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Article

Isolation and Quantification of Dialkylmercury Species by Headspace Solid Phase Microextraction and Gas Chromatography with Atomic Emission Detection

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Foi desenvolvida uma metodologia para quantificar compostos dialquilmercúricos usando Microextração em Fase Sólida em Headspace (HS-SPME) e Cromatografia Gasosa com Detecção por Emissão Atômica (GC-AED). Os parâmetros para detecção de Hg foram otimizados usando planejamento fatorial e superfícies de resposta. Experimentos univariados foram empregados para determinar as condições de HS-SPME; as melhores fibras foram 75 µm de Carboxen / PDMS e 65 µm de PDMS / DVB. Porém, as primeiras foram descartadas pela extensa degradação térmica dos analitos na dessorção. O procedimento otimizado permite detectar os analitos em amostras aquosas com limite de detecção de 1,7 e 0,2 ng L^{-1} para dimetil- and dietilmercúrio, respectivamente. As curvas analíticas são lineares nas faixas de 36 a 180 ng L^{-1} (Me_2Hg) e 38 a 190 ng L^{-1} (Et_2Hg), com limite de quantificação de 38 ng L^{-1} (Me_2Hg) e 29 ng L^{-1} (Et_2Hg) e coeficientes de correlação de 0,998 para Me_2Hg e 0,999 para Et_2Hg.

A methodology to quantify dialkylmercury compounds using Headspace Solid Phase Microextraction (HS-SPME) and Gas Chromatography with Atomic Emission Detection (GC-AED) was developed. The parameters for Hg detection were optimized by factorial design and response surfaces. Univariate experiments were employed to determine the HS-SPME conditions; 75 µm Carboxen / PDMS and 65 µm PDMS / DVB were the best fibers. However, the former was excluded from further experiments due to extensive thermal degradation of analytes during desorption. The optimized procedure allowed detection of the analytes from aqueous samples with LOD of 1.7 ng L^{-1} and 0.2 ng L^{-1} for dimethyl- and diethylmercury, respectively. The analytical curves are linear in the range from 36 to 180 ng L^{-1} (Me_2Hg) and 38 to 190 ng L^{-1} (Et_2Hg), with LOQ of 38 ng L^{-1} (Me_2Hg) and 29 ng L^{-1} (Et_2Hg) and correlation coefficients of 0.998 for Me_2Hg and 0.999 for Et_2Hg.

Keywords: HS-SPME, GC-AED, dimethylmercury, diethylmercury, factorial design

Introduction

Microorganisms can convert Hg^{+2} into organomercury compounds such as methylmercury, $(CH_3)Hg^+$, and dimethylmercury, $(CH_3)_2Hg$. These species are among the most dangerous environmental contaminants. Concentrations as low as 0.04 µg L^{-1} of methylmercury have been reported to be harmful to some species, and organomercuric species can be up to hundred times more toxic than the inorganic forms.[1] Therefore, proper assessment of environmental mercury contamination demands sensitive and selective analytical methods capable of speciating the several possible forms of this metal. Although procedures involving selective reduction of the analytes, coupled to detection techniques such as CV-AAS (Cold Vapor - Atomic Absorption Spectrometry), have been employed,[2] methodologies involving chromatographic separation of the Hg species by HPLC,[3] CE[4] or, more frequently, GC[5] are more usual. Traditionally, the ECD was the detector of choice;[6] however, since it is not specific for organomercury compounds, more recently devices such as the Atomic Emission Detector (AED)[7] have been favored. Compared to the ECD and other spectroscopic chromatographic detectors, AED has a remarkably higher sensitivity for organometallic compounds.[8] However, especially for detection of metal compounds, a careful optimization of

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the GC-AED operational conditions has to be done: e.g., the He plasma support gas has to be simultaneously mixed with oxygen (to eliminate carbon deposits in the discharge tube generated from the fragmentation of organic eluates)[9] and hydrogen (to prevent the build-up of refractory metal oxides, causing peak tailing, loss of sensitivity and memory effects).[10] The overall procedure for GC-AED optimization can be complex and time-consuming, considering the number of parameters (helium flow, pressures of the oxygen and hydrogen gases mixed into the plasma and the temperatures of transfer line and the plasma cavity) and the possible interdependence between these parameters (which demand multivariate optimization strategies).

Since some of the target species (especially CH_3Hg^+) are ionic, their conversion to volatile non-ionic derivatives is necessary before their separation and detection by GC.[5] Also, isolation from the matrix and pre-concentration of the organomercuric compounds and their derivatives is necessary for their determination. Although the derivatization can be performed *via* Grignard reactions,[11] simpler alkylation procedures using sodium tetraethyl-, tetrapropyl- or tetraphenylborate are presently preferred since they can be carried out directly in aqueous matrixes.[12] However, conditions such as: nature and concentration of the alkylating agent, solution pH, ionic strength of reaction media, reaction time and temperature[13] should be optimized to achieve quantitative and reliable results. As for the isolation of organomercury compounds and their derivatives from aqueous and biological samples, Solid Phase Microextraction (SPME) can be regarded as one of the best available choices due to its inherent speed and reliability.[14,15] Several operational variables also should be studied for the optimization of SPME procedures – extraction mode, nature of extracting fiber coating and others.[16] Some of the variables affecting the performance of SPME – such as temperature and media ionic strength – also influence the course of the derivatization process. In most of the recent procedures for determination of organomercury species the derivatization and extraction steps are simultaneously optimized and carried out.[17] However, considering the different physico-chemical processes involved in these steps, both direction and magnitude of the operational variable effects, common to both, can be radically different and their execution in parallel can reduce overall method sensitivity and precision. Consequently, separate extraction optimization and derivatization parameters can lead to a expressive improvement on the resulting complete analytical methodology. Therefore, in this work the conditions for headspace extraction by SPME of two neutral organomercury species – dimethyl- (Me_2Hg) and diethylmercury (Et_2Hg) – were established by univariate process while their detection by GC-AED was also optimized, employing a multivariate approach.

Experimental

Gas Chromatograph

GC-AED system was a HP-6850 gas chromatograph equipped with a G-2350A Atomic Emission Detector (Agilent Technologies, Wilmington, DE, USA) and fitted with a 25 m × 0.32 mm × 0.17 μm HP-1 capillary column and a HP-7683 automated injector (Agilent). Helium was used both as carrier (1.8 mL min^{-1}) and plasma support (make-up) gas; high purity hydrogen and oxygen were also used as plasma doping gases. GC-AED chromatograms were obtained monitoring the 253.65 nm Hg emission line. For the optimization of the AED operation, the oven temperature was programmed from 45 °C to 100 °C at 5 °C min^{-1}. For these experiments, direct injection (using the automated injector) of 0.2 μL (split ratio of 1:20) of alkylmercury solutions was used, with the injector kept at 260 °C. For the manual SPME analysis, the injector was operated in splitless mode for 5 min, and then purged with helium at 150 mL min^{-1}. During GC-AED optmization studies, the injector temperature was preliminarly set to 230 °C and the desorption time was 5 min. For the HS-SPME optimization, the injector temperature was varied from 150 °C to 260 °C; 150 °C was selected for the remainder experiments. The oven temperature was programmed as follows: 2 min at 45 °C, then heated at 20 °C min^{-1} up to 105 °C.

Chemicals and materials

Acetone solutions (6 μg mL^{-1}) of Me_2Hg and Et_2Hg (Alpha Products, Houston, TX) were used for the AED optimization experiments. Analytical grade NaCl and acetone (Synth Química, Rio de Janeiro, Brazil) were also employed. The SPME fibers: 100 μm PDMS (P100), 85 μm polyacrylate (PACR), 65 μm PDMS / DVB (DVB) and 75 μm Carboxen / DVB (CAR) and the holder for manual operation were all purchased from Supelco (Bellefont, PA, USA). Prior to use, the fibers were conditioned according to supplier's recommendations. All extractions were performed with the samples contained in 16 mL septum-sealed glass vials (Supelco), and under 1200 rpm magnetic agitation.

Optimization of the GC-AED system for mercury detection

A multivariate approach was adopted to optimize the main operational parameters: He make-up flow ($\mathbf{F_{He}}$);

pressures of plasma doping oxygen ($\mathbf{p_{O2}}$) and hydrogen ($\mathbf{p_{H2}}$); temperatures of transfer line ($\mathbf{T_{TL}}$) and plasma cavity ($\mathbf{T_{PC}}$) of the GC-AED for mercury detection. Triplicate injections of dialkylmercury standard solutions under varied operational conditions arranged according to a 2^5 factorial design experiment were carried out. The values for the parameters were: $\mathbf{F_{He}}$ = 130 and 200 mL min^{-1}; $\mathbf{p_{02}}$ = 30 and 50 psi; $\mathbf{p_{H2}}$ = 10 and 20 psi; $\mathbf{T_{TL}}$ and $\mathbf{T_{PC}}$ = 230 °C and 280 °C. **S/N** ratios both for Me_2Hg and Et_2Hg peaks in all runs were defined as the response, and the effects of the variables were calculated using Matlab 6 (Mathworks Inc., Natick, MA, USA). Additional experiments were carried out to plot a $\mathbf{S/N \times F_{He} \times p_{O2}}$ surface response to further assess the optimum values for these two variables.

HS-SPME method for dialkylmercury compounds

Conventional univariate studies were carried out to select the best SPME fiber, the desorption temperature and time, extraction temperature, ionic strength of the extracting media and extraction time. All experiments were performed in triplicate. To select the best fiber for the further experiments, 5 mL of a 6 ng mL^{-1} solution of the test compounds contained in 16 mL septum-sealed vials was stirred at ambient temperature for 5 min for sample/headspace equilibration. After the pre-equilibration a SPME fiber was exposed to the vial headspace for 10 min, and the extracted analytes were immediately desorbed and analyzed by GC-AED. The effect of the desorption temperature was assessed for DVB and CAR fibers using the same procedure, but with injector temperatures ranging from 150 °C to 260 °C. To study the effect of sample temperature, extractions of the test solution with DVB fiber and temperatures of 25 °C, 45 °C and 60 °C were carried out. The effect of the extracting media ionic strength was assessed using extractions with DVB fibers under the same conditions, but using as solvent for the test samples NaCl solutions with concentrations up to 36% m/v. Finally, the extraction profiles for Me_2Hg and Et_2Hg were evaluated with DVB extractions of test samples containing 9% NaCl over the time range between 3 min and 45 min.

Sensitivity, precision and limits of detection and quantitation for Me_2Hg and Et_2Hg were calculated with analytical curves estimated using extractions of test samples with concentrations ranging from 36 ng L^{-1} to 180 ng L^{-1} (Me_2Hg) and 38 ng L^{-1} to 190 ng L^{-1} (Et_2Hg) under the optimized GC-AED and HS-SPME conditions (GC-AED: $\mathbf{F_{He}}$ = 154 psi mL min^{-1}; $\mathbf{p_{02}}$ = 24 psi; $\mathbf{p_{H2}}$ = 10 psi; $\mathbf{T_{TL}}$ and $\mathbf{T_{PC}}$ = 230 °C; HS-SPME: 15 min extraction with DVB fiber at 25 °C and injector temperature of 150 °C).

Results and Discussion

Table 1 shows the effects of the studied operational variables ($\mathbf{F_{He}}$, $\mathbf{p_{O2}}$, $\mathbf{p_{H2}}$, $\mathbf{T_{TL}}$ and $\mathbf{T_{PC}}$) on the S/N ratios measured for the Et_2Hg chromatographic peak, obtained after processing the factorial design experiment. Since the behavior and tendencies observed for Me_2Hg were equivalent to those of Et_2Hg, only the data and results for the later will be presented and discussed here. The second-order interactions between these variables are also shown in this table. To simplify the discussions, larger-order interactions were not considered. Adopting 95% as the confidence level, only the effects of $\mathbf{F_{He}}$ (–26.6) and $\mathbf{p_{02}}$ (–24.1) on the S/N ratios were significant. As they are negative, this indicates that the S/N ratio increase when F_{He} and p_{O2} are reduced in the studied range. As for the other variables, their effects were not representative and therefore they were fixed at the lower level for the remainder of this study. The presence of some statistically significant 2nd order interactions ($\mathbf{p_{H2} \times p_{H2}}$ and $\mathbf{T_{TL} \times T_{PC}}$) confirms the necessity of multivariate approaches for optimization of operational conditions in GC-AED systems, since they indicate that these variables do not act independently on the response.

To determine the optimum values of $\mathbf{F_{He}}$ and $\mathbf{p_{02}}$, additional experiments were performed and the results

Table 1. Effects of helium make-up flow ($\mathbf{F_{He}}$ / mL min^{-1}), pressure of oxygen ($\mathbf{p_{O2}}$ / psi), pressure of hydrogen ($\mathbf{p_{H2}}$ / psi), temperature of transfer line ($\mathbf{T_{TL}}$ / °C) and temperature of plasma cavity ($\mathbf{T_{PC}}$ / °C) on the GC-AED S/N ratio for Hg and second-order interactions[a] of these variable measured after factorial design experiments

		Variables				
		$\mathbf{F_{He}}$	$\mathbf{p_{H2}}$	$\mathbf{p_{O2}}$	$\mathbf{T_{TL}}$	$\mathbf{T_{PC}}$
1st Order Effects		***–26.6*** [b]	5.7	***–24.1***	–1.8	–0.6
2nd Order Interactions	$\mathbf{F_{He} \times ...}$	-	–5.9	1.0	4.8	5.7
	$\mathbf{p_{H2} \times ...}$	–5.9	-	***–8.6***	1.5	3.9
	$\mathbf{p_{O2} \times ...}$	1.0	***–8.6***	-	–0.5	–0.7
	$\mathbf{T_{TL} \times ...}$	4.8	1.5	–0.5	-	***14.7***

[a]Interactions with 3rd and 4th orders not considered; Values in ***bold italic*** indicates statistically significance within a 95% confidence level.

obtained fitted to a $\mathbf{S/N} \times \mathbf{F_{He}} \times \mathbf{p_{O2}}$ response surface. This response surface is shown in Figure 1. The **S/N** ratio is maximized with lower pressures of O_2 added to the plasma. For the He make-up flow, the dependence is more complex and there is a minimum for $F_{He} \approx 100$ mL min^{-1}; better **S/N** ratios are obtained either with higher or lower He flows. Since the mechanism of signal generation in GC-AED is extremely complex,[18] justification of these tendencies in terms of possible processes occurring in the plasma zone is impracticable. For the remaining experiments, $\mathbf{p_{02}}$ was lowered only to 24 psi. Although better **S/N** ratios are attainable using lower pressures of oxygen, during routine operation it was observed that this causes a substantial reduction of the lifetime of the detection cells, due to the build-up of carbonaceous deposits. For $\mathbf{F_{He}}$,154 mL min^{-1} was selected, to simultaneously maximize the **S/N** ratio and the detection cell lifetime (which is also reduced with low He make-up flows).

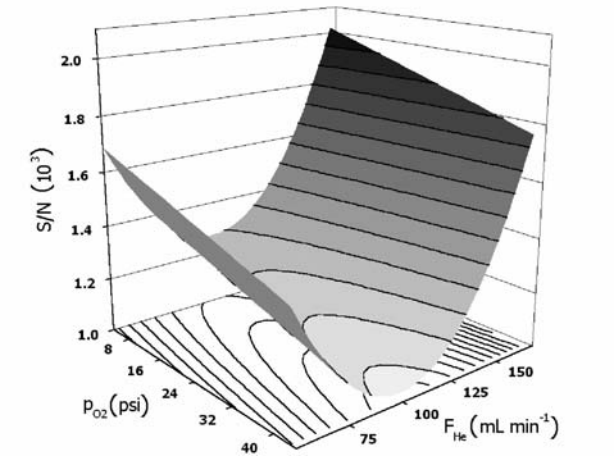

Figure 1. Response surface ($\mathbf{S/N} \times \mathbf{p_{O2}} \times \mathbf{F_{He}}$) for the Et_2Hg chromatographic peak.

Figure 2 compares the average peak areas obtained after extractions using different SPME fibers. For both compounds, the order of extraction efficiency is PACR << P100 < DVB < CAR. This particular sequence can be explained in terms of both the polarity and the volatility of the analytes. Dialkylmercury compounds are non-polar species; their affinity with a polar coating such as polyacrylate is expected to be low, which is confirmed by the limited extraction efficiency with this fiber. In contrast, the extracted amounts are substantially higher for P100 fibers, due to the non-polar coating (polydimethylsiloxane) of this fiber; which up to the present has been the fiber most frequently employed for extractions of the alkylmercury compounds.[19-21] Higher efficiencies are possible with the DVB and CAR fibers. Since the coatings of these fibers are dispersions of solids (divinylbenzene and Carboxen 1006, respectivelly) in polydimethylsiloxane, both adsorption and partition occurs during the extraction.[22] For all fibers except CAR, extraction efficiency for Et_2Hg was higher than that of Me_2Hg. Since the CAR fiber is selective for lighter, more volatile analytes, the improvement on the extracted amounts for Me_2Hg was expected. Considering their improved extraction efficiencies towards the analytes, both CAR and DVB fibers were selected for the further experiments.

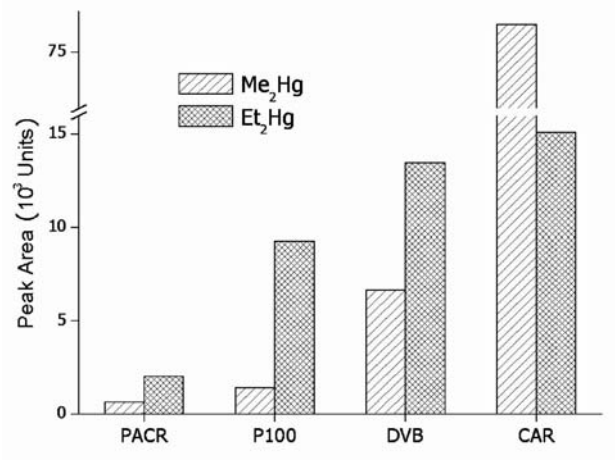

Figure 2. Peak areas for Me_2Hg and Et_2Hg found after HS-SPME using varied fibers.

The necessity of careful optimization of injector temperatures for chromatographic analyses of organomercury compounds is well known, since these analytes are thermally labile.[23] The effect of desorption temperature on the peak areas is shown in Figures 3 and 4. The pattern of the variation of peak area of the analytes with the desorption temperature for DVB fibers is different from that of CAR: for both analytes on DVB, increasing the temperature causes a linear decrease in the peak areas. For CAR, the peak areas are maximized with desorption temperatures of *ca.* 220 °C. Some additional information can result from inspection of the chromatograms shown in Figure 4. The main feature of these chromatograms is the presence of peaks with $t_R \approx 1.40$ min, which are attributed to decomposition products of the dialkylmercury species (mainly Hg^0).[24] Comparing the chromatograms obtained with desorption temperature of 150 °C, it can be seen that the decomposition peak is barely visible for DVB fiber, although it is still intense for CAR fibers. For higher injector temperatures, decomposition peaks appear for both fibers, being the more intense in the DVB chromatogram, and with a height similar to the Me_2Hg peak in the CAR chromatogram. These observations suggest that the thermal degradation of dialkylmercury

compounds is enhanced when they are adsorbed over the fiber coatings. Decomposition of R_2Hg species adsorbed over carbon-based materials has already been described in the literature.[25] For CAR, even at the comparatively low temperature of 150 °C artifact peaks already appear; the increase in the peak areas of the dialkylmercury compounds observed up to *ca.* 220 °C can be regarded as a result of improved desorption of the extracted analytes which, in that temperature range, seems to exceed the loss of adsorbed R_2Hg caused by thermal decomposition. Compared to CAR, adsorption and desorption from DVB fibers is faster[26] and the improvement on the desorption rate with temperature is expected to be marginal. Therefore, the only visible effect of higher desorption temperature on DVB is an increase on the rate of thermal degradation. Finally, from Figure 4 an expressive peak tailing on the chromatograms corresponding to CAR fibers is also evident for all studied desorption temperatures. This is the usual situation when these fibers are employed[27] and it is also a consequence of the previously mentioned slow desorption of extracted materials. In view of the absence of significant thermal decomposition, DVB fibers were selected for the remaining experiments, even considering the higher extraction efficiency of CAR, especially towards Me_2Hg. The injector temperature was set to 150 °C, to avoid loss of analytes by thermal decomposition.

As for the dependence between extraction temperature and extraction efficiency, it was observed that the efficiency decreases with the temperature. In the range between 25 °C and 60 °C, the peak areas were reduced in 81% (Me_2Hg) and 73% (Et_2Hg). Higher temperatures lead to an increase the speed of transfer of volatile analytes from the sample to the headspace, but simultaneously the fiber coating/headspace distribution constant is reduced,[28] causing

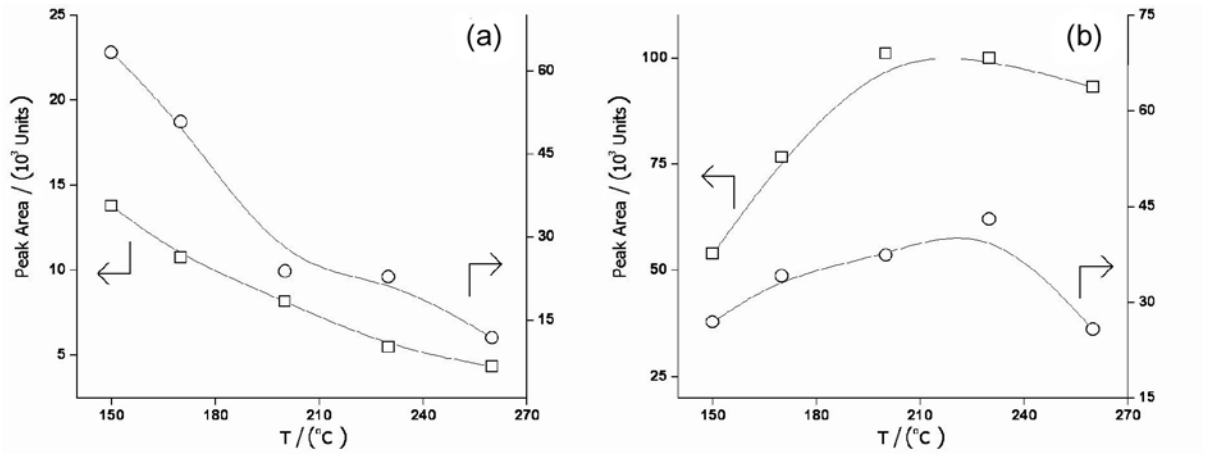

Figure 3. Dependence between peak areas and desorption temperature for Me_2Hg (○) and Et_2Hg (□), after HS-SPME using DVB (a) and CAR (b) fibers.

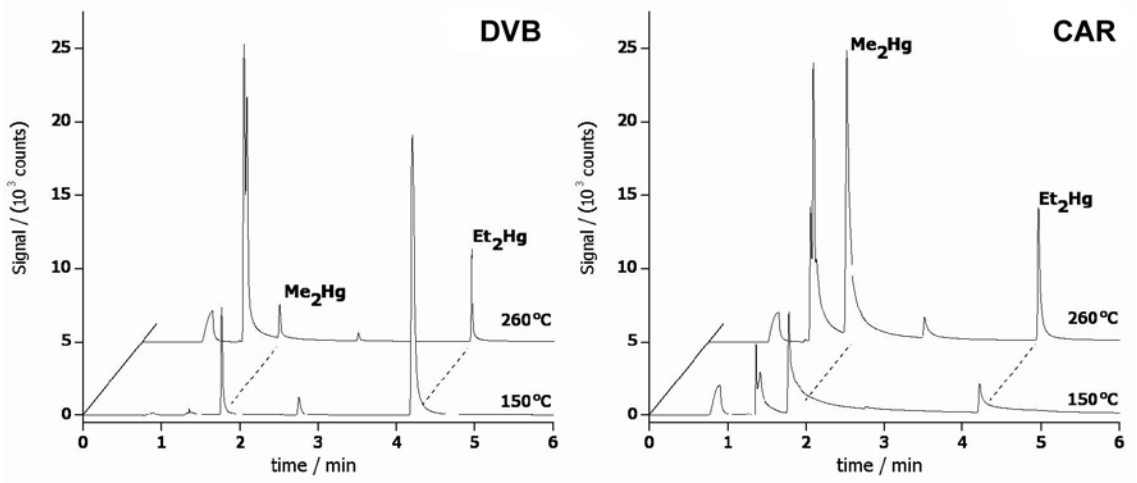

Figure 4. Typical GC-AED chromatograms for HS-SPME extractions using DVB or CAR fibers and desorption temperatures of 150 °C and 260 °C.

for the observed behaviour. Therefore, all remaining work was carried out at the laboratory temperature, (25 ± 1) °C. The effect of different NaCl concentrations in the extracting media is shown in Figure 5. For Et_2Hg, the extraction efficiency is roughly constant up to 18% NaCl, decreasing for more saline media; for Me_2Hg, efficiency peaks at 9% NaCl. The usual behavior in HS-SPME, especially for non-polar analytes such as dialkylmercury compounds, is a constant increase of the extraction efficiency with the NaCl concentration.[28] The different effect of NaCl concentration seen here was already been reported for SPME,[29] but was attributed to the quenching of the derivatization process, ethylation. The appearance of a similar effect here shows that an excessive NaCl concentration in the media can also reduce the extraction efficiency, independent of its effect upon the alkylation of organomercury species. This effect can be attributed to the possible formation of ionic, non-volatile and stable complexes between the R_2Hg species and chloride ions.[30] Higher concentrations of NaCl would shift the reaction equilibrium towards the formation of the complex, decreasing the concentration of free extractable dialkylmercury species. Considering the observed profiles, 9% NaCl was added to the extracting media for the remainder of this work. Extraction time profiles obtained for HS-SPME of dialkylmercury solutions containing 9% NaCl revealed that equilibrium is reached after *ca.* 15 min extraction for Me_2Hg and between 20 min and 30 min for Et_2Hg. However, for practical reasons, since the increase in the area of the later after 15 min is negligible, 15 min was adopted as the extraction time.

The sensitivity, precision and detectability for R_2Hg quantitation using the optimized extraction and detection method can be evaluated from the data presented on Table 2. The precision for both analytes in the concentration range studied, expressed as the regression coefficients of the analytical curves, can be regarded as adequate (r = 0.998 for Me_2Hg and 0.999 for Et_2Hg). The **F**-values for the regression lines, 715 and 2002, respectively, are higher than the 95% significance critical value of 19.2, confirming the linearity of the data in this range. The limits of detection and quantitation, 1.7 ng(Hg) L^{-1} for Me_2Hg and 0.2 ng(Hg) L^{-1} for Et_2Hg, are adequate for application to most of the environmental or clinical samples[29] and in general better than figures reported for similar studies regarding determination of Hg species in water samples:[32] e.g., 412 µg(Hg) L^{-1} (diphenylmercury using SPME-HPLC-UV); 3.1 ng(Hg) L^{-1} (methylmercury using SPME-GC-MS) and 3.7 ng(Hg) L^{-1} (generic alkylmercury using SPME-GC-ICPMS).

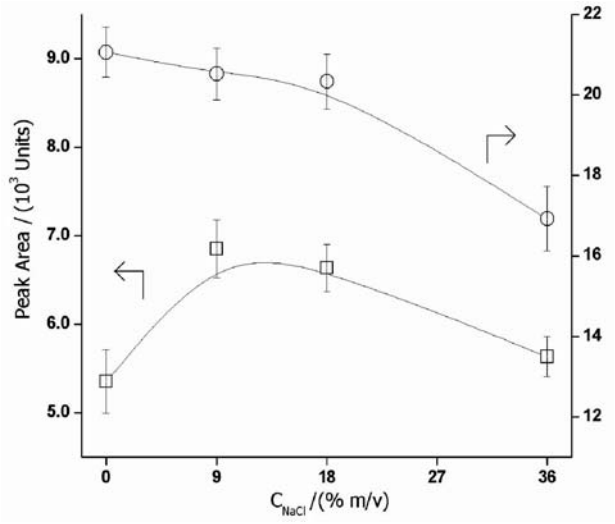

Figure 5. Dependence between peak areas and NaCl concentration in the samples for Me_2Hg (○) and Et_2Hg (□), after HS-SPME using DVB fibers.

Table 2. Figures of merit for the determination of R_2Hg using the optimized HS-SPME / GC-AED method: slopes a, intercepts b, correlation coefficients r and regression standard errors s_{tot} of the analytical curves, corresponding regression F-test parameter F and absolute limits of detection LOD[a] and quantitation LOQ[b], in ng(Hg) L^{-1}

Parameter	Me_2Hg	Et_2Hg
a	1.45 ± 0.05	14.6 ± 0.5
b	34 ± 6	−49 ± 58
r	0.998	0.999
s_{tot}	6.2	55.6
F	715	1002
LOD[a]	1.7	0.2
LOQ[b]	38	29

[a]Estimated from signal-to-noise (**S/N**) ratio and defined as the concentration of analyte generating a peak with height equal to 3 × (**S/N**)[31]; [b]Estimated from the regression data and defined as $10 \times \mathbf{s_{tot}} / \mathbf{a}$.[31]

Conclusions

Headspace SPME together with GC-AED was found to be specially suited for isolation, separation and detection of dialkylmercury compounds. The optimization of the operational parameters affecting the AED operation for Hg detection provided a significant enhancement of the sensitivity and detectability for this element. Coupled with HS-SPME, this technique is able to detect and quantify amounts of these analytes in the ng L^{-1} range. As for the HS-SPME method, use of PDMS-DVB fibers provide a significant enhancement of the sensitivity, compared to the PDMS fibers usually employed. The thermal instability of these species point to the need of a careful optimization of the extraction parameters, notably the desorption temperature and time.

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