Analysis of Foods for As, Cd, Cr, Hg and Pb by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS)

Current Method
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Introduction

- This method is for the analysis of food for the following elements: arsenic, cadmium, chromium, lead and mercury.
- The technique used for determining the above elements is Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). This instrument is to be operated in the helium collision cell mode to eliminate interference from isobaric polyatomic species via kinetic energy discrimination.
- The method assumes the use of one of the following Agilent ICP-MS instruments: 7500ce, 7500cx, 7700x.

Sample Preparation by Microwave Digestion

- Homogenize sample or required number of subsamples using appropriate laboratory grinders/mills.
- Analytical portion 0.4 - 5.0 g. Generally, use the equivalent of about 0.5 dry material. The maximum mass will depend on the specific microwave digestion vessel. For most food samples this will be between 0.5 and 2 g. Less than the maximum mass should be used for samples high in salt content to avoid matrix interference. A maximum analytical portion of 5 g should not be exceeded to avoid excessive dilution of the nitric acid. Use 1 g reagent water for method blanks (MBKs). Adding 1 g of reagent water first can help control exothermic reactions during the acid addition and digestion for dry samples and dry reference materials.
- Add 8 mL (11.3 g) HNO_3.
    - Acid should be added drop wise for the first few mL until it can be established that the sample will not react violently.
    - Some foods, especially those high in sugar, will react with nitric acid within several minutes. Some finely ground spices (especially turmeric) will react immediately and violently with nitric acid. Always add acid drop wise to dry, finely ground samples until you can be sure that the reaction will not proceed too rapidly. If foaming or reaction with the acid is observed, let the vessels sit uncovered in a class 100 clean hood for 20 minutes or until reaction subsides. If a clean hood is unavailable, place caps on vessels without pressing down fully or, if so equipped, cap vessels but loosen the pressure relief nut (with the safety membrane) to allow pressure to escape. If, however, it appears that excessive foaming would result in the sample-acid mixture expanding out of the vessel then cap the vessel and tighten to appropriate torque to prevent loss of sample or acid.
    - Acid purity will vary between manufacturers and grade. Use double distilled grade for lowest method blank values. The trade name for double distilled grade will vary by manufacturer.

    - Weighing acid using a top loading balance and Teflon® FEP wash bottle is suggested.
- Add 1 mL high purity H_2O_2. Cap and place in microwave system.
- Digest in microwave. Ramp power over 25 minutes until 200 °C is reached. Maintain this temperature for 15 minutes.
- Cool to < 50 °C and remove carousel from oven.
- Dilute to approximately 100 mL with reagent water and add 1 mL (1.2 g) double distilled HCl. Add more reagent water for a final volume of 200 mL or 202 g.
    - Gravimetric dilution is recommended into an acid washed 250 mL polyethylene bottle or other suitable plastic container. Mass of 200 mL 2% HNO_3 – 0.5% HCl solution is approximately 202 g.
    - Acid concentration in sample digests will be approximately 2% HNO_3 and 0.5% HCl. The HCl will help stabilize Hg.
    - Glass should not be used for dilution or storage of solutions because of possible contamination.
- Total dilution factor will be approximately 100 to 400 depending on the analytical portion. This dilution will help eliminate matrix interference. Dilution factor of less than this is not recommended unless it can be demonstrated that matrix interference has been adequately compensated for.
- Include at least 3 method blanks with each batch to assess contamination.
- Include at least 1 reference material certified for each analyte with each batch.
- Include at least one fortified analytical portion (FAP).
    - Fortify such that the resulting analytical solution will contain an additional 0.2 - 0.5 ng/mL Hg and 0.5 - 1.0 ng/mL As, Cd, Cr and Pb.

Instrument Setup

- Tune instrument according to manufacturer's instructions (tuning guide) in normal (no gas) mode. Be sure to meet specifications for sensitivity, oxide ratio and doubly charged ratio. Tuning for low oxide and low double charged species is important for minimizing interference.
- Switch to helium collision cell mode and make the necessary adjustments according to manufacturer's instructions (tuning guide). Be sure to meet specifications for sensitivity, oxide ratio, doubly charged ratio and interference removal. Adjust instrument parameters to minimize the ratio of the signals at mass 51 to 59 while aspirating a tuning solution containing 1% HCl and 1 µg/L Co. The signal at mass 51 is from $^{35}Cl^{16}O^+$, an interfering polyatomic species formed in the plasma. The signal at mass 59 is from Co.
- Ensure that the reaction cell and various lens voltage parameters are set properly to minimize other isobaric polyatomic interferences such as:
    - Mass 52 from ArC^+, Mg_2^+ and $ClOH^+$
    - Mass 75 from $ArCl^+$ and $CaCl^+$
- Set up method to include the following analytical isotopes:
    - The 5 elements of interest: ^{52}Cr, ^{75}As, ^{111}Cd, , ^{201}Hg, $^{206,207,208}Pb$
    - Internal standard elements: ^{103}Rh, ^{193}Ir, ^{209}Bi
        - If for some reason any of these elements can not be used then use one of the following elements as internal standards (IS).

Recommend IS Isotope	Optional IS Isotopes
^{103}Rh	^{105}Pd, ^{72}Ge, ^{74}Ge
^{193}Ir	^{195}Pt, ^{197}Au
^{209}Bi	^{205}Tl, ^{175}Lu

    - Potentially interfering elements: ^{146}Nd, ^{147}Sm
    - Optional additional masses to monitor: ^{53}Cr, ^{110}Cd, ^{114}Cd, ^{182}W, ^{202}Hg
        - These additional masses to monitor are optional but recommended. In the event of unexpected results or of suspected interference, the additional isotope information may help confirm the finding as the analyte and not the result of uncorrected interference.
- Program method to operate in the helium collision cell mode for all analytes. This mode will help minimize polyatomic interferences.
    - Note that the helium collision cell mode will not eliminate double charged species interference. This type of interference needs a correction factor determined on the day of analysis and entered into the method.
- Use three points per peak, three integrations and the following minimum integration time for each element:
    - 0.2 sec integration time: Cr, Pb
    - 0.5 sec integration time: As, Cd
    - 1.0 sec integration time: Hg

Determination of As, Cd, Cr, Hg and Pb by ICP-MS

- Pre-Analysis Scan in Semi-Quant or Raw Counts Mode
    - Analyze analytical solutions (one replicate from each sample) for As, Cd, Cr, Hg, Pb and any element that might be used as an internal standard. Use 1% HNO_3 for the internal standards uptake line for this scan. This pre-analysis scan checks for the presence of internal standard elements and high levels of analyte elements which will require additional dilution.
        - This will require some knowledge of the relation between counts and concentration if the raw counts mode is used.
        - Level of an internal standard element is considered significant if the counts in the analytical solution would contribute 2% or more of the counts in an analytical run for an internal standard isotope.
            - ◊ A correction must be applied or a different internal standard element must be chosen if internal standard element is present at a significant level.
        - Instrument mode can be counts mode or semi-quant mode.
        - It can be advantageous to also check for Nd and Sm which can cause a positive interference at mass 75 and interfere with arsenic quantitation via formation of double charged species.

        - It can be advantageous to also check for the following elements that are sometimes present at high concentrations and might cause various types of interference: Na, Mg, Ca, K, Fe, Zn, and Sr. Analytical solutions with these elements present at tens or hundreds of mg/L might need to be analyzed at additional dilutions. Knowing this up front and preloading the autosampler can save time by preventing the need for a separate reanalysis.
            - ◊ Elements present at high concentration can decrease analyte response due to ionization suppression especially for elements with a high first ionization potential.
            - ◊ Elements present at high concentration will increase the possibility of interfering molecular species such as $^{40}Ca^{35}Cl^{+}$ and $^{40}Ar^{35}Cl^{+}$ interference at m/z 75 (As) and $^{35}ClOH^{+}$ and $^{26}Mg_2^{+}$ interference at m/z 52 (Cr).
        - Some samples may contain low levels of tungsten which can interfere with ^{202}Hg via formation of $^{186}W^{16}O$. The lack of W must be confirmed if ^{202}Hg is used. Thus ^{201}Hg is recommended.
- Analysis – Quantitation mode
    - Configure instrument for He collision cell mode. Use 3-4 volts kinetic energy discrimination (difference between octapole bias and quadrupole bias) and 3-5 mL He. All analytes must be analyzed in this mode.
    - Use the following analyte isotopes: ^{75}As, ^{52}Cr, ^{111}Cd, ^{201}Hg and ^{206}Pb, ^{207}Pb ^{208}Pb.
        - Calculate sum for lead isotopes to account for isotopic variation between the standard solutions and the samples.
    - Standardize instrument with multi-element standards containing As, Cd, Cr, Hg and Pb at concentrations of approximately 0.1, 0.5, 1.0, and 10 ng/mL in matrix of 2% HNO_3 – 0.5% HCl.
        - These are suggested concentrations. The lowest concentration solution can be < 0.1 ng/mL (such as 0.05 ng/mL) if the instrument has sufficient sensitivity. The highest concentration solution can be < 10 ng/mL (such as 5 ng/mL) if samples are not expected to be high in a particular analyte.
        - The maximum Hg concentration should be 1.0 ng/mL otherwise very long wash out times will be required.
        - This acid matrix will approximate the acid concentration in the digest solutions.
    - Suggested internal standard isotope for ^{75}As, ^{52}Cr, and ^{111}Cd: ^{103}Rh
        - If for some reason ^{103}Rh can not be used other suggested internal standard isotopes are: ^{105}Pd, ^{72}Ge, or ^{74}Ge
        - Be aware of the following interferences:
            - ◊ Sr will interfere with ^{105}Pd via $SrOH^{+}$. This interference should be largely eliminated by the He collision cell mode.
            - ◊ Fe will interfere with ^{72}Ge via $^{56}Fe^{16}O^{+}$. This interference should be largely eliminated by the He collision cell mode.
            - ◊ Se will interfere with ^{74}Ge because of ^{74}Se. This isobaric isotope interference is not eliminated by the He collision cell mode. However, this interference would be expected to be very small

because of the low abundance of ^{74}Se (0.9%). This interference could be a problem with Se fortified foods.

- Suggested internal standard for ^{201}Hg: ^{193}Ir
  - If for some reason ^{193}Ir can not be used then other suggested internal standard isotopes are: ^{195}Pt or ^{197}Au
- Suggested internal standard for $^{206,207,208}Pb$: ^{209}Bi.
  - If for some reason ^{209}Bi can not be used then other suggested internal standard isotopes are: ^{205}Tl or ^{175}Lu
- The concentration of mono-isotopic internal standard elements (Rh, Au, Bi) should be approximately 25 ng/mL in the internal standard solution. This will result in about 50,000 cps on an Agilent 7500ce instrument.
  - Adjust the concentration of other internal standard elements according to their isotopic abundance. Signals of about 50,000 to 100,000 cps should be obtained for internal standard isotopes.
  - Prepare the internal standard solution in 2% HNO_3 – 0.5% HCl.
- If As is going to be determined then prepare the internal standard solution to contain 20% v/v isopropyl alcohol and diluting the rest with 2% HNO_3 – 0.5% HCl.
  - For example: Add 10 mL isopropyl alcohol and approximately 30 mL 2% HNO_3 – 0.5% HCl to a clean 50 mL plastic tube. Pipette aliquots of internal standard stock solutions and dilute to 50 mL with 2 % HNO_3 – 0.5% HCl.
  - Use electronic grade isopropyl alcohol.
  - Isopropyl alcohol will increase sensitivity of As by a factor or 2 to 3 via the so-called "carbon effect". The presence of carbon in the plasma enhances the ionization efficiency of some high ionization potential elements such as As.
  - Isopropyl alcohol will also normalize the carbon content between the standards and analytical solutions and even out the effects of residual carbon not oxidized during the digestion.
  - Note that the alcohol will greatly increase the formation of ArC^+ which will interfere with ^{52}Cr resulting in a higher detection limit for Cr. Therefore, if alcohol will be used to enhance the As signal, make sure the He collision cell parameters at set to eliminate ArC^+ interference on ^{52}Cr.
- Suppression of internal standard isotope response usually indicates some type of matrix effect is present
  - Dilute any analytical solution where the internal standard signal differs by more than 40% from the calibration blank.
  - Start with a 1:1 dilution using 2% HNO_3 – 0.5% HCl for diluent.
- A correction factor will have to be applied to ^{75}As if Nd or Sm is present at concentrations high enough to cause a signal greater than the detection limit at mass 75 due to doubly charged species. Calculate these factors before starting analysis:
  - Analyze a 5 or 10 µg/L Nd and Sm separately (single element solutions)
  - Calculate the Nd correction factor from the ratio of the signal at mass 75 and the signal for ^{146}Nd.

        - Calculate the Sm correction factor from the ratio of the signal at mass 75 and the signal for ^{147}Sm.
        - Enter these correction factors for Nd and Sm on ^{75}As using the Method Edit function.
        - Correction factor will change slightly on a day to day basis because it is affected by plasma conditions.
    - Fortified Analytical Solution (FAS): Fortify an aliquot of analytical solution ("the digest"). Fortification level should be twice the native level or an amount that will result in 0.2- 0.5 ng/mL Hg and 0.5 - 1.0 ng/mL As, Cd, Cr and Pb which ever is greater. This can be conveniently prepared by pipetting 10 mL of solution into an autosampler tube or clean plastic test/centrifuge tube and then adding the appropriate amount of standards.
        - The FASs can be prepared during the analytical run. Append the Sequence Table as you prepare each FAS and add to the autosampler.
        - Prepare one FAS for each sample type/matrix.

Analytical Quality Control

- Set up and tune instrument to meet laboratory's specification for sensitivity, oxide ratio and doubly charged ratio.
- Insure that the reaction cell and various lens voltage parameters are set properly to minimize isobaric interference at mass 52 from ArC and $ClOH^+$ and at mass 75 from $ArCl^+$.
- Minimum correlation coefficient for standard curve is 0.998.
- Verify standardization by analyzing a check solution immediately after standardization, after every 10 analytical solutions, and at the end of the run. Check Solution Recovery must be 100 ± 10%. One of the mid-level standard solutions can be used as the check solution.
- Dilute any analytical solutions with analyte levels above the highest standard.
    - Use 2% HNO_3 – 0.5% HCl for diluent.
- If Nd or Sm are present at > 0.5 µg/L in the analytical solution, demonstrate lack of interference from doubly charged species by analyzing an interference check solution consisting of 5 µg/L Nd, Sm.
    - Results at ^{75}As should be <LOQ if interference equations were determined properly.
- Control limits for the reference material (True Value Recovery) is 100 ± 20% from certified value. Suggested reference materials include but are not limited to:
    - NIST Apple Leaves SRM 1515 (Pb, Hg).
    - NIST Tomato Leaves SRM 1573a (As, Cd, Cr, Hg).
    - NIST Pine Needles SRM 1575a (Cd, Hg, Pb).
    - NIST Durum Wheat Flour RM 8436 (Cd, Cr).
    - NIST Corn Bran RM 8433 (Pb).
    - NIST Rice Flour SRM 1568a (As).
    - NIST Spinach Leaves SRM 1570a (As, Cd, Hg).
    - NIST Oyster Tissue SRM 1566b (As, Cd, Hg, Pb).

- Control limits for the fortified analytical portion (FAP Recovery) is 100 ± 20%.
- Control limits for the fortified analytical solution (FAS Recovery) is 100 ± 10%.
- Report the limit of quantification LOQ) with all sample results (including reference materials) (See EAM for limit determination procedures; available from www.fda.gov/eam).
- Instrument detection limits (IDLs) must be determined before starting any analysis. (See EAM for limit determination procedures; available from http://www.fda.gov/eam).