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Title: Determination of Metals by ICP-MS		
Revision: 03	Replaces: CLG-TM3.02	Effective: 08/24/2012

Contents

A. INTRODUCTION

1. Summary of Procedure

   Sample is digested with concentrated nitric acid in a microwave digestion apparatus. The sample digest is diluted, fortified with internal standards, and analyzed using inductively coupled plasma mass spectrometry (ICP-MS).

2. Applicability

   This method is suitable for quantification of the analytes listed below.

   Table 1 – Applicable Analytes

Metal	Applicable Matrices	Applicable Species	MLA (ppb)
Lead (Pb)	Liver, kidney, muscle, processed products	Beef, pork, poultry, Catfish (muscle only)	≥ 25
Cadmium (Cd)	Liver, kidney, muscle, processed products	Beef, pork, poultry, Catfish (muscle only)	≥ 10
Selenium (Se)	muscle, processed products	Beef, pork, poultry	≥ 500
Manganese (Mn)	muscle, processed products	Beef,pork, poultry	≥ 200
Molybdenum (Mo)	muscle, processed products	Beef, pork, poultry	≥ 50
Thallium (Tl)	muscle, processed products	Beef, pork,poultry	≥ 50
Cobalt (Co)	muscle, processed products	Beef, pork, poultry	≥ 25

Note: Refer to 21CFR for tolerance values set by FDA and 40CFR for tolerance values set by EPA.

B. EQUIPMENT

Note: Equivalent equipment may be substituted.

1. Apparatus
   a. Analytical balance - sensitive to 0.1 mg, Mettler, PG403-S
   b. MarsXpress Microwave and MarsXpress Digestion System, CEM.

c. MarsXpress Microwave Digestion vessels - 55 mL capacity liners, vent plugs, and screw caps, CEM, cat. nos. 574125 and 212020.

d. Vacuum Concentration/Drying apparatus - Microvap accessory set for MarsXpress system, CEM.

e. Vacuum Scrubber Module and Dual Head Vacuum Pump, 120V/60Hz, CEM.

f. Stirring rods (optional) - Teflon or polypropylene, Lab Depot, cat. no. F377390001.

g. Volumetric flasks - polypropylene or polymethylpentane, 50, 100, 1000 mL, class A, Lab Depot cat. no. 5460P-50, 5640P-100, 5640P-1L .

h. Volumetric flasks - glass, 10 - 1000 mL, as needed for preparation of standards, reagents class A, VWR cat. no. 89000-398 (10 mL) - 89000-412 (1000 mL).

i. Micropipettors - fixed or variable, covering ranges 10 - 5000 µL.

j. Bottles - polypropylene, 100 and 250 mL, Fisher, cat no. 02-893A, and 02-893B.

k. Centrifuge tubes - polypropylene, 50 mL, Fisher Scientific, cat. no. 06-443-18.

l. Argon gas, high purity grade (99.99%).

m. Syringe filter (optional) - Acrodisc CR 13 mm, with 0.2 µm PTFE Membrane, Gelman Laboratory, VWR, cat no. 28143-982.

n. Milestone Trace Clean (optional).

o. Helium gas, ultra high purity ≥ 99.999%

p. Hydrogen gas, ultra high purity ≥ 99.999%

2. Instrumentation

a. Inductively Coupled Plasma Mass Spectrometer - Agilent model 7500 ce, equipped with an Octapole Reaction System (ORS).

C. REAGENTS AND SOLUTIONS

Note: Equivalent reagents / solutions may be substituted. The stability time frame of the solution is dependant on the expiration date of the components used or the listed expiration date, whichever is soonest.

1. Reagents

a. Deionized water (DI water) - for cleaning only.

b. Millipure water - Deionized water polished to ASTM CAP/NCCLS Type 1 specifications or better (resistance ≥ 18 megaohms).

c. Nitric acid (HNO_3) - concentrated. Ultra-pure grade (Optima by Fisher or Double

Distilled by GFS) recommended. For samples, standards and reagents.

d. Nitric acid (HNO_3) – concentrated. Metal analysis or ICP grade (e.g., OmniTrace by EMD). For cleaning only.

e. Sodium Hydroxide (NaOH) - reagent grade.

f. Mass spectrometer tuning solution (10 µg/L) - Lithium, Yttrium, Cerium, Thallium, and Cobalt in 2% HNO_3, Cat No. 5184-3566, Agilent Technologies.

2. Solutions

a. 25% NaOH solution (for evaporation scrubber):

Weigh 250 grams of NaOH into a 1 L volumetric flask and bring up to volume with water.

b. 2% HNO_3 solution:

Dilute concentrated HNO_3 1:50 with millipure water (e.g., 20 mL/1L). Prepare and store in polypropylene bottles.

Note: Additional HNO_3 concentrations may be necessary based on commercial standards.

D. STANDARD(S)

Note: Equivalent standards / solutions may be substituted. Purity and counterions are to be taken into account when calculating standard concentrations. The stability time frame of the solution is dependant on the expiration date of the components used or the listed expiration date, whichever ends sooner.

Note: All standards and solutions may be stored at room temperature.

1. Standard Information

All elemental standard and internal standard solutions are prepared from commercial reference standards, which are available at concentrations of 1,000 or 10,000 mg/L (µg/mL). Reference standards must be ICP-MS grade.

a. Elemental standard solutions

Table 2 - Analytical Standard Information

Company	**Name**	**Catalog No.**
Inorganic Ventures, Lakewood, NJ.	Cadmium	CGCD10-1
Inorganic Ventures, Lakewood, NJ	Cobalt	CGCO10-1
Inorganic Ventures, Lakewood, NJ	Lead	CG-PB10-1
Inorganic Ventures, Lakewood, NJ	Manganese	CGMN10-1
Inorganic Ventures, Lakewood, NJ	Molybdenum	CGMO10-1
Inorganic Ventures, Lakewood, NJ	Selenium	CGSE10-1
Inorganic Ventures, Lakewood, NJ	Thallium	CGTL10-1

Company	Name	Catalog No.
SCP Science, Champlain, NY	Cadmium	140-061-480
SCP Science, Champlain, NY	Cobalt	140-061-270
SCP Science, Champlain, NY	Lead	140-061-820
SCP Science, Champlain, NY	Manganese	140-061-250
SCP Science, Champlain, NY	Molybdenum	140-060-420
SCP Science, Champlain, NY	Selenium	140-061-340
SCP Science, Champlain, NY	Thallium	140-061-810

Table 3- Internal Standard Information

Company	Name	Catalog No.
SCP Science, Champlain, NY	Bismuth	140-061-831
SCP Science, Champlain, NY	Gallium	140-061-311
SCP Science, Champlain, NY	Germanium	140-060-320
SCP Science, Champlain, NY	Indium	140-061-491
SCP Science, Champlain, NY	Rhodium	140-062-451
SCP Science, Champlain, NY	Scandium	140-061-210
SCP Science, Champlain, NY	Terbium	140-061-651
SCP Science, Champlain, NY	Yttrium	140-061-391

2. Preparation of Standard Solution(s)

Important: Metals may be leached from glass by nitric acid. Store all standard solutions in polypropylene or other inert containers. If glassware is used, it should be cleaned with nitric acid and dedicated for trace metals analyses. Standards prepared in glassware should be used immediately or transferred to suitable containers for storage.

a. Internal standard (ISTD), (5000 µg/L):

Add volumes of reference standard solution(s) (see table 7) equivalent to 500 µg (e.g., 500 µL of a 1000 mg/L solution) to a 100 mL volumetric flask and dilute to 100 mL with 2% HNO_3. Mix.

Note: Other internal standards can be used as long as the element is not contained in the sample, the mass number is similar to that of the analyte, and the ionization potential is similar to that of the analyte.

b. Calibration Standards

Calibration standards are required for constructing a multipoint standard curve covering the range of analyte concentrations anticipated in samples.

Prepare intermediate standards by making dilutions of commercially available standard solutions into a concentration of HNO_3 similar to the commercial solution. Suggested concentrations, based on use of 1000 mg/L standards, are:

i. 10,000 µg/L:

Pipet 100 µL of 1000 mg/L standard to a 10 mL volumetric flask and dilute to volume.

ii. 1000 µg/L:

Pipet 10.0 mL of 10,000 µg/L solution (i) to a 100 mL volumetric flask and dilute to volume.

iii. 100 µg/L:

Pipet 1.00 mL of 10,000 µg/L solution (i) to a 100 mL volumetric flask and dilute to volume.

Prepare calibration standards by making appropriate dilutions of intermediate standards with 2% HNO_3 and adding sufficient 5000 µg/L ISTD to result in a final ISTD concentration of 5 µg/L. Prepare these standards using polymeric volumetric flasks.

The Table below lists some suggested concentrations for calibration standards and recommended volumes and concentrations of solutions required for preparation of 100 mL volumes of each.

Table 4 – Calibration Standards

Calibration STD Conc. [Sample Conc* in ()]	Amount used x Intermediate Standard concentration	Amount ISTD
Calibration Blank (0 ppb)	(2% HNO_3 Only)	100 µL
0.05 µg/L (5 ppb)	50 µL x 100 µg/L	100 µL
0.10 µg/L (10 ppb)	100 µL x 100 µg/L	100 µL
0.20 µg/L (20 ppb)	200 µL x 100 µg/L	100 µL
0.50 µg/L (50 ppb)	500 µL x 100 µg/L	100 µL
1.00 µg/L (100 ppb)	100 µL x 1000 µg/L	100 µL
2.00 µg/L (200 ppb)	200 µL x 1000 µg/L	100 µL
5.00 µg/L (500 ppb)	500 µL x 1000 µg/L	100 µL
10.00 µg/L (1000 ppb)	1000 µL x 1000 µg/L	100 µL

* Equivalent Analyte concentration in a sample in ppb, assuming a sample concentration of 0.01 g/mL (0.5 g/50 mL) in final extract.

c. Quality Control Standards

Prepare Quality Control standards from commercially available standard solution(s), e.g. 10,000 µg/L, obtained from a *different source* than that used to prepare Calibration Standards. Two types of quality control standard must be prepared:

i. QC Standard:

Prepare a combined metals standard having concentrations near the midpoint of the calibration curve, but different from those used in any calibration standard. Prepare in same manner calibration standards are prepared, diluting with 2% HNO_3 and adding sufficient 5000 µg/L ISTD to result in a final ISTD concentration of 5 µg/L.

ii. Intermediate Fortification Standard:

Use 10,000 µg/L metals standard solutions (D.2.b.i) to prepare an intermediate multimetal fortification standard with concentrations about 500 times the MLA, diluting to volume with 2% HNO_3.

Table 5 - Intermediate Fortification Standard Solution (IFSS) in 2% HNO_3

Analyte	**Standard Concentration** (µg/mL)	**Volume** (µL)/100 mL	**IFSS Concentration** (ng/mL)
Pb	10000	125	12500
Cd	10000	50	5000
Co	10000	125	12500
Mo	10000	250	25000
Tl	10000	250	25000
Mn	10000	1000	100000
Se	10000	2500	250000

iii. Fortification Standard:

Place 500 µL of the intermediate fortification standard in a 50 mL polymeric volumetric flask and dilute to volume with 2% HNO_3. 100 µL of the Fortification Standard added to control tissue is equivalent to the MLA of the metals (100 µL = 25 ppb Pb, 10 ppb Cd, 25 ppb Co, 50 ppb Mo, 50 ppb Tl, 200 ppb Mn, and 500 ppb Se) based on a 0.5 g sample weight. Note: Standards containing other concentration ratios of metals may be used if desired.

E. SAMPLE PREPARATION

Note: Since trace amounts of analyte metals are ubiquitous in the environment and may be present in dust particles, efforts should be made to avoid external contamination. All areas/materials involved in sample preparation and analysis should be kept as dust-free as possible to minimize the chance of contamination.

Samples must be thoroughly blended to assure uniformity prior to removal of a test

portion.

F. ANALYTICAL PROCEDURE

1. Preparation of Controls and Samples

   a. Weigh homogenized samples, and blank tissue sufficient to prepare the negative control(s), positive control(s), and check samples (approximately 0.5 g for muscle tissues, 0.5 - 1 g for liver and kidney[1]) to the nearest 0.01 g into a clean[2] microwave vessel liner. Teflon or polypropylene stirring rods may be used to manipulate samples.

      Note: Truly blank tissues may not be available. Use previously analyzed tissues having low analyte levels for this purpose.

      [1]Caution! Mixing sample types or sample weights may produce unacceptably large variations in pressures developed during digestion, possibly resulting in damage to vessels if unvented caps are used. In order to maintain relatively constant digestion conditions in all unvented vessels, analyst should digest like quantities of similar sample matrices in each batch.

      [2]Vessel liners, caps, stoppers, and tubing must be cleaned after each use to reduce the possibility of cross-contamination. Refer to Section J.2 for recommended cleaning procedure.

   b. Prepare positive control(s) by adding Fortification Standard to the tissue blank within the quantitation range.

2. Extraction Procedure

   a. Microwave Digestion

      i. Add 5 mL of concentrated HNO_3, Ultra-pure grade, to each vessel.

      ii. Assemble the vessel according to the manufacturer's instructions.

      iii. Place assembled vessels into the microwave according to the manufacturer's instructions.

      iv. Program oven with parameters demonstrated to safely and effectively digest samples (producing a clear digest when diluted). Recommended parameters are listed in the table below. It may be necessary to adjust these parameters to accommodate variations between individual instruments.

Power:	1200 Watts*
Ramp time:	10 minutes
Final temperature:	180 °C

Temperature hold time:	10 minutes
Cool down time:	10 minutes

*If there are less than eight vessels in the microwave the wattage can be lowered.

v. Initiate oven program and digest samples.

vi. Allow vessels to cool, then transfer to a fume hood and allow vessels to equilibrate to room temperature.

vii. Slowly open the vent fittings and vent to atmospheric pressure, then disassemble vessels.

b. Microwave Evaporation

i. Place vessel liners into the evaporation carousel and assemble according to the manufacturer's instructions. Note: Manufacturer recommends use of PTFE syringe filters with the evaporation manifold for trace metal analysis (optional).

ii. Place the evaporation assembly into the microwave.

iii. Program oven to reduce solution volumes to approximately 1 mL. Typical program parameters are listed below.

Power:	600 Watts
Ramp time:	5 minutes
Final temperature:	120 °C
Temperature hold time:	3.5 minutes*
Cool down time:	10 minutes

*Typical value required when 8 vessels are used. Hold times required to achieve a final volume of 1 mL for any given number of vessels must be determined experimentally.

Note: If the microwave is capable of determining an evaporation plateau temperature, a temperature drop of $\Delta T \approx 7$ °C may be used to control the final volume. This approach is more variable, but does not require adjustment for tissue type, tissue weight, or number of samples.

iv. Initiate oven program and evaporate samples.

v. Once vessels have cooled to room temperature, remove the evaporation assembly from the oven and dismantle. Flush the evaporation manifold with DI water.

c. Extraction Preparation

i. If the solution volume remaining in the vessel liner is <1 mL, add concentrated HNO_3 to bring volume to approximately 1 mL. Note: residual acid volumes of up to 2.5 mL are acceptable, but should be avoided if possible. Pour extract solution into a 50 mL plastic tube containing approximately 10 mL millipure water.

ii. Quantitatively transfer residual digest by rinsing the liner 3 - 4 times with millipure water, adding each rinse to the extract in the tube. Keep total rinse volume < 35 mL.

iii. Add 50 µL of 5000 µg/L ISTD solution to the extract.

iv. Bring extract volume to 50 mL with millipure water.

v. Cap tube and invert several times to mix.

Note: The percentage of dissolved solids in the 50 mL extract, which is higher than that recommended by instrument manufacturer, can be reduced by increasing the dilution volume. Analyst must balance detrimental effects of high dissolved solids content (matrix effects, instrument contamination) against detrimental effects resulting from environmental contamination and lower analyte concentrations when considering this. If additional dilutions are made, care must be taken to maintain acid strength at ~2% and ISTD concentration at 5 µg/L. Adjust standard curve concentrations accordingly, if necessary.

3. Instrumental Settings – ICP-MS Analysis

Note: The instrument parameters may be optimized to ensure system suitability.

a. Tuning

i. Prior to sample analysis check the instrument's tuning parameters by analyzing the Mass Spectrometer Tuning Solution as specified by the manufacturer. Check the sensitivity, % RSD, % oxide, % doubly charged, peak shape, and resolution.

ii. If these parameters are outside the manufacturer's specifications, retune the instrument.

b. ICP-MS Parameters

Set up instrument to monitor isotopes of appropriate metals, and selected internal standards.

Table 6. ICP-MS Metal Isotopes

Metal	**Isotope** ***Isotope used for quantitation**	**Gas Mode Used**
Manganese	55*	He

Metal	**Isotope** ***Isotope used for quantitation***	**Gas Mode Used**
Cobalt	59*	He
Selenium	78*, 82	H_2
Molybdenum	95, 98*	He
Cadmium	111*, 112,114	He
Thallium	203, 205*	He
Lead	206*, 207*, 208*	He

Note: These metals can be analyzed in no gas mode (no reaction cell), but may require the use of interference correction equation(s).

Table 7. ICP-MS Internal Standard Isotopes

Internal Standard	**Isotope**
Scandium	45
Gallium	69, 71
Germanium	72
Yttrium	89
Rhodium	103
Indium	115
Terbium	159
Bismuth	209

c. Instrument calibration

i. Analyze a calibration blank followed by at least 3 calibration standards (D.2.b) covering the range of interest. Using linear regression analysis, plot relative response (response relative to ISTD response) vs. concentration in µg/L and determine slope (m), intercept (b), and correlation coefficient (r) of the calibration curve. This can be automatically performed by the ICP-MS software. Correlation coefficient r must be ≥0.995, or calibration must be repeated.

ii. Analyze the calibration blank and a QC standard (D.2.c.i.) immediately after the calibration curve. The response of the blank should be similar to that observed when initially analyzed. The calculated metals concentrations in the QC standard must be within ± 10% of their accepted value. If these conditions are not met, the calibration sequence must be repeated until results are acceptable.

4. Injection sequence / Sample Set

   a. Calibration blanks and QC standards must be included in the sample analysis sequence after at least every 12 consecutive samples analyzed, and also at the end of the sample sequence to verify instrument performance over the course of the run.

   b. If response of any sample exceeds highest standard in the calibration curve, make an appropriate dilution in 2% HNO_3 and add ISTD to maintain a 5 µg/L concentration, then re-analyze.

   c. A sample set consists of the following:

      Note: Each sample set must contain one QA sample/20 samples.

      i. Negative control.

      ii. Positive control(s).

      iii. Samples.

G. CALCULATIONS / IDENTIFICATION

Note: Instrument software can be programmed to perform all necessary calculations.

1. Using values for m, b determined for the calibration curve (F.3.c), determine selected analyte concentration (CE, in µg/L) in any extract having a relative response R using:

   C_E (µg/L) = C_E, µg/L = (R-b)/m

   Note: If sample is found to contain molybdenum, instrument software must be set to compensate for contribution of molybdenum oxide to the 111 isotope used for quantitation of cadmium in the sample.

2. Calculate selected analyte concentrations in digested controls and samples (C_S) using:

$$C_S \text{ (ppb)} = \frac{C_E \times V_E \times D}{W}$$

Where
C_E = Analyte concentration in final extract, in µg/L
V_E = Final sample extract volume in milliliters
D = Dilution factor (Diluted volume/aliquot volume), if secondary dilution was made.
W = Sample Weight in grams.

3. Calculate Relative % Difference (RPD) for duplicate results using:

$$RPD = \frac{|C1 - C2| \times 200}{(C1 + C2)}$$

Where:
C1 = first duplicate's concentration.
C2 = second duplicate's concentration.

4. Calculate recoveries of fortified controls and check samples using

$$\%Rec = \frac{(C_F - C_B) \times W \times 100}{V_{FS} \times C_{FS}}$$

Where
C_F, C_B = Analyte concentrations determined for the fortified sample and the blank tissue from which it was prepared, in ppb (ng/g).
W = Weight of fortified control, in grams.
V_{FS} = Volume of fortification standard added, in mL.
C_{FS} = Concentration of fortification standard, in µg/L.

H. SAFETY INFORMATION AND PRECAUTIONS

1. Required Protective Equipment — Safety glasses, lab coat, protective gloves.

2. Hazards

Procedure Step	*Hazard*	*Recommended Safe Procedures*
Nitric Acid	Strong oxidizer. May be fatal if swallowed or inhaled. Extremely corrosive. Contact with skin or eyes may cause severe burns and permanent damage.	Perform operations using concentrated acid in fume hood. Use protective eyewear, gloves and clothing. Store in approved acid safety cabinet away from basic or other reactive materials.
Microwave Digester	Possible explosion hazard	Follow manufacturer recommendations
Metals Standards	Poisonous if ingested.	Do not pipet by mouth

3. Disposal Procedures

Follow local, state and federal guidelines for disposal.

I. QUALITY ASSURANCE PLAN

1. Performance Standard

Table 8 - Performance Standard

Analyte	*Analytical Range (ppb)*	*Acceptable Recovery (%)*
Cadmium	≥ 10	71-132
Lead	≥ 25	81-127
Selenium	≥ 500	72-146
Manganese	≥ 200	81-110
Molybdenum	≥ 50	90-115
Thallium	≥ 50	95-118
Cobalt	≥ 25	86-107

For each sample set:

a. The instrument calibration meets specifications in section F.3.c.

b. The recovery calculated for the positive control meets specifications in the above table.

c. If a positive control duplicate is run, the calculated RPD is ≤ 20%.

d. All calibration blanks injected show consistent responses, and all QC standards are within ± 10% of the accepted value.

e. For each sample within the set, the internal standard response is within ± 50% of the average instrument calibration internal standard response.

2. Critical Control Points and Specifications

None known

3. Intralaboratory Check Samples

a. System, minimum contents.

i. Frequency: One per week per analyst when samples analyzed.

ii. Records are to be maintained.

b. Acceptability criteria.

Refer to I. 1.

If unacceptable values are obtained, then:

i. Investigate following established procedures.

ii. Take corrective action as warranted.

4. Sample Condition upon Receipt: Cold

J. APPENDIX

1. References

   a. Agilent 7500 ICP-MS Hardware Manual, G1833-90004, January 2001.

   b. CEM XP-1500 Plus Vessel Accessory Sets and Autovent Option Instruction for Use, 600493, Rev. 5, 8/01.

   c. CEM Vacuum Concentration/Drying Accessory Set Instructions for Assembly and Use, 600484, Rev. 1, 6/99.

   d. CEM Mars Operation Manual, 600122, Rev 2, February, 2006.

   e. EPA Method 6020, Inductively Coupled Plasma-Mass Spectrometry, Revision 0, September 1994.

2. Cleaning Vessel Liners

   The following procedures are suitable for removal of residual adsorbed residues from Teflon liners used in this method. Other procedures are available and may be used if demonstrated to be effective.

   Option 1

   a. Add approximately 10 mL of conc. HNO_3 (metal analysis grade) to each digestion vessel liner.

   b. Assemble vessels as specified by manufacturer.

   c. Place in microwave.

   d. Digest at 600W, ramp to 150 °C over 10 minutes, then hold at temperature for 10 minutes.

   e. Cool vessels to room temperature, then disassemble.

   f. Rinse vessel liners and caps with millipure water several times to remove all traces of acid.

   g. Place in a clean environment to dry.

   h. Reference: CEM VesselCleaning09.doc, 04/09

   Option 2

   Using a Milestone Trace Clean apparatus and conc. metal analysis grade HNO_3:

   a. Place the microwave vessel liners and caps into the apparatus.

   b. Start the method program as per the manufacturer suggestion.

   c. After the apparatus has cooled, remove the liners and caps.

d. Rinse with Millipure water.

e. Place in a clean environment to dry.

K. APPROVALS AND AUTHORITIES

1. Approvals on file.

2. Issuing Authority: Director, Laboratory Quality Assurance Division.