Spectrochimica Acta Part B 64 (2009) 506–512

Contents lists available at ScienceDirect

Spectrochimica Acta Part B

journal homepage: www.elsevier.com/locate/sab

Methylmercury determination using a hyphenated high performance liquid chromatography ultraviolet cold vapor multipath atomic absorption spectrometry system ☆

Reinaldo C. Campos [a,*], Rodrigo A. Gonçalves [a], Geisamanda P. Brandão [a], Marlo S. Azevedo [a], Fabiana Oliveira [b], Julio Wasserman [b]

[a] Department of Chemistry, Pontifical Catholic University of Rio de Janeiro, Rua Marques de S Vicente 225, 22453-900 Rio de Janeiro, Brazil
[b] Institut of Geosciences, Fluminense Federal University, Av. Gal. Milton Tavares de Souza, s/n; 24.210-340, Niteroi, Rio de Janeiro, Brazil

ARTICLE INFO

Article history:
Received 2 December 2008
Accepted 18 May 2009
Available online 24 June 2009

Keywords:
Methylmercury
Hyphenation
Multipath atomic absorption spectrometry

ABSTRACT

The present work investigates the use of a multipath cell atomic absorption mercury detector for mercury speciation analysis in a hyphenated high performance liquid chromatography assembly. The multipath absorption cell multiplies the optical path while energy losses are compensated by a very intense primary source. Zeeman-effect background correction compensates for non-specific absorption. For the separation step, the mobile phase consisted in a 0.010% m/v mercaptoethanol solution in 5% methanol (pH = 5), a C_{18} column was used as stationary phase, and post column treatment was performed by UV irradiation (60 °C, 13 W). The eluate was then merged with 3 mol L^{-1} HCl, reduction was performed by a $NaBH_4$ solution, and the Hg vapor formed was separated at the gas–liquid separator and carried through a desiccant membrane to the detector. The detector was easily attached to the system, since an external gas flow to the gas–liquid separator was provided. A multivariate approach was used to optimize the procedure and peak area was used for measurement. Instrumental limits of detection of 0.05 μg L^{-1} were obtained for ionic (Hg^{2+}) and $HgCH_3^+$, for an injection volume of 200 μL. The multipath atomic absorption spectrometer proved to be a competitive mercury detector in hyphenated systems in relation to the most commonly used atomic fluorescence and inductively coupled plasma mass spectrometric detectors. Preliminary application studies were performed for the determination of methyl mercury in sediments.

1. Introduction

Since its introduction, cold vapor atomic absorption spectrometry (CV AAS) has become the most popular method for total mercury determination at trace levels. Its sensitivity, simplicity and low cost have contributed to its wide acceptance for total mercury determination in biological and environmental samples. However, in relation to mercury speciation analysis, due the very low concentrations of some mercury species, the limits of detection (LOD) obtained with CV AAS were not always sufficient. This is specially the case for hyphenated techniques, considering the low sample volumes associated with chromatographic techniques, implying the use of pre-concentration strategies [1–3]. Other more sensitive detectors, such as inductively coupled plasma mass spectrometry (ICP-MS) and atomic fluorescence spectrometry (AFS) have become the choice in speciation studies [4–12]. Moreover, ICP-MS brings the possibility of isotopic discrimination [13], which can be of great importance in special cases [14–19]. Nevertheless, ICP-MS is still expensive to purchase and run, and in speciation analysis running costs are stressed by the long analysis time. Hence, the adoption of a sensitive but more affordable Hg detector is desirable. In this sense, CV AFS appeared as a natural choice [5,9–12], since it is more sensitive than CV AAS, and much less expensive than ICP-MS. Consequently, CV AFS has been used for the determination of mercury species at very low levels in several environmental studies [20–25]. However, much care must be taken in relation to the possibility of quenching: Water vapor must be strictly avoided and the maximum sensitivity is attained with the use of high-purity gases, such as argon or preferably helium. CV AAS has found its way to the scene of sub ng L^{-1} determination of Hg since the introduction of a dedicated mercury detector, using a multipath absorption cell and Zeeman-effect background correction [26], whose sensitivity is in the same range of AFS detectors. Thus, the present work investigates the hyphenation of this detector with high performance liquid chromatography for methylmercury determination.

☆ This paper was presented at the 10th Rio Symposium on Atomic Spectrometry, held in Salvador-Bahia, Brazil, 7–12 September 2008, and is published in the special issue of Spectrochimica Acta Part B, dedicated to that conference.

* Corresponding author. Fax: +55 21 35271309.
E-mail address: rccampos@puc-rio.br (R.C. Campos).

0584-8547/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.sab.2009.05.021

2. Experimental

2.1. Instrumentation

The measurements were performed with a high performance liquid chromatography-uv-cold vapor atomic absorption spectrometry (HPLC-UV-CV-AAS) assembly (Fig. 1) consisting of a GT1310A isocratic pump (Agilent, Böblingen, Germany), an injection valve (Rheodyne, California, USA) with a 200 µL stainless steel sample loop, a reversed-phase analytical column packed with Exsil ODS (RP C18, 250 mm × 4.6 mm, 5 µm), an UV speciation heated coil (S570U100, PS Analytical, Orpington, Kent, England), operating at 13 W and 60 °C, a ME gas liquid separator (PS Analytical) and a RA-915$^+$ mercury spectrometer (LUMEX, Saint Petersburg, Russia). The chromatographic separation was performed at room temperature. The reducing and acidifying solutions were merged with the column eluate using peristaltic pumps type ISM787A (Ismatec, Glattbrugg, Switzerland), and Tygon tubes (1.14 mm). The mixing chemifold and the reaction coils (length = 93 cm, inner diameter = 0.8 mm) were made of Teflon^R. The mercury vapor was dried with nitrogen using a MD-110-12FP-4 desiccant membrane, 27.5 cm long (Perma Pure, Toms River, USA). The RA-915$^+$ mercury spectrometer was used as detector. In this instrument, a multipath cell increases the optical path to a length equivalent to 3 m. A very intense Hg discharge lamp contributes to a stable baseline and a magnetic field applied to the lamp together with a polarizer permits the use of Zeeman-effect background correction. Since the mercury spectrometer has been operated in the liquid analysis mode, the flow promoted by its internal pump should be equilibrated by an extra flow of gas in order to keep the liquid inside the gas–liquid separator at the right level. For practical reasons, argon (AGA, Rio de Janeiro, Brazil) was used, but nitrogen or even air could also have been used. This operation was manually made, taking the liquid level inside the gas–liquid separator as the reference for finding the proper flow of the external gas. All gases were made mercury free by passing them through gold traps conveniently positioned in the system. A personal computer was used to collect the data which were treated using Excel (Microsoft, Redmond, USA). For the extraction of methylmercury, a Thornton model T50 ultrasonic bath (Inpec Eletrônica Ltda, Vinhedo, Brasil) was used, operating at 40 kHz.

2.2. Materials, reagents, solutions and samples

All reagents were of analytical reagent grade. Ultra pure water (resistivity > 18.0 MΩ cm), obtained from a Master System apparatus (Gehaka, S. Paulo, Brazil) was used throughout. Analytical grade HNO_3 and HCl (both Vetec, Rio de Janeiro, Brazil) were purified by sub-boiling distillation using a PTFE sub-boiling apparatus (Hans Kuerner, Rosenheim, Germany). The 1000 mg L^{-1} mercury (Hg II) standard solution was prepared by diluting Titrisol (Merck, Darmstadt, Germany) ampoules with 0.2% v/v HNO_3. A 1000 mg L^{-1} methylmercury (as Hg) stock solution was prepared from its chloride salt (Carlo Erba, Milan, Italy): The salt was accurately weighed (±0.0001 g) and dissolved with 50% v/v ethanol; further dilutions to the indicated concentrations were made with water; 1.000 µg L^{-1} intermediary solutions of both mercury species were prepared daily and used to prepare the calibration solutions. The calibration solutions were prepared just before their introduction into the injector, in order to avoid losses. For the mobile phase methanol (Vetec, Duque de Caxias, Brazil), and mercaptoethanol (Vetec) were used. Acetic acid (Vetec) and ammonium acetate (Vetec) were used for buffering. Sodium borohydride (Vetec), dissolved in 0.5% NaOH (Vetec) was used as reducing agent. This solution was prepared daily. Dichloromethane and KOH (both Vetec) were used for methylmercury extraction. For the preliminary application studies a sediment certified reference material (IAEA 405, IAEA, Vienna, Austria), as well as surface sediment samples were used. They were collected with an Ekman grab sampler, conditioned in plastic bags, labeled and taken to the laboratory. They were kept frozen until the analysis. All plastic and glassware were washed with tap water, immersed in neutral Extran (48 h), rinsed with tap and deionized water, and immersed in 20% v/v HNO_3 for, at least, 24 h. Before use, these materials were thoroughly rinsed with ultrapure water and oven dried at 40 °C, avoiding any contact with metallic surfaces and dust contamination. Contamination was always checked by a strict blank control.

2.3. Procedures

2.3.1. Instrumental determination of methylmercury

The mobile phase consisted in a 0.01% v/v mercaptoethanol solution in 5% v/v methanol, with the pH adjusted to 5 by the addition of 0.06% v/v acetic acid + 0.15% ammonium acetate. The mobile phase flow rate was 1.4 mL min^{-1}. Calibration and sample solutions were manually injected (200 µL) using a hypodermic syringe; 1.5% $NaBH_4$ in 0.5% NaOH was used as reducing agent and 3 mol L^{-1} HCl as acidifying solution with a flow rate of 2.2 mL min^{-1}. The dry nitrogen flow through the desiccating membrane was 80 mL min^{-1}. An argon flow of 240 mL min^{-1} was necessary to keep the liquid in the gas–liquid separator in the right level. Aqueous standards, prepared just before their injection by adequate dilutions of the 1.000 µg L^{-1} solutions were used for calibration. Since each run took about 20 min (for methylmercury determination only), this procedure had no deleterious effect in the total analysis time. Peak area was used for measurement. Data collected in the personal computer were transferred to Excel data sheets, and chromatogram profiles were obtained. Integration consisted in localizing the peaks by the retention time and summing up the absorbance of the individual data in the integration interval. At least 200 points were counted, relative to the smallest (blank) peaks.

2.3.2. Sediment characterization and methyl mercury extraction and determination

The organic matter content in the sediments was determined after calcination at 400 °C for 6 h. The procedure for methylmercury extraction from the sediments was based on the method of Ramalhosa et al. [27]. Sample masses up to 300 mg were weighed in conical ended screw capped 50-mL tubes (Sarstedt, Nümbrecht, Germany). Two mL of a 25% m/v KOH solution in methanol were added and the

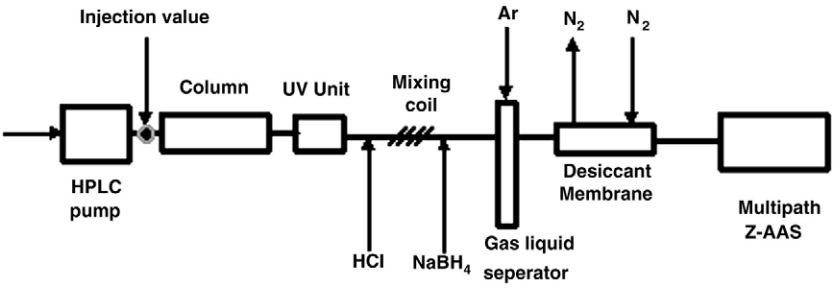

Fig. 1. Block diagram of the hyphenated system.

mixture sonicated for 3 h. Then 6 mL of CH_2Cl_2 were added, followed by the slow addition of 1.5 mL of concentrated HCl, and the extraction of the neutral methylmercury chloride to the organic phase accomplished by mechanical shaking (10 min). Five milliliters of the organic layer was taken with a pipette, and transferred to another tube where 10 mL of pure water was added; the methylmercury was transferred to the aqueous phase evaporating CH_2Cl_2 by bubbling nitrogen (80 mL min^{-1}) through the organic layer. The remaining aqueous solution was injected in the hyphenated system. All procedures were performed in a fume hood. For organic matter rich sediments the methanolic KOH extraction was performed twice. A flow-chart of the procedure is shown in Fig. 2. Calibration with aqueous solution matches the matrix of the final sample solution.

3. Results

3.1. Characterization of the Zeeman multipath AAS detector

In order to characterize the detector in relation to its detection capability, a calibration curve was performed using a batch vapor generator and Hg^{2+} aqueous calibration solutions, ranging from 2.5 to 20 μg L^{-1}. The sample volume was 50 mL and $SnCl_2$ was used as reductant. No gold trap or desiccant has been used. Air (0.5 L min^{-1}) was used as carrier gas, and the equipment operated in the water analysis mode. The curve was linear in the studied range ($r>0.999$), and the calibration curve was described by a $y=bx$ equation ($b=11.9\pm0.1$), since the intercept was not significantly different from zero [28]. The instrument displays absorbance in arbitrary units. The limit of detection calculated as the concentration equivalent to three times the standard deviation of the calibration curve blank ($n=10$) was 0.4 ng L^{-1} (peak height), which is equivalent to 14 pg transported to the detector, since the transport yield of the generator has previously been measured as 70%. Limits of detection of the same order can also be attained by AFS detectors, but using argon or helium as carrier gas, and strictly avoiding any water vapor in the excitation cell, in order to prevent quenching.

3.2. Multivariate optimization of the hyphenated system

For this study, a 50 μg L^{-1} Hg aqueous solution in ultrapure water was used. Any stability problem was overcome by preparing this

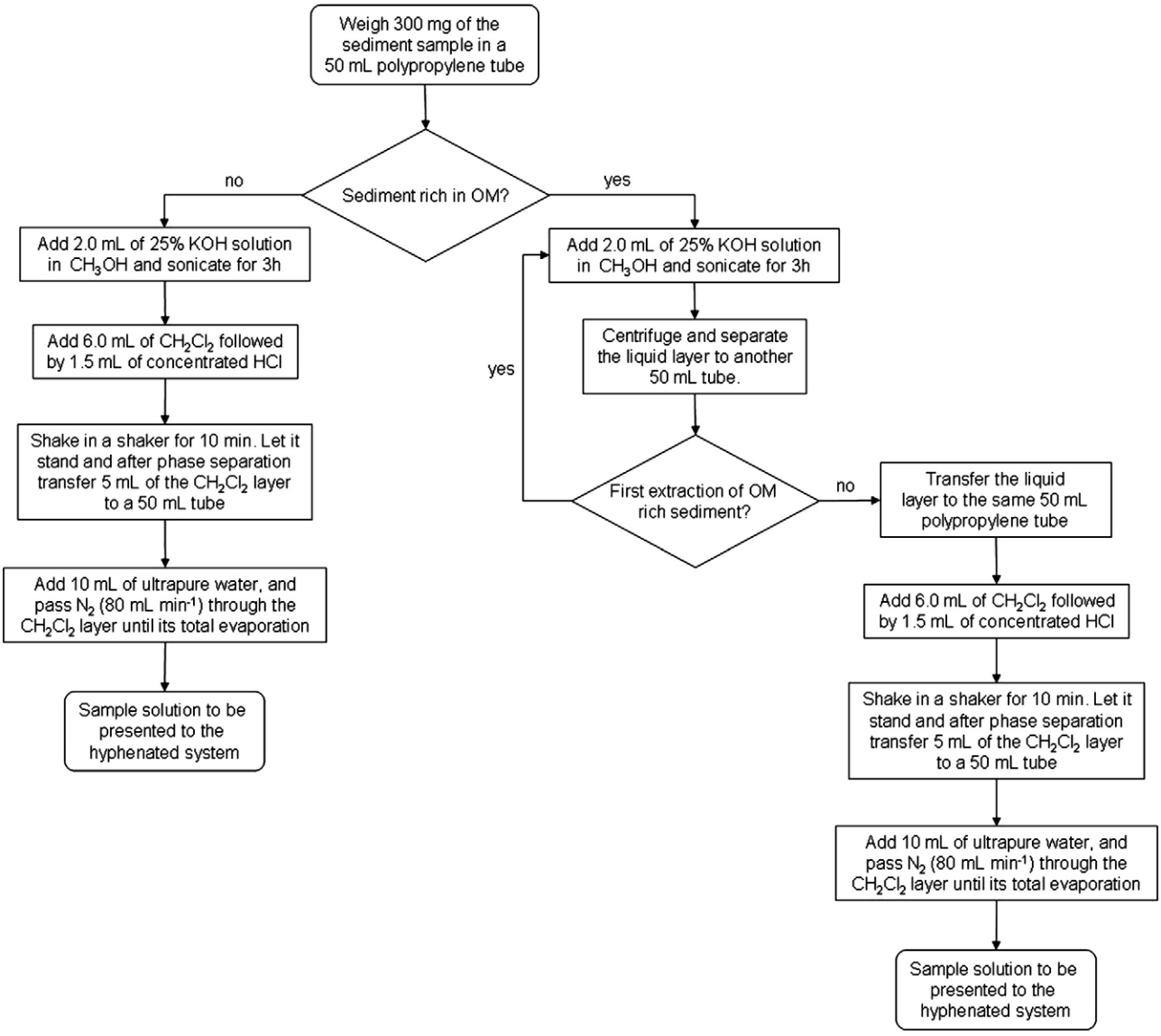

Fig. 2. Flow chart for the methylmercury extraction procedure from sediments.

solution by adequate dilution of the 1000 µg L^{-1} standard solution just before its injection. A response surface method was used for obtaining the optimal conditions. This method permits the estimation of the interactions and quadratic effects. A D optimal design was chosen since more complex models (e.g non-linear) can be evaluated. In contrast to factorial designs, the D optimal matrix is non-orthogonal, and the estimated effects are correlated. This kind of design is independent from the type of model (first order, quadratic, etc) to be adjusted. The investigated responses were resolution [29] and t_2, which is the retention time of methylmercury species. The optimization targeted a resolution of 2.5, at a minimum value for t_2. Preliminary experiments defined the investigated ranges. Five variables were studied at three different levels in duplicate analysis, implying $2\times32=64$ experiments. Table 1 shows the investigated factors, and the values of the three different levels. The $NaBH_4$ and HCl flow rates were joined in only one factor in order to keep the number of experiments reasonable, taking into account the long analysis time. The $NaBH_4$ and HCl flow rate ratio was always 1. The experiments were performed in 4 blocks, and all the measurements in each block were made in only one day, by the same operator, in the same assembly. The experiments of the different blocks were normalized for comparison. For the resolution (Res), the model obtained is described by Eq. (1)

$$Res = 2.54-0.081D-0.55A^2 + 0.28E^2 + 0.076AC \quad (1)$$

A, B, C, D and E represent the studied factors as displayed in Table 1. Although highly significant, this model explains only 42% of the variability ($R^2=0.42$). The analysis of predict versus actual values plot (from where R^2 comes from) shows that the low R^2 value is due to the poor adjustment of the extreme points, what is corroborated by the normal plot of the residuals. From this model, the most relevant parameter for the resolution is the mercaptoethanol concentration. Factors B (mobile phase flow rate) and C (HCl concentration) are not relevant. Fig. 3 shows the surface responses for the resolution fixing B at 1.05 mL min^{-1} and C at 3.00 mol L^{-1}. For the methylmercury retention time (t_2) the model obtained is described by Eq. (2). This model explains 96% of the variability ($R^2=0.96$). Fig. 4 shows a response surface for t_2.

$$t_2 = 1453-48.41A-200B + 28.18C-22.90D + 57.02B^2-40.22C^2 -26.45AD-21.63CD + 31.26DE \quad (2)$$

Considering values in the studied range and the optimization targets (resolution = 2.5 and t_2 minimum), a series of possible solutions has been obtained. Those with extreme values for A, D and E were not considered due to the lack of fit of the resolution model for extreme values (B and C were not relevant). Thus the optimized values for the studied variables were (A) 0.01% v/v for the mercaptoethanol concentration (in 5% v/v methanol, pH = 5.0); (B) 1.4 mL min^{-1} for the mobile phase flow rate; (C) 3 mol L^{-1} for the HCl concentration; (D) 1.5% m/v for the $NaBH_4$ concentration in 0.5% NaOH and (E) a reducing and HCl solution flow rate of 2.2 mL min^{-1}. These values led to a desirability of

Table 1
Investigated factors and their respective values at the three different investigated levels.

Variables		Levels/values		
		−1	0	+1
A	Mercaptoethanol conc. (% v/v)	0.009	0.010	0.011
B	Mobile phase flow rate (mL min^{-1})	1	1.25	1.50
C	HCl concentration (mol L^{-1})	2	3	4
D	$NaBH_4$ concentration (% m/v)	1	1.5	2.0
E	$NaBH_4$ and HCl flow rate (mL min^{-1})	1.6	2.4	3.1

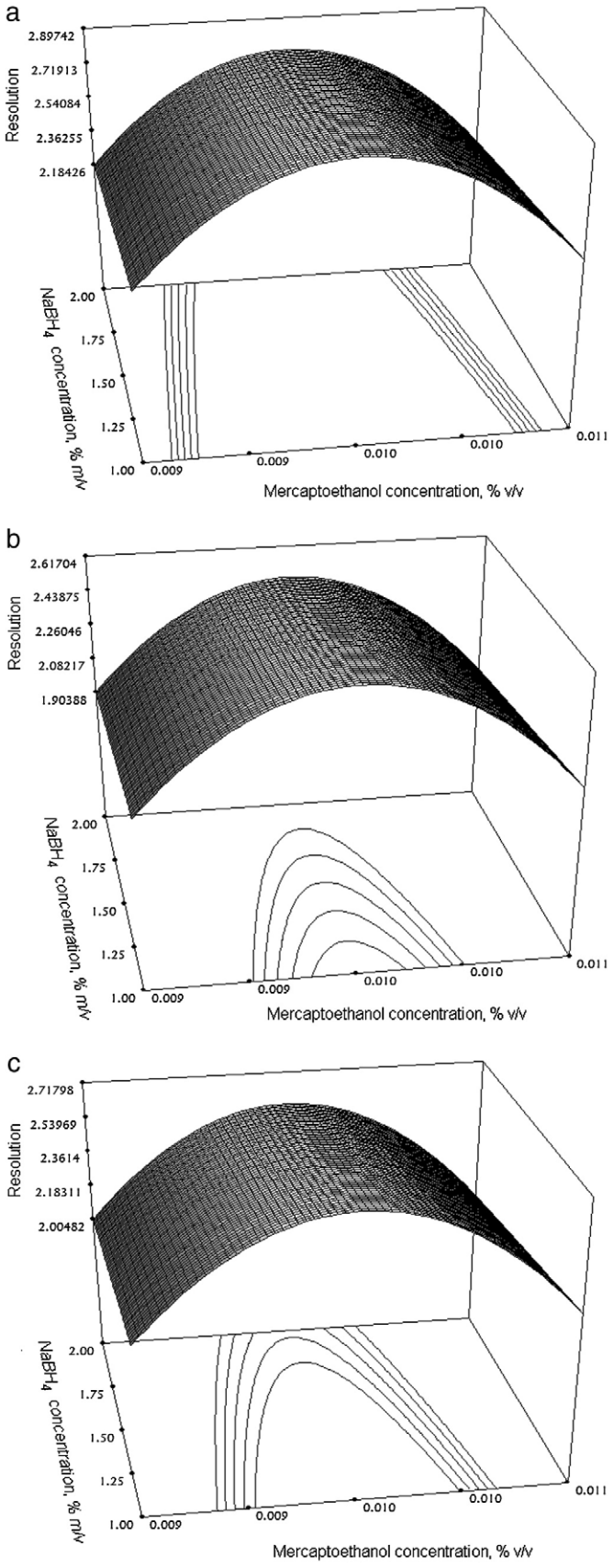

Fig. 3. Surface responses for the resolution at fixed values of mobile phase flow rate (1.05 mL min^{-1}) and HCl concentration (3.00 mol L^{-1}). $NaBH_4$ and HCl flow rates (mL min^{-1}) of 1.6 (a), 2.3 (b) and 2.8 (c).

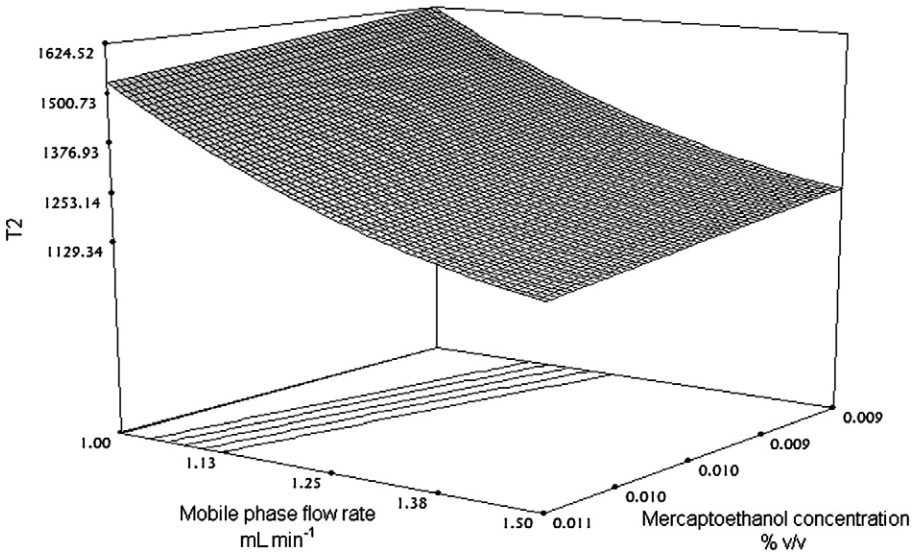

Fig. 4. Response surface for the methylmercury retention time (t_2) at fixed values of mobile phase flow rate (1.05 mL min^{-1}) and HCl concentration (3.00 mol L^{-1}). $NaBH_4$ and HCl flow rates of 2.2 mL min^{-1}.

0.85. Fig. 5a shows a chromatogram obtained for 5 μg L^{-1} calibration solutions of inorganic (HgII) and methylmercury solutions, while a calibration curve is displayed in Fig. 5b. The instrumental limit of detection, calculated according to IUPAC ($3S_B$, $n=5$) was 0.05 μg L^{-1} for both mercury species. This figure is in accordance to what is expected taking into account the instrumental LOD of the detector calculated in 3.1. This is also equivalent to 0.01 ng, similar to the value found by Ramalhosa et al. [27], using an HPLC-UV-CV-AFS system. For Hg speciation analysis systems using separation by HPLC and no pre-concentration step, lower LOD can only be reached if DIN ICP-MS or CV ICP-MS is used as detector [30,31]. The coefficient of variation for 5 successive injections of a 5 μg L^{-1} calibration solution (both species) was 5%.

3.3. Preliminary application studies

3.3.1. Determination of methylmercury in a certified sediment reference sample

The determination of methylmercury in sediments is of importance since a correct risk evaluation, due to the presence of mercury in this environmental compartment is dependent on the knowledge of the

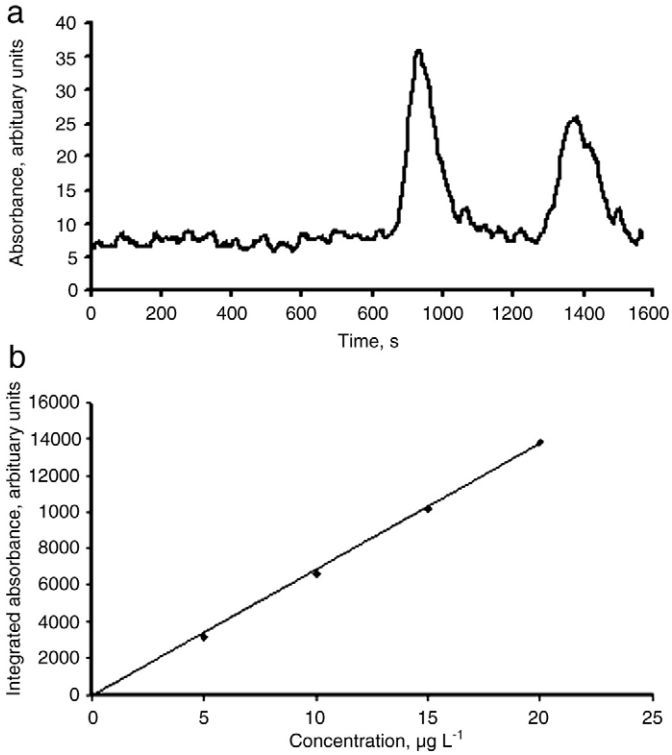

Fig. 5. Mercury (II) and methylmercury determination using the HPLC-UV-CV-AAS system with the multipath AAS detector at the optimized conditions: (a) chromatogram profile for ionic and methylmercury obtained with a 5 μg L^{-1} mixed calibration solution; (b) calibration curve for methylmercury ($y=686x\pm8$, $R^2=0.998$).

concentration of this species [32]. Among a series of procedures [33], hyphenated techniques using an analyte-specific atomic spectrometric detector was the analytical system of choice in many occasions [27,34–37]. The methylmercury extraction from the IAEA 404 sediment CRM differs just slightly from that proposed by Ramalhosa et al. [27], and is described in Section 2.3. The only differences have been that a definite volume of the organic layer was taken (5 mL) and the volume of water used to recover the methylmercury to the aqueous medium was only 10 instead of 30 mL. This was done to achieve a better limit of detection for the whole analytical procedure. In order to avoid any methylmercury loss during the evaporation step, the N_2 flux was strictly controlled. Usually, methyl mercury responds for a minor fraction of the total mercury content in sediments, and even a slight artifact conversion of inorganic mercury into methylmercury can lead to erroneous methylmercury evaluation [14,16,19,38]. In the present procedure, undesired methyl mercury formation is avoided by the use of CH_2Cl_2: Note that in the first step (Fig. 2), both organic and inorganic mercury are co-extracted by the methanolic KOH solution. After the addition of HCl to this solution, further extraction step with CH_2Cl_2 promotes the complete separation of the organic from the inorganic species since only the neutral methyl mercury chloride formed is transferred to the CH_2Cl_2 layer, while the inorganic mercury, in the form of $HgCl_4^{2-}$ is left in the methanolic fraction. Thus, any transformation of Hg (II) into methyl mercury along the further analysis steps is avoided. At first, a 5 μg L^{-1} aqueous solution was submitted to the whole analytical procedure, and recoveries close to 100% were found, supporting the adequacy of calibration against aqueous standards. The calibration curve was linear up to at least 25 μg L^{-1} and R^2 values > 0.99 were obtained. The method limit of detection calculated as the concentration equivalent to three times the standard deviation of the method blank was 1.4 μg kg^{-1} considering a sediment mass of 300 mg. This means a limit of quantification of 4.6 μg kg^{-1}. The sediment CRM has certified values of 810 μg kg^{-1} (total Hg) and 5.49 ± 0.53 μg kg^{-1} for methyl mercury. It corresponds to the usual situation found in the environment, where methylmercury represents only a small fraction of the total mercury and, consequently, interconversion is critical in relation to the formation of methylmercury during the analytical procedure. The good concordance between found (5.4 ± 0.5 μg kg^{-1}, $n = 5$) and certified values confirmed the accuracy of the method for this sample.

3.3.2. Determination of methylmercury in other sediment samples

In order to investigate the applicability of the procedure to sediment samples with characteristics other than the investigated CRM, recovery studies were performed. Three types of sediments were used: a sandy, a carbonatic and an organic-matter (OM) rich sediment (OM = 12%). Methylmercury (50 μL; 1000 μg L^{-1}) was spiked to the samples after sample weighing, and the spiked samples were submitted to the whole analytical procedure. Analysis was performed in duplicate. In contrast to the other samples, poor recovery (50%) was observed for the OM-rich sediment. It has been a muddy sediment, with predominance of silt (40%) and clay (40%), and $D_{85} = 80$ μm. Drying and further pulverization of this sediment did not remediate the problem. Poor recoveries remained even after introducing one more CH_2Cl_2 extraction step and increasing the volume of the methanolic KOH and HCL solutions or the volume of the HCl solution only. However, when the methanolic KOH extraction step was duplicated and each resulting solution independently extracted by CH_2Cl_2, and the two extracts pooled for evaporation, a 98% recovery has been obtained. These results showed that for this sediment sample, a procedure that was efficient for the quantitative extraction of methyl mercury of a CRM sediment sample could not be directly employed.

4. Conclusions

The multipath Zeeman AAS detector proved an excellent alternative to AFS in the determination of mercury in HPLC hyphenated systems. The interface was easily made by just linking the gas outlet of the gas–liquid separator to the instrument inlet via a drying membrane. The instrument operated at the water analysis mode and an external gas flow directed to the gas–liquid separator was necessary to compensate the flow promoted by the internal pump of the equipment. No special gas is necessary. The data stored in a PC could be easily exported to Excel sheets for integration calculation. Multivariate optimization of the hyphenated system led to an instrumental LOD of 0.01 ng for both inorganic and methylmercury species, similar to systems that use AFS for detection. Zeeman-effect background correction efficiently compensates for non-specific absorbance. In contrast to Hg measurements performed by AFS, quenching is not a problem in AAS. This means that even air can be used as carrier gas and the presence of some water vapor in the atom cell is not critical, making the routine operation of the system much simpler. The mercaptoethanol concentration was the most sensitive variable related to species separation. This solution must be prepared daily and its concentration closely checked, otherwise large variations in the retention times and resolution will be observed. Twenty-minute runs were sufficient for the complete integration of the methyl mercury peak. The system was used for the determination of methyl mercury in a CRM sediment sample after methyl mercury extraction. Good concordance was observed between found and certified values. However, this was not the case for an organic matter-rich sediment, for which a modification of the original extraction procedure was necessary. Thus, caution must be taken in the generalization of extraction procedures, even when they are supported by CRM analysis, if the CRM does not represent the composition of the samples to be analyzed. It is also important to note that calibration by analyte addition does not necessarily compensate for incomplete extraction. Comprehensive studies of methyl mercury extraction encompassing different kind of sediments are still required.

References

[1] R. Eiden, R. Falter, B. Augustin-Castro, H.F. Scholer, Distillation, on-line RP C18 preconcentration and HPLC-UV-PCO-CVAAS as a new combination for the determination of methylmercury in sediments and fish tissue, Fresenius J. Anal. Chem. 357 (1997) 439–441.
[2] J. Qvarnström, Q. Tu, W. Frech, C. Lüdke, Flow injection-liquid chromatography-cold vapour atomic absorption spectrometry for rapid determination of methyl an inorganic mercury, Analyst 125 (2000) 1193–1197.
[3] M.T. Chun, A. de Diego, F.M. Martin, O.F.X. Donard, Rapid and quantitative microwave-assisted recovery of methylmercury from standard reference sediments J, Anal. At. Spectrom. 12 (1997) 629–635.
[4] C. Chiou, S. Jiang, K.S.K. Danadurai, Determination of mercury compounds in fish by microwave-assisted extraction and liquid chromatography-vapor generation-inductively coupled plasma mass spectrometry, Spectrochim. Acta Part B 56 (2001) 1133–1142.
[5] Y. Mao, G. Liu, G. Meichel, Y. Cai, G. Jiang, Simultaneous speciation of monomethylmercury and monoethylmercury by aqueous phenylation and purge-and-trap preconcentration followed by atomic spectrometry detection, Anal. Chem. 80 (2008) 7163–7168.
[6] M.S. da Rocha, A.B. Soldado, E. Blanco, A. Sanz-Medel, Speciation of mercury using capillary electrophoresis coupled to volatile species generation-inductively coupled plasma mass spectrometry, J. Anal. At. Spectrom. 16 (2001) 951–956.
[7] J. Morton, V.A. Carolanb, P.H.E. Gardiner, The speciation of inorganic and methylmercury in human hair by high-performance liquid chromatography coupled with inductively coupled plasma mass spectrometry, J. Anal. At. Spectrom. 17 (2002) 377–381.
[8] Q. Tu, J. Qian, W. Frech, Rapid determination of methylmercury in biological materials by GC-MIP-AES or GC-ICP-MS following simultaneous ultrasonic assisted in situ ethylation and solvent extraction, J. Anal. Atom. Spectrom. 15 (2000) 1583–1588.
[9] E. Ramalhosa, S. Río Segade, E.P.C. Vale, A. Duarte, Microwave treatment of biological samples for methylmercury determination by high performance liquid chromatography-cold vapour atomic fluorescence spectrometry, Analyst 126 (2001) 1583–1587.
[10] C. Tseng, C.R. Hammerschmidt, W.F. Fitzgerald, Determination of methylmercury in environmental matrixes by on-line flow injection and atomic fluorescence spectrometry, Anal. Chem. 76 (2004) 7131–7136.
[11] K.C. Bowles, S.C. Apte, Determination of methylmercury in sediments by steam distillation/aqueous-phase ethylation and atomic fluorescence spectrometry, Anal. Chim. Acta 419 (2000) 145–151.
[12] Y. Cai, Speciation and analysis of mercury, arsenic, and selenium by atomic fluorescence spectrometry, Trends Anal. Chem 19 (2000) 62–66.

[13] P. Rodríguez-González, J.M. Marchante-Gayón, J.I.G. Alonso, A. Sanz-Medel, Isotope dilution analysis for elemental speciation: a tutorial review, Spectrochim. Acta Part B 60 (2005) 151–207.
[14] H. Hintelmann, R. Falter, G. Ilgen, R.D. Evans, Determination of artifactual formation of monomethylmercury (CH_3Hg^+) in environmental samples using stable Hg^{2+} isotopes with ICP-MS detection: calculation of contents applying species specific isotope addition, Fresenius J. Anal. Chem. 358 (1997) 363–370.
[15] G.M.M. Rahman, H.M.'Skip' Kingston, Development of a microwave-assisted extraction method and isotopic validation of mercury species in soils and sediments, J. Anal. At. Spectrom. 20 (2005) 183–191.
[16] L. Lambertsson, E. Lundberg, M. Nilsson, W. Frech, Applications of enriched stable isotope tracers in combination with isotope dilution GC-ICP-MS to study mercury species transformation in sea sediments during in situ ethylation and determination, J. Anal. At. Spectrom. 16 (2001) 1296–1301.
[17] R.C.R. Martín-Doimeadios, E. Tessier, D. Amouroux, R. Guyoneaud, R. Duranb, P. Caumette, O.F.X. Donard, Mercury methylation/demethylation and volatilization pathways in estuarine sediment slurries using species-specific enriched stable isotopes, Mar. Chem. 90 (2004) 107–123.
[18] E. Ciceri, S. Recchia, C. Dossi, L. Yang, R.E. Sturgeon, Validation of an isotope dilution, ICP-MS method based on internal mass bias correction for the determination of trace concentrations of Hg in sediment cores, Talanta 74 (2008) 642–647.
[19] J. Qvarnstrom, W. Frech, Mercury species transformations during sample pretreatment of biological tissues studied by HPLC-ICP-MS, J. Anal. At. Spectrom. 17 (2002) 1486–1491.
[20] C.H. Conaway, S. Squire, R.P. Mason, A.R. Flegal, Mercury speciation in the San Francisco Bay estuary, Mar. Chem. 80 (2003) 199–225.
[21] H. Bagheri, A. Gholami, Determination of very low levels of dissolved mercury(II) and methylmercury in river waters by continuous flow with on-line UV decomposition and cold-vapor atomic fluorescence spectrometry after pre-concentration on a silica gel-2-mercaptobenzimidazol sorbent, Talanta 55 (2001) 1141–1150.
[22] T. Stoichev, R.C.R. Martin-Doimeadios, E. Tessier, D. Amouroux, O.F.X. Donard, Improvement of analytical performances for mercury speciation by on-line derivatization, cryofocussing and atomic fluorescence spectrometry, Talanta 62 (2004) 433–438.
[23] J.H. Trefry, R.P. Trocine, M.L. McElvaine, R.D. Rember, L.T. Hawkins, Total mercury and methylmercury in sediments near offshore drilling sites in the Gulf of Mexico, Environ. Geol. 53 (2007) 375–385.
[24] M. Valega, A.I. Lillebø, M.E. Pereira, W.T. Corns, P.B. Stockwell, A.C. Duarte, M.A. Pardal, Assessment of methylmercury production in a temperate salt marsh (Ria de Aveiro Lagoon, Portugal), Mar. Pollut. Bull. 56 (2008) 153–158.
[25] M.C. Bisinoti, W.F. Jardim, Production of organic mercury from Hg^0: experiments using microcosm, J. Braz. Chem. Soc. 14 (2003) 244–248.
[26] http://www.ohiolumex.com/product/ra915.shtml.
[27] E. Ramalhosa, S. Río Segade, E. Pereira, C. Vale, A. Duarte, Simple methodology for methylmercury and inorganic mercury determinations by high-performance liquid chromatography–cold vapour atomic fluorescence spectrometry, Anal. Chim. Acta 448 (2001) 135–143.
[28] R. Caulcutt, R. Boddy, Statistics for Analytical Chemists, Chapman and Hall, London, 1983.
[29] D.C. Harris, Quantitative Chemical Analysis, 6th ed.W. H. Freeman and Company, New York, 2003.
[30] C. Wan, C. Chen, S. Jiand, Determination of mercury compounds in water samples by liquid chromatography–inductively coupled plasma mass spectrometry with and in situ nebulizer/vapor generator, J. Anal. At. Spectrom. 12 (1997) 683–687.
[31] S.C.K. Shum, H.M. Pang, R.S. Houk, Speciation of mercury and lead compounds by microbore column liquid chromatography-inductively coupled plasma mass spectrometry with direct injection nebulization, Anal. Chem. 64 (1992) 2444–2450.
[32] U. Skyllberg, A. Drott, L. Lambertsson, E. Bjorn, T. Karlsson, T. Johnson, S.-A. Heinemo, H. Holmstrom, Net methylmercury production as a basis for improved risk assessment of mercury contaminated sediments, Ambio 36 (2007) 437–441.
[33] C.F. Harrington, The speciation of mercury and organomercury compounds by using high-performance liquid chromatography, Trends Anal. Chem. 19 (2000) 167–179.
[34] J. Canario, P. Antunes, J. Lavrado, C. Vale, Simple method for monomethylmercury determination in estuarine sediments, Trends Anal. Chem. 23 (2004) 10–11.
[35] R. Falter, G. Ilgen, Coupling of the RP C18 pre-concentration HPLC-UV-PCO-system with atomic fluorescence detection for the determination of methylmercury in sediment and biological tissue, Fresenius J. Anal. Chem. 358 (1997) 407–410.
[36] E. Ramalhosa, S. Río Segade, E. Pereira, C. Vale, A. Duarte, Microwave-assisted extraction for methylmercury determination in sediments by high performance liquid chromatography-cold vapour-atomic fluorescence spectrometry, J. Anal. At. Spectrom. 16 (2001) 643–647.
[37] J.C.A. Wuillouda, R.G. Wuillouda, A.P. Vonderheide, J.A. Caruso, Gas chromatography/plasma spectrometry—an important analytical tool for elemental speciation studies, Spectrochim. Acta Part B 59 (2004) 755–792.
[38] C.R. Hammerschmidt, W.F. Fitzgerald, Formation of artifact methylmercury during extraction from a sediment reference material, Anal. Chem. 73 (2001) 5930–5936.