



Determination of Mercury in Fish by Cold Vapor Atomic Fluorescence Spectroscopy

Introduction

Mercury is a toxic element whose harmful effects are well documented and understood. The consumption of fish is the primary source of mercury absorption for the general public. Because fish can bio-accumulate mercury, unacceptably high levels of mercury appear in fish taken from pristine waters. Fish tissue sometimes can be more than 100,000 times more concentrated in mercury than their indigenous waters. Often warnings related to fish in specified bodies of water or to certain species of fish limit recommended consumption for the entire population or those at high risk such as women of child-bearing age. This technical note describes the determination of mercury in fish tissue using the *Hydra AF* Mercury Analyzer.



Experimental

Sample Preparation

A variety of canned tuna samples were purchased along with a lyophilized dogfish reference material (DORM-2) provided by the National Research Council of Canada. The digestion procedure was modified from Digestion I found in the U.S. EPA Method 1631 Appendix. Each of the tuna samples were opened and liquids drained. Samples were patted dry between paper towels. For each sample two separate aliquots of approximately 0.25 grams were transferred to 50 mL polypropylene test tubes. To each aliquot 5.0 ml of 3:1 (w/w) $\text{H}_2\text{SO}_4:\text{HNO}_3$ was added and sat at room temperature for 2 hours. Next the samples were heated to 80°C for 40 minutes. At this point all samples were liquefied. Then 15.0 ml of 6N HCL, 3.0 ml of 0.1N BrCl solution and 4.0 ml of de-ionized water were added. The BrCl solution is the same that is identified on U.S. EPA Method 245.7. After mixing, the samples were heated to 60°C for 60 minutes. The sample solution was yellow in color and clear without any precipitate. Before analysis, each sample was diluted 1:10 with 2% (w/v) HCl and 0.1ml of hydroxylamine hydrochloride was added to remove free bromine.

For calibration standards 4.0 ml of standard was added to polypropylene test tubes. All sample reagents were added to each standard cup except for the 4.0 ml of de-ionized water. Table I summarizes the digestion procedure.

Table I: Digestion Summary

Step	Reagent	Standard Volume (ml)	Sample Volume (ml)	After Addition
1	Standard	4.0	0.0	
2	3:1 H ₂ SO ₄ :HNO ₃	5.0	5.0	2 Hrs @ room then 40min @80°C
3	6 N HCl	15.0	15.0	
4	0.1N BrCl	3.0	3.0	
5	Water	0.0	4.0	60 min @60°C
6	2% HCl	1:10 dilution	1:10 dilution	
7	30% Hydroxylamine hydrochloride	0.1	0.1	

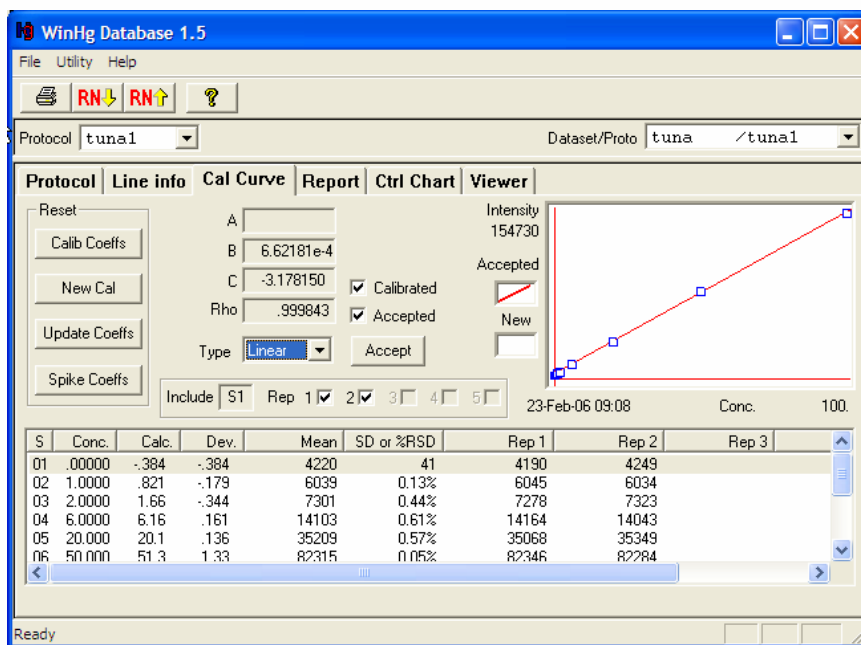
Sample Analysis

The **Hydra AF** was employed in the direct fluorescence mode for the analysis. Instrument conditions were:

Parameter	Value
Carrier gas	0.3 LPM Argon
Pump Speed	5 ml/min
Rinse time	60 secs.
Uptake time	20 secs.
Integration	45 secs.
Reductant	10% SnCl ₂ in 10% HCl (w/w)

The original concentration of the calibration standards were entered into the calibration table. Original concentrations were 0.00, 1.00, 2.00, 6.00, 20.00, 50.00, & 100.0 ppb. The actual concentration of the standards was (standard volume/final volume)/(dilution) or (4.0 ml/27ml)/10 or 0.0148 the original concentration. The calibration fit is shown in Figure I. The linearity is excellent with a correlation coefficient of 0.9998.

Figure I: Linear Calibration Fit



This calibration curve was employed to determine the concentration of mercury in digested sample solutions. To calculate the concentration in the original sample results were multiplied by 4.0 (the volume of standard added) and divided by the sample weight (approximately 0.25gm). The certified reference material was further diluted (1:2) with 2% HCl to bring it into calibration range. Results for a variety of tuna appear in Table II. Two replicates for each aliquot were measured.

Table II: Results for Canned Tuna & Dogfish Reference

Sample	Aliquot	Rep 1 (ppm)	Rep 2 (ppm)	Replicate Mean (ppm)	Sample Mean (ppm)	Recovery (%)
Carvalho Coast	A	0.101	0.101	0.101	0.200	
	B	0.297	0.302	0.300		
*Carvalho Min.	A	0.291	0.292	0.292	0.315	
	B	0.339	0.338	0.338		
BB Solid Wt.	A	0.553	0.557	0.555	0.578	
	B	0.605	0.599	0.602		
CS Solid Wt.	A	0.467	0.456	0.462	0.565	
	B	0.665	0.671	0.668		
BB Prime	A	0.317	0.319	0.318	0.320	
	B	0.321	0.321	0.321		
S&S Solid Wt.	A	0.408	0.404	0.406	0.408	
	B	0.411	0.410	0.410		
3D Solid Wt.	A	0.399	0.398	0.398	0.440	
	B	0.482	0.483	0.482		
SKT Lt.	A	0.083	0.084	0.084	0.170	
	B	0.258	0.254	0.256		
NP (Yellowfin)	A	0.138	0.139	0.138	0.137	
	B	0.136	0.137	0.136		
SKT Solid Wt.	A	0.499	0.501	0.500	0.477	
	B	0.454	0.453	0.454		
DORM-2	A	4.62	4.60	4.61	4.54	99.3
	B	4.46	4.46	4.46		96.1

*This tuna claimed to be low in mercury

Conclusions

The **Hydra AF** Mercury Analyzer provides accurate and reproducible results for the determination of mercury in fish tissue. Hydra's high sensitivity permits the determination of mercury even in fish where anticipated mercury content is low. Sample preparation using the sulfuric/nitric acid mixture followed by oxidation with bromine monochloride results in complete dissolution without the need for microwave digestion.

References

1. Appendix to Method 1631, Total Mercury in Tissue, Sludge, Sediment, and Soil by Acid Digestion and BrCl Oxidation, EPA-821-R-01-013, January 2001
2. Method 245.7, Mercury in Water by Cold Vapor Atomic Fluorescence Spectrometry, EPA-821-R-01-008, January 2001