



# Elemental Analysis Manual

## for Food and Related Products

The following is a section of the Elemental Analysis Manual for Food and Related Products.

For additional information and to view other sections of the manual, visit the Elemental Analysis Manual for Food and Related Products web page at http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm2006954.htm.

U.S. Department of Health & Human Services

FDA U.S. Food and Drug Administration

# Elemental Analysis Manual

## for Food and Related Products

# 3.6 Performance

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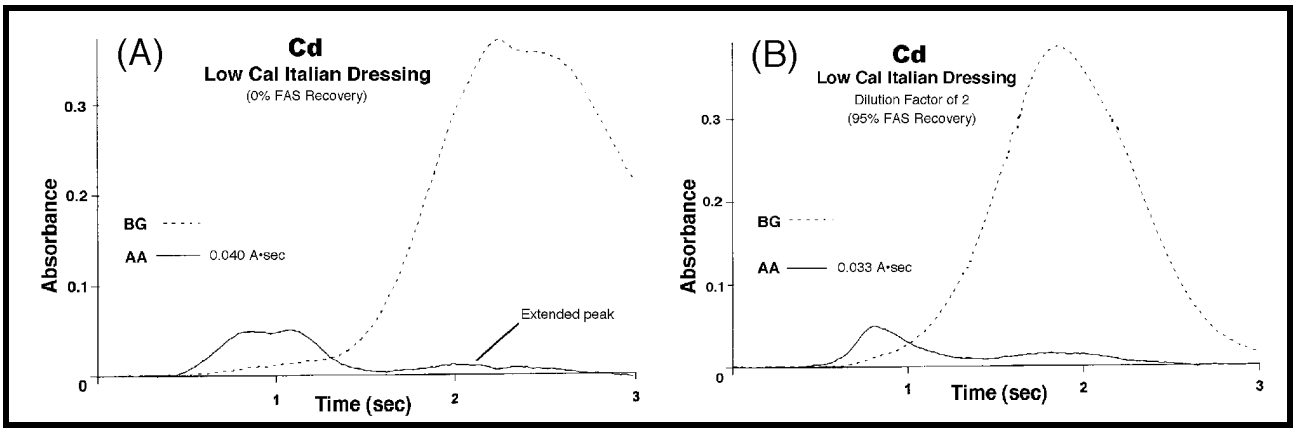
## 3.6.1 INSTRUMENT PERFORMANCE

### 3.6.1.1 GRAPHITE FURNACE ATOMIC ABSORPTION SPECTROMETER

**Interferences** - Several types of interferences are associated with graphite furnace atomic absorption spectrometry (GF-AAS) and can be classified into three major divisions: spectral, matrix, and memory. An instrument with the capability to graphically display absorbance versus time is required to evaluate interferences.

Spectral interference is the result of absorption of light by an element or molecule that is not the analyte of interest or from black body radiation. Spectral interference caused by another element is rare with GF-AAS because of the narrow atomic line widths emitted by source lamps and the narrow absorption profiles.

Molecular species, however, can produce broadband absorption profiles. The use of matrix modifiers, optimized furnace temperature programs and Zeeman effect background correction can help minimize the effect of this nonspecific absorption. Black body emission from the hot graphite tube can also produce spectral interference. Not using atomization temperatures higher than is necessary to volatilize the analyte and maintaining proper furnace alignment can minimize this type of interference.




**3.6 Figure 1. Detection of Matrix Interference.** Matrix interference caused by high salt or mineral content of foods: (A) Detected by low recovery of fortified analytical solution (FAS), (B) Correction by dilution of analytical solution. BG = Background Absorbance; AA = Atomic Absorbance.

Matrix interferences are caused by matrix components in the analytical portion that inhibit formation of free analyte atoms during the furnace program's atomization step. Components in the matrix may also contribute to pre-atomization volatilization of analyte. The use of platform atomization (instead of tube wall) provides a more constant temperature environment for volatilization of analyte atoms. This environment is more conducive to the formation of free analyte atoms and helps to minimize matrix interference. The use of 5% hydrogen in the argon gas during the dry and char steps reduces interference from high levels of chloride[1]. Matrix interference is still a challenge to overcome with many foods even with these aids for reducing interferences. High salt and high mineral content foods are a particular analytical challenge for GF-AAS (3.6 Figure 1). Some examples of these foods are condiments (mustard, ketchup etc.), pickles, cheese and processed meats. Matrix interference can be detected by poor fortification recovery of the FAP and FAS quality control analyses. If the fortification recovery is outside the acceptable range, then a matrix effect should be suspected and the analytical solution must be

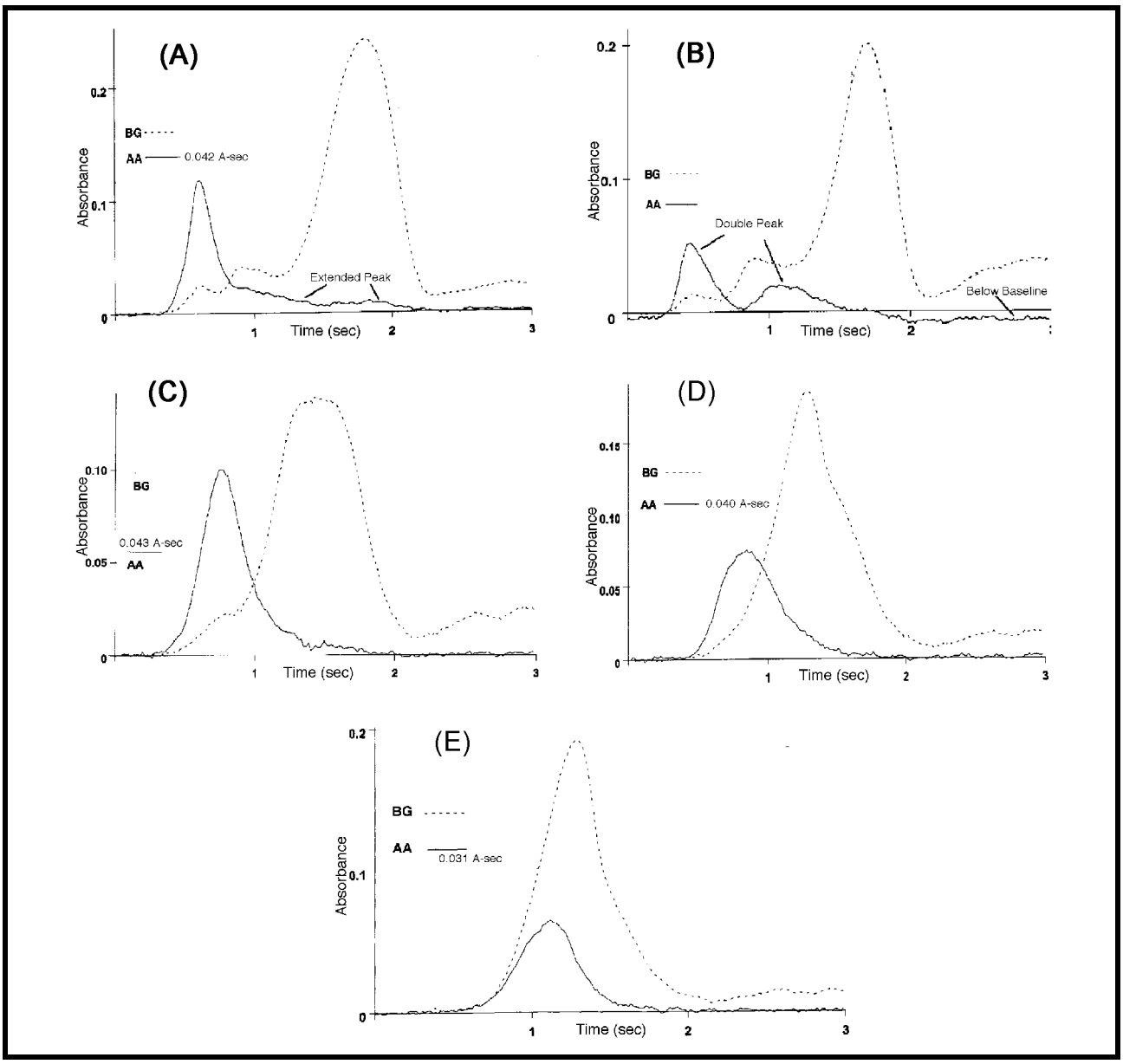
diluted and re-analyzed or analyzed by method of standard additions. See 3.6 Figure 2.

In addition to low fortification recovery, an irregular peak profile (sec. 3.6.1.2) might also indicate matrix interference. Analysts should examine analyte peaks for each sample and compare with the standard solution peaks (3.6 Figure 3A). Irregularities to recognize include excessively broadened peaks (low peak height to peak area ratio) (3.6 Figure 3B), doubled or multiple analyte peaks (3.6 Figures 2B and 3C) or a peak's appearance time shifted from expected (3.6 Figures 4A and 4B). For example, American cheese usually exhibits low fortification recovery due to the presence of relatively high levels of salt and other minerals (*i.e.*, phosphate) which hinder the rapid atomization of both cadmium and lead resulting in a very broad peak profile (3.6 Figures 4C and 4D). The peak height to peak area ratio will typically be as much as 40-60% lower than the ratio for the standard solutions. A 0.8 g analytical portion of American cheese will typically result in a 75% percent recovery of lead for the FAS. The slope of the standard addition curve will be approximately 75% of the slope of a check solution that has a matrix of just nitric acid.

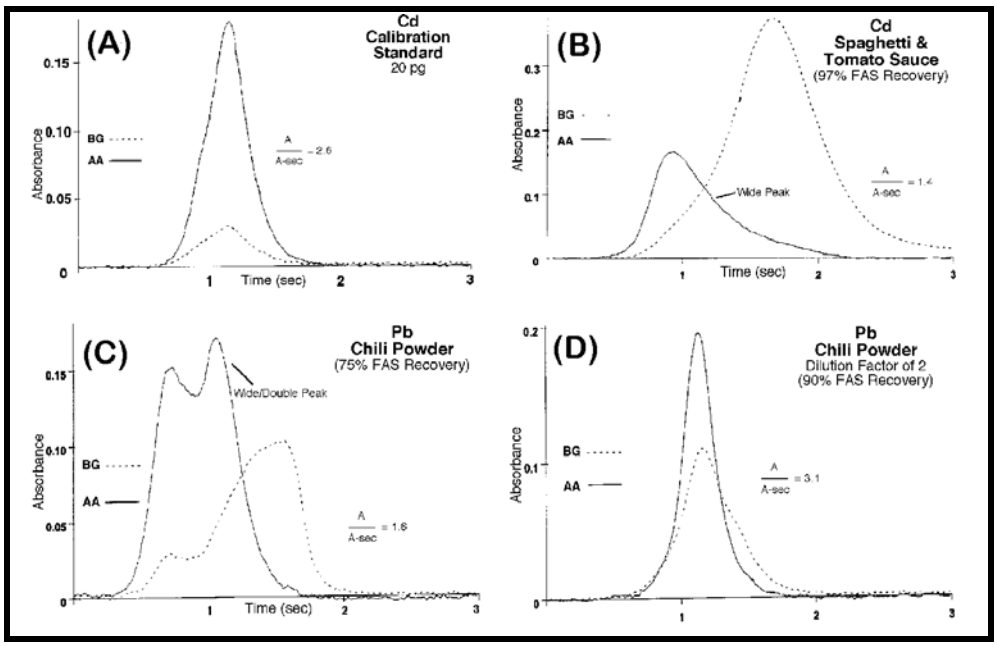
Since furnace programs are usually optimized using a standard solution (no matrix effect), the read step might need to be lengthened to include the entire broadened analyte peak obtained from the food. Ensure that analyte peak tails return to the baseline before the end of the integration/read step (3.6 Figures 5A and 5B). A standard solution peak might return to base line after 2-3 sec whereas some food samples might require 3-4 sec.

Another phenomenon that has been observed with high-salt samples is pre-atomization analyte loss during the char step. This phenomenon causes an analytical result that is biased low. This loss can occur even when using a matrix modifier and a conservative char temperature. Pre-atomization loss is indicated by an absorbance less than zero at the beginning of the read cycle and ending with the peak's tail dipping below the baseline. Samples displaying this behavior need to be diluted and re-analyzed (3.6 Figures 5C-5E).

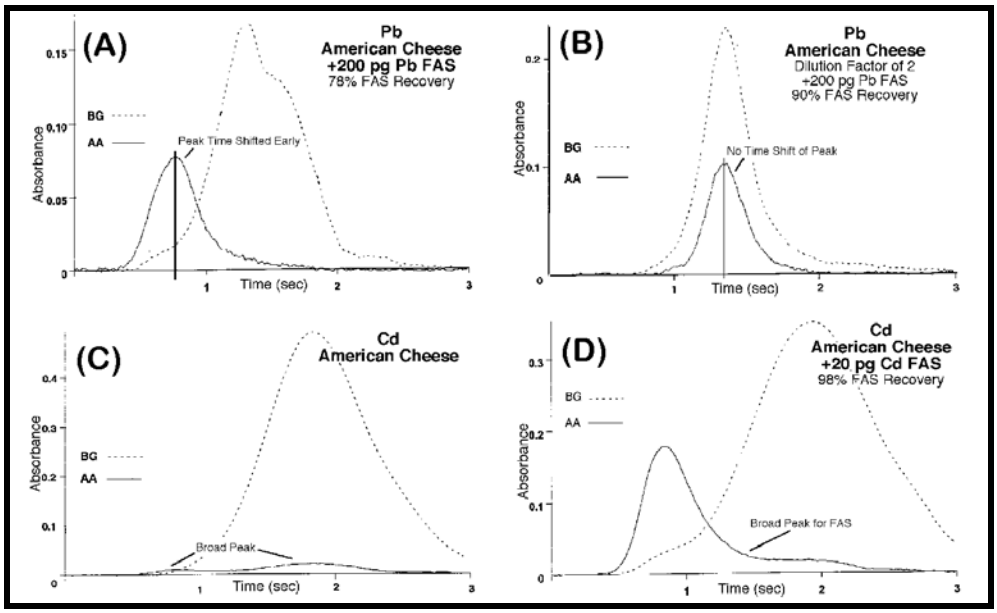
Analytes at high levels in the analytical solution may not be volatilized out of the graphite furnace. This residual analyte may have a "memory effect" on the next measurement resulting in a falsely high result (*i.e.*, false positive or high bias). In addition, foods high in salt and minerals can have a matrix memory effect on the next measurement. Use of a clean-out step of a few seconds at maximum temperature should minimize these problems.



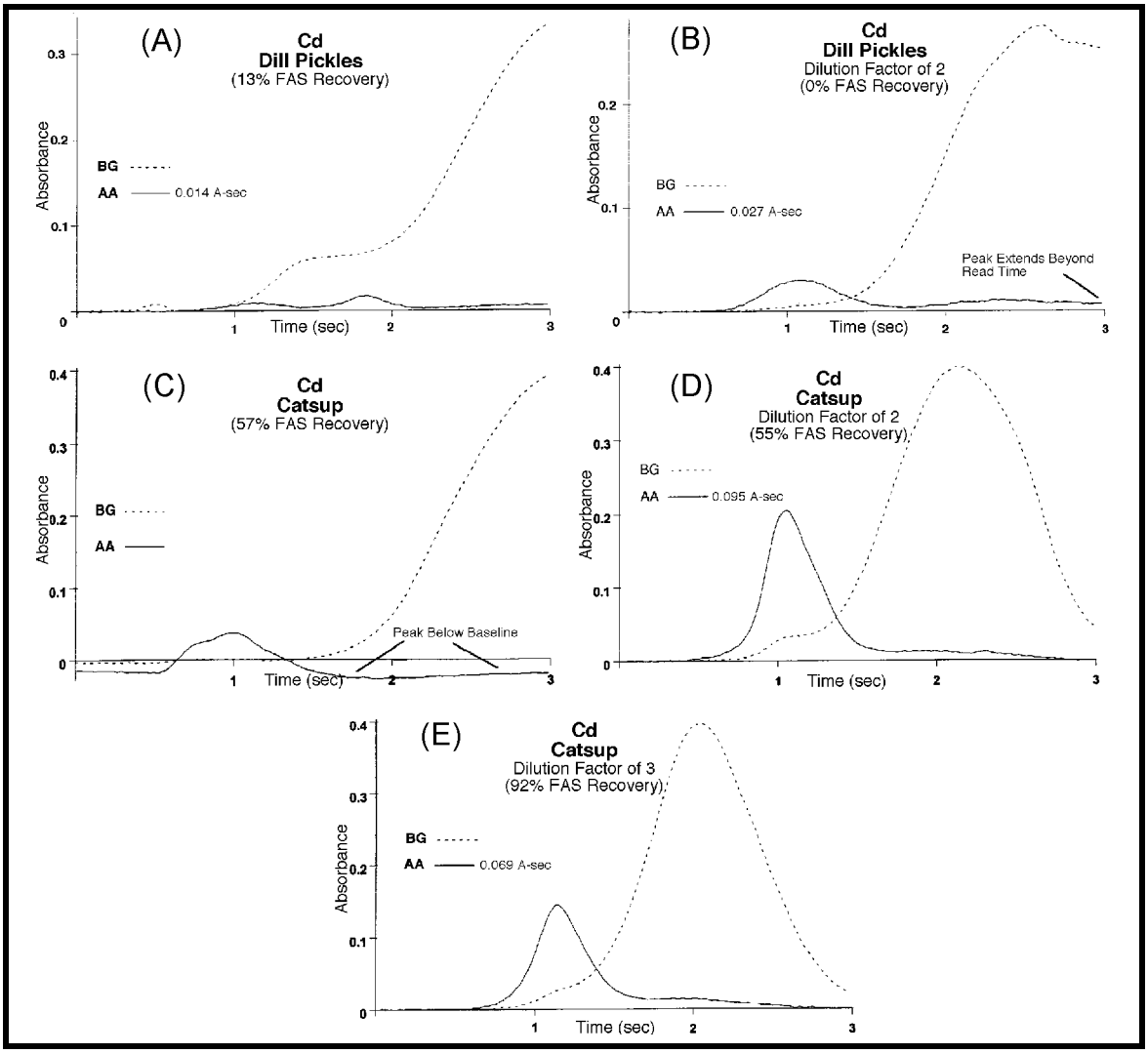

**3.6 Figure 2. Manifestation of Matrix Interference.** Matrix interference indicated by irregular peak profile, low recovery of fortified analytical solutions (FAS) and low recovery of expected value for spinach reference material (NIST SRM 1570): (A) DF 2, 42% RM Recovery, 0% FAS Recovery, (B) DF 2, +400 pg Pb FAS, 0% FAS Recovery, (C) DF 3, 75% RM Recovery, [21% FAS Recovery], (D) DF 4, 102% RM Recovery, [97% FAS Recovery], (E) DF 5, 101% RM Recovery, [95% FAS Recovery]. BG = Background Absorbance; AA = Atomic Absorbance.




**3.6 Figure 3. Irregular Peak Profiles.** Matrix interference indicated by irregular peak profile: (A) Calibration standard with ideal peak profile, (B) Excessively wide peak, (C) Excessively wide and double peak with low FAS Recovery, (D) Correction of wide and double peak by dilutions. BG = Background Absorbance; AA = Atomic Absorbance.




**3.6 Figure 4. Time Shifted Peaks.** Matrix interference indicated by irregular peak profile due to relatively high levels of salt and other minerals: (A) Peak appearance time shift, (B) Correction of peak appearance time shift by dilution, (C) Broad peak profile due to high level of phosphate from food matrix, (D) Interference by high concentration of mineral element(s) indicated by low FAS Recovery. BG = Background Absorbance; AA = Atomic Absorbance.




**3.6 Figure 5. Additional Irregular Peaks.** Matrix interference indicated by peak tail extension and pre-atomization analyte loss: (A) and (B) Analyte tail does not return to baseline before end of integration/read step, (C) Pre-atomization loss indicated by absorbance less than zero at beginning of read step and absorbance less than zero (below baseline) at end of read step, (D) and (E) Correction of pre-atomization loss by dilution. BG = Background Absorbance; AA = Atomic Absorbance.

**Peak Profile** - For GF-AAS, peak profile (shape of absorbance vs. time graph) is used to evaluate the quality of the analyte atomization. The profiles of the standard solutions should be very close to the manufacturer's example of an ideal peak for the particular instrument/furnace/tube/element combination. The ratio of peak height to area (H/A) provides for an objective way to judge peak profiles. A narrow peak will have a larger H/A. A wide peak will have a smaller H/A and indicates a slow release of analyte. Matrix suppression should be suspected if the H/A ratio for an analytical solution is <80% of the ratio for a standard solution. If the release is slow enough (low H/A), some analyte might still be present in the tube after the

end of the read step resulting in the peak not returning to baseline. Ensure that the read time is sufficient for all analytical solutions. A low H/A can also indicate a doubled peak. Doubled peaks are another manifestation of matrix interference. The degree of matrix interference will be quantitatively assessed by the FAS recovery.

**Instrument Setup** - Clean optical windows and replace when the absorbance is 50% greater than manufacturer's specification for new windows.

Inspect and replace tube and platform if necessary. A tube/platform will last between 200-800 firings depending on instrument manufacturer, atomization temperature and sample type. Consult instrument's laboratory records information on tube history. Tubes should be replaced when characteristic mass has increased 10-15%, when peak profiles have degraded (longer tails, doubled peaks, wider peaks) or when laboratory records indicate tube is approaching the end of its performance lifetime. Starting an analysis with a new tube is better than having tube performance degrade during an analytical run.

Check optical alignment of furnace and alignment of autosampler tip.

After at least 30 minutes instrument warmed up, record lamp energy for future reference. Suspect a problem if lamp energy is <90% of expected.

Perform instrument sensitivity check. If characteristic mass specification cannot be met, then the standard solution was improperly made or there is a problem with the instrument or furnace program.

Perform instrument stability check. If short term precision is >5% RSD, determine and correct problem.

**Pre-standardization Checks** -

1. Instrument sensitivity check—Adequate instrument sensitivity is demonstrated by analyzing a standard solution and calculating characteristic mass, $m_o$ (see §3.2.1). Choose a standard that results in 0.05–0.1 A-sec. This daily $m_o$ must be within 20% of the expected value as calculated from accumulated $m_o$ results for the same set of conditions. If proper sensitivity cannot be demonstrated, determine and correct problem before standardization.
2. Instrument stability check—Instrument stability is demonstrated by analyzing a standard solution a minimum of 5 times. Choose a standard that results in 0.05–0.1 A-sec. The resulting RSD of absorbance signals must be ≤5%. If RSD >5%, determine and correct problem before standardization.

**Standardization Verification** - To ensure accuracy, instrument standardization is verified initially, during and after an analytical run.

1. Initial standardization verification—Analyze ICS and standard blank immediately following instrument standardization. Results for ICS recovery must be 100 ± 5% of expected value. Analyze standard blank after ICS to check for carry over. Results for the standard blank must be less than the ASDL. If either of these conditions is not met, diagnose and correct the problem(s) and re-standardize GF-AAS instrument.
2. Continuing standardization verification—To verify lack of instrumental drift, analyze a check solution at a frequency of 10% of analytical solutions and at end of analytical run. Control limits for check solutions are 100 ± 10% of expected concentrations. If control limits

are not met then analysis must be discontinued, cause of deviation determined and instrument re-standardized. All analytical solutions following the last acceptable check solution must then be re-analyzed. This procedure ensures that all groups of 10 or less analytical solution analyses are bracketed by valid standardization verification checks.

**Analysis Checks** -

1. Precision—All measurement results of analytical solutions, standard solutions, standard additions, and quality control solutions are based on the mean of at least 2 replicate injections of the solution from the same autosampler cup. Precision between the injections must be 7% RSD or less for all analytical solutions with ≥0.012 A-sec. If control limits are not met then re-analyze the analytical solution. If the repeat analysis is still out of control, then suspect instrument problem or matrix interference. Diagnose the problem, make necessary adjustments and re-analyze the analytical solution.
2. Standard curve—The value for the correlation coefficient (r) must be ≥0.998. Highest standard must be within the LDR if a linear algorithm is used. A value less than this control limit is an indication of a problem with preparation or standardization due to one or more standard solutions or the standard blank. If display of the standard curve (A-sec vs. concentration) indicates which standard solution is bad, provide re-standardization data for that standard solution. Otherwise, re-standardize with all standard solutions. If re-standardization does not fix the problem, then prepare new standard solutions and re-standardize GF-AAS instrument.
3. Standard additions—The value for correlation coefficient (r) must be ≥0.995. A-sec of all analytical solutions plus additions must be within the LDR.

Dilute any analytical solutions with standard blank if integrated absorbance is greater than highest standard solution. Estimate degree of dilution needed from absorbance.

There are limitations to the amount of matrix effect correctable by quantification using the method of standard additions. If the slope of an analytical solution standard addition curve is <50% of the slope standard addition curve of a standard blank (or a standard solution without any matrix effect such as the ICS), then the analytical solution should be diluted by a factor of 2 with standard blank and re-analyzed.

### 3.6.1.2 COLD VAPOR ATOMIC ABSORPTION SPECTROMETER

**Interferences** - Mercury contamination from reagents, containers, miscellaneous laboratory supplies, and mercury vapor in laboratory air may cause erroneously high results unless suitable contamination control procedures are used. Contamination was minimized during validation of this method by using disposable, plastic laboratory containers and pipette tips that did not need acid-cleaning, using ultra pure acids, acid-cleaning 2-L Teflon® containers in which reagents were prepared, acid-cleaning ware and preparing reagents as close in time as possible to the time of use, and purging stannous chloride reducing solution with argon to remove mercury contamination. Other procedures that were found necessary to minimize contamination from laboratory air included putting caps and caps in place as much as possible, using laboratory hoods only when needed to exhaust acid fumes, and placing ultra pure acids in a secondary

sealed container to minimize transport of mercury vapor through reagent bottle walls. These procedures were adequate to keep mercury contamination in solutions below approximately 0.002 µg/L during the course of analysis and make possible accurate quantification of ≥0.01 mg/kg levels in seafood. Additional quality control procedures, more rigorous than those in this method, must be used if lower concentrations need to be quantified accurately.

Nonspecific absorption due to molecular species in the absorption cell may produce erroneously high results. Molecular gases are minimized by using ultra high purity argon to carry mercury vapor through the atomic absorption cell and ensuring that all connections in the carrier gas path are tight. Water vapor is removed from the carrier stream by passing it through drying device. Other dissolved gases are removed by shaking the hot, decomposition acid mixture and allowing it to degas.

Spectral interferences due to direct line overlap of other elements are rare in atomic absorption and are further minimized by the vapor generation step in which mercury, but not other elements, is reduced to atomic vapor that absorbs radiation at 253.7 nm.

**Instrument setup** - Set up instrument, turn on power, and warm-up instrument as directed in operator manuals provided by manufacturer. Three or more hours may be necessary to warm up electronics and detector and ensure absence of drift during analysis of solutions containing mercury concentrations <1 µg/L. Turn on and warm up Hg lamp ≥30 minutes before analyzing solutions.

Inspect peristaltic pump tubing and replace it with new tubing if flat or worn spots are observed. Start gas and liquid flows and ensure that liquid flow through uptake tubing, gas-liquid separator, and drain tubing is as described in operator manuals. Condition new tubing for 30-60 minutes before analyzing sample solutions by pumping acid concentrations equal to those that will be pumped through tubes during analyses. Old analytical solutions from previously digested and analyzed samples may be combined and used to condition new sample uptake tubing. If necessary, re-adjust clamp tension on pump tubing after tubing is conditioned.

When instrument warm-up is achieved, zero the instrument, then immediately analyze a standard blank once and a standard solution with high concentration 2 or more times. Visually inspect instrument response profiles and calculate instrument sensitivity and percent relative standard deviation of the high concentration standard solution. Measure pump speed (revolutions/minute) and solution uptake rate (mL/minute) using a graduated cylinder and stopwatch. Adjust operating conditions if necessary.

**Pre-standardization Checks** -

1. *Instrument sensitivity check* — Adequate instrument sensitivity is demonstrated by analyzing a standard solution and calculating instrument sensitivity, Â. Choose a standard in the middle standard calibration range. This instrument sensitivity must be within 20% of the instrument manufacturer's specification. If proper instrument sensitivity cannot be demonstrated, determine and correct problem before standardization.
2. *Instrument stability check* — Instrument stability must be demonstrated by analyzing a standard solution a minimum of 5 times. Choose a standard that results in the middle of

the linear range. The resulting RSD of absorbance signals must be ≤2%. If RSD >2%, determine and correct problem before standardization.

**Standardization verification** - To ensure accuracy of standardization, instrument standardization is verified initially, during and after an analytical run and by the analysis of a reference material.

1. *Initial standardization verification* — Analyze the low and high concentration standard solutions and standard blank as check solutions immediately following instrument standardization. Check solution recovery for low and the high standard solutions must be 95-105%. Results for the standard blank must be less than the ASDL. If either of these conditions is not met, diagnose and correct the problem(s) and re-standardize instrument.
2. *Continuing standardization verification* — To verify lack of instrumental drift, analyze a check solution at a frequency of 10% of analytical solutions and at end of analytical run. Control limits for check solutions are 100 ± 10% of expected concentrations. If control limits are not met analysis must be discontinued, cause of deviation determined and instrument re-standardized. All analytical solutions following the last acceptable check solution must be re-analyzed. This procedure ensures all groups of 10 or less analytical solution analyses are bracketed by valid standardization verification checks.

**Analysis Checks** -

1. *Standard curve* — The value for correlation coefficient of determination (r) must be ≥0.998. The highest standard must be within the LDR. A value less than this control limit is an indication of a problem with preparation or standardization due to one or more standard solutions or the standard blank. If display of the standard curve (absorbance vs. concentration) indicates which standard solution is bad, provide re-standardization data for that standard solution. Otherwise, re-standardize with all standard solutions. If re-standardization does not fix the problem, then prepare new standard solutions and re-standardize instrument.
2. *Peak Profile* — Examine the peak profile (shape) of each analytical solution. The profiles of the standard solutions should be very close to the manufacturer's example of an ideal peak.
3. *Carry-over* — Verify absence of carry-over of mercury(II) ion from previous solutions by analyzing standard blank.

### 3.6.1.3 INDUCTIVELY COUPLED PLASMA-ATOMIC EMISSION SPECTROMETER

**Interferences**[2] - Spectral interferences associated with inductively coupled plasma-atomic emission spectrometry (ICP-AES) are caused by background emission from continuous or recombination phenomena, stray light from line emission of high concentration elements, overlap of a spectral line from another element, or unresolved overlap of molecular band spectra.

Subtracting background emission is usually necessary for most analytical emission lines. Spectral scans (wavelength verses intensity) in analyte wavelength region may indicate when alternate emission lines are desirable because of severe spectral interference. Spectral scans will also show whether the most appropriate estimate of background emission is provided by an interpolation from measurements on one or both sides of the analyte peak. Locations selected for background intensity measurements will be determined by the complexity of spectrum adjacent to a wavelength peak. Locations used for routine measurement must be free of off-line spectral interference (inter-element or molecular) or adequately corrected to reflect the same change in background intensity as occurs at the wavelength peak.

Spectral overlap may be avoided by using alternate wavelengths or can be compensated for by correcting for inter-element contributions, which involves measuring interfering elements. Extensive information on interferences at various wavelengths and resolutions is available in Boumans' Tables[3] and Winge's Atlas[4]. Users may apply inter-element correction factors determined on their instruments within tested concentration ranges to compensate (off-line or on-line) for effects of interfering elements. When inter-element corrections constitute a major portion of an emission signal, accuracy may be greatly reduced. For the element levels typically found in foods, spectral overlap is not likely[3-5] except for phosphorus, calcium, iron, zinc, aluminum and titanium. These elements should be included in the analyte list even if quantitative results are not needed so that inter-element corrections can be applied.

Interference effects must be evaluated for each instrument. To determine appropriate location for off-line background correction, an analyst must scan on either side adjacent to the analytical wavelength and record apparent emission intensity from all other method analytes. On-line and off-line spectral interference effects must be determined and documented for all method analytes and corrections must be performed on all analyses. Tests to determine spectral interference must be done using analyte concentrations that will adequately describe interference. Expansion of the scan's scaling may be necessary to observe the interference or ascertain its absence. For most elements, 100 mg/L single element solutions are sufficient although higher concentrations may be necessary for some mineral elements (e.g., calcium). Failure to correct for spectral interference can result in false positive or false negative results. Uncorrected interfering peaks occurring on or very close to the analyte peak can result in false-positives or positive bias. Uncorrected interfering peaks occurring on or very close to a background correction wavelength can cause negative bias or even negative results.

Physical interferences are effects associated with sample nebulization and transport processes. Changes in viscosity and surface tension can cause significant inaccuracies, especially in analytical solutions containing high dissolved solids or high acid concentrations. Physical interferences can be reduced by diluting the analytical solution. Chemical interferences include molecular-compound formation, ionization effects, and solute-vaporization effects. Normally, these effects are not significant with ICP-AES. If observed, they can be minimized by careful selection of operating conditions, matrix matching, and using method of standard-additions. Chemical interferences are highly dependent on matrix type and specific analyte element determined.

Memory interferences occur when analytes from a previously measured analytical solution contribute to analyte signals currently being measured in an analytical solution. Memory effects

can result from analyte deposition on nebulizer uptake tubing or from build-up of material in the plasma torch and spray chamber. The site where these effects occur is element dependent and can be minimized by flushing with a standard blank between analytical solutions. Monitoring for memory interferences is performed during an analytical run and suitable rinse times are to be established to control their affect on analyte measurements. Rinse times necessary for a particular element must be determined before analysis. Determination of a sufficient rinse time may be achieved by aspirating a standard solution containing elements corresponding to either the upper end of their LDRs or concentrations ten times those usually encountered. A normal aspiration time should be used, followed by analysis of the standard blank at designated intervals. The length of time required to reduce an analyte's signal to within a factor of two of the ASDLs should be used as the rinse time if more than the minimum 60 sec is required. Until required rinse time is established, a 60 sec rinse period is recommended between analytical solutions and standards. If memory interference is suspected, analytical solutions should be re-analyzed using a longer rinse period.

**Instrument setup** - Each laboratory must determine optimum instrument parameters for radio frequency (RF) power, view height, argon flow rates and sample uptake rate. Analyst should be aware that small changes in RF power, view height and argon flow rates can greatly affect instrument performance and inter-element correction factors. Inspect sample introduction system including nebulizer, torch, injector tube and uptake tubing for salt deposits and dirt that would restrict solution flow and affect instrument performance. Inspection frequency will depend on work load and analytical solution composition. Inspect system at each use and clean as needed. Allow instrument to become thermally stable before standardization and analyses. This usually requires at least 20 to 30 minutes of operation. After instrument warm-up, perform optical profiling. Optical profiling is performed with a built-in mercury lamp, a 2 mg/L Mn solution, or a procedure recommended by instrument manufacturer. If laboratory has a sequential type ICP-AES instrument, perform wavelength calibration according to manufacturer's instruction.

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*Safety Note: Inductively coupled plasmas emit ultraviolet radiation during operation and must be viewed with proper eye protection.*

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**Pre-standardization checks** - Instrument sensitivity and precision check-Ensuring the instrument is operating correctly is essential before spending time standardizing or analyzing samples. Instrument sensitivity and short-term precision must be demonstrated before proceeding with standardization. Analyze one of the standard solutions, or a separate solution made for this check, for 5 replicate integrations. Monitor the emission counts (or emission ratio) of a selected element (*e.g.*, 2 mg/L Mn). Calculate the mean and RSD of the emission counts. The mean emission counts should be within 20% of the historical mean indicating good sensitivity. The RSD should be less than 5% indicating good precision. Failure of either the sensitivity or precision check usually indicates a solution introduction problem. Correct the problem before proceeding.

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*Note: A special solution dedicated to this daily task may be used routinely. The element used for this check can be different from analyte(s). The daily mean emission counts and RSD should be recorded for future reference.*

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**Standardization verification** - To ensure consistent instrument performance and accuracy, IDL and instrument standardization are verified initially. Instrument standardization is also verified during and after an analytical run.

1. *IDL verification* — immediately after standardization, determine IDLs. Analyze the standard blank 5 times (separate analyses with normal autosampler rinse in between). The IDLs must be within 3 times the normally obtained IDL values. Record IDLs for future reference.
2. *Initial standardization verification* — Analyze ICS and standard blank immediately following instrument standardization and IDL verification. Results for ICS recovery must be 100 ± 5% of expected value. Analyze standard blank after ICS to check for carry over. Results for the standard blank must be less than the ASDL. If either of these conditions is not met, diagnose and correct the problem(s) and re-standardize ICP-AES instrument. Note: If the fortification solution was used to prepare ICS and ICS is out of control, an error in fortification of the FAP should be suspected and may require the FAP to be re-prepared.
3. *Continuing standardization verification* — To verify lack of instrumental drift, analyze a check solution at a frequency of 10% of analytical solutions and at end of analytical run. Control limits for check solutions are 100 ± 10% of expected concentrations. If control limits are not met analysis must be discontinued, cause of deviation determined and instrument re-standardized. All analytical solutions following the last acceptable check solution must be re-analyzed. This procedure ensures all groups of 10 or less analytical solution analyses are bracketed by valid standardization verification checks.

**Analysis Checks** -

1. *Precision* — All measurement results of analytical solutions, diluted analytical solutions, standard solutions, and quality control solutions shall be based on the mean of at least 3 replicate integrations. Precision of replicate integrations must be 7% RSD or less for analytes above ASQL in all analytical solutions. If control limits are not met then re-analyze the analytical solution. If the repeat analysis is still out of control then suspect either an instrument problem or matrix interference. Diagnose problem, make necessary adjustments and re-analyze analytical solution. There may be either a problem with the sample introduction system or a physical interference with the analytical solution. Flushing the sample introduction system for several minutes and diluting analytical solution by a factor of 2 may resolve the problem. If the RSD still fails then diagnose the problem and fix before proceeding.
2. *Standard curve* — The highest standard solution must be within the LDR. Values for correlation coefficients (r) must be ≥0.998. A value less than this control limit indicates problem with preparation or standardization due to one or more standard solutions or the standard blank. If display of the standard curve (intensity vs. concentration) indicates which standard solution is bad, re-standardization that standard solution. Otherwise re-standardize ICP-AES instrument. If re-standardization does not fix the problem, then prepare new standard solutions and re-standardize instrument.
3. *Alternate wavelength precision* — If possible, use multiple alternate wavelengths for

each analyte. For all measurement results >ASQL, the concentration found at the primary wavelength must agree within ±10% relative difference of the concentration found at the secondary wavelengths for each element. A relative difference >10% can be due to instrument problems or matrix/spectral interference in the analytical solution. If check solutions are within control limits, dilute the analytical solution and re-analyze. If the diluted analytical solution is still out of control, an alternate analytical method must be used.

4. *Wavelength scan* — Each analytical solution is checked for spectral interference by performing a wavelength scan. An intensity (emission counts) verses wavelength scan is recorded for each element for each analytical solution. Depending on ICP-AES instrument software, these scans can be incorporated into the ICP-AES analytical run or performed in a separate "scan" run. An appropriate standard solution must be scanned and the result overlaid with the scan of the analytical solution. A standard solution close in element concentration to the analytical solution should be chosen. A broad or double peak indicates an unresolved peak that may result in a positive bias. Interfering peaks could be from elements not being quantified. Peaks in the area of the background correction point(s) may result in a negative bias. Background correction points must be in an area(s) free from other peaks.

### 3.6.1.4 INDUCTIVELY COUPLED PLASMA-MASS SPECTROMETER

**Interferences** - Several types of interferences are associated with inductively coupled plasma-mass spectrometry (ICP-MS). Instrument operators must be familiar with the various interferences, the means of detecting interferences and the ways to eliminate or minimize them. Typical quadrupole-based instruments do not have the resolution capabilities to resolve interferences that are less than one nominal mass unit such as $^{75}As$ (mass 74.9216) and $^{40}Ar^{35}Cl$ (mass 74.9312).

1. *Elemental isobaric interference* — is caused by an isotope of an element other than the analyte element that forms a singly charged ion with the same nominal mass-to-charge ratio as the analyte isotope. The recommended isotopes for elements determined by this method are free from elemental isobaric interference. An example of elemental isobaric interference is $^{114}Cd$ and $^{114}Sn$. However, none of the recommended isotopes in this method suffers from an elemental isobaric interference.
2. *Doubly charged species isobaric interference* — is actually a special case of elemental isobaric interference caused by an isotope of an element other than the analyte element that forms a doubly charged ion with the same nominal mass-to-charge ratio as the analyte isotope. The only doubly charged species of concern for this method are $^{150}Sm^{++}$ and $^{150}Nd^{++}$. The mass to charge ratio is 75 for $^{150}Sm^{++}$ and $^{150}Nd^{++}$, which is the same mass to charge ratio used to determine arsenic. Tuning the instrument to minimize doubly charged species is helpful. However, if neodymium or samarium is present in an analytical solution, correction factors must be applied. Otherwise, arsenic results could have a positive bias.
3. *Polyatomic isobaric interference* — is caused by molecular species with the same

nominal mass-to-charge ratio as the analyte isotope. These ions can be formed in the plasma, the interface or the reaction cell. The sources for these molecular ions are the plasma (Ar), the atmosphere (C, O, N, $CO_2$), the matrix ($H_2O$) and the sample. The main polyatomic interferences on arsenic are $^{40}Ar^{35}Cl$ and $^{40}Ca^{35}Cl$. These could be significant because many processed foods are high in salt and dairy products are naturally high in calcium. Additionally, HCl is added to help stabilize Hg so chloride will be present in all analytical solutions. The main polyatomic interference on the recommended Cd isotope ($^{111}Cd$) is $^{95}Mo^{16}O$. The effect should be negligible if the instrument is tuned properly (low oxide formation) and the fact that Mo levels in foods are very low. Lead and mercury should not be affected by polyatomic isobaric interferences in most food and dietary supplement analyses.

4. *Matrix interference* — is caused by various properties of the analytical solution such as dissolved solids content and viscosity. High dissolved solids can affect nebulizer operation, cause deposits on the interface cones, and affect ionization efficiency. Cone deposits will cause the response to drift over time. High dissolved solids/salt in an analytical solution will suppress ionization of elements with high ionization potentials (>9 ev) more than other elements. Therefore, an internal standard element that has a similar ionization potential (IP) as the analyte will probably compensate for this suppression better than one with a greatly different IP. Another type of matrix effect is suppression caused by the space charge effect. Lighter elements will tend to be "knocked around" and not pass through the ion lenses as efficiently as heavier elements. An internal standard element close in mass to the analyte element will help with this type of interference.
   a given element in the food analytical solutions verses the standards thus leading to inaccurate results. Analytical solutions must be limited to <0.2% (2000 mg/L) dissolved solids. Suppression of the internal standard isotope usually indicates that some type of matrix effect is present. Dilution is required for any analytical solution if the internal standard signal differs by more than 40% from the calibration blank. Although internal standards can compensate for matrix effects, there is a limit to the amount of correction applied before the accuracy of the measurement suffers. Poor fortification recovery of the FAP and FAS quality control analyses can also indicate matrix interference. If the fortification recovery is outside the acceptable range, then a matrix effect should be suspected and the analytical solution must be diluted and reanalyzed or analyzed by method of standard additions.

   The net effe

5. *Memory effect interference* — is caused by a high concentration of an element in an analytical solution that does not fully rinse out of the sample introduction system during the programmed rinse time. Sufficient rinse time must be allowed in the autosampler program to rinse out the highest concentrations of elements expected. Analyzing a blank after the highest standard will confirm if the rinse time is long enough. Mercury is especially prone to memory effects. Therefore, the highest Hg standard should be no higher than 1 µg/L.

**Instrument setup** - Each laboratory must determine optimum instrument parameters for radio frequency (RF) power, sampling depth, argon flow rates, collision cell gas flow rate, lens voltages and sample uptake rate. Analyst should be aware that small changes in RF power, sampling depth and argon flow rates could greatly affect the instrument performance. Inspect

sample introduction system including nebulizer, torch, pump tubes and sampler cone. Inspection frequency will depend on work load and analytical solution composition. Inspect system at each use and clean as needed. Clean cones when deposits are noticed. Allow instrument to become thermally stable before standardization and analyses. This usually requires at least 20-30 minutes of operation. After instrument warm-up, perform tuning. Set up method and autosampler sequence table. Determine any corrections factors needed and enter in method.

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*Safety Note: Inductively coupled plasmas emit ultraviolet radiation during operation and must be viewed with proper eye protection.*

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**Pre-standardization checks** - Instrument sensitivity and precision check — Ensuring that the instrument is operating correctly is essential before spending time standardizing or analyzing samples. Instrument sensitivity and short-term precision must be demonstrated before proceeding with standardization. Run the "Tune Report" after tuning the instrument and while still aspirating the tune solution. Results for sensitivity, oxide lever, double charged species, peak axis and peak height should meet laboratory's or manufacturer's specifications. Analyze one of the midlevel standard solutions and check that the RSD is ≤5% indicating good precision. Failure of either the sensitivity or precision check usually indicates a solution introduction problem. Correct the problem before proceeding.

**Standardization verification** - To ensure accuracy, IDL and instrument standardization are verified initially. Instrument standardization is also verified during and after an analytical run.

1. *Standard curve* — Values for correlation coefficients (r) must be ≥0.998. A value less than this control limit indicates problem with preparation or standardization due to one or more standard solutions or the standard blank. If display of the standard curve (intensity vs. concentration) indicates which standard solution is bad, re-standardization that standard solution. Otherwise re-standardize ICP-MS instrument. If re-standardization does not fix the problem, then prepare new standard solutions and re-standardize instrument.
2. *IDL verification* — immediately after standardization, determine IDLs. Analyze the standard blank 5 times (separate analyses with normal autosampler rinse in between). The IDLs must be within 3 times the normally obtained IDL values.
3. *Initial standardization verification* — Analyze ICS and standard blank immediately following instrument standardization and IDL verification. Results for ICS recovery must be 100 ± 5% of expected value. Analyze standard blank after ICS to check for carry over. Results for the standard blank must be less than the ASDL. If either of these conditions is not met, diagnose and correct the problem(s) and re-standardize ICP-MS instrument. *Note: If the fortification solution was used to prepare ICS and ICS is out of control, an error in fortification of the FAP should be suspected and may require the FAP to be re-prepared.*
4. *Continuing standardization verification* — To verify lack of instrumental drift and carry over, analyze a check solution and the standard blank at a frequency of 10% of analytical solutions and at end of analytical run. Control limits for check solutions are 100 ± 10% of expected concentrations. Control limits for standard blanks are ≤ASDL. If control limits

are not met analysis must be discontinued, cause of deviation determined and instrument re-standardized. All analytical solutions following the last acceptable check solution and standard blank must be re-analyzed. This procedure ensures all groups of 10 or less analytical solution analyses are bracketed by valid standardization verification checks.

**Analysis Checks** -

1. *Precision* — All measurement results of analytical solutions, diluted analytical solutions, standard solutions, and quality control solutions shall be based on the mean of 3 replicate integrations. Precision of replicate integrations is usually 7% RSD or less for concentrations above ASQL in all analytical solutions. If control limits are not met then re-analyze the analytical solution. If the repeat analysis is still out of control, then suspect instrument problem or matrix interference. Diagnose problem, make necessary adjustments and re-analyze analytical solution. There may be either a problem with the sample introduction system or a physical interference with the analytical solution. Flushing the sample introduction system for several minutes and diluting analytical solution by a factor of 2 may resolve the problem. If the RSD still fails then diagnose the problem and fix before proceeding.
2. *Standard additions* — If quantification is performed by the method of standard additions, the value for correlation coefficient (r) must be ≥0.995.

## 3.6.2 METHOD PERFORMANCE

**Reference Material (RM)** - RM results are used to assess accuracy. Assessing accuracy using a z-score (§3.5.3) is the preferred procedure. However, for simplicity, control limits are usually used and set at an RM recovery of 100 ± 20% unless the RM's reference uncertainties (at 95% confidence level) are greater than 20%. For each element of interest, there must be an established value for that element at a concentration above LOQ. If three or more RMs are analyzed then only two-thirds of an element's RM recovery results must meet the control limit. Repeat analysis of all batch analytical solutions if control limit is exceeded. If RM recovery fails again, reject batch results and repeat digestion and analysis of all samples in batch. When appropriate RMs are unavailable other quality control measures are used to judge acceptance of batch analytical results (e.g., FAP recovery, FMB recovery).

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*Note: Failing the control limit should be highly unusual because a laboratory's experience analyzing a RM should establish predictable results. Whenever a new RM is investigated, treat it initially as an unknown and if accuracy is a problem, identify and correct the cause(s) of the problem(s) before the RM is used as a control material for judgment of batch quality. In-house RMs with established values are acceptable.*

*Note: Choice of RM depends on availability but should be similar to the sample matrix. Unfortunately, suitable RMs may not be obtainable. Non-certified element concentrations provided on a certificate may be used for quality control if the laboratory has established the ability to meet the acceptance criteria.*

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**Fortified analytical portion (FAP)** - FAP results are used to assess analyte recovery and matrix induced interference. Control limits are usually a FAP recovery of 100 ± 20%. A poor recovery can indicate analyte loss during preparation, physical/transport interference and spectral interference. Prepare two replicate portions, one portion is the unfortified analytical portion (UAP) and the other portion, the FAP, is fortified with each analyte before digestion. Fortify by pipetting no more than a total of 1 mL (0.5 mL for CV-AAS) of fortification solution(s) (may be measured gravimetrically) into the digestion vessel. Fortification level is based on prior knowledge of analyte concentration in the sample or typical reported levels for the food. Fortification should be 100-200% of expected analyte level. Foods with low analyte levels or unknown analyte levels should be fortified so that analyte concentration in the analytical solution is approximately midpoint of LDR or for ICP-AES between 10 times ASQL and 40 mg/L. Analyze an FAP for each food type for which there is no FAP recovery data on record using the method. If FAP recovery results are unacceptable, re-analyze the FAP analytical solution. If FAP recovery fails again then the analyst must use other batch analytical results to evaluate the quality of the analysis and determine if the batch samples must be re-analyzed. Unreliable FAP recovery results may occur due to measurement imprecision when the fortification level is less than 100% of the native level. When fortification levels are too low, the FAP recovery may be considered invalid due to an inappropriate fortification level. In this case, other quality control measures may be used to judge acceptance of batch analytical results (*e.g.*, RMs, FMBs). Depending on the other quality control results and the purpose of the analysis (*i.e.*, survey or enforcement) another FAP may need to be analyzed using an appropriate fortification level based on the sample's analyte concentration.

**Fortified analytical solution (FAS)** - A FAS is used to assess matrix-induced interference. Control limits are usually a FAS recovery of 100 ± 10%. If FAS is out of control, suspect matrix interference, dilute analytical solution by a factor of 2 or more and re-analyze. FAS fortification, recovery check, dilution and re-analysis can usually be performed automatically by the instrument's software and autosampler. As an alternative to dilution, if FAS recovery was ≥50%, analytical solution can be analyzed by method of standard additions. If recovery was <50% then there is a possibility that the standard additions technique may not be able to compensate for the large matrix interference present.

**Fortified method blank (FMB)** - FMB checks accuracy of the fortification procedure without any matrix effects. Control limits are usually a FMB recovery of 100 ± 10%. If FMB is out of control, an error in fortification should be suspected and FMB needs to be prepared again and re-analyzed. The FMB is an optional quality control sample but can be helpful in verifying the fortification procedure and reveal pipet malfunctions and dilution errors.

**Laboratory MBK ($MBK_L$)** - Mean of analyte concentration measurements (to at least 3 significant digits) of at least 5 independently prepared MBKs (unfortified) rounded to a two significant-digit number. $MBK_L$ should be established using MBK results accumulated from many independent analyses over extended periods (i.e., months). $MBK_L$ is determined for each analyte-method-instrument combination. $MBK_L$ represents the analyte level expected during routine analyses and MBKs analyzed with a batch of samples are compared to $MBK_L$. $MBK_L$ is subtracted from all analytical solutions results.

**Laboratory MBK critical value ($MBK_C$)** - Mean of analyte concentration measurements (to at least 3 significant digits) of the MBKs (unfortified) used to establish $MBK_L$ plus 2 times the standard deviation of the these MBKs rounded up to the next greatest two significant-digit number. $MBK_C$ is used to judge the quality of MBKs analyzed with each batch of samples.

$$MBK_C = MBK_L + (2 \cdot s)$$

where, *s* is the standard deviation of the MBKs used to establish $MBK_L$.

**Batch method blanks (MBK)** - MBK results are used to assess contamination from the laboratory environment and reagents. A batch's MBK results are compared to the expected level of $MBK_L$. A batch's MBK results are acceptable when at least two-thirds of the MBK results are $\leq MBK_C$. Batch MBKs exceeding this $MBK_C$ should be uncommon. The more frequently batch MBKs exceed $MBK_C$, the more attention should be directed to identifying and correcting the cause of contamination or to consider reestablishing $MBK_L$ and $MBK_C$.

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