U.S. Department of Health & Human Services

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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 https://www.fda.gov/food/laboratory-methods-food/elemental-analysis-manual-eam-food-and-related-products

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Elemental Analysis Manual

for Food and Related Products

2.3 Analytical Solution Preparation

Version 3.0 (September, 2021)

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GLOSSARY and ACRONYMS

This section discusses sample preparation for atomic spectrometric analysis and is provided for general information. Instructions and procedures presented here should not be used in place of anything specified in the EAM methods but may be useful for method development and other research. Non-destructive techniques such as energy dispersive x-ray fluorescence and instrumental neutron activation analysis are not the focus of this section.

Analytical test solutions introduced to atomic spectrometers must be prepared so that their composition falls within standardization parameters and any interference problems are mitigated. The preparation may be trivial, requiring nothing more than a simple dilution, or complex, requiring procedures such as extractions, digestions, and/or speciation steps.

The determination of the mass fraction of elemental analytes in an analytical sample (commonly referred to as *total* elemental analysis) typically requires the isolation of the elemental components from or decomposition of the analytical portion(s) followed by atomic spectroscopy.

The determination of the mass fraction of element containing molecular analytes in an analytical sample (commonly referred to as elemental *speciation* analysis) typically requires that the molecular analyte(s) of interest are isolated from other components followed by chromatographic separation coupled to atomic spectroscopy.

For the purposes of this discussion, the emphasis will be on food analysis and the most common sample preparation requirements of atomic spectrometry - production of aqueous solutions with fairly well-characterized limits for total dissolved solids and/or total organic content. This applies for the atomic spectrometers most commonly used in FDA laboratories (ICP-AES and ICP-MS) as well as those that remain viable in EAM Methods but are not currently in use (FAAS and GFAAS).

2.3.1. DIRECT ("Dilute and Shoot")

Direct analysis of neat liquids can be applied to samples such as ready-to-drink (particulate free) fruit juices, carbonated beverages, sports drinks, or melted popsicles. Foods such as these typically have relatively low levels of dissolved solids and total organic carbon (TOC) and can often be directly introduced to a spectrometer. The ionization source (*i.e.*, the flame, graphite furnace or plasma) provides sufficient energy to decompose small amounts of simple organic components (which are ultimately removed in the gas phase exhaust).

Direct analysis after simple dilution (with water or dilute nitric acid) can be applied to samples such as salt-based spices, instant beverage powders, and particulate free frozen juice concentrates. Foods such as these are fully soluble in water or dilute acid and can often be introduced directly after dilution. Dilution factors should be carefully selected to reduce the levels of interfering material (e.g., salt, total dissolved solids, TOC, etc.).

When diluting a new food type, experimentation with extra analytical portions may be necessary to learn whether it can be analyzed without digesting and, if so, determine the appropriate solvent (to solubilize the analytes of interest) and dilution level (to reduce the matrix).

When diluting, solubility may be enhanced with sonication and/or gentle heating. If undissolved particulate material remains and it is known that it does not contain significant amounts of the elements of interest, then the solution can be filtered (to remove this material so it does not clog the spectrometer's sample introduction components). Note however that any filtering procedure must be evaluated for potential contamination or unintended removal of analytes of interest.

Direct analysis is more susceptible to non-spectral interference from concomitant elements (*e.g.*, carbon enhanced ionization matrix effects). Undigested analytical solutions may be more susceptible to differences in sample introduction efficiency, ionization efficiency, as well both plasma and spectral matrix effects (*e.g.*, aerosol formation efficiency, easily ionizable element effects, charge transfer effects, polyatomic interferences). Careful matrix matching of standard solutions can help mitigate these effects.

2.3.2. EXTRACTION (Remove from Matrix)

In some methods, elements (or elemental species) are extracted from the sample matrix. Following are several relevant points:

- Extraction efficiency and yield can be elusive but they need to be sufficient for the intended purpose.
- Gentle heating and/or sonication can be useful.
- Centrifugation and/or filtration may sometimes be necessary to prevent undissolved, suspended particulates from being introduced to the spectrometer.
- In general, extraction solutions present a higher risk, relative to digested solutions, of matrix effects (via spectrometer introduction dynamics and/or plasma and spectral interferences or enhancements).
- The choice of extraction solvent and parameters depend on both the analytes of interest and the analytical sample composition.
- To determine total extractable elements, strong acid extraction solvents, such as 1-5 M nitric acid, typically maximize recovery.
- For elemental speciation, care must be taken to prevent the interconversion of the elemental species of interest. For this reason, mildly acidic extraction solvents, such as 0.3 M nitric acid, water, or even mildly basic solvents such as TMAH are most frequently chosen.
- When evaluating extraction parameters in method development, extraction efficiency can be evaluated by comparing extraction amount to the total element analysis of a separate analytical portion (prepared via digestion).

2.3.3. MICROWAVE DIGESTION (Eliminate Matrix)

Most foods can not be analyzed directly and require acid digestion to remove matrix and bring the analytes into solution. The most common means to do this with minimum risk of acquiring environmental contamination is to use laboratory-grade microwave ovens which raise the digest to high temperature and pressure in closed vessels specifically designed for this purpose. Systems are available with a range of design, safety and performance features. Software programs monitor temperature and pressure and control energy input to sustain safe and advantageous decomposition conditions. In addition to enabling digest temperatures to be raised, closed vessels (including vent and reseal types) keep sufficient acid (*i.e.*, minimal vapor loss) and lower the risk of environmental contamination.

Acid digestions can be carried out in open vessels using relatively inexpensive equipment (*e.g.*, hot plates, hot blocks, heating manifolds) but digest temperatures are limited at atmospheric pressure. 2.3 Table 1 shows, for a few representative food types, that the minimum temperatures needed for rapid food decomposition are well above the boiling range for nitric acid (83 ºC for pure acid; 120 ºC for 68%). This process (*i.e.*, at atmospheric pressure) is usually relatively slow, time consuming, and with increased risk of contamination (via entering into open vessels).

2.3 Table 1. Minimum Temperatures Needed for Rapid Decomposition of Foods (Using Nitric Acid)

Food component	Temperature range
Starches, sugars, simple hydrocarbons	150-160 °C
Plant materials	160-180 °C
Animal tissues	170-190 °C
Fats, oils	190-210 °C

2.3.3.1. Acid(s)

In common practice, digestions are virtually always accomplished via oxidative decomposition using nitric acid, possibly with addition of hydrogen peroxide (typically ~1:14 molar ratio [or ~1:8 weight ratio] 30% H_2O_2 to conc. HNO_3).

Other acids are generally unnecessary for decomposition of foods and supplements but can be useful in unique circumstances (*e.g.*, unusual matrices, research, forensic food adulteration investigation). HCl can help keep elements in solution which might otherwise (in dilute nitric solution alone) form insoluble salts (*e.g.*, Al, Pt, Au) and/or volatile species (e.g. Hg, I). It would typically be added after decomposition, when the digest solution is diluted. HF may be required if significant amounts of analyte(s) of interest are tied up in silicates but provides little advantage for food and is generally avoided for safety. While $HClO_4$ is excellent for decomposing organic material, it carries safety risks requiring significant training and expensive laboratory equipment. H_2SO_4 is generally avoided because it can lead to formation of insoluble sulfates, sulfides and sulfur-based polyatomic interferences.

2.3.3.2. Analytical Portion Mass

Analytical portion mass is typically in the 0.5-1.0 g range. Larger amounts would logically counter the (undesirable) effects of heterogeneity but this could result in unsafe digestion vessel internal pressures and increased matrix effects. In routine practice, a laboratory will either have experience with a matrix and already know an appropriate analytical portion mass or will need to 'start low' with a cautiously small mass then gradually increase, if desired.

For any new matrix or one with questionable/unknown composition, analytical portion mass should initially be limited to the smaller of 0.5 g or ½ of a digestion vessel's maximum safe mass ($Mass_{max}$). This applies also to high-energy foods such as fats and oils. With experience, it may be found safe and acceptable to increase mass in subsequent analyses.

Closed vessel manufacturers typically specify $Mass_{max}$ (as dry mass) for plant and animal tissue-based foods. $Mass_{max}$ can also be approximated using 2.3 Equation 1 if the vessel's maximum energy release and the food's energy-to-mass ratio (energy:mass) are known. Energy:mass (often given as kcal/100 g) for many foods are available [1] [2]but may also be discerned using 2.3 Equation 2 using the calories and serving size listed on the nutrition panel of a food's label. Analytical portion masses that would be acceptable for sealed vessels will generally be appropriate also for vent-&-reseal type vessels of similar volume capacity. 2.3 Table 2 provides $Mass_{max}$ calculated for selected foods when using 90 mL & 800 psi digestion vessels.

$$Mass_{max}(g) = \frac{Vessel\ Max\ Energy\ (kcal)}{Energy\text{-}to\text{-}mass\ Ratio\left(\frac{kcal}{g}\right)} \qquad \text{2.3 Equation 1}$$

$$\frac{kcal}{g} = \frac{label\ \text{"calories" per serving}\ (kcal)}{serving\ mass\ (g)}$$ 2.3 Equation 2

Example - nutrition bar and digestion vessel rating 6 kcal:

Per label, 210 Calories (i.e., kilocalories) for a 50g serving size. Energy:mass=4.2 kcal/g (2.3 Equation 2) and $Mass_{max}$ *=1.4 g (2.3 Equation 1). The recommendation for a new matrix is the smaller of 0.5 g or ½* $Mass_{max}$ *(i.e., analyze 0.5 g of the nutrition bar).*

$$\frac{210\ kcal}{50\ g} = 4.2\left(\frac{kcal}{g}\right)$$ (via 2.3 Equation 1)

$$\frac{Vessel\ Max\ Energy\ (kcal)}{Energy\text{–}to\text{–}mass\ ratio\left(\frac{kcal}{g}\right)} = \frac{6\ kcal}{4.2\left(\frac{kcal}{g}\right)} = 1.4g$$ (via 2.3 Equation 2)

2.3 Table 2. $Mass_{max}$ for Selected Foods (Sealed Vessels)[a]

Food	$Mass_{max}$ (g)	Food	$Mass_{max}$ (g)
American cheese	1.2	Lettuce, Iceberg	5
Beef liver	2.4	Nuts, mixed	0.8
Peaches, canned	5	Peanut butter	0.8
Spaghetti, canned	5	Raisin bran cereal	1.6
Bread (white)	1.8	Fruit cocktail, canned	5
Spinach	5	Mustard, catsup, pickles	n/a[b]

[a] Use less mass until experience gained with these or similar foods.

[b] $Mass_{max}$ not applicable because mass is limited due to interference from salt. Typical masses are ~3 g for mustard/catsup and ~4g for pickles.

2.3.3.3. Digestion Procedure

Below are general procedural guidelines given for informational purposes only. They are not meant to over-ride instructions contained in any method. Reagent amounts are typical for food and capacity assumed to be 90 mL for 800 psi closed vessels or 55-110 mL for vent-and-seal vessels. Adjust parameters and amounts, as needed, if specifications differ and/or for non-food matrices.

Analysts must understand the functional and safety procedures associated with all equipment used and take precautions to minimize risk of contamination (*i.e.*, use high-purity water and acids, clean items, work in a clean hood, etc.).

- Transfer analytical portion into a digestion vessel liner (avoid placing material on liner walls; mass typically recorded to ≤0.001g).
- Optional - Add 1 mL water. (See note below for highly reactive matrices)
- Add nitric acid (typically ~11.3 g or ~8.0 mL; wash down any material on vessel wall).

For highly reactive matrices (e.g., dry food and some CRM materials), it can be useful to add (prior to adding nitric acid) up to ~1 g water to moderate (slow down) the reaction. Add no more than the analytical portion mass or 10% of the total reagent volume.

For strong exothermic reactions when nitric acid is added (e.g., if high in sugar), cap and seal the vessel immediately after adding the acid to prevent loss of sample material and/or nitric acid. Allow the exothermic reaction to complete and cool (ok to let sit overnight). Vent before the microwave heating (this is particularly important for sealed vessels to prevent blowout). Limiting mass will help to prevent membrane rupture and vessel blowout.

- After any initial reactions subside (≥20 min), add 1 mL 30% H_2O_2.
- Seal vessel.
- Swirl or shake vessel to wet/disperse sample material (prior to microwave heating but after any initial vigorous reaction has subsided).
- Run microwave program (For most foods, 25-min ramp to 200°C & sustain 15 min is sufficient)
- Cool to ≤50° C then slowly vent (ideally in an exhausting clean hood).
- Inspect and evaluate solution. If digestion is complete, then continue.
- Transfer and dilute (such that elemental levels are in working range and matrix effects are minimized).
- Inspect digestion vessel for malfunction (*e.g.*, missing or ruptured safety membrane, loose cap, distorted cap or vent plug, breach in vessel wall). If malfunction is observed, discard digest solution.

2.3.3.4. Incomplete Digestion

After digestion, the solution should be clear and colorless or slightly yellow. A yellow-orange color is from dissolved NO_x which can be liberated by adding a few drops 30% H_2O_2. Incomplete digestion is indicated if there is significant turbidity (from particulates) and/or a yellow-green or brown color (indicative of incomplete decomposition of organic substances). If incomplete digestion is suspected, check for malfunction of the microwave digestion system (lack of safety membrane, safety membrane cap not tight, wrong oven program, etc.). If a malfunction is identified, the affected digest portions should be discarded and replacement portions digested. If no malfunction is identified, then evaluate and consider options such as discussed below.

Turbidity comes from particulates. These are more easily visualized if a bright light is shined through the bottom of a digestion vessel liner but even then it can be challenging to know when the amount of particulate material is "significant". A small amount of fine particulate material is not uncommon and generally not problematic. It is usually nitric acid-insoluble material such as titanium dioxide (*i.e.*, an ingredient), silica (commonly present with foods such as spinach), or salts.

Provided the particulates do not contain significant amounts of the analytes of interest, even large amounts of particulates may not be problematic. However, they would need to be centrifuged with decantation or filtered out to prevent issues associated with the spectrometer.

If the particulates need to be dissolved, hydrofluoric acid can be used for insoluble material such as silica but this requires additional safety precautions and its removal prior to introduction to the

spectrometer. Hydrochloric acid (at a final concentration of 0.5-1%) added during or shortly after dilution can inhibit the formation of some insoluble salts (most notably for mercury, by forming the readily soluble $HgCl_2$ rather than relatively insoluble Hg_2Cl_2, HgS or the volatile Hg^0).

Incomplete decomposition of organic substances usually requires an additional microwave digestion run, possibly with additional acid. Reviewing plots of observed temperature and pressure versus time and microwave energy application can be helpful when diagnosing digestion problems. Correct the problem and extend the digestion or discard and analyze a replicate analytical portion using adjusted parameters.

Temperature and pressure plots vs time and microwave energy application can be useful to diagnose incomplete digestion.

- pressure limit reached but not target temperature - vent and repeat heating program
- target temperature not reached and no pressure limit - add more nitric (but ≤20% of original amount) and repeat the heating program.
- temperature limit reached before temperature ramp completes (exothermic reaction, short cycle times) - repeat heating program (possibly with longer temperature ramp time, e.g., 40 minutes).
- heating program stopped short (uncontrolled exothermic reaction causes pressure or temperature increase so fast that program terminates) - discard solution, repeat using less mass and/or longer time for initial digestion after addition of acid but before heating.

2.3.3.5. Digestion Vessel Cleaning

Digestion vessels should be acid cleaned prior to each digestion. When used for the first time or after an incomplete digestion (i.e., dark yellow/brown digest), they should also be cleaned (prior to the acid cleaning) with liquid laboratory-grade detergent.

Detergent cleaning — Disassemble vessels and soak for at least 2 hours in a solution of liquid laboratory-grade detergent and hot water. Thermowells should be wiped with a paper towel and detergent solution. Rinse thoroughly with warm tap water then reagent water. Allow to dry in a clean area (preferably Class 100).

Acid cleaning — Add 10 mL nitric acid to each digestion vessel and microwave according to the Clean Program in 2.3 Table 3 (or as suggested by the manufacturer). After cooling to <50 °C vent slowly in a fume hood. Disassemble and rinse off vessel covers and liners with copious quantities of reagent water (triple wash at minimum). Dry in a clean area (preferably Class 100). Outside surfaces of vessels may be dried with laboratory tissues. If vessels are not used immediately after drying, store assembled in a Class 100 clean area or other appropriate contamination free environment.

Additional information on cleaning may be provided by the manufacturer(s) of the microwave digestion equipment. Be careful to not clean using anything that can scratch the vessel walls which are relatively soft and can be easily marred.

2.3 Table 3. Digestion Vessel Clean Program

Parameter:	Value
Maximum Power (Watts)	1200

Control Pressure (psi)	Not Used
Ramp Time (min)	10
Hold Time (min)	3
Control Temperature (°C)	200

2.3.4. SPECIATION (Isolate Chemical Form)

(Under development.)

2.3.5. HISTORY

EAM 2.3 Table 4. History

Version	Revisions Made	Effective Date
1.0	*Analytical Portion to Analytical Solution*	June 2008 (hard copy only)
2.0	Major re-organization. Title changed to *Digestion and Separation*; added subsection 2.3.2 with links to sample preparation procedures in various methods; Converted to pdf for web posting.	September 2014
3.0	Major reorganization and expansion of the section's scope. Title changed to *Analytical Solution Preparation*; added extraction section; expanded microwave digestion discussion with subsections; added *Speciation* placeholder to be drafted later; added *History* section.	September 2021

References

[1] S. F. W. a. K. H. Souci, in *Food Composition and Nutrition Tables, 5th Ed.*, Boca Raton, FL, CRC Press, 1994.

[2] "Food Data Central," USDA, [Online]. Available: https://fdc.nal.usda.gov/. [Accessed September 2021].