BY DAVID PFEIL

Mercury Determination in Fish by Cold Vapor Atomic Fluorescence Spectroscopy

Mercury is a toxic element with harmful effects that 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 a fully automated cold vapor atomic fluorescence analyzer.

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 USEPA Method 1631 Appendix. Each of the tuna samples was opened and liquids drained. Samples were patted dry between paper towels. For each sample two separate aliquots of approximately 0.25 gram were transferred to 50mL polypropylene test tubes. To each aliquot we added 5.0mL of 3:1 (w/w) H_2SO_4:HNO_3 and let stand at room temperature for 2 hours. Next the samples were heated to 80°C for 40 minutes. At this point all samples are liquefied. We added 15.0mLs of 6N HCL, 3.0mLs of 0.1N BrCl solution and 4.0mLs of de-ionized water. The BrCl solution is the same that is identified on USEPA Method 245.7. After mixing, the samples were heated to 60°C for 60 minutes. Samples were yellow in color and clear solutions without any precipitate. Before analysis, each sample was diluted 1:10 with 2 percent (w/v) HCl and 0.1mL of hydroxylamine hydrochloride was added to remove free bromine.

For calibration standards, 4.0mLs of standard was added to polypropylene test tubes. All sample reagents were added to each standard cup, except for the 4.0mLs of de-ionized water. Table 1 summarizes the digestion procedure.

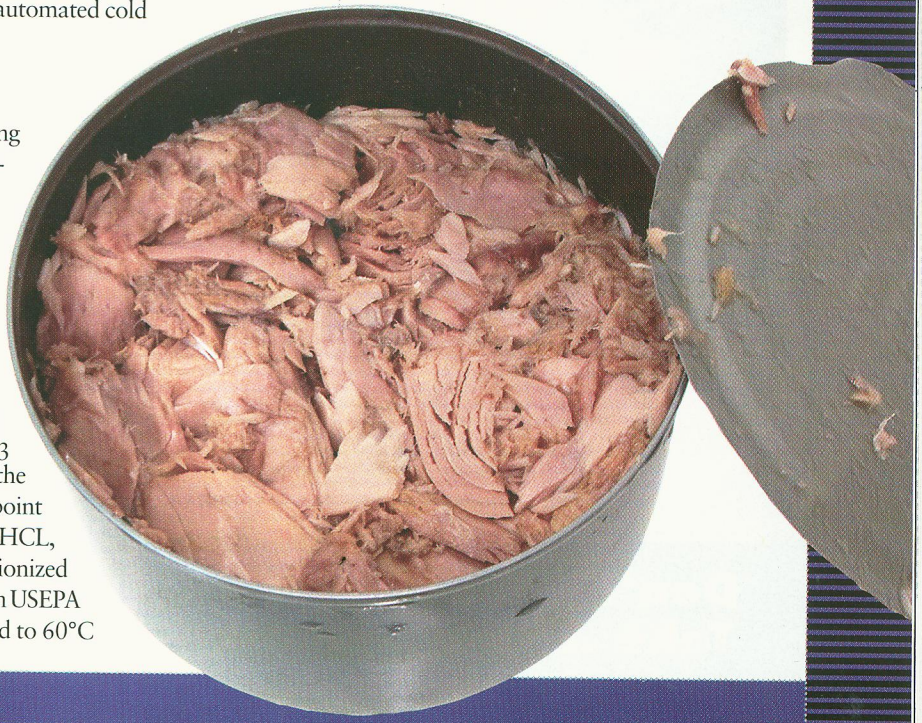

Table 1: **DIGESTION SUMMARY**

Step	Reagent	Standard Volume (mL)	Sample Volume (mL)	After Addition
1	Standard	4.0	0.0	
2	3:1 H_2SO_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	60min @ 60°C
6	2% HCl	1:10 dilution	1:10 dilution	
7	30% Hydroxylamine hydrochloride	0.1	0.1	

Analysis

Another analyzer that uses a dual detector approach for routine work in the sub-parts-per-thousand (ppt) to high parts-per-billion (ppb) range was also employed. It has been designed for compliance with EPA methods 1631 and 245.7 and with European Standards EN-13506 and EN-12338. This analyzer provides a prescreen mode to protect the high sensitivity gold amalgamators from contamination. It was also employed in the direct fluorescence mode for the analysis. Instrument conditions were:

Carrier gas: 0.3 LPM Argon
Pump Speed: 5 mL/min
Rinse time: 60 secs.
Uptake time: 20 secs.
Integration: 45 secs.
Reductant: 10 percent $SnCl_2$ in 10 percent HCl (w/w)

The original concentration of the calibration standards was entered into the calibration table. Original concentrations were 0.00, 1.00, 2.00, 6.00, 20.00, 50.00, and 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 1. The linearity is excellent with a correlation coefficient of 0.9998.

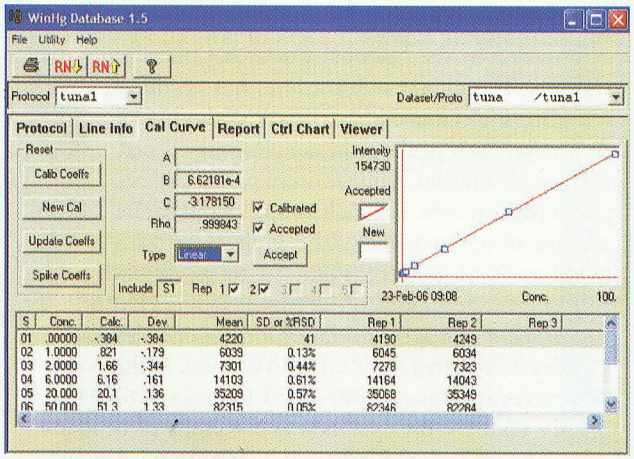

Fig. 1: Linear Calibration Fit

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 percent HCl to bring it into calibration range. Results for a variety of tuna appear in Table 2. Two replicates for each aliquot were measured.

Conclusions

The first analyzer provides accurate and reproducible results for the determination of mercury in fish tissue. Its high sensitivity

Table 2:
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

will permit 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:

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

Method 245.7, Mercury in Water by Cold Vapor Atomic Fluorescence Spectrometry, EPA-821-R-01-008, January 2001

David Pfeil is the ICP and Hg product manager for Teledyne Leeman Labs, a Hudson, N.H.-based developer of atomic spectroscopy. Reach him at 1-800-634-9942.