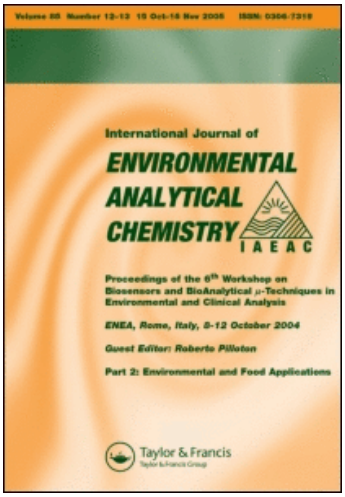

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DETERMINATION OF ORGANOMERCURY AND MERCURY IN ENVIRONMENTAL SAMPLES BY FLOW INJECTION ATOMIC FLUORESCENCE SPECTROPHOTOMETRY

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Flow injection analysis (FIA) involving the on-line oxidation of organomercury species to Hg^{2+}, followed by reduction to Hg^0 with acidified tin(II)chloride has been successfully coupled to an atomic fluorescence spectrophotometer (AFS) for determination of total organomercury in environmental samples. The method gave accurate and reproducible results for the certified dogfish samples DORM-1 and DOLT-1. A suitable organomercury extraction procedure for use with the method was established. The FIA-AFS method may also be used for determination of total mercury in environmental samples. The limit of detection (LOD) for organomercury was 200 pg and 2 pg for inorganic mercury using a 100 µl injection. The system is dedicated, cost-effective, sensitive and simple to use with a throughput of 17 analyses per hour.

KEY WORDS: Organomercury, mercury fluorescence, flow injection analysis.

INTRODUCTION

The determination of organomercury in environmental matrices has been possible for 30 years[1,2], but until recently inexpensive dedicated systems offering high sensitivity and good reproducibility have not been available. The use of chromatographic techniques for speciating organomercurials is well documented, but in the case of fish and sediment samples methylmercury (MeHg) is virtually exclusively the organic form in which mercury occurs[3,4] although ethylmercury is sometimes found in low concentration in sediments[5]. To obviate the need for expensive chromatographic techniques which therefore often do not separate anything in these samples, flow injection analysis (FIA) can be substituted to determine total organomercury. The use of an effective digestion and extraction of organomercury species from the matrix and any inorganic mercury present is naturally a pre-condition for assuring that such a flow injection system will be a success. As such extractions are essential, even for many of the chromatorgraphic

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methods, no additional sample preparation time results from the use of a flow-injection system.

The determination of total organomercury (methylmercury) in certified reference materials DORM-1 and DOLT-1 (dogfish muscle and liver respectively) and fresh fish and sediment samples is possible using a FIA-AFS system with suitable pre-extraction of the organomercury into cysteine. The method is inexpensive, dedicated, easy to perform and may be automated.

MATERIALS AND METHODS

Reagents

The methylmercuric chloride stock solution (250 mg l^{-1} CH_3Hg^+) was prepared by dissolving the compound in a 0.001 M cysteine solution. Standard solutions were prepared by dilution with 0.001 M cysteine to take account of matrix effects with samples and stored in quartz glass vials with mini-inert valves (Supelco, Saffron Walden, UK) to prevent analyte loss. Inorganic Hg(II) standard solutions were prepared in 3% HCl by dilution of a 1000 mg l^{-1} $HgCl_2$ stock solution (Spectrosol, BDH, UK). All reagents for FIA were of analytical grade (Table 1). The oxidising and reducing solutions were made fresh every 2 weeks. The acidic reductant was stored in a brown glass bottle. Solvents used for extraction were glass-distilled grade (Rathburn Chemicals, Walkerburn, Scotland).

Extraction of organomercury

Samples were extracted by 3 methods (Table 2). Approximately 0.3 g of dogfish (*Squalus acanthias*) certified reference materials (CRMs) DORM-1 (dogfish muscle) and DOLT-1 (dogfish liver, National Research Council, Canada) were used to determine the most suitable extractions. For fresh samples of eel (*Anguilla anguilla*) and roach (*Rutilus rutilus*) up to 5 g wet weight of well macerated material was used. Two of the methods (B and C) involved a mild digestion of the sample with tetramethylammonium chloride prior to extraction. Method A is a modification of Longbottom's method[7] and Method C is based on that described by Hintelmann *et al.*[5]. Sodium thiosulphate or cysteine was

Table 1 Analytical parameters for organomercury and mercury(II) determination by FIA-AFS.

Parameter/component	*Specification*
Injection volume	100 µl
Carrier stream	Water
Oxidising stream	25 g l^{-1} potassium peroxodisulphate and 1.2 g l^{-1} copper sulphate in 0.25 M sulphuric acid Flowrate 0.4 ml min^{-1}, reaction coil volume 0.5 ml
Reducing stream	100 g tin(II)chloride dissolved in 200 ml HCl, made up to 1 l with water Flowrate 0.5 ml min^{-1}, reaction coil volume 1.0 ml
Argon gas flow rates	Tubular membrane drying gas 2000 ml min^{-1} AFS sheath gas 300 ml min^{-1} AFS carrier gas 250 ml min^{-1}

Table 2 Organomercury extraction protocols for the three methods used.

Method	*Procedure*
A	1. 5 ml 0.5 M $CuSO_4$ and 10 ml 3 M KI in 1 M H_2SO_4 added to sample and shaken for 30 min. 2. 5.0 ml toluene added, shaken for 5 min, centrifuged at 2000 rpm for 2 min. Toluene layer containing organomercurials removed†. 3. Sample re-extracted with 5.0 ml toluene and aliquots combined. 4. 1.0 ml 0.001 M cysteine or $S_2O_3^{2-}$ added and shaken for 5 min. Aqueous extract presented to detector.
B	1. 5 ml 20% (w/v) tetramethylammonium chloride added to sample and heated in closed vial at 60°C for 4 h. Sample cooled 2. Procedure as for Method A
C	1. As for Step 1 of Method B. 2. Sampled cooled and acidified with 5 ml 6 M HCl. 3. 10.0 ml 0.00025 M dithizone in chloroform added and shaken for 15 min. Sample filtered; organic phase transferred to test tube. 4. Shaken with 1 ml 1:1 5% $NaNO_2$: 0.01 M HCl, 0.01 M H_2SO_4, 0.1 M NaCl (mixed immediately before use) until solution changes from green to orange. 5. Aqueous layer removed and chloroform washed with 1 ml water and removed to clean test-tube. 6. Organomercury back-extracted into 1.0 ml 0.001 M cysteine or $S_2O_3^{2-}$. Aqueous phase analysed.

†In all extractions the exact volume of solvent removed was noted for correction due to sample loss

used in the final extraction step. A 0.001 M cysteine solution was found to be the most suitable for the operating conditions.

Digestion for total mercury analysis

Fish samples were digested using a Milestone 1200 Mega microwave digester (Milestone, Bergamo, Italy) in pressurised digestion bombs. Samples were weighed out as described above and reacted with a digestion matrix of 2 ml 30% H_2O_2, 7 ml HNO_3 and 1 ml water for 30 min before being subjected to a digestion regime recommended by the microwave manufacturers. Digestates were made up to 50 ml with water and analysed.

The flow injection—atomic fluorescence system

The flow injection system (Figure 1) consisted of an injection port and pump (Waters, UK) which carried the sample on-line to a strong oxidising agent within a reaction coil. The oxidising agent converts any organomercury species present to Hg^{2+}. Reduction of the mercuric ions was achieved in a further coil in which acidic stannous chloride is mixed with the sample. The elemental mercury generated was separated from the liquid phase by a quartz glass gas-liquid separator (PS Analytical, Sevenoaks, UK) specifically designed for the analysis of mercury and carried to the atomic fluorescence detector (10.023 Merlin Detector, PS Analytical) for determination. Drying of the mercury-containing vapour before detection was achieved with a hygroscopic tubular membrane

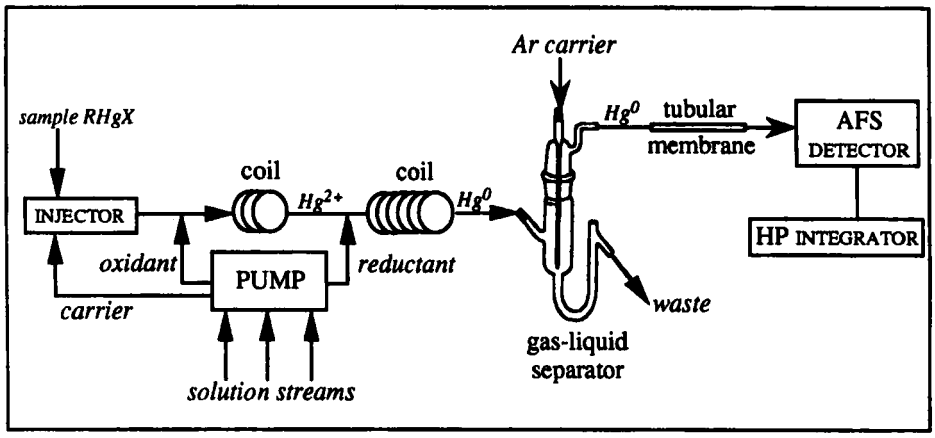

Figure 1 The flow injection—atomic fluorescence detection system.

(Perma Pure, New Jersey, USA). The detector output was recorded on a Hewlett Packard 3396A integrator and peak heights were measured. All flow injection tubing and joints were of PTFE or Tygon. Details of the optimised flow injection solutions and operating parameters are given in Table 1.

RESULTS AND DISCUSSION

System optimisation

The effectiveness of each aspect of the flow injection chemistry was examined by varying one parameter at a time and noting the detector response to standard methylmercury solutions. The initial configuration tested was based on that described by Hintelmann *et al.*[5] for an HPLC-AFS system which utilised an alkaline reductant solution (Table 3) and sodium thiosulphate to extract organomercury species. However, it was found that without an HPLC column and with the reaction coil lengths available, a black precipitate was formed in the reductant coil and the gas-liquid separator after a couple of hours of operating the system. This led to unexpected noise and multiple peaks (Figure 2), suggesting incomplete conversion of organomercury species to Hg^0 and blocking of the tubing. Thiosulphate was therefore considered unsuitable for use in the flow injection system and the chemistry was re-assessed with cysteine as the final organomercury extractant. Very broad and unreproducible peaks resulted when an alkaline reducing stream was used (Figure 2) and small amounts of black precipitate still formed. The use of an acidic reducing stream removed these problems and the system was finally optimised to the conditions in Table 1.

The carrier solution was varied from an ammonium acetate buffer solution to 6 M HCl and various methanol-water mixtures (Table 3). No significant improvements were found for these solutions over water which was subsequently used to keep the system as simple and cheap as possible. The system optimised for organomercury analysis also worked successfully for total mercury analyses. The drying of the Hg^0 carrying argon vapour after the gas-liquid separator is essential to prevent quenching of the fluorescence signal

Table 3 Ranges of components tested for the optimization of the chemistry of the flow injection system.

Parameter	*Component*	*Range*
Carrier stream	Methanol-water	0–30% methanol
Oxidising stream	Sulphuric acid	0.25–0.5 M
	Copper sulphate	0–3.2 g l^{-1}
	Potassium peroxodisulphate	25–50 g l^{-1}
	Reaction coil volume	0, 0.5 and 1.0 ml
	Flow rate	0.4 and 0.5 ml min^{-1}
Reducing stream	tin(II)chloride acid system	15–100 g l^{-1} in 30–200 ml l^{-1} HCl
	tin(II)chloride alkaline system	15 g l^{-1} in 48 g l^{-1} NaOH
	Reaction coil volume	0, 0.5 and 1.0 ml

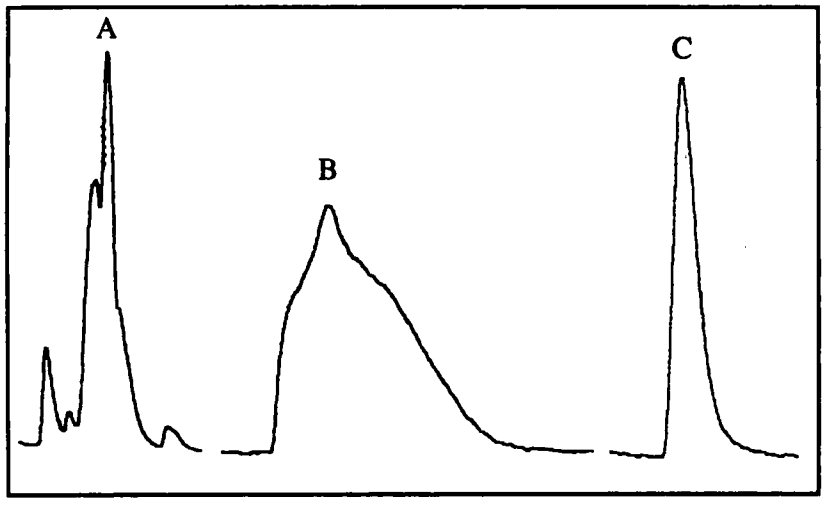

Figure 2 Effects of the chemistry of the flow injection system on detector response to single injections of 100 µl of 100 µg l^{-1} CH_3Hg^+ standard.
Peak A, alkaline reductant, CH_3HgCl made up in 0.001 M sodium thiosulphate; **B**, alkaline reductant, CH_3HgCl in 0.001 M cysteine; **C**, optimised acidic reductant, CH_3HgCl in 0.001 M cysteine.

by water vapour. The efficiency of traditional drying agents such as magnesium perchlorate or calcium chloride declines rapidly, requiring frequent changing of toxic and difficult to handle materials. Other drying methods including glass fibre filters, concentrated sulphuric acid or dry ice in ethanol[5] are tedious and need constant attention. The use of a hygroscopic tubular membrane was simple and virtually maintenance-free[6], effectively removing water vapour from the system described.

System performance

Using the optimised system a linear detector response was found for the range 5–1000 µg l^{-1} CH_3Hg^+ in 0.001 M cysteine. The upper limit of linearity was not determined, but the limit of detection (LOD) was 2 µg l^{-1} (n = 10, RSD = 6.4%),

equivalent to 0.2 ng CH_3Hg^+ in absolute terms. The RSD for measurements at 25 μg l^{-1} (10 replicates) was 3.7%. For fresh fish samples the LOD allows determination of 0.4 μg kg^{-1} CH_3Hg^+ on a wet-weight basis. Inorganic mercury(II) standards in 3% HCl gave a linear response over the tested range of 0.05–1000 μg l^{-1} with an LOD of 0.02 μg l^{-1} (n = 10, RSD = 4.3%, 2 pg Hg^{2+} absolute). The use of 0.001 M cysteine as the matrix for MeHg standard solutions is essential as it is used in the extraction of organomercury from real samples and takes account of an important matrix effect which diminishes the detector response. Methylmercury standards made up in water alone are also unstable, necessitating regular preparation of fresh standards. Analysis time is rapid at under 4 min per sample injected, allowing duplicate determination of about eight samples per hour.

Extraction efficiencies

The three extraction methods were compared for their ability to extract organomercury from the certified reference materials DORM-1 and DOLT-1 and separate any inorganic mercury from the extract. Methylmercury standards in 0.001 M cysteine were also subjected to each extraction method to determine recoveries. Method C gave good results for DORM-1 (Table 4), a material with 92% of the total mercury present as organomercury. However, with DOLT-1 which has only 36% of mercury as organomercury, it was apparent that the method was effectively extracting all mercury species from the sample matrix, but not separating the inorganic species from the organomercurials. This is because the dithizone was not releasing the inorganic mercury extracted in the separation step using acidified $NaNO_2$/NaCl. Method A gave more consistent but low recoveries in the order of 45%, probably because of the absence of the tetramethylammonium digestion step prior to extraction. Method B proved the most reliable and effective with extraction efficiencies of 70–80%. The mild digestion of samples is therefore an important step in the extraction process. Recoveries of methylmercury extracted from a standard 250 μg l^{-1} solution in 0.001 M cysteine using Method B were in the range 92–111%.

Determination of samples

The certified reference materials DORM-1 and DOLT-1 were analysed for organomercury after extraction by Method B and for total mercury after microwave digestion. Good agreement with certified values was found for both materials after correction for organomercury extraction efficiency (Table 5). Work is presently being

Table 4 Organomercury extraction efficiencies for CRMs using the three methods.

Method	*Percent extraction efficiency†*	
	DORM-1	*DOLT-1*
A	45	48
B	72	81
C	99	355

†All extractions and analyses conducted in triplicate, i.e. n = 9.

Table 5 Concentrations of organo-mercury and total mercury determined in DORM-1 and DOLT-1.

CRM	*Organomercury (μg kg^{-1})*		*Total mercury (μg kg^{-1})*	
	Determined†	*Certified*	*Determined*	*Certified*
DORM-1	727 ± 55	731 ± 60	766 ± 43	798 ± 74
DOLT-1	89 ± 5	80 ± 11	248 ± 17	225 ± 37

†Mean and standard deviation of three extractions using Method B, each analysed in triplicate.

carried out on sediment samples, but until recently[4] a reference organomercury sediment material has not been available. This will aid in further validating the method.

CONCLUSIONS

The FIA-AFS method described has been optimised for the analysis of total organomercury and total mercury in fish samples after the use of suitable extraction and digestion procedures. The most suitable organomercury extraction procedure involved a mild tetramethylammonium digestion, followed by extraction into toluene and back-extraction into 0.001 M cysteine. The use of an all acidic flow injection chemistry in conjunction with cysteine removed the problems of precipitation and resulting poor peak shape and reproducibility experienced when an alkaline reductant and sodium thiosulphate were used. Preliminary results indicate the success of the method for use in the determination of organomercury and total mercury in marine reference materials DORM-1 and DOLT-1. Further steps are being taken to fully validate the method for determination of organomercury in other environmental matrices such as sediments and water. The major advantages of the method are its simplicity and relatively low cost.

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