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Microwave-assisted extraction for methylmercury determination in sediments by high performance liquid chromatography-cold vapour-atomic fluorescence spectrometry

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A simple and rapid procedure for methylmercury extraction from sediments based on microwave-assisted alkaline digestion with methanolic potassium hydroxide was optimized on parameters such as microwave power, extraction time and sample size. Organomercury species were extracted with dichloromethane in hydrochloric acid medium and back-extracted into ultra-pure water. The sediment extracts were injected into an analytical system composed of high-performance liquid chromatography-ultraviolet-post-column oxidation-cold vapour-atomic fluorescence spectrometry (HPLC-UV-PCO-CV-AFS) for methylmercury determination. Quantitative methylmercury recoveries were obtained when 0.15 g of sediment were suspended into 6 ml of 25% m/v methanolic potassium hydroxide and the slurry was exposed to microwave irradiation at 84 W for 2 min. The detection limit of proposed method was 12 ng g^{-1} while the relative standard deviation was less than 5%. The method was validated by the analysis of two sediment certified reference materials and the methylmercury concentrations found were in good agreement (95% confidence level) with the certified values.

Introduction

Mercury is one of the most dangerous contaminants in the environment due to its accumulation in aquatic organisms and bioamplification phenomena through the trophic chain. Nevertheless, the relative toxicity of mercury depends on its chemical form, methylmercury being one of the most toxic and also the most commonly occurring form of organic mercury present in the environment.[1]

Methylmercury can be found in the environment as a consequence of the methylation of inorganic mercury by sulfate reducing or methanogenic bacteria and transmethylation reactions with organometallics.[2] Therefore, the assessment of methylmercury concentration, namely in sediments, is very important to the interpretation of biogeochemical cycles of mercury in the aquatic environment.

One of the main problems in methylmercury determination in sediments is the extraction of this organomercury specie from a complex matrix. Furthermore, methylmercury concentration usually does not exceed 1.5% of the total mercury present in sediments.[3] The principal requirements of an extraction method are the complete separation of the analyte from the interfering matrix and adequate concentration methodology of the analyte up to a detectable concentration level without analyte loss, sample contamination or changes in speciation. The most commonly used extraction methods for methylmercury determination in sediments are based on the Westöö procedure.[4,5] The first step is the extraction of mercury species using potassium bromide and sulfuric acid saturated with copper(II) sulfate or hydrochloric acid. Methylmercury is separated by successive extractions with organic solvents (toluene or benzene) and back-extracted with an aqueous complexing agent (cysteine or thiosulfate). Other methodologies, such as alkaline digestion,[6–10] distillation[11,12] and supercritical fluid extraction[13] have been developed for the extraction of mercury species as alternatives to the Westöö procedure. However, such sample preparation methods all suffer from some common disadvantages, such as laborious procedures, solvent- and time-consuming problems and the possibility of originating artefacts.

The acceleration of the conventional extraction methods of methylmercury from sediments by the use of ultrasound energy allows workers to decrease dramatically the extraction time from 1–6 h to 45 min.[14] However, such decrease in time can be more pronounced when using microwave-assisted extraction. Tseng *et al.*[15,16] achieved quantitative methylmercury recovery in 3 min using nitric acid or hydrochloric acid as extractant. Quantitative recoveries were also obtained in 10 min by Vázquez *et al.*[17] when methylmercury extraction was carried out in a microwave oven and the solvents used were hydrochloric acid and toluene.

The most common method of mercury speciation is gas chromatography (GC) followed by electron capture detection (ECD).[14,17] The low selectivity associated with this detector for organomercury species is increased by replacing the ECD with atomic absorption spectrometry (AAS),[15] microwave induced plasma atomic emission spectrometry (MIP-AES),[13] atomic fluorescence spectrometry (AFS)[7] or inductively coupled plasma mass spectrometry (ICP-MS).[18] Besides, the use of high-performance liquid chromatography (HPLC) for mercury speciation has some advantages in comparison to GC: simplified sample preparation and no derivatization of samples is required. HPLC has also been coupled to several detection techniques such as AAS,[19] AFS[2,20] or ICP-MS.[21]

This work reports the original application of an easy to implement and high throughoutput procedure for sample

preparation based on the utilisation of microwave energy and alkaline digestion. The procedure was then combined with high-performance liquid chromatography-ultraviolet-post-column oxidation-cold vapour-atomic fluorescence spectrometry (HPLC-UV-PCO-CV-AFS) for the determination of methylmercury in sediments. The extraction efficiency of a methanolic potassium hydroxide solution was studied for different power applied and exposure time. The performance of the proposed method was evaluated by the analysis of two certified reference materials of sediment.

Experimental

Instrumentation

A schematic diagram of the HPLC-UV-PCO-CV-AFS system is shown in Fig. 1. A sample was injected into a 200 µl loop and mixed with the mobile phase at an elution rate of 0.63 ml min^{-1}. The eluate from the HPLC column was then mixed with a stream (1.4 ml min^{-1}) of ultra-pure water and the mercury compounds were oxidized in the oxidation coil by UV-irradiation. The resulting inorganic mercury was reduced to elemental mercury by a stream (1.7 ml min^{-1}) of the reducing agent in a reduction coil and emerged into a gas–liquid separator. Mercury vapour was purged from the solution in the gas–liquid separator with an argon stream (65 ml min^{-1}) and swept through the moisture traps into the AFS detector. A second stream of argon (98 ml min^{-1}), denoted as shield gas, entered also directly into the detector.

The chromatographic system included a HPLC pump module (Knauer, Berlin, Germany), a six-port injection valve (Rheodyne, California, USA) equipped with a 200 µl Peek sample loop and a reversed-phase analytical column packed with Nucleosil ODS (RP C_{18}, 25 cm × 4.6 mm, 5 µm). All separations were performed at room temperature under isocratic conditions. The post-column oxidation system consisted of a UV-irradiation lamp (8 W, 254 nm) (Camag, Muttenz, Germany) surrounded by a 3 m coil. The lamp was placed in a box for eye protection. A reduction coil (2 m), two flow-meters and a quartz gas–liquid separator (PS Analytical, Orpington, Kent, England) were used for mercury cold vapour generation. The solutions were introduced into the system by four-channel peristaltic pumps (Ismatec, Zürich, Switzerland) through Tygon tubes (R 3603). The mixing joints and both reaction coils were made of 0.50 mm id Teflon. The drying of mercury vapour generated in the gas–liquid separator was carried out in a sulfuric acid trap (0 °C) connected to a calcium chloride trap (7 cm × 1 cm id). A PS Analytical Model 10.023 Merlin atomic fluorescence spectrometer (PS Analytical) was used as mercury detector.

A CEM Model MDS-81D microwave oven (600 W maximum output) with glass tubes of 22 ml and Gilson shakers (Gilson, Villiers le Bel, France) were used for sample preparation.

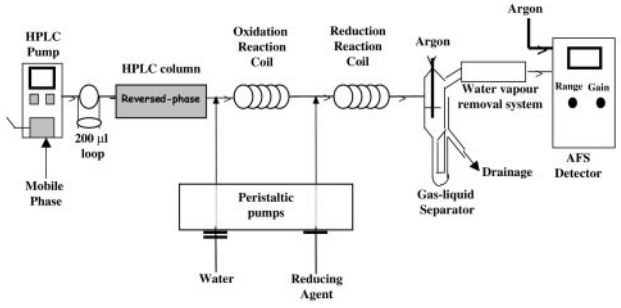

Fig. 1 Schematic diagram of the HPLC-UV-PCO-CV-AFS system for determination of methylmercury.

Reagents, standards and reference materials

All reagents and standards were prepared in ultra-pure water produced in a Milli-Q Model 185 system. The chemicals were of analytical-reagent grade as well as mercury-free and used without further purification. The mobile phase was a mixture of methanol (Merck, liquid chromatography, Darmstadt, Germany) and water (5+95, v/v) containing 0.01% v/v 2-mercaptoethanol (Merck, p.a.) buffered at pH 5 with 0.06% v/v acetic acid (Merck, p.a.) and 0.15% m/v ammonium acetate (Merck, p.a.). The mobile phase was filtered through 0.2 µm membranes (NL 16, Schleicher & Schuell, Dassel, Germany) and de-gassed in an ultrasonic bath for 30 min prior to use.

The reducing agent, 3% m/v tin(II) chloride in 15% v/v hydrochloric acid, was prepared daily by the dissolution of the appropriate amount of mercury-free tin(II) chloride (Merck, p.a.) in mercury-free hydrochloric acid (Merck) on a hot-plate. The solution was brought to volume with ultrapure water, filtered through 0.45 µm membranes (Millipore, Bedford, MA, USA) and purified from mercury by bubbling with nitrogen for 2 h.

Stock solutions of mercury nitrate (1000 mg l^{-1}, Spectrosol, BDH, Poole, England) and methylmercury chloride (1000 mg l^{-1} in mercury, Alpha Products, Karlsruhe, Germany) were used weekly to prepare a working standard solution of 10 mg l^{-1} (as Hg) of each individual species in water. The lower working standard solutions were prepared daily in ultra-pure water. They were stored cool at 4 °C.

25% m/v methanolic potassium hydroxide solution was prepared daily by the dissolution of potassium hydroxide (Merck, p.a.) in methanol (Merck, liquid chromatography). Dichloromethane was obtained from Merck (liquid chromatography).

Two sediment reference materials with different certified contents of methylmercury were used to validate the proposed method. BCR 580 was obtained from the Community Bureau of Reference (BCR, Brussels, Belgium) and IAEA 356 was obtained from the International Atomic Energy Agency (IAEA, Vienna, Austria). The sediment extracts were filtered through 0.2 µm membranes (PVDF PP, LIDA, Kenosha, USA) prior to injection.

Figures of merit

The analytical performance of the HPLC-UV-PCO-CV-AFS technique was evaluated using methylmercury and inorganic mercury standards. The retention times were 19 and 24 min for methylmercury and inorganic mercury, respectively. The detection limit for both mercury species, 10 ± 2 pg, corresponding to a concentration of 51 ± 9 ng dm^{-3}, was calculated from calibration curves in the range of 100–800 ng l^{-1} and based on the amount necessary to yield a net signal equal to three times the standard deviation of the blank. The relative standard deviation (n = 4) for a 300 ng l^{-1} methylmercury standard was less than 1%.

Extraction procedure

A sample of 0.05–0.25 g was weighted in glass tubes and 2–10 ml of 25% m/v methanol–potassium hydroxide were then added. The tubes were capped and the slurry was homogenised by magnetic agitation and subjected to microwave irradiation for 30–210 s at 60–96 W. The solution was allowed to cool to room temperature. The alkaline extract was mechanically shaken in glass separating funnel for 10 min with 6 ml of dichloromethane and 1.5–7.5 ml of concentrated hydrochloric acid. With this procedure organomercury compounds were extracted into the organic phase, whereas inorganic mercury remained in the aqueous phase as chloro complexes. A dichloromethane aliquot was transferred into another tube and the slurry was again treated with 6 ml of dichloromethane

for 10 min. Finally, the dichloromethane layers were combined and organomercurials were solvent-exchanged into 35 ml of ultra-pure water by evaporation of the organic solvent with a current of nitrogen. This final solution was injected into the HPLC-UV-PCO-CV-AFS system. Blanks and methylmercury standards were subjected to the same procedure in order to check possible contamination, losses of analyte or interconversion of species.

Results and discussion

Methylmercury stability in a microwave field

The effect of microwave energy on methylmercury behaviour depends on the physico-chemical properties of the solvent employed. In this work, a 25% m/v methanolic potassium hydroxide solution was used as extractant since quantitative recoveries have been already reported for methylmercury extraction from sediments using this solvent without microwave irradiation.[6–8] Furthermore, when sediments rich in organic matter are to be analyzed, acid leaching releases only a certain fraction of methylmercury.[8,10] In order to check the stability of methylmercury in this extraction medium during microwave-assisted treatment, 2 ml of a 25% m/v methanolic potassium hydroxide solution spiked with 7.5 ng of methylmercury were submitted to different powers, ranging from 60 to 96 W for 210 s. The results obtained were compared to the ones obtained for 2 ml of the same solution unexposed to microwave irradiation. All experiments were performed in two replicates and each replicate was measured twice. The recovery calculated as the quotient between methylmercury concentration determined with and without microwave treatment was higher than 90% in all cases. Furthermore, no analytical signal corresponding to inorganic mercury was ever detected. The disadvantages reported by other authors[22] in relation to the use of microwave irradiation to accelerate methylmercury extraction, such as the conversion of methylmercury to inorganic mercury due to the use of hydrochloric acid or nitric acid as extractant, and the evaporation losses caused by the heating effect during microwave irradiation, were eliminated by the use of methanolic potassium hydroxide as extractant.

Optimization of microwave-assisted extraction procedure

The following variables, related to the extraction efficiency in a microwave field, were optimised in order to achieve quantitative recoveries for methylmercury from sediments: microwave power, extraction time and sample amount. A BCR 580 reference sediment was used for this purpose. As already mentioned, all experiments were performed in two replicates and each replicate was measured twice. A six-point standard additions method was always used in the determination step, in order to avoid possible matrix interferences.

Firstly, the effect of microwave power and extraction time on methylmercury recovery was studied. For this, several experiments were carried out, in which a sample of 0.25 g was treated with 2 ml of 25% m/v methanolic potassium hydroxide in a microwave oven for 30–210 s at a power ranging from 60 to 96 W. As shown in Fig. 2, high methylmercury recoveries were observed with high extraction times (~180 s) when working at low powers (60 W) or with low extraction times (~70 s) at high powers (96 W). When working at intermediate powers (~80 W), the best methylmercury recoveries were obtained for an extraction time around 90–150 s. When using conditions different from those, the obtained methylmercury recoveries were low. A microwave power of 84 W was selected for further works because the highest methylmercury recovery was obtained at this power and was practically constant for extraction times between 90 and 150 s.

Secondly, the influence of sample amount on methylmercury recovery was investigated. Different sample amounts ranging from 0.05 to 0.25 g were weighted, treated with 2 ml of 25% m/v methanolic potassium hydroxide and exposed to microwave irradiation at 84 W for 120 s. It was found that methylmercury recovery increased with decreasing sample amount from 0.25 to 0.05 g (Fig. 3). A recovery of 109 ± 18% (mean ± standard deviation) was reached by using 0.05 g of sample. The handling of very small amounts of sub-samples could cause high variations between replicates, causing high standard deviations. This problem would be more pronounced when field samples were analysed, which are often less homogeneous than reference materials. Therefore, the use of large sample sizes would be necessary to improve the analytical accuracy.

The amount of sample, the volume of 25% m/v methanolic potassium hydroxide and the volume of concentrated hydrochloric acid were multiplied by a factor, while the dichloromethane volume was maintained constant at 6 ml. Methylmercury recovery was quantitative (95 ± 7%) when a factor of 3 was used (0.15 g of sample–6 ml of 25% m/v methanolic potassium hydroxide–4.5 ml of concentrated hydrochloric acid), but it was only 45 ± 4% for a factor of 5 (0.25 g of sample–10 ml of 25% m/v methanolic potassium hydroxide–7.5 ml of concentrated hydrochloric acid), due to the formation of larger potassium chloride amount. Such an occurrence of KCl precipitate hinders the extraction of the organomercurials to the dichloromethane phase. For these conditions, there was a failed attempt to improve the recovery by increasing the volume of dichloromethane.

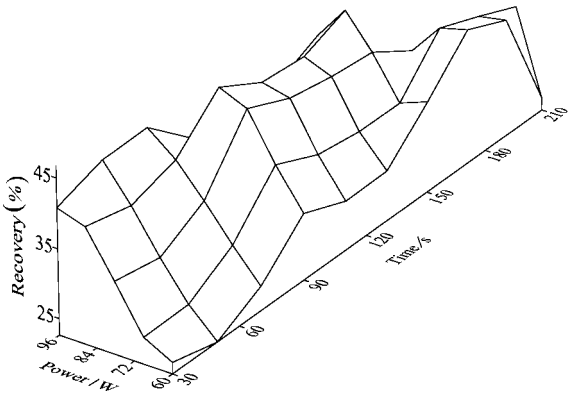

Fig. 2 Optimization of microwave power and extraction time for methylmercury recovery from BCR 580 reference sediment.

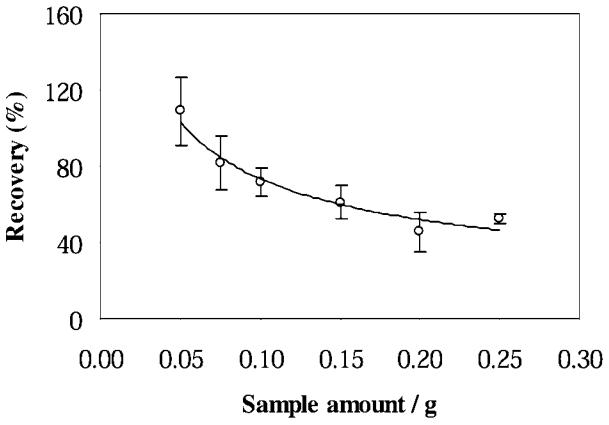

Fig. 3 Effect of sample amount on methylmercury recovery from BCR 580 reference sediment.

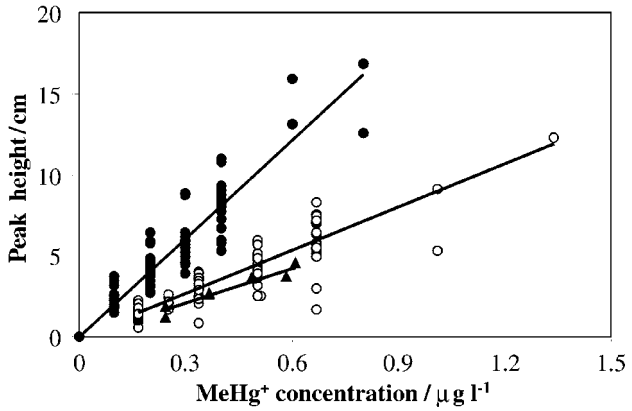

Fig. 4 Historical series of analytical values for methylmercury obtained from aqueous standards (●) and spiked sediment reference materials (BCR 580 (○) and IAEA 356 (▲)).

Matrix effects in sediment extracts

The existence of matrix interference was checked in order to study the possibility of the direct determination of methylmercury in sediment extracts. For this purpose a historical series of analytical values were plotted, which included aqueous standards, as well as solutions of two certified reference sediments spiked with methylmercury. Since the sample already originated an analytical signal even when no methylmercury was added, it was then necessary to subtract the signal of the sample from the signals obtained with the spiked solutions. The resulting difference was attributed to the signal of the standard. As shown in Fig. 4, when the additions of methylmercury standard were increased, the difference between methylmercury signals obtained from the aqueous standards and the spiked reference sediments also increased. It could be seen as a considerable spread but it should not be forgotten that the samples of each CRM were subjected to different treatments.

The slopes and the intercepts of the curves corresponding to aqueous standards and the BCR 580 and IAEA 356 reference materials are shown in Table 1. It can be observed that the 95% confidence interval for the slope of the aqueous standards did not include the slopes obtained with the spiked extracts of reference sediments. Besides, the 95% confidence interval for the true intercept of the aqueous standards included zero. Therefore a linear model passing through the origin was fitted to all three cases. Table 1 also shows that the slopes obtained with the linear model passing through the origin are significantly different (>95% confidence) in all of the three cases which demonstrates the existence of strong matrix effects on the analytical response. The direct determination of methylmercury in sediment extracts using the calibration method with aqueous standards was not possible and the standard additions method was always preferred in order to avoid matrix interferences. Taking into account the need for using the standard addition method, the sample throughput of the methodology was around four samples in every six h of operation.

Table 1 Slope and intercept values corresponding to the analytical response from aqueous standards and sediment extracts spiked with methylmercury

Sample	Slope[23] $b \pm CL^a$/cm l μg^{-1}	Intercept[23] $a \pm CL^b$/cm
Model $y=a+b.x$		
Aqueous standard	19.143 ± 2.036	0.388 ± 0.624
Model $y=b.x$		
Aqueous standard ($n=75$)	20.245 ± 1.003	
BCR 580 ($n=102$)	8.852 ± 0.431	
IAEA 356 ($n=7$)	7.070 ± 0.657	

[a] $CL=t\ ESD/\sqrt{\sum(x_i-\bar{x})^2}$ (without passing through the origin); $CL=t\ ESD/\sqrt{\sum x^2}$ (passing through the origin). [b] $CL=t\ ESD\sqrt{[1/n+\bar{x}^2/\sum(x_i-\bar{x})^2]}$ being ESD = √(residual sum of squares/residual degrees of freedom).

Table 2 Methylmercury determination in certified reference materials

Reference material	Certified value[a], $\bar{x} \pm t\ s/\sqrt{n}$/ng g^{-1}	Found value[ab], $\bar{x} \pm t\ s/\sqrt{n}$/ng g^{-1}
BCR 580	75.5 ± 3.7	69.5 ± 8.8
IAEA 356	5.46 ± 0.39	5.53 ± 0.21

[a] Average value ± confidence limit ($p=0.05$). [b] $n=3$.

Validation of the analytical method

Two certified reference materials of sediment containing different amounts of methylmercury, BCR 580 and IAEA 356, were analyzed to evaluate the performance of the proposed method (0.15 g sample, 84 W, 120 s). The detection limit of the analytical method for methylmercury determination in sediments was 12 ng g^{-1}, calculated as three times the standard deviation of the blank. As a consequence of the very low level of methylmercury in the IAEA 356 reference material, the analysis required the treatment of four 0.15 g portions of this reference material following the extraction procedure described and the mixing of the dichloromethane extracts obtained for each portion. Organomercury species were back-extracted into 15 ml of deionized water. The certified and determined values of methylmercury concentration, as well as the confidence limits, in both reference materials are given in Table 2. It can be seen that the contents found by the proposed method were in good agreement (*t*-test, 95% confidence level) with the certified values. Fig. 5 shows a typical chromatogram of a sample of BCR 580 without addition and a sample of the same material added with 0.17 ng ml^{-1} of methylmercury standard. The relative standard deviation was always less than 5% ($n=3$).

Conclusions

Microwave-assisted alkaline digestion with methanolic potassium hydroxide is a fast, simple and efficient sample preparation method for mercury speciation analysis in

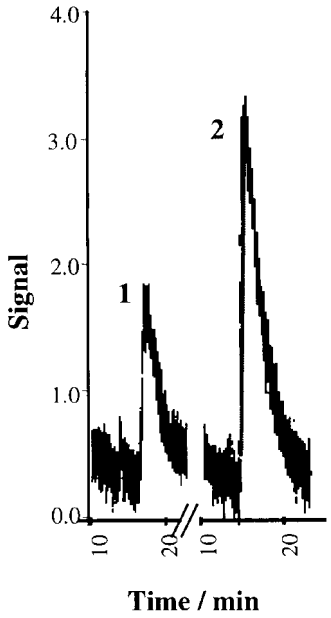

Fig. 5 Chromatogram of the BCR 580 reference sediment. Peak 1, methylmercury, peak 2, methylmercury and standard addition of 0.17 ng ml^{-1} methylmercury standard.

sediments. The proposed extraction procedure reduces markedly the time required from 3 h to 2 min in relation to similar conventional methods.[7] Furthermore, one of the advantages of microwave extraction devices is the possibility of conducting many simultaneous extractions. The use of a reflux condenser based on the open microwave digestion of the sample was not required since losses by evaporation of the extractant and target analytes were not produced.[15] Methylmercury concentration was determined in the sediment extracts by HPLC-UV-PCO-CV-AFS. The analysis of two sediment certified reference materials verified the efficiency, precision and accuracy of the complete method for methylmercury determination in sediments.

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