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Methylmercury determination in biological samples by derivatization, solid-phase microextraction and gas chromatography with microwave-induced plasma atomic emission spectrometry

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Abstract

A method for the extraction and gas chromatographic determination of methylmercury in biological matrices is presented. By combining the advantages of two extraction techniques—microwave-assisted extraction (MAE) and solid-phase microextraction (SPME)—the separation of methylmercury from biological samples is possible. Specifically, the procedure involves microwave extraction with 3 *M* hydrochloric acid, followed by aqueous-phase derivatization with sodium tetraphenylborate and headspace SPME with a silica fibre coated with polydimethylsiloxane (PDMS). For optimization of the derivatization–SPME procedure, a central composite experimental design with $\alpha = 1.682$ and two central points was used to model gas-chromatographic peak areas as functions of pH, extraction temperature and sorption time. A desirability function was then used for the simultaneous optimization for methylmercury and Hg(II). The optimal derivatization−SPME conditions identified were close to pH 5, temperature 100 °C, and sorption time 15 min. The identification and quantification of the extracted methylmercury is carried out by gas chromatography with microwave-induced plasma atomic emission spectrometry detection. The validity of the new procedure is shown by the results of analyses of certified reference materials.

Keywords: Atomic emission spectrometry; Headspace analysis; Solid-phase microextraction; Experimental design; Organomercury compounds; Methylmercury

1. Introduction

The separation, detection and determination of metal species and their organometallic compounds present the analyst with many challenges. Significant recent advances in quantitation of metal species have given rise to an increasing literature on sample preparation and chromatography-based analytical methodology. In the case of mercury, the need for quantitative differentiation of individual species is shown by the many studies [1] that have found organic mercury forms, especially methylated forms, to be considerably more toxic than inorganic ions, and that bioaccumulation in the food chain (facilitated by lipophilicity [2]) can lead to methylated forms constituting up to 90% of the total mercury concentration in fish flesh. At present, several sophisticated hyphenated techniques are available for separation and quantification of organomercury compounds. The most widely used methods are based on gas chromatography coupled with element-selective detection techniques such as inductively coupled

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plasma mass spectrometry (ICP-MS) [3–6], microwave-induced helium plasma atomic emission spectrometry (MIP-AES) [7,8] and atomic fluorescence spectroscopy [9,10]. In all cases, one of the most important issues is sample preparation, because the compounds present in the matrix are generally non-volatile species and need to be extracted and converted to volatile species prior to quantitation.

One of the commonly used derivatization methods is aqueous ethylation with sodium tetraethylborate ($NaBEt_4$) [11–16]. However, in a validation study of this procedure using isotopically-labelled methylmercury and GC–ICP-MS it was found that, in halide-containing solutions, transformation of methylmercury (MeHg) into elemental mercury occurred during ethylation [15]. This does not happen with propylation by sodium tetrapropylborate ($NaBPr_4$) [17]. The advantages of another agent that gives good results [18–20], sodium tetraphenylborate ($NaBPh_4$), have been discussed by Cai et al. [20]. In this work, we derivatized organomercury compounds with $NaBPh_4$ because phenylation can take place in the aqueous phase and extraction can simultaneously be performed. Also, $NaBPh_4$ is more stable than $NaBEt_4$ [21] and problems of passivation in the capillary column are avoided in gas chromatographic analysis.

Solid-phase microextraction (SPME), first developed by Arthur and Pawliszyn in 1990 [22], is now widely used for extraction of metal species. Cai et al. [14] described the first analytical procedure for the determination of MeHg and labile Hg^{2+} using in situ aqueous ethylation with $NaBEt_4$ followed by headspace SPME sampling and GC–MS. SPME by direct immersion [23] or headspace sampling with a poly(dimethylsiloxane)-coated silica fibre has subsequently been successfully used for determination of inorganic mercury and methylmercury in water [24,25], sediments [24,26,27], soils [28,29], human body fluids [30] and other biological samples [23,31].

In this paper, we describe a fast and easy method for the quantitation of methylmercury using microwave energy followed by derivatization with sodium tetraphenylborate, extraction of the derivative by headspace SPME, and determination by the highly sensitive and selective GC–MIP-AES technique. Derivatization and extraction conditions were optimized using an appropriate experimental design. The optimised method was then used to analyse various certified reference materials.

The advantages of the developed analytical process are:

1. The combination of leaching by microwave energy with a suitable derivatization procedure and headspace SPME avoids the need for previous capillary column treatment with inorganic salts before GC–MIP-AES, does not require organic solvent consumption and also reduces analysis time and the number of sample manipulation steps and improves chromatographic characteristics [limits of detection (LOD) and quantitation (LOQ)] for the derivatized compounds.
2. Using the experimental design approach, not only the optimal operational conditions for different compounds could be achieved, but a great deal of information about the effects of each factor on the recovery is obtained while the minimum number of experiments is performed. A major and clear point from this study is that a general derivatization–SPME procedure can be designed for isolation and preconcentration of the mercury species studied.

2. Experimental

All organomercury concentrations reported in this paper are expressed as mass of mercury (Hg) per unit mass or volume.

2.1. Instruments

The microwave extractor system was a MES 1000 (CEM, Matthews, NC, USA) equipped with a solvent detection system and capable of simultaneously extracting 12 samples in PTFE-lined extraction vessels under identical conditions of temperature and pressure. An inboard pressure control system was installed for monitoring and controlling pressure conditions inside the extraction vessels.

SPME fibres and fibre holders for manual sampling were obtained from Supelco (Bellefonte, PA, USA). Fibres with 100-μm-thick poly(dimethylsiloxane) (PDMS) or 65-μm-thick poly(dimethylsilox-

ane–divinylbenzene) (PDMS–DVB) coatings were used. Both kinds of fibre were conditioned before use by heating in a gas chromatograph injection port (60 min at 250 °C for PDMS and 30 min at 260 °C for PDMS–DVB). Samples were stirred during extraction using an Agimatic-E laboratory hotplate/stirrer (Selecta, Barcelona, Spain) and PTFE-coated stir bars 3 mm in diameter and 7 mm long (Supelco).

The gas chromatograph was an HP 5890A Series II apparatus (Hewlett-Packard, Palo Alto, CA, USA) coupled to a Hewlett-Packard 5921A microwave-induced plasma atomic emission spectrometer. Data acquisition and processing were carried out by means of a Hewlett-Packard 9144 Chemstation running Chemstation software. A split-splitless capillary injection port was used. The GC separations were carried out on a 30 m×0.32 mm I.D. DB-5ms capillary column with a film thickness of 0.25 μm obtained from J&W Scientific (Folsom, CA, USA). The optimized GC separation conditions were as follows: injection port temperature, 200 °C; desorption time, 2 min; column head pressure 140 kPa; column flow, 3.2 ml min^{-1}; initial oven temperature, 90 °C; initial time, 3 min; ramp rate, 30 °C min^{-1}; final oven temperature, 270 °C; final time, 10 min; transfer line temperature, 280 °C. The optimized AES parameters were these: wavelength, 254 nm for a mercury line; helium make-up flow-rate, 180 ml min^{-1}; ferrule purge vent, 20 ml min^{-1}; scavenger gases, 350 kPa for hydrogen and 200 kPa for oxygen; helium supply purge, 205 kPa; spectrometer purge flow-rate, 2 ml min^{-1}; cavity temperature, 280 °C.

2.2. Reagents, standards and solutions

Methylmercury chloride (98.5% pure), ethylmercury chloride (99%), mercury chloride (99.5%), sodium hydroxide (99%), hydrochloric acid (30%) and sodium tetraphenylborate (99.5%) were purchased from Merck (Darmstadt, Germany). Methanol (gradient HPLC grade) was purchased from Scharlau (Barcelona, Spain).

Stock 1.2 g l^{-1} solutions of methylmercury chloride and ethylmercury chloride in methanol and mercury chloride in water were prepared. Working standard solutions (0.1×10^{-3} g l^{-1} for methylmercury chloride and ethylmercury chloride, and 2.5 g l^{-1} for mercury chloride) were prepared by appropriate dilution of the stock solutions with water. The final working standard solutions had a very low percentage of methanol. All solutions were stored in the dark at 5 °C when not in use. The derivatization reagent, 1% aqueous $NaBPh_4$ solution, was prepared daily. Water was purified by a Milli-Q water purification system (Millipore, Bedford, MA, USA).

Helium (99.9995% pure; Carburos Metálicos, La Coruña, Spain) was used as both carrier and make-up gas. Oxygen and hydrogen (99.9995% pure; Carburos Metálicos) were used to enhance the combustion of the organic compounds and to improve baseline stability, respectively.

The certified reference materials TORT-1, DOLT-2 and DORM-2 were obtained from the National Research Council of Canada (NRCC) and CRM 464 from Standards Measurement and Testing Programme of the European Union.

Glassware was rinsed with Milli-Q water, decontaminated overnight in 10% nitric acid solutions, and then rinsed again.

2.3. Headspace solid-phase microextraction procedure

Mercury compounds in biological samples must be released form the matrix prior to SPME. Published procedures include: (a) treatment with HOAc–NaOAc buffer (pH 3) for 96 h [27]; (b) addition of methanolic KOH solution followed by shaking in an ultrasonic bath for 3 h [14]; and (c) subcritical water extraction [29]. In this work, the mercury compounds were leached from biological samples using a previously reported procedure based on microwave energy [32,33]. Approximately 0.2 g of the biological material (TORT-1) was accurately weighed in the PTFE-lined extraction vessel, 10 ml of 3 *M* hydrochloric acid was added, the extraction vessel was closed (a new rupture membrane was used for each extraction) and the vessel was heated at 100 °C in a microwave oven at full power for 10 min, after which the sample carousel was removed from the oven and cooled in a water bath.

To select the sample volume, preliminary studies were made [34]. This variable has no significant influence on sorption when it was tested between 10

and 70 ml. Due to these results, 10 ml of total volume was considered.

For SPME sampling, 2-ml aliquots of the sample extract obtained by MAE were placed into 22-ml glass vials and pH was adjusted to the desired value in accordance with the experimental design used for optimization. The volume was made up to 8 ml with water, to keep constant the analyte concentrations. The magnetic stirring bar and 2 ml of 1% $NaBPh_4$ solution were added. Then the vial was immediately sealed with a cap and a pre-punched septum, placed inside a heating bath over a magnetic plate, and allowed to equilibrate. Temperature was adjusted to the desired value in accordance with the experimental design used for optimization, and a PDMS SPME fibre was then lowered into the headspace of the vial. After a predetermined sampling time (also established in the experimental design), the PDMS fibre was withdrawn and inserted into the GC injector for thermal desorption at 200 °C for 2 min.

For quantification, ethylmercury was used as internal standard. For calibration, mercury standards and ethylmercury were added to 6 ml Milli-Q water in a 22-ml vial, and the mixture was brought to desired optimum value of pH in accordance with the experimental design and made up to 8 ml with water. Then, 2 ml of $NaBPh_4$ were added, the quantities of the standards being such as to afford final concentrations of 0–10 ng ml^{-1} (mercury standards) or 0.5 ng ml^{-1} (ethylmercury). The mixture was then treated as described above for headspace SPME sampling.

2.4. Experimental design

In the light of the literature [14,26] and a preliminary study [34], the headspace SPME parameters optimized for derivatization–extraction of MeHg and Hg^{2+} were pH, adsorption time and temperature. PDMS phase is not resistant to media with pH below 4 or above 10 [35]. For this reason, pH values between 4 and 8 were considered for the experimental design.

Extraction-time profile curves for organomercurial compounds in aqueous solution (signal peak area vs. extraction time) at 70 °C were previously studied between 3 and 45 min [34]. Although equilibrium has been reached for all the analytes at 27 min, in the present paper extraction temperature was tested between 60 and 100 °C in order to obtain a shortest acceptable extraction time (studied between 3 and 27 min).

Their joint and several influences were studied by means of a central composite experimental design allowing a second-degree polynomial to be fitted to data from 16 experiments, i.e. 2^k+2k+n for $k=3$ variables and $n=2$ extra points at the centre of the design. This design amounts to a full 2^3 factorial design augmented with six star points plus two central points. The function fitted was

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{33} x_3^2 \quad (1)$$

where x_1 is pH, x_2 is extraction temperature, x_3 is sorption time and the response y is gas-chromatographic peak area. The low, central and high levels of the factorial design are listed in Table 1 together with the values of the star points located at ± 1.68 steps from the centre of the experimental domain.

Experimental design combined with desirability function was applied for the optimization of the SPME of the phenylated compounds, PhMeHg and Ph_2Hg, in order to obtain optimum operating conditions for routine work [36,37].

Experiments were conducted in randomized order

Table 1
Factor levels and star points in the central composite design

Factor	Central level	Step	Lower level	Upper level	Star points ($\alpha = 1.68$)	
					$-\alpha$	$+\alpha$
pH	6	1	5	7	4	8
Temperature (°C)	80	12	68	92	60	100
Time (min)	15	7	8	22	3	27

to avoid systematic errors All statistical calculations were carried out using Statgraphics Plus for Windows [38] or Nemrod for Windows 95 [39].

3. Results and discussion

3.1. Choice of SPME fibre

The choice of a particular SPME fibre coating depends on the chemical structure of the derivatized analyte. In this work, consideration of the volatility and polarity of the $NaBPh_4$-derivatized analytes [21] and of fibre coating led to evaluation of two fibre types, one coated with 100-μm-thick PDMS and the other coated with 65-μm-thick PDMS–DVB. In preliminary experiments, it was found that the PDMS fibre performed well with MeHg, EtHg and Hg^{2+} derivatized to the volatile and less polar species MePhHg, EtPhHg and Ph_2Hg. The PDMS–DVB fibre was less efficient for MePhHg and EtPhHg than the PDMS fibre, and gave no Ph_2Hg peak upon chromatography. A comparative study of organomercuric peak area obtained by different fibre is shown in Fig. 1.

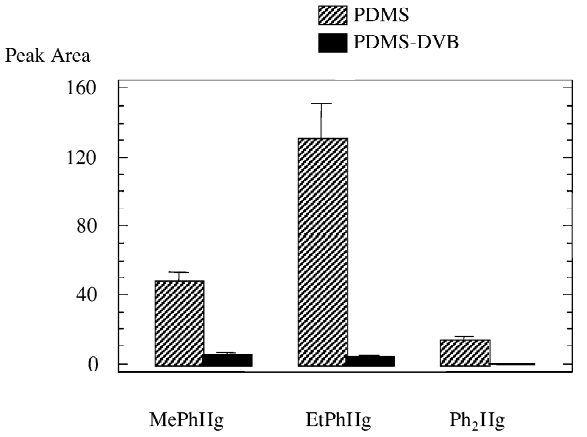

Fig. 1. Comparison of organomercuric peak area obtained by GC–MIP-AES with different fibre coatings. Concentration of organomercuric compounds: 50 ng ml^{-1}; extraction time: 27 min; sampling temperature: 70 °C; stirring rate: 500 r.p.m.; desorption temperature: 200 °C; desorption time: 2 min.

3.2. Factorial design. Estimation of the response surfaces

Analysis of the derivatization–SPME results was carried out in two steps: first the GC peak area was modelled as a function of pH, time and temperature for each analyte, and then the responses for MePhHg and Ph_2Hg were used in a global desirability function to find the simultaneous optimal conditions for the extraction and derivatization process. The optimization criteria for SPME procedure of the considered mercury compounds were peak areas to yield recoveries between 90 and 100%. Table 2 shows the experimental design matrix and the peak area results for MePhHg and Ph_2Hg. Response surface analysis based on multiple regression and analysis of variance (ANOVA) at 5% significance level, showed different results for the two studied compounds. Table 3 lists the coefficients obtained for Eq. (1), and Fig. 2 shows Pareto charts of the standardized effects. For MePhHg, no factor or interaction had a significant effect (the most nearly significant term was the interaction between extraction temperature and sorption time, which was negatively correlated with peak area), while for Ph_2Hg extraction temperature and sorption time caused strong statistically significant increases in peak area. Fig. 3 shows the chromatographic peak area responses of MePhHg (Fig. 3a) and Ph_2Hg (Fig. 3b) as functions of these two variables for the pH value in the centre of the domain. Examination of these and analogous plots for all the other possible cases in which one variable is kept constant and the other two are modified showed different trends for the peak area of the two compounds.

In view of this result, we adopted a multicriterion decision-making approach. First, the response functions for MePhHg and Ph_2Hg were transformed into desirability functions by a maximization function for d_{Me} as follows

$$d_{Me} = \begin{cases} 0 & y_{Me} \le y_{Me}^{(min)} \\ \left(\dfrac{y_{Me} - y_{Me}^{(min)}}{y_{Me}^{(max)} - y_{Me}^{(min)}} \right)^2 & y_{Me}^{(min)} \le y_{Me} \le y_{Me}^{(max)} \\ 1 & y_{Me} \ge y_{Me}^{(max)} \end{cases} \quad (2)$$

where $y_{Me}^{(min)} = 61.64$ (the smallest MePhHg peak area in Table 2) and $y_{Me}^{(max)} = 90$ (the smallest regarded as fully acceptable), and using a bilateral function for d_{Ph}

Table 2
Design matrix and chromatographic peak area response values

Run no.	pH	Temperature	Time	Peak area	
				MePhHg	Ph_2Hg
1	−1	−1	−1	65.34	0.00
2	+1	−1	−1	80.73	0.00
3	−1	+1	−1	87.73	65.92
4	+1	+1	−1	101.10	77.10
5	−1	−1	+1	78.71	31.41
6	+1	−1	+1	100.07	9.52
7	−1	+1	+1	85.09	240.94
8	+1	+1	+1	63.51	141.82
9	$-\alpha$	0	0	71.00	153.66
10	$+\alpha$	0	0	99.96	104.81
11	0	$-\alpha$	0	61.64	31.61
12	0	$+\alpha$	0	97.79	159.24
13	0	0	$-\alpha$	66.29	0.00
14	0	0	$+\alpha$	84.40	234.08
15	0	0	0	73.55	93.38
16	0	0	0	68.96	88.35

Table 3
Coefficients of Eq. (1)

Compound	β_0	β_1	β_2	β_3	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
MePhHg	71.056	5.656	5.373	1.680	5.510	3.472	1.927	−5.620	−3.622	−9.117
Ph_2Hg	95.154	−14.058	51.219	49.378	3.210	−8.744	−1.102	−8.256	−16.524	24.851

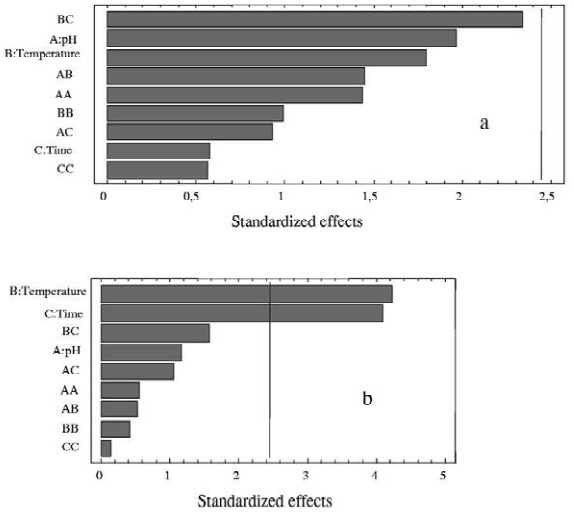

Fig. 2. Pareto chart of the standardized effects of pH, extraction temperature, sorption time and the interactions of Eq. (1) on the chromatographic peak areas of MePhHg (a) and Ph_2Hg (b).

$$d_{Ph} = \begin{cases} 0 & y_{Ph} \le y_{Ph}^{(min)} \text{ or } y_{Ph} \ge y_{Ph}^{(max)} \\ \left(\dfrac{y_{Ph} - y_{Ph}^{(min)}}{y_{Ph}^{(target)} - y_{Ph}^{(min)}} \right)^{0.5} & y_{Ph}^{(min)} \le y_{Ph} \le y_{Ph}^{(target)} \\ \left(\dfrac{y_{Ph}^{(max)} - y_{Ph}}{y_{Ph}^{(max)} - y_{Ph}^{(target)}} \right)^{8} & y_{Ph}^{(target)} \le y_{Ph} \le y_{Ph}^{(max)} \end{cases} \quad (3)$$

where $y_{Ph}^{(min)} = 0$ (the smallest Ph_2Hg peak area in Table 2), $y_{Ph}^{(max)} = 240.94$ (the largest), and the target $y_{Ph}^{(target)} = 110$. A bilateral desirability was used for Ph_2Hg because recoveries greater than 100% appeared in some analyses of TORT-1. The combined desirability function $D = (d_{Me} d_{Ph})^{1/2}$ was then maximized numerically. The predicted SPME conditions for MePhHg and Ph_2Hg at the optimum were found near the boundary of the experimental domain in the neighbourhood of pH 5, extraction temperature $T = 100\,°C$ and sorption time $t = 15$ min (indicated by arrows in Fig. 4). The two-dimensional plots of

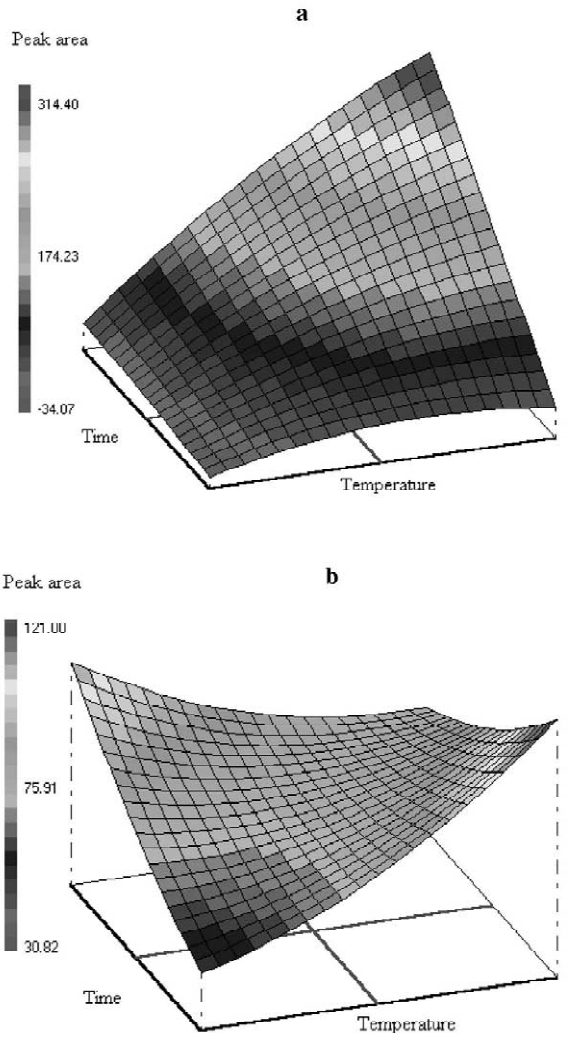

Fig. 3. Response surface by plotting extraction temperature versus sorption time estimated for MePhHg (a) and Ph_2Hg (b).

global desirability are shown in Fig. 4. The regions in grey correspond to null values for *D* when level factors are not suitable to be chosen.

3.3. Calibration, limit of detection, limit of quantification and precision

Using the optimized conditions, calibration curves were constructed by means of three procedures: using external standards, using internal standard and by standard addition. In all cases, four concentrations obtained by appropriate dilution of standards were each run in triplicate. Linear regression of chromatographic peak area on analyte concentration afforded equations that showed good linearity over the concentration ranges studied, 0.2–3.0 ng ml^{-1} for MeHg and 0.1–8.0 ng ml^{-1} for Hg^{2+} (Table 4), and were validated by linearity and lack of fit *F*-tests following ANOVA. The results obtained using external calibration and internal calibration were of similar quality for both MePhHg and Ph_2Hg, but the standard addition method showed much poorer fit. Internal standard calibration was accordingly used in all further experiments, and since the internal standard (EtHg) undergoes all sample preparation steps, it corrects for variation in derivatization and sorption yields.

Table 5 lists other method quality parameters. The limit of detection (LOD) was calculated as $3s_{y/x}/m$, i.e. three times the standard deviation estimated in the regression analysis divided by the slope of the calibration line, and the limit of quantitation as $10s_{y/x}/m$. Repeatability and reproducibility were evaluated by means of a series of five analyses of TORT-1 performed on the same day and another five carried out on different dates.

3.4. Analysis of certified reference materials

The accuracy of the new method to determine MeHg was tested by analysis of the certified reference materials TORT-1, DOLT-2, DORM-2 and CRM 464, which have different methylmercury contents. The measured concentrations are in good agreement with the certified values (Table 6). In the same table, the results obtained from a polluted cockle have also been included.

Fig. 5 shows typical chromatograms obtained for TORT-1 (a) and a blank experiment (b). In the chromatogram of Fig. 5a, the presence of two peaks corresponding to MePhHg and Ph_2Hg can be observed. This indicates that it was possible to use $NaBPh_4$ as a derivatizating reagent to the MeHg species after isolating the Hg^{2+} species. However, higher results of total Hg than the certified values were found in most cases after the correction with

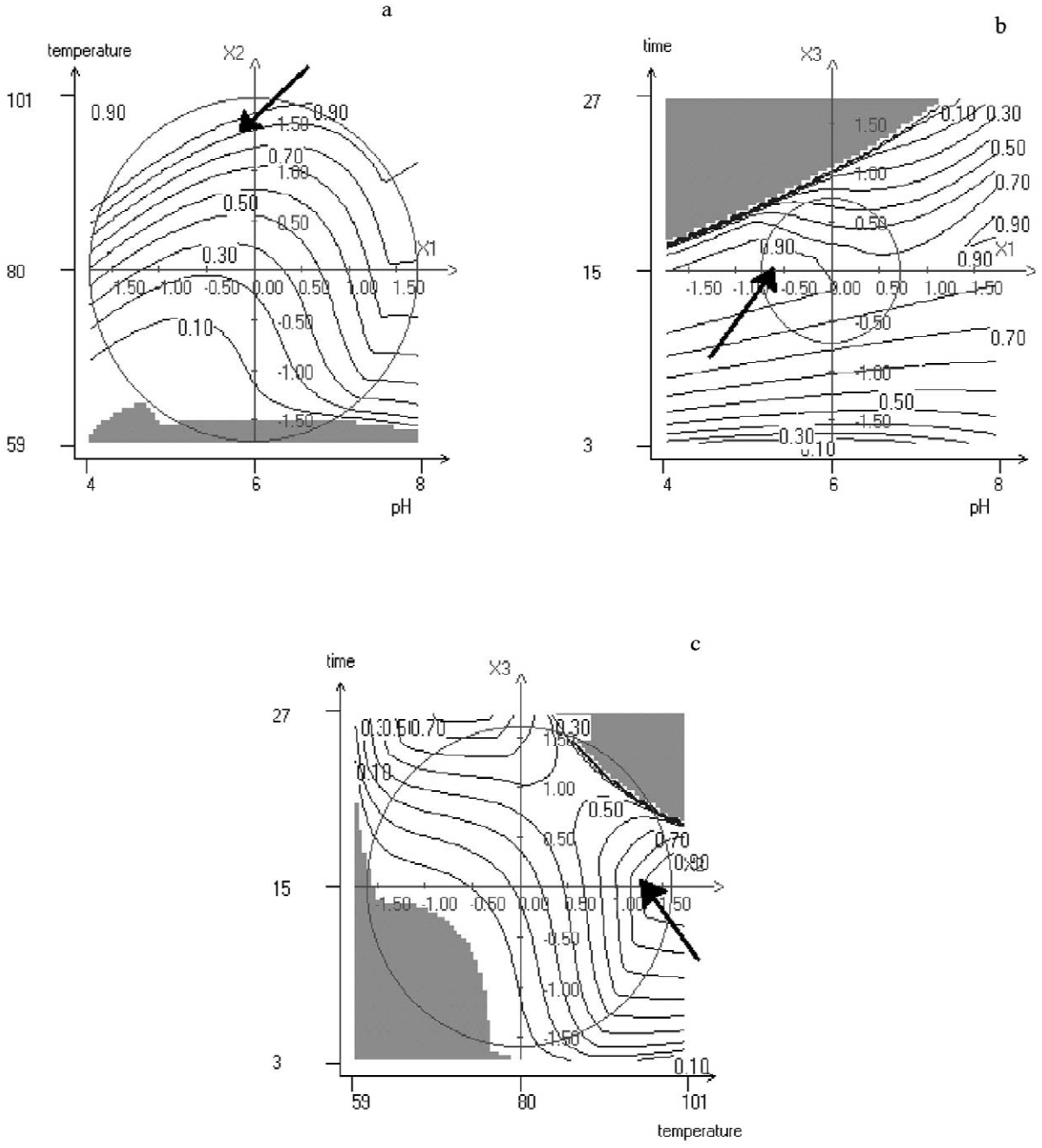

Fig. 4. Contour plots of the global desirability D in the $t=15$ min, $T=100$ °C and pH 5 planes (Fig. 4a, b and c, respectively); the circles indicate the experimental domain in these planes, and the shaded areas regions in which $D=0$.

Table 4
Calibration results

Calibration procedure	Compound	Linear regression equation	Correlation (R^2)
External standard	MePhHg	$y=49.5x+0.997$	0.9991
	Ph_2Hg	$y=19.1x+6.14$	0.9977
Internal standard[a]	MePhHg	$y=0.671x+0.001$	0.9997
	Ph_2Hg	$y=0.269x+0.088$	0.9979
Standard addition[b]	MePhHg	$y=101x+62.2$	0.9904
	Ph_2Hg	$y=102.8x+253$	0.9861

[a] With ethylmercury chloride as internal standard.
[b] To TORT-1 reference material.

Table 5
Limits of detection and quantification, and precision, of determination of organomercury compounds by SPME–GC–MIP-AES

Compound	LOD (ng ml^{-1} as metal)	LOQ (ng ml^{-1} as metal)	Repeatability, RSD (%, $n=5$)	Reproducibility, RSD (%, $n=5$)
MePhHg	0.12	0.40	6.7	8.6
Ph_2Hg	0.86	2.86	12.9	16.3

blank signal. Significant artefact formation during analysis was reported by Demuth and Heumann [15], where transformation of methylmercury into Hg elemental and Hg^{2+} was found during the ethylation step in halide containing solutions. The Hg^{2+} derivatization procedure with $NaBPh_4$ requires further studies of possible species transformations during chemical pre-treatments of the sample. In a future work, the best strategy to verify this possible artefact formation and the validation of speciation methods would be to spike with isotopically-labelled element compounds in connection with a GC–ICP-MS system.

Table 6
Results of determination of methylmercury in different certified reference materials and in a real sample

Reference material	Certified value[a] (μg g^{-1} as Hg)	Measured value[b] ($n=6$) (μg g^{-1} as Hg)	Recovery (%)[b] ($n=6$)
TORT-1	0.128±0.014	0.129±0.006	100.8±5.0
DOLT-2	0.693±0.053	0.718±0.067	103.7±9.6
DORM-2	4.47±0.32	4.49±0.25	100.4±5.5
CRM 463	3.04±0.16	3.15±0.23	103.7±7.5
Cockle	–	0.96±0.08	–

[a] Means and 95% confidence limits.
[b] Means±standard deviations for n independent analyses.

4. Conclusions

In situ aqueous phenylation and headspace SPME are highly suitable for extraction of mercury compounds from biological samples for GC analysis. Optimization of the parameters affecting the derivatization–SPME process was readily achieved using a central composite experimental design; joint optimization of the extraction recoveries of MePhHg and Ph_2Hg by means of an overall desirability function led to the identification of optimal experimental conditions in the neighbourhood of: pH 5, extraction temperature 100 °C, and sorption time 15 min. When combined with determination of the extracted analytes by gas chromatography with microwave-induced plasma atomic emission spectrometry detection, the new derivatization–SPME

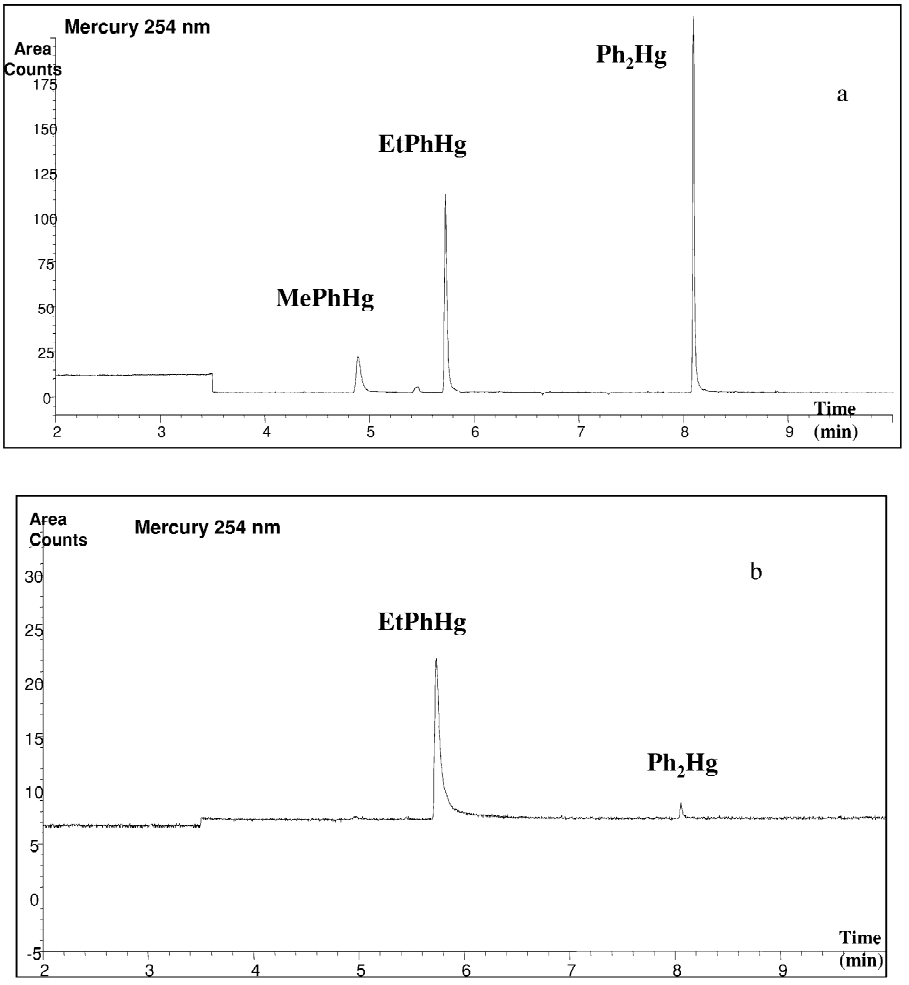

Fig. 5. A typical chromatogram of TORT-1 obtained under SPME–GC–AES optimized conditions (a) and a chromatogram obtained by the analysis of blank (b). Chromatographic conditions are given in the text.

method constitutes a means of quantitating methylmercury that is sensitive and selective and exhibits good linearity, precision and detection limits.

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