

Colorado Department of Public Health and Environment



Laboratory Services Division Inorganic Chemistry Laboratory

**Metals, Inductively Coupled Plasma – Mass Spectrometry,
(ICP-MS), Fish Tissues**

Revision 2

AUGUST 2005

TITLE

Metals, ICP-MS, Fish Tissue

REFERENCES

Determination of Metals in Fish Tissue by Inductively Coupled Plasma – Atomic Emission Spectrometry. EPA Method 200.11, Revision 1.3, April 1987.

“Less is Better: Laboratory Chemical Management for Waste Reduction,” available from American Chemical Society Department of Government Regulations and Science Policy, 115 16th St. N.W., Washington, D.C. 20036, 202.872.4477.

“Waste Management Manual for Laboratory Personnel,” available from American Chemical Society Department of Government Regulations and Science Policy, 115 16th St. N.W., Washington, D.C. 20036, 202.872.4477.

METHOD

Strong base digestion followed by Inductively Coupled Plasma – Mass Spectrometry.

PRINCIPLE

A sample of fish tissue is dissociated using tetramethylammonium hydroxide, low heat, and vortex mixing. The resulting colloidal suspension is partially oxidized with the addition of hydrogen peroxide. The metals are then solubilized by acidification with nitric acid and heat. The sample is diluted with deionized water to 50 mL, vortex mixed, and filtered. The final solution is analyzed by inductively coupled plasma – mass spectrometry (ICP-MS) using calibration standards prepared with the digestion reagents.

SAMPLE

At least one gram of wet fish tissue or 0.2 g previously dried fish (e.g. reference material). The determined metal concentration may be reported in microgram/gram (ug/g) wet tissue weight, or the weight of a dried portion of tissue may be used to calculate a ug/g dry tissue weight. This method is applicable to the analyses of antimony (Sb), arsenic (As), beryllium (Be), cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), selenium (Se), thallium (Tl), and zinc (Zn). Since Sb and Cr are unstable in the fish matrix analysis solution, these elements should be analyzed within 24 hours of completion of the digestion procedure.

SAFETY

Consult MSDS for appropriate handling of chemicals. Wear gloves and safety glasses with goggles or face shield when working with concentrated bases and acids. Precautions should also

be taken to minimize potential bacterial infections from handling and dissecting fish. Basic housekeeping and sanitation practices and use of gloves is recommended.

EQUIPMENT

1. Dissecting board: Polyethylene or other inert, nonmetallic material.
2. Forceps: Plastic, Teflon or Teflon coated.
3. Surgical-quality knives: Ceramic, Teflon, or high-purity stainless steel.
4. Tissue grinder: Disposable 50-mL conical plastic.
5. Centrifuge tubes: Disposable polypropylene, 50-mL for standards preparation, fillet digestion and dilution.
6. Oak Ridge centrifuge tubes: Disposable polysulfone, 30-mL for whole-fish digestion.
7. Vacuum filter unit: Disposable 50-mL centrifuge tube with 0.22-um membrane filter, e.g. Millipore Steriflip.
8. Culture tubes: Disposable polystyrene, 17 x 100-mm.
9. Storage bottles: Narrow-mouth Teflon FEP (fluorinated ethylene propylene) bottle for long-term (1 – 2 week) storage of standards and references.
10. Wash bottles: One-piece stem, Teflon FEP.
11. Volumetric flasks (Class A): 100-mL and 250-mL, for preparing standards and references.
12. Volumetric pipettes (Class A): 5-mL and 25-mL, for preparing standards and references.
13. Pipettors: BioHit™, with metal-free tips, various sizes capable of delivering 0.2 uL to 500 uL.
14. Drying oven: Gravity convection oven, with thermostatic control capable of maintaining $\pm 5^{\circ}\text{C}$.
15. Vortex mixer.
16. ICP-MS instrument and peripherals described in separate Standard Operating Procedure.
17. Analytical balance.

REAGENTS

Reagent grade chemicals shall be used in all tests unless otherwise indicated. It is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficient high purity to permit its use without lessening the accuracy of the determination.

1. Reagent water equivalent to ASTM Type 1 (ASTM D-1193) with greater than 18 megaohm/cm resistivity.
2. Hydrogen peroxide (H₂O₂), 50%, stabilized, certified purity.
3. Nitric acid (HNO₃), 16M concentrated, "Trace Metal" grade or better.
4. Tetramethylammonium hydroxide [(CH₃)₄NOH], TMAH, 25% aqueous solution, electronic grade 99.9999% (metals basis). Alfa-Aesar #20932 or equivalent.

Reagent Preparation

Note: Label all solutions appropriately with analyst initials, date of preparation, solutions description and concentration, special storage instructions and hazards as appropriate.

1. Single element stock standards, 10 mg/L each individual element; antimony (Sb), arsenic (As), beryllium (Be), cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), selenium (Se), silver (Ag), thallium (Tl), and zinc (Zn). Certified stock solutions commercially (High-Purity Standards, Charleston, SC, or equivalent source) prepared from high-purity metals or salts.
2. Multiple-element stock standard, 20 mg/L each above 10 elements combined. Certified stock solution commercially (High-Purity Standards, Charleston, SC, or equivalent source) prepared from high-purity metals or salts.
3. Multiple-element dilute standard, 2 mg/L. Dilute 5 mL of the above multiple-element stock standard and 2.5 mL 16M HNO₃ to 50 mL with reagent water.
4. Calibration Standards. To five 50-mL centrifuge tubes, add 1 mL each TMAH and 0.5 mL each 50% H₂O₂. Add the appropriate volumes of multiple-element stock or dilute standard as indicated in Table 1 below. Allow the solutions to stand open for 30 minutes to vent released oxygen. Add 2.5 mL 16M HNO₃ and dilute to 50 mL with reagent water.

Table 1. Recommended Calibration Standards

<i>Calibration Level</i>	<i>Standard</i>	<i>Standard Added (mL)</i>	<i>Final Concentration (µg/L)</i>
5	20-mg/L STOCK	0.50	200
4	20-mg/L STOCK	0.25	100

<i>Calibration Level</i>	<i>Standard</i>	<i>Standard Added (mL)</i>	<i>Final Concentration (µg/L)</i>
3	2-mg/L DILUTE	0.25	10
2	2-mg/L DILUTE	0.025	1
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5. Tuning Solution (TS-2) stock, 10 mg/L each of cerium (Ce), lithium (Li), yttrium (Y), and thallium (Tl). Certified stock solution commercially (High-Purity Standards, Charleston, SC, or equivalent source) prepared from high-purity metals or salts.
 - a) TS-2 working solution. Dilute 0.25 mL each of the TS-2 stock solution and 10-mg/L U single-element standard to 250 mL with 1-% v/v HNO₃.

6. Tune Check Solution (TS-3) stock, 10 mg/L each of beryllium (Be), cobalt (Co), indium (In), lead (Pb), and magnesium (Mg). Certified stock solution commercially (High-Purity Standards, Charleston, SC, or equivalent source) prepared from high-purity metals or salts.
 - a) TS-3 working solution. To a 50-mL centrifuge tube, add 1 mL TMAH and 0.5 mL 50% H₂O₂. Add 0.25 mL of the TS-3 stock. Allow the solution to stand open for 30 minutes to vent released oxygen. Add 2.5 mL 16M HNO₃ and dilute to 50 mL with reagent water.

7. Internal Standard Solution (ISTD) stock, 10 mg/L each of lithium-6 (Li⁶), scandium (Sc), germanium (Ge), yttrium (Y), indium (In), terbium (Tb), and bismuth (Bi). Certified stock solution commercially (Agilent Technologies, Wilmington, DE, or equivalent source) prepared from high-purity metals or salts.
 - a) ISTD working solution. Dilute 25 mL of the ISTD stock solution to 250 mL with 1-% v/v HNO₃.

8. Quality Control Standard (QCS). Commercially (High-Purity Standards, Charleston, SC, or equivalent source) prepared mixed element standards: QC-7 (1000 mg/L K; 100 mg/L Ag, Al, B, Ba, Na; 50 mg/L Si), QC-19 (100 mg/L As, Be, Ca, Cd, Co, Cr, Cu, Fe, Mg, Mn, Mo, Ni, Pb, Sb, Se, Ti, Tl, Zn), or QC-26 a combined standard solution of QC-7 and QC-19. The QCS must be from a source independent of that used to prepare the calibration standards.
 - a) QCS working solution. To a 50-mL centrifuge tube, add 1 mL TMAH and 0.5 mL 50% H₂O₂. Add 0.05 mL of QC-26 (or 0.05 mL each of QC-7 and 19). Allow the solution to stand open for 30 minutes to vent released oxygen. Add 2.5 mL 16M HNO₃ and dilute to 50 mL with reagent water.

9. Laboratory Control Standards (LCS) or method reference. Certified mixed element reference solutions commercially (Environmental Resource Assoc., Arvada, CO, or equivalent source) prepared concentrate. Prepare a working solution within the concentration range of the calibration standards at the same time and with the same procedure as the fish samples. The LCS must be from a source independent of that used to prepare the calibration standards.

10. Interference Check Solution (ICS). Certified mixed element solution commercially (Environmental Resource Assoc., Arvada, CO, or equivalent source) prepared concentrate. Prepare a working solution so that the interferants, Al, Ca, Mg, and Fe are near 50 - 100 mg/L, and the analytes, As, Cd, Cr, Se, and Ag are near 0.02 mg/L.
 - a) ICS-A. To a 50-mL centrifuge tube, add 1 mL TMAH and 0.5 mL 50% H₂O₂. Add 0.5 mL of a solution containing 5000 mg/L each of Al, Ca, Fe, and Mg (e.g. INFCS-4 mix from High Purity). Allow the solution to stand open for 30 minutes to vent released oxygen. Add 2.5 mL 16M HNO₃ and dilute to 50 mL with reagent water.
 - b) ICS-AB. To a 50-mL centrifuge tube, add 1 mL TMAH and 0.5 mL 50% H₂O₂. Add 0.5 mL of a solution containing 5000 mg/L each of Al, Ca, Fe, and Mg (e.g. INFCS-4 mix from High Purity), and 0.05 mL of the 20-mg/L multiple-element stock standard. Allow the solution to stand open for 30 minutes to vent released oxygen. Add 2.5 mL 16M HNO₃ and dilute to 50 mL with reagent water.
11. Calibration Blank, Initial (ICB) and Continuing (CCB), 5% HNO₃, used as the zero calibration standard and to check the zero level of the calibration curve. To a 50-mL centrifuge tube, add 1 mL TMAH and 0.5 mL 50% H₂O₂. Allow the solution to stand open for 30 minutes to vent released oxygen. Add 2.5 mL 16M HNO₃ and dilute to 50 mL with reagent water.
12. Laboratory reagent/method blank solution, 5% HNO₃, contains all reagents used in processing the samples. To a 50-mL centrifuge tube, add 1 mL TMAH and 0.5 mL 50% H₂O₂ and process through the entire digestion.
13. Laboratory Fortified Blank (LFB). To a 50-mL centrifuge tube, add 1 mL TMAH and 0.5 mL 50% H₂O₂. Add 0.50 mL of 2-mg/L multiple-element dilute standard solution. Allow the solution to stand open for 30 minutes to vent released oxygen. Add 2.5 mL 16M HNO₃ and dilute to 50 mL with reagent water. to 50 mL of matrix blank solution and mix well.
14. Pulse to Analog (P/A) Calibration solution, 100 ug/L of all method analyte elements. Dilute 2mL each of the ISTD stock solution, 10 mg/L U, and 10 mg/L thorium (Th), and 0.2 mL of the QC-26 solution to 200 mL with 1-% v/v HNO₃.

PROCEDURE

1. Skinless fillet samples are homogenized in a tissue grinder and/or by chop-and-mix if the fillet is too large for the grinder.
2. Use ceramic knife to chop fillet into pieces small enough (10 – 15 g) to fit into the grinder. For whole fish, chop and homogenize viscera.

3. After all the pieces are homogenized, mix together using the ceramic knife and cutting board. For whole fish, mix homogenized viscera into homogenized fillet.
4. Homogenized and mixed samples are placed in labeled 50-mL centrifuge tubes and frozen at -20°C or below until analyzed. For large fish, place as much as possible into a 50-mL centrifuge tube and store the remainder in a freezer bag.
5. Place a labeled 50-mL centrifuge tube in a 100-mL beaker and tare both containers. Transfer a 1- to 2-g aliquot of fish tissue to this centrifuge tube and record the weight to the nearest 10 milligrams. For whole-fish samples, transfer the aliquot of tissue to a similarly tared Oak Ridge tube.
6. Prepare a matrix duplicate by transferring an additional 1- to 2-g aliquot of that fish tissue to another labeled centrifuge tube.
7. Prepare a matrix spike by transferring an additional 1- to 2-g aliquot of that fish tissue to another labeled centrifuge tube and adding 0.05 mL of the 20-mg/L multiple-element stock standard.
8. Transfer a 0.2-g aliquot of dried fish reference material to a labeled 50-mL centrifuge tube.
9. Add a volume of TMAH equal to the weight of the fish tissue (1.0 mL TMAH = 1.0 gram tissue). Replace and tighten the cap securely.
10. Prepare a Method Blank by transferring 1.0 mL of reagent water and 1.0 mL of TMAH to a labeled 50-mL centrifuge tube. Process this blank through the entire digestion.
11. Prepare a Method (aqueous) Reference by transferring 1.0 mL of reagent water, 1.0 mL of TMAH and the appropriate aliquot of stock reference to a labeled 50-mL centrifuge tube. Process this reference through the entire digestion.
12. Place all samples, blank and references in an open rack and heat for one hour at $65 \pm 5^{\circ}\text{C}$.
13. After the first hour of heating, remove the samples from the oven, retighten the caps if loose, and mix the samples using a vortex mixer. Return the samples to the oven and heat for an additional hour.
14. After the second hour of heating, again vortex mix each sample and place the rack of mixed samples in an ice water bath for 30 minutes. Add 0.5 mL of cold 50% H_2O_2 to each sample. Immediately recap the tube and tighten the cap securely. Treat each sample individually before proceeding to the next sample. Keep the samples in the ice water bath until all samples have been treated. Refrigerate the capped samples overnight.

15. The following day, vortex mix and then add 2.5 mL 16M HNO₃ to each sample. Recap the tube and vortex mix, treating each sample individually before proceeding to the next sample. Return the samples to the oven, and heat for one hour at 100°C.
16. Cool the samples to room temperature, make up to 50 mL with reagent water, and mix well. Final acid concentration is 5%.
17. For whole fish digested in the Oak Ridge tubes, transfer the contents to a labeled 50-mL centrifuge tube. Use multiple rinses of the Oak Ridge tube with reagent water to make up the volume in the 50-mL centrifuge tube and mix well.
18. Attach each sample tube to a Millipore Steriflip, or similar, vacuum filtration unit, and filter each sample into a clean, labeled 50-mL centrifuge tube.
19. Tune the ICP-MS according to that Standard Operating Procedure, using the prescribed tune solutions.
20. Load the sample identification numbers into the appropriate sequence template (e.g. fish.s or bigfish.s), which analyze the samples with the appropriate method (e.g. fish.m or bigfish.m).

INTERPRETATION AND REPORTING

1. All Quality Control criteria listed in the ICP-MS Standard Operating Procedure are followed. The fish reference material has the Quality Control recovery limits of 70 – 100%.
2. The method detection limits (MDL) and practical quantitation limits (PQL) in ug/g are determined for each analyte using a natural or spiked fish matrix.
3. Report the results as ug metal / g wet fish tissue as calculated below:

$$\mu\text{g/g wet weight} = \left(\frac{\mu\text{g} / \text{L} * 0.05 \text{ L}}{\text{wet weight fish(g)}} \right)$$

If a dry-weight value is requested, determine the dry weight from a separate 1- to 2-g aliquot of fish tissue dried 48 hours at 90°C.

POLLUTION PREVENTION

1. Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operation. The USEPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Laboratory personnel should feasibly use pollution prevention

techniques to manage waste generation. When wastes cannot be reduced at the source, the USEPA recommends recycling as the next best option.

2. The quantity of chemicals purchased should be based on expected usage during their shelf lives and disposal cost of unused material. Volumes of prepared reagents should reflect anticipated usage and reagent stability.
3. For information about pollution prevention applicable to laboratories and research institutions, consult "Less is Better: Laboratory Chemical Management for Waste Reduction," available from American Chemical Society Department of Government Regulations and Science Policy, 115 16th St. N.W., Washington, D.C. 20036, 202.872.4477.

WASTE MANAGEMENT

1. The USEPA requires that the laboratory waste management practice be conducted with all applicable rules and regulations. Excess reagents, chemicals, and method process wastes should be characterized and disposed of in an acceptable manner. The agency urges laboratories to protect the environment by minimizing and controlling all releases from hood and bench operations. The agency also urges laboratories to fully comply with any liquid waste discharge permits and regulations and to comply with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions.
2. For further information on waste management consult the "Waste Management Manual for Laboratory Personnel," available from American Chemical Society Department of Government Regulations and Science Policy, 115 16th St. N.W., Washington, D.C. 20036, 202.872.4477.
3. The appropriate disposal procedure for each laboratory chemical solution used in this analysis is summarized in Table 2.

Table 2. Chemical Solution Disposal Procedures

<i>Solution</i>	<i>Disposal</i>
Single element stock standards, 10 mg/L	Containerize. Dispose as RCRA hazardous waste. Arsenic (D004), Barium (D005), Cadmium (D006), Chromium (D007), Lead (D008), Selenium (D010), and Silver (D011).
Multiple-element stock standard, 20 mg/L	Containerize. Dispose as RCRA hazardous waste. Arsenic (D004), Cadmium (D006), Chromium (D007), Lead (D008), Selenium (D010), and Silver (D011).
Multiple-element dilute standard, 2 mg/L	Containerize. Dispose as RCRA hazardous waste. Corrosive (D002), Cadmium (D006) and Selenium (D010).
Calibration standard level 5 (L-5).	Neutralize. Dispose to sanitary sewer

<i>Solution</i>	<i>Disposal</i>
Calibration standard level 4 (L-4).	Neutralize. Dispose to sanitary sewer
Calibration standard level 3 (L-3)	Neutralize. Dispose to sanitary sewer
Calibration standard level 2 (L-2)	Neutralize. Dispose to sanitary sewer
Calibration standard level 1 (L-1)	Neutralize. Dispose to sanitary sewer
Tune Check Solution (TS-2) stock, 10 mg/L	Dispose to sanitary sewer
TS-2 working solution.	Neutralize. Dispose to sanitary sewer
Tune Check Solution (TS-3) stock, 10 mg/L	Containerize. Dispose as RCRA hazardous waste. Lead - (D008).
TS-3 working solution.	Neutralize. Dispose to sanitary sewer
Internal Standard Solution (ISTD) stock, 10 mg/L	Dispose to sanitary sewer.
ISTD working solution.	Neutralize. Dispose to sanitary sewer
Quality Control Standards (QCS) QC-7.	Containerize. Dispose as RCRA hazardous waste. Silver - (D011) and (D005) Barium.
Quality Control Standards (QCS) QC-19.	Containerize. Dispose as RCRA hazardous waste. Arsenic (D004), Cadmium (D006), Chromium (D008), Lead (D008) and Selenium (D010).
Quality Control Standards (QCS) QC-26.	Containerize. Dispose as RCRA hazardous wastes. Arsenic (D004), Barium (D005), Cadmium (D006), Chromium (D007), Lead (D008), Selenium (D010), and Silver (D011).
QCS working solution.	Containerize. Dispose as RCRA hazardous waste. Corrosive (D002), Cadmium (D006) and Selenium (D010).
Laboratory Control Standards (LCS) or references	Check concentrations against TCLP limits. Neutralize and dispose to sanitary sewer or containerize and dispose as RCRA hazardous waste.
Interference Check Solution (ICS).	Dispose to sanitary sewer.
Calibration Blank, Initial (ICB) and Continuing (CCB),	Neutralize. Dispose to sanitary sewer
Laboratory reagent/matrix blank solution	Neutralize. Dispose to sanitary sewer
Laboratory Fortified Blank (LFB).	Neutralize. Dispose to sanitary sewer
Pulse to Analog (P/A) Calibration Solution.	Neutralize. Dispose to sanitary sewer
Sample Residual (aqueous)	Check concentrations against TCLP limits. Neutralize and dispose to sanitary sewer or containerize and dispose as RCRA hazardous waste.

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