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# Experiences with the Analytical Procedures for the Determination of Methylmercury in Environmental Samples

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**The analytical method using ethylation, gas chromatography–cold vapor atomic fluorescence spectroscopy (GC–CV AFS), combined with different pretreatment procedures, has been used for the determination of methylmercury (MeHg) in various aqueous and solid environmental samples. Different types of samples with varying matrices and MeHg concentration levels require different pretreatment procedures in order to isolate/separate MeHg from the sample matrix, and to overcome interferences in the ethylation reaction process. Limitations of different pretreatment procedures (solvent extraction into methylene chloride, distillation under nitrogen flow and alkaline digestion) are discussed and recommendations are given. The influence of the quality of the ethylating reagent on the analytical results is also examined.**

**Keywords: Methylmercury, derivatization, ethylation, gas chromatography, digestion, sediment, natural water, cold vapour, atomic fluorescence spectroscopy**

## INTRODUCTION

The great importance of the biogeochemical cycle of methylmercury (MeHg), the most toxic form of mercury (Hg), has promoted many scientists to develop new methods/pretreatment procedures for the determination of MeHg in various types of samples/matrices.[1–12] It has been a challenge to cope with both problems connected to ultra-low Hg levels (picogram amounts) and problems arising from various complex matrices. This is particularly true for laboratories aiming to set up procedures for MeHg determination in different environmental samples. The choice of an appropriate and sensitive detection system (e.g. cold vapor atomic absorption spectrometry—CV AAS; cold vapour atomic fluorescence spectrometry—CV AFS; or gas chromatography–electron capture detection—GC/ECD) may to some extent be governed by availability in the respective laboratory. However, it is vitally important to choose an appropriate and validated pretreatment procedure to isolate/separate MeHg from the sample matrix, in order to have an efficient and accurate determination of MeHg in the matrix of concern.

Newly developed pretreatment procedures, e.g. distillation, KOH/methanol digestion and extraction with methylene chloride, are presently starting to replace some of the more traditional isolation procedures, e.g. acid leaching/solvent extraction.[13] The new pretreatment steps are often also combined with a derivatization step[4,9,14] converting the MeHg to a form easily separated from other Hg species. Assessments of performance characteristics of the respective procedures have been reported previously.[4–7] The matrix influence is still great for some sample types and a better control of the derivatization reaction is also achievable. In this paper, we report our experience of pretreatment procedures, especially from the distillation and alkaline digestion steps, which we hope will help other laboratories in the analysis of MeHg in environmental samples.

## EXPERIMENTAL

### Pretreatment/isolation procedure and detection technique

An extraction procedure using methylene chloride ($CH_2Cl_2$) to extract MeHg chloride from water samples has been used according to the method described by Bloom[4] and Horvat *et al.*[8] In brief, the following procedure was used. About 40–50 ml of the sample was weighed directly into a 125-ml Teflon bottle and 5 ml KCl-saturated 10% HCl and 40 ml $CH_2Cl_2$ were added. Bottles were

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placed on a mechanical shaker and shaken overnight. After shaking, the water and $CH_2Cl_2$ phases were allowed to separate and the water was removed with a 5 ml pipette. Milli-Q water (50 ml) was then added and the $CH_2Cl_2$ was evaporated by heating the bottles to about 60 °C in a fume hood. After the $CH_2Cl_2$ had been visibly removed, the sample was purged for 5 min with high-purity nitrogen to ensure complete removal.

A distillation procedure[7,8] has been applied for both aqueous and solid material/biological samples. Aqueous samples (40–50 ml) were acidified with $H_2SO_4$/KCl mixture and distilled in 60 ml Teflon vials with lids equipped with $\frac{1}{8}$ in (3 mm) Teflon tubing. The distillation vials were placed in an aluminum block which was heated to 145 °C, whereas the receivers were cooled in an ice bath. The distillation was run at a nitrogen flow rate of 60 ml $min^{-1}$ for 3–6 h until 85 to 90% of the distillate was collected: the time depended on the sample size, flow rate and temperature. Distillation of solid materials was also performed by the same approach although smaller vials (22 ml) were used. In this case, a small amount (usually 1 g for sediments) was weighed into the vial and water was added along with the acidifying mixture. MeHg in solid samples was always determined on a wet weight basis and later corrected for water content determined in a separate sample.

Finally, an alkaline (KOH/methanol) digestion procedure[4,7] has been used for solid/biological material. About 1 g of the sample was placed in a Teflon vial and digested for 6 h at 60 °C in 10 ml 25% KOH in methanol. The digestate was diluted to 18.2 ml with methanol. A small (typically 100–200 µl) aliquot was used for direct analysis.

After pretreatment, derivatization by aqueous-phase ethylation followed by isothermal GC–CV AFS detection, were performed. This experimental procedure has been described in detail elsewhere.[4,5,7,8] In short, the MeHg in the sample (after pretreatment according to the procedures described above) was ethylated to methylethylmercury in an acetate buffer solution. Inorganic Hg in the sample was converted to diethylmercury. These species are volatile and can be purged out of the solution by bubbling nitrogen through the reaction vessel. The Hg species (ethylation derivatives) were collected on Carbotrap columns. The different organic Hg compounds were then desorbed onto a Chromosorb (Supelco) GC column by gentle heating of the Carbotrap column. After the Hg forms had been separated they passed through a pyrolytic column consisting of a 20-cm quatz glass tube partly filled with quartz wool and heated to about 800 °C. All Hg species were thereby converted to $Hg^0$ and could be detected using CV AFS.[15] The experimental details were similar to those described elsewhere.[4,5,7,8]

### Calibration and quality control

An analytical calibration curve consisting of more than five data points, normally ranging from 30 to 100 pg, was run every day using standard solutions prepared from pure $CH_3HgCl$(s). Procedural blanks, duplicate analyses and recovery checks of standard additions were run for each batch of samples. Reference materials, when available, were also included as part of the routine analysis.

### Sample collection, preservation and handling

Acid-cleaned Teflon and Pyrex-type glass bottles were used for collecting samples. Fresh solid samples, such as sediment and soil, and biological material samples were kept in a freezer at −18 °C until analysis. Water samples were preserved with 5 ml $l^{-1}$ suprapur HCl if the extraction procedure was to be used, or by adding 10 ml $l^{-1}$ 9 M $H_2SO_4$ and 5 ml $l^{-1}$ 2.68 M KCl to the sample, when the distillation procedure was to be applied. Water samples were stored at +4 °C in the dark.

## RESULTS AND DISCUSSION

Verification of the analytical procedures was performed using analysis of Certified Reference Materials, the method of standard addition and comparison of the analytical results obtained after applying different pretreatmet procedures on the sample.

Distillation MeHg spike recoveries from three stream waters with varying content of total organic carbon (TOC) (15–35 mg $l^{-1}$) and one from Milli-Q water, were examined in relation to different amounts of MeHg added. Figure 1 shows a good correlation between the spiked amount of MeHg and the MeHg concentration found in the stream waters and in the Milli-Q water sample, within the concentration range studied. The mean value of the recovery of spiked

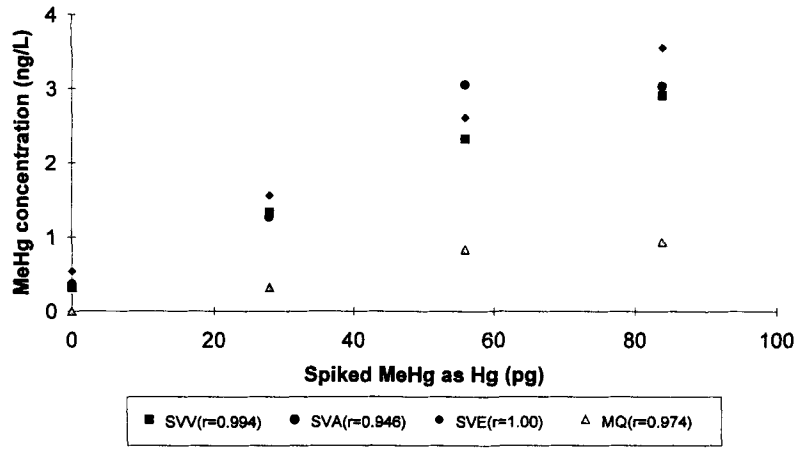


**Figure 1** Standard addition curves for distilled stream water samples with varying TOC (15–35 mg $l^{-1}$) and a Milli-Q water sample. The correlation coefficient ($r$) between the spiked amount and the MeHg concentration found, is given for the respective type of sample. SVV, SVA and SVE denotes samples taken in different humic-rich streams in northern Sweden.

MeHg from stream waters is 90.8 ± 12.6% ($n = 9$, coefficient of variance = CV = 13.9%), when *ca* 85% of the water sample was distilled (i.e. the distillation recovery). In Fig. 2, distillation and spike recoveries are shown for samples with varying TOC. No systematic difference can be detected and the recovery seems to be independent of the TOC of the sample. However, the mean value of recovery from Milli-Q water is much lower: 68.2 ± 11.6% ($n = 6$). In a previous study of MeHg in lake waters[8] it was found that a significant part of the MeHg contained in a sample may undergo decomposition towards the end of the distillation, if more than 85% of the sample is distilled. The lower spike recovery in Milli-Q waters suggests that the spiked MeHg is less stable in Milli-Q water than in stream waters, in which the MeHg is bound to the naturally occurring organic substances. This may explain the result we found in the present study. It is therefore recommended not to use too great amounts of MeHg for spiking natural water samples (i.e. less than two to five times of the amount of MeHg found in the water sample), since it is critical that the speciation of the spiked MeHg does not differ in composition from the MeHg originally present in the sample.

In Fig. 3, a comparison of the analytical results using distillation and alkaline digestion pretreatment procedures for the determination of MeHg in fresh zooplankton samples is presented. The correlation of the measured concentrations using

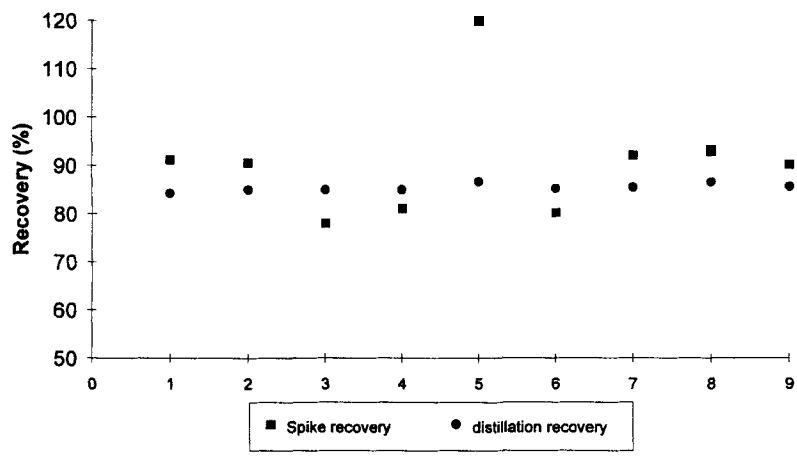


**Figure 2** Comparison of spike recoveries (defined as the fraction of the MeHg originally present in the distilled sample that is recovered in the distillate) and distillation recoveries (defined as the volume of sample transferred in the distillation process) from stream water samples with varying TOC (15–35 mg $l^{-1}$).

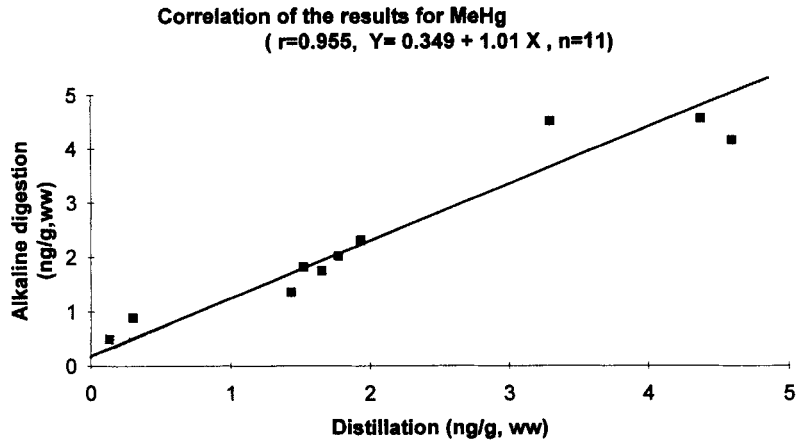


**Figure 3** Correlation of the MeHg levels obtained using distillation and alkaline digestion pretreatments of various zooplankton samples.

these two procedures is acceptable ($n=11$; $r=0.955$; alkaline method $=0.349+1.01\times$ distillation method). The lower value obtained by distillation is mainly caused by lower recovery compared with alkaline digestion (Table 1) The MeHg recovery in distillation is not linearly related to the amount of distillate collected, since a part of the MeHg may undergo decomposition when distilling more than 90% of the sample.[8] This may certainly cause analytical errors and requires a precise control of the distillation in order to collect optimal amounts of the distillate, i.e. 85–90% of the orignal sample volume. In general, the alkaline digestion procedure gives consistent and high recoveries of MeHg in various solid or biological samples, and needs less labor compared with the distillation procedure. However, as the digested sample interferes with the ethylation, only a small aliquot ($ca$ <0.15%) of the solution can be taken for the ethylation reaction and subsequent measurement of MeHg. The application of the alkaline digestion procedure to a solid sample requires a higher level of MeHg in the sample, preferably more than 1 ng $g^{-1}$.

**Table 1** Results of spike recoveries from different matrices after standard addition using distillation and alkaline digestion procedures

| Sample | Distillation recovery (%) | Alkaline digestion recovery (%) |
|---|---|---|
| Sediment IAEA-256 | 81 ± 2 ($n=2$) | |
| Fish sample | 80.5 ± 11.5 ($n=4$) | 98.5 ± 3.4 ($n=4$) |
| Zooplankton | 79 ± 1 ($n=2$) | 104 ± 12 ($n=2$) |

For water samples, a comparison of the recovery of MeHg by solvent extraction and distillation procedures has been discussed in detail

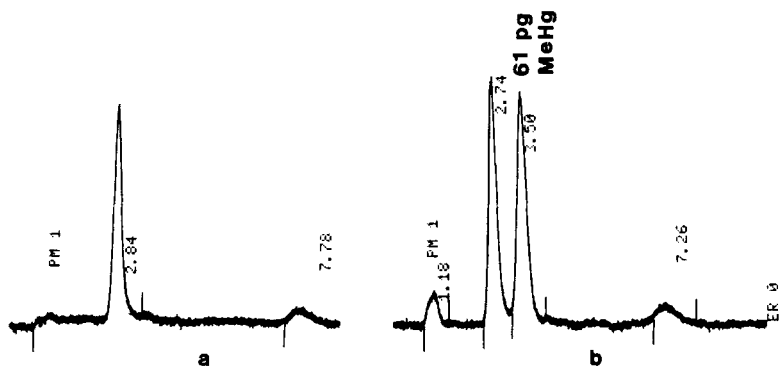


**Figure 4** Chromatograms obtained after aqueous-phase ethylation/isothermal GC–CV AFS detection, at a 90 ± 3 °C GC column temperature. A blank sample run is shown in chromatogram **a**, and a natural water sample in **b**. Retention times: minutes 1.18 min corresponds to $Hg^0$; 2.74 and 2.84 min, to the same unknown compound originating from the ethylationg reagent; 3.50 min to 61 pg Hg as $CH_3HgCl$; and 7.26 and 7.78 min to inorganic Hg.

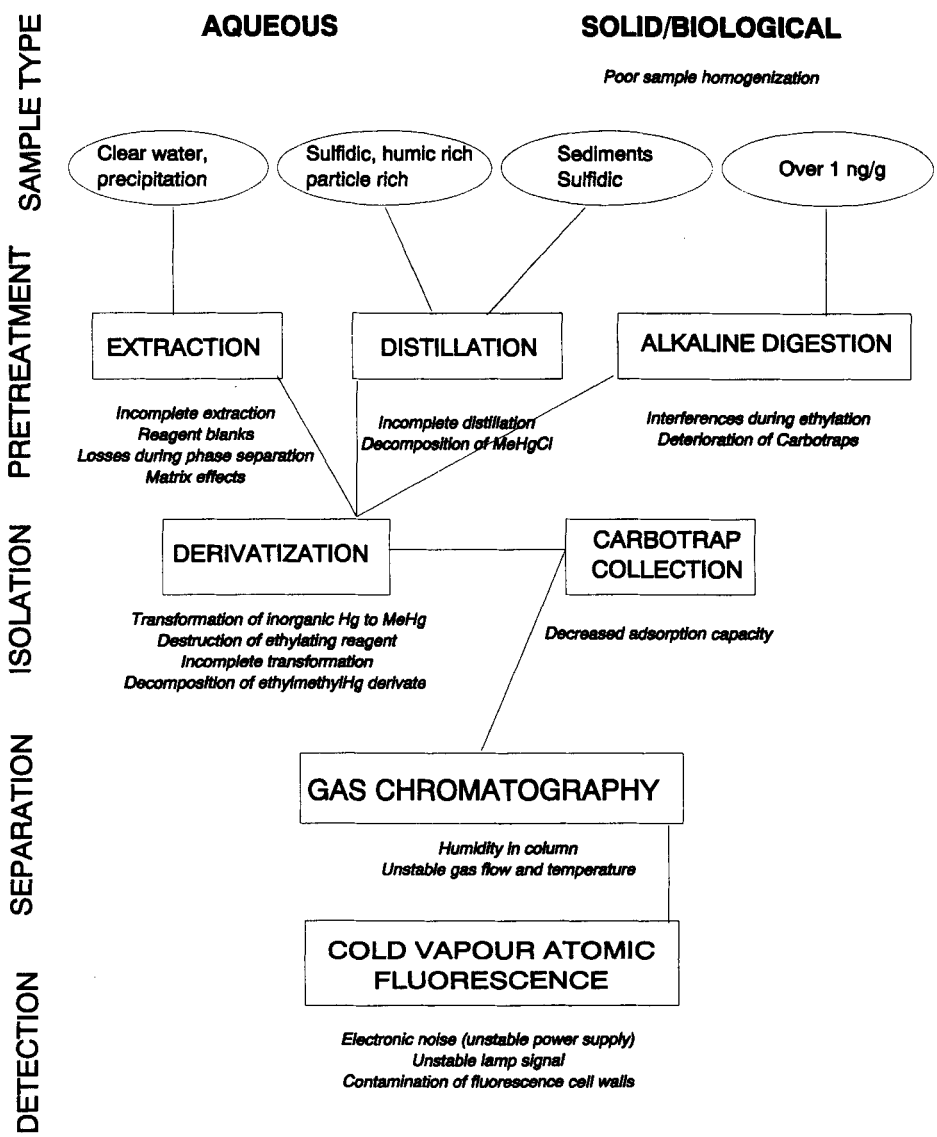


**Figure 5** Schematic flow chart of recommendations for the selecton of methods for determiation of MeHg in various environmental samples. Common sources of errors in each step are indicated in italic style.

elsewhere.[8] The advantage of the solvent extraction proccedure is that it is easy to perform and less laborious. The extraction recovery is lower than the distillation recovery (extraction recovery: $79 \pm 14\%$, $n = 15$). The main disadvantage with the solvent extraction is low recovery and low reproducibility when analyzing MeHg in anoxic and humic-rich water samples, as well as water samples with high levels of particulate matter.

It is our experience that the quality of the ethylating reagent (sodium tetraethylborate, $NaBEt_4$) differs between various batches. In some of the batches, an unknown compound of the ethylating reagent gave a high peak which could overlie the MeHg peak in the chromatogram (Fig. 4). It was found necessary to control the He flow rate and the temperature of the GC column (*ca* 90 °C) very carefully in order to separate the MeHg signal from the signal originating in the ethylating reagent. The variations in the quality of the ethylating reagent demands the performance of rigorous tests using standard calibrations, spike recoveries and standard additions each time a new batch of $NaBEt_4$ is introduced.

There are many sources of error in each step of

the determination of MeHg in environmental samples. Matrix effects may cause errors, such as incomplete extraction/distillation/digestion, species transformation or losses during treatment. Errors may occur during the derivatization step as well as when detecting the MeHg.[15] In order to be able to analyze environmental samples for MeHg with good accuracy and precision, it is an absolute necessity to use strict quality control procedures for each type of sample analyzed. The efficiency of the pretreatment steps (extraction, distillation and digestion) are best controlled using spiking experiments and standard addition techniques. In some cases, it is necessary to vary the experimental parameters used in the different techniques, such as distillation, nitrogen flow rate or the proportions of samples and methylene chloride used for the extraction, in order to test the efficiencies and identify the optimum conditions. It is also valuable to use different pretreatment techniques of the same sample and a test of the quality of the methods. Aqueous samples can be both extracted and distilled whereas biological samples of sediments can be both distilled and digested in alkaline KOH. Matrix effects during derivatization are sometimes caused by sample interferences with the ethylating reagent. In this case, spiking of the sample with known quantities of MeHg may prove to be a good test. It is also recommended to analyze aliquots of different volumes of the sample in order to determine whether matrix effects are causing analytical errors.

The general problem of poor sample homogenization of fresh samples, such as soil, sediment or zooplankton samples, should also be mentioned. A significant bias due to variation in, for example, the water content may always occur. These problems can be avoided by careful sample homogenization or, if not possible, by analyzing a number of samples so that an estimate of the variations can be obtained.

As each pretreatment has its own advantages and limitations, it is very important to use an appropriate pretreatment/isolation procedure and detection system to obtain an efficient and accurate determination of MeHg in the matrix of concern. In Fig. 5, a schematic flow chart of recommendations on the determination of MeHg in various environmental samples is given, as well as the possible sources of errors in each step.

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