Method 200.11

Determination of Metals in Fish Tissue by Inductively Coupled Plasma-Atomic Emission Spectrometry

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TABLES

NOTICE

This document has been peer and administratively reviewed within EPA. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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Method 200.11
Determination of Metals in Fish Tissue by Inductively Coupled Plasma-Atomic Emission Spectrometry

1. SCOPE AND APPLICATION

1.1 This method is an inductively coupled plasma (ICP) atomic emission spectrometric procedure for use in the determination of naturally occurring and accumulated toxic metals in the edible tissue portion (fillet) of the fish. The tissue must be taken from a fresh, not previously frozen, fish to prevent loss of analyte or contamination of the tissue due to cell lysis and resulting fluid exchange. The method is not intended to be used for the analysis of dried fish tissue. This method is applicable to the analyses of the following metals:

Metal	Chemical Abstract Services Registry Numbers (CAS RN)
Aluminum (Al)	7429-90-5
Antimony (Sb)	7440-36-0
Arsenic (As)	7440-38-2
Beryllium (Be)	7440-41-7
Cadmium (Cd)	7440-43-9
Chromium (Cr)	7440-47-3
Copper (Cu)	7440-50-8
Lead (Pb)	7439-92-1
Nickel (Ni)	7440-02-0
Selenium (Se)	7782-49-2
Thallium (Tl)	7440-28-0
Zinc (Zn)	7440-66-6

1.2 This method also may be used for the spectrochemical analyses of other elements commonly found in fish tissue. Specific analytes included are the following:

Analyte	Chemical Abstract Services Registry Numbers (CAS RN)
Calcium (Ca)	7440-70-2
Iron (Fe)	7439-89-6
Magnesium (Mg)	7439-95-4
Phosphorus (P)	7723-14-0
Sodium (Na)	7440-23-5

1.3 Specific instrumental operating conditions are given and should be used whenever possible. However, because of the differences between various makes and models of spectrometers, the analyst should follow the instrument manufacturer's instructions in adapting the instrument's operation to approximate the recommended conditions given in this method.

1.4 Table 1 lists the recommended wavelengths with locations for background correction for the metals presently included in this method. Also listed in Table 1 are the method detection limits (MDLs) (1) for certain metals determined in fish tissue using conventional pneumatic nebulization for sample introduction into the ICP.

1.5 Once the tissue samples have been collected, approximately 20 fish fillet samples including the mandatory quality control samples can be analyzed using this method during the 1.5 day work period required to complete the analysis.

2. SUMMARY OF METHOD

2.1 A 1 to 2 gram sample of fish tissue is taken from a fresh (not previously frozen) fish and transferred to a preweighed, labeled polysulfone Oak Ridge type centrifuge tube. The tissue is dissociated using tetramethylammonium hydroxide (2, 3), low heat and vortex mixing. The resulting colloidal suspension is cooled in an ice bath and then partially oxidized with the addition of hydrogen peroxide while allowing the sample to stand overnight at room temperature. The following day the metals are solubilized by acidification with nitric acid and heat, and then diluted with deionized, distilled water to a weight/volume ratio equal to 1 gram fish tissue per 10 mL of solution. The diluted sample is vortex mixed, centrifuged and finally the acidified aqueous solution is analyzed by direct aspiration background corrected ICP atomic emission spectrometry. The determined metal concentration is reported in microgram/gram (µg/g) wet fish tissue weight.

2.2 The basis of the determination step of the method is the measurement of atomic emission by optical spectroscopy. The sample is nebulized and the aerosol that is produced is transported to the plasma torch where excitation occurs. Characteristic atomic-line emission spectra are produced by a radio-frequency ICP. The spectra are dispersed by a grating spectrometer and the intensities of the lines are monitored by photomultiplier tubes. The photocurrents from the photomultiplier tubes are processed and controlled by a computer system. Background correction is required to compensate for the variable background contribution of fish matrix and reagents to the analyte determination. The location recommended for background correction for each analyte is given in Table 1.

3. DEFINITIONS

3.1 Fish tissue - the skinless edible muscle tissue of the fish commonly referred to as the fillet.

3.2 Calibration blank - a volume of deionized, distilled water containing all reagents used to prepare the tissue for analyses. (See 7.10).

3.3 Calibration check standard - a single standard solution containing all dissolution reagents and each analyte at known concentration used to verify the calibration (See 7.11.1).

3.4 Quality control sample - a solution obtained from a source different from that used to prepare the standard stock solution (7.7) having known concentration values to be used to verify the single metal stock solutions (See 10.2).

3.5 Laboratory control standard - a standard solution containing all analytes of interest at known concentration, spiked into the reagents matrix and carried through the entire analytical scheme as a sample (See 10.5).

3.6 Method blank - a solution of the reagent matrix carried through the entire analytical scheme as a sample (See 10.4).

4. INTERFERENCES

4.1 Occurrences of chromium contamination of biological samples from the use of stainless steel have been reported in the literature (4). Use of special cutting implements and dissecting board made from materials that are not of interest is recommended. Knife blades made of titanium with Teflon handles have been successfully used.

4.2 Sample contamination and losses are held to a minimum because the collected sample is preserved, processed and analyzed from the same polysulfone centrifuge tube. However, antimony and chromium are not stable in the fish matrix analysis solution and therefore, the sample should be analyzed within 24 hours after completion of the preparation procedure (See 11.2 to 11.7).

4.3 The processed sample ready for analysis will contain a precipitate and possibly floatable solids as a surface layer partially covering the analysis solution. Nevertheless, physical occlusion of metals in these solids is not expected. Percent recovery of known spike concentrations for all metals is near or exceeds 90% (See 13.2)

4.4 Since all samples are diluted to the same weight volume ratio (1 gram/10 mL), all samples have a similar concentration of the major constituents in the matrix. The major constituent elements (Ca, K, Mg, Na and P) in the fish tissue matrix that are measured do not suppress analyte signal intensities nor cause interelement spectral interferences for the wavelengths and analytical conditions recommended. However, these elements represent less than 1/10 of the 2% dissolved solids aspirated. Since the unmeasured constituents account for the majority of the matrix, it is suspected that they cause the shifts in background intensity and

molecular band contribution to wavelength signal near 190 nanometers (nm). Although background correction adjacent to the wavelength will compensate for the majority of the broad band interferences, it has been demonstrated that the use of correction factors based on the reading molecular band signal at 386.17 nm can be useful in providing the additional correction needed for the thallium wavelength (190.8 nm).

4.5 It is reported that dissolved solids exceeding 1500 to 2000 mg/L can cause a reduction in signal intensities. When spiked fish tissue samples are additionally diluted by a factor of 4 to reduce the dissolved solids to 0.5% or less, most of the observed signal suppression biases of 5% to 15% are eliminated. However, with dilution the MDLs are raised and the precision and accuracy of low level analyses are affected. Since the suppression effect on each element is nearly constant over the narrow concentration range of interest, the slight negative bias experienced is considered acceptable for the advantage of lower detection limits.

4.6 The number of interelement spectral interferences in the fish tissue matrix is minimal. Listed below are all interelement correction factors determined for the wavelengths and background correction locations recommended in this method. Obviously, these factors are only applicable to the instrument used in the development of this method. However, they can be used as a guide and are evidence that except for spiked samples, most fish tissue analyses would not require interelement correction factors. It should be noted that if a listed interferent is present at a concentration of 10 µg/g or less, its apparent concentration on the analyte channel is less than the analyte's determined MDL.

INTERELEMENT CORRECTION FACTORS

Analyte	Interferent	Factor
As	Al	+ .0080
As	Be	- .0027
As	Ni	- .0056
Cr	Cu	- .0007
Cr	Ni	+ .0006
Cr	Fe	- .0003
Pb	Al	- .0234
Pb	Cu	+ .0008
Sb	Cr	+ .0150
Sb	Ni	- .0087
Se	Fe	- .0205
Tl	Molecular	+ .0036
Zn	Cu	+ .0013
Zn	Ni	+ .0039

A 1 µg/g concentration of interferent would either add to or subtract from the analyte an apparent concentration in µg/g equal to the value of the correction factor.

4.7 The following "off-the-line" background correction locations should be avoided because of existing spectral interference.

4.7.1 The low side (- 0.07 nm) of the 190.8 nm thallium wavelength has a spectral interference from phosphorus.

4.7.2 Background correction on the low side of the 193.7 nm arsenic wavelength below - 0.06 nm may result in a severe negative bias.

4.7.3 The high side (+ 0.07 nm) of the 196.0 nm selenium wavelength has a severe undefined spectral interference originating from the tetramethylammonium hydroxide.

4.7.4 Background correction on the low side of the 259.9 nm iron wavelength below - 0.06 nm may result in spectral interference from 259.8 nm iron wavelength.

4.7.5 The low side (- 0.05 nm) of the 308.2 nm aluminum wavelength has a spectral interference from argon.

4.7.6 The low side (- 0.04 nm) of the 213.8 nm zinc wavelength read in the 2nd order has a weak spectral interference from magnesium.

5. SAFETY

5.1 All personnel handling environmental samples known to contain or to have been in contact with human waste should be immunized against known disease causative agents.

5.2 Precautions should also be taken to minimize potential bacterial infections from handling and dissecting fish. Basic good housekeeping and sanitation practices and use of rubber or plastic gloves is recommended.

5.3 Mobile and remote sampling locations should be equipped with a communication system to summon help in case of an emergency. It is recommended that field personnel not work alone.

5.4 Material safety data sheets for all chemical reagents should be available to and understood by all personnel using this method. Specifically, tetramethylammonium hydroxide (25%), hydrogen peroxide (50%) and concentrated nitric acid are moderately toxic and extremely irritating to skin and mucus menbranes. Use these reagents in a hood whenever possible and if eye or skin contact occurs, flush with large volumes of water. Always wear safety glasses or a shield for eye protection when working with these reagents.

6. APPARATUS AND EQUIPMENT

6.1 Tissue dissecting equipment

6.1.1 Dissecting Board: Polyethylene or other inert, nonmetallic material, any non-wetting, easy-to-clean or disposable surface is suitable. Adhesive backed Teflon or plastic film may be convenient to use.

6.1.2 Forceps: Plastic, Teflon or Teflon coated.

6.1.3 Surgical Blades: Disposable stainless steel with stainless steel or plastic handle. (See 4.1)

6.1.4 Scissors: Stainless steel.

6.1.5 Plastic bags with water tight seal, metal free.

6.1.6 Label tape: Self-adhesive, vinyl coated marking tape, solvent resistant, usable for temperatures from + 121° C to - 23° C.

6.1.7 Polyvinyl chloride or rubber gloves, talc-free.

6.2 Labware - All glassware, polysulfone and Teflon containers must be soaked and washed with detergent, rinsed with tap water, soaked in (1 + 1) nitric acid (7.3.1) rinsed again with tap water followed by deionized, distilled water (7.1). The use of chromic acid must be avoided.

6.2.1 Glassware: Class A volumetric flasks and pipets of various volumes.

6.2.2 Micropipets: Reusable graduated lambda micropipets with a 0.1 mL capacity.

6.2.3 Oak Ridge type centrifuge tubes: 30 mL capacity, polysulfone tube with polypropylene screw closure (available from most suppliers of laboratory equipment).

6.2.4 Storage bottles: Narrow-mouth bottles, Teflon FEP (fluorinated ethylene propylene) with Tefzel ETFE (ethylene tetrafluorethylene) screw closure, 125 mL and 250 mL capacities.

6.2.5 Wash bottle: One-piece stem, Teflon FEP bottle with Tefzel ETFE screw closure, 125 mL capacity.

6.3 Sample processing equipment

6.3.1 Pipet suction apparatus: Chrome plated metal with rubber adapter, made for use with lambda and other reusable micropipets. Clay Adams 4555, Curtin Matheson Scientific CAT 059-709 or equivalent.

6.3.2 Rinse stand and clamp to hold pipet suction apparatus.

6.3.3 Test tube rack: Polycarbonate tube size 25-30 mm, 3 x 8 array.

6.3.4 Dish pan: Pan of molded high density polyethylene, with an interior dimension of 14" x 12".

6.3.5 Single pan balance: Balance capable of weighing to the nearest 0.01 gram.

6.3.6 Analytical balance: Balance capable of weighing to the nearest 0.0001 gram.

6.3.7 Vortex mixer: Vortex mixer with neoprene mixing head and built-in rheostat control.

6.3.8 Centrifuge: Steel cabinet with guard bowl, capable of reaching 2000 r.p.m. compatible with centrifuge tubes (6.2.3), electric timer and brake.

6.3.9 Drying oven: Gravity convection oven, with thermostatic control capable of maintaining 65° C ± 5° C with an interior dimension no smaller than 14" x 6" x 6".

6.4 Analytical instrumentation

6.4.1 The ICP instrument may be a simultaneous or sequential spectrometer system that uses ionized argon gas as the plasma. However, the system and the processing of background corrected signals must be computer controlled. The instrument must be capable of meeting and complying with the requirements and description of the technique given in Section 2.2 of the method. The instrument must be equipped with a nebulizer capable of accepting 2% dissolved solids.

6.4.2 A variable speed peristaltic pump is required to deliver both standard and sample solutions to the nebulizer.

6.4.3 The use of mass flow controllers to regulate the argon flow rates, especially through the nebulizer, are highly recommended. Their use will provide more exacting control of reproducible plasma conditions.

7. REAGENTS AND CONSUMABLE MATERIAL

7.1 Deionized, distilled water: Prepare by passing distilled water through a mixed bed of cation and anion exchange resins. Use deionized, distilled water for the preparation of all reagents and as dilution or rinse water. The purity of this water must be equivalent to ASTM Type II reagent water of Specification D 1193 (5).

7.2 Hydrogen peroxide (H_2O_2)(CAS RN 7722-84-1), 50%, stabilized purity certified.

7.3 Nitric acid (HNO_3), conc. (sp.gr. 1.41) (CAS RN 7697-37-2), ACS reagent grade or equivalent. Redistilled acid is acceptable.

7.3.1 Nitric acid, (1+1): Add 500 mL conc. HNO_3 (7.3) to 400 mL deionized, distilled water (7.1) and dilute to 1 liter.

7.4 Hydrochloric acid (HCl), conc. (sp. gr. 1.19, CAS RN 7647-01-0); ACS reagent grade or equivalent.

7.4.1 Hydrochloric acid, (1+1): Add 500 mL conc. HCl (7.4) to 400 mL deionized, distilled water (7.1) and dilute to 1 liter.

7.5 Tetramethylammonium hydroxide [$(CH_3)_4NOH$], (CAS RN 75-59-2), TMAH 25% aqueous solution, electronic grade 99.9999% (metals basis) ALFA #20932 or equivalent.

7.6 Sodium hydroxide (NaOH) (CAS RN 1310-73-2), ACS reagent grade or equivalent.

7.7 Standard stock solutions may be purchased or prepared from ultra-high purity grade chemicals or metals. All salts must be dried for 1 h at 105° C unless specified otherwise.
(CAUTION: Wash hands thoroughly after handling.)
Typical stock solution preparation procedures follow:

7.7.1 Aluminum solution, stock (1 mL = 1000 µg Al) - Dissolve 0.100 gram aluminum metal in an acid mixture of 4 mL (1 + 1) HCl (7.4.1) and 1 mL conc. HNO_3 (7.3) in a beaker. Warm gently to effect solution. When solution is complete, transfer quantitatively to a 100 mL volumetric flask and dilute to the mark with deionized, distilled water (7.1). Store the solution in a screwcap Teflon FEP storage bottle (6.2.4).

7.7.2 Antimony solution, stock (1 mL = 1000 µg Sb) - Dissolve 0.2669 gram potassium antimonyl tartrate [$K(SbO)C_4H_4O_6$] (CAS RN 11071-15-1) in deionized, distilled water (7.1) and dilute to 100 mL in a volumetric flask. Store the solution in a screwcap Teflon FEP storage bottle (6.2.4).

7.7.3 Arsenic solution, stock (1 mL = 1000 µg As) - Dissolve 0.1320 gram arsenic trioxide (As_2O_3) (CAS RN 1327-53-3) in 20 mL deionized, distilled water (7.1) containing 0.4 g sodium hydroxide (NaOH) (7.6). Acidify the solution with 2 mL conc. HNO_3 (7.3) and dilute to 100 mL in a volumetric flask with deionized, distilled water. Store the solution in a screwcap Teflon FEP storage bottle (6.2.4).

7.7.4 Beryllium solution stock (1 mL = 500 µg Be) - Do not dry. dissolve 0.9830 gram beryllium sulfate ($BeSO_44H_2O$) in deionized, distilled water, (7.1), add 1.0 mL conc. HNO_3 (7.3) and dilute to 100 mL in a volumetric flask with deionized, distilled water. Store the solution in a screwcap Teflon FEP storage bottle (6.2.4).

7.7.5 Cadmium solution stock (1 mL = 1000 µg Cd) - Dissolve 0.100 gram cadmium metal in 4 mL conc. HNO_3 (7.3), dilute to 100 mL in a volumetric flask with deionized, distilled water. Store the solution in a screwcap Teflon FEP storage bottle (6.2.4).

7.7.6 Calcium solution stock (1 mL = 1000 µg Ca) - Suspend 0.2498 gram calcium carbonate ($CaCO_3$) dried at 180° C for 1 hr before weighing, in deionized, distilled water (7.1). Dissolve cautiously by adding dropwise a minimum amount of (1+1) HCl (7.4.1). Add 10.0 mL (1+1) HCl (7.4.1) and dilute to 100 mL in a volumetric flask with deionized, distilled water (7.1). Store the solution in a screwcap Teflon FEP storage bottle (6.2.4).

7.7.7 Chromium solution, stock (1 mL = 1000 µg Cr) - Dissolve 0.1923 gram chromium trioxide (CrO_3) in deionized, distilled water (7.1). When solution is complete, acidify with 1 mL conc. HNO_3 (7.3) and dilute to 100 mL in a volumetric flask with deionized, distilled water (7.1). Store the solution in a screwcap Teflon FEP storage bottle (6.2.4).

7.7.8 Copper solution, stock (1 mL = 1000 µg Cu) - Dissolve 0.100 gram copper metal in 2 mL conc. HNO_3 (7.3). Dilute to 100 mL in a volumetric flask with deionized, distilled water (7.1). Store the solution in a screwcap Teflon FEP storage bottle (6.2.4).

7.7.9 Iron solution, stock (1 mL = 1000 µg Fe) - Dissolve 0.100 gram iron metal in 4 mL (1+1) HCl (7.4.1). Dilute to 100 mL in a volumetric flask with deionized, distilled water (7.1). Store the solution in a screwcap Teflon FEP storage bottle (6.2.4).

7.7.10 Lead solution, stock (1 mL = 1000 µg Pb) - Dissolve 0.1613 gram lead nitrate [$Pb(NO_3)_2$] in a minimum amount of (1+1) HNO_3 (7.3.1). Add 5 mL conc. HNO_3 (7.3). Dilute to 100 mL in a volumetric flask with deionized, distilled water (7.1). Store the solution in screwcap Teflon FEP storage bottle (6.2.4).

7.7.11 Magnesium solution, stock (1 mL = 1000 µg Mg) - Dissolve 0.100 gram magnesium metal in 2 mL (1+1) HCl (7.4.1) and dilute to 100 mL in a volumetric flask with deionized, distilled water (7.1). Store the solution in a screwcap Teflon FEP storage bottle (6.2.4).

7.7.12 Nickel solution, stock (1 mL = 1000 µg Ni) - Dissolve 0.100 gram nickel metal in 5 mL hot conc. HNO_3 (7.3). Cool and dilute to 100 mL in a volumetric flask with deionized, distilled water (7.1). Store the solution in a screwcap Teflon FEP storage bottle (6.2.4).

7.7.13 Phosphorus solution, stock (1 mL = 1000 µg P) - Dissolve 0.3745 gram ammonium phosphate, monobasic [$(NH_4)H_2PO_4$] (CAS RN 7722-76-1) in deionized, distilled water (7.1) and dilute to 100 mL in a volumetric flask. Store the solution in a screwcap Teflon FEP storage bottle (6.2.4).

7.7.14 Potassium solution, stock (1 mL = 1000 µg K) - Dissolve 0.1907 gram potassium chloride (KCl) previously dried at 110° C for 3 hrs, in deionized, distilled water (7.1) and dilute to 100 mL in a volumetric flask. Store the solution in a screwcap Teflon FEP storage bottle.

7.7.15 Selenium solution, stock (1 mL = 1000 µg Se) - Dissolve 0.1414 gram selenium dioxide (SeO_2) in deionized, distilled water (7.1) and dilute to 100 mL in a volumetric flask. Store the solution in a screwcap Teflon FEP storage bottle (6.2.4).

7.7.16 Sodium solution, stock (1 mL = 1000 µg Na) - Dissolve 0.2542 gram sodium chloride (NaCl) in deionized, distilled water (7.1). Add 1.0 mL conc. HNO_3 (7.3) and dilute to 100 mL in a volumetric flask with deionized, distilled water (7.1). Store the solution in a screwcap Teflon FEP storage bottle (6.2.4).

7.7.17 Thallium solution, stock (1 mL = 1000 µg Tl) - Dissolve 0.1303 gram thallous nitrate ($TlNO_3$) in deionized, distilled water (7.1). Add 1.0 mL conc. HNO_3 (7.3) and dilute to 100 mL in a volumetric flask with deionized, distilled water (7.1). Store the solution in a screwcap Teflon FEP storage bottle (6.2.4).

7.7.18 Zinc solution, stock (1 mL = 1000 µg Zn) - Dissolve 0.100 gram zinc metal in 5 mL conc. HNO_3 (7.3). Dilute to 100 mL with deionized, distilled water (7.1). Store the solution in a screwcap Teflon FEP storage bottle (6.2.4).

7.8 Prepare four 100 mL mixed standard solutions by combining aliquots from the appropriate individual stock solutions (7.7) in volumetric flasks and diluting to the mark with deionized, distilled water (7.1). Prior to preparing the mixed standard solutions, each stock solution should be analyzed to determine purity and should be compared to a quality control check sample (10.2) to verify its concentration. For the wavelength and background correction positions recommended, prepare the mixed standard solution using the following listed aliquot volumes of the individual stock standards. Transfer the prepared mixed standard solutions in screwcap Teflon FEP storage bottles (6.2.4).

7.8.1 Mixed standard solution I (Volume = 100.0 mL)

Analyte	Stock Solution	Aliquot Vol., mL	Analyte Conc., µg/mL
Al	7.7.1	10.0	100
Ca	7.7.6	10.0	100
Cd	7.7.5	2.0	20
Cu	7.7.8	1.0	10
Mg	7.7.11	10.0	100
Sb	7.7.2	5.0	50
Se	7.7.15	5.0	50

7.8.2 Mixed standard solution II (Volume = 100.0 mL)

Analyte	Stock Solution	Aliquot Vol., mL	Analyte Conc., µg/mL
As	7.7.3	10.0	100
Cr	7.7.7	5.0	50

7.8.3 Mixed standard solution III (Volume = 100.0 mL)

Analyte	Stock Solution	Aliquot Vol., mL	Analyte Conc., µg/mL
Na	7.7.16	10.0	100
Pb	7.7.10	10.0	100
Tl	7.7.17	5.0	50
Zn	7.7.18	5.0	50

7.8.4 Mixed standard solution IV (Volume = 100.0 mL)

Analyte	Stock Solution	Aliquot Vol., mL	Analyte Conc., µg/mL
Be	7.7.4	2.0	10
Fe	7.7.9	10.0	100
K	7.7.14	20.0	200
Ni	7.7.12	2.0	20
P	7.7.13	10.0	100

7.9 Prepare four instrument calibration standards, each in 100 mL volumetric flask by adding in the following order 10 mL TMAH (7.5) and 5 mL H_2O_2 (7.2) to 10 mL of each of the four mixed standard solutions (7.8)(See Note 1). Allow the four solutions to stand open for 30 minutes to vent released oxygen. After standing, add 5 mL of conc. HNO_3 (7.3) to each solution and dilute to the mark with deionized, distilled water (7.1). Transfer the prepared calibration standards in screwcap Teflon FEP storage bottles (6.2.4).

Note 1: Prior to adding the TMAH, H_2O_2 and HNO_3 to calibration standard IV (7.9.4), add 1 mL of (1 + 1) HCl (7.4.1) and mix. The addition of HCl prevents the formation of a precipitate. Also, when H_2O_2 is added to calibration standard IV, it must be added dropwise to prevent sudden and violent effervescence.

7.9.1 Calibration Standard I (Volume 100.0 mL)

Analyte	Conc., µg/mL
Al	10.0
Ca	10.0
Cd	2.0
Cu	1.0
Mg	10.0
Sb	5.0
Se	5.0

7.9.2 Calibration Standard II (Volume = 100.0 mL)

Analyte	Conc., µg/mL
As	10.0
Cr	5.0

7.9.3 Calibration Standard III (Volume = 100.0 mL)

Analyte	Conc., µg/mL
Na	10.0
Pb	10.0
Tl	5.0
Zn	5.0

7.9.4 Calibration Standard IV (Volume = 100.0 mL) (See Note 1)

Analyte	Conc., µg/mL
Be	1.0
Fe	10.0
K	20.0
Ni	2.0
P	10.0

7.10 Prepare a calibration blank by diluting the combination solution of 10 mL TMAH (7.5), 5 mL H_2O_2 (7.2) and 5 mL conc. HNO_3 (7.3) to 100 mL in a volumetric flask with deionized, distilled water (7.1). Store the calibration blank in a screwcap Teflon FEP storage bottle (6.2.4).

7.11 Prepare a calibration check standard stock solution in a 100 mL volumetric flask by combining the following listed aliquot volumes of the individual stock standards and diluting to the mark with deionized, distilled water (7.1). Transfer the stock solution in a screwcap Teflon FEP storage bottle (6.2.4).

Analyte	Stock Solution	Aliquot Vol., mL	Analyte Conc., µg/mL
Al	7.7.1	1.0	10.0
As	7.7.3	1.0	10.0
Be	7.7.4	2.0	10.0
Ca	7.7.6	2.0	20.0
Cd	7.7.5	1.0	10.0
Cr	7.7.7	1.0	10.0
Cu	7.7.8	1.0	10.0
Fe	7.7.9	1.0	10.0
K	7.7.14	10.0	100.0
Mg	7.7.11	2.0	20.0
Na	7.7.16	2.0	20.0
Ni	7.7.12	1.0	10.0
P	7.7.13	10.0	100.0
Pb	7.7.10	1.0	10.0
Sb	7.7.2	1.0	10.0
Se	7.7.15	1.0	10.0
Tl	7.7.17	1.0	10.0
Zn	7.7.18	1.0	10.0

7.11.1 At the time of calibration prepare the calibration check standard (3.3) in a 100 mL volumetric flask by adding in the following order, 10 mL TMAH (7.5), 5 mL H_2O_2 (7.2) and 5 mL conc. HNO_3 (7.3) to 10 mL of the calibration check standard stock solution (7.11) and diluting to the mark with deionized, distilled water (7.1). Transfer the calibration check standard in a screwcap Teflon FEP storage bottle (6.2.4).

Analyte	Calibration Check Std. Conc., µg/mL
Al	1.0
As	1.0
Be	1.0
Ca	2.0
Cd	1.0
Cr	1.0
Cu	1.0
Fe	1.0
K	10.0
Mg	2.0
Na	2.0
Ni	1.0
P	10.0
Pb	1.0
Sb	1.0
Se	1.0
Tl	1.0
Zn	1.0

7.12 Prepare the laboratory control standard stock solution in a 200 mL volumetric flask by combining the following listed aliquot volumes of the individual stock solution and diluting to the mark with deionized, distilled water (7.1). Transfer the laboratory control standard solution in a screwcap Teflon FEP storage bottle (6.2.4).

Analyte	Stock Solution	Aliquot Vol., mL	Analyte Conc. µg/mL
Al	7.7.1	10.0	50
As	7.7.3	10.0	50
Be	7.7.4	1.0	2.5
Cd	7.7.5	1.0	5.0
Cr	7.7.7	2.0	10
Cu	7.7.8	5.0	25
Ni	7.7.12	5.0	25
Pb	7.7.10	5.0	25
Sb	7.7.2	5.0	25
Se	7.7.15	10.0	50
Tl	7.7.17	5.0	50
Zn	7.7.18	10.0	50

7.13 Prepare an instrument wash acid solution by diluting 50 mL of conc. HNO_3 (7.3) to 1 liter with deionized, distilled water (7.1). Store in a convenient manner. This solution is to be used to flush the solution uptake system and nebulizer between standards and samples.

7.14 Ice, crushed

8. SAMPLE COLLECTION, PRESERVATION AND STORAGE

8.1 Fish samples are collected using a variety of equipment, methods and techniques such as trot lines, trawls, seines, dredges, nets, ichthyocides and electrofishing. The technique used must be free from contamination by metals. For example, permanganate may be used to detoxify Rotenone but should not come in contact with the fish to be analyzed (6).

8.2 Appropriate individual tissue samples should be taken soon after collection of the fish and must be taken prior to freezing(7). If dissection of the tissue cannot be performed immediately after collection, each fish should be placed in a plastic bag, sealed and placed on ice or refrigerated at approximately 4° C.

8.3 Prior to dissection, the fish should be rinsed with metal-free water and blotted dry. Dissection should be performed within 24 hours of collection. Each individual fillet sample should also be rinsed with metal-free water blotted dry, placed in a preweighed, labeled polysulfone centrifuge tube (6.2.3) and frozen at - 20° C or below (dry ice).

8.4 Skinless fillet samples of approximately 1-2 gm (1cm x 0.5 cm x 2 cm) should be cut from the fish using a special implement (See 4.1) and handled with plastic forceps (8,9).

8.5 A maximum holding time for frozen samples has not been determined.

9. CALIBRATION AND STANDARDIZATION

9.1 Specific wavelengths and background correction locations given in Table 1 and instrument operating conditions given in Table 2 should be used whenever possible. However, because of the differences among various makes and models of spectrometers, the analyst should follow the instrument manufacturer's instructions in adapting the instrument's operation to approximate the recommended operating conditions. Other wavelengths and background correction locations may be substituted if they can provide the needed sensitivity and are corrected for spectral interference.

9.2 Allow the instrument to become thermally stable before beginning. This usually requires at least 30 minutes of operation prior to calibration.

9.3 Profile the instrument and adjust the plasma to a previously established condition by regulating the argon flow rate through the nebulizer while monitoring the intensity ratio of selected atom/ion wavelengths [e.g., Cu(I) 324.75 nm/Mn(II) 257.61 nm]

9.4 Calibrate the instrument according to the instrument manufacturer's instructions using the prepared calibration blank (7.10) and calibration standards (7.9).

9.5 The following operational steps should be used for both standards and samples.

9.5.1 Using a peristalic pump introduce the standard or sample to nebulizer at a uniform rate (e.g., 1.2 mL $min.^{-1}$).

9.5.2 To allow equilibrium to be reached in the plasma, aspirate the standard or sample solution for 30 seconds after reaching the plasma before beginning integration of the background corrected signal.

9.5.3 Use the average value of four, 4 seconds background corrected integration periods as the atomic emission signal to be correlated to analyte concentration.

9.5.4 Between each standard or sample, flush the nebulizer and solution uptake system with the wash acid solution (7.13) for a period of 60 seconds.

9.6 Analyze the calibrations check standard (7.11.1) and blank (7.10) immediately following calibration, at the end of the analyses and periodically throughout the sample run. The analyzed value of the calibration check standard should be within an interval of 95% to 105% of the expected value. If the value is outside the interval, the instrument should be recalibrated and all samples following the last acceptable calibration check standard should be reanalyzed.

10. QUALITY CONTROL

10.1 Each laboratory using this method is required to operate a formal quality control (QC) program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the analysis of spiked samples as a continuing check on performance. The laboratory is required to maintain performance records that define the quality of data thus generated. Specific minimum QC requirements consist of:

10.1.1 Verify the purity and concentration of the single metal stock standard solutions (See 10.2).

10.1.2 Determine the method detection limit for each analyte of interest (See 10.3).

10.1.3 Analysis of method blanks to detect introduction of reagent and labware contamination (See 10.4).

10.1.4 Analysis of a laboratory control standard to demonstrate continuing laboratory performance (See 10.5).

10.1.5 Demonstration of the ability to generate acceptable data of known accuracy and precision with this method (See 10.6).

10.2 Prepare the quality control sample (3.4) in the same acid matrix as the diluted aliquot of the stock standard solution to be verified. The concentration of the analyte should be the same in both solutions and be within the range of 1 to 10 µg/mL. The concentration and purity of each stock single metal solution must be verified before preparation of the mixed standards (7.8). The concentration of the mixed standard solutions (7.8, 7.11 and 7.12) should be verified with a quality control sample (3.4) every 3 months.

10.3 The method detection limit (MDL) in µg/g must be determined for each of the following analytes: Al, As, Be, Cd, Cr, Cu, Ni, Pb, Sb, Se, Tl, and Zn. Except for As, Cu and Zn, the MDLs for all analytes must be determined in the fish tissue matrix. Because of background concentrations in fish tissue, the MDL determination of As, Cu and Zn should be completed by spiking the method blank (3.6) matrix. The MDL determinations should be made using seven replicate samples prepared as described in the procedure (11.) and with each sample analyzed from a separate and newly prepared calibration curve. The concentration of the spike in the sample should be approximately 3 times the estimated detection limit. The determined MDL values tested in Table 1 can be used as a guide. (Actual solution concentration in µg/mL are 10% the listed values.) Appropriate dilutions of the laboratory control standard stock solution (7.12) may be used for spiking.

10.4 A method blank (3.6) is to be analyzed with each group of samples. Prepare the method blank by transferring 1.0 mL TMAH (7.5) to a clean preweighed, labeled 30 mL polysulfone Oak Ridge type centrifuge tube (6.2.3). Carry the blank through the entire procedure (11.) as a 1.0 gram sample ending with a final solution volume of 10 mL. The method blank values for the following metals: Al, As, Be, Cd, Cr, Cu, Ni, Pb, Sb, Se, Tl, and Zn should be below the metal's respective MDL. If the method blank indicates contamination, attention should be given to the cleaning procedure and the purity of the reagents should be verified.

10.5 A laboratory control standard (LCS) (3.5) is to be analyzed with each group of samples. The LCS should contain the following metals: Al, As, Be, Cd, Cr, Cu, Ni, Pb, Sb, Se, Tl and Zn each

at a concentration of approximately 10 times its respective MDL. To prepare the LCS, pipet 0.1 mL of the laboratory control stock standard (7.12) into a clean preweighed, labeled 30 mL polysulfone Oak Ridge type centrifuge tube (6.2.3). Add 1 mL of TMAH (7.5) and carry the LSC through the entire procedure (11.) as a sample ending with a final volume of 10 mL. The analyzed values should be within ± 2 standard deviations of an established mean value determined from seven prior replicate analyses. Data in Table 3 may be used as a guide until a sufficient number of replicates have been determined. If an analyzed value is greater than ± 2 standard deviations, it is outside the warning limits. If it is greater than ± 3 standard deviations, the analysis is out of control. When the analysis is out of control, take appropriate steps (10.2) to verify the concentration of the LCS stock standard and calibration standards. Also, take steps to ensure random contamination is not operative.

10.6 To demonstrate precision and accuracy select one fish from each group of samples (20 or less) and at the time of dissection collect three adjacent 1.0 ± 0.1 gram fillets. Prepare and analyze two of the fillet samples as duplicates to determine precision. Spike the third fillet sample with a 0.1 mL aliquot of the laboratory control stock standard to estimate the accuracy of the analysis.

10.6.1 To measure the precision of the analysis, the relative difference (RD) between the duplicate analyses of Cu, Zn and other measurable metals is compared to a previously established critical relative difference (CRD) determined from 15 prior duplicate analyses of the same type of tissue and species of fish. The RD between sample duplicates is determined by dividing their difference in concentration by their mean concentration. The CRD can be calculated using the following equation:

$$CRD = 3.27\left[\frac{\sum_{i=1}^{n}\frac{Ri}{\bar{X}i}}{n}\right],$$

where: Ri = is the calculated difference between the duplicates in each set,

$\bar{X}i$ = is the mean value for the duplicate set, and

n = is the number (15) of duplicate sets analyzed.

10.6.2 To estimate accuracy the mean value of the duplicate analyses (10.6.1) is subtracted from the spiked fillet value and percent recovery of the spike is determined. A spike recovery outside the interval of 90% to 110% of the expected values for the metals Al, Cd, Pb and Zn, can be used to alert the analyst that the accuracy of the analysis is out of control. Dilute an aliquot of the spiked sample 1 + 1 with the calibration blank solution and reanalyze the sample. Increased percent recovery to within the desired interval indicates a physical, dissolved solids interference and that the accuracy is within the expected limits of the method. Recovery of all spiked metals should not be less than 85% or more than 115%.

11. PROCEDURE

11.1 At the start of sample processing, remove the cap from the preweighed, labeled centrifuge tube (6.2.3) containing the sample and reweigh the tube to determine the weight of the tissue by difference. This can be done using a single pan balance; wipe the outside of the centrifuge tube with a Kimwipe or suitable paper tissue and place the tube upright in a tared 100 mL Griffin beaker. The weight of the tissue should be between 1 and 2 grams and expressed to the nearest 10 milligrams. Record the tissue weight.

11.2 Using a 2 mL graduated pipet add a volume of 25% tetramethylammonium hydroxide (TMAH) (7.5) equal to the weight of the tissue (1 mL TMAH = 1 gram tissue). The aliquot of TMAH should be to the nearest tenth of a mL equal to the tissue weight (e.g., 1.6 mL of TMAH for 1.62 grams of tissue). With the TMAH added, replace and tighten the cap securely. (This will minimize the odor caused in heating the sample mixture.) Place the sample in an open rack for adequate heating and place the rack in a drying oven preheated to 65° C ± 5° C and warm the sample for one hour.

11.3 After an hour of heating, remove the sample from the oven, retighten the cap if loose, and mix the sample for a few seconds using a vortex mixer set at median power setting. Return the sample to the drying oven and heat for an additional hour.

11.4 After the second hour of heating, again vortex mix the sample and place the mixed sample in an ice water bath for 30 minutes. This can be done by placing the entire sample rack in a pan of ice water of sufficient volume to envelop the base of the tube to just above the level of the sample liquid. After 30 minutes, remove the cooled sample from the bath and add 0.5 mL of cold 50% hydrogen peroxide (7.2). Immediately recap the tube and tighten the cap securely. DO NOT MIX THE SAMPLE. The action of the peroxide will begin soon after its addition, indicated by foaming inside the

tube. It is essential that the cap be securely tightened to prevent losses. If the peroxide action is extremely vigorous, return the sample to the ice water bath to slow the reaction. If more than one sample is being prepared, treat each sample individually before proceeding to the next sample. Allow the capped sample to stand overnight at room temperature for the available oxygen to react and to complete the limited oxidation process.

11.5 The following morning, vortex mix and then acidify the sample with conc. nitric acid (7.3) to between 4% and 5% (v/v) acid. The volume of nitric acid added to each sample is based on the final volume of sample. The final sample volume is calculated by multiplying the wet tissue weight by 10. Using a 1 mL graduated pipet add the appropriate volume of nitric acid as indicated in the following table:

Weight of Tissue, g	Final Sample Volume, mL	Volume of Conc. HNO_3 Added, mL
0.80 - 1.04	8 to 10	0.4
1.05 - 1.24	10 to 12	0.5
1.25 - 1.44	12 to 14	0.6
1.45 - 1.64	14 to 16	0.7
1.65 - 1.84	16 to 18	0.8
1.85 - 2.04	18 to 20	0.9
2.05 - 2.24	20 to 22	1.0

After the acid addition, recap the tube and vortex mix the sample. Return the tube to the drying oven preheated to 100° C and heat the sample for an hour to solubilize the metals before proceeding. Note: After the acid is added, solids will fall out of solution and a precipitate will form. This is normal and to be expected.

11.6 After the period of solubilization, cool the tube to room temperature. Uncap the tube and place the tube on the single pan balance in a tared 100 mL Griffin beaker. Adjust the final volume of the sample by adding deionized, distilled water from a "squeeze" wash bottle (6.2.5) while weighing the tube to an appropriate weight to maintain the contant weight/volume ratio of 1 gram/10 mL. The appropriate weight is calculated by multiplying the wet tissue weight by 10 and adding the product to the recorded weight of the empty tube.

11.7 After dilution is completed, recap the tube and vortex mix the sample. After mixing, centrifuge the sample at 2000 r.p.m. for 10 minutes. After centrifuging, the sample may contain floatable solids as a surface layer as well as the precipitate. Also, some

particles may adhere to the wall of the tube. This condition is normal and should not cause concern unless the analysis solution actually contains suspended material. In this situation the sample may require filtration through glass wool and also necessitate filtration of the method blank (See 10.2) to verify the absence of contamination from the glass wool. The sample is now ready for analysis. Analyze the sample within 24 hours of preparation (See 4.2).

11.8 Aspirate the sample into the ICP using the same operating conditions used in calibration (9.) while making certain the precipitate is not disturbed and inadvertently aspirated. If the surface of the analysis solution is partially covered with floatable solids, proceed by removing the tip of the aspiration tube from the wash solution (7.13) and allow an air bubble segment to form in the sample uptake line. Reverse the pump flow and while back pumping the air bubble insert the aspiration tube past the floatable solids into the sample solution. Change the pump flow back to uptake direction and aspirate the sample.

11.9 If a determined analyte of interest exceeds 10 µg/g dilute the sample (1 + 3) with calibration blank solution (7.10) and reanalyze the sample to verify the determined concentration.

12. CALCULATIONS

12.1 If dilutions are performed, the appropriate factor must be applied to sample values.

12.2 Data read from the instrument in µg/mL should be rounded to the thousandth place.

12.3 To express the data in concentrations of µg/g wet tissue weight multiply the rounded µg/mL data by a factor of 10.

12.4 Report µg/g wet tissue weight data up to three significant figures.

12.5 Do not report data below the determined MDL.

13. PRECISION AND ACCURACY (Single laboratory, EMSL-Cincinnati)

13.1 The analyses data presented in Tables 4, 5 and 6 were generated without the use of heating the sample to solubilize the metals after the addition of nitric acid. The heating step was added during the evaluation of the method primarily to improve the nature and appearance of the processed sample and to facilitate ICP analyses. Although the complete usefulness of the heating step is not known at this time, it is anticipated that an increase in dissolved solids will occur.

13.2 Table 4 lists accuracy data from two groups of seven bluegill fillet samples. All fillets were taken from the same fish, and spiked with 12 analytes (Al, As, Be, Cd, Cr, Cu, Ni, Pb, Sb, Se, Tl and Zn) at two different concentration levels. The concentration

of each analyte added to the first group of fillets was approximately 40% of the spike concentration added to the second group. The spike concentration selected for each analyte was a convenient concentration for pipetting and for most analytes similar multiple of the determined MDL concentration. (See Table 1 for a listing of the determined MDL concentration for each analyte.) The spike concentration used for Cu and Zn were higher MDL multiples because of naturally occurring concentrations in the bluegill fillet. These data are expressed as the mean concentration of the recovered spike and percent recovery based on the theoretical value. Seven separate calibrations were used in these determinations with one sample from each group being analyzed on each calibration.

13.3 Table 5 lists the precision data that corresponds to the accuracy data given in Table 4. These data are expressed as standard deviation and relative standard deviation around the mean concentration of the recovered spike.

13.4 Table 6 lists the mean, standard deviation, relative standard deviation, median and range values from the analyses of 14 unspiked fillet samples. These fillets were used as controls and taken from the same bluegill fish used in the accuracy and precision study described in 13.2 and 13.3.

13.5 Table 7 lists comparative data to a vigorous nitric acid-hydrogen peroxide digestion. Twelve fillets were taken from the same bluegill fish that had been exposed for four days to the following metal concentrations:

Metal	Nominal Concentration, mg/L
Al	0.5
Be	0.5
Cd	1
Cu	0.5
Ni	2
Pb	5
Sb	1
Zn	2

The first group of four fillets was processed according to procedure given in this method.

The second group of four fillets was processed as described in procedure of this method with the exception that the samples were not heated during the acid solubilization step.

The third group of four fillets was processed using a vigorous nitric acid-hydrogen peroxide digestion with the final dilution containing 5% (v/v) hydrochloric acid.

13.6 Table 8 lists the mean, standard deviation and percent recovery data from the analysis of four 0.25 gram aliquots of NBS SRM 1566 Oyster Tissue. These analyses were completed utilizing the heating step following the addition of nitric acid.

14. REFERENCES

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Table 1. Recommended Wavelengths with Locations For Background Correction and Method Detection Limits (MDL)

Analyte	Wavelength,[1] nm	Location for Bkgd. Correction	MDL, µg/g Wet Tissue Weight
Al	308.215	+ 0.061 nm	0.3
As	193.696	+ 0.061 nm	0.4*
Be	313.042	- 0.061 nm	0.02
Ca	315.887	+ 0.061 nm	-
Cd	226.502	+ 0.061 nm	0.02
Cr	205.552 X 2	- 0.030 nm	0.05
Cu	324.754	- 0.061 nm	0.05*
Fe	259.940	+ 0.061 nm	-
K	766.491	- 0.061 nm	-
Mg	279.079	- 0.061 nm	-
Na	588.995	+ 0.061 nm	-
Ni	231.604 X 2	- 0.030 nm	0.08
P	214.914 X 2	+ 0.030 nm	-
Pb	220.353	+ 0.061 nm	0.2
Sb	206.883	+ 0.061 nm	0.2
Se	196.026	- 0.061 nm	0.6
Tl	190.864	+ 0.061 nm	0.5
Zn	213.856 X 2	+ 0.030 nm	0.07*

(1) Wavelength X 2 indicates wavelength is read in second order.
(*) MDL determined in method blank matrix.

Table 2. Inductively Coupled Plasma Instrument Operating Conditions

Forward rf power	1100 watts
Reflected rf power	< 5 watts
Viewing height above work coil	16 mm
Argon supply	Liquid argon
Argon pressure	40 psi
Coolant argon flow rate	19 L min^{-1}
Aerosol carrier argon flow rate	630 cc min^{-1}
Auxillary (plasma) argon flow rate	300 cc min^{-1}
Sample uptake rate controlled to	1.2 mL min^{-1}

Table 3. Laboratory Control Standards
Precision and Accuracy Data[1]
Concentration, µg/g

	LEVEL #1			LEVEL #2		
ANALYTE	THEO. VALUE	ANALYSIS MEAN	RSD[2]	THEO. VALUE	ANALYSIS MEAN	RSD[2]
Al	2.00	1.98	7.3%	5.00	4.93	1.7%
As	2.00	2.05	6.5%	5.00	5.15	2.3%
Be	0.10	0.10	5.4%	0.25	0.25	1.7%
Cd	0.20	0.21	1.9%	0.50	0.51	1.7%
Cr	0.40	0.41	4.3%	1.00	1.00	2.4%
Cu	0.80	0.80	2.3%	2.00	1.98	0.7%
Ni	1.00	0.97	3.0%	2.50	2.45	1.0%
Pb	1.00	1.01	7.5%	2.50	2.47	2.2%
Sb	1.00	0.99	5.9%	2.50	2.53	2.8%
Se	1.00	1.01	19.0%	2.50	2.56	5.0%
Tl	2.00	2.01	5.5%	5.00	5.02	2.3%
Zn	4.00	3.96	1.2%	10.0	9.85	0.9%

1. The data are the result of analyzing one laboratory control standard solution at each level on seven separate calibrations.
2. RSD is relative standard deviation.

Table 4. Accuracy Data in Bluegill Fillet
Concentration, µg/g Wet Tissue Weight

ANALYTE	LEVEL #1			LEVEL #2		
	THEO. SPIKE VALUE	ANALYSIS MEAN	PERCENT RECOVERED	THEO. SPIKE VALUE	ANALYSIS MEAN	PERCENT RECOVERED
Al	2.00	1.75	88%	5.00	4.42	88%
As	2.00	1.91	96%	5.00	4.72	94%
Be	0.10	0.10	100%	0.25	0.25	100%
Cd	0.20	0.19	95%	0.50	0.46	92%
Cr	0.40	0.35	88%	1.00	0.89	89%
Cu	0.80	0.75	94%	2.00	1.85	93%
Ni	1.00	0.90	90%	2.50	2.21	88%
Pb	1.00	0.92	92%	2.50	2.21	88%
Sb	1.00	0.94	94%	2.50	2.27	91%
Se	1.00	1.13	113%	2.50	2.60	104%
Tl	2.00	2.13	107%	5.00	4.85	97%
Zn	4.00	3.72	93%	10.00	8.70	87%

Table 5. Precision Data in Bluegill Fillet
Concentration, µg/g Wet Tissue Weight

	LEVEL #1			LEVEL #2		
ANALYTE	ANALYSIS MEAN(1)	STD. DEV.	RSD(2)	ANALYSIS MEAN(1)	STD. DEV.	RSD(2)
Al	1.75	0.081	4.6%	4.42	0.195	4.4%
As	1.91	0.142	7.4%	4.72	0.203	4.3%
Be	0.10	0.004	4.0%	0.25	0.005	2.0%
Cd	0.19	0.005	2.6%	0.46	0.018	3.9%
Cr	0.35	0.020	5.7%	0.89	0.038	4.3%
Cu	0.75	0.018	2.4%	1.85	0.048	2.6%
Ni	0.90	0.017	1.9%	2.21	0.050	2.3%
Pb	0.92	0.081	8.8%	2.21	0.107	4.8%
Sb	0.94	0.057	6.1%	2.27	0.107	4.8%
Se	1.13	0.178	15.7%	2.60	0.268	10.3%
Tl	2.13	0.088	4.1%	4.85	0.145	3.0%
Zn	3.72	0.199	5.3%	8.70	0.342	3.9%

1. Analysis mean of the spike value tested in Table 4.
2. RSD is relative standard deviation.

Table 6. Analyses Data of Bluegill Fillets[1]
Concentration, µg/g Wet Tissue Weight

ANALYTE	ANALYSIS MEAN	STD. DEV.	RSD[2]	CALCULATED MEDIAN	RANGE
As	0.64	0.189	29.5%	0.66	0.28 - 0.97
Ca	192	130	67.7%	132	113 - 511
Cu	0.16	0.033	20.6%	0.16	0.10 - 0.22
Fe	0.98	0.132	13.4%	0.94	0.76 - 1.17
K	3980	94	2.4%	4010	3750 - 4060
Mg	313	10	3.2%	313	294 - 332
Na	255	12	4.7%	256	236 - 276
P	2220	81	3.6%	2220	2130 - 2390
Zn	4.49	0.280	6.2%	4.57	3.89 - 4.87

1. 14 fillets taken from one fish.
2. RSD is relative standard deviation.

Table 7. Comparative Data to Nitric Acid - Hydrogen Peroxide Digestion
Concentration, µg/g Wet Tissue Weight

	TMAH PREPARATION				ACID DIGESTION	
	HEATED		UNHEATED			
ANALYTE	ANALYSIS MEAN	STD. DEV.	ANALYSIS MEAN	STD. DEV.	ANALYSES MEAN	STD. DEV.
Al	< 0.3	--	< 0.3	--	2.88	1.22
As	0.43	0.075	< 0.4	--	< 0.3	--
Be	0.22	0.156	0.07	0.026	0.13	0.096
Ca	170	68	111	17	131	64
Cd	0.07	0.024	0.05	0.029	0.06	0.019
Cr	< 0.05	--	< 0.05	--	< 0.05	--
Cu	0.29	0.106	0.23	0.069	0.25	0.088
Fe	1.21	0.328	1.04	0.179	1.60	0.240
K	3470	33	3540	63	3430	136
Mg	268	5.1	272	9.3	266	12
Na	92.4	7.79	96.0	5.90	110	12
Ni	0.22	0.067	0.16	0.045	0.19	0.070
P	2120	69	2060	52	1900	52
Pb	1.44	1.02	0.63	0.631	1.00	0.755
Sb	< 0.2	--	< 0.2	--	< 0.2	--
Se	< 0.6	--	< 0.6	--	< 0.6	--
Tl	< 0.5	--	< 0.5	--	< 0.5	--
Zn	3.72	0.509	3.71	0.347	3.40	0.500

Table 8. Analyses Data - NBS SRM 1566 Oyster Tissue
Concentration, µg/g Dry Weight

ANALYTE	PUBLISHED CERTIFIED VALUE	ANALYSIS MEAN	STD. DEV.	PERCENT RECOVERED	DILUTION(1) MEAN	PERCENT RECOVERED
As	13.4 ± 1.9	12.9	0.39	96%	12.3	91%
Ca	1500 ± 200	1290	73	86%	1360	91%
Cd	3.5 ± 0.4	3.11	0.029	89%	3.14	90%
Cr	0.69 ± 0.27	< 0.05	—	--	N.D.(2)	—
Cu	63.0 ± 3.5	59.0	0.54	94%	60.0	95%
Fe	195 ± 34	134	3.0	69%	141	72%
K	9690 ± 50	8620	115	89%	9500	98%
Mg	1280 ± 90	1120	5	88%	1220	95%
Na	5100 ± 300	4550	48	89%	4830	95%
Ni	1.03 ± 0.19	0.77	0.093	75%	N.D.	—
P	8100*	7110	52	88%	7340	91%
Pb	0.48 ± 0.04	0.53	0.303	110%	N.D.	—
Se	2.1 ± 0.5	2.47	0.656	118%	N.D.	--
Zn	852 ± 14	743	4.7	87%	830	97%

1. The dilution mean is the reanalyses of the prepared aliquots combined and diluted 1 + 3 with calibration blank solution. Reported concentration adjusted for dilution.

2. N.D. - Not detected below MDL.

* Phosphorus value not certified.

DATE: March 30, 1987

SUBJECT: Disposition of Comments, Deliverable 1320[A], EMSL-Cincinnati No. 733, Method 200.11 Determination of Metals in Fish Tissue by Inductively Coupled Plasma-Atomic Emission Spectrometry

FROM: Theodore D. Martin, Research Chemist
Inorganic Analyses Section
Physical and Chemical Methods Branch

TO: Robert L. Booth, Director
Environmental Monitoring and Support
Laboratory - Cincinnati

Method 200.11 Determination of Metals in Fish Tissue by Inductively Coupled Plasma-Atomic Emission Spectrometry was completed on March 6, 1987, and distributed to twelve reviewers for concurrent technical and administrative review. Nine of the twelve reviewers returned comments by the required date of March 20, 1987. All reviewers found the method acceptable, requiring only minor revisions as noted with comments in the margins of the text.

The final revision (1.3) of the method, based on the comments received that did not require speculative interpretation, was completed March 26, 1987. The most notable change was the incorporation in the solubilization procedure of a heating step following the addition of nitric acid. Although this change does not appear to significantly improve recoveries, it did facilitate the spectrometric analysis of the processed sample. The change is discussed in the precision and accuracy section of the method.

EMSL-CI:TDMartin:lbh:STC:rm.554:x7312:0600d:3/30/87

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF RESEARCH AND DEVELOPMENT
ENVIRONMENTAL MONITORING AND SUPPORT LABORATORY
CINCINNATI, OHIO 45268

DATE:

SUBJECT: Transmittal of Environmental Monitoring and Support Laboratory - Cincinnati (EMSL-Cincinnati) Deliverable No. 1320[A], EMSL-Cincinnati No. 733, "Chemical Methods for Extraction of Inorganic Pollutants from Biological Tissue"

FROM: Robert L. Booth, Director
Environmental Monitoring and Support
Laboratory - Cincinnati

TO: William A. Whittington, Director
Office of Water Regulations and Standards (WH-551)
Office of Water Programs

Attached are two copies of Method 200.11 "Determination of Metals in Fish Tissue by Inductively Coupled Plasma-Atomic Emission Spectrometry" which is being submitted in fulfillment of Deliverable Item No. 1320[A] "Chemical Methods for Extraction of Inorganic Pollutants from Biological Tissue" authored by Theodore Martin, Eleanor Martin, and Gerald McKee of the Inorganic Analyses Section, Larry Lobring, Quentin Pickering, and William Horning of the Aquatic Biology Section, EMSL-Cincinnati.

The purpose of this research was to develop and standardize an analytical method for the measurement of metals in fish tissue that is less time consuming, more precise and equally accurate as the existing procedure. The method currently recommended for fish tissue analyses is a difficult, whole fish analyses that requires a wet digestion with sulfuric acid, dry ashing of 450° C, wet digestion with nitric acid and a dissolution step followed by atomic absorption measurement. Most regional laboratories are using their own variations of this method that eliminates some of these steps. The attached method requires a dissociation step using tetramethylammonium hydroxide, heat, oxidation with hydrogen peroxide, dissolution with nitric acid and measurement by inductively coupled plasma atomic emission spectrometry. This method is applicable for the analyses of aluminum, antimony, arsenic, beryllium, cadmium, calcium, chromium, copper, iron, lead, magnesium, nickel, phosphorus, selenium, sodium, thallium and zinc. It contains single laboratory precision, accuracy and method detection limit data we generated using the method and a section detailing the quality assurance practices we believe are considered necessary to produce valid data.

This product has been prepared according to Agency format and satisfies the objective for the analysis of edible fish fillets for the elements specified. This method is less time consuming, more precise and as accurate as the Agency recommended method. We plan to distribute this method to the

regional laboratories for comment prior to further method development. We plan on continuing research on this technique for additional types of tissue and more analytes. If your staff or others are interested in discussing any of the technical details of the report, please contact Gerald McKee at 684-7372 or Ted Martin at 684-7312.

Attachment: (2)
As Stated

cc: Larry Jensen without attachment
Vaun Newill without attachment
Donald Ehreth with attachment
Courtney Riordan with attachment
Elenora Karicher with attachment
Edmund Notzon with attachment
Frederick Leutner with attachment
Charles Plost with attachment
Cornelius Weber with attachment
Betty Thomas with attachment
James Lichtenberg with attachment
Gerald McKee with attachment
Kathie Fieler with two copies of memorandum and attachment