National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 3252

Protein Drink Mix

This Standard Reference Material (SRM) is intended primarily for validation of methods for determining proximates, fatty acids, cholesterol, vitamins, elements, and amino acids in protein drink mixes and similar materials. This SRM can also be used for quality assurance when assigning values to in-house reference materials. The SRM is a blend of commercial protein drink mixes. A unit of SRM 3252 consists of five heat-sealed aluminized pouches, each containing approximately 10 g of material.

Certified Mass Fraction Values: The certified mass fraction values for fatty acids and cholesterol, elements, and vitamins in SRM 3252, reported on a dry-mass 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 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 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, reported on a dry-mass basis, are provided for additional fatty acids (Table 4), additional elements (Table 5), additional vitamins, carnitine, and myo-inositol (Table 6), proximates and calories (Table 7), amino acids (Table 8), and caffeine, theobromine, and total polyphenols (Table 9). 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 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 [1]. Reference 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].

Expiration of Certification: The certification of **SRM 3252** 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, K.E. Sharpless, and L.J. Wood of the NIST Chemical Sciences Division and W. Koschute of the Grocery Manufacturers Association (GMA, Washington, DC).

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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Robert L. Watters, Jr., Director
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Analytical measurements at NIST were performed by J.F. Browning, J. Camara, K.D. Chieh, W.C. Davis, G.E. Hahm, R. Oflaz, M.M. Phillips, J.S. Pritchett, R.L. Paul, B.J. Porter, S.A. Rabb, M.M. Schantz, J.R. Sieber, L.T. Sniegoski, M.J. Welch, L.J. Wood of the NIST Chemical Sciences Division and B.E. Lang and B.C. Nelson of the NIST Biosystems and Biomaterials Division. Analyses for value assignment were also performed by the following laboratories participating in a GMA Food Industry Analytical Chemists Committee (FIACC) interlaboratory comparison exercise: Conagra Foods, Omaha, NE; Covance (Asia) Pte. Ltd., Singapore; Covance Laboratories, Inc., Battle Creek, MI; Covance Laboratories, Inc., Madison, WI; Del Monte Foods, Walnut Creek, CA; Eurofins Central Analytical Laboratories, Metairie, LA; Eurofins Chemical Control SRL, Cuneo, Italy; Eurofins Scientific, Des Moines, IA; General Mills, Inc., Golden Valley, MN; Hormel Foods Corporation, Austin, MN; Illinois Institute of Technology, Bedford Park, IL; Krueger Food Laboratories, Billerica, MA; Land O'Lakes, Arden Hills, MN; Schwan Food Company, Salina, KS; Silliker, Markham, ON, Canada; Silliker Illinois Analytical Laboratory, Crete, IL; Silliker México, Queretaro, Qro, Mexico; The J.M. Smucker Co., Orville, OH; and The National Food Laboratory, Livermore, CA.

NOTICE TO USERS: SRM 3252 IS INTENDED FOR LABORATORY USE ONLY, NOT FOR HUMAN CONSUMPTION.

INSTRUCTIONS FOR STORAGE AND USE

Storage: The SRM should be stored at controlled room temperature (20 °C to 25 °C) in the original unopened packets. For elemental analyses, the packet can be resealed, stored at room temperature, and test portions removed and analyzed until the material reaches its expiration date. For organic analyses, the packet can be resealed, stored at room temperature, and test portions removed and analyzed for two weeks after the packet was initially opened.

Use: Before use, the contents of the packet should be mixed thoroughly. Allow the contents to settle for one minute prior to opening to minimize the loss of fine particles. To relate analytical determinations to the certified values in this certificate, the test portion mass indicated in the description of the NIST analyses for each group of analytes below should be used. The minimum sample mass related to the NIST analytical determinations is described in the "Source, Preparation, and Analysis" section. 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.

Determination of Moisture: Moisture content of SRM 3252 was determined at NIST by (1) freeze-drying to constant mass over 7 d; (2) drying over magnesium perchlorate in a desiccator at room temperature for 20 d; and (3) drying for 3 h in a forced-air oven at 80 °C. Unweighted results obtained using all three techniques were averaged to determine a conversion factor of (0.9502 ± 0.0038) gram dry mass per gram as-received mass, which was used to convert data from an as-received to a dry-mass basis; the uncertainty shown on this value is an expanded uncertainty. An uncertainty component for the conversion factor (0.2 %) obtained from the moisture measurements is incorporated in the uncertainties of the certified and reference values, reported on a dry-mass basis, that are provided in this certificate.

SOURCE, PREPARATION, AND ANALYSIS[(1)]

Source and Preparation: The SRM is a blend of commercially available protein drink mixes. The commercial products were transferred to High-Purity Standards (Charleston, SC) where the material was blended and packaged. The protein drink mix was heat-sealed in approximately 10 g aliquots inside nitrogen-flushed 4-mil polyethylene bags, which were then sealed inside nitrogen-flushed Mylar bags along with two packets of silica gel each. Following packaging, SRM 3252 was irradiated (Neutron Products, Inc., Dickerson, MD) to an absorbed dose of 6.3 kGy to 8.4 kGy.

Analytical Approach for Determination of Fatty Acids and Cholesterol: Value assignment of the mass fractions of fatty acids in SRM 3252 was based on the combination of measurements made at NIST and collaborating laboratories, where appropriate. NIST provided measurements by using gas chromatography (GC) with flame ionization detection (FID). Value assignment of the cholesterol mass fraction was based on measurements made by NIST using an isotope dilution (ID) GC method with mass spectrometry (MS).

[(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.

NIST Analyses for Fatty Acids: Mass fractions of fatty acids were determined by GC-FID from two 1.0 g test portions from each of ten packets of SRM 3252. The analytes and internal standards were extracted into a mixture of methanol and toluene containing butylated hydroxytoluene (BHT, 1 g/L) as an antioxidant by sonication for 30 min. After centrifuging, the solvent was removed and fresh toluene containing BHT was added. The extraction was repeated for a total of three cycles, and all supernatants were combined and concentrated to approximately 2 mL under nitrogen. The concentrated extract was combined with 1 mL of MethPrep II (0.2 N methanolic [m-trifluoromethylphenyl] trimethylammonium hydroxide, Alltech, Deerfield, IL). Samples were mixed for 1 min and allowed to equilibrate for at least 1 h 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, 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 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 duplicate 0.5 g test portions from each of three packets of SRM 3252 weighed into Pyrex 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, 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.

Analytical Approach for Determination of Elements: Value assignment of the mass fractions of the elements in SRM 3252 was based on the combination of measurements from up to four different analytical methods at NIST and collaborating laboratories, where appropriate. NIST provided measurements by using inductively coupled plasma optical emission spectrometry (ICP-OES), inductively coupled plasma mass spectrometry (ICP-MS), wavelength dispersive X-ray fluorescence spectrometry (WDXRF), and instrumental neutron activation analysis (INAA).

NIST Analyses for Ba, Ca, Fe, I, K, Mg, Mn, Mo, Na, P, Se, and Sr using ICP-OES and/or ICP-MS: The mass fractions of barium, calcium, iron, magnesium, manganese, phosphorus, potassium, sodium, and strontium were measured by ICP-OES. The mass fractions of barium, iodine, molybdenum, selenium, and strontium were measured by ICP-MS. For each technique, duplicate 0.4 g test portions were taken from each of 10 packets of SRM 3252. Samples were digested in a microwave sample preparation system using nitric acid. For the determination of iodine by ICP-MS, samples were made basic using NH_4OH. Quantification for ICP-OES and ICP-MS was based on the method of standard additions.

NIST Analyses for Ca, Cu, Fe, K, Mn, Na, P, and Zn using WDXRF: Mass fractions of calcium, copper, iron, potassium, manganese, sodium, phosphorus, and zinc were measured by WDXRF in duplicate 4.0 g test portions taken from each of six packets of SRM 3252. Briquettes were prepared for each sample, and the K-$L_{2,3}$ characteristic X-ray lines of all elements were used for quantification.

NIST Analyses for Al, Ca, Cl, Cr, Fe, I, K, La, Mg, Mn, Mo, Na, Se, Sm, and Zn using INAA: Mass fractions of aluminum, calcium, chlorine, chromium, iodine, iron, lanthanum, magnesium, manganese, molybdenum, potassium, samarium, selenium, sodium, and zinc were measured by INAA in individual disks that were prepared from 0.2 g test portions taken from each of ten packets of SRM 3252. Samples, standards, and controls were packaged individually in clean polyethylene bags and irradiated individually. For determination of aluminum, calcium, chlorine, iodine, magnesium, manganese, potassium, and sodium, samples were irradiated at 20 MW for 60 s and nuclides were counted for 15 min after a 10 min decay. For determination of chromium, iron, lanthanum, molybdenum, samarium, selenium, and zinc, samples were irradiated at 20 MW for 8 h and nuclides were counted for 5 d following a 2 h decay or for 8 h following more than a 14 d decay.

Analytical Approach for Determination of Vitamins: Value assignment of the mass fractions of the vitamins in SRM 3252 was based on the combination of results provided by various analytical methods at NIST and collaborating laboratories. NIST provided measurements by using isotope dilution (ID) with liquid chromatography (LC) and mass spectrometry (MS) or tandem mass spectrometry (MS/MS).

NIST Analyses for Thiamine, Riboflavin, Niacinamide, Niacin, Pantothenic Acid, Pyridoxamine, and Pyridoxine: Mass fractions of thiamine, riboflavin, niacinamide, niacin, pantothenic acid, pyridoxamine, and pyridoxine were measured by ID-LC-MS/MS in duplicate 5 g test portions taken from each of ten packets of SRM 3252. The analytes and internal standards were extracted into ammonium acetate at pH 2.6 by stirring at 100 °C for 30 min. Samples were centrifuged following the digestion and an aliquot of the supernatant was analyzed 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. 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 10.

NIST Analyses for Biotin: Biotin was measured in two 1.0 g test portions taken from each of ten packets of SRM 3252. $^{2}H_{2}$-biotin was added as an internal standard. An aqueous solution of formic acid was added to the samples, which were then subjected to mechanical shaking for 30 min. Samples were centrifuged, and biotin and $^{2}H_{2}$-biotin were extracted on solid-phase extraction cartridges and eluted with a water/methanol solution containing formic acid for positive ion mode ID-LC-MS analysis. An isocratic LC method with a water/methanol/formic acid mobile phase and a C18 reversed-phase column were used for the determination of biotin. Biotin and $^{2}H_{2}$-biotin were monitored at *m/z* 245 and *m/z* 247, respectively. Calibrants were prepared gravimetrically, at levels intended to approximate the levels of the vitamins in the SRM following extraction. A single internal standard solution was used for the calibrants and samples.

NIST Analyses for Choline and Carnitine: Mass fractions of choline and carnitine were measured in duplicate 1.0 g test portions taken from each of ten packets of SRM 3252. $^{2}H_{9}$-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 in 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 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. A single internal standard solution was used for the calibrants and samples.

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 3252. 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 a bootstrap procedure [3–4].

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 lauric acid and myristic acid 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: 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 NIST, the mean of the mean values from NIST results were used. 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. For biotin, the calculation of assigned values is the mean of the NIST results with confirmation provided by collaborating laboratories. For niacin, niacinamide, and pyridoxine, the calculation of assigned values is the mean of the NIST results with confirmation provided by the determined total vitamin value, based on the combination of data from NIST and collaborating laboratories.

Certified Mass Fraction Values for Fatty Acids as Free Fatty Acids: Each certified mass fraction value is the combined mean from the mean of NIST GC-FID data and the median of the mean of data provided by collaborating laboratories. Methods reported by collaborating laboratories included GC-FID as well as hydrolysis with derivatization and LC. 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 ISO/JCGM Guide and k is a coverage factor corresponding to approximately 95 % confidence [2–4]. The measurands are the mass fractions of selected fatty acids in protein drink mix as listed in Table 1. Metrological traceability is to the SI derived units of mass fraction (expressed as g/100 g).

Certified Mass Fraction Value for Cholesterol: The certified mass fraction value for cholesterol is the mean of results obtained by NIST using ID-GC/MS. The uncertainty provided with the 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 represents the combined uncertainty consistent with the ISO/JCGM Guide and k is a coverage factor corresponding to approximately 95 % confidence [2]. The uncertainty for cholesterol incorporates a Type A component for the difference between the certification set of data and a confirming set of data and Type B components for purity of the reference compound, completeness of hydrolysis, and stability of cholesterol in base. The measurand is the mass fraction of cholesterol in protein drink mix as listed in Table 1. Metrological traceability is to the SI derived units of mass fraction (expressed as mg/g).

Table 1. Certified Mass Fraction Values for Fatty Acids (as Free Fatty Acids) and Cholesterol in SRM 3252

	Common Name	Mass Fraction (g/100 g)	Coverage Factor, k
Octanoic Acid (C8:0)	Caprylic Acid	0.0097 ± 0.0014	2.00
(Z)-9-Tetradecenoic Acid (C14:1)	Myristoleic Acid	0.0081 ± 0.0015	2.00
(Z)-9-Hexadecenoic Acid (C16:1 n-7)	Palmitoleic Acid	0.01649 ± 0.00069	2.00
Total cis-C18:1 Fatty Acids		1.186 ± 0.072	2.00

	Mass Fraction (mg/g)	Coverage Factor, k
Cholesterol	0.5077 ± 0.0056	2.45

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. 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 multiple methods and their respective uncertainties consistent with the ISO/JCGM Guide and k is a coverage factor corresponding to approximately 95 % confidence [2–4]. The measurands are the mass fractions of selected elements in protein drink mix as listed in Table 2. Metrological traceability is to the SI derived units of mass fraction (expressed as mg/kg).

Table 2. Certified Mass Fraction Values for Elements in SRM 3252

	Mass Fraction (mg/kg)			Coverage Factor k
Barium (Ba)[a,b]	3.12	±	0.14	2.00
Calcium (Ca)[a,c,d,e]	17840	±	990	2.00
Copper (Cu)[c,e]	36.36	±	0.74	2.00
Iron (Fe)[a,c,d,e]	381	±	13	2.00
Magnesium (Mg)[a,d,e]	6337	±	174	2.00
Manganese (Mn)[a,c,d,e]	11.12	±	0.48	2.00
Molybdenum (Mo)[a,d]	1.19	±	0.20	2.00
Phosphorus (P)[a,c,e]	17210	±	710	2.00
Potassium (K)[a,c,d,e]	11550	±	530	2.00
Selenium (Se)[b,d]	0.596	±	0.037	2.00
Sodium (Na)[a,c,d,e]	6820	±	170	2.00
Strontium (Sr)[a,b]	13.65	±	0.79	2.00
Zinc (Zn)[c,d,e]	235	±	12	2.00

[a] NIST ICP-OES
[b] NIST ICP-MS
[c] NIST WDXRF
[d] NIST INAA
[e] Collaborating laboratories. Reported methods included atomic absorption spectroscopy (AAS), ICP optical emission spectroscopy (ICP-OES), ICP-MS, ion chromatography with suppressed conductivity detection, and colorimetry.

Certified Mass Fraction Values for Vitamins: Each certified mass fraction value is the combined mean from the mean of results from analyses provided by NIST and the median of the mean of results provided by collaborating laboratories, where appropriate. 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 ISO/JCGM Guide and k is a coverage factor corresponding to approximately 95 % confidence [2–4]. The uncertainty for thiamine incorporates an additional component for possible bias observed in an unspiked sample. The measurands are the mass fractions of selected vitamins in protein drink mix as listed in Table 3. Metrological traceability is to the SI derived units of mass fraction (expressed as mg/kg).

Table 3. Certified Mass Fraction Values for Vitamins in SRM 3252

	Mass Fraction (mg/kg)	Coverage Factor, k
Thiamine (Vitamin B_1)[a,b]	12.3 ± 1.6	2.00
Riboflavin (Vitamin B_2)[a,b]	28.7 ± 2.8	2.00
Niacinamide (Vitamin B_3)[a]	269.7 ± 4.4	2.08
Niacin (Vitamin B_3)[a]	7.33 ± 0.26	2.09
Total Vitamin B_3 as Niacinamide[a,b,c]	287 ± 21	2.00
Pantothenic Acid (Vitamin B_5)[a,b]	150 ± 12	2.00
Pyridoxine (Vitamin B_6)[a]	29.2 ± 1.6	2.09
Total Vitamin B_6 as Pyridoxine[a,b,d]	29.1 ± 2.7	2.00
Biotin[e]	4.43 ± 0.19	2.02
Choline[e]	1328 ± 17	2.07

[a] NIST ID-LC-MS/MS

[b] Collaborating laboratories. Reported methods included microbiological assay, digestion with fluorescence detection, extraction with reversed phase LC and fluorescence detection, extraction with LC-MS, and an autoanalyzer.

[c] Total vitamin B_3 is the sum of niacin and niacinamide; the mass fraction of niacin was mathematically converted to niacinamide by multiplying by the ratio of the relative molecular masses of niacin and niacinamide.

[d] Total vitamin B_6 is the sum of pyridoxal, pyridoxamine, and pyridoxine; the mass fractions of pyridoxal and pyridoxamine were mathematically converted to pyridoxine by multiplying the mass fraction by the ratio of the relative molecular masses of pyridoxal, pyridoxamine, and pyridoxine.

[e] NIST ID-LC-MS

Reference Values for Fatty Acids (as Free Fatty Acids): Each reference mass fraction value is the mean from the combination of the mean results from analyses at NIST and the median of the mean of results provided by collaborating laboratories, where appropriate. The uncertainty provided is an expanded uncertainty about the mean or median 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 ISO/JCGM Guide and k is a coverage factor corresponding to approximately 95 % confidence [2]. The uncertainties for lauric acid and myristic acid also incorporate an additional component for possible inhomogeneity. The measurands are the mass fractions of selected fatty acids in protein drink mix as listed in Table 4 as determined by the indicated method. Metrological traceability is to the SI derived units of mass fraction (expressed as g/100 g).

Table 4. Reference Mass Fraction Values for Fatty Acids (as Free Fatty Acids) in SRM 3252

	Common Name	Mass Fraction (g/100 g)	Coverage Factor, *k*
Butanoic Acid (C4:0)[a]	Butyric Acid	0.0235 ± 0.0098	2.23
Hexanoic Acid (C6:0)[a]	Caproic Acid	0.0168 ± 0.0026	2.18
Decanoic Acid (C10:0)[a]	Capric Acid	0.0219 ± 0.0025	2.13
Dodecanoic Acid (C12:0)[a,b]	Lauric Acid	0.0276 ± 0.0076	2.00
Tetradecanoic Acid (C14:0)[a,b]	Myristic Acid	0.093 ± 0.027	2.00
Pentadecanoic Acid (C15:0)[a]		0.0129 ± 0.0011	2.13
Hexadecanoic Acid (C16:0)[a]	Palmitic Acid	1.131 ± 0.063	2.13
Heptadecanoic Acid (C17:0)[a]	Margaric Acid	0.0126 ± 0.0011	2.13
Octadecanoic Acid (C18:0)[a]	Stearic Acid	0.778 ± 0.048	2.13
Total trans-C18:1 Fatty Acids[a]		0.68 ± 0.20	2.13
(Z)-9-Octadecenoic Acid (C18:1 n-9)[a]	Oleic Acid	1.026 ± 0.077	2.23
(Z)-11-Octadecenoic Acid (C18:1 n-7)[a]	Vaccenic Acid	0.0839 ± 0.0077	2.36
(Z,Z)-9,12-Octadecadienoic Acid (C18:2 n-6)[a]	Linoleic Acid	1.126 ± 0.086	2.20
Total cis-C18:2 Fatty Acids[a]		1.18 ± 0.14	2.16
Total trans-C18:2 Fatty Acids[a]		0.030 ± 0.010	2.20
Total trans-C18:2 Conjugated Fatty Acids[a]		0.0093 ± 0.0021	3.18
(Z,Z,Z)-9,12,15-Octadecatrienoic Acid (C18:3 n-3)[a]	α-Linolenic Acid	0.122 ± 0.016	2.14
Eicosanoic Acid (C20:0)[a]	Arachidic Acid	0.0242 ± 0.0020	2.13
(Z)-11-Eicosenoic Acid (C20:1 n-9)[b]	Gondoic Acid	0.0070 ± 0.0004	2.09
Docosanoic Acid (C22:0)	Behenic Acid	0.0310 ± 0.0048	2.13
Tetracosanoic Acid (C24:0)[a]	Lignoceric Acid	0.0203 ± 0.0018	2.20
Saturated Fatty Acids[a]		2.173 ± 0.094	2.13
cis-Monounsaturated Fatty Acids[a]		1.255 ± 0.063	2.13
cis-Polyunsaturated Fatty Acids[a]		1.30 ± 0.10	2.13
Total Trans Fatty Acids[a]		0.71 ± 0.22	2.13
Total Omega-3 Fatty Acids[a]		0.122 ± 0.011	2.20
Total Omega-6 Fatty Acids[a]		1.179 ± 0.062	2.18

[a] Collaborating laboratories. Reported methods included GC-FID as well as hydrolysis with derivatization and LC.
[b] NIST GC-FID

Reference Mass Fraction Values for Elements: Each reference mass fraction value is the mean result of NIST analyses by INAA. 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 ISO/JCGM Guide and k is a coverage factor corresponding to approximately 95 % confidence [2]. The measurands are the mass fractions of selected elements in protein drink mix as listed in Table 5 as determined by the indicated method. Metrological traceability is to the SI derived units of mass fraction (expressed as mg/kg).

Table 5. Reference Mass Fraction Values for Elements in SRM 3252

	Mass Fraction (mg/kg)	Coverage Factor, k
Aluminum (Al)	76.6 ± 4.8	2.18
Chlorine (Cl)	7160 ± 690	2.16
Chromium (Cr)	1.06 ± 0.10	2.12
Iodine (I)[(a)]	1.84 ± 0.20	2.16
Lanthanum (La)	0.0742 ± 0.0031	2.09
Samarium (Sm)	0.0170 ± 0.0014	2.09

[(a)] Confirmation by NIST ICP-MS.

Reference Mass Fraction Values for Vitamins, Carnitine, and myo-Inositol: Each reference mass fraction value is the mean result of NIST analyses or the median of the mean of results provided by collaborating laboratories. The uncertainty provided is an expanded uncertainty about the mean or median 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 ISO/JCGM Guide and k is a coverage factor corresponding to approximately 95 % confidence [2]. For values based on more than one data source, the combined uncertainty incorporates the observed difference between the results from the methods and their respective uncertainties, consistent with the ISO/JCGM Guide [2–4]. The uncertainty for pyridoxamine incorporates an additional component for calibration variability. The measurands are the mass fractions of selected vitamins, carnitine, and myo-Inositol in protein drink mix as listed in Table 6 as determined by the indicated method. Metrological traceability is to the SI derived units of mass fraction (expressed as mg/kg).

Table 6. Reference Mass Fraction Values for Vitamins, Carnitine, and myo-Inositol in SRM 3252

	Mass Fraction (mg/kg)	Coverage Factor, k
Ascorbic Acid (Vitamin C)[(a)]	940 ± 100	2.13
Pyridoxamine (Vitamin B_6)[(b)]	0.0605 ± 0.0063	2.00
Folic Acid[(c)]	7.6 ± 1.9	2.57
Vitamin B_{12}[(d)]	0.108 ± 0.026	2.57
Carnitine[(e)]	4.76 ± 0.12	2.09
myo-Inositol[(c)]	186 ± 40	4.30
Retinol[(f)]	23.8 ± 1.5	2.31
α-Tocopherol[(f)]	370 ± 86	2.36
δ-Tocopherol[(f)]	6.1 ± 1.5	4.30

[(a)] Collaborating laboratories. Reported methods included extraction with LC and electrochemical detection, extraction with LC and absorbance detection, extraction with LC and fluorescence detection, AOAC 984.26 automated flow method for ascorbic acid, and dichloroindophenol titration for ascorbic acid.
[(b)] NIST ID-LC-MS/MS
[(c)] Collaborating laboratories. Reported methods included microbiological assay.
[(d)] Collaborating laboratories. Reported methods included LC-DAD and microbiological assay.
[(e)] NIST ID-LC-MS
[(f)] Collaborating laboratories.

Reference Mass Fraction Values for Proximates and Calories: Each reference mass fraction value is the median of the mean of results provided by collaborating laboratories. The uncertainty provided is an expanded uncertainty about the median 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 ISO/JCGM Guide and k is a coverage factor corresponding to approximately 95 % confidence [2]. The measurands are the mass fractions of selected proximates in protein drink mix as listed in Table 7 as determined by the indicated method. Metrological traceability is to the SI derived units of mass fraction (expressed as g/100 g). The measurand is the caloric content listed in Table 7 as determined by the indicated method. Metrological traceability is to the SI derived units for energy (expressed as kcal/100 g).

Table 7. Reference Mass Fraction Values for Proximates and Calories in SRM 3252

	Mass Fraction (g/100 g)	Coverage Factor, *k*
Ash[(a)]	10.77 ± 0.10	2.06
Protein[(b)]	66.92 ± 0.61	2.06
Carbohydrates[(c)]	15.31 ± 0.99	2.14
Total Dietary Fiber	6.22 ± 0.95	2.23
Fat (as the sum of fatty acids as triglycerides)	5.81 ± 0.29	2.13

	Energy (kcal per 100 g)	Coverage Factor, *k*
Calories[(d)]	381.2 ± 2.2	2.01

[(a)] Ash was determined by collaborating laboratories using weight loss after ignition in a muffle furnace and thermogravimetric analysis.

[(b)] Nitrogen was determined by collaborating laboratories using Kjeldahl and combustion (LECO). A factor of 6.25 was used to convert nitrogen results to protein.

[(c)] Carbohydrates were determined by collaborating laboratories by difference (solids less the sum of protein, fat, and ash).

[(d)] The reference 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 381.2 kcal per 100 grams.

Reference Mass Fraction Values for Amino Acids: Each reference mass fraction value is the median of the mean results provided by collaborating laboratories. The uncertainty provided is an expanded uncertainty about the median 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 ISO/JCGM Guide and k is a coverage factor corresponding to approximately 95 % confidence [2]. The measurands are the mass fractions of selected amino acids in protein drink mix as listed in Table 8 as determined by the method used by the collaborating laboratories. Metrological traceability is to the SI derived units of mass fraction (expressed as g/100 g).

Table 8. Reference Mass Fraction Values for Amino Acids in SRM 3252

	Mass Fraction (g/100 g)	Coverage Factor, k
Alanine	2.87 ± 0.59	3.18
Arginine	3.55 ± 0.52	3.18
Aspartic Acid	7.0 ± 1.4	3.18
Cystine	0.87 ± 0.23	12.7
Glutamic Acid	13.4 ± 3.0	3.18
Glycine	1.93 ± 0.50	3.18
Histidine	1.49 ± 0.18	3.18
Isoleucine	3.42 ± 0.82	3.18
Leucine	6.2 ± 1.2	3.18
Lysine	4.84 ± 0.78	3.18
Methionine	1.426 ± 0.058	4.30
Phenylalanine	3.19 ± 0.47	3.18
Serine	3.85 ± 0.94	3.18
Threonine	3.38 ± 0.67	3.18
Tyrosine	2.66 ± 0.32	3.18
Valine	3.7 ± 1.1	3.18

Reference Mass Fraction Values for Additional Measurands: Each reference mass fraction value is the median of the mean results provided by collaborating laboratories. The uncertainty provided is an expanded uncertainty about the median 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 ISO/JCGM Guide and k is a coverage factor corresponding to approximately 95 % confidence [2]. The measurands are the mass fractions of caffeine, theobromine, and total polyphenols in protein drink mix as listed in Table 9 as determined by the method used by the collaborating laboratories. Metrological traceability is to the SI derived units of mass fraction (expressed as mg/kg).

Table 9. Reference Mass Fraction Values for Additional Measurands in SRM 3252

	Mass Fraction (mg/kg)	Coverage Factor, k
Caffeine	174.4 ± 2.5	3.18
Theobromine	2315 ± 93	4.30
Total Polyphenols (Gallic Acid Equivalents)	7800 ± 1500	4.30

Table 10. ID-LC-MS/MS Transitions Monitored for Vitamins

Compound	Precursor Ion (*m/z*)	→ Product Ion (*m/z*)	Internal Standard	IS Precursor Ion (*m/z*)	→ IS Product Ion (*m/z*)
Thiamine	266	42	$^{13}C_3$-Thiamine	269	42
		123			123
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
Niacin	124	52	$^{2}H_4$-Niacinamide	127	53
		53			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
Pyridoxine	170	77	$^{13}C_4$-Pyridoxine	174	81
		80			83
		134			138
		152			156

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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; e-mail srminfo@nist.gov; or via the Internet at http://www.nist.gov/srm.