Determination of Methylmercury in Human Hair:
A Comparison of Digestion Methods

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Introduction

Mercury is a well-known human health hazard and is routinely monitored in public health studies in order to assess its effects. Total mercury is frequently measured to assess human exposure, due to the relative ease of analysis; however, this information does not always provide sufficient information to accurately assess the source of exposure and/or risk to a person or community.

Because there is no definitive correlation factor establishing the ratio of methylmercury to total mercury in biological samples, it is important to measure methylmercury in human biomonitoring studies. Minimally invasive sample collection is also desirable in most instances; therefore, human blood, urine, and hair are typically collected for analysis. Urine is not an acceptable biomonitoring matrix for methylmercury since methylmercury is nearly completely absorbed through the gut (Gochfeld, 2003). Therefore hair is particularly valuable, as it provides an exposure timeline, rather than just the instantaneous snapshot of body burden provided by blood analysis.

One commonly employed digestion technique for the determination of methylmercury in tissue samples is a potassium hydroxide and methanol (KOH/MeOH) digestion (Bloom 1992). The KOH/MeOH digestion when used for hair is often subject to matrix interferences as noticed by low matrix spike recoveries at analysis and increased results when analyzed at a dilution. Though the samples can typically be diluted 100 fold and exhibit acceptable matrix spike recoveries, this is not a feasible approach as it also increases the method reporting limit (MRL) by a factor of 100.

When the MRL is elevated that high, many of the results are then near or below the MRL, making them estimates and therefore not a useful biomonitoring tool. In this study, an alternative digestion technique using nitric acid (HNO_3) originally developed for the digestion of insects was applied to the digestion of hair samples and then compared to the commonly used KOH/MeOH digestion procedure for the analysis of methylmercury in hair samples (Hammerschmidt, 2005).

Materials and Methods

Analysis was done following EPA Method 1630. The samples were adjusted to a pH of 4.5 - 5, and then ethylated using 0.050 mL of a 1% sodium tetraethylborate in 2% potassium hydroxide solution (Bloom, 1989). The ethylated samples were brought up to zero headspace with reagent water in a septa topped vial and placed on the autosampler for analysis. All analyses were performed using a Brooks Rand Labs MERX methylmercury autoanalyzer.

Sample Preparation & Digestion Methods

	Potassium Hydroxide in Methanol (KOH/MeOH)	Nitric Acid (HNO_3)
Sample Mass	100 mg	100 mg
Digestion Solution	25% KOH in Methanol (1 mL)	4M Nitric Acid (20 mL)
Digestion Temperature/Time	65°C for 4 hours	65°C for 24 hours
Diluent	Methanol	None
Final Volume	2.5 mL	20 mL
Analysis Volume	0.030 mL	0.500 mL
MDL/MRL	1.0/3.0 ng/g	0.5/1.5 ng/g

Figure 1: The two digestion schemes used are outlined in this table. The notable differences between the two methods are the digestion solution used and the final volume.

Results

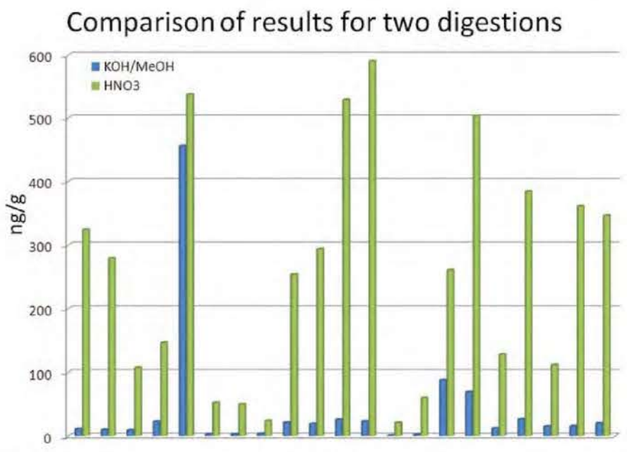

Figure 2a: Hair samples from 21 individuals were digested by both the KOH/MeOH and the HNO_3 digestion methods and were analyzed at the respective default volumes. The analysis for both methods was performed on the same instrument on the same day. The results from both methods are shown. On average, the HNO_3 results are 88% higher than results for the same sample digested with KOH/MeOH. The result from the HNO_3 digest was greater than the result from the KOH/MeOH digest for every sample analyzed.

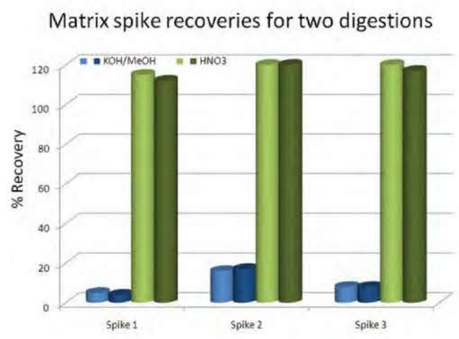

Figure 3a: Three of the samples displayed in Figure 2a were selected for matrix spikes. The matrix spike was digested in duplicate by each method and analyzed with the samples at the default volume. The recovery of the matrix spikes for the KOH/MeOH digestion were all less than 20%, whereas the recovery for the HNO_3 digestion were all greater than 100%.

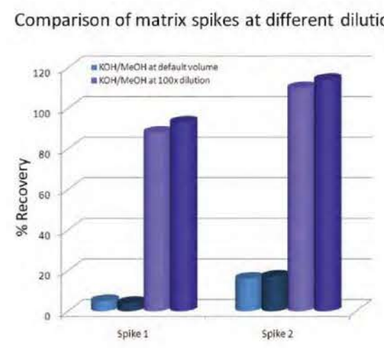

Figure 3b: A matrix spike and matrix spike duplicate were prepared by KOH/MeOH digestion. The digests were analyzed at default volume and at a 100x dilution and were analyzed on the same day. The spike recoveries change drastically from 2-3% at default volume to 88-114% when analyzed at 100x. This demonstrates the level of interference present at default volume.

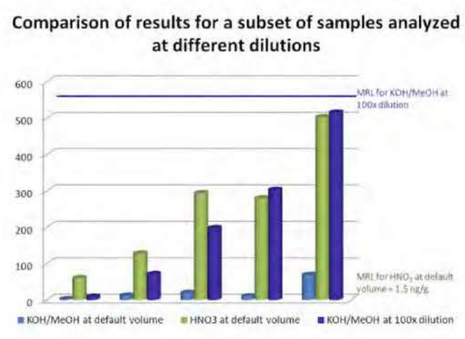

Figure 2b: A subset of the hair samples prepped with KOH/MeOH were analyzed at a 100x dilution. The sample results for the KOH/MeOH digest increased when greater dilutions of the digest were analyzed, eventually yielding results similar to the HNO_3 digestion. However, the larger dilution caused the MRL to increase to over 500 ng/g, which is greater than a majority of the hair results. The HNO_3 digestion requires no dilution so the MRL remains 1.5 ng/g.

Other Biota Sample Types

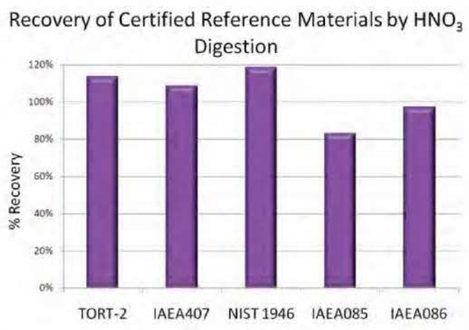

Figure 4: As the HNO_3 digestion is a relatively new technique, further tests were done to assess its accuracy as well as applicability to other tissue samples. Recovery of certified reference materials was assessed. TORT-2 (lobster hepatopancreas), IAEA407 and NIST 1946 (fish tissue), IAEA085 (spiked human hair), and IAEA086 (unspiked human hair) were digested by the HNO_3 method in quadruplicate. The average recoveries are shown. Based on the results, the HNO_3 digestion is suitable for a variety of biological matrices.

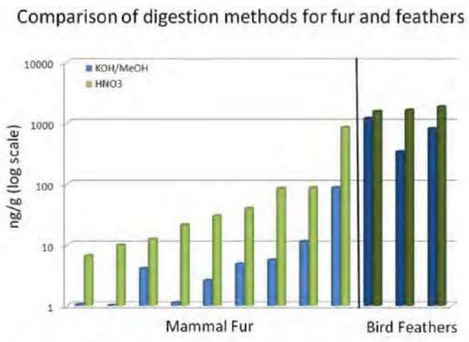

Figure 5: To further explore the potential of the HNO_3 digestion method for other biological samples that have shown matrix interferences, the KOH/MeOH and HNO_3 digestion methods were performed on mammal fur and bird feathers. The mammal fur had results similar to that of the human hair with the HNO_3 results an order of 87% greater than the KOH/MeOH results. The bird feathers displayed a similar trend but the discrepancy between the results was lower at only 53% greater.

Discussion

Two digestion methods were compared: 25% potassium hydroxide in methanol and 4M nitric acid. Both methods are based on procedures described in the peer-reviewed literature and were assessed for accuracy, reliability, and ease of preparation for hair samples. After digestion, all samples were analyzed following EPA Method 1630 (CV-GC-AFS).

The KOH/MeOH digestion method exhibited significant matrix interference, as demonstrated by the recoveries of matrix spikes. Significant dilution of the KOH/MeOH digestion did yield results that were no longer impacted by interference, but the large dilution resulted in a significant increase of the MRL to a level near or above the level of many of the samples.

The nitric acid digestion method did not exhibit a substantial level of matrix interference, and excellent recoveries were achieved for matrix spikes and reference materials even when no dilution was made.

Though the two digestion methods are equally easy to prepare, the HNO_3 method was a more accurate method for the analysis of hair, fur, and feather samples as they were all able to be analyzed at default volume with no significant matrix interferences.

Literature Cited

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