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Closed-Ampule Digestion Procedure for the Determination of Mercury in Soil and Tissue Using Cold Vapor Atomic Fluorescence Spectrometry

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Sensitive, reliable and precise methods of sample preparation are needed for the determination of mercury in environmental (soils and sediments) and biological (fish, blood and other tissue) samples, especially in aquatic systems. The majority of mercury entering natural waters is incorporated into sediments and soils (Floyd and Sommers 1971) and taken up by fish through the food chain. Analysis of total-Hg in environmental and biological samples requires two processes (i) the conversion of organic forms of Hg to Hg^{2+} (inorganic mercury) in solution by various digestion/oxidation procedures and (ii) the reduction of solution Hg^{2+} to its gaseous form (Hg^{o}) for analysis. Various digestion procedures have been described (Swift and Campbell 1993; Bloom 1989, 1992; Sadiq et al. 1991; Van Delft and Vos 1988; Bloom and Crecelius 1983, 1987; Sullivan and Delfino 1982; Floyd and Sommers 1975; Armstrong and Uthe 1971) for the determination of total-Hg.

Previous open-vessel digestion/oxidation techniques use a variety of acids (HCl, HNO_3, and H_2SO_4) for digestion followed by potassium permanganate/persulphate oxidants and analysis by cold vapor atomic absorption spectrometry (CVAAS), (Szakács et al. 1980; Van Delft and Vos 1988). Open-vessel digestion results in considerable loss of organic mercury compounds, particularly for samples with high organic mercury content by volatilization of organomercury compounds at elevated temperatures or by incomplete oxidation of the sample matrix at ambient temperature (Van Delft and Vos 1988). Strong oxidants such as $KMnO_4$ K_2SO_4 and $K_2Cr_2O_7$ used by some investigators (Melton et al. 1971; Floyd and Sommers 1975; Szakács et al. 1980; lannuzzi and Wenning 1995) appear to incompletely oxidize high organic matter soils, contaminate samples and exhibit high blank contamination levels giving poor precision. Also efforts to lower the detection limits using the permanganate/persulfate oxidation method have for the most part been unsuccessful (Swift and Campbell 1993). These earlier methods are also complex and use large amounts of glassware. Swift and Campbell (1993) digested samples in a closed teflon vessel designed for microwave use with a nitric/hydrochloric acid mixture and a microwave heating-assisted acid dissolution technique. The method detection limit (MDL) obtained from this procedure was, however, much higher than the MDL obtained from the closed-ampule procedure proposed in this paper.

A digestion/oxidation procedure is needed that is straightforward, clean, uses less

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laboratory equipment and chemicals and allows for a large number of samples to be prepared simultaneously. The closed-ampule digestion technique described in this paper involves preparation of samples with only one acid (nitric acid) and water in 10-ml pre-scored gold band ampules (Wheaton, Milliville, NJ), which are sealed and placed in an autoclave in a water bath for 1 hr at 105°C. This sealed ampule digestion/oxidation technique prevents loss of organic mercury compounds by volatilization as all reactions occur in a closed system. The closed vessel also allows the heat and pressure to break down the carbon-mercury bond in one easy step. Once samples are prepared in the closed vessel procedure, they are stable indefinitely for all Hg losses. The preparation of samples in ampules are quite effective and allows for large number of samples to be prepared simultaneously and efficiently. The samples are analyzed by a CVAFS mercury detection system. Mercury recovery in sediments and tissue samples using this method are shown to be higher than those using other acid mixtures.

MATERIALS AND METHODS

Prescored 10-mL glass gold band ampules (Wheaton) were used in the preparation of samples. Trace-metal grade concentrated HCI and HNO_3 used were of certified ACS grade and obtained comme.icially from Fisher Scientific. Quality control standards for soil/sediment (NIST sediment, 60 ng/g, 8406) and fish/tissue (NBS 1566a Oyster Tissue, 64 ng/g)) were obtained from the National Institute of Standards and Technology (Gaithersburg, MD). All deionized water used was produced from a Barnstead B-pure system located in a Hg-clean room. Standard reference curves were prepared by analyzing 0, 100, 250 and 400 ng/L standards. Working standards used in total Hg analysis were prepared daily from a stock solution (100 ng/mL) which is also made on a daily basis. The stock solution was obtained from a commercially available mercury standard 1000 µg/mL, SPEX Industries, Edison, NJ).

Surface soil and sediment samples high in organic matter (e.g., peat, marls and marly peat) were collected using a stainless steel spade, trowel or Ekman dredge. The samples were stored in wide-mouth polyethylene specimen cups (125 mL, Fisher Scientific). Subsurface soil and sediment samples were collected in polycarbonate core tubes.

Soils rich in organic material were made into a slurry by blending (using an Osterizer) 125 cc of soil with 30 50 mL distilled water for 3 min to homogenize the sample. Five (5) mL of this slurry was diluted with 45 mL of 0.6N HCI in a clean specimen cup and left for 1 hr to neutralize any carbonates. Another 5 mL of the slurry was weighed then dried overnight in a drying oven. The weight of the dried soil is obtained and used to obtain the concentration of mercury in the soil normalized to the dry weight. From the diluted mixture, 1.0 mL was placed in a 10 mL glass ampule with 2 mL of concentrated nitric acid (Jones et al. 1995). The sample was then left under a fume hood for 20 min, flame sealed, placed in a water bath and autoclaved for 1 hr at 105°C. Dry sediment samples were first sieved using a stainless steel 80.µm sieve and the < 80-µm size sediment retained for analysis. Approximately 0.4 g of sediment is weighed into a 10 mL glass ampule with 2 mL HNO_3 and 1 mL H_2O added to the ampule and processed as above.

Mosquito fish (Gambusia sp.) samples were collected using a dip net, placed in zip-lock sample bags, labelled and stored in a cooler with ice (while in the field). In the laboratory, sediment, soil and fish samples are stored in a freezer at -20°C until ready for analysis (Southeast Environmental Research Program (SERP) Internal Standard Operating Procedure 1994).

Whole fish that weighed less than 0.5 g were digested intact, while larger fish were prepared by taking tissue plugs (using a stainless steel biopsy needle, 4 mm in diameter) from the left dorsal muscle. Three tissue plugs (with a combined weight of approximately 0.4 g) were taken from different parts of the muscle to obtain a representative sample. It is important to use less than 0.4 g of fish tissue and dry sediment since larger amounts could cause the ampules to explode in the autoclave. The samples were digested in 10 mL glass ampules with 2 mL HNO_3 and 1 mL H_2O. The open ampules were then left under a fume hood for 20 min then sealed and autoclaved for 1 hr at 105°C.

Digestion of NIST sediment standard and NBS oyster tissue standard samples in sealed ampules with various acids were compared to determine accuracy and precision (%RSD) of the methodology. The sediment standard and tissue standard were subjected to the following digestion procedures and analyzed to determine the best total-Hg recovery. The samples were prepared by weighing out approximately 0.1 g of the sediment standard and 0.01 g of the tissue standard in I0-mL glass ampules, respectively, and digested by adding each of the following, (1) 3 mL HNO_3 (2) 3 mL HCI (3) 2 mL HNO_3 and 1 nmL HCI and (4) 2 mL HNO_3 and 1 mL H_2O. The ampules were then sealed, autoclaved and prepared for analysis. Another method using 3mL H_2SO_4 was used to digest the soil and tissue standards, however, in both cases, after being autoclaved the digestate liquid was black from organic carbonation. As a result this method was rejected.

The liquid digestates were diluted (usually 1:20) with 0.12N HCI solution in 20-mL polyethylene vials and then seven replicates were automatically analyzed (see below). Mercury concentrations were obtained by referring to standard reference curves.

All Hg analyses were done using a Merlin Plus, PS Analytical (Kent, UK) cold vapor atomic fluorescence spectrometer (AFS) system. The system consists of an autosampler, vapor generator, mercury fluorescence detector and a PC-based integrator package. In the cold vapor technique the digested sample reacts with acidic stannous chloride to convert Hg (II) present in the samples to Hg°. The Hg° vapor is then removed from solution in a gas liquid separator by argon and the mercury detected by atomic fluorescence. The mercury vapor is channeled through a chimney past a light source and photomultiplier tube which are at right angles to each other (PS Analytical 1992). As the \'apor absorbs the mercury light it fluoresces at the same wavelength (usually 254 nm). The AFS consists of a specific high intensity mercury lamp (developed by Cathodeon Ltd., Nuffield Road, Cambridge), with a fixed 254 nm filter and masking system. This design allows for the efficient isolation of the required excitation and emission wavelengths for mercury and helps minimize of interference effects (PS Analytical 1992). The optimized operating conditions of the AFS System vary depending on whether ultratrace levels or high levels of mercury are to be measured (Jones at al. 1995).

RESULTS AND DISCUSSION

Several methods with the closed-ampule digestion were compared and the results shown in Tables 1 and 2. Four replicate samples (prepared according to the internal SOP using both sediment and oyster tissue standards) were analyzed in duplicate for each method. Table 1 shows the expected value for total-Hg in the soil/sediment NIST standard (based on the recommended value of 60 ng Hg/g), the calculated concentrations obtained, recovery and precision (%RSD) for each method. Table 2 shows the expected value for total-Hg in the NBS Oyster Tissue standard and the concentrations obtained from the four methods described above. For both sediment

Table 1. Analyses of NIST Sediment Standard using various methods. Sediment standard value 60 ng Hg/g

Methods	Mean† ng Hg/g	Recovery (%R)	Precision (% RSD)
3 mL HNO_3	56.6	92-100	3.7
3 mL HCl	59.3	96-101	2.1
2 mL HNO_3 + 1 mL HCl	33.4	51-60	7.1
2 mL HNO_3 + 1 mL H_2O	56.9	95-105	2.6
‡Peat soil	155.4	95-105	1.1
‡River sediment	542.8	95-105	1.1
‡Volcanic soil	883.9	95-105	3.1

† mean of four replicate samples analyzed in duplicate
% RSD = percent relative standard deviation
‡ Sediments and soils analyzed using 2 mL HNO_3 + 1 mL H_2O

Table 2. Analyses of NBS Oyster Tissue using various methods. Oyster standard value 64.2 ± 6.7 ng Hg/g

Methods	Mean† ng Hg/g	Recovery (%R)	Precision % RSD
3 mL HNO_3	70.7	99 - 120	8.4
3 mL HCl	69.7	96 - 110	21.5
2 mL HNO_3 + 1 mL HCl	68.6	46 - 150	41.6
2 mL HNO_3 + 1 mL H_2O	72.31	95 - 113	3.6
‡Fish Tissue	526.69	95 - 110	0.6

† mean of 4 samples analyzed in duplicate
% RSD = percent relative standard deviation
‡ Fish tissue analyzed using 2 mL HNO_3 + 1 mL H_2O

and oyster analyses, good recoveries (92% - 113%) were obtained for all the methods except for method 3 (2 rnL HNO_3 and 1 mL HCl). Large %RSD were found for tissue using method 2 (3 mL HCl) and method 3 (HNO_3/HCl combination). It can be seen that method 4, the 2 mL HNO_3 and 1 mL H_2O combination is the desirable method for both sediment and tissue analyses.

The use of nitric acid alone in this procedure makes it possible to be cleaned up for background Hg by sub-boiling point distillation. $KMnO_4$ and $K_2S_2O_8$ cannot be cleaned up by distillation or baking due to their chemical instabilities. If one uses the EPA 245.6 method, there has to be a mechanism for large blank corrections due to $KMnO_4$ ($K_2S_2O_8$

is relatively clean). In the closed-ampule method this correction will be much lower.

The real sample data (Tables 1 and 2) shows the results of the mosquito fish, Gambusia sp. peat soils, river sediments and volcanic soils using the closed ampule digestion method. The percent recovery (obtained from the ratio of the calculated concentration over the expected concentration) was consistent over time (usually within a 95 - 105% range) and the precision for each sample was much greater than observed with the standards.

A comprehensive quality assurance plan with a standard operating procedure (SOP) has been developed for use with this method. Using the SOP a method detection limit (MDL) was calculated to characterize the abilities and limitation of the proposed method. A method detection limit (MDL) of 4.3 ng Hg/g for soil/sediment and 3.2 ng Hg/g for fish samples was achieved using US EPA methods. Precision and accuracy were determined by analyzing a fish and soil sample seven times. The standard deviation of the seven concentrations is multiplied by the students' *t* value for a 99% confidence level and a standard deviation estimate with *n*-1 degrees of freedom is *t* = *3.14* for seven replicates (US EPA 1993). The fish sample had a mean of 54.2 ng Hg/g with a RSD of 3.5%. The EPA 245.6 method for determining mercury in fish tissue is a reliable and successful method, however, this is a two-step procedure in which the tissue digestion step is performed in a water bath at 58-60°C (low temperature), followed by a room temperature oxidation in the presence of $KMnO_4/K_2S_2O_8$, overnight. If this same procedure were to be adapted for acids alone, there would be incomplete digestion due to the survival of the carbon-mercury bond. The closed-ampule procedure allows the heat and pressure to break down the carbon-mercury bond in one easy step. A soil sample rich in organic matter (85% total organic matter) showed a mean of 83.32 ng Hg/g with a RSD 1.5%.

Closed ampule digestion of environmental and biological samples described in this paper has been used successfully in the determination of total-Hg. The closed system allows for complete digestion/oxidation of environmental and biological samples by concentrated nitric acid and water. The preparation of samples requires only a small amount of material compared to previous methods where 30 - 300 g of material was needed. The process described is simple, straightforward and uses less apparatus. The preparation of samples in closed I0-mL glass ampules efficiently. Recoveries of 95% to 110% are consistently obtained when analyzing environmental and biological samples. The specialized mercury detector with the AFS system has provided detection limits at extremely low levels for soil, sediment, fish and tissue samples.

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