Potential Bias in Arsenic Speciation Results for Tissues Prepared by EPA Method 1632

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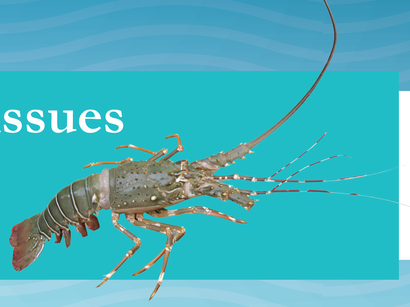


Introduction

EPA Method 1632a[1] describes two digestion methods for tissue samples analyzed by hydride generation - cyrogenic trapping - atomic absorption spectrometry (HG-CT-AAS). Both digestion methods are deemed equally acceptable in the method for detection of iAs, As(III), MMA, and DMA.

Arsenic Species
In order of decreasing toxicity
Inorganic Arsenic - iAs
Arsenite - As(III)
Arsenate - As(V)
Monomethylarsonic acid - MMA
Dimethylarsinic acid - DMA
Trimethylarsine oxide - TMAO
Arsenobetaine - AsB

However, release of certified reference materials (CRM) that have certified values for arsenic species has highlighted some drawbacks of the EPA method, specifically the NaOH preparation for tissues. It was found that digesting many sample types with sodium hydroxide (NaOH) produced a high bias to DMA results. It was further discovered that this was observed in samples that had significant concentrations of AsB, such as fin fish. Since this initial observation, more CRMs have become available or are now certified for a broader range of As species, and these more recent CRMs were used to validate this finding (Figure 1).

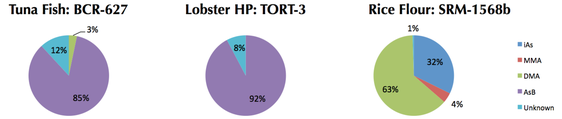


Figure 1. Composition of CRMs recently made available by different manufacturers. European Commission IRMM BCR-626 total As 4.8 mg/kg. National Research Council of Canada's TORT-3 total As 59.5 mg/kg. NIST 1568b total As 0.285 mg/kg. All three CRMs were certified for arsenic species in the last half of 2013.

This study used a combination of CRMs, blank spikes (BS), and samples of varying matrices to determine the extent of this bias and any future course of action for specific sample types. This testing confirmed that conversion of AsB during the NaOH digestion caused high bias for DMA. Furthermore, the hydrochloric acid (HCl) preparation did not cause high bias, and provides more consistent results when digesting plant materials.

Methods

Following the general procedure from EPA Method 1632a, samples, CRMs, and BSs were prepared in both 2 M HCl and 2 M NaOH (Figure 2) and analyzed by HG-CT-AAS (Figure 3). CRMs and BS were prepared and analyzed in triplicate. For all data, the average of the triplicate result is shown. The RSDs for the triplicate analyses were all less than 15%. In addition, all extracts were analyzed by HPLC-ICP-MS for confirmation of results.

Figure 2. Sample preparation procedure by EPA Method 1632a.

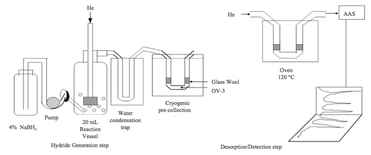


Figure 3. HG-CT-AAS analysis apparatus.

Results

Results for CRMs digested with 2 M NaOH and 2 M HCl were both analyzed by HG-CT-AAS (Figure 4). Two of the CRMs showed a similar pattern of an extremely high bias to the DMA results when the NaOH sample preparation was used. However, the result was much closer to the certified value when the HCl sample preparation was used.
Both sample preparation methods produced results closer to the certified values when analyzed by HPLC-ICP-MS (Figure 5). Levels of DMA observed for CRMs BCR-627 and TORT-3 when digested with NaOH are clearly biased high, at about 1600% and 820% recovery, respectively, when analyzed by HG-CT-AAS.

Note that the "true" value used for DMA in CRM TORT-3 is assumed to be approximately the difference between the total As and AsB certified values. Though it is not likely that the balance is all DMA, the value shown is the maximum amount of DMA that could be present. The result for the analysis of DMA by HPLC-ICP-MS when digested with NaOH was also higher than the "true" value but much closer to it (Figure 5).

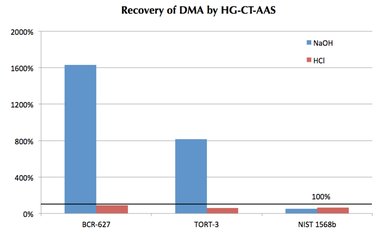


Figure 4. DMA recovery of CRMs by HG-CT-AAS for each digestion method.

The results for the Rice Flour CRM, NIST 1568b, were similar when prepared with either NaOH or HCl (Figure 5), though the NaOH digestion routinely yielded lower recoveries while the HCl sample preparation method typically produced acceptable recoveries.

Though it appeared that AsB was converting to DMA, the HPLC-ICP-MS results did not confirm that hypothesis for either digestion method. Figure 5 shows the CRM recoveries when prepared with the NaOH digestion. The recoveries for CRMs analyzed by HPLC-ICP-MS are much closer to 100% than the recoveries from the same sample preparation when analyzed by HG-CT-AAS. The recoveries for the samples digested with HCl (not shown) were even closer to 100% for all CRMs.

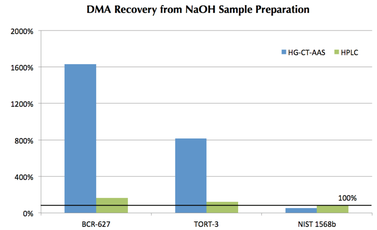


Figure 5. DMA recovery in CRMs by HG-CT-AAS and HPLC-ICP-MS, digested with NaOH.

A side-by-side comparison of AsB BS results (Figure 6) illustrates the large disparity in apparent DMA results by the two extraction methods when analyzed by HG-CT-AAS. To determine that this phenomenon was not occurring during the analytical process, AsB was also spiked directly into the sample bubbler and analyzed for DMA, yielding no detectable results (not shown). Figure 6 shows nearly quantitative recovery of AsB blank spikes as DMA when prepared with NaOH, versus no DMA detection when prepared with HCl.

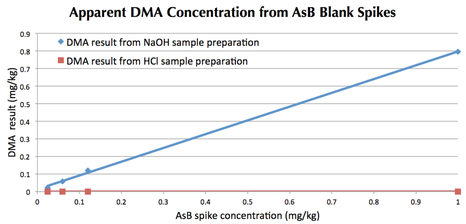
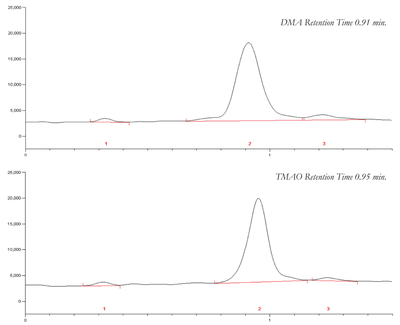


Figure 6. Apparent DMA results produced by AsB blank spikes, by digestion method, when measured by HG-CT-AAS (mg/kg)



Figures 7-8. Chromatograms of DMA and TMAO blank spikes analyzed by HG-CT-AAS.

No references of direct conversion of AsB to DMA could be found in the published literature. The best hypothesis was that an intermediate species was formed during the NaOH digestion of samples with significant concentrations of AsB, and this species was being detected as DMA during analysis by HG-CT-AAS. Trimethylarsine oxide (TMAO) is a known breakdown product of AsB[2] and when a TMAO blank spike was analyzed by HG-CT-AAS, it was detected at the same retention time as DMA (Figures 7-8), with an average recovery of 88%. This presents another important issue with EPA Method 1632a: samples that contain TMAO initially, with or without digestion, will also produce high-biased data for DMA.

Compared to HG-CT-AAS, an HPLC-ICP-MS chromatogram (Figure 9) shows multiple species in a single run, and can differentiate between them. Though TMAO and AsB may still co-elute, DMA is clearly separated from other species, demonstrating HPLC-ICP-MS to be a superior alternative method for the speciation of arsenic in certain tissue samples.

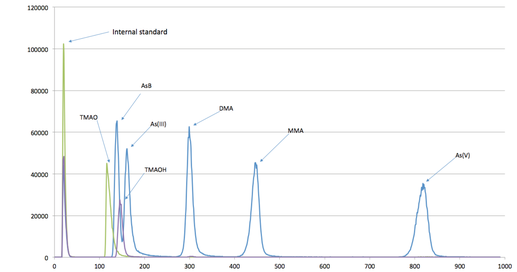


Figure 9. Typical HPLC-ICP-MS chromatogram showing multiple arsenic species.

Once the problem of AsB causing a high bias for DMA had been recognized in the results for CRMs, real world samples of various matrix types were compared (Table 1). Similar trends to CRM results were seen in this data. Outliers that were higher by the HCl preparation method are highlighted in red, with concentrations increasing by 43-74% for fish gills, organs, and algae. Increased concentration for the algae sample mirrors what was seen for the rice CRM, which had no AsB. The remaining fin fish samples all had significantly lower concentrations of DMA by the HCl digestion, just as was seen in the comparable CRMs.

Sample Matrix	NaOH Preparation (mg/kg)	HCl Preparation (mg/kg)	RPD
fish	0.272	0.097	95%
gills	0.13	0.202	43%
fish organs	0.122	0.265	74%
biota	0.639	0.151	124%
biota	1.04	0.451	79%
algae	0.381	0.613	47%
fish	1.16	0.181	146%
fish	1.03	0.332	102%
fish	4.41	3.73	17%
mussel	2.53	0.515	132%

Table 1. Comparison of DMA results for various biota samples prepared by both digestion methods.

Conclusions

- The NaOH sample preparation method specified in EPA Method 1632a should not be used to digest most biological samples for the determination of DMA.
- The HCl sample preparation method specified in EPA Method 1632a is an adequate procedure for the determination of iAs, As(III), MMA, and DMA.
- Any sample suspected of having detectable levels of TMAO should not be analyzed for DMA by HG-CT-AAS.
- Development of CRMs of various matrices and with certified values for multiple species of arsenic should continue to be a priority.

References

[1] "Chemical Speciation of Arsenic in Water and Tissue by Hydride Generation Quartz Furnace Atomic Absorption Spectrometry" EPA Method 1632 Revision A (2001).
[2] V.K. Sharma, M. Sohn. Environmental International 35 (2009) 743-759.