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# Selective determination of alkylmercury compounds in solid matrices after subcritical water extraction, followed by solid-phase microextraction and GC–MS†

Andreas Beichert, Susanne Padberg and Bernd W. Wenclawiak*
Analytical Chemistry, University of Siegen, Adolf-Reichwein-Str., D-57068 Siegen, Germany

**A method for the extraction and determination of methylmercury (MeHg) in solid matrices is presented. Combining the advantages of two extraction techniques—subcritical water extraction (subWE) and solid-phase microextraction (SPME)—selective separation of MeHg from soils is possible. The procedure is based on extraction with subcritical water without using organic solvents, followed by *in situ* aqueous-phase derivatization with sodium tetraethylborate and headspace SPME with a silica fiber coated with poly(dimethylsiloxane). The optimization of the extraction parameters is described. The identification and quantification of the extracted alkylmercury compounds from spiked soil samples is performed by GC–MS after thermal desorption. Copyright © 2000 John Wiley & Sons, Ltd.**

**Keywords: methylmercury; subcritical water extraction; solid-phase microextraction; soil samples**

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## INTRODUCTION

Due to the toxicity of methylmercury (MeHg), a reliable analysis for this species in a variety of native samples is still a challenging task.

For selective separation of MeHg, different isolation procedures such as solvent extraction, distillation, ion-exchange, aqueous-phase ethylation and alkaline digestion are used in various combinations. Intercomparison studies of separation procedures and detection techniques for mercury speciation in environmental samples have given an overview of common techniques and their reliability.[1–9] For low-level mercury speciation it is of the utmost importance to develop a fundamentally different method to control these analytical methods. The method for MeHg separation should provide sufficient sensitivity and selectivity in complex matrix samples, eliminating or minimizing possible interferences.[10]

Because the ethylation step, which is widely used in various separation techniques, may be prone to interferences, particularly from humic substances and chloride,[11] aqueous distillation is commonly used for separation of low concentrations of methylmercury; this eliminates negative matrix interferences. On the other hand, the possible generation of a monomethylmercury artifact as a result of the action of naturally occurring organic substances on inorganic mercury, particularly in humic-rich soil samples, during the aqueous distillation procedure has been discussed.[12–14] Therefore a new method for the determination of methylmercury in soil samples is desirable, which avoids interferences as well as the formation of artifactual methylmercury. Minimum sample handling is necessary to preserve the original species present in the sample.[10]

The determination of methylmercury in soil samples by subcritical water extraction (subWE) combined with headspace solid-phase microextraction (SPME) after ethylation, followed by GC-MS detection, is investigated in this study. The principle of subWE is based on the large reduction in the polarity of liquid water that occurs at higher temperatures. Miller and Hawthorne state that 'the ability of water to extract nonpolar substances is

* Correspondence to: Prof. Dr Bernd W. Wenclawiak, University of Siegen, Analytical Chemistry I, Adolf-Reichwein-Str., D-57068 Siegen, Germany.
E-mail: wenclawiak@chemie.uni-siegen.de
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linked to the fact that the dielectric constant of water can be reduced significantly with increasing temperature'.[15] The polarity of water is controlled by pressure, and mainly by temperature. By increasing the temperature the polarity of water can be reduced. Pressure is needed to maintain water in the liquid state. Polar and slightly polar substances are extracted at moderate temperatures. SubWE requires no organic solvent and eliminates the need for waste removal. The simple handling steps are: (1) the sample is placed in an extraction cell; (2) the cell is filled with water; (3) the cell is closed, making sure that a gas-cushion is present in it; and (4) the cell is heated in an oven.

Until now subWE has been used mainly for the selective separation of organic substances from solid materials.[15–19]

SPME is based on the equilibrium extraction of analytes from aqueous or gaseous matrices onto an appropriate polymeric coating of a miniature cylindrical fiber.[20,21] The sorbed (preconcentrated) compounds are subsequently released from the fiber in the GC injection liner by thermal desorption.

Combining the advantages of both extraction techniques—no use of organic solvents, fast and selective separation procedure and simple handling—we describe the separation of MeHg from solid matrices.

## EXPERIMENTAL

In this study MeHg was extracted from soil (solid matrix) with subcritical water. An aliquot of the aqueous layer was used for ethylation to produce ethylmethylmercury,[22,23] (MeHgEt), which was extracted via headspace SPME while the *in situ* derivatization continued. By sampling from the headspace, only the species of interest are extracted with no interfering matrix analytes. The alkylmercury compounds were determined by GC–MS after thermal desorption (Fig. 1).

For selective determination, extraction parameters have to be defined and optimized. The extraction kinetics and the influence of matrix effects for both methods were investigated. Finally the whole extraction procedure was applied for the determination of MeHg in real soil samples.

## Instrumentation

### Subcritical water extraction

Subcritical water extraction was performed using a stainless steel extraction cell (Minnesota Valve and Fitting, Eden Praire, MN, USA) 64 mm long and 7 mm i.d., with end caps. The cell is rated for a maximum pressure of 496 bar. The extraction cell was heated in an HP 5890 gas chromatographic oven (Hewlett Packard, Waldbronn, Germany).

### Solid-phase microextraction

The SPME fiber holder and the fiber were obtained from Supelco (Deisenhofen, Germany). Fibers were coated with 100 $\mu$m poly(dimethylsiloxane) (PDMS) and used for all experiments. Glass vials (2 ml) closed with PTFE-coated silicone rubber septa were used as SPME extraction containers.

### GC–MS detection

Mercury compounds were identified by an HP 6890 gas chromatograph with an HP MSD 5972 quadrupole detector (Hewlett Packard, Waldbronn, Germany). GC was performed on a 30 m $\times$ 0.32 mm $\times$ 0.25 $\mu$m Supelcowax 10 column supplied by Supelco. Helium was the carrier gas.

## Reagents and materials

### MeHg standard solution

The MeHg standard solution (400 $\mu g e^{-1}$) was prepared weekly from a 4.45 ppm stock solution of methylmercury chloride (Riedel-de Haën, Seelze, Germany). The solution was stored at 4 °C in the dark. The working solution was prepared daily.

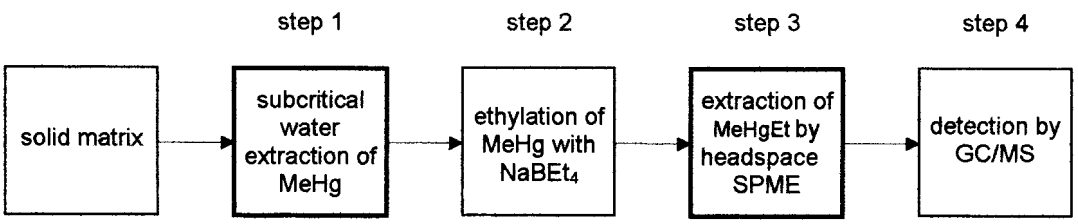


**Figure 1** Analytical procedure for MeHg extraction and determination from solid material.

 

**Solutions for ethylation**[23]
Sodium tetraethylborate (98% purity) was purchased from Strem Chemicals (Newburgport, MA, USA). A 1% solution was freshly prepared with deionized water before each analysis.

Buffer solutions with pH values between 4 and 5.5 were obtained by mixing appropriate amounts of sodium acetate (2 M) and glacial acetic acid (2 M) in deionized water, giving a final volume of 1 l. For stabilization 5 g of 1 M hydrochloric acid was added.

**Sample material**
The extraction of MeHg from a real soil matrix was performed with homogenized dried soil samples of a para-brown earth.

## Procedure

After the extraction parameters were optimized (see below), the whole procedure for the determination of MeHg in soil samples include four steps (Fig. 1).

**Step 1**
A 1 g sample of homogenized dried soil material was weighed into the extraction cell, and 2 ml water spiked (mixed) with the MeHg standard solution (400 $\mu$g $l^{-1}$) was added. After being capped, the cell was placed vertically in the preheated GC oven for the static extraction step. The cell was removed from the oven after a defined time and cooled with tapwater.

**Steps 2 and 3**
Subsequently ethylation and SPME were performed simultaneously. A 100 $\mu$l aliquot of the extractant water was mixed with 50 $\mu$l of the acetate buffer solution (pH 4.9) in a 2 ml glass vial, placed on a magnetic stirring bar and the vial was closed with a PTFE-coated septum. $NaBEt_4$ solution (100 $\mu$l) was added after piercing the septum with a syringe. The 2 ml glass vial for *in situ* derivatization and SPME was chosen because of the higher efficiency in extraction time in comparison with 16 ml vials.[24] The hole in the septum was used to insert the needle of the SPME holder. The PDMS fiber was exposed to the headspace of the stirred solution. Ethylation and extraction occurred simultaneously at a constant temperature of 30 °C. The derivatization was finished after 10 min,[22,23] but the total exposure time for the fiber was defined after investigation of the equilibrium time (see below).

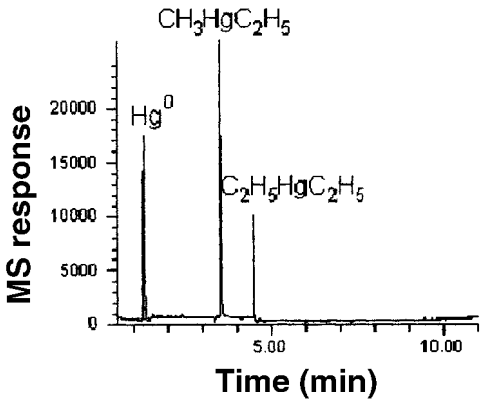


**Figure 2** Chromatogram of a MeHg standard, extracted with headspace SPME after ethylation.

**Step 4**
The fiber was removed from the vial and transferred into the GC injection port. Thermal desorption was performed in the splitless mode for 1 min at 250 °C. The GC was operated in a temperature program mode: 40 °C for 2 min, increased to 260 °C at a rate of 20 °C $min^{-1}$ and then held for 20 min. Within 5 min mercury(0), methylethylmercury (MeHgEt) and diethylmercury ($HgEt_2$) were separated and identified (Fig. 2). Identification was performed in the scan mode, quantification was performed in the selected ion monitoring (SIM) mode set at $m/z$ = 199, 200, 202 for mercury(0), $m/z$ = 214, 215, 217 for MeHgEt and $m/z$ = 202, 231, 258 for $HgEt_2$. A heating step followed, for cleaning of the column.

To ensure high extraction efficiency of the whole method each extraction step was optimized (Table 1).

## RESULTS

### Optimization of the extraction parameters

MeHg extracted from solutions and natural soil samples by varying the extraction conditions was quantified by GC–MS.

For optimization of subWE parameters, MeHg from standard solutions as well as from natural soil samples was subsequently extracted, derivatized and determined with headspace SPME.

This study used a three step approach to

 

**Table 1** Optimization of extraction parameters

| MeHg by headspace SPME/GC–MS from: | Parameter | Method | Results after optimization |
|---|---|---|---|
| Separation/determination of | | | |
| 1 Standard solution | (a) Extraction time | Extraction kinetics: 10, 15, 25, 35 min | 15 min by stirring the solution at a constant temperature of 30 °C |
| | (b) Linear detection range | Calibration: 0, 1, 5, 10, 20, 40 ppb | Detection limit: 1.3 ppb RSD: 6% |
| 2 Standard solution after subWE | (a) Extraction time | Extraction kinetics: 30, 40, 50 min | 30 min extraction at 165 °C |
| | (b) Extraction temperature | Extraction kinetics: 165 °C, 180 °C, 200 °C | |
| 3 Spiked soil samples after subWE | (a) Linear detection range | Calibration: 0, 1, 5, 10, 40 ppb | Detection limit: 5 ppb |

investigate the applicability of subWE followed by SPME for the determination of MeHg (Table 1).

## Method 1

*(a) SPME—extraction time*

The principle of SPME is based on the adjustment of an equilibrium between the analyte concentrations in the liquid phase or in the headspace and the solid-phase fiber coating, but full quantitative extraction of the analyte does not take place, i.e. an equilibrium exists. As long as conditions for the method remain constant (*t*, *T*, vial volume, fiber) the method is reproducible. Volatile compounds transfer more efficiently from water to headspace to the fiber coating than from water directly to the fiber coating. Additionally in headspace SPME, the mass transfer from the liquid phase to the headspace is speeded up by constantly stirring the liquid phase, generating a continuously fresh surface. Because derivatized ($NaB_4$) MeHg is volatile and nonpolar, and therefore has a greater affinity for the nonpolar PDMS fiber, in our method MeHg is ethylated during headspace SPME.

The time for the extraction equilibration of headspace SPME for MeHg was studied by extracting 100 $\mu$l ethylated MeHg (MeHgEt) standard solution (400 $\mu g\ l^{-1}$). The samples were stirred at a constant temperature of 30 °C for 10, 15, 25 and 35 min. Figure 3 shows the relative extraction yield ($n = 9$) of MeHgEt with increasing extraction time.

After 15 min of sampling, most MeHgEt was extracted. The minimal increase beyond 15 min did not justify a longer extraction time. By equilibrations under SPME, conditions (*t*, *T*, vial volume, fiber) were kept constant for delivering reproducible extractions and quantifiable data. Complete desorption of the adsorbed MeHgEt after 1 min at

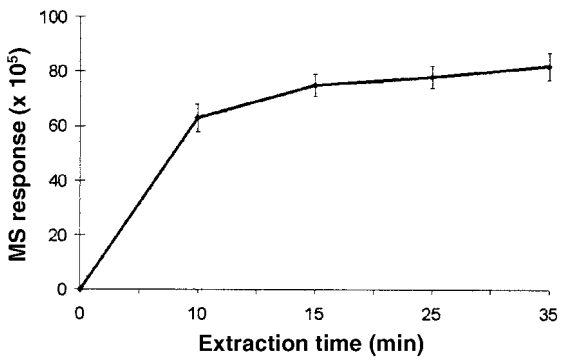


**Figure 3** Influence of sampling time on SPME efficiency.

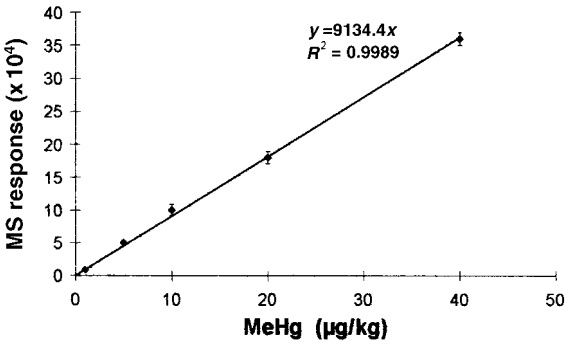


**Figure 4** Calibration curve of MeHg extracted by headspace SPME.

250 °C (splitless mode) was controlled by repeating the thermal desorption step with the same fiber. No more MeHgEt was identified by GC.

*(b) SPME—linear detection range*
The reliable determination of low-level MeHg concentrations requires control of the linear detection range. Portions (100 $\mu$l) of different MeHg standard solutions containing 1, 5, 10, 20 and 40 ppb MeHg were ethylated and extracted. The calibration curve (Fig. 4) is based on analysis ($n = 3$) of the standard solution.

A good linear range was achieved from the detection limit to 40 ppb. The detection limit was determined as 1.3 ppb, calculated as the threefold standard deviation of three replicate measurements. The reproducibility of the SPME method was evaluated by nine subsequent measurements with 6% RSD.

**Method 2**
*(a) SubWE/SPME—extraction time*
The effect of subWE time on the recovery of MeHg was studied. Replicate samples (2ml each $n = 3$) of MeHg standard solution (400 $\mu$g $l^{-1}$) were extracted at 200 °C, which corresponded to a pressure of 15 bar, for extraction times of 30, 40 and 50 min. The extraction yield decreased with increasing extraction time (Fig. 5).

After 30 min only 3% of the MeHg was unaccounted for. Considering the low quantities this involved, was acceptable. Longer extraction times increased decomposition and were less favorable.

*(b) SubWE/SPME—extraction temperature*
By increasing to extraction temperature, the solubility of the analytes is enhanced. But decomposition effects at higher extraction temperatures have to be controlled. Therefore 2 ml of MeHg standard solution was extracted for 30 min at 165 °C, 180 °C and 200 °C. By increasing the extraction temperature from 165 °C to 200 °C the recovery decreases by about 40%. Comparing the recovery of MeHg extraction from standard solution only by SPME, the decrease in the recovery at 165 °C is only by 3%. Lower extraction temperatures will extend the extraction time.

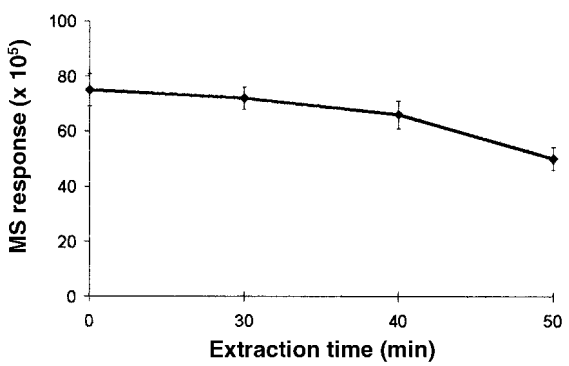


**Figure 5** Decrease in MeHg concentration with increasing subWE time.

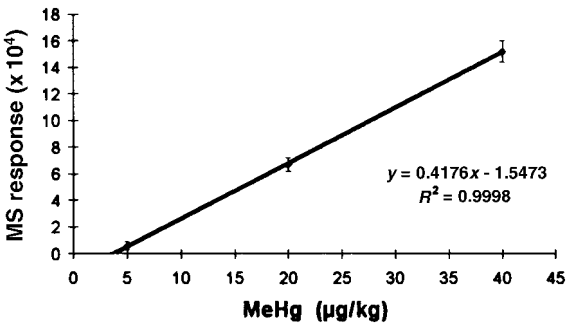


**Figure 6** Calibration for the determination of MeHg in soil samples by subWE followed by headspace SPME and GC–MS.

## Soil sample analyses

**Method 3**
*(a) SubWE / SPME of soil sample—detection range*
SPME of alkylmercury compounds has been reported mainly from biological and aqueous samples.[25–31] This extraction technique has not yet been applied to the determination of methylmercury from soil samples. The reasons might include problems of sample preparation of such inhomogeneous material, caused by the content of organic substances. Such a sample preparation step is required for SPME, because the analyte has to be in the aqueous or gaseous phase. This was achieved in this study by subWE of MeHg from soil samples.

After optimizing the extraction parameters the whole extraction procedure for the separation of MeHg from soil material was evaluated by analyzing homogenized dried soil samples of a para-brown earth. Soil samples (1g) were spiked with 5, 20 and 40 $\mu$g $l^{-1}$ of MeHg standard solution in 2 ml deionized water. MeHg was extracted using the following optimized extraction parameters

SubWE: 30 min at 165 °C

SPME: 15 min headspace sampling, stirring at 30 °C

The detection limit of 5.0 ppb for the selective determination of MeHg in spiked soil samples by subWE followed by SPME and GC–MS was calculated on the basis of the calibration curve (Fig. 6). The recovery was unexpectedly low. Further experiments showed that the surface of the stainless steel extraction cells is at least one reason for this low result.

Under the optimized extraction conditions it is possible to determine MeHg soil samples by subWE followed by headspace SPME and GC–MS detection. The developed procedure, combining the advantages of two extraction techniques, is a fast and reliable method. However, the determination of MeHg at low concentrations in soil samples requires further studies, mainly in the subWE area.

## REFERENCES


1. Quevauviller P. *Fresenius' J. Anal. Chem.* 1997; **358**: 419.
2. Quevauviller P, Fortunati GU, Filipelli M, Baldi F, Bianchi M, Muntau H. *Appl. Organomet. Chem.* 1996; **10**: 537.
3. Padberg S, Iverfeld A, Lee YH, Baldi F, Filipelli M, May K, Stoeppler M. Determination of Low-level Methylmercury Concentrations in Water and Complex Matrices by Different Analytical Methods. A Methodological Intercomparison. In *Mercury Pollution*, Watras CJ, Huckabee JW (eds). Lewis Publishers, Boca Raton: 1994; 567–580.
4. Horvat M, Bloom N, Liang L. *Anal. Chim. Acta* 1993; **281**: 135.
5. Horvat M, Liang L, Azemard S, Mandic V, Villeneuve JP, Coquery M. *Fresenius' J. Anal. Chem.* 1997; **358**: 411.
6. Padberg S, Burow M, Stoeppier M. *Fresenius' J. Anal. Chem.* 1993; **346**: 686.
7. Quevauviller P, Fortunati GU, Fillipelli M, Bortoli A, Muntau H. *Appl. Organomet. Chem.* 1998; **12**: 531.
8. Sànchez Uría JE, Sanz-Medel A. *Talanta* 1998; **47**: 509.
9. Bloom NS, Horvat M, Watras CJ. *Water Air Soil Pollut.* 1995; **80**: 1257.
10. Vela NP, Caruso JA. *J. Anal. At. Spectrom.* 1993; **8**: 787.
11. Bowles KC, Apte SC. *Anal. Chem.* 1998; **70**: 395.
12. Hintelmann H, Falter R, Ilgen G, Evans RD. *Fresenius' J. Anal. Chem.* 1997; **358**: 363.
13. Bloom NS, Colman JA, Barber L. *Fresenius' J. Anal. Chem.* 1997; **358**: 371.
14. Quevauviller P, Horvat M. *Anal. Chem.* 1999; **71**: 155.
15. Miller DJ, Hawthorne SB. *Anal. Chem.* 1998; **70**: 1618.
16. Yang Y, Belghazi M, Lagadec A, Miller DJ, Hawthorne SB. *J. Chromatography, A* 1998; **810**: 149.
17. Hageman KJ, Mazeas L, Grabanski CB, Miller DJ, Hawthorne SB. *Anal. Chem.* 1996; **68**: 3892.
18. Yang Y, Bowadt S, Hawthorne SB, Miller DJ. *Anal. Chem.* 1995; **67**: 4571.
19. Hawthorne SB, Yang Y, Miller DJ. *Anal. Chem.* 1994; **66**: 2912.
20. Pawliszyn J. *Solid-phase Microextraction, Theory and Practice*. Wiley–VCH: New York, 1997.
21. Zhang Z, Yang MJ, Pawliszyn J. *Anal. Chem.* 1994; **66**: 844.
22. Rapsomanikis S, Donard OFX, Weber JA. *Anal. Chem.* 1986; **58**: 35.
23. Bloom NS. *Can. J. Fish. Aquat. Sci.* 1989; **46**: 1131.
24. Eisert R, Pawliszyn J. *Crit. Rev. Anal. Chem.* 1997; **27**: 103.
25. Mothes S, Wennrich R. *J. High Res. Chromatogr.* 1999; **22**: 181.
26. Okeyo P, Rentz SM, Snow NH. *J. High Res. Chromatogr.* 1997; **20**: 171.
27. Dunemann L, Hajimiragha H, Bererow J. *Fresenius' J. Anal. Chem.* 1999; **363**: 466.
28. Moens L, de Smaele T, Dams R. *Anal. Chem.* 1997; **69**: 1604.
29. Bin H, Gui-bin J, Zhe-ming N. *J. Anal. At. Spectrom.* 1998; **13**: 1141.
30. de Smaele T, Moens L, Sandra P, Dams R. *Mikrochim. Acta* 1999; **130**: 241.
31. Cai Y, Monsalud S, Feurton KG, Jaffé R, Jones RD. *Appl. Organomet. Chem.* 1998; **12**: 565.


 