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Determination of Monomethylmercury Cation in Sediments by Vacuum Distillation Followed by Hydride Derivatization and Atomic Fluorescence Spectrometric Detection

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This paper presents a method capable of quantitatively separating sub-nanogram amounts of monomethylmercury cation ($MeHg^+$) and dimethylmercury from sediments by vacuum distillation at 40 °C and 6 μm Hg pressure followed by hydride derivatization and atomic fluorescence spectrometric detection. Concentrations of $MeHg^+$ in Great Bay Estuarine sediments ranged from 2.2 to 7.3 ng g^{-1} (dry weight) with a 4.7 ng g^{-1} average for samples taken over nine weeks of the 1996 summer. The RSD for replicate determinations of a homogenized estuarine sediment is typically less than 6%. The detection limit for the routine determinations on $MeHg^+$ is 0.2 ng g^{-1} dry weight of sediment. We validated the method by determining $MeHg^+$ concentration in reference sediment S-19, by confirming our method against an established extraction method, and by recovering 85% of 10 ng MeHgCl spiked into estuarine sediments. This paper also includes significant improvements in the hydride derivatization method for mercury compounds relative to previous work with respect to faster analysis time and lower detection limits. © 1997 John Wiley & Sons, Ltd.

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INTRODUCTION

Determination of monomethylmercury cation ($MeHg^+$) in sediments is still a difficult problem.[1] Commonly used methods include acid extraction, base extraction and atmospheric-pressure distillation.[2–6] Acid extraction is the most controversial approach because it did not quantitatively release $MeHg^+$ from sediment in two studies,[2,6] but was successful in a third one.[4] Basic extraction sometimes gives matrix problems with ethylation derivatization.[6] Atmospheric-pressure distillation is effective, but requires careful attention to the purge-gas flow rate and percentage completion of the distillation.[5] It is important to note that five of seven laboratories that provided the data used in the consensus $MeHg^+$ concentration determination in the only available sediment certified reference material (CRM) IAEA-356[2] utilized atmospheric-pressure distillation. The other two laboratories used alkaline digestion, and one of these used distillation as a secondary separation step prior to derivatization. Development of new and reliable methods for speciation of mercury compounds in difficult sediment matrices is clearly advantageous.

Separation from, and quantification of $MeHg^+$, in sediments presents several analytical challenges. The first task is to separate $MeHg^+$ from its binding to humic matter, proteins and particulate matter.[6,7] The second problem occurs when the inorganic $Hg_{tot}/MeHg^+$ ratio is very high. The ratio is typically at least 100 : 1,[6,8,9] and for sediment S-19 it is about 1700 : 1.[10,11] When Hg(II) is not separated from $MeHg^+$, it may be difficult to quantify the much weaker $MeHg^+$ signal. In addition, ethylation derivatization with high Hg(II) concentrations can convert some of it into the ethyl derivative of $MeHg^+$.[6] The third challenge is non-mercury interferences in extracts that may make derivatization of $MeHg^+$

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non-quantitative and/or irreproducible. This problem occurs in alkaline extractions,[6] but not in atmospheric-pressure distillation[6] or toluene/thiosulfate extraction.[4]

The two major goals of this research were development of new vacuum-distillation method for quantifying $MeHg^+$ in sediments, and improvement of hydride derivatization for speciation of mercury compounds. Vacuum distillation for separation of $MeHg^+$ from sediments produces distillates which can be reproducibly quantified by hydride generation–cold vapor atomic fluorescence spectrometry (HG–CVAFS). We observed few matrix interferences and, with one exception, no interference from high concentrations of Hg(II). We validated the method by using a reference sediment and an extraction technique. Recoveries for 10 ng of MeHgCl spiked into sediment using vacuum distillation were 85% (±6%). Improvements to our hydride derivatization method,[12, 13] which originated with the groups of Filippelli[14] and Craig,[15] include decreased analysis time, more efficient water removal and lowered detection limits.

EXPERIMENTAL

Glassware, plasticware and reagents

All glassware, PTFE tubing and impingers used in the AFS system were cleaned prior to use following the procedure of Horvat *et al.*[6] The first step, which was done only once, consisted of leaching them in concentrated HNO_3 (85 °C) for 24 h. After cooling and rinsing with doubly distilled deionized water (DD H_2O), the items were heated overnight in 1% HCl (70 °C). The second step was also used for general cleaning. Other glassware (scintillation vials, beakers etc.) was soaked in 7% HNO_3 (rough bath) overnight, and then in a second solution of 7% HNO_3 (final bath) for an additional 12 h. Glassware was then rinsed with DD H_2O and dried in an oven (70 °C).

DD H_2O distilled from a Corning Mega-pure still was used in all experiments. Aqueous 12% $NaBH_4$ (w/v) was prepared from 99% $NaBH_4$ (Aldrich) in a beaker. The solution was stirred for 15 min, filtered with a 0.2 μm polycarbonate Nuclepore filter, diluted to a 6% solution with water, and purged with mercury-free nitrogen for 3 h to remove traces of Hg^0. Hydrochloric acid (HCl) was trace-metal grade (Fisher Scientific). Reagents were analytical-grade, unless otherwise specified.

Standards

Stock solutions containing 1000 μg ml^{-1} were made up as follows (all masses and concentrations of mercury compounds in this paper are based on Hg^0). Hg(II) standard was made by oxidizing Hg^0 in 1 ml concentrated nitric acid, and then diluting to 100 ml in DD H_2O. MeHgCl (Alfa, Danvers, MA, USA) was purified from traces of Hg(II) in the following manner: 1 g of MeHgCl was dissolved in 10 ml CH_2Cl_2 and extracted twice with 5 ml of a solution containing 1.2 M HCl that was saturated in potassium chloride (KCl). The separated CH_2Cl_2 layer was dried with anhydrous sodium sulfate, poured off and evaporated for 2–5 h in a fume-hood. MeHgCl (purified) and Me_2Hg (Alfa) were dissolved in methanol (CH_3OH) to make the stock solutions. Working standards (10 μg ml^{-1}) and calibration standards (0.050 μg ml^{-1}) were made by successive dilution of stock solutions. MeHgCl and Hg(II) standards were diluted in 0.1 M HCl and Me_2Hg standards were diluted in MeOH. Working standards remained stable for one month and calibration standards for one week. Standards were injected into the reaction flask using a 20-μl adjustable Gilson Pipetman.

Apparatus for HG–CVAFS

All PTFE tubing was 3.1 mm o.d. (1.0 mm i.d.) unless otherwise specified. As shown in Fig. 1, two Cole–Parmer flow meters (A) controlled the flow rate of helium carrier gas. A Hamilton three-way valve (B) (No. 86727) was used to switch between high and low flow rates. A gold trap (C) (Brooks–Rand) removed Hg^0 from the carrier gas. The hydride generation flask (F) is a PTFE impinger (Savillex), 6.5 cm tall × 3.0 cm diameter (30 ml total volume). The injection port (E) is a modified $\frac{1}{8}$ in × $\frac{1}{8}$ in (3.2 mm × 3.2 mm) Teflon® PFA union T (Cole Parmer No. H-06374-61) fitted with a 7.0 mm PTFE lined septum (SGE, type CS), and was located above the reaction flask. It was connected to the reaction flask with PTFE tubing, which exited 2 cm from the bottom of the reaction flask (just above the surface of the solution). A Hamilton four-way valve (D) (No. 86731) directed the flow of carrier gas to purge or bypass the reaction flask. A PTFE impinger (G), identical to the hydride generation flask, was

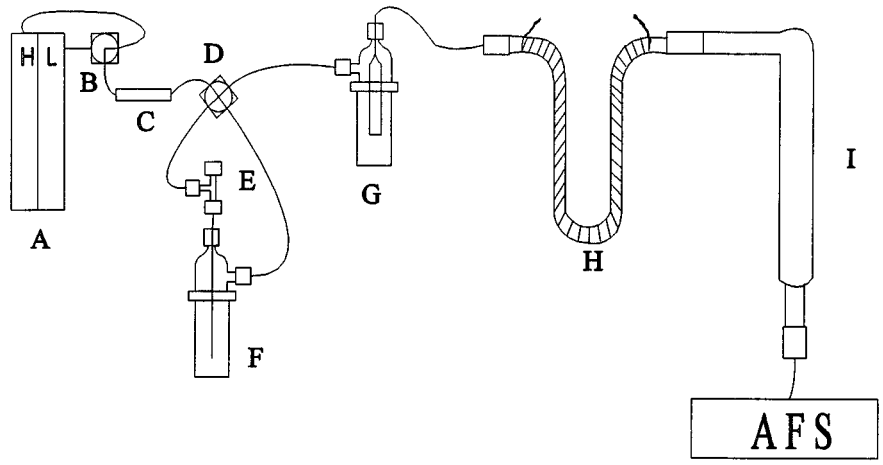

Figure 1 Optimized HG–CVAFS system including flow meters (A), three-way valve (B), gold trap (C), four-way valve (D), injection port (E), reaction flask (F), water trap (G), cryogenic trap/column (H) and furnace (I).

used to remove water from the gaseous sample stream. In this case, however, inlet and outlet were reversed, with the flow entering the side and exiting through the top of the impinger. A 5 cm length of PTFE shrink-tubing (6.0 mm i.d.) attached to PTFE tubing (inside the impinger) was the exit from the water trap. The opening of the larger tubing was 4 cm from the bottom of the trap. The water trap was submerged 5 cm into a dry ice/acetone bath (-70 °C).

The column, packing material, furnace, and connections were as described previously,[12] except for the following modifications. The length of the U-shaped pyrex column (H) was 35 cm. The furnace was L-shaped with an unheated inlet (6 cm $\times$ 3.0 mm i.d.) and an outlet 17 cm in length with a 6.0 mm i.d. The last 2 cm of the furnace outlet were unheated. A Brooks–Rand CVAFS-2 was used for the detection of Hg^0. Data acquisition and integration were performed by a Labview (National Instruments) program.

Operating procedures for hydride generation–cold vapor atomic fluorescence spectrometry (HG–CVAFS)

The reaction medium, purge time, $NaBH_4$ volume and helium flow rates were determined by simplex optimization. Given flow rates are for the column at -196 °C and would be lower at room temperature. A 0.3 M NaCl solution (5 ml) was introduced into the reaction flask from a Repipet II dispenser. Standards and/or sample extracts were then added to the reaction flask and it was reconnected to the system. The column was placed in liquid nitrogen and the helium carrier gas flow was switched to 250 ml min^{-1} and directed through the reaction flask. Then 0.3 ml of 6% $NaBH_4$ was injected through the septum of the reaction flask and the stirred solution was purged for 3.5 min. The helium flow was then switched to 150 ml min^{-1} and to bypass the reaction flask. The Variac for the column was turned on to 10 V, the liquid nitrogen was removed, and the data acquisition program was begun. After the analytes had eluted from the column (3 min), the Variac was set to 20 V for 2 min to clean the column prior to the next run. The reaction flask was removed, rinsed once with 0.1 M HCl, and twice with 0.3 M NaCl. The impinger used for the water trap was cleaned about every ten runs, by rinsing with 0.1 M HCl followed by DD H_2O. Retention times and temperatures of elution for the three analytes were: Hg(II) (1.05 min, 25 °C); $MeHg^+$ (1.75 min, 44 °C); and Me_2Hg (2.25 min, 54 °C). The temperature in the trap after the 2 min cleaning time was 165 °C.

Operating procedure for Hg^0 as a calibrant

Hg^0 was sometimes used as a calibrant[16] by taking known headspace volumes over a pool of Hg^0 in a sealed vial at constant temperature and purging them through the dry reaction flask onto the cold trap. Other aspects of the procedure were as in the preceding section.

Sediment samples

Weekly samples were collected from the *Spartina alterniflora* region of Chapman's Marsh, which is located near the mouth of the Squam-
scott River in the Great Bay Estuary, NH, USA. The top 5 cm of sediment, including roots, was collected in acid-washed containers. Sediment was sieved through a 2.36-mm copper mesh to remove most roots and large organic matter, homogenized by mixing and stored in acid-washed scintillation vials in the dark at 4 °C. These sediment samples were analyzed within 12 h, unless otherwise noted. For long-term storage, sediment was frozen (− 20 °C) in the dark in acid-washed scintillation vials. Sediment S-19 (Commission of European Communities) is a dry freshwater sediment[10] that was used as received. The sediment was stored in the dark in a sealed brown glass bottle at room temperature. $MeHg^+$ and total mercury (Hg_{tot}) concentrations in sediment S-19 were determined in it in the course of an interlaboratory study.[10, 11]

Spike recoveries

Two methods were used to test recovery of $MeHg^+$ during vacuum distillation in the absence of sediments. In the first, MeHgCl was spiked into the reaction flask and distilled without addition of reagents. In the second, MeHgCl was spiked into the reagent used for extraction before distillation and taken through the entire procedure (see below).

For recoveries from sediments MeHgCl (10 ng) spikes were added to weighed sediment samples and vortexed (VWR Vortex II, setting 6) for 1 min to homogenize. Recoveries by distillation or extraction were determined immediately (see below). Me_2Hg (10 ng) was spiked into unmodified wet sediment and vacuum-distilled (see below) without addition of reagents. For Me_2Hg recoveries the distillate was rinsed from the trap with *ca* 10 ml of 1% (w/v) KOH/MeOH.

Apparatus for the vacuum distillation

The vacuum-distillation system (Fig. 2) consisted of a round-bottom reaction flask (R), two glass U-shaped traps (T1, T2), a cylindrical pump trap (PT), two high-vacuum valves (S1, S2) (PTFE, Kontes, with Viton® O-rings) and a vacuum pump (Cenco Hyvac®). All connections were greaseless ground-glass ball and socket (35/25 joint size) type with O-ring seals and stainless steel pinchclamps. The reaction flask (R) was a standard 50 ml Pyrex round-bottom flask (24/40). The rest of the system was custom-made by Anderson Glass-blowing (Fitzwilliam, NH, USA). The glass tubing from the ball and socket joints was 13 mm o.d. × 3 mm wall thickness. The first trap (T1) widened to 20 mm o.d. in the lower half and was filled with 4 mm glass beads to 5 cm above the bottom of the bend. It was crimped to a diameter of 3 mm (on the inlet side) so that solutions could be poured out of the trap while the glass beads were held back. The second trap (T2) is a uniform 13 mm o.d. throughout, and also contains 4 mm glass beads. Both traps are 20 cm high. The pump trap (PT) is cylindrical (20.5 cm × 4 cm diameter) with the outlet tube to the pump 3.5 cm from the bottom of the trap.

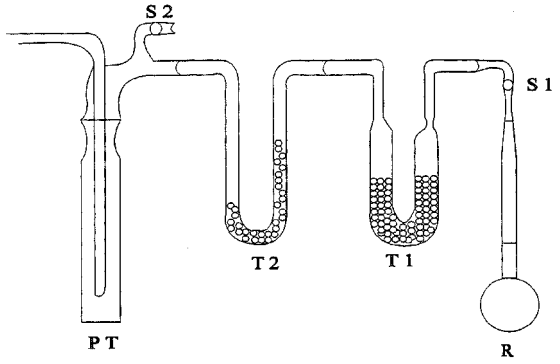

Figure 2 Vacuum-distillation apparatus including reaction flask (R), stopcocks (S1 and S2), cryogenic traps (T1 and T2) and vacuum-pump trap (PT).

Procedure for vacuum distillation

Sediment (0.3–0.5 g dry weight) was placed in a round-bottom flask. If the samples were to be spiked for recovery experiments, it was done at this time. To the flask was added 3.5 ml of a solution containing 2.5 M H_2SO_4, 0.5 M NaCl and 0.01 M $CuCl_2$. The flask was closed with a ground-glass stopper and vortexed (VWR Vortex II, setting 6) for 1 min. The stopper was secured on the flask with a Keck® clamp, and the sample was sonicated (Branson 2200 L sonicator) at 60 °C for 30 min. The flask was attached to the

the sample heated in a 40 °C water bath. Before or at this time, stopcock S2 was closed, the vacuum pump was turned on, and the pump trap was cooled to −196 °C in liquid nitrogen. Traps T1 and T2 were also placed in liquid nitrogen, and the system was allowed to cool and evacuated for 5 min. Stopcock S1 was opened to the reaction flask and evacuated for 1.0 h at 6 μmHg. Care had to be taken during this time that the level of liquid nitrogen in trap T1 did not change significantly. The vacuum pump was turned off and stopcock S2 was opened to return the system to atmospheric pressure. All Dewars containing liquid nitrogen were removed so that oxygen would not condense in the cold traps. Trap T1 was removed from the system, placed in a room-temperature waterbath, and a rinse solution (*ca* 5 ml of 0.1 M HCl) was added. After the trap had warmed to room temperature (about 5 min), the contents were poured into a scintillation vial. Two more 5-ml rinses of the trap were added to the sample. The solution volume was calculated by its mass and the MeHgCl concentration determined by HG–CVAFS.

Toluene/thiosulfate extraction

This was a slight modification of the procedure developed by Filippelli.[4] Sediment (0.3–0.5 g dry weight) was placed in a 40-ml glass centrifuge tube. After the addition of 5 ml of a solution containing 2.5 M H_2SO_4, 1% (w/v) NaCl and $CuSO_4$, the open tube was heated in a boiling waterbath (100 °C) for 15 min. The solution was cooled and 5 ml of toluene was added. After the tube had been sealed with a Teflon-lined screw cap, the sample was vortexed for 1 min and heated in a boiling waterbath for 15 min. The tube was centrifuged (International Clinical Centrifuge, Model CL) at 6000 rpm for 2 min to separate the layers. The toluene layer was removed, and placed in a 20-ml scintillation vial containing 5 ml of aqueous 0.01 M $Na_2S_2O_3$. The sample was sealed with a Teflon®-lined cap, vortexed for 1 min, and the toluene layer was removed. Then 0.500 ml of 0.2 M H_3BO_3/$NaB(OH)_3$ buffer (pH 9.2) was added to prevent acidification and possible decomposition of the $Na_2S_2O_3$. This aqueous layer was boiled down to *ca* 1 ml in a 200 °C sandbath to remove traces of toluene. The solution was cooled, and diluted to the original volume with DD H_2O before determination of the $MeHg^+$ concentration using HG–CVAFS.

Clean-up step for sediment S-19 distillate

Due to the *ca* 9000 ng (500 ng ml^{-1}) of inorganic Hg(II) in the S-19 distillate, a clean-up step was necessary for proper determination of the $MeHg^+$ concentration. After vacuum distillation of the sediment as described above, the solution obtained (*ca* 18 ml of 0.1 M HCl) was extracted into toluene (2×5 ml + 1×2.5 ml). The toluene layers were combined, back-extracted with 12.5 ml of 0.01 M $Na_2S_2O_3$, and the toluene layer removed. The aqueous layer was boiled down to *ca* 5 ml in a 200 °C sandbath to remove traces of toluene. MeHgCl (10 ng) was spiked into 18 ml of 0.1 M HCl and extracted as above to determine the extraction efficiency for the clean-up step.

RESULTS

Calibration data and limits of detection

The calibration data and detection limits for the mercury compounds show that the HG–CVAFS method is very reliable and sensitive (Table 1). The AFS detection limit is 0.2 pg (3σ) and detection limits while running samples are included in Table 1. The detection limit for Hg(II) is higher than that for the other mercury compounds because only Hg(II) and Hg^0 are contaminants in our laboratory. Correlation coefficients (R^2) for the slopes of calibration curves are generally greater than 0.99. The RSD for replicated standards in the response range typically used (0–1.5 ng) is *ca* 5%.

Spike recoveries

In the absence of sediments, spike recoveries of 30 ng MeHgCl added to the reaction flask and

Table 1 Typical calibration curve data and detection limits for mercury vapor (Hg^0), inorganic mercury [Hg(II)], monomethylmercury cation ($MeHg^+$) and dimethylmercury (Me_2Hg)

Compound	Slope (area/ng)	Detection limit (pg)[a]
$Hg^{(0)}$	26.9	0.3
Hg(II)	21.4	13
$MeHg^+$	22.6	0.3
Me_2Hg	28	0.3

[a] Based on $3\times$ the standard deviation of the blank value.

distilled in the absence of reagents were 97% (±5%) (n=4). When 5 ng MeHgCl spikes were carried through the entire extraction–distillation procedure, recoveries were 98% (±3%) (n=3). These results clearly show that $MeHg^+$ does not decompose during the distillation step alone, or during the entire extraction–distillation method.

We did six spike recoveries (Table 2) for the vacuum distillation of $MeHg^+$ from estuarine sediments over the time period of this study. The percentage recoveries varied from 79% to 92% with an average of 84.7%. Other $MeHg^+$ recoveries are from a toluene/thiosulfate extraction on one estuarine sediment (63.6%) and for the clean-up step of S-19 freshwater reference sediment (77.8%). The vacuum-distillation recovery of Me_2Hg from an unmodified estuarine sediment was 67.4%.

Determination of $MeHg^+$ in sediments

$MeHg^+$ concentrations in estuarine sediments collected from Chapman's Marsh are listed in Table 3 and a typical chromatogram in Fig. 3. All concentrations are uncorrected for extraction efficiency. The average $MeHg^+$ concentration was 4.75 (±1.76) ng g^{-1} dry weight, and the detection limit for routine determinations was 0.2 ng g^{-1} dry weight. (Unless stated otherwise, all concentrations are per sediment dry weight.) The wet/dry weight ratio for the sediments ranged from 3.4 to 4.8, with an average of 4.3. The RSD for replicate measurements of a sediment sample was generally below 6%, with the higher values attributed to a lack of homogeneity. The distillates from estuarine sediments contained between 0.5 and 1.0 ng ml^{-1} of Hg(II), which did not interfere in the determination of $MeHg^+$ concentrations.

Absence of Me_2Hg in Chapman's Marsh sediments

Others[17, 18] have found Me_2Hg in sediments, and Puk and Weber[12] found it in *Spartina alterniflora* from Chapman's Marsh using vacuum distillation. Because 2.5 M H_2SO_4 would at least partially decompose Me_2Hg,[19] we cannot determine it simultaneously with $MeHg^+$ by vacuum distillation. In addition, it is preferable to seek Me_2Hg in unmodified samples because of its volatility. We found no Me_2Hg by vacuum distillation of unchanged sediment in this study, despite favorable spike recoveries (Table 2).

DISCUSSION

Comments on relative sensitivity and matrix effects

The HG–CVAFS system contains a number of steps including derivatization and atomization to Hg^0 before AFS detection. If, for example, hydride derivatization of $MeHg^+$ or atomization of MeHgH were incomplete or some MeHgH had decomposed, the sensitivity to $MeHg^+$ would decrease. Because Hg^0 was stable and needed neither derivatization nor atomization, it was considered to be an absolute calibrant of the system.[16] The response of the HG–CVAFS system to Hg^0 was used for quality control, and we made no corrections in responses of other calibration standards. Typical sensitivity ratios (mercury compound/Hg^0) are 0.85 for Hg(II) and $MeHg^+$, and 1.0 for Me_2Hg. The 0.85 $MeHg^+/Hg^0$ sensitivity ratio meant that $MeHg^+$ was derivatized to MeHgH with near 100% efficiency, and that the MeHgH was trapped, and eluted from, the column without

Table 2 Percentage recoveries of a 10 ng spike of MeHgCl and Me_2Hg by methods used in this paper

Speciation method	Recovery ± RSD (%)	No. of determinations
Vacuum distillation[a]	84.7 ± 6.5	6
Vacuum distillation of Me_2Hg	67.4 ± 12.3	2
Toluene/thiosulfate extraction[a]	63.6 ± 11.0	3
Clean-up extraction (S-19 reference sediment)[a]	77.8 ± 0.1	2

[a] For $MeHg^+$.

Table 3 Concentrations of monomethylmercury cation ($MeHg^+$) in Chapman's Marsh sediment (ng/g dry weight) as determined by vacuum distillation

Sampling date	$MeHg^+$ RSD	No. of determinations
10 July 1996	3.60 ± 5.8	5
10 July 1996[a]	2.79 ± 2.5	3
17 July 1996	5.54 ± 5.0	3
24 July 1996[b]	7.35 ± 0.2	2
31 July 1996	4.40 ± 4.0	3
7 August 1996	2.24 ± 20.9	3
14 August 1996	5.38 ± 27.3	3
28 August 1996	3.83 ± 5.2	3

[a] This value was determined using a toluene/thiosulfate extraction on sediment which was frozen (− 20 °C) until 4 September 1996.
[b] These values were determined on 28 July 1996 with intermediate storage at 4 °C.

significant decomposition.

We determined $MeHg^+$ concentration of sediment distillates and extracts by the method of standard additions. Matrix effects were not severe, but the response varied between 65% and 90% of that in 0.3 M NaCl (Table 1). The correlation coefficients (R^2) for standard additions were generally greater than 0.99, and the RSD for replicate measurements was less than 10%. Daily variation in sensitivity between samples, if the matrix was similar, was also less than 10%. We used standard additions because a relatively small change in sensitivity can induce a much greater change in the calculated value for $MeHg^+$ concentration in the original sediment.

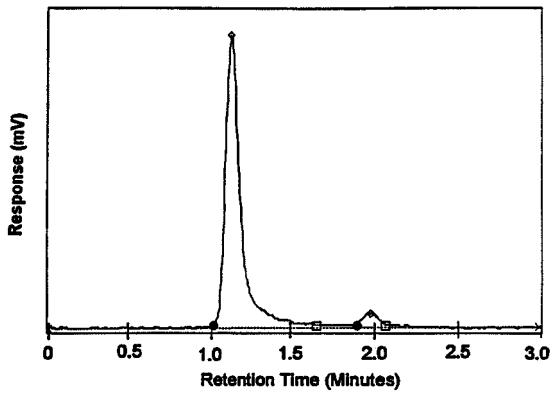

Figure 3 Typical chromatogram of a distillate from a Great Bay Estuarine sediment. Distillate aliquots contained a range of 30 to 75 pg $MeHg^+$. Retention times are 1.1 min for Hg(II) and 2.0 min for $MeHg^+$.

Medium for vacuum distillation

Each component of the extractant containing 2.5 M H_2SO_4, 0.5 M NaCl and 0.01 M $CuCl_2$ serves a specific purpose for release of $MeHg^+$ from the sediment or during the vacuum distillation step. H^+ and Cu^{2+} both release $MeHg^+$ from ligands or other adsorbing sites in sediments by competing with it. The ligand Cl^- bonds to $MeHg^+$ to help release it from sediment sites, and aids in the distillation step by forming volatile MeHgCl and non-volatile $HgCl_4^{2-}$.

Cu^{2+} in the extractant serves a second important function by forming CuS, which has a very low solubility product constant of 1×10^{-37}. This prevents distillation of H_2S, which, if present, would cause a large decrease in sensitivity for $MeHg^+$ determination using the HG–CVAFS system. Alternatively, one could purge the distillate with nitrogen to remove H_2S prior to the determination of $MeHg^+$. This procedure does not volatilize MeHgCl under the conditions we use, but we prefer to avoid this extra step.

A recent study of vapor/aqueous solution equilibrium constants of MeHgCl showed[20] that the equilibrium is virtually unchanged between pH 0 to 9, and that high Cl^- concentrations do not decrease the vapor-phase fraction. Therefore, the presence of 2.5 M H_2SO_4 and 0.5 M NaCl should have little effect on the vapor-phase $MeHg^+$ concentration. The H_2SO_4 concentration is essential to mass transport because it causes the sediment to remain wet throughout the distillation. At lower concentrations of H_2SO_4 the sediment dried out quickly during the distillation, and spike recoveries were lower and much less reproducible. Apparently, a surface layer of dry sediment prevents the further volatilization of MeHgCl.

Validation of vacuum distillation method for determination of $MeHg^+$ in sediments

Consensus concentrations in freshwater sediment S-19 are 91.0 μg g^{-1} (±11.0 μg g^{-1}) for total Hg,[11] and 53.1 ng g^{-1} (±8.5 ng g^{-1}) for $MeHg^+$.[10] The *ca* 1700:1 $Hg_{tot}/MeHg^+$ ratio necessitated an extraction clean-up step for this sediment distillate because it contained about 10% of total mercury originally in the sediment. Although we did not do detailed experiments, we estimate that the $Hg_{tot}/MeHg^+$ in sediments of ratio greater than 1000:1 would make quantitation of $MeHg^+$ with vacuum distillation alone

very difficult. After a correction for the extraction efficiency (Table 2) of the clean-up step only, we determined a $MeHg^+$ concentration of 51.24 ng g^{-1} (±4.40 ng g^{-1}) in sediment S-19.

We employed the toluene/thiosulfate extraction developed by Filippelli[4] as a comparison method for the vacuum distillation of the 10 July sediment. We determined uncorrected $MeHg^+$ concentrations of 2.79 (±0.07) ng g^{-1} for the extraction and 3.60 (±0.21) ng g^{-1} for vacuum distillation. When these numbers are corrected to account for the $MeHg^+$ spike recoveries (Table 2), the values are 4.39 (±0.11) ng g^{-1} for extraction and 4.25 (±0.25) ng g^{-1} for vacuum distillation. A *t*-test at the 95% confidence level shows that there is no significant difference between the results obtained by the two methods (P=0.62).

$MeHg^+$ concentrations in estuarine sediments

Our 2.2–7.4 ng g^{-1} range of $MeHg^+$ concentrations in Chapman's Marsh of the Great Bay Estuary is similar to concentrations found in other marine sediments. Examples include <0.1 to 30 ng g^{-1},[9] <15 ng g^{-1} (one exception),[8] 12 ng g^{-1},[17] 4.46 ng g^{-1},[2] and several sediments ranging from 0.16 to 8.47 ng g^{-1}.[6]

Despite the tremendous chemical and biological changes in Chapman's Marsh from 1984 to 1986 documented by Hines *et al*.,[21] we find no evidence for a significant change in $MeHg^+$ concentration for the 1996 summer. One-way ANOVA (95% confidence) for $MeHg^+$ concentration by collection date indicates that observed concentration differences are random (P=0.85). Variables such as salinity, tide, sulfate reduction and depth, which may affect $MeHg^+$ concentration, were not controlled in these experiments.

Is freeze-drying a valid pre-treatment?

Successful vacuum distillation of MeHgCl at 40 °C and 6 μm Hg as described above suggests that freeze-drying of environmental samples is likely to result in losses. MeHgCl's 0.58 mm vapor pressure of sublimation at 20 °C[22] and equilibrium vapor/water constant of 3.8×10^{-5} at 25 °C[20] are reasons for the successful vacuum distillation of MeHgCl. Other papers also indicate that MeHgX (X^{n-} is the anion of $MeHg^+$, usually Cl^-) is volatile in environmental samples. For example, Pillay *et al*.[23] confirmed a 16–64% loss of total mercury by freeze-drying from unspiked fish and plankton/algae, but did not detect loss from sediment. One likely reason for the absence of detected loss from sediments is that fish (and probably plankton/algae) are usually predominantly the quite-volatile MeHgX,[24] while most mercury in sediments is less-volatile Hg(II).[6, 8, 9] Alternatively, MeHgX in some environmental samples may not be volatile due to the presence of sulfur-containing ligands.[25] One recent paper,[26] in contrast, suggests (with only modest statistical validity) that freeze-dried mussels do not appear to lose significant amounts of MeHgX. The important lesson is that researchers should perform recovery experiments with freeze-drying of environmental samples to validate this pre-treatment.

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REFERENCES

1. J. H. Weber, Analytical methods for the determination of mercury(II) and methylmercury compounds: the problem of speciation. In: *Mercury and Its Effects on Environment and Biology*, Vol. 34. *Metal Ions in Biological Systems*, Sigel, H. and Sigel, A. (eds), Marcel Dekker, New York, 1997, pp. 1–19.
2. M. Horvat, V. Mandić, L. Liang, N. S. Bloom, S. Padberg, Y.-H. Lee, H. Hintelmann and J. Benoit, *Appl. Organomet. Chem.* **8**, 533 (1994).
3. R. G. Fischer, S. Rapsomanikis, M. O. Andreae and F. Baldi, *Environ. Sci. Technol.* **29**, 993 (1995).
4. M. Filippelli, *Appl. Organomet. Chem.* **8**, 687 (1994).
5. Y.-H. Lee, J. Munthe and Å. Iverfeldt, *Appl. Organomet. Chem.* **8**, 659 (1994).
6. M. Horvat, N. S. Bloom and L. Liang, *Anal. Chim. Acta* **281**, 135 (1993).
7. H. Hintelmann, P. M. Welbourn and R. D. Evans, *Wat. Air Soil Pollut.* **80**, 1031 (1995).
8. C. Gagnon, E. Pelletier, A. Mucci and W. F. Fitzgerald, *Limnol. Oceanogr.* **41**, 428 (1996).
9. J. M. Bubb, T. P. Williams and J. N. Lester, *Water Sci. Technol.* **28**, 329 (1993).
10. P. Quevauviller, G. U. Fortunati, M. Filippelli, F. Baldi, M. Bianchi and H. Muntau, *Appl. Organomet. Chem.* **10**, 537 (1996).
11. H. Emteborg, E. Björklund, F. Ödman, L. Karlsson, L. Mathiasson, W. Frech and D. C. Baxter, *Analyst* (*London*) **121**, 19 (1996).

12. R. Puk and J. H. Weber, *Anal. Chim. Acta* **292**, 175 (1994).
13. J. H. Weber and R. Puk, *Appl. Organomet. Chem.* **8**, 709 (1994).
14. M. Filippelli, F. Baldi, F. E. Brinckman and G. J. Olson, *Environ. Sci. Technol.* **26**, 1457 (1992).
15. P. J. Craig, D. Mennie, N. Ostah, O. F. X. Donard and F. Martin, *Analyst* (*London*) **117**, 823 (1992).
16. E. Saouter and B. Blattmann, *Anal. Chem.* **66**, 2031 (1994).
17. D. Wallschläger, H. Hintelmann, R. D. Evans and R.-D. Wilken, *Wat. Air Soil Pollut.* **80**, 1325 (1995).
18. P. Quevauviller, O. F. X. Donard, J. C. Wasserman, F. M. Martin and J. Schneider, *Appl. Organomet. Chem.* **6**, 221 (1992).
19. R. Puk and J. H. Weber, *Appl. Organomet. Chem.* **8**, 293 (1994).
20. C. Meuleman, C. C. Laiño, P. Lansens and W. Baeyens, *Water Res.* **27**, 1431 (1993).
21. M. E. Hines, S. L. Knollmeyer and J. B. Tugel, *Limnol. Oceanogr.* **34**, 578 (1989).
22. F. Kai, K. Tajima, R. Yamada, Y. Noda, T. Yunoue and H. Huang, *Nippon Kagaku Kaishi* 536 (1991).
23. K. K. S. Pillay, C. C. Thomas, jr., J. A. Sondel and C. M. Hyche, *Anal. Chem.* **43**, 1419 (1971).
24. N. S. Bloom, *Can. J. Fish. Aquat. Sci.* **49**, 1010 (1992).
25. P. D. LaFleur, *Anal. Chem.* **45**, 1534 (1973).
26. B. Lasorsa and S. Allen-Gill, *Wat. Air Soil Pollut.* **80**, 905 (1995).

