National Institute of Standards and Technology
U.S. Department of Commerce

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10 November 2022

Standard Reference Material® 1549a

Whole Milk Powder

CERTIFICATE OF ANALYSIS

Purpose: The certified values delivered by this Standard Reference Material (SRM) are intended for validating methods for determining elements, vitamins, and cholesterol in whole milk powder and similar materials and for quality assurance when assigning values to in-house control materials.

Description: SRM 1549a consists of five heat sealed aluminized packets, each containing approximately 10 g of material.

Certified Values: The NIST certified values provided in Table 1 are traceable to the International System of Units (SI) derived unit of mass fraction, expressed as milligrams per kilogram or milligrams per gram. The values are reported on an as-received basis [1].

Table 1. Certified Values for Various Measurands in SRM 1549a

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

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

	Mass Fraction[(a)] (mg/g)
Cholesterol	0.981 ± 0.071

[(a)] 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].

[(b)] Niacinamide was the only form of vitamin B_3 detected.

[(c)] NIST measured pyridoxal and pyridoxamine individually, and pyridoxamine was mathematically converted to pyridoxal by multiplication by the ratio of the relative molecular masses.

Non-Certified Values: Non-certified values for elements, vitamins, carnitine, choline, fatty acids as free fatty acids, proximates, calories, and amino acids in SRM 1549a are provided in Appendix A.

Period of Validity: The certified values delivered by SRM 1549a are valid within the measurement uncertainty specified until **01 July 2025.** The certified values are nullified if the SRM is stored or used improperly, damaged, contaminated, or otherwise modified.

Maintenance of Certified Values: NIST will monitor this SRM over the period of its validity. If substantive technical changes occur that affect the certification, NIST will issue an amended certificate through the NIST SRM website (https://www.nist.gov/srm) and notify registered users. SRM users can register online from a link available on the NIST SRM website or fill out the user registration form that is supplied with the SRM. Registration will facilitate notification. Before making use of any of the values delivered by this material, users should verify they have the most recent version of this documentation, available through the NIST SRM website (https://www.nist.gov/srm).

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Additional Information: Additional information is provided in Appendices B and C.

Safety: SRM 1549a is intended for research use only; not for human consumption.

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 of NIST methods in Appendix C. 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.

Material Acquisition 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 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, as described in Appendix C.

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 using liquid chromatography (LC) with or without ID and MS or tandem mass spectrometry (MS/MS) and provided by collaborating laboratories, where appropriate, as described in Appendix C.

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) as described in Appendix C.

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. Methods reported by collaborating laboratories are described in Appendix C. 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) [6].

Homogeneity Assessment: The homogeneity of fatty acids, cholesterol, elements, and vitamins was assessed at NIST using the methods and test portion sizes described in Appendix C. 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.

REFERENCES

[1] Beauchamp, C.R.; Camara, J.E.; Carney, J.; Choquette, S.J.; Cole, K.D.; DeRose, P.C.; Duewer, D.L.; Epstein, M.S.; Kline, M.C.; Lippa, K.A.; Lucon, E.; Molloy, J.; Nelson, M.A.; Phinney, K.W.; Polakoski, M.; Possolo, A.; Sander, L.C.; Schiel, J.E.; Sharpless, K.E.; Toman, B.; Winchester, M.R.; Windover, D.; *Metrological Tools for the Reference Materials and Reference Instruments of the NIST Material Measurement Laboratory*; NIST Special Publication (NIST SP) 260-136, 2021 edition; U.S. Government Printing Office: Washington, DC (2021); available at https://nvlpubs.nist.gov/nistpubs/SpecialPublications/NIST.SP.260-136-2021.pdf (accessed Nov 2022).

[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/en/committees/jc/jcgm/publications (accessed Nov 2022); 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 Nov 2022).

[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/en/committees/jc/jcgm/publications (accessed Nov 2022).

[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).

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[7] 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).

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

Certificate Revision History: 10 November 2022 (Non-certified value for solids removed; updated format; editorial changes); 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); 23 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).

Certain commercial equipment, instruments, or materials may be identified in this Certificate of Analysis 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.

Users of this SRM should ensure that the Certificate of Analysis in their possession is current. This can be accomplished by contacting the Office of Reference Materials 100 Bureau Drive, Stop 2300, Gaithersburg, MD 20899-2300; telephone (301) 975-2200; e-mail srminfo@nist.gov; or the Internet at https://www.nist.gov/srm.

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APPENDIX A

Non-Certified Values: Non-certified values are suitable for use in method development, method harmonization, and process control but do not meet the NIST criteria for certification [1] nor provide metrological traceability to the SI or other higher-order reference system. They are the best estimates of the true values based on available data. The values are provided with an uncertainty that may reflect only measurement reproducibility, may not include all sources of uncertainty, and/or may reflect a lack of sufficient statistical agreement among multiple analytical methods.

Table A1. Non-Certified Values for Various Measurands in SRM 1549a

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

	Mass Fraction[a] (g/100 g)
Ash	5.625 ± 0.045
Protein	25.64 ± 0.31
Carbohydrates	38.43 ± 0.95
Fat (as the sum of fatty acids as Free Fatty Acids)	26.98 ± 0.66

	Energy (kcal/100 g)[a,b]
Calories[b]	502.2 ± 5.7

	Mass Fraction[a] (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

[a] 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 95 % confidence.

[b] 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.

Analytical Approach for Determination of Elements and Vitamins: Value assignment of the mass fractions of the elements and vitamins was described in the Certificate of Analysis.

Analytical Approach for Determination of Proximates, Calories, and Amino Acids: Value assignment of the mass fractions of the proximates, calories, and amino acids in SRM 1549a was based on the combination of results provided by collaborating laboratories as described in Appendix C.

Table A2. Non-Certified Mass Fraction Values for Fatty Acids as Free Fatty Acids in SRM 1549a[(a)]

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

[(a)] 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 95 % confidence.

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 various analytical methods at NIST using gas chromatography (GC) with flame ionization detection (FID) and GC-MS and provided by collaborating laboratories, where appropriate, as described in Appendix C.

Maintenance of Non-Certified Values: NIST will monitor this material to the end of its period of validity listed on the first page of the Certificate of Analysis. If substantive technical changes occur that affect the non-certified values during this period, NIST will update this Appendix. Before making use of any of the values delivered by this material, users should obtain the most recent version of this documentation, available free of charge through the https://www.nist.gov/srm website.

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APPENDIX B

Contributors to the Development and Value Assignment of SRM 1549a

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Collaborating Laboratories Contributing Data to Value Assignment through Grocery Manufacturers Association (GMA) Food Industry Analytical Chemists Committee (FIACC) Interlaboratory Study (ILS)
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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)
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APPENDIX C

NIST Methods Used for Value Assignment of Elements

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.

NIST Methods Used for Value Assignment of Vitamins

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 C1.

Table C1. ID LC MS/MS Transitions Monitored for Vitamins

Compound	Precursor Ion → Product Ion (*m/z*)		Internal Standard	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
Niacinamide	123	53	$^{2}H_4$-Niacinamide	127	56
		78			81
		80			84
Pantothenic Acid	220	41	$^{13}C_3$,^{15}N-Pantothenate Acid	224	41
		43			43
		72			76
		90			94
Pyridoxamine	169	77	$^{2}H_3$-Pyridoxamine	172	79
		134			136
		152			155
Pyridoxal	245	41	$^{2}H_3$-Pyridoxal	171	43
		67			70
		94			97
		150			153

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^{1}HNMR. 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^{1}HNMR. A single internal standard solution was used for the calibrants and samples.

NIST Methods Used for Value Assignment of Cholesterol

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.

NIST Methods Used for Value Assignment of Fatty Acids

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 samples were mixed for 30 s while still warm. Saturated aqueous sodium chloride solution was added, and the samples were 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.

Table C2. Methods Reported by Collaborating Laboratories Contributing Data to Value Assignment

	Analyte(s)	Method
Fatty Acids	All	GC-FID
Elements	Ca, Mg, K, Na	AAS and ICP-OES
	Mn, Zn	AAS, ICP-OES, and ICP-MS
	P	Absorption spectrophotometry, Colorimetry, ICP-OES, and ICP-MS
Vitamins	Biotin, Vitamin B_{12}	Microbiological assay
Proximates	Ash	Mass loss after ignition in muffle furnace
	Protein	Nitrogen determination using Kjeldahl, combustion; thermal conductivity (factor of 6.38)
	Carbohydrates	Calculation, [solids – (protein + fat (as the sum of fatty acids) + ash)]
	Calories	Calculation, [9×Fat + 4×Protein + 4×Carbohydrates], the mean caloric content is 499.1 kcal/100 g
Amino Acids	All	Hydrolysis with derivatization and LC

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