

# Simple methodology for methylmercury and inorganic mercury determinations by high-performance liquid chromatography–cold vapour atomic fluorescence spectrometry

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## Abstract

high-performance liquid chromatography (HPLC)–cold vapour atomic fluorescence spectrometry (CV-AFS). The use of UV oxidation of the organic forms of mercury, the tin(II) chloride reduction in acidic medium of mercury and the introduction of a water vapour trap before the H<sub>2</sub>SO<sub>4</sub>/CaCl<sub>2</sub> reagent in a by detection limit (DL) of 10 MeHg and inorganic mercury by apparatus was operationally optimised through the modified simplex method leading to an increase of the signal by a factor of 2. The method performance has been tested by determining the MeHg concentration in a sediment certified reference material (CRM) and the results were statistically indistinguishable from the certificate value ( $\alpha = 0.05$ ) both for the mean value and

Methylmercury; Cold vapour atomic fluorescence spectrometry; Liquid chromatography; UV radiation; Simplex optimisation

*Keywords:*

## 1. Introduction

Mercury in natural environments may be present in several inorganic and organic forms, being methylmercury (MeHg) the dominant toxic occurring organomercury specie in environmental matrices. In order to assess fluxes, bioavailability and toxicity of the different mercury forms, the chemical species present in the environmental compartments should be determined with a high degree of analytical confidence.

Several analytical methods for separating mercury species based either on gas chromatography (GC) or high-performance liquid chromatography (HPLC) coupled with element-specific detection for mercury have been developed in the last few years. Coupled with GC, a large variety of mercury detection and quantification methods have been used: electron capture detection (ECD) [1]; microwave induced plasma (MIP) [2–4]; atomic fluorescence spectrometry (AFS) [5–7]; atomic absorption spectrometry (AAS) [8,9]; inductively coupled plasma-mass spectrometry (ICP-MS) [10,11]; and glow discharge atomic emission spectrometry (GD-AES) [12]. Coupled with HPLC, the detection and quantification methods

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for mercury speciation have included: ICP-MS [13]; photometry [14]; cold vapour atomic absorption spectrometry (CV-AAS) [15]; cold vapour atomic fluorescence spectrometry (CV-AFS) [16,17]; atomic emission spectrometry (AES) [18,19]; and atmospheric pressure ionisation mass spectrometry (API-MS) [20]. Although GC has been the most widely used separation technique in mercury speciation studies, it is necessary to perform column passivation by repeated mercury salt injections [21] and derivatisation of mercury compounds to form volatile species [22]. HPLC allows an easier sample treatment, elimination of the derivatisation step, separation of mercury compounds at laboratory temperature, injection of large volumes of sample, easy performance and automation. One disadvantage of HPLC systems is the highest detection limits (DLs), usually ranging between 20 and 1000 pg [15,16,23,24].

The most commonly used detection techniques for organomercury compounds determination by HPLC are the CV-AAS and the CV-AFS. The mobile phase is mixed with a reducing agent, e.g. tin(II) chloride ( $\text{SnCl}_2$ ) in an alkaline solution [16,25,26] or sodium tetrahydroborate ( $\text{NaBH}_4$ ) [15,17,27,28]. The main drawback in the use of  $\text{NaBH}_4$  in the reduction step is the generation of hydrogen simultaneously with the production of elemental mercury. Hydrogen, besides requiring careful safety precautions, quenches the mercury fluorescence signal, thus acting as an interference. The reduced mercury vapour formed in the reduction step is then separated in a gas-liquid separator cell and stripped by an inert gas directly into the flow cell of the detector. Usually, when  $\text{SnCl}_2$  is used for mercury reduction, organomercury compounds should be transformed into inorganic mercury prior to reduction. Several oxidising solutions, e.g. potassium persulfate [16,25,29], a mixture of potassium bromate and bromide [30,31], as well as, UV radiation [15] have been used for organomercury compounds oxidation. When  $\text{NaBH}_4$  is used as reducing agent, the subsequent oxidation step seems to be unnecessary since this compound is efficient in decomposing inorganic and organic mercury to elemental form [32].

A simple HPLC-based methodology, with  $\text{SnCl}_2$  reduction in acidic medium, for the determination of methylmercury and inorganic mercury, with a DL of around 10 pg without any pre-concentration step, is proposed in this work. The composition of the mobile

phase and the type of oxidation (chemical oxidation versus UV irradiation) were selected, and the flow rates of the mobile phase and of the reducing agent, length of reduction reaction coil, flow rates of carrier and shield gas were optimised by a modified simplex algorithm. The high performance of the methodology has been confirmed by its application to the analysis of a certified reference material (CRM) sediment reference material.

## 2. Experimental

### 2.1. Assembling of instrumentation

A schematic view of the HPLC-CV-AFS system used in this work is presented in Fig. 1, including a reversed-phase HPLC system for the separation of different mercury species and a CV-AFS for element-specific detection.

The chromatographic system consisted of a HPLC pump (Knauer, Berlin, Germany; 10 cm<sup>3</sup>), a six-port injection valve (Type Rheodyne; six-port) (Rheodyne, California, USA) equipped with a 200  $\mu\text{l}$  PEEK loop and a reversed-phase analytical column packed with Nucleosil ODS (RPC18, 25 cm  $\times$  4.6 mm, 5  $\mu\text{m}$ ). The separated mercury species eluted from the HPLC column were either oxidised to inorganic mercury by wet chemical oxidation (potassium bromate/bromide) or UV irradiation (CAMAG lamps, either 8 or 15 W, 254 nm) (Camag, Muttenz, Germany) in an oxidation reaction coil. This coil was switched every 4 months, when the system was operating around 8 h a day. The reducing agent, tin(II) chloride in acidic solution, 3% (m/v)  $\text{SnCl}_2$  in 15% HCl (v/v), was chosen, following the procedure reported by Mena and McLeod [31] and Bloxham et al. [30]. The reducing agent was pumped and mixed with the solution eluting from the oxidation reaction coil into the reduction reaction coil. Peristaltic pumps (Ismatec Reglo; four channels) (Ismatec, Zürich, Switzerland) injected both the oxidising and reducing solutions through Tygon tubes (R 3603). The mixing joints and both reaction coils were made of 0.50 mm (i.d.) Teflon. From the reduction reaction coil, the mixture was then pumped to a quartz gas-liquid separator cell (12 cm<sup>3</sup>) (PSAnalytical, Kent, England). An argon stream (carrier flow) stripped the elemental mercury from the solution, into

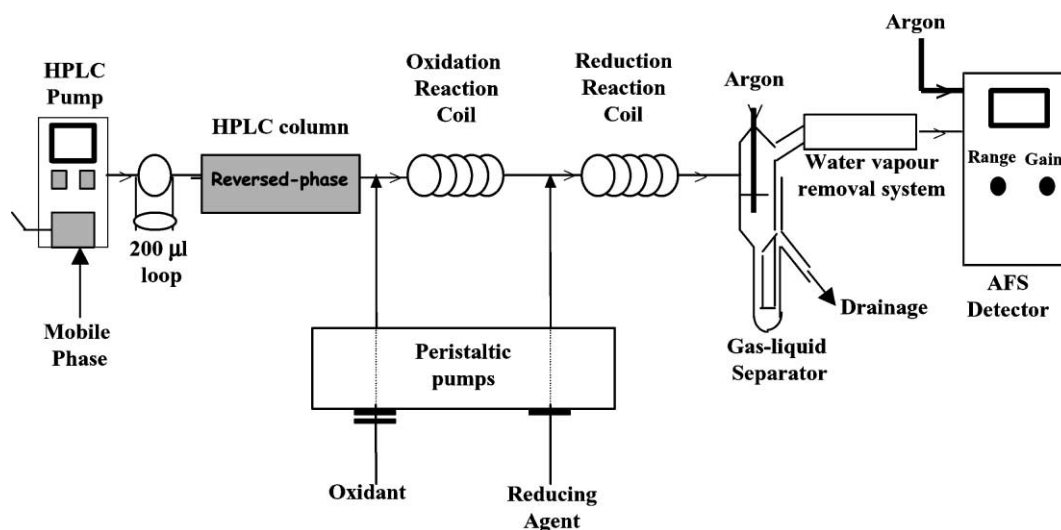


Fig. 1. HPLC–CV–AFS system assembled to perform the separation and detection of methylmercury and inorganic mercury.

the detector cell of the AFS (10.023 Merlin Detector, PSAAnalytical, Kent, England) and a second stream of argon, denoted by shield gas, also entered directly into the detector. The removal of water vapour was achieved with either a hygroscopic tubular membrane (Perma Pure) or an acid sulphuric trap connected to a calcium chloride trap.

## 2.2. Reagents

All the analytical-reagent grade chemicals were used without further purification. When possible Hg-free reagents were used, all standards and reagents were prepared in ultra-pure water produced in a Milli-Q model 185 system. The mobile phase was a mixture of methanol (Merck, liquid chromatography, Darmstadt, Germany) and ultra-pure water, containing 2-mercaptoethanol (Merck, p.a., Darmstadt, Germany) buffered at pH 5 with 0.06% (v/v) acetic acid (Merck, p.a., Darmstadt, Germany) and 0.15% (m/v) ammonium acetate (Merck, p.a., Darmstadt, Germany). The mobile phase was filtered through 0.2- $\mu\text{m}$  membranes (NL 16, Schleicher & Schuell, Dassel, Germany) and degassed in an ultrasonic bath for 30 min prior to use.

The oxidising solution (0.5% (m/v) KBr + 0.14% (m/v) KBrO<sub>3</sub> in 2 mol dm<sup>-3</sup> HCl) [30,31] was freshly prepared and filtered by 0.45- $\mu\text{m}$  membranes (Millipore, Bedford, USA). The reducing agent,

tin(II) chloride Hg-free, was obtained from Merck (p.a., Darmstadt, Germany). A solution of 3% (m/v) in 15% (v/v) Hg-free HCl was prepared daily, filtered by 0.45- $\mu\text{m}$  membranes (Millipore, Bedford, USA) and purified with a stream of nitrogen for 2 h. Stock solutions of mercury nitrate (1000 mg dm<sup>-3</sup>, Spectrosol BDH, Poole, England) and methylmercury chloride (1000 mg dm<sup>-3</sup> in mercury, Alpha Products, Karlsruhe, Germany) was used weekly to prepare a working solution of 10 mg dm<sup>-3</sup> (as Hg) of each individual species in ultra-pure water. Lower working standards were also prepared daily in ultra-pure water.

## 2.3. Sequential simplex method

Some initial conditions were settled, such as the type of oxidation used, the composition of mobile phase and the water vapour removal system, before starting the optimisation of the system. In order to reach the optimal conditions (the maximum mean signal-to-noise ratio) of the assembled continuous-flow system, the optimisation of the HPLC–CV–AFS system was performed by using the sequential simplex method [33]. The parameters taken into account in the optimisation procedure were; flow rates of mobile phase; water and reducing agent; length of reduction reaction coil; and flow rates of carrier and shield argon. The response variable evaluated was the

methylmercury fluorescence signal obtained for a  $2 \mu\text{g dm}^{-3}$  aqueous solution. This concentration was considered acceptable in comparison to other HPLC optimisation works:  $40 \mu\text{g dm}^{-3}$  [16];  $250 \mu\text{g dm}^{-3}$  [15], and  $10 \times 10^3 \mu\text{g dm}^{-3}$  [20]. The experiments were performed in three replicates. No other organomercury species were studied in this work because the most probable organomercury compound found in our environmental samples was methylmercury.

## 2.4. Extraction procedure

The extraction and cleanup procedures employed in the analysis of the CRM sediment reference material (CRM 580) were similar to the ones employed by Caricchia et al. [21] and Liang et al. [34], respectively. The method consisted of extracting 0.25 g of the sediment sample with 2 ml of KOH/CH<sub>3</sub>OH (25%, m/v) in an ultrasonic bath. After cooling, a 6 cm<sup>3</sup> volume of CH<sub>2</sub>Cl<sub>2</sub> was added to the digestate and then 1.5 cm<sup>3</sup> of concentrated HCl was added slowly. The mixture was capped and shaken for 10 min. The mixture was poured into a separating funnel and after the phases had separated, the lower solvent phase CH<sub>2</sub>Cl<sub>2</sub> was collected in a glass tube. For most of the situations, the digestate was washed once with another 6 cm<sup>3</sup> of CH<sub>2</sub>Cl<sub>2</sub>. Then 35 cm<sup>3</sup> of ultra-pure water was added to the solvent phase in the glass tube, being the organic phase evaporated by bubbling nitrogen. The determinations were done by the standard addition method, with four additions in duplicate.

## 3. Results and discussion

### 3.1. Setting of experimental conditions

#### 3.1.1. Wet oxidation as oxidation procedure

The wet oxidation was performed at three temperatures (30, 50 and 65°C) and in four coil lengths (3.4, 10.0, 13.4 and 19.0 m). The formation of bromine, which is an oxidising agent, was easily observed by the yellow colour of the solution. The wet oxidation recovery, measured as the ratio between MeHg<sup>+</sup> and Hg(II) peaks heights, ranged from 37 to nearly 74% when temperature varied from 30 to 65°C, with a coil length of 3.4 m. In fact, with this coil, the methylmercury signal increased almost two-fold when temperature

varied from 30 to 50°C and remained the same at 65°C. Since complete oxidation was not observed, an attempt was made for longer length of the oxidation coil. At 30°C, the oxidation recovery increased until 85% when coil length varied from 3.4 to 10.0 m, reaching a plateau until 19.0 m. At temperatures higher than 50°C, overheating of the solutions was observed.

#### 3.1.2. UV radiation as oxidation procedure

When experimenting UV radiation as oxidation procedure, a water flow was introduced in the system before the oxidation reaction coil in order to reduce the vaporisation of methanol, which would cause fluorescence quenching. Furthermore, such operational conditions caused a smooth reduction process and a reliable analytical signal. The oxidation recovery of methylmercury obtained with an UV radiation lamps of 8 and 15 W, and using three coil lengths (1.0, 3.3 and 10.0 m) is shown in Fig. 2. For coil lengths around 1 m, both lamps produced incomplete oxidation (between 24 and 45%) and the oxidation could only be considered complete for 3-m reaction coils either for lamps of 8 and 15 W.

#### 3.1.3. Composition of mobile phase

The principal variables associated with the composition of mobile phase, which could influence the retention time of mercury compounds, are: pH, amounts of methanol and 2-mercaptoethanol, which is used as organic modifier. In our experiments, a buffer

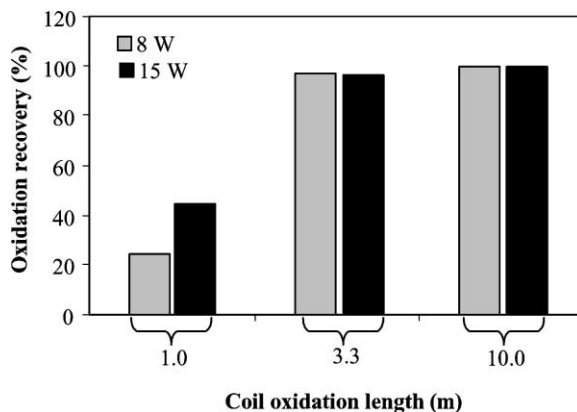


Fig. 2. Oxidation recovery of methylmercury promoted by UV radiation (8 and 15 W) using three coil lengths (1.0, 3.3 and 10.0 m).

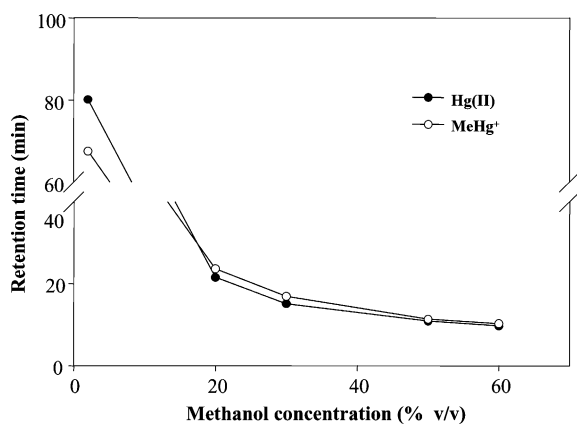


Fig. 3. Retention times of methylmercury and inorganic mercury using different quantities of methanol in mobile phase (0.01%, v/v of 2-mercaptoethanol).

solution of pH = 5 was selected [13,15,35] for different amounts of methanol (2–60%, v/v). The separation of Hg(II) and MeHg<sup>+</sup> was only effective for low percentages of methanol (Fig. 3). Peak splitting and peak broadening was not observed under these conditions. The retention time of those mercurial species varied less than 3% when 2-mercaptoethanol concentration varied between 0.0007 and 0.5% (v/v). The highest value of methylmercury height peak was obtained for 0.010% (v/v) of 2-mercaptoethanol.

#### 3.1.4. Water vapour removal system

The hygroscopic tubular membrane usually chosen to remove water from Hg vapour when using AFS detection has a low performance for long working periods owing to saturation. Since daily regeneration [36] would not be recommendable for routine works, hygroscopic tubular membrane was replaced by a water vapour removal system proposed by Falter and Ilgen

Table 1  
Operational working conditions

Parameter	Value
Mobile phase composition	5% (v/v) Methanol + 0.01% (v/v) 2-mercaptoethanol at pH 5
Type of oxidation	UV (8 W)
Length of oxidation coil	3 m
Reducing agent	3% (m/v) SnCl <sub>2</sub> in 15% (v/v) HCl
Water removal system	H <sub>2</sub> SO <sub>4</sub> (0°C) + CaCl <sub>2</sub> (7 cm × 1 cm i.d.)

[17]. This system consists of cooling the gas–liquid separator cell and the reduction coil to 0°C with ice to lower eluent vaporisation during the elemental mercury generation process. A sulphuric acid cool trap (0°C) and a calcium chloride trap (7 cm × 1 cm, i.d.) were placed daily in the system after the gas–liquid separator cell. The two traps were justified, since a considerable amount of water was vaporized in the analytical process and it should be removed before reaching the detector. If the calcium chloride trap was used alone, the solid would become rapidly pasty. The sulphuric acid trap was very efficient because at the end of the day the increment in the volume of the solution was notorious, due to the presence of water. The calcium chloride trap, besides removing any residual water vapour, was maintained also to protect the cell detector from any acid vapour.

After all these preliminary experiments, the experimental conditions were selected as shown in Table 1.

#### 3.2. Sequential simplex method

The modified simplex optimisation method [33] was applied to six variables (flow rates of the mobile phase, water, reducing agent, carrier and shield argon, and the length of the reduction coil). The step size,

Table 2  
Step size, reference value and decimals of the optimised variables

Variable	Step size	Reference value	Lower limit	Upper limit	Decimals
Mobile phase flow rate (cm <sup>3</sup> min <sup>-1</sup> )	0.1	0.6	0.5	0.8	2
Water flow rate (cm <sup>3</sup> min <sup>-1</sup> )	0.2	1.5	0.5	2.0	1
Reducing agent flow rate (cm <sup>3</sup> min <sup>-1</sup> )	0.2	1.5	0.5	2.0	1
Argon carrier flow rate (cm <sup>3</sup> min <sup>-1</sup> )	100	200	0	300	0
Argon shield flow rate (cm <sup>3</sup> min <sup>-1</sup> )	100	200	0	300	0
Reduction coil length (cm)	50	226	100	300	2

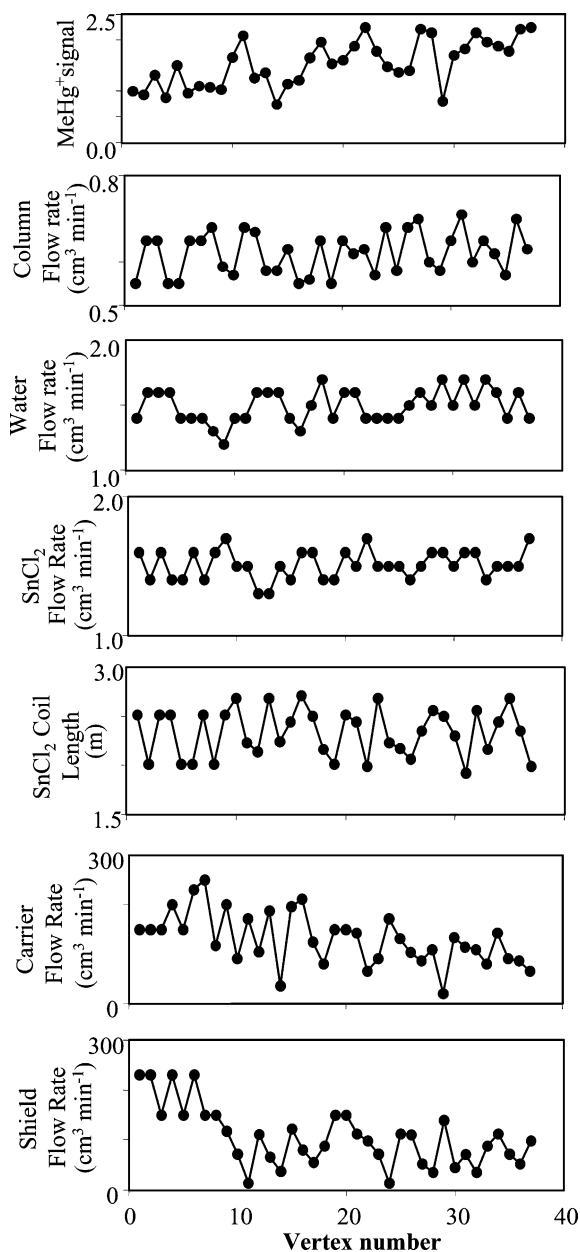


Fig. 4. Evolution of  $\text{MeHg}^+$  signal and variables along the simplex method.

reference value and decimals used in the multi-simplex procedure for each variable are presented in Table 2. The simplex progressed towards the optimum  $\text{MeHg}^+$  value as shown in Fig. 4, which shows also the

evolution of the variables along experiments. This evolution confirms small increases of the signal for a relatively large combination of variables. The carrier argon flow rate was the only variable that showed a tendency for a linear relationship with  $\text{MeHg}^+$  signal, for flow rates higher than  $60 \text{ cm}^3 \text{ min}^{-1}$  ( $\text{MeHg}^+$  signal =  $-64 \times 10^{-4} \cdot Q_{\text{carrier}} + 2.46$ ;  $r = 0.71$ ; S.D. for the slope and intercept =  $11 \times 10^{-4}$  and  $0.16$ , respectively;  $n = 35$ ). The optimum value of the carrier flow rate was around  $65 \text{ cm}^3 \text{ min}^{-1}$ . Other variables (flow rates of the mobile phase, water, reducing agent and shield gas and length of the reduction coil) did not show any particular pattern, originating similar  $\text{MeHg}^+$  responses for a large combination of values.

A total of 37 experiments were carried out. A chromatogram of a mixed standard of  $\text{MeHg}^+$  ( $2 \mu\text{g dm}^{-3}$ ) and inorganic mercury ( $2 \mu\text{g dm}^{-3}$ ) used in the simplex optimisation is shown in Fig. 5. The maximum  $\text{MeHg}^+$  response (2.24) was obtained with the experimental conditions described in Table 3, which lead to optimised retention times of 19 and 24 min for methylmercury and inorganic mercury, respectively. After the optimisation was concluded, the  $\text{MeHg}^+$  signal has been increased by a factor of 2 in relation to the initial conditions.

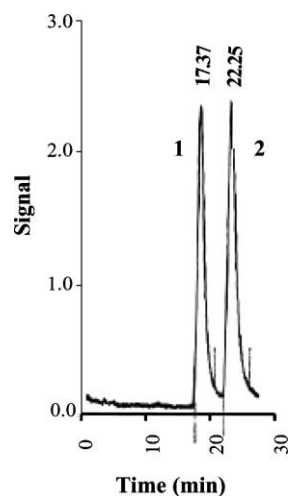


Fig. 5. Chromatogram of the mixed standard used in the simplex optimisation: peak 1, methylmercury; peak 2, inorganic mercury. Concentration of both species:  $2 \mu\text{g dm}^{-3}$ .

Table 3  
Optimised conditions

Variable	Optimised values
Mobile phase flow rate (cm <sup>3</sup> min <sup>-1</sup> )	0.63
Water flow rate (cm <sup>3</sup> min <sup>-1</sup> )	1.4
Reducing agent flow rate (cm <sup>3</sup> min <sup>-1</sup> )	1.7
Argon carrier flow rate (cm <sup>3</sup> min <sup>-1</sup> )	65
Argon shield flow rate (cm <sup>3</sup> min <sup>-1</sup> )	98
Reduction coil length (m)	1.99

### 3.3. Figures of merit

To determine the DL, the AFS detector was set to work at its maximum sensitivity (range = 1000 and gain = 10.0).

Repeatability of the analytical signal with 95% confidence level was determined as the product  $t_{\sqrt{2}}s$  [37] using four injections of a 300 ng dm<sup>-3</sup> (as Hg) MeHg<sup>+</sup> standard. The mean of the peak height was 6.38 cm and the repeatability was equal to 0.26 cm, which means the coefficient of variation ( $(s/\bar{x}) \times 100$ ) is lower than 1%.

The DL was calculated from the calibration curves in the range of 100–800 ng dm<sup>-3</sup> and based on the amount (or concentration) necessary to yield a net signal equal to three times the S.D. of the blank. The DL was  $10 \pm 2$  pg (mean  $\pm$  S.D.), corresponding to concentrations of  $51 \pm 9$  ng dm<sup>-3</sup>. Those values were lower than most of the results reported using other techniques (Table 4). The lowest DLs are reported when preconcentration coupled to a CV-AFS detector [17] and ICP-MS with a direct injection nebulizer [44] or cold vapor generation [13] was used. The DL obtained in this work is about half of the value reported by Hintelmann and Wilken [16], with a similar detection system. This improvement may be due to differences in the reducing agent employed, in the composition of mobile phase chosen and in the water vapour removal system used.

The DL found for Hg(II) was similar to those determined for MeHg<sup>+</sup>, since the oxidation percentage obtained in this work ranged from 95 to 100%.

### 3.4. Application of the methodology

The analytical performance of the methodology proposed in this work was evaluated by the analysis of

Table 4  
Methylmercury DL reported in previous HPLC works

Method	DL of MeHg <sup>+</sup> (ng)
HPLC-Amperometric/ Coulometric [24]	0.16–1.0
HPLC-UV-VIS [38,39]	0.10–25
HPLC-CV-AAS [15,40]	$80 \times 10^{-3}$ to 1.0
HPLC-CV-AAS <sup>a</sup> [41,42]	$20 \times 10^{-3}$ to 0.10
HPLC-CV-AFS [16]	$20 \times 10^{-3}$
HPLC-CV-AFS <sup>a</sup> [17]	$8.0 \times 10^{-3}$
HPLC-ACP-AES [19]	70
HPLC-ICP-AES [18]	$46 \times 10^3$
HPLC-CV-ICP-AES [18]	7.4–9.4
HPLC-CV-MIP-AES [43]	$35 \times 10^{-3}$
HPLC-DIN <sup>b</sup> -ICP-MS [44]	$7.0 \times 10^{-3}$
HPLC-USN <sup>c</sup> -ICP-MS [45]	0.14
HPLC-PN <sup>d</sup> -ICP-MS [23]	0.70
HPLC-CV-PN <sup>d</sup> -ICP-MS [13,23]	$3.0 \times 10^{-3}$ to $60 \times 10^{-3}$
This work	$10 \times 10^{-3}$

<sup>a</sup> With preconcentration.

<sup>b</sup> DIN: direct injection nebulizer.

<sup>c</sup> USN: ultrasonic nebulizer.

<sup>d</sup> PN: pneumatic nebulizer.

a certified reference sediment (CRM 580) using alkaline digestion. Several works have shown concern over the possible formation of MeHg artefacts during certain analytical procedures, such as distillation [46,47] and alkaline dissolution. The amount of MeHg formed was very small, varying from about 0.005 to 0.1% of the spiked Hg<sup>2+</sup> [46]. Liang and Lazoff [48] also demonstrated that the artefact formation in alkaline digestion/solvent extraction procedure is insignificant for sediment samples.

The extraction of mercury compounds was carried out in an ultrasonic bath. This treatment was performed at two different times (45 min and 3 h), as shown in Table 5. The 45 min treatment yielded low recoveries, while a good agreement with the certified

Table 5  
Results of determination of methylmercury in CRM 580 by solvent extraction after alkaline digestion performed in ultrasonic bath (mean value  $\pm$  S.D.)

Ultrasonic bath treatment	[MeHg <sup>+</sup> ] (ng g <sup>-1</sup> as Hg)		Recovery (%)
	Found	Certified	
45 min	$42.6 \pm 2.5$	$75.5 \pm 5.5$	$56.6 \pm 3.3$
3 h	$70.6 \pm 1.4$	$75.5 \pm 5.5$	$93.6 \pm 1.8$

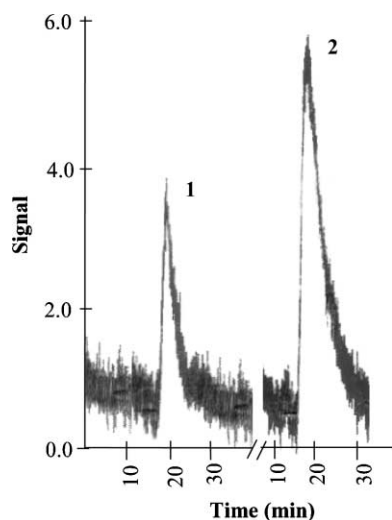


Fig. 6. Chromatogram of the CRM 580: peak 1, methylmercury; peak 2, methylmercury  $\pm$  standard addition of  $0.49 \mu\text{g dm}^{-3}$  methylmercury standard.

value for methylmercury was only obtained with the 3 h treatment. The concentration of methylmercury determined in the CRM was not statistically different ( $\alpha = 0.05$ ) from its certified value both for the S.D. and the mean values. A chromatogram of a CRM 580 sample without addition and a sample of the same material added with  $0.49 \mu\text{g dm}^{-3}$  of  $\text{MeHg}^+$  standard, is shown in Fig. 6. Since the peak of methylmercury is the only analytical signal observed, the extraction method can be considered selective for the organic forms of mercury. The higher noise of the baseline observed when compared to the chromatogram of the mixed standard, is due to working at the maximum sensitivity of the detector. The obtained peak for methylmercury was smaller than it was expected considering the aqueous standards, which could be due to a possible matrix effect. Taking into account the need for using the standard addition method, the sample throughput of the methodology was around four samples for every 6 h of operation, if four standard additions were performed in duplicate.

#### 4. Conclusion

A low DL methodology ( $10 \pm 2 \text{ pg}$ ) for separation and determination of  $\text{MeHg}^+$  and  $\text{Hg(II)}$  has been

accomplished by HPLC-UV-CV-AFS. The UV oxidation of organomercury compounds discards the addition of any chemicals to the sample and can be performed at laboratory temperature in smaller coil lengths when comparing to the wet oxidation method. The tin(II) chloride reduction of mercury avoids the production of hydrogen that causes quenching of mercury fluorescence. The water vapour trap based on  $\text{H}_2\text{SO}_4/\text{CaCl}_2$  allows operation for longer periods of time without interruption. However, it is needed to replenish the drying tubes daily. The analytical conditions were optimised with a modified simplex method leading to an increase of the signal by a factor of 2. The method performance has been tested by determining the methylmercury concentration in a certified reference sediment and the result obtained was not statistically different from the certified value ( $\alpha = 0.05$ ). The sample throughput of the proposed methodology was around four samples for 6 h of operation, when using four standard additions in duplicate.

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