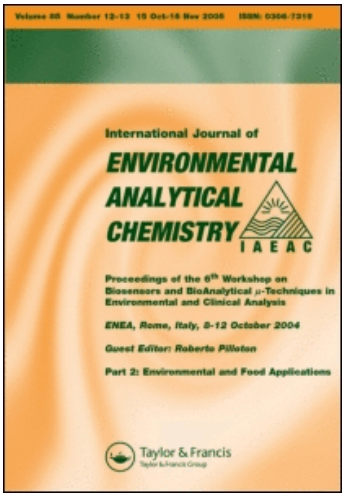

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EVALUATION OF SOME ISOLATION METHODS FOR ORGANOMERCURY DETERMINATION IN SOIL AND FISH SAMPLES BY CAPILLARY GAS CHROMATOGRAPHY—ATOMIC FLUORESCENCE SPECTROMETRY

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Three extraction methods, acidic KBr/$CuSO_4$ isolation-methylene chloride extraction, acidic KBr/$CuSO_4$ isolation-methylene chloride extraction with an alkaline digestion pretreatment, and an extraction method at a milder condition with citrate buffer and dithizone in chloroform, were studied for methylmercury and ethylmercury determination in soils, sediments and fish samples by the recently developed capillary gas chromatography—atomic fluorescence spectrometry system (GC-AFS). The acidic KBr/$CuSO_4$-methylene chloride extraction and the acidic KBr/$CuSO_4$-methylene chloride extraction with an alkaline digestion pretreatment were shown to be the effective methods for soils/sediments and fish samples analysis, respectively. The presence of ethylmercury species in soils of the Florida Everglades, observed with the acidic KBr/$CuSO_4$ isolation and methylene chloride extraction procedure, was further confirmed with the dithizone complexation/extraction procedure. The GC-AFS analytical method offers high sensitivity and selectivity for the determination of organomercury halides. The GC column maintenance, a critical step for organomercury halides analysis using GC, is also discussed.

Keywords: Methylmercury; ethylmercury; GC-AFS; soil; sediment; fish

INTRODUCTION

Sample preparation procedure is a critical step in the valid determination of organomercury in environmental samples. For soil, sediment and biological samples, the complete release of organomercury from the matrix is the first and most significant step of the whole sample preparation procedure, since organ-

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omercury compounds are well known to bind strongly to the matrix. A valid sample pretreatment method should enable the quantitative release of the target analytes from the matrix without alteration of the species during the preparation procedure. Many methods have been developed for biological sample analysis, particularly for fish tissue.[1] Most of these methods use the principles developed by Westöö,[2] in which samples are homogenized in water, acidified with hydrochloric acid, and treated with benzene to extract methylmercury chloride (MeHgCl) from the aqueous phase. Then MeHgCl in the benzene phase is separated from interfering co-extracted impurities by back-extraction with aqueous cysteine solution. The aqueous solution is acidified to break up the MeHg-thiol complex and the MeHgCl is again extracted into benzene. This procedure has been modified and used for determination of MeHg in soil and sediment samples.[3–6]

Alkaline hydrolysis has been frequently used as a pretreatment step for organomercury analysis in different environmental samples, especially in biological materials,[7–9] to achieve a homogenized solution. This solution is either used directly, for analysis by techniques such as derivatization-purge-trap coupled with atomic absorption spectrometry (AAS) or atomic fluorescence spectrometry (AFS),[7,10,11] or is subjected to a procedure similar to that for acidic liberation/organic solvent extraction mentioned above.[12,13]

Because of the suspected instability of organomercurials under very acidic conditions and the poor spike recoveries for several organomercury species obtained by the widely used HCl/toluene technique, an alternative extraction procedure at a milder condition was recently reported by Hintelmann et al..[14,15] In brief, soil samples were buffered at pH 2 with a citrate buffer and organomercury compounds were extracted with dithizone in chloroform. Organomercury-dithizone complexes were destroyed by a nitrite/acid mixture. The organomercury species remaining in the organic phase were then extracted into an aqueous thiosulfate solution which was finally analyzed using a high performance liquid chromatography coupled with an AFS detection (HPLC-AFS).

Another currently used sample preparation technique is based on a steam distillation. This method has been used to separate organomercury compounds from sediment, water, and biological samples.[11,16–18] The distillate was subjected to analysis with either cold vapor atomic absorption (CVAA),[16–18] gas chromatography/electron capture detector (GC-ECD),[18] or the aqueous ethylation-purge/trap-AFS method.[11]

Although many sample preparation procedures have been developed, the controversy in terms of reliability of the procedures has not been resolved. The steam distillation, for instance, has been claimed to show advantages over the solvent extraction with regards to the recovery and the detection limit, and with-

out the use of organic solvents.[11] However, recent investigations showed that the distillation procedure used to separate MeHg from both lake water and lake sediment samples generates artificial MeHg aided by the presence of natural organic substances.[19] In addition, the procedure of aqueous ethylation with $NaBEt_4$ was found to induce MeHg from inorganic mercury present in sediment, when the extract obtained with alkaline digestion was directly subjected to analysis by the aqueous ethylation-purge-trap and GC-AFS.[11]

A variety of analytical methods have been developed for the determination of MeHg species in environmental and biological samples. Most of these methods employ separation by standard chromatographic techniques and subsequent determination by an appropriate and sensitive detection system, including AAS, AFS, ECD, and various types of atomic emission spectrometry (AES), utilizing inductively coupled plasma (ICP) or microwave induced plasma (MIP).[1,20] Two methods in particular have been used for routine analysis of MeHg: GC-ECD[1,12,21,22] and aqueous ethylation of MeHg with sodium tetraethylborate ($NaBEt_4$), followed by purge and trap and detection by either AAS[7,23] or AFS.[10,11] Both techniques have disadvantages when applied to environmental and biological samples. The ECD detection is simple and convenient, but the halogen-bearing compounds coextracted with organomercury can interfere with the determination because of the non-specificity of the ECD. Although the ethylation-purge-trap method provides a good detection limit for MeHg analysis, its application is limited because it does not distinguish between ethylmercury (EtHg) and inorganic mercury (Hg^{2+}). Alternative techniques which offer high sensitivity, high selectivity, and the capacity for the determination of different species of organomercury compounds, are capillary GC and HPLC coupled with an AFS detection.[3,14] By using these recently developed techniques, EtHg has been observed in both soil and sediment samples.[3,4,15]

Generally, the choice of a sample preparation procedure is based on the analytical method used. Although a modified acidic leaching/organic solvent extraction method has been developed and used for the determination of organomercury compounds in soil and sediment samples by the capillary GC-AFS technique,[3,6] there are still no comprehensive reports concerning sample treatments for the determination of organomercury species in soil, sediment and biological samples for capillary GC-AFS analysis. Furthmore, comparisons of different methods are essential in terms of the reliability of the analytical procedures. The purpose of this study was to evaluate several commonly used extraction procedures for the determination of MeHg and EtHg from soil and fish samples using the highly sensitive and selective GC-AFS technique. Three techniques were chosen: the traditional acidic leaching/organic solvent extraction,[3,5] the acidic leaching/organic solvent extraction with an alkaline digestion pretreat-

ment,[8,13] and the extraction method at a milder condition reported by Hintelmann et al..[14,15] These procedures have been modified or improved, as discussed below, in order to be used for the subsequent analysis with GC-AFS. The methods for GC column maintenance, which are always crucial for the determination of organomercury halide by GC, are also discussed. By using the different extraction techniques, the results of this study provide further evidence for the recently observed presence of EtHg in soils and sediments of the Florida Everglades.[3,4]

EXPERIMENTAL

Apparatus

Organomercury analyses were performed using the P.S. Analytical mercury speciation system model PSA 10.723. This is an integrated gas chromatography—mercury atomic fluorescence instrument which is comprised of an Ai Cambridge, UK, model GC 94 gas chromatograph equipped with a CTC A200S autosampler, an optic injector module and coupled to the PSA Merlin Detector via a pyrolysis oven held at 800°C. The DB-1 (J & W Scientific) fused silica analytical column had dimensions of 15 m × 0.53-μm i.d. (Megabore) and a 1.5-μm film thickness. The column temperature was held at 50°C for 1 min, programmed at 30°C/min to 140°C, which was held for 3 min, then programmed at 30°C/min to a final temperature of 200°C, and held for 3 min. A split/splitless injector was used in the splitless mode and maintained at 250°C. The carry gas and make-up gas flows were 4.0 mL/min of helium and 60 mL/min of argon, respectively.

For the PSA Merlin detection system, the sheath gas flow was 150 mL/min of argon. Other parameter settings were the same as previously reported.[3,6] Data was acquired by a real-time chromatographic control and data acquisition system (E-Lab, Version 4. 10R, OMS Tech Inc. USA). The detection limit was defined as the amount of mercury giving a peak area equal to three times the standard deviation of the baseline noise.

A PS Analytical Merlin Mercury Fluorescence System was used for total mercury analysis. Details of the procedure used has been reported elsewhere.[6]

Reagents and Materials

Deionized water produced by a Barnstead B-Pure system was used in all aqueous solutions. Optima grade methylene chloride, certified ACS grade potassium bromide, copper sulfate, sodium thiosulfate, anhydrous citric acid, sodium nitrite,

sodium hydroxide, sodium chloride, dithizone, and trace metal grade concentrated sulfuric acid and hydrochloric acid were from Fisher Scientific. Chloroform was HPLC grade (Burdick & Jackson Laboratory, INC., MI, USA). All mercury standards were purchased from Ultra Scientific. Standard stock solutions of methyl-, ethylmercury chloride (MeHgCl, EtHgCl) were prepared by dissolving appropriate amounts of the standards in methanol. These solutions were stored in dark brown glass bottles at room temperature (20°C) and diluted with methylene chloride or water to give working standards or spiking standards of desired concentrations when required.

The acidic potassium bromide solution was prepared by dissolving 180 g of KBr in 500 mL water and adding 50 mL sulfuric acid in 100 mL water. After cooling to room temperature the solutions were mixed and made up to 1 L with water. Copper sulfate (1 M) and sodium thiosulfate (0.01 M) solutions were prepared by dissolving appropriate amounts of the salts in water.

The preparation of the reagents used in Hintelmann et al.'s method was followed as reported.[14] In short, the citrate buffer consisted of citric acid (21 g/L) and sodium hydroxide (8 g/L) and was adjusted to pH 2 with 1% hydrochloric acid. The dithizone extractant (0.25 mM) was prepared in chloroform. A 1:1 mixture of 5% of sodium nitrite and an acid solution consisting of hydrochloric acid (0.01 M), sulfuric acid (0.01 M) and sodium chloride (0.1 M) was used to destroy the dithizone-mercury complexes. The two solutions were mixed immediately before use.

Sample Preparation Procedures

Soil, sediment and fish samples were collected from the Florida Everglades using the procedures reported previously.[6] Four samples which contained both MeHg and EtHg species were chosen for the present comparative study. The samples were homogenized to an uniform consistency with a blender (Osterizer) prior to extraction. In addition, a standard reference material (DORM-2, fish tissue) obtained from the National Research Council of Canada, was also used in this study.

For simplicity, the sample preparation methods utilizing acidic isolation/solvent extraction, acidic isolation/solvent extraction with alkaline hydrolysis pretreatment, and the method reported by Hintelmann et al. are referred to as M1, M2, and M3, respectively. Details of the extraction procedures for soil, sediment and fish samples analysis are summarized in Figures 1 and 2. M1 used for sediment and soil extraction was slightly modified when used for fish analysis. Because of the formation of an emulsion in the aqueous phase when 1 mL of sodium thiosulfate solution was added into the CH_2Cl_2 extract, 0.5 mL pro-

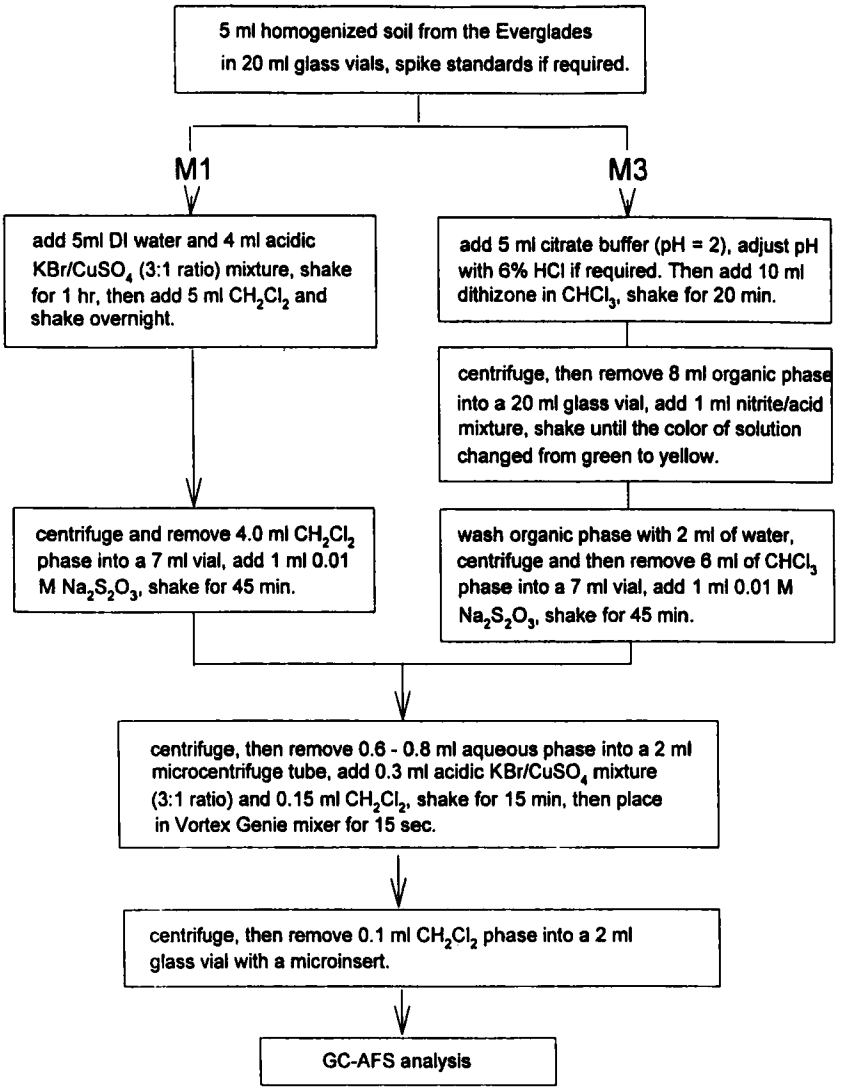

FIGURE 1 Schematic diagram of the procedure for MeHg and EtHg analysis in soils and sediments.

panol were added at this step. M2 employed a similar procedure to that used in M1, except a pretreatment with alkaline digestion was used. M3, which was initially designed for the determination of organomercury species in sediments using HPLC-AFS,[15] was also modified in this study. Instead of the direct analysis of organomercury in the sodium thiosulfate solution by HPLC-AFS, organ-

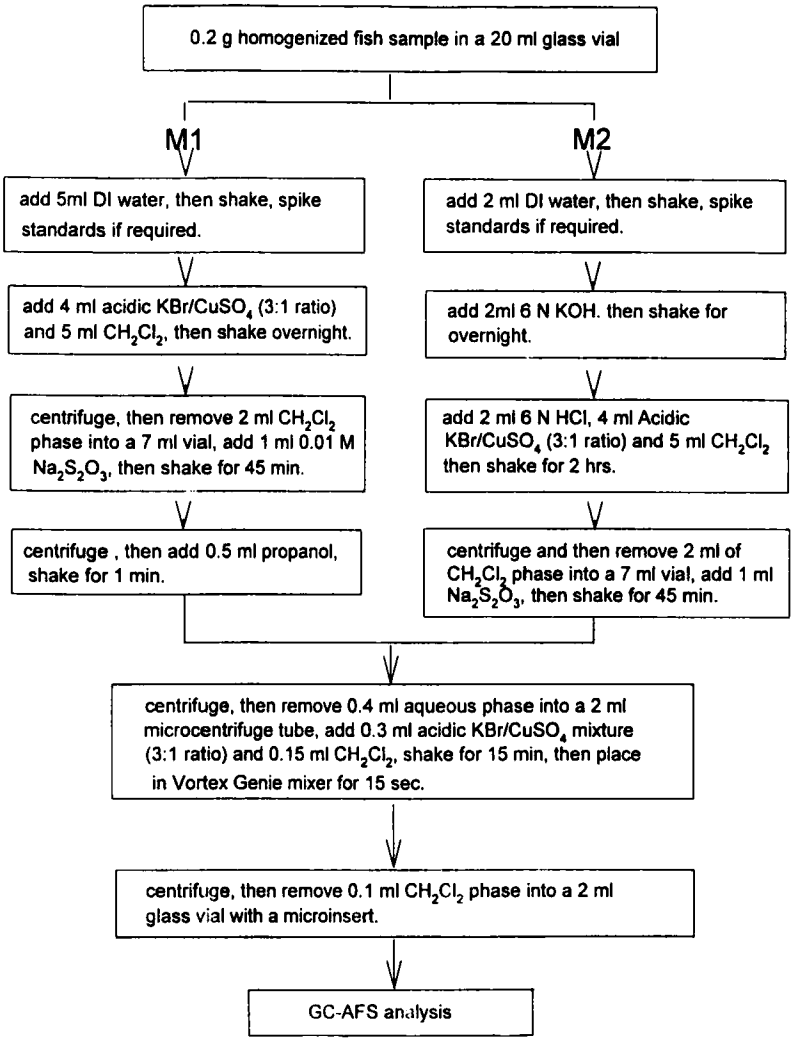

FIGURE 2 Schematic diagram of the procedure for MeHg and EtHg analysis in fish tissue.

omercury species were back-extracted into CH_2Cl_2 with the assistance of acidic $KBr/CuSO_4$ and analyzed using GC-AFS.

Generally, triplicate extractions were performed for both spiked and unspiked samples. Calibration was performed by standard addition. Procedural blanks were performed in the same manner as the real samples except without added sample. The standards for quantitation were made by adding 0.8 mL DI water, 0.2 mL MeHgCl and EtHgCl working solution (prepared in water, 5 pg/μL as

Hg), 0.3 mL acidic $KBr/CuSO_4$ mixture (3:1 ratio) and 0.2 mL CH_2Cl_2, into a 2 mL microcentrifuge tube. The contents were shaken for 15 min, mixed for 15 sec on a Vortex Genie mixer, and then centrifuged. A 0.10–0.15 mL organic phase was dried over anhydrous sodium sulfate and placed into a 2 mL autosampler vial with a microinsert. Injections of 1–5 μL were analyzed by GC/AFS. Quantitation was based on the peak areas.

RESULTS AND DISCUSSION

Sediment Extraction

Since the alkaline digestion was initially developed and showed special advantages for the analysis of biological samples such as hair, blood, fish and soft tissues,[8] only M1 and M3 were chosen for soils and sediments analysis. Table I shows the results of total mercury (HgT), MeHg, and EtHg determination in soil and sediment samples using M1 and M3. For M1, recoveries of MeHg and EtHg achieved from spiked samples were satisfactory for both the Everglades soils and sediments. Both MeHg and EtHg were found in these samples. It should be noted that the organomercury spiking concentration was at ng/g as Hg, which was the same concentration levels for MeHg and EtHg species found in the unspiked samples. For M3, the pH of the matrix is critical.[24] It was found that 5 mL of citrate solution were not enough to buffer the 5 mL of slurry of the Everglades soils. The pH of the slurry needed to be adjusted with 6% of hydrochloric acid. As shown in Table I, samples S1 and S2, extracted without further pH adjustment with 6% of hydrochloric acid, had extremely low spiking recoveries for both MeHg and EtHg species. With pH adjustment using HCl solution (samples S3 and S4), the spiking recoveries of MeHg, and particularly EtHg were increased significantly. Both MeHg and EtHg were also found in the Everglades soil samples S3 and S4 with M3, though the concentrations were lower than that found with M1. It is interesting to note that the presence of EtHg species in soils of the Florida Everglades, observed with M1, was further confirmed with M3.

Hintelmann et al.[15] have tested the spiked recoveries of eight organic mercury compounds from soil samples. In their experiments, 2 g of soil was spiked with 2 μg of each organomercury and extracted using M3. The recoveries were found to be 63 $\pm$ 5.7% and 66 $\pm$ 9.1% for MeHg and EtHg, respectively. Unfortunately no spiking recovery at lower levels (ng/g) was reported in their paper. The lower concentrations of MeHg and EtHg and the lower recoveries of MeHg found in the Everglades soils with M3 suggest that the organomercury

TABLE I Results of total mercury (HgT), MeHg, and EtHg determination in soil samples from the Florida Everglades using M1 and M3

	HgT (ng/g)	*Organic Hg spiking level (ng/g)*	*MeHg*		*EtHg*	
			Concentration (ng/g)	*Recovery (%)*	*Concentration (ng/g)*	*Recovery (%)*
M1						
S-1	192.5 ± 2.9	2.3	2.25 ± 0.03	77.7 ± 2.9	0.55 ± 0.02	63.5 ± 5.0
S-2	NA	6.9	5.41 ± 0.02	126.6 ± 8.8	5.01 ± 0.57	96.3 ± 9.4
S-3	95.4 ± 9.5	1.5	1.43 ± 0.04	87.7 ± 2.4	0.91 ± 0.03	79.7 ± 1.3
S-4	107.0 ± 14.2	1.7	1.53 ± 0.17	108.6 ± 3.4	4.55 ± 0.23	74.7 ± 3.1
M3						
S-1[a]	192.5 ± 2.9	2.3	ND	7.6 ± 0.6	ND	8.1 ± 0.3
S-2[a]	NA	34.6	ND	6.6 ± 1.6	ND	7.8 ± 1.5
S-3	95.4 ± 9.5	1.5	0.65 ± 0.16	30.0 ± 7.1	0.64 ± 0.07	87.4 ± 8.3
S-4	107.0 ± 14.2	1.7	0.30 ± 0.05	54.9 ± 1.0	0.61 ± 0.05	91.6 ± 10.5

NA: not analyzed.
ND: not detectable.
M1: acidic KBr/$CuSO_4$ isolation method.
M3: extraction under milder conditions (see text for details).
a: without further pH adjustment after addition of buffer solution.

species cannot be extracted quantitatively at low ppb levels under mild conditions. Another possibility is that M3, which was designed for sediment analysis with HPLC/AFS, requires further optimization for the matrix used in this study and the GC-AFS detection.

Acidic $KBr/CuSO_4$ mixture was used in M1 for isolation of organomercury compounds from soils and sediments. The advantages of using this mixture as an extractant, versus hydrochloric acid alone, have been well documented.[8,25] Briefly, organomercury bound with the soil and sediment matrix was released by the combined action of the acid and cupric ions; the formed organomercury bromide derivatives (RHgBr) were then extracted into the methylene chloride layer. Bromide was chosen as the halide source owing to the more favorable distribution between the organic and the aqueous phase for RHgBr compounds compared to other halides.[8] Cupric ions, on the other hand, were used to promote the release of the organomercury compounds from matrix.[8,11] In contrast to M1, citrate buffer (pH = 2) was used in M3. Although dithizone that forms strong complexes with organomercurials aids in enhancing the leaching of organomercury from its matrix, it appears that the cleavage for the strong binding between organomercury and its matrix was not sufficient at pH 2.

The results of this study indicates that in order to achieve valid and accurate results for the determination of organomercury compounds, spiking recovery has to be done by spiking the sample with the target analytes at a concentration level similar to that present in the unspiked sample. Compared to M3, M1 seems to be a more efficient extraction procedure for the MeHg and EtHg determination in soil and sediment samples. This is especially true when soils and sediments with a low organomercury concentration (ng/g level) are analyzed.

Fish Extraction

The results of HgT, MeHg, and EtHg determination in homogenized fish and a certified reference material (DORM-2) using methods M1 and M2 are summarized in Table II. Satisfactory spiking recoveries were obtained for all the samples analyzed using both M1 and M2. While MeHg was detected in both fish from the Everglades and the Dorm-2, EtHg was not found. The concentrations of MeHg found in DORM-2 were 4.74 $\pm$ 0.18 and 5.09 $\pm$ 0.28 μg/g as Hg for M1 and M2, respectively, both in good agreement with the certified data (4.47 $\pm$ 0.32 μg/g as Hg). The M1 method used for fish and DORM-2 was the same extraction procedure as that used for soils and sediments except for the addition of 0.5 mL of propanol into the aqueous sodium thiosulfate phase during the clean-up step of the extraction. As fish tissue could not be dissolved in the

TABLE II Results of total mercury (HgT), MeHg, and EtHg determination in homogenized fish samples using M1 and M2 methods

		HgT	*Organic Hg spiking level*	*MeHg*		*EtHg*	
				Concentration	*Recovery (%)*	*Concentration*	*Recovery (%)*
M1							
Fish[a]	(ng/g)	103.9 ± 0.9	375	58.9 ± 9.1	84.4 ± 6.2	ND	80.4 ± 4.4
DORM-2	(μg/g)	4.64 ± 0.26[b]	5.5	4.74 ± 0.18[c]	63.9 ± 1.3	ND	69.5 ± 2.4
M2							
Fish	(ng/g)	103.9 ± 0.9	375	78.5 ± 11.6	86.8 ± 5.9	ND	86.4 ± 5.0
DORM-2	(μg/g)	4.64 ± 0.26	5.2	5.09 ± 0.28	79.9 ± 2.1	ND	98.5 ± 3.5

M1: acidic KBr/$CuSO_4$ isolation method.
M2: acidic KBr/$CuSO_4$ isolation method with alkaline digestion.
a: fish sample was collected from the Florida Everglades.
b: certified value.
c: certified MeHg value: 4.47 ± 0.32 μg/g as Hg.

acidic KBr/$CuSO_4$ mixture, the sample had to be well homogenized prior to extraction.

As shown in Table II, although similar spiking recoveries in fish samples were obtained by using both M1 and M2, the concentration of MeHg found in the samples was lower for M1 than for M2. This could be attributed to insufficient homogenization of the fish tissue for M1. M2, on the other hand, provided an efficient homogenization and a more uniform sample distribution because of the use of an alkaline digestion step. This could also result in a more efficient leaching of organomercury from sample matrix due to breakdown of protein and lipid material in the matrix during digestion. Cysteine was often used in the alkaline digestion step to complex the organomercury compounds and protect them from possible disintegration.[8,13] However, no such degradation for either MeHg or EtHg was observed in our study using 3M of potassium hydroxide as digestion reagent.

GC-AFS Analysis

For all the extraction procedures compared above, organomercury bromides obtained after the last extraction step were subjected to GC-AFS analysis. It has been shown that the AFS offers a high sensitivity for mercury analysis compared to other detection techniques.[3,10] Absolute detection limit determined in this study was 0.2 pg as Hg for both MeHg and EtHg. Details on the GC-AFS instrument setup has been described elsewhere.[3,6]

Although GC-AFS is a very selective and sensitive technique, some practical problems need attention. Such is the case of GC column maintenance, which always is an important step when GC method is used for organomercury halide analysis.[26] Some methods for column treatment have been described previously.[27] Several other procedures were tested in this study and found to be very useful in reducing column deterioration. Water, even in trace amounts, can cause serious problem during GC analysis of organomercurials. For the particular method used here, the aqueous phase from which the organomercury bromides were extracted into the methylene chloride phase was a sodium thiosulfate solution. The sulfur compounds brought into the injection port by the injection clearly affected the separation of organomercury compounds in the column. In order to avoid introducing the aqueous phase into the injection port, the methylene chloride layer separated at the last step was dried over a small amount of anhydrous sodium sulfate, packed in a 200 μL pipet tip. The dried methylene chloride phase was collected directly into a glass insert, which was placed in a 2 mL auotosampler vial. Another step taken was to increase the temperature of the injection port from 200 to 300°C when column maintenance was needed

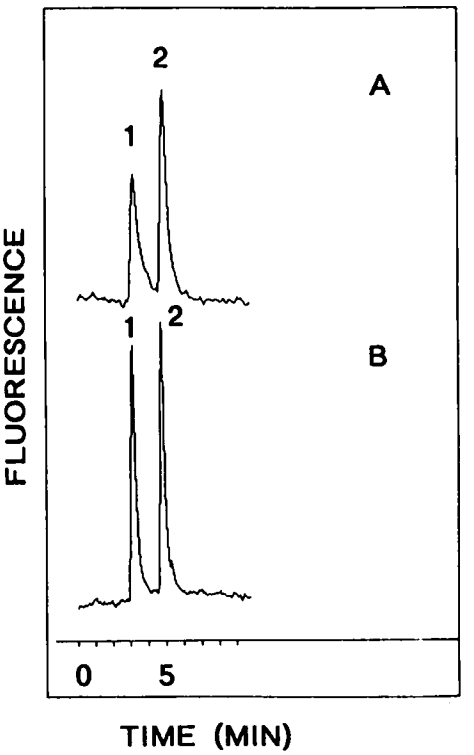

FIGURE 3 Effect of the temperature of the injection port on the separation efficiency with GC/AFS. Chromatograms obtained after about two months of real sample analysis. A, 200°C; B, 300°C. Peak identifications: 1, MeHg; 2, EtHg.

(extended retention time, decreased sensitivity, band broadening etc.). With this procedure, separation efficiency of the column was increased. Figures 3A and B show two typical chromatograms obtained at 200 and 300°C of injection temperature. The exact reason for the improvement of column performance by increasing the temperature of the injection port is not evident from the available data, however, it is plausible that the interferences in the extract are deposited at the front of the column and/or the injection port and can strongly interact with organomercury bromides. This may affect or even prevent the elution of the organomercury compounds in the column.[5] The increased temperature in the injection port would decrease the interactions between organomercury and the interferences and enhance the transport of target compounds onto the column, subsequently improving its separation efficiency. It is interesting to note that no degradation of organomercury bromides was observed during the analysis even if the temperature of the injection port was raised to 350°C. Indeed,

both MeHgBr and EtHgBr have been reported to be relatively thermally stable (the bond dissociation energies of the Hg-C bond are 258.6 ± 8 and 252.3 ± 13 kJ/mol for MeHgBr and EtHgBr, respectively).[28] This enables the analysis of MeHgBr and EtHgBr to be performed at these relatively high temperatures.

CONCLUSIONS

Several extraction procedures were studied for organomercury (MeHg and EtHg) determination in soils, sediments, and fish samples by using GC-AFS. For soils and sediments, acidic KBr/$CuSO_4$ isolation with methylene chloride extraction, a procedure modified from the traditional acidic leaching/organic solvent extraction, was more effective than the extraction procedure under milder conditions with a citrate buffer and dithizone in chloroform. This is particularly true when the concentrations of MeHg and EtHg are at low ppb level. The presence of EtHg in the Everglades soils, observed with the acidic KBr/$CuSO_4$ isolation and methylene chloride extraction procedure, was further confirmed with the method reported by Hintelmann et al.[14,15] For fish sample analysis, the acidic KBr/$CuSO_4$/methylene chloride extraction with an alkaline digestion pretreatment was shown to be an effective extraction procedure. GC-AFS offers a highly sensitive and selective analytical method for the determination of organomercury halides. The GC column, with proper maintenance, will last more than three months with up to 50 injections of soil sample extracts each working day. Since serious limitations have been found with both the distillation and the ethylation procedures used to analyze both MeHg and EtHg species in soils, sediments, and biological samples, the modified acidic isolation/organic solvent extraction combined with the GC-AFS technique provides a useful analytical tool for organomercury speciation and an alternative for the currently used methods.

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