United States Department of Agriculture
Food Safety and Inspection Service, Office of Public Health Science

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Title: Detection of Thallium, Arsenic and Mercury using ICP-AES		
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Contents

A. INTRODUCTION

1. Theory

   Samples are dried, defatted by extraction with petroleum ether, and digested by heating with concentrated nitric acid. The digest is diluted and centrifuged to remove residual particulates, and an aliquot is analyzed using inductively coupled plasma-atomic emission spectrometry (ICP-AES).

2. Applicability

   This procedure is applicable for screening for thallium, arsenic, and mercury at or above concentrations of 25 ppm in meat and food products containing meat.

B. EQUIPMENT

Note: Equivalent equipment may be substituted.

Apparatus

a. Balance - top loading, sensitive to 0.01 g.

b. Vycor® crucible - 50 mL.

c. Oven - forced draft, capable of maintaining 90-100 °C temperatures.

d. Hot plate - adjustable temperature.

e. Centrifuge tubes - polypropylene, 15 mL, with caps.

f. Centrifuge - Cat. No. T6000B, Sorvall.

g. Filter paper - 70 mm circle, Cat. No. 4, Whatman.

h. Volumetric flasks - 10, 100, 1000 mL.

3. Instrumentation

   a. ICP spectrometer - Perkin-Elmer Optima 3000 DV, with ICP Winlab Software.

C. REAGENTS AND SOLUTIONS

1. Reagents

   Note: Equivalent reagents may be substituted.

   a. Deionized water.

   b. Petroleum ether - ACS or better grade.

   c. Nitric acid (HNO_3) - redistilled, 69 - 70% w/w (>98% w/V).

d. Hydrochloric acid (HCl) - concentrated.

2. Solutions

a. 2% Nitric Acid, w/v:

Add 1 volume of 70% HNO_3 to 49 volumes of deionized water and mix.

b. 40% Nitric Acid, w/v:

Add 1 volume of 70% HNO_3 to 1.5 volumes deionized water and mix.

c. 1 N HCl:

Add 1 volume of concentrated HCl to 11 volumes of deionized water and mix.

D. STANDARDS

1. Source: Certified ICP standards for arsenic, mercury, thallium, and yttrium are available at nominal concentrations of 1000 or 10,000 µg/mL from SCP Science, Champlain, NY.

2. Preparation

a. Yttrium Internal Standard (ISTD) solution, (2 µg/mL):

Pipet 2.0 mL of 1000 µg/mL yttrium standard into a 1 L volumetric flask. Dilute to volume with 1N HCl.

b. Fortification standard, (100 µg/mL):

Pipet 10.0 mL of 1000 µg/mL arsenic, mercury, and thallium standards (or 1.00 mL of 10,000 µg/mL standard) into a 100 mL volumetric flask. Dilute to volume with 2% HNO_3.

c. Calibration standard, (3-5 µg/mL):

Note: Equivalent to 30 - 50 ppm in sample, based on 1.00 g sample weight.

Pipet 3.0-5.0 mL of 100 µg/mL metals fortification standard into a 100 mL volumetric flask. Dilute to volume with 40% HNO_3.

Note: 40% acid is used to approximate acid content of samples, since instrument response for analytes, especially mercury, was observed to decrease with increasing acid content.

3. Storage and Stability

a. Store all standards in sealed containers at room temperature.

b. Stability: The primary source of concentration change for these standards is due to evaporation, since As, Hg, and Tl are stable in HNO_3 at µg/mL concentrations.

i. Certified standard solutions (D.1) may be used up to their expiration dates.

ii. Fortification and calibration standards are known to be stable for at least 10 weeks.

E. SAMPLE PREPARATION

1. Sample Blending

   Blend samples thoroughly using a blender or food processor. Refrigerate until needed.

2. Extraction

   a. Weigh 1.0 ± 0.1 g test sample to nearest 0.01 g into a 50 mL Vycor® crucible.

      Note: Prepare negative and positive controls at this time. Weigh two matrix blanks. Add 250 µL of the 100 µg/mL fortification standard to one of them.

   b. Flatten sample to maximize surface area. Dry in oven for at least 1 hour at 95 ± 5 °C.

   c. Remove crucible from oven and allow to cool. While the sample is still warm, add 5 mL of petroleum ether and swirl to dissolve any fat. Decant the solvent to a waste container.

   d. Repeat the extraction with an additional 5 mL of petroleum ether. Place the crucible in a hood to and allow residual solvent to evaporate.

   e. Add 4 mL of 70% HNO_3 to crucible and place on a hot plate. Adjust temperature of hot plate so that digestion of visible solids requires 15-20 minutes for an average sample. Digest samples until visible solids have dissolved. Avoid overheating or prolonged digestion past the point of dissolution.

   f. When digestion is complete, remove crucible from the hot plate and allow to cool to room temperature.
      Stopping Point: Sample digest may be covered and left overnight.

   g. Filter the sample digest through filter paper into a 15 mL polypropylene centrifuge tube. Rinse the crucible with small amounts of deionized water, using these to wash the filter paper. Adjust final volume of solution to 10 mL with deionized water. Cap the tube and mix well. Refrigerate for at least 1 hour
      Stopping Point. Solutions can be stored overnight if necessary.

   h. Observe the solution. Most will contain some suspended particulate matter. Centrifuge at 2000 - 2500 rpm for approximately 5 minutes to clarify.

   i. If solution is not clear following centrifugation step, pass through filter paper again into a clean 15 mL polypropylene tube. Samples are ready for ICP analysis.

F. ANALYSIS

1. ICP Setup

   a. Set up ICP sampling pump with a T-fitting to mix ISTD with all solutions analyzed. Flow of ISTD solution should comprise approximately 50% of total input flow.

   b. Program system software to quantitate on the basis of response relative to the ISTD concentration.

   c. Calibrate the ICP using a 0 µg/mL (40% HNO_3 solution) calibration blank and a 3-5 µg/mL calibration standard. Monitor wavelengths as shown in table below.

Metal	Wavelength
Yttrium	371.030
Thallium	351.924
Arsenic	193.696
Mercury	253.652

2. Analysis Sequence

   a. Analyze external standard at least two times to verify instrument sensitivity and stability. Replicate STD/ISTD ratios should agree within 10%.

   b. Analyze blank, fortified control(s), and test samples.
   Note: Re-analyze external standard as often as necessary during sample run to verify consistency of relative response.

G. CALCULATIONS

1. Program Instrument to calculate analyte concentrations based on a single point standard curve using the general formula:

   Amount Detected, ppm = A*B/C*W, where

   A = Relative (to ISTD) response of control/sample.
   B = Concentration of External Standard, expressed as ppm in 1.00 g matrix.
   C = Relative response for External Standard.
   W = Sample weight in grams.

2. Calculate Amount Detected for each analyte in all controls and samples.

3. Determine recoveries for the fortified control by dividing Amount Detected by the fortification level.

a. Determine a cutoff level for each analyte by multiplying the amount detected in the fortified control by 0.75.

4. Report samples having a calculated amount ≥ cutoff level as screen positives at a 25 ppm level.

5. Screen results may be reported only if:

a. The recovery for the fortified control falls within the acceptable range as set in section I.1.

b. The blank control calculates to be negative.

H. SAFETY INFORMATION AND PRECAUTIONS

1. Required Protective Equipment - Safety glasses and lab coat.

2. Hazards

Item	*Hazard*	*Recommended Safe Procedures*
Concentrated. Acids - HCl, HNO_3	May be fatal if swallowed or fumes 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 acids in approved safety cabinet away from basic or other reactive materials.
Hg, As, Tl standards and standard solutions	Highly poisonous if ingested. Hg, Tl are also toxic via contact with skin or lungs, and are strong irritants. Acidic solvent used (see above for hazards)	Handle concentrated solutions with care. Avoid ingestion, inhalation, or dermal contact. Use protective eyewear, gloves and clothing
Petroleum ether Extraction step E.2.c.	Highly flammable liquid and vapor. Harmful or fatal if swallowed or inhaled. May affect central nervous system, cause irritation to skin, eyes, and respiratory tract.	Perform extraction in fume hood away from sources of ignition. Wear protective gloves when handling or transferring solvent.
E.2.e Sample dissolution	Hot plate may cause burns if touched. Conc. HNO_3 may bump or spatter when heated, resulting in possibility of chemical burns	Perform operations using concentrated acid in fume hood, use protective eyewear, gloves and clothing.

3. Disposal Procedures

Procedure Step	*Hazard*	*Recommended Safe Procedures*
Acids and acid solutions	See above	Dispose according to appropriate local, state, and federal regulations.
Hg, As, Th standards	See above	Dispose according to appropriate local, state, and federal regulations.

I. QUALITY ASSURANCE PLAN

1. Performance Standards

   a. Controls:

Analyte	*Target Concentration*	*Acceptable recovery (control)*
Thallium	≥ 25 ppm	75 - 120%
Arsenic	≥ 25 ppm	75 - 120%
Mercury	≥ 25 ppm	60 - 110%

   b. Familiarization and Check Samples

      i. No False Negatives at concentrations ≥ 25 ppm

      ii. No False positives at concentrations ≤ 12.5 ppm.

2. Readiness To Perform (FSIS Training Plan)

   a. Familiarization

      i. Phase I: Standards-- Calibrate the ICP using a 0 µg/mL (40% HNO_3 solution) calibration blank and a 3-5 µg/mL calibration standard, in duplicate on 3 different days.

      Monitor wavelengths as shown in table below.

Metal	*Wavelength*
Yttrium	*371.030*
Thallium	*351.924*
Arsenic	*193.696*
Mercury	*253.652*

ii. Phase II: Fortified samples.

A minimum of 3 negative and 18 positive (fortified at 25 ppm) samples analyzed over at least 3 different days. Use the recovery of the first positive sample analyzed each day as the basis for determination of screen positive/negative results for remaining samples.

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

iii. Phase III: Check samples for analyst accreditation.
At least 8 blind check samples, Set must include 2 to 3 blanks. Positives must be fortified at levels between 25 - 50 ppm. Each sample set must include a separate blank and recovery to be used as the basis for determining positive/negative results for check samples.

iv. Calculate recoveries and false positive/negative rates for all samples analyzed in Phases II and III. Report analytical findings to Supervisor or Quality Assurance Manager (QAM).

v. Approval from QAM is required to commence official analysis.

b. Acceptability criteria.

Refer to section I.1. above.

3. Intralaboratory Check Samples

a. System, minimum contents.

i. Frequency: One per week when analyses are performed.

b. Records are to be maintained by the analyst and reviewed.

c. Acceptability criteria.

If unacceptable values are obtained, then:

i. Stop official analyses by that analyst.

ii. Take corrective action.

4. Sample Acceptability and Stability

a. Minimum sample size: 250 g.

b. Sample storage: Refrigerate or freeze.

i. Time: Analytes stable indefinitely.

ii. Condition: Samples must be unspoiled on receipt.

5. Sample Set

a. Sample Set must include:

i. At least one negative and one positive control prepared from a blank food

matrix.

ii. Test samples to be analyzed.

6. Sensitivity

a. Minimum proficiency level (MPL): 25 ppm.

J. WORKSHEET

Reserved.

K. APPENDIX

Reserved.

L. APPROVALS AND AUTHORITIES

Approvals on file.

Issuing Authority: Laboratory Quality Assurance Division (LQAD).