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Certification of Standard Reference Material® 2386

Avocado Powder

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Abstract

The National Institute of Standards and Technology (NIST) recently released Standard Reference Material (SRM) 2386 Avocado Powder which has values assigned for over 70 analytes. This material is intended to be used for the evaluation of methods for the determination of elements, vitamins, amino acids, fatty acids, and proximates in this and similar matrices. The material was purchased pre-packaged from a commercial vendor and data was obtained from NIST and interlaboratory comparison exercises. A description of the material, sample preparations, results, and data analysis are discussed in the following report.

Keywords

Amino Acids; Avocado; Elements; Fat-Soluble Vitamins; Fatty Acids; Moisture; Proximates; Reference Material; Water-Soluble Vitamins.

Table of Contents

List of Tables

List of Figures

1. Introduction

In 1997 and 2000, NIST held workshops to identify needs of the food industry and federal regulators. Among other things, NIST was asked to continue production of food-matrix SRMs in various sectors of the AOAC food composition triangle [1] for use by laboratories making measurements in support of nutrition labeling. These laboratories need a means for demonstrating method validity and accuracy when analyzing food products to generate data for nutrition labels. SRM 2386 fills a void in sector 2 of the food triangle as a high fat material with substantive nutritional content (Fig. 1). In addition, SRM 2386 would be only the second powdered material in the upper sectors of the food triangle.

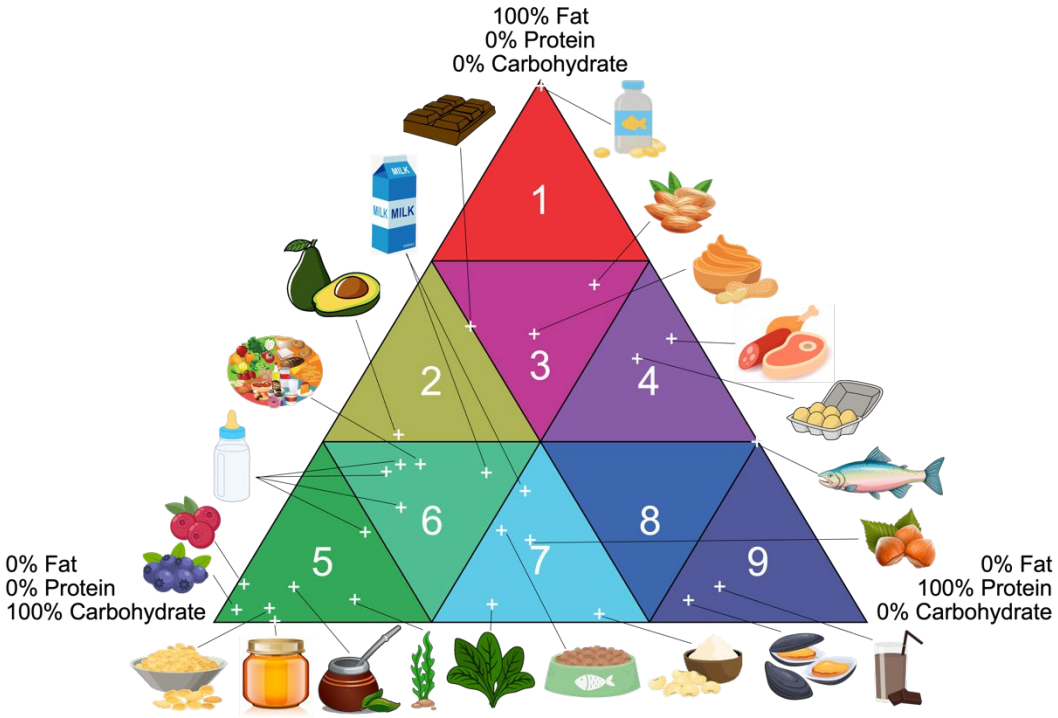


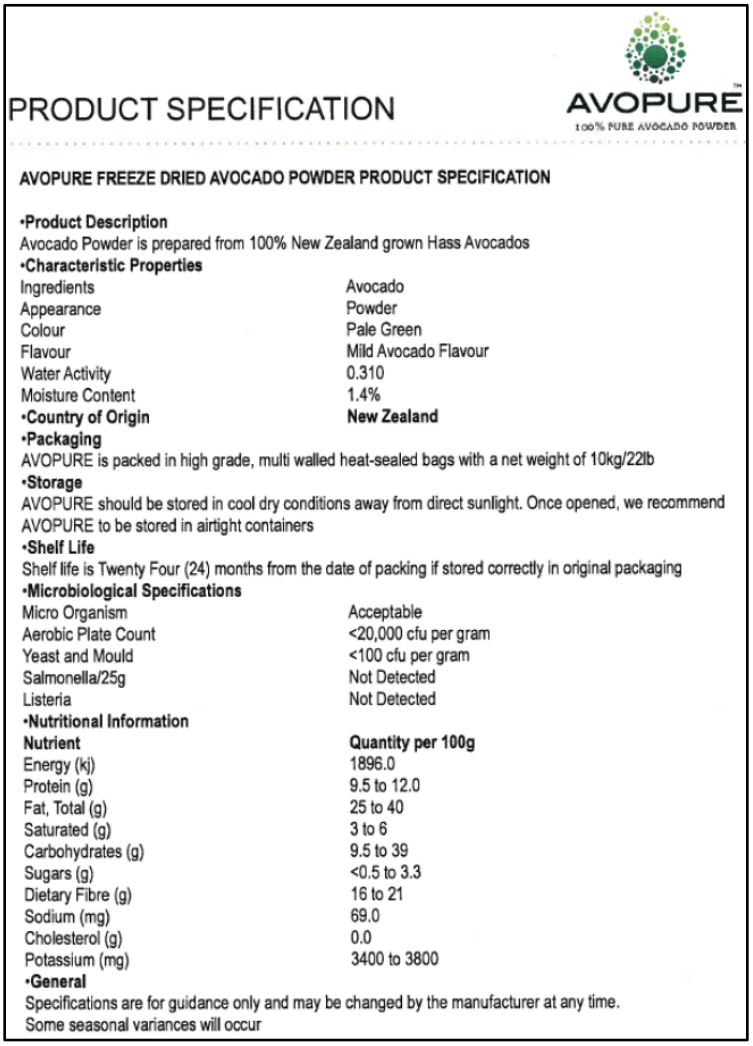
Fig. 1. NIST adaptation of the AOAC food composition triangle.

The white "+" depict the location of available food-matrix reference materials.

2. Material

2.1. Acquisition & Packaging

SRM 2386 Avocado Powder was obtained from Avopure, a division of Avocado Oil NZ Ltd (Tauranga, New Zealand). Twenty kilograms of freeze-dried avocado powder was packaged at the Avopure facility into 2000 10-gram multi-walled, heat-sealed pouches (see Fig. 2). The material was received in a shipment of three large cardboard containers, each containing four inner boxes for a total of 12 boxes.



PRODUCT SPECIFICATION

AVOPURE
100% PURE AVOCADO POWDER

AVOPURE FREEZE DRIED AVOCADO POWDER PRODUCT SPECIFICATION

•Product Description
Avocado Powder is prepared from 100% New Zealand grown Hass Avocados

•Characteristic Properties

Ingredients	Avocado
Appearance	Powder
Colour	Pale Green
Flavour	Mild Avocado Flavour
Water Activity	0.310
Moisture Content	1.4%
•Country of Origin	**New Zealand**

•Packaging
AVOPURE is packed in high grade, multi walled heat-sealed bags with a net weight of 10kg/22lb

•Storage
AVOPURE should be stored in cool dry conditions away from direct sunlight. Once opened, we recommend AVOPURE to be stored in airtight containers

•Shelf Life
Shelf life is Twenty Four (24) months from the date of packing if stored correctly in original packaging

•Microbiological Specifications

Micro Organism	Acceptable
Aerobic Plate Count	<20,000 cfu per gram
Yeast and Mould	<100 cfu per gram
Salmonella/25g	Not Detected
Listeria	Not Detected

•Nutritional Information

Nutrient	**Quantity per 100g**
Energy (kj)	1896.0
Protein (g)	9.5 to 12.0
Fat, Total (g)	25 to 40
Saturated (g)	3 to 6
Carbohydrates (g)	9.5 to 39
Sugars (g)	<0.5 to 3.3
Dietary Fibre (g)	16 to 21
Sodium (mg)	69.0
Cholesterol (g)	0.0
Potassium (mg)	3400 to 3800

•General
Specifications are for guidance only and may be changed by the manufacturer at any time.
Some seasonal variances will occur

Fig. 2. Product Specification for Avopure Freeze-Dried Avocado Powder.

2.2. Irradiation

SRM 2386 was irradiated by Neutron Products, Inc. (Dickerson, MD) in the original cardboard containers from the manufacturer. The target for the absorbed dose was (6.0 to 10.0) kGy. The actual absorbed doses measured by Neutron Products were (6.4 to 10.0) kGy (see Fig. 3).

CERTIFICATE OF IRRADIATION

IRRADIATION LOT NUMBER: 07520334

DATE(S) OF IRRADIATION: July 30, 2015 & August 3, 2015

CUSTOMER
NIST
100 Bureau Drive
MS 8462
Gaithersburg, Maryland 20899

PURCHASE ORDER NO.:
15-646-L434

PRODUCT DESCRIPTION AND CUSTOMER LOT NUMBER
Avocado Powder
SRM #2386

NUMBER OF CARTONS IN IRRADIATION LOT
3

ABSORBED DOSE SPECIFIED		ABSORBED DOSE MEASURED	
MINIMUM	6.0 kGy	MINIMUM	6.4 kGy
MAXIMUM	10.0 kGy	MAXIMUM	10.0 kGy

ABSORBED DOSE MEASURED BY FWT60-00 DOSIMETERS. UNCERTAINTY OF MEASUREMENT ± 3.4% AT A 95% CONFIDENCE LEVEL.

COMMENTS

for Kimberly A Harmon
Carol M. Campbell
QC Manager, Radiation Processing Services
Date August 4, 2015

neUTROn pRODUCTS inc

Fig. 3. Certificate of Irradiation for SRM 2386 Avocado Powder.

2.3. Storage

The packets of SRM 2386 have been stored at room temperature (18 to 22) °C at NIST since their receipt.

3. Experimental Procedures

3.1. NIST Methods and Procedures

3.1.1. Moisture Content

Moisture content was determined at NIST using three independent methods. Independent, unopened samples from each of six boxes were used for the determination of moisture by freeze-drying and independent, unopened samples from each of 12 boxes were used for desiccator drying and forced air oven drying. A summary of drying results is shown in the results and discussion section.

3.1.1.1. Freeze Drying

Two aliquots of material from each of six freshly opened packets of SRM 2386 were transferred to Pyrex weighing bottles that had been previously heated at 110 °C, cooled to constant weight, and weighed (m_b). The weighing bottle was capped, and mass of the material plus weighing bottle was recorded (m_w). All weighings were conducted on the same balance, the calibration of which was confirmed with calibrated masses prior to use. The bottles were passed through a static eliminator prior to each weighing. All weights were determined and recorded to ± 0.00001 g.

Dryings were performed using a Virtis Advantage Plus Freeze Dryer (SP Scientific) using a standard drying program with a minimum temperature of -40 °C and approximate pressure of 2.66 Pa (200 mTorr). Uncapped samples were frozen at -40 °C for 20 h; the temperature was then increased to -10 °C and held for 7 d. At the end of the seven-day drying cycle, the vacuum was released, and the sample bottles were capped and transferred to a desiccator containing freshly opened magnesium perchlorate for at least one hour before weighing. Samples were removed from the desiccator, weighed, and the results (m_d) recorded.

3.1.1.2. Desiccator Drying

Single aliquots from each of 12 freshly opened packets were placed in pre-weighed, glass weighing vessels (m_b) to an approximate depth of 1 cm. The packets were rotated to mix prior to sampling. The vessels were again weighed (m_w) and placed in a desiccator over magnesium perchlorate ($Mg(ClO_4)_2$). The samples were removed from the desiccator on day 7, weighed, returned to the desiccator, and the results (m_d) recorded. The samples were weighed and the weights recorded again on days 14, 21, and 28. All weighings were performed using the same balance serviced and calibrated annually by Mettler. Prior to each use, calibration is verified by using standard masses ranging from (0.5 to 20) g that are traceable to the SI through the standard mass set maintained by the Inorganic Chemical Metrology Group.

3.1.1.3. Forced Air Drying

Single aliquots from each of 12 freshly opened packets were placed in pre-weighed, glass weighing vessels (m_b) to an approximate depth of 1 cm. The packets were rotated to mix prior to sampling. The vessels were again weighed (m_w) and placed in a forced-air drying oven set at 80 °C with caps removed. After 1 h, the samples were removed, capped, and allowed to cool to room temperature in a desiccator. Cooled samples were removed from the desiccator, weighed, and the results (m_d)

recorded. All weighings were performed using the same balance serviced and calibrated annually by Mettler. Prior to each use, calibration is verified by using standard masses ranging from (0.5 to 20) g that are traceable to the SI through the standard mass set maintained by the Inorganic Chemical Metrology Group.

3.1.1.4. Moisture Calculation

The overall moisture results were calculated assuming that all mass losses were due to loss of moisture alone using the following equations:

$$\text{Moisture content} = 100\,\frac{m_{\mathrm{w}}-m_{\mathrm{d}}}{m_{\mathrm{w}}-m_{\mathrm{b}}} \quad (1)$$

$$U_{95}(\text{Moisture content}) = 2.2\sqrt{u_{\mathrm{a}}^2 + u_{\mathrm{b1}}^2 + u_{\mathrm{b2}}^2 + u_{\mathrm{b3}}^2} \quad (2)$$

where u_{a} is the standard deviation for the samples ($n = 6$ or $n = 12$) and $u_{\mathrm{b}i}$ are the standard uncertainties of the three weighings, each estimated to be ± 0.01/√3 mg. For each $u_{\mathrm{b}i}$ this value is converted to moisture content by division of the mean sample mass value. The expanded uncertainty value, U_{95}, is expressed at an approximate confidence level of 95 % by choosing the expansion factor 2.2, calculated based on degrees of freedom.

3.1.2. Elements

A summary of elements measured for value assignment in SRM 2386 is listed in Table 1.

Table 1. Methods Used for Elemental Determinations.

Element	NIST Methods
Boron (B)	TNPGAA
Cadmium (Cd)	ID ICP-MS
Calcium (Ca)	ICP-OES
Copper (Cu)	ICP-OES
Iron (Fe)	ICP-OES
Magnesium (Mg)	ICP-OES
Manganese (Mn)	ICP-OES
Molybdenum (Mo)	
Phosphorus (P)	ICP-OES
Potassium (K)	ICP-OES
Selenium (Se)	
Sodium (Na)	ICP-OES
Zinc (Zn)	ICP-OES

ICP-OES Inductively Coupled Plasma Optical Emission Spectrometry
ID ICP-MS Isotope Dilution Inductively Coupled Plasma Mass Spectrometry
TNPGAA Thermal Neutron Prompt Gamma-Ray Activation Analysis

3.1.2.1. ICP-OES Analysis

Mass fractions of Ca, Cu, Fe, K, Mg, Mn, Na, P, and Zn in SRM 2386 were determined at NIST using ICP-OES. Two 0.5 g aliquots were taken from each of 10 packets of SRM 2386 and were

placed into Teflon microwave vessels. Four 0.5 g aliquots of both SRM 1845a Whole Egg Powder and SRM 1577c Bovine Liver were prepared along with the samples for quality assurance, although both controls were not used for all elements. All samples were analyzed in as-received condition. Twelve procedural reagent blanks were also prepared along with the samples. Concentrated nitric acid (HNO_3, 10 mL) was added to each vessel, and indium (0.25 mL of a 100 mg/kg In solution) and scandium (0.5 mL of a 100 mg/kg Sc solution) were added as internal standards to improve the precision of the instrumental measurements. The In solution was prepared in-house from Indium Corporation of America Lot # JK 1171 to a final concentration of 1.5 % (volume fraction) HNO_3. The Sc solution was prepared from SRM 3148a Scandium (Sc) Standard Solution to a final concentration of 1.5 % (volume fraction) HNO_3. All weighings were performed using a Mettler AT261 Delta Range analytical balance serviced and calibrated annually by Mettler. Prior to each use, calibration is verified by using standard masses ranging from (0.5 to 20) g that are traceable to the International System of Units (SI) through the standard mass set maintained by the Inorganic Chemical Metrology Group.

All prepared samples, controls, and blanks were digested using a CEM MARS microwave sample preparation system according to the microwave procedure in Table 2. After microwave digestion, solutions were transferred to Teflon beakers and were heated on a hot plate with a surface temperature of approximately 175 °C until the volume was reduced to near dryness. Samples were then diluted using 1.5 % (volume fraction) HNO_3. Because the samples of SRM 2386 appeared to contain undigested fat, additional concentrated HNO_3 and 1 mL of concentrated perchloric acid ($HClO_4$) were added to each sample. The solutions were covered for a minimum of 4 h to reflux then heated on a hot plate with a surface temperature of approximately 205 °C until the volume was reduced to near dryness. Samples were then diluted to 30 g using 1.5 % (volume fraction) HNO_3 and transferred to polyethylene bottles.

All samples were prepared using redistilled grade HNO_3 from Veritas and ACS grade $HClO_4$ from Mallinckrodt. Samples and acids were diluted using 18 MΩ·cm water. All dilute acid concentrations are expressed in volume fractions with respect to the concentrated acid.

Table 2. Microwave Settings for Digestion of SRM 2386 Samples for Elemental Analysis.

Step	Power (W)	Power Setting (%)	Ramp Time (min)	Control Pressure (PSI)	Temperature (°C)	Hold Time (min)
1	800	100	25	800	150	25
2	1600	100	25	800	190	15

Analyte mass fractions were calculated by the method of standard additions to compensate for any matrix effects. Samples were diluted to approximate analyte mass fractions. From each dilution, two aliquots were taken, and a matrix matched spike was added to one. The sample mass fraction dilutions, mass fractions of the matrix matched spike solution added to the second aliquot, and the total mass fraction expected in the spiked solution are listed in Table 3.

Table 3. Standards and Approximate Mass Fractions for Determination of Elements.

Element	Symbol	Source SRM		Mass Fraction in Sample Solution (mg/kg)	Mass Fraction Added (Spike) (mg/kg)	Total Mass Fraction in Spiked Aliquot (mg/kg)
		SRM Number	Lot Number			
Calcium	Ca	3109a	130213	0.3	0.4	0.7
Copper	Cu	3114	120618	0.3	0.6	0.9
Iron	Fe	3126a	51031	0.6	0.7	1.3
Potassium	K	3141a	51220	0.9	1	1.9
Magnesium	Mg	3131a	140110	0.3	0.5	0.8
Manganese	Mn	3132	50429	0.2	0.4	0.6
Sodium	Na	3152a	10728	1	1	2
Phosphorus	P	3139a	60717	1.5	2	3.5
Zinc	Zn	3168a	120629	0.6	1	1.6

Two inductively coupled plasma optical emission spectrometers were used for analysis: A Perkin-Elmer Optima 3300 Dual View and a Perkin-Elmer Optima 5300 Dual View. The analytes in the sample, control, and blank solutions were measured according to the parameters in Table 4.

Table 4. ICP-OES Parameters Used to Measure Elements.

Element	Symbol	Wavelength (nm)	Plasma View	Integration Time (s)	Read Time (s)	Number of Runs
Calcium	Ca	317.933	Axial	0.1	1	2
Copper	Cu	224.700	Axial	0.1	1	2
Iron	Fe	238.204	Axial	0.1	1	2
Potassium	K	766.550	Radial	0.1	1	2
Magnesium	Mg	285.213	Axial	0.025	1	2
Manganese	Mn	257.610	Axial	0.1	1	2
Sodium	Na	589.478	Radial	0.1	1	2
Phosphorus	P	213.615	Axial	0.1	1	3
Zinc	Zn	206.200	Axial	0.1	1	2
Indium	In	230.606	Axial	0.1	1	2
Scandium	Sc	361.383	Axial Radial	0.025 0.1	1	2

Four instrumental measurements were averaged for each sample aliquot and each spiked aliquot. After exporting raw data to Microsoft Excel, final mass fractions were calculated using the method of standard additions.

3.1.2.2. ID ICP-MS Analysis

The mass fraction of Cd in SRM 2386 was determined at NIST using ID ICP-MS [2,3,4]. Two 0.5 g aliquots were taken from each of 6 packets of SRM 2386 and were placed into Teflon microwave vessels. Samples were allowed to equilibrate with room temperature for sixteen hours before processing. The aluminized packets of SRM 2386 were cut open with Teflon scissors and the dry avocado cakes were crushed and mixed by squeezing against the sides of the aluminized packets. Portions were transferred to clean aluminum weighing boats *via* an aluminum spatula, until test portions with a nominal mass of 0.5 g were obtained. The boats were transferred to a balance and masses were recorded to ± 0.00001 g. The boats were removed from the balance and the test

portions were transferred to a microwave digestion vessel. Aliquots of SRM 2384 Baking Chocolate and SRM 1577c Bovine Liver were prepared along with the samples for quality assurance. Known amounts of a ^{111}Cd spike solution were added to each test portion of SRM 2386, SRM 2384, SRM 1577c, and to the standards processed as samples (Standard as Sample, SAS) control samples, spike calibration samples, and procedural blanks by mass difference using a capped plastic syringe. The mass of each added spike solution portion was recorded to ± 0.00001 g. SAS controls were prepared in clean microwave vessels and spike calibration samples were prepared in clean 30 mL low density polyethylene (LDPE) bottles. Test portions, SAS controls, and spike calibration samples were spiked so that approximately 0.6 ng of ^{111}Cd spike was added for every 1 ng Cd in the sample, resulting in ^{111}Cd/^{112}Cd, ^{111}Cd/^{113}Cd, and ^{111}Cd/^{114}Cd ratios of 2.7, 5.6, and 2.4, respectively. Procedural blanks were composed of smaller amounts of ^{111}Cd (≈0.6 ng) added to clean vessels in the same manner as test portions. Concentrated HNO_3 (4 g) was used to wash spike solution down from the sides of the microwave vessel after each addition of spike solution to each test portion, SAS control, and procedural blank (hereafter referred to as *samples*). The working ^{111}Cd spike solution in 2 % volume fraction HNO_3 was prepared by gravimetric dilution of a master stock solution of enriched ^{111}Cd (96.5 %, Oak Ridge assay) prepared from ^{111}CdO obtained from Oak Ridge National Laboratory. The Cd isotopic composition of this spike solution was experimentally verified by ICP-MS measurement. The masses of the sample test portions and added spike solutions were obtained on a calibrated 5-place Mettler XP205 balance and were recorded electronically. The specialized instruments and labware used in this analysis are described in Table 5.

The samples were pre-digested on a hot plate in a class 10 clean room for 2 h. Following predigestion, the vessels were cooled to ambient temperature and an additional 10 g HNO_3 were added. Vessels were transferred to a MARS Microwave Reaction System and digested according to the parameters listed in Table 2. Vessels were cooled to ambient temperature, removed from the microwave oven, and the contents transferred back to the hot plates in order to boil off the digestion acid. Solutions appeared a deep blue color. The digests were evaporated to near dryness and re-dissolved in one to two drops of concentrated HNO_3 followed by approximately 4 g of 2 % (volume fraction) HNO_3 to produce clear solutions. Samples were quantitatively transferred to Nalgene bottles and diluted with 2 % (volume fraction) HNO_3 to a mass fraction of approximately 3.5 µg/kg ^{111}Cd.

All samples were prepared using optima grade (Thermo Fisher Scientific, Waltham, MA) HNO_3. High-purity water was prepared in-house by sub-boiling distillation using a conditioned, quartz still with deionized water as feedstock. All dilute acid concentrations are expressed in volume fractions with respect to the concentrated acid.

SRM 3108 Cadmium (Cd) Standard Solution (lot # 130116) served as a primary standard by gravimetric dilution to obtain the desired Cd mass fraction. An additional primary standard solution was prepared from the high purity Cd of SRM 746 Cadmium-Vapor Pressure (99.999+ percent purity, NIST and vendor assay). A (0.3 to 0.4) g piece of the metal was cleaned with an acid etch, dried, and weighed to ± 0.000005 g with a calibrated 6-place Mettler AT 20 balance. An air buoyancy correction of 0.999988 and a purity correction of 0.99999 were applied to the measured mass of the metal. The metal was dissolved quantitatively and diluted gravimetrically to obtain the desired mass fraction. The masses of the standard solution dilutions were obtained on a calibrated 5-place Mettler XP205 balance and were recorded electronically.

Table 5. Instruments and Labware Used in the Determination of Cadmium (Cd).

Instrument/Labware	Manufacturer
Isotemp Standard Laboratory Oven	Fisher Scientific, Pittsburgh, PA
XP205 balance	Mettler-Toledo, Columbus, OH
AT 20 balance	Mettler-Toledo, Columbus, OH
MARS Microwave Reaction System	CEM, Mathews, NC
Mars EasyPrep Vessels (TFM™-polytetrafluoroethylene Teflon)	CEM, Mathews, NC
5 mL and 10 mL plastic syringe	Henke Sass Wolf GmbH, Tuttlingen, Germany
30 mL LDPE bottles	Nalge Nunc, Rochester, NY
4 mL HDPE scintillation vials	Scientific Commodities, Lake Havasu City, AZ
XseriesII ICP-MS	ThermoFisher Scientific, Madison, WI
ESI SC-2DX autosampler	Elemental Scientific, Omaha, NE
100 µL/min PFA-ST microconcentric nebulizer	Elemental Scientific, Omaha, NE
Peltier-cooled impact bead spray chamber	ThermoFisher Scientific, Madison, WI

HDPE High density polyethylene
PFA Perfluoroalkoxy alkane

The amount of ^{111}Cd in the spike solution was calibrated against the primary Cd standards using reverse ID ICP-MS using the following functional relationship for calculations:

$$c_{\mathrm{y}} = \frac{1}{m_y}\left\{m_z c_z \left[\frac{((Ab\ z)_z (k_{b\prime})(R_{y/z})_{b\prime}) - (Ab\ y)_z}{(Ab\ y)_y - (k_{b\prime})(R_{y/z})_{b\prime}(Ab\ z)_y}\right]\right\} \quad (3)$$

In this expression, *y* refers to the spike, *z* refers to the standard, *m* to mass, *Ab z* to abundance of the reference isotope (i.e., ^{112}Cd, ^{113}Cd, ^{114}Cd), *Ab y* to abundance of the spike isotope (i.e., ^{111}Cd), *k* to the correction factor for mass bias, *R* to ratio, *b'* to the spike calibration blend (standard spiked with enriched isotope) corrected for dead time, and *c* to amount content (µmol/g).

Spike calibration samples were prepared concurrent with the analytical samples to have mass ratios similar to the analytical samples. The spike samples were diluted to produce the same ICP-MS count rate as the analytical samples. Two aliquots from each of two separate primary standard solution preparations were added to weighed spike solution aliquots resulting in four calibration samples.

Mass spectrometric analyses were performed on a ThermoFisher Scientific X series II ICP-MS equipped with matrix tolerant (Xt) cones and operated at 1400 W. Solution was introduced *via* a peristaltic pump into a low-flow (100 µL/min) PFA microconcentric nebulizer. The nebulizer was fitted to an impact-bead spray chamber cooled to 2 °C. Samples were analyzed in both standard mode and collision cell kinetic energy discrimination mode (CC/KED mode). For CC/KED mode, a cell gas of 8 % mole fraction hydrogen in balance helium was introduced at a rate of 4.00 mL/min, the hexapole bias was operated at -20 V, and the quadrupole bias was set at -17 V. Measurements were conducted using peak jump data acquisition with one point per peak. Five blocks of data, each one minute in duration, were acquired per sample, and the mean intensity ratios were used for computations. Measured intensities were corrected for dead-time and interference (as required) and the intensity ratios were corrected for mass bias and drift. Detector dead-time was experimentally determined using natural Gd solutions with mass fractions that

resulted in count rates spanning the count rate range from (1×10^5 to 1×10^6) counts per second (cps). The measured dead time was 35 ns. For Cd, a solution of pure Cd with nominal natural isotopic composition was used to measure the mass bias correction factor. (Note: ^{111}Cd and ^{112}Cd are considered absolutely stable; ^{113}Cd has a half-life of 7.6×10^{15} y and though ^{114}Cd has been predicted to be radioactive, decay has not been observed due to an extremely long half-life). The mass bias factor was measured at the beginning of the analysis sequence. The mass bias factor was then used to correct the measured ratio of a spike calibration sample measured immediately afterward. The spike calibration sample had an isotopic ratio similar to the spiked test portions, was remeasured throughout the analysis, and was used to correct the blanks, remaining calibration samples, test portions and controls for mass bias and any subsequent instrument drift. Drift was assessed every three samples and a correction applied assuming temporal linearity.

Signal intensities for Cd and interfering ions were measured at dwell times as described in Table 6. High-purity solutions of Zr, Mo, In, and Sn were also measured at the start of each analysis and used to evaluate and correct for spectral interference. For example, the measured count rate at mass 111 was corrected for the intensity of the $^{94}Zr^{16}O^{1}H$ interference in the sample by multiplying the measured ^{91}Zr signal intensity in the sample by the measured natural isotopic $^{94}Zr/^{91}Zr$ ratio and multiplying that by the $^{94}Zr^{16}O^{1}H/^{94}Zr$ ratio measured in the pure solution of Zr at the start of the analysis. The same process was followed to determine the intensity of the $^{95}Mo^{16}O$, and $^{94}Mo^{16}O^{1}H$ interferences at mass 111 in the sample. Likewise, masses 112, 113, and 114 were also corrected for potential interferences as described in Table 6. Cd mass fractions were calculated in the spiked samples from corrected $^{111}Cd/^{112}Cd$, $^{111}Cd/^{113}Cd$, and $^{111}Cd/^{114}Cd$ intensity ratios, and the results averaged.

Table 6. ICP-MS Dwell Times for Target and Interfering Ions.

Ion	Dwell time	Potential Interferences
^{111}Cd	10 ms	$^{94}Zr^{16}O^{1}H$, $^{95}Mo^{16}O$, $^{94}Mo^{16}O^{1}H$
^{112}Cd	20 ms	$^{96}Zr^{16}O$, $^{95}Mo^{16}O^{1}H$, $^{96}Mo^{16}O$, ^{112}Sn
^{113}Cd	20 ms	$^{96}Zr^{16}O^{1}H$, $^{96}Mo^{1}6O^{1}H$, $^{97}Mo^{16}O$, ^{113}In
^{114}Cd	20 ms	$^{97}Mo^{16}O^{1}H$, $^{98}Mo^{16}O$, ^{114}Sn
^{90}Zr	5 ms	
^{91}Zr	5 ms	
^{95}Mo	5 ms	
^{97}Mo	5 ms	
^{98}Mo	5 ms	
^{115}In	5 ms	
^{117}Sn	5 ms	
^{118}Sn	5 ms	

The functional relationship below was used to calculate the ID ICP-MS mass fraction results:

$$c_{\mathrm{x}} = \frac{1}{m_x}\left\{m_y c_y \left[\frac{(Ab\ y)_y - (k_b)(R_{y/x})_b (Ab\ x)_y}{((Ab\ x)_x (k_b)(R_{y/x})_b) - (Ab\ y)_x}\right] - blank\right\} \qquad (4)$$

In this expression, *x* refers to the sample, *y* refers to the spike, *m* to mass, *Ab x* to abundance of the reference isotope (i.e., ^{112}Cd, ^{113}Cd, ^{114}Cd), *Ab y* to abundance of the spike isotope (i.e., ^{111}Cd), *k* to the correction factor for mass bias, *R* to ratio, *b* to the sample blend (sample spiked with enriched isotopes) corrected for dead time and interference, *blank* to procedure blank (µmol), and *c* to

amount content (μmol/g). The amount content in μmol/g was converted to mass fraction (mg/kg) by multiplying by the atomic weight [5].

A third nominal 1 g test portion was sampled from each of the SRM 2386 packets for moisture determination. The moisture determination samples were weighed directly into clean, dry glass weighing bottles for which the tare mass had been recorded. The portions of SRM 2386 were dried for 1 h in a forced air convection oven at 80 °C. Samples were cooled to room temperature in a desiccator and the loss in mass measured. The SRM 2386 samples were dried a second time under the same conditions to confirm that the samples had reached a constant mass. For Cd mass fractions reported on a dry mass basis, results from test portions were corrected for moisture using the mean mass loss measured for each respective packet. The mass loss after 1 h of drying did not differ significantly from the mass loss measured after an additional hour of drying (absolute average difference of 0.077 % loss) and so the two results were averaged.

3.1.2.3. TNPGAA Analysis

The mass fraction of B in SRM 2386 was determined at NIST using TNPGAA [2,3,4]. Two 0.75 g aliquots were taken from each of 6 packets of SRM 2386 and were pressed into pellets using a 13 mm stainless steel die and hydraulic press at 10,000 pounds' (6.89×10^7 Pa) force for (3 to 5) s. Prior to sampling, the material in each pouch was mixed by gentle side-to-side motion and rotation for approximately 1 min. Each pellet was heat-sealed into a bag of fluorinated ethylene propylene (FEP) Teflon prior to analysis. Initially, only 1 pellet was prepared from material in each pouch, but due to concerns about possible boron contamination of the die, a second set of six pellets were prepared using a different die. Three aliquots of SRM 1573a Tomato Leaves were prepared along with the samples for quality assurance. Procedural blanks were also prepared along with the samples, including an empty Teflon bag and a pressed disk of Whatman 42 filter paper. A Mettler Toledo XP205DR analytical balance, with calibration verified using Troemner calibrated masses, was used for weighing in the preparation of samples, controls, and standards. All samples were sealed in Teflon bags for analysis.

Standards used for calibration were legacy boron standards prepared by pipetting boric acid solution onto filter paper, which have been used many times previously for certification of boron in biological and agricultural materials using TNPGAA. Three standards, containing 62.1 μg B, 75.9 μg B, and 68.5 μg B, were used in this investigation. Additional standards, prepared from mixtures of tris(hydroxymethyl)aminomethane (TRIS) and a gravimetrically diluted solution of SRM 3107 Boron (B) Standard Solution, were used to assess the effect of hydrogen mass fraction on boron sensitivity. The TRIS material was crushed to a fine powder using a Spex Mixer mill. A portion was then weighed into a mixing vial and doped with about 0.2 g of the boron solution added via a plastic pipette (weighed before and after deposition). The powder was allowed to dry for three to four days in a clean hood and was then homogenized in the mixer mill. Pellets containing approximately 750 mg of doped material were then prepared as described earlier. All standards were sealed in Teflon bags for analysis.

Samples, standards, and controls were analyzed using the TNPGAA, vertical beam VT-5 facility located at the NIST Center for Neutron Research (NCNR) [6]. Targets were irradiated in an air-filled sample chamber; samples and standards were irradiated for 10 min each and controls were irradiated for 20 min. A 139 mg titanium foil was irradiated at regular intervals in order to monitor any variation in the neutron fluence rate and sample positioning within the beam over the course

of the investigation. An empty Teflon bag was irradiated overnight to measure boron background arising from neutron capture by shielding materials. A pressed disk of Whatman 42 filter paper was also irradiated as a standards blank.

3.1.3. Water-Soluble Vitamins and Related Measurands

A summary of water-soluble vitamins analyzed in SRM 2386 is provided in Table 7.

Table 7. Methods Used for Vitamin Determinations.

Analyte	NIST Method
Ascorbic acid (vitamin C)	LC-UV
Thiamine (vitamin B_1)	ID-LC-MS/MS
Riboflavin (vitamin B_2)	ID-LC-MS/MS
Niacinamide (vitamin B_3)	ID-LC-MS/MS
Niacin (vitamin B_3)	ID-LC-MS/MS
Total vitamin B_3	ID-LC-MS/MS
Pantothenic acid (vitamin B_5)	ID-LC-MS/MS
Pyridoxal (vitamin B_6)	ID-LC-MS/MS
Pyridoxine (vitamin B_6)	ID-LC-MS/MS
Total vitamin B_6	ID-LC-MS/MS
Choline	ID-LC-MS/MS
Carnitine	ID-LC-MS/MS

LC-UV Liquid Chromatography with UV Absorbance Detection
ID-LC-MS/MS Isotope Dilution Liquid Chromatography with Tandem Mass Spectrometry Detection

3.1.3.1. Ascorbic Acid (Vitamin C)

The mass fraction of ascorbic acid (vitamin C) in SRM 2386 was determined at NIST using LC-UV and employing an internal standard, as modified from an earlier study [7]. Three 2 g aliquots were taken from each of 10 packets of SRM 2386 and were dissolved in (25 to 30) g of 0.1 mol/L hydrochloric acid (HCl). Three 2 g aliquots of SRM 1849a Infant/Adult Nutritional Formula I (milk-based) were prepared along with the samples for quality assurance. A 4-pyridoxic acid (4-PA) solution was prepared for use as an internal standard using pure material obtained from Sigma-Aldrich (St. Louis, MO) by dissolving 477.38 mg 4-PA in 1111.2507 g of 0.1 mol/L HCl. A 15-g aliquot of the 4-PA solution was added gravimetrically to each sample followed by 2 g of a 40 % solution of metaphosphoric acid to stabilize the vitamin C. About (0.5 to 1) g of dithiothreitol (DTT) solution (100 mg in 10 mL of 0.5 mol/L potassium phosphate dibasic) was added to the solution to convert dihydroascorbic acid to total ascorbic acid. The solution was sonicated for 30 min followed by centrifugation (1000 g_n) at room temperature for 15 min. A 1-mL aliquot of the solution was removed and filtered using both a 0.45 μm and a 0.22 μm nylon filter prior to LC-UV analysis.

Four stock solutions of vitamin C (Sigma-Aldrich) were prepared by dissolving the compound in 0.1 mol/L HCl. Four calibration standards were independently prepared from these solutions and were run during the analyses of SRM 2386. The purity of the vitamin C solution was determined to be (99.71 ± 0.10) % using LC-UV at 243 nm; the uncertainty represents the standard deviation of single measurements of four independently prepared solutions. Purity was also assessed using quantitative proton nuclear magnetic (qNMR) resonance spectroscopy using an internal standard

approach as (99.68 ± 0.17) %. Because qNMR is a higher order method for purity assessment of neat materials at NIST, the purity value from this method was used to correct the mass fraction of the vitamin C calibration solutions used in SRM 2386 evaluations. Quantitation was based on the internal standard approach using averaged response factors. Mass fractions (expressed in mg/kg) were calculated from the ratio of peak areas and the detector response factors. An exemplar LC-UV chromatogram of an extract of SRM 2386 is shown in Fig. 4.

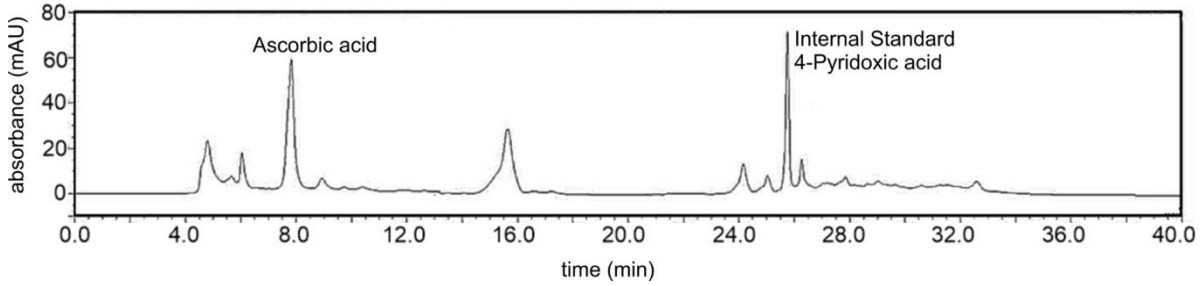


Fig. 4. Exemplar LC-UV Chromatogram of Ascorbic Acid (Vitamin C) Avocado Powder.

3.1.3.2. B Vitamins

Mass fractions of thiamine, riboflavin, niacinamide, niacin, total vitamin B_3, pantothenic acid, pyridoxal, pyridoxine, and total vitamin B_6 in SRM 2386 were determined at NIST by ID-LC-MS/MS. Two (1 to 1.5) g aliquots were taken from each of 10 packets of SRM 2386 and were placed into 125 mL polypropylene HotBlock digestion vessels. The contents of each packet of SRM 2386 were well mixed prior to sampling for extraction by applying external pressure to the open packet to break up pieces. Four 2 g aliquots each of SRM 1845a Whole Egg Powder from two separate packets and four (10 to 12) g aliquots of SRM 2387 Peanut Butter were prepared along with the samples for quality assurance. Three blank samples were prepared along with the samples containing (1) only the internal standard solutions, (2) only the sample of SRM 2386, and (3) only the extraction solvent. These samples were diluted to the approximate volume of the other samples and carried through the extraction process to identify any potential biases that may occur. A nominal 1 g aliquot of the mixed internal standard solution (described below) and 30 mL of an extraction solvent (0.1 mol/L ammonium acetate in water, adjusted to pH 2.6 with HCl) were added to all samples, controls, and blanks.

Samples were loosely capped and placed in a HotBlock with continuous stirring using a magnetic stir bar. Prior to certification, the extraction procedure was optimized for extraction temperature and number of extractions (see Section 4.3.2). A single cycle of HotBlock heating at 100 °C for 30 min was selected for extraction of vitamins for the certification measurements. Following digestion, contents of the digestion vessel were transferred to a 50 mL polyethylene centrifuge tube, and the digestion vessel was rinsed with a small aliquot (< 5 mL) of extraction solvent. Magnetic stir bars remained in the digestion cups. The samples were centrifuged for 15 min at (1000 g_n), and an aliquot of the supernatant was removed and filtered through a 0.45 µm regenerated cellulose (RC) filter into an autosampler vial for analysis by LC-MS/MS.

Samples, calibrants, controls, and blanks were analyzed by using an Agilent Series 1290 LC equipped with an Agilent Series 6410 Triple Quadrupole MS with electrospray ionization in the positive ion mode. The system was composed of a mobile phase degasser, binary pump, autosampler, and mass selective detector. The instrument was tuned prior to certification. A

Cadenza CD-C18 column (250 × 4.6 mm i.d., 3 µm particles) from Silvertone Sciences (Philadelphia, PA) was used for the analyses without a guard cartridge. The gradient elution program shown in Table 8 was used with a flow rate of 0.8 mL/min. Mobile phase A consisted of 20 mM ammonium formate in water adjusted to pH 4.0 with formic acid, and mobile phase B was methanol. A 10 µL injection volume was used for all samples. The mass spectrometer was operated at a nebulizer pressure of 1.03×10^{-5} Pa (15 psig), a drying gas flow of 11 L/min, a drying gas temperature of 300 °C, a capillary voltage of 4000 V, and a dwell time of 100 ms.

Table 8. LC Gradient Profile Used for Analysis of B Vitamins.

Time (min)	% A	% B
0	100	0
6	100	0
20	50	50
20.1	0	100
30	0	100
30.1	100	0
50	100	0

Calibration solutions were prepared from neat materials as described in Table 9. Purity of these reference standards has been evaluated by NIST using LC-UV and these purities were used to correct the reported mass fractions for each analyte. Isotopically labelled vitamin analogues were used as internal standards as described in Table 10.

Table 9. Calibration Materials used for Determination of B Vitamins.

Compound	Source	Lot Number
Thiamine chloride hydrochloride	U.S. Pharmacopeia (Rockville, MD)	#O1F236
Riboflavin	U.S. Pharmacopeia (Rockville, MD)	#N0C021
Niacinamide	U.S. Pharmacopeia (Rockville, MD)	#N0E024
Niacin	Sigma (St. Louis, MO)	#1173748
Calcium pantothenate	U.S. Pharmacopeia (Rockville, MD)	#O1H081
Pyridoxal hydrochloride	Sigma (St. Louis, MO)	#021M1809V
Pyridoxine hydrochloride	U.S. Pharmacopeia (Rockville, MD)	#Q0G409

Table 10. Isotopically Labelled Standards used for Determination of B Vitamins.

Labeled Compound	Source	Lot Number
Thiamine chloride (4,5,4-methyl-$^{13}C_3$)	Cambridge Isotope Laboratories (Andover, MA)	#PR-16731
Riboflavin ($^{13}C_4$, $^{15}N_2$)	Isosciences (King of Prussia, PA)	#SJ-2007-284A1
Niacinamide (2,4,5,6-$^{2}H_4$)	Isosciences (King of Prussia, PA)	#DS2-2005-202A1
Niacin ($^{2}H_4$)	Isosciences (King of Prussia, PA)	#DS2-2004-126A1
Calcium pantothenate monohydrate (β-alanyl-$^{13}C_3$,^{15}N)	Cambridge Isotope Laboratories (Andover, MA)	#PR-16732A
Pyridoxal hydrochloride ($^{2}H_3$)	Isosciences (King of Prussia, PA)	#LN9-2012-028A2
Pyridoxine hydrochloride (4,5-bis(hydroxymethyl)-$^{13}C_4$)	Cambridge Isotope Laboratories (Andover, MA)	#PR-16338

All stock calibrant and internal standard solutions were prepared in 0.1 mol/L ammonium acetate in water, adjusted to pH 2.6 with HCl. A stock solution containing each labeled vitamin was prepared for use in spiking SRM 2386 samples and controls, and for combination with calibration

solutions to determine response factors. Diluted and mixed solutions were prepared in 0.1 mol/L ammonium acetate in water, adjusted to pH 2.6 with HCl. Calibrant and internal standard solutions were stored in the refrigerator (4 °C) when not in use.

An AT261 Delta Range analytical balance calibrated according to standard procedures, was used for weighing in the preparation of samples, controls, and standards. All solvents used were HPLC grade. All other salts and acids used in sample and mobile phase preparation were reagent grade. All sample and solution preparation were conducted under reduced lighting to minimize potential vitamin degradation.

Quantification was performed in multiple reaction monitoring (MRM) mode using the timetable, transitions, fragmentor voltages, and collision energies listed in Table 11 for the vitamins and their respective internal standards.

Table 11. Multiple Reaction Monitoring Conditions for B Vitamins.

Time (min)	Compound (Abbreviation)	Precursor Ion (m/z)	Product Ion (m/z)	IS Precursor Ion (m/z)	IS Product Ion (m/z)	Fragmentor (V)	Collision Energy (eV)
8.0	Niacin (B_3)	124.0	52.1	128.0	53.0	120	48
			53.0		56.1		32
			78.0		81.0		22
			80.0		84.0		20
11.0	Thiamine (B_1)	266.1	42.1	270.1	42.1	110	52
			81.0		81.1		30
			123.1		123.1		10
14.0	Pyridoxal (B_6)	168.1	41.2	171.1	43.1	110	44
			67.1		70.1		30
			94.1		97.1		22
			150.0		153.1		10
	Pyridoxine (B_6)	170.1	77.0	174.1	81.1	120	38
			80.1		83.1		40
			134.0		138.0		18
			152.1		156.1		10
16.0	Niacinamide (B_3)	123.1	53.1	127.1	56.1	120	30
			78.0		81.0		22
			80.0		84.1		20
17.5	Pantothenic Acid (B_5)	220.0	41.1	224.0	41.1	110	48
			43.1		43.1		30
			72.1		76.0		16
			90.1		94.1		10
22.0	Riboflavin (B_2)	377.2	43.1	383.2	43.1	146	38
			172.1		175.1		38
			198.0		202.1		38
			243.1		249.1		18

An exemplar ID-LC-MS/MS with MRM chromatogram for an extract of SRM 2386 is shown in Fig. 5.

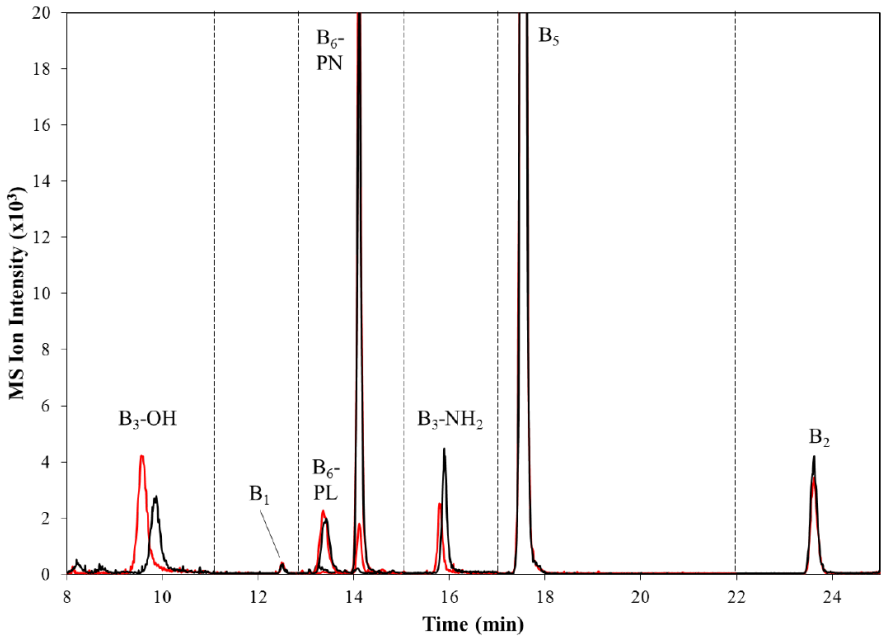


Fig. 5. Exemplar ID-LC-MS/MS Chromatogram for B Vitamins.

Transitions for vitamin ions are shown in black, transitions for isotopically labeled internal standards are shown in red. Only traces for most intense transitions are displayed.

3.1.3.3. Choline and Carnitine

The mass fractions of choline and carnitine in SRM 2386 were determined at NIST using ID-LC-MS/MS. Two 1 g aliquots were taken from each of 10 packets of SRM 2386 and were placed into 50 mL polyethylene centrifuge tubes. The contents of each packet of SRM 2386 were well mixed prior to sampling for extraction by applying external pressure to the open packet to break up pieces and thorough mixing with a metal spatula. Four 1 g aliquots from two different packets of SRM 1849a Infant/Adult Nutritional Powder I (milk-based) were prepared along with the samples for quality assurance. Three blank samples were prepared containing (1) only the internal standard solutions, (2) only the sample of SRM 2386, and (3) only the extraction solvent. These samples were diluted to the approximate volume of the other samples and carried through the extraction process to identify any potential biases that may occur. An aliquot of the mixed internal standard solution (≈1.43 g d_9-choline and ≈0.81 g d_9-carnitine, exact mass known) and a portion (≈30 mL) of extraction solvent (1 mol/L aqueous HCl) were added. Internal standard solutions were prepared from choline chloride (trimethyl-d_9, Lot #PR- 16783) obtained from Cambridge Isotope Laboratories (Andover, MA) and from DL-carnitine HCl (trimethyl-d_9, Lot #Z324P21) obtained from C/D/N Isotopes (Pointe-Claire, QC, Canada). All stock calibrant and internal standard solutions were prepared in HPLC grade water. A stock solution containing each labeled analyte was prepared for use in spiking SRM 2386 samples and controls, and for combination with calibration solutions to determine response factors. Diluted and mixed solutions were prepared in HPLC grade water. Calibrants and internal standard solutions were stored in the refrigerator (4 °C) when not in use. A calibrated Mettler AT261 Delta Range analytical balance was used for weighing in the preparation of samples, controls, and standards.

Samples were shaken and vortexed for 20 s to ensure thorough mixing. The entire 30 mL sample was transferred from the polyethylene tube into a Teflon microwave vessel and hydrolyzed under 1600 W of microwave radiation using a Microwave Assisted Reaction System (MARS) with HP-500 Plus vessels from CEM Corporation (Matthews, NC). Prior to certification, the extraction procedure was optimized for microwave hold temperature, hold time, acid concentration, and need for a post-hydrolysis enzyme treatment. The optimum settings were chosen for certification measurements based on the highest extraction yield for choline. Samples were heated to 150 °C over 15 min and held at 150 °C for 15 min with a maximum pressure of 2.76×10^{-5} Pa (40 psi). Samples were then cooled and transferred back to 50 mL polyethylene centrifuge tubes. The pH of each sample was adjusted to be in the range of (3.8 to 4.0) using a 50 % solution (w/w) of sodium hydroxide and the pH was confirmed visually using pH paper. The samples were centrifuged for 15 min, and the supernatant was filtered through a 0.45 µm RC filter. Approximately (4 to 5) drops of sample extract were combined with ≈1.5 mL of HPLC grade water in an autosampler vial.

Choline bitartrate (Lot #0112016V) and (±)-carnitine hydrochloride (Lot #0001333675) were obtained from Sigma (St. Louis, MO). (NOTE: Choline chloride is very hygroscopic and should not be used as a reference standard for choline measurements.) Purity of these reference standards has been evaluated by NIST using liquid chromatography with ultraviolet absorbance detection, differential scanning calorimetry, quantitative proton nuclear magnetic resonance spectroscopy, and Karl Fisher analysis, and these purities were used to correct the reported mass fractions for each analyte. All solvents used were HPLC grade. Phospholipase D (from *Arachis hypogaea* (peanut), Type II, lyophilized powder, ≥ 60 units/mg protein) and Triton X-100 used in the extraction optimization study for evaluation enzymatic hydrolysis were obtained from Sigma. Hydrochloric acid and sodium hydroxide used in the hydrolysis were reagent grade.

Samples were analyzed by using an Agilent Series 1290 Infinity II LC equipped with an Agilent Series 6410 Triple Quadrupole MS with electrospray ionization in the positive ion mode. The system was composed of a mobile phase degasser, binary pump, autosampler, and mass selective detector. The instrument was tuned prior to certification. A Scherzo SMC18 column (250 × 4.6 mm i.d., 3 µm particles) from Silvertone Sciences (Philadelphia, PA) was used for the analyses without a guard cartridge. The gradient elution program listed in Table 12 was used with a flow rate of 0.5 mL/min. Mobile phase A consisted of 3 mmol/L ammonium formate in water, and mobile phase B was 25 mmol/L ammonium formate in 80:20 water:acetonitrile (volume fraction). A 5.0 µL injection volume was used for all samples. The mass spectrometer was operated at a nebulizer pressure of 15 psig, a drying gas flow of 6 L/min, a drying gas temperature of 300 °C, a capillary voltage of 4000 V, and a dwell time of 100 ms.

Table 12. LC Gradient Profile Used for Analysis of Choline and Carnitine.

Time (min)	% A	% B
0	100	0
11	0	100
27	0	100
27.1	0	0
45	0	0

Quantification was performed in multiple reaction monitoring (MRM) mode using the timetable, transitions, fragmentor voltages, and collision energies listed in Table 13 for choline, carnitine,

and their respective internal standards. An exemplar ID-LC-MS/MS with MRM chromatogram for an extract of SRM 2386 is shown in Fig. 6.

Table 13. Multiple Reaction Monitoring Conditions for Choline and Carnitine.

Time (min)	Compound	Precursor Ion (m/z)	Product Ion (m/z)	IS Precursor Ion (m/z)	IS Product Ion (m/z)	Fragmentor (V)	Collision Energy (eV)
6.0	Carnitine	162.12	60.1 103.0	171.17	69.2 103.0	110	20 16
12.0	Choline	105.12	58.1 60.1	113.17	66.2 69.2	110	32 20

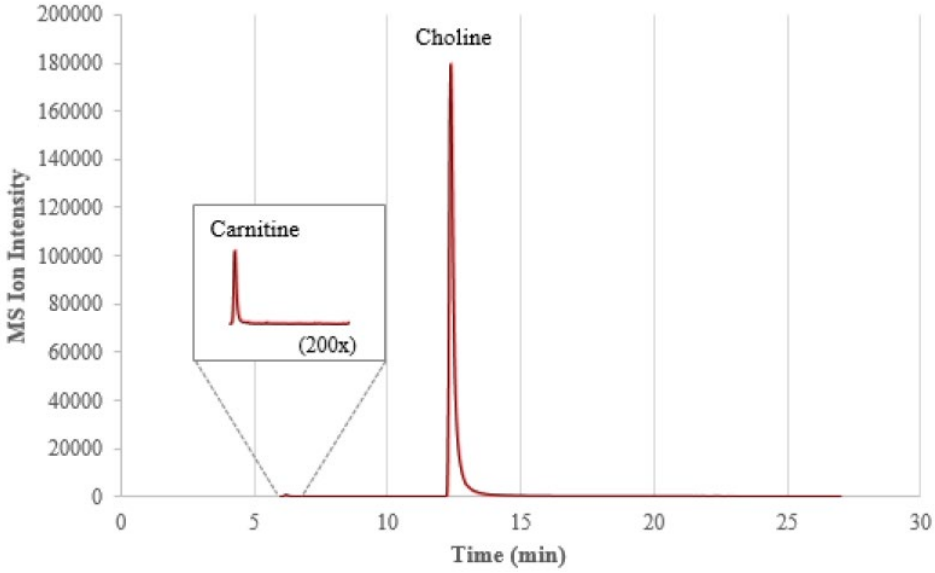


Fig. 6. Exemplar ID-LC-MS/MS Chromatogram for Choline and Carnitine.

Transitions for choline and carnitine ions are shown in black, transitions for isotopically labeled internal standards are shown in red. Transitions for each analyte are nearly identical to the corresponding transitions for isotopically labeled internal standards.

3.1.4. Fatty Acids

The mass fractions of selected fatty acids (as free fatty acids) in SRM 2386 were determined at NIST by a method involving Soxhlet extraction, thermal transesterification with *m*-trifluoromethylphenyl trimethylammonium hydroxide derivatization agent, and gas chromatography with flame ionization detection (GC-FID). For extraction, duplicate 0.5 g portions of SRM 2386 from 12 unopened packets were analyzed. Along with the samples, duplicate 0.5 g portions of SRM 1845a Whole Egg Powder and a set mass (between 0.1 g and 2 g) of calibration solution were prepared for quality control. Samples were prepared and extracted over an eight-day period by Soxhlet extraction. All solid samples (SRM 2386 and SRM 1845a) were stored in 50-mL polypropylene centrifuge tubes at 4 °C between weighings. All aliquots were added to approximately 3 g of hydromatrix (Agilent Technologies, Wilmington, DE) in a Whatman cellulose extraction thimble (GE Healthcare Life Sciences, Marlborough, MA) and 0.5 mL of an internal standard solution was added to the extraction thimble by weight via gas-tight syringe. A whole-method blank control was prepared by adding only the internal standard solution to

hydromatrix. An additional 3 g of hydromatrix was added to all mixtures, followed by ≈ 0.5 mL of HPLC-grade water. Each mixture was stirred with a clean spatula before extraction. The cellulose extraction thimbles were Soxhlet extracted for (20 to 22) h using approximately 250 mL of solvent containing 80 % (volume fraction) hexanes with 30 mg/L (nominal) butylated hydroxytoluene (BHT; Sigma-Aldrich) and 20 % acetone.

Calibration solutions were prepared from SRM 2377 Fatty Acid Methyl Esters in 2,2,4-Trimethylpentane. SRM 2377 was used to prepare six calibration solutions. Three calibration solutions were prepared by direct gravimetric additions of SRM 2377 to the Soxhlet extraction thimbles. For a broad concentration range, a diluted calibration solution was created by a gravimetric dilution (approximately 1:10) of SRM 2377 into 2,2,4-trimethylpentane. The single diluted stock solution was then used to create three additional calibration solutions, which were prepared by direct gravimetric additions to the Soxhlet extraction thimbles. Tridecanonin (C13:0 triglyceride) and methyl octacosanoate (C28:0 fatty acid methyl ester) were obtained from Sigma-Aldrich, Inc. (St Louis, MO) and were gravimetrically added to MTBE and the resulting solution was used as an internal standard (IS) spiking solution.

After extraction, the solutions were transferred from round-bottom flasks to Turbovap vessels and were concentrated under nitrogen to near dryness. Approximately 1 mL of toluene was volumetrically added to all samples and the solutions were mixed thoroughly before being transferred to 4 mL amber glass vials. All extracted samples were stored at 4 °C until further analysis. One day after the final set of extractions, a 1-mL ampoule of Meth-Prep II derivatization solution (W.R. Grace & Co., Columbia, MD) was added to all vials and the vials were shaken for (10 to 15) s and allowed to sit at room temperature for 1 h. For GC-FID analysis, 1 mL of each derivatized solution was added to autosampler vials (high concentration solution) and a 100 µL aliquot of each sample and control solution was volumetrically diluted 1:10 with toluene in additional autosampler vials. No dilution of the calibration solutions was necessary.

An Agilent 7890A GC-FID (Agilent Technologies) was used for analysis with a 0.25 mm × 100 m SP2560 (poly(biscyanopropyl siloxane)) fused-silica capillary column (Supelco, Bellefonte, PA) with 0.25 µm film thickness. The instrumental method was adapted from AOAC Official Method 996.06 Fat (Total, Saturated, and Unsaturated) in Foods [8]. A 1 µL injection was performed with a split ratio of 120:1, split flow of 120 mL/min, and injector temperature of 275 °C. The carrier gas used was helium with a flow rate of 1 mL/min (calculated average linear velocity 18.168 cm/sec). The oven program begins at 100 °C and is held for 4 min after injection. The temperature is then increased by 3 °C/min to 240 °C, which is then held for 20 min. The flame ionization detector settings include a temperature of 250 °C, hydrogen flow of 44 mL/min, air flow of 400 mL/min, and makeup (nitrogen) gas flow of 29 mL/min. The instrumental analysis was controlled using Open Lab ChemStation Rev C.01.04 (Agilent Technologies). Example chromatograms for the SRM 2386 extract and for a calibration solution are displayed in Fig. 7.

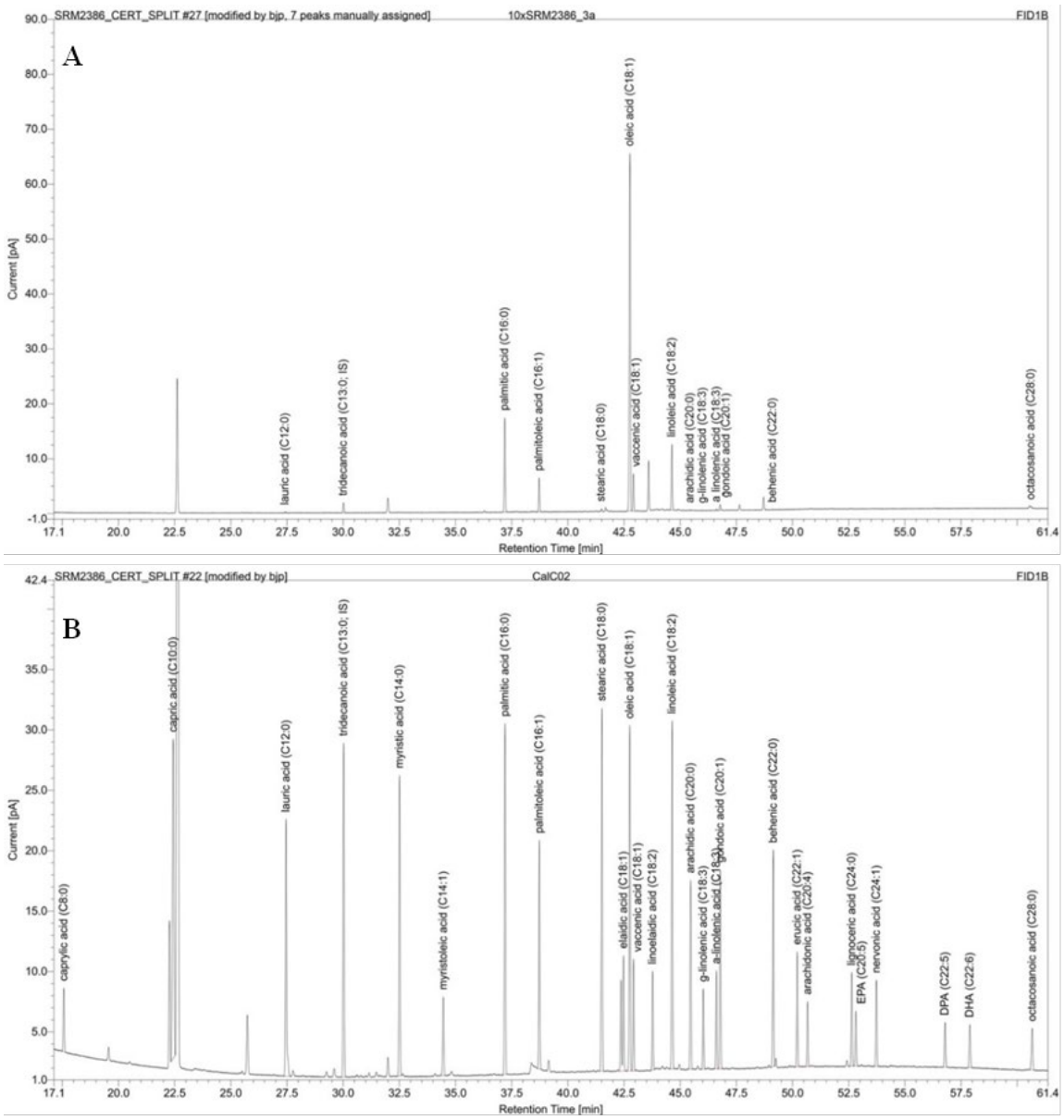


Fig. 7. Exemplar Chromatograms of Fatty Acids.

The chromatogram in panel A is for the SRM 2386 extract; that in panel B is for a calibration solution. Detected compounds are labeled.

Each sample was extracted in duplicate over three separate days and measured over three days. Samples were analyzed in random order, with every (3 to 4) samples or controls bracketed by calibrants. Quantitation was based on linear regression of internal standard-normalized response using tridecanonin (C13:0 triglyceride) as the internal standard. The methyl octacosanoate (C28:0 fatty acid methyl ester) material could not be used since its peak overlapped a peak in the SRM 2386 extracts that was not present in the control or calibration solutions.

Per industry standard, mass fractions of fatty acids are reported as grams of equivalent free fatty acid per 100 grams sample. Table 14 lists the factors for converting from fatty acid methyl ester (directly measured in this study) to free fatty acids, taken from AOAC Official Method 996.06 [8].

Table 14. Factors for Converting Fatty Acid Methyl Ester to Free Fatty Acid Percentages.

Fatty Acid	Factor	Fatty Acid	Factor	Fatty Acid	Factor
α-linolenic acid	0.9520	EPA	0.9957	myristoleic acid	0.9417
arachidic acid	0.9570	erucic acid	0.9602	nervonic acid	0.9632
arachidonic acid	0.9560	γ-linolenic acid	0.9520	oleic acid	0.9527
behenic acid	0.9604	gondoic acid	0.9568	palmitic acid	0.9481
capric acid	0.9247	lauric acid	0.9346	palmitoleic acid	0.9477
caprylic acid	0.9114	lignoceric acid	0.9963	stearic acid	0.9530
DHA	0.9590	linoelaidic acid	0.9524	transvaccenic acid	0.9527
DPA	0.9593	linoleic acid	0.9524	vaccenic acid	0.9527
elaidic acid	0.9527	myristic acid	0.9421		

Hydromatrix and boiling stones used for Soxhlet were first solvent rinsed with hexanes and air-dried. All solvents used for standard preparation, sample preparation, and extraction were HPLC-grade or better.

3.2. GMA FIAC Interlaboratory Studies

The Grocery Manufacturers Association's Food Industry Analytical Chemists (GMA FIAC) Share Group distributed Candidate SRM 2386 in two interlaboratory studies. The quantitative results from these studies are reported here in full. The reported results from each participating organization have been assigned an arbitrary numeric code.

Laboratories participating in the Fall 2015 GMA Study and the Spring 2016 GMA Study reported values for many fatty acids. All participants in either study who reported their methods used GC-FID for separation and detection. Table 15 lists the study(ies) in which participants reported fatty acid results and whether they reported using a hydrolysis and derivatization method.

Table 15. Reported Usage of Hydrolysis and Derivatization Methods for Fatty Acids.

Code[a]	Fall 2015 Study	Spring 2016 Study
2	Yes	*b*
3	No	*b*
4	Yes	No
5	No	Yes
6	No	*b*
7	Yes	Yes
9	*b*	Yes
10	*c*	*b*
12	*b*	Yes
13	*c*	No
16	*b*	Yes
18	Yes	Yes
22	*b*	No
24	Yes	*b*
25	*b*	No
26	No	*b*
27	No	*b*
28	*b*	No
29	*b*	Yes

a Arbitrary participant identification code
b Did not report fatty acid results in study
c Reported fatty acid results but did not provide method information

3.2.1. Fall 2015 GMA Study

In August 2015, GMA FIAC Share Group distributed Candidate SRM 2386 in an interlaboratory study. Participants were asked to measure analytes of a total nutrient panel (proximates, fatty acids, vitamins, minerals, amino acids, phytosterols) in test portions taken from two individual packets of SRM 2386. Results were reported by the participants listed in Table 16.

Table 16. Participants in the Fall 2015 GMA Study.

Company	Location	Country
Mereiux NutriSciences Brasil	Sao Paolo	Brazil
Nestlé Brasil Ltda.	Sao Paolo	Brazil
Silliker Canada Co	Markham, ON	Canada
Covance (Asia) Pte. Ltd.	The Synergy	Singapore
Covance Inc.	Harrogate North Yorkshire	United Kingdom
Con Agra Foods	Omaha, NE	USA
Covance Inc.	Battle Creek, MI	USA
Covance Inc.	Madison, WI	USA
Del Monte Foods	Walnut Creek, CA	USA
Eurofins Scientific	Des Moines, IA	USA
General Mills Inc.	Golden Valley, MN	USA
Hormel Foods	Austin, MN	USA
Krueger Food Labs	Chelmsford, MA	USA
Land O' Lakes	Arden Hills, MN	USA
Nestle Quality Assurance Center	Dublin, OH	USA
NSF International	Ann Arbor, MI	USA
Schwan Food Company	Salina, KS	USA
The JM Smucker Co.	Orrville, OH	USA
The National Food Laboratory	Livermore, CA	USA

3.2.2. Spring 2016 GMA Study

In January 2016, the GMA FIAC Share Group distributed Candidate SRM 2386 in a second interlaboratory study. Participants were asked to measure fatty acids in test portions taken from two individual packets of SRM 2386. Results were reported by the participants listed in Table 17.

Table 17. Participants in the Spring 2016 GMA Study.

Company	Location	Country
Covance (Asia) Pte. Ltd.	The Synergy	Singapore
Covance Inc.	Harrogate North Yorkshire	United Kingdom
Covance Inc.	Madison, WI	USA
Del Monte Foods	Walnut Creek, CA	USA
Eurofins Central Analytical Laboratories	Metairie, LA	USA
Eurofins Scientific	Des Moines, IA	USA
Hormel Foods	Austin, MN	USA
Krueger Food Labs	Chelmsford, MA	USA
Land O' Lakes	Arden Hills, MN	USA
Nestle Quality Assurance Center	Dublin, OH	USA
Schwan Food Company	Salina, KS	USA

3.3. Statistical Approaches for Value Assignment

Statistical analysis was provided by the NIST Statistical Engineering Division (SED). Where more than one method was available for a measured analyte, the estimated value is a weighted mean of the method estimates available for this analyte. The weighted mean used is the Dersimonian-Laird estimate [9], the uncertainty of which is estimated using a bootstrap procedure based on a Gaussian random effects model for the between-method effects [10,11,12,13]. If only one method is available for an analyte, then that method estimate is the analyte estimate.

The uncertainties of all values except ash incorporate a relative uncertainty of 0.9 % due to moisture correction. In addition, values for some analytes incorporate an uncertainty component due to possible inhomogeneity. To address issues of possible inhomogeneity of the SRM, both analyses of variance with 5 % significance level and graphical analyses were run on NIST data where box information was available. For some measurands, the uncertainty incorporates a component for possible inhomogeneity based on the standard deviation as described in the individual results and discussion sections below.

Very marked differences are often observed between the results from the different laboratories participating in an interlaboratory study. For each interlaboratory study, the method estimate for that study for each analyte is the weighted median of the individual laboratory means for that analyte, where the weights are based on a Laplace random effects model [20]. For this SRM, the weighted median is equal to or very close to the unweighted median of laboratory means for most analytes. The uncertainty of the weighted median is estimated using a bootstrap procedure based on a Laplace random effects model for the between-laboratory and within-laboratory effects [10-14]. The weights and uncertainty of the weighted median are based in part on the uncertainties of the individual laboratory means. Here, the uncertainty assigned to each laboratory mean is the standard deviation of that mean. If a laboratory reported only one measurement for an analyte, then for the purposes of the computation that value is assigned an uncertainty equal to the maximum of the uncertainties reported by the other laboratories for that analyte.

A number of extreme outlier measurements from the interlaboratory studies were flagged by the analysts and omitted from the calculations. The deviance of these measurements from the others exceeded the usual variation, often differing by an order of magnitude or more. Other measurements may be questionable but could not be determined to be unrepresentative extreme outliers because of the sparseness and variation of the rest of the data.

Some of the estimates and uncertainties in this report are purposely listed with more significant digits than is scientifically warranted. The relevant technical experts trim any estimates and uncertainties to the number of significant digits that are scientifically warranted prior to inclusion on the Certificate of Analysis as either certified or non-certified values [15].

4. Results and Discussion

4.1. Moisture

4.1.1. NIST Results

The change in mass as a function of time in the desiccator is displayed in Fig. 8, demonstrating that the rate of change decreases after 14 days. Based on this data, a minimum of a 14 days should be used for determination of moisture by desiccator drying. Moisture results from the three NIST methods are tabulated in Table 18, including summary statistics where *N* = number of values and SD = standard deviation of values.

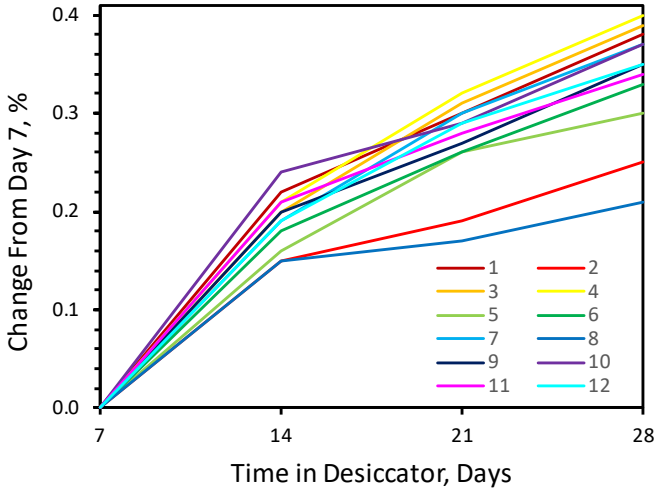


Fig. 8. Change in Percent Moisture of SRM 2386 as a Function of Time in Desiccator.

Table 18. NIST Results for Moisture, %.

	Freeze Drying[a]			Desiccator[b]				FAIR[c]	Combined[d]	
Box	**A**	**B**	**Mean[e]**	**7 Day**	**14 Day**	**21 Day**	**28 Day**		**Mean**	**SD**
1				5.24	5.46	5.54	5.62	5.91	5.69	0.32
2	3.78	3.80	3.79	3.57	3.72	3.76	3.82	4.24	3.92	0.28
3				5.22	5.42	5.53	5.61	5.87	5.65	0.32
4	5.37	5.46	5.42	5.2	5.41	5.52	5.6	5.98	5.60	0.33
5				3.61	3.77	3.87	3.91	4.31	4.04	0.38
6	3.83	4.04	3.93	3.22	3.40	3.48	3.55	4.02	3.78	0.33
7				5.11	5.30	5.41	5.48	5.77	5.54	0.34
8	3.80	3.97	3.88	3.36	3.51	3.53	3.57	4.17	3.85	0.33
9				3.22	3.42	3.49	3.57	3.97	3.70	0.38
10	5.46	5.36	5.41	5.15	5.39	5.44	5.52	5.87	5.56	0.27
11				5.18	5.39	5.46	5.52	5.84	5.61	0.31
12	5.37	5.38	5.37	5.28	5.47	5.57	5.63	5.91	5.58	0.28
***N*:**	6	6	6	12	12	12	12	12	12	
Mean:	4.60	4.67	4.63	4.45	4.64	4.72	4.78	5.15	4.88	
SD:	0.88	0.81	0.84	0.94	0.95	0.97	0.98	0.90	0.90	

a Freeze drying for 7 days @ -40° C
b Desiccator drying over magnesium perchlorate
c Forced air drying for 1 h @ 80° C
d Combination 14 day desiccator drying, forced air drying, and mean of freeze-drying replicates
e Mean of two replicates from a single package

Moisture results from the three NIST methods are visualized in Fig. 9 and Fig. 10. The circles in the graphics represent the results from desiccator drying, the red triangles represent the results from forced air drying, and the green squares represent the results from freeze drying. The mean moisture is indicated by the solid line, and the dashed lines bound the interval Mean ± 2×SD. While all drying methods produce similar values, the within-method variability is greater than expected based on experience with other food materials. As a result, the uncertainty on assigned values will be sufficiently large to encompass the within-packet moisture variability.

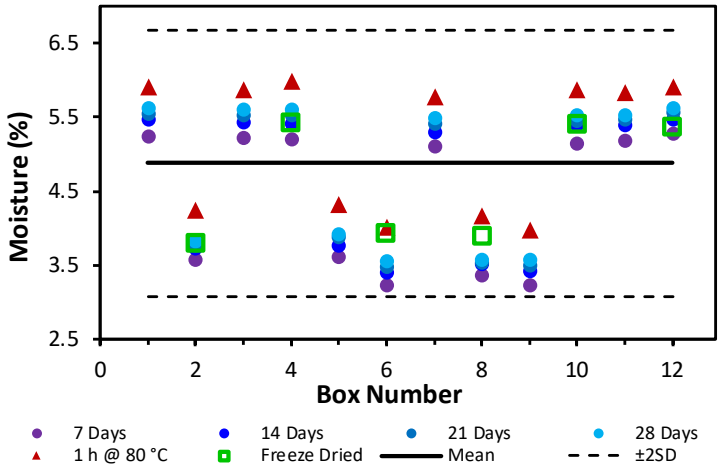


Fig. 9. Percent Moisture of SRM 2386 as a Function of Box Number.

The results of moisture determination as a function of initial sample weight are displayed in Fig. 10. The green squares represent the results from day 14 desiccator drying, the red triangles represent the results from forced air oven drying, and the circles represent the results from freeze drying. The mean moisture is indicated by the solid line, and the dashed lines bound the interval Mean ± 2×SD. A correlation between the percent moisture and sample weight may indicate poor performance of a drying method for specific sample. However, over the range used, the weight of the SRM 2386 sample has little to no effect on the percent moisture determined.

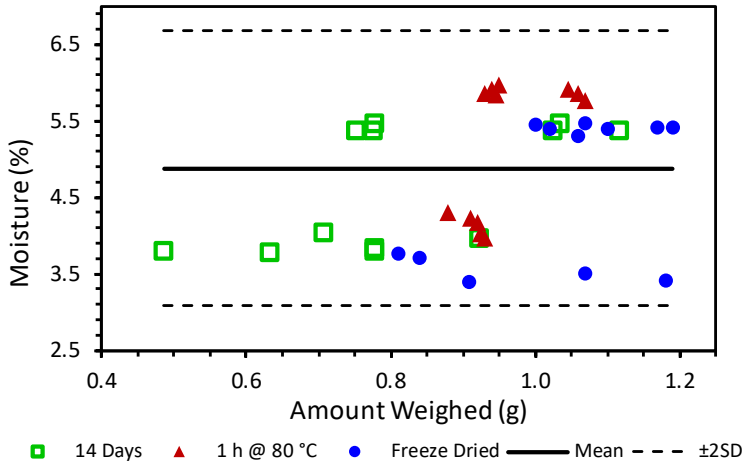


Fig. 10. Percent Moisture of SRM 2386 as a Function of Sample Weight.

4.1.2. GMA Results

Laboratories participating in the Fall 2015 GMA Study reported moisture results as percent total solids, which was converted to percent moisture by subtraction of percent total solids from 100 %. Table 19 lists the results and methods reported for moisture in the Fall 2015 GMA Study.

Table 19. Fall 2015 GMA Study Results for Moisture, %.

	Packet		Summary[a]		
Lab	**A**	**B**	**Mean**	**SD**	**Method**
2	6.25	6.18	6.22	0.05	Vacuum oven
3	5.13	4.46	4.79	0.47	Forced-air oven
4	3.97	3.77	3.87	0.14	Vacuum oven
5	7.56	7.45	7.51	0.08	Forced-air oven
7	5.50	5.50	5.50	0.00	Vacuum oven
10	4.80		4.80		*not reported*
11	4.50		4.50		*not reported*
13	5.62		5.62		*not reported*
16	3.20	3.20	3.20	0.00	Vacuum oven
18	6.88		6.88		Vacuum oven
24	6.76	6.95	6.86	0.13	Vacuum oven
25	7.46	7.48	7.47	0.01	Forced-air oven
26	4.48	4.53	4.51	0.04	Vacuum oven
27	3.39	3.41	3.40	0.01	Vacuum oven
		N	14	10	
		Mean, Pooled SD	5.37	0.17	
		SD	1.45		

a Reported as percent total solids, converted to percent moisture by subtraction from 100 %.

4.1.3. Value Assignment and Dry-Mass Conversions

The assigned value for moisture content was determined using three NIST techniques and data from the Fall 2015 GMA Study. The four method estimates were combined using the Dersimonian-Laird weighted mean [9] to estimate a dry-mass proportion of (0.9516 ± 0.0178) gram dry-mass per gram as-received mass, which was used to convert data from an as-received to a dry-mass basis. The uncertainty shown on this value is an approximate 95 % level of confidence expanded uncertainty, U_{95}. This uncertainty incorporates a component for possible inhomogeneity based on the standard deviation of box means, as a division into two groups was apparent in all the NIST moisture estimation methods.

The moisture correction is achieved by multiplying the as-received measurements by a conversion factor equal to the inverse of the dry-mass proportion. A relative uncertainty component for the conversion factor (0.9 %) obtained from the moisture measurements is incorporated in the uncertainties of the estimated analyte values (except ash), reported on a dry-mass basis.

4.2. Elements

All elemental results determined at NIST were determined on a dry-mass basis. Results provided by the Fall 2015 GMA Study were provided on an as-received basis but converted to a dry-mass basis for the Certificate of Analysis (COA).

4.2.1. Boron

The NIST TNPGAA results for boron (B), on a dry-mass basis, are summarized in Table 20, along with the moisture results collected during these experiments. The table also provides several summary values: N = number of values, Mean = mean of values, SD = standard deviation of values, and Pooled SD = square-root of the sum of the squared within-box standard deviations. The quality assurance measurement results were concordant with the certified value delivered by the control material, SRM 1573a.

Table 20. Summary of Results for Boron (B), mg/kg.

	Boron (mg/kg)				Moisture
Box	**A**	**B**	**Mean**	**SD**	**%**
2	175	177	176.0	1.4	3.78
4	165	168	166.5	2.1	5.37
6	179	179	179.0	0.0	3.83
8	178	176	177.0	1.4	3.80
10	165	164	164.5	0.7	5.46
12	171	165	168.0	4.2	5.37
		***N*:**	6		6
		Mean, Pooled SD:	171.8	2.1	4.60
		SD:	6.2		0.88

The NIST boron results as a function of the sample box number are displayed in Fig. 11. The blue circles in the figure represent the results for the first replicate and the red squares the second replicate. The mean mass fraction is indicated by the solid line, and the dashed lines bound the interval Mean ± 2×SD.

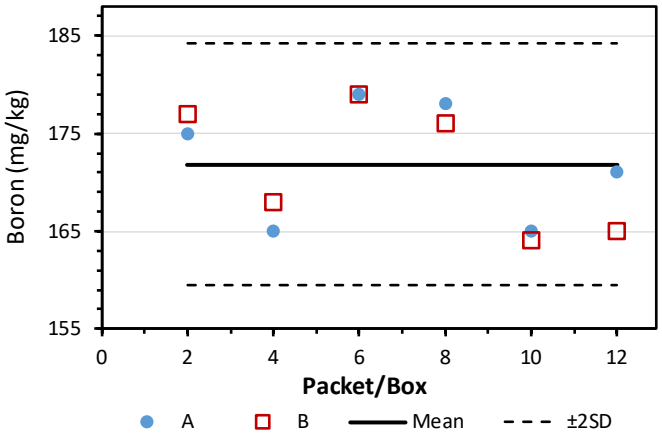


Fig. 11. Boron (B) Dry-Mass Basis Mass Fraction as a Function of Box Number.

Table 21 details the uncertainty components and calculations. The most difficult uncertainty to estimate in the analysis of hydrogenous materials by TNPGAA is the effect of hydrogen (H) scattering on element sensitivities. Element sensitivities in thin hydrogenous targets can be enhanced by up to 10 % compared to those measured in a thicker target [16]. Filter paper standards have been used in the past to measure boron in agricultural materials due to the similarity in H content. For example, both the control material, SRM 1573a Tomato Leaves, and the filter paper standards used in this investigation yielded hydrogen count rates of about (95 ± 10) cps. However, due to high moisture content, the H count rates of the SRM 2386 samples averaged around (140 ± 5) cps. To estimate the effects of neutron scattering, 750 mg boron standard pellets prepared from two mixtures of TRIS and boron spectrometric solution were measured. The H count rate of these pellets fell into the range of (160 to 170) cps. The average boron sensitivity of these pellets was found to differ from the average boron sensitivity measured in filter paper standards by only about 1 %. To be conservative, a 1 % uncertainty from H scattering effects was thus added to the total uncertainty.

Table 21. Uncertainty Budget for TNPGAA Analysis of Boron (B).

Component	Description	u_{rel}	Units
Sample measurement	$s/\sqrt{n}$, where s is standard deviation of the sample data replication and n is the number of samples analyzed.	1.03	%
Standard replication	$s/\sqrt{n}$, where s is standard deviation of the standard data replication and n is the number of standards analyzed.	0.71	%
Weighing of samples	Uncertainty in weighing/average weight of sample: 100*0.01 mg /750 mg	0.001	%
Sample positioning Flux variation	Estimated from the standard deviation of repeated measurements of a titanium foil.	0.5	%
H scattering effects	Estimated as approximately 1 % from the difference between boron standards of different H count rate and geometry.	1	%
Standard quantity	Uncertainty in spectrometric standard mass fraction (given on the certificate)/2, assuming the certificate uncertainty is an expanded uncertainty with coverage factor of 2.	0.1	%
Delivery of standard	Standard solution was determined by mass, and weighed to ±0.1 mg, so % uncertainty in solution mass for 100 mg of solution is 100*0.01/100.	0.01	%
Blank correction	Estimated as 10 % of the blank correction.	0.06	%
u_{rel}	Combined relative uncertainty	1.68	%
k	Student's t 95 % coverage factor for 5 degrees of freedom	2.57	%
U_{95rel}	$k*u_{rel}$, relative expanded uncertainty at a 95 % level of confidence	4.32	%
U_{95}	171.8 mg/kg * U_{95rel} / 100	7.42	mg/kg

4.2.2. Cadmium

The NIST ID ICP-MS results for cadmium (Cd) on an as-received and dry-mass basis are summarized in Table 22, along with the moisture results collected during these experiments. The table also provides several summary values: N = number of values, Mean = mean of values, SD = standard deviation of values, and Pooled SD = square-root of the sum of the squared within-box standard deviations.

Table 22. Summary of Results for Cadmium (Cd), mg/kg.

	As-Received Basis (mg/kg)				Dry-Mass Basis (mg/kg)				Moisture
Box	**A**	**B**	**Mean**	**SD**	**A**	**B**	**Mean**	**SD**	**(%)**
1	0.13221	0.13191	0.13206	0.00021	0.14045	0.14014	0.14030	0.00022	5.87
2	0.14419	0.14390	0.14405	0.00021	0.15075	0.15045	0.15060	0.00021	4.35
4	0.13020	0.12996	0.13008	0.00017	0.13865	0.13840	0.13853	0.00018	6.10
6	0.13279	0.13313	0.13296	0.00024	0.14107	0.14143	0.14125	0.00025	5.87
7	0.13214	0.13262	0.13238	0.00034	0.14052	0.14103	0.14078	0.00036	5.96
12	0.13223	0.13215	0.13219	0.00006	0.14062	0.14054	0.14058	0.00006	5.97
		***N*:**	6			N	6		6
		Mean, Pooled SD:	0.13395	0.00022		Mean, Pooled SD	0.14200	0.00023	5.69
		SD:	0.00504			SD	0.00431		0.66

The NIST cadmium results as a function of the sample box number are displayed in Fig. 12. The blue circles in the figure represent the results for the first replicate and the red squares the second replicate. The mean mass fraction is indicated by the solid line, and the dashed lines bound the interval Mean ± 2×SD.

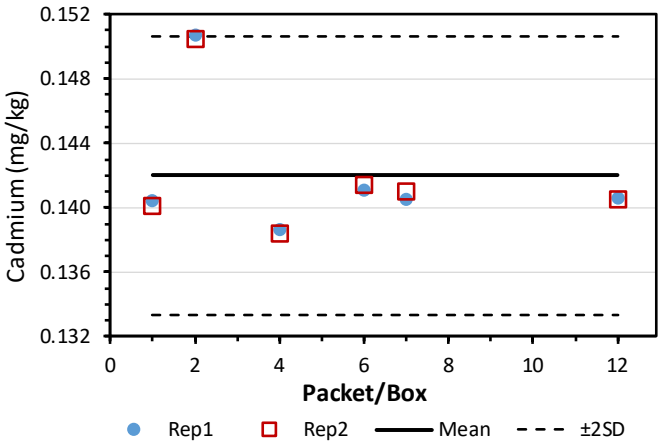


Fig. 12. Cadmium (Cd) Dry-Mass Mass Fraction as a Function of Box Number.

Table 23 details the uncertainty components and calculations. ANOVA indicates significant between-bottle differences at a $p = 0.05$ significance level for results reported on both as-received and dry-mass bases ($p = 9.4\times10^{-9}$ and 3.4×10^{-8}); thus the results for the A and B sub-samples were averaged and six observations were included in the calculation of the mean and standard deviation.

After applying the correction for moisture, the %RSD improves to 3.04 indicating that differences in the moisture content between packets explains some of the observed variability, but not all. The observed variability between the packets/boxes is greater than that expected for the ID ICP-MS measurement process for Cd at this concentration level (estimated to be 1% relative U, approximate level of confidence of 95%), and indicates material heterogeneity for Cd. The quality

assurance measurement results were concordant with the certified values delivered by the control materials, SRM 2384 and SRM 1577c.

Table 23. Uncertainty Budget for ID ICP-MS Analysis of Cadmium (Cd).

Component	x_i	$u(x_i)$	Units	c_i	$c_i u(x_i)$	v_i	RelCon (%)
Rep	1.000	0.033	1	1.41E-01	4.62E-03	5	96.50
blank	0.00000022	0.00000009	µmol	-2.47E+02	-2.14E-05	2	<0.01
m_x	0.48448	0.00015	g	-2.91E-01	-4.36E-05	∞	0.01
DMCF	0.9404	0.0028	1	-1.49E-01	-4.20E-04	∞	0.80
m_y	0.66971	0.00015	g	2.10E-01	3.15E-05	∞	<0.01
c_y	0.0005435	0.0000011	µmol/g	2.59E+02	2.97E-04	∞	0.40
$(Aby)_y$	0.96497	0.00050	1	1.48E-01	7.41E-05	∞	0.02
$(Abx)_y$	0.00588	0.00025	1	-3.79E-01	-9.47E-05	∞	0.04
$(Abx)_x$	0.28730	0.00070	1	-5.92E-01	-4.14E-04	∞	0.78
$(Aby)_x$	0.12800	0.00020	1	2.33E-01	4.65E-05	∞	0.01
k_b	1.0000	0.0015	1	-1.73E-01	-2.61E-04	∞	0.30
$(Ry/x)_b$	2.5539	0.0074	1	-6.74E-02	-5.02E-04	∞	1.10
AtWt	112.4110	0.0040	µg/µmol	1.25E-03	5.01E-06	∞	<0.01
				u(total):	0.0047		
				k_{95}:	2.57		
				U_{95}(total):	0.0121		

x_i	Typical value of the component
$u(x_i)$	Standard uncertainty of x_i
c_i	Sensitivity coefficient for the component in the measurement model
v_i	Effective degrees of freedom for component
RelCon	$100(c_i u(x_i)/u(\text{total}))^2$, relative contribution of the component to the total standard uncertainty
Rep	Sample repeatability, using a prediction interval estimated as the (standard deviation of the mean of the dry mass basis mass fraction results)($\sqrt{(6+1)}$).
blank	Procedure blank correction: estimated as the (standard deviation of the mean of procedure blank determinations.)
m_x	Sample mass: (±0.00030 g tolerance of the 5-place balance)/2.
DMCF	Moisture correction (100 - Moisture %)/100: (absolute value of the difference between the mean of the current result and that in Section 4.1.1)/2
m_y	mass of added spike solution: (±0.00030 g tolerance of the 5-place balance)/2.
c_y	Spike solution amount content calibrated by reverse ID comprised of the combined content ($U_{95,\text{rel}}$ between (0.15 & 0.2) %, the half width of the difference between the mean results of two primary standard solutions and the relative standard deviation of the mean of 5 spike calibration samples.
$(Aby)_y$	Abundance of spike isotope in the spike solution: (Oak Ridge certified U_{95})/2.
$(Abx)_y$	Abundance of sample isotope in the spike solution: (Oak Ridge certified U_{95})/2.
$(Abx)_x$	Abundance of sample isotope in the sample: (uncertainty reported in Reference [17])/2.
$(Aby)_x$	Abundance of spike isotope in the sample: (uncertainty reported in reference [17])/2.
k_b	Mass bias correction factor: (u of replicate mass bias uncertainty combined with an experienced-based $U_{95,\text{rel}}$ of ± 1 %)/2.
$(Ry/x)_b$	Ratio of intensity at spike mass to intensity at sample mass in an unknown spiked with enriched isotope: (± 0.2 % for dead time correction combined with the average % difference for replicate ICP-MS measurements)/2.
AtWt	atomic weight. (u reported in reference [17])/2.
u(total)	$\sum(c_i u(x_i))^2$, the combined standard uncertainty for the measurement
k_{95}	Student's two-tailed 95 % level of confidence expansion factor for 5 degrees of freedom
U_{95}(total)	$k_{95} \times u$(total), 95 % level of confidence expanded uncertainty for the measurement

4.2.3. Calcium, Copper, Iron, Potassium, Magnesium, Manganese, Sodium, Phosphorus, and Zinc

Results for Ca, Cu, Fe, K, Mg, Mn, Na, P, and Zn at NIST by using ICP-OES were reported on a dry-mass basis and are summarized in the following sections. All results have been corrected for the mean blank values from their corresponding runs by subtracting the mean total micrograms found in the blanks from the total micrograms found in each individual sample. In all cases, the quality assurance measurement results were concordant with the certified values delivered by the control materials, SRM 1845a Whole Egg Powder and SRM 1577c Bovine Liver.

4.2.3.1. Calcium

The NIST ICP-OES results for calcium (Ca) and all Ca values reported by the participants in the Fall 2015 GMA Study are summarized in Table 24. The table also provides several summary values: *N* = number of values, Mean = mean of values, SD = standard deviation of values, and Pooled SD = square-root of the sum of the squared within-box or within-participant standard deviations.

Table 24. Summary of Results for Calcium (Ca), mg/kg.

NIST ICP-OES

Box	A	B	Mean	SD
1		749.8	749.8	
2	777.3	766.3	771.8	7.8
3	718.9	747.3	733.1	20.0
4	758.3	736.6	747.4	15.3
5	749.4	736.8	743.1	8.9
6	731.2	741.0	736.1	6.9
7	763.2	749.7	756.4	9.5
8		735.3	735.3	
9	751.9	749.5	750.7	1.7
10	753.4	752.3	752.9	0.7
		***N*:**	10	
		Mean, Pooled SD:	747.7	10.7
		SD:	11.6	

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Lab	A	B	Mean	SD	Method
1	786	785	785.5	0.7	*not reported*
2	744	730	737.0	9.9	ICP-OES
3	739	758	748.5	13.4	ICP-OES
4	882	965	923.5	58.7	AAS
5	793	762	777.5	21.9	ICP-MS
6	748	800	774.0	36.8	ICP-OES
7	667	609	638.0	41.0	ICP-OES
10	713		713.0		*not reported*
11	739		739.0		*not reported*
13	758		758.0		*not reported*
16	759	759	759.0	0.0	AAS
18	770		770.0		ICP-OES
24	804	781	792.5	16.3	AAS
26	777	762	769.5	10.6	ICP-OES
27	785	774	779.5	7.8	AAS
		***N*:**	15		
		Mean, Pooled SD:	764.3	26.4	
		SD:	58.2		

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The NIST calcium results as a function of the sample box number are displayed in Fig. 13. The blue circles in the figure represent the results for the first replicate and the red squares the second replicate. The mean mass fraction is indicated by the solid line, and the dashed lines bound the interval Mean ± 2×SD.

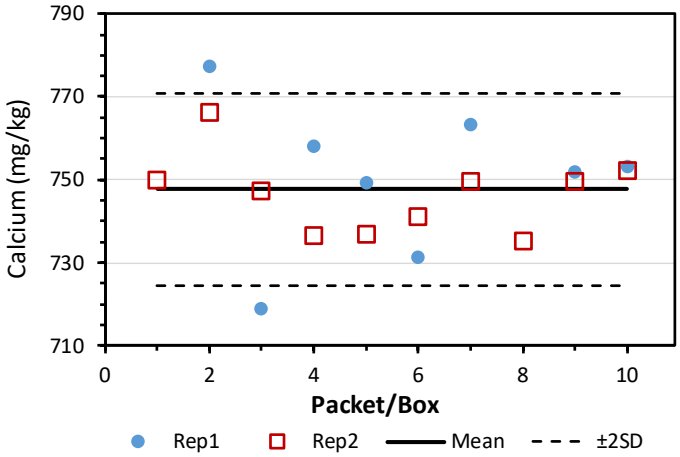

Fig. 13. Calcium (Ca) Mass Fraction as a Function of Box Number.

4.2.3.2. Copper

The NIST ICP-OES results for copper (Cu) and all Cu values reported by the participants in the Fall 2015 GMA Study are summarized in Table 25. The table also provides several summary values: *N* = number of values, Mean = mean of values, SD = standard deviation of values, and Pooled SD = square-root of the sum of the squared within-box or within-participant standard deviations.

Table 25. Summary of Results for Copper (Cu), mg/kg.

NIST ICP-OES

Box	A	B	Mean	SD
1	15.63	15.45	15.54	0.13
2	16.59	16.47	16.53	0.08
3	16.72	16.56	16.64	0.11
4	15.60	15.63	15.61	0.02
5	16.82	15.25	16.03	1.11
6	16.40	17.43	16.92	0.73
7	15.85	16.09	15.97	0.17
8	15.94	15.46	15.70	0.35
9	17.06	16.84	16.95	0.16
10	16.21	16.16	16.18	0.04
		***N*:**	10	
		Mean, Pooled SD:	16.21	0.44
		SD:	0.53	

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Lab	A	B	Mean	SD	Method
1	15.2	14.6	14.90	0.42	*not reported*
2	16.0	16.2	16.10	0.14	ICP-OES
4	18.9	18.2	18.55	0.49	AAS
5	15.9	15.9	15.90	0.00	ICP-MS
6	15.2	15.8	15.50	0.42	ICP-OES
7	7.8	5.9	6.85	1.34	ICP-MS
10	16.4		16.40		*not reported*
11	18.0		18.00		*not reported*
13	17.5		17.50		*not reported*
16	15.0	15.0	15.00	0.00	AAS
18	15.0		15.00		ICP-OES
26	14.9	15.1	15.00	0.14	ICP-OES
27	17.7	17.7	17.70	0.00	AAS
		***N*:**	13		
		Mean, Pooled SD:	15.57	0.52	
		SD:	2.91		

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The NIST copper results as a function of the sample box number are displayed in Fig. 14. The blue circles in the figure represent the results for the first replicate and the red squares the second

replicate. The mean mass fraction is indicated by the solid line, and the dashed lines bound the interval Mean ± 2×SD.

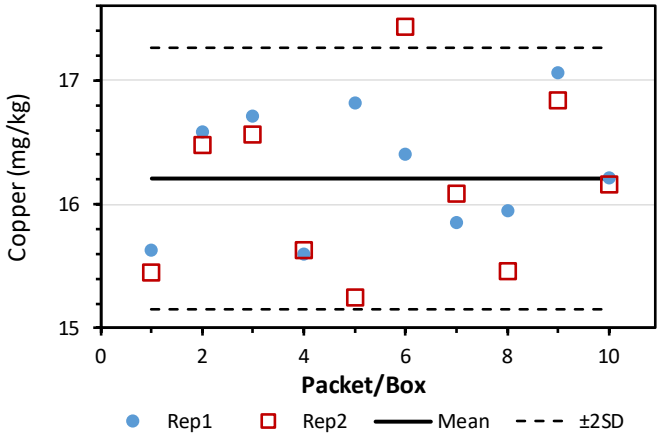

Fig. 14. Copper (Cu) Mass Fraction as a Function of Box Number.

4.2.3.3. Iron

The NIST ICP-OES results for iron (Fe) and all Fe values reported by the participants in the Fall 2015 GMA Study are summarized in Table 26. The table also provides several summary values: *N* = number of values, Mean = mean of values, SD = standard deviation of values, and Pooled SD = square-root of the sum of the squared within-box or within-participant standard deviations. Statistical outliers identified using Dixon's Q-test were excluded from further calculations.

Table 26. Summary of Results for Iron (Fe), mg/kg.

NIST ICP-OES

Box	A	B	Mean	SD
1	32.16	32.67	32.41	0.36
2	33.64		33.64	
3		34.36	34.36	
4	32.67	31.22	31.95	1.03
5	32.14		32.14	
6	33.10	35.18	34.14	1.47
7	33.23	36.56	34.89	2.35
8	33.46	32.79	33.13	0.47
9	35.74	34.42	35.08	0.93
10		32.84	32.84	
		***N*:**	10	
		Mean, Pooled SD:	33.46	1.29
		SD:	1.14	

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Lab	A	B	Mean	SD	Method
1	34.1	37.4	35.75	2.33	*not reported*
2	32.6	33.3	32.95	0.49	ICP-OES
3	31.9	33.7	32.80	1.27	ICP-OES
4	40.2	37.3	38.75	2.05	AAS
5	32.5	32.4	32.45	0.07	ICP-MS
6	33.6	35.0	34.30	0.99	ICP-OES
7	57.0	28.0	42.50	20.51	*not reported*
10	33.2		33.20		*not reported*
11		39.1	39.10		*not reported*
13	33.8		33.80		*not reported*
16	23.0	23.0	23.00	0.00	AAS
18	39.2		39.20		ICP-OES
24	41.0	38.0	39.50	2.12	AAS
26	28.8	28.3	28.55	0.35	ICP-OES
27	33.3	33.0	33.15	0.21	AAS
		***N*:**	15		
		Mean, Pooled SD:	34.60	6.31	
		SD:	4.88		

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The NIST iron results as a function of the sample box number are displayed in Fig. 15. The blue circles in the figure represent the results for the first replicate and the red squares the second replicate. The mean mass fraction is indicated by the solid line, and the dashed lines bound the interval Mean ± 2×SD.

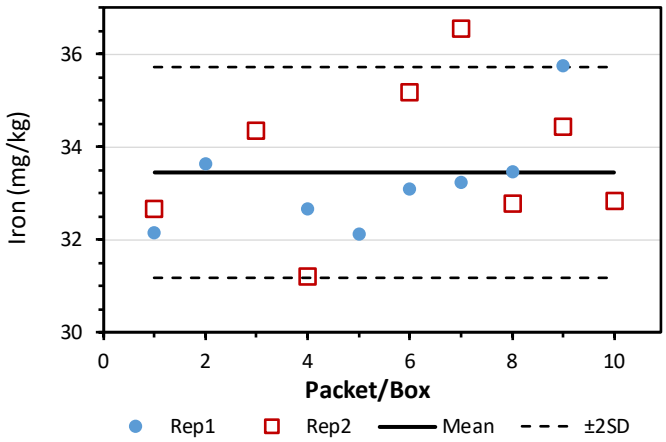

Fig. 15. Iron (Fe) Mass Fraction as a Function of Box Number.

4.2.3.4. Potassium

The NIST ICP-OES results for potassium (K) and all K values reported by participants in the Fall 2015 GMA Study are summarized in Table 27. The table also provides several summary values: *N* = number of values, Mean = mean of values, SD = standard deviation of values, and Pooled SD = square-root of the sum of squared within-box or within-participant standard deviations.

Table 27. Summary of Results for Potassium (K), mg/kg.

NIST ICP-OES

Box	A	B	Mean	SD
1	32031	37868	34950	4128
2	42509	39670	41089	2008
3	41743	41317	41530	0301
4	35472	37562	36517	1478
5	40475	35706	38091	3372
6	41249	38983	40116	1603
7	39835	39211	39523	0442
8	38511	37947	38229	0399
9	42543	38184	40364	3082
10	38248	38798	38523	0389
		***N*:**	10	
		Mean, Pooled SD:	38893	2175
		SD:	2062	

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Lab	A	B	Mean	SD	Method
1	37000	37000	37000	0	*not reported*
2	32200	32400	32300	141	ICP-OES
3	34310	35430	34870	792	ICP-OES
4	34000	34400	34200	283	AAS
5	33100	33300	33200	141	ICP-MS
6	36800	39570	38185	1959	ICP-OES
7	31100	30700	30900	283	ICP-MS
10	32300		32300		*not reported*
11	31500		31500		*not reported*
13	33700		33700		*not reported*
16	32332	32252	32292	57	AAS
18	32100		32100		ICP-OES
24	31600	31400	31500	141	AAS
26	28700	28800	28750	71	ICP-OES
27	35499	35409	35454	64	AAS
		***N*:**	15		
		Mean, Pooled SD:	33217	653	
		SD:	2434		

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The NIST potassium results as a function of the sample box number are displayed in Fig. 16. The blue circles in the figure represent the results for the first replicate and the red squares the second replicate. The mean mass fraction is indicated by the solid line, and the dashed lines bound the interval Mean ± 2×SD.

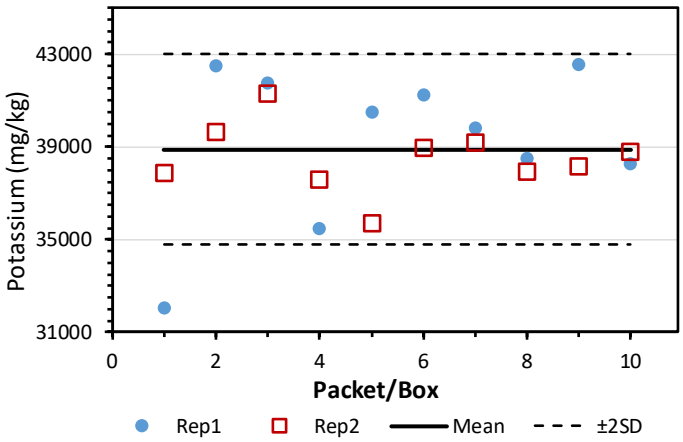

Fig. 16. Potassium (K) Mass Fraction as a Function of Box Number.

4.2.3.5. Magnesium

The NIST ICP-OES results for magnesium (Mg) and all Mg values reported by participants in the Fall 2015 GMA Study are summarized in Table 28. The table also provides several summary values: *N* = number of values, Mean = mean of values, SD = standard deviation of values, and Pooled SD = square-root of the sum of squared within-box or within-participant standard deviations. Statistical outliers identified using Dixon's Q-test were excluded from further calculations.

Table 28. Summary of Results for Magnesium (Mg), mg/kg.

NIST ICP-OES

Box	A	B	Mean	SD
1	1739	1887	1813	0105
2	1950	1946	1948	0003
3	2042	1987	2015	0038
4	1918	1874	1896	0031
5	1904		1904	
6	1917	1890	1903	0019
7	1829	1914	1871	0060
8	1794	2404	2099	0432
9	2019	2769	2394	0531
10	2497	2354	2426	0101
		***N*:**	10	
		Mean, Pooled SD:	2027	235
		SD:	217	

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Lab	A	B	Mean	SD	Method
1	2060	2060	2060	0	*not reported*
2	1970	1920	1945	35	ICP-OES
4	1790	1760	1775	21	AAS
5	1910	1910	1910	0	ICP-MS
6	2060	2150	2105	64	ICP-OES
7	1600	1460	1530	99	ICP-OES
10	1730		1730		*not reported*
11	1690		1690		*not reported*
13	1850		1850		*not reported*
16	2131	2150	2141	13	AAS
18	1850		1850		ICP-OES
24	1900	1900	1900	0	AAS
26	2030	2030	2030	0	ICP-OES
27	1713	1678	1696	25	AAS
		***N*:**	14		
		Mean, Pooled SD:	1872	40	
		SD:	177		

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The NIST magnesium results as a function of the sample box number are displayed in Fig. 17. The blue circles in the figure represent the results for the first replicate and the red squares the second replicate. The mean mass fraction is indicated by the solid line, and the dashed lines bound the interval Mean ± 2×SD.

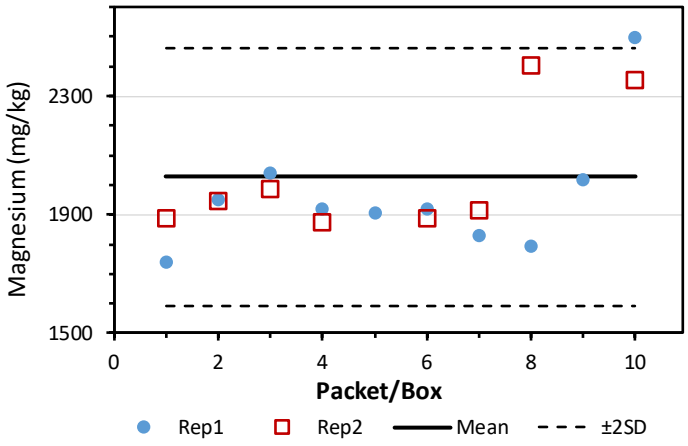

Fig. 17. Magnesium (Mg) Mass Fraction as a Function of Box Number.

4.2.3.6. Manganese

The NIST ICP-OES results for manganese (Mn) and all Mn values reported by the participants in the Fall 2015 GMA Study are summarized in Table 29. The table also provides several summary values: *N* = number of values, Mean = mean of values, SD = standard deviation of values, and Pooled SD = square-root of the sum of the squared within-box or within-participant standard deviations.

Table 29. Summary of Results for Manganese (Mn), mg/kg.

NIST ICP-OES

Box	A	B	Mean	SD
1	10.34	10.34	10.34	0.00
2	11.11	10.89	11.00	0.16
3	11.00	10.90	10.95	0.07
4	10.35	10.27	10.31	0.05
5	10.50	10.28	10.39	0.16
6	10.79	11.33	11.06	0.38
7	10.62	10.73	10.68	0.08
8	10.67	10.54	10.60	0.09
9	11.15	11.02	11.08	0.09
10	10.75	10.60	10.68	0.10
		***N*:**	10	
		Mean, Pooled SD:	10.71	0.15
		SD:	0.30	

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Lab	A	B	Mean	SD	Method
1	10.60	10.80	10.70	0.14	*not reported*
2	10.40	10.60	10.50	0.14	ICP-OES
4	12.00	11.70	11.85	0.21	AAS
5	10.10	10.30	10.20	0.14	ICP-MS
6	8.46	8.85	8.66	0.28	ICP-OES
7	8.80	7.90	8.35	0.64	ICP-MS
10	10.50		10.50		ICP-OES
11	10.70		10.70		*not reported*
13	10.50		10.50		*not reported*
18	<10				ICP-OES
27	10.00	10.00	10.00	0.00	ICP-MS
		***N*:**	10		
		Mean, Pooled SD:	10.20	0.29	
		SD:	1.02		

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The NIST manganese results as a function of the sample box number are displayed in Fig. 18. The blue circles in the figure represent the results for the first replicate and the red squares the second replicate. The mean mass fraction is indicated by the solid line, and the dashed lines bound the interval Mean ± 2×SD.

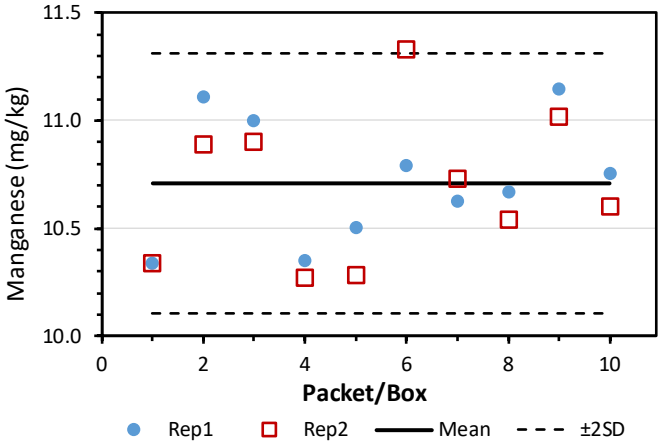

Fig. 18. Manganese (Mn) Mass Fraction as a Function of Box Number.

4.2.3.7. Sodium

The NIST ICP-OES results for sodium (Na) and all Na values reported by the participants in the Fall 2015 GMA Study are summarized in Table 30. The table also provides several summary values: *N* = number of values, Mean = mean of values, SD = standard deviation of values, and Pooled SD = square-root of the sum of the squared within-box or within-participant standard deviations.

Table 30. Summary of Results for Sodium (Na), mg/kg.

NIST ICP-OES

Box	A	B	Mean	SD
1	680	816	748	96
2	903	919	911	11
3	983	907	945	54
4	833	816	825	12
5	825	789	807	26
6	904	910	907	4
7	857	838	847	14
8	818	831	825	9
9	920	918	919	2
10	852	831	841	15
		***N*:**	10	
		Mean, Pooled SD:	857	37
		SD:	61	

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Lab	A	B	Mean	SD	Method
1	967	964	966	2	*not reported*
2	837	836	837	1	ICP-OES
3	810	890	850	57	ICP-OES
4	1310	1200	1255	78	AAS
5	851	858	855	5	ICP-MS
6	756	807	782	36	ICP-OES
7	866	857	862	6	ICP-MS
10	846		846		*not reported*
11	842		842		*not reported*
13	847		847		*not reported*
16	903	910	907	5	AAS
18	860		860		ICP-OES
24	1460	1330	1395	92	AAS
26	1010	1040	1025	21	ICP-OES
27	917	917	917	0	AAS
		***N*:**	15		
		Mean, Pooled SD:	936	42	
		SD:	170		

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The NIST sodium results as a function of the sample box number are displayed in Fig. 19. The blue circles in the figure represent the results for the first replicate and the red squares the second replicate. The mean mass fraction is indicated by the solid line, and the dashed lines bound the interval Mean ± 2×SD.

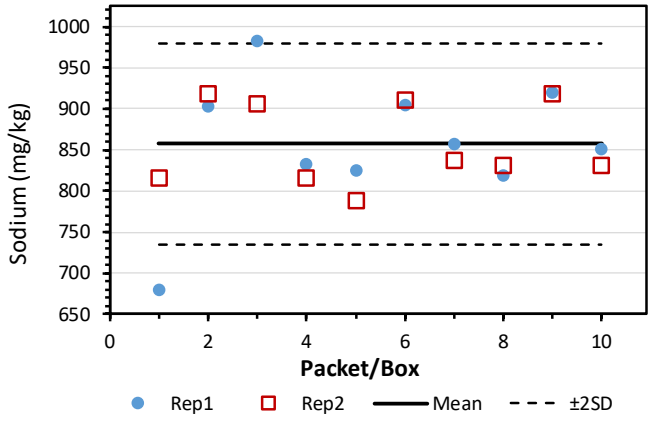

Fig. 19. Sodium (Na) Mass Fraction as a Function of Box Number.

4.2.3.8. Phosphorus

The NIST ICP-OES results for phosphorus (P) and all P values reported by the participants in the Fall 2015 GMA Study are summarized in Table 31. The table also provides several summary values: *N* = number of values, Mean = mean of values, SD = standard deviation of values, and Pooled SD = square-root of the sum of the squared within-box or within-participant standard deviations. Statistical outliers identified using Dixon's Q-test were excluded from further calculations.

Table 31. Summary of Results for Phosphorus (P), mg/kg.

NIST ICP-OES

Box	A	B	Mean	SD
1	2982	3467	3224	343
2	3683	4020	3851	238
3	3099	3714	3406	435
4	3410	3367	3388	30
5	3455	3359	3407	68
6	3645	3701	3673	40
7	3501	3471	3486	22
8		3375	3375	
9	3720	3763	3742	30
10	3427	3467	3447	29
		***N*:**	10	
		Mean, Pooled SD:	3500	204
		SD:	193	

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Lab	A	B	Mean	SD	Method
1	3610	3600	3605	7	*not reported*
2	3560	3520	3540	28	ICP-OES
3	3420	3600	3510	127	ICP-OES
4	2920	2950	2935	21	colorimetry
5	3540	3500	3520	28	ICP-MS
6	3670	4010	3840	240	ICP-OES
7	3100	2870	2985	163	ICP-OES
10	3420		3420		*not reported*
11	3940		3940		*not reported*
13	3550		3550		*not reported*
16	3425	3462	3444	26	colorimetry
18	3260		3260		ICP-OES
24	31700	31400	*outlier*		colorimetry
26	3700	3700	3700	0	AAS
27	4260	4260	4260	0	ICP-MS
			***N*:**	14	
			Mean, Pooled SD:	3536	102
			SD:	348	

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The NIST phosphorus results as a function of the sample box number are displayed in Fig. 20. The blue circles in the figure represent the results for the first replicate and the red squares the second replicate. The mean mass fraction is indicated by the solid line, and the dashed lines bound the interval Mean ± 2×SD.

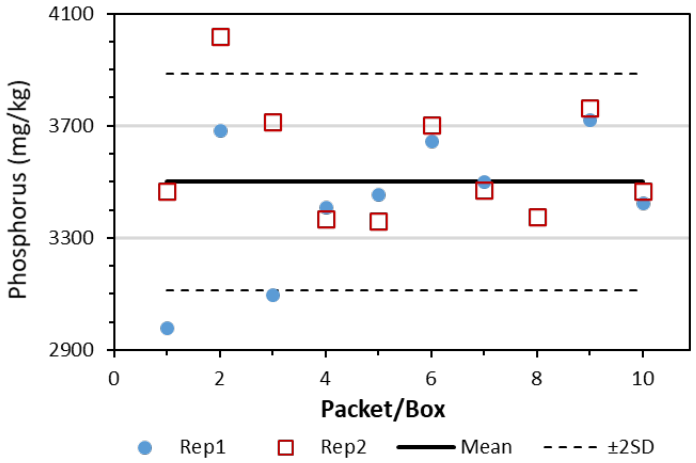

Fig. 20. Phosphorus (P) Mass Fraction as a Function of Box Number.

4.2.3.9. Zinc

The NIST ICP-OES results for zinc (Zn) and all Zn values reported by the participants in the Fall 2015 GMA Study are summarized in Table 32. The table also provides several summary values: *N* = number of values, Mean = mean of values, SD = standard deviation of values, and Pooled SD = square-root of the sum of the squared within-box or within-participant standard deviations.

Table 32. Summary of Results for Zinc (Zn), mg/kg.

NIST ICP-OES

Box	A	B	Mean	SD
1	33.77	34.44	34.11	0.47
2	36.70	37.59	37.15	0.63
3	36.48	36.92	36.70	0.31
4	34.33	33.67	34.00	0.47
5	39.12	33.48	36.30	3.99
6	35.30	36.25	35.78	0.67
7	34.94	34.72	34.83	0.16
8	34.26	33.20	33.73	0.75
9	37.72	37.58	37.65	0.10
10	33.70	34.98	34.34	0.91
		***N*:**	10	
		Mean, Pooled SD:	35.46	1.37
		SD:	1.44	

Fall 2015 GMA Study

Lab	A	B	Mean	SD	Method
1	35.5	35.8	35.7	0.2	*not reported*
2	35.9	36.0	36.0	0.1	ICP-OES
4	44.3	41.2	42.8	2.2	AAS
5	36.5	36.3	36.4	0.1	ICP-MS
6	40.0	41.8	40.9	1.3	ICP-OES
7	30.0	26.0	28.0	2.8	ICP-OES
10	35.5		35.5		*not reported*
11	36.8		36.8		*not reported*
13	37.9		37.9		*not reported*
16	34.0	34.0	34.0	0.0	AAS
18	35.1		35.1		ICP-OES
24	33.0	33.0	33.0	0.0	AAS
26	37.1	36.0	36.6	0.8	ICP-OES
27	38.4	38.7	38.6	0.2	AAS
		***N*:**	14		
		Mean, Pooled SD:	36.2	1.2	
		SD:	3.5		

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ICP-OES Inductively Coupled Plasma Optical Emission Spectroscopy

The NIST zinc results as a function of the sample box number are displayed in Fig. 21. The blue circles in the figure represent the results for the first replicate and the red squares the second

replicate. The mean mass fraction is indicated by the solid line, and the dashed lines bound the interval Mean ± 2×SD.

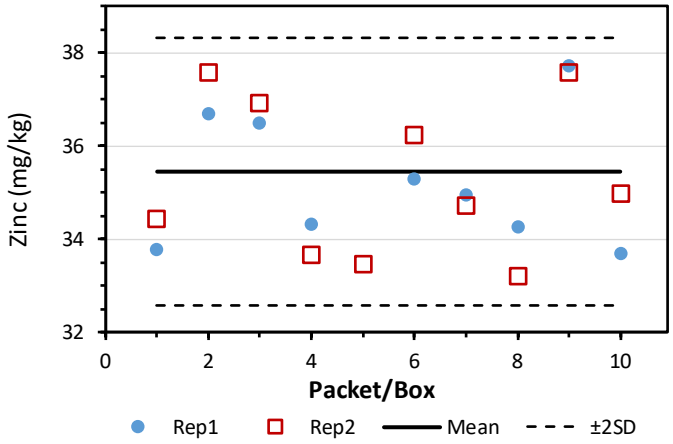

Fig. 21. Zinc (Zn) Mass Fraction as a Function of Box Number.

4.2.3.10. Uncertainty Budget

Table 33 lists the uncertainty budget for Ca, Cu, Fe, K, Mg, Mn, Na, P, and Zn.

Table 33. Uncertainty Budget for ICP-OES Analysis of Ca, Cu, Fe, K, Mg, Mn, Na, P, Zn.

Uncertainty	Basis	Type	DF
Sample Replication, s_{sample}	The uncertainty due to sample preparation and measurement is estimated by calculating the standard deviation of the mean. (n = 16, 19, or 20)	A	15,18,19
Blank Replication, s_{blank}	The uncertainty due to blank preparation and measurement is estimated by calculating the standard deviation of the mean. (n = 12)	A	11
Moisture Correction, $s_{moisture}$	The uncertainty due to the moisture correction is estimated by calculating the standard deviation of the mean then converting percent moisture to mass. (n = 4)	A	3
Primary Standard, u_s	The uncertainty associated with the primary standards is calculated to be the expanded uncertainty divided by the expansion factor, k, obtained from the Certificate of Analysis for each SRM used as the standard addition spike.	B	> 60
Weighing of Standards, u_{b1}	The uncertainty for each weighing of the standard is ± 0.01 mg based on the certificate of calibration for the balance. This uncertainty is normalized by division by √3.	B	∞
Weighing of Samples, u_{b2}	The uncertainty for each weighing of the sample is ± 0.01 mg based on the certificate of calibration for the balance. This uncertainty is normalized by division by √3.	B	∞

4.2.4. Molybdenum

All molybdenum (Mo) values reported by laboratories participating in the Fall 2015 GMA Study are summarized in Table 34. The table also provides several summary values: *N* = number of values, Mean = mean of values, SD = standard deviation of values, and Pooled SD = square-root of the sum of the squared within-participant standard deviations.

Table 34. Summary of Results for Molybdenum (Mo), mg/kg.

Lab	A	B	Mean	SD	Method
1	0.0850	0.0830	0.0840	0.0014	*not reported*
5	0.0840	0.0810	0.0825	0.0021	ICP-MS
18	0.0680		0.0680		ICP-OES
27	<0.5	<0.5			ICP-MS
		***N*:**	3		
		Mean, Pooled SD:	0.0782	0.0018	
		SD:	0.0088		

ICP-MS Inductively Coupled Plasma Mass Spectrometry
ICP-OES Inductively Coupled Plasma Optical Emission Spectroscopy

4.2.5. Selenium

All selenium (Se) values reported by laboratories participating in the Fall 2015 GMA Study are summarized in Table 35. The table also provides several summary values: *N* = number of values, Mean = mean of values, SD = standard deviation of values, and Pooled SD = square-root of the sum of the squared within-participant standard deviations.

Table 35. Summary of Results for Selenium (Se), mg/kg.

Lab	A	B	Mean	SD	Method
1	0.046	0.043	0.0445	0.0021	*not reported*
3	712	746			ICP-MS
5	<0.4	<0.4			ICP-MS
7	<0.1	<0.1			other
10	0.4000		0.4000		*not reported*
13	0.0400		0.0400		*not reported*
18	0.0140		0.0140		ICP-OES
27	0.0860	0.0840	0.0850	0.0014	ICP-MS
		***N*:**	5		
		Mean, Pooled SD:	0.1167	0.0018	
		SD:	0.1604		

ICP-MS Inductively Coupled Plasma Mass Spectrometry
ICP-OES Inductively Coupled Plasma Optical Emission Spectroscopy

4.2.6. Value Assignment

As described in Section 3.3, the available data for each measurand was used to provide an estimate of the mass fraction present in SRM 2386 where x is the mean and $U_{95}(x)$ is the 95% confidence interval. The summary of these estimates for elements is provided in Table 36, along with a summary of the methods used to arrive at these estimates. A blank in the table indicates that no data from that method was available for determination of the estimate. Analysis of variance at a 5 % significance level showed statistically significant heterogeneity in some cases, and the uncertainties for Cd, Mg, Mn, and Na values containing NIST results incorporate an additional component for possible heterogeneity.

Table 36. Summary of Estimates for Elements in SRM 2386, mg/kg.

			Based on	
Analyte	x	$U_{95}(x)$	**NIST Methods**	**Fall 2015 GMA Methods[a]**
Boron	171.833	14.422	TNPGAA	
Cadmium	0.14201	0.00988	ID-ICP-MS	
Calcium	776.61	62.32	ICP-OES	ICP-OES, ICP-MS, AAS
Copper	16.22	0.60	ICP-OES	ICP-OES, ICP-MS, AAS
Iron	33.57	1.38	ICP-OES	ICP-OES, ICP-MS, AAS
Magnesium	1999.25	540.99	ICP-OES	ICP-OES, ICP-MS, AAS
Manganese	10.72	0.70	ICP-OES	ICP-OES, ICP-MS, AAS
Molybdenum	0.0867	0.0168		ICP-OES, ICP-MS
Phosphorus	3591.30	214.30	ICP-OES	ICP-OES, ICP-MS, AAS, Colorimetry
Potassium	36440.80	5011.22	ICP-OES	ICP-OES, ICP-MS, AAS
Selenium	0.0460	0.0278		ICP-OES, ICP-MS
Sodium	866.53	138.93	ICP-OES	ICP-OES, ICP-MS, AAS
Zinc	36.57	2.62	ICP-OES	ICP-OES, ICP-MS, AAS

a Not all laboratories reported methods used.

AAS Atomic Absorption Spectroscopy
ICP-OES Inductively Coupled Plasma Optical Emission Spectrometry
ICP-MS Inductively Coupled Plasma Mass Spectrometry
ID ICP-MS Isotope Dilution Inductively Coupled Plasma Mass Spectrometry
TNPGAA Thermal Neutron Prompt Gamma-Ray Activation Analysis

4.3. Vitamins

All vitamin results determined at NIST and by the Fall 2015 GMA Study were reported on an as-received basis and converted to a dry-mass basis using the moisture correction described in Section 3.1.1.4 for reporting on the COA. Results from GMA studies include those vitamins that were quantitatively determined by at least two participants. Results reported as "0" or "<" values are not used in the statistical summaries. Values that are at least 10-fold greater than the median of the quantitative values (most likely reflecting unit conversion errors) are also not used in the summaries.

4.3.1. Vitamin C (Ascorbic Acid)

The NIST LC-UV results for ascorbic acid are summarized in Table 37. The table also provides several summary values: *N* = number of values, Mean = mean of values, SD = standard deviation of values, and Pooled SD = square-root of the sum of the squared within-box standard deviations. The quality assurance measurement results were concordant with the certified value delivered by the control material, SRM 1849a.

Table 37. Summary of NIST Results for Ascorbic Acid (Vitamin C), mg/kg.

	Packet 1					Packet 2					Packet 3					Total	
Box	**A**	**B**	**C**	**Mean**	**SD**	**A**	**B**	**C**	**Mean**	**SD**	**A**	**B**	**C**	**Mean**	**SD**	**Mean**	**SD**
1	173	174	171	172.7	1.5	176	175	180	177.0	2.6	174	175	170	173.0	2.6	174.2	2.4
2	175	182	175	177.3	4.0	185	186	183	184.7	1.5	173	177	179	176.3	3.1	179.4	4.6
3	170	179	184	177.7	7.1	174	171	182	175.7	5.7	172	175	181	176.0	4.6	176.4	1.1
4	184	177	180	180.3	3.5	176	176	181	177.7	2.9	172	175	182	176.3	5.1	178.1	2.0
5	175	183	177	178.3	4.2	171	176	174	173.7	2.5	186	180	184	183.3	3.1	178.4	4.8
6	179	177	174	176.7	2.5	170	175	180	175.0	5.0	184	176	178	179.3	4.2	177.0	2.2
7	172	175	171	172.7	2.1	177	177	180	178.0	1.7	178	171	177	175.3	3.8	175.3	2.7
8	175	171	184	176.7	6.7	177	170	178	175.0	4.4	180	176	171	175.7	4.5	175.8	0.8
9	183	185	182	183.3	1.5	186	185	185	185.3	0.6	183	186	185	184.7	1.5	184.4	1.0
10	173	174	169	172.0	2.6	178	177	170	175.0	4.4	176	179	179	178.0	1.7	175.0	3.0
			***N*:**	10					10					10		10	
			Mean, Pooled SD:	176.8	4.0				177.7	3.5				177.8	3.6	177.4	2.8
			SD:	3.6					4.1					3.7		3.0	

The NIST ascorbic acid results as a function of box number are displayed in Fig. 22. The blue diamonds represent the triplicate results for samples from the first packet, the red triangles represent the triplicates from the second packet, and the green circles represent the triplicates from the third packet. The mean mass fraction is indicated by the solid line, and the dashed lines bound the interval Mean ± 2×SD. While the within-packet triplicates appear bunched, the within-sample variability as estimated by the within-sample pooled standard deviations are of very similar magnitude to the between-sample standard deviations. SRM 2386 appears homogenous with regard to ascorbic acid content.

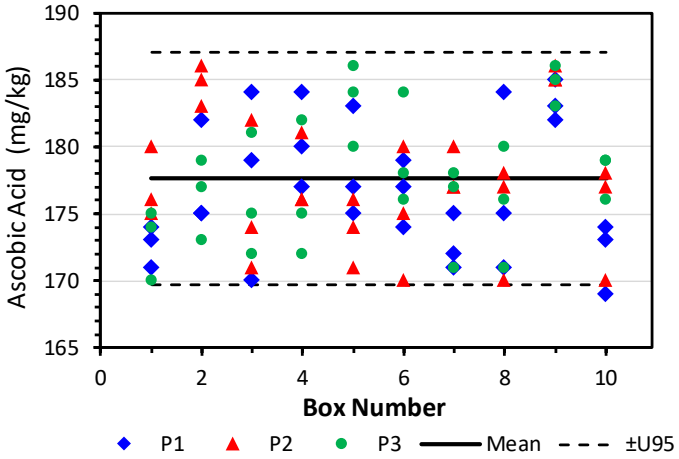

Fig. 22. Ascorbic Acid (Vitamin C) Mass Fraction as a Function of Box Number.

Ascorbic acid values reported by the participants in the Fall 2015 GMA Study are summarized in Table 38. The results of the study were highly variable and summary statistics could not be calculated.

Table 38. Fall 2015 GMA Study Results for Ascorbic Acid (Vitamin C), mg/kg.

Lab[a]	A	B	Mean	SD	Method
2	<10	<10			LC-UV
3	1.2	1.3	1.3	0.1	LC-UV
4	<10	<10			Other
5	5.9	5.6	5.8	0.2	*not reported*
6	<5	<5			DCPIP
7	6.2		6.2		*not reported*
10	<10				*not reported*
13	1700		1700		*not reported*
16	490	510	500	14	DCPIP
26	<0.4	<0.4			LC-FL

DCPIP Titration with Dichlorophenol Indophenol Detection
LC-UV Liquid Chromatography with Ultraviolet Absorbance Detection
LC-FL Liquid Chromatography with Fluorescence Detection

4.3.2. B Vitamins

Vitamins were extracted from samples of SRM 2386 for 30 min by sonication without added heat and by using a HotBlock at 60 °C and 100 °C, for up to three extraction cycles, and the recoveries using different conditions compared. Consistent extraction yields were observed at all extraction temperatures for thiamine, riboflavin, niacin, and pantothenic acid. Increased recovery was observed, however, for niacinamide, pyridoxal, and pyridoxine at elevated temperatures. No significant increases in extraction yield were observed with increasing number of extraction cycles for any of the measurands. The results of the optimization experiments are summarized in Fig. 23. For value assignment, the vitamins were extracted using a HotBlock at 100 °C.

For quantification, mass fractions of thiamine, riboflavin, niacin, niacinamide, pantothenic acid, pyridoxine, and pyridoxal in the samples were bracketed with calibration solutions. A response factor was calculated for each transition in each injection, and an average response factor (RF) was determined for each transition using:

$$RF = \frac{(A_{\mathrm{a}})(m_{\mathrm{IS}})}{(A_{\mathrm{IS}})(m_{\mathrm{a}})} \tag{5}$$

where: A_{a} peak area of the analyte,
A_{IS} peak area of the internal standard,
m_{IS} mass of the internal standard, and
m_{a} mass of the analyte.

Very low signal to noise was observed for some transitions, and those transitions were not used in determination of average RFs. Relative standard deviation (RSD) for five injections of five calibration solutions was good for all transitions of riboflavin (2.8 to 4.6) %, niacin (4.4 to 5.4) %, and pantothenic acid (1.8 to 3.3) %, three transitions of pyridoxine (3.7 to 6.0) %, two transitions of niacinamide (4.1 to 4.9) %, and one transition of pyridoxal (5.3 %). Variability (RSD) was slightly higher, yet still acceptable, for one transition each for thiamine (9.7 %), niacinamide (7.5 %), and pyridoxine (9.5 %). High variability was observed for two transitions of thiamine (18.6 to 27.3) % and three transitions of pyridoxal (14.7 to 16.1) %. One transition for thiamine giving high variability (27.3 %) was not used in calculation of sample mass fractions due to low signal to noise observed in the calibrants. For all vitamins, the variability in the analysis of the samples is comparable to or greater than the variability in the calibration.

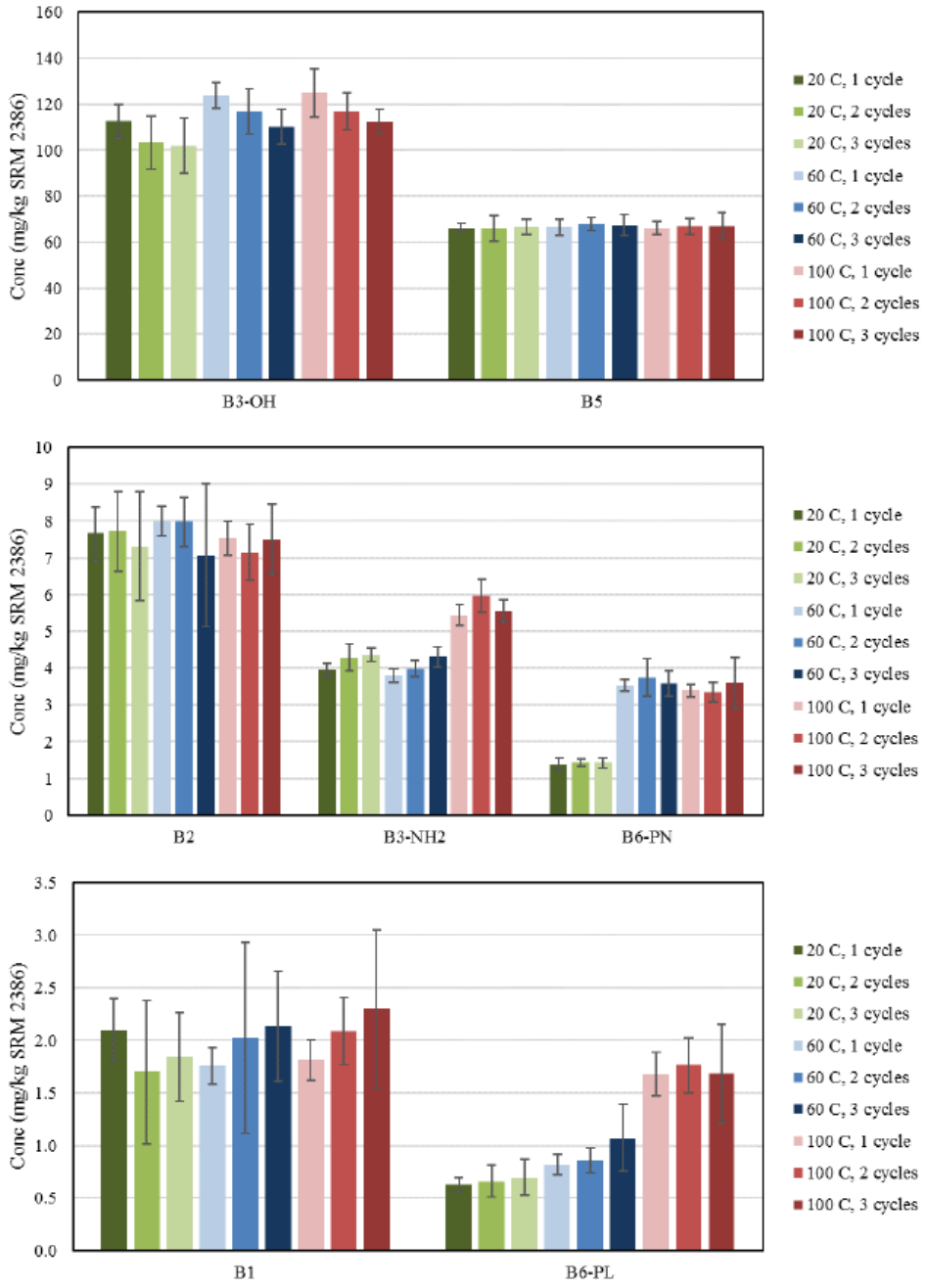

Fig. 23. Optimization of Extraction Temperature and Number of Cycles for B Vitamins.

Error bars represent the standard deviation of three measurements.

A large peak was identified in the *m/z* 269.1 → *m/z* 81 transition for labeled thiamine in a blank sample, which contained only SRM 2386 with no internal standard spike. As a result, this transition was not used in the determination of thiamine mass fraction in the samples. No other peaks were identified in any blank samples.

Averages of peak areas over all samples or calibrants were used for each transition. Averages of masses and/or mass fractions were used to estimate the levels in the samples or calibrants. The uncertainty in peak integration was assumed to be 1 %. The uncertainty in weight on a g-scale

balance was assumed to be 0.005 %. Uncertainty in purity of calibrant materials was assumed to be 5 % when the uncertainty was not previously established. The combined measurement uncertainties were between 1.25% and 2.02% for all transitions of all analytes.

The mass fraction results for each compound in each sample were determined as the mean of the value from each transition with adequate signal to noise in the samples and calibrants using the measurement equation:

$$x = \frac{(A_{\mathrm{a,s}})(A_{\mathrm{IS,c}})(m_{\mathrm{IS,s}})(m_{\mathrm{a,c}})(p_{\mathrm{a}})}{(A_{\mathrm{a,c}})(A_{\mathrm{IS,s}})(m_{\mathrm{IS,c}})(m_{\mathrm{s}})} \quad (6)$$

$A_{\mathrm{a,s}}$ peak area of the analyte in the sample,
$A_{\mathrm{IS,c}}$ peak area of the internal standard in the calibrant,
$m_{\mathrm{IS,s}}$ mass of the internal standard in the sample,
$m_{\mathrm{a,c}}$ mass of the analyte in the calibrant,
p_{a} purity of the analyte in the calibrant,
$A_{\mathrm{IS,s}}$ peak area of the internal standard in the sample,
$A_{\mathrm{a,c}}$ peak area of the analyte in the calibrant,
$m_{\mathrm{IS,c}}$ mass of the internal standard in the calibrant, and
m_{s} mass of the sample.

Measured values for thiamine, riboflavin, and pantothenic acid were consistent with the certified values for the SRM 1845a control, however the values for niacinamide and pyridoxal were higher than expected. Measured values for thiamine, riboflavin, niacin, pantothenic acid, pyridoxine, and total vitamin B_6 were consistent with the certified values for the SRM 2387 control, however the measured values for niacinamide and pyridoxal were also higher than expected. These observations may have been related to the more robust extraction condition used for SRM 2386 (heating at 100 °C for 30 min) compared to those used for the original value assignment of control materials. The vitamins in the controls were also declared to be in the free, unbound form.

The NIST ID LC-MS/MS results for the various B vitamins are summarized in the following sections.

4.3.2.1. Thiamine (Vitamin B_1)

The NIST ID-LC-MS/MS results for thiamine (vitamin B_1) and the thiamine values reported by the participants in the Fall 2015 GMA Study are summarized in Table 39. The table also provides several summary values: *N* = number of values, Mean = mean of values, SD = standard deviation of values, and Pooled SD = square-root of the sum of the squared within-box or within-participant standard deviations.

Table 39. Summary of Results for Thiamine (Vitamin B_1), mg/kg.

NIST ID-LC-MS/MS

Box	A	B	Mean	SD
1	1.516	1.716	1.616	0.141
2	1.644	1.787	1.716	0.101
3	1.727	1.973	1.850	0.174
4	1.785	1.656	1.721	0.091
5	1.514	1.626	1.570	0.079
6	1.730	1.857	1.794	0.090
7	1.942	1.792	1.867	0.106
8	1.708	1.778	1.743	0.049
9	1.727	1.905	1.816	0.126
10	1.599	1.633	1.616	0.024
		***N*:**	10	
		Mean, Pooled SD:	1.731	0.106
		SD:	0.104	

Fall 2015 GMA Study

Lab	A	B	Mean	SD	Method
5	1.63	1.60	1.62	0.02	LC-MS or LC-MS/MS
6	1.98	1.96	1.97	0.01	LC-FL
7	3.58	3.38	3.48	0.14	Digestion-fluorescence
10	1.80		1.80		*not reported*
13	2.00		2.00		*not reported*
18	1.90		1.90		Digestion-fluorescence & AA
		***N*:**	6		
		Mean, Pooled SD:	2.13	0.08	
		SD:	0.68		

AA Autoanalyzer
LC-FL Liquid Chromatography with Fluorescence Detection
LC-MS Liquid Chromatography with Mass Spectrometry
LC-MS/MS Liquid Chromatography with Tandem Mass Spectrometry
ID-LC-MS/MS Isotope Dilution Liquid Chromatography with Tandem Mass Spectrometry

The NIST thiamine results as a function of box number are displayed in Fig. 24. The blue circles in the figure represent the results for the first replicate and the red squares the second replicate. The mean mass fraction is indicated by the solid line, and the dashed lines bound the interval Mean ± 2×SD.

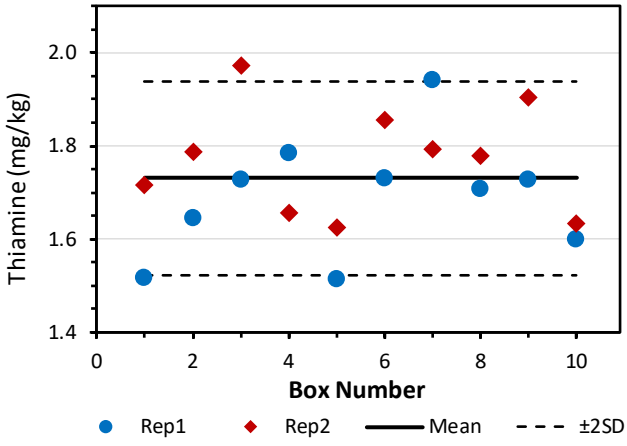

Fig. 24. Thiamine (Vitamin B_1) Mass Fraction as a Function of Box Number.

4.3.2.2. Riboflavin (Vitamin B_2)

The NIST ID-LC-MS/MS results for riboflavin (vitamin B_2) and the riboflavin values reported by the participants in the Fall 2015 GMA Study are summarized in Table 40. The table also provides several summary values: *N* = number of values, Mean = mean of values, SD = standard deviation of values, and Pooled SD = square-root of the sum of the squared within-box or within-participant standard deviations.

Table 40. Summary of Results for Riboflavin (Vitamin B_2), mg/kg.

NIST ID-LC-MS/MS

Box	A	B	Mean	SD
1	6.881	6.586	6.734	0.209
2	7.502	7.530	7.516	0.020
3	7.472	7.807	7.640	0.237
4	6.894	6.965	6.930	0.050
5	7.497	7.834	7.666	0.238
6	7.638	7.333	7.486	0.216
7	7.212	7.538	7.375	0.231
8	7.070	7.164	7.117	0.066
9	7.391	7.575	7.483	0.130
10	7.145	7.056	7.101	0.063
		***N*:**	10	
		Mean, Pooled SD:	7.305	0.169
		SD:	0.316	

Fall 2015 GMA Study

Lab	A	B	Mean	SD	Method
5	5.34	5.32	5.33	0.01	LC-MS
6	6.15	5.92	6.04	0.16	Extraction-LC-FL
7	9.06	6.00	7.53	2.16	Digestion-fluorescence
10	11.10		11.10		*not reported*
13	13.30		13.30		*not reported*
18	9.60		9.60		Digestion-fluorescence
		***N*:**	6		
		Mean, Pooled SD:	8.82	1.25	
		SD:	3.08		

LC-FL Liquid Chromatography with Fluorescence Detection
LC-MS Liquid Chromatography with Mass Spectrometry
ID-LC-MS/MS Isotope Dilution Liquid Chromatography with Tandem Mass Spectrometry

The NIST riboflavin results as a function of box number are displayed in Fig. 25. The blue circles in the figure represent the results for the first replicate and the red squares the second replicate. The mean mass fraction is indicated by the solid line, and the dashed lines bound the interval Mean ± 2×SD.

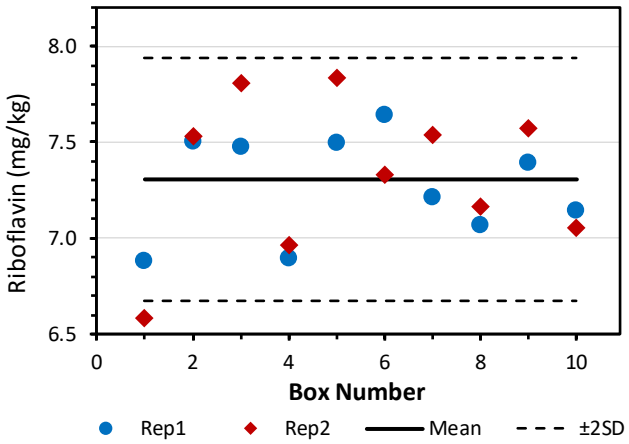

Fig. 25. Riboflavin (Vitamin B_2) Mass Fraction as a Function of Box Number.

4.3.2.3. Niacinamide (Vitamin B_3)

The NIST ID-LC-MS/MS results for niacinamide (vitamin B_3) and the niacinamide values reported by the participants in the Fall 2015 GMA Study are summarized in Table 41. The table also provides several summary values: N = number of values, Mean = mean of values, SD = standard deviation of values, and Pooled SD = square-root of the sum of the squared within-box standard deviations.

Table 41. Summary of Results for Niacinamide (Vitamin B_3), mg/kg.

NIST ID-LC-MS/MS

Box	A	B	Mean	SD
1	6.468	6.948	6.708	0.340
2	6.320	6.268	6.294	0.036
3	5.867	5.926	5.897	0.042
4	6.656	6.327	6.491	0.232
5	5.693	5.815	5.754	0.086
6	6.167	5.968	6.067	0.141
7	5.891	6.123	6.007	0.164
8	6.623	6.670	6.647	0.033
9	5.777	5.854	5.816	0.055
10	6.677	6.720	6.698	0.030
		***N*:**	10	
		Mean, Pooled SD:	6.238	0.152
		SD:	0.377	

Fall 2015 GMA Study

Lab	A	B	Mean	SD	Method
5	<0.2	<0.2			LC-MS or LC-MS/MS
6	6.13	5.47	5.80	0.47	LC-FL

LC-FL Liquid Chromatography with Fluorescence Detection
LC-MS Liquid Chromatography with Mass Spectrometry
LC-MS/MS Liquid Chromatography with Tandem Mass Spectrometry
ID-LC-MS/MS Isotope Dilution Liquid Chromatography with Tandem Mass Spectrometry

The NIST niacinamide results as a function of box number are displayed in Fig. 26. The blue circles in the figure represent the results for the first replicate and the red squares the second replicate. The mean mass fraction is indicated by the solid line, and the dashed lines bound the interval Mean ± 2×SD.

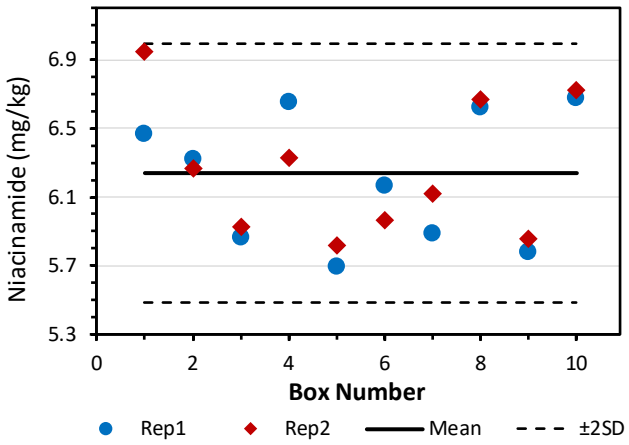

Fig. 26. Niacinamide (Vitamin B_3) Mass Fraction as a Function of Box Number.

4.3.2.4. Niacin (Vitamin B_3)

The NIST ID-LC-MS/MS results for niacin (vitamin B_3) and the niacin values reported by the participants in the Fall 2015 GMA Study are summarized in Table 42. The table also provides several summary values: *N* = number of values, Mean = mean of values, SD = standard deviation of values, and Pooled SD = square-root of the sum of the squared within-box or within-participant standard deviations.

Table 42. Summary of Results for Niacin (Vitamin B_3), mg/kg.

NIST ID-LC-MS/MS

Box	A	B	Mean	SD
1	84.06	86.74	85.40	1.90
2	96.21	96.81	96.51	0.42
3	97.71	96.35	97.03	0.96
4	84.75	82.94	83.85	1.28
5	96.89	95.82	96.36	0.76
6	101.14	96.91	99.03	2.99
7	93.18	92.58	92.88	0.42
8	77.75	81.02	79.39	2.32
9	94.32	94.94	94.63	0.44
10	84.83	84.36	84.60	0.34
		N:	10	
		Mean, Pooled SD:	90.97	1.47
		SD:	6.95	

Fall 2015 GMA Study

Lab	A	B	Mean	SD	Method
5	107.6	108.0	107.8	0.3	LC-MS or LC-MS/MS
6	70.2	76.8	73.5	4.7	LC-FL
7	132.0	131.0	131.5	0.7	*not reported*
10	117.0		117.0		*not reported*
13	123.0		123.0		*not reported*
18	114.0		114.0		Microbiological
		***N*:**	6		
		Mean, Pooled SD:	111.1	2.7	
		SD:	20.1		

LC-FL Liquid Chromatography with Fluorescence Detection
LC-MS Liquid Chromatography with Mass Spectrometry
LC-MS/MS Liquid Chromatography with Tandem Mass Spectrometry
ID-LC-MS/MS Isotope Dilution Liquid Chromatography with Tandem Mass Spectrometry

The NIST niacin results as a function of box number are displayed in Fig. 27. The blue circles in the figure represent the results for the first replicate and the red squares the second replicate. The mean mass fraction is indicated by the solid line, and the dashed lines bound the interval Mean ± 2×SD.

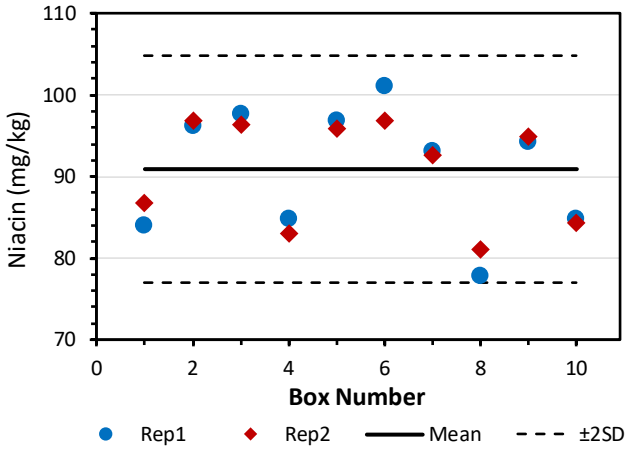

Fig. 27. Niacin (Vitamin B_3) Mass Fraction as a Function of Box Number.

4.3.2.5. Total Vitamin B_3 as Niacinamide

The NIST ID-LC-MS/MS results for total vitamin B_3 and the total vitamin B_3 values reported by the participants in the Fall 2015 GMA Study are summarized in Table 43. The table also provides several summary values: *N* = number of values, Mean = mean of values, SD = standard deviation of values, and Pooled SD = square-root of the sum of the squared within-box or within-participant standard deviations. Total vitamin B_3 was calculated as the mass fraction of niacinamide plus 0.992 times the mass fraction of niacin. The factor 0.992 is the ratio of the molar masses of the two compounds: 122.1 g/mol for niacinamide and 123.1 g/mol for niacin.

Table 43. Summary of Results for Total Vitamin B_3 as Niacinamide, mg/kg.

NIST ID-LC-MS/MS

Box	A	B	Mean	SD
1	89.85	92.99	91.42	2.22
2	101.76	102.30	102.03	0.38
3	102.79	101.50	102.15	0.91
4	90.72	88.60	89.66	1.50
5	101.81	100.86	101.34	0.67
6	106.50	102.10	104.30	3.11
7	98.32	97.96	98.14	0.26
8	83.75	87.04	85.39	2.33
9	99.34	100.03	99.68	0.49
10	90.83	90.40	90.61	0.30
		***N*:**	10	
		Mean, Pooled SD:	96.47	1.55
		SD:	6.58	

Fall 2015 GMA Study

Lab	A	B	Mean	SD	Method
5	107.6	108.0	107.8	0.3	LC-MS or LC-MS/MS
7	132.0	131.0	131.5	0.7	*not reported*
		***N*:**	2		
		Mean, Pooled SD:	119.7	0.5	
		SD:	16.8		

LC-MS Liquid Chromatography with Mass Spectrometry
LC-MS/MS Liquid Chromatography with Tandem Mass Spectrometry

The NIST results for total vitamin B_3 as a function of box number are displayed in Fig. 28. The blue circles in the figure represent the results for the first replicate and the red squares the second replicate. The mean mass fraction is indicated by the solid line, and the dashed lines bound the interval Mean ± 2×SD.

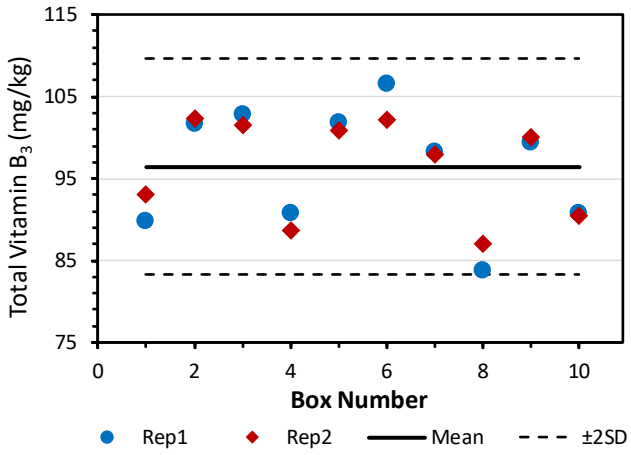

Fig. 28. Total Vitamin B_3 Mass Fraction as a Function of Box Number.

4.3.2.6. Pantothenic Acid (Vitamin B_5)

The NIST ID-LC-MS/MS results for pantothenic acid (vitamin B_5) and the pantothenic acid values reported by the participants in the Fall 2015 GMA Study are summarized in Table 44. The table also provides several summary values: *N* = number of values, Mean = mean of values, SD = standard deviation of values, and Pooled SD = square-root of the sum of the squared within-box or within-participant standard deviations.

Table 44. Summary of Results for Pantothenic Acid (Vitamin B_5), mg/kg.

NIST ID-LC-MS/MS

Box	A	B	Mean	SD
1	57.56	58.11	57.84	0.39
2	67.64	67.51	67.58	0.09
3	67.65	68.70	68.18	0.74
4	56.78	56.93	56.86	0.11
5	67.79	66.86	67.33	0.66
6	67.41	67.16	67.29	0.18
7	65.76	65.92	65.84	0.11
8	57.12	57.02	57.07	0.07
9	66.12	65.70	65.91	0.30
10	59.15	59.09	59.12	0.04
		***N*:**	10	
		Mean, Pooled SD:	63.30	0.36
		SD:	4.89	

Fall 2015 GMA Study

Lab	A	B	Mean	SD	Method
5	6.5	5.9	6.2	0.4	LC-MS or LC-MS/MS
7	70.2	47.4	58.8	16.1	*not reported*
10	72.2		72.2		*not reported*
13	67.5		67.5		*not reported*
18	63.0		63.0		microbiological
		***N*:**	5		
		Mean, Pooled SD:	53.5	11.4	
		SD:	26.9		

LC-MS Liquid Chromatography with Mass Spectrometry
LC-MS/MS Liquid Chromatography with Tandem Mass Spectrometry
ID-LC-MS/MS Isotope Dilution Liquid Chromatography with Tandem Mass Spectrometry

The NIST pantothenic acid results as a function of box number are displayed in Fig. 29. The blue circles in the figure represent the results for the first replicate and the red squares the second replicate. The mean mass fraction is indicated by the solid line, and the dashed lines bound the interval Mean ± 2×SD.

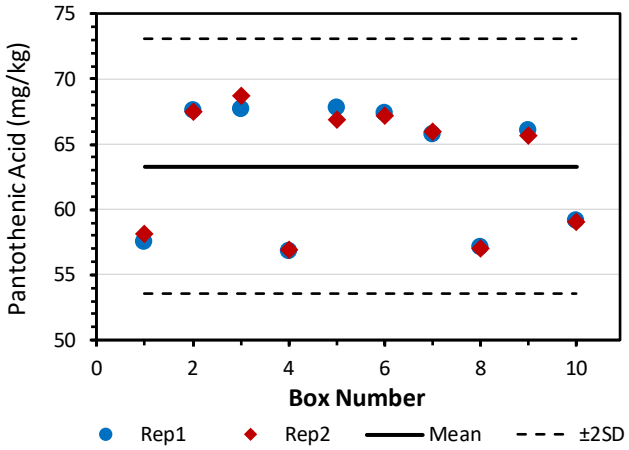

Fig. 29. Pantothenic Acid (Vitamin B_5) Mass Fraction as a Function of Box Number.

4.3.2.7. Pyridoxal (Vitamin B_6)

The NIST ID-LC-MS/MS results for pyridoxal (vitamin B_6) and the pyridoxal values reported by a participant in the Fall 2015 GMA Study are summarized in Table 45. The table also provides several summary values: *N* = number of values, Mean = mean of values, SD = standard deviation of values, and Pooled SD = square-root of the sum of the squared within-box standard deviations.

Table 45. Summary of Results for Pyridoxal (Vitamin B_6), mg/kg.

NIST ID-LC-MS/MS

Box	A	B	Mean	SD
1	1.346	1.479	1.412	0.094
2	1.910	1.820	1.865	0.063
3	1.903	1.820	1.862	0.059
4	1.383	1.294	1.339	0.063
5	1.694	1.789	1.741	0.067
6	2.006	1.869	1.937	0.097
7	2.029	2.160	2.094	0.093
8	1.345	1.471	1.408	0.089
9	2.101	2.161	2.131	0.042
10	1.384	1.460	1.422	0.053
		***N*:**	10	
		Mean, Pooled SD:	1.721	0.074
		SD:	0.303	

Fall 2015 GMA Study

Lab	A	B	Mean	SD	Method
5	1.3	1.2	1.23	0.11	LC-MS or LC-MS/MS

LC-MS Liquid Chromatography with Mass Spectrometry
LC-MS/MS Liquid Chromatography with Tandem Mass Spectrometry
ID-LC-MS/MS Isotope Dilution Liquid Chromatography with Tandem Mass Spectrometry

The NIST pyridoxal results as a function of box number are displayed in Fig. 30. The blue circles in the figure represent the results for the first replicate and the red squares the second replicate. The mean mass fraction is indicated by the solid line, and the dashed lines bound the interval Mean ± 2×SD.

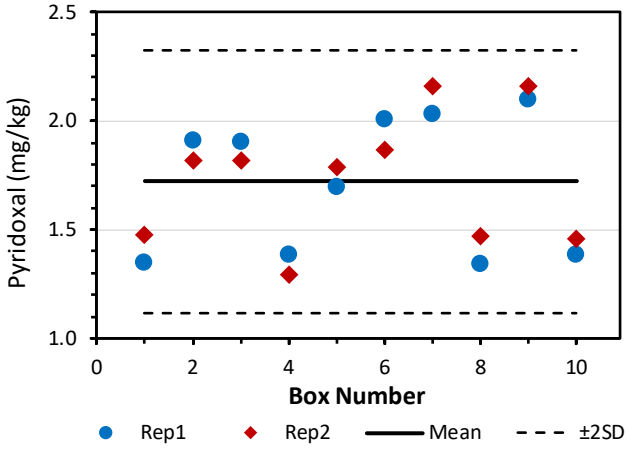

Fig. 30. Pyridoxal (Vitamin B_6) Mass Fraction as a Function of Box Number.

4.3.2.8. Pyridoxine (Vitamin B_6)

The NIST ID-LC-MS/MS results for pyridoxine (vitamin B_6) and the pyridoxine values reported by the participants in the Fall 2015 GMA Study are summarized in Table 46. The table also provides several summary values: *N* = number of values, Mean = mean of values, SD = standard deviation of values, and Pooled SD = square-root of the sum of the squared within-box standard deviations.

Table 46. Summary of Results for Pyridoxine (Vitamin B_6), mg/kg.

NIST ID-LC-MS/MS

Box	A	B	Mean	SD
1	3.256	3.246	3.251	0.007
2	3.599	3.636	3.618	0.026
3	3.654	3.567	3.611	0.061
4	3.235	3.201	3.218	0.024
5	3.652	3.520	3.586	0.094
6	3.557	3.478	3.518	0.056
7	3.520	3.448	3.484	0.051
8	3.142	3.146	3.144	0.003
9	3.551	3.485	3.518	0.047
10	3.409	3.178	3.294	0.163
		***N*:**	10	
		Mean, Pooled SD:	3.424	0.070
		SD:	0.179	

Fall 2015 GMA Study

Lab	A	B	Mean	SD	Method
5	3.16	3.07	3.12	0.06	LC-MS or LC-MS/MS

LC-MS Liquid Chromatography with Mass Spectrometry
LC-MS/MS Liquid Chromatography with Tandem Mass Spectrometry
ID-LC-MS/MS Isotope Dilution Liquid Chromatography with Tandem Mass Spectrometry

The NIST pyridoxine results as a function of box number are displayed in Fig. 31. The blue circles in the figure represent the results for the first replicate and the red squares the second replicate. The mean mass fraction is indicated by the solid line, and the dashed lines bound the interval Mean ± 2×SD.

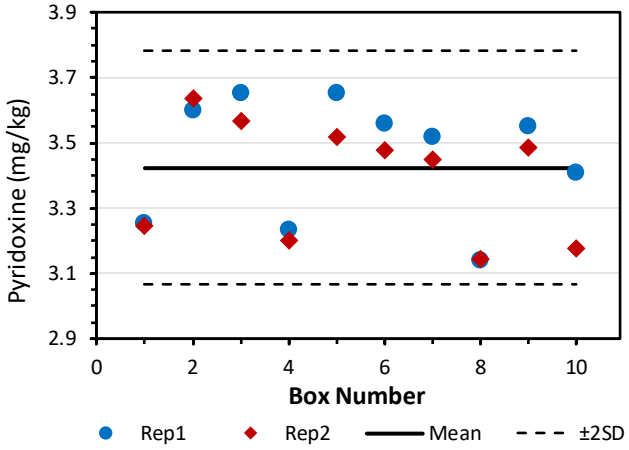

Fig. 31. Pyridoxine (Vitamin B_6) Mass Fraction as a Function of Box Number.

4.3.2.9. Total Vitamin B_6

The NIST ID-LC-MS/MS results for total vitamin B_6 and the total vitamin B_6 values reported by the participants in the Fall 2015 GMA Study are summarized in Table 47. The table also provides several summary values: *N* = number of values, Mean = mean of values, SD = standard deviation of values, and Pooled SD = square-root of the sum of the squared within-box or within-participant standard deviations. Total vitamin B_6 was calculated as the mass fraction of pyridoxine plus 1.017 times the mass fraction of pyridoxal. The factor 1.017 is the ratio of the molar masses of the two compounds: 170.0 g/mol for pyridoxine and 167.2 g/mol for pyridoxal.

Table 47. Summary of Results for Total Vitamin B_6, mg/kg.

NIST ID-LC-MS/MS

Box	A	B	Mean	SD
1	4.618	4.743	4.680	0.089
2	5.532	5.479	5.505	0.038
3	5.580	5.409	5.495	0.121
4	4.635	4.511	4.573	0.088
5	5.367	5.330	5.348	0.026
6	5.588	5.369	5.478	0.154
7	5.573	5.634	5.603	0.043
8	4.503	4.635	4.569	0.093
9	5.678	5.673	5.675	0.004
10	4.811	4.656	4.733	0.109
		***N*:**	10	
		Mean, Pooled SD:	5.166	0.089
		SD:	0.464	

Fall 2015 GMA Study

Lab	A	B	Mean	SD	Method
3	0.48	0.48	0.48	0.00	LC-MS or LC-MS/MS
5	4.47	4.22	4.35	0.18	LC-MS or LC-MS/MS
6	5.70	5.80	5.75	0.07	LC-FL
7	8.37	8.57	8.47	0.14	*not reported*
10	5.82		5.82		*not reported*
13	9.01		9.01		*not reported*
18	24.97		24.97		Microbiological
		***N*:**	7		
		Mean, Pooled SD:	8.41	0.12	
		SD:	7.83		

LC-FL Liquid Chromatography with Fluorescence Detection
LC-MS Liquid Chromatography with Mass Spectrometry
LC-MS/MS Liquid Chromatography with Tandem Mass Spectrometry
ID-LC-MS/MS Isotope Dilution Liquid Chromatography with Tandem Mass Spectrometry

The NIST results for total vitamin B_6 as a function of box number are displayed in Fig. 32. The blue circles in the figure represent the results for the first replicate and the red squares the second replicate. The mean mass fraction is indicated by the solid line, and the dashed lines bound the interval Mean ± 2×SD.

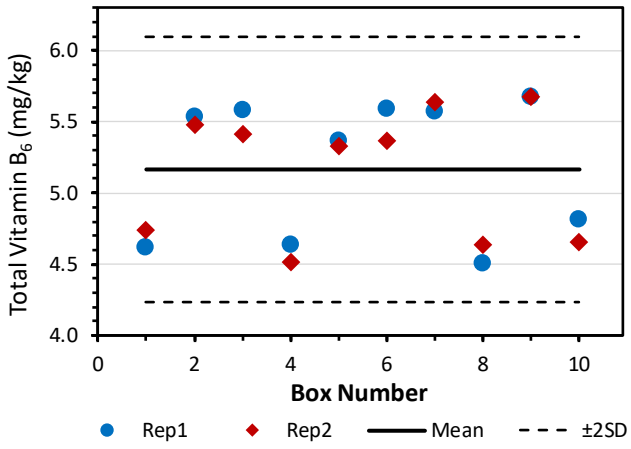

Fig. 32. Total Vitamin B_6 Mass Fraction as a Function of Box Number.

4.3.3. Choline and Carnitine

Choline and carnitine were extracted from samples of SRM 2386 using microwave-assisted hydrolysis with 1 mol/L HCl at various temperatures ranging from (110 to 160) °C using a 15-min hold time (Fig. 33). The choline mass fraction levels increased steadily with increased temperature from (110 to 150) °C, with a slight decrease in mass fraction observed at the highest hold temperature setting of 160 °C. Minimal changes in carnitine mass fraction levels were obtained with increased temperature.

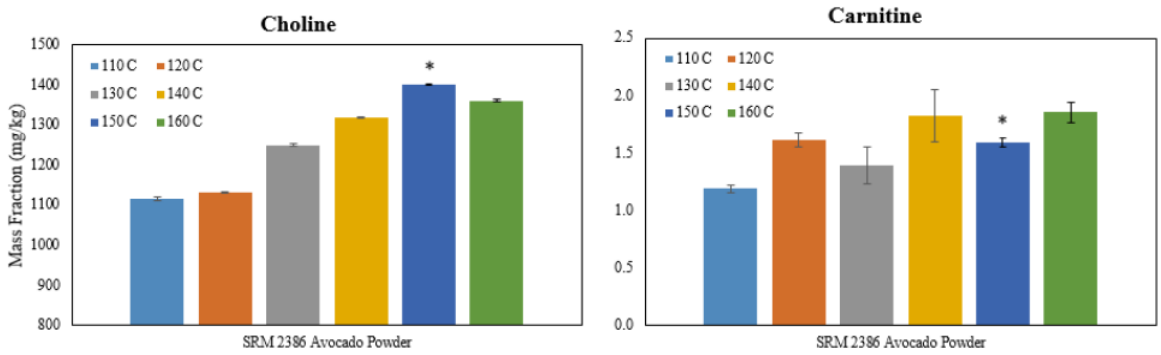

Fig. 33. Optimization of Hold Temperature for Choline and Carnitine Extraction.

The asterisk denotes the conditions used for the certification measurements. Error bars represent the standard deviation of the measured mass fraction levels (N=3).

Choline and carnitine were extracted from samples of SRM 2386 using microwave-assisted hydrolysis with a hold temperature of 140 °C and variable hold times for samples prepared with 1 mol/L HCl and 2 mol/L HCl (Fig. 34). Changes in hold time and acid concentration did not produce significant changes in measured choline or carnitine mass fractions, so the shortest time and lowest acid concentration were chosen for simplification of the certification sample preparation.

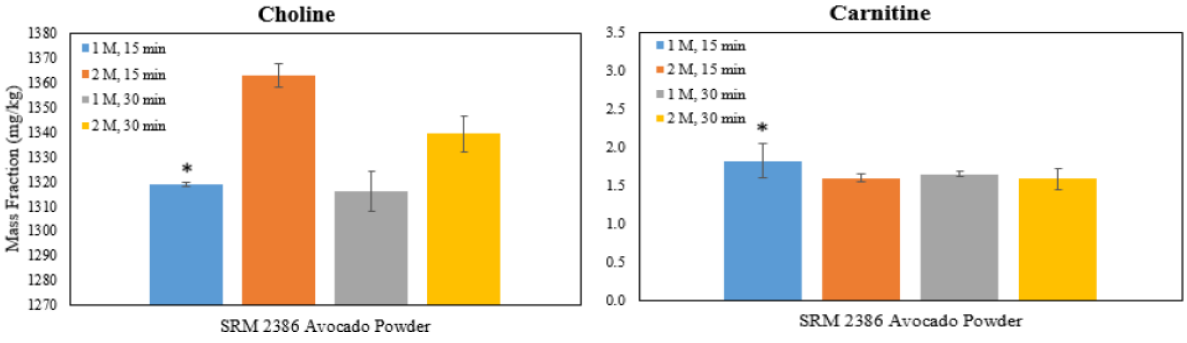

Fig. 34. Optimization of Hold Time and Fraction Acid for Choline and Carnitine Extraction.

The asterisk denotes the conditions used for the certification measurements. Error bars represent the standard deviation of the measured mass fraction levels (N=3).

Most forms of choline are susceptible to acid hydrolysis, but the choline found in phosphocholine may require phospholipase enzyme hydrolysis to free the choline ion from the phospholipid backbone. Following microwave-assisted hydrolysis using parameters determined above and pH adjustment, samples were treated with ≈100 µL of Triton X-100, a surfactant used to improve recovery of choline esters. A 300 µL aliquot of the sample was combined with 1 mL of phospholipase D solution (20 U/mL in 0.25 mol/L sodium acetate plus 0.05 mol/L calcium chloride solution) in a 15 mL polyethylene centrifuge tube and incubated in a water bath at 37 °C for 15 min. The samples were then diluted to ≈10 mL with water, centrifuged, and the supernatant filtered through a 0.45 µm regenerated cellulose (RC) filter, and recovery compared with the same samples without enzyme treatment (Fig. 35). Choline recovery decreased with the use of post-hydrolysis enzyme treatment and the recovery of carnitine did not depend on the use of the treatment. As a result, the post-hydrolysis enzyme treatment was not performed for preparation of samples for certification measurements.

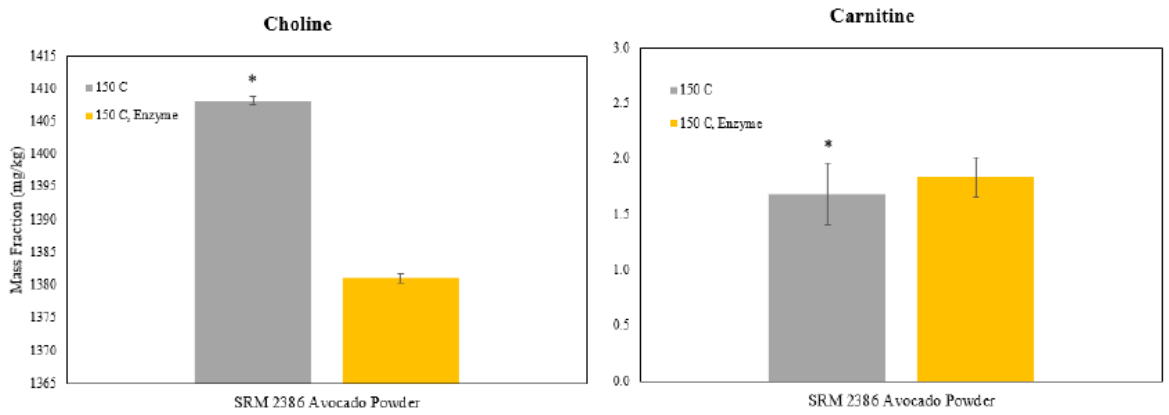

Fig. 35. Impact of Post-Hydrolysis Enzyme Treatment on Recovery for Choline and Carnitine.

The asterisk denotes the conditions used for the certification measurements. Error bars represent the standard deviation of the measured mass fraction levels (N=3).

For quantitation, mass fractions of choline and carnitine in the samples were bracketed with calibration solutions. Each calibration solution for SRM 2386 was injected 5 times; those for the control (SRM 1849a) were injected 2 times. A response factor (RF) was calculated for each injection using Eq. (5). The relative standard deviation (RSD) for five injections of calibration solutions was good for choline (2.33 to 2.37) % and acceptable for carnitine (4.51 to 6.44) %. The RSD for two injections of calibration solutions for the control was excellent for both choline (0.60 to 0.75) % and carnitine (0.50 to 0.61) %.

Averages of peak areas over all samples or calibrants were used for each transition. Averages of masses and/or mass fractions were used to estimate the levels in the samples or calibrants. The uncertainty in peak integration was assumed to be 1 %. The uncertainty in weight on a g-scale balance was assumed to be 0.005 %. Uncertainty in purity of calibrant materials was assumed to be 5 % when the uncertainty was not previously established. The combined measurement uncertainty was estimated to be 2.39 % for both transitions of choline. The observed between-sample relative measurement precision (RSD) was 2.36 %. Unidentified peaks were detected in the MRM channels for labeled and unlabeled choline in blank samples containing only extraction solvent. Peaks from five injections of the blank samples were integrated for each choline MRM transition. The resulting average area was about 0.09 % relative to the areas of labeled and

unlabeled choline in the SRM 2386 samples. These observations suggest that 2.5 % is an appropriate RSD for the choline measurements.

For carnitine, the between-sample RSD within each transition was an acceptable 10.0 %. However, combining results between transitions increased the variability because the means for the transitions differ: (2.30 ± 0.25) mg/kg for *m/z* 162 → *m/z* 60.1 compared to (1.79 ± 0.18) mg/kg for *m/z* 162 → *m/z* 103. An examination of the MRM transition ratios between the calibration solution and the samples indicates that the carnitine signal in one of the channels is biased. Because only two transitions were monitored, however, the biased transition cannot be identified. The above results for carnitine may thus be biased, high or low, by about one-half of the between-transition difference, or approximately (0.26 ± 0.15) mg/kg.

The mass fraction results for each compound in each sample were determined as the mean of the value from each transition with adequate signal to noise in the samples and calibrants using Eq. (6). The quality assurance measurement results were concordant with the certified values delivered by the control material, SRM 1849a. The NIST ID LC-MS/MS results are summarized in the following sections.

4.3.3.1. Choline

The NIST ID-LC-MS/MS results for choline and the choline values reported by the participants in the Fall 2015 GMA Study are summarized in Table 48. The table also provides several summary values: *N* = number of values, Mean = mean of values, SD = standard deviation of values, and Pooled SD = square-root of the sum of the squared within-box standard deviations.

Table 48. Summary of Results for Choline, mg/kg.

NIST ID-LC-MS/MS

Box	Rep1	Rep2	Mean	SD
1	1364	1357	1361	5
2	1445	1430	1438	11
3	1434	1441	1438	5
4	1351	1363	1357	8
5	1430	1421	1426	6
6	1428	1430	1429	1
7	1409	1410	1410	1
8	1372	1358	1365	10
9	1418	1402	1410	11
10	1380	1379	1380	1
		***N*:**	10	
		Mean, Pooled SD:	1401	7
		SD:	33	

Fall 2015 GMA Study

Lab	A	B	Mean	SD	Method
5	1440	1430	1435	7	LC-MS or LC-MS/MS
7	1210	1250	1230	28	Other
10	1240		1240		*not reported*
13	815		815		*not reported*
		***N*:**	4		
		Mean, Pooled SD:	1180	21	
		SD:	261		

LC-MS Liquid Chromatography with Mass Spectrometry
LC-MS/MS Liquid Chromatography with Tandem Mass Spectrometry
ID-LC-MS/MS Isotope Dilution Liquid Chromatography with Tandem Mass Spectrometry

The NIST choline results as a function of box number are displayed in Fig. 36. The blue circles in the figure represent the results for the first replicate and the red squares the second replicate. The mean mass fraction is indicated by the solid line, and the dashed lines bound the interval Mean ± 2×SD.

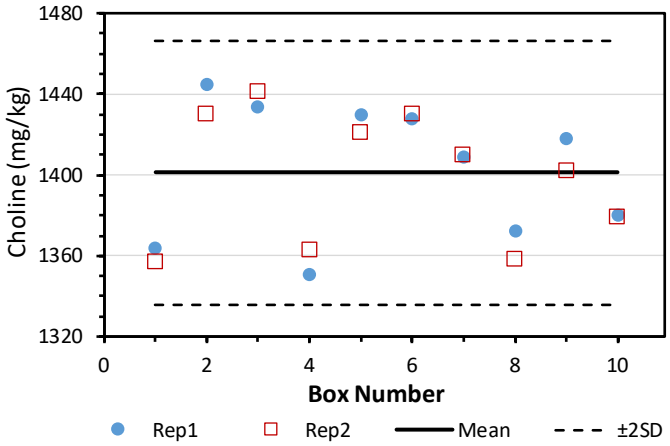

Fig. 36. Choline Mass Fraction as a Function of Box Number.

4.3.3.2. Carnitine

The NIST ID-LC-MS/MS results for carnitine and all of the carnitine values reported by the participants in the Fall 2015 GMA Study are summarized in Table 49. The table also provides several summary values: *N* = number of values, Mean = mean of values, SD = standard deviation of values, and Pooled SD = square-root of the sum of the squared within-box standard deviations.

Table 49. Summary of Results for Carnitine, mg/kg.

NIST ID-LC-MS/MS

Box	A	B	Mean	SD
1	1.810	1.840	1.825	0.021
2	2.150	2.270	2.210	0.085
3	2.170	2.200	2.185	0.021
4	1.710	1.850	1.780	0.099
5	2.080	2.180	2.130	0.071
6	2.170	2.290	2.230	0.085
7	2.350	2.380	2.365	0.021
8	1.810	1.830	1.820	0.014
9	1.970	2.040	2.005	0.049
10	1.940	1.900	1.920	0.028
		***N*:**	10	
		Mean, Pooled SD:	2.047	0.058
		SD:	0.205	

Fall 2015 GMA Study

Lab	A	B	Method
5	<5	<5	Hydrolysis, derivatization, LC
10	0		*not reported*
13	0		*not reported*

LC Liquid Chromatography
ID-LC-MS/MS Isotope Dilution Liquid Chromatography with Tandem Mass Spectrometry

The NIST carnitine results as a function of box number are displayed in Fig. 37. The blue circles in the figure represent the results for the first replicate and the red squares the second replicate. The mean mass fraction is indicated by the solid line, and the dashed lines bound the interval Mean ± 2×SD.

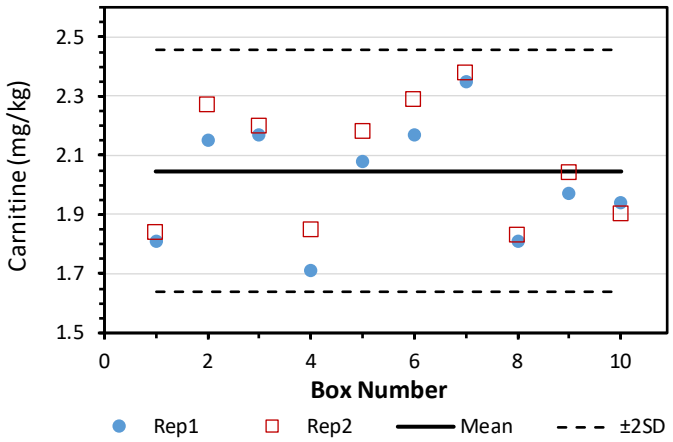

Fig. 37. Carnitine Mass Fraction as a Function of Box Number.

4.3.4. Biotin

All biotin values reported by laboratories participating in the Fall 2015 GMA Study are summarized in Table 50. The table also provides several summary values: *N* = number of values, Mean = mean of values, SD = standard deviation of values, and Pooled SD = square-root of the sum of the squared within-participant standard deviations.

Table 50. Summary of Results for Biotin, mg/kg.

Lab	A	B	Mean	SD	Method
5	<0.02	<0.02			LC-MS or LC-MS/MS
7	0.155	0.214	0.185	0.042	Microbiological
10	0.080		0.080		*not reported*
13	0.080		0.080		*not reported*
		***N*:**	3		
		Mean, Pooled SD:	0.115	0.042	
		SD:	0.060		

LC-MS Liquid Chromatography with Mass Spectrometry
LC-MS/MS Liquid Chromatography with Tandem Mass Spectrometry

4.3.5. myo-Inositol

All *myo*-inositol values reported by laboratories participating in the Fall 2015 GMA Study are summarized in Table 51. The table also provides several summary values: *N* = number of values, Mean = mean of values, SD = standard deviation of values, and Pooled SD = square-root of the sum of the squared within-participant standard deviations.

Table 51. Summary of Results for myo-Inositol, mg/kg.

Lab	A	B	Mean	SD	Method
10	3820		3820		*not reported*
13	3950		3950		*not reported*
		***N*:**	2		
		Mean:	3885		
		SD:	92		

4.3.6. Total Folate

All total folate values reported by laboratories participating in the Fall 2015 GMA Study are summarized in Table 52. The table also provides several summary values: *N* = number of values, Mean = mean of values, SD = standard deviation of values, and Pooled SD = square-root of the sum of the squared within-participant standard deviations.

Table 52. Summary of Results for Total Folate, mg/kg.

Lab	A	B	Mean	SD	Method
7	2.33		2.33		Microbiological
10	2.19		2.19		*not reported*
13	2.41		2.41		*not reported*
18	1.71		1.71		LC-MS or LC-MS/MS
		***N*:**	4		
		Mean:	2.16		
		SD:	0.31		

LC-MS Liquid Chromatography with Mass Spectrometry
LC-MS/MS Liquid Chromatography with Tandem Mass Spectrometry

4.3.7. Retinol (Vitamin A)

All retinol (vitamin A) values reported by laboratories participating in the Fall 2015 GMA Study are summarized in Table 53. The table also provides several summary values: *N* = number of values, Mean = mean of values, SD = standard deviation of values, and Pooled SD = square-root of the sum of the squared within-participant standard deviations.

Table 53. Summary of Results for Retinol (Vitamin A), mg/kg.

Lab	A	B	Mean	SD	Method
2	0.130	0.080	0.105	0.035	Saponification, LC-Abs
3	0	0			Extraction
4	0.620	0.520	0.570	0.071	Saponification, extraction, LC-Abs
5	0.180	0.130	0.155	0.035	Saponification, extraction, LC-Abs
6	<1000	<1000			Saponification, extraction, LC-Abs
10	<0.30				*not reported*
11	0.601		0.601		*not reported*
13	<0.30				*not reported*
18	1.670		1.670		Saponification, LC-Abs
26	<12	<12			Extraction
27	<0.5	<0.5			Extraction
		***N*:**	5		
		Mean, Pooled SD:	0.620	0.050	
		SD:	0.563		

LC-Abs Liquid Chromatography with Absorbance Detection

4.3.8. β-Carotene

All of the β-carotene values reported by laboratories participating in the Fall 2015 GMA Study are summarized in Table 54. The table also provides several summary values: *N* = number of values, Mean = mean of values, SD = standard deviation of values, and Pooled SD = square-root of the sum of the squared within-participant standard deviations.

Table 54. Summary of Results for b-Carotene (Provitamin A), mg/kg.

Lab	A	B	Mean	SD	Method
3	35	28			*not reported*
5	<0.24	<0.24			*not reported*
10	0.250		0.250		*not reported*
13	0.220		0.220		*not reported*
18	0.144		0.144		LC-Abs
27	<0.5	<0.5			*not reported*
		***N*:**	3		
		Mean:	0.205		
		SD:	0.063		

LC-Abs Liquid Chromatography with Absorbance Detection

4.3.9. Tocopherols (Vitamin E)

All of the tocopherol values reported by laboratories participating in the Fall 2015 GMA Study are summarized in Table 55. The table also provides several summary values: *N* = number of values, Mean = mean of values, SD = standard deviation of values, and Pooled SD = square-root of the sum of the squared within-participant standard deviations.

Table 55. Summary of Results for Tocopherols (Vitamin E), mg/kg.

	α-Tocopherol				β-Tocopherol		γ-Tocopherol		
Lab	**A**	**B**	**Mean**	**SD**	**A**	**B**	**A**	**B**	**Method**
2	47.50	50.20	48.85	1.91					Saponification, LC-FL
4	<30	<30							Saponification, extraction, LC-Abs
5	27.	25	26.00	1.41					Saponification, extraction, LC-FL
6	288	284							Saponification, extraction, LC-FL
7	47.70		47.70		18.50		4.75		*not reported*
10	23.30		23.30		3.04		3.65		*not reported*
18	27.30		27.30		2.35		3.73		Saponification, LC-FL
27	21.13	21.93	21.53	0.57					LC
		***N*:**	6		3		3		
		Mean, Pooled SD:	32.45	1.41	7.96		4.04		
		SD:	10.15		10.54		0.71		

LC-Abs Liquid Chromatography with Absorbance Detection
LC-FL Liquid Chromatography with Fluorescence Detection

4.3.10. Vitamin K

All of the vitamin K values reported by laboratories participating in the Fall 2015 GMA Study are summarized in Table 56. The table also provides several summary values: *N* = number of values, Mean = mean of values, SD = standard deviation of values, and Pooled SD = square-root of the sum of the squared within-participant standard deviations.

Table 56. Summary of Results for Vitamin K, mg/kg.

Lab	A	B	Mean	SD	Method
5	0.386	0.385	0.386	0.001	Extraction, LC-FL
10	0.325		0.325		*not specified*
13	0.120		0.120		*not specified*
27	<0.2	<0.2			LC
		***N*:**	3		
		Mean, Pooled SD:	0.277	0.001	
		SD:	0.161		

LC Liquid Chromatography
LC-FL Liquid Chromatography with Fluorescence Detection

4.3.11. Value Assignment

As described in Section 3.3, the available data for each measurand was used to provide an estimate of the mass fraction present in SRM 2386 where x is the mean and $U_{95}(x)$ is the 95% confidence interval. The summary of these estimates for vitamins is provided in Table 57, along with a summary of the methods used to arrive at these estimates. A blank in the table indicates that no data from that method was available for determination of the estimate. For ascorbic acid, riboflavin, niacin, niacinamide, total vitamin B_3, pantothenic acid, pyridoxal, pyridoxine, total vitamin B_6 as pyridoxine, choline, and carnitine, the uncertainty incorporates a component for possible inhomogeneity based on the standard deviation.

Table 57. Summary of Estimates for Vitamins in SRM 2386, mg/kg.

			Based on	
Analyte	x	$U_{95}(x)$	**NIST Method**	**Fall 2015 GMA Methods[a]**
Ascorbic acid	186.44	361.76[b]	LC-UV	LC-FL, LC-UV, DCPIP
Ascorbic acid	186.4449	10.5938	LC-UV	
Biotin	0.084	0.112[b]		LC-MS or LC-MS/MS, Microbiological
Carnitine	2.151	0.461	ID-LC-MS/MS	
Choline	1468.39	141.50	ID-LC-MS/MS	Extraction-based LC-MS or LC-MS/MS
myo Inositol	4082.568	372.830		*Not reported*
Niacin	106.82	30.64	ID-LC-MS/MS	LC-MS, LC-MS/MS, Extraction-LC, Microbiological
Niacinamide	6.555	0.872	ID-LC-MS/MS	LC-MS, LC-MS/MS, Extraction-LC
Pantothenic acid	66.56	11.26	ID-LC-MS/MS	LC-MS, LC-MS/MS, Microbiological
Pyridoxal	1.809	0.676	ID-LC-MS/MS	
Pyridoxine	3.598	0.414	ID-LC-MS/MS	
Retinol	0.599	0.743[b]		Saponification, Extraction, LC-UV
Riboflavin	7.68	1.35	ID-LC-MS/MS	LC-MS, Digestion-FL, Extraction-FL
Thiamine	1.82	0.17	ID-LC-MS/MS	AA, LC-MS, LC-MS/MS, LC-FL, Digestion-FL
Total Folates	2.375	0.569		LC-MS or LC-MS/MS, Microbiological
Total Vitamin B_3	101.44	24.03	ID-LC-MS/MS	LC-MS, LC-MS/MS, Extraction-LC-FL, Microbiological
Total Vitamin B_6 as Pyridoxine	5.43	2.06	ID-LC-MS/MS	LC-MS, LC-MS/MS, LC-FL, Microbiological
Vitamin K	0.342	0.271		Extraction, LC-FL
α-Tocopherol	28.005	12.797		Saponification, Extraction, LC-UV, LC-FL
β-Carotene	0.247	17.499[b]		LC-UV
β-Tocopherol	3.195	21.387[b]		Saponification, Extraction, LC-UV, LC-FL
γ-Tocopherol	3.920	1.383		Saponification, Extraction, LC-UV, LC-FL

a Not all laboratories reported methods used.
b The expanded uncertainty is larger than the value, indicating a large level of variability. Any interval for the value should be truncated at zero.

AA	Autoanalyzer
DCPIP	Titration with Dichlorophenol Indophenol Detection
ID-LC-MS/MS	Isotope Dilution Liquid Chromatography with Tandem Mass Spectrometry
FL	Fluorescence
LC	Liquid Chromatography
LC-UV	Liquid Chromatography with UV Absorbance Detection
LC-FL	Liquid Chromatography with Fluorescence Detection
LC-MS	Liquid Chromatography with Mass Spectrometry
LC-MS/MS	Liquid Chromatography with Tandem Mass Spectrometry

4.4. Fatty Acids

No fatty acids were consistently detected in the duplicate analysis of the blank sample, although a detectable quantity of oleic acid, palmitic acid, and stearic acid methyl esters were found in the first blank analysis, potentially due to carryover from the high concentration of the first calibration solution. The carry-over mass from the previous calibrant accounts for less than 1 % of the mass in the calibration solution and should not affect calculations. No quantity of these compounds was detectable in the second analysis of the same blank solution. The contribution of the potential blank contamination is minimal to all fatty acids except stearic acid and should not affect the overall quantitation of most of the fatty acids. Up to 14 % of the steric acid result may be due to blank contamination.

In previous studies of fatty acids, measurement of both a concentrated and dilute solution of the sample extract was required to determine both high- and low-level fatty acids. In this study, concentrated and dilute solutions of the extracts were measured but due to the high level of background signal and the high concentration of most fatty acids, the results for the concentrated samples are not reported. The analyte signal for some compounds in multiple samples was below a 3.3 signal-to-noise cut-off for limits of detection and are also not reported. The Soxhlet setup for one of the box 1 samples was spilled resulting in the loss of sample mass. The results from this sample are technical outliers and are not reported.

The quality assurance measurement results for most of the free fatty acids were concordant with the non-certified values delivered by the control material, SRM 1845a.

All fatty acids results determined at NIST and by the Fall 2015 and Spring 2016 GMA Studies were reported on an as-received basis and converted to a dry-mass basis using the moisture correction described in Section 3.1.1.4 for reporting on the COA. Results from GMA studies include those fatty acids that were quantitatively determined by at least two participants. Results reported as “0” or “<” values are not used in the statistical summaries. Values that are at least 10-fold greater than the median of the quantitative values (most likely reflecting unit conversion errors) are also not used in the summaries.

4.4.1. Lauric Acid (C12:0)

The NIST GC-FID results and the lauric acid values reported by the participants in the Fall 2015 GMA Study and Spring 2016 GMA Study are summarized in Table 58. The table also provides several summary values: *N* = number of values, Mean = mean of values, SD = standard deviation of values, and Pooled SD = square-root of the sum of the squared within-box or within-participant standard deviations.

Table 58. Summary of Results for Lauric Acid, %.

NIST GC-FID				
Box	**A**	**B**	**Mean**	**SD**
1	0.046		0.046	
2	0.116	0.043	0.080	0.052
3	0.072	0.122	0.097	0.035
4	0.066	0.055	0.061	0.008
5	0.081	0.069	0.075	0.008
6	0.088	0.094	0.091	0.004
7	0.046	0.070	0.058	0.017
8	0.104	0.057	0.081	0.033
9	0.053	0.059	0.056	0.004
12		0.113	0.113	
		***N*:**	10	
		Mean, Pooled SD:	0.076	0.026
		SD:	0.021	

Fall 2015 GMA Study				
Lab	**A**	**B**	**Mean**	**SD**
2	0.003	0.004	0.004	0.001
3	0.016		0.016	
4				
5	<0.01	<0.01		
6	<0.01	<0.01		
7	0.050	0.040	0.045	0.007
10	<0.005			
12				
13	<0.01	<0.01		
16				
18				
22				
24	0.010	0.010	0.010	0.000
25				
27	0.003	0.003	0.003	0.000
28				
29				
		***N*:**	5	
		Mean, Pooled SD:	0.016	0.004
		SD:	0.017	

Spring 2016 GMA Study			
A	**B**	**Mean**	**SD**
0	0		
0.020	0.020	0.020	0.000
0.005	0.004	0.005	0.001
0.040	0.020	0.030	0.014
0	0		
0.010	<0.01	0.010	
<0.01	<0.01		
<0.01			
<0.007			
<0.01			
<0.01	<0.01		
		4	
		0.016	0.008
		0.011	

The NIST lauric acid results as a function of box number are displayed in Fig. 38. The blue circles in the graphics represent the results for the first replicate and the red squares the second replicate. The mean mass fraction is indicated by the solid line, and the dashed lines bound the interval Mean ± 2×SD.

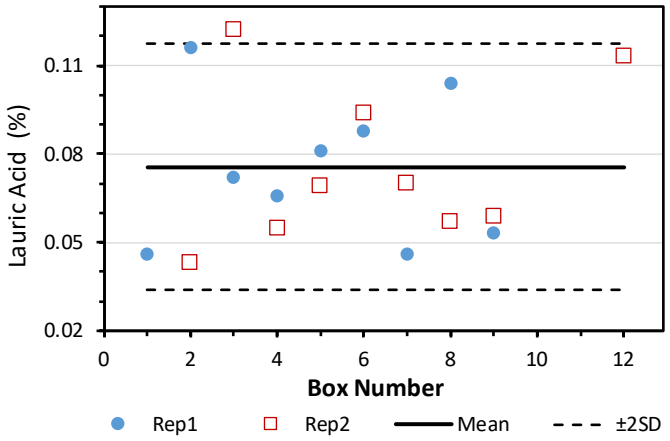

Fig. 38. Lauric Acid Mass Fraction as a Function of Box Number.

4.4.2. Myristic Acid (C14:0)

Myristic acid values reported by laboratories participating in the Fall 2015 GMA Study and the Spring 2016 GMA Study are summarized in Table 59. The table also provides several summary values: *N* = number of values, Mean = mean of values, SD = standard deviation of values, and Pooled SD = square-root of the sum of the squared within-participant standard deviations.

Table 59. Summary of Results for Myristic Acid, %.

	Fall 2015 GMA Study				Spring 2016 GMA Study			
Lab	**A**	**B**	**Mean**	**SD**	**A**	**B**	**Mean**	**SD**
2	0.016	0.014	0.015	0.001				
3	0.019	0.018	0.019	0.001				
4					0.013	0.000	0.007	0.009
5	<0.01	<0.01			0.010	0.010	0.010	0.000
6	0.012	0.013	0.013	0.001				
7	0.020	0.020	0.020	0.000	0.018	0.018	0.018	0.000
10	0.011		0.011					
12					0.020	0.030	0.025	0.007
13	0.010		0.010		0.015	0.009	0.012	0.004
16					0.010	0.020	0.015	0.007
18	0.015		0.015		0.010	0.010	0.010	0.000
22					0.011		0.011	
24	0.020	0.020	0.020	0.000				
25					0.012		0.012	
26	0.012	0.012	0.012	0.000				
27	0.016	0.017	0.017	0.000				
28					0.011		0.011	
29					0.011	<0.01	0.011	
		***N*:**	10				11	
		Mean, Pooled SD:	0.015	0.001			0.013	0.005
		SD:	0.004				0.005	

4.4.3. Palmitic Acid (C16:0)

The NIST GC-FID results and the palmitic acid values reported by the participants in the Fall 2015 GMA Study and Spring 2016 GMA Study are summarized in Table 60. The table also provides several summary values: *N* = number of values, Mean = mean of values, SD = standard deviation of values, and Pooled SD = square-root of the sum of the squared within-box or within-participant standard deviations.

Table 60. Summary of Results for Palmitic Acid, %.

NIST GC-FID				
Box	**A**	**B**	**Mean**	**SD**
1	2.998		2.998	
2	3.007	3.071	3.039	0.045
3	3.084	3.078	3.081	0.004
4	3.054	3.008	3.031	0.033
5	3.030	2.918	2.974	0.079
6	3.099	3.034	3.067	0.046
7	3.019	3.019	3.019	0.000
8	3.102	3.172	3.137	0.049
9	3.057	3.023	3.040	0.024
12	2.966	3.073	3.020	0.076
		***N*:**	10	
		Mean, Pooled SD:	3.041	0.047
		SD:	0.046	

Fall 2015 GMA Study				
Lab	**A**	**B**	**Mean**	**SD**
2	3.445	3.536	3.491	0.064
3	3.052	3.105	3.079	0.037
4	4.710	4.610	4.660	0.071
5	3.020	3.040	3.030	0.014
6	2.732	2.787	2.760	0.039
7	3.260	3.220	3.240	0.028
9				
10	3.110		3.110	
12				
13	3.022		3.022	
16				
18	3.277		3.277	
22				
24	3.220	3.220	3.220	0.000
25				
26	4.034	4.106	4.070	0.051
27	3.949	3.652	3.800	0.210
28				
29				
		***N*:**	12	
		Mean, Pooled SD:	3.396	0.081
		SD:	0.536	

Spring 2016 GMA Study			
A	**B**	**Mean**	**SD**
2.990	2.970	2.980	0.014
3.110	3.150	3.130	0.028
3.612	3.233	3.423	0.268
4.570	4.650	4.610	0.057
3.190	3.250	3.220	0.042
3.603	3.594	3.599	0.006
3.230	3.180	3.205	0.035
2.850	2.800	2.825	0.035
3.040		3.040	
3.420		3.420	
3.190		3.190	
3.310	3.310	3.310	0.000
		12	
		3.329	0.094
		0.455	

The NIST palmitic acid results as a function of box number are displayed in Fig. 39. The blue circles in the graphics represent the results for the first replicate and the red squares the second replicate. The mean mass fraction is indicated by the solid line, and the dashed lines bound the interval Mean ± 2×SD.

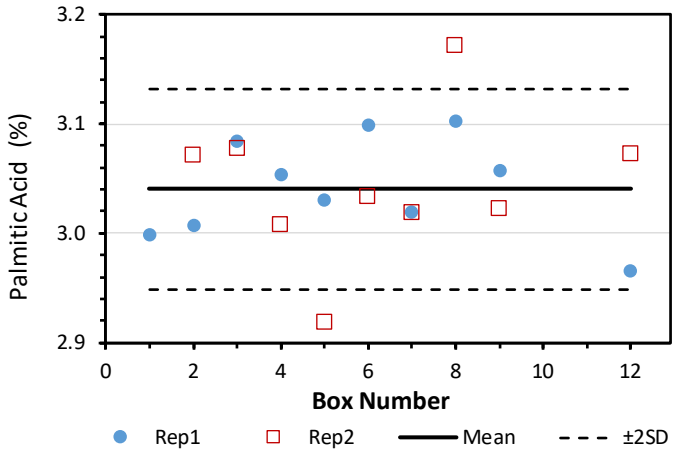

Fig. 39. Palmitic Acid Mass Fraction as a Function of Box Number.

4.4.4. Palmitoleic Acid (C16:1-9c)

The NIST GC-FID results and the palmitoleic acid values reported by the participants in the Fall 2015 GMA Study and Spring 2016 GMA Study are summarized in Table 61. The table also provides several summary values: *N* = number of values, Mean = mean of values, SD = standard deviation of values, and Pooled SD = square-root of the sum of the squared within-box or within-participant standard deviations.

Table 61. Summary of Results for Palmitoleic Acid, %.

NIST GC-FID				
Box	**A**	**B**	**Mean**	**SD**
1	1.095		1.095	
2	1.124	1.147	1.136	0.016
3	1.154	1.151	1.153	0.002
4	1.119	1.103	1.111	0.011
5	1.133	1.081	1.107	0.037
6	1.149	1.138	1.144	0.008
7	1.112	1.110	1.111	0.001
8	1.165	1.173	1.169	0.006
9	1.136	1.119	1.128	0.012
12	1.105	1.148	1.127	0.030
		***N*:**	10	
		Mean, Pooled SD:	1.128	0.018
		SD:	0.023	

Fall 2015 GMA Study				
Lab	**A**	**B**	**Mean**	**SD**
2	1.164	1.184	1.174	0.014
3	1.081	1.101	1.091	0.014
4	1.650	1.620	1.635	0.021
5				
6	0.987	1.006	0.997	0.013
7	1.080	1.080	1.080	0.000
9				
10	1.073		1.073	
12				
13	1.058		1.058	
16				
18	1.124		1.124	
22				
24	1.110	1.110	1.110	0.000
25				
26	1.401	1.419	1.410	0.013
27	1.352	1.253	1.302	0.070
28				
		***N*:**	11	
		Mean, Pooled SD:	1.187	0.028
		SD:	0.190	

Spring 2016 GMA Study			
A	**B**	**Mean**	**SD**
0.887	0.882	0.885	0.004
1.060	1.080	1.070	0.014
1.194	1.092	1.143	0.072
1.540	1.660	1.600	0.085
1.100	1.100	1.100	0.000
1.120	1.110	1.115	0.007
1.000	0.980	0.990	0.014
1.050		1.050	
1.180		1.180	
1.100		1.100	
		10	
		1.123	0.043
		0.187	

The NIST palmitoleic acid results as a function of box number are displayed in Fig. 40. The blue circles in the graphics represent the results for the first replicate and the red squares the second replicate. The mean mass fraction is indicated by the solid line, and the dashed lines bound the interval Mean ± 2×SD.

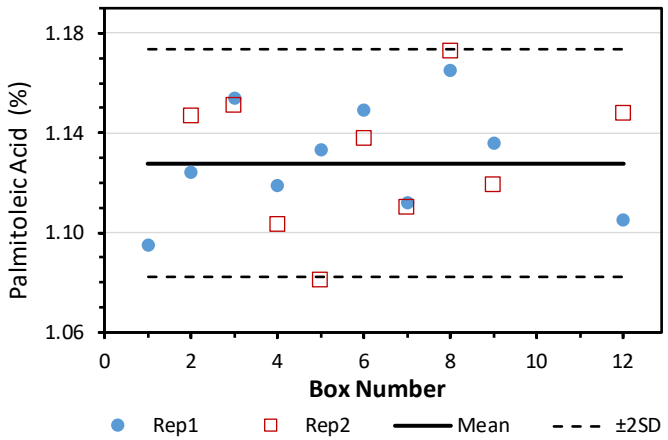

Fig. 40. Palmitoleic Acid Mass Fraction as a Function of Box Number.

4.4.5. Stearic Acid (C18:0)

The NIST GC-FID results and the stearic acid values reported by the participants in the Fall 2015 GMA Study and Spring 2016 GMA Study are summarized in Table 62. The table also provides several summary values: *N* = number of values, Mean = mean of values, SD = standard deviation of values, and Pooled SD = square-root of the sum of the squared within-box or within-participant standard deviations.

Table 62. Summary of Results for Stearic Acid, %.

NIST GC-FID				
Box	**A**	**B**	**Mean**	**SD**
1				
2	0.087	0.102	0.095	0.011
3	0.097	0.108	0.103	0.008
4	0.100	0.101	0.101	0.001
5	0.096	0.120	0.108	0.017
6	0.084	0.081	0.083	0.002
7	0.099	0.101	0.100	0.001
8	0.108	0.102	0.105	0.004
9	0.094	0.107	0.101	0.009
12	0.101	0.102	0.102	0.001
		N:	9	
		Mean, Pooled SD:	0.099	0.008
		SD:	0.007	

Fall 2015 GMA Study				
Lab	**A**	**B**	**Mean**	**SD**
2	0.110	0.109	0.110	0.001
3	0.188	0.192	0.190	0.003
4	0.140	0.130	0.135	0.007
5	0.081	0.082	0.082	0.001
6	0.084	0.085	0.085	0.001
7	0.120	0.120	0.120	0.000
9				
10	0.090		0.090	
12				
13	0.087		0.087	
16				
18	0.106		0.106	
22				
24	0.120	0.140	0.130	0.014
25				
26	0.106	0.106	0.106	0.000
27	0.116	0.103	0.110	0.009
28				
29				
		***N*:**	12	
		Mean, Pooled SD:	0.112	0.006
		SD:	0.030	

Spring 2016 GMA Study			
A	**B**	**Mean**	**SD**
0.220	0.229	0.225	0.006
0.118	0.097	0.108	0.015
0.150	0.150	0.150	0.000
0.100	0.120	0.110	0.014
0.104	0.092	0.098	0.008
0.120	0.100	0.110	0.014
0.100	0.100	0.100	0.000
0.088		0.088	
0.100		0.100	
0.095		0.095	
0.101	0.101	0.101	0.000
		11	
		0.117	0.010
		0.039	

The NIST stearic acid results as a function of box number are displayed in Fig. 41. The blue circles in the graphics represent the results for the first replicate and the red squares the second replicate. The mean mass fraction is indicated by the solid line, and the dashed lines bound the interval Mean ± 2×SD.

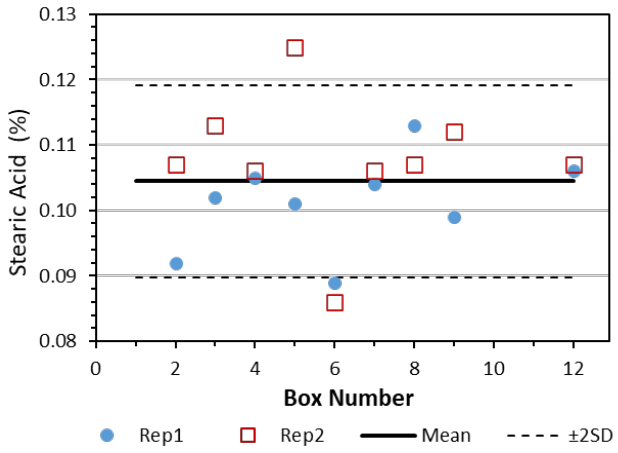

Fig. 41. Stearic Acid Mass Fraction as a Function of Box Number.

4.4.6. Oleic Acid (C18:1-9c)

The NIST GC-FID results and the oleic acid values reported by the participants in the Fall 2015 GMA Study and Spring 2016 GMA Study are summarized in Table 63. The table also provides several summary values: *N* = number of values, Mean = mean of values, SD = standard deviation of values, and Pooled SD = square-root of the sum of the squared within-box or within-participant standard deviations.

Table 63. Summary of Results for Oleic Acid, %.

NIST GC-FID				
Box	**A**	**B**	**Mean**	**SD**
1	12.335		12.335	
2	12.051	12.378	12.215	0.231
3	12.410	12.316	12.363	0.066
4	12.570	12.357	12.464	0.151
5	12.219	11.676	11.948	0.384
6	12.497	12.269	12.383	0.161
7	12.430	12.414	12.422	0.011
8	12.466	12.698	12.582	0.164
9	12.398	12.239	12.319	0.112
12	11.925	12.314	12.120	0.275
N:			10	
Mean, Pooled SD:			12.315	0.203
SD:			0.181	

Fall 2015 GMA Study				
Lab	**A**	**B**	**Mean**	**SD**
3	13.113	13.290	13.202	0.125
4	18.540	18.390	18.465	0.106
7	12.500	12.440	12.470	0.042
9				
10	12.754		12.754	
12				
13	12.376		12.376	
16				
18	17.569		17.569	
22				
24	12.710	12.640	12.675	0.049
26	18.187	18.190	18.189	0.002
29				
***N*:**			8	
Mean, Pooled SD:			14.712	0.079
SD:			2.805	

Spring 2016 GMA Study			
A	**B**	**Mean**	**SD**
13.310	12.960	13.135	0.247
18.690	18.830	18.760	0.099
12.800	12.820	12.810	0.014
17.998	17.992	17.995	0.004
12.920	12.720	12.820	0.141
11.750	11.550	11.650	0.141
12.300		12.300	
14.000	13.000	13.500	0.707
15.300	15.300	15.300	0.000
		9	
		14.252	0.276
		2.548	

The NIST oleic acid results as a function of box number are displayed in Fig. 42. The blue circles in the graphics represent the results for the first replicate and the red squares the second replicate. The mean mass fraction is indicated by the solid line, and the dashed lines bound the interval Mean ± 2×SD.

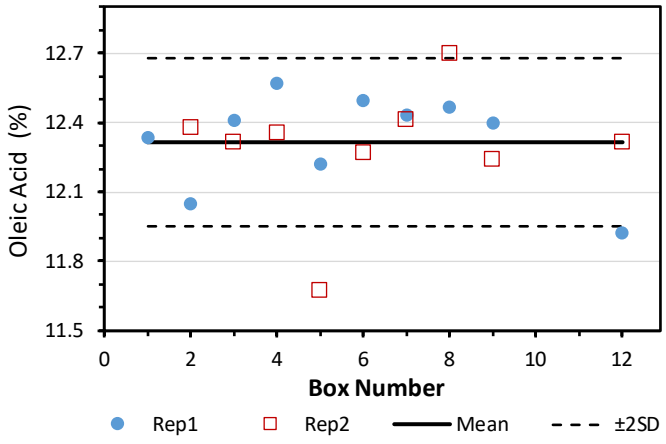

Fig. 42. Oleic Acid Mass Fraction as a Function of Box Number.

4.4.7. Vaccenic Acid (C18:1-11c)

The NIST GC-FID results and the vaccenic acid values reported by the participants in the Fall 2015 GMA Study and Spring 2016 GMA Study are summarized in Table 64. The table also provides several summary values: *N* = number of values, Mean = mean of values, SD = standard deviation of values, and Pooled SD = square-root of the sum of the squared within-box or within-participant standard deviations.

Table 64. Summary of Results for Vaccenic Acid, %.

NIST GC-FID					Fall 2015 GMA Study					Spring 2016 GMA Study			
Box	**A**	**B**	**Mean**	**SD**	**Lab**	**A**	**B**	**Mean**	**SD**	**A**	**B**	**Mean**	**SD**
1	1.167		1.167		3	3.213	3.285	3.249	0.051				
2	1.172	1.191	1.182	0.013	4	1.840	1.840	1.840	0.000	3.600	3.850	3.725	0.177
3	1.191	1.206	1.199	0.011	7	1.120	1.120	1.120	0.000				
4	1.186	1.172	1.179	0.010	9					1.980	1.960	1.970	0.014
5	1.177	1.129	1.153	0.034	10	1.145		1.145					
6	1.202	1.190	1.196	0.008	12					1.140	1.140	1.140	0.000
7	1.177	1.171	1.174	0.004	13	1.191		1.191		1.274	1.268	1.271	0.004
8	1.219	1.216	1.218	0.002	16					1.170	1.140	1.155	0.021
9	1.188	1.173	1.181	0.011	22					1.120		1.120	
12	1.152	1.192	1.172	0.028	24	1.150	1.150	1.150	0.000				
N:			10		25					1.260		1.260	
Mean, Pooled SD:			1.182	0.017	26	1.455	1.467	1.461	0.008				
SD:			0.018		28					1.180		1.180	
					***N*:**			7				8	
					Mean, Pooled SD:			1.594	0.023			1.603	0.080
					SD:			0.775				0.902	

The NIST vaccenic acid results as a function of box number are displayed in Fig. 43. The blue circles in the graphics represent the results for the first replicate and the red squares the second replicate. The mean mass fraction is indicated by the solid line, and the dashed lines bound the interval Mean ± 2×SD.

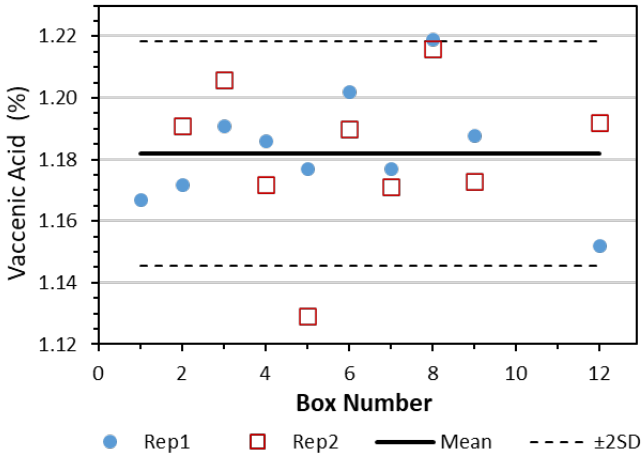

Fig. 43. Vaccenic Acid Mass Fraction as a Function of Box Number.

4.4.8. Total *cis*-C18:1 Fatty Acids

The NIST GC-FID results and the total *cis*-C18:1 fatty acid values reported by the participants in the Fall 2015 GMA Study and Spring 2016 GMA Study are summarized in Table 65. The table also provides several summary values: N = number of values, Mean = mean of values, SD = standard deviation of values, and Pooled SD = square-root of the sum of the squared within-box or within-participant standard deviations.

Table 65. Summary of Results for Total cis-C18:1 Fatty Acids, %.

NIST GC-FID				
Box	**A**	**B**	**Mean**	**SD**
1	13.502		13.502	
2	13.223	13.569	13.396	0.245
3	13.601	13.522	13.562	0.056
4	13.756	13.529	13.643	0.161
5	13.396	12.805	13.101	0.418
6	13.699	13.459	13.579	0.170
7	13.607	13.585	13.596	0.016
8	13.685	13.914	13.800	0.162
9	13.586	13.412	13.499	0.123
12	13.077	13.506	13.292	0.303
		***N*:**	10	
		Mean, Pooled SD:	13.497	0.218
		SD:	0.195	

Fall 2015 GMA Study				
Lab	**A**	**B**	**Mean**	**SD**
2	19.725	19.401	19.563	0.229
3	16.326	16.575	16.451	0.176
4	20.380	20.230	20.305	0.106
5				
6	12.145	12.406	12.276	0.185
7	13.660	13.590	13.625	0.049
9				
10	13.925		13.925	
12				
13	13.597		13.597	
16				
18	17.569		17.569	
22				
24	15.940	15.800	15.870	0.099
25				
26	19.693	19.669	19.681	0.017
27	16.323	15.036	15.679	0.910
28				
29				
		***N*:**	11	
		Mean, Pooled SD:	16.231	0.348
		SD:	2.767	

Spring 2016 GMA Study			
A	**B**	**Mean**	**SD**
16.910	16.800	16.855	0.078
15.430	15.530	15.480	0.071
20.306	17.812	19.059	1.764
20.680	20.800	20.740	0.085
13.970	13.990	13.980	0.014
19.317	19.302	19.310	0.011
16.880	16.570	16.725	0.219
13.400		13.400	
15.300		15.300	
16.600		16.600	
15.300	15.300	15.300	0.000
		11	
		16.614	0.630
		2.288	

The NIST total *cis*-C18:1 fatty acid results as a function of box number are displayed in Fig. 44. The blue circles in the graphics represent the results for the first replicate and the red squares the second replicate. The mean mass fraction is indicated by the solid line, and the dashed lines bound the interval Mean ± 2×SD.

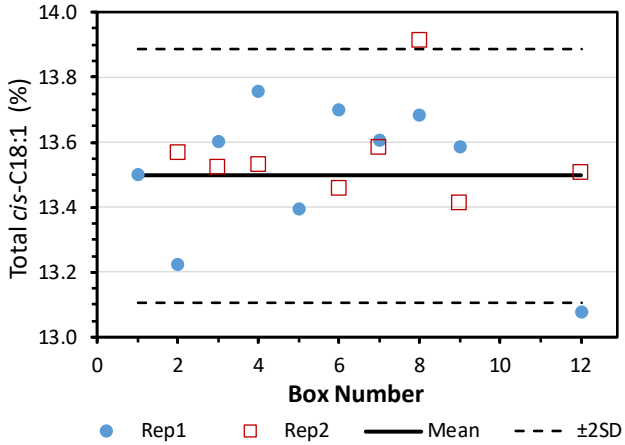

Fig. 44. Total *cis*-C18:1 Fatty Acids Mass Fraction as a Function of Box Number.

4.4.9. Total *trans*-C18:1 Fatty Acids

The total *trans*-C18:1 fatty acids values reported by laboratories participating in the Fall 2015 GMA Study and the Spring 2016 GMA Study are summarized in Table 66. The table also provides several summary values: *N* = number of values, Mean = mean of values, SD = standard deviation of values, and Pooled SD = square-root of the sum of the squared within-participant standard deviations.

Table 66. Summary of Results for Total trans-C18:1 Fatty Acids, %.

	Fall 2015 GMA Study				Spring 2016 GMA Study			
Lab	**A**	**B**	**Mean**	**SD**	**A**	**B**	**Mean**	**SD**
2	0.014	0.008	0.011	0.004				
4	0.030	0.040	0.035	0.007	0.037	0.192	0.115	0.110
6		0.010	0.010					
7	0.130	0.130	0.130	0.000	0.012	0.008	0.010	0.003
10	0.038		0.038					
12					0.110	0.120	0.115	0.007
13	0.013		0.013		0.006	0.006	0.006	0.000
16					0.010	0.050	0.030	0.028
18	0.008		0.008					
22					0.011		0.011	
24	0.040	0.040	0.040	0.000				
25					0.016		0.016	
26	0.006	0.006	0.006	0.000				
28					0.024		0.024	
29					0.028	0.029	0.029	0.001
		***N*:**	9				9	
		Mean, Pooled SD:	0.032	0.004			0.039	0.046
		SD:	0.039				0.043	

4.4.10. Linoleic Acid (C18:2-9,12c)

The NIST GC-FID results and the linoleic acid values reported by the participants in the Fall 2015 GMA Study and Spring 2016 GMA Study are summarized in Table 67. The table also provides several summary values: *N* = number of values, Mean = mean of values, SD = standard deviation of values, and Pooled SD = square-root of the sum of the squared within-box or within-participant standard deviations.

Table 67. Summary of Results for Linoleic Acid, %.

NIST GC-FID				
Box	**A**	**B**	**Mean**	**SD**
1	2.080		2.080	
2	2.086	2.126	2.106	0.028
3	2.131	2.139	2.135	0.006
4	2.151	2.082	2.117	0.049
5	2.093	1.997	2.045	0.068
6	2.152	2.109	2.131	0.030
7	2.091	2.099	2.095	0.006
8	2.159	2.190	2.175	0.022
9	2.114	2.102	2.108	0.008
12	2.080	2.123	2.102	0.030
		***N*:**	10	
		Mean, Pooled SD:	2.109	0.034
		SD:	0.034	

Fall 2015 GMA Study				
Lab	**A**	**B**	**Mean**	**SD**
3	2.101	2.152	2.127	0.036
4	3.060	3.040	3.050	0.014
5				
6	1.817	1.858	1.838	0.029
7	2.100	2.090	2.095	0.007
9				
10	2.108		2.108	
12				
13	2.083		2.083	
16				
18	2.218		2.218	
22				
25				
26	2.630	2.627	2.629	0.002
28				
29				
		***N*:**	8	
		Mean, Pooled SD:	2.268	0.022
		SD:	0.385	

Spring 2016 GMA Study			
A	**B**	**Mean**	**SD**
2.040	2.040	2.040	0.000
2.280	2.290	2.285	0.007
3.090	3.150	3.120	0.042
2.100	2.100	2.100	0.000
2.445	2.439	2.442	0.004
2.150	2.120	2.135	0.021
2.080		2.080	
2.330		2.330	
2.160		2.160	
2.350	2.340	2.345	0.007
		10	
		2.304	0.018
		0.316	

The NIST linoleic acid results as a function of box number are displayed in Fig. 45. The blue circles in the graphics represent the results for the first replicate and the red squares the second replicate. The mean mass fraction is indicated by the solid line, and the dashed lines bound the interval Mean ± 2×SD.

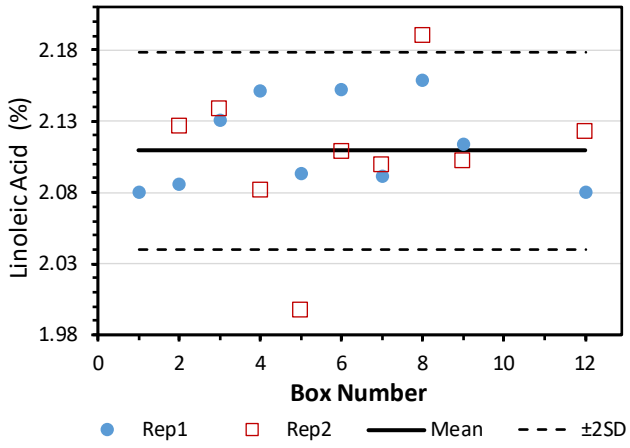

Fig. 45. Linoleic Acid Mass Fraction as a Function of Box Number.

4.4.11. Total *cis*-C18:2 Fatty Acids

The total *cis*-C18:2 fatty acids values reported by laboratories participating in the Fall 2015 GMA Study and the Spring 2016 GMA Study are summarized in Table 68. The table also provides several summary values: *N* = number of values, Mean = mean of values, SD = standard deviation of values, and Pooled SD = square-root of the sum of the squared within-participant standard deviations.

Table 68. Summary of Results for Total cis-C18:2 Fatty Acids, %.

	Fall 2015 GMA Study				Spring 2016 GMA Study			
Lab	**A**	**B**	**Mean**	**SD**	**A**	**B**	**Mean**	**SD**
2	0.014	0.008	0.011	0.004				
4	0.030	0.040	0.035	0.007	0.037	0.192	0.115	0.110
6		0.010	0.010					
7	0.130	0.130	0.130	0.000	0.012	0.008	0.010	0.003
10	0.038		0.038					
12					0.110	0.120	0.115	0.007
13	0.013		0.013		0.006	0.006	0.006	0.000
16					0.010	0.050	0.030	0.028
18	0.008		0.008					
22					0.011		0.011	
24	0.040	0.040	0.040	0.000				
25					0.016		0.016	
26	0.006	0.006	0.006	0.000				
28					0.024		0.024	
29					0.028	0.029	0.029	0.001
		***N*:**	9				9	
		Mean, Pooled SD:	0.032	0.004			0.039	0.046
		SD:	0.039				0.043	

4.4.12. Total *trans*-C18:2 Fatty Acids

The total *trans*-C18:2 fatty acids values reported by laboratories participating in the Fall 2015 GMA Study and the Spring 2016 GMA Study are summarized in Table 69. The table also provides several summary values: *N* = number of values, Mean = mean of values, SD = standard deviation of values, and Pooled SD = square-root of the sum of the squared within-participant standard deviations.

Table 69. Summary of Results for Total trans-C18:2 Fatty Acids, %.

	Fall 2015 GMA Study				Spring 2016 GMA Study			
Lab	**A**	**B**	**Mean**	**SD**	**A**	**B**	**Mean**	**SD**
2	0	0						
3	0	0						
4	4.440	4.460			0	0.060	0.060	
5	<0.01							
6	0.012	<0.01						
7	0.01	0.010	0.010	0.000	0.015	0.018	0.017	0.002
9					4.000	3.660		
10	0.011		0.011					
12					0.010	0.010	0.010	0.000
13	<0.01				0	0		
16					0.020	0.010	0.015	0.007
18	0.003		0.003					
24	0.110	0.050	0.080	0.042				
26	0.009	0.015	0.012	0.004				
27	4.340	3.940						
***N*:**			5				4	
Mean, Pooled SD:			0.023	0.025			0.025	0.004
SD:			0.032				0.023	

4.4.13. γ-Linolenic Acid (C18:3-6,9,12c)

The γ-linolenic acid values reported by laboratories participating in the Fall 2015 GMA Study and the Spring 2016 GMA Study are summarized in Table 70. The table also provides several summary values: *N* = number of values, Mean = mean of values, SD = standard deviation of values, and Pooled SD = square-root of the sum of the squared within-participant standard deviations.

Table 70. Summary of Results for γ-Linolenic Acid, %.

	Fall 2015 GMA Study				Spring 2016 GMA Study			
Lab	**A**	**B**	**Mean**	**SD**	**A**	**B**	**Mean**	**SD**
2	0.006		0.006					
3	0.032	0.017	0.025	0.011				
5					0.04	0.04	0.040	0.000
6	0.012	0.017	0.015	0.004				
7	0.010	0.010	0.010	0.000	0.009	0.007	0.008	0.001
10	0.010		0.010					
12					0.01	0.01	0.010	0.000
13	<0.01				0.012	0.006	0.009	0.004
16					<0.01	<0.01		
18					0.01	0.01	0.010	0.000
22					<0.01			
24	<0.01	<0.01						
25					<0.007			
26	0.006	0.009	0.008	0.002				
27	0.019	0.017	0.018	0.001				
28					<0.01			
		***N*:**	7				5	
		Mean, Pooled SD:	0.013	0.005			0.015	0.002
		SD:	0.007				0.014	

4.4.14. α-Linolenic Acid (C18:2-9,12c)

The NIST GC-FID results and the α-linolenic acid values reported by the participants in the Fall 2015 GMA Study and Spring 2016 GMA Study are summarized in Table 71. The table also provides several summary values: *N* = number of values, Mean = mean of values, SD = standard deviation of values, and Pooled SD = square-root of the sum of the squared within-box or within-participant standard deviations.

Table 71. Summary of Results for α-Linolenic Acid, %.

NIST GC-FID				
Box	**A**	**B**	**Mean**	**SD**
1	0.219		0.219	
2	0.214	0.221	0.218	0.005
3	0.222	0.227	0.225	0.004
4	0.222	0.220	0.221	0.001
5	0.218	0.200	0.209	0.013
6	0.226		0.226	
7	0.219	0.217	0.218	0.001
8	0.224	0.229	0.227	0.004
9	0.219	0.216	0.218	0.002
12	0.205	0.215	0.210	0.007
		***N*:**	10	
		Mean, Pooled SD:	0.219	0.006
		SD:	0.006	

Fall 2015 GMA Study				
Lab	**A**	**B**	**Mean**	**SD**
2	0.185	0.185	0.185	0.000
3	0.192	0.194	0.193	0.001
4				
5				
6	0.157	0.160	0.159	0.002
7	0.180	0.180	0.180	0.000
9				
10	0.192		0.192	
12				
13	0.185		0.185	
16				
18	0.217		0.217	
22				
24	0.190	0.190	0.190	0.000
25				
26	0.217	0.220	0.219	0.002
27	0.263	0.236	0.249	0.019
28				
29				
		***N*:**	10	
		Mean, Pooled SD:	0.197	0.007
		SD:	0.025	

Spring 2016 GMA Study			
A	**B**	**Mean**	**SD**
0.193	0.199	0.196	0.004
0.200	0.200	0.200	0.000
0.189	0.173	0.181	0.011
0.340	0.350	0.345	0.007
0.190	0.190	0.190	0.000
0.196	0.193	0.195	0.002
0.200	0.200	0.200	0.000
0.196		0.196	
0.219		0.219	
0.202		0.202	
0.234	0.232	0.233	0.001
		11	
		0.214	0.005
		0.046	

The NIST α-linolenic acid results as a function of box number are displayed in Fig. 46. The blue circles in the graphics represent the results for the first replicate and the red squares the second replicate. The mean mass fraction is indicated by the solid line, and the dashed lines bound the interval Mean ± 2×SD.

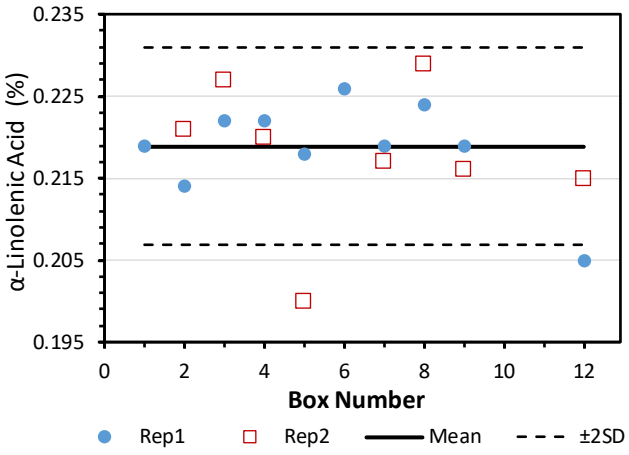

Fig. 46. α-Linolenic Acid Mass Fraction as a Function of Box Number.

4.4.15. Arachidic Acid (C20:0)

The arachidic acid values reported by laboratories participating in the Fall 2015 GMA Study and the Spring 2016 GMA Study are summarized in Table 72. The table also provides several summary values: *N* = number of values, Mean = mean of values, SD = standard deviation of values, and Pooled SD = square-root of the sum of the squared within-participant standard deviations.

Table 72. Summary of Results for Arachidic Acid, %.

	Fall 2015 GMA Study				Spring 2016 GMA Study			
Lab	**A**	**B**	**Mean**	**SD**	**A**	**B**	**Mean**	**SD**
2	0.014	0.011	0.013	0.002				
3	0.012	0.021	0.017	0.006				
4		0.050	0.050		0.015	0.026	0.021	0.008
6	0.017	0.017	0.017	0.000				
7	0.010	0.010	0.010	0.000	0.011	0.011	0.011	0.000
10	0.014		0.014					
12					0.010	0.020	0.015	0.007
13	0.014		0.014		0.027	0.030	0.029	0.002
16					0.030		0.020	0.014
18	0.015		0.015		0.020	0.020	0.020	0.000
22					0.010		0.010	
24	0.020	0.020	0.020	0.000				
25					0.015		0.015	
26	0.015	0.018	0.017	0.002				
27	0.021	0.019	0.020	0.001				
28					0.011		0.011	
29					0.011	0.012	0.012	0.001
		***N*:**	11				10	
		Mean, Pooled SD:	0.019	0.003			0.016	0.007
		SD:	0.011				0.006	

4.4.16. Total *cis*-C20:1 Fatty Acids

The NIST GC-FID results and the total *cis*-C20:1 fatty acid values reported by the participants in the Fall 2015 GMA Study and Spring 2016 GMA Study are summarized in Table 73. The table also provides several summary values: *N* = number of values, Mean = mean of values, SD = standard deviation of values, and Pooled SD = square-root of the sum of the squared within-box or within-participant standard deviations.

Table 73. Summary of Results for Total cis-C20:1 Fatty Acids, %.

NIST GC-FID				
Box	**A**	**B**	**Mean**	**SD**
1	0.037		0.037	
2	0.046	0.035	0.041	0.008
3	0.040	0.043	0.042	0.002
4	0.041	0.036	0.039	0.004
5	0.034	0.039	0.037	0.004
6	0.043	0.039	0.041	0.003
7	0.035	0.036	0.036	0.001
8	0.035	0.031	0.033	0.003
9	0.040	0.033	0.037	0.005
12	0.033	0.045	0.039	0.008
		***N*:**	10	
		Mean, Pooled SD:	0.038	0.005
		SD:	0.003	

Fall 2015 GMA Study				
Lab	**A**	**B**	**Mean**	**SD**
2	0.042	0.046	0.044	0.003
3	0.043	0.042	0.043	0.001
4	0.340	0.330		
5				
6	0.037	0.038	0.038	0.001
7	0.050	0.050	0.050	0.000
10	0.041		0.041	
12				
13	0.040		0.040	
16				
18	0.039		0.039	
22				
24	0.050	0.040	0.045	0.007
25				
26	0.549	0.561	0.555	0.008
27	0.055	0.050	0.052	0.003
28				
29				
		***N*:**	11	
		Mean, Pooled SD:	0.116	0.005
		SD:	0.170	

Spring 2016 GMA Study			
A	**B**	**Mean**	**SD**
0.040	0.050	0.045	0.007
0.060	0.060	0.060	0.000
0.045	0.042	0.044	0.002
0.050	0.050	0.050	0.000
0.059	0.056	0.058	0.002
0.050	0.050	0.050	0.000
0.040	0.040	0.040	0.000
0.039		0.039	
0.046		0.046	
0.040		0.040	
0.044	0.043	0.044	0.001
		11	
		0.047	0.003
		0.007	

The NIST total *cis*-C20:1 fatty acid results as a function of box number are displayed in Fig. 47. The blue circles in the graphics represent the results for the first replicate and the red squares the second replicate. The mean mass fraction is indicated by the solid line, and the dashed lines bound the interval Mean ± 2×SD.

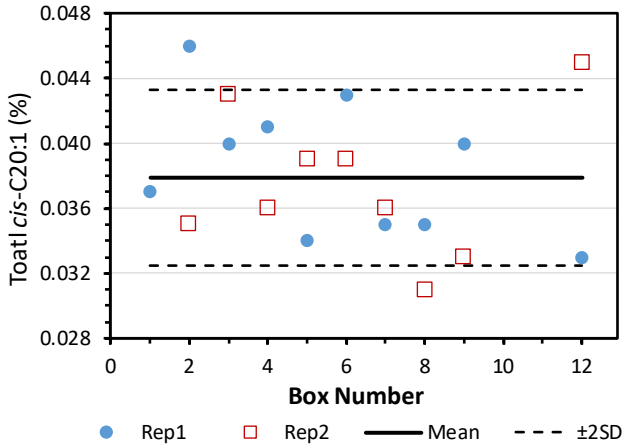

Fig. 47. Total *cis*-C20:1 Fatty Acids Mass Fraction as a Function of Box Number.

4.4.17. Eicosadienoic Acid (C20:2-11,14c)

The eicosadienoic acid values reported by laboratories participating in the Fall 2015 GMA Study and the Spring 2016 GMA Study are summarized in Table 74. The table also provides several summary values: *N* = number of values, Mean = mean of values, SD = standard deviation of values, and Pooled SD = square-root of the sum of the squared within-participant standard deviations.

Table 74. Summary of Results for Eicosadienoic Acid, %.

	Fall 2015 GMA Study				Spring 2016 GMA Study			
Lab	**A**	**B**	**Mean**	**SD**	**A**	**B**	**Mean**	**SD**
2	0	0						
3	0.023	0.025	0.024	0.001				
4	0.370	0.390			0.020	0.020	0.020	0.000
6	<0.01	<0.01						
7	<0.01	<0.01			0	0		
9					0.28	0.23		
10	<0.005							
12					<0.01			
13	<0.01				0			
16					<0.01			
18	0.011		0.011					
22					<0.01			
24	0.020	0.020	0.020	0.000				
25					<0.007			
26	0.275	0.272						
27	0.006	0.007	0.006	0.000				
28					<0.01			
29					<0.01	<0.01		
		***N*:**	4				1	
		Mean, Pooled SD:	0.015	0.001			0.020	0.000
		SD:	0.008					

4.4.18. Behenic Acid (C22:0)

The behenic acid values reported by laboratories participating in the Fall 2015 GMA Study and the Spring 2016 GMA Study are summarized in Table 75. The table also provides several summary values: *N* = number of values, Mean = mean of values, SD = standard deviation of values, and Pooled SD = square-root of the sum of the squared within-participant standard deviations.

Table 75. Summary of Results for Behenic Acid, %.

	Fall 2015 GMA Study				Spring 2016 GMA Study			
Lab	**A**	**B**	**Mean**	**SD**	**A**	**B**	**Mean**	**SD**
2	0.081	0.114	0.098	0.023				
3	0.133	0.132	0.133	0.001				
4					0.200	0.220	0.210	0.014
5					0.020	0.020	0.020	0.000
6	<0.01	<0.01						
7	0.02	0.02	0.020	0.000	0.416	0.227		
10	0.009		0.009					
12					0.02	0.01	0.015	0.007
13	0.011		0.011		0	0		
16					<0.01	<0.01		
18					0.040	0.020	0.030	0.014
24	0.130	0.130	0.130	0.000				
27	0.020	0.020	0.020	0.000				
		***N*:**	6				4	
		Mean, Pooled SD:	0.054	0.000			0.069	0.011
		SD:	0.060					

4.4.19. Lignoceric Acid (C24:0)

The lignoceric acid values reported by laboratories participating in the Fall 2015 GMA Study and the Spring 2016 GMA Study are summarized in Table 76. The table also provides several summary values: *N* = number of values, Mean = mean of values, SD = standard deviation of values, and Pooled SD = square-root of the sum of the squared within-participant standard deviations.

Table 76. Summary of Results for Lignoceric Acid, %.

	Fall 2015 GMA Study				Spring 2016 GMA Study			
Lab	**A**	**B**	**Mean**	**SD**	**A**	**B**	**Mean**	**SD**
3	0.018	0.019	0.019	0.001				
2	0.023	0.029	0.026	0.004				
4	0.030	0.030	0.030	0.000	0.020	0.020	0.020	0.000
5					0.110	0.090	0.100	0.014
6	<0.01	<0.01						
7	0.060	0.060	0.060	0.000	0.022	0.016	0.019	0.004
10	0.084		0.084					
12					0.020	0.020	0.020	0.000
13	0.025		0.025		0.012	0.012	0.012	0.000
16					0.020	0.020	0.020	0.000
18	0.020		0.020		0.020	0.020	0.020	0.000
22					0.019		0.019	
24	0.030	0.030	0.030	0.000				
25					0.027		0.027	
26	0.018	0.018	0.018	0.000				
27	0.027	0.044	0.036	0.012				
28					0.020		0.020	
		***N*:**	10				10	
		Mean, Pooled SD:	0.035	0.005			0.028	0.006
		SD:	0.021				0.026	

4.4.20. Total ω-3 Fatty Acids

The total ω-3 fatty acid values reported by laboratories participating in the Fall 2015 GMA Study and the Spring 2016 GMA Study are summarized in Table 77. The table also provides several summary values: *N* = number of values, Mean = mean of values, SD = standard deviation of values, and Pooled SD = square-root of the sum of the squared within-participant standard deviations.

Table 77. Summary of Results for Total ω-3 Fatty Acids, %.

	Fall 2015 GMA Study				Spring 2016 GMA Study			
Lab	**A**	**B**	**Mean**	**SD**	**A**	**B**	**Mean**	**SD**
2	0.20	0.20	0.20	0.00				
3	0.19	0.24	0.22	0.03				
4	0.34	0.33	0.34	0.01	0.24	0.29	0.27	0.04
5	0.33	0.32	0.33	0.00	0.25	0.25	0.25	0.00
6	0.17	0.17	0.17	0.00				
7	0.19	0.19	0.19	0.00	0.20	0.18	0.19	0.01
9					0.34	0.35	0.35	0.01
10	0.2		0.20					
12					0.19	0.19	0.19	0.00
13	0.195		0.20		2.649	2.717		
16					0.2	0.2	0.20	0.00
18					0.2	0.2	0.20	0.00
22					0.20		0.20	
24	0.19	0.19	0.19	0.00		0.219	0.22	
25								
26	1.27	1.19						
28					0.214		0.21	
29					0.246	0.232	0.24	0.01
		***N*:**	9				11	
		Mean, Pooled SD:	0.22	0.01			0.23	0.01
		SD:	0.06				0.05	

4.4.21. Total ω-6 Fatty Acids

The total ω-6 fatty acid values reported by laboratories participating in the Fall 2015 GMA Study and the Spring 2016 GMA Study are summarized in Table 78. The table also provides several summary values: *N* = number of values, Mean = mean of values, SD = standard deviation of values, and Pooled SD = square-root of the sum of the squared within-participant standard deviations.

Table 78. Summary of Results for Total ω-6 Fatty Acids, %.

	Fall 2015 GMA Study				Spring 2016 GMA Study			
Lab	**A**	**B**	**Mean**	**SD**	**A**	**B**	**Mean**	**SD**
2	2.37	2.34	2.36	0.02				
3	2.16	2.19	2.18	0.03				
4	3.43	3.43	3.43	0.00	2.07	2.20	2.14	0.09
5	2.08	2.09	2.09	0.01				
6	1.83	1.87	1.85	0.03				
7	2.11	2.10	2.11	0.01	2.37	2.12	2.25	0.18
9					3.51	3.50	3.51	0.01
10	2.12		2.12					
12					2.12	2.13	2.13	0.01
13	2.08		2.08		2.45	2.45	2.45	0.01
16					2.16	2.12	2.14	0.03
18					2.03	2.00	2.02	0.02
22					2.08		2.08	
24	2.16	2.15	2.16	0.01				
25					2.33		2.33	
26	2.92	2.92	2.92	0.00				
28					2.16		2.16	
29					2.35	2.34	2.35	0.01
		***N*:**	10				11	
		Mean, Pooled SD:	2.33	0.02			2.32	0.07
		SD:	0.48				0.41	

4.4.22. Saturated Fat

The saturated fat values reported by laboratories participating in the Fall 2015 GMA Study and the Spring 2016 GMA Study are summarized in Table 79. The table also provides several summary values: *N* = number of values, Mean = mean of values, SD = standard deviation of values, and Pooled SD = square-root of the sum of the squared within-participant standard deviations.

Table 79. Summary of Results for Saturated Fat, %.

	Fall 2015 GMA Study				Spring 2016 GMA Study			
Lab	**A**	**B**	**Mean**	**SD**	**A**	**B**	**Mean**	**SD**
2	3.84	3.72	3.78	0.08				
3	3.44	3.49	3.46	0.03				
4	4.88	4.82	4.85	0.04	3.46	3.46	3.46	0.00
5	3.23	3.25	3.24	0.01	3.20	3.23	3.22	0.02
6	2.91	2.97	2.94	0.04				
7	3.57	3.50	3.54	0.05	4.23	3.63	3.93	0.42
9					4.72	4.79	4.76	0.05
10	3.32		3.32					
12					3.44	3.48	3.46	0.03
13	3.17		3.17		3.81	3.784	3.80	0.02
16					3.45	3.36	3.41	0.06
18	3.29		3.29		3.09	3.03	3.06	0.04
22					3.45		3.45	
24	3.57	3.49	3.53	0.06				
25					3.58		3.58	
26	4.25	4.33	4.29	0.06				
27	4.19	3.89	4.04	0.21				
28					3.36		3.36	
29					3.71	3.73	3.72	0.01
		***N*:**	12				12	
		Mean, Pooled SD:	3.62	0.09			3.60	0.15
		SD:	0.54				0.44	

4.4.23. *cis*-Monounsaturated Fat

The *cis*-monounsaturated fat values reported by laboratories participating in the Fall 2015 GMA Study and the Spring 2016 GMA Study are summarized in Table 80. The table also provides several summary values: *N* = number of values, Mean = mean of values, SD = standard deviation of values, and Pooled SD = square-root of the sum of the squared within-participant standard deviations.

Table 80. Summary of Results for cis-Monosaturated Fat, %.

	Fall 2015 GMA Study				Spring 2016 GMA Study			
Lab	**A**	**B**	**Mean**	**SD**	**A**	**B**	**Mean**	**SD**
2	20.99	20.66	20.83	0.23				
3	17.47	17.74	17.61	0.19				
4	22.23	22.07	22.15	0.11	17.38	17.33	17.36	0.04
5	14.90	15.00	14.95	0.07	16.55	16.67	16.61	0.08
6	13.19	13.47	13.33	0.20				
7	14.87	14.81	14.84	0.04	21.57	18.97	20.27	1.84
9					22.36	22.53	22.45	0.12
10	15.04		15.04					
12					15.18	15.21	15.20	0.02
13	14.70		14.70		20.65	20.61	20.63	0.03
16					18.09	17.77	17.93	0.23
18	17.95		17.95		13.81	13.62	13.72	0.13
22					14.50		14.50	
24	17.18	17.03	17.11	0.11				
25					16.50		16.50	
26	21.68	21.68	21.68	0.00				
27	17.77	16.39	17.08	0.98				
28					17.80		17.80	
29					16.60	16.50	16.55	0.07
		***N*:**	12				12	
		Mean, Pooled SD:	17.27	0.35			17.46	0.62
		SD:	2.94				2.59	

4.4.24. *cis*-Polyunsaturated Fat

The *cis*-polyunsaturated fat values reported by laboratories participating in the Fall 2015 GMA Study and the Spring 2016 GMA Study are summarized in Table 81. The table also provides several summary values: *N* = number of values, Mean = mean of values, SD = standard deviation of values, and Pooled SD = square-root of the sum of the squared within-participant standard deviations.

Table 81. Summary of Results for cis-Polyunsaturated Fat, %.

	Fall 2015 GMA Study				Spring 2016 GMA Study			
Lab	**A**	**B**	**Mean**	**SD**	**A**	**B**	**Mean**	**SD**
2	2.57	2.54	2.56	0.02				
3	2.39	2.48	2.44	0.06				
4	3.80	3.93	3.87	0.09	2.30	2.43	2.37	0.09
5	2.41	2.42	2.42	0.01	2.54	2.55	2.55	0.01
6	1.99	2.04	2.02	0.03				
7	2.35	2.33	2.34	0.01	2.56	2.29	2.43	0.19
9					3.70	3.73	3.72	0.02
10	2.32		2.32					
12					2.35	2.37	2.36	0.01
13	2.28		2.28		5.141	5.203	5.17	0.04
16					2.38	2.34	2.36	0.03
18	2.35		2.35		2.23	2.2	2.22	0.02
22					2.29		2.29	
24	2.38	2.38	2.38	0.00				
25					2.55		2.55	
26	4.21	4.14	4.17	0.05				
27	3.02	2.77	2.90	0.18				
28					2.37		2.37	
29					2.60	2.58	2.59	0.01
		***N*:**	12				12	
		Mean, Pooled SD:	2.67	0.07			2.75	0.07
		SD:	0.67				0.86	

4.4.25. Total *trans*-Fat

The total *trans*-fat values reported by laboratories participating in the Fall 2015 GMA Study and the Spring 2016 GMA Study are summarized in Table 82. The table also provides several summary values: *N* = number of values, Mean = mean of values, SD = standard deviation of values, and Pooled SD = square-root of the sum of the squared within-participant standard deviations.

Table 82. Summary of Results for Total trans-Fat, %.

	Fall 2015 GMA Study				Spring 2016 GMA Study			
Lab	**A**	**B**	**Mean**	**SD**	**A**	**B**	**Mean**	**SD**
2	0.040	0.040	0.040	0.000				
3	0.018	0.019	0.019	0.001				
4	4.480	4.570			0.050	0.260	0.155	0.148
5	<0.01	<0.01						
6	0.020	0.010	0.015	0.007				
7	0.150	0.140	0.145	0.007	0.060	0.050	0.055	0.007
9					4.000	3.660		
10	0.090		0.090					
12					0.120	0.130	0.125	0.007
13	0.013		0.013		0.039	0.039	0.039	0.000
16					0.060	0.070	0.065	0.007
18	0.060		0.060		0.030	0.030	0.030	0.000
22					0.026	0.000	0.013	0.018
24	0.170	0.120	0.145	0.035				
25								
26	0.045	0.048	0.047	0.002				
27	4.350	3.650						
28					0.024		0.024	
29					0.028	0.029	0.029	0.001
		***N*:**	9				9	
		Mean, Pooled SD:	0.064	0.015			0.059	0.053
		SD:	0.052				0.049	

4.4.26. Value Assignment

As described in Section 3.3, the available data for each measurand was used to provide an estimate of the mass fraction present in SRM 2386 where x is the mean and $U_{95}(x)$ is the 95% confidence interval. The summary of these estimates for fatty acids is provided in Table 83, along with a summary of the methods used to arrive at these estimates. A blank in the table indicates that no data from that method was available for determination of the estimate.

Table 83. Summary of Estimates for Fatty Acids in SRM 2386, %.

			Based on		
Analyte	x	$U_{95}(x)$	**NIST Methods**	**Fall 2015 GMA Methods[a]**	**Spring 2016 GMA Methods[a]**
C12:0 Lauric Acid	0.0353	0.0493[b]	GC-FID	GC-FID	GC-FID
C14:0 Myristic Acid	0.0143	0.0038		GC-FID	GC-FID
C16:0 Palmitic Acid	3.277	0.1668	GC-FID	GC-FID	GC-FID
C16:1-9c Palmitoleic Acid	1.1862	0.0361	GC-FID	GC-FID	GC-FID
C18:0 Stearic Acid	0.1053	0.0059	GC-FID	GC-FID	GC-FID
C18:1-9c Oleic Acid	12.9468	0.5322	GC-FID	GC-FID	GC-FID
C18:1-11c Vaccenic Acid	1.2428	0.1146	GC-FID	GC-FID	GC-FID
Total *cis*-C18:1	15.9919	2.3364	GC-FID	GC-FID	GC-FID
Total *trans*-C18:1	0.0169	0.0205[b]		GC-FID	GC-FID
C18:2-9,12c Linoleic Acid	2.2193	0.0705	GC-FID	GC-FID	GC-FID
Total *cis*-C18:2	2.2192	0.0609		GC-FID	GC-FID
C18:3-9,12,15c α-Linolenic Acid	0.2144	0.0222	GC-FID	GC-FID	GC-FID
C18:3-6,9,12c γ-Linolenic Acid	0.0105	0.0049		GC-FID	GC-FID
C20:0 Arachidic Acid	0.0168	0.0035		GC-FID	GC-FID
Total *cis*-C20:1	0.0435	0.0045	GC-FID	GC-FID	GC-FID
C20:2-11,14c Eicosadienoic Acid	0.0231	0.1917[b]		GC-FID	GC-FID
C24:0 Lignoceric Acid	0.0255	0.0085		GC-FID	GC-FID
Saturated Fat	3.6558	0.1890		GC-FID	GC-FID
cis-Monounsaturated Fat	17.9171	1.1130		GC-FID	GC-FID
cis-Polyunsaturated Fat	2.5194	0.2362		GC-FID	GC-FID
Total *trans*-Fat	0.0434	0.0270		GC-FID	GC-FID
Total Omega-3 Fatty Acids	0.2181	0.0222		GC-FID	GC-FID
Total Omega-6 Fatty Acids	2.2604	0.1607		GC-FID	GC-FID

a Not all laboratories reported methods used.
b The expanded uncertainty is larger than the value, indicating a large level of variability. Any interval for the value should be truncated at zero.
GC-FID Gas Chromatography with Flame Ionization Detection

4.5. Proximates

Results for proximates provided by the Fall 2015 GMA Study were provided on an as-received basis but converted to a dry-mass basis for the Certificate of Analysis (COA), except ash.

4.5.1. Total Fat (Sum of Fatty Acids as Triglycerides)

The total fat values reported by laboratories participating in the Fall 2015 GMA Study are summarized in Table 84. The table also provides several summary values: *N* = number of values, Mean = mean of values, SD = standard deviation of values, and Pooled SD = square-root of the sum of the squared within-participant standard deviations.

Table 84. Summary of Results for Total Fat, %.

Lab	A	B	Mean	SD	Method
2	28.18	28.69	28.44	0.36	Sum of fatty acids as triglycerides
3	24.41	24.83	24.62	0.30	Sum of fatty acids as triglycerides
4	35.39	35.39	35.39	0.00	Sum of fatty acids as triglycerides
5	20.50	20.70	20.60	0.14	Sum of fatty acids as triglycerides
6	31.89	32.02	31.96	0.09	Sum of fatty acids as triglycerides
7	35.90	35.75	35.83	0.11	Sum of fatty acids as triglycerides
18	24.80		24.80		Sum of fatty acids as triglycerides
24	24.41	24.23	24.32	0.13	Sum of fatty acids as triglycerides
25	29.96	29.78	29.87	0.13	Sum of fatty acids as triglycerides
26	31.93	31.63	31.78	0.21	Sum of fatty acids as triglycerides
27	30.67	28.24	29.46	1.72	Sum of fatty acids as triglycerides
		***N*:**	11		
		Mean, Pooled SD:	28.82	0.57	
		SD:	2.92		

4.5.2. Ash

The ash values reported by laboratories participating in the Fall 2015 GMA Study are summarized in Table 85. The table also provides several summary values: *N* = number of values, Mean = mean of values, SD = standard deviation of values, and Pooled SD = square-root of the sum of the squared within-participant standard deviations.

Table 85. Summary of Results for Ash, %.

Lab	A	B	Mean	SD	Method
2	7.16	7.16	7.16	0.00	Weight loss
3	6.51	6.85	6.68	0.24	Weight loss
4	5.80	5.71	5.76	0.06	Weight loss
5	7.31	7.45	7.38	0.10	Weight loss
6	7.54	7.55	7.55	0.01	Weight loss
7	14.10	14.06	14.08	0.03	Weight loss
10	15.20	12.10	13.65	2.19	*not reported*
13	13.40		13.40		*not reported*
16	5.90	6.10	6.00	0.14	*not reported*
18	6.79		6.79		Weight loss
24	7.68	7.70	7.69	0.01	Weight loss
25	8.09	8.09	8.09	0.00	Weight loss
26	8.20	8.35	8.28	0.11	Weight loss
27	7.19	7.18	7.19	0.01	Weight loss
		***N*:**	14		
		Mean, Pooled SD:	8.55	0.64	
		SD:	1.54		

4.5.3. Protein

The protein values reported by laboratories participating in the Fall 2015 GMA Study are summarized in Table 86. The table also provides several summary values: *N* = number of values, Mean = mean of values, SD = standard deviation of values, and Pooled SD = square-root of the sum of the squared within-participant standard deviations.

Table 86. Summary of Results for Protein, %.

Lab	A	B	Mean	SD	Method
2	9.94	9.01	9.48	0.66	Kjeldahl, factor 6.25
3	9.02	9.34	9.18	0.23	Combustion - Leco
4	9.04	8.75	8.90	0.21	Kjeldahl, factor 6.25
5	9.11	9.02	9.07	0.06	Kjeldahl
6	10.09	9.99	10.04	0.07	Combustion - Leco
7	9.79	9.95	9.87	0.11	*Not reported*
10	9.72		9.72		*Not reported*
11	9.61		9.61		*Not reported*
13	10.40		10.40		*Not reported*
16	9.10	9.10	9.10	0.00	Kjeldahl
18	9.73		9.73		Combustion - Leco; Kjeldahl, factor 6.25
24	9.55	9.38	9.47	0.12	Kjeldahl
25	8.17	8.17	8.17	0.00	Kjeldahl
26	9.64	9.70	9.67	0.04	Combustion - Leco
27	9.42	9.48	9.45	0.04	Combustion - Leco
		***N*:**	14		
		Mean, Pooled SD:	9.46	0.24	
		SD:	0.29		

4.5.4. Carbohydrates

The carbohydrate values reported by laboratories participating in the Fall 2015 GMA Study are summarized in Table 87. The table also provides several summary values: *N* = number of values, Mean = mean of values, SD = standard deviation of values, and Pooled SD = square-root of the sum of the squared within-participant standard deviations.

Table 87. Summary of Results for Carbohydrates, %.

Lab	A	B	Mean	SD	Method
2	47.96	49.32	48.64	0.96	Solids-(protein+fat+ash)
3	54.93	54.51	54.72	0.30	Solids-(protein+fat+ash)
4	45.80	46.38	46.09	0.41	Solids-(protein+fat+ash)
5	54.40	54.40	54.40	0.00	Solids-(protein+fat+ash)
7	48.71	48.76	48.74	0.04	Solids-(protein+fat+ash)
10	45.90		45.90		*not reported*
11	38.90		38.90		*not reported*
13	39.30		39.30		*not reported*
18	51.80		51.80		Solids-(protein+fat+ash)
24	41.69	41.69	41.69	0.00	Solids-(protein+fat+ash)
25	29.41	29.57	29.49	0.11	Solids-(protein+fat+ash)
26	45.75	45.79	45.77	0.03	Solids-(protein+fat+ash)
27	49.33	51.69	50.51	1.67	Solids-(protein+fat+ash)
		***N*:**	13		
		Mean, Pooled SD:	45.84	0.67	
		SD:	1.99		

4.5.5. Total Dietary Fiber

The total dietary fiber values reported by laboratories participating in the Fall 2015 GMA Study are summarized in Table 88. The table also provides several summary values: *N* = number of values, Mean = mean of values, SD = standard deviation of values, and Pooled SD = square-root of the sum of the squared within-participant standard deviations.

Table 88. Summary of Results for Dietary Fiber, %.

Lab	A	B	Mean	SD	Method
2	21.02	21.31	21.17	0.21	Other
3	21.08	21.27	21.18	0.13	AOAC 985.29
4	20.60	20.40	20.50	0.14	AOAC 985.29
5	18.30	18.70	18.50	0.28	AOAC 985.29
6	30.30	28.80	29.55	1.06	*not reported*
7	8.00	8.00	8.00	0.00	*not reported*
10	21.10		21.10		*not reported*
13	20.10		20.10		*not reported*
16	18.20	21.30	19.75	2.19	AOAC 985.29
18	23.80		23.80		AOAC 985.29
24	21.95	21.74	21.85	0.15	AOAC 985.29
25	16.91	16.91	16.91	0.00	AOAC 985.29
		N:	12		
		Mean, Pooled SD:	20.20	0.82	
		SD:	2.40		

4.5.6. Total Sugars

The total sugars values reported by laboratories participating in the Fall 2015 GMA Study are summarized in Table 89. The table also provides several summary values: *N* = number of values, Mean = mean of values, SD = standard deviation of values, and Pooled SD = square-root of the sum of the squared within-participant standard deviations.

Table 89. Summary of Results for Total Sugars, %.

Lab	A	B	Mean	SD	Method
2	1.90	1.90	1.90	0.00	LC-RI
3	2.58	2.92	2.75	0.24	LC-ELSD
5	2.90	3.00	2.95	0.07	LC-amperometric
7	2.60	2.68	2.64	0.05	*not reported*
10	2.30		2.30		*not reported*
11	2.40		2.40		*not reported*
13	2.50		2.50		*not reported*
16	1.63	1.66	1.65	0.02	LC-RI
18	2.53		2.53		LC-RI
24	2.87	2.95	2.91	0.06	LC-ELSD
26	2.21	2.16	2.19	0.04	LC-RI
27	6.34	5.98	6.16	0.25	LC-RI
		***N*:**	12		
		Mean, Pooled SD:	2.74	0.13	
		SD:	0.66		

LC-amperometric Liquid Chromatography with Amperometric Detection
LC-ELSD Liquid Chromatography with Evaporative Light Scattering Detection
LC-RI Liquid Chromatography with Refractive Index Detection

4.5.7. Calories

The calorie values reported by laboratories participating in the Fall 2015 GMA Study are summarized in Table 90. The table also provides several summary values: N = number of values, Mean = mean of values, SD = standard deviation of values, and Pooled SD = square-root of the sum of the squared within-participant standard deviations.

Table 90. Summary of Results for Calories, kcal/100 g.

Lab	A	B	Mean	SD	Method
2	487.0	487.0	487.0	0.0	9(fat)+4(protein)+4(carbohydrate)
3	475.0	479.0	477.0	2.8	9(fat)+4(protein)+4(carbohydrate)
4	538.0	539.0	538.5	0.7	9(fat)+4(protein)+4(carbohydrate)
5	449.0	449.0	449.0	0.0	9(fat)+4(protein)+4(carbohydrate)
7	431.0	430.0	430.5	0.7	9(fat)+4(protein)+4(carbohydrate)
10	441.0		441.0		*not reported*
11	508.0		508.0		*not reported*
13	400.0		400.0		*not reported*
18	469.0		469.0		9(fat)+4(protein)+4(carbohydrate)
24	518.0	520.0	519.0	1.4	9(fat)+4(protein)+4(carbohydrate)
25	420.0	419.0	419.5	0.7	9(fat)+4(protein)+4(carbohydrate)
26	508.9	506.6	507.8	1.6	9(fat)+4(protein)+4(carbohydrate)
27	511.0	499.0	505.0	8.5	9(fat)+4(protein)+4(carbohydrate)
		***N*:**	13		
		Mean, Pooled SD:	473.2	3.1	
		SD:	23.6		

4.5.8. Value Assignment

As described in Section 3.3, the available data for each measurand was used to provide an estimate of the mass fraction present in SRM 2386 where x is the mean and $U_{95}(x)$ is the 95% confidence interval. The summary of these estimates for proximates is provided in Table 91, along with a summary of the methods used to arrive at these estimates. A blank in the table indicates that no data from that method was available for determination of the estimate.

Table 91. Summary of Estimates for Proximates in SRM 2386.

				Based on
Analyte	x	$U_{95}(x)$	**Units**	**Fall 2015 GMA Methods[a]**
Ash	7.46	1.42	%	Weight loss on drying
Protein	9.96	0.37	%	Kjeldahl, Leco Combustion (Factor of 6.25)
Fat	30.95	3.64	%	Sum of fatty acids as triglycerides
Carbohydrates	48.43	4.39	%	Calculation [Solids-(protein+fat+ash)]
Total Dietary Fiber	21.86	2.64	%	AOAC 985.29
Total Sugars	2.64	0.51	%	LC-RI, LC-ELSD, LC-AMP
Calories	501.26	30.69	kcal/100 g	Calculation [9(fat)+4(protein)+4(carbohydrate)]

a Not all laboratories reported methods used.
LC-RI Liquid Chromatography with Refractive Index Detection
LC-ELSD Liquid Chromatography with Evaporative Light Scattering Detection
LC-AMP Liquid Chromatography with Amperometric Detection

4.6. Amino Acids

Results for amino acids provided by the Fall 2015 GMA Study were provided on an as-received basis but converted to a dry-mass basis for the Certificate of Analysis (COA). All participants who reported method information for amino acids used liquid chromatography following hydrolysis and derivatization. Some laboratories did not report the method used.

4.6.1. Fall 2015 GMA Study

The amino acid values reported by laboratories participating in the Fall 2015 GMA Study are summarized in Table 92. The table also provides several summary values: *N* = number of values, Mean = mean of values, SD = standard deviation of values, and Pooled SD = square-root of the sum of the squared within-participant standard deviations.

Table 92. Summary of Results for Amino Acids, %.

	Alanine				Arginine				Aspartic Acid			
Lab	**A**	**B**	**Mean**	**SD**	**A**	**B**	**Mean**	**SD**	**A**	**B**	**Mean**	**SD**
3	0.402	0.408	0.405	0.004	0.360	0.380	0.370	0.014	0.676	0.685	0.681	0.006
5	0.460	0.470	0.465	0.007	0.367	0.371	0.369	0.003	0.770	0.780	0.775	0.007
7	0.494	0.495	0.495	0.001	0.397	0.408	0.403	0.008	0.846	0.833	0.840	0.009
10	0.468		0.468		0.451		0.451		0.787		0.787	
13	0.490		0.490		0.500		0.500		0.820		0.820	
***N*:**			5				5				5	
Mean, Pooled SD:			0.465	0.005			0.419	0.009			0.780	0.008
SD:			0.036				0.056				0.061	

	Cystine				Glutamic Acid				Glycine			
Lab	**A**	**B**	**Mean**	**SD**	**A**	**B**	**Mean**	**SD**	**A**	**B**	**Mean**	**SD**
3					0.926	0.941	0.934	0.011	0.357	0.367	0.362	0.007
5					1.030	1.050	1.040	0.014	0.400	0.420	0.410	0.014
7	0.093	0.080	0.087	0.009	1.150	1.141	1.146	0.006	0.454	0.459	0.457	0.004
10	0.113		0.113		1.080		1.080		0.432		0.432	
13	0.080		0.080		1.120		1.120		0.420		0.420	
***N*:**			3				5				5	
Mean, Pooled SD:			0.093	0.009			1.064	0.011			0.416	0.009
SD:			0.017				0.083				0.035	

	Histidine				Isolucine				Leucine			
Lab	**A**	**B**	**Mean**	**SD**	**A**	**B**	**Mean**	**SD**	**A**	**B**	**Mean**	**SD**
3	0.135	0.142	0.139	0.005	0.210	0.212	0.211	0.001	0.470	0.475	0.473	0.004
5	0.180	0.190	0.185	0.007	0.260	0.300	0.280	0.028	0.550	0.580	0.565	0.021
7	0.233	0.223	0.228	0.007	0.374	0.367	0.371	0.005	0.613	0.613	0.613	0.000
10	0.171		0.171		0.370		0.370		0.609		0.609	
13	0.200		0.200		0.410		0.410		0.670		0.670	
***N*:**			5				5				5	
Mean, Pooled SD:			0.185	0.006			0.328	0.017			0.586	0.012
SD:			0.033				0.081				0.074	

Lysine

Lab	A	B	Mean	SD
3	0.287	0.299	0.293	0.008
5	0.440	0.460	0.450	0.014
7	0.641	0.535	0.588	0.075
10	0.439		0.439	
13	0.530		0.530	
		***N*:**	5	
		Mean, Pooled SD:	0.460	0.044
		SD:	0.111	

Methionine

A	B	Mean	SD
0.105	0.102	0.104	0.002
0.172	0.169	0.171	0.002
0.134		0.134	
0.170		0.170	
		4	
		0.145	0.002
		0.032	

Phenylalanine

A	B	Mean	SD
0.278	0.279	0.279	0.001
0.310	0.330	0.320	0.014
0.368	0.354	0.361	0.010
0.350		0.350	
0.400		0.400	
		5	
		0.342	0.010
		0.046	

Proline

Lab	A	B	Mean	SD
3	0.277	0.286	0.282	0.006
5	0.410	0.390	0.400	0.014
7	0.444	0.460	0.452	0.011
10	0.413		0.413	
13	0.420		0.420	
		***N*:**	5	
		Mean, Pooled SD:	0.393	0.011
		SD:	0.065	

Serine

A	B	Mean	SD
0.428	0.440	0.434	0.008
0.490	0.490	0.490	0.000
0.509	0.494	0.502	0.011
0.465		0.465	
0.490		0.490	
		5	
		0.476	0.008
		0.027	

Threonine

A	B	Mean	SD
0.288	0.293	0.291	0.004
0.370	0.390	0.380	0.014
0.385	0.381	0.383	0.003
0.359		0.359	
0.380		0.380	
		5	
		0.359	0.009
		0.039	

Tyrosine

Lab	A	B	Mean	SD
3	0.206	0.206	0.206	0.000
5	0.270	0.280	0.275	0.007
7	0.280	0.270	0.275	0.007
10	0.307		0.307	
13	0.330		0.330	
		***N*:**	5	
		Mean, Pooled SD:	0.279	0.006
		SD:	0.047	

Valine

A	B	Mean	SD
0.267	0.271	0.269	0.003
0.360	0.410	0.385	0.035
0.495	0.506	0.501	0.008
0.485		0.485	
0.530		0.530	
		5	
		0.434	0.021
		0.107	

4.6.2. Value Assignment

As described in Section 3.3, the available data for each measurand was used to provide an estimate of the mass fraction present in SRM 2386 where x is the mean and $U_{95}(x)$ is the 95% confidence interval. The summary of these estimates for amino acids is provided in Table 93, along with a summary of the methods used to arrive at these estimates.

Table 93. Summary of Estimates for Amino Acids in SRM 2386, %.

			Based On
Analyte	x	$U_{95}(x)$	**Fall 2015 GMA Methods[a]**
Alanine	0.492	0.043	Hydrolysis and Derivatization with LC
Arginine	0.423	0.076	Hydrolysis and Derivatization with LC
Aspartic acid	0.827	0.077	Hydrolysis and Derivatization with LC
Cystine	0.091	0.036	Hydrolysis and Derivatization with LC
Glutamic acid	1.135	0.107	Hydrolysis and Derivatization with LC
Glycine	0.441	0.042	Hydrolysis and Derivatization with LC
Histidine	0.194	0.045	Hydrolysis and Derivatization with LC
Isoleucine	0.389	0.103	Hydrolysis and Derivatization with LC
Leucine	0.640	0.092	Hydrolysis and Derivatization with LC
Lysine	0.473	0.144	Hydrolysis and Derivatization with LC
Methionine	0.160	0.057	Hydrolysis and Derivatization with LC
Phenylalanine	0.368	0.058	Hydrolysis and Derivatization with LC
Proline	0.434	0.070	Hydrolysis and Derivatization with LC
Serine	0.515	0.035	Hydrolysis and Derivatization with LC
Threonine	0.399	0.044	Hydrolysis and Derivatization with LC
Tyrosine	0.289	0.056	Hydrolysis and Derivatization with LC
Valine	0.510	0.135	Hydrolysis and Derivatization with LC

a Not all laboratories reported methods used.

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Appendix A. Acronyms

AA	Autoanalyzer
AAS	Atomic Absorption Spectroscopy
COA	Certificate of Analysis
cps	counts per second
DCPIP	Titration with Dichlorophenol Indophenol Detection
FIAC	Food Industry Analytical Chemists
FL	Fluorescence
GC-FID	Gas Chromatography with Flame Ionization Detection
GMA	Grocery Manufacturers Association
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
ICP-OES	Inductively Coupled Plasma Optical Emission Spectrometry
ID ICP-MS	Isotope Dilution Inductively Coupled Plasma Mass Spectrometry
ID-LC-MS/MS	Isotope Dilution Liquid Chromatography with Tandem Mass Spectrometry Detection
IS	Internal Standard
LC	Liquid Chromatography
LC-ELSD	Liquid Chromatography with Evaporative Light Scattering Detection
LC-MS/MS	Liquid Chromatography with Tandem Mass Spectrometry Detection
LC-RI	Liquid Chromatography with Refractive Index Detection
LC-UV	Liquid Chromatography with UV Absorbance Detection
LDPE	Low Density Polyethylene
MARS	Microwave Assisted Reaction System
MRM	Multiple Reaction Monitoring
NIST	National Institute of Standards and Technology
PFA	Perfluoroalkoxy Alkane
RC	Regenerated Cellulose
RF	Response Factor
RSD	Relative standard deviation
SAS	Standard as Sample
SI	International System of Units
SRM	Standard Reference Material
TRIS	tris(hydroxymethyl)aminomethane
TNPGAA	Thermal Neutron Prompt Gamma-Ray Activation Analysis

Appendix B. Updates

1) Replaced incorrect Table 43 Total Vitamin B_3 as Niacinamide, Section 4.3.2.5.
2) Added Appendix A: Acronyms.
3) Updated Figure 1 to a 2023 version.
4) Numerous format changes to bring document into compliance with NIST current standard format.