# Considerations for a reduced-level mercury laboratory and determination of ultratrace level total mercury in water by atomic fluorescence detection: Part 1

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THE BIOACCUMULATION of mercury in aquatic/riparian food webs has been the impetus for unraveling the biogeochemical cycles of mercury and developing more sensitive methods of detection. In aquatic ecosystems in which piscivorous fish have readily detectable levels of accumulated mercury in their tissue, the surrounding water will typically test less than the detection limits of current cold vapor atomic absorption analytical methods. The typical detection limit for mercury in water using permanganate oxidation/cold vapor atomic absorption (U.S. EPA Method 245.2) is presently 200 ng/L (ppt), while the lowest ambient water quality criterion for mercury is 12 ng/L.[1] Evaluation of mercury bioaccumulation in food webs in relation to the ambient environment is a considerable challenge involving the need to develop more sensitive analytical techniques for locating the sources of this potentially toxic metal in the environment.

Several laboratories have developed fluorescence detection methods that lower the detection limits for total mercury concentration to the low and sub-part per trillion range (ultratrace levels or UTL). The U.S. EPA's Region IV Environmental Services Div. (Athens, GA) has prepared a special reduced-level mercury environment laboratory and is modifying existing UTL methods for organic mercury speciation and total mercury. The objective is to generate methods that yield high-quality results and large sample throughput for water, tissue, and sediments.

This paper describes the physical requirements and considerations for the laboratory and discusses a modification of the digestion and detection procedure described in the Yorkshire Water Authority Method[2] for total mercury in water. The method presented here is the basis for U.S. EPA Method 245.7 being published in more detail by the U.S. EPA Environmental Monitoring Systems Laboratory (Cincinnati, OH).*

*Laboratory considerations*

Because of its high vapor pressure, mercury is generally ubiquitous at low levels in almost any laboratory environment. This is especially true of older laboratories in which mercury thermometers have been broken and reagents or samples containing mercury have been spilled. In order to achieve valid ultratrace level analysis (UTLA), the potential for contamination of samples by the surrounding laboratory environment must be minimized. Mercury levels in the ambient laboratory air must be reduced such that samples undergoing analytical preparation will not become contaminated during the limited exposure to air.

In the authors' facility, an existing laboratory configured with a connecting decontamination room was modified to create a reduced-level mercury environment. The laboratory was previously used for reagent storage and sample processing for metal analysis. The decontamination room was converted into a support area for reagent preparation and labware cleaning. The support area also serves as a convenient intermediate clean area before entrance into the laboratory where sample digestion and analysis are performed (UTLA laboratory). A recirculating air-cleaning system, with activated carbon filters to remove mercury vapor from the air, was installed in the UTLA laboratory. A cooling and heating system using activated carbon-filtered air was installed to isolate the laboratory from the building air system. The intake air port on the hood was also fitted with activated carbon filters. Ideally, the laboratory should maintain positive pressure; however, this could not be achieved with the current laboratory design.

The benches, furniture, and labware were all new. Furnishing ultratrace level laboratories with surplus equipment from other laboratories is not recommended unless the equipment can be decontaminated. Tacky mats were placed in the support area and UTLA laboratory entrances to prevent tracking in particles containing mercury. Personnel access to the laboratory is limited in order to minimize the amount of outside air and particles entering the laboratory.

A rigorous cleaning routine is followed in the laboratory and constant attention is given to potential sources of mercury to avoid contamination in UTL analysis. New labware is cleaned and tested for mercury contamination prior to use. Storage of boxed products in the UTLA laboratory is avoided because paper products (such as laboratory wipes and cardboard boxes) have been found to be sources of contamination. Other equipment used in analysis (such as pipet tips and laboratory wrapping film) is also tested for mercury contamination prior to use. No stock mercury standards are stored in the UTLA laboratory and no standards with concentrations greater than 10 ppm are allowed in the support area. Labware that must be stored is sealed in plastic. The neck and caps of reagents are sealed with Parafilm® (American National Can, Greenwich, CT) to prevent mercury deposition and contamination.

The UTLA laboratory air is monitored weekly for mercury levels using two pairs of traps, made of an acid/bromate/bromide mixture, that are exposed to the laboratory atmosphere for one week. This exposure time was chosen because shorter intervals do not trap enough mercury to be consistently detectable. One pair of traps is exposed on the bench near the analytical equipment, and the other pair is exposed in the hood in which samples are prepared for analysis. Mercury levels are typically 10 to 20 ppt (by weight in the trap solution) for the bench traps, while levels in the hood traps are approximately 5 ppt higher. In the 12 months from September 1993 to September 1994, the lowest mercury levels were 4.0 ppt for the bench traps and 4.8 ppt for the hood traps (hood filters were replaced that week). The highest mercury levels were 26 ppt for the bench traps and 36 ppt for the hood traps. These increased levels correspond to a period of increased activity in building maintenance. The data are extremely useful for monitoring for laboratory contamination and degradation in the efficiency of the air filtration system. When laboratory trap results are higher than normal, the air filters are rotated to expose new surface area of the activated carbon and to rectify air channeling around the carbon particles in the filters. Alternatively, the hood filters are replaced. The information from these traps is empirical and cannot be related to specific concentrations of mercury in the laboratory air at this time.

Other areas in the building are monitored monthly using traps that are exposed for 24 hr (longer time periods result in mercury trapped at levels higher than the UTLA working range). The building traps typically have mercury levels of 30 to 60 ppt for 24-hr exposures. Comparing the results of the building traps to the lower results of the UTLA laboratory traps, while considering exposure times, illustrates the effectiveness of the laboratory modifications. The mercury concentration of the UTLA laboratory air is presently being analyzed with the objective of comparing these new results to the trap data and evaluating the validity of the traps.

*Experimental*

Precautions are required in the field while collecting samples for UTL mercury analysis. Sample collection considerations and protocol are beyond the scope of this paper, but are discussed elsewhere.[3,4]

Total mercury in surface water samples was determined by cold digestion with hydrochloric acid and a bromate/bromide mixture. The procedure produces bromine monochloride, which, in the presence of excess bromide ions and acid, is converted to free bromine. The free bromine degrades organic mercury compounds in the sample,[5,6] and stannous chloride is then reacted with the digested sample to reduce the mercury to $Hg^0$. The $Hg^0$ is transported as vapor to a fluorescence detector by a stream of high-purity argon gas.

*Reagents and reagent preparation*

ASTM Type I water[7] is used as the water referenced in this method.

1. Argon: High-purity (99.999%) argon is used and an activated carbon trap is placed in line to remove impurities.
2. Hydrochloric acid: Trace metal-grade (TMG) or purer acid is used. Because the mercury content in acids varies greatly, a reliable source must be located and the mercury concentration in the acid routinely monitored. TMG HCl (Mallinckrodt Chemicals, Inc., Paris, KY) has proved to be satisfactory by consis-

*The mention of trade names or commercial products is for illustration purposes only and does not constitute recommendation of these products by the U.S. EPA.

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iently containing 6 ppt mercury or less.

3. Potassium bromate and potassium bromide: Trace mercury is volatilized by heating in a muffle furnace at 250 °C for a minimum of 8 hr.
4. Bromate/bromide solution: Prepared weekly by dissolving 1.39 g potassium bromate and 5.95 g potassium bromide in 500 mL of water.
5. Hydroxylamine hydrochloride solution (5% wt/wt): Prepared weekly by dissolving 2.5 g of hydroxylamine hydrochloride in 50 mL of water.
6. Stannous chloride solution (2% in 10% HCl): Prepared by adding water to a 1-L flask followed by 100 mL of concentrated HCl and 20.0 g of stannous chloride. The solution is stirred to dissolve the stannous chloride and brought to volume with water. Trace mercury is removed from this solution by sparging with argon for 30 min immediately before use. This solution is prepared daily and covered during analysis to prevent oxidation.
7. Wash (reagent blank): Prepared daily by adding water to a 2-L flask followed by 100 mL of concentrated HCl and 40 mL of the bromate/bromide solution, then brought to volume. The wash is decolorized with 2.0 mL of the 5% hydroxylamine hydrochloride solution.
8. Mercury stock solution (1.0 mg/mL): A mercury stock solution of certified concentration was purchased from Fisher Scientific (Pittsburgh, PA).

Reusable labware is rigorously cleaned between uses. A proven cleaning method is to soak the labware overnight in Micro® (International Products Corp., Trenton, NJ) cleaning solution. The labware is then rinsed four times and soaked overnight in an acid/bromate/bromide mixture containing approximately 5% HCl and enough bromate/bromide solution to produce a yellow color indicative of free bromine. This mixture will trap mercury from the air and will also produce bromine vapor; therefore, this container must be closed. Enough of the 5% hydroxylamine hydrochloride solution is added to decolorize the mixture, and the labware is then rinsed six times with water.

The risk of contaminating samples during digestion and analysis is especially high at these low levels. To monitor for this occurrence, each sample is digested twice and each of these digestions is analyzed twice. When large differences in results suggest contamination, the samples are digested and analyzed again in the same manner.

The samples are prepared by transferring 50 mL of sample to a graduated disposable centrifuge tube, followed by 2.5 mL of concentrated HCl and 1.0 mL of the bromate/bromide solution. Reagent blanks and traps are prepared by adding the acid and bromate/bromide solution to 50 mL of water. The samples are allowed to digest at least 30 min. Calibration standards and reference materials are prepared in the same manner as the samples and with the same batch of reagents.

If the yellow color from the bromate/bromide solution does not persist, solution is added in 1.0-mL increments until color is present during the entire digestion period to ensure complete digestion. A corresponding blank is prepared to match the additional reagent concentration in the sample. After the digestion period has elapsed, 0.05 mL of the 5% hydroxylamine hydrochloride is added as a prereductant. The samples are then ready for analysis.

### *Instrumentation and analysis*

*Figure 1* is an illustration of the instrument configuration. A hydride generator and mercury-specific fluorescence detector (PS Analytical Ltd., Sevenoaks, Kent, U.K.) are used in conjunction with software for IBM (Armonk, NY)-compatible computer control. A peristaltic pump directs sample and reagent flows through a switching valve and into a gas/liquid separator or to a waste line. Wash and reductant are directed to the separator when the sampling sequence is inactive. During sampling, the switching valve directs sample and reductant into the separator and the wash is directed to waste. Argon is bubbled through the separator (carrier gas) and transports the mercury vapor through a dryer tube and into the detector. The dryer tube removes water vapor, which causes scattering of the analytical signal.

Perma Pure dryer tubes (Perma Pure Products, Farmingdale, NJ) have proved to be successful at removing water vapor without degrading the sensitivity of the analysis. To improve the peak shape, the carrier gas is supplemented with sheath gas as the mercury is swept into the detector. The use of an autosampler was explored, but has been unsuccessful to date due to low level contamination and carryover.

The software-controlled timing sequence consists of four steps. Step one is a delay that allows time for the line to flush with sample and for the sample to reach the switching valve. Step two begins with the valve switching from wash to sample and allows time for the sample to reach the separator and react with the stannous chloride. The reduced mercury vapor is then swept into the detector by a stream of argon gas. Step three is the analysis time during which the peak reaches its maximum and the height of the peak is measured. In step four, the switching valve directs wash into the separator, sample is directed to waste, and the detector signal returns to the baseline level.

Typical parameters for analysis are as follows. Sample flow: 6 mL/min; wash flow: 6 mL/min; reductant flow: 3 mL/min; step 1: 5 sec; step 2: 25 sec; step 3: 30 sec; step 4: 60 sec; argon flows: 160 cc/min carrier, 150 cc/min sheath, 3 L/min dryer tube.

Argon and liquid flows should be uniform in order to maintain a stable baseline. The addition of a mass flow controller for the gas and a variable speed peristaltic pump for the liquids provides the improved control, reproducibility, and stability that are required of the system.

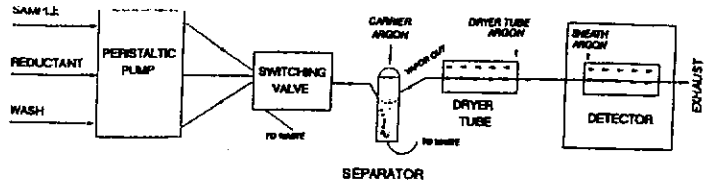


Figure 1 *Instrument configuration for ultratrace level mercury analysis.*

Table 1

**Recovery of total mercury from reference materials and spikes**

| Identification | Avg. % recovery | # Analyses |
|---|---|---|
| Reference Material WS024 | 93.0 | 10 |
| Reference Material WS029 | 94.4 | 24 |
| Reference Material WS030 | 96.8 | 10 |
| Reference Material WS031 | 101.5 | 36 |
| Reference Material R293 | 100.3 | 11 |
| Reference Material 1641 B | 98.1 | 84 |
| Reference Material 1641 C | 101.3 | 18 |
| Sample spikes <10 ppt | 99.1 | 30 |
| Sample spikes >10 ppt | 84.9 | 7 |
| All sample spikes | 96.4 | 37 |

### *Calibration and quality control*

A calibration blank and a minimum of four calibration standards are prepared to span the concentration range of interest. The lowest standard is prepared at a concentration near the method detection limit (1 ppt) when the range of interest is less than 10 ppt to demonstrate the ability to analyze at this level. Two reference materials are analyzed with each set of digestions to demonstrate that the digestion is adequate and the calibration standards are prepared correctly. At least one of the references contains a known amount of an organic mercury compound.

A linear correlation coefficient of 0.995 or greater for the curve is considered acceptable. The protocol for each set of analyses entails generation of the calibration curve followed by analysis of a blank, the references, then another blank, and a set of samples. A blank and a calibration check (a calibration standard or reference) are analyzed after every 10 samples. The runs are concluded with a blank and a calibration check.

Statistical parameters for evaluating the quality of results are integrated into the authors' quality assurance/quality control (QA/QC) program and are continually updated as data are generated. For QA/QC purposes, at least 10% of the samples are duplicated and spiked. The spiking solution contains a known amount of mercury, 50% of which is methylmercuric chloride. Data for recovery of mercury from the reference materials and spikes are presented in *Table 1*. The recovery of mercury from the reference materials must be within 10% of the true value to proceed with sample analysis. Precision data for sample replication are presented in *Table 2*. These results illustrate the difficulty in obtaining high-quality data in mercury analysis below 10 ppt. Duplicate sample digestions resulted in a relative percent difference of 16.2 for the analysis of 32 samples.

This laboratory participated in a study with three other laboratories to measure UTL mercury concentrations in split samples. Each laboratory received five samples that were each divided into two bottles before distribution. The data generated in this study are presented in *Table 3*. The results for sample number 3 clearly indicate the need for extreme caution when collecting, splitting, and analyzing samples for UTL analysis.

### *Conclusion*

The brominating digestion described here works well for complete digestion of surface water samples and detection of mercury at ultratrace levels. The method detection limit[8] is approximately 1 ppt with the authors' current method and instrumentation. Ultratrace level detection methods and instrumentation are continually being modified and refined to lower the levels of detection. For example, two methods for concentrating samples are being examined at this laboratory. The first method involves the use of a gold amalgam trap specifically designed for use with the authors' instrumentation.

Table 2

**Precision data for replicate digestions of water samples**

| | All reps. | Results ≥10 ppt | Results <10 ppt |
|---|---|---|---|
| Average RPD* | 16.7 | 8.3 | 19.9 |
| # Analyses | 176 | 48 | 128 |

*Relative percent difference = (|rep$_1$–rep$_2$|÷ avg. of reps) × 100.