National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 3290

Dry Cat Food

This Standard Reference Material (SRM) is intended primarily for use in validating methods for determining proximates, vitamins, elements, fatty acids, and amino acids in dry cat food and similar matrices. This SRM can also be used for quality assurance when assigning values to in-house control materials. This SRM is a blend of commercially available dry cat foods. A unit of SRM 3290 consists of five heat-sealed, aluminized pouches, each containing approximately 10 g of material.

Certified Mass Fraction Values: The certified mass fraction values of elements and vitamins, reported on a dry-mass basis, are provided in Tables 1 and 2. A NIST certified value is a value for which NIST has the highest confidence in its accuracy in that all known or suspected sources of bias have been investigated or taken into account [1]. Analyses for value assignment were performed by NIST and collaborating laboratories. Certified values were calculated as the mean of the mean values from NIST methods and the median of the mean of results provided by collaborating laboratories, where appropriate. All values were combined without weighting. 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 elements and vitamins (Tables 3 and 4), and for fatty acids, proximates, calories, and amino acids (Tables 5 through 7). A NIST reference value is a non-certified value that is the best estimate of the true value based on available data; however, the value does not meet the NIST criteria for certification [1] and is provided with associated uncertainties that may reflect only measurement reproducibility, may not include all sources of uncertainty, or may reflect a lack of sufficient statistical agreement among multiple analytical methods. The reference mass fraction values were derived from results reported by NIST and/or collaborating laboratories.

Information Mass Fraction Values: Information mass fraction values for several elements are provided in Table 8 as additional information on the composition of the material. A NIST information value is considered to be a value that may be of interest to the SRM user, but insufficient information is available to assess the uncertainty associated with the value, and therefore no uncertainty is provided [1]. Values are reported on a dry-mass basis in mass fraction units [5]. Information values cannot be used to establish metrological traceability.

Expiration of Certification: The certification of **SRM 3290** is valid, within the measurement uncertainty specified, until **01 October 2025**, provided the SRM is handled and stored in accordance with 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 W. Koshute of the Grocery Manufacturers Association (GMA, Washington, DC).

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Steven J. Choquette, Director
Office of Reference Materials

Analyses at NIST were performed by G.C. Caceres, J.E. Camara, G.E. Hahm, B.E. Lang, J.L. Molloy, K.E. Murphy, R.L. Paul, M.M. Phillips, S.A. Rabb, L.J. Wood, and L.L. Yu of the NIST Chemical Sciences Division, B.C. Nelson of the NIST Biosystems and Biomaterials Division, and J. Brown Thomas, J.F. Browning, K.D. Chieh, S.K.R. Chinthalapati, A. Lee, R. Oflaz, B.J. Porter, M.M. Schantz, and J.R. Sieber formerly of NIST.

Analysts at the following laboratories performed measurements that contributed to the value assignment of nutrients in SRM 3290 Dry Cat Food as part of the GMA Food Industry Analytical Chemists Committee (FIACC) interlaboratory comparison exercise: Campbell Soup Company (Camden, NJ), ConAgra Foods Analytical Laboratory (Omaha, NE), Covance Laboratories, Inc. (Battle Creek, MI; Madison, WI; Singapore), Del Monte Foods (Walnut Creek, CA), Eurofins Central Analytical Laboratories (Metairie, LA), Eurofins Scientific (Des Moines, IA), General Mills Inc. (Golden Valley, MN), Hormel Foods (Austin, MN), Krueger Food Labs (Billerica, MA), Land O' Lakes (Arden Hills, MN), Schwan Food Company (Salina, KS), Silliker Food Science Center (Crete, IL), Silliker Mexico (Mexico City, Mexico), Silliker Shanghai, Ltd. (Shanghai, China), Silliker Canada (Markham, ON, Canada), The J.M. Smucker Co. (Orrville, OH), and The National Food Laboratory (Livermore, CA).

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

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

NOTICE TO USERS: SRM 3290 IS INTENDED FOR RESEARCH USE; NOT FOR HUMAN OR ANIMAL 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 controlled room temperature, and test portions removed for analysis 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 within 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 or reference 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 or reference values using procedures described in reference 6.

Determination of Moisture: Moisture content of SRM 3290 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 21 d; and (3) drying in a forced-air oven at 80 °C for 3 h. Unweighted results obtained using all three techniques were averaged to determine a conversion factor of (0.9564 ± 0.0015) 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. A relative uncertainty component for the conversion factor (0.08 %) 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: SRM 3290 is a blend of commercially available dry cat foods. The commercial products were transferred to High-Purity Standards (Charleston, SC) in the bags in which they were received. The material was ground, blended, and packaged in 10 g aliquots in heat-sealed 4 mil polyethylene bags then sealed inside nitrogen-flushed aluminized plastic bags along with two silica gel packets. Following packaging, SRM 3290 was irradiated (Neutron Products, Inc., Dickerson, MD) to an absorbed dose of 6.3 kGy to 8.4 kGy.

Analytical Approach for Determination of Elements: Value assignment of the mass fractions of the elements in SRM 3290 was based on the combination of measurements from at least two different analytical methods at NIST or a single NIST result and results obtained by collaborating laboratories, where appropriate. NIST provided measurements by using inductively coupled plasma optical emission spectrometry (ICP-OES), inductively coupled

[(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.

plasma mass spectrometry (ICP-MS), isotope dilution inductively coupled plasma mass spectrometry (ID ICP-MS), instrumental neutron activation analysis (INAA), radiochemical neutron activation analysis (RNAA), and wavelength dispersive X-ray fluorescence spectrometry (WDXRF).

NIST Analyses for As, Ba, Ca, Cr, Fe, I, K, Mn, Mo, Ni, P, Na, Se, Sr, and Zn using ICP-OES and/or ICP-MS: Mass fractions of barium, calcium, iron, potassium, manganese, phosphorus, potassium, sodium, strontium, and zinc were determined by ICP-OES. Mass fractions of arsenic, barium, chromium, iodine, molybdenum, nickel, selenium, and strontium were determined by ICP-MS. For each technique, duplicate 0.5 g test portions were taken from each of either six or ten packages of SRM 3290 and digested in closed vessels using nitric acid in a microwave sample preparation system. For the determination of iodine by ICP-MS, ammonium hydroxide was added to samples after digestion to raise the pH. Quantification for all analytes was based on the method of standard additions using the SRM 3100 series single element standard solutions.

NIST Analyses for Cd and Pb using ID ICP-MS: Mass fractions of cadmium and lead were determined by ID ICP-MS using duplicate, nominal 1 g test portions taken from each of six packages of SRM 3290. Samples were spiked with isotopically enriched ^{111}Cd and ^{206}Pb and were digested in nitric acid using a microwave sample preparation system as described in references 7 and 8. Sample digests were evaporated to near dryness and a portion was reconstituted in dilute nitric acid for analysis. Lead was measured in standard mode, whereas cadmium was measured in collision cell/kinetic energy discrimination mode. Quantification was based on standard solutions prepared from SRM 746 *Cadmium-Vapor Pressure*, SRM 3108 *Cadmium (Cd) Standard Solution*, SRM 3128 *Lead (Pb) Standard Solution*, and a commercial metal sample using isotope dilution.

NIST Analyses for Al, Ca, Cl, Cr, Fe, I, K, Mg, Mn, Mo, Na, Se, and Zn using INAA: Mass fractions of aluminum, calcium, chlorine, chromium, iron, iodine, potassium, magnesium, manganese, molybdenum, sodium, selenium, and zinc were determined by INAA using duplicate 0.25 g test portions taken from each of ten packages of SRM 3290. For the analysis of chromium, iron, molybdenum, selenium, and zinc, powders were pressed into cylindrical pellets, and samples, standards, and controls were packaged individually in clean polyethylene bags and irradiated individually at 20 MW for 8 h with a 180-degree inversion after 4 h. Nuclides were counted for 6 h after decays of 7 d for molybdenum, for 8 h after decays of 14 days for iron, selenium, and zinc, and for 4 h after decays of 21 d for chromium. For analysis of aluminum, calcium, chlorine, iodine, magnesium, manganese, potassium, and sodium, each sample, standard, or control material was individually irradiated together with one flux monitor foil for 60 s at a reactor power of 20 MW. Nuclides were counted for 5 min to 10 min after decays of 10 min to 15 min. Quantification is based on validated pure metal foils, pure compounds, or SRM 3100 single element standard solutions.

NIST Analyses for As using RNAA: The mass fraction of arsenic was determined by RNAA from single, nominal 0.3 g test portions taken from each of six packets of SRM 3290, and in each of five packets one month later. Test portions were pressed into pellets, packaged individually in clean polyethylene bags, and irradiated individually at 20 MW for 6 h. Samples, standards, and controls were combined with ^{77}As prior to chemical separation, then dissolved in nitric acid or a mixture of nitric and perchloric acids, and As was separated from the matrix as described in reference 9. The 559 keV line from decay of ^{76}As was used for quantification. The 239 keV line from decay of ^{77}As was evaluated for yield determination.

NIST Analyses for Ca, Cl, Cu, Fe, K, Mg, Mn, Na, P, and S using WDXRF: Mass fractions of calcium, chlorine, copper, iron, magnesium, manganese, sodium, phosphorous, potassium, and sulfur were measured by WDXRF using duplicate 4.0 g test portions taken from each of six packets of SRM 3290. Briquettes were prepared for each sample. The K-$L_{2,3}$ characteristic X-ray lines of all elements were used for quantification.

Analytical Approach for Determination of Vitamins: Value assignment of the mass fractions of the vitamins in SRM 3290 was based on the combination of measurements made using analytical methods at NIST and collaborating laboratories, where appropriate. NIST provided measurements by using liquid chromatography (LC) with absorbance detection, isotope dilution (ID) with LC and mass spectrometry (MS) or tandem mass spectrometry (MS/MS), and LC-ICP-MS.

NIST Analyses for Ascorbic Acid using LC-Absorbance: The mass fraction of ascorbic acid (vitamin C) was measured in triplicate 2 g test portions taken from each of six packets of SRM 3290. The sample was dissolved in 30 g of 0.1 mol/L HCl. 4-Pyridoxic acid was added as an internal standard, metaphosphoric acid was added to stabilize the vitamin C in solution, and dithiothreitol was added to convert dihydroascorbic acid to total ascorbic acid. The solutions were sonicated for 30 min, centrifuged, then filtered prior to LC analysis. Separations were performed on a C18 column with a gradient mobile phase of potassium phosphate (dibasic) and acetonitrile and absorbance detection at 243 nm. Ascorbic acid and 4-pyridoxic acid were monitored at 243 nm and 260 nm, respectively. The purity of the neat calibrant material was determined at NIST using LC-absorbance at 243 nm. A single internal standard solution was used for the calibrants and samples.

NIST Analyses for Thiamine, Riboflavin, Niacinamide, Niacin, Pantothenic Acid, Pyridoxal, Pyridoxamine, and Pyridoxine using ID-LC-MS/MS: Mass fractions of thiamine, riboflavin, niacinamide, niacin, pantothenic acid, pyridoxal, pyridoxamine, and pyridoxine were measured by ID-LC-MS/MS in duplicate 2.5 g test portions taken from each of 10 packets of SRM 3290. The analytes and internal standards were extracted into ammonium acetate (adjusted to pH 2.6 with hydrochloric acid) by rotary mixing for 30 min. Samples were centrifuged 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 purity of neat calibrant materials was determined at NIST using LC-absorbance, Karl Fischer titration, thermogravimetric analysis, and differential scanning calorimetry. The analyte and internal standard transitions monitored for ID-LC-MS/MS are listed in Table 9.

NIST Analyses for Biotin using LC-MS: The mass fraction of biotin was measured in two 1.0 g test portions taken from each of 10 packets of SRM 3290. $^{2}H_{2}$-biotin was added as an internal standard. An aqueous solution of 1.5 % formic acid (volume fraction) 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 (SPE) cartridges and eluted with a water/methanol solution containing formic acid for positive-ion mode LC-MS analysis. An isocratic LC method with a water/methanol/formic acid mobile phase and a C18 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. The purity of the neat calibrant material was determined at NIST using quantitative proton nuclear magnetic resonance spectroscopy. A single internal standard solution was used for the calibrants and samples.

NIST Analyses for Folic Acid using LC-MS/MS: The mass fraction of folic acid was measured in two 1.0 g test portions taken from each of 10 packets of SRM 3290. $^{13}C_{5}$-folic acid was added as an internal standard. A neutral extraction buffer was added to the samples, which were then subjected to mechanical shaking for 2 h. Samples were centrifuged and the supernatant was filtered prior to positive-ion mode LC-MS/MS analysis. A gradient method with a water/acetonitrile/formic acid mobile phase and a C18 column were used for LC-MS/MS determination. The transitions *m/z* 442 → 295 and *m/z* 447 → 295 were monitored for folic acid and $^{13}C_{5}$-folic acid, respectively. Calibrants were prepared gravimetrically, with concentrations assigned spectrophotometrically, 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 Analysis for Free Cyanocobalamin using LC-ICP-MS: The mass fraction of free cyanocobalamin was measured in two 2.0 g test portions taken from each of ten packets of SRM 3290. Cyanocobalamin was extracted into deionized water, samples were centrifuged, and the supernatants were filtered through 0.45 µm nylon filters. Yttrium was added as an internal standard. A mobile phase of ethylenediaminetetraacetic acid in methanol and water and a C18 column were used for LC-ICP-MS analysis. Cobalt and cyanocobalamin were monitored at *m/z* 59, and yttrium was monitored at *m/z* 89. The purity of the neat calibrant material was determined at NIST using LC-absorbance.

NIST Analyses for Choline and Carnitine using LC-MS: Mass fractions of choline and carnitine were measured in duplicate 0.5 g test portions taken from each of 10 packets of SRM 3290. Internal standards of d_9-choline and d_9-carnitine were added to the samples. The analytes and internal standards were extracted and hydrolyzed by microwave digestion in 1 mol/L hydrochloric acid followed by enzyme treatment with phospholipase D prior to 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 d_9-choline were monitored at *m/z* 104 and *m/z* 113, respectively. Carnitine and d_9-carnitine were monitored at *m/z* 162 and *m/z* 171, respectively. Calibrants were prepared gravimetrically, at levels intended to approximate the levels of choline and carnitine in the SRM following extraction. The purity of neat calibrant materials was determined at NIST using quantitative proton nuclear magnetic resonance spectroscopy. 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 3290 was based on the combination of measurements made at NIST and by collaborating laboratories, where appropriate. NIST provided measurements using gas chromatography with flame ionization detection (GC-FID).

NIST Analyses for Fatty Acids: Mass fractions of fatty acids were determined by GC-FID from two 1 g test portions from each of 10 packets of SRM 3290. The dry cat food and internal standards [myristic-d_{27} and stearic-d_{35} in toluene containing 1 g/L butylated hydroxytoluene (BHT)] were extracted into a methanol:toluene (1:10 volume fraction) solution by sonication, vortexing, centrifuging, and finally transferring the solvent. The steps were repeated twice more using only an addition of toluene containing BHT. The final volume of 30 mL of toluene and 1 mL of methanol

was evaporated to 2 mL. At this point, 1 mL of MethPrep II [0.2 N methanolic (m-trifluoromethylphenyl) trimethylammonium hydroxide, Alltech (Deerfield, IL)] was added volumetrically to the vial. The vials were then manually mixed for approximately 1 min and allowed to equilibrate for at least 1 h prior to transfer to an autosampler vial for GC-FID analysis. The GC-FID was equipped with a 0.25 mm × 100 m biscyanopropyl polysiloxane fused silica capillary column. Calibrants were prepared gravimetrically from SRM 2377 *Fatty Acid Methyl Esters in Isooctane*, at levels intended to approximate the levels of the fatty acids in the SRM following extraction. The same 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, fatty acids, vitamins, elements, and amino acids on test portions taken from each of two packets of SRM 3290. 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 elements, water-soluble vitamins, and fatty acids was assessed at NIST using the methods and test portion sizes described above. Analyses of variance showed statistically significant heterogeneity in some cases, and the uncertainties for biotin and free cyanocobalamin incorporate an additional component for possible heterogeneity. Homogeneity of constituents measured solely by collaborating laboratories (e.g., proximates, amino acids) was not assessed, although the data were treated as though these analytes were homogeneously distributed.

Value Assignment: For calculation of assigned values for analytes that were measured only by NIST, the mean of the mean values from NIST results was used. The collaborating laboratories reported the individual results for each of their analyses for a given analyte. The mean of each laboratory's results was then determined. For calculation of assigned values for analytes that were measured only by the collaborating laboratories, the median of the laboratory means was used. For analytes that were also measured by NIST, the median of the individual collaborating laboratory means and the means of the individual sets of NIST data were averaged, as appropriate. For niacin, niacinamide, pyridoxamine, pyridoxal, 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 Elements: Each certified mass fraction value is the combined mean from the means of results from analyses provided by NIST and the median of the mean 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 [2–4]. The measurand is the total mass fraction for each element listed in Table 1 on a dry-mass basis. Metrological traceability is to the International System of Units (SI) derived unit for mass fraction (expressed as milligrams per kilogram).

Table 1. Certified Mass Fraction Values for Elements in SRM 3290

	Mass Fraction (mg/kg)		
Arsenic (As)[a,b]	0.146	±	0.013
Barium (Ba)[a,c]	12.39	±	0.32
Calcium (Ca)[c,d,e,f]	11230	±	600
Cadmium (Cd)[g]	0.0384	±	0.0013
Chlorine (Cl)[e,f]	8350	±	200
Chromium (Cr)[a,f]	30.01	±	0.37
Copper (Cu)[d,e,f]	18.35	±	0.64
Iron (Fe)[c,d,e,f]	364	±	15
Lead (Pb)[g]	0.440	±	0.029
Magnesium (Mg)[d,e,f]	981	±	84
Manganese (Mn)[c,d,e,f]	74.0	±	6.8
Molybdenum (Mo)[a,f]	3.38	±	0.25
Phosphorus (P)[c,d,e]	9320	±	510
Potassium (K)[c,d,e,f]	7660	±	250
Selenium (Se)[a,f]	0.548	±	0.048
Sodium (Na)[c,d,e,f]	5500	±	230
Strontium (Sr)[a,c]	15.63	±	0.36

[a] NIST ICP-MS
[b] NIST RNAA
[c] NIST ICP-OES
[d] Collaborating laboratories. Reported methods included atomic absorption spectroscopy (AAS), ICP-OES, ICP-MS, and colorimetry.
[e] NIST WDXRF
[f] NIST INAA
[g] NIST ID ICP-MS

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 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 [2–4]. The uncertainty for biotin incorporates an additional component for possible inhomogeneity. The measurand is the total mass fraction for each vitamin listed in Table 2 on a dry-mass basis. Metrological traceability is to the SI derived unit for mass fraction (expressed as milligrams per kilogram).

Table 2. Certified Mass Fraction Values for Vitamins in SRM 3290

	Mass Fraction (mg/kg)		
Thiamine (Vitamin B_1)[a,b]	26.5	±	3.0
Riboflavin (Vitamin B_2)[a,b]	39.8	±	5.6
Niacinamide (Vitamin B_3)[a]	27.99	±	0.22
Niacin (Vitamin B_3)[a]	181.0	±	3.1
Total Vitamin B_3 as Niacinamide[a,b,c]	218	±	21
Free Pyridoxamine (Vitamin B_6)[a]	0.737	±	0.050
Pyridoxal (Vitamin B_6)[a]	0.877	±	0.025
Pyridoxine (Vitamin B_6)[a]	27.38	±	0.24
Total Vitamin B_6 as Pyridoxine[a,b,d]	30.1	±	3.2
Biotin[b,e]	1.42	±	0.23
Folic Acid[a,b,f]	6.0	±	1.0
Choline[e]	2627	±	12
Carnitine[e]	59.2	±	1.3

[a] NIST ID-LC-MS/MS

[b] Collaborating laboratories. Reported methods included extraction with LC-MS, extraction with RPLC and fluorescence detection, absorption spectrophotometry, and microbiological assay.

[c] NIST measured niacinamide and niacin individually, the mass fraction of niacin was mathematically converted to niacinamide by multiplication by the ratio of the relative molecular masses of niacin and niacinamide.

[d] NIST measured pyridoxamine, pyridoxal, and pyridoxine individually, the mass fractions of pyridoxal and pyridoxamine were mathematically converted to pyridoxine by multiplication by the ratio of the relative molecular masses of pyridoxamine, pyridoxal, and pyridoxine.

[e] NIST ID-LC-MS

[f] Metrological traceability is established through the molar absorptivity of the compound.

Reference Mass Fraction Values for Elements: Each reference mass fraction value is the mean of the mean results from analyses provided by NIST and the median of the mean 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 [2]. The measurands are the mass fractions listed in Table 3, on a dry-mass basis, as determined by the methods indicated. Metrological traceability is to the SI derived unit for mass fraction (expressed as milligrams per kilograms) as realized by the methods used.

Table 3. Reference Mass Fraction Values for Elements in SRM 3290

	Mass Fraction (mg/kg)		
Iodine (I)[a,b]	3.38	±	0.54
Nickel (Ni)[b]	17.08	±	0.72
Zinc (Zn)[c,d]	226	±	33

[a] NIST INAA
[b] NIST ICP-MS
[c] NIST ICP-OES
[d] Collaborating laboratories. Reported methods included AAS and ICP-OES.

Reference Mass Fraction Values for Vitamins: Each reference mass fraction value is the mean of the mean results from analyses provided by NIST and the median of the mean results provided by collaborating laboratories, where appropriate. The uncertainty provided with each value is an expanded uncertainty about the mean or median to cover the measurand with approximately 95 % confidence [2]. The uncertainty for free cyanocobalamin incorporates an additional component for possible inhomogeneity. The measurands are the mass fractions listed in Table 4, on a dry-mass basis, as determined by the methods indicated. Metrological traceability is to mass fraction (expressed as milligrams per kilograms) as realized by the methods used.

Table 4. Reference Mass Fraction Values for Vitamins in SRM 3290

	Mass Fraction (mg/kg)		
Pantothenic acid (Vitamin B_5)[a,b]	54	±	11
Free Cyanocobalamin (Vitamin B_{12})[c]	0.095	±	0.025
Total Vitamin B_{12} by Microbiological Assay[d]	0.176	±	0.056
Ascorbic Acid (Vitamin C)[e]	100.5	±	1.4
α-Tocopherol (Vitamin E)[f]	602	±	55

[a] NIST ID-LC-MS/MS
[b] Collaborating laboratories. Reported methods included extraction with LC-MS and microbiological assay.
[c] NIST LC-ICP-MS
[d] Collaborating laboratories.
[e] NIST LC-absorbance
[f] Collaborating laboratories. Reported methods included saponification with LC and fluorescence detection and normal phase LC.

Reference Mass Fraction Values for Fatty Acids as Free Fatty Acids: Each reference mass fraction value is the mean of the mean results from analyses provided by NIST and the median of the mean results provided by collaborating laboratories, where appropriate. The uncertainty provided with each value is an expanded uncertainty about the mean or median to cover the measurand with approximately 95 % confidence [2]. The measurand is the mass fraction for each fatty acid listed in Table 5, on a dry-mass basis, as determined by the methods indicated. Metrological traceability is to mass fraction (expressed as grams per 100 grams) as realized by the methods used.

Table 5. Reference Mass Fraction Values for Fatty Acids (as Free Fatty Acids) in SRM 3290

	Common Name	Mass Fraction (g/100 g)
Octanoic Acid (C8:0)[a,b]	Caprylic Acid	0.00192 ± 0.00073
Dodecanoic Acid (C12:0)[a,b]	Lauric Acid	0.0076 ± 0.0026
Tetradecanoic Acid (C14:0)[a,b]	Myristic Acid	0.106 ± 0.016
(*Z*)-9-Tetradecenoic Acid (C14:1)[a,b]	Myristoleic Acid	0.01756 ± 0.00061
Pentadecanoic Acid (C15:0)[b]		0.01255 ± 0.00070
Hexadecanoic Acid (C16:0)[a,b]	Palmitic Acid	2.60 ± 0.38
Heptadecanoic Acid (C17:0)[b]	Margaric Acid	0.0193 ± 0.0014
Heptadecenoic Acid (C17:1)[b]	Margoleic Acid	0.0106 ± 0.0020
Octadecanoic Acid (C18:0)[a,b]	Stearic Acid	0.69 ± 0.10
(*Z*)-9-Octadecenoic Acid (C18:1 n-9)[a,b]	Oleic Acid	4.02 ± 0.12
(*Z*)-11-Octadecenoic Acid (C18:1 n-7)[a,b]	Vaccenic Acid	0.2149 ± 0.0078
Total *cis*-C18:1 Fatty Acids[a,b]		4.203 ± 0.097
Total *trans*-C18:1 Fatty Acids[b]		0.0491 ± 0.0076
(*Z,Z*)-9,12-Octadecadienoic Acid (C18:2 n-6)[a,b]	Linoleic Acid	2.88 ± 0.15
Total *cis*-C18:2 Fatty Acids[b]		2.958 ± 0.069
Total *trans*-C18:2 Fatty Acids[b]		0.0251 ± 0.0088
(*Z,Z,Z*)-9,12,15-Octadecatrienoic Acid (C18:3 n-3)[a,b]	α-Linolenic Acid	0.163 ± 0.012
(*Z,Z,Z*)-6,9,12-Octadecatrienoic Acid (C18:3 n-6)[a,b]	γ-Linolenic Acid	0.0199 ± 0.0012
Octadecatetraenoic Acid (C18:4 n-3)[b]	Stearidonic Acid	0.0133 ± 0.0028
Eicosanoic Acid (C20:0)[a,b]	Arachidic Acid	0.0224 ± 0.0013
(*Z*)-11-Eicosenoic Acid (C20:1 n-9)[a]	Gondoic Acid	0.0393 ± 0.0014
Total *cis*-C20:1 Fatty Acids[b]		0.0473 ± 0.0099
(*Z,Z*)-11,14-Eicosadienoic Acid (C20:2 n-6)[b]		0.0220 ± 0.0073
(*Z,Z,Z,Z*)-8,11,14,17-Eicosatetraenoic Acid (C20:4 n-3)[a,b]	Arachidonic Acid	0.069 ± 0.012
(*Z,Z,Z,Z,Z*)-5,8,11,14,17-Eicosapentaenoic Acid (C20:5 n-3)[a,b]	EPA	0.0736 ± 0.0065
Docosanoic Acid (C22:0)[a,b]	Behenic Acid	0.0152 ± 0.0057
(*Z*)-13-Docosenoic Acid (C22:1 n-9)[a]	Erucic Acid	0.00225 ± 0.00032
Total *cis*-C22:4 Fatty Acids[b]		0.0167 ± 0.0036
(*Z,Z,Z,Z,Z*)-7,10,13,16,19-Docosapentaenoic Acid (C22:5 n-3)[a,b]	DPA	0.01470 ± 0.00098
(*Z,Z,Z,Z,Z,Z*)-4,7,10,13,16,19-Docosahexaenoic Acid (C22:6 n-3)[a,b]	DHA	0.0540 ± 0.0019
(*Z*)-15-Tetracosenoic Acid (C24:1)[a]	Nervonic Acid	0.00277 ± 0.00028
Saturated Fatty Acids[b]		3.72 ± 0.13
cis-Monounsaturated Fatty Acids[b]		4.94 ± 0.11
cis-Polyunsaturated Fatty Acids[b]		3.43 ± 0.14
Total Trans Fatty Acids[b]		0.081 ± 0.024
Total Omega-3 Fatty Acids[b]		0.312 ± 0.055
Total Omega-6 Fatty Acids[b]		3.089 ± 0.082

[a] NIST GC-FID

[b] Collaborating laboratories. Reported methods included GC-FID.

Reference Mass Fraction Values for Proximates and Calories: Each reference mass fraction value is the median of the mean values provided by collaborating laboratories. The uncertainty provided with the value is an expanded uncertainty about the mean to cover the measurand with approximately 95 % confidence [2]. The measurands are the mass fractions listed in Table 6, on a dry-mass basis, as determined by the methods indicated. Metrological traceability is to mass fraction (expressed as grams per 100 grams) as realized by the methods used. For calories, the measurand is the caloric content (expressed as kilocalories per 100 grams), listed in Table 6 on a dry-mass basis as determined by the method indicated and metrological traceability is to the scale realized by that method for energy.

Table 6. Reference Mass Fraction Values for Proximates and Calories in SRM 3290

	Mass Fraction (g/100 g)		
Ash[a]	7.343	±	0.062
Protein[b]	32.77	±	0.30
Carbohydrates[c]	46.30	±	0.87
Crude Fiber[d]	4.18	±	0.64
Total Dietary Fiber	11.99	±	0.84
Fat (sum of fatty acids as triglycerides)	12.60	±	0.42
Fat (gravimetric)[e]	13.95	±	0.24
	Energy (kcal per 100 g)		
Calories[f]	431.3	±	2.3

[a] Ash was determined by collaborating laboratories using weight loss after ignition in muffle furnace.
[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] Crude fiber was determined by collaborating laboratories using AOAC 962.09, AOCS Ba6a-05, and Ankom filter bag method.
[e] Gravimetric fat was determined by collaborating laboratories using AOAC 922.06, acid hydrolysis, and A.H. Mojonnier method.
[f] 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 carbohydrates, respectively, the mean caloric content is equal to 429.7 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 using hydrolysis followed by derivatization and LC. The uncertainty provided with the value is an expanded uncertainty about the mean to cover the measurand with approximately 95 % confidence [2]. The measurands are the mass fractions listed in Table 7, on a dry-mass basis, as determined by the methods indicated. Metrological traceability is to mass fraction (expressed as grams per 100 grams) as realized by the methods used.

Table 7. Reference Mass Fraction Values for Amino Acids in SRM 3290

	Mass Fraction (g/100 g)		
Alanine	2.06	±	0.22
Arginine	1.78	±	0.29
Aspartic Acid	2.40	±	0.15
Cystine	0.39	±	0.16
Glutamic Acid	5.27	±	0.30
Glycine	2.17	±	0.18
Histidine	0.658	±	0.098
Hydroxyproline	0.65	±	0.15
Isoleucine	1.20	±	0.22
Leucine	2.74	±	0.19
Lysine	1.68	±	0.26
Methionine	0.88	±	0.14
Phenylalanine	1.38	±	0.12
Proline	2.20	±	0.30
Serine	1.41	±	0.13
Taurine	0.236	±	0.067
Threonine	1.18	±	0.10
Tyrosine	1.00	±	0.25
Valine	1.40	±	0.24

Information Mass Fraction Values for Elements: Each information mass fraction value, reported on a dry-mass basis, is the mean result from analyses provided by NIST. No uncertainty is provided because there is insufficient information available for its assessment. Information values cannot be used to establish metrological traceability.

Table 8. Information Mass Fraction Values for Selected Elements in SRM 3290

	Mass Fraction (mg/kg)
Aluminum (Al)[(a)]	400
Sulfur (S)[(b)]	6400

[(a)] NIST INAA
[(b)] NIST WDXRF

Table 9. 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
		81			81
		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	128	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
Pyridoxal	168	41	$^{13}C_4$-Pyridoxine	171	43
		67			70
		94			97
		150			153
Pyridoxine	170	77	$^{13}C_4$-Pyridoxine	174	81
		80			83
		134			138
		153			156
Pyridoxamine	169	77	$^{2}H_3$-Pyridoxamine	172	79
		134			136
		152			155

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.; Phinney, K.W.; Polakoski, M.; Possolo, A.; Sharpless, K.E.; Sieber, J.R.; 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, 2020 Edition; U.S. Government Printing Office: Washington, DC (2020); available at https://nvlpubs.nist.gov/nistpubs/SpecialPublications/NIST.SP.260-136-2020.pdf (accessed Jul 2021).

[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 (2008); available at https://www.bipm.org/en/publications/guides (accessed Jul 2021); 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 Jul 2021).

[3] JCGM 101:2008; *Evaluation of Measurement Data — Supplement 1 to the "Guide to Expression of Uncertainty in Measurement" — Propagation of Distributions Using a Monte Carlo Method*; Joint Committee for Guides in Metrology (2008); available at https://www.bipm.org/en/publications/guides (accessed Jul 2021).

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

[5] Thompson, A.; Taylor, B.N.; *Guide for the Use of the International System of Units (SI)*; NIST Special Publication 811; U.S. Government Printing Office: Washington, DC (2008); available at https://www.nist.gov/pml/special-publication-811 (accessed Jul 2021).

[6] Sharpless, K.E.; Lippa, K.A.; Duewer, D.L.; Rukhin, A.L.; *The ABCs of Using Standard Reference Materials in the Analysis of Foods and Dietary Supplements: A Practical Guide*; NIST Special Publication 260-181; U.S. Government Printing Office: Washington, DC (2014); available at https://nvlpubs.nist.gov/nistpubs/SpecialPublications/NIST.SP.260-181.pdf (accessed Jul 2021).

[7] Murphy, K.E.; Vetter, T.W.; *Recognizing and Overcoming Analytical Error in the Use of ICP-MS for the Determination of Cadmium in Breakfast Cereal and Dietary Supplements*; Anal. Bioanal. Chem., Vol. 405, pp. 4579–4588 (2013).

[8] Murphy, K.E.; Beary, E.S.; Rearick, M.S.; Vocke, R.D.; *Isotope Dilution Inductively Coupled Plasma Mass Spectrometry (ID ICP-MS) for the Certification of Lead and Cadmium in Environmental Standard Reference Materials*; Fresenius J. Anal. Chem., Vol. 368, pp. 362–370 (2000).

[9] Paul, R.L.; *Determination of Arsenic in Food and Dietary Supplement Standard Reference Materials by Neutron Activation Analysis*; Anal. Chem., Vol. 83, pp. 152–156 (2011).

Certificate Revision History: 09 July 2021 (Removed reference value for retinol (vitamin A) based on observed instability; downgraded certified values for fatty acids to reference values to properly reflect traceability and moved from Tables 1 and 4 to Table 5; re-numbered tables accordingly; editorial changes); 05 February 2018 (Addition of certified values for cadmium and lead; addition of reference values for nickel and ascorbic acid (vitamin C); update of reference value for isoleucine to correct a rounding error; editorial changes); 22 April 2016 (Updated footnotes for choline and carnitine in Table 2; editorial changes); 19 November 2015 (Original certificate date).

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