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Exploring the Working Range of Automated Standard Dilution Analysis of Nutrient Elements in Foods by Inductively Coupled Plasma Optical Emission Spectrometry

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Abstract

Inductively coupled plasma optical emission spectrometry (ICP-OES) is an important tool for measuring nutrient elements in food. ICP-OES methods typically determine analytical concentrations using external standard calibration, but can be susceptible to matrix effects. The method of standard additions does not suffer from matrix effects but is time consuming and labor intensive. Automated standard dilution analysis (SDA) allows for online matrix matched calibration without preparing individual standard additions for each sample matrix. This approach may solve both time and matrix issues and has been described in the literature as an attractive alternative to standard additions. The working range of the method for nutrient elements, however, is an understudied feature of SDA that may be a potential drawback to routine analysis of foods.

We evaluated automated SDA performance through the analysis of 10 reference materials and four fortified (i.e., spiked) foods spanning the AOAC food triangle. We evaluated the working range, accuracy, and precision for analyses of nutrient elements in foods. Accepted accuracy (80–120% recovery) was achieved for 10 nutrient elements, Ca, Cu, Fe, K, Mg, Mn, Na, P, S, and Zn, when the analytical solution concentration to standard concentration ratio was less than 10. This equates to a working range for each element spanning at least two orders of magnitude. Removing outliers, Z scores (n = 95) ranged from -1.8 to 0.88, and the average recovery (n = 85) from fortification experiments was 97 +/- 12% (2σ). Therefore, automated SDA applied to ICP-OES may be used for nutrient elemental analyses in samples with difficult matrices such as foods.

Introduction

The foods and beverages people consume have a profound effect on human health, and a healthy diet can help reduce the risk of chronic disease.[1] The U.S. Food and Drug Administration's (FDA) mission to maintain a safe and nutritious food supply is met in part by monitoring food and dietary supplements for both toxic and nutritional elements.[2] Elemental analysis of food provides data that FDA uses to make science-based decisions in support of its mission to ensure safety. These data are necessary,

whether the elements are called nutrients, metals, chemicals, ingredient, native, etc., and whether the levels are considered deficient, healthy, toxic, normal, added, etc.[2]

The FDA uses inductively coupled plasma optical emission spectrometry (ICP-OES) to measure nutrient element concentrations in foods.[3, 4] The current, routine method for nutrient element analysis of foods involves closed vessel microwave assisted digestion and the analysis of 23 total elements by ICP-OES.[5] This method uses external standard calibration to determine solution concentrations and cautions that matrix matching by standard additions may be necessary for complex matrices.[6] In addition to the FDA method, several validated inductively coupled plasma-based methods exist and are used for regulatory analysis of nutrient elements in foods.[7-12] These methods also feature external standard calibration and may be susceptible to matrix effects, especially when the method is applied to sample types not included in the method validation. As a result, there is a need for developing and validating accurate and efficient matrix matched approaches to calibration for nutrient elements in foods because preparing standard additions is a labor- and time-intensive process.

Standard dilution analysis (SDA) is an alternative calibration approach to standard additions that combines internal standardization and matrix matched calibration.[13] SDA was first introduced using two solutions per sample replicate, solution one (S1) and solution two (S2), where both solutions were composed of equal amounts of sample, and S1 and S2 were made up with standard and internal standard solution and blank solution, respectively. S2 was added to S1 manually, and time resolved data were collected using the instrument software.[13] Recently, efforts have been made to use the instrument autosampler to automatically prepare and mix S1 and S2.[14] The preparation and mixing of S1 and S2 during automated SDA were initially carried out with a homemade mixing chamber and later optimized using a simple two channel pinch valve.[14, 15]

SDA or automated SDA has been successfully applied to the analysis of beverages and wines, foodstuffs, concentrated acids, pharmaceutical samples, and samples prepared with complex matrices (e.g., elevated levels of easily ionizable elements).[14-19] These reports describe how SDA is comparable to standard additions and often outperforms internal standardization and external calibration depending on

the complexity of the matrix.[13, 14, 17, 20] To the authors' best knowledge, however, SDA has yet to be validated on matrices spanning the 9 sectors of the AOAC food triangle defined according to fat, carbohydrate, and protein content. Additionally, the working range of automated SDA has yet to be defined or studied for nutrient elements.

In this work, automated SDA was performed similar to what was described by Jones et al.,[15] where the working range, precision, accuracy, and limits of quantitation of the method were evaluated.[21] The automated SDA approach with a simple pinch valve allows for immediate matrix matched calibration and analysis of sample digests without separate preparation of standard additions for every sample matrix. Method optimization and discussion of results were based on 10 nutrient elements, Ca, Cu, Fe, K, Mg, Mn, Na, P, S, and Zn in ten reference materials and four fortified foods that span the AOAC food triangle.[22] The working range of the method was explored relative to trends observed from the ratio of analytical solution concentration to standard concentration.

Materials and Methods

Reagents and instrumentation

Nitric acid, hydrochloric acid, and hydrogen peroxide (Optima grade) were purchased from ThermoFisher Scientific (Waltham, MA, USA). Single element and custom blend, multielement standard solutions were purchased from Inorganic Ventures (Christiansburg, VA, USA) or High Purity Standards (North Charleston, SC, USA). All solutions were prepared gravimetrically using deionized water (MilliQ Element, Millipore, Billerica, MA, USA).

Samples were homogenized with a knife mill (GM 300, Retsch, Haan, NRW, Germany) or a disposable grinding chamber (IKA, Wilmington, NC, USA). Microwave digestion was performed using a CEM MARS 6 system (CEM Corporation, Matthews NC, USA). Digests were analyzed using an Agilent 5900 ICP-OES (Agilent Technologies, Santa Clara, CA, USA). Automated SDA was performed using a 12V three-way solenoid pinch valve fitted for tubing with 1/32" ID × 3/32" OD (Cole-Palmer, Vernon Hills, IL, USA). Table 1 lists instrumental and automated SDA parameters.

Microwave assisted digestion

Homogenized samples and reference materials were digested according to Elemental Analysis Manual 4.7.[23, 24] Briefly, 0.5 g sample portions were digested with 8 mL HNO_3 and 1 mL H_2O_2. Temperature was ramped to 200°C over 25 minutes followed by a 15-minute hold at temperature and then cooled to room temperature. For vegetable oil, 0.25 g sample portions were taken to avoid excessive pressure buildup from the digestion of a 100% fat sample. Digests were gravimetrically diluted to approximately 100 mL for a final acid concentration of approximately 5% HNO_3 and 0.5% HCl, with a nominal 200x dilution factor, except for vegetable oil digests with a nominal 400x dilution factor. Table 2 lists samples used in this study and their position in the AOAC food triangle.

Fortified samples

Fortified analytical portions (FAP)s were prepared at three levels in triplicate at approximately 50, 100, and 250% the native concentration for each of the 10 elements evaluated in the study. Fortifications (i.e., spikes) were added prior to digestion. When native concentrations were below the LOD, samples were fortified at approximately 5×, 10×, and 25× LOD. The native concentrations and LODs used to determine appropriate fortification concentrations were based on results from external standard calibration analyses performed prior to automated SDA experiments. Results in this study are shown for analytical solution concentrations greater than the analytical solution quantitation level (ASQL) determined using automated SDA.

Automated standard dilution analysis

Standard concentrations were prepared in solution one (S1) at 50 mg/kg for K and Na, 10 mg/kg for Ca, Mg, P, and S, and 1 mg/kg for Cu, Fe, Mn, and Zn. Standard concentrations were chosen to match the middle point of the external standard calibration curve from our in-house method for nutrient element analysis by ICP-OES. This procedure was similar to selecting a fortification (i.e., spiking) concentration for samples with unknown concentrations.[23] Lu was prepared as internal standard one (IS1) in S1, and In was prepared as internal standard 2 (IS2) in solution 2 (S2). IS1 and IS2 were prepared at a concentration of 1 mg/kg, and S1 and S2 were made up to 1L

for a final acid concentration of 5% HNO_3 and 0.5% HCl. Accurate results have been shown for manual standard dilution analysis with In as the internal standard at a concentration of 1 mg/kg. Varying the internal standard concentration from 1 to 5 mg/kg in manual standard dilution analysis did not significantly affect the accuracy.[17] Therefore, we selected 1 mg/kg as the concentration of our two internal standards for automated standard dilution analysis and did not evaluate accuracy as a function of varying internal standard concentration.

Throughout the instrumental analysis, the autosampler continually drew up sample solution, and S1 or S2 were sequentially added via a y-joint in the pump tubing, diluting the sample 1:1 with increasing and decreasing amounts of standard and blank. All signals for analytes and internal standards were collected simultaneously by the polychromator. The alternating addition of S1 and S2 was accomplished with a 12V solenoid pinch valve timed to close one port at a time every 60s.[15] A 120V AC to 12V DC transformer plugged into an automatic timer supplied power to the switch valve at 60s intervals (Fig. 1). Raw intensity data were exported with instrument software. Data processing was carried out with R and *Tidyverse* packages.[25, 26] All R code and relevant output is provided in the supplementary material.

Theory

Automated standard dilution analysis theory

Solution concentrations were determined according to eq. 1,[14] where C_A^{soln}, C_A^{std}, and S_I^{max}, is the concentration of the analyte in the sample solution after digestion, the concentration of the analyte in the standard solution, and the maximum signal of IS1, respectively. The *intercept* and *slope* in eq. 1 were determined from the linear regression model generated from plotting the signal of the analyte against the signal of IS1. S_I^{max} was determined from plotting the signal of IS1 against the signal of IS2, fitting a linear regression model, and generating the *y*-intercept.[14]

$$C_A^{soln} = \frac{intercept\, C_A^{std}}{slope \quad S_I^{max}} \qquad \text{(eq. 1)}$$

Reference material Z score determinations

Z scores were determined according to eq. 2,[27] where X_{meas} is the measurement result, X_{ref} is the reference value found on the certificate of analysis, and σ = (σ_{meas}^2 + σ_{ref}^2)$^{1/2}$ where σ_{meas} is the uncertainty of the measurement and σ_{ref} is the uncertainty of the reference value. Reference material measurement results were compared with certified values given on the certificates.

$$Z = \frac{(X_{meas} - X_{ref})}{\sigma} \quad \text{(eq. 2)}$$

The total standard uncertainty for reference material certified values (σ_{ref}) was obtained from the certificate. Because the uncertainties were listed as expanded uncertainties at the 95% confidence level, they were divided by the coverage factor listed on the certificate (e.g., 2) to obtain standard uncertainties at approximately a 67% confidence level for use in Z score calculations. Total standard uncertainty for measurement results (σ_{meas}) was defined at 10% when greater than the LOQ.

Results and Discussion

Determining automated standard dilution analysis parameters

The online mixing of standard and blank with the sample provides 100 matrix matched calibration data points according to the 100 instrumental readings taken per analysis (Table 1). The periodic profile from the gradual increase and decrease of analytical signal due to the alternating additions of S1 and S2 was repeatable across an instrumental sequence (Fig. 2a). For each nutrient element, the values for *slope*, *intercept*, and S_I^{max} are used to determine the concentration of the analyte in the sample solution according to eq. 1. Time resolved data from automated standard dilution analysis of NIST 1577c are displayed for Zn in Fig. 2a-c. The RSDs (n = 9) for *slope*, *intercept*, and S_I^{max}, were 1.8, 5.2, and 1.1%, respectively. This confirms automated SDA, with a simple pinch valve, is a repeatable calibration method. The variation in *slope* and *intercept* may be attributable to differences in dilution factors from the digestion in addition to variation from the instrument and the introduction of solution with the automated SDA rig.

Limits of detection and quantification

Limits of detection (LOD)s and limits of quantification (LOQ)s were determined according to the analysis of 30 method blanks from three separate digestions. One blank for Fe was discarded due to contamination. The analytical solution detection limit (ASDL) and ASQL were defined as 3σ and 10σ, respectively, where σ is the standard deviation of the blank concentration equivalents (Table 3). LODs and LOQs were determined assuming a dilution factor of 200 from the digestion. The LODs for the 10 nutrient elements ranged from 0.39 mg/kg (Zn) to 110 mg/kg (Na), and LOQs ranged from 1.3 mg/kg (Zn) to 370 mg/kg (Na) (Table 3).

The LODs are similar for elements with the same standard concentration (e.g., Cu, Fe, Mn, and Zn; Ca, Mg, P, and S; and K and Na), and LODs are higher when standard concentrations are higher (e.g., Ca, Mg, P, and S compared to Cu, Fe, Mn, and Zn). According to Jones et al., the varying of limits of detection as a function of the concentration of the standard solution is due to the fact a higher concentration standard leads to more uncertainty in values near zero. At lower concentrations of standard, the estimate of the noise level as the detection level is approached is better sampled because the maximum concentration in the SDA calibration is lower.[15] For the purposes of this study, the LODs and LOQs for the target analyte concentrations in foods were adequate to evaluate the working range of the method for the 10 selected nutrient elements.

Defining the working range of automated standard dilution analysis

At elevated levels of analytical solution concentration, there is little change in analyte signal during automated SDA. When the concentration of the analyte in the sample solution is significantly higher than the standard concentration, the SDA region of the plot looks more like a constant plateau than a dynamic change in signal from the oscillating addition of standard and blank in S1 and S2 respectively. Conversely, when the standard concentration is much higher than the concentration of the analyte in the sample solution, the change in analytical signal is exaggerated, matching the decrease and increase of the IS1 signal, where the signal reaches an equilibrium at normalized intensities near 0.

Consider Zn in three different samples: NIST 1566b, NIST 1577c, and a fortified analytical portion (FAP) of vegetable oil (Fig. 3). The native concentration of Zn in vegetable oil was below the LOD. The certified values for Zn in NIST 1566b and NIST 1577c are 1424, and 181.1 mg/kg, respectively, and the vegetable oil sample was fortified with Zn at a concentration of 2.6 mg/kg. Given the digestion dilution factor of approximately 400, the analytical solution concentration of Zn in the fortified vegetable oil sample should be approximately 0.007 mg/kg. With dilution factors of approximately 200, the analytical solution concentrations for Zn in NIST 1566b and NIST 1577c should be approximately 7 and 0.9 mg/kg, respectively.

The heights of the signal plateaus reached after the addition of blank from S2 represents the decreasing order of analytical solution concentrations (Fig. 3). The two extremes, NIST 1566b and the fortified vegetable oil sample, illustrate analytical solution concentrations outside the working range for the Zn standard concentration used in the study. Analytical recoveries for Zn in NIST 1566b, NIST 1577c, and the fortified vegetable oil sample were 162 +/- 24% (2σ), 110 +/- 3% (2σ), and 61 +/- 6% (2σ), respectively. The poor recovery for the fortified vegetable oil sample was due to an analytical solution concentration at the ASQL and a large difference in standard and analytical solution concentration where the standard concentration was approximately 200 times greater than the analytical solution concentration. The poor recovery for NIST 1566b was due to a large difference between the standard and analytical solution concentrations where the analytical solution concentration was approximately 13 times greater than the standard solution concentration. A simple parameter representing the working range of automated SDA would prove useful to avoid manually going through every SDA plot generated to confirm the analysis is performed within the working range of the method.

Determining a metric for the working range of automated standard dilution analysis

To determine a representative parameter for the working range of automated SDA, the accuracy of fortified (i.e., spiked) foods and reference materials were compared to the ratio of analytical solution concentration to standard concentration.

There's a positive trend between analytical recovery and the ratio of analytical solution concentration to standard concentration (Figs. 4a–b). Based on these data, a threshold ratio of 10 was set as the cap of the working range for automated SDA. This suggests accurate results are obtainable at ratios below 10.

Consider results for Zn, where S2 was prepared with a Zn concentration of 0.98 mg/kg. Accurate results were found for Zn ratios between 0.01 and 3.7. This equates to solution concentrations of 0.0098 and 3.6 mg/kg, a range spanning three orders of magnitude, and recoveries of 94 +/- 24% (2σ) and 101 +/- 4% (2σ), respectively (Table 4). Similar results were observed for Fe, where S2 was prepared to have a standard concentration of 0.98 mg/kg. Accurate results were observed at ratios between 0.022 and 7.0. This equates to analytical solution concentrations of 0.022 and 6.9 mg/kg, and recoveries of 91 +/- 12% (2σ) and 108 +/- 9% (2σ), respectively (Table 4).

Exceptions for this trend, where recoveries were outside the 80–120% range, include the following elements and samples: Ca in two fortified (i.e., spiked) corn flakes samples; K in a fortified vegetable oil sample and NIST 1568b; Mn in NRC Dorm-4; Na in a fortified vegetable oil sample; P in a fortified vegetable oil sample; and Zn in a fortified vegetable oil sample. These results had analytical solution concentrations ranging from 1 to 3 × ASQL, and analytical solution concentration to standard concentration ratios ranging from 0.01 to 0.1. This suggests results near the ASQL, with low analytical solution concentration to standard concentration ratios, may need to be reevaluated by lowering the standard concentration in S1.

Accuracy of automated standard dilution analysis for 10 nutrient elements

The accuracy of automated SDA was determined according to the analysis of a suite of reference materials and spiked (i.e., fortified) food samples spanning the AOAC food triangle (Tabs. 2, S1, S2, Figs. 5, 6). Reference material Z scores for all elements with an analytical solution to standard concentration ratio less than 10 (n = 102) ranged from -4.2 to 1.7 with a median Z score of -0.16 (Table S1, Fig. 5). Removing seven outliers resulted in a range from -1.8 to 0.88 and a median of -0.16. Outliers were identified as Z scores outside 1.5 times the interquartile range above the upper quartile and below the lower quartile (i.e., points outside the whiskers of the boxplot in Fig. 5b).

Accounting for outliers, all Z scores were between -2 and 2, at 10% total uncertainty. Therefore, the described method with automatic standard dilution analysis features an uncertainty no worse than 10% for the sample matrices evaluated.

Fortification recoveries for all elements with an analytical solution to standard concentration less than 10 (n = 92) ranged from 54 to 110%, and the average recovery was 95 +/- 18% (2σ) (Table S2, Fig. 6). Removing seven outliers resulted in a range from 82 to 110%, and an average recovery of 97 +/- 12% (2σ). Outliers were identified as fortification recoveries outside 1.5 times the interquartile range above the upper quartile and below the lower quartile (i.e., points outside the whiskers of the boxplot in Fig. 6b).

Contamination and sample inhomogeneity during sample preparation was the largest contributor to imprecision (Fig. 6d). Another contributing factor was noise when determining concentrations at the LOQ. Contamination is a lab-specific issue that affects all methods. Although the contamination was unfortunate, it is not indicative of a specific weakness for automated SDA. For example, 11 of the 19 FAP sample results with RSDs greater than 10% were from a breaded chicken fortification (Table S2, Fig. 6d). Of these 11, 9 were from the level 1 breaded chicken fortification. Consider Fe as a specific example, the amount of Fe fortified was 4.9, 5.8, and 3.8 mg/kg for replicate 1, 2, and 3, respectively, and the LOQ was 3.7 mg/kg (Table 3). A combination of contamination and operating near the LOQ led to the lack of precision (RSDs > 20%).

Except for K in Dorm-4 with an analytical solution concentration of 59.5 mg/kg and an analytical solution to standard concentration ratio of 1.2, the identified outliers from the reference material and fortification analyses were a result of low analytical solution to standard concentration ratios and analytical solution concentrations near the ASQL. Therefore, a proposed workflow for analyzing samples with unknown concentrations would include selecting a standard concentration similar to what was used in this study (Table 4), determining the method ASDL and ASQL, then measuring the analytical solution concentration and comparing the concentration to the standard concentration. Based on the results of this study, no further modifications are necessary if the solution concentration is greater than the ASQL and the ratio is between 0.1 and 10 (Figs. 4a–b). However, if the ratio is greater than 10, then the procedure should be

repeated using a higher standard concentration. Similarly, if the ratio is less than 0.1, then the procedure may need to be repeated using a lower standard concentration. Ultimately, at trace concentrations, the method will be limited to the sensitivity of the technique itself no matter the concentration of the standard.

Time and labor advantages of automated standard dilution analysis

The reference material and fortification results suggest automated SDA is an accurate method for nutrient element analysis of foods. Automated SDA is a suitable alternative to standard additions for matrix matched analysis of foods, with an added labor and time advantage of automatic online calibration. To illustrate these advantages, consider the time it took to run the samples presented in this study. Three batches of 40 digestions were run on one instrument sequence. The total time to run all 120 solutions was 14 hours, and the time required to run one solution was 6 minutes. Once samples were digested, the only additional sample preparation required was preparing S1 and S2 and setting up the instrument. Considering time estimates for sample uptake (35 seconds), plasma stabilization (15 seconds), read time (10 seconds), and rinse (30 seconds) to run one solution on the instrument using a conventional instrumental setup, a typical 4-point standard addition curve for one sample replicate would take approximately 6 minutes. This is equal to the time required to run one sample replicate by automated SDA which provides 100 standard additions based on the oscillating addition of standard and blank (Fig. 2). The time and labor advantages of automated SDA is obvious when taking into account the effective elimination of preparing standard addition curves prior to instrumental analysis.

Conclusions

Automated SDA is an efficient, accurate, and precise matrix matched approach to calibration. The working range of automated SDA and ICP-OES spans at least two orders of magnitude for 10 nutrient elements: Ca, Cu, Fe, K, Mg, Mn, Na, P, S, and Zn. In addition to eliminating matrix effects, automatic SDA effectively eliminates the preparation of calibration curves. This may significantly improve sample throughput for

routine analyses. Therefore, automated SDA and ICP-OES may prove useful for regulatory analysis of nutrient elements in foods.

Conflicts of interest

The authors declare no competing interests.

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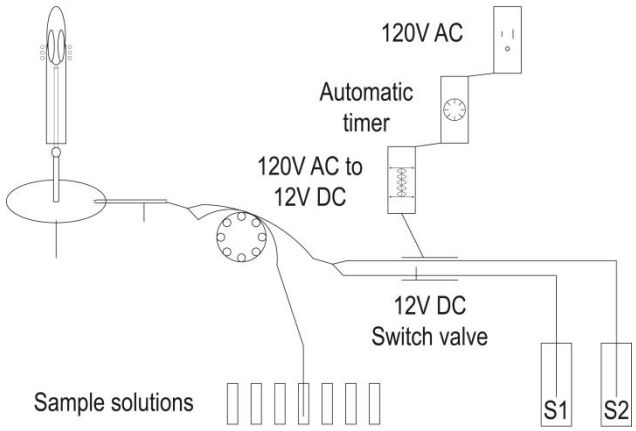

Figure 1. Schematic of the sample introduction for automated standard dilution analysis.

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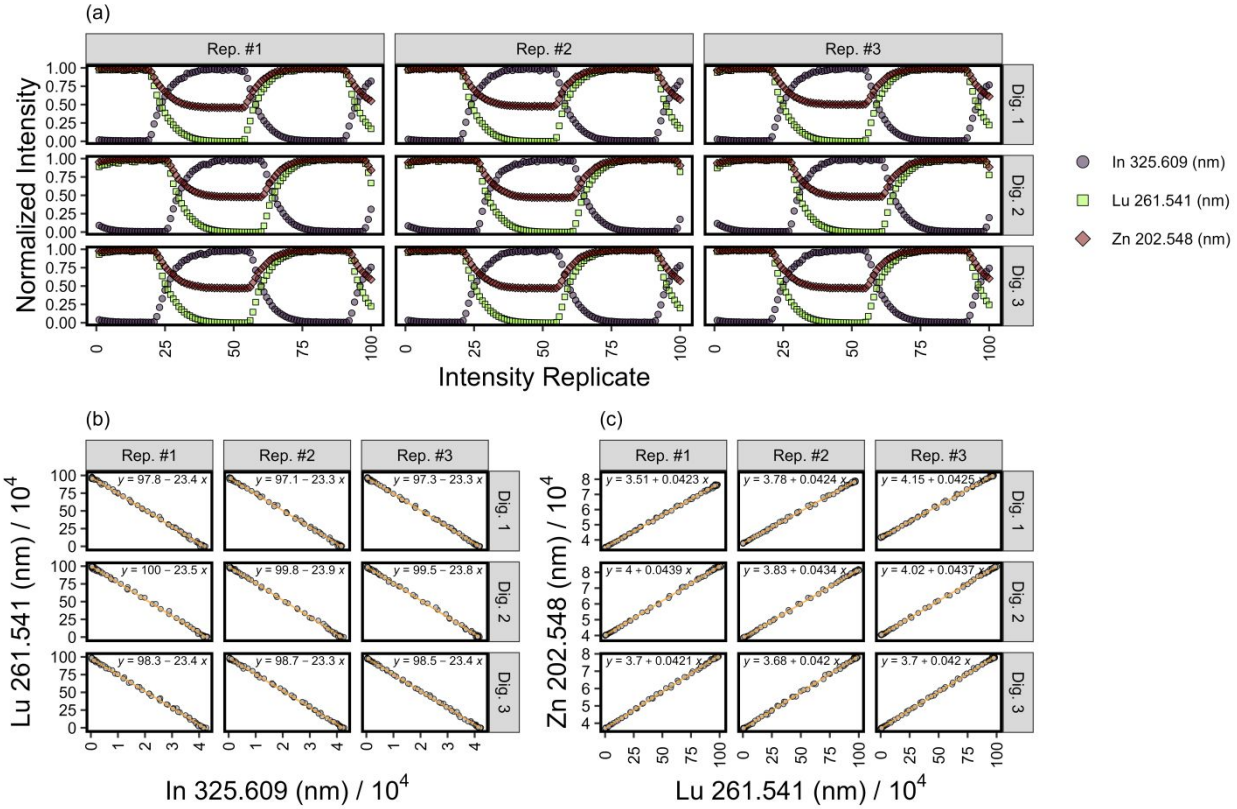

Figure 2. Time resolved data from automated standard dilution analysis of NIST 1577c. Three digestions were analyzed in triplicate. All replicates were analyzed during one instrumental sequence. Signals for Zn are shown as the analyte, and Lu and In are IS1 and IS2, respectively. (a) Time-resolved intensity data for In, Lu, and Zn. The intensity replicates are the 100 1 s read time replicates. Signals were normalized to the max intensity during the individual analysis. (b) IS1 vs. IS2 plots for each replicate and relevant regression parameters. The intensity was scaled by 10^4. (c) Analyte vs. IS1 for each replicate and relevant regression parameters. The intensity was scaled by 10^4.

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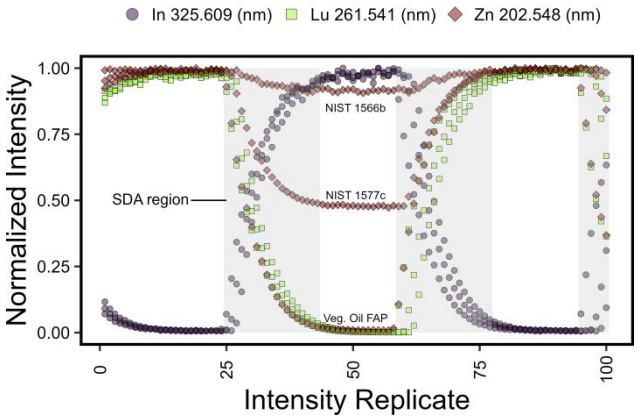

Figure 3. Overlayed time resolved data from automated standard dilution analysis of a single digestion replicate of NIST 1566b, NIST 1577c, and a fortified analytical portion (FAP) of vegetable oil. Shaded regions represent time periods of mixing between sample solution with S1 and S2, i.e., "SDA region".[13] Signals were normalized to the max intensity during the analysis.

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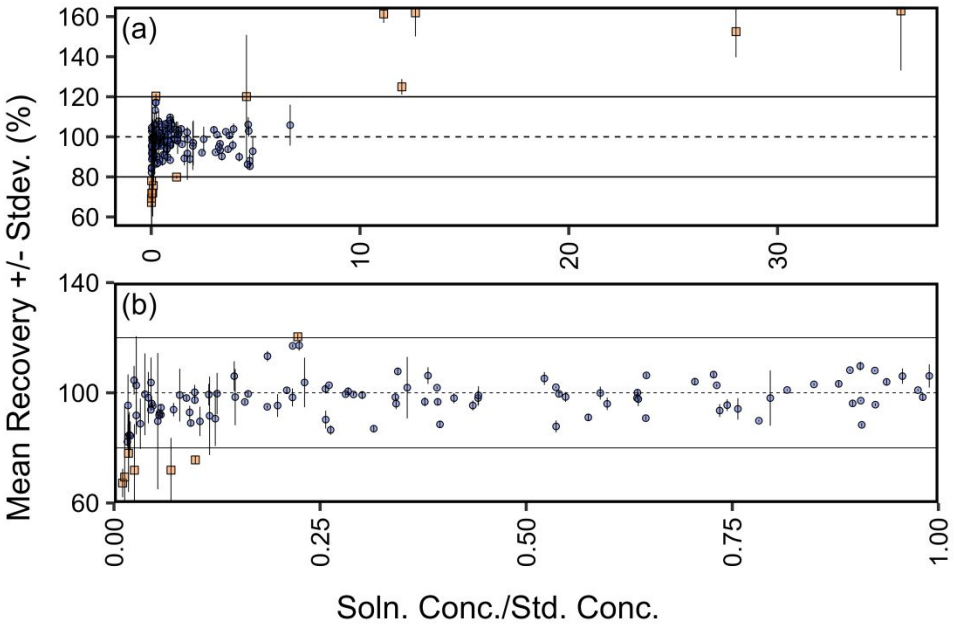

Figure 4. Analytical recovery for all elements from the analysis of reference materials and fortified (i.e., spiked) samples viewed as a function of (a) the ratio of analytical solution concentration to standard concentration, (b) the ratio of analytical solution concentration to standard concentration, focusing on ratio values from 0 to 1. Circles and squares represent analytical recoveries within and outside 80–120%, respectively.

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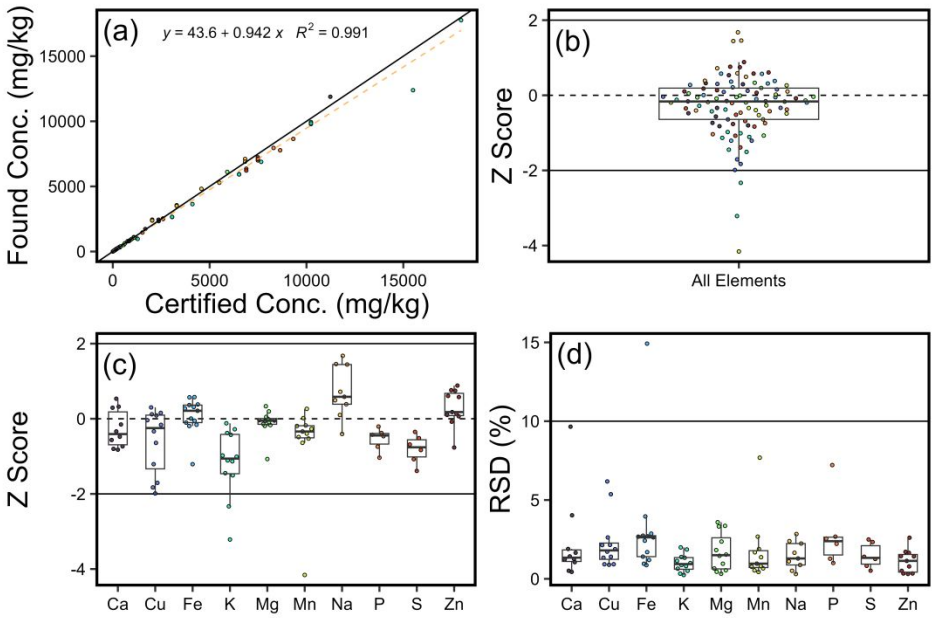

Figure 5. Results (n = 102) from the analysis of 10 different reference materials. (a) Relevant regression parameters from plotting sample concentrations determined using automated standard dilution analysis against reference material certified values. (b) Summary statistics for Z scores from all elements described by a boxplot. (c) Summary statistics for Z scores described by a boxplot for each element. (d) Relative standard deviation (RSD) summary statistics described by a boxplot according to each element.

(12.7 cm width, 1.5 column)

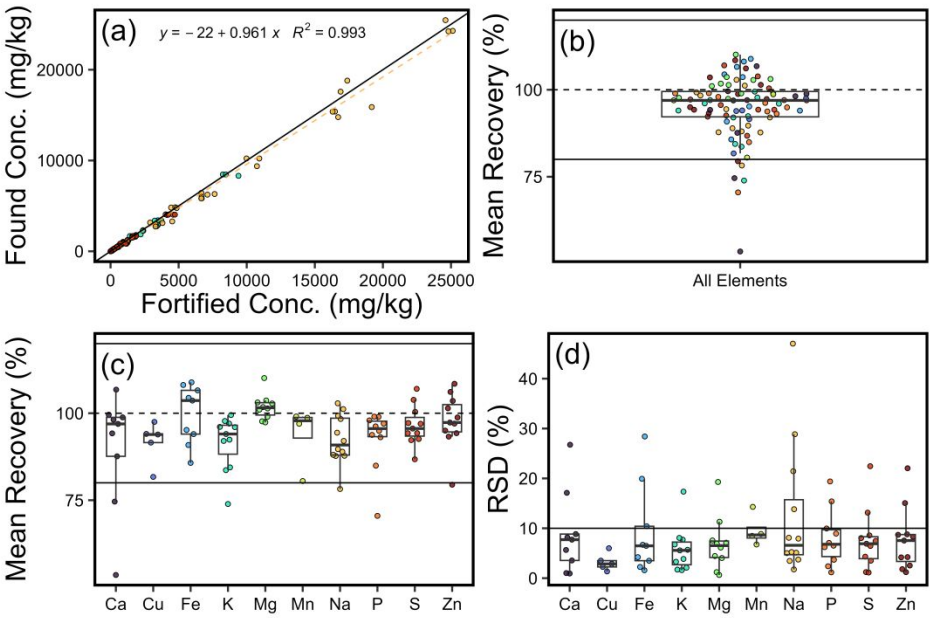

Figure 6. Results from the analysis of four different fortified foods spiked at three levels each. (a) Relevant regression parameters from plotting sample concentrations from individual replicates (n = 277) determined using automated standard dilution analysis plotted against fortified concentrations. (b) Summary statistics (n = 92) for analytical recoveries from all elements described by a boxplot. (c) Summary statistics for analytical recovery described by a boxplot for each element. (d) Relative standard deviation (RSD) summary statistics described by a boxplot according to each element.

(12.7 cm width, 1.5 column)

Table 1. Relevant instrumental and automated standard dilution analysis parameters.

Instrumental parameter	Operating condition
Radio frequency power (kW)	1.20
Sample uptake delay (s)	100[a]
Rinse time (s)	30[a]
Stabilization time (s)	30
Nebulization gas flow rate (L/min)	0.70
Plasma gas flow rate (L/min)	12.0
Auxiliary gas flow rate (L/min)	1.00
Spray chamber	Cyclonic, double pass (glass)
Nebulizer	Flow blurring, OneNeb™
Viewing	SVDV
Read/integration time (s)	1
Instrument replicates	100
Background correction	Fitted background correction (FBC)
Elements and wavelengths (nm)	Ca 315.887, Cu 327.395, Fe 238.204, K 766.491, Mg 279.800, Mn 257.610, Na 588.995, P 213.618, S 181.972, Zn 202.548
Pump tubing	White/white; 1.02 mm I.D.
Switch valve time (s)	60

[a]Slow pump

Table 2. Sample and reference materials according to their position in the AOAC food triangle.

Reference Material or Food Sample	AOAC Food Triangle Sector
NIST 1566b Oyster Tissue	7[28]
NIST 1568b Rice Flour	5[29]
NIST 1577c Bovine Liver	8[29]
NIST 1845a Whole Egg Powder	4[29]
NIST 2976 Mussel Tissue	8[29]
NIST 3233 Fortified Breakfast Cereal	5[29]
NIST 3290 Dry Cat Food	8[29]
NRC DOLT-4 Dogfish Liver	NA[a]
NRC DORM-4 Fish Protein	NA[a]
FDA Cocoa Powder	NA[a]
Breaded Chicken	7
Corn Flakes	5
Italian Dressing	2
Vegetable Oil	1

[a]Fat, carbohydrate, and protein content were not provided on the certificate of analysis and no reference could be found for the position on the AOAC triangle. Therefore, the position on the AOAC food triangle could not be determined

Table 3. Figures of merit rounded to two significant figures. The limits of detection (LODs) and limits of quantification (LOQs) were determined assuming a dilution factor of 200.

Element and wavelength (nm)	ASDL (mg/kg)	ASQL (mg/kg)	LOD (mg/kg)	LOQ (mg/kg)
Ca 315.887	0.029	0.095	5.8	19
Cu 327.395	0.0034	0.011	0.70	2.3
Fe 238.204	0.0055	0.018	1.1	3.7
K 766.491	0.52	1.7	110	350
Mg 279.800	0.051	0.17	10	35
Mn 257.610	0.0021	0.0069	0.42	1.4
Na 588.995	0.54	1.8	110	370
P 213.618	0.029	0.096	5.9	20
S 181.972	0.067	0.22	14	45
Zn 202.548	0.0019	0.0064	0.39	1.3

Table 4. Defined accurate working ranges for 10 nutrient elements.

Element	Standard conc. (mg/kg)	Minimum ratio[a]	Maximum ratio[b]	Minimum solution conc. (mg/kg)	Maximum solution conc. (mg/kg)	Minimum sample conc. (mg/kg)	Maximum sample conc. (mg/kg)
Ca	9.8	0.039	6.7	0.38	66	76	13000
Cu	0.98	0.013	1.3	0.013	1.3	2.5	250
Fe	0.98	0.022	7.0	0.022	6.9	4.3	1400
K	49	0.061	1.7	3.0	83	600	17000
Mg	9.8	0.026	3.0	0.25	29	51	5900
Mn	0.98	0.0083	0.41	0.0081	0.40	1.6	80
Na	49	0.049	3.8	2.4	190	480	37000
P	9.8	0.076	4.9	0.74	48	150	9600
S	9.8	0.023	3.4	0.23	33	45	6700
Zn	0.98	0.010	3.7	0.0098	3.6	2.0	730

[a]Minimum analytical solution concentration to standard concentration ratio observed with a recovery from a fortified (i.e., spiked) sample or reference material within 80–120% recovery
[b]Maximum analytical solution concentration to standard concentration ratio observed with a recovery from a fortified (i.e., spiked) sample or reference material within 80–120% recovery

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