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Speciation analysis of mercury in seawater from the lagoon of Venice by on-line pre-concentration HPLC–ICP-MS

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ABSTRACT

A method based on the coupling of HPLC with ICP-MS with an on-line pre-concentration micro-column has been developed for the analysis of inorganic and methyl mercury in the dissolved phase of natural waters. This method allows the rapid pre-concentration and matrix removal of interferences in complex matrices such as seawater with minimal sampling handling. Detection limits of 0.07 ng L^{-1} for inorganic mercury and 0.02 ng L^{-1} for methyl mercury have been achieved allowing the determination of inorganic mercury and methyl mercury in filtered seawater from the Venice lagoon. Good accuracy and reproducibility was demonstrated by the repeat analysis of the certified reference material BCR-579 coastal seawater. The developed HPLC separation was shown to be also suitable for the determination of methyl mercury in extracts of the particulate phase.

1. Introduction

The most common methods currently in use for the speciation analysis of mercury species are chromatography typically Gas Chromatography (GC) or High Performance Liquid Chromatography (HPLC) coupled to an elemental specific detector such as inductively coupled plasma-mass spectrometry (ICP-MS) [1]. GC coupled with ICP-MS currently has some of the lowest reported detection limits [2] for mercury species with detection limits of 0.027 pg g^{-1} for methyl mercury (CH_3Hg) and 0.27 pg g^{-1} for inorganic mercury (Hg^{2+}) with solid phase microextraction (SPME) pre-concentration. Other detection methods such as atomic fluorescence spectroscopy with solid phase extraction [3] can reach detection limits as low as 0.01 ng L^{-1} for CH_3Hg and is suitable for the analysis of mercury species in ocean water [4]. However, the drawback of GC is that the species have to be rendered volatile and this requires a derivatisation step first with either Grignard reagents or more recently tetraalkyborate compounds [5] which can be time consuming and can sometimes result in species transformations [6], because of this another method for comparison is desirable.

HPLC on the other hand requires no derivatisation step, as the species do not need to be volatile before injection

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[7], simplifying the sample preparation considerably. However, to reach the detection limits required for environmental analysis, a pre-concentration step is necessary, the various pre-concentration methods used have been reviewed [8] and include on-line [7], and off-line [9] pre-concentration on various materials including C-18 micro-columns [10,11] and sulfhydryl cotton [12]. However, to successfully separate mercury species by HPLC, ion pairing agents such as L-cysteine [13,14] are required, which when coupled with vapour generation and ICP-MS gives detection limits of between 0.03 and 0.11 ng mL^{-1}. HPLC–ICP-MS with off-line pre-concentration [15] reached detection limits of 5.2 ng L^{-1} for Hg^{2+} and 5.6 ng L^{-1} for CH_3Hg, recently microbore HPLC–ICP-MS has been used for the speciation analysis of mercury [16], the use of a 1.0 mm i.d. analytical column operating at a flow rate of 70 μL min^{-1} significantly reduced the amount of solvent reaching the plasma leading to interesting sensitivity gains. This approach achieved detection limits of 11 ng L^{-1} for Hg^{2+} and 23 ng L^{-1} for CH_3Hg with no pre-concentration, however the large dead volume of the ICP-MS sample introduction system prevented the authors from fully exploiting the sensitivity that microbore HPLC should bring.

In this work a mid-bore (2.1 mm i.d.) HPLC column has been used, as the flow rates for these columns are most suitable for coupling with the low flow (<500 μL min^{-1}) higher sensitivity concentric nebulisers now available on the market. The reduced internal diameter means lower flow rates can be used, meaning that less solvent is introduced into the plasma increasing mass sensitivity, the mid-bore column geometry has the additional advantage of suffering less from dead volume effects, when compared to microbore HPLC. The use of L-cysteine and 2-mercaptoethanol in the mobile phase means that organic solvents and the problems related with them are avoided.

The replacement of the sample injection loop with a micro-column meant that large volumes (up to 5 mL) could be injected onto the pre-concentration column then eluted onto the analytical column, as the direct injection of 5 mL of sample onto the column could compromise the chromatographic resolution. This approach also allowed rapid on-line sample pre-concentration and matrix removal with minimal sample handling by the analyst for matrices as complex as seawater. Injection of the entire pre-concentrated sample instead of an aliquot as is the case for off-line pre-concentration resulted in low detection limits with minimal matrix effects while avoiding complex sample handling steps such as derivatisation.

The method was applied to seawater collected from the lagoon that surrounds the city of Venice. Monitoring of mercury in and around the city of Venice is important as it is World Heritage Site located uniquely in a coastal lagoon that from the 1950s through to the late 1980s was heavily contaminated with mercury by chlor-alkali process discharges from the nearby Marghera chemical works [17]. As fishing is still an important economic activity in this body of water, careful monitoring of the water quality of this delicate ecosystem is required, and the number of samples necessary for this means that rapid, and sensitive methods for monitoring important pollutants are required.

Table 1 – HPLC–ICP-MS operating conditions

Agilent 7500 is ICP-QMS	
Forward power	1450 W
Plasma gas flow	15 L min^{-1}
Auxiliary gas flow	1 L min^{-1}
Carrier gas flow	1.09 L min^{-1}
Sample depth	5 mm
Monitoring masses	*m/z* 184, 202 (1 point per peak)
Acquisition mode	Time resolved analysis
Integration time per mass	0.5 s
Spray chamber temperature	2 °C
Agilent 1100 series HPLC	
Column	100 × 2.1 mm Alltima HP C-18 3 μm particle size
Flow rate	0.2 mL min^{-1}
Injection volume	0.1–5 mL

2. Experimental

2.1. Instrumentation

The ICP-QMS used in this work was an Agilent 7500is (Agilent Technologies, Yokogawa Analytical Systems, Tokyo, Japan) fitted with a standard quartz spray chamber and a PolyPro-ST concentric nebuliser (Elemental Scientific Inc. Omaha, USA). This was coupled to an Agilent 1100 series HPLC pump (Agilent, Waldbronn, Germany) fitted with a manual injection valve (9125, Rheodyne, CA, USA) with a 100 μL (PEEK) sample loop (Alltech, Deerfield IL, USA), or an Opti-lynx™ 100 μL micro-column filled with a C-18 silica based packing material (Alltech, Deerfield IL, USA) instead of a sample loop. The mercury species were separated isocratically on a 100 × 2.1 mm Alltima HP C-18 3 μm column (Alltech, Deerfield IL, USA) at a flow rate of 0.2 mL min^{-1}, with a mobile phase of 0.5% L-cysteine (m/v) and 0.05% 2-mercaptoethanol (v/v) dissolved in ultra-pure water. The instrumental conditions are summarised in Table 1. The masses monitored were *m/z* 202, the most abundant mercury isotope and 184, a tungsten isotope to check for interference peaks from the formation of $^{184}W\ ^{18}O$.

2.2. Standards, reagents and materials

Mercury (II) chloride, methylmercury (II) chloride and 2-mercaptoethanol were purchased from Sigma–Aldrich (Milan, Italy) and the L-cysteine was purchased from VWR International (Milan, Italy). Stock standard solutions of approximately 1000 mg L^{-1} (as mercury) mercury chloride and CH_3Hg chloride were prepared by weight from the respective salts. Mercury chloride was dissolved in 1% (v/v) hydrochloric acid (Suprapur grade, Merck, Darmstadt, Germany) in a 100 mL amber glass bottle (Schott, Mainz, Germany); CH_3HgCl was dissolved in 10 mL of methanol (gradient UpS grade, Romil, Cambridge, UK) in a 100 mL amber glass bottle and made up to volume with 1% (v/v) hydrochloric acid, both solutions were stored refrigerated in the dark until required [18]. Working standards were made by serially diluting the stock standards with ultra-pure water in acid washed amber glass bottles (Schott, Duran, Mainz, Germany), samples were stored and

diluted in acid washed amber glass vials with PTFE liners (Supelco, Bellefonte, PA, USA). The water (18.2 MΩ) was generated by a Pure Lab Ultra water system (Elga Lab Water, High Wycombe, UK). Syringe filters when used were 0.45 μm cellulose acetate of 17 mm diameter (Alltech, Deerfield, IL, USA). The 0.2 μm cellulose acetate filters used for filtering the sea water samples were obtained from Sartorius (Germany), the accuracy and reproducibility of the method was checked by repeat analysis of the certified reference material BCR 579 coastal seawater certified for total mercury (IRMM, Geel, Belgium).

3. Results and discussion

3.1. Optimisation of the chromatographic separation

To maintain maximum sensitivity for the detector (in this case ICP-MS) it was decided to avoid methods using organic solvents. Of these the use of L-cysteine as an ion pairing agent seemed the most promising [13,19]. Fig. 1a shows a chromatogram of a mixed standard of 1 $\mu g L^{-1}$ of inorganic mercury and 1 $\mu g L^{-1}$ of CH_3Hg separated with a mobile phase of 0.5% L-cysteine at a flow rate of 0.2 mL min^{-1}, Fig. 1b shows the same standard separated with the same column and flow rate but with the addition of 0.05% (v/v) 2-mercaptoethanol to the mobile phase. It can be seen from Fig. 1b that the addition of this reagent has little effect on the area of the inorganic mercury peak, but has caused an increase in the peak height of the CH_3Hg peak, and a sharpening of both analytical peaks. The effect of further increases in the amount of 2-mercaptoethanol in the mobile phase can be seen in Fig. 2, this clearly shows that the addition of 2-mercaptoethanol increases the retention time for both analytes, but any increase above 0.05% (v/v) results in a significant loss in chromatographic resolution.

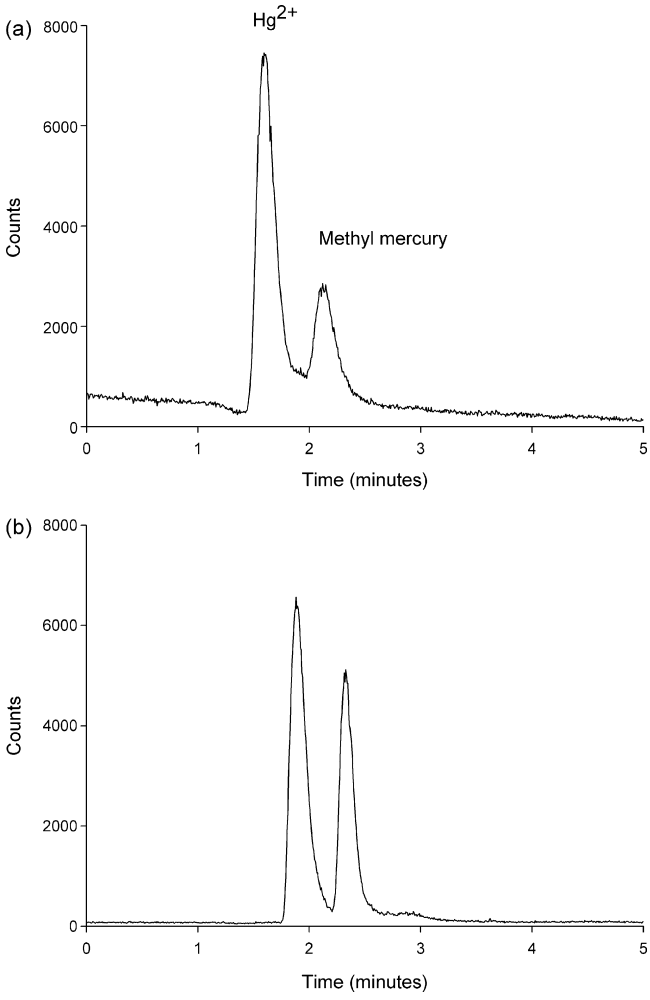

Fig. 1 – (a) The separation of a 1 $\mu g L^{-1}$ (100 μL injection) mixed inorganic mercury and CH_3Hg standard on a 100 × 2.1 mm Alltima HP C-18 3 μm HPLC column at a flow rate of 0.2 mL min^{-1} with a mobile phase of 0.5% (v/v) L-cysteine. (b) The separation of a 1 $\mu g L^{-1}$ (100 μL injection) mixed inorganic mercury and CH_3Hg standard on a 100 × 2.1 mm Alltima HP C-18 3 μm HPLC column at a flow rate of 0.2 mL min^{-1} with a mobile phase of 0.5% (m/v) L-cysteine and 0.05% (v/v) 2-mercaptoethanol.

3.2. Optimisation of the pre-concentration technique

To improve the sensitivity in order to detect mercury species at environmental levels, it was decided to include a pre-concentration technique. Aizpùn et al. [10] reported the use of a C-18 column modified with 2-mercaptoethanol to pre-concentrate the mercury species off-line, we decided to modify this method to an on-line method so that the entire pre-concentrated volume would be injected onto the column. This was achieved by replacing the 100 μL sample loop with a pre-concentration micro-column. The micro-column in this case is an Opti-Lynx™ trap cartridge with a bed volume of 100 μL, with an internal diameter of 4.6 mm and a length of 5.0 mm packed with a C-18 stationary phase. The sample is manually loaded onto the column using a standard glass HPLC syringe via the sample injection port, with the valve in the load position, sample elution is achieved by switching the valve to inject and the HPLC mobile phase elutes the analytes from the micro-column and transports them to the analytical column. Fig. 3 shows a chromatogram of a 100 μL injection of a 100 ng L^{-1} per species (as mercury) mixed standard of inorganic mercury and CH_3Hg prepared in 1% (v/v) HCl, and the same standard after the injection of a 1 mL aliquot onto the pre-concentration column before chromatographic separation. The peak areas for inorganic mercury and CH_3Hg after pre-concentration are increased by 10 and 6 times, respectively, which corresponds to the increased volume injected for inorganic mercury, but CH_3Hg appears to be pre-concentrated but with a roughly 50–60% efficiency.

Improvement of the pre-concentration of CH_3Hg was investigated by loading the pre-concentration column with higher concentrations of the individual reagents present in the mobile phase, this was done by injecting more concentrated solutions of 2-mercaptoethanol or L-cysteine onto the pre-concentration column to increase the ion pairing capacity. The effect of adding either 2-mercaptoethanol or L-cysteine to the standards was tried to increase the concentration of thiol–mercury complexes in solution. The results are summarised in Tables 2a and 2b as percent (%) recover-

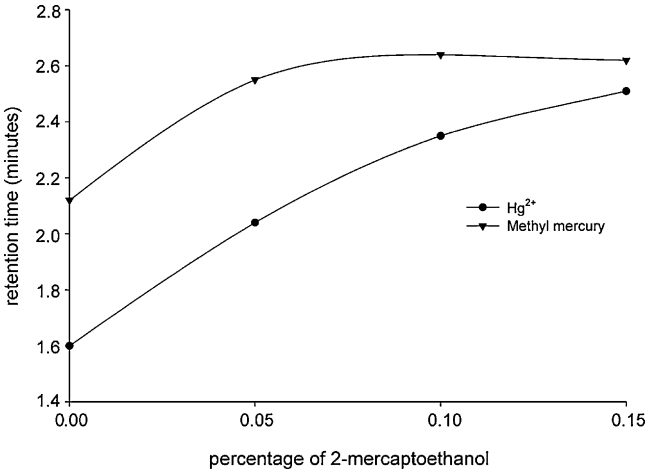

Fig. 2 – The effect of increasing the concentration of 2-mercaptoethanol in the mobile phase on the separation of inorganic and CH_3Hg by HPLC using a 100 × 2.1 mm Alltima HP C-18 3 μm HPLC column at a flow rate of 0.2 mL min^{-1} with a mobile phase of 0.5% (m/v) L-cysteine and increasing concentrations of 2-mercaptoethanol.

ies compared to the integration results of standards injected under the same standard conditions as listed above (the pre-concentration column preconditioned with the mobile phase and standards prepared in 1% (v/v) HCl). The percent recovery is calculated as the (integration results new conditions/integration results with standard conditions) × 100.

The results in Table 2a shows that loading the column with more reagents such as L-cysteine or 2-mercaptoethanol has a detrimental effect on the pre-concentration capability of the column, and that washing the column with 1 mL of ultra-pure water before use to remove them also had a negative effect demonstrating that the compounds present in the mobile phase play an important part in the pre-concentration mechanism.

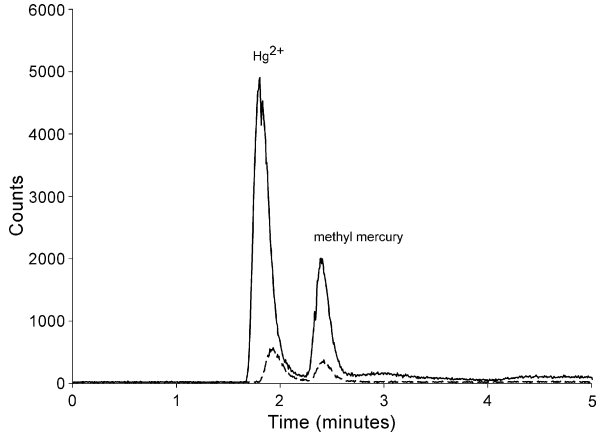

Fig. 3 – A chromatogram of a 100 μL injection of a 100 ng L^{-1} per species (as mercury) mixed standard of inorganic mercury and CH_3Hg prepared in 1% (v/v) HCl (small peaks, dashed line), and the same standard after the injection of a 1 mL aliquot onto the pre-concentration column before chromatographic separation (large peaks, solid line).

Table 2a – Recovery (%) of the mercury species with different column pre-treatments compared to their pre-concentration with column pre-conditioning with the HPLC mobile phase (0.5% (m/v) L-cysteine and 0.05% (v/v) 2-mercaptoethanol) with the species standards prepared in 1% (v/v) HCl

Pre-concentration column pre-treatment	Percent recovery (%)	
	Hg^{2+}	CH_3Hg
Injection of 1 mL of 0.2% (v/v) 2-mercaptoethanol	81	38
Injection of 1 mL of 1% (m/v) L-cysteine	16	11
Injection of 1 mL ultra-pure water	89	21

Table 2b – Recovery (%) of the mercury species in different standard matrices on a C-18 micro-column pre-conditioned with the mobile phase

Standard matrix	Percent recovery (%)	
	Hg^{2+}	CH_3Hg
Standard prepared in 0.05% (v/v) 2-mercaptoethanol	46	21
Standard prepared in 0.5% (m/v) L-cysteine	45	72
Standard prepared in 0.2% (v/v) HCl	136	114
Standard prepared in water	155	348

Table 2b shows that making the standards in 2-mercaptoethanol or L-cysteine showed no improvement, with a net reduction in analyte recovery demonstrating that inorganic mercury and CH_3Hg bind to the ion pairing reagents by forming on column complexes with L-cysteine and 2-mercaptoethanol immobilized on the stationary phase, rather than forming complexes in solution that then have an affinity for the stationary phase. The results in dilute hydrochloric acid and water show the only pre-concentration improvement, showing that mercury and above all CH_3Hg binds to thiols at neutral or a slightly acidic pH. This is in agreement with Percy et al. [14] who reported that at a pH between 5.0 and 8.0 cysteine is present as a zwitterion with the carboxyl group deprotonated (pK_a 1.95), the amino group protonated (pK_a 9.05) and the sulfhydryl group protonated. Our results are further supported by the findings of Rabenstein and Fairhurst [20] who reported that the sulfhydryl group binds CH_3Hg most strongly with a formation constant for CH_3Hg cysteine complexes of 5.0×10^{15} but that at pH < 2 this complex disassociates due to competition of protons for the sulfhydryl group.

In Fig. 4 the effect of sample volume (injection volume) on the pre-concentration of a mixed 10 ng L^{-1} Hg^{2+} (closed circle symbols) and CH_3Hg standard (open circle symbols) in ultra-pure water is reported, showing a linearity up to a pre-concentration volume of 20 mL for methyl mercury when the standards are made in ultra-pure water, but the Hg^{2+} profile is curved demonstrating that the break through volume maybe close to 20 mL. It proved to be impractical to inject larger volumes accurately with a 1 mL syringe making it difficult to obtain a precise determination of the break through volume,

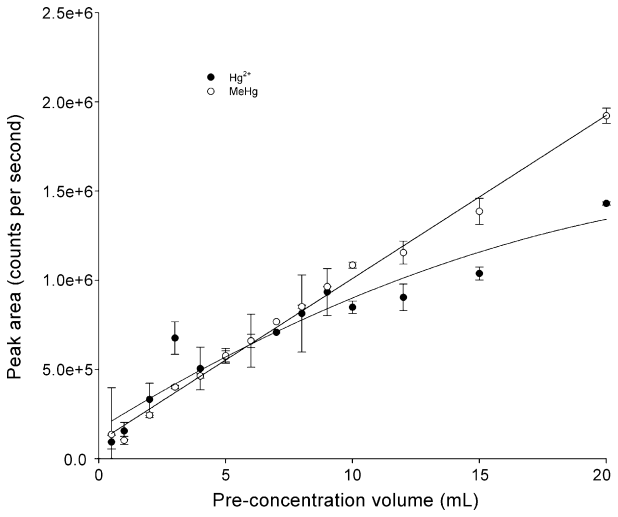

Fig. 4 – The effect of sample volume (injection volume) on the pre-concentration of a mixed 10 ng L^{-1} Hg^{2+} (closed circle symbols) and CH_3Hg standard (open circle symbols) in ultra-pure water ($n = 3$, error bars of 2S.D.).

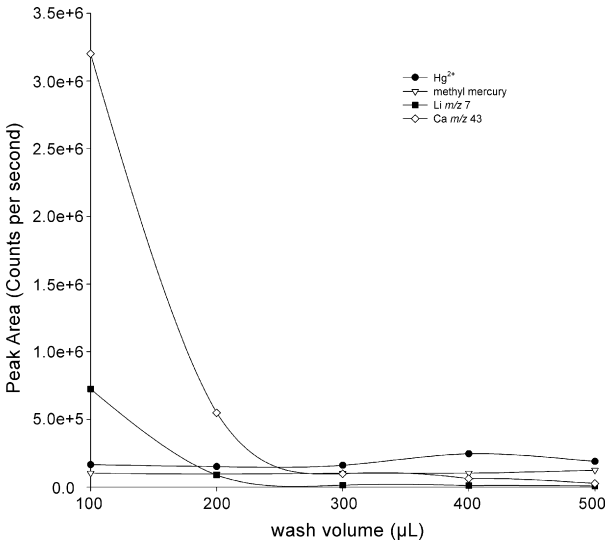

Fig. 5 – The effect of column wash volume on the recovery of mercury species (Hg^{2+} closed circles, CH_3Hg inverted open triangle symbols) from the pre-concentration column and elimination of the seawater matrix (Li filled square symbols, Ca open diamond symbols).

due to small errors in the amount of sample taken up each time and human error resulting in smaller volumes injected than expected. Using larger syringes made it more difficult to push the liquid onto the column, the largest practical syringe volume was found to be 5 mL, the back pressure generated by the pre-concentration column when using syringes above this volume caused the removable needles being used to lose liquid causing inaccuracies in the amount injected. The back pressure generated also proved to be too high for the use of a peristaltic pump to load the column with the low pressure fittings available in the laboratory.

The pre-concentration volume possible for real samples was then investigated by spiking a filtered seawater sample (filtered with a 0.2 μm membrane filter) with a mixed 10 ng L^{-1} Hg^{2+} and CH_3Hg standard and injecting it undiluted onto the pre-concentration column. A large characteristic sodium emission was observed in the bullet region of the plasma when the column was not washed after injection of a 1 mL sample of seawater, due to elution of the seawater matrix. Different wash volumes with ultra-pure water between 100 and 500 μL were investigated; removal of the seawater matrix was monitored by measuring Ca at *m*/z 43 and Li at *m*/z 7, and recovery of the mercury species by measuring the peak areas of the repeated 1 mL injections of the standard in seawater. The results are reported in Fig. 5, these show that Li is eliminated after a wash volume of 300 μL and 500 μL is required to return the Ca signal to baseline levels. Observing the plasma showed the sodium emission disappeared after washing with 200 μL, but the levels of Na present saturated the detector at wash volumes below 300 μL, making it impractical to use *m*/z 23 for monitoring of the washing process. The mercury recoveries after washing were unchanged so 500 μL was adopted as the washing volume.

To maintain the low blank levels necessary and avoid carry over between samples, the sample syringe was washed three times between samples or standards, the first wash was with 1% (v/v) HCl and the last 2 washes were with ultra-pure water in 2 different sample bottles so a cleanliness gradient was effectively achieved for the syringe washing solutions. The first wash solution instead of being discharged to waste was injected into the injection valve while in the inject position, to clean the injection port and internal flow lines that were not being effectively cleaned by the mobile phase. For the column washing for seawater samples, a separate 500 μL cleaned glass syringe was used to exclusively inject the column washing solution of ultra-pure water taken from the third wash solution bottle to avoid adding mercury to that already pre-concentrated on the column.

Having demonstrated that the washing protocols were effective, it was attempted to find the breakthrough volumes for Hg^{2+} and CH_3Hg in undiluted seawater, the results can be seen in Fig. 6, demonstrating that the break through volume for Hg^{2+} maybe close to 20 mL, but the curve for CH_3Hg is linear up to 20 mL demonstrating that much higher volumes can be pre-concentrated than those that can be injected using a syringe, indicating that these columns may be suitable for use to pre-concentrate the mercury species present off-line.

3.3. *Calibration and analytical figures of merit*

As mercury is only stable for short time periods when it is in unacidified solutions [21,18], the sample and standard handling protocol of Planchon et al. [22] was applied with the modification that all the standards were made in amber glass bottles. Mixed analytical standards between 0 and 100 ng L^{-1} were made fresh in ultra-pure water from acidified mother solutions (the concentrations of these were periodically checked against a certified mercury standard), samples were stored at −20 °C before analysis and were analysed immediately after defrosting without any acidification.

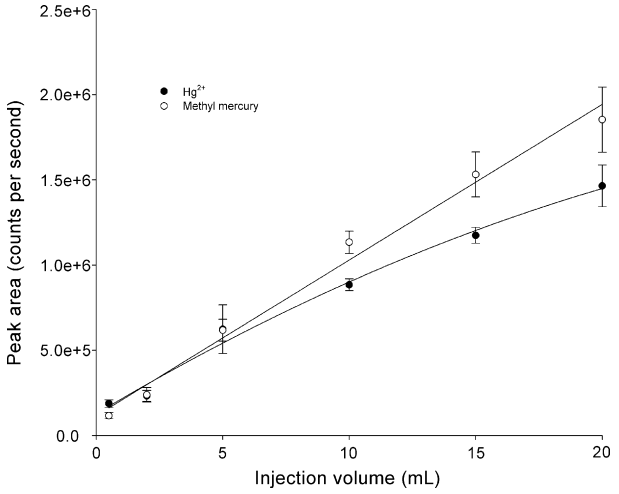

Fig. 6 – The effect of sample volume (injection volume) on the pre-concentration of a mixed 10 ng L^{-1} Hg^{2+} (closed circle symbols) and CH_3Hg standard (open circle symbols) in undiluted filtered seawater ($n = 3$, error bars of 2S.D.).

The figures of merit for injections of 20 mL of standard are reported in Table 3a. The reproducibility tests for the standard injections were carried out on new freshly made standards, as under these conditions the standards are only stable for an hour at most. From Table 3a it can be seen that the external calibration is linear over the calibration range and that the detection limit for CH_3Hg is better than that for inorganic mercury. The explanation for this is that the detection limit for inorganic mercury is blank limited as there is inorganic mercury present in all the reagents used during analysis. To test the accuracy of the external calibration a standard additions calibration for Hg^{2+} and CH_3Hg in a filtered seawater sample was carried out using the same calibration range and injection volumes, the results are reported in Table 3b, a separate spike of 10 ng $L^{-1}$was made on the same sample and repeatedly injected to find the spike recovery compared to the external calibration curve. In addition to this the accuracy and reproducibility of the method was checked by repeat analysis of the certified reference material BCR 579 coastal seawater certified for total mercury. Analysis of this material revealed that the mercury was wholly present as inorganic mercury, so an aliquot was spiked with 2 ng L^{-1} of CH_3Hg to check the spike recovery for this analyte. The results can be seen in Table 4, and show that with an external calibration method

Table 3a – Analytical figures of merit for the HPLC–ICP-MS with the micro-column pre-concentration method over a calibration range of 0–100 ng L^{-1} in ultra-pure water

Figures of merit in pure water	Hg^{2+}	CH_3Hg
Regression slope of linear range 0–100 ng L^{-1} (cps/ng L^{-1})	56,241	102,424
Linear regression coefficient (r^2)	0.9993	0.9994
Precision of peak area, 10 ng L^{-1} (%R.S.D.) ($n = 3$)	13.1	28.6
Limit of detection (3 × S.D. of concentration for a 0.5 ng L^{-1} standard) ($n = 5$)	0.07	0.02
Blank equivalent concentration (ng L^{-1})	1.18	0.21

Table 3b – Analytical figures of merit for the HPLC–ICP-MS with the micro-column pre-concentration method over a calibration range of 0–100 ng L^{-1} in filtered unacidified seawater

Figures of merit in seawater	Hg^{2+}	CH_3Hg
Regression slope of linear range 0–100 ng L^{-1} (cps/ng L^{-1})	64,600	90,782
Linear regression coefficient (r^2)	0.9968	0.9986
Precision of peak area, 10 ng L^{-1} (%R.S.D.) ($n = 3$)	8.9	5.5
Limit of detection (3 × S.D. of concentration for a 0.5 ng L^{-1} standard) ($n = 5$)	0.12	0.03
Blank equivalent concentration (ng L^{-1})	0.78	0.07

Table 4 – Spike recovery for an aliquot of filtered unacidified seawater spiked at 10 ng L^{-1} with Hg^{2+} and CH_3Hg versus an external calibration with standards made in ultra-pure water, and the accuracy and reproducibility of repeat injections of the certified reference material BCR 579 spiked with 2.0 ng L^{-1} of CH_3Hg versus an external calibration with standards made in ultra-pure water and a matrix matched calibration with standards made in filtered undiluted seawater

Sample	Hg^{2+} spike recovery (%)	CH_3Hg spike recovery (%)
10 ng L^{-1} spike ($n = 3$) ± 1S.D.	108 ± 4	84 ± 3
Sample	**Hg^{2+} (ng L^{-1})**	**CH_3Hg (ng L^{-1})**
BCR 579[a] ($n = 5$) versus an external calibration	2.21[b] ± 0.55	1.85[c] ± 0.23
BCR 579[a] ($n = 5$) versus a matrix matched calibration	1.86[b] ± 0.34	2.1[c] ± 0.14

[a] BCR 579 coastal seawater reference material certified value 1.85 ± 0.2 ng L^{-1}.
[b] (Result ± 1S.D.).
[c] CH_3Hg spiked at 2.0 ng L^{-1} result ± S.D.

Table 5 – Analysis of the particulate and dissolved phases of water samples from Venetian canals for mercury species

Sample number	Volume filtered (mL)	Mean concentration in extract (ng L^{-1}) (S.D.) $n = 3$		Mean concentration in the particulate phase (ng L^{-1})		Mean concentration in the dissolved phase (ng L^{-1}) (S.D.) $n = 2$	
		Hg^{2+}	CH_3 Hg^+	Hg^{2+}	CH_3Hg^+	Hg^{2+}	CH_3Hg^+
1	510	N.D.	48.0 (0.5)	N.D.	0.29	0.24 (0.07)	0.06 (0.02)
2	500	N.D.	66.3 (2.1)	N.D.	0.40	0.54 (0.05)	0.13 (0.01)
3	500	N.D.	72.1 (0.1)	N.D.	0.46	0.38 (0.07)	0.07 (0.02)
Filter blank	500	3.2 (1.2)	4.9 (2.2)				

over estimates the Hg^{2+} content of the spiked samples and under estimates the CH_3Hg content of the spiked samples, which is reflected in the results for the spiked BCR 579 reference material. This suggests that there is a matrix effect on the pre-concentration phase that needs to be corrected for. The standard additions calibration (matrix matched calibration), when used to quantitate the Hg^{2+} and CH_3Hg levels in the reference material spiked with CH_3Hg give excellent agreement with the certified and spike values, respectively, showing that matrix matching the standards with seawater adequately corrects the matrix effects previously identified.

3.4. *Sample analysis*

To see if the method was suitable for monitoring mercury levels in the Venice lagoon three samples of surface water from canals close to the University were analysed for inorganic and CH_3Hg. The samples were collected in clean glass bottles and were transported to the laboratory for immediate analysis. Aliquots of the samples (20 mL) were filtered and injected onto the pre-concentration column; the column was then washed with 500 μL of ultra-pure water to remove the seawater matrix.

To investigate the concentration of CH_3Hg associated with the particulate phase, approximately 500 mL of the 3 separate unfiltered, unacidified samples of Venice canal water were filtered using the filters specified above. These samples had been left deliberately unacidified to avoid disturbing the equilibrium between the particulate and dissolved phases. The filters were transferred into 15 mL amber glass vials and the CH_3Hg present was extracted from the particulate matter immobilized on the filter using 6 mL of an extraction solution of 7% (v/v) HCl and 1% (v/v) 2-mercaptoethanol with an ultrasonic bath set to 60 °C with a sonication extraction time of 30 min. The extract was filtered using 0.45 μm syringe filters and diluted 1:1 with ultra-pure water. A calibration blank and the mixed mercury species standards (calibration range 0–500 ng L^{-1} of inorganic mercury and CH_3Hg) were made up in the diluted matrix (3.5% (v/v) HCl and 0.5% (v/v) 2-mercaptoethanol) in acid washed 25 mL amber glass bottles and were found to be stable for a week. As samples containing 2-mercaptoethanol cannot be pre-concentrated using the micro-column method described above, a 100 μL PEEK sample loop was fitted to the HPLC injection valve for sample introduction of the sample extracts. The results are summarised in Table 5, and show that after an effective pre-concentration of the particulate phase on a filter, the CH_3Hg concentration is easily quantifiable with a good precision (<1% R.S.D.). Our results for the particulate phase range from 0.29 to 0.46 ng L^{-1} which although from a small number of samples are similar to those found by Bloom et al. [17] who found values ranging from 0.05 to 0.27 ng L^{-1}, the results of this author for CH_3Hg in the dissolved phase (filtered with 0.45 μm filters) are similar to ours with values ranging from 0.02 to 0.10 ng L^{-1}. The results for the mercury levels in the dissolved phase show that the method is sensitive enough to detect inorganic and methyl mercury levels in the Venice lagoon, although methyl mercury levels are close to our detection limits so these analyses may need to be carried out with larger volumes during monitoring campaigns. This will require the use of higher pressure fittings for the peristaltic pump, or the use of a syringe pump or HPLC pump to load the pre-concentration columns off-line before use.

4. Conclusions

Methods for the determination of inorganic and CH_3Hg in the dissolved (filtered before analysis) phase of natural waters and for the determination of CH_3Hg in the particulate phase of natural waters has been developed. The use of a micro-column in place of the sample loop in the injection valve allowed the rapid and reproducible pre-concentration of dissolved mercury species and the removal of possible matrix interferences present in seawater (such as Na and Ca) prior to their determination. This method has been successfully applied to samples from the Venetian lagoon, an important environment at the northern end of the Adriatic Sea. The results found for Hg^{2+} agree well with a certified reference material, BCR 579, coastal seawater, certified for mercury and the results for dissolved levels of CH_3Hg are similar to those reported in the literature for this environment. This method is not suitable for the direct determination of CH_3Hg in unfiltered samples, so the filtrate was collected for samples of up to 500 mL. Extraction of this filtrate enabled the determination of CH_3Hg associated with the particulate phase present at levels below 1 ng L^{-1}. Although this methodology does not reach the detection limits of GC–ICP-MS with SPME or purge and trap pre-concentration that have been used for the determination of mercury species in pristine environments; it is suitable for the determination of mercury species in large numbers of samples from polluted aquatic environments, as the time required for pre-concentration and matrix removal is less than a minute per replicate.

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