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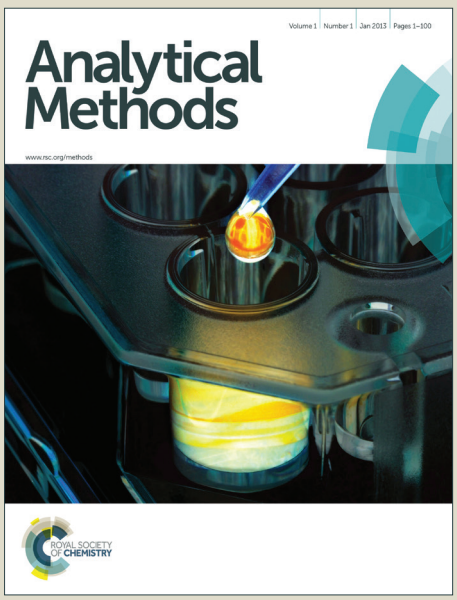

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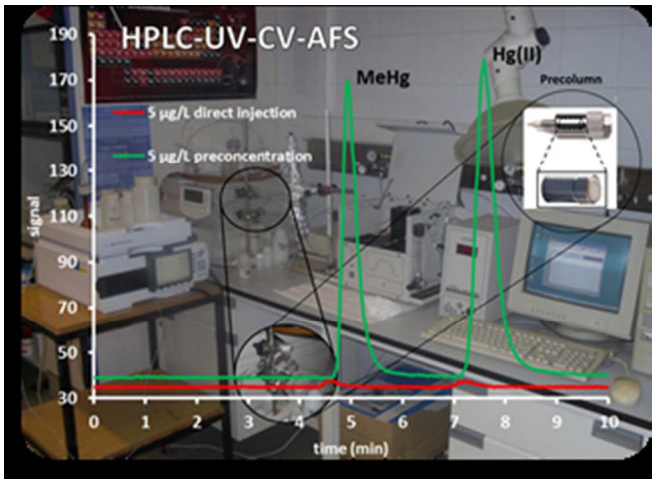

The online preconcentration and speciation of mercury in waters developed method involves low sample volume and simple sample pre-treatment obtaining good recoveries regardless of the water matrix composition.
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Mercury (II) and methylmercury determination in waters by liquid chromatography hyphenated to cold vapour atomic fluorescence spectrometry after online short-column preconcentration

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ABSTRACT

This paper reports a method developed for the simultaneous determination of methylmercury ($MeHg^{+}$) and mercury (II) (Hg^{2+}) species in waters by liquid chromatography coupled to online UV irradiation and cold vapour atomic fluorescence spectroscopy (LC-UV-CV-AFS) after online short-column preconcentration. This work focused on systematic studies of several variables to establish the maximum species recoveries, preconcentration factors and good reproducibility. The optimum results obtained were the following: 2-mercaptoethanol 0.07 mmol L^{-1} as a complexing agent, precolumn conditioning with the mobile phase: a mixture of 80 % of Methanol (MeOH) and 20 % of the following buffer: 0.0015 mol L^{-1} ammonium pyrrolidine dithiocarbamate (APDC) and 0.01 mol L^{-1} ammonium acetate (NH_4CH_3COO) at pH 5.5, 2 cm precolumn length and 2 mL min^{-1} sample flow.

The method was applied to three water samples with different mineralisation content. Various tests, based on spikes, were performed on each sample. A breakthrough volume of 4 mL was found. Recovery values of 72±3% and 81±5% for $MeHg^{+}$ and Hg^{2+}, respectively, were obtained regardless of the matrix composition, and the PF values were 30 and 32 for $MeHg^{+}$ and Hg^{2+}, respectively.

The accuracy of the preconcentration method was verified by analysing a certified reference material. The detection limits (LDs) obtained were 15 ng L^{-1} for $MeHg^{+}$ and 2 ng L^{-1} for Hg^{2+}. The quantification limits (LQs) were 50 ng L^{-1} for both species. The established analytical online preconcentration method is suitable for the quantification of mercury species in a wide range of environmental waters.

Keywords: mercury speciation, waters, LC-UV-CV-AFS, preconcentration.

INTRODUCTION

The determination of mercury species in environmental samples is of global concern, because of their natural persistence in the environment and the distinct mechanisms whereby they change their chemical form [1], which affects their distribution and toxicity. The most relevant species in the environment are elemental mercury (Hg^{0}), mercury (II) (Hg^{2+}), monomethylmercury (CH_3Hg^{+}, $MeHg^{+}$), dimethylmercury (DMeHg) and monoethylmercury ($EtHg^{+}$). Organic mercury compounds tend to be much more toxic than mercury (II), and mercury (II) is more toxic than the elemental form. $MeHg^{+}$ is the form in which mercury accumulates and biomagnifies in the aquatic food chain due to its high liposolubility [2]. It is absorbed, transported through biological membranes and accumulated on nerve cells. Due to the decrease in production and use of organomercurials, methylmercury ($MeHg^{+}$) is by far the most common organomercury compound in the environment [3].

Mercury is released into the environment from both natural sources and as a result of human activities. Once it has entered the environment, mercury cycles occur between air, land and water. In these cycles, mercury species may be converted [1]. A relevant transformation process in aquatic environments is mercury (II) conversion into

monomethylmercury by microorganisms and microalgae [4]. Therefore, water is one of the most relevant studied environmental compartments. It not only has a great impact on the environment, but also on human health because safe water is essential to human activity.

The European Water Directive [5], which seeks to establish a framework for the protection of groundwater and surface waters, includes mercury and its compounds in a list of priority and hazardous substances. Therefore, it is one of the elements to be considered in evaluations of the status of physico-chemical water quality. However, at present, the European Drinking Water Directive considers only total mercury concentration, and establishes the parametric value of 1 μg L^{-1}. [6]

Mercury concentrations in waters are expected to be very low [7]. Besides, methylmercury levels tend to be much lower than those of mercury (II), due to decomposition of organic compounds by solar UV light and the difficulty of methylation reactions in the aqueous phase. The mean reported for Hg concentration in water is 2 ng L^{-1}. [8] $MeHg^{+}$ concentration corresponds to a 1% of this value, and the rest is mercury (II). The concentration of mercury is normally in the range of 1–20 ng L^{-1} in open-ocean water, while up to 100 ng L^{-1} is usually found in coastal water, owing to anthropogenic discharges [9]. In the literature, analytical methods using CV-AFS or CV-AAS detectors without a preconcentration step have limits of quantification higher than the Hg concentrations in waters [3, 10–13]. Therefore, because of the extremely low concentrations of mercury in this type of samples, highly sensitive, simple and rapid methods are required. Consequently, preconcentration systems need to be developed.

Several extraction and preconcentration methods have been reported for the enrichment of mercury species applied mainly in environmental waters. The main approaches for the preconcentration of trace elements from water are liquid-liquid extraction (LLE) [14]

and solid phase extraction (SPE). Comparatively, SPE is more environmentally friendly, as it is free of toxic organic extraction reagent. Most importantly, its stronger tolerance to complex matrices endows it with better capability of online application [9]. In solid phase extraction as a preconcentration step, C18 cartridges have been the most widely used stationary phase, both directly and after derivatisation [15–24] with a wide range of complexing agents, most of which contain sulphur, such as 2-mercaptoalcohols, dithiocarbamates, dithizones, triazenes, even bacteria [15–32]. A wide variety of eluting agents have also been used to desorb mercury species from the stationary phase, such as acidic solutions, thiourea solutions, mobile phases with organic– modifiers, aqueous solutions with a reagent containing sulphur, even a mixture of these kinds of solutions with an organic solvent, among others [9, 14–35].

After elution, a separation procedure has sometimes been applied. In some cases, gas-chromatography or liquid chromatography was performed to separate mercury species [9, 15–18, 20–22, 24, 25, 29, 33, 36]. In others, selective retention or elution of mercury species was carried out using different complexing agents or eluting agents for each species [19, 26, 28, 30, 31, 34, 35]. A wide variety of detectors have been used, either for offline preconcentration or online flow injection preconcentration. Ultraviolet detection (UV), ICP-MS and atomic absorption or fluorescence spectrometry with cold vapour generation (CV-AAS and CV-AFS) are the most relevant systems of detection reported [9, 15–24, 26–36]. ICP-MS is the most sensitive of these detectors. However, an online pre-concentration system coupled to CV-AFS could provide similar analytical performance by using a simpler set-up and with a lower investment.

As the reported methods for the mercury preconcentration are mainly applied to natural waters, such as sea, river, spring, lake, rain and underground waters, among others, there is a lack of studies applied to drinking waters. A few studies are applied to tap

water. Thus, the aim of this paper is to develop an online method for mercury (II) and methylmercury determination by high-performance liquid chromatography hyphenated to cold vapour atomic fluorescence spectrometry after short-column preconcentration. The established method was applied to determine mercury species in drinking water samples of different matrix composition, including a certified reference material of wastewater.

EXPERIMENTAL

Instrumentation

The LC system consisted of a quaternary pump and degasser (Agilent Technologies 1100, Waldbronn, Germany), equipped with a manual stainless steel sampler injector (Rheodyne Model 7725i) and a 100 µL sample loop.

The separation of mercury species (Hg^{2+} and $MeHg^{+}$) was achieved in an analytical RP-C18 column (ODS Hypersyl 250 mm × 4.6 mm id, 5 µm, Thermo Hypersil-Keystone).

After separation, a photo-oxidation step was performed in a 12-meter length × 0.5 mm id PTFE tube coiled around a UV lamp with 150 W of power irradiation (Heraeus TQ 150).

The reduction step was achieved in a cold vapour generator (CV) 10004 (P.S. Analytical, Orpington, UK), in which the effluent was mixed with the reducing agent. The metallic mercury vapour that was obtained reached the gas-liquid separator, from which it was dragged into the detector by an argon stream (300 mL min^{-1}) and dried in a PermaPure membrane with nitrogen (2.5 L min^{-1}). Measurements were made using a Merlin Mercury Atomic Fluorescence Detector model 10023 (P.S. Analytical).

Reagents and Standards

Only analytical grade reagents were used. The standards and reagents in this study were prepared with doubly deionised water (Elix&Rios 5–15MΩ cm^{-1}, Total Organic Carbon <30 μg L^{-1}) obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA).

An mercury (II) stock standard solution of 1000 mg L^{-1} was prepared by dissolving appropriate amounts of mercury chloride, $HgCl_2$ (Merck, Darmstadt, Germany) in 1% (V/V) HNO_3, from nitric acid 69% (Panreac, Hiperpur). A methylmercury stock standard solution of 1000 mg L^{-1} was prepared by dissolving appropriate amounts of CH_3HgCl (Carlo Erba, Milan, Italy) in 3% methanol (Panreac, p.a.). All stock standard solutions were stored at 4°C. The working standard solutions were prepared daily from the stock standard solutions by appropriate dilution.

For the cold vapour generation, $SnCl_2$ solution was prepared daily from tin chloride 2-hydrate (Panreac, p.a.) to a 1.5% concentration, in 4% of HCl, from hydrochloric acid 35% (Panreac, Hiperpur).

The mobile phase was prepared daily by dissolving appropriate amounts of ammonium pyrrolidine dithiocarbamate, APDC (Fluka, p.a.), and ammonium acetate, NH_4CH_3COO (Merck, p.a.) in water. The pH was adjusted to 5.5 with diluted acetic acid (Panreac, p.a.) and then filtered on 0.45 μm filter paper (Millipore type HA). The final mobile phase composition was a mixture of 80 % of MeOH LC gradient grade (Panreac, p.a.) and the prepared buffer: 0.0015 mol L^{-1} APDC and 0.01 mol L^{-1} NH_4CH_3COO.

For the preconcentration step, 2-mercaptoethanol and APDC (Fluka, p.a.) were used as a complexing agent for mercury species in working solutions and water samples, taking appropriate amounts.

Certified reference material (CRM) of wastewater effluent acidified with HNO_3 to about pH 2 to stabilise the trace amounts (ERM-CA713) was used for quality control. It was

obtained from the Institute for Reference Materials and Measurements of the European Commission's Joint Research Centre, Geel, Belgium.

Samples

Three samples, tap water and weak and strong mineralised bottled waters, were filtered through a filter with 0.22 μm pore size. The origin, pH and conductivity values for each sample after filtration are shown in Table 1, together with some anion and cation content determined by anionic exchange chromatography and ICP-OES, respectively. Final solutions of 0.5 μg L^{-1} and 5 μg L^{-1} for the two mercury species with the appropriate amount of complexing agent were prepared by making up the volume with the corresponding water matrix: double deionised water, weak and strong mineralised bottled water or tap water, prior to the preconcentration step.

Preconcentration system

A previously developed and validated LC-UV-CV-AFS method for the separation of mercury species was adapted. The experimental conditions of the hyphenated technique are described in Ibáñez-Palomino et al. [3].

In order to couple the online preconcentration system prior to the LC-UV-CV-AFS, the original sample loop was replaced with a short RP C18 precolumn with the same characteristics as the separation column: ODS Hypersyl 10, 20 or 50 mm × 4.6 mm id, 5 μm, Thermo Hypersil-Keystone, which was connected by an isocratic LC pump (Agilent Technologies 1260, Waldbronn, Germany) and a six channel valve (Rheodyne Model 7000 6-port). This system alternates the sample flow and the mobile phase passing through the precolumn, which allows the loading of different sample volumes to the precolumn, so as to preconcentrate mercury species. When the valve is in the load

position, the sample passes through the precolumn and mercury species are adsorbed on the stationary phase. In the inject position, the mobile phase passes through the precolumn and elutes the retained mercury species to the LC-UV-CV-AFS system for determination. Figure 1 shows a schematic diagram of the online preconcentration system coupled to LC-UV-CV-AFS for the determination of trace mercury species in water samples.

The samples were quantified by means of an external calibration curve from methylmercury and mercury (II) standards from 2.5 μg L^{-1} to 750 μg L^{-1}. They were prepared by appropriate dilution in MeOH:APDC 80:20 and they were injected in the LC-UV-CV-AFS system using the 100 μL loop represented in Figure 1.

RESULTS AND DISCUSSION

To set the working standard concentration for the preconcentration studies, detection and quantification limits of the previously established LC-UV-CV-AFS method [3] were assessed again with the current instrumental conditions. The detection limits (calculated as 3 SD_{BLANK}/slope; n = 23) were 0.53 and 0.57 μg L^{-1} for $MeHg^{+}$ and Hg^{2+}, respectively. The quantification limits (calculated as 10 SD_{BLANK}/slope; n = 23) were 1.80 and 1.90 μg L^{-1} for $MeHg^{+}$ and Hg^{2+}, respectively. The values were of the same order of magnitude of those previously reported. Linearity range was observed to be lineal up to 750 μg L^{-1}. [3]

Different tests using several replicates of working standard solution containing mercury species at a concentration of 5 μg L^{-1}, which is slightly higher than the limit of quantification, were performed to establish the preconcentration method. Even if the preconcentration system increased the signal for the working standards, a lack of reproducibility and strong memory effects were observed. Thus, systematic studies of

several variables were undertaken to assess the load volume, preconcentration factors (PF) and recoveries. PFs were calculated as the ratio between the concentration obtained after preconcentration and the initial concentration. Recovery values were calculated as the ratio between the experimental concentration obtained and the theoretical.

Assessment of the preconcentration step

Initial preconcentration tests working with standards showed a lack of reproducibility of the signal or even no detection of the species in the elution step when no complexing agent was added to the working standard solutions. Thus, the use of a complexing agent which is able to retain mercury species was studied.. Two complexing agents, APDC and 2-mercaptoethanol, were tested as commonly used in the literature [17, 22] at concentrations 2 mmol L^{-1} and 14 mmol L^{-1}, respectively. Working standard solutions of 0.05, 0.5 and 5 μg L^{-1} of $MeHg^{+}$ and Hg^{2+} were prepared with the complexing agent, and were preconcentrated in different working sessions.

When working with APDC, the presence of low peak signals at retentions times different from those attributed to the mercury species in the separation method has been observed with a lack of reproducibility. Even if a significant retention can be achieved with APDC, an incomplete elution of the species as well as memory effects was observed. When using 2-mercaptoethanol as complexing agent, both species were eluted at the expected retention times, with a good signal and overcoming the previous observed problems. Thus, further studies were performed using 2-mercaptoethanol as complexing agent to establish its concentration.

Concentrations of 2-mercaptoethanol from 0.07 to 140 mmol L^{-1} were tested. Different sample volumes of these standard solutions were preconcentrated in three working sessions using a 1 cm-long precolumn. When the highest concentration of 2-

mercaptoethanol (140 mmol L^{-1}) was used, different signals that did not correspond to neither $MeHg^{+}$ nor Hg^{2+} were observed. These additional signals could be due to the formation of undesired complexes of $Hg(CH_3OH)$:mercaptoethanol [37]. Concentrations of the complexing agent from 0.07 to 14 mmol L^{-1} did not show any additional signals, apart from mercury species. Figure 2 shows the breakthrough volume obtained. As can be seen, at the lower 2-mercaptoethanol concentration, higher sample volumes could be loaded in the precolumn before achieving the breakthrough point. Consequently, higher preconcentration factors were obtained. Thus, 0.07 mmol L^{-1} was selected as the working concentration.

Conditioning the precolumn with 2-mercaptoethanol 0.07 mmol L^{-1} caused a decrease in peak intensity, because the retention of the free thiol groups in the C18 precolumn decreased the amount of stationary phase available for the retention of $MeHg^{+}$ and Hg^{2+} complexes. Thus, in further experiments, the precolumn was only conditioned with mobile phase.

The sample loading at different flows was also assessed to study the possible impact of this variable on mercury species signals. Two different sample volumes, 2 and 5 mL, were preconcentrated at five different flows, from 1 to 5 mL min^{-1} using a 1 cm-long precolumn. The peak signals obtained at 1 and 2 mL min^{-1} flow were of the same order of magnitude, but from 3 mL min^{-1} flow, the signal of both species decreased gradually. When the flow rate was increased, the contact time was not enough to achieve equilibrium between the mobile and stationary phases. Thus, 2 mL min^{-1} was selected for further assays.

The effect of precolumn length was studied to assess the retention capability of mercury species in the stationary phase. Three columns of different lengths were selected: 1, 2 and 5 cm. Two working standard solutions of $MeHg^{+}$ and Hg^{2+} at concentrations of 0.5

and 5 µg L^{-1} of both species were initially prepared in 2-mercaptoethanol 0.07 mmol L^{-1}. Increasing volumes of these solutions were tested until the breakthrough point. As an example, Figure 3 represents the mercury species concentrations obtained in the preconcentration of a given volume in working solutions of 5 µg L^{-1}. As can be observed, the 1 cm-long precolumn breakthrough volume for both species was lower than 8 mL. However, in 2 and 5 cm-long precolumns, these volumes increased up to 14-18 mL. In all cases, the breakthrough volumes were higher for Hg^{2+} than for $MeHg^{+}$, due to the higher affinity of this species for the C18 stationary phase.

Preconcentration factors and recoveries at the breakthrough volume including the standard deviation are plotted in Figure 4A for both species in each precolumn. Higher preconcentration factors were obtained when 2 and 5 cm precolumns were used, due to the fact that their retention capability is higher than that of the 1 cm precolumn. Regarding percent recoveries, similar values were obtained among the three precolumns and they ranged from 60 to 80%.

Even if preconcentration factors provided by 2 and 5 cm precolumns were suitable, the observed chromatographic behaviour of both systems was different, as shown in Figure 4B. Direct injection of 5 µg L^{-1} standard has also been included for comparison purposes. As can be seen, mercury (II) did not present Gaussian behaviour when the breakthrough volume was preconcentrated in a 5 cm precolumn. This effect could be because the precolumn is long enough for the mercury (II) separation process to start before the analytical column is reached. Thus, it can be concluded that a 2 cm column is most suitable for the preconcentration method.

Application in water samples

Once the most appropriate conditions for online preconcentration had been selected, the water samples were tested. Three water samples of increasing complexity (weak mineralisation, strong mineralisation and tap water) were characterised following the procedure described in section "Samples". To ensure if the samples could have or not trace amounts of mercury, total mercury content was determined in all matrices by ICP-MS, and the Hg content was under the detection limit (0.05 μg L^{-1}). Samples were spiked at three levels: low-level (0.5 μg L^{-1} of both species), medium-level (0.5 μg L^{-1} of $MeHg^{+}$ and 5 μg L^{-1} of Hg^{2+}) and high-level (5 μg L^{-1} of both species). The samples were then preconcentrated until the breakthrough volume was achieved for each matrix. Due to a matrix effect, both breakthrough volumes and preconcentration factors were lower in water samples (≈ 7 mL and ≈ 50, respectively) than in double deionised water (16 mL and ≈ 120, respectively). This effect may be due to a possible competition of other substances present in water samples in addition to mercury with the precolumn stationary phase, which can lead to a decrease in its retention capacity. However, recovery values of both species were of same order of magnitude as those previously described in section "Precolumn length" and ranged from 67 to 86%, regardless of the type of water, which may indicate that this parameter is independent from matrix composition. Higher PF and recoveries for Hg^{2+} were also observed.

From the data obtained, it was considered that the most suitable breakthrough volume for routine analysis is the obtained for the most complex matrix (tap water) and for the least retained species ($MeHg^{+}$), which are the worst retention conditions: 4 mL. This volume allows us to work under reproducible conditions with good recoveries, regardless of the type of sample and the concentration levels.

Table 2 shows the preconcentration factor, recovery, mean values and standard deviation for a 4 mL preconcentration volume. The overall average represents the mean

of each replicate. The PF values were 30±1 and 32±3 for $MeHg^{+}$ and Hg^{2+}, respectively. The recovery values were 72% $MeHg^{+}$ and 81% for Hg^{2+} and the RSD means were below 15%. As it can be seen, methylmercury recoveries are always lower than those obtained for the Hg^{2+}. The possible justification to this behaviour is that MeHg+-mercaptoethanol complexes present less affinity for C18 than the Hg2+ ones. The higher affinity of Hg2+ for the C18 could be due to the stoichiometry of the formed complex. Hg2+ forms 1:2 complexes with 2-mercaptoethanol and APDC whereas MeHg+ forms 1:1 complexes. The 1:2 complex presents more retention in C18 than 1:1 complex because it has more sulphur atoms in the structure, which are the main responsible of the retention process in C18.

Considering that the waters that were analysed had different matrixes, the standard deviations obtained were suitable and the similarity between the PF and recovery values demonstrates the robustness of the established conditions for the online preconcentration system.

Thus, Table 3 summarises the optimum conditions for the determination of $MeHg^{+}$ and Hg^{2+} by LC-UV-CV-AFS following online preconcentration.

Analytical figures of merit

Accuracy

The method's accuracy was assessed by the analysis of a certified reference material (CRM). To our knowledge there are no CRMs for Hg^{2+} and $MeHg^{+}$ species in natural waters. Most of the CRMs available for total mercury consist of spiked water samples [7]. To evaluate the preconcentration method, the most suitable CRMs would be waters with a total mercury level close to the limit of quantification of the analytical technique without the preconcentration step. It was only found wastewater with certified values

for the total content of 10 elements including mercury (ERM-CA713, 1.84±0.11 μg Hg L^{-1}). Total Hg content was analysed in the CRM by CV-AFS, which provided a mercury concentration of 1.81±0.03 μg L^{-1} (n=3). No significant difference was found between the certified and experimental total content (*t*-test at 95% confidence level).

Mercury species in the CRM were analysed by direct injection and after the online preconcentration step, using the previously established optimised conditions. A total of 4 mL of wastewater were preconcentrated and the PF obtained in section "Application in water samples" (see Table 2) were applied. The analyses were performed in triplicate. Table 4 summarises the results obtained by the two speciation methods. Regarding the direct injection method, the concentration of methylmercury was below the limit of detection, whereas the concentration of mercury (II) in the CRM was close to the limit of quantification.

In the preconcentration method, both species were well-quantified. Regarding the sum of species, a *t*-test (95% confidence level) was performed with respect to the certified value. No significant difference was found. The results show that the preconcentration method can quantify all mercury species, and the sum of them can be used to determine the total mercury content in water.

Limits of detection and quantification

Limits of detection and quantification for the online preconcentration method were assessedexperimentally by injecting standard solutions from 1 to 500 ng L^{-1}. Hg^{2+} was detected at about 2 ng L^{-1} whereas $MeHg^{+}$ was detected at about 15 ng L^{-1}. Experimental limits of quantification were 50 ng L^{-1}.

Limit of detection and quantification concentrations were considerably lower than those obtained by the direct injection method: values were in the order of μg L^{-1}, compared to values in the order of tens of ng L^{-1}, using a non-expensive technique.

However, the preconcentration of samples with a low complexity matrix would decrease the limits of detection and quantification in the online preconcentration method, by using a higher load volume.

Table 5 compares the detection limits and recoveries obtained in this paper with those previously reported in the literature using similar methodology. The recoveries obtained are comparable, and the detection limits are of the same order of magnitude when the total amount of mercury detected is considered. As expected, the detection limits obtained with ICP-MS are lower than those obtained with AAS or AFS. Nevertheless, CV-AFS provides suitable analytical performance, is user-friendly and requires lower investment and maintenance costs than ICP-MS, so it is a good approach in daily routine laboratory analysis.

CONCLUSIONS

An online preconcentration method for $MeHg^{+}$ and Hg^{2+} determination, the most relevant mercury species present in the environment, was developed using a 2 cm ODS Hypersil (C18; reverse phase) precolumn in the preconcentration step. These precolumns are commercially available and widely used in routine analysis laboratories. The method requires a low volume (4 mL) and a simple sample pre-treatment (addition of 2-mercaptoehtanol 0.07 mmol L^{-1}). The online preconcentration-LC-UV-CV-AFS system provides recoveries of 72±3% and 81±5% for $MeHg^{+}$ and Hg^{2+}, respectively, which were obtained regardless of the matrix composition.

The sum of the species in the proposed method matched with total mercury content. The limits of detection and quantification established are suitable for analytical performance using environmental samples. Thus, the method is widely applicable, highly precise and accurate, and could be useful for $MeHg^{+}$ and Hg^{2+} determinations, in response to any future legislation on mercury species.

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452 Table 1. Characteristics of the water samples tested.

	Weak mineralised water	Strong mineralised water	Tap water
pH	6.8	7.8	8.1
Conductivity ($\mu S\ cm^{-1}$)	66	767	547
Cl^- ($mg\ L^{-1}$)	1.8	7.1	34.1
F^- ($mg\ L^{-1}$)	0.06	0.16	0.10
NO_3^- ($mg\ L^{-1}$)	1.7	0.56	5.6
SO_4^{2-} ($mg\ L^{-1}$)	5.5	120	43.5
Ca^{2+} ($mg\ L^{-1}$)	3.2	94	52.9
Mg^{2+} ($mg\ L^{-1}$)	3.5	43	9.0
Na^+ ($mg\ L^{-1}$)	1.6	7.7	20.7
K^+ ($mg\ L^{-1}$)	1.4	2.5	3.3

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Table 2. Preconcentration parameters obtained for each species in water samples for a 4 mL preconcentration volume.

Species	Sample (water)	PF[a]	Recovery [b] (%)	RSD [b] (%)
$MeHg^{+}$	Double deionised	29±2	73±5	2
	Weak mineralised	30±2	74±6	8
	Strong mineralised	30±1	67±9	13
	Tap	27±4	74±2	2
	Overall Average	30±1	72±3	4
Hg^{2+}	Double deionised	32±2	80±4	5
	Weak mineralised	34±1	86±4	5
	Strong mineralised	34±2	84±5	6
	Tap	35±5	87±9	10
	Overall Average	32±3	81±5	6

[a] Preconcentration factor.

[b] n=3

Table 3. Final selected conditions for online preconcentration of $MeHg^{+}$ and Hg^{2+} by LC-UV-CV-AFS.

Optimum conditions	
Precolumn conditioning	Mobile phase
Complexing agent	2-mercaptoethanol 0.07 mmol L^{-1}
Sample flow	2 mL min^{-1}
Precolumn length	2 cm
Preconcentration volume	4 mL

Table 4. Methylmercury and mercury (II) concentration obtained in ERM-CA713 (certified value: 1.84±0.11 μg Hg L^{-1}) by direct injection and online preconcentration.

Species	Direct injection		Online preconcentration	
	C(μg L^{-1})	RSD (%)	C(μg L^{-1})	RSD (%)
$MeHg^{+}$	< LD	-	0.28±0.01	2.9
Hg^{2+}	1.71±0.02	1.2	1.72±0.06	3.6
Sum of species	1.71±0.02	1.2	2.00±0.06	3.0

Table 5. Online preconcentration of mercury species in water samples.

Mercury species	Matrix	Complexing agent	Retention/Elution	Instrumental method	Absolute LD (pg)	Recoveries (%)	Reference
$MeHg^{+}$ Hg^{2+}	Bottled water Tap water	2-mercaptoethanol 0.07 mM	Retention in a C18 microcolumn and elution with MeOH:ADPC 1.5 mM pH 5.5 (80:20)	LC-UV-CV-AFS	60 8	69-86	Present method
$MeHg^{+}$ $EtHg^{+}$ $PhHg^{+}$ Hg^{2+}	Human urine	APDC 2 mM	Retention in a C18 microcolumn and elution with MeOH:ACN:APDC 1.5 mM (38:30:32)	LC-CV-AAS	526.5 351 585 292.5	92-106	[15]
$MeHg^{+}$ Hg^{2+}	Brackish water	APDC 2 mM	Retention in a C18 microcolumn and elution with MeOH:APDC 1.5 mM (50:50)	LC-CV-AAS	1.7 3.4	-	[17]
$MeHg^{+}$ Hg^{2+}	Seawater from lagoon	2-mercaptoethanol	Retention in a microcolumn C18 modified with 2-mercaptoethanol and elution with H_2O with 0.5 % L-cysteine and 0.05 % 2-mercaptoethanol	LC-ICP-MS	0.6 2.4	84-108	[20]
$MeHg^{+}$ $EtHg^{+}$ Hg^{2+}	Sea water	-	Retention in a cation exchange microcolumn and elution with MeOH:L-cysteine 10 mM pH 8 (4:96)	LC-ICP-MS	0.48 0.24 1.26	87-102	[9]

FIGURE CAPTIONS

Figure 1. Schematic representation of the online preconcentration system hyphenated to LC-UV-CV-AFS: (a) of the sample on the precolumn, (b) Elution of the sample to the separation column.

Figure 2. Breakthrough volume obtained versus complexing agent concentration in a 5 µg L^{-1} $MeHg^{+}$ and Hg^{2+} standard. Precolumn length: 1 cm.

Figure 3. Mercury species concentrations obtained versus volume preconcentrated on working solutions of 5 µg L^{-1}.

Figure 4. $MeHg^{+}$ and Hg^{2+} recoveries, preconcentration factors (A) and chromatograms obtained (B) from a 5 µg L^{-1} standard at the breakthrough volume in each precolumn, together with a direct injection of this standard.