Determination of Mercury in Fish Tissue, a Rapid, Automated Technique for Routine Analysis.

Application Note

Atomic Absorption

Authors

S. J. Evans,

M. S. Johnson,

R. T. Leah

School of Biology

University of Liverpool

UK

Introduction

Mercury is recognized as an environmental contaminant and its organic compounds, especially methylmercury, are known to be capable of damaging the central nervous system. Since the tragedy of Minamata Bay in Japan [1] most concern has centred on the presence of mercury in fish since seafood is a major source of this element. Consequently, the last 25 years have seen many analytical surveys of lakes and sea-fishing grounds.

Liverpool University has recently undertaken a survey of eighteen sites in the Irish Sea of the concentration of mercury in seven species of fish, representing different trophic levels in the marine food chain. This survey has required the dissection and analysis of some 8,000 fish over a period of 15 months, so there has been a need to develop rapid techniques, requiring minimum technician time and suited to the routine analysis of large numbers of fish muscle samples. Care has also been taken to minimize costs. A procedure is outlined below for the batch analysis of mercury in 250 fish samples. Some of the interferences and problems encountered during the development of the method are discussed.

Cold vapor determination of mercury using atomic absorption spectroscopy is a very sensitive and specific technique. Conventional flame AAS suffers from poor sensitivity, with a concentration of 5 mg l^{-1} in solution producing an absorbance of only 0.0044. This is not sensitive enough for the analysis of biological samples. In 1968, Hatch and Ott [2] described a method whereby mercury ions, in an acidified digest, could be reduced to neutral mercury atoms by stannous chloride, these atoms being stripped from solution by a flow of air. The mercury vapor is then presented to an absorption cell in the light path of an AA instrument. This cold vapor technique is now an accepted analytical method for the determination of trace levels of mercury.

Helsby [3] has published a review of the development and applications of this technique.

Agilent Technologies

The Agilent Model 76 Vapor Generation Accessory

The instrumentation in use consists of an Agilent Techtron AA-1275 Atomic Absorption Spectrophotometer, an Agilent PSC Model 55 Programmable Sample Changer, and an Agilent Model 76 Vapor Generation Accessory. The AA-1275 and PSC-55 are linked with an Apple IIe Microcomputer for full automatic operation, data capture and processing.

The VGA-76 is a continuous flow vapor generator and can be coupled to an automatic sampler [4]. It is also designed to use solutions containing high concentrations of acid. A peristaltic pump pushes the reducing agent and the sample through a mixing coil to a gas/liquid separator. Here, mercury vapor (or hydrides in determinations of arsenic, selenium and antimony) are stripped from solution by a stream of inert gas (argon or nitrogen) and swept into a flow cell positioned in the AAS burner compartment. This system produces a continuous analytical signal, not a transient peak as with earlier vapor generation systems. Absorbances are measured in the integration mode instead of the peak area or peak height mode.

Since the intention was to use a simple nitric acid digestion procedure (see Digestion Procedures) the early commissioning of the VGA-76 involved the use of mercury standards prepared in different concentrations of nitric acid. The first parameter investigated was the delay time. This is the time in seconds which elapses between the probe of the autosampler reaching the fully down position and the absorbance reading becoming stable. For a 0.02 µg/mL mercury standard in 40% v/v nitric acid the delay time required is 55 seconds (Figure 1). The same concentration of mercury in 20%, 30% and 50% v/v nitric acid requires the same delay time, but higher concentrations of mercury take a few seconds longer to reach a steady signal. The delay time now used routinely is 70 seconds.

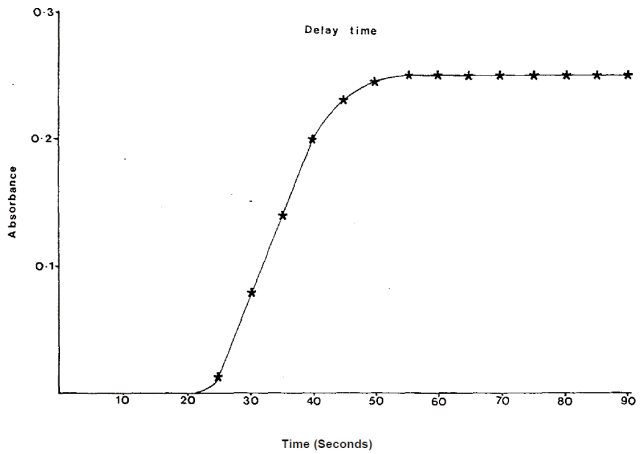

Figure 1.

The VGA-76 Operation Manual recommends the use of Digestion Procedures sodium borohydride as the reducing agent in the determination of mercury, the solution containing 0.3% NaBH4 in 0.5% NaOH. It has been found in the present study that while sodium borohydride provides a slightly more sensitive response than stannous chloride (25% SnCl2 w/v in 20% v/v HCl) for nitric acid standard solutions, a severe interference exists in the analysis of fish sample digests.

Figure 2 shows the response to mercury standards, prepared in 40% v/v nitric acid, obtained with sodium borohydride and stannous chloride as alternative reductants. We conclude that stannous chloride should be used routinely because of the interference observed with $NaBH_4$.

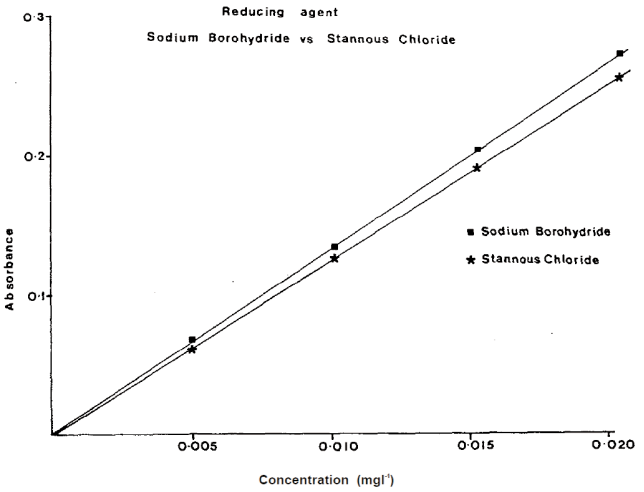

Figure 2.

Many previous methods were time-consuming partly because HNO_3 levels were reduced by evaporation prior to analysis. We wished to reduce the time taken as well as the opportunity for contamination by eliminating this step so the analytical response to mercury standards prepared in different nitric acid concentrations was investigated. Figure 3 shows calibration curves for mercury standards prepared in 30%, 40% and 50% v/v nitric acid. As can be seen there is virtually no difference in the calibration curves. Absorbances were much lower at very high concentrations of HNO_3 so 40% v/v acid was chosen as the target final concentration for routine analysis.

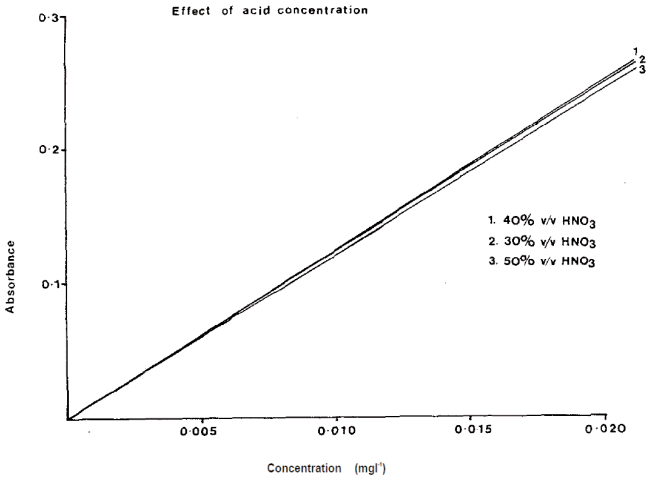

Figure 3.

With the parameters listed below it is possible to analyze 134 samples per day. Routine relative standard deviations are less than 0.5%.

Typical Parameters for Routine Analysis of Mercury in Fish Tissues (Using Agilent Model VGA-76, PSC-55, and AA-1275)

Reducing agent	25% w/v SnCl2 in 20% v/v HCl
Typical flow rate	1 mL per minute
Distilled water	Typical flow rate 1 mL per minute
Sample	Typical flow rate 6.5 mL per minute
Inert gas	Nitrogen
Standards	0.010, 0.020, 0.050, 0.075 and 0.090 µg/mL prepared in 40% v/v nitric acid
Typical absorbances	0.125, 0.250, 0.620, 0.840, 0.980
Delay time	70 seconds
Rinse time	30 seconds
Integrate hold	4 seconds with 4 replicate readings
Wavelength	253.7 nm
Abs. exp. factor	x 1

One significant problem encountered when determining mercury has been the fluctuation in the temperature of the laboratory. An increase in laboratory temperature leads to an increase in sensitivity of the method. To combat this problem an automatic reslope calibration is performed after every seven sample analyses.

Digestion Procedures

There are many published methods for the digestion of fish tissue. Most involve the use of three reagents, with one method requiring the use of no less than six reagents. Velghe et al. [5] describe a method whereby a small piece of fish tissue is mixed with potassium permanganate and sulphuric acid, and then digested on a hot-plate. After digestion, the excess permanganate is reduced with hydroxylamine hydrochloride. The process suggested by Munns and Holland [6] involves a double digestion of 5 g of sample. The first digestion uses a combination of sulphuric acid, nitric acid and sodium molybdate. The second step uses a 1:1 nitric acid/perchloric acid mixture. Sullivan and Delfino [7] used a sulphuric acid/ nitric acid mixture to digest the fish tissue at room temperature for 24 hours. Hydrogen peroxide is then added and the mixture heated to 265 °C. After cooling, potassium permanganate is added to maintain an oxidizing medium. This, eventually, being reduced with hydroxylamine solution.

As stated earlier, the survey of the Irish Sea required analysis of 8,000 fish samples over a period of 15 months, so it was highly desirable to use a relatively simple digestion procedure involving one reagent, and the simplest possible glassware. Extensive analysis of biological samples at Liverpool University indicated that simple digestion by nitric acid was adequate, without the prolonged refluxing and volume reduction practised by other workers using just nitric acid, (Ministry of Agriculture, Food and Fisheries – MAFF internal method). This method, described in Table 1 and now in routine use, has been tested by spiking samples with mercury and by inter-laboratory cross-calibration studies. A long sequence of quality control, reference tissue analyses was used to confirm its reliability (see Table 4).

Recoveries of mercury from spiked fish samples were 92–102%. The inter-laboratory calibration study, with the Ministry of Agriculture, Food and Fisheries laboratory at Burnham on Crouch, Essex, centred on 21 samples from three marine and one freshwater fish species. The 21 samples contained varying amounts of fat or oil and muscle mercury concentrations in the range 0.13–1.27 mg kg^{-1} fresh weight. The results showed recoveries in the range of 95–104% of those obtained by MAFF, the mean recovery being 96.2%. These recoveries are consistent with the standards of interlaboratory calibration for mercury analysis – Munns and Holland [6], and are acceptable when compared with other studies – Uthe et al. [8]. The lowest recoveries were associated with fish containing more oil/fat than average.

As stated earlier, mercury standards prepared in 40% v/v nitric acid provide excellent results in terms of sensitivity and reproducibility when sodium borohydride is used as the reducing agent. However, trials with digests of fish tissue give totally unacceptable recoveries of the order of less than 5–30%. Moreover, analysis of the same digests on different days showed remarkable variations with time. This problem does not exist when stannous chloride is used as the reductant. Table 2 shows that sodium borohydride appears to release mercury quantitatively in the absence of fish tissue (for example from acid-matched standards). Table 3 shows the percentage recoveries obtained by sodium borohydride and stannous chloride in the analysis of a lyophilized dogfish muscle tissue. The recoveries given by sodium borohydride appear to be negatively correlated with the quantity of fish flour digested. Such an effect was not apparent when stannous chloride was used. For further reading on the use of stannous chloride for mercury determination by the US EPA approved methodology [9].

Table 1. Digestion Procedure for Fish Muscle Samples as Developed at the University of Liverpool

1. Weigh a 24 × 150 mm Pyrex, numbered, digestion tube.
2. Place 1–5 g of homogenised fish muscle into the tube (amount used depends on anticipated mercury concentration).
3. Add 10 mL of concentrated nitric acid. Allow to stand overnight.
4. Transfer the digestion tube to an aluminium block placed on a hot-plate. Adequate temperature control was achieved by using a standard aluminium topped laboratory hotplate, shielded from the fume-cupboard updraft by aluminium foil. Adequate refluxing, with negligible loss of mercury, occurs because the top of the test tube acts as an air-cooled condenser. Seventy-two tubes can be heated on each hotplate. Raise the temperature to 125 °C over a period of three hours. Allow to gently reflux for a further four hours, then cool.
5. Weigh the tube plus digest.
6. Add 15 mL of distilled water. Ensure mixing by use of a rotary vibrator (Vortex).
7. Transfer the sample to an 18 × 150 mm autosampler tube. The PSC-55 carousel can hold 67 samples for analysis.

The final concentration of mercury is calculated from the three weight values using an average value for the specific gravity of the remaining digest. Some of the residual error results from the variability in this final specific gravity because of variation in loss of moisture and decomposition of the acid. The saving in time by using weight instead of volume is large at the expense of minimal additional error.

Table 2. Typical Absorbance Values for Stannous Chloride and Sodium Borohydride Standards Prepared in 40% v/v Nitric Acid

Hg conc (mg L^{-1})	0.005	0.010	0.020
$SnCl_2$	0.062	0.125	0.250
$NaBH_4$	0.067	0.133	0.265

*Table 3. The Effects of Reducing Agents on the Recovery of Mercury From Fish Flour Containing 1.62 mg kg^{-1} Hg Dry Weight**

Sample weight dig	$SnCl_2$ mg kg^{-1} Hg	% recovery	$NaBH_4$ mg kg^{-1} Hg	% recovery
0.1 g	1.58	97.5	0.370	22.8
0.2 g	1.62	100.0	0.037	2.3
0.5 g	1.58	97.5	0.003	<1.0

* Standard tissue provided by MAFF, Burnham on Crouch.

Table 4. Analytical Quality in the Quantitative Recovery of Mercury From Standardized Fish Muscle Homogenate

Material	Freeze-dried fish muscle homogenate supplied by the International Atomic Energy Authority in Monaco, (Reference Material MA-A–2 TM).		
Reported Hg value	0.47 ± 0.02 mg kg-1 dry weight (mean ± s. error)		
Liverpool	Conditions – 57 separate assays of the University homogenate at 4–7 day intervals over a results: period of 10 months.		
	Mean recovery (mg kg^{-1})		
	Value	**S. error**	**(95%) Confidence interval (± 2σ)**
	0.462	0.004	0.454–0.470
	Mean recovery (%)		
	Value	**S. error**	**(95%) Confidence interval (± 2σ)**
	98.4	0.75	96.9–99.9

Conclusions

The VGA 76 Vapor Generation Accessory, in conjunction with a PSC-55 Autosampler, provides a rapid and sensitive system for the automated determination of mercury in fish tissue. It is possible to gain a satisfactory level of precision and accuracy using nitric acid digestion and simple glassware. The inter-laboratory exercises and the analysis of commercially available fish flours suggest that mercury is quantitatively recovered by our digestion procedure.

Acknowledgements

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