National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 1549a

Whole Milk Powder

This Standard Reference Material (SRM) is intended primarily for evaluation of methods for determining proximates, fatty acids, cholesterol, vitamins, elements, and amino acids in whole milk powder and similar materials. This SRM can also be used for quality assurance when assigning values to in-house reference materials. The SRM is a whole milk powder prepared by a commercial manufacturer. A unit of SRM 1549a consists of five heat-sealed aluminized packets, each containing approximately 10 g of material.

Certified Mass Fraction Values: Certified mass fraction values for cholesterol, elements, and vitamins in SRM 1549a, reported on an as-received basis, are provided in Tables 1 through 3. 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 taken into account [1]. Analyses for value assignments were performed by NIST and collaborating laboratories. Certified values were calculated as the mean of the mean values from NIST methods and the median of the mean 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 additional elements, additional vitamins, choline, carnitine, proximates, fatty acids, calories, and amino acids in SRM 1549a, reported on an as-received basis, are provided in Tables 4 through 8. 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 an uncertainty 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 1549a** is valid, within the measurement uncertainty specified, until **01 July 2025**, 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.

Coordination of the technical measurements leading to the certification of this SRM was performed by M.M. Phillips and L.J. Wood of the NIST Chemical Sciences Division, K.E. Sharpless of the NIST Special Programs Office, and S. Ehling of the Grocery Manufacturers Association (GMA, Washington, DC).

Support for assignment of values for vitamin D_3 and 25-hydroxyvitamin D_3 was provided by the National Institutes of Health, Office of Dietary Supplements (NIH-ODS). Technical consultation was provided by J.M. Betz (NIH-ODS).

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.

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Certificate Revision History on Last Page

Steven J. Choquette, Director
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Analytical measurements at NIST were performed by C. Bryan, C.Q. Burdette, W.C. Davis, C. Luvonga, B.E. Lang, M.M. Phillips, L.J. Wood, and L.L. Yu of the NIST Chemical Sciences Division; Y. Nuevo Ordóñez, R. Oflaz, D.J. O'Kelly, B.J. Porter, M.M. Schantz, L.T. Sniegoski, B.E. Tomlin, and M.J. Welch formerly of NIST.

Analyses for value assignment were also performed by the following laboratories participating in a GMA Food Industry Analytical Chemists Committee (FIACC) interlaboratory comparison exercise: Abbott Nutrition (Columbus, OH); ConAgra Foods (Omaha, NE); Covance, Inc. (Madison, WI); Del Monte Foods (Walnut Creek, CA); Eurofins Central Analytical Laboratories (Metairie, LA); Eurofins Chemical Control (Des Moines, IA); Eurofins Scientific (Des Moines, IA); Eurofins S&S (Hanover, MD); General Mills, Inc. (Golden Valley, MN); Hormel Foods Corporation (Austin, MN); Krueger Food Laboratories (Billerica, MA); Land O'Lakes (Arden Hills, MN); National Center for Food Safety and Technology (Summit-Argo, IL); Schwan Food Company (Salina, KS); Silliker (Madison, WI); The J.M. Smucker Co. (Orrville, OH); The National Food Laboratory (Livermore, CA).

NOTICE AND WARNING TO USERS

SRM 1549a IS INTENDED FOR RESEARCH USE; NOT FOR HUMAN CONSUMPTION.

INSTRUCTIONS FOR STORAGE AND USE

Storage: The SRM should be stored under refrigeration (2 °C to 8 °C) in the original unopened packets. For elemental analyses, the packet can be opened, test portions removed and analyzed, then the packet resealed until the material reaches its expiration date. For organic analyses, the packet can be resealed, stored under refrigeration, and test portions removed and analyzed for three weeks after the packet was initially opened.

Use: Before use, a packet should be allowed to warm to room temperature, and the contents of the packet should be mixed thoroughly by shaking the packet. Allow the contents to settle for one minute prior to opening to minimize the loss of fine particles. For certified values to be valid, test portion size should be based on descriptions below (See "Source, Preparation, and 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 whole milk powder. The contents of six bags, each containing 27 kg of powdered whole milk, were blended and packaged by High-Purity Standards (Charleston, SC). The milk powder was sealed in approximately 10 g aliquots in Mylar bags that had been flushed with nitrogen.

Analytical Approach for Determination of Cholesterol: Value assignment of the cholesterol mass fraction was based on measurements made by NIST using a method based on isotope dilution (ID) gas chromatography (GC) with mass spectrometry (MS).

NIST Analyses for Cholesterol: The mass fraction of cholesterol was measured using the ID-GC-MS method developed at NIST for serum cholesterol [6] and modified for the determination of cholesterol in food matrices using AOAC International Official Method 996.06 for hydrolysis [7]. Three sets of samples were prepared, each consisting of triplicate 0.5 g test portions from each of three packets of SRM 1549a weighed into screw-capped test tubes. An aliquot of a solution containing a known mass of the internal standard, cholesterol-$^{13}C_3$, was added to each tube. Cholesterol esters were hydrolyzed by heating the samples in an alcohol-KOH solution for 1 h at 100 °C. Cholesterol was extracted into hexane, and a portion of the hexane extract was evaporated to dryness prior to addition of N,O-bis(trimethylsilyl)acetamide to convert cholesterol to the trimethylsilyl (TMS) derivative. GC-MS was performed using a 30 m (phenyl/methyl polysiloxane, 5/95 mole fraction) non-polar fused silica column directly interfaced to the ion source. Cholesterol was determined in the electron ionization mode with selected ion monitoring at *m/z* 458 and *m/z* 461 for the unlabeled and labeled cholesterol-TMS, respectively. Calibrants were prepared gravimetrically, from SRM 911c *Cholesterol*, at levels intended to approximate the level of the cholesterol in the SRM following extraction. A single internal standard solution was used for the calibrants and samples. Calculations are based on linear regression analysis for the calibrants.

[(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.

Analytical Approach for Determination of Elements: Value assignment of the mass fractions of the elements in SRM 1549a was based on the combination of measurements provided by NIST using inductively coupled plasma optical emission spectrometry (ICP-OES), inductively coupled plasma mass spectrometry (ICP-MS), instrumental neutron activation analysis (INAA), and provided by collaborating laboratories, where appropriate.

NIST Analyses for Ba, Ca, Cu, Fe, I, K, Mg, Mn, Mo, Na, Ni, P, Sr, and Zn by ICP-OES and/or ICP-MS: Mass fractions of barium, calcium, magnesium, phosphorus, potassium, sodium, strontium, and zinc were measured by ICP-OES. Mass fractions of barium, copper, iron, manganese, molybdenum, nickel, and strontium were measured by ICP-MS. For each technique, duplicate 0.5 g test portions were taken from each of 10 packets of SRM 1549a. Samples were digested in a microwave sample preparation system either with or without subsequent hot-plate digestion using nitric acid, a nitric acid/hydrofluoric acid mixture, or a nitric acid/perchloric acid mixture. The mass fraction of iodine was measured by ICP-MS in single 0.3 g test portions taken from each of six packets and in each of four packets a year later. Samples were digested in aqueous tetramethylammonium hydroxide using a microwave sample preparation system. Quantitation for all elements was based on the method of standard additions using the SRM 3100 series single element standard solutions.

NIST Analyses for Se and Zn by INAA: The mass fractions of selenium and zinc were measured by INAA in duplicate 0.22 g test portions taken from each of six packets of SRM 1549a. Powders were pressed into cylindrical pellets, and samples, standards, and controls were packaged individually in clean polyethylene bags and irradiated individually at 20 MW. Samples, controls, and standards, prepared from SRM 3100 series single element standard solutions, were irradiated for 4 h. Irradiation capsules were then inverted 180 degrees, and materials were irradiated another 4 h. Selenium and zinc were counted for 8 h after a decay of more than 90 d.

Analytical Approach for Determination of Vitamins: Value assignment of the mass fractions of the vitamins in SRM 1549a was based on the combination of results provided from various analytical methods at NIST and collaborating laboratories, where appropriate. NIST provided measurements by using liquid chromatography (LC) with or without ID and MS or tandem mass spectrometry (MS/MS).

NIST Analyses for Riboflavin, Niacinamide, Pantothenic Acid, Pyridoxamine, and Pyridoxal by LC-MS or ID-LC-MS: The mass fraction of riboflavin was measured by LC-MS in duplicate 2.5 g test portions taken from each of 10 packets of SRM 1549a. Mass fractions of niacinamide, pantothenic acid, pyridoxamine, and pyridoxal were measured by ID-LC-MS in duplicate 2.5 g test portions taken from each of 10 packets of SRM 1549a. $^{2}H_{4}$-niacinamide, $^{13}C_{3}$,^{15}N-pantothenic acid, $^{2}H_{3}$-pyridoxamine, and $^{2}H_{3}$-pyridoxal were added as internal standards. The analytes and internal standards were extracted into dilute acetic acid for analysis by positive ion mode LC-MS. A gradient method with an ammonium formate buffer/methanol mobile phase and a C18 column were used for LC-MS determination of the vitamins. Niacinamide and $^{2}H_{4}$-niacinamide were measured at *m/z* 123 and *m/z* 127, respectively. Pantothenic acid and $^{13}C_{3}$,^{15}N-pantothenic acid were measured at *m/z* 220 and *m/z* 224, respectively. Pyridoxamine and $^{2}H_{3}$-pyridoxamine were measured at *m/z* 169 and *m/z* 172, respectively. Pyridoxal and $^{2}H_{3}$-pyridoxal were measured at *m/z* 168 and *m/z* 171, respectively. Riboflavin was measured at *m/z* 377, using $^{13}C_{3}$,^{15}N-pantothenic acid as an internal standard. 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 (TGA), quantitative proton nuclear magnetic resonance spectroscopy (q^{1}HNMR), and differential scanning calorimetry (DSC). A single internal standard solution was used for the calibrants and samples.

NIST Analyses for Riboflavin, Niacinamide, Pantothenic Acid, Pyridoxamine, and Pyridoxal by ID-LC-MS/MS: Mass fractions of riboflavin, niacinamide, pantothenic acid, pyridoxamine, and pyridoxal were measured by ID-LC-MS/MS in duplicate 2 g test portions taken from each of six packets of SRM 1549a. $^{13}C_{4}$,$^{15}N_{2}$-Riboflavin, $^{2}H_{4}$-niacinamide, $^{13}C_{3}$,^{15}N-pantothenic acid, $^{2}H_{3}$-pyridoxamine, and $^{2}H_{3}$-pyridoxal were added as internal standards. The analytes and internal standards were extracted into dilute acetic acid for analysis by positive-ion mode ID-LC-MS/MS. A gradient method with an ammonium formate buffer/methanol 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, TGA, q^{1}HNMR, and DSC. 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 9.

NIST Analyses for Choline and Carnitine: Mass fractions of choline and carnitine were measured in duplicate 1 g samples taken from each of 10 packets of SRM 1549a. $^{2}H_{9}$-choline chloride and $^{2}H_{9}$-carnitine hydrochloride were added as internal standards. The analytes and internal standards were extracted and hydrolyzed by microwave digestion into dilute hydrochloric acid for analysis by positive-ion mode LC-MS. A gradient method with an ammonium formate/acetonitrile mobile phase and a mixed-mode C18 column were used for ID-LC-MS determination.

Choline and $^{2}H_9$-choline were measured at *m/z* 104 and *m/z* 113, respectively. Carnitine and $^{2}H_9$-carnitine were measured at *m/z* 162 and *m/z* 171, respectively. 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 q^1HNMR. A single internal standard solution was used for the calibrants and samples.

NIST Analyses for Vitamin D_3 and 25-Hydroxyvitamin D_3: Mass fractions of vitamin D_3 (cholecalciferol) and 25-hydroxyvitamin D_3 were measured in duplicate 2.0 g to 3.0 g test portions taken from each of ten packets of SRM 1549a. Vitamin D_3-$^{13}C_5$ and 25-hydroxyvitamin D_3-$^{13}C_5$ were added as internal standards. Prior to extraction, the samples of SRM 1549a were incubated with lipase at 40 °C for 2 h to hydrolyze the fats. Ethanol containing butylated hydroxytoluene (BHT) and potassium carbonate was added to each sample, and the analytes and internal standards were extracted into hexane containing BHT by overnight stirring. The samples were centrifuged, the supernatants were decanted, and an additional aliquot of hexane containing BHT 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. One additional cycle of sonication and rotary mixing was conducted, for a total of three extractions. Magnesium sulfate was added to the pooled organic layers. Following vortex mixing and centrifugation, the organic layer was decanted and evaporated to dryness under nitrogen. The analytes were derivatized with 4-phenyl-1,2,4-triazoline-3,5-dione (PTAD) and reconstituted in a mixture of methanol and ethyl acetate 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 ID-LC-MS/MS determination. Vitamin D_3+PTAD and vitamin D_3-$^{13}C_5$+PTAD were measured at transitions *m/z* 560 → *m/z* 298 and *m/z* 565 → *m/z* 298, respectively. 25-Hydroxyvitamin D_3+PTAD and 25-hydroxyvitamin D_3-$^{13}C_5$+PTAD were measured at transitions *m/z* 558 → *m/z* 298 and *m/z* 563 → *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_3 calibrant material was determined by the manufacturer and confirmed at NIST using spectrophotometry. The purity of the neat 25-hydroxyvitamin D_3 calibrant material was determined at NIST using LC-absorbance, Karl Fischer titration, TGA, and q^1HNMR. A single internal standard solution was used for the calibrants and samples.

Analytical Approach for Determination of Fatty Acids: Value assignment of the mass fractions of fatty acids in SRM 1549a was based on the combination of measurements made using two extraction procedures and two different analytical methods at NIST and by collaborating laboratories, where appropriate. NIST provided results using gas GC with flame ionization detection (FID) and GC-MS.

NIST Analyses for Fatty Acids by GC-FID: Mass fractions of fatty acids were determined by GC-FID from two 0.4 g to 0.8 g test portions from each of 10 packets of SRM 1549a. The milk powder was added to pressurized-fluid extraction (PFE) cells that were half filled with Hydromatrix (Varian, Palo Alto, CA). The milk powder was mixed with the Hydromatrix and additional Hydromatrix was added to fill the cell. The mixtures were spiked with an internal standard (IS) solution containing octacosanoic acid and myristic-d_{27} acid and 0.2 g water. Following PFE with hexane:acetone (4:1 volume fraction), extracts were combined with methanolic (*m*-trifluoromethylphenyl) trimethylammonium hydroxide (1:1 volume fraction), vortexed, and allowed to stand for at least 30 min prior to analysis by GC-FID. GC-FID was performed using a 0.25 mm × 100 m biscyanopropyl polysiloxane-fused silica capillary column. Calibrants were prepared gravimetrically from SRM 2377 *Fatty Acid Methyl Esters in 2,2,4-Trimethylpentane*, at levels intended to approximate the levels of the fatty acids in the SRM following extraction. A single internal standard solution was used for the calibrants and samples. Calculations are based on average response factors for the calibrants.

NIST Analyses for Fatty Acids by GC-MS: Mass fractions of fatty acids were determined by GC-MS from two 0.7 g to 0.9 g test portions from each of six packets of SRM 1549a. The milk powder was combined with wet Hydromatrix and transferred to a glass extraction thimble containing glass wool. An internal standard solution containing octacosanoic acid and myristic-d_{27} acid was added, and samples were extracted for 22 h using a methylene chloride:methanol (2:1 volume fraction) solution. Following extraction, extracts were concentrated, methanolic sodium hydroxide was added, and the sample was heated at 100 °C for 5 min with gentle shaking every 10 min. The sample was cooled to room temperature, methanolic BF_3 was added, and the samples were heated to 100 °C for 30 min. Butylated hydroxytoluene (an antioxidant) in hexane was added, and the sample was mixed for 30 s while still warm. Saturated aqueous sodium chloride solution was added, and the sample was mixed for 1 min and cooled to room temperature. The hexane layer was removed to another tube, and the hexane extraction was repeated twice. The three hexane layers were combined, and a portion was transferred to an autosampler vial for GC-MS analysis. GC-MS was performed using a 0.25 mm × 60 m fused silica capillary column containing cyanopropyl/phenylpolysiloxane (50/50 mole fraction) phases. Calibrants were prepared gravimetrically from SRM 2377 *Fatty Acid Methyl Esters in 2,2,4-Trimethylpentane*, at levels intended to approximate the levels of the fatty acids in the SRM following extraction. The internal standard solution was used for the calibrants and samples. Calculations are based on average response factors for the calibrants.

Collaborating Laboratories' Analyses: The GMA FIACC laboratories were asked to use their usual methods to make single measurements of proximates, calories, vitamins, elements, and amino acids on test portions taken from each of two packets of SRM 1549a. Because of variability among data provided by laboratories participating in an interlaboratory comparison exercise, the median of laboratory means is used, with the uncertainty estimated using the median absolute deviation (MADe) [8].

Homogeneity Assessment: The homogeneity of fatty acids, cholesterol, elements, and vitamins was assessed at NIST using the methods and test portion sizes described above. Analysis of the variance showed statistically significant heterogeneity in some cases, and the uncertainties for barium, copper, iron, magnesium, molybdenum, nickel, phosphorus, strontium, zinc, niacinamide, pantothenic acid, pyridoxal, pyridoxamine, total vitamin B_6 as pyridoxal, carnitine, choline, cholecalciferol, and cholesterol all incorporate an uncertainty 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 means of the mean values from NIST results were 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 collaborating 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 collaborating laboratory means, as appropriate.

Certified Mass Fraction Value for Cholesterol: The certified mass fraction for cholesterol is the mean of results obtained by NIST using ID-GC-MS. Values are expressed as $x \pm U_{95\%}(x)$, where x is the certified value and $U_{95\%}(x)$ is the expanded uncertainty of the certified value. The true value of the analyte lies within the interval $x \pm U_{95\%}(x)$ with 95 % confidence. To propagate this uncertainty, treat the certified value as a normally distributed random variable with mean x and standard deviation $U_{95\%}(x)/2$ [2–4]. The measurand is the total mass fraction of cholesterol in milk powder as listed in Table 1 on an as-received basis. Metrological traceability is to the SI measurement unit for chemical mass fraction, expressed as milligrams per gram.

Table 1. Certified Mass Fraction Value for Cholesterol in SRM 1549a

	Mass Fraction (mg/g)
Cholesterol	0.981 ± 0.071

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 mean of results provided by collaborating laboratories, where appropriate. Values are expressed as $x \pm U_{95\%}(x)$, where x is the certified value and $U_{95\%}(x)$ is the expanded uncertainty of the certified value. The true value of the analyte lies within the interval $x \pm U_{95\%}(x)$ with 95 % confidence. To propagate this uncertainty, treat the certified value as a normally distributed random variable with mean x and standard deviation $U_{95\%}(x)/2$ [2–4]. The measurands are the total mass fractions of elements in milk powder as listed in Table 2 on an as-received basis. Metrological traceability is to the SI measurement unit for chemical mass fraction, expressed as milligrams per kilogram.

Table 2. Certified Mass Fraction Values for Elements in SRM 1549a

	Mass Fraction (mg/kg)
Barium (Ba)[a,b]	0.566 ± 0.039
Calcium (Ca)[a,c]	8810 ± 240
Magnesium (Mg)[a,c]	892 ± 62
Manganese (Mn)[b,d]	0.184 ± 0.024
Phosphorus (P)[a,e]	7600 ± 500
Potassium (K)[a,c]	11920 ± 430
Selenium (Se)[c,f]	0.242 ± 0.026
Sodium (Na)[a,c]	3176 ± 58
Strontium (Sr)[a,b]	2.14 ± 0.19
Zinc (Zn)[a,d,f]	33.8 ± 2.3

[a] NIST ICP-OES
[b] NIST ICP-MS
[c] Collaborating laboratories. Reported methods included AAS and ICP-OES.
[d] Collaborating laboratories. Reported methods included AAS, ICP-OES, and ICP-MS.
[e] Collaborating laboratories. Reported methods included absorption spectrophotometry, colorimetry, ICP-OES, and ICP-MS.
[f] NIST INAA

Certified Mass Fraction Values for Vitamins: Each certified mass fraction value is the combined mean from the mean of results from analyses by NIST. Values are expressed as $x \pm U_{95\%}(x)$, where x is the certified value and $U_{95\%}(x)$ is the expanded uncertainty of the certified value. The true value of the analyte lies within the interval $x \pm U_{95\%}(x)$ with 95 % confidence. To propagate this uncertainty, treat the certified value as a normally distributed random variable with mean x and standard deviation $U_{95\%}(x)/2$ [2–4]. The measurands are the total mass fractions of vitamins in milk powder as listed in Table 3 on an as-received basis. Metrological traceability is to the SI measurement unit for chemical mass fraction, expressed as milligrams per kilogram.

Table 3. Certified Mass Fraction Values for Vitamins in SRM 1549a

	Mass Fraction (mg/kg)
Riboflavin (Vitamin B_2)[a,b]	10.6 ± 1.9
Niacinamide (Vitamin B_3)[a,c,d]	5.91 ± 0.39
Pantothenic Acid (Vitamin B_5)[a,c]	33.7 ± 2.7
Pyridoxal[a,c]	1.72 ± 0.16
Pyridoxamine[a,c]	0.259 ± 0.023
Total Vitamin B_6 as Pyridoxal[a,c,e]	1.97 ± 0.16

[a] NIST ID-LC-MS/MS
[b] NIST LC-MS
[c] NIST ID-LC-MS
[d] Niacinamide was the only form of vitamin B_3 detected.
[e] NIST measured pyridoxal and pyridoxamine individually, and pyridoxamine was mathematically converted to pyridoxal by multiplication by the ratio of the relative molecular masses.

Reference Mass Fraction Values for Elements: Each reference mass fraction value is the mean result of NIST analyses using ICP-MS. Values are expressed as $x \pm U_{95\%}(x)$, where x is the estimated value and $U_{95\%}(x)$ is the expanded uncertainty of the value. The method-specific value of the analyte lies within the interval $x \pm U_{95\%}(x)$ with about a 95 % confidence [2-4]. The measurands are the mass fractions of elements in milk powder as listed in Table 4, on an as-received basis, as determined by the method indicated. Metrological traceability is to the SI measurement unit for chemical mass fraction, expressed as milligrams per kilogram.

Table 4. Reference Mass Fraction Values for Elements in SRM 1549a

	Mass Fraction (mg/kg)		
Copper (Cu)	0.638	±	0.049
Iodine (I)	3.34	±	0.30
Iron (Fe)	1.85	±	0.73
Molybdenum (Mo)	0.377	±	0.072
Nickel (Ni)	0.068	±	0.014

Reference Mass Fraction Values for Vitamins: Each reference mass fraction value is the mean result of NIST analyses or the median of the mean results provided by collaborating laboratories. Values are expressed as $x \pm U_{95\%}(x)$, where x is the estimated value and $U_{95\%}(x)$ is the expanded uncertainty of the value. The method-specific value of the analyte lies within the interval $x \pm U_{95\%}(x)$ with about a 95 % confidence [2-4]. The measurands are the total mass fractions of vitamins in milk powder as listed in Table 5, on an as-received basis, as determined by the method indicated. Metrological traceability is to the measurement processes and standards used by NIST and collaborating laboratories.

Table 5. Reference Mass Fraction Values for Vitamins, Carnitine, and Choline in SRM 1549a

	Mass Fraction (mg/kg)		
Biotin[(a)]	0.152	±	0.016
Carnitine[(b)]	173.1	±	8.6
Choline[(b)]	998	±	63
Vitamin B_{12}[(a)]	0.032	±	0.002
Cholecalciferol (Vitamin D_3)[(c)]	0.00188	±	0.00035
25-Hydroxyvitamin D_3[(c)]	0.00053	±	0.00005

[(a)] Collaborating laboratories. Reported methods included microbiological assay.
[(b)] NIST ID-LC-MS
[(c)] NIST ID-LC-MS/MS

Reference Mass Fraction Values for Fatty Acids as Free Fatty Acids: Each reference mass fraction value is the combined mean from the mean of results from analyses by NIST and the median of the mean of results provided by collaborating laboratories, where appropriate. Values are expressed as $x \pm U_{95\%}(x)$, where x is the estimated value and $U_{95\%}(x)$ is the expanded uncertainty of the value. The method-specific value of the analyte lies within the interval $x \pm U_{95\%}(x)$ with about a 95 % confidence [2-4]. The measurands are the total mass fractions of fatty acids in milk powder as listed in Table 6, on an as-received basis, as determined by the methods indicated. Metrological traceability is to the measurement processes and standards used by NIST and collaborating laboratories.

Table 6. Reference Mass Fraction Values for Fatty Acids (as Free Fatty Acids) in SRM 1549a

	Common Name	Mass Fraction (g/100 g)
Butanoic Acid (C4:0)[(a)]	Butyric Acid	0.868 ± 0.096
Hexanoic Acid (C6:0)[(a)]	Caproic Acid	0.573 ± 0.028
Octanoic Acid (C8:0)[(a,b,c)]	Caprylic Acid	0.311 ± 0.008
Dodecanoic Acid (C12:0)[(a,b,c)]	Lauric Acid	0.764 ± 0.084
Tridecanoic Acid (C13:0)[(a)]		0.029 ± 0.004
Tetradecanoic Acid (C14:0)[(a,b,c)]	Myristic Acid	2.49 ± 0.19
Pentadecanoic Acid (C15:0)[(a)]		0.265 ± 0.017
Hexadecanoic Acid (C16:0)[(a,b,c)]	Palmitic Acid	6.65 ± 0.44
(*Z*)-9-Hexadecenoic Acid (C16:1 n-7)[(a,b,c)]	Palmitoleic Acid	0.385 ± 0.025
(*E*)-9-Hexadecenoic Acid (C16:1-9t)[(a)]	*trans*-Palmitelaidic Acid	0.091 ± 0.015
Heptadecanoic Acid (C17:0)[(a)]	Margaric Acid	0.171 ± 0.013
(*Z*)-9-Heptadecenoic Acid (C17:1 n-8)[(a)]	Margaroleic Acid	0.056 ± 0.005
Octadecanoic Acid (C18:0)[(a,b,c)]	Stearic Acid	2.57 ± 0.18
(*Z*)-9-Octadecenoic Acid (C18:1 n-9)[(a,b,c)]	Oleic Acid	4.83 ± 0.50
(*Z,Z*)-9,12-Octadecadienoic Acid (C18:2 n-6)[(a,b,c)]	Linoleic Acid	0.659 ± 0.057
(*Z,Z,Z,Z,Z*)-7,10,13,16,19-Docosapentaenoic Acid (C22:5)[(b)]	DPA	0.014 ± 0.001

[(a)] Collaborating laboratories. Reported methods included GC-FID.
[(b)] NIST GC-FID
[(c)] NIST GC-MS

Reference Mass Fraction Values for Proximates and Calories: Each reference mass fraction value is the median of the mean results provided by collaborating laboratories. Values are expressed as $x \pm U_{95\%}(x)$, where x is the estimated value and $U_{95\%}(x)$ is the expanded uncertainty of the value. The method-specific value of the analyte lies within the interval $x \pm U_{95\%}(x)$ with about a 95 % confidence [2-4]. For proximates, the measurands are the total mass fractions in milk powder as listed in Table 7, on an as-received basis, as determined by the methods indicated. For calories, the measurand is the caloric content in milk powder as listed in Table 7, on an as-received basis, as determined by the method indicated. Metrological traceability is to the measurement processes and standards used by collaborating laboratories.

Table 7. Reference Values for Proximates and Calories in SRM 1549a

	Mass Fraction (g/100 g)		
Solids[(a)]	96.92	±	0.26
Ash[(b)]	5.625	±	0.045
Protein[(c)]	25.64	±	0.31
Carbohydrates[(d)]	38.43	±	0.95
Fat (as the sum of fatty acids as Free Fatty Acids)	26.98	±	0.66
	Energy (kcal per 100 g)		
Calories[(e)]	502.2	±	5.7

[(a)] Collaborating laboratories. Reported methods include drying in a forced-air oven, drying in a vacuum oven, and thermogravimetric analysis.
[(b)] Collaborating laboratories. Reported methods include weight loss after ignition in a muffle furnace.
[(c)] Collaborating laboratories. Reported methods include Kjeldahl, combustion, and thermal conductivity to determine nitrogen. A factor of 6.38 was used to convert nitrogen results to protein.
[(d)] Collaborating laboratories. Calculated by difference (solids less the sum of protein, fat, and ash).
[(e)] The value for calories is the median of lab mean caloric calculations from the interlaboratory comparison exercise. If the mean proximate values above are used for calculation, with caloric equivalents of 9, 4, and 4 for fat (as the sum of fatty acids), protein, and carbohydrate, respectively, the mean caloric content is 499.1 kcal/100 g.

Reference Mass Fraction Values for Amino Acids: Each reference mass fraction value is the median of the mean results provided by collaborating laboratories using hydrolysis followed by derivatization and LC. Values are expressed as $x \pm U_{95\%}(x)$, where x is the estimated value and $U_{95\%}(x)$ is the expanded uncertainty of the value. The method-specific value of the analyte lies within the interval $x \pm U_{95\%}(x)$ with about a 95 % confidence [2-4]. The measurands are the total mass fractions of amino acids in milk powder as listed in Table 8, on an as-received basis, as determined by the method indicated. Metrological traceability is to the measurement processes and standards used by collaborating laboratories.

Table 8. Reference Mass Fraction Values for Amino Acids in SRM 1549a

	Mass Fraction (g/100 g)
Alanine	0.845 ± 0.084
Arginine	0.89 ± 0.14
Aspartic Acid	1.96 ± 0.06
Cystine	0.18 ± 0.02
Glutamic Acid	5.34 ± 0.22
Glycine	0.46 ± 0.04
Histidine	0.617 ± 0.083
Isoleucine	1.12 ± 0.20
Leucine	2.41 ± 0.25
Lysine	2.05 ± 0.12
Methionine	0.68 ± 0.10
Phenylalanine	1.21 ± 0.11
Serine	1.42 ± 0.02
Threonine	1.09 ± 0.06
Tryptophan	0.29 ± 0.02
Tyrosine	1.12 ± 0.06
Valine	1.34 ± 0.26

Table 9. LC-MS/MS Transitions Monitored for Vitamins

Compound	Precursor Ion (*m/z*)	→ Product Ion (*m/z*)	Internal Standard (IS)	IS Precursor Ion (*m/z*)	→ IS Product Ion (*m/z*)
Riboflavin	377	43	$^{13}C_4,^{15}N_2$-Riboflavin	383	43
		172			175
		198			202
		243			249
Niacinamide	123	53	$^{2}H_4$-Niacinamide	127	56
		78			81
		80			84
Pantothenic Acid	220	41	$^{13}C_3,^{15}N$-Pantothenic Acid	224	41
		43			43
		72			76
		90			94
Pyridoxamine	169	77	$^{2}H_3$-Pyridoxamine	172	79
		134			136
		152			155
Pyridoxal	168	41	$^{2}H_3$-Pyridoxal	171	43
		67			70
		94			97
		150			153

REFERENCES

[1] May, W.; Parris, R.; Beck, C.; Fassett, J.; Greenberg, R.; Guenther, F.; Kramer, G.; Wise, S.; Gills, T.; Colbert, J.; Gettings, R.; MacDonald, B.; *Definition of Terms and Modes Used at NIST for Value-Assignment of Reference Materials for Chemical Measurements*; NIST Special Publication 260-136; U.S. Government Printing Office: Washington, DC (2000); available at https://www.nist.gov/system/files/documents/srm/SP260-136.PDF (accessed May 2020).

[2] JCGM 100:2008; *Evaluation of Measurement Data — Guide to the Expression of Uncertainty in Measurement* (GUM 1995 with Minor Corrections); Joint Committee for Guides in Metrology (JCGM) (2008); available at https://www.bipm.org/utils/common/documents/jcgm/JCGM_100_2008_E.pdf (accessed May 2020); see also Taylor, B.N.; Kuyatt, C.E.; *Guidelines for Evaluating and Expressing the Uncertainty of NIST Measurement Results*; NIST Technical Note 1297; U.S. Government Printing Office: Washington, DC (1994); available at https://www.nist.gov/pml/nist-technical-note-1297 (accessed May 2020).

[3] JCGM 101:2008; *Evaluation of Measurement Data – Supplement 1 to the "Guide to the Expression of Uncertainty in Measurement" — Propagation of Distributions using a Monte Carlo Method*; JCGM (2008); available at https://www.bipm.org/utils/common/documents/jcgm/JCGM_101_2008_E.pdf (accessed May 2020).

[4] Efron, B.; Tibshirani, R.J.; *An Introduction to the Bootstrap*; Chapman & Hall, London, UK (1993).

[5] Sharpless, K.E.; Duewer, D.L.; *Standard Reference Materials for Analysis of Dietary Supplements*; J. AOAC Int., Vol. 91, pp. 1298–1302 (2008).

[6] Ellerbe, P.; Meiselman, S.; Sniegoski, L.T.; Welch, M.J.; White, V.E.; *Determination of Serum Cholesterol by a Modification of the Isotope Dilution Mass Spectrometric Definitive Method*; Anal. Chem., Vol. 614, pp. 1718–1723 (1989).

[7] AOAC Official Method 996.06; *Official Methods of Analysis*, 18th ed.; AOAC International, Rockville, MD (2000).

[8] Huber, P.J; *Robust Statistics*; John Wiley, New York (1981).

Certificate Revision History: 29 May 2020 (Removal of certified value for ascorbic acid based on observed instability; certified values for fatty acids changed to reference values to properly reflect traceability and moved from Table 1 to Table 6; reference values for fatty acids moved from Table 7 to Table 6; some tables were re-numbered accordingly; correction to the measured isomer of heptadecenoic acid from n-7 to n-8; correction to the name of cysteine to cystine; editorial changes); **22 August 2018** (Change of expiration date; removal of values for myristoleic acid, vaccenic acid, α-linolenic acid, capric acid, and thiamine based on observed instability; correction of values for lauric acid, myristic acid, palmitic acid, iron, butyric acid, tridecanoic acid, margaric acid, margaroleic acid, cystine, glutamic acid, and glycine due to rounding errors; editorial changes); **28 October 2016** (Addition of values for cholecalciferol and 25-hydroxyvitamin D_3; conversion of fatty acid values from triglycerides to free fatty acids; editorial changes); **21 October 2014** (Update of values for Vitamin B_6; editorial changes); **13 August 2013** (Original certificate date).

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