Analysis of Fish for Total Mercury

Standard Operating Procedure SOP No. HC520B.SOP

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1.0 Scope and Application

This method is only for total mercury measurement after dissolving the fish tissue in concentrated nitric acid under high pressure and temperature using microwave digestion system. The mercury in solution will be analyzed using a nondispersive atomic fluorescence spectrometer. Digestion procedure for samples is described by Feng et al. (1994) and the procedure for instrumental measurement of mercury is given by Bloom and Fitzgerald. Procedures for collection, homogenization, and data reporting are covered by other appropriate NBS/GLSC SOPs.

Note: This method replaces HC520A.SOP.

2.0 Summary of Method

This method is only for total mercury measurement after dissolving the fish tissue in concentrated nitric acid under high pressure and temperature using a microwave digestion system. The mercury in solution will be analyzed using a nondispersive atomic fluorescence spectrometer. Digestion procedure for samples is described by Feng et al. (1994) and the procedure for instrumental measurement of mercury is given by Bloom and Fitzgerald. Procedures for collection, homogenization, and data reporting are covered by other appropriate NBS/GLSC methods.

3.0 Interferences

3.1 Contamination in the laboratory will be minimized by processing the samples in an epoxy-coated plastic chamber equipped with HEPA filers to achieve Class 100 laminar flow conditions. Milli-Q water and quartz-redistilled acids will be used for sample digestion.

3.2 All the labware (glass or teflon will be decontaminated using the 9-step procedure described by Nriagu et al. (1993). Some of the key steps include degreasing with soap, sequential washing with acetone, concentrated nitric and hydrochloric acid, soaking in warm (40-50°C) 2 M nitric acid for 3 days, and rinsing thoroughly with Milli-Q water. After use, the labware is soaked in 6 M hydrochloric acid for three days, followed by warm nitric acid for three days and then rinsed with Milli-Q water. The final rinse is done in the HEPA-equipped chamber, and all the containers are stored in acid-washed, triple plastic bags. Cleaned volumetric flasks will be filled with 1.0 M nitric acid and triple bagged for storage.

4.0 Safety

Mercury in the pure form is toxic. Both nitric and sulfuric acid in the concentrated form will cause severe chemical burn on tissue and require use of safety glasses when handling, even at dilutions containing 20% of the concentrated form. The hazards of each chemical and reagent used in this method have been generally defined, but each chemical compound used should be treated as a potential health hazard. A reference file of material safety data sheets is available at the U of M Lab and in NBS to all personnel involved in chemical analysis.

5.0 Apparatus and Chemicals

5.1 Teflon-lined pressure bombs, 90 mL

5.2 Sphex CDS 7000 Microwave digestion unit

5.3 Cold vapor atomic fluorescence spectrometer, Rand Corp.,Seattle, WA.

5.4 Freeze dryer, model 25 SRC, Virtis, Gardiner, NY

5.5 Balance, Top loading, Sartorius, 1204 (0.01 g) or better

5.6 Ultra pure acids (nitric and hydrochloric)

5.7 Milli-Q water

5.8 Stannous chloride, ACS grade, Fisher Scientific

5.9 Mercury standard stock solution, Perkin Elmer

5.10 Aluminum weighing pan (6 X 2 cm)

5.11 Whatman quartz 47 mm filter membrane, acid washed, #1851-047

6.0 Digestion Procedure

6.1. Homogenized study fish samples are stored frozen in glass screw cap containers until analyzed. Fish samples are thawed just prior to being weighed for digestion. For each sample from the study, check, duplicate, or spiked samples about one gram of homogenized tissue is weighed into a pre-weighed digestion tube. Record both empty weighing pan and weighing pan plus wet tissue weights which will be used to determine dry weight.

6.2 Digestion

6.2.1 Add 10 mL of concentrated nitric acid to the sample in the digestion vessel and leave the mixture at room temperature for 30 minutes. Then place the teflon vessel containing the sample into the double outer liner of the digestion bomb, cap with a sensor head and pressure rupture disc. Place the sealed vessel in the microwave carousel. Prepare the remaining samples of the set in the same manner. The set must also contain a blank, spike, duplicate, and reference samples.

6.2.2 After connecting the sensor cables to a port in the oven cavity, the power level and time for each digestion stage are programmed into the computer controller. To minimize violent reactions the oven temperature is slowly ramped to the set temperature over 20-30 minutes. When the temperature reaches 190°C and pressure 180 psi, heat the sample for an additional 15 minutes. After the digestion step is completed digestion bombs are cooled to room temperature.

6.2.3 Each cooled digestion bomb is opened and the clear liquid (fish dissolved in nitric acid) is diluted to 25 mL using mercury free Milli-Q water. Residues in sample vessel after digestion is often indicative of incomplete or improper digestion and the sample batch are re-digested. Sample filtration is not required in completely digested fish tissues.

7.0 Analysis

7.1 An aliquot (0.5 to 1.0 mL) of the digested sample is added to 10 mL of Milli-Q water in a glass reduction chamber. The mixture is purged with ultra-pure argon for 10 minutes. One mL of stannous chloride is then added to the mixture to reduce the mercury, and the elemental mercury formed is stripped (with argon) and collected on gold-coated quartz grains. The trapped mercury is subsequently desorbed thermally and measured on a Tekran CVAFS Mercury Analyzer Model 2500. The output, as peak height or peak area, is recorded by means of an HP 3396A Integrator.

7.2 Calibration

7.2.1 The calibration curve is prepared by reducing standard solutions containing 1, 2, 4 and 6 ng of mercury. This is done by using 0.5, 1.0, 2.0 and 3.0 mL of standard solution containing 2.0 μg/L (or 2.0 ng/mL) Hg. Each aliquot of the standard solution is added to 10 mL of Milli-Q water in a glass reduction chamber. The mixture is purged with ultra-pure argon for 10 minutes. One mL of stannous chloride is then added to the mixture to reduce the mercury, and the elemental mercury formed is stripped (with argon) and collected on gold-coated quartz grains. The trapped mercury is subsequently desorbed thermally and measured on a Tekran CVAFS Mercury Analyzer Model 2500. The outputs for the four standard samples, recorded by an HP 3396A Integrator, are used to derive the average slope for the standard curve in terms of atomic fluorescence units per 1.0 ng of Hg.

7.2.2 Calibration of the spectrometer is with a minimum of four serial diluted standards of mercury covering the range expected in the samples that have been taken through the digestion and reduction procedure. The calibration range for most samples is expected to be from about 0.1 - 3 ng of Hg. If concentrations in the fish are lower the calibration range will be moved downward since the detection limit of the instrument is 0.01 ng/L of dissolved Hg.

7.3 Determining the method detection limit

7.3.1 The method detection limit will be determine using the USEPA method of seven (40 CFR) using spiked fish tissue or other acceptable matrix containing less than 1 ng of mercury.

7.3.2 When a peak is visible below the established method detection limit a concentration will be reported but result will be flagged using established EPA codes. When no peak is detected for mercury the results will be reported as zero.

7.4 Result

7.4.1 Results will be reported in ng/g (wet weight) or as stated in the data standards being established by USEPA. Mercury data, including results from calibration, check, duplicates, spikes, and reference samples will be submitted to NBS-GLSC where they will be checked by Jim Hickey for consistency with EPA standards and completeness. Acceptance criteria is shown in Tables 7.1 and 7.2 of the NBS Analytical Quality Assurance Project Plan for IAG DW14947692-02-0. NBS will submit mercury results to USEPA using data standards that are currently being finalized.

8.0 References

8.1 Bloom, N.S. and W.F. Fitzgerald. 1988. Determination of volatile mercury species at the picogram level by low-temperature gas chromatograph with cold-vapor atomic fluorescence detection. Anal. Chim. Acta 208: 151-161

8.2 Feng, Y. and R.S. Barratt. 1994. Digestion of dust samples in a microwave oven. Sci. total Environ. 143: 157-161

8.2 Nriagu, J.O., Lawson, G., Wong, H.K.T. and J.M. Azcue. 1993. A protocol for minimizing contamination in the analysis of trace metals in Great Lakes waters. J. Great Lakes Res. 19: 175-182