A rapid and robust method for the organic mercury determination in Hg mine waters

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ABSTRACT
A new, rapid, simple and robust method for the organic mercury determination in Hg mine waters has been developed. Organic mercury species were extracted in CH_2Cl_2 as bromide derivatives in a single step and aliquots of the organic phase were evaporated retaining organic mercury in a N-acetyl-L-cysteine solution. Finally, the organic mercury was determined by using the direct mercury analyser DMA-80. The suitability of the proposed method to be applied to real samples was evaluated by methylmercury recovery studies in spiked waters from an abandoned mining area in Asturias. Recoveries higher than 92% were achieved for all the tested matrices. In addition, no influence of iron and sulphate was observed as a consequence of the short time required for extraction and quantification. Preparation of spiked samples and analysis of an acceptable number of replicates could be carried out in the same day. This has proved to be a noticeable advantage since matrix effects were not significant, keeping the simplicity of the method.

INTRODUCTION
The change in speciation of mercury from inorganic to methylated forms is the first step in the aquatic bioaccumulation process. Methylation can occur non-enzymatically or through microbial action. Once methylmercury (MeHg) is released, it can be introduced into the food chain by rapid diffusion and tightly binding to proteins. As a result of food-chain biomagnification, highest levels are found in the tissues of such predatory species as freshwater trout, pike, walleye, bass and ocean tuna, swordfish and shark. The bioconcentration factor, i.e., the ratio of the concentration of MeHg in fish tissue to that in water, is usually between 10000 and 100000. Levels of selenium in the water may affect the availability of mercury for uptake into aquatic biota (WHO, 1990).
Water chemistry is likely to be an important factor controlling bioaccumulation rate and the concentration of MeHg in fish. Hg and MeHg concentrations, water temperature, dissolved oxygen (DO), dissolved organic carbon (DOC), pH, total suspended solids (TSS), particulate organic carbon (POC), particulate organic nitrogen (PON), and sulphate concentrations are some of the main chemical variables that might influence both methylation of Hg and the uptake and accumulation of MeHg by fish from sediment and water-column (Benoit *et al*., 2003). DOC and pH are probably the most important chemical variables affecting MeHg accumulation by fish (Mason *et al*., 2000), although many other chemical parameters that influence Hg speciation in the water column and finally affect the bioavailability of MeHg to biota, could also be contributing factors. Physical parameters of watershed-surroundings can alter the amount of MeHg formed in the aquatic environment but they are unlikely to have direct impact on bioaccumulation of MeHg in aquatic organisms.
In natural waters, two main mercury species can be identified: inorganic mercury (Hg^{2+}) and methylmercury (CH_3Hg^+) (Martínez *et al*., 2000). MeHg levels in water are usually much lower that those found for inorganic mercury.
Although other organic mercury species can be present in natural samples, monomethylmercury is clearly predominant in natural waters, soils and sediments representing nearly all organic mercury in these environments. Consequently, the evaluation of the organic mercury fraction in water samples can provide very useful and practical information about its potential toxicity to fish species.
The general trend in the development of methods for the determination of organic mercury species from water samples has been towards a number of established stages: preconcentration, extraction, derivatisation to form adequate species able to be separated by a chromatographic technique and quantification by a suitable detection technique such as ECD, CVAAS or CVAFS (Sánchez and Sanz-Medel, 1998). These methods are able to determine mercury speciation in natural waters but they can be considered as time consuming due to they required large number of stages. Unfortunately, they are unavailable for most of conventional laboratories due to the required highly sophisticated equipment.
Despite of these speciation methods, extraction procedures to isolate the organic mercury fraction offer simplicity and they can be easily applied to environmental samples. Most extraction methods are based on

an acid or alkaline treatment followed by the proper extraction using an organic solvent (Sánchez and Sanz-Medel, 1998). Some of these methods are affected by the artificial formation of MeHg during their application (Hintelmann *et al*., 1997). However, methods employing bromide complexation present the lowest artifact formation degree (Bloom *et al*.,1997). In this work, the use of $CuBr_2$ as a new releasing agent of organic mercury species is proposed. This reagent combines the bromide complexing action with the Hg displacing capacity of Cu (II), that competes with mercury ions for binding sites.
In the last years, several companies have developed specific mercury analysers, which can directly determine mercury contents in solid samples. One of them is the DMA-80 from Milestone (Sorisole, Italy) used in this work. This analyser is based on the electrothermal atomization of mercury in samples, followed by its preconcentration in an Au-amalgamator and its detection by atomic absorption spectrometry at 253.65 nm. It offers low detection limits, good reproducibility and the possibility of changing operational conditions as a function of the nature and quantity of sample. The use of quartz boats also allows us to determine mercury concentrations in liquid solutions with a good precision at similar concentration level than classical cold vapour atomic absorption spectrometry (CVAAS) but using a considerably lower amount of liquid sample.
This report describes a procedure for the determination of the organic mercury fraction in Hg mine waters, using successfully the DMA-80 mercury specific analyser advantages. The proposed method is based on our previous method developed for soils, adapted through the optimisation of some experimental conditions (Fernández-Martínez & Rucandio, 2005). Furthermore, the applicability of the method was evaluated through the quantitative performance of the $CuBr_2$ extraction for the determination of organic mercury fraction in some real matrices from an old Hg mining area in Asturias (Spain).

AREA OF STUDY

Mercury mining activities in Asturias (North-Western Spain) constituted a significant industry until 1974. Abandoned mercury mining works in Asturias are distributed all over the region, but the most important of them are located in two main mining districts (Mieres and Pola de Lena) in the central part of the region, closely to its capital. The most important sites in these two districts are: “El Terronal” and “Los Rueldos” in Mieres, and “La Soterraña” in Pola de Lena (the first two had their own metallurgical plant on-site). The environmental legacy of the mining and metallurgical works -developed in a time where the ecological concern was almost inexistent-, appears in the form of abundant spoil heaps of different age and dimensions, as well as rests of buildings and subsequent Hg-polluted soils, plants and watercourses. Some mine drainage and leachates from spoil heaps, which are eventually incorporated to streams tributaries of major rivers, show acidic conditions, such as the case of Los Rueldos mine (Loredo *et al*., 2005). Some of these sites have been partly remediated (e.g. the encapsulation of El Terronal spoil heap) (Ordóñez *et al*., 2004) or are currently under study to do so, but most of these hot spots keep spreading their adverse effects to their local environment (Loredo *et al*., 1999).
For the purpose of this study, three water samples, belonging to a periodic sampling program developed in the studied area, were chosen. These samples are referenced as points 2, 6 and 8. Point 2 is located downstream from the spoil heap and the pyrometallurgical installations of La Soterraña mine. Sample No. 6 was taken in San Tirso River, downstream the old metallurgical plant and the -now encapsulated- spoil heap at El Terronal mining site. Finally, Point 8 corresponds to a pond outside the old Los Rueldos Hg mine, where acid mine drainage is accumulated. All the samples were taken in April 2005, and a complete physicochemical characterisation of the collected waters was implemented.

EXPERIMENTAL

Reagents

All reagents were of analytical-reagent grade with the exception of CH_2Cl_2 (HPLC grade). Ultrapure water from a Milli-Q system (Millipore Bedford, MA, USA) was used throughout.
A 0.3 M $CuBr_2$ solution was prepared by dissolving the appropriate amount in 5% v/v HCl. A 1% w/v N-Acetyl-L-Cysteine (**NAC**) solution was prepared by dissolving this reagent in ultrapure water.
A 1000 mg L^{-1} stock solutions of methylmercury chloride and inorganic mercury was obtained from Merck (Darmstadt, Germany) and working standard solutions were prepared freshly from them by sequential dilution with 0.5 N HNO_3.

Cleaning Procedure

A specific cleaning procedure was designed and applied with the purpose of avoiding contamination among samples. This procedure comprised a number of steps:

1. All glassware was washed vigorously with a conventional soap and rinsed with abundant tap water.
2. The material was immersed in a 25% v/v nitric acid solution overnight.
3. Then glassware was rinsed three times with ultrapure water and dried in an oven at 105 ºC.
4. Clean material was stored in appropriate containers in the dark and taken before use.

Instruments, apparatus and materials

The DMA-80 analyser is shown schematically in Figure 1. The solid or liquid sample is weighed and introduced into the sample boat and then put into the autosampler. The sample is initially dried and then thermally decomposed in a continuous flow of oxygen. Combustion products are carried off and further decomposed in a hot catalyst bed. Mercury vapours are trapped on a gold amalgamator and subsequently desorbed for quantification. The mercury content is determined using atomic absorption spectrophotometry

at 254 nm. All processing steps are self-contained within the DMA unit and the results are displayed using a control terminal.

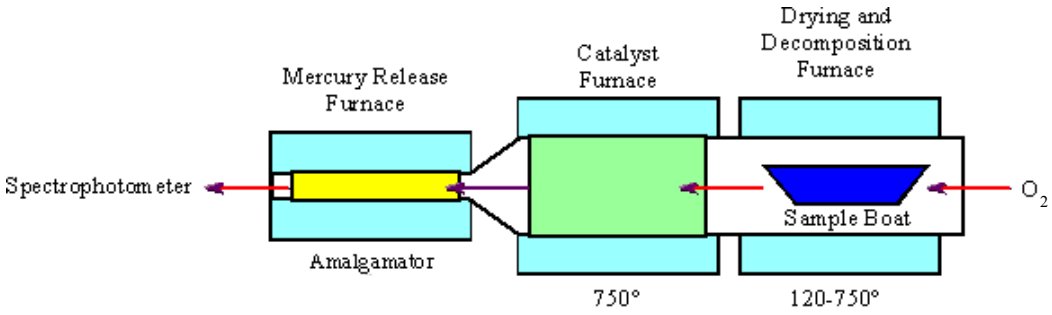

Figure 1: Schematic diagram of the mercury specific analyser DMA-80

Other equipment used were an end-over-end shaker (Bunsen ARR-8) with variable speed, an ultrasonic bath (SELECTA ULTRASONS 9L) with switch-timer clockwise, a bench-top centrifuge (Eppendorf 5804) and a drying thermostatized oven (Proeti S.A.) with a maximum adjustable temperature of 200 ºC.
50 mL glass centrifuge tubes of 29 mm maximum diameter equipped with twisting taps and acid-resistant joints were used for the extraction experiments.

Experimental procedure
Analyses were performed by using 200 mL water samples aliquots. The extraction method is schematically represented in Figure 2. The water sample was transferred to a 250 mL conical separating funnel and then 10 mL c.c. HCl, 10 mL 0.3 M $CuBr_2$ and 10 mL CH_2Cl_2 were added. The sample was vigorously shaken for 2 minutes and left until the organic and aqueous phases were separated. Organic phase was quantitatively recovered into a centrifuge tube and then centrifuged at 3200 rpm for 5 minutes. Aliquots of 200 µL-organic phase were accurately taken by means of HPLC syringes and transferred to quartz boats filled with 100 µL of 1% v/v NAC solution. Then evaporation of the organic solvent was carried out into a fume hood for 25 minutes. After the evaporation was completed, samples were introduced in the autosampler and then 200 µL of a 5% v/v HNO_3 solution were added. This addition prevents organic mercury losses during analysis and allows to obtain comparable results for replicates of the same sample. Finally, samples were analysed by the DMA-80 using operational conditions listed in Table 1.

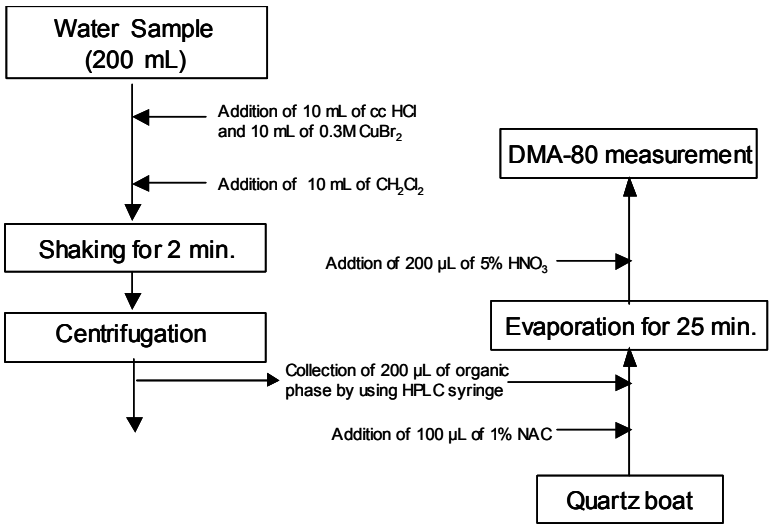

Figure 2: Extraction method for the determination of the organic mercury fraction

Table 1: Operational conditions

Parameter	*Value*
Oxygen Flow Rate	4 bar
Drying temperature	300 ºC
Drying time	180 s
Decomposition temperature	850 ºC
Decomposition time	180 s
Purge time	60 s
Amalgamation heating time	12 s
Recording time	30 s

RESULTS AND DISCUSSION

Method Development

As a first step, ultrapure water samples were spiked by addition of a known amount of a MeHg solution. In addition, an excess of inorganic mercury, in a selected Hg^{2+}/MeHg ratio of 10:1, was also added in order to assess the specificity of the proposed method to extract selectively the organic mercury species.. The obtained results are shown in Table 2.

Table 2: Recovery of MeHg in spiked ultrapure water

Sample	$[MeHg^{+}]$ added ($\mu g\ L^{-1}$)	$[Hg^{2+}]$ added ($\mu g\ L^{-1}$)	n	$[Hg]_{ORG}$ found ($\mu g\ L^{-1}$)	Recovery of $MeHg^{+}$ (%)
Ultrapure water	10	100	6	9.46 ± 0.27	94.6 ± 2.7

From these results, it can be deduced almost quantitative recoveries of spiked MeHg were achieved being the mean value about 95% with relative standard deviations lower than 5%. On the other hand, in spite of having an excess of inorganic mercury, it seems that extraction of inorganic mercury does not occur in these conditions, since found concentration did not exceed the added MeHg. This proves the selectivity of CH_2Cl_2 for dissolving MeHg bromide complexes remaining inorganic mercury species in the aqueous phase. The process permits the separation of the MeHg and preconcentration of this form with a factor of 20 which can be increased, it is necessary, by taking higher volumes of sample.

Application to water matrices from an old mining area

Since certified reference waters from mercury mining areas are not available, three samples were chosen as it is described in the *Area of Study* section. The total mercury concentrations in these samples were analysed and the results were below the detection limit of the method. Therefore, the suitability of the method to be applied to waters from contaminated sites was studied by analysing spiked Hg mine water samples. After filtering the taken water samples, spiked samples were prepared by addition of different volumes of Hg^{2+} and MeHg solutions. Analyses of samples were carried out as described in the *Experimental Procedure* section. Mean results are shown in Table 3.

Table 3: Recovery of MeHg in spiked mining area waters

Sample	$[MeHg^{+}]$ added ($\mu g\ L^{-1}$)	$[Hg^{2+}]$ added ($\mu g\ L^{-1}$)	n	$[Hg]_{ORG}$ found ($\mu g\ L^{-1}$)	Recovery of $MeHg^{+}$ (%)
Point 2	10	100	3	9.21 ± 0.49	92.1 ± 4.9
Point 6	10	100	3	10.11 ± 0.43	101.1 ± 4.3
Point 8	10	100	3	9.43 ± 0.25	94.3 ± 2.5

Results from Table 3 showed quantitative MeHg recoveries, where recovery percentages were higher than 92% for the three tested matrices. In addition, no inorganic mercury was co-extracted in the organic phase. From a point of view of the interfering compounds, some critical parameters from the physicochemical characterisation data must be considered for the accurate and appropriate method use (Table 4). They were selected as influential factors in MeHg extraction processes according to the revised literature. In this sense, it has been reported that inorganic mercury can be extracted together with organic mercury species by complexation with iodide ions (Decadt *et al*., 1985). This means that water matrices containing high quantities of complexing agents such as iodide, could promote the extractability of Hg^{2+} in organic solvents, but it does not occur with these Hg mine samples.

Table 4: Significant physicochemical data of collected samples

Sample	I^- (mg L^{-1})	Fe(III) (mg L^{-1})	SO_4^{2-} (mg L^{-1})
Point 2	< 0.02	0.12	41
Point 6	< 0.02	0.04	206
Point 8	< 0.02	788	115

Several authors have observed transformations from alkylmercury (both methyl and ethyl) to inorganic mercury under strongly acidic conditions in solutions with high contents of Fe(III) (Yan *et al*., 2003). These transformations can alter significantly mercury speciation in waters and the effect is increased with time. It has been established that transformations usually start to be observed in 8 hours after acidification of sample (Yan *et al*., 2003). In our study, samples from points 2 and 6 did not present significant concentrations of Fe(III). However samples from point 8 exhibited a very high Fe(III) concentration and consequently it was predictable that MeHg losses could take place. The good recoveries obtained in all cases confirmed the lack of MeHg losses for these samples. The apparent absence of transformations is probably due to the short time required to extract organic mercury species.
On the other hand, some incubation studies have demonstrated that the presence of sulphate in waters enhance the production of MeHg from inorganic mercury (Gilmour *et al*., 1992). It seems that the rate of production and the final concentration of MeHg increase with the sulphate concentration and contact time. In the present work the three selected points presented significant concentrations of sulphate, specially points 8 and 6. Once again, the high speed of treatment and analysis seems to be the reason for the imperceptible influence of the sulphate presence.

CONCLUSIONS

A new methodology has been developed for the organic mercury determination in Hg mine waters. The use of $CuBr_2$ solution as extracting agent showed to be very appropriate to extract quantitatively organic mercury species as bromide derivatives. Quantitative recoveries were achieved when ultrapure and Hg mine water samples were spiked with methylmercury. The high speed of application and analysis showed to be one of the main advantage of the proposed method, making possible the analysis of a considerable number of samples per day and, on the other hand, allowing to keep the simplicity of the method by minimising matrix effects. It was not necessary to carry out additional steps in order to avoid the influence of factors usually affecting other conventional methods for determining mercury speciation in waters. In this sense, no influences of sulphate and iron were observed when freshly prepared spiked samples were extracted. Therefore, the proposed method can be very advisable to be applied to contaminated water samples from mining or industrial areas in order to evaluate the environmental risk of these environments.

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