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Determination of Methylmercury in Fish and Aqueous Samples Using Solid-Phase Microextraction Followed by Gas Chromatography–Atomic Fluorescence Spectrometry

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An analytical method is described for methylmercury determination in fish and aqueous samples using solid-phase microextraction (SPME) followed by gas chromatography–atomic fluorescence spectrometry (GC–AFS). The procedure involves aqueous-phase derivatization of methylmercury species with sodium tetraethylborate in a sample vial and subsequent extraction with a silica fiber coated with poly(dimethylsiloxane). The mercury derivatives are desorbed in the splitless injection port of a gas chromatograph and subsequently analyzed by GC–AFS. The headspace SPME procedure is used and parameters affecting the extraction, adsorption and desorption are evaluated. Results for methylmercury analysis in standard reference material (DORM-2) and fish samples are presented. © **1998 John Wiley & Sons, Ltd.**

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INTRODUCTION

Solid-phase microextraction (SPME), a simple and fast sample preparation technique developed recently by Pawliszyn and co-workers,[1–4] has been used for the determination of numerous trace pollutants in a variety of environmental and biological samples.[1–11] It involves the extraction of volatile or semivolatile organic compounds directly from aqueous or gaseous samples onto a fused-silica fiber that is coated with an appropriate stationary phase. While the fiber is exposed to the sample, the analytes partition from the sample matrix into the stationary phase until equilibrium is reached. The fiber is withdrawn, and then directly transferred into a GC injector for thermal desorption and analysis.

Mercury is a very important environmental contaminant. Methylmercury (MeHg), the most toxic species of mercury found in environmental and biological samples, is of particular concern since this compound can cause severe neurological damage to humans and wildlife.[12–17] The determination and monitoring of mercury is a special challenge in the field of heavy-metal analysis.[15] As public awareness regarding the toxicity and the environmental impact of mercury contamination increases, the demand for a simple, fast and reliable analytical method which can distinguish between organic and inorganic forms of mercury also increases.

Several analytical techniques for MeHg determination have been reported.[15] Gas chromatography (GC) with electron capture detection (ECD) was traditionally used for the determination and speciation of organomercury in many environmental and

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biological samples.[15,18] The classic method for extracting and separating methylmercury involves liberation, isolation by multiple liquid–liquid extraction with benzene or toluene, and subsequent analysis by GC–ECD.[18] However, one of the drawbacks of this technique is that the halogen-bearing compounds co-extracted with organomercury interfere with the determination because of the nonspecificity of the ECD. An alternative method has recently been developed for MeHg and ethylmercury (EtHg) speciation by employing a modified liquid–liquid extraction procedure and followed by gas chromatography–atomic fluorescence spectrometry (GC–AFS) detection.[19,20] Another method that has been widely used for MeHg analysis is the aqueous ethylation with sodium tetraethylborate ($NaBEt_4$), followed by purge-and-trap and atomic absorption spectrometry (AAS)[21,22] or by AFS[23,24] detection. The use of $NaBEt_4$ as an alkylation reagent has significant advantages since the derivatization reaction can be performed in the aqueous phase, consequently reducing the analytical time and eliminating the need for organic solvent extraction. Among the various detection techniques used for mercury determination and speciation such as AFS, AAS, mass spectrometry (MS)[5] and inductively coupled plasma–atomic emission absorption (ICP–AES),[25] AFS has shown to be the most sensitive technique.[19,20,23,24]

Mercury species have been analyzed using SPME followed by MS[5,10] and electrochemical methods.[9] Because of the important advantages offered by AFS and SPME, the combination of these techniques has the potential to provide a fast, simple and sensitive analytical tool for MeHg determination in environmental and biological samples. In the present report, an analytical procedure for the determination of MeHg in aqueous and fish samples using aqueous ethylation with $NaBEt_4$, subsequent SPME sampling, then GC–AFS detection, is described. The headspace SPME procedure is used and the parameters affecting the extraction, adsorption and desorption procedures are evaluated. Results for methylmercury analysis in standard reference material (DORM-2) and fish samples are presented.

EXPERIMENTAL

Apparatus

Mercury analysis was performed using a P.S. Analytical mercury speciation system model PSA 10.723. This is an integrated gas chromatography–mercury atomic fluorescence instrument which comprises an Ai Cambridge (UK) model GC 94 gas chromatograph equipped with a CTC A200S autosampler and an optic injector module, coupled to the PSA Merlin detector via a pyrolysis oven held at 800 °C. A fused silica analytical column with dimensions of 15 m × 0.53 mm i.d. (Megabore), coated with a 1.5 μm film thickness of DB-1 (J&W Scientific) was used. The column temperature was held at 40 °C for 30 s, programmed at 30 °C min^{-1} to 85 °C, held at 85 °C for 1 min, then programmed at 20 °C min^{-1} to a final temperature of 200 °C, and held there for 1 min. A split/splitless injector was used in the splitless mode and maintained at 150 °C. The carrier gas and make-up gas flows were 4.0 ml min^{-1} of helium and 60 ml min^{-1} of argon, respectively. For the PSA Merlin detection system, the sheath gas flow was 150 ml min^{-1} of argon. Other parameter settings were the same as those reported previously.[19,20] Data were acquired by a real-time chromatographic control and data-acquisition system (E-Lab, Version 4.1OR, OMS Tech Inc., USA).

The SPME fiber holder for manual use and the fiber coated with a 100 μm thickness of poly(dimethylsiloxane) were obtained from Supelco Inc. (Bellefonte, PA, USA). This holder was designed to be used with a re-usable, replaceable, Supelco SPME fiber assembly. Glass vials (10 ml) with Teflon-coated silicone rubber septa were used throughout the experiments. The SPME extractions were performed with magnetic stirring, with a Teflon-coated magnetic stirring bar, to ensure the proper mixing of the sample solution.

Reagents and materials

Deionized (DI) water produced by a Barnstead B-Pure system was used in all aqueous solutions. Optima-grade methanol, certified ACS-grade potassium bromide, copper sulfate, potassium hydroxide, anhydrous sodium sulfate, sodium acetate and trace-metal-grade concentrated sulfuric acid, acetic acid and hydrochloric acid were from Fisher Scientific (Pittsburgh, PA, USA).

All mercury standards were purchased from Ultra Scientific (N. Kingstown, RI, USA). Standard stock solutions of methylmercury chloride (MeHgCl) were prepared by dissolving appropriate amounts of the standards (>95%) in methanol. These solutions were stored in dark brown glass bottles at room temperature (20 °C).

Sodium tetraethylborate was purchased from

Strem Chemicals (Newburgport, MA, USA). A fresh solution of 1% (w/v) $NaBEt_4$ was prepared daily in deionized water. A buffer at pH 4.5 was prepared by mixing appropriate amounts of sodium acetate (0.2 M) and acetic acid (0.2 M).

The standard reference material, DORM-2 (dogfish muscle), was obtained from the National Research Council of Canada (NRCC), Ottawa, ON, Canada. The certified value of MeHg in DORM-2 is 4.47 ± 0.32 μg g^{-1} as Hg. Fish samples were collected in a small pond located at Florida International University, University Park, Miami.

Procedure

After the fish samples had been homogenized with a blender, 200 mg of homogenized sample, 2 ml of DI water and 2 ml of 6 M KOH were placed in a 20 ml glass vial and shaken for 4 h.

For water sample analysis, the sampling vial was prepared by placing the magnetic stirring bar, 6.8 ml of DI water and 1.2 ml of acetate buffer solution (pH 4.5) in a 10 ml glass vial, then 200 μl of MeHg standard (10 pg μl^{-1} as Hg), and 200 μl of 1% $NaBEt_4$ solution were added and the vial was closed immediately. For fish sample analysis, 8 ml of buffer solution, a 200 μl aliquot of the fish extract and 200 μl of 1% $NaBEt_4$ solution were placed in the 10 ml vial. The fiber was drawn into the needle of the SPME fiber holder (for manual use), then the needle was used to pierce the septum of the sample vial. The fiber was lowered into the headspace by depressing the plunger. The fiber was about 0.3 cm above the surface of the stirred solution. In this way, the ethylation and extraction processes were performed simultaneously. Afterwards, the fiber was retracted into the needle and immediately inserted into the GC injector for thermal desorption.

In an attempt to sample methylmercury bromide using headspace SPME, the magnetic stirring bar, 6 ml of DI water, 1 ml of acidic KBr solution and 1 ml of MeHg standards (5 pg μl^{-1} as Hg) were added and the vial was closed immediately. A similar procedure was employed to the ethylation–headspace SPME method.

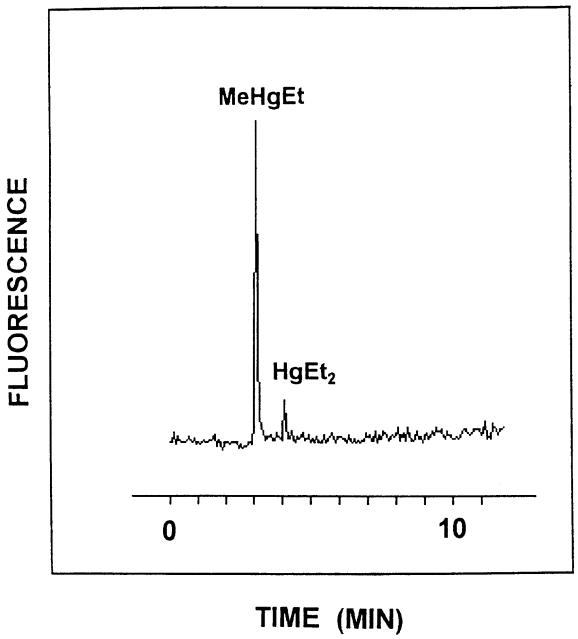

Figure 1 Typical chromatogram obtained using the ethylation–headspace SPME method for MeHg analysis.

RESULTS AND DISCUSSION

Optimization of the variables

It is well known that MeHgBr and MeHgCl partition favorably into organic solvents from aqueous solutions under acidic conditions. This extraction scheme has been widely used for organomercury analysis by GC with different detectors,[18–20] but alternative sample preparation methods are needed. As an initial attempt, MeHgBr was determined by headspace SPME. A very small amount of MeHgBr was found to be sampled, indicating an insufficient volatility of this compound which results in a low partition coefficient between headspace and aqueous phase. Direct aqueous-phase SPME is not realistic because of the strongly acidic condition used and the matrix effects for real-sample analysis.[5]

The derivatization of MeHgBr with $NaBEt_4$ can significantly improve the partitioning of MeHg between fiber coating and sample matrix, since the fully alkylated mercury species have greater affinity for the nonpolar poly(dimethylsiloxane) coating. Although the ethylated products are very volatile (b.p. of $HgEt_2$ = 159 °C), it was found that a DB-1 column operated in normal conditions (without a cryofocusing step) gave a good separation. Figure 1 shows a typical chromatogram obtained using ethylation–headspace SPME for MeHg

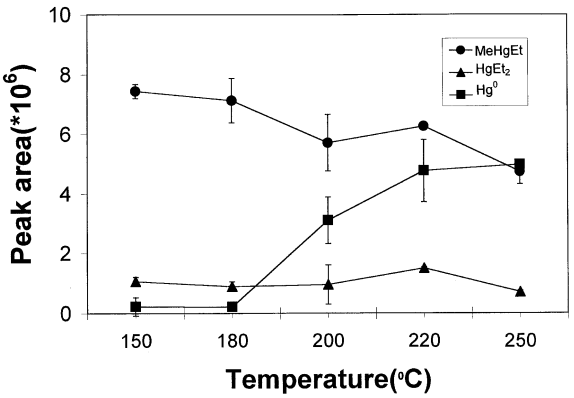

Figure 2 The effect of injection port temperature on the desorption of MeHg.

analysis. The $HgEt_2$ peak resulted from the impurities in reagents and/or contaminant introduced during the procedures.

The temperature of the injection port is critical for mercury analysis with GC since an unsuitable temperature can cause thermal decomposition of mercury derivatives or insufficient desorption.[5,24] The effects of temperature on the desorption of MeHg from the SPME fiber were studied. The desorption time used in these experiments was 20 s. As shown in Fig. 2 the desorption temperature has significant effects on the responses for both MeHgEt and Hg^0. The peak area of MeHgEt decreased with the temperature from 150 to 250 °C, while Hg^0 increased sharply. This result clearly indicates that MeHgEt is decomposed to Hg^0 at temperatures higher than 180 °C, so a desorption temperature of 150 °C was used for the subsequent experiments. It appears that the decomposition of MeHgEt is also dependent on the injection-port design since no decomposition was found, even at 220 °C in previous work using GC–MS.[5]

The desorption time was also evaluated but, as no significant difference in the response for MeHgEt was observed between 10 and 40 s, all the experiments were performed with a desorption time of 20 s.

The analyte derivatization reaction and extraction were carried out simultaneously for two reasons.

(1) As discussed above, the mercury halide compounds have much lower partition coefficients compared with fully alkylated mercury species; consequently, they are difficult to extract from the headspace. Indeed, no mercury halide peaks were observed on the chromatograms.
(2) Extraction of the ethylated mercury while it is being formed shifts the equilibrium of the reaction towards ethyl derivative formation. The time needed for both ethylation and extraction was studied. It was found that a period of 5 min was sufficient for the reaction and extraction equilibrium to be reached.

The effect of reaction and adsorption temperature was assessed at temperatures ranging from 20 to 46 °C. No significant effects were observed between 20 and 40 °C. However, the peak area of Hg^0 was increased at 46 °C, indicating that decomposition of MeHg and/or inorganic mercury (Hg^{2+}) occurred under these conditions. Therefore, the reaction and adsorption could be performed conveniently at room temperature (20 °C) without need of a heating device.

The absolute detection limit of the GC–AFS instrument, calculated as three times the standard deviation of the baseline noise, is 0.02 pg (as Hg).[19,20] The concentration detection limit is a function of the sample size that can be used in the procedure. In the present study, 8.4 ml water samples were used. For fish analysis, 200 mg of sample was dissolved in 4 ml of KOH solution and, from this solution, 200 µl was analyzed. The concentration detection limits calculated for water and fish samples were 3.0 ng l^{-1} and 6.6 ng g^{-1} wet weight, respectively. The reproducibility was assessed for water samples by running six replicates; the relative standard deviation (RSD) was 9.1%. A good dynamic linear range was achieved from the detection limit to 1100 ng l^{-1} (Fig. 3).

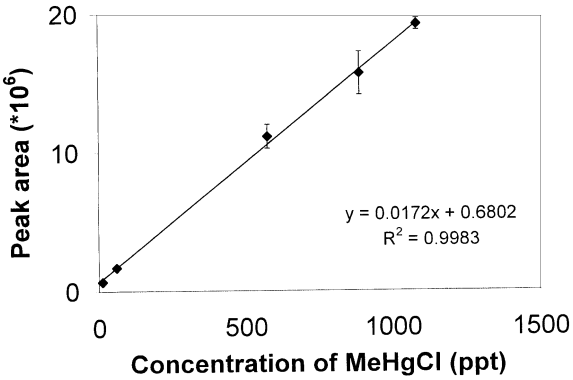

Figure 3 Calibration curve obtained using the ethylation–headspace SPME method for MeHg analysis.

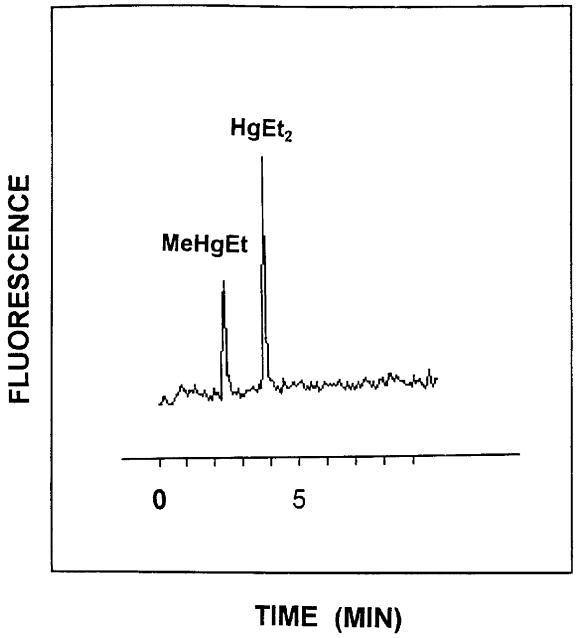

Figure 4 Typical chromatogram obtained for fish analysis using the ethylation–headspace SPME method.

Real-sample analysis

The analytical technique was evaluated by analyzing a fish sample and a standard reference material (DORM-2). The method of standard addition was used to account for the matrix effects. Figure 4 shows a typical chromatogram obtained for fish analysis. The concentration of MeHg in this fish sample was found to be 70.8 ± 7.9 ng g^{-1} ($n = 2$) wet weight, which was in good agreement with that obtained using a liquid–liquid extraction procedure.[26] The MeHg concentration found in DORM-2 was 4.06 ± 0.14 μg g^{-1} ($n = 3$), which is close to the certified value.

In summary, the proposed technique using derivatization with $NaBEt_4$ followed by headspace SPME and GC–AFS detection has been demonstrated to be a fast and reliable screening method for MeHg contamination in fish. However, this method is not sufficiently sensitive for MeHg analysis in real water samples, which usually contain MeHg at low ppt (parts per trillion) levels. The sensitivity of this method could be improved by employing a fiber with a more selective coating.

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REFERENCES

1. C. L. Arthur and J. Pawliszyn, *Anal. Chem.* **62**, 2145 (1990).
2. D. Louch, S. Motlagh and J. Pawliszyn, *Anal Chem.* **64**, 1187 (1992).
3. Z. Zhang and J. Pawliszyn, *Anal. Chem.* **65**, 1843 (1993).
4. D. W. Potter and J. Pawliszyn, *Environ. Sci. Technol.* **28**, 298 (1994).
5. Y. Cai and J. M. Bayona, *J. Chromatogr. Sect. A* **696**, 113 (1995).
6. Y. Morcillo, Y. Cai and J. M. Bayona, *J. High Resol. Chromatogr.* **18**, 767 (1995).
7. T. Gorecki and J. Pawliszyn, *Anal. Chem.* **68**, 3008 (1996).
8. K. G. Furton, J. C. Bruna and J. R. Almirall, *J. High Resol. Chromatogr.* **18**, 625 (1995).
9. F. Guo, T. Gorecki, D. Irish and J. Pawliszyn, *Anal. Commun.* **33**, 361 (1996).
10. C. M. Barshick, S. A. Barshick, M. L. Mohill, P. F. Britt and D. H. Smith, *Rapid Commun. Mass Spectrom.* **10**, 341 (1996).
11. B. L. Wittkamp, S. B. Hawthorne and D. C. Tilotta, *Anal. Chem.* **69**, 1197 (1997).
12. R. P. Mason and W. F. Fitzgerald, *Nature (London)* **347**, 457 (1990).
13. G. A. Gill and K. W. Bruland, *Environ. Sci. Technol.* **24**, 1392 (1990).
14. C. T. Driscoll, C. Yan, C. L. Schofield, R. Munson and J. Holsapple, *Environ. Sci. Technol.* **28**, 136A (1994).
15. P. J. Craig, in: *Organometallic Compounds in the Environment*, Craig, P. J. (ed.), Longman, Harlow, UK, 1986, p. 66.
16. R. P. Mason, J. R. Reinfelder and F. M. M. Morel, *Environ. Sci. Technol.* **30**, 1835 (1996).
17. M. Trudel and J. Rasmussen, *Environ. Sci. Technol.* **31**, 1716 (1997).
18. G. Westöö, *Acta Chem. Scand.* **20**, 2131 (1966).
19. A. Alli, R. Jaffé and R. Jones, *J. High Res Chromatogr.* **17**, 745 (1994).
20. Y. Cai, A. Alli, R. Jaffé and R. Jones, *Anal. Chim. Acta* **334**, 251 (1996).
21. S. Rapsomanikis, O. F. X. Donard and J. H. Weber, *Anal. Chem.* **58**, 35 (1986).
22. R. Fisher, S. Rapsomanikis and M. O. Andreae, *Anal. Chem.* **65**, 763 (1993).
23. N. Bloom, *Can. J. Fish. Aquat. Sci.* **46**, 1131 (1989).
24. L. Liang, M. Horvat and N. S. Bloom, *Talanta* **41**, 371 (1994).
25. H. Hintelmann, R. Douglas Evans and J. Y. J. Villeneuve, *Anal. At. Spectrom.* **10**, 619 (1995).
26. Y. Cai, G. Tang, R. Jaffé and R. Jones, *Intern. J. Environ. Anal. Chem.*, in press.