Analysis of Shellfish Tissue for Cadmium, Mercury and Nickel

Application Note

Atomic Absorption

Author

Jonathan H. Moffett

Introduction

Huge amounts of toxic effluents are being dumped either directly or indirectly into the world's oceans. The ocean's organisms concentrate these toxic chemicals with unfortunate effects on humans. Since the tragedy at Minamata Bay, Japan, in the 1950s, the levels of toxic metals in seafood have been carefully monitored [1]. This is especially important around coastal industrial and mining areas as well as river mouths.

Shellfish such as oysters and mussels are bottom-dwelling non-mobile filter feeders. These factors mean that shellfish are very good environmental indicators and can locate sources of pollution.

The screening of fish must be rapid and accurate. A method for digesting fish for mercury determinations by cold vapor has been described [1]. As mercury is very volatile, the digestion method should be applicable to other toxic heavy metals.

A freeze dried sample of shellfish tissue was supplied and a method was required for digestion and analysis.

Agilent Technologies

Experimental

Reagents

All reagents were AR grade. Water was deionized distilled (DD) water (18 M-ohm grade).

Standards

Standards were diluted from 1000 mg/L commercial atomic absorption solutions in deionized distilled (DD) water with dilute nitric acid as stabilizer. Working concentrations used are shown in Table 1.

Table 1. Working Concentration of Standards

Cd	5.00 µg/L in 0.08 M HNO_3
Ni	50.0 µg/L in 0.08 M HNO_3
Hg	0.50 µg/L in 0.3 M HNO_3/0.04% (m/v) $K_2Cr_2O_7$

Modifier

Ammonium dihydrogen orthophosphate ($NH_4H_2PO_4$) was used as a chemical modifier for cadmium [2]. $NH_4H_2PO_4$ (1 g) was dissolved in DD water (100 mL). The solution was found to have an appreciable cadmium signal. Trace metals were removed by using an Agilent Bond Elut SCX (strong cation exchange) column (kit B). Nitric acid (30 mL of 2 M) was drawn through the column using a water vacuum pump. The column was then washed using DD water (30 mL). The column was not allowed to dry out. The modifier solution was allowed to elute through under gravity with the first 2 mL being discarded. The eluant was caught directly in a washed sample vial and placed in the modifier position of the PSD-96 carousel. The overlayed cadmium signals before and after elution can be seen in Figure 1, in which the eluted signal is at baseline level. This method of purification has not yet been fully optimized.

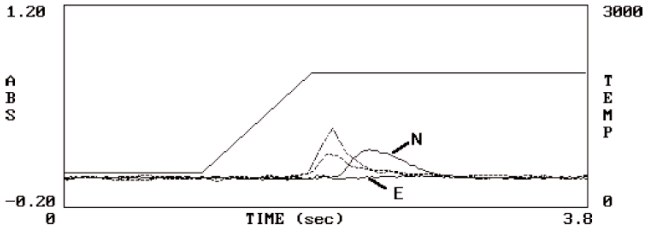

Figure 1. Noneluted (N) and eluted (E) modifier atomic (—) and background (- -) signals.

Reference Sample

A National Institute of Science and Technology (NIST) standard reference material, bovine liver (1577), was used as a reference sample.

Sample Preparation

Two digestion procedures were considered. The first digestion method was shown to be suitable for mercury in fish tissue [1]. In this method a sample (1g) was digested in concentrated nitric acid (10 mL). This method was eventually used for this study.

The second method was used to determine As, Sb and Se in various environmental matrices [3]. This method is a modified version of the first method by including hydrogen peroxide.

NIST 1577

Concentrated nitric acid (10 mL) was added to a known mass of liver (1 g) in a test tube [1]. After heating for three hours at 125 °C, the warm yellow solution was quantitatively transferred to a 50-mL volumetric flask. When made up to the mark with DD water, fat solidified as a precipitate. This did not appear to affect results.

Shellfish Sample

One fish sample was cut into halves using stainless steel scissors. Each half was digested separately. The digestion quantities above were scaled down by a factor of 10 because each half weighed about

0.1 g. Heating times were not changed. The solutions were transferred to 5-mL volumetric flasks. These were subsequently diluted as needed.

All masses and final volumes of the samples are shown in Table 2.

Table 2. Mass and Final Volume of Digested Samples

		Final Volume (mL)	
Sample	**Mass (g)**	**(Cd, Ni)**	**(Hg)**
NIST 1577	1.0026	50	
Shellfish 1	0.1373	100	125
Shellfish 2	0.1026	100	125

Instrumentation

For the determination of nickel and cadmium, an Agilent SpectrAA-300GZ atomic absorption spectrometer with Zeeman background correction, and fitted with a PSD-96 sampler, was used. Atomization for cadmium was from a pyrolytic forked platform. Atomization for nickel was from the wall of a pyrolytic coated partition tube. Argon was the inert gas.

For the determination of mercury, an Agilent SpectrAA-20ABQ atomic absorption spectrometer fitted with a VGA-76 vapor generation accessory and an MCA-90 mercury concentration accessory were used [4].

Lamps were standard Agilent hollow cathode lamps for each of the elements.

Recovery studies for nickel and cadmium were performed using Quality Control Protocol (QCP V2.0). Signal graphics were captured using Signal Graphics Library (SGL V1.01).

Instrument parameters are given in Tables 3a, 3b and 3c.

Results and Discussion

Cadmium

The aqueous calibration graph for cadmium is shown in Figure 2.

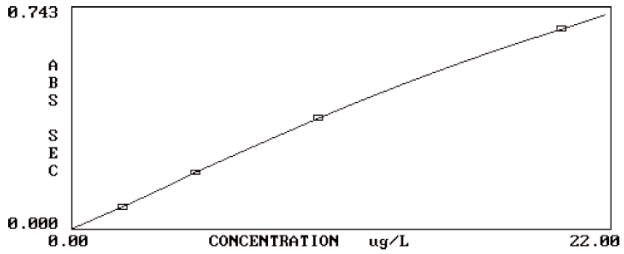

Figure 2. Cadmium calibration graph.

Replicate atomization signal peaks of NIST 1577, shellfish 1 and shellfish 2 for cadmium are shown in Figures 3, 4 and 5 respectively. The peak shapes all compare well. They are well-formed and the peak background signal is well within the capability of the instrument's corrector.

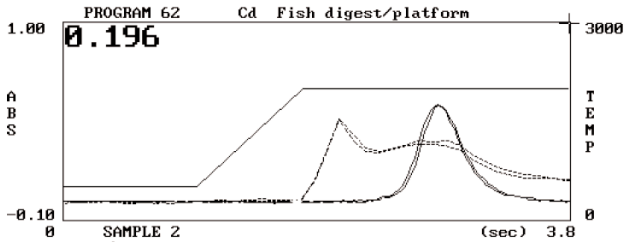

Figure 3. Cadmium signal - NIST 1577.

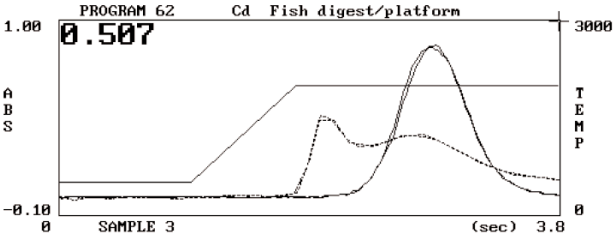

Figure 4. Cadmium signal - shellfish 1.

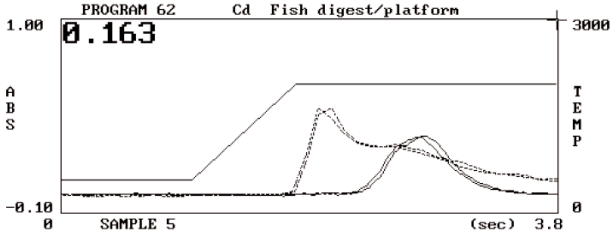

Figure 5. Cadmium signal - shellfish 2.

Table 3a. Instrument Parameters for the Determination of Cadmium

Program 62: Cd fish digest/platform

Instrument mode	Absorbance
Calibration mode	Concentration
Measurement mode	Peak area
Lamp position	3
Lamp current (mA)	4
Slit width (nm)	0.5
Slit height	Normal
Wavelength (nm)	228.8
Sample introduction	Sampler automixing
Time constant	0.05
Measurement time (s)	1.0
Replicates	2
Background correction	On
Maximum absorbance	0.70

Furnace parameters

Step no.	Temperature (C)	Time (s)	Gas flow (L/min)	Gas type	Read command
1	350	20.0	3.0	Normal	No
2	500	15.0	3.0	Normal	No
3	500	10.0	3.0	Normal	No
4	500	1.0	3.0	Normal	No
5	2000	0.8	0.0	Normal	Yes
6	2000	2.0	0.0	Normal	Yes
7	2500	2.0	3.0	Normal	No

Sampler parameters

Volumes (µL)

	Solution	Blank	Modifier
Blank	–	20	5
Standard 1	2	18	5
Standard 2	5	15	5
Standard 3	10	10	5
Standard 4	20	5	
Sample 5	15	5	

		Recalibration rate	0		
		Reslope rate	0		
Multiple inject	No	Hot inject	Yes	Pre inject	No
		Temperature	150		
		Inject rate	5		

Table 3b. Instrument Parameters for the Determination of Nickel

Program 66: Ni fish digest/wall

Instrument mode	Absorbance
Calibration mode	Concentration
Measurement mode	Peak area
Lamp position	2
Lamp current (mA)	6
Slit width (nm)	0.2
Slit height	Normal
Wavelength (nm)	232.0
Sample introduction	Sampler automixing
Time constant	0.05
Measurement time (s)	1.0
Replicates	2
Background correction	On
Maximum absorbance	1.00

Furnace parameters

Step no.	Temperature (C)	Time (s)	Gas flow (L/min)	Gas type	Read command
1	150	20.0	3.0	Normal	No
2	700	15.0	3.0	Normal	No
3	700	10.0	3.0	Normal	No
4	700	1.0	3.0	Normal	No
5	2400	0.9	0.0	Normal	Yes
6	2400	2.0	0.0	Normal	Yes
7	2500	2.0	3.0	Normal	No

Sampler parameters

Volumes (µL)

	Solution	Blank	Modifier
Blank	–	20	
Standard 1	2	18	
Standard 2	5	15	
Standard 3	10	10	
Standard 4	20		
Sample	10	10	

		Recalibration rate	0		
		Reslope rate	0		
Multiple inject	No	Hot inject	Yes	Pre inject	No
		Temperature	120		
		Inject rate	5		

Table 3c. Instrument Parameters for the Determination of Mercury

Program 5: Hg	
Instrument mode	Absorbance
Calibration mode	Concentration
Measurement mode	Peak height
Lamp position	3
Lamp current (mA)	4
Slit width (nm)	0.5
Wavelength (nm)	253.7
Sample introduction	Manual
Delay time	0
Time constant	0.05
Measurement time (s)	40.0
Replicates	1
Background correction	Off
Air flow	0.00
MCA-90 parameters	
Drain (s)	20
Collect (s)	180
Heat (s)	20

The result of the NIST 1577 analysis for cadmium tends to support the validity of the digestion and calibration procedures. Table 4a shows the result is very close to the certified value.

The wide difference between shellfish 1 and shellfish 2 for cadmium cannot be explained from this study alone. The discrepancy is caused either by contamination or by inhomogeneous distribution of cadmium in the original sample, with contamination being the most likely cause.

Nickel

The aqueous calibration graph for nickel is shown in Figure 6.

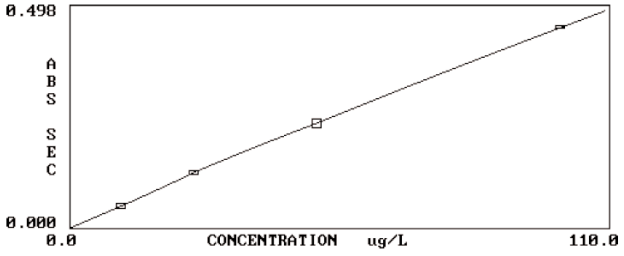

Figure 6. Nickel calibration graph.

Replicate atomization signal peaks of each of the samples for nickel are shown in Figures 7, 8 and 9. As for cadmium, peaks are well-formed, while background is negligible for nickel. NIST 1577 sample has a larger background peak because it was diluted less than the shellfish samples.

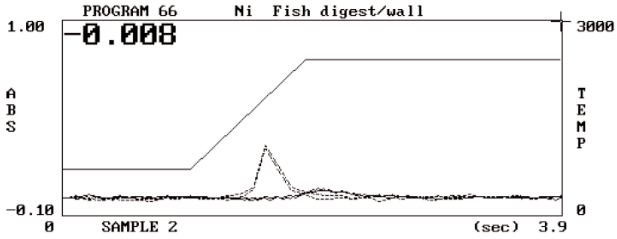

Figure 7. Nickel signal - NIST 1577.

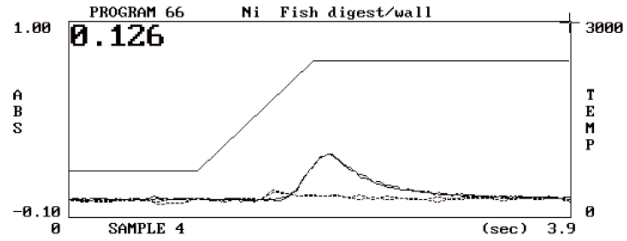

Figure 8. Nickel signal - shellfish 1.

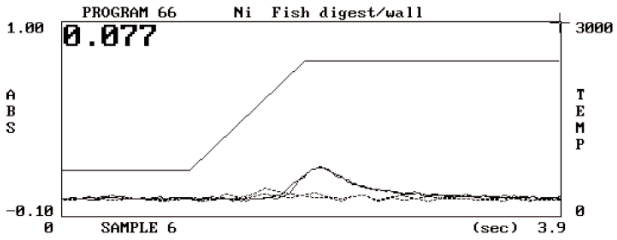

Figure 9. Nickel signal - shellfish 2.

The close result between shellfish 1 and shellfish 2 for nickel indicates homogeneous distribution in the original sample. NIST 1577 does not have a certified level of nickel.

Recovery Studies

QCP software allows a QC spike recovery by spiking the final solution with standard solution during injection into the furnace. The recovery (%R) is calculated as below:

$$\%R = (SSR-SR)/SA \times 100$$

where

SSR = Spiked sample result

SR = Sample result

SA = Spike added

If recovery is between 85% to 115% the aqueous calibration graph is normally considered to be a close match for the sample solution. A recovery of less than 40% usually indicates either a severe matrix effect or else measurement close to or greater than the maximum absorbance of the element using a Zeeman effect instrument. The recovery results for the samples are given in Table 4a, 4b and 4c.

Table 4a. Summary of Analytical Results for Cadmium in Shellfish

Sample	Soln conc (µg/L)	Conc found (µg/L)	Certif value (µg/L)	RSD (%)	recov (%)
NIST 1577	5.17	0.26	0.27 ± 0.04	0.4	94.5
NIST 1577	5.16	0.26		1.5	86.2
shellfish 1	14.08*	10.25*		0.3	28.3
shellfish 1	13.85*	10.09*		1.2	35.8
shellfish 2	4.34	4.23		3.3	85.5
shellfish 2	4.32	4.21		1.8	83.9

* is a software label to indicate the measured absorbance exceeded the maximum recommended Zeeman absorbance.

Table 4b. Summary of Analytical Results for Nickel in Shellfish

Sample	Soln conc (µg/L)	Conc found (µg/L)	Certif value (µg/L)	RSD (%)	recov (%)
NIST 1577	0.5	0.0	not certified	99.9	83.9
NIST 1577	-1.7	-0.1		47.2	102.5
shellfish 1	25.9	18.9		0.3	99.6
shellfish 1	25.2	18.4		1.0	95.1
shellfish 2	15.8	15.4		2.3	91.2
shellfish 2	15.8	15.4		6.7	79.3

Table 4c. Summary of Analytical Results for Mercury in Shellfish

Sample	Soln conc (µg/L)	Conc found (µg/L)	Certified value (µg/L)
NIST 1577	0.332	0.0166	0.016 ± 0.002
shellfish 1	0.344	0.313	
shellfish 2	0.372	0.453	

The recoveries for cadmium are good except for both aliquots of shellfish 1. The instrument software has flagged the results in Table 4a with an asterisk. This indicates that the maximum absorbance (0.7) for cadmium has been exceeded. The unexpectedly high result for shellfish 1 strongly suggested contamination of this sample and further studies (including dilution or reduced sample volumes) were not performed.

The results for shellfish 2 are below the maximum absorbance limit and the recoveries are very good.

The recoveries are also good for nickel except for one of shellfish 2 results. However, a portion of the same solution which immediately preceded this one had an excellent recovery.

A complete recovery study should also include a matrix spike which is the addition of analyte to a sample before digestion. The limited amount of sample available did not permit such study.

Mercury

The low levels of mercury in solution required the use of the MCA-90 [4,5]. This measurement consumes about 20 mg of sample. The amount of sample did not allow spike recovery studies. The close result of NIST 1577 with the certified value and the similar results for shellfish 1 and 2 suggests that the measured results are accurate.

Conclusion

The close agreement of the NIST 1577 results with the certified results indicates that the digestion procedure used was entirely satisfactory for the volatile elements mercury and cadmium. It seems reasonable that the same would be true for the shellfish. The final volumes in Table 2 should be adjusted to measure the expected levels in individual samples. They were suitable for the levels of the metals in NIST 1577 and shellfish 2.

The generally good recovery study results for the shellfish indicate the results are accurate. Certainly the calibration procedure is valid. Confirming the accuracy of any sample analysis requires a considerable amount of work. Procedures should include: long-term trend studies, inter-laboratory studies and using (if available) a certified reference fish sample.

References

1. S. J. Evans, M. S. Johnson, and, R. T. Leah. Varian Instruments At Work AA-60, May **1986**.
2. L. M. Beach. Varian Instruments At Work AA-90, June **1989**.
3. L. M. Beach. Varian Instruments At Work AA-105, February **1992**.
4. J. H. Moffett. Varian Instruments At Work AA-104, December **1991**.
5. L. M. Beach. Varian Instruments At Work AA-108, October **1992**.

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