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Determination of total mercury in biological samples using flow injection CVAAS following tissue solubilization in formic acid

Masahiko Kan [a], Scott N. Willie [b,*], Christine Scriver [b], Ralph E. Sturgeon [b]

[a] *Environmental Information Measurement Sciences, Hokkaido University of Education Sapporo, Sapporo 002-8502, Japan*
[b] *Institute for National Measurement Standards, National Research Council Canada, Ottawa, Ont., Canada K1A 0R6*

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Abstract

Total mercury in biological samples was determined by flow injection (FI) cold vapour atomic absorption spectrometry (CVAAS) following tissue solubilization with formic acid. A mixture of potassium bromide and potassium bromate was used to decompose organomercury compounds prior to their reduction with sodium borohydride. A gold amalgam system was used to achieve lower detection limits when required. National Research Council Canada certified reference materials dogfish liver (DOLT-3), dogfish flesh (DORM-2) and lobster hepatopancreas (TORT-2), as well as oyster tissue (NIST SRM 1566b) and mussel tissue (NIST SRM 2976) were used to assess the accuracy of the method. The method of standard additions provided the most accurate results. Limit of detection (LOD) for Hg in the solid sample of 0.001 and 0.01 $\mu g\,g^{-1}$ were achieved with and without amalgamation, respectively. The precision of measurement for 1.6 $ng\,ml^{-1}$ methylmercury was 2.7% using the amalgam system.

Keywords: Mercury; Biological samples; Formic acid solubilization; Flow injection CVAAS

1. Introduction

Mercury is a toxic species that is the subject of much concern; consequently, significant effort has been made to develop methods for its determination in environmental and biological samples. Cold vapor atomic absorption spectrometry (CVAAS) is a sensitive and conventional instrumental analytical method used extensively for this purpose [1]. Inorganic mercury ions (Hg^{2+}) are reduced to metallic mercury (Hg^0) in the reduction system, with subsequent cold vapor detection by atomic absorption. When incorporated into a flow injection (FI) system, the procedure can be automated with rapid sample throughput, however, sample preparation can remain the rate-limiting step in the overall analysis. Some progress has been made to avoid lengthy sample preparation steps through the availability of mercury analyzers that permit direct heating of a solid sample, although acid digestion at elevated temperature is more frequently used to destroy the sample matrix and decompose organomercury compounds prior to analysis.

A rapid and simple sample preparation method has been reported using tetramethylammonium hydroxide (TMAH) to solubilize biological tissues [2]. This procedure has been used to determine total mercury using FI–CVAAS with on-line decomposition of organomercury using potassium permanganate. TMAH has also been applied to the speciation of Hg in tissues: the determination of inorganic mercury using CVAAS [3]; inorganic and methylmercury using microwave-assisted extraction in combination with hydride generation–cryogenic trapping–gas chromatography–electrothermal atomic absorption spectrometric detection (HG–CT–GC–ETAAS) [4]; methylmercury using furnace atomization plasma emission (FAPES) [5]; and gas chromatography with atomic fluorescence detection [6]. Of note in the later study, species interconversion of methylmercury into dimethylmercury was found to occur during sample extraction. It should also be noted that biolog-

* Corresponding author. Fax: +1 613 993 2451.
E-mail address: scott.willie@nrc-cnrc.gc.ca (S.N. Willie).

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ical solutions solubilized with TMAH produce an unpleasant odor that requires adequate ventilation [7].

Recently, formic acid has been reported as an effective reagent to solubilize solid biological samples for the determination of trace metals by ETAAS [8]. The sample preparation procedure is not as simple as the TMAH procedure reported by Tao et al. [2], however, it is less time consuming and labor intensive than conventional acid digestion, as the solubilization can be performed in the same plastic bottle the sample is to be stored in.

In this study, a simple method using formic acid to solubilize biological tissue samples is described for the determination of total mercury by FI–CVAAS. For samples with a low concentration of mercury, a gold amalgam system was used to lower the detection limit. In many of these samples, methylmercury is the dominant mercury species. Bromine chloride has been successfully used to decompose organomercury compounds [9] and has been applied to the determination of total Hg in water by atomic fluorescence spectrometry [10,11]. This approach was used here.

2. Experimental

2.1. Apparatus

A Perkin-Elmer (Norwalk, CT, USA) Model FIMS flow injection mercury system, a Perkin-Elmer AS-90 autosampler, and a Perkin-Elmer amalgam system were used in this study. The 253.7 nm wavelength was used for detection.

The FIMS tubing was configured as recommended by the manufacturer. A sample loop was connected to the 5-port valve that permitted the loop contents to be switched in or out of the carrier stream as required. The reductant and carrier were mixed downstream from the valve using a three-channel manifold. Pump 1 was used to fill the sample loop and pump 2 controlled the carrier, reductant and waste flows. Tygon peristaltic pump tubing was used: sample, 1.52 mm i.d. (8 ml min^{-1} at 100 rpm); carrier and reductant, 1.14 mm i.d. (5 ml min^{-1} at 120 rpm); waste 3.18 mm i.d. (30 ml min^{-1} at 120 rpm).

2.2. Reagents

All chemicals were of analytical reagent grade unless specified otherwise. High purity water (18 MΩ cm) was obtained from a Nanopure deionization system (Barnstead–Thermolyne, Dubuque, IA, USA). Sodium borohydride (0.1 M) solution was prepared daily (Alfa Chemicals Inc., Newburyport, MA, USA) in 5 mM sodium hydroxide. Hydrochloric acid (0.25 M) was used as carrier solution. Bromide–bromate reagent (1.2 M potassium bromide–0.2 M potassium bromate) was prepared daily by mixing equal volumes of 2.4 M potassium bromide aqueous solution and 0.4 M potassium bromate aqueous solution. An inorganic mercury standard stock solution was prepared by dissolution of mercury chloride (Gold Star, Alfa Chemicals, Ward Hill, MA, USA) in 5 M nitric acid. Working standards were prepared by serial dilution with high purity water and hydrochloric acid. A methylmercury standard stock solution was prepared by dissolving methylmercury chloride (Alpha Division, Danvers, MA, USA) in propan-2-ol. Working standards were prepared by serial dilution with high purity methanol. Formic acid (Anachemia, Montreal, QC, Canada) and an antifoaming reagent, antifoam "B" (BDH, Toronto, Ont., Canada), were used as received.

The certified reference materials from the National Research Council Canada (NRC), dogfish liver (DOLT-3), dogfish flesh (DORM-2) and lobster hepatopancreas (TORT-2) and NIST (Gaithersburg, MD) oyster tissue (SRMs 1566b) and mussel tissue (SRMs 2976) were used to assess the accuracy of the method.

Table 1
FIMS conditions and program for amalgam system

FIMS conditions	
Sample volume	1000 μl
Carrier	0.25 M HCl
Reductant	0.1 M $NaBH_4$–5 mM NaOH
Purging gas	300 ml min^{-1} argon
Carrier gas	75 ml min^{-1} argon

FIMS program

Step no.	Time (s)	Pump 1 (rpm)	Pump 2 (rpm)	Valve position	Read	Heat
Prefill	40	100	120	Fill		
Step 1	25	100	120	Fill		
Step 2	60	Off	120	Inject		
Step 3	10	Off	40	Fill		
Step 4	10	Off	40	Fill	On	On
Step 5	20	Off	40	Fill		

2.3. Mercury measurement

The FIMS conditions and program are shown in Tables 1 and 2, with and without the amalgam system, respectively. After the sample loop was filled with sample in step 1, the injection valve was switched to introduce the sample into the carrier stream (0.25 M hydrochloric acid) where

Table 2
FIMS conditions and program without amalgamation

FIMS conditions	
Sample volume	200 μl
Carrier	0.25 M HCl
Reductant	0.1 M $NaBH_4$–5 mM NaOH
Purging gas	100 ml min^{-1} Argon

FIMS program

Step no.	Time (s)	Pump 1 (rpm)	Pump 2 (rpm)	Valve position	Read
Prefill	10	100	120	Fill	
Step 1	20	100	120	Fill	
Step 2	30	100	120	Inject	On

it was subsequently merged with sodium borohydride in the manifold. The mercury vapor was separated in the gas–liquid separator (Perkin-Elmer Model B019-3772) and transferred by the argon carrier into the quartz cell for detection or to the amalgam system for collection. Both the peak height and the peak area were measured. Inorganic or organic mercury standard solutions could be used for calibration.

In step 2 of the amalgam system program (Table 2), the Hg vapor was directed to the gold/platinum gauze where it was subsequently desorbed when halogen lamps were used (step 4) to heat the trap. The mercury vapor was transported to the detection cell in a 75 ml min^{-1} flow of argon carrier gas.

2.4. Use of antifoam

Three drops of the antifoam "B" was found to be sufficient to eliminate foaming of the sample mixture as it moved through the FI system. Excessive foaming reduces the effectiveness of the gas–liquid separator as well as creates uncertainty in filling the sample loop.

2.5. Sample preparation

Solutions of solubilized biological tissue were obtained by weighing nominal 1 g sub-samples into 50 ml pre-cleaned screw-capped poly(propylene) bottles and adding 40 ml of formic acid. The bottles were placed in an ultrasonic bath for a few hours at 50 °C as described by Scriver et al. [8]. These solutions have been shown to be stable for at least several months [8]. For analysis, working solutions were obtained by transferring a 2 g aliquot of these samples to an autosampler vial where three drops of the Antiform "B" and 6 ml of bromide–bromate reagent were added, water was added if dilution was required. Following addition of the bromide–bromate reagent, the solution would turn cloudy within 10 min. This did not cause measurement problems with the determination of Hg if the solutions were analyzed within one hour. Settling of the precipitate would occur and the absorbance reading would decrease if these working solutions were left undisturbed on the autosampler tray for >60 min and then sampled into the FIAS.

For low concentrations of Hg requiring measurement using the amalgam system, 3 ml of solubilized sample and 9 ml of bromide–bromate reagent were used to dilute the sample. Conditions were optimized using standards of methylmercury, containing formic acid, antifoam "B" and bromide–bromate reagent.

2.6. Results and discussion

2.6.1. Optimization of conditions

2.6.1.1. Effect of bromide–bromate reagent. The utilization of chlorine gas has been reported for the decomposition of organomercury in water [13], and halogens have long been known to sever carbon–mercury bonds [14]. Potassium bromide–potassium bromate has found acceptance as an effective reagent for destruction of organomercury compounds and this approach has become incorporated into an EPA method [12]. The effect of the ratio of bromide–bromate reagent to formic acid was studied. Without the addition of bromide–bromate reagent, a signal only slightly above the blank result was obtained for 4.5 ng ml^{-1} methylmercury in formic acid. Between 2 and 6 ml of bromide–bromate reagent per milliliter of formic acid was found to provide an optimum signal. Ratios greater than 6:1 of bromine–bromate:formic acid were not investigated. In order to ensure sufficient bromine–bromate, a 3:1 ratio of bromide–bromate reagent to the volume of the solubilized sample was used for subsequent measurements.

The concentration of the stock solution of the bromide–bromate reagent was chosen such that the solubilities were not exceeded and precipitation of the mixture would not occur on storage.

2.7. Volume of formic acid

The bromide–bromate reagent requires an acidic medium for efficient destruction of the organomercury compounds [7–9]. Fig. 1 shows the effect of varying the volume of formic acid on the response from a methylmercury standard. A final volume of 20 ml was maintained while a constant 3:1 ratio of bromide–bromate:formic acid were varied from 1.5 to 15 ml and 0.5 to 5 ml, respectively. Constant results for methylmercury were found over this range. Sufficient acidity from the formic acid, as well as the bromide–bromate solution, appears to be present in these solutions for effective decomposition.

2.8. Figures of merit

2.8.1. Without amalgamation

The limit of detection (LOD) was calculated to be 2 ng (peak area) and 0.6 ng (peak height), based on the three times the standard deviation of the response from the reagent blank ($n = 10$). These values correspond to 0.04 and 0.01 μg g^{-1} of total mercury in the solid sample, for the measurement

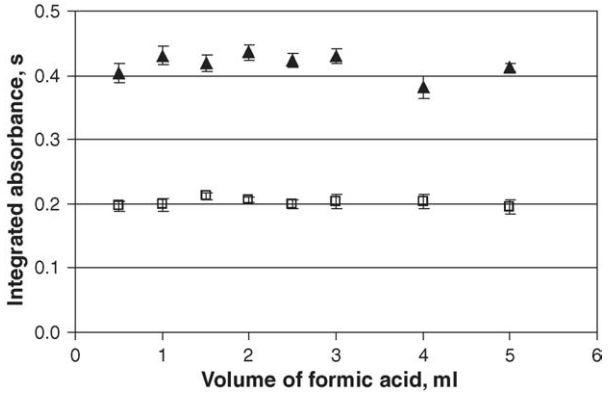

Fig. 1. Effect of the volume of formic acid on the integrated absorbance: (▲) 4.5 ng ml^{-1}; (□) 2.3 ng ml^{-1} methylmercury.

Table 3
Analytical results for certified reference materials ($\mu g\,g^{-1}$)

Sample	Certified ($\mu g\,g^{-1}$)	This work[a] ($\mu g\,g^{-1}$)	
		Peak area	Peak height
DOLT-3	3.37 ± 0.14	3.12 ± 0.15	3.03 ± 0.24
DORM-2	4.64 ± 0.26	4.44 ± 0.67	3.68 ± 0.75
TORT-2	0.27 ± 0.06	0.34 ± 0.03	0.27 ± 0.03
NIST SRM 1566b	0.0371 ± 0.0013	0.048 ± 0.010[b]	0.047 ± 0.009[b]
NIST SRM 2976	0.0610 ± 0.0036	0.064 ± 0.004[b]	0.070 ± 0.007[b]

[a] Mean value ± standard deviation ($n = 3$ or 4) obtained by the method of standard additions.
[b] Values obtained with the amalgamation system.

of the peak area and peak height, respectively. The relative standard deviation of absorbance for 4.5 ng ml^{-1} methylmercury spiked into a reagent blank solution was 1.7% for the peak area and 1.3% for the peak height ($n = 6$). The precision of replicate measurement for the solubilized biological samples was determined to be 4.8% ($n = 4$, peak area) as the relative standard deviation using a solution of DOLT-3 at a concentration 80-fold above the detection limit.

2.9. With amalgamation

The volume of the sample loop was increased from 0.2 to 1 ml for use with the amalgamation system. Three milliliters of sample were combined with 9 ml of bromide–bromate reagent for measurement. A limit of detection of 0.5 ng (peak area) and 0.1 ng (peak height) were obtained ($n = 8$); corresponding to 0.005 $\mu g\,g^{-1}$ (peak area), and 0.001 $\mu g\,g^{-1}$ (peak height) of total mercury in the solid samples. The relative standard deviation of absorbance for 1.6 ng ml^{-1} methylmercury ($n = 7$) was 2.7% for the peak area and 3.4% for the peak height. The precision of replicate measurement (peak area) for the solubilized biological samples was determined to be 13% ($n = 4$) as the relative standard deviation using a solution of NIST CRM 2976 at a concentration about 10-fold above the detection limit.

3. Analytical results

The accuracy of the method was evaluated by analyzing a suite of certified reference materials. The certified reference materials from the NRC, DOLT-3, DORM-2 and TORT-2 were used, as well as reference materials from NIST, SRM 1566b and SRM 2976. The results are summarized in Table 3. The determined values for total mercury overlap with the uncertainties in the certified values, although the mean of the peak area measurements were generally closer to the certified mean than the peak height measurements. These results were obtained using calibration by the method of the standard additions. An external calibration curve method could not be utilized because the slopes of the standard addition curves for the references materials and the slope of the aqueous calibration curve were slightly different, for example, the slope for a standard addition to DOLT-3 was 20 % less than that for the calibration curve.

4. Conclusions

Considerable research is directed towards improving analytical instrumentation, however, simplifying the sample preparation procedure can be equally, if not more valuable, by reducing the cost and complexity of the analysis. Total mercury in biological samples was successfully determined with FI–CVAAS following solubilization with formic acid. A speciation scheme to separately determine organic and inorganic mercury in these samples was not attempted, as the use of sodium borohydride precludes this. Bromide–bromate was required to quantitatively destroy the organomercury fraction. The solutions did not have the pungent odor associated with the TMAH solubilized solutions [7], however, TMAH solutions can be quantitated against an aqueous calibration curve [3]. This method does not require extensive sample preparation and could be used to rapidly survey samples against a calibration curve if the highest accuracy is not required. However, it has been shown that accurate results can be achieved using the method of standard additions for calibration.

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