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Title: Determination of Arsenic by Atomic Absorption Spectroscopy		
Revision: 05	Replaces: CLG-ARS.04	Effective: 06/20/2016

Contents

A. INTRODUCTION

1. Summary of Procedure

   The sample is charred in a furnace, and ashed to remove the remaining organic residue. The sample ash is dissolved in hydrochloric acid and reacted with sodium borohydride to convert the arsenic to a volatile hydride. The reaction mixture is purged with a stream of argon through a gas/liquid separator, where the liquid is pumped to waste. The argon carrier transports the separated arsenic hydride to a heated quartz absorption cell for measurement by atomic absorption spectrophotometry.

2. Applicability

   This method is applicable to liver, kidney, muscle, fish of the order Siluriformes (catfish) muscle, and egg products at levels ≥ 0.2 ppm.

   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. Balance - accurate to ± 0.02 g, Sartorius, B1419-52A.

   b. Crucibles - Vycor® transparent, 50 mL, Corning, #1 294050b0.

   c. Muffle furnace and controller - capable of maintaining a temperature of 500 ± 50 °C, Thermolyne, #FA 1740 and #CP53640.

   d. Hot plate - capable of maintaining a surface temperature of 120 ± 10 °C, Thermolyne, #HPA2245M.

   e. Bottles - polyethylene, 125 mL or 250 mL suitable for storing standards, Nalge, #20030004 and #2003008.

   f. Centrifuge tubes - graduated, polypropylene with screw cap, 50 mL, Becton Dickinson Labware FALCON® Brand Blue Max™, #2098.

   g. Ultrasonic cleaner - Branson, 8821 OMT.

   h. Magnetic stirrer - Thermolyne, S7225.

   i. Magnetic stirring bar - Scientific Products, S8314-25.

   j. Stirring rod - polypropylene, Nalge, #61 690010.

   k. Dispensers - Repipet®, 5, 10, and 20 mL, Barnstead Thermolyne 3005A, 3010A, and 3020A.

l. Robot Coupé® food processor - Robot Coupé® U.S.A., Inc., 39236-6627.

2. Instrumentation

a. Atomic absorption spectrophotometer (AAS) equipped with background correction capability and data handling system, Perkin-Elmer Model AAnalyst 300.

b. Electrodeless discharge lamp (EDL), Perkin-Elmer #3050860.

Note: Hollow cathode lamp, single element (arsenic (As)), Perkin-Elmer, #N3050105 may be used if desired.

c. Flow injection analysis system (FIAS) working in the metal hydride mode, Perkin-Elmer Model FIAS 400.

d. Autosampler - Perkin-Elmer Model AS-90.

C. REAGENTS AND SOLUTIONS

Note: Equivalent reagents / solutions may be substituted. The stability time frame of the solution is dependant on the expiration dates of the compounds used.The maximum length of time that a working reagent shall be used is 1 year unless the laboratory has produced extension data.

1. Reagents

a. Magnesium nitrate hexahydrate (Mg(NO3)2 • 6H2O) - reagent grade, Mallinckrodt AR ® ACS.

b. Hydrochloric acid (HCl) - concentrated, Mallinckrodt AR®.

c. Nitric acid (HNO3) - concentrated, Mallinckrodt AR®.

d. Potassium iodide ((KI) - Mallinckrodt AR®, ACS.

e. L-ascorbic acid - reagent grade, Mallinckrodt AR®, ACS.

f. Sodium hydroxide (NaOH) - reagent grade, Mallinckrodt AR®, ACS.

g. Sodium borohydride ($NaBH_4$) - pellets, Aldrich Chemical Company, 45,289-0.

2. Solutions

Note: Use distilled deionized water unless otherwise noted.

a. $Mg(NO_3)_2$ solution, 50% w/v:

Dissolve 500 g $Mg(NO_3)_2 \cdot 6H_2O$ in 500 mL distilled water and dilute to 1 L with distilled water.

b. HCl solution, 4.5N:

Mix 372 mL concentrated HCl with 500 mL of water and dilute to 1 L with water.

c. HCl solution, 10% v/v:

Mix l00 mL concentrated HCl with 500 mL of water and dilute to 1 L with water.

d. HNO_3 solution, 50% v/v:

Prepare mixture of one part concentrated HNO_3 and one part water.

e. 10% KI/ascorbic acid solution w/v:

Dissolve 20 g of KI and 5 g of L-ascorbic acid in 200 mL 10% HCl (C.2.c.).

f. $NaBH_4$•NaOH solution:

Weigh 0.5 g NaOH and 2 g of $NaBH_4$ into a 1 L volumetric flask. Dilute to volume with distilled water and mix well. Let stand until dissolved and mix well. Solution is prepared daily.

D. STANDARDS

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. In-house prepared standards shall be assigned an expiration date that is no later than the expiration date of the earlilest expiring component or no later than the stability stated in the method, whichever ends soonest. The maximum length of time that an in-house prepared standard shall be used is 1 year unless the laboratory has produced extension data.

1. Standard Information

a. Arsenic, 1000 µg/mL inorganic As, Alfa Catalog Chemicals, Morton Thiokol lnc. #8805.

b. Organic arsenic as Arsenilic acid, 100% purity, Fisher #1389.

2. Preparation of Standard Solution(s)

a. Organic As stock solution (1000 µg/mL):

Dissolve 0.2897g Arsenilic acid in distilled, deionized H_2O and dilute to 100 mL.

b. Intermediate As standard (100 µg/mL):

Dilute 10 mL of either 1000 µg/mL arsenic standard solution (D.1.a. or D.2.a.) to 100 mL with 10% HCl. Store at room temperature for 1 year.

c. Working standards

Pipet 0, 1, 2, 3, 4, and 5 mL of the 100 µg/mL intermediate As standard into separate 100 mL volumetric flasks. Dilute to volume with 10% HCl to give 0, 1, 2, 3, 4, and 5 µg/mL standards respectively. Store at room temperature for 1 year.

d. Calibration standards

To prepare calibration solutions of 0.0, 0.2, 0.4, 0.6, 0.8, and 1.0 ppm, pipet 1 mL of each working standard (0, 1, 2, 3, 4, and 5 µg/mL) into six clean 50 mL polypropylene centrifuge tubes. Add 9 mL of 4.5N HCl, 35 mL of 10% HCl followed by 5 mL of 10% KI/ascorbic acid solution to make a final volume of 50 mL. Mix well and let stand for 1 hour. Store at room temperature for 1 year.

E. SAMPLE PREPARATION

1. Muscle

Trim off as much fat as possible. Use a Robot Coupe® or equivalent commercial grade food processor to thoroughly blend the tissue (Use of a worm type chopper with plate opening no greater than 1/8" may be substituted. Mix thoroughly after chopping).

2. Liver or kidney

Trim off as much connective tissue as possible. Place tissue into a blending jar and blend until the tissue is homogenized. Do not blend continuously for periods exceeding 1 minute. Excessive blending may overheat the tissue. Allow tissue to cool between blendings.

F. ANALYTICAL PROCEDURE

1. Preparation of Controls and Samples

a. Weigh 5.0 ± 0.1 g of homogenized sample into a 50 mL Vycor® crucible. (Smaller sample sizes not less than 1.0 ± 0.1 g may be used.)

Also, weigh out additional 5.0 ± 0.1 g portions of a known blank tissue into a 50 mL Vycor® crucible for each of the following Quality Control samples as needed:

i. A tissue blank - One needed for each analytical batch.

ii. A recovery sample - One recovery for every set up to twenty samples. Fortify the recovery sample with 1.00 mL of the 3.0 µg/mL working standard. Each recovery sample is equivalent to 0.6 ppm of arsenic in tissue.

iii. An internal check sample - If required, once per week per analyst for an analytical batch. Unknown fortification by another analyst with 1.0 mL of

any of the working standards.

iv. Prepare a reagent blank to be included with each analytical batch.

2. Extraction Procedure

a. Depending upon sample weight, add 3 - 6 mL of 50% $MgNO_3$ solution to the sample and mix thoroughly with a polypropylene stirring rod.

b. Place the sample into a cool (< 80 °C) muffle furnace and raise the temperature of the oven according to the following furnace control program:

Furnace Controller Program*

Step 1	Ramp = 3 °C/min	Level =	100 °C	Dwell =	360 min
Step 2	Ramp = 3 °C/min	Level =	150 °C	Dwell =	60 min
Step 3	Ramp = 3 °C/min	Level =	500 °C	Dwell =	480 min
Step 4	Ramp = end				

*The sample must not be heated so rapidly that it ignites.

Remove the samples from the oven and cool to room temperature.

c. Add 1 - 4 mL 50% HNO_3 solution to the ash while washing down the sides of the crucibles (Make sure ash is thoroughly wetted). Take ash to dryness on a hot plate. Precautions must be taken to avoid splattering of liquid from the crucible. Return the sample to the muffle furnace and raise the temperature to 500 ± 50 °C. Maintain the sample at this temperature for 1 hour.

Note: Repeat this step if any carbon residue remains in the crucible.

d. Remove the sample from the muffle furnace and cool to room temperature under a hood.

e. Add 10 mL of 4.5N HCl. If ash fails to dissolve after the addition of the HCl then place sample into an ultrasonic bath to dissolve.

f. Transfer the solution from the crucible to a clean 50 mL polypropylene (PPE) test tube using two portions of 10% HCl to a final volume of 45 mL.

g. To this solution add 5 mL of 10% KI/ascorbic acid solution and mix well. Let stand for at least 60 minutes.

h. Analyze sample by using AAS according to Section F.3.

i. If the instrumental response for the sample exceeds the response for the most concentrated standard, dilute the sample and reanalyze it. If upon reanalysis the amount found in the sample exceeds tolerance then repeat the sample analysis from the beginning of the method.

3. Instrumental Settings

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

Set up the AAS according to the manufacturer's instructions.

a. Operating parameters for Perkin-Elmer #AAnalyst 300.

Lamp:	As EDL
Wavelength:	193.7 nm
Slit:	0.7 nm
Cell Temperature:	900 °C

b. Operating parameters for FIAS-400.

Step#	Time (s)	Pump l (rpm)	Pump 2 (rpm)	Valve	Read (s)
Prefill	15	120	120	Fill	
1	10	100	120	Fill	
2	15	0	120	Inject	15

The sample is introduced to the FIA valve manually or by an autosampler. When the valve is in the fill position, the injection loop is filled with sample solution carried by pump 1. When the valve is in the injection position, an exact reproducible sample volume is injected into the carrier stream. The sample and the carrier stream travel to the chemifold. Pump 2 carries the $NaBH_4$•NaOH solution to the chemifold, where it is mixed with the sample. The resultant reaction reduces the analyte to its hydride form. The reacted mixture is purged with a stream of argon through a gas/liquid separator, where the liquid is pumped to waste. The argon carrier transports the separated arsenic hydride to the absorption cell for measurement.

Measure the absorption of the As in the samples.

4. Sample Set

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

a. Reagent Blank

b. Tissue blank

c. Recovery sample

d. Check Sample if needed

e. Samples

G. CALCULATIONS

Note: Calculations may be performed by built in data system.

1. By using the appropriate regression algorithm, construct a standard curve of the As concentration vs. the absorption for the external standards.

   Using the external standard regression curve, compute the As concentration of any recovery or internal check sample, in ppm. The final concentration of any recovery or internal check sample must be corrected by subtracting any tissue blank contribution according to the following equation:

   Rec. or Int. Chk. As Conc. = Calc. As Conc. – Calc. Tissue Blk. Conc.

   Using the external standard regression curve, compute the As concentration for each sample, in ppm. The final concentration of any sample must be corrected by subtracting any reagent blank contribution according to the following equation:

   Sample As Conc. = Calc. As Conc. – Calc. Reag. Blk. Conc.

   Note: Reagent blank is required to help determine presence of trace contaminants from glassware or reagents.

   Then correct for recovery, according to the following equation:

   Recovery correction:

   $$\text{ppm correction} = \frac{\text{ppm in sample}}{\text{Fortified tissue recovery fraction}}$$

   Where fortified tissue recovery fraction is the % Recovery expressed as a fraction. i.e. 98.7% would be 0.987 expressed as a fraction.

2. Instrument software does the calculations, r-value must be ≥ 0.995.

H. SAFETY INFORMATION AND PRECAUTIONS

1. Required Protective Equipment - Safety glasses, plastic gloves, laboratory coat, heat-resistant gloves, and crucible tongs.

2. Hazards

Procedure Step	*Hazard*	*Recommended Safe Procedures*
$Mg(NO_3)_2$	Skin, eye, and respiratory irritant.	Use only in chemical fume hood. Wear suitable protective clothing, gloves, and eye/face protection.
$NaBH_4$	Flammable. Toxic by inhalation, ingestion, or skin absorption. Extremely destructive to upper respiratory track, eyes and skin.	Use only in chemical fume hood. Wear suitable protective clothing, gloves and eye/face protection.
HCl	Skin, eye, and respiratory irritant.	Prepare solutions in a well-ventilated area such as a fume hood and dispense using repipettors wherever possible. Wear plastic gloves.
HNO_3	Corrosive. Contact with liquids can result in burns and severe skin, eye, and respiratory irritation.	Same as HCl
NaOH	Corrosive. Contact with liquids can result in burns and severe skin, eye, and respiratory irritation.	Same as HCl
Equipment		
Muffle furnace	Hot!	Wear heat-resistant gloves. Use crucible tongs to remove and insert crucible.

*Manufacturers Material Safety Data Sheet (MSDS) should be obtained and kept on file for complete safety information.

3. Disposal Procedures

Follow federal, state and local regulations

I. QUALITY ASSURANCE PLAN

1. Performance Standard

Analyte	*Analytical Range (ppm)*	*Acceptable Recovery(%)*	*Acceptable Repeatability (CV)*	*Reproducibility*
As(organic)	0.2 - 1.0	70 -110	≤ 10	≤ 20
As(inorganic)	0.2 - 1.0	80 - 110	≤ 10	≤ 20

2. Critical Control Points and Specifications

	Record	*Acceptable Control*
a.	Sample weight	5 ± 0.1 g
b.	Initial muffle furnace temperature	Cool < 80 °C
c.	Muffle furnace temperature increase	Slowly
d.	Final muffle furnace temperature	500 ± 50 °C
e.	Completeness of ashing	No visible carbon residue
f.	Reagent blank	Absorbance should produce a response < 0.1 ppm. If greater, check for cleanliness and contamination of glassware and reagents.

g. Standards — New commercial standards should be verified against old standards. Agreement should be within ± 10%.

3. Intralaboratory Check Samples

a. System, minimum contents.

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

ii. Records are 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: Not spoiled or rancid.

J. APPENDIX

1. Minimum Level of Applicability (MLA): 0.2 ppm.

K. APPROVALS AND AUTHORITIES

1. Approvals on file.

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