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Title: Determination of Arsenic by Atomic Absorption Spectroscopy		
Revision: 04	Replaces: CLG-ARS.03	Effective: 4/02/2010

Contents

A. INTRODUCTION

1. Theory

   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, catfish muscle, and egg products at levels ≥ 0.2 ppm.

B. EQUIPMENT

Note: Equivalent apparatus and instrumentation 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 and solutions may be substituted.

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: An equivalent standard may be substituted.

1. Source

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

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.

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.

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.

3. Storage and Stability (if not included with preparation).

a. Intermediate, working and calibration standards may be stored at room temperature.

b. Stability:

Intermediate As standard: 1 year.

Working standards: 1 year.

Calibration standards: 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. Ashing Procedure and Sample Transfer.

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 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.

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

c. 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.

d. 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.

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

f. 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.

g. 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.

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

i. Analyze sample by using AAS according to Section F.2.

j. 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.

2. Instrumental Conditions

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.

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

Procedure Step	*Hazard*	*Recommended Safe Procedures*
$NaBH_4$•NaOH	See above	Remaining reagent along with the instrument waste solution is disposed following all Federal, State, and Local environmental laws.
$Mg(NO_3)_2$	See above	Follow all Federal, state and local environmental laws.

Acids	See above	Dispose following all Federal, state and local environmental laws.
Arsenic Lamp		Dispose following all Federal requirements and environmental laws.

*Manufacturers MSDS should be obtained and kept on file for complete disposal information

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. Readiness To Perform

a. Familiarization

i. Phase I: Standards- Standard curve on each of 3 consecutive days, which will include the following:

(a) 0.0 ppm

(b) 0.2 ppm

(c) 0.4 ppm

(d) 0.6 ppm

(e) 0.8 ppm

(f) 1.00 ppm

Note: $r \geq 0.995$

ii. Phase II: Fortified samples- Duplicate replicates at 0, 0.2, 0.4, 0.6, 0.8 and 1.0 ppm on each of 3 different days using blanks of appropriate matrix. The blank must produce a response $\leq$ 0.1 ppm.

Note: Phase I and Phase II may be performed concurrently.

iii. Phase III: Check samples for analyst accreditation.

(a) Eight unknown samples, a blank and a recovery fortified at 0.6 ppm. One of the unknown samples will be fortified at 0 ppm and the remaining unknown samples will be fortified at 0.2 ppm to 1.0 ppm.

(b) Samples submitted by the Quality Assurance Manager (QAM), Supervisor or designee.

(c) Report analytical findings to QAM.

(d) Authorization from QAM and/or Supervisor is required to

commence official analysis.

b. Acceptability criteria.

Refer to I. 1.

4. Intralaboratory Check Samples

a. System, minimum contents.

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

ii. Records are maintained by the analyst and available for review by the Supervisor and Quality Assurance Manager.

b. Acceptability criteria.

Refer to I. 1.

If unacceptable values are obtained, then:

i. Stop all official arsenic analyses by that analyst.

ii. Take corrective action.

5. Sample Acceptability and Stability

a. Matrix: Liver, kidney, muscle (animal or catfish), and processed egg products.

b. Sample receipt size: Minimum 50 g.

c. Condition upon receipt: Not spoiled or rancid.

d. Sample storage:

i. Time: 6 months.

ii. Condition: Frozen (< -10 °C).

6. Sample Batch

a. Reagent Blank

b. Tissue blank

c. Recovery Sample

d. Check sample if needed

e. Samples

7. Analyst Capability

Minimum proficiency level (MPL): 0.2 ppm.

J. WORKSHEET

[RESERVED]

K. APPENDIX

[RESERVED]

L. APPROVALS AND AUTHORITIES

1. Approvals on file.

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