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Methylmercury Determination by Purge and Trap–GC–FTIR–AAS after $NaBH_4$ Derivatization of an Environmental Thiosulfate Extract

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A method of detecting methylmercury species (MeHg) and dimethylmercury (DMM) has been studied. MeHg was transformed prior to its determination as methylmercury hydride (MMH) by use of $NaBH_4$. The two volatile forms of organic mercury were detected by a purge and trap (PT) unit in-line with a gas chromatograph (GC) connected with a Fourier transform infrared spectrometer (FTIR) which was also in-line with an atomic absorption spectrometer (AAS). Environmental samples were analyzed by this technique. MeHg was detected in thiosulfate extracts of fish and sediment, and MeHg and DMM directly in water samples. Picogram levels of sensitivity were obtained, the limit of detection being 100 pg for MeHg and 50 pg for DMM. The calibration graph was linear for both compounds up to 10 ng as Hg.

Keywords: Methylmercury, dimethylmercury, purge and trap GC–FTIR–AAS, hydride generation, fish, water, sediment

INTRODUCTION

Recent studies on organic mercury compounds in the environment have shown that a variety of possible forms of this metal exist.[1,2] There is therefore a need to detect these forms specifically and at picogram levels because in many matrices, such as natural waters and in organisms at lower trophic levels of the food chain, methylmercury concentrations are very low. Various methods have been used giving good results,[3–12] but different methods are needed to compare the results obtained. In this paper we have improved our previous work on the detection of methylmercury and organic mercury using PT–GC–FTIR[13] by adding AAS determination in-line with IR determination for a higher sensitivity. In fact, nondestructive IR detection is used to measure mercury present in a molecule which will then be detected by AAS. Quevauviller *et al.* have recently used hydride generation to detect methylmercury (MeHg) and dimethylmercury (DMM) in sediments,[14] and Puk and Weber[15] have studied this hydride derivatization for mercury in estuarine samples of *Spartina alterniflora* and *Zostera marina* L. with good results.

EXPERIMENTAL

Apparatus

A Nicolet 20 SXB Fourier Transform Interferometer (FTIR), equipped with a GC–FTIR optical bench accessory and a mercury–cadmium telluride (MCT) infrared detector, was used in-line with a gas chromatograph (Carlo Erba HRGC 5300 Mega Series). A purge and trap apparatus (PT) (Chrompack) was used in-line with the GC–FTIR.

The PT was programmed as follows: precooling time 1 min, purge time 5 min at 20 °C with a flow rate (nitrogen) of 60 cm^3 min^{-1}, injection time 1 min at 100 °C. A wide-bore fused silica column (CP Sil8 50 m × 0.53 mm i.d. with 2 μm film thickness) was used in-line in the PT–GC–FTIR apparatus isothermically at 30 °C with a nitrogen flow rate of 30 cm^3 min^{-1}. The PT–GC–FTIR was connected via a wide-bore column to a quartz cell 30 cm long, of 1 mm i.d., and thereafter to a Perkin–Elmer Model 372 atomic absorption spectrometer with a mercury hollow cathode lamp operated at 6 mA (spectral band pass, 2.0 nm) and at a resonance wavelength of 253.7 nm. The mercury vapor has detected in a windowless glass cell (20 cm long and of 4 mm

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i.d.). The quartz cell was heated at 750 °C when DMM was to be detected.

Reagents

Distilled, deionized water (DDW) was used. A 0.01 M solution of sodium thiosulfate ($Na_2S_2O_3.5H_2O$) was prepared by dissolving 0.2482 g in 100 cm^3 of DDW. A solution of 1% (w/v) sodium borohydride ($NaBH_4$) (Carlo Erba) in DDW was used as the derivatizing agent.

Standards

A stock solution of methylmercury chloride (BDH) was prepared by dissolving 0.1251 g in 100 cm^3 of ethanol to obtain a concentration of 1000 μg cm^{-3} as Hg and this was stored at −10 °C. A stock standard solution of dimethylmercury (10 μl; Aldrich) was mixed with 100 cm^3 of ethanol to obtain a concentration of 276 μg cm^{-3} as Hg. Standard mercury working solutions (100 ng cm^{-3}) were prepared by appropriate dilutions of the stock solutions with DDW and were stored at 5 °C.

Procedure

Fish samples

A 0.5 g dried fish sample in a 15 cm^3 polyethylene screw-capped glass test-tube was heated in a boiling water bath with 1 cm^3 of concentrated hydrochloric acid (37%, w/v) for 10 min until total dissolution of the sample occurred. After cooling and adding 10 cm^3 of DDW, the test-tube was centrifuged for 1 min at 6000 rpm. The water layer was transferred to another test-tube and 5 cm^3 of toluene was added.

The vial was then stoppered, shaken for 1 min and centrifuged for 1 min at 6000 rpm. The toluene layer was transferred using a micropipette into a 10 cm^3 screw-capped vial and 1 cm^3 of 0.01 M sodium thiosulfate solution was added and vortexed for 30 s. A 50 μl aliquot of this thiosulfate solution containing organic mercury compounds was added to a 5 cm^3 PT vial, containing 5 cm^3 of thiosulfate solution and 100 μl of 1% $NaBH_4$. The vial was promptly purged in the PT apparatus for 5 min with a nitrogen gas flow rate of 60 cm^3 min^{-1} at ambient temperature. The volatile mercury species were trapped in a 0.53 mm wide-bore column held at −120 °C and injected automatically onto the column by heating the trap at 100 °C. The volatile mercury species were separated from other gaseous compounds, detected by the in-line FTIR and determined after thermal decomposition by cold-vapor AAS.

An alternative method consists of digestion of 1 g of sample with 10 cm^3 methanolic KOH (25% w/v) and analyzing directly the final solution. A 200 μl aliquot was added to 5 cm^3 of thiosulfate solution with 100 μl of $NaBH_4$ solution and the solution was purged in the PT apparatus. The results obtained were similar to the previous HCl extraction.

Water samples

Portions (5 cm^3) of solutions containing organomercurials were purged directly by adding 100 μl of 1% $NaBH_4$ solution.

Sediments

To a 0.5 g portion of sediment in a 15 cm^3 screw-cap polyethylene glass test-tube was added 5 cm^3 of 2.5 M H_2SO_4; after gas pressure had developed another 4 cm^3 of H_2SO_4 was added and the tube was then heated in a boiling water bath for 10 min. After cooling and centrifuging at 6000 rpm the water layer was transferred to another glass test-tube. The water layer was extracted with 5 cm^3 of toluene by shaking for 1 min followed by centrifuging. Then the toluene layer was placed into a 10 cm^3 screw-cap vial containing 5 cm^3 of thiosulfate solution. After shaking for 30 s, the toluene layer was discarded and the thiosulfate solution was transferred into a 25 cm^3 beaker and heated on a hotplate (200 °C) to evaporate the solution to 0.66 cm^3 and to eliminate toluene traces from the solution. After cooling, the thiosulfate solution was transferred to a PT vial, 100 μl of 1% $NaBH_4$ solution was added and the solution purged in the PT apparatus. The volatile organic mercury species were then detected by cold-vapor AAS.

RESULTS AND DISCUSSION

The aim of this study was to detect MeHg and DMM with a new aqueous derivatization at the picogram level. We have focused our attention on these two species because they are believed to be the mobile chemical forms of mercury in the environment.

GC–FTIR in-line with AAS detection was very

important for verifying possible interfering substances and also to determine the retention time of the analyte by injection of a large quantity and detection by its IR spectrum. MeHg istantaneously reacts with $NaBH_4$ to produce methylmercury hydride (MMH), which was easily purged from the solution. MMH was separated by gas chromatography and detected at the 100 ng level by FTIR and at the picogram level by AAS. Optimization of the reaction conditions was studied by varying pH, $NaBH_4$ concentration, purging flow rate and stripping time. From pH 1 to 14 no pH effect was observed on MMH formation. Similarly the addition of from 10 µl to 1 cm^3 of $NaBH_4$ solution to the reaction vessel did not produce any difference in MMH recovery.

The peak heights of MMH and DMM were studied as a function of the pyrolytic cell temperature (Fig. 1). For DMM the optimum temperature was 600 °C and at higher temperatures no increase in the signal was observed. For MMH unexpected results were obtained. MMH could be detected by AAS without any pyrolysis effect on its AAS absorption peak height. Figure 2 shows the recovery of MMH and DMM present in a solution using different purging flow rates over a five minute period.

The linearity of the PT-GC-FTIR-AAS method for detection of spiked thiosulfate solutions ranges from 100 pg to 10 ng for MMH and 50 pg to 8 ng for DMM (Fig. 3). The accuracy was verified by analysis of a reference material with

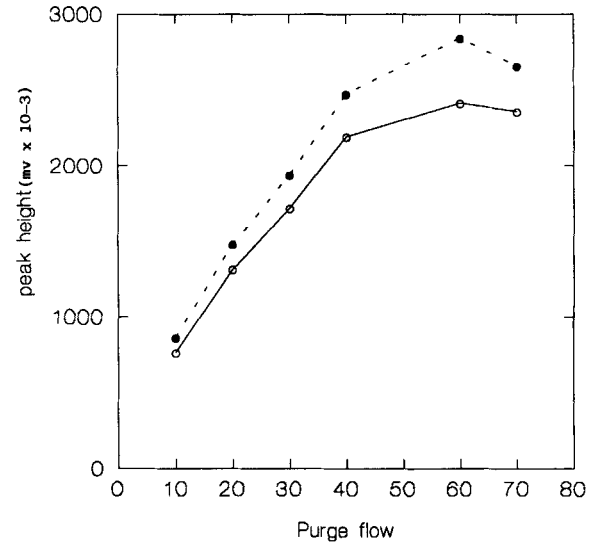

Figure 2 Peak height of 10 ng of MMH and 8 ng of DMM with different 5 min purge flow rates. --- DMM, —— MMH.

GF-AAS detection[16] for comparison. The results are shown in Table 1 and good agreement was obtained.

We also attempted to detect MeHg in a sediment. Experiments were performed with a lyophilized sediment. We used different methods of extraction: (a) with methanolic 25% (w/v) KOH hydrolysis and successive toluene extraction into 2.5 M H_2SO_4 media; (b) with 5 M HCl; and (c) with 2.5 M H_2SO_4 of samples spiked with different amounts of MeHg. A quantitative recovery of

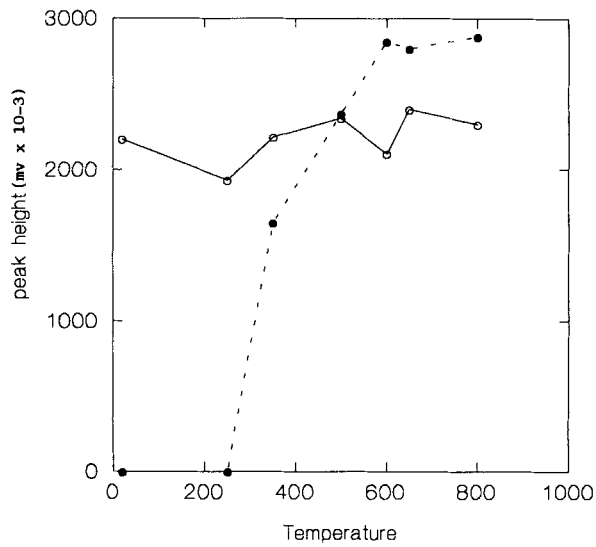

Figure 1 Peak height of 10 ng MMH and 8 ng DMM at different pyrolysis temperatures --- DMM, —— MMH.

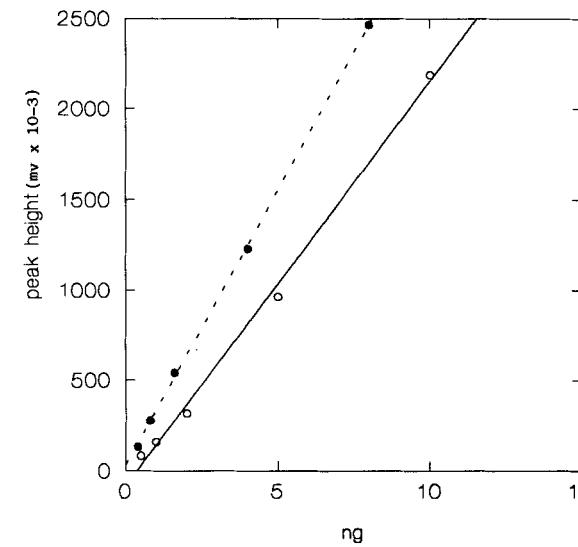

Figure 3 Calibration curve of MMH and DMM by PT-GC-FTIR-AAS. --- DMM, —— MMH.

Table 1 Comparison of methylmercury concentration (μg g^{-1}, dry weight) in Reference Material determined by PT-GC-FTIR-AAS and GF-ASS

	Methylmercury content (μg g^{-1}) as Hg			
		PT-GC-FTIR-AAS[a]		
Sample	GF AAS[a]	HCl extract	KOH extract	Certified value
BCR tuna fish CRM 463	2.60 ± 0.12	2.85 ± 0.15	2.55 ± 0.18	2.83 ± 0.22
BCR tuna fish CRM 464	5.37 ± 0.32	5.15 ± 0.19	4.97 ± 0.28	5.09 ± 0.29

[a] Mean of five determinations.

Table 2 Methylmercury content in various sediment samples

Sample	Location	Methylmercury (ng g^{-1} as Hg)[a]
SP1	La Spezia gulf	3.7 ± 0.29
SP2	La Spezia gulf	0.5 ± 0.07
ROS/A	Rosignano S.	3.7 ± 0.31
ROS/B	Rosignano S.	9.1 ± 0.54
IAEA 356[b]		4.8 ± 0.33

[a] Mean of five determinations.
[b] Certified value: 4.91 ± 0.48 ng g^{-1}.

spiked MeHg was observed only for 25 M H_2SO_4 extraction (Fig. 4). MeHg was very stable at this H_2SO_4 concentration and heating the sample at 100 °C for more than 1 h did not change the recovery using the H_2SO_4 extraction technique. MeHg concentrations in some marine sediments were determined. The results obtained are listed in Table 2.

In summary, a new and very sensitive technique has been developed to detect MeHg in environmental matrices at a subnanogram level, either in a thiosulfate extract or directly in water. Figure 5 shows a typical chromatogram obtained by this technique from a water sample containing

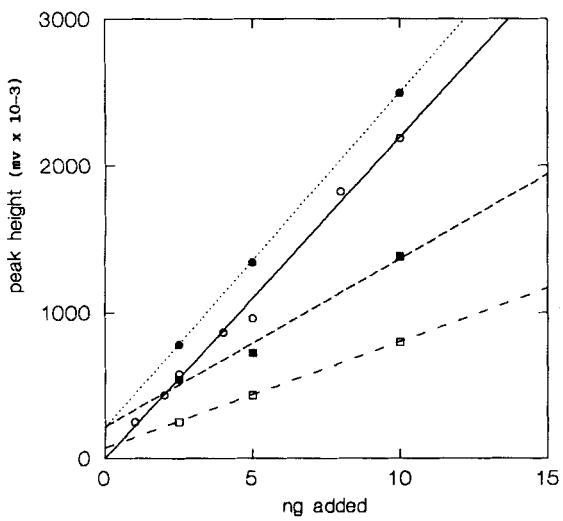

Figure 4 Methylmercury recovery of spiked La Spezia sediment with different extraction procedures: methanolic 25% KOH, 5 M HCl and 2.5 M H_2SO_4 respectively; STD, thiosulfate standard solution. ····· H_2SO_4, --- HCl, - - - KOH, —— STD.

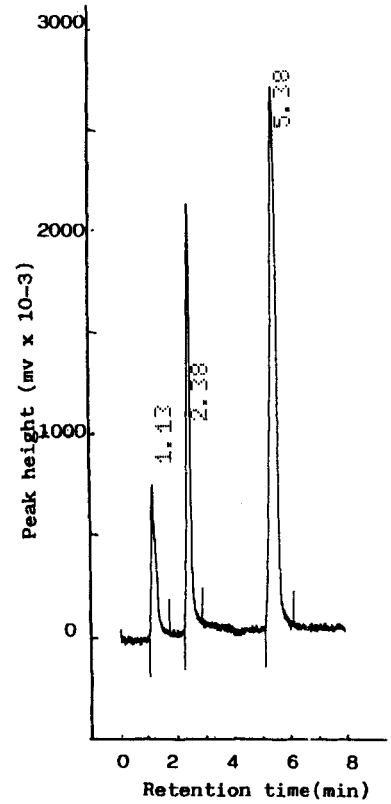

Figure 5 Typical gas chromatogram obtained from a water sample containing 10 ng of MMH and 8 ng of DMM.

both species. It is clear that, in the thiosulfate extract, only MeHg was detected. There is also present a peak due to elemental mercury produced by $NaBH_4$ with inorganic mercury and probably by some MeHgH dismutation.

We now intend to verify the possibility of detecting MeHg directly in aqueous extracts, in particular in samples that are difficult to analyse such as sediments.

REFERENCES

1. F. Baldi, M. Pepi and M. Filippelli, *Appl. Environ. Microbiol.* **59**, 2479 (1993).
2. M. Filippelli and F. Baldi, *Appl. Organomet. Chem.* **6**, 487 (1993).
3. G. Westöö, *Acta Chem. Scand.* **22**, 2277 (1968).
4. L. Fishbein, *Chromatogr. Rev.* **13**, 83 (1970).
5. L. Magos, *Analyst (London)* **96**, 847 (1971).
6. C. J. Cappon and J. C. Smith, *Anal. Chem.* **39**, 365 (1977).
7. P. Jones and G. Nikless, *Analyst (London)* **103**, 1121 (1978).
8. I. M. Davies, *Anal. Chim. Acta* **102**, 189 (1978).
9. P. J. Craig and P. A. Morton, *Mar. Pollut. Bull* **14**, 408 (1983).
10. Y. H. Lee, *J. Environ. Anal. Chem.* **29**, 2663 (1987).
11. N. Bloom and W. F. Fitzerald, *Anal. Chim. Acta* **208**, 151 (1988).
12. S. Fisher, S. Rapsomanikis and M. O. Andreae, *Anal. Chem.* **65**, 763 (1993).
13. M. Filippelli, F. Baldi, E. F. Brinckman and G. J. Olson, *Environ. Sci. Technol.* **26**, 1457 (1992).
14. P. Quevauviller, O. F. X. Donard, J. C. Wasserman, F. M. Martin and J. Schneider, *Appl. Organomet. Chem.* **6**, 221 (1992).
15. R. Puk and J. H. Weber, *Anal. Chim. Acta* in press.
16. M. Filippelli, *Anal. Chem.* **59**, 116 (1987).