National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 3235

Soy Milk

This Standard Reference Material (SRM) is intended primarily for validation of methods for determining vitamins, elements, proximates, fatty acids, and amino acids in soy milk and similar materials. This SRM can also be used for quality assurance when assigning values to in-house reference materials. The SRM is a soy milk prepared by a commercial manufacturer. A unit of SRM 3235 consists of 10 ampoules, each containing approximately 10 mL of material.

The development of SRM 3235 was a collaboration between the National Institute of Standards and Technology (NIST) and the National Institutes of Health Office of Dietary Supplements (NIH-ODS).

Certified Mass Fraction Values: The certified mass fraction values for elements and vitamins in SRM 3235, reported on an as-received basis, are provided in Tables 1 and 2. A NIST certified value is a value for which NIST has the highest confidence in its accuracy in that all known or suspected sources of bias have been investigated or taken into account [1]. Analyses for value assignment were performed by NIST and collaborating laboratories. Certified values were calculated as the mean of the mean values from NIST methods and the median or mean of the means of results provided by collaborating laboratories, where appropriate. The associated uncertainties are expressed at an approximately 95 % level of confidence [2–4].

Reference Mass Fraction Values: Reference mass fraction values for iron, additional vitamins, proximates and calories, fatty acids, and amino acids in SRM 3235, reported on an as-received basis, are provided in Tables 3 through 7. A NIST reference value is a noncertified value that is the best estimate of the true value based on available data; however, the value does not meet the NIST criteria for certification [1] and is provided with associated uncertainties that may reflect only measurement reproducibility, may not include all sources of uncertainty, or may reflect a lack of sufficient statistical agreement among multiple analytical methods. The reference mass fraction values were derived from results reported by NIST and/or collaborating laboratories.

Expiration of Certification: The certification of **SRM 3235** is valid, within the measurement uncertainty specified, until **01 October 2026**, provided the SRM is handled and stored in accordance with the instructions given in this certificate (see "Instructions for Storage and Use"). The certification is nullified if the SRM is damaged, contaminated, or otherwise modified.

Maintenance of SRM Certification: NIST will monitor this SRM over the period of its certification. If substantive technical changes occur that affect the certification before the expiration of this certificate, NIST will notify the purchaser. Registration (see attached sheet or register online) will facilitate notification.

Support for the development of SRM 3235 was provided in part by NIH-ODS. Technical consultation was provided by J.M. Betz of NIH-ODS.

Overall direction and coordination of the technical measurements leading to the certification of this SRM were performed by M.M. Phillips and L.J. Wood of the NIST Chemical Sciences Division and K.E. Sharpless of the NIST Special Programs Office.

Analytical measurements at NIST were performed by J.F. Browning, C.Q. Burdette, K.D. Chieh, B.E. Lang, M.M. Phillips, B.J. Porter, C.A. Rimmer, J.B. Thomas, and L.J. Wood of the NIST Chemical Sciences Division.

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Analyses for value assignment were also performed by the following laboratories participating in a GMA Food Industry Analytical Chemists Share Group (FIACSG) interlaboratory comparison exercise: Chelab Silliker, (Resana, Italy); Conagra Foods (Omaha, NE); Covance Laboratories, Inc. (Battle Creek, MI); Covance Laboratories, Inc. (Harrogate, North Yorkshire, United Kingdom); Covance Laboratories, Inc. (Madison, WI); Del Monte Foods (Walnut Creek, CA); Eurofins Nutrition Analysis Center (Des Moines, IA); General Mills, Inc. (Golden Valley, MN); Hormel Foods Corporation (Austin, MN); Krueger Food Laboratories (Billerica, MA); Land O'Lakes (Arden Hills, MN); Nestlé Brasil (São Paulo, Brazil); Nestlé Centroamerica (Antigua, Guatemala); Nestlé Malaysia (Petaling Jaya, Malaysia); Nestlé Nederland BV (Nunspeet, Netherlands); Nestlé Quality Assurance Center (Dublin, OH); Schwan Food Company (Salina, KS); Silliker Ibérica (Barcelona, Spain); Silliker Illinois Analytical Laboratory (Crete, IL); The Coca Cola Company, Asia Pacific Technical Center (Shanghai, China); The J.M. Smucker Co. (Orrville, OH); The National Food Laboratory (Livermore, CA).

Statistical analysis was provided by J.H. Yen of the NIST Statistical Engineering Division.

Support aspects involved in the issuance of this SRM were coordinated through the NIST Office of Reference Materials.

NOTICE AND WARNING TO USERS

SRM 3235 IS INTENDED FOR RESEARCH USE, NOT FOR HUMAN CONSUMPTION.

INSTRUCTIONS FOR STORAGE AND USE

Storage: The original unopened ampoules of SRM 3235 should be stored under refrigeration (4 °C). Once the ampoule is opened, the long-term stability of all analytes in SRM 3235 is unknown. Therefore, the certification only applies to the initial use and the same results are not guaranteed if the remaining liquid is used longer than two days after opening.

Use: Prior to removal of a test portion for analysis, the contents of an ampoule of material should be mixed thoroughly. For certified values to be valid, a 4 g to 5 g test portion should be used for analysis. Results obtained in analyses should include their own estimates of uncertainty and can be compared to the certified values using procedures described in reference 5.

SOURCE, PREPARATION, AND ANALYSIS[(1)]

Source and Preparation: The SRM is a soy milk prepared by a commercial manufacturer. The product was packaged into single-use, 10-mL glass ampoules, each containing 10 mL of liquid.

Analytical Approach for Determination of Elements: Value assignment of the mass fractions of the elements in SRM 3235 was based on the combination of results from NIST and collaborating laboratories, where appropriate. NIST provided measurements by using inductively coupled plasma optical emission spectrometry (ICP-OES).

NIST Analyses for Ca, Cu, Fe, K, Mg, Mn, Na, P, and Zn Using ICP-OES: Mass fractions of calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc were measured by ICP-OES. Duplicate 5 g test portions were taken from each of 10 ampoules of SRM 3235 and were digested in a microwave sample preparation system using nitric acid. Quantitation was based on the method of standard additions using SRM 3100 series single element standard solutions.

Analytical Approach for Determination of Vitamins: Value assignment of the mass fractions of the vitamins in SRM 3235 was based on the combination of results from NIST and collaborating laboratories, where appropriate. NIST provided measurements by using isotope dilution (ID) with liquid chromatography (LC) and tandem mass spectrometry (MS/MS) or LC with absorbance or fluorescence detection.

NIST Analyses for Riboflavin, Pantothenic Acid, and Pyridoxine Using ID-LC-MS/MS: Mass fractions of riboflavin, pantothenic acid, and pyridoxine were measured by ID-LC-MS/MS in duplicate 4.5 g test portions taken from each of ten ampoules of SRM 3235. The analytes and internal standards were extracted into ammonium acetate at pH 2.6 for analysis by positive ion mode ID-LC-MS/MS. A gradient method with an ammonium formate buffer/methanol

[(1)] Certain commercial equipment, instruments or materials are identified in this certificate to adequately specify the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

mobile phase and a C18 column were used for ID-LC-MS/MS determination of the vitamins. Calibrants were prepared gravimetrically at levels intended to approximate the levels of the vitamins in the SRM following extraction. The purity of neat calibrant materials was determined at NIST using LC-absorbance, Karl Fischer titration, thermogravimetric analysis, and differential scanning calorimetry. A single internal standard solution was used for the calibrants and samples. The analyte and internal standard transitions monitored for ID-LC-MS/MS are listed in Table 8.

NIST Analyses for Vitamin D_2 Using ID-LC-MS/MS: The mass fraction of vitamin D_2 (ergocalciferol) was measured in duplicate 5 g test portions taken from each of ten ampoules of SRM 3235. Vitamin D_3-$^{13}C_5$ was added as an internal standard. Prior to extraction, the samples of SRM 3235 were sonicated with 1 % ethylenediamine tetraacetic acid (mass fraction) for 1 h. Dipotassium oxalate solution (35 %, mass fraction) was added to each sample, and the analyte and internal standard were extracted into 5:7 tert-butylmethylether:petroleum ether (volume fraction) by rotational agitation for 1 h. The samples were centrifuged, the supernatants were decanted, and an additional aliquot of 5:7 tert-butylmethylether:petroleum ether (volume fraction) was added. Samples were extracted further by a combination of sonication and rotary mixing, then centrifuged, and the supernatants combined with those from the previous extraction. Two additional cycles of sonication and rotary mixing were conducted, for a total of four extractions. The pooled organic layers were dried using magnesium sulfate, and following centrifugation evaporated to dryness under nitrogen. The analytes were derivatized with 4-phenyl-1,2,4-triazoline-3,5-dione (PTAD) and reconstituted in ethanol containing butylated hydroxytoluene (BHT) for analysis by positive-ion mode LC-MS/MS. A gradient method with a water/methanol mobile phase and a pentafluorophenyl column were used for LC-MS/MS determination. Vitamin D_2+PTAD and vitamin D_3-$^{13}C_5$+PTAD were measured at transitions *m/z* 572 → *m/z* 298 and *m/z* 565 → *m/z* 298, respectively. Calibrants were prepared gravimetrically at levels intended to approximate the levels of the vitamins in the SRM following extraction. The purity of the neat vitamin D_2 calibrant material was determined at NIST using spectrophotometry at 265 nm (molar extinction coefficient of 19,400 L $mol^{-1}cm^{-1}$). A single internal standard solution was used for the calibrants and samples.

NIST Analyses for Tocopherols Using Liquid Chromatography with Fluorescence Detection: Mass fractions of δ-tocopherol and γ-tocopherol were measured in duplicate 3 g to 5 g test portions taken from each of ten ampoules of SRM 3235. Ethanolic tocol was added as an internal standard. Prior to extraction, the sample was suspended in water and dipotassium oxalate solution (35 %, mass fraction) was added. The analytes and internal standard were extracted from the sample into 10:5:7 ethanol:tert-butylmethylether:petroleum ether (volume fraction) by rotational agitation for 15 min. The samples were centrifuged, the supernatants were decanted, and an additional aliquot of ethanol:tert-butylmethylether:petroleum ether was added. Samples were extracted further by 15 min of mixing/rotation. Two additional cycles of sonication were conducted, for a total of four extractions. The pooled organic layers were washed twice with water, evaporated to dryness under nitrogen, and resuspended in a mixture of ethanol and ethyl acetate for analysis by LC-fluorescence. An isocratic method with a water/methanol mobile phase and a C30 column were used for LC-fluorescence determination. The separation was monitored using a fluorescence detector at an excitation wavelength of 295 nm and an emission wavelength of 330 nm. Calibrants were prepared gravimetrically at levels intended to approximate the levels of the tocopherols in the SRM following extraction. The purity of the δ-tocopherol calibrant material was determined at NIST using spectrophotometry at 297 nm (molar extinction coefficient of 3,762 L $mol^{-1}cm^{-1}$) in combination with LC-absorbance at 297 nm. The purity of the γ-tocopherol calibrant material was determined at NIST using spectrophotometry at 298 nm (molar extinction coefficient of 3,808 L $mol^{-1}cm^{-1}$) in combination with LC-absorbance at 297 nm. A single internal standard solution was used for the calibrants and samples.

NIST Analyses for Tocopherols Using Liquid Chromatography with Absorbance Detection: Mass fractions of δ-tocopherol and γ-tocopherol were measured in duplicate 5 g test portions taken from each of six ampoules of SRM 3235. Ethanolic tocol was added as an internal standard. The analytes and internal standard were extracted from the sample into hexane by sonication for 1 h followed by overnight rotational agitation. The samples were centrifuged, the supernatants were decanted, and an additional aliquot of hexane was added. Samples were extracted further by 2 h of sonication. One additional cycle of sonication was conducted, for a total of three extractions. The pooled organic layers were evaporated to dryness under nitrogen and resuspended in ethanol containing BHT for analysis by LC-absorbance. An isocratic method with a water/methanol mobile phase and a C30 column were used for LC-absorbance determination. The separation was monitored using an absorbance detector at 298 nm. Calibrants were prepared gravimetrically at levels intended to approximate the levels of the tocopherols in the SRM following extraction. The purity of the calibrant materials was determined at NIST using LC-absorbance at 297 nm. A single internal standard solution was used for the calibrants and samples.

Collaborating Laboratories' Analyses: The GMA FIACSG laboratories were asked to use their usual methods to make single measurements of fatty acids, proximates, calories, elements, vitamins, and amino acids on test portions taken from each of two ampoules of SRM 3235. Because of variability among data provided by laboratories

participating in this interlaboratory comparison exercise, the median of laboratory means was used, with the uncertainty estimated using the median absolute deviation (MAD) [6].

Homogeneity Assessment: The homogeneity of elements and vitamins in the SRM was assessed at NIST using the methods and test portion sizes described above. Analysis of the variance showed statistically significant heterogeneity for iron, riboflavin, ergocalciferol, γ-tocopherol, and δ-tocopherol, and the uncertainties for these values incorporate an additional component for possible heterogeneity. Homogeneity of constituents measured solely by collaborating laboratories (e.g., proximates, amino acids) was not assessed, although the data were treated as though these analytes were homogeneously distributed.

Value Assignment: For calculation of assigned values for analytes that were measured only by NIST, the mean of the mean values from NIST results was used. The collaborating laboratories reported the individual results for each of their analyses for a given analyte. The mean of each laboratory's results was then determined. For calculation of assigned values for analytes that were measured only by the GMA FIACSG laboratories, the median of the laboratory means was used. For analytes that were also measured by NIST, the mean of the individual sets of NIST data were averaged with the median of the individual GMA FIACSG laboratory means, as appropriate.

Certified Mass Fraction Values for Elements: Each certified mass fraction value is the combined mean from the mean of results from analyses by NIST and the median of the means of results provided by collaborating laboratories. The uncertainty provided with each value is an expanded uncertainty about the mean to cover the measurand with approximately 95 % confidence. The expanded uncertainty is calculated as $U = ku_c$, where u_c incorporates the observed difference between the results from the methods and their respective uncertainties consistent with the JCGM Guide and with its Supplement 1, and k is a coverage factor corresponding to approximately 95 % confidence [2–4]. The measurand is the total mass fraction for each element listed in Table 1. Metrological traceability is to the SI derived unit for mass fraction (expressed as milligrams per kilogram) on an as-received basis.

Table 1. Certified Mass Fraction Values for Elements in SRM 3235

	Mass Fraction (mg/kg)			Coverage Factor, k
Calcium (Ca)[a,b]	1219	±	38	2.00
Copper (Cu)[a,c]	0.976	±	0.022	2.00
Magnesium (Mg)[a,b]	170.4	±	4.9	2.00
Manganese (Mn)[a,c]	1.907	±	0.094	2.00
Phosphorus (P)[a,c]	392	±	28	2.00
Potassium (K)[a,b]	1357	±	20	2.00
Sodium (Na)[a,b]	488.2	±	7.0	2.00
Zinc (Zn)[a,b]	2.58	±	0.19	2.00

[a] NIST ICP-OES

[b] Collaborating laboratories. Reported methods included atomic absorption spectroscopy (AAS) and ICP-OES.

[c] Collaborating laboratories. Reported methods included AAS, ICP-OES, and ICP-MS.

Certified Mass Fraction Values for Vitamins: Each certified mass fraction value is the combined mean from the mean of results from analyses by NIST and the median of the means of results provided by collaborating laboratories. The uncertainty provided with each value is an expanded uncertainty about the mean to cover the measurand with approximately 95 % confidence. The expanded uncertainty is calculated as $U = ku_c$, where u_c incorporates the observed difference between the results from the methods and their respective uncertainties consistent with the JCGM Guide and with its Supplement 1, and k is a coverage factor corresponding to approximately 95 % confidence [2–4]. The uncertainties for riboflavin, ergocalciferol, γ-tocopherol, and δ-tocopherol also incorporate an additional uncertainty component for possible inhomogeneity. The measurand is the total mass fraction for each vitamin listed in Table 2. Metrological traceability is to the SI derived unit for mass fraction (expressed as milligrams per kilogram) on an as-received basis.

Table 2. Certified Mass Fraction Values for Vitamins in SRM 3235

	Mass Fraction (mg/kg)			Coverage Factor, k
Riboflavin (Vitamin B_2)[a,b,c]	2.23	±	0.40	2.00
Pantothenic Acid (Vitamin B_5)[a,d]	0.92	±	0.16	2.00
Ergocalciferol (Vitamin D_2)[a,e]	0.0120	±	0.0024	2.00
γ-Tocopherol (Vitamin E)[f,g,h]	7.5	±	1.6	2.00
δ-Tocopherol (Vitamin E)[f,g,h]	6.1	±	1.1	2.00

[a] NIST ID-LC-MS/MS
[b] This value represents the free (unbound) form of the vitamin.
[c] Collaborating laboratories. Reported methods included microbiological assay, autoanalyzer, LC-fluorescence, and LC-MS or LC-MS/MS.
[d] Collaborating laboratories. Reported methods included microbiological assay and LC-MS or LC-MS/MS.
[e] Collaborating laboratories. Reported methods included LC-absorbance and LC-MS or LC-MS/MS.
[f] NIST LC-absorbance
[g] NIST LC-fluorescence
[h] Collaborating laboratories. Reported methods included LC-absorbance and LC-fluorescence.

Reference Mass Fraction Value for Iron: The reference mass fraction value is the mean of the mean of results from ICP-OES analyses by NIST and the median of the means of results provided by collaborating laboratories. Methods reported by collaborating laboratories included AAS, ICP-OES, and ICP-MS. The uncertainty provided is an expanded uncertainty about the mean to cover the measurand with approximately 95 % confidence. The expanded uncertainty is calculated as $U = ku_c$, where u_c represents the combined uncertainty consistent with the JCGM Guide and with its Supplement 1, and k is a coverage factor corresponding to approximately 95 % confidence [2–4]. The uncertainty for iron also incorporates an additional uncertainty component for possible inhomogeneity. The measurand is the mass fraction for iron as determined by the methods indicated. Metrological traceability is to the SI derived unit for mass fraction (expressed as milligrams per kilogram) on an as-received basis.

Table 3. Reference Mass Fraction Values for Elements in SRM 3235

	Mass Fraction (mg/kg)			Coverage Factor, k
Iron (Fe)	5.2	±	1.2	2.00

Reference Mass Fraction Values for Vitamins: Each reference mass fraction value is the mean from the combination of the mean results from NIST and the median of the means of results provided by collaborating laboratories, where appropriate. The uncertainty provided is an expanded uncertainty about the mean to cover the measurand with approximately 95 % confidence. The expanded uncertainty is calculated as $U = ku_c$, where u_c represents the combined uncertainty consistent with the JCGM Guide and with its Supplement 1, and k is a coverage factor corresponding to approximately 95 % confidence [2–4]. The measurand is the mass fraction for each vitamin listed in Table 4 as determined by the method indicated. Metrological traceability is to the SI derived unit for mass fraction (expressed as milligrams per kilogram) on an as-received basis.

Table 4. Reference Mass Fraction Values for Vitamins in SRM 3235

	Mass Fraction (mg/kg)	Coverage Factor, k
Pyridoxine (Vitamin B_6)[a,b]	0.02573 ± 0.00061	2.11
Cyanocobalamin (Vitamin B_{12})[c]	0.0147 ± 0.0011	2.36
Retinol (Vitamin A)[d]	0.662 ± 0.079	2.10
Phylloquinone (Vitamin K)[e]	0.0370 ± 0.0035	4.30

[a] NIST ID-LC-MS/MS
[b] This value represents the free (unbound) form of the vitamin.
[c] Collaborating laboratories. Reported methods included microbiological assay and LC-absorbance.
[d] Collaborating laboratories. Reported methods included LC-absorbance following saponification and/or extraction.
[e] Collaborating laboratories. Reported methods included LC.

Reference Values for Proximates and Calories: Each reference value is the median of the means of results provided by collaborating laboratories. The uncertainty provided is an expanded uncertainty about the mean to cover the measurand with approximately 95 % confidence. The expanded uncertainty is calculated as $U = ku_c$, where u_c represents the combined uncertainty consistent with the JCGM Guide and with its Supplement 1, and k is a coverage factor corresponding to approximately 95 % confidence [2,3]. For proximates, the measurands are the mass fractions of proximates listed in Table 5 as determined by the methods indicated. Metrological traceability is to the SI derived unit for mass fraction (expressed as grams per 100 grams) on an as-received basis for proximates. For calories, the measurand is the caloric content listed in Table 5 as determined by the method indicated. Metrological traceability is to the SI derived unit for energy (expressed as kilocalories per 100 grams) on an as-received basis for calories.

Table 5. Reference Values for Proximates and Calories in SRM 3235

	Mass Fraction (g/100 g)	Coverage Factor, k
Solids[a]	8.30 ± 0.05	2.13
Ash[b]	0.66 ± 0.04	2.13
Protein[c]	2.57 ± 0.06	2.11
Carbohydrates[d]	3.20 ± 0.37	2.16
Fat (as the sum of fatty acids as triglycerides)	1.70 ± 0.05	2.16
Total Sugars[e]	2.19 ± 0.15	2.36
	Energy (kcal per 100 g)	**Coverage Factor, k**
Calories[f]	39 ± 1	2.18

[a] Solids were determined by collaborating laboratories using drying in a forced-air oven and drying in a vacuum oven.
[b] Ash was determined by collaborating laboratories using weight loss after ignition in a muffle furnace.
[c] Nitrogen was determined by collaborating laboratories using Kjeldahl and combustion (LECO). A factor of 5.71 was used to convert nitrogen results to protein.
[d] Carbohydrates were determined by collaborating laboratories by difference (solids less the sum of protein, fat, and ash).
[e] Total sugars were determined by collaborating laboratories using LC with refractive index detection, LC with amperometric detection, or LC with evaporative light scattering detection.
[f] Calories were determined by collaborating laboratories as the median of lab means using caloric calculations, the sum of caloric equivalents of 9, 4, and 4 for fat (as the sum of fatty acids as triglycerides), protein, and carbohydrate, respectively. If the mean proximate values above are used for calculation, the mean caloric content is 38 kcal per 100 grams.

Reference Mass Fraction Values for Fatty Acids as Free Fatty Acids: Each reference mass fraction value is the median of the means of results provided by collaborating laboratories using GC-FID following hydrolysis and derivatization. The uncertainty provided with each value is an expanded uncertainty about the mean to cover the measurand with approximately 95 % confidence. The expanded uncertainty is calculated as $U = ku_c$, where u_c is the combined uncertainty, consistent with the JCGM Guide and with its Supplement 1, and k is a coverage factor corresponding to approximately 95 % confidence [2,3]. The measurand is the mass fraction for each fatty acid listed in Table 6 as determined by the methods indicated. Metrological traceability is to the SI derived unit for mass fraction (expressed as grams per 100 grams) on an as-received basis.

Table 6. Reference Mass Fraction Values for Fatty Acids (as Free Fatty Acids) in SRM 3235

	Common Name	Mass Fraction (g/100 g)	Coverage Factor, k
Hexadecanoic Acid (C16:0)	Palmitic Acid	0.1800 ± 0.0059	2.20
(Z)-9-Octadecenoic Acid (C18:1 n9)	Oleic Acid	0.336 ± 0.020	2.23
Total *cis*-C18:1		0.355 ± 0.022	2.26
(Z,Z)-9,12-Octadecadienoic Acid (C18:2 n6)	Linoleic Acid	0.876 ± 0.034	2.45
Total *cis*-C18:2		0.880 ± 0.024	2.20
(Z,Z,Z)-9,12,15-Octadecatrienoic Acid (C18:3 n3)	α-Linolenic Acid	0.1430 ± 0.0030	2.20
Saturated Fat		0.266 ± 0.014	2.14
cis-Monounsaturated Fat		0.350 ± 0.010	2.14
cis-Polyunsaturated Fat		1.010 ± 0.028	2.14
Total ω-3 Fatty Acids		0.1400 ± 0.0045	2.18
Total ω-6 Fatty Acids		0.870 ± 0.023	2.18

Reference Mass Fraction Values for Amino Acids: Each reference mass fraction value is the median of the means of results provided by collaborating laboratories using hydrolysis followed by derivatization and LC. The uncertainty provided is an expanded uncertainty about the mean to cover the measurand with approximately 95 % confidence. The expanded uncertainty is calculated as $U = ku_c$, where u_c represents the combined uncertainty consistent with the JCGM Guide and with its Supplement 1, and k is a coverage factor corresponding to approximately 95 % confidence [2,3]. The measurand is the mass fraction of each amino acid listed in Table 7 as determined by the method indicated. Metrological traceability is to the SI derived unit for mass fraction (expressed as grams per 100 grams) on an as-received basis.

Table 7. Reference Mass Fraction Values for Amino Acids in SRM 3235

	Mass Fraction (g/100 g)	Coverage Factor, k
Alanine	0.122 ± 0.003	2.36
Arginine	0.22 ± 0.02	2.36
Aspartic Acid	0.33 ± 0.01	2.36
Glutamic Acid	0.53 ± 0.02	2.36
Glycine	0.117 ± 0.007	2.36
Histidine	0.070 ± 0.007	2.36
Isoleucine	0.13 ± 0.02	2.36
Leucine	0.217 ± 0.005	2.36
Lysine	0.18 ± 0.02	2.36
Methionine	0.037 ± 0.004	2.45
Phenylalanine	0.142 ± 0.007	2.36
Proline	0.141 ± 0.008	2.36
Serine	0.150 ± 0.004	2.36
Threonine	0.109 ± 0.006	2.36
Tryptophan	0.040 ± 0.002	2.57
Tyrosine	0.109 ± 0.005	2.36

Table 8. LC-MS/MS Transitions Monitored for Vitamins

Compound	Precursor Ion → Product Ion (m/z)		Internal Standard (IS)	IS Precursor Ion → IS Product Ion (m/z)	
Riboflavin	377	43	$^{13}C_4$,$^{15}N_2$-Riboflavin	383	43
		172			175
		198			202
		243			249
Pantothenic Acid	220	41	$^{13}C_3$,^{15}N-Pantothenic Acid	224	41
		43			43
		72			76
		90			94
Pyridoxine	170	77	$^{13}C_4$-Pyridoxine	174	81
		80			83
		134			138
		152			156

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[6] Huber, P.; *Robust Statistics*, John Wiley, Hoboken, NJ (1981).

Users of this SRM should ensure that the Certificate of Analysis in their possession is current. This can be accomplished by contacting the SRM Program: telephone (301) 975-2200; fax (301) 948-3730; email srminfo@nist.gov; or via the Internet at http://www.nist.gov/srm.