Chapter 11
Analyzing Food Samples—Inorganic Chemicals

Sean M. Ryan

Introduction

In 2009, over 300 different varieties and types of foods were being analyzed in the United States Food and Drug Administration (FDA) total diet study (TDS) program for inorganic chemicals/elements. However, not every element is determined in every item. Presently, 16 elements are routinely determined in these foods. Previously, as many as 24 elements were determined, but for various reasons, some of these elements have been dropped from the analytical list.

A total of 5 different analytical techniques are used to determine the 16 elements of interest. Four of these analytical techniques require the sample to be digested and dissolved in an acidic aqueous solution prior to introduction of the sample into the analytical instrument. Three different sample preparation techniques are used for these digestions. The fifth technique, the analysis of mercury, does not require sample digestion.

The procedures described within are general outlines and do not include all techniques and cautions. The full set of operational instructions can be found within the references listed below and are available from the FDA Kansas City Laboratory.

S.M. Ryan, M.S. (✉)
US Food and Drug Administration, 11630 W 80th Street,
Lenexa, KS 66214, USA
e-mail: sean.ryan@fda.hhs.gov

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Sample Preparation Techniques

Ternary Acid Digestion

This digestion scheme provides the avenue for the multiple determinations of elements in total diet items using Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) [1–3] and Hydride Generation Atomic Absorption Spectrometry (HGAAS) [4] techniques.

Arsenic, calcium, copper, iron, magnesium, manganese, phosphorus, potassium, selenium, sodium, and zinc are determined after the samples have been digested in a mixture of nitric, perchloric and sulfuric acids. Other elements including aluminum, antimony, cobalt, chromium, molybdenum, strontium, tin, titanium, and vanadium will be or have been previously determined in solutions prepared by using this technique. Approximately 260 total diet items are analyzed using this technique.

Depending upon the amount of lipids/solids and percent moisture, between 2.00 and 20.0 g of well-homogenized sample are placed within a quartz Kjeldahl flask. A small amount of deionized water may be used to wash down the sample into the flask. This is then followed by an acid mixture (4:1:1 by volume) of nitric, perchloric and sulfuric acids. It is advantageous to cover the Kjeldahl flask with a clean plastic beaker and allow the solution to react over night at room temperature. Following the Standard Operating Procedure [2] (SOP), carefully heat until only a clear solution of sulfuric acid remains. Initially, the more easily oxidized portion is attacked by nitric acid. Once all the nitric acid is consumed and/or boiled off, the temperature rises to the boiling point of perchloric acid. Perchloric acid attacks the more difficult oxidizable materials (such as fats). Eventually, all the perchloric acid is then boiled off, the temperature rises again and a clear solution of boiling concentrated sulfuric acid remains.

As the nitric acid is consumed, some samples may begin to char, which will adversely affect the recoveries of certain elements. Charring may be controlled by careful, small additions of nitric acid. Should it be necessary to add nitric acid at this point, it must be done with extreme caution, as rapid additions will result in violent expulsion of the solution. Once the digestion is complete, with the sample remaining in only sulfuric acid, it may be cooled and carefully diluted to a known volume with deionized water. After the initial dilution to volume, the digest may be volumetrically split for the separate ICP-AES and GFAAS determinations. Follow all appropriate laboratory contamination control procedures [5].

For the ternary acid digestion, each analytical batch consists of one method blank and one matrix spike/matrix spike duplicate sample fortified with the elements of interest, and 17 analytical samples. This gives a total of 20 sample flasks per batch. The first batch should also include the nonfortified analytical sample, preferably prepared in duplicate, which is then repeatedly chosen for the matrix spike/matrix spike duplicate pairs used in the remaining analytical batches.

Ternary Acid Digestion for Iodine

Iodine is determined after the samples have been digested in a mixture of nitric, perchloric, and sulfuric acids [1, 6]. All total diet items are analyzed for iodine. This digestion scheme provides the avenue for the determination of iodine in total diet items using ultraviolet-visible spectrophotometry [5] (UV-VIS) techniques. Iodine, in various forms, is oxidized to iodate (or higher oxides) during the digestion and is then prereduced to iodide by arsenic (III) in acid solution. Iodide catalyzes the reduction of cerium (IV) [yellow] to cerium (III) [colorless] by arsenic (III) in a somewhat involved process, which is enhanced by the presence of chloride. The disappearance of the yellow color of cerium (IV) is monitored at 420 nm.

Depending upon the amount of lipids/solids and percent moisture, between 1.00 and 2.1 g of well-homogenized sample are placed within a dry quartz Kjeldahl flask. A small amount of deionized water may be added to the dry reference material. (Never add any more than 1 ml of water per gram of reference material sample. Do not add any water to any analytical samples). This is then followed by 10 ml of nitric acid, 20 ml of perchloric acid, and 5.5 ml of sulfuric acid. It is advantageous to cover the Kjeldahl flask with a clean plastic beaker and allow the solution to react over night at room temperature.

The samples are heated and refluxed, using a cold finger condenser placed into the neck of the Kjeldahl flask. After the reflux period is complete (minimum of 1 h), the heat is turned off, the solutions allowed to cool, and the condenser is rinsed with deionized water, directing the rinsings into the flask. The condensers are then removed and the heaters are turned up to reinitiate boiling. The solutions are now heated to drive off all nitric and perchloric acids, leaving only sulfuric acid. Completion of the digestion is indicated when a condensation ring begins to rise up the neck of the flask. Iodine is easily lost during digestion sequences, so it is important that all procedures described in the reference [6] are followed meticulously. Proper weighing and handling techniques are critical to the success of the digestion. Also, assure that appropriate contamination control procedures are observed.

For the ternary acid digestion for iodine, each analytical batch consists of 100 total diet food items, six method blanks, at least one National Institute of Standards and Technology (NIST) reference material, five working standard solutions, one to two diluting solutions, one initial calibration verification (ICV) standard and six matrix spike/matrix spike duplicate sample pairs fortified with known quantities of potassium biiodate. This batch should also include the nonfortified analytical sample, preferably prepared in duplicate, which is then repeatedly chosen for use of the matrix spike/matrix spike duplicate pairs used in the remaining analytical batches.

Nitric Acid Solubilization and Direct Ashing Preparation for Cadmium, Lead and Nickel

This digestion scheme provides the avenue for the determinations of cadmium, lead, and nickel [7] in total diet study items using heated Graphite Furnace Atomic Absorption Spectrometry [8] (GFAAS). Lead, cadmium, and nickel are determined after the samples have been digested with nitric acid, the nitric acid driven off, and the samples then oxidized further, using a stepwise furnace program, ramping up to 470 °C. Lead, in all total diet items, is determined using this technique and cadmium and nickel are determined in about 260 total diet items.

Depending of the type of sample, from 1 to 9 g of sample are weighed into a quartz beaker [9]. Suggested weights for sample types are shown in the referenced SOP [7]. A small amount of nitric acid and 1 ml of 200 mg/ml magnesium nitrate are added and the beaker is covered with a vented lid to prevent contamination. Magnesium nitrate is utilized to provide oxygen for conversion of the elements to low volatility oxides. It is advantageous to allow the sample to sit overnight before heating, to initiate digestion and reduce the chance of violent reaction upon heating. The covered beakers are placed on a hotplate at a low temperature and allowed to digest until vigorous reaction ceases and the samples are completely wetted. Careful observation is important at this point to manipulate temperatures and beakers to avoid spattering.

Once the sample solutions cease to exhibit vigorous reaction, the beakers are cooled and placed in a convection oven, where the temperature is gradually increased through a series of steps to continue the digestion and drive off the acid solution. After the prescribed solubilization steps are accomplished in the convection oven, the beakers are cooled and transferred to a muffle furnace where they are subjected to a programmed heating ramp to perform the dry ashing of the samples. The maximum suggested temperature of the muffling operation is 470 °C. At this temperature, the metallic oxides will not volatilize. The appearance of the ashed samples at this point should be light gray to white, with no remaining carbon. If ashing appears incomplete at this point, further treatment will be required, as described in the SOP. The sample residue remaining in the beakers can now be dissolved, with heating, with a small amount of dilute acid and diluted volumetrically for determination on the GFAAS.

For the nitric acid solubilization/direct ashing digestion, each analytical batch consists of one method blank and one matrix spike/matrix spike duplicate sample fortified with the elements of interest, and 17 analytical samples for a total of 20 sample flasks per batch. The first batch should also include the nonfortified analytical sample, preferably prepared in duplicate, which is then repeatedly chosen for use of the matrix spike/matrix spike duplicate pairs used in the remaining analytical batches.

Sample Analysis Techniques

Inductively Coupled Plasma Atomic Emission Spectrophotometry (ICP-AES)

Calcium, chromium, copper, iron, magnesium, manganese, molybdenum, phosphorus, potassium, sodium, and zinc are determined and reported using an axial view ICP-AES [3]. A radial view ICP-AES may also be used, although the sensitivity will be lower. Arsenic, cadmium, nickel and selenium are also determined using this technique, but generally these elements are not reported using this method.

As described in the ternary acid digestion [2], the sample preparation results in a 10 % sulfuric acid solution. Prepare all calibration standards similarly. Follow instrument manufacturer's recommended conditions and consult the reference [3] for the specific instrumental parameters. The use of an internal standard is preferred. Yttrium, indium, or scandium may be used for internal standards.

Following the initial instrumental calibration of all elements of interest, an initial calibration blank (ICB) is followed by another independently prepared initial calibration verification (ICV) standard. This ICV standard should be prepared independently from the standards used in the calibration curves. Ideally, this ICV standard would be prepared or obtained from sources different than those used for the calibration standards. The ICV is used primarily to verify the accuracy of the standard calibration curve. Quality control criteria should be set for the recovery of analytes determined in the ICV and linearity of the calibration standards. Generally, 100 ± 10 % of the known ICV reference value is acceptable and a correlation coefficient (r) of >= 0.9975 of the calibration curves are in order. These two criteria must be met in order to proceed further.

Blocks of ten samples each (including method blanks, reference materials, samples, and matrix spikes) are followed by continuing calibration blanks (CCB) and continuing calibration verification standards (CCV). The CCV can be any standard, preferably with known concentrations near the midpoint of the calibration curve. Criteria must also be set for CCV standard, typically 100 ± 10 or 15 %. The CCV is used primarily to verify that instrumental drift of the calibration curve has not occurred. Criteria for ICBs and CCBs can also be set, if needed. Should any element fail the recovery criteria, the analyses must be stopped and only the results preceding the last acceptable CCV can be reported. The problem must be investigated and remedied before the analyses can be restarted. Criteria should also be set for the recovery of reference material and matrix spikes. Generally, 100 ± 20 or 25 % recoveries are typical. Duplicate analyses results, for results greater than the limit of quantification (LOQ) should be <= 30 % Relative Percent Difference (RPD) [10].

Although modified slightly for each analytical technique, each of the other analytical techniques has similar quality control requirements.

Hydride Generation Atomic Absorption Spectrometry (HGAAS) Determination of Selenium and Arsenic

Selenium and arsenic are determined sequentially from a solution aliquot obtained from the ternary acid digestion using a hydride generation atomic absorption technique [4]. A known volume of hydrochloric acid is added to a known volume of the resultant 10 % sulfuric acid sample solution. Selenium (VI) is reduced to selenium (IV) under these conditions. The sample solution is introduced into the instrument sampling loop, and is mixed with a basic solution of sodium borohydride. Selenium in the sample is reduced to gaseous selenium hydride, the gas passed into a gas/liquid separator, and the resultant dried gas introduced into a heated quartz cell, where elemental selenium results. Selenium is then determined by atomic absorption spectrometry.

Once selenium has been successfully determined, a known amount of ascorbic acid/potassium iodide solution is added to the remaining solution. Mix and allow this solution to stand overnight in order to reduce the arsenic (V) to arsenic (III). Calibration standards should be treated identically. Arsenic is then determined similarly to selenium.

Prepare all calibration standards in a matrix of 10 % sulfuric acid and 6 % hydrochloric acid. Follow instrument manufacturer's recommended conditions and consult the reference [4] for the specific instrumental parameters. Quality control procedures, similar to those above in inductively coupled plasma section, should be followed. Consult the reference [4] for the exact quality control requirements.

UV-VIS Spectrophotometry Determination for Iodine

Ce(IV) ions are reduced by As(III) and the reaction is catalyzed by iodide (I^-) in an acid solution [6, 11, 12]. The reduction of the yellow Ce(IV) to colorless Ce(III) is followed spectrophotometrically at 420 nm. The inverse absorbance is proportional to the concentration of iodide in the samples.

Potassium biiodate, primary standard grade, is used for the preparation of all stock and working iodine calibration standards. Prepare all calibration standards in deionized water and carry all calibration standards through the digestion procedure. Failure to digest calibration standards will result in a poor standard curve. Follow instrument manufacturer's recommended conditions and consult the reference [6, 12] for the specific instrumental parameters. Quality control procedures, similar to those above in inductively coupled plasma section, should be followed. Consult the reference [6] for the exact quality control requirements.

Determination of Lead, Cadmium, and Nickel by GFAAS

Lead, cadmium, and nickel are each determined individually using Graphite Furnace Atomic Absorption Spectroscopy (GFAAS) [8]. As described in the nitric acid

solublization direct ashing digestion [7], the sample preparation results in a 5 % nitric acid solution. Prepare all calibration standards similarly. Follow instrument manufacturer's recommended conditions and consult the reference [3] for the specific instrumental parameters. Each element analyzed will require differing instrument conditions, analytical wavelengths, graphite tubes, and matrix modifiers.

Peak area rather than peak height is the preferred method of measurement. Other species (such as chlorides, sulfates, sulfites etc.) have slightly different atomization temperatures that result in peak broadening, rendering simple peak height measurements questionable. Background correction should always be used, and Zeeman background correction is to be preferred.

All appropriate laboratory contamination control procedures should be followed as lead, nickel, and cadmium are common laboratory contaminants when determined at the exceeding low levels required for total diet analyses. Quality control procedures, similar to those above in inductively coupled plasma section, should be followed. Consult the reference [8] for the exact quality control requirements.

Determination of Total Mercury

Unlike the previous four determinations for elements in the US TDS, analysis for mercury does not require the sample be digested/solubilized [13, 14]. Historically, analysis of total mercury in the total diet program involved a wet block digestion procedure followed by analysis using cold vapor atomic absorption. This older procedure and SOP is still available upon request [15]. This preparation procedure and analysis has now been replaced. An automated direct mercury analyzer, the Teledyne Hydra-C Automated Direct Mercury Analyzer, is now the instrument used for the analysis of mercury in the FDA TDS. The procedure used for mercury analysis is based upon the U.S. Environmental Protection Agency method 7473.

The analytical process [14] involves combusting of the sample in an atmosphere of oxygen at high temperature. The gases formed are passed through a heated catalyst that removes halogens, nitrogen oxides and sulfur oxides. The remaining combustion products including elemental mercury are swept into a gold amalgamation tube. The amalgamation tube captures all the mercury and then the tube is heated to release the mercury. The mercury vapor is then swept into a cold vapor atomic absorption spectrometer and the mercury determined. Quality control procedures, similar to those above in inductively coupled plasma section, should be followed. Consult the reference [13] for the exact quality control requirements.

Inductively Coupled Plasma Mass Spectrophotometry (ICPMS)

One of the goals of the FDA TDS program is to replace many of the previously mentioned preparation techniques and instruments with one sample preparation and one instrumental analysis determination [16]. Sample preparation using microwave

oven technology followed by ICPMS determination of elements will likely meet this goal. All of the previously mentioned preparations and determinations (with the exception of iodine and perhaps mercury) will likely in the future be replaced and samples digested by microwave and determined by ICPMS only. Any additional elements added at a later date can easily be determined by this procedure.

The amount of sample digested will likely be reduced from the amount presently used. This will result in even greater challenges in obtaining a representative homogeneous sample for microwave sample preparation than is experienced today. The focus of investigational work may be shifted from analytical means to sample preparation.

References

1. This procedure should only be attempted by personnel familiar with the hazards associated with hot, boiling, concentrated acids. Proper use of safety equipment is absolutely necessary, particularly in dealing with the safe use of perchloric acid. Violent reactions can sometimes occur during or prior to heating, especially with samples containing alcohols or high amounts of lipids. Follow all safety procedures indicated in all laboratory procedures and references within
2. KAN-LAB-MET.99: Ternary acid digestion, Laboratory Procedure. Food and Drug Administration, Kansas City District
3. KAN-LAB-MET.92: Determination of elements by ICPAES, Laboratory Procedure. Food and Drug Administration, Kansas City District
4. KAN-LAB-MET.96: Determination of arsenic and selenium by AA Hydride Generation Instrumentation, Laboratory Procedure. Food and Drug Administration, Kansas City District
5. KAN-LAB-MET.90: Elemental analysis – contamination control, Laboratory Procedure. Food and Drug Administration, Kansas City District
6. KAN-LAB-MET.95: Determination of iodine in foods, Laboratory Procedure. Food and Drug Administration, Kansas City District
7. KAN-LAB-MET.97: Nitric acid solubilization and direct ashing procedure for lead, cadmium and nickel, Laboratory Procedure. Food and Drug Administration, Kansas City District
8. KAN-LAB-MET.93: Determination of lead, cadmium and nickel by GFAAS, Laboratory Procedure. Food and Drug Administration, Kansas City District
9. Quartz tall form beakers, 150 mL, with vented covers, Quartz Scientific Inc., or equivalent
10. Relative Percent Difference = 100 X (O − D)/(1/2(O + D) where O is the original and D is the duplicate
11. Blaha JG (1986) The determination of iodine from total diet foods, Laboratory Information Bulletin #3045, USFDA, vol 2(5)
12. La Chat QuickChem® Method 10-136-09-1-A, Determination of iodine in 0.2 M potassium hydroxide by flow injection analysis, 17 July 2001
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14. Leeman Labs, Inc, Teledyne Instruments, Hydra-C, automated analyzer for the direct determination of mercury by thermal decomposition and gold amalgamation, Operator's manual, 7 Oct 2008
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16. KAN-LAB-MET.100: Determination of elements by ICPMS, Laboratory Procedure. Food and Drug Administration, Kansas City District