Dilli and Sutikno 1984; Elaseer and Nickless 1994; Gomez-Ariza et al.

1998) and atomic emission detection (AES) for organoselenium compounds (Campillo et al. 2007, 2009; Dietz et al. 2004), flame photometric detection (FPD), atomic emission detection and photoionization detection (PID) for organosulfur compounds (Campillo et al. 2009; Gerbersman et al. 1995; Haberhauer-Troyer et al. 1999; Hunter and Kuykendall 2004; Kataoka et al. 2000) and fluorine-induced chemiluminescence detection for volatile species containing reduced sulfur, selenium and tellurium (Burra et al. 2010; Swearingen et al. 2004, 2006). Also mass spectrometry (MS) can be used as a general detector (Cao et al. 2001; Gomez-Ariza et al. 1998; Meija et al. 2002) or made more selective in MS selected ion monitoring mode (Kapsimali and Zachariadis 2009). FID detection is simple, requiring no sample derivatization, inexpensive and the most common detector available on gas chromatographs. However, FID has poor selectivity and only moderate sensitivity for organo-metalloidal compounds (Hunter and Kuykendall 2004). ECD offers good selectivity and detection limit, but requires a derivatization step for changing target analytes to a form that can be sensitively detected by ECD. MS detection system offers very high assurance of sample identity and low detection limits, but the cost of MS can be problem for some laboratories. Fluorine-induced chemiluminescence offers a selective and sensitive means for organo-sulfur, -selenium, and -tellurium compounds with a linear range of three orders of magnitude for the target analytes under study here. This detection system has been extensively used for determination of different form of organometalloids in the headspace of different bacterial cultures (Burra et al. 2010; Chasteen et al. 1990; Swearingen et al. 2004; Van Fleet-Stalder and 1998).

Since the matrices of environmental samples are often complex and the concentration of analytes is also often very low, sample preparation steps play an important role in the determination of these chemical species. Liquid-liquid extraction (LLE), solid-phase extraction (SPE), and purge and trap with cryogenic trapping in a glass tube or different adsorbents (P&T) are the widely used methods for extraction and preconcentration of volatile organic species. However, LLE is time consuming and tedious and requires large amounts of high purity and therefore high cost and potentially hazardous organic solvents. SPE uses much less solvent than LLE, but requires column conditioning and is relatively expensive. P&T can also suffer from severe losses of volatile compounds. For avoiding the disadvantages of the LLE, SPE, and P&T, solid-phase microextraction (SPME) has been introduced as a solventless technique for extraction, preconcentration and introduction of the analytes from sample in one step (Abalos et al. 2002; Bueno and Pannier 2009; Burra et al. 2010; Busto and Guasch 2002; Dietz et al. 2004; Swearingen et al. 2004, 2006). However, SPME also has some problems such as high fiber cost, fiber fragility, a possibility of sample carry-over between runs, and a decline of extraction efficiency with time (Palit et al. 2005; Prosen and Zupan-Kralj 1999; Psillakis and Kalogerakis 2003).

Recently, liquid phase microextraction (LPME) has been introduced as an efficient alternative to traditional methods for sample preparation and extraction of organic compounds. LPME is a single-step extraction method that has a very high sample to solvent ratio which leads to a higher enrichment factor of target analytes. LPME is fast, simple, inexpensive and since very little solvent is used, there is minimal cost and exposure to toxic organic solvents. Different types of this technique such as hollow fiber based LPME, fiber in tube-LPME, single drop-LPME, head space-LPME, directly suspended droplet microextraction and LPME by solidification of floating drop, dispersive liquid-liquid extraction and ultrasound assisted emulsification extraction have been introduced and applied for extraction and preconcentration of different analytes, as highlighted in several reviews (Dadfarnia and Shabani 2010; Ghasemi et al. 2011; Pena-Pereira et al. 2010; Rezaee et al. 2010; Sarafraz-Yazdi and Amiri 2010). Some of these methods suffer from serious losses problem for volatile compounds.

The aim of this work was to develop a simple, fast and precise methodology based on the liquid phase microextraction for determination of dimethyl sulfide (DMS), dimethyl disulfide (DMDS), dimethyl trisulfide (DMTS), dimethyl diselenide (DMDSe) and dimethyl telluride (DMTe) as most common volatile organochalcogens produced by microorganisms, in biospheric water samples and bacterial cultures—using GC with fluorine-induced chemiluminescence detection.

MATERIAL AND METHODS

Reagents and bacteria cultures

Chemicals used in this study included DMDS (CH_3SSCH_3), DMDSe ($CH_3SeSeCH_3$), DMTS (CH_3SSSCH_3), DMS (CH_3SCH_3), acetonitrile and hexane were purchased from Sigma-Aldrich (St. Louis, MO, USA). DMTe (CH_3TeCH_3) from Organometallics Inc. (East Hampstead, NH, USA) was used without further purification. Stock solutions of target analytes were prepared by appropriate dilution with acetonitrile and stored at 4°C in the refrigerator. Fresh working solutions were prepared daily by diluting different amounts of the standard stock solutions in deionized water (RiOs 3 water purification system, Millipore; Billerica, MA, USA) to required concentrations.

Reagents used in bacterial cultures include Bacto™ tryptone (Becton Dickson, Sparks, MD, USA), yeast extract (EMD Chemicals, Gibbstown, NJ, USA) and sodium chloride (BDH Chemicals, West Chester, PA, USA). A metalloid-resistant, Gram positive, spore-forming, gelatinase-positive bacterium identified as *Bacillus* sp., LHVE was isolated from Huerquehue National Park in southern Chile and grown anaerobically in Luria Bertani (LB) medium (Sambrook et al.1989). LB medium consists of bacto tryptone, yeast extract and sodium chloride. Precultures of LHVE were prepared anaerobically by inoculating liquid LB medium with a single colony from an LB-agar plate containing LHVE followed by incubation in a water bath regulated at 37°C for 24 hours (Burra et al. 2010).

Instrumentation

A Hewlett-Packard 5890 series II GC with a Sievers 300 fluorine-induced sulfur chemiluminescence detector (originally sold by Ionics Instruments, Boulder, CO, USA) was used in the analysis with data integrated by a Hewlett Packard 3396 series III integrator. A 30-m, 0.32-μm internal diameter capillary column with a DB-1 stationary phase and a 5.0-μm chromatographic film was used (J&W Scientific, Folsom, CA, USA). Helium was the carrier gas with a flow rate of 1ml·min^{-1}. A splitless injection mode was used for all sample analysis with injector temperature of 275°C and the split valve opened at 1min to improve peak shape. The oven temperature program was: 30°C, held for 1min followed by ramp 25°C·min^{-1} to 250°C and then held for 2min.

Analytical procedure

A 9.5ml aliquot of the aqueous sample solutions prepared as above containing 5% of NaCl were transferred in a 10-ml Erlenmeyer flask. Then 30μl of hexane were added and the flask was hermetically sealed with a stopper and put into a water bath placed on a hot plate-stirrer. During the extraction, the sample solution was stirred constantly. After 15min of extraction, the stirrer was turned off and after a 1-min wait, a 1μl aliquot of the hexane on the top of the aqueous solution was taken with a 10μl microsyringe (Hamilton Co., Reno NV, USA) and injected splitless into the hot injector of the GC for analysis. Before each extraction, the microsyringe was washed at least 10 times with solvent in order to eliminate the bubble in the barrel at the end of the plunger.

RESULTS AND DISCUSSION

Optimization of liquid phase microextraction

In this study experiments were designed to develop a simple, fast and efficient microextraction procedure for some volatile organochalcogens. Under proper stirring of the aqueous phase, a circulatory flow was created in both phases. Figure 1 shows the schematic flow pattern in the microextraction system used. This flow consists of both a horizontal-rotational component and a vertical-toroidal component. The vertical component tends to stretch the organic layer down into the aqueous phase. Therefore the organic layer formed a steady vortex shape and fine droplets of organic layer separated from the tip of the vortex and dispersed in the aqueous phase. During circulation, the fine droplets periodically rise and rejoin the organic layer. This continuous circulation of the droplets in solution increases the contact area of the phases and also intensifies the mass transfer of target analytes between these phases. Increasing the contact area can increase the mass transfer rate and extraction efficiency.

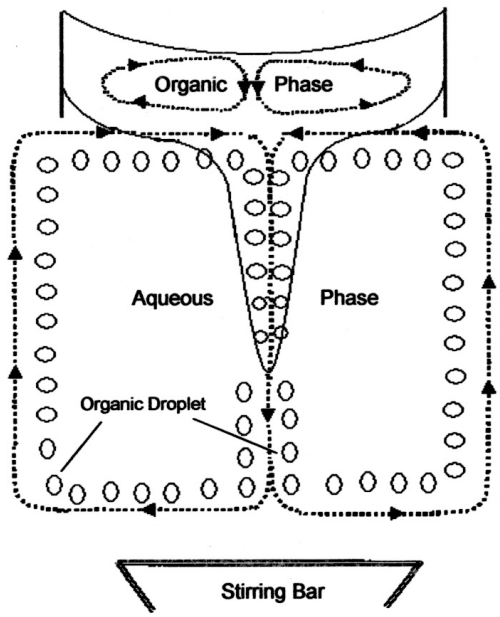

Figure 1. Schematic flow patterns in the two phase microextraction systems. Toridal flow is illustrated conceptually with dotted lines and arrows. The rotational component is omitted for clarity.

Factors affecting the extraction efficiency such as extracting solvent and its volume, extraction time, extraction temperature, stirring speed and salt effect were investigated and optimized. The optimization study was carried out by changing one variable at a time. A mixed working solution spiked with organochalcogens standards was used to perform the optimization studies. The chromatographic peak area was selected as the measure of extraction efficiency under different experimental conditions. Throughout these studies, the injected volume of the extracted analytes into the GC was kept constant at 1μl and all results were reported as an average of three replicate measurements.

Selection of extracting solvent

Selection of suitable extracting solvent is of great importance in order to obtain efficient extraction for a target analyte. An extracting solvent must have several characteristics: it should have a good affinity for target compounds, a low solubility in water, a lower density than water and good gas chromatographic behavior with the analytical GC column used for separation. On the basis of these considerations n-hexane, n-octane, acetonitrile, and cyclohexane were tested in the preliminary experiments. Among these different extracting solvents, n-hexane showed the best extraction efficiency. In addition this solvent showed no detectable signal with the fluorine-induced chemiluminescence detector.

Effect of extracting solvent volume

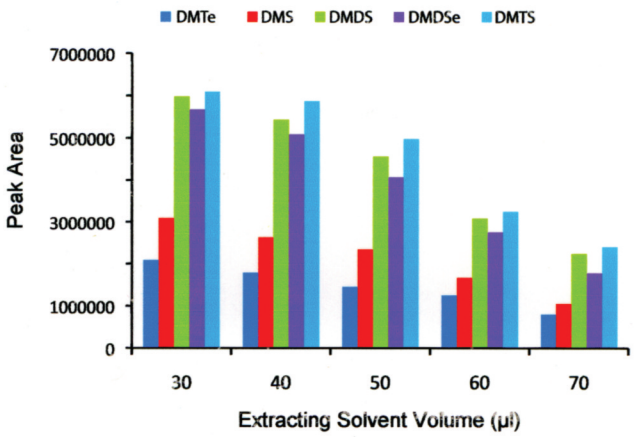

Figure 2. Effect of extracting solvent volume on target compound peak area.

The volume of extracting solvent placed in contact with the aqueous phase can obviously affect the extraction efficiency. The effect of organic solvent volume was investigated in the range of 30-70µl. The results are shown in Figure 2. As can be seen, the peak area of the target analytes decreased with increasing the volume of extracting solvent due to dilution effect. This behavior can also be explained by considering the enrichment factor equation:

$$E = C^{O}/C_0^{aq} = \frac{K_t}{1 + K_t\left(\frac{V^{O}}{V^{aq}}\right)}$$

where, C^{O} and C_0^{aq} are the analyte concentration in organic phase and its initial concentration in the aqueous phase, respectively. V^{O}/V^{aq} is the volume ratio of organic phase to the aqueous phase and Kt is the distribution coefficient at time of t. This equation shows that for a constant aqueous volume, the enrichment factor has a reverse relationship with volume of organic phase. The volumes smaller than 30µl were avoided due to dissolution of organic phase in aqueous phase and because of the difficulty of sample manipulation which led to a reduction in precision. The volume of 30µl was selected for further studies.

Effect of extraction temperature

Temperature is a major parameter affecting extraction efficiency. Increasing the temperature facilitates mass transfer of the analyte and increases the efficiency of the extraction. On the other hand, high temperatures can decrease the extraction efficiency due to loss of volatile analytes and increasing analyte solubility. The effect of temperature on the peak area of target analytes was investigated in the range of 20-50°C. The results (Figure 3) showed that, except for DMS, extraction efficiency increased with increasing temperature up to 40°C and then decreased slightly. For DMS, the extraction efficiency decreased after

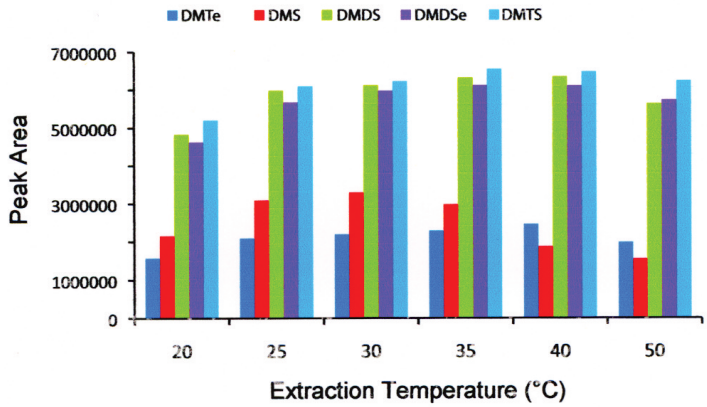

Figure 3. Effect of extraction temperature on target compound peak area.

30°C probably due to an increase in volatility of this analyte (B.P. of DMS: 38°C). Therefore a temperature of 30°C was selected for further studies.

Stirring speed

Stirring the aqueous phase creates a circular flow in both phases. This can intensify mass transfer of analytes and therefore increase the extraction efficiency. Also this circular flow induces a vortex state for the organic layer blending it into the aqueous phase. This can increase the contact surface area of the two immiscible phases and improve the extraction efficiency. The effect of stirring speed on the extraction efficiency was investigated in the range of 500-1600rpm (these speeds were nominal as determined by the stirrer's

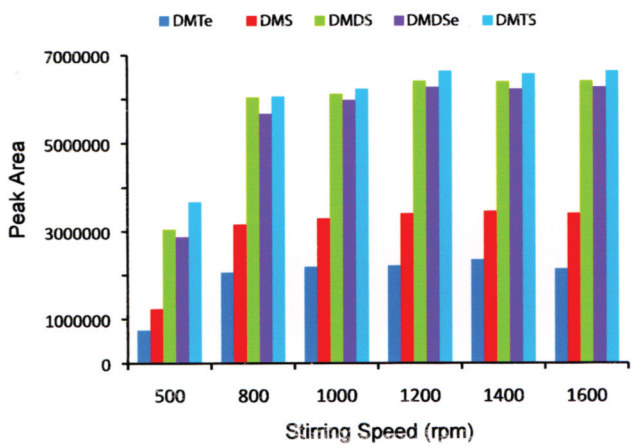

Figure 4. Effect of stirring speed on target compound peak area.

manual setting (Model 700 Hot plate/stirrer, VWR, Radnor, PA, USA). The results (Figure 4) show that the peak area of the target analytes increased with increasing of stirring speed up to 1200rpm and then leveled off. The stirring rate of 1200rpm was selected for subsequent experiments.

Salt effect

Addition of salts can improve the extraction efficiency due to reduction of the emulsification effect and the solubility of target analytes in aqueous phase. The effect of salt addition in LPME procedure was investigated by comparing the peak area of the target analytes in the presence of different concentrations of NaCl (0-15% w/v). The results (Figure 5) showed that the extraction efficiency increased with increasing the salt concentration up to 5% and then leveled off. Therefore the aqueous NaCl concentration was adjusted to 5% in all further experiments.

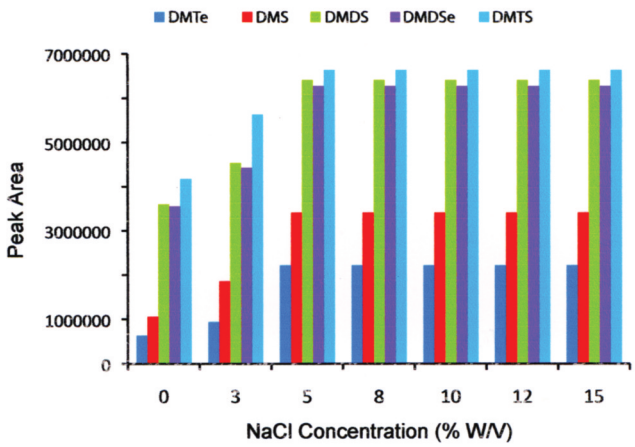

Figure 5. Effect of salt addition on target compound peak area.

Effect of extraction time

Extraction time is another important factor that may have significant effect on the extraction efficiency. The effect of extraction time on the relative peak area of analytes was investigated in the range of 5-25min. The results showed that the extraction efficiency of target analytes increased with increasing the extraction time up to 15min and then remained constant, indicating that an equilibrium state was reached. Therefore the extraction time of 15min was selected for further studies.

Evaluation of method performance

Calibration curves were drawn using 10ml of target analytes and five spiking levels of all analytes. For each level, three replicate extractions were performed at optimal conditions selected as described above (solvent volume: 30µl; extraction time: 15min; stirring rate: 1200rpm; sample temperature: 30°C and aqueous phase ionic strength: 5% w/v of NaCl). The corresponding correlation coefficients (R^2), dynamic linear ranges (DLR), and limit of detections (LOD, based on three times signal to noise ratio), were calculated for all target analytes and summarized in Table 1. The precision of the method was evaluated through investigation the repeatability (intraday precision) and reproducibility (interday precision). The repeatability was evaluated over five replicates spiked at 20$\mu g \cdot l^{-1}$ for DMDS, DMDSe and DMTS and 100$\mu g \cdot l^{-1}$ for DMS and DMTe within one day. The reproducibility was evaluated over five replicates, spiked at same level per work day for three days. The repeatability and reproducibility, expressed as relative standard deviations (RSD) were between 5.5-7.5% and 7.3-9.1%, respectively. The enrichment factor (EF), calculated as the ratio between the analyte concentration in the organic phase and initial concentration of the target analyte in the aqueous phase, were in the range 100-250 for all these target analytes.

Table 1. Figures of merit for determination of organochalcogens with this method.

Analyte	DLR[a] ($\mu g \cdot l^{-1}$)	R^2 [b]	LOD[c] ($ng \cdot l^{-1}$)	RSD[d] (%)	RSD[e] (%)	EF[f]
DMDS	3-60	0.9983	13	5.6	7.5	250
DMDSe	6-120	0.9961	5	5.0	7.3	200
DMTS	5-150	0.9985	15	5.5	7.4	200
DMS	100-350	0.9981	220	7.5	9.1	100
DMTe	40-500	0.9980	620	6.4	9.0	150

[a] Dynamic linear range
[b] Correlation coefficient
[c] Limit of detection from extracted aqueous solution (3S/N)
[d] Repeatability, intraday precision
[e] Reproducibility, interday precision
[f] Enrichment factor

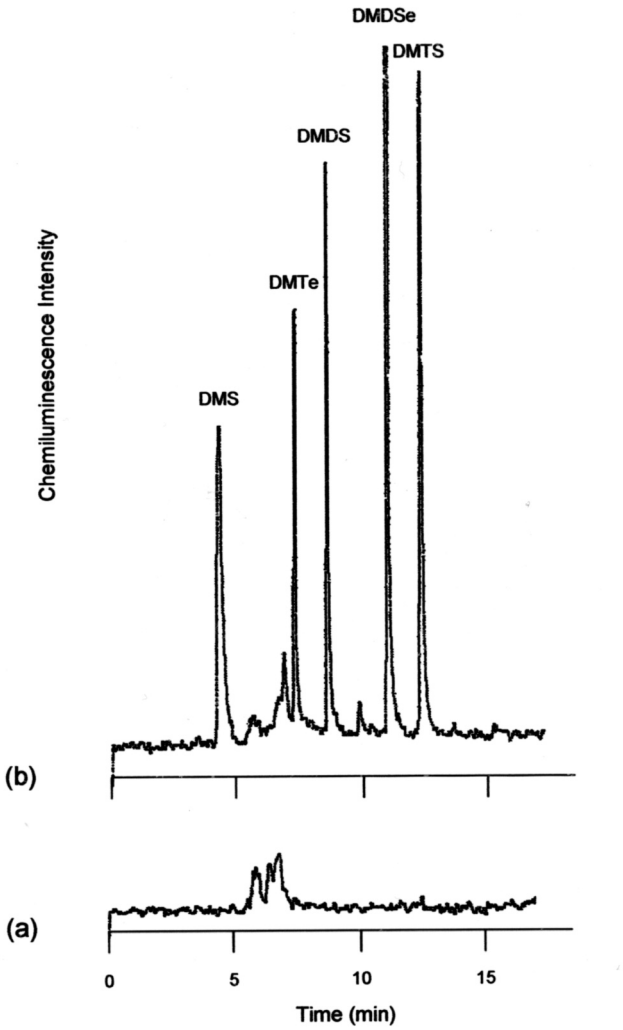

Figure 6. Chromatogram of (a) unspiked lake water (b) spiked lake water with 20$\mu g \cdot l^{-1}$ DMDS, DMTS, DMDSe, and 200$\mu g \cdot l^{-1}$ DMS and DMTe after liquid phase extraction.

Real sample analysis

To test the applicability of the proposed method for determination of organochalcogens in real samples, the developed method was applied to the determination of target compounds in live liquid cultures of LHVE, a metalloid-resistant bacterium, laboratory tap water and lake water samples (Sam Houston Memorial Museum Lake, Huntsville Texas, USA). The water samples were collected in amber glass bottles. The bottles were rinsed several times with the water samples and then, filled until overflow to prevent loss of volatile compounds to headspace. The water samples were stored at the temperature of 4°C until their analysis. These samples were filtered through 42.5mm pore size cellulose acetate filters (Whatman brand from VWR) before analysis. No target compounds were detected in the real samples (detection limits <50pg on column for all target compounds). Therefore, spiked bacterial culture and water samples were used for investigation of matrix effects. Figure 6 shows the chromatogram obtained for the spiked and non-spiked lake water sampled at optimum working conditions. The level of spiking ranged from 20 to 200μg target compound$\cdot l^{-1}$ sample solution and is specified in that figure's legend. Table 2 shows the obtained results and relative recovery for determination of the interested compounds in spiked samples. Relative recoveries were calculated as the ratio of the response in real and distilled water samples, spiked with same amount of analytes. Acceptable recoveries (95-108%) demonstrated that the matrices of bacterial culture, tap water and lake water samples had no effect on the performance of the presented method.

CONCLUSIONS

A simple and rapid liquid phase microextraction method coupled gas chromatography-fluorine induced chemiluminescence detection system has been developed for determination of organochalcogens in water and

Table 2. Determination of target analytes in real samples.

Analyte	Added ($\mu g \cdot l^{-1}$)	Found ($\mu g \cdot l^{-1}$)[a]		
		Bacterial culture (%R[b])	Tap water (%R)	Lake water(%R)
DMDS	5	4.8 ± 0.3 (96)	5.2 ± 0.4 (104)	5.4 ± 0.3 (108)
	20	21.0 ± 1 (105)	20.7 ± 0.9 (103)	21.2 ± 0.8 (106)
DMDSe	5	5.2 ± 0.4 (104)	5.5 ± 0.6 (110)	5.4 ± 0.5 (108)
	20	21.0 ± 0.9 (105)	21.6 ± 0.8 (108)	20.6 ± 0.4 (103)
DMTS	5	5.3 ± 0.6 (106)	5.2 ± 0.9 (104)	5.3 ± 0.6 (106)
	20	21.0 ± 11 (105)	20.6 ± 0.8 (103)	20.6 ± 0.7 (103)
DMS	100	95 ± 7 (95)	97 ± 4 (97)	98 ± 6 (98)
	200	198 ± 6 (99)	194 ± 6 (97)	194 ± 5 (97)
DMTe	100	96 ± 6 (96)	98 ± 5 (98)	97 ± 5 (97)
	200	197 ± 4 (98)	195 ± 6 (97)	196 ± 4 (98)

[a] Results: Mean ± standard deviation of five replicate determinations
[b] R: Recovery

bacterial culture samples. The developed method has many advantages including simplicity, rapidity, low cost, good repeatability and reproducibility, high sensitivity and good recovery. The detection limits of $ng \cdot l^{-1}$ were achieved with a sample volume of only 10ml under optimized working conditions.

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REFERENCES

Abalos, M., X. Prieto, J.M. Bayona. 2002. Determination of volatile alkyl sulfides in wastewater by headspace solid-phase microextraction followed by gas chromatography-mass spectrometry. Journal of Chromatography A 963: 249-257.

Bueno, M., F. Pannier. 2009. Quantitative analysis of volatile selenium metabolites in normal urine by headspace solid phase microextraction gas chromatography–inductively coupled plasma mass spectrometry. Talanta 78: 759–763.

Burra, R., G.A. Pradenas, R.A. Montes, C.C. Vasques, T.G. Chasteen. 2010. Production of dimethyl triselenide and dimethyl diselenenyl sulfide in the headspace of metalloid-resistant bacillus species grown in the presence of selenium oxyanions. Analytical Biochemistry 369: 217-222.

Busto, O., J. Guasch. 2002. Application of solid phase microextraction to the determination of sulfur compounds with low volatility in wines. Journal of Chromatography A 954: 211-219.

Campillo, N., R. Peñalver, M. Hernandez-Cordoba, C. Perez-Sirvent, M.-J. Martinez-Sanchez. 2007. 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. Journal of Chromatography A 1165: 191-199.

Campillo, N., R. Peñalver, I. López-García, M. Hernández-Córdoba. 2009. Headspace solid-phase microextraction for the determination of volatile organic sulphur and selenium compounds in beers, wines and spirits using gas chromatography and atomic emission detection. Journal of Chromatography A 1216: 6735–6740.

Cao, T.H., R.A. Cooney, M.M. Woznichak, S.W. May, R.F. Browner. 2001. Speciation and identification of organoselenium metabolites in human urine using inductively coupled plasma mass spectrometry and tandem mass spectrometry. Analytical Chemistry 73: 2898-2902.

Chasteen, T.G., R. Bentley. 2003. Biomethylation of selenium and tellurium: Microorganisms and plants. Chemical Reviews 103: 1-26.

Chasteen, T.G., G.M. Silver, J.W. Birks, R. Fall. 1990. Fluorine-induced chemiluminescence detection of biologically methylated tellurium, selenium, and sulfur compounds. Chromatographia 30: 181–185.

Dadfarnia, S., A.M.H. Shabani. 2010. Recent development in liquid phase microextraction for determination of trace level concentration of metals. Analytica Chimica Acta 658: 107–119.

Dietz, C., J. Sanz Landaluze, P. Ximenez-Embun, Y. Madrid-Albarran, C. Camara. 2004. Volatile organo selenium speciation in biological matter by solid phase microextraction-moderate temperature multicapillary gas chromatography with microwave induced plasma atomic emission spectrometry detection. Analytica Chimica Acta 501: 157-167.

Dilli, S., I. Sutikno. 1984. Analysis of selenium at the ultra-trace level by gas chromatography. Journal of Chromatography 300: 265-302.

Elaseer, A., G. Nickless. 1994. Determination of selenium by gas chromatography--electron-capture detection using a rapid derivatization procedure. Journal of Chromatography A 664: 77-87.

Frankenberger, W.T., S. Benson. (Eds.). 1994. Selenium in the Environment. 456p. Marcel Dekker, New York.

Gerbersman, C., R. Lobinski, F.C. Adams. 1995. Determination of volatile sulfur compounds in water samples, beer and coffee with purge and trap gas chromatography-microwave-induced plasma atomic emission spectrometry. Analytica Chimica Acta 316: 93-104.

Ghasemi, E., M. Sillanpaa, N.M. Najafi. 2011. Headspace hollow fiber protected liquid-phase microextraction combined with gas chromatography-mass spectroscopy for speciation and determination of volatile organic compounds of selenium in environmental and biological samples. Journal of Chromatography A 1218: 380-386.

Gomez-Ariza, J.L., J.A. Pozas, I. Giraldez, E. Morales. 1998. Speciation of volatile forms of selenium and inorganic selenium in sediments by gas chromatography-mass spectrometry. Journal of Chromatography A 823: 259-277.

Haberhauer-Troyer, C., E. Rosenberg, M. Grasserbauer. 1999. Evaluation of solid-phase microextraction for sampling of volatile organic sulfur compounds in air for subsequent gas chromatographic analysis with atomic emission detection. Journal of Chromatography A 848: 305-315.

Haygarth, P. 1994. Global importance and global cycling of selenium. In: Selenium in the Environment (ed. W.T. Frankenberger, Jr., S. Benson), pp. 1-27. Marcel Dekker, New York.

Hunter, W.J., L.D. Kuykendall. 2004. Determination of dimethylselenide and dimethyldiselenide by gas chromatography-photoionization detection. Journal of Chromatography A 1038: 295-297.

Kapsimali, D.C., G.A. Zachariadis. 2009. Headspace and direct immersion solid phase microextraction procedures for selenite determination in urine, saliva and milk by gas chromatography mass spectrometry. Journal of Chromatography B 877: 3210-3214.

Kataoka, H., H.L. Lord, J. Pawliszyn. 2000. Applications of solid-phase microextraction in food analysis. Journal of Chromatography A 880: 35–62.

Meija, J., M. Montes-Bayon, D.L. Le Duc, N. Terry, J.A. Caruso. 2002. Simultaneous monitoring of volatile selenium and sulfur species from Se accumulating plant (wild type and genetically modified) by GC/MS and GC/ICPMS using solid-phase microextraction for sample introduction. Analytical Chemistry 74: 5837-5844.

Nogueira, C.W., V.C. Borges, G. Zeni, J.B.T. Rocha. 2005. Organochalcogens effects on δ-aminolevulinate dehydratase activity from human erythrocytic cells in vitro. Toxicology 191: 169-178.

Palit, M., D. Pardasni, A.K. Gupta, D.K. Dubey. 2005. Application of single drop microextraction for analysis of chemical warfare agents and related compounds in water by gas chromatography/mass spectrometry. Analytical Chemistry 77: 711-717.

Pena-Pereira, F., I. Lavilla, C. Bendicho. 2010. Liquid-phase microextraction techniques within the framework of green chemistry. TrAC Trends in Analytical Chemistry 29: 617-628.

Pinel-Raffaitin, P., C. Pecheyran, D. Amouroux. 2008. New volatile selenium and tellurium species in fermentation gases

produced by composting duck manure. Atmospheric Environment 42: 7786-7794.
Prosen, H., L. Zupancic-Kralj. 1999. Solid-phase microextraction. TrAC Trends in Analytical Chemistry 18: 272-282.
Psillakis, E., N. Kalogerakis. 2003. Developments in liquid-phase microextraction. TrAC Trends in Analytical Chemistry 22: 565-574.
Rezaee, M., Y. Yamini, M. Faraji. 2010. Evolution of dispersive liquid–liquid microextraction method. Journal of Chromatography A 1217: 2342-2357.
Sambrook, J., E. Fritsch, T. Maniatis. 1989. Molecular Cloning: A Laboratory Manual, Second Edition. Cold Spring Harbor Laboratory, New York.
Sarafraz-Yazdi, A., A. Amiri. 2010. Liquid-phase microextraction. TrAC Trends in Analytical Chemistry 29: 1-14.
Swearingen, J.W., Jr., M.A. Araya, M.F. Plishker, C.P. Saavedra, C.C. Vásquez, T.G. Chasteen. 2004. Identification of biogenic organotellurides in *Escherichia coli* K-12 headspace gases using solid-phase microextraction and gas chromatography. Analytical Biochemistry 331: 106–114.
Swearingen, J.W., Jr., D.P. Frankel, D.E. Fuentes, C.P. Saavedra, C.C. Vasquez, T.G. Chasteen. 2006. Identification of biogenic dimethyl selenodisulfide in the headspace gases above genetically-modified *Escherichia coli*. Analytical Biochemistry 348: 115-122.
Van Fleet-Stalder, V., T.G. Chasteen. 1998. Using fluorine-induced chemiluminescence to detect organo-metalloids in the headspace of phototrophic bacterial cultures amended with selenium and tellurium. Journal of Photochemistry and Photobiology 43: 193–203.