Development of Improved Sample Preparation Methods for Determination of Methyl Mercury in Solids

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Introduction and Background

Mercury (Hg) and its environmental effects have become topics of concern in recent years. Anthropogenic sources and natural processes release Hg into the environment. Hg cycles through the environment and can ultimately be converted into different species of mercury, including toxic monomethyl mercury (MMHg). MMHg biomagnifies up the food chain; therefore, it is important to be able to quantify low levels of MMHg, particularly in soils and sediments where most of the methylation can occur.

Many sample preparation methods for the determination of MMHg in soils and sediments are costly and time consuming. The purpose of this study was to investigate and develop more efficient methods for the preparation of soil and sediment samples for MMHg analysis. A common method for preparing soil and sediment samples for MMHg determination is a time consuming back-extraction using dichloromethane (DCM). For this study, two alternative sediment/soil sample digestion procedures for MMHg were investigated and compared to the DCM back-extraction: a digestion using a 25% solution of potassium hydroxide in methanol (KOH/Me), and a digestion using a 9 M sulfuric acid (H_2SO_4) solution. There are many advantages to be gained by development of an alternative preparation method (Table 1a and 1b).

Figure 1: The Brooks Rand Labs MERX™ automated methyl mercury system

Preparation Methods

DCM Back-Extraction Methods

- Weight out approximately 2.5 g of sample
- Add 2 mL 1 M $CuSO_4$ and 10 mL 18% KBr/ 5% H_2SO_4 to each sample
- Allow samples to leach for 1 hour
- Add approximately 20 g DCM
- Shake vigorously and put on shaker tray for 1 hour
- Centrifuge for 10 minutes at 3200 rpm
- Filter sample into rinsed and labeled 125-mL Teflon bottles
- Add approximately 50 mL of preheated reagent water and 2-3 boiling chips
- Place samples on hotplate set to 70°C for 6 hours

Alternative Sample Preparation Methods

- Approximately 1 g of sample weighed into a clean glass vial
- 5 mL of 9 M H_2SO_4 or 10 mL of 25% KOH/Me added to each sample
- Digested at 90 °C for 4 hours
- Diluted to a final volume of 20 mL with reagent water for 9 M H_2SO_4 prep or methanol for 25% KOH/Me prep.
- CRMs and MS/MSDs were prepared with each set of samples

DCM Back-Extraction Method — Table 1a

Pros	Cons
• Quantitatively extracts MMHg from interferences that may be present in sample • Validated Brooks Rand Labs method	• DCM is a suspected carcinogen • Requires use of: ✓ Multiple container types ✓ Funnels ✓ Phase-separation paper ✓ Centrifuge ✓ 2.5 g sample • Takes 8-10 hours to complete preparation of a 20 sample batch

New Preparation Techniques — Table 1b

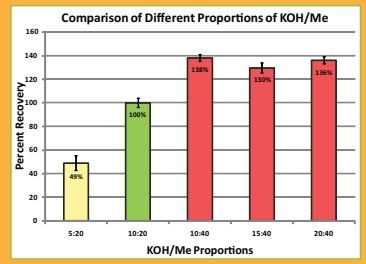
Pros	Cons
• Uses only 20-mL glass vials, reagents, oven, and 1 g of sample • Eliminates use of DCM • Takes 2-3 hours to complete preparation of a 20 sample batch • Potential to: ✓ Increase throughput ✓ Save time and money	• Does not solely extract MMHg; therefore limited by Hg(II) concentrations in samples • Not yet validated by Brooks Rand Labs • Poor QA results

Analysis Method

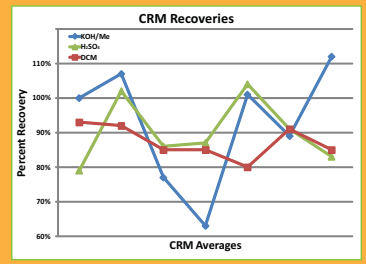
All samples were analyzed via CVAFS on Brooks Rand Labs MERX™ MMHg autoanalyzer (Figure 1). Acetate buffer (0.6 mL) was added to each sample and then individual samples were adjusted to pH 4.5-5.0 using a 50% KOH solution, if needed. Sodium tetraethylborate was added to each sample to volatilize the mercury species. The volatile species were then pre-concentrated on a Tenax™ trap, desorbed by heat, and flushed through an isothermal gas chromatography (GC) column.

Following chromatographic separation, the Hg species passed through a pyrolytic column, thus reducing all of the species to Hg^0, which was quantified using atomic fluorescence spectrometry (See Figure 2). The limiting factor for selection of analytical aliquot size is Hg (II) concentrations, in order to prevent gross contamination of the instrumentation. Initial experiments indicated very high concentrations of Hg(II) were extracted when the alternative procedures were used; therefore, all samples were initially screened at a significant dilution. Samples were then reanalyzed at a greater aliquot volume depending on the Hg(II) levels.

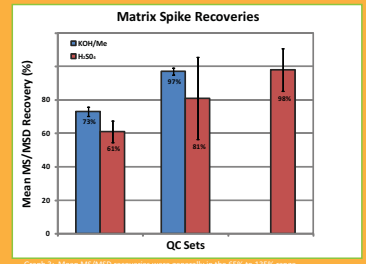
Results



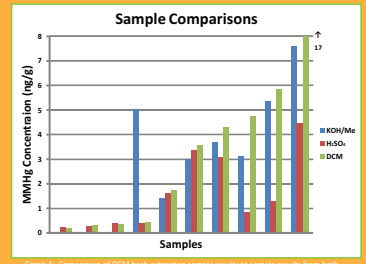
Graph 1: Comparison of CRM (CC-580) recoveries for varying proportions of 25% KOH/Me. Sample sets are labeled by volume of KOH/Me added prior to digestion and final dilution volume with methanol. The ideal proportion proved to be 10 mL of KOH/Me with a final dilution volume of 20 mL.



Graph 2: Both methods produced quantifiable CRM recoveries with the typical control limits of 65 - 135%, which was consistent with CRM recoveries typically seen from the DCM back-extraction method.



Graph 3: Mean MS/MSD recoveries were generally in the 65% to 135% range.



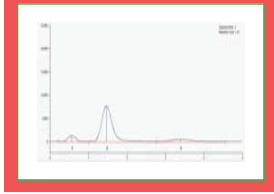
Graph 4: Comparison of DCM back-extraction sample results to sample results from both experimental methods.

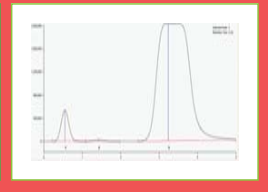
Conclusion

Two simple digestion schemes for MMHg in sediment samples were tested and compared to Brooks Rand Labs' standard methodology of the DCM back-extraction preparation.

- Both experimental methods produced CRM and MS/MSD recoveries within the typical control levels of 65 to 135%, consistent with DCM results. This demonstrates that both experimental methods were able to extract MMHg from the sample with acceptable accuracy.
- Results from soil and sediment samples prepared by the H_2SO_4 Method compared best to DCM Method results when the sample concentrations were less than 3.5 ng/g.
- Sample results from the KOH/Me Method compared well to the DCM Method results for samples with concentrations between 3.5 - 6 ng/g.

Although both experimental sample preparations were able to successfully extract MMHg from the sample, they were unable to solely extract MMHg. Consequently, samples needed to be analyzed at a significant dilution to avoid Hg(II) contamination of the instrument. This resulted in an increased detection limit and fewer quantifiable results.





Next Steps

Future research for the KOH/Me method will include diluting the samples with water instead of methanol, which would increase the amount that could be analyzed, thus yielding a higher number of quantifiable results. Similarly, the H_2SO_4 method would also be diluted with water to a final volume of 40 mL instead of 20 mL. This would eliminate the variability seen when samples are below the quantifiable level and would hopefully produce more consistent sample results when compared to the DCM results. A holding time study will also be conducted to investigate the length of time in which samples will produce accurate recoveries.

References

- Antunes P, Cerdeira I, Lavrado J, Vale C. Simple method of monomethylmercury determination in estuarine sediments. *Trends in Analytical Chemistry*. 2004;23(10-11):799-806.
- SOP #BR-0312, Revision 12a, "Determination of Methyl Mercury by Aqueous Phase Ethylation, Trap Pre-Collection, Isothermal GC Separation, and CVAFS Detection: BRL Procedure for EPA Method 1630 (Waters) and EPA Method 1630, Modified (Solids)," Brooks Rand Labs, Seattle WA, September 2008.
- Chandrasekhar, TM; Yu, R. "Analysis of methyl mercury in sediment and tissue," Revised by K. Tate. HG-003-2.2, February 2005.
- De Diego A, Donard O, Martin F, Tseng C. Rapid and quantitative microwave-assisted recovery of methylmercury from standard reference sediments. *JAAS*. 1997;12:629-635.
- Marvin-DiPasquale M, USGS – Menlo Park. Personal communication, 2008.

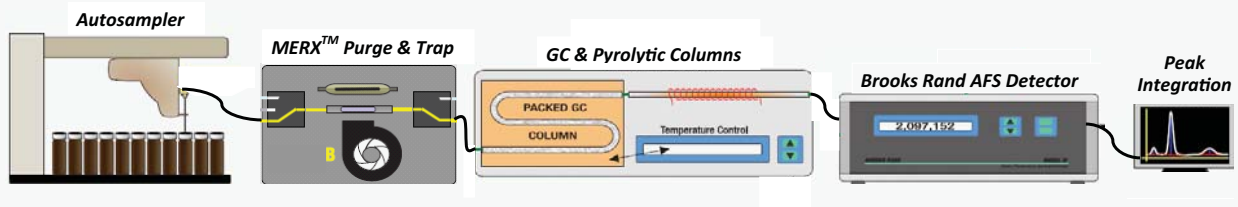


Figure 2