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Title: Determination of Arsenic by Atomic Absorption Spectrophotometry.		
Revision: .03	Replaces: .02	Effective: 12/01/01

Contents

A. INTRODUCTION

The sample is dried in an oven, then 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.

This method is suitable for the analysis of trace quantities of arsenic in liver, kidney, and/or muscle tissue. This method is also suitable for the analysis of egg products.

B. EQUIPMENT

Note: Equivalent apparatus and instrumentation may be substituted.

1. Apparatus
   a. Balance - accurate to ± 0.02 g, Sartorius, B1419-52A.
   b. Mechanical oven - capable of maintaining a temperature of 95 ± 5 °C.
   c. Crucibles - Vycor® transparent, 50 mL, Corning, #1 294050b0.
   d. Muffle furnace and controller - capable of maintaining a temperature of 500 ± 50 °C, Thermolyne, #FA 1740 and #CP53640.
   e. Hot plate - capable of maintaining a surface temperature of 120 ± 10 °C, Thermolyne, #HPA2245M.
   f. Bottles - polyethylene, 125 mL or 250 mL suitable for storing standards, Nalge, #20030004 and #2003008.
   g. Centrifuge tubes - graduated, polypropylene with screw cap, 50 mL, Becton Dickinson Labware FALCON® Brand Blue Max™, #2098.
   h. Ultrasonic cleaner - Branson, 8821 OMT.
   i. Magnetic stirrer - Thermolyne, S7225.
   j. Magnetic stirring bar - Scientific Products, S8314-25.
   k. Stirring rod - polypropylene, Nalge, #61 690010.
   l. Dispensers - Repipet®, 5, 10, and 20 mL, Barnstead Thermolyne 3005A, 3010A, and 3020A.
   m. Robot Coupé® food processor - Robot Coupé® U.S.A., Inc., Jackson MS 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% wlv:

      Dissolve 500 g $Mg(NO_3)_2 \cdot 6H_2O$ in 500 mL distilled water and dilute to 1 liter 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% vlv:

      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 1L volumetric flask. Dilute to volume with distilled water and mix well. Let stand until dissolved and mix well. New solution is prepared for each set.

D. STANDARDS

Note: An equivalent standard may be substituted.

1. Source

a. Arsenic, 1000 µg/mL inorganic As, Alfa Catalog Chemicals, Morton Thiokol lnc.#88051.

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.l.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 l00 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 4 - 9 mL of 4.5N HCl (the volume is equal to the volume of acid used to dissolve the ash in step F. l.f minus 1 mL, the volume of the standard). Then add 10% HCl to make a total volume of 45 mL followed by 5 mL of 10% KI/ascorbic acid to make a final volume of 50 mL. Mix well and let stand for 1 hour.

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.

   Freeze samples if they are not to be analyzed within one week otherwise refrigerate immediately upon receipt. It is convenient to use flat plastic freezer bags to freeze samples in slabs 1 to 2 cm thick.

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 ± 0.01 g may be used.) Depending upon sample weight, add 3 - 6 mL of 50% $MgNO_3$ solution to the sample and mix thoroughly with a polypropylene stirring rod. Also, weigh a tissue blank, a recovery sample and prepare a reagent blank. For the recovery, use 1.0 mL of 3 µg/mL working standard to fortify 5 g of sample, equivalent to 0.6 ppm.

   b. Dry in an oven 90 - 100 °C until the sample is thoroughly dry (approximately 6 hours or overnight).

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	Level = 50 °C	Dwell = 60 min
Step 2	Ramp = 3 °C	Level =100 °C	Dwell = 90 min
Step 3	Ramp = 3 °C	Level =150 °C	Dwell = 90 min
Step 4	Ramp = 3 °C	Level =200 °C	Dwell = 60 min
Step 5	Ramp = 3 °C	Level =250 °C	Dwell = 60 min
Step 6	Ramp = 3 °C	Level =350 °C	Dwell = 60 min
Step 7	Ramp = 3 °C	Level =500 °C	Dwell = 480 min
Step 8	Ramp = end	Level =50 °C	Dwell = 0 min

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

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

A second ashing step is required to remove any remaining carbon residue. Usually the ash only needs to be thoroughly wetted with HNO3 once to produce a carbon-free ash. Repeat the following step if needed.

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.

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

f. Add 5 -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

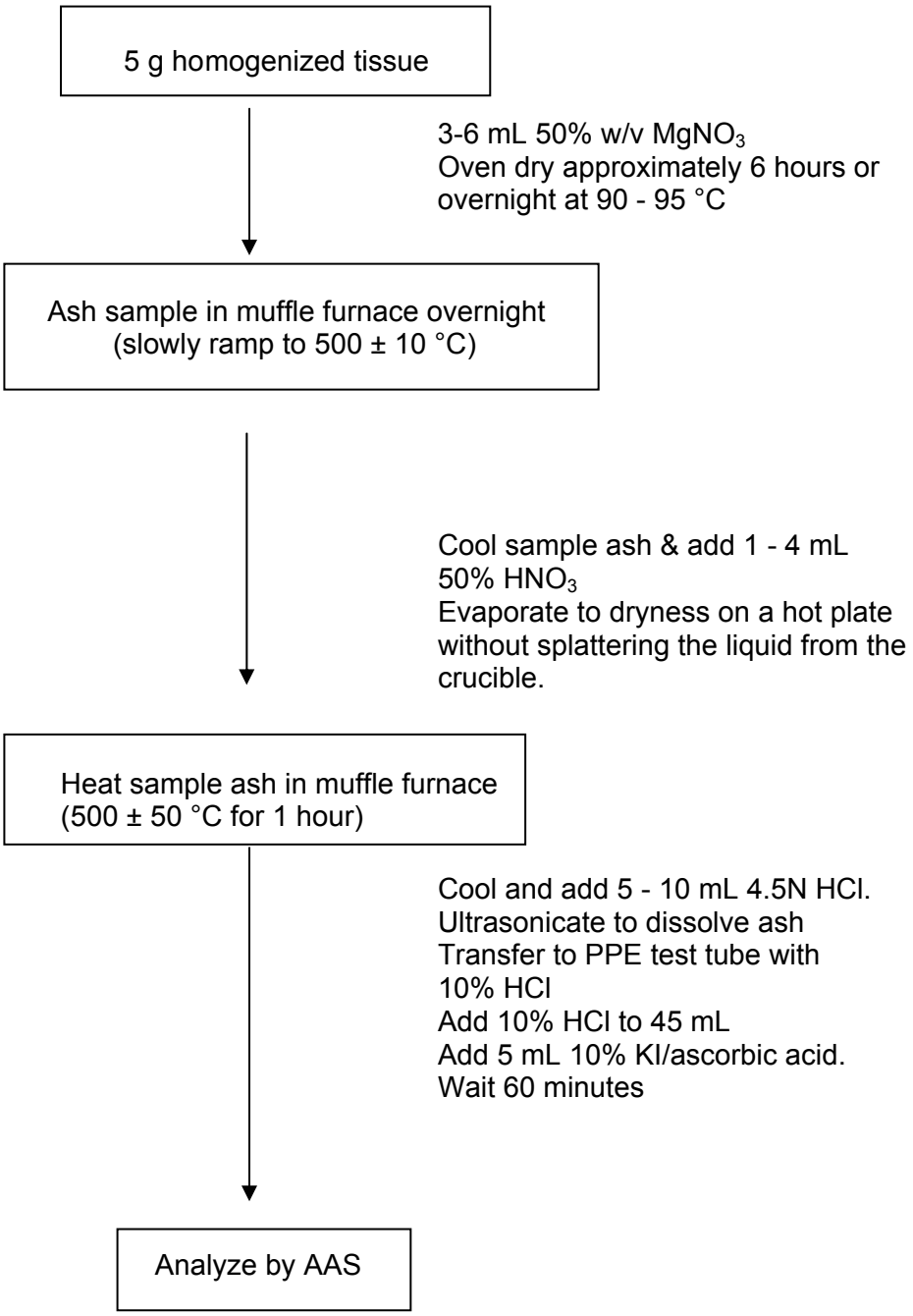
b. Operating parameters for FIAS-400.

Step#	Time (s)	Pump I (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.

3. Flowchart


5 g homogenized tissue
3-6 mL 50% w/v $MgNO_3$
Oven dry approximately 6 hours or overnight at 90 - 95 °C
Ash sample in muffle furnace overnight (slowly ramp to 500 ± 10 °C)
Cool sample ash & add 1 - 4 mL 50% HNO_3
Evaporate to dryness on a hot plate without splattering the liquid from the crucible.
Heat sample ash in muffle furnace (500 ± 50 °C for 1 hour)
Cool and add 5 - 10 mL 4.5N HCl.
Ultrasonicate to dissolve ash
Transfer to PPE test tube with 10% HCl
Add 10% HCl to 45 mL
Add 5 mL 10% KI/ascorbic acid.
Wait 60 minutes
Analyze by AAS

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 (x) for each sample, in ppm. Then correct for recovery, according to the following equation:

   Recovery correction:

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

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

H. HAZARD ANALYSIS

1. Method Title - Determination of Arsenic by Atomic Absorption Spectrophotometry.

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

3. Hazards

*Reagents**	*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.

4. Disposal Procedures

*Reagent**	*Hazard*	*Recommended Safe Procedures*
$NaBH_4$•NaOH	See above	Left over reagent is acidified (pH 3-5) (BH_4 to BOH) and flushed down the sink with plenty of water. The instrument waste solution (already acidic) is neutralized and flush down the sink with plenty of water. Follow all Federal, State, and Local environmental laws.
$Mg(NO_3)_2$	See above	Follow all Federal, state and local environmental laws.
Acids	See above	Neutralize and flush down the sink. Observe all Federal, state and local 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

The Measurement Uncertainty and Method Detection Limit should be recalculated yearly or whenever a major change in the method occurs. For example: Change in personnel analyzing samples or new equipment received—detector, spectrometer, column.

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
f.	Completeness of ashing	No visible carbon residue
g.	Reagent blank	Absorbance should produce a response < 0.1 ppm. If greater, check for cleanliness and contamination of glassware and reagents.
h.	Standards	New commercial standards should be verified against old standards. Agreement should be within ± 10%.

3. Readiness To Perform (FSIS Training Plan)

   a. Familiarization

      i. Phase I: Standards - Duplicate set of standard curves on each of 3 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) 1.0 ppm

         Note: $r \geq 0.995$

ii. Phase II: Fortified samples - Duplicate replicates at 0, 0.2, 0.4, 0.6, 0.8, 0.8, and 1.0 ppm over a period of 3 different days using blanks of appropriate matrix. Process the same blank on all three days. 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) Fourteen unknown samples, including a blank and a recovery fortified at 0.6 ppm. Unknown samples to be fortified between 0 to 1.0 ppm.

(b) Samples arranged through the FSIS Accredited Laboratory Program (ALP).

(c) Report analytical findings to ALP.

(d) Notification from ALP is required to commence official analysis.

b. Acceptability criteria.

Refer to section I. 1., Performance Standards

4. Intralaboratory Check Samples

a. System, minimum contents.

i. Frequency: Initially, minimum of 1 check sample per set, reduced to 1 per week per analyst. This sample is an internal check sample that has been analyzed at least 10 times to obtain a "running" average.

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

(a) All replicate findings

(b) All percent recoveries

(c) All blanks

(d) Control charts

(e) For all recoveries and blanks, the running average, standard deviation and coefficient of variation on the last acceptable ten samples

b. Acceptability criteria.

Refer to section I. 1., Performance Standards

If unacceptable values are obtained, then:

i. Stop all official analyses by that analyst.

ii. Take corrective action.

5. Interlaboratory Check Sample Program

   a. Frequency: 1 every other month.

   b. Samples: Procured, prepared, and provided by FSIS. (may be incurred or fortified).

   c. Report analytical findings to provider according to their specifications.

   If unacceptable values are obtained, then:

   i. Stop all official analyses by that analyst.

   ii. Take corrective action.

6. Sample Acceptability and Stability

   a. Matrix: Liver, kidney, muscle, 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)

7. Sample Set

   a. Reagent blank

   b. Tissue blank

   c. Recovery -- tissue blank fortified at concentration of interest

   d. Incurred check sample, 1 per week (optional: 1 with each set)

   e. Samples

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

8. Sensitivity

   Minimum proficiency level (MPL): 0.2 ppm

J. WORKSHEET

Note: Computer generated worksheet may be used.

The following example of a worksheet may be photocopied.

Determination Arsenic Analysis by Atomic Absorption Spectrophotometry

Analyst _____ Date Started _____ Date Completed _____

Instrument Conditions (AAS #4100):
Wavelength: 193.7 nm
Slit Width: 0. 7 nm
Expansion: 10 nm
Quartz Cell Temperature: 900°C
Reductant: 0.2% $NaBH_4$ in 0.05% NaOH
Carrier Solution: 10% HCl (v/v)
Fuel Flow: 2.5 L/min

STD. Concentration: _____
Matrix Spike: _____
% Recovery: _____
%Running 10 Rec: _____
% Running 10 Chk: _____
STD Curve Corr. Coef.:
($r \geq 0.995$)

Tube #	Sample Identification	Tissue Code	Sample Weight	Final Vol. (mL)	Mg/Kg or ppm found	Result Corrected (ppm found – reagent blk)	Recovery Corrected	Results	Comments
1	Control Blk								
2	Fortified Rec.								
3	Check 1								
4	Check 2								
5	Reagent Blk								
6									
7									
8									
9									
10									
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Bruce Cottingham	10/30/01

Approval signatures on file