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Flow injection-liquid chromatography-cold vapour atomic absorption spectrometry for rapid determination of methyl and inorganic mercury

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A previously described system for determining low concentrations of mercury species in environmental samples using flow injection high-performance liquid chromatography cold vapour atomic absorption spectrometry (FI-HPLC-CVAAS) has been further developed with respect to time of analysis, long term signal stability, memory effects, detection limits, and environmental friendliness. Methyl and inorganic mercury were determined without pre-treatment in brackish water and in digested biological certified reference materials, DOLT-2 and TORT-2. Results were compared with those obtained by gas chromatography microwave-induced plasma atomic emission spectroscopy (GC-MIP-AES) using either butylation with a Grignard reagent or ethylation with sodium tetraethylborate. With the FI-HPLC-CVAAS system, absolute detection limits are 1.7 pg and 3.4 pg for methyl and inorganic mercury, respectively. Mercury species in a sample can be determined at the 0.4 ng l^{-1} level within 5 min. For lower concentrations the time for analysis has to be increased.

Introduction

Today's instrumentation for direct determination of mercury species in environmental samples provides insufficient sensitivity and, for this reason, a number of different hyphenated techniques have to be employed. Species are normally isolated from the matrix and pre-concentrated. Before determination, pre-treatment with reagents and separation by chromatography is needed. The many different steps involved in the process of speciation result in time-consuming and complicated methods demanding high skills of operation. Therefore, routinely performed speciation measurements are at present scarce, although there is a great need for such analysis as it provides much more information than total determinations. A large number of methods for determining mercury species in environmental and biological samples have been developed and the most recent review on this subject was in 1998 by Morita *et al.*[1]

The currently most popular methods use gas chromatographic (GC) separation of the mercury species followed by selective detection with atomic absorption spectrometry (AAS), atomic emission spectrometry (AES), atomic fluorescence spectrometry (AFS), or mass spectrometry (MS). The time needed for separation of relevant mercury species with capillary GC is quite long,[2,3] but it can be substantially reduced with multi-capillary GC.[4] Enrichment and sample pre-treatment is, however, still the most time consuming step, in particular for complex samples with low concentrations.

In a relatively simple situation, as for the determination of mercury species in natural water samples, enrichment can, for example, be performed by solid-phase, liquid[5,6] or liquid–liquid extraction.[7] Enrichment requires typically more than 60 min for low ppt concentrations of mercury species.

Extracted species need to be converted to forms that can be easily separated by the GC. Frequently employed is ethylation in an aqueous phase followed by collection of volatilised derivatives on a chromatographic column. This step could be time-consuming, but, for samples with low salt content, ethylation can be performed directly thereby combining enrichment and derivatisation. Grignard reagents are also used to form suitable derivatives for GC separation. Mercury species need in this case to be present in an organic phase. This approach includes addition of several reagents and centrifugation and it appreciably increases analysis time.

With HPLC, species need no derivatisation for efficient separation, which simplifies and quickens sample pre-treatment. In addition, HPLC instrumentation allows for on-line enrichment of species by using a pre-concentration column. This step can be performed in parallel to mercury detection. When HPLC is coupled to atomic spectrometry, detection is not straightforward as it involves separation of mercury from the liquid eluate. Several works describe the performance of mercury species separation by HPLC.[8–11] Although only methyl and inorganic mercury have been mainly found in natural samples, methods are used for a large number of species, for example 4-methyl phenyl mercury or methoxyethylmercury,[12] which require more efficient chromatographic separation and longer analysis times. Detection by atomic spectrometric techniques includes CVAAS,[10,11,13] CVAFS[12] and MIP-AES.[8]

None of the above-described HPLC systems combine rapid determination, simple on-line sample pre-concentration and low detection limits. The aim of this work was to develop a simple, fast and automatic system providing low detection limits, which could be used for routine work for mercury speciation in environmental samples. As a starting point for this research, we used the instrumentation described by Yin *et al.*[14]

Experimental

Instrumentation and methods

The FI-HPLC-CVAAS instrument is a further development of a previously described system.[14] The instrumental set up is

schematically shown in Fig. 1. A computer controlled syringe pump (Kloehn 50300, Kloehn Company Ltd, Las Vegas, NV, USA), equipped with an eight-port valve and a 10 or 5 ml syringe was used to drive the sample solution through a C-18 filled pre-concentration column [1, Fig. 1] made from PEEK®. The syringe was alternately filled with portions of 0.2 ml water sample and 0.1 ml ammonium pyrrolidinedithiocarbamate (APDC) complexing agent solution in acetate buffer until a total volume of maximum 9 ml. With the injection valve in load position, the mixture was then pumped through the pre-concentration column at 2.5 ml min^{-1}. For larger total volumes than 9 ml this procedure was automatically repeated. After pre-concentration, the syringe was filled and rinsed with water, which was pumped the same way as the sample mixture. Between different sample injections, the syringe was washed with acidic solution and water to prevent memory effects. After pre-concentration, the electrically driven six-port injection valve (Knauer, Germany) was switched to the inject position and the mercury species were backwards-eluted from the pre-concentration column and separated on a 53 × 4.5 mm, 5 μm C-18 chromatographic column [2, Fig. 1] (Bischoff chromatography, Germany). A 3 + 1 methanol–water (v/v) eluent containing 1.5 mM APDC was used and the pump's flow rate was 1 ml min^{-1} (Knauer, Germany). In a 200 μl coil following a T-connection the column eluate was mixed with a reducing reagent, 1% (w/v) $NaBH_4$ in 0.5% (w/v) NaOH, pumped at a flow rate of 0.5 ml min^{-1}. In the gas–liquid separator [3, Fig. 1] the volatile mercury species were removed from the liquid phase by purging with argon at 25 ml min^{-1} while the liquid was pumped to waste at a nominal rate of 9 ml min^{-1}. Argon gas carried the mercury species through a thermolysis cell made of quartz (inner diameter 5 mm, length 20 mm) heated to about 800 °C and filled in the centre 15 mm with MgO crystals surrounded by quartz wool to facilitate formation of metallic mercury.[15] A Nafion® drying tube, length 900 mm, inner diameter 1.0 mm and outer diameter 1.4 mm, (Perma Pure, Tons River, NJ, USA), was installed prior to the cuvette. The Nafion tube was positioned in a polyethylene plastic tube (inner diameter 3.0 mm, outer diameter 6.0 mm) and drying argon was flushed at 200 ml min^{-1} opposing the sample flow. Except for the argon flows, a Compaq 433 computer controlled the system. The cuvette for absorbance measurements at 253.7 nm and the pumps controlling the flow of the reducing solution and waste from the gas–liquid separator are a part of the flow injection mercury system FIMS-400 (Perkin-Elmer, Germany). Peak area was used for evaluation. Capillaries connected to the syringe pump were made of Teflon with 0.5 mm inner diameter.

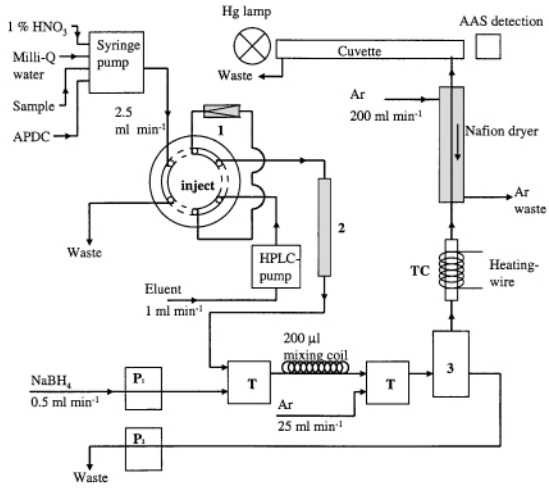

Fig. 1 Schematic diagram of the FI-HPLC-CVAAS system: 1 = pre-concentration column, 2 = separation column, 3 = gas–liquid separator, inject = six-port injection valve in inject position, T = T-mixer, P_1, P_2 = peristaltic pumps, TC = thermolysis cell filled with MgO.

PEEK capillaries with 0.11 mm inner diameter were used in the high pressure part of the system.

The GC-MIP-AES methods used for comparative measurements have been described in previous works.[16–18]

Reagents

The chemicals used were of analytical-reagent grade and sometimes purified further prior to use. Methanol was of HPLC-grade (JT Baker, Deventer, Holland). The complexing agent solution consisted normally of 2 mM ammonium pyrrolidinedithiocarbamate, APDC (Aldrich, Steinheim, Germany) in 10 mM ammonium acetate (Merck, Darmstadt, Germany), pH 6. This solution was purified over Chelite S resin (Serva, Heidelberg Germany). For biological samples a concentration of 50 mM of APDC was used. Solutions of APDC and sodium borohydride (Merck, for synthesis > 96%, Germany) in sodium hydroxide (EKA, Spånga, Sweden) were freshly prepared at least every third day. Solutions of 20% TMAH (tetramethyl ammonium hydroxide, Sigma, Sweden) and 0.5 mol l^{-1} DDTC (sodium diethyldithiocarbamate 99%+ ACS grade, Aldrich, Steinheim, Germany) were prepared by dissolving the salt in Milli-Q water (Millipore Milli-Q water system, Bedford, MD, USA) followed by purification over Chelite S resin. A solution containing 1% (w/v) of sodium tetraethylborate (Strem chemicals, Newburyport, USA) and 2% (w/v) of potassium hydroxide (Merck, Darmstadt, Germany) was freshly prepared under nitrogen every five days. The solution was kept frozen until use. A buffer solution of pH 4.9 was prepared by dissolving 272 g of sodium acetate (Merck, Darmstadt, Germany) and 118 ml of glacial acetic acid (Merck, Darmstadt, Germany) in Milli-Q water to a final volume of 1 l. The buffer was purified using Chelite S resin. Synthetic seawater was prepared using NaCl, $MgSO_4 \cdot 7H_2O$ and $NaHCO_3$ according to Grasshoff,[19] and the final salinity was 34‰.

Preparation of standard solutions. Inorganic mercury standards were prepared by stepwise diluting a 1000 mg l^{-1} certified standard ($HgCl_2$, Referensmaterial AB, Ulricehamn, Sweden) with Milli-Q water. Diluted samples were prepared freshly and, depending on the procedure applied, acidified (about 0.02% w/w of each) with suprapure nitric and hydrochloric acid (sub-boiling distilled in an all-quartz apparatus, Heraeus Quarzschmelze, Hanau, Germany). A 100 mg l^{-1} methyl mercury stock standard solution was prepared by dissolving the appropriate amount of CH_3HgCl (Merck, Darmstadt, Germany) in 3 ml of 99% ethanol and by dilution to 100.0 ml with Milli-Q water. Methyl mercury working standards were prepared by diluting the stock standard solution with Milli-Q water.

Sample preparation

HPLC. Mercury standards in Milli-Q- and brackish water were directly injected into the HPLC system. Twenty to 200 mg of certified reference material (TORT-2 and DOLT-2) were digested in an ultrasonic bath for 1–2 h in 1–2 ml of 20% TMAH,[20] treated with a pH 7 phosphate buffer (Merck, Darmstadt, Germany) and 4.4 mol l^{-1} acetic acid, and diluted in Milli-Q water before injection. The digestion procedure was the same for all methods.

Butylation. Milli-Q water or the digested sample was buffered to pH 9 with borate buffer and 4 mol l^{-1} HCl, followed by addition of 1.0 ml of 0.5 mol l^{-1} DDTC. After samples had been shaken for 5 min, 1.0 ml of toluene was added and the samples were shaken for an additional 5 min followed by centrifugation for 5 min at 3200 rpm. The toluene phase was

transferred into another centrifuge tube standing in an ice–water bath and butylated as described by Snell *et al.*[16]

Ethylation. Six ml of CH_2Cl_2 was added to 30 ml brackish water or digested diluted reference material, followed by dropwise addition of 4 M or concentrated HCl, respectively, to adjust the pH below 2. The samples were shaken vigorously for half an hour and then centrifuged for 30 min at 3000 rpm. The CH_2Cl_2 with extracted methyl mercury was then pipetted into a 50 ml polypropylene centrifuge tube. The above extraction procedure was repeated 3 times and the organic phases were combined. Then approximately 30 ml of Milli-Q water was added. The sample was purged with Ar at a flow rate of 80 ml min^{-1} to remove the organic solvent, leaving the extracted methyl mercury in the water matrix. This solution was transferred to a 125 ml glass flask along with 200 μl of pH 4.9 acetate buffer, and 50 μl of 1% $NaBEt_4$ was added. The sample was purged for 15 min with Ar at a flow rate of 80 ml min^{-1}. The purged analytes passed through the Nafion dryer tube and were trapped in a quartz tube (170 mm length, 2.5 mm inner diameter) packed with 100 mg of Tenax TA, mesh 60/80 (Supelco, Bellefonte, PA, USA). The flow rate of the Ar dryer gas was 200 ml min^{-1}. After trapping, the quartz tube was connected to the GC. The trap was rapidly heated at 200 °C by a specially built furnace. A stream of He at a flow rate of 300 ml min^{-1} carried the desorbed mercury species from the Tenax into the GC column. The method of standard additions was used for calibration. The brackish water matrix had to be eliminated before adding the ethylation reagent otherwise recovery is poor as shown elsewhere.[17] Because the extraction discriminates inorganic mercury it can not be determined with this method.

Results and discussion

Instrumental and methodological modifications

With a previously described system[14] using a peristaltic pump, APDC and sample solutions were mixed on line and introduced into the pre-concentration column. The pressure build up from the latter made it difficult to control and balance the two mixing flows. Under these conditions the volume of liquid passing through the pre-concentration column could vary depending on the pressure. In addition, with the peristaltic pump, memory effects from analyte species adsorbed on the pump tubing could be seen. In the work presented here, we used a syringe pump (see Fig. 1) to reduce the above mentioned problems. Rinsing the pump with diluted HNO_3 after sample introduction virtually eliminates memory effects. Syringe pumps are also known for high accuracy and precision of delivered sample volumes, which are both typically better than 0.1%.[21]

Previously an acetonitrile–water–methanol eluent was used for chromatographic separation.[14] Here we removed acetonitrile from the procedure and just used methanol–water to avoid hazardous organic waste. In order to shorten the analysis time, a shorter column was employed with sufficient efficiency to separate methyl- from inorganic mercury and with a total elution time of 5 min.

Before phase separation, a 200 μl mixing coil was implemented to increase time for the reaction between eluted species and $NaBH_4$. Since the system was only optimised for methyl- and inorganic mercury no further addition of reagents, such as HNO_3,[10,14] was needed for optimum sensitivity.

Methyl mercury forms volatile methyl mercury hydride, which is released from the gas–liquid separator before reduction to metallic mercury by $NaBH_4$ has taken place.[14,22–24] Therefore, on-line thermolysis in the presence of heated MgO was performed. This salt facilitates quantitative conversion of alkyl mercury species to elemental mercury.[15]

We installed a semi-permeable Nafion membrane dryer tube after the thermolysis cell to remove water moisture before mercury was introduced into the cuvette.[25] This resulted in improved baseline stability and precision. Signals for a blank determination and for dried argon are shown in Fig. 2. As the noise for both signals is similar, it can be concluded that it reflects mainly the source flicker noise, and the measuring cell's transmission flicker factor is therefore negligible when using the Nafion dryer. Without the Nafion drying tube there was increased baseline noise and drift such that the absorbance increased by about 0.03 over a 5 min period.

When the Nafion tube was placed before the thermolysis cell practically no signal for methyl mercury was detected, whereas no change in signal intensity was observed for inorganic mercury. With the Nafion tube positioned after the thermolysis cell similar sensitivities were obtained for methyl and inorganic mercury. This means that the eluted methyl mercury complex was insignificantly reduced to its elemental form prior to thermolysis. As has been discussed elsewhere[22] in the reduction scheme of alkyl mercury to elemental mercury by $NaBH_4$, alkyl mercury hydride is formed as an intermediate and this is practically the only mercury species volatilised from the gas–liquid separator used here. Methyl mercury hydride is likely to be trapped in the structure of the Nafion membrane. In separate experiments in connection with *in-situ* ethylation of water samples, see Experimental section, we observed no losses of the less reactive ethyl methyl mercury when using a Nafion dryer.

Analyte species trapping, transport and detection

Efficiency of enrichment. Injecting 50 μl of 10 ng ml^{-1} methyl and inorganic mercury directly without enrichment into the eluent stream resulted in characteristic masses of 15.5 pg for both species, see Table 1. By comparing these results with characteristic masses including pre-concentration, 19.9 and 19.3 pg for methyl and inorganic mercury, respectively, the enrichment efficiency for mercury species in Milli-Q water is estimated to be close to 80%. Reducing the liquid flow rate from the syringe pump to the pre-concentration column did not increase the sensitivity. It was thought that the increased adsorption of mercury species complexes on the Teflon capillary from the syringe pump to the injector counteracts an increased trapping efficiency on the pre-concentration column. Pressure build up from the pre-concentration column did limit sample flow rates to 2.5 ml min^{-1}.

Formation and release of volatile mercury species. We compared characteristic masses for water standards and metallic mercury both introduced into the eluent stream. From this the efficiency of formation of volatile mercury species in the gas–liquid separator was calculated to be 24% for an optimised flow rate of the carrier gas, 25 ml min^{-1}. When the flow rate was lowered to 14 ml min^{-1}, efficiencies decreased to 12% and 9% for inorganic and methyl mercury, respectively. Higher carrier

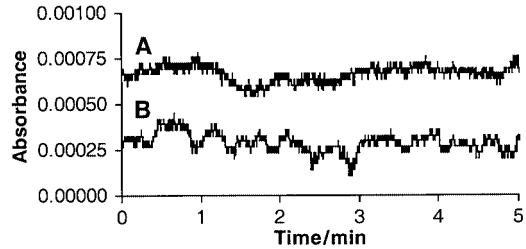

Fig. 2 Noise at the 253.7 nm wavelength with A, only dried argon flowing through the cuvette and B, a blank injection under analytical conditions.

gas flows will decrease the sensitivity of the method as a result of shorter residence times of atoms in the cuvette.

Overall efficiency. The overall efficiency of the FI-HPLC-CVAAS was tested by comparative measurements of water standards introduced before the pre-concentration column and elemental mercury in air introduced directly into the cuvette. For these measurements, characteristic mass ratios for methyl and inorganic mercury are 7.29 and 7.07. This results in an overall efficiency of about 14%.

Figures of merit and instrumental performance Table 2 shows a comparison of results obtained with the FI-HPLC-CVAAS and GC-MIP-AES for a number of samples. For the latter technique, *in-situ* ethylation[17,18] and butylation with a Grignard reagent[16] was used for sample pre-treatment. The agreement for methyl mercury determination in the brackish water by HPLC and the ethylation procedure was within the limit of precision, indicating that both methods are accurate. For the HPLC method the total analysis time per sample is in this case 30 min and both methyl and inorganic mercury can be determined. For the ethylation method the analysis time per sample is about 180 min, as methyl mercury has to be separated from the matrix by an extraction procedure prior to ethylation.

Using the HPLC method, digested reference materials in TMAH were buffered and diluted with Milli-Q water prior to determination. With the reference methods, mercury species in the digested sample had to be extracted to an organic phase and derivatised prior to determination.

As can be seen from Table 2, with the three methods used, all results are in good agreement within the certified values. This shows that the different methods do not give rise to significant systematic errors during speciation of mercury in these tested materials.

The characteristic mass for water standards (0.05–1.00 ng ml^{-1}), including all analytical steps, was 19.3 ± 1.7 and 19.9 ± 1.7 pg for inorganic and methyl mercury, respectively, where the ± values represent one standard deviation, see Table 1. Data reflect mean values (n = 3–5) from six measuring occasions obtained at different days during one month. From these results we conclude that the system shows reasonable long-term stability as reflected by standard deviation of measurements over 30 days. Notably, the signals' sensitivity is rather independent of the chemical form of the mercury species determined, indicating that the overall efficiency of the analytical procedure is the same for both mercury species.

The relative standard deviation of signal repeatability for 1 ml of 250 ng l^{-1} mercury species in the water standard was 2.9% for methyl and 5.7% for inorganic mercury. The absolute detection limit based on three times the standard deviation of the 250 ng l^{-1} standard was 1.7 and 3.4 pg for methyl and inorganic mercury, respectively. This translates to relative detection limits of 0.034 ng l^{-1} for methyl and 0.068 ng l^{-1} for inorganic mercury in a 50 ml sample.

Fig. 3 shows signals for methyl and inorganic mercury in a brackish water sample with salinity of 5‰ from the Baltic Bay. For this measurement 50 ml of sample was introduced. The concentrations of methyl and inorganic mercury found in this sample were 0.11 and 5.3 ng l^{-1}, respectively, using the method of standard additions. As can be seen from Table 1, in brackish water the characteristic mass for methyl mercury is significantly lower than for inorganic mercury and for this type of sample the method of standard additions has to be applied to obtain accurate results. In a preliminary study, synthetic seawater spiked with mercury species was injected prior to the pre-concentration column giving the same sensitivity as for the

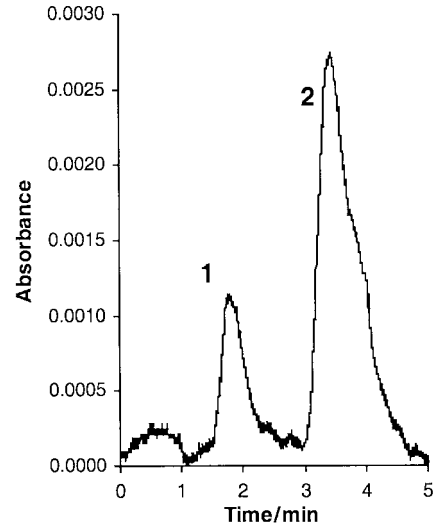

Fig. 3 Analytical signals close to the detection limit; 1, methyl mercury (0.1 ng l^{-1}) and 2, inorganic mercury (5 ng l^{-1}).

Table 1 Characteristic masses, pg, for samples introduced at various locations of the FI-HPLC-CVAAS

		$MeHg^+$	Hg^{2+}	Hg^0
Prior pre-concentration column	Milli-Q water[a]	19.9 ± 1.7	19.3 ± 1.7	
	Brackish water[b]	16.9 ± 0.4	23.2 ± 0.6	
Introduction to eluent stream	Milli-Q water[c]	15.5 ± 0.2	15.5 ± 0.4	
Introduction to eluent stream	Air			3.7
Introduction to Nafion dryer	Air			2.7

[a] The ± values represent one standard deviation from the mean for 20 measurements during one month at six occasions. [b] The ± values represent the uncertainty in the slope of the calibration graph calculated by the line estimation formula of Excel. [c] The ± values represent one standard deviation from the mean for 5 measurements on one occasion.

Table 2 Mercury species concentrations determined for different sample types with three different methods. The ± values represent one standard deviation of the mean for three determinations

	FI-HPLC-CVAAS		GC-MIP-AES after butylation		GC-MIP-AES after ethylation	Certified values	
	$MeHg^+$	Hg^{2+}	$MeHg^+$	Hg^{2+}	$MeHg^+$	$MeHg^+$	Hg_{total}
Tort-2, Lobster hepatopancreas/ng g^{-1}	157 ± 12	123 ± 10	160 ± 6	123 ± 12	150 ± 8	152 ± 13	270 ± 60
Dolt-2, Dogfish liver/ng g^{-1}	728 ± 55	1439 ± 62	727 ± 31	1320 ± 58	682 ± 37	693 ± 53	2140 ± 280
Brackish water 5‰ salinity/ng l^{-1}	0.11 ± 0.002	5.3 ± 0.19	—	—	0.12 ± 0.009	—	—

brackish water. This indicates that the method can also be used for the determination of mercury species in seawater samples.

Conclusions

The combined effect of several modifications of FI-HPLC-CVAAS has resulted in a system with a unique combination of performance characteristics with respect to detection limit, rapidity of analysis and simplicity. The detection limits for methyl and inorganic mercury are 1.7 pg and 3.4 pg respectively, the time for one analysis is 5 min and sample preparation includes no extraction, derivatisation or external enrichment. In addition, the method does not produce hazardous organic waste.

By using a monolithic pre-concentration column[26] higher flow rates during pre-concentration could probably be used with maintained high enrichment efficiency. This will reduce analysis time for samples with mercury concentrations below 0.4 ng l^{-1}, which otherwise would need prolonged enrichment time. Total automation of the system could be achieved for routine analysis.

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