Determination of Mercury in Fish: A Low-Cost Implementation of Cold-Vapor Atomic Absorbance for the Undergraduate Environmental Chemistry Laboratory

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Cold Vapor Analysis of Mercury in Fish

Mercury has long been known to be harmful to humans, particularly children and developing fetuses. The neurotoxic effects of the metal are worse for young children, but will adversely affect all people. The primary route of exposure to mercury tends to be fish consumption. Due to the biomagnifying properties of organic mercury, top of the food chain saltwater fish tend to have the highest concentrations of mercury. These fish also tend to be the most attractive for human consumption. The Food and Drug administration monitored mercury levels in commercial seafood from 1990-2010 and found average mercury concentrations of 0.99 ppm in swordfish and 0.39 ppm in tuna. You will analyze samples of both of these types of fish and determine if seafood purchased at local supermarket is typical of that found by the FDA.

Safety and Waste Disposal

All of the solutions used for this experiment contain strong acids and end up contaminated with mercury. Wear gloves and goggles at all times while working with them and dispose of them in the designated waste container when you are finished. The digestion step involves boiling nitric and sulfuric acids in order to destroy the fish tissue. It will have the same effect on your flesh if you come in contact with it. Perform this procedure in a fume hood and treat the mixture with extreme caution.

Procedure

You should prepare and analyze one sample of each fish as well as do your assigned part of the calibration measurements. The sections of this experiment do not necessarily need to be performed in order. For example, the calibration measurements can be performed while your sample is digesting.

Fish sample preparation

1. If the fish has not already been homogenized, place it in a beaker and process thoroughly with the tissue blender until the mixture is uniform throughout.

2. Transfer an approximately 10 g portion, carefully weighed, of homogenized fish to a 250 mL round bottom flask. Use a Pasteur pipette to rinse the neck of the flask with 5 mL of deionized water.
3. Add several boiling stones, 20 mg of V_2O_5, and 20 mL of 50% HNO_3/50% H_2SO_4 to the flask.
4. Connect the flask to a condenser, make sure the cooling water is running, and boil the mixture gently for 15 minutes.
5. Pour 15 mL of deionized water into the condenser to rinse it. Add 2 drops of 30% hydrogen peroxide through the condenser, and rinse with another 15 mL of deionized water.
6. Remove the flask from the condenser and cool it to room temperature in running water.
7. Transfer the contents of the flask quantitatively to a 100 mL volumetric flask by rinsing with several portions of deionized water and dilute to volume.

Standard solution analysis

1. Pre-measure 100 mL of diluting solution in the graduated cylinder marked 'D' and 20 mL of reducing solution in the graduated cylinder marked 'R'.
2. Use a micropipette to transfer a 1000 µL aliquot of 1 µg/mL Hg standard solution into the analysis flask. Add the diluting solution and swirl to mix.
3. Add the reducing solution and insert the stopper with aerator into the flask.
4. Press "Read" on the spectrometer and flip the air switch to on to start the flow.
5. While the air is flowing, premeasure diluting solution and reducing solution for the next analysis.
6. When the star disappears from the spectrometer screen, note the absorbance reading, pour the solution into the waste, and rinse with a small amount of deionized water.
7. Repeat the analysis using aliquots of 800, 600, 400, 200, and 0 µL of Hg standard solution. Measure the absorbance of all the solutions in triplicate.

Fish sample analysis

1. Pre-measure 75 mL of diluting solution in the graduated cylinder marked 'D' and 20 mL of reducing solution in the graduated cylinder marked 'R'.
2. Use a volumetric pipette to transfer a 25 mL aliquot of digested fish solution into the analysis flask. Add the diluting solution and swirl to mix.
3. Add the reducing solution and insert the stopper with aerator into the flask.
4. Press "Read" on the spectrometer and flip the air switch to on to start the flow.
5. While the air is flowing, premeasure diluting solution and reducing solution for the next analysis.
6. When the star disappears from the spectrometer screen, note the absorbance reading, pour the solution into the waste, and rinse with a small amount of deionized water.
7. Repeat the analysis until you have measured the absorbance of each fish sample three times.

Report

Make a calibration curve by graphing absorbance vs. μg of Hg added to the analysis flask for each standard measurement. Use the calibration curve to determine μg of Hg in each fish aliquot and calculate the total μg of Hg in the digested fish sample. Calculate the concentration of Hg in the fish using the following formula:

$$\text{ppm Hg} = \mu\text{g Hg/g sample}$$

The EPA recommends a maximum mercury intake of 0.3 μg/kg per day for adults (0.1 μg/kg for pregnant women). What is the maximum amount of mercury a 150 lb adult should consume daily? Calculate the amount of mercury in a 3 oz serving of the fish you analyzed. Would it be safe to eat a serving of this fish each day?

Cold Vapor Analysis of Mercury in Fish
Instructor Notes

Safety and Waste Disposal

All of the solutions used for this experiment contain strong acids and end up contaminated with mercury. Gloves and goggles should be worn at all times while working with them. Waste should be packaged and disposed of with caustic, heavy-metal containing waste. The digestion step involves boiling nitric and sulfuric acids. This step should be done in a fume hood and the students should be closely supervised.

Required Time

This experiment has been run in a three-hour laboratory section by a class of eight students, working in groups of three. We prefer not to allow groups any larger so that all the students can be actively involved in the analysis. The calibration measurements can be made while the fish samples are being digested and diluted. Measurements of the fish samples, which take about 2 minutes per replicate, are the limiting step for completing the lab. Instructors with larger classes could consider having each group analyze only one of the fish samples, so that more groups could be formed without increasing the number of measurements.

Solutions

Nitric and sulfuric acids should be trace metal analysis grade. All other chemicals can be reagent grade. Deionized (18 MΩ) water should be used for all solutions. All glassware should be acid-washed with 1 M HNO_3 and rinsed with deionized water prior to use.

Reducing Solution

Carefully add 50 mL H_2SO_4 to about 300 mL H_2O. Cool to room temperature and add 15 g NaCl, 15 g hydroxylamine sulfate and 25 g $SnCl_2$. Dilute to 500 mL.

Diluting Solution

To a 1 L volumetric flask containing about 500 mL of H_2O, add 58 mL HNO_3 and 67 mL H_2SO_4. Dilute to volume with H_2O.

50% HNO_3/H_2SO_4

Mix equal volumes of concentrated HNO_3 and H_2SO_4.

1 N H_2SO_4

For preparing the 1 µg/mL working Hg standard solution at the beginning of the workday. Dilute 14 mL of concentrated H_2SO_4 to 500 mL.

1000 µg/mL Mercury Standard

We used a Fluka TraceCERT AA standard solution of 1000 µg/mL Hg dissolved in 12% nitric acid.

1 µg/mL Working Hg Standard Solution

Use a micropipettor to dilute 100 µL of 1000 µg/mL mercury standard to 100 mL in a volumetric flask with 1 N H_2SO_4. Prepare fresh at the beginning of each day.

Mercury Scrubbing Solution (0.25% iodine in 3% KI)

Mix 4.5 g KI and 0.375 g I_2 in 150 mL of distilled water in the scrubber flask.

Suggested Samples

The FDA reports finding the highest levels of mercury in king mackerel, shark, swordfish, and Tilefish. Some varieties of tuna have also been found to have relatively high mercury levels. We chose frozen, boneless, skinless steaks of swordfish and tuna. Both had measureable levels of mercury, within the range reported by FDA. Use of fish samples with skin and/or bones would be an interesting complication in the experiment, requiring students to think about whether to remove them and how their presence may effect interpretation of the results.

Required Materials

The analysis is performed on an atomic absorbance spectrometer equipped with a mercury hollow cathode lamp at 253.7 nm. The spectrometer is setup with the following parameters: Lamp Current = 6 mA, Integration Time = 60 s, 1 repetition, Calibration = Nonlinear (1), Mode = Peak Height (3). We used a Perkin Elmer 3100 AA system. On another instrument, the student instructions may need to be changed (specifically steps 4 and 6 in both the standard and sample analysis sections).

Air flow through the analysis system was preset by means of a needle valve to about 2 L/min and controlled with an on/off valve.

The necessary quantities of glassware and solutions are listed below.

For each fish sample digestion:

250 mL round bottom flask and condenser

100 mL volumetric flask

Boiling stones

Deionized water

20 mg V_2O_5

20 mL 50% HNO_3/50% H_2SO_4

2 drops 30% H_2O_2

For analyses (to be shared by all students)

100 mL graduated cylinder marked "D" for diluting solution

100 mL graduated cylinder marked "R" for reducing solution

For each standard analysis

1000 µL micropipettor with tip (tip can be used for all standards)

100 mL diluting solution

20 mL reducing solution

1 mL or less 1 µg/mL Hg working standard solution

Deionized water to rinse flask

For each fish sample analysis

25 mL volumetric pipette

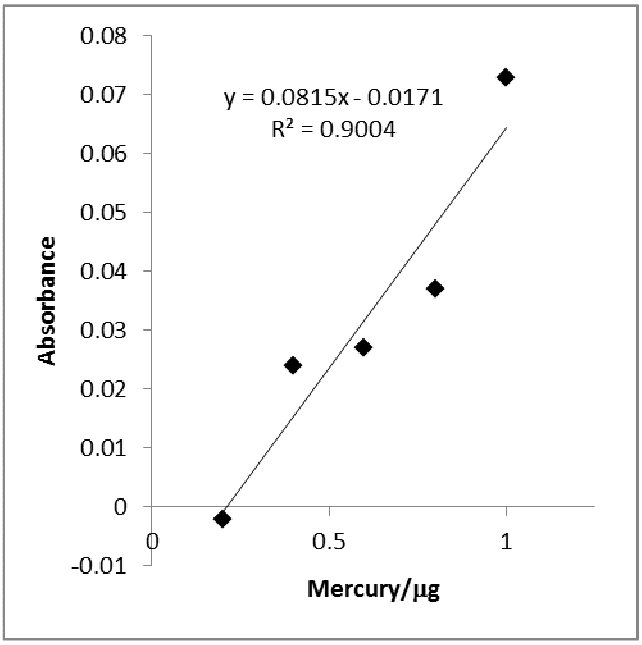
75 mL diluting solution

20 mL reducing solution

Deionized water to rinse flask

Expected Results

The calibration curve generated using this method shows some scatter in the data points around the best-fit line. The apparent curvature of the points on the graph presented in the manuscript was an artifact present that time only. On other occasions, as shown at right, the calibration did not exhibit the "s" shape apparent in Figure 1 of the manuscript, leading us to conclude that there is not a systematic deviation from linearity with this method.



The standard deviation of repeated blank measurements is routinely 0.01 absorbance units, when determined from data sets consisting of either three or ten repeated measurements. Standard and sample measurements made in triplicate have standards ranging from 0.005 to 0.03 absorbance units. The lower precision compared to the blanks likely has to do with the fact that we used house air for our cold vapor system and the flow rate may not be consistent from one sampling to the next.

The measured mercury concentrations presented here are above the detection limit for the method, but not above the limit of quantitation. We take the opportunity to discuss with our students the importance of checking to see if sample measurements are within the working range of the calibration and the fact that compromises must be made to balance data quality with available budget. We also discuss strategies for improving the method performance, such as using air from a tank with regulator or using a cell with a longer path length in order to increase

the absorbance. Instructors wishing to spend a bit more money on the analysis may want to adopt one of these strategies.

The mercury concentrations in our fish samples (about 0.3 ppm) were at the low end of the range observed by the FDA for these species (up to 2 ppm for tuna and 3 ppm for swordfish). Nevertheless, students will still find that the amount of mercury in a 3 oz serving (25-29 μg) is higher than the recommended daily intake for a 150 lb person (about 20 μg).

Method Chemistry

This experiment involves significantly more chemical reagents than many analytical methods taught in the undergraduate laboratory. This situation illustrates the complexity of performing analyses on samples in a biological matrix. The purpose of the various reagents is described here.

Before the analysis can be conducted, the mercury must be extracted from the fish samples. The flesh of the fish is digested by wet ashing in refluxing sulfuric and nitric acids. Oxidation of the remaining organic material is completed by the addition of 30% hydrogen peroxide. Oxidation by both the hot acid and H_2O_2 is catalyzed by the V_2O_5 present in the reaction flask. The strongly oxidizing environment also serves to convert all mercury present to Hg^{2+}. This method therefore measures the total mercury content of the fish.

During the analysis, the sample solution is treated with a reducing mixture of hydroxylamine sulfate and tin(II) chloride. The hydroxylamine serves primarily to reduce residual oxidizing agents in the sample, while Sn^{2+} reduces the mercury to the elemental form, according to the reaction below, for which E^0 is +0.7 V.

$$Sn^{2+} + Hg^{2+} \rightarrow Sn^{4+} + Hg^0$$

The elemental mercury is then swept out of solution by the stream of air and passes through the analytical cell where its absorbance is measured.

The air is then bubbled through a solution containing iodine and potassium iodide. In this solution, the mercury is reoxidized to Hg^{2+} by I_2 and complexes with I^- to form the soluble $[HgI_4]^{2-}$. This scrubbing step removes mercury from the air stream before it is vented to the exhaust hood. We discuss with our students the importance of using analytical methods that minimize hazardous effluent, a task which is particularly important when the analyte itself is a toxic heavy metal.

Cold Vapor Analysis of Mercury in Fish
Apparatus Assembly

1. A 10 cm gas cell with sodium chloride windows ordinarily used for HCl vibrational spectroscopy was borrowed from the physical chemistry laboratory and attached to the AA burner head with plastic cable ties as shown in Figure 1.
2. Two Erlenmeyer flasks with sidearms were fitted with rubber stoppers through which gas dispersion tubes had been passed as shown in Figure 2. One of these flasks is the analysis flask. The other holds the iodine/iodide scrubbing solution.
3. A drying tube was filled with desiccant to remove water. We used magnesium perchlorate because that was suggested in the EPA method, but you may want to use something else if you are uncomfortable handling the perchlorate. Molecular sieves are a good substitute, provided they are regenerated on a regular basis to retain their capacity.
4. Vinyl tubing was used to connect the components of the system in the following order: air supply, analysis flask, drying tube, cell, scrubbing flask. The complete system is shown in Figure 3.

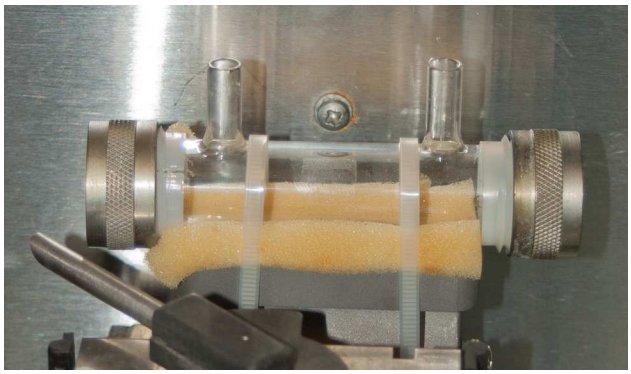


Figure 1. Gas cell attached to burner head.

Figure 2. Erlenmeyer flask fitted with gas dispersion tube for analyte and iodine scrubbing solutions.

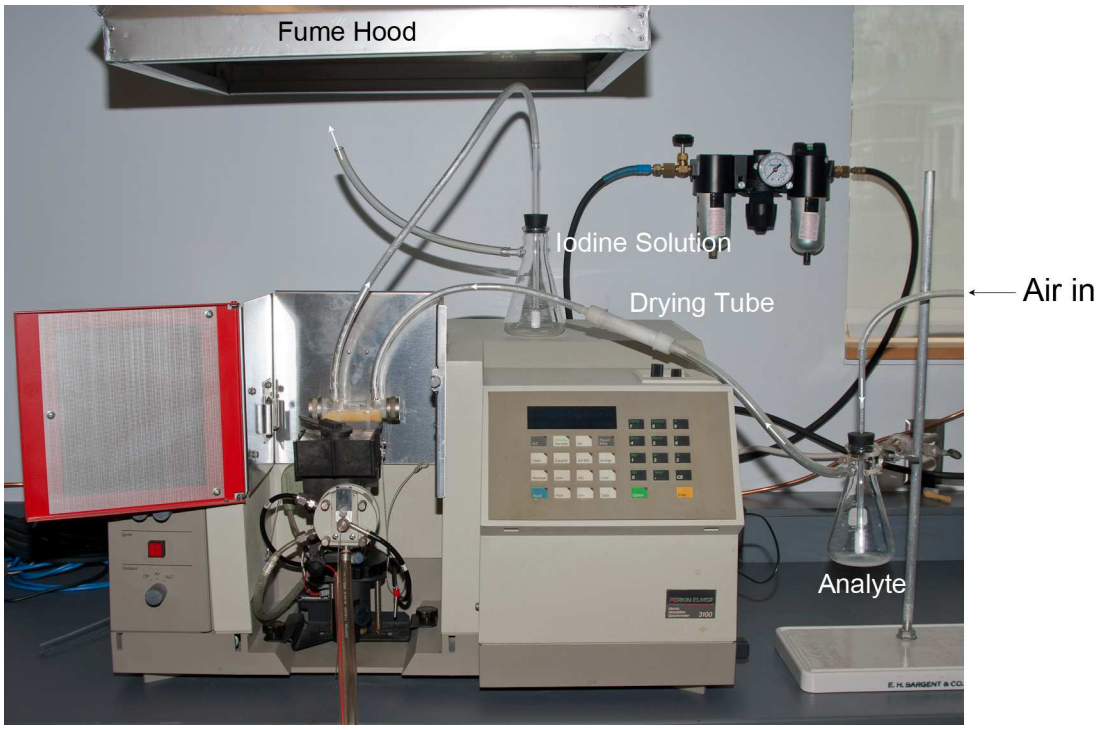


Figure 3. Cold vapor system installed in AA spectrometer. Arrows show the direction of air flow in tubing.

CAS Registry Numbers

Nitric acid (7697-37-2)

Sulfuric acid (7664-93-9)

Sodium chloride (7647-14-5)

Hydroxylamine sulfate (10039-54-0)

Tin(II) chloride (7772-99-8)

Mercury AA Standard, contains

- Nitric acid (7697-37-2)
- Water (7732-18-5)
- Mercury(II) nitrate (7783-34-8)

Magnesium perchlorate (10034-81-8)

Potassium iodide (7681-11-0)

Iodine (7553-56-2)

Vanadium(V) oxide (1314-62-1)

Hydrogen peroxide (7722-84-1)

Potential Hazards

Mercury AA standard: Mercury solutions are toxic. In addition, this solution is prepared in diluted sulfuric acid. It is therefore caustic and should be handled with gloves. Care should be taken to avoid spillage.

Nitric acid: Nitric acid is a strong, oxidizing acid. It should be handled with gloves and care should be taken to avoid spillage.

Sulfuric acid: Sulfuric acid is a strong acid. It should be handled with gloves and care should be taken to avoid spillage.

Boiling concentrated nitric and sulfuric acids: This mixture is extremely hazardous. It will rapidly destroy any organic material with which it comes in contact. It should be handled only in a fume hood. Students should wear gloves, goggles and a lab coat, and should be closely supervised. Material Safety Data Sheets for these two acids can be found at the following links:

Nitric acid: https://us.vwr.com/stibo/hi_res/8303833.pdf
Sulfuric acid: https://us.vwr.com/stibo/hi_res/8923786.pdf

Hydroxylamine sulfate: Hydroxylamine sulfate is corrosive and an irritant. It should be handled with gloves and care should be taken to avoid spillage.

Magnesium perchlorate: Magnesium perchlorate is a strong oxidizer. It should be handled with care and kept away from organics and reducing agents.

Iodine: Iodine is corrosive and a severe irritant. Handle with gloves and avoid contact with exposed skin/eyes. Iodine sublimes slightly at room temperature and should be handled in a fume hood.

Vanadium(V) oxide: Vanadium (V) oxide is extremely toxic. It should be handled with gloves.

Hydrogen peroxide: 30% Hydrogen peroxide is toxic and an oxidizer. It should be handled with gloves and care should be taken to avoid spillage. Avoid contact with exposed skin/eyes.