



# Certificate of Analysis

## Standard Reference Material<sup>®</sup> 2387

### Peanut Butter

This Standard Reference Material (SRM) is intended primarily for use in validating methods for determining proximates, fatty acids, calories, vitamins, elements, amino acids, aflatoxins, and acrylamide in peanut butter and similar matrices. This SRM can also be used for quality assurance when assigning values to in-house reference materials. The SRM is a creamy peanut butter prepared by a manufacturer of peanut butter products. A unit of SRM 2387 consists of three jars of peanut butter containing 170 g each.

**Certified Mass Fraction Values:** Certified mass fraction values of fat, selected fatty acids, elements, and tocopherols in SRM 2387 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 mass fraction values in this material were calculated as the mean of the mean values from NIST methods and the median or mean of the measurements made by collaborating laboratories, where appropriate. The associated uncertainties are expressed at an approximately 95 % level of confidence [2,3,6]. Values are reported on an as-received (not dry-mass) basis in mass fraction units [4].

**Reference Mass Fraction Values:** Reference mass fraction values for additional proximates, fatty acids, amino acids, calories, total dietary fiber, vitamins, aflatoxins, and acrylamide are provided in Tables 4 through 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 [1] and is provided with an associated uncertainty that may reflect only measurement reproducibility, may not include all sources of uncertainty, or may reflect a lack of sufficient statistical agreement among multiple analytical methods. Reference mass fraction values were derived from results reported by NIST and collaborating laboratories. Values are reported on an as-received (not dry-mass) basis in mass fraction units [4].

**Expiration of Certification:** The certification of **SRM 2387** is valid, within the measurement uncertainty specified, until **31 December 2019**, provided the SRM is handled and stored in accordance with the instructions given in this certificate (see “Instructions for Storage and Use”). This 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 H.B. Chin, I-P. Ho, and D.W. Howell of the National Food Processors Association (NFPA, Dublin, CA and Washington, DC).

Analytical measurements at NIST were performed by C.Q. Burdette, M.M. Phillips, C.S. Phinney, B.J. Porter, K.E. Sharpless, and L.J. Wood of the NIST Chemical Sciences Division, and B.E. Lang of the NIST Biosystems and Biomaterials Division.

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

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*Certificate Revision History on Last Page*

Analyses for value assignment were also performed by the following laboratories participating in a NFPA Food Industry Analytical Chemists Subcommittee (FIACS) interlaboratory comparison exercise: Beech-Nut Nutrition Corporation; Canajoharie, NY, USA; Campbell Soup Company; Camden, NJ, USA; Covance, Inc., Madison, WI, USA; General Mills, Inc., Minneapolis, MN, USA; Hormel Foods Corporation, Austin, MN, USA; Kraft Foods, Glenview, IL, USA; Krueger Food Laboratories, Inc.; Cambridge, MA, USA; Nabisco, Inc., East Hanover, NJ, USA; Nestlé USA, Dublin, OH, USA; Novartis Nutrition Corporation, St. Louis Park, MN, USA; Ralston Purina Company, St. Louis, MO, USA; U.S. Department of Agriculture, Food Composition Laboratory, Beltsville, MD, USA; and Woodson-Tenent Laboratories, Memphis, TN, USA. Additional laboratories providing measurements for value assignment of aflatoxins included: U.S. Food and Drug Administration, Atlanta, GA, USA; Neogen Corporation, Lansing, MI, USA; U.S. Department of Agriculture, Agricultural Marketing Service, Blakely, GA, USA; and Trilogy Analytical Laboratory, Washington, MO, USA. Analyses for value assignment of acrylamide were also performed by the following laboratories participating in a Joint Institute for Food Safety and Applied Nutrition (JIFSAN) Acrylamide Working Group interlaboratory comparison exercise: American Oil Chemists Society, Champaign, IL, USA; Covance Laboratories, Madison, WI, USA; Eurofins Scientific, Memphis, TN, USA; Federal Institute for Risk Assessment (BfR), Berlin, Germany; U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, College Park, MD, USA; U.S. Food and Drug Administration, Office of Regulatory Affairs, Lenexa, KS, USA; Food Research Institute, University of Wisconsin, Madison, WI, USA; General Mills, Inc., Minneapolis, MN, USA; Health Canada, Ottawa, ON, Canada; Livsmedelsverket (National Food Administration), Helsinki, Finland; National Food Processors Association, Washington, DC, USA; Nestlé, Lausanne, Switzerland; Procter and Gamble, Cincinnati, OH, USA; Swiss Quality Testing Services, Dietikon, Switzerland; and The National Food Laboratory, Dublin, CA, USA.

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

**NOTICE TO USERS:** SRM 2387 IS INTENDED FOR LABORATORY USE ONLY, NOT FOR HUMAN CONSUMPTION.

#### **INSTRUCTIONS FOR STORAGE AND USE**

**Storage:** Until required for use, the peanut butter should be frozen at  $-20\text{ }^{\circ}\text{C}$  ( $0\text{ }^{\circ}\text{F}$ ).

**Use:** Prior to removal of a test portion for analysis, a jar of peanut butter should be thawed under refrigeration overnight. The contents of a jar should be mixed thoroughly prior to removal of a test portion. The following masses used for NIST analyses should be used as the minimum sample size to ensure valid results: 5 g to 7 g for tocopherols; 1 g for fat and fatty acids; 0.5 g for elements; and 10 g for water-soluble vitamins.

#### **SOURCE, PREPARATION, AND ANALYSIS**

**Source and Preparation:** SRM 2387 is creamy peanut butter containing roasted peanuts, sugar, partially hydrogenated vegetable oils (48 % rapeseed, 40 % cottonseed, and 12 % soybean oil), and salt, and was prepared for NIST as part of a larger production run. Raw, shelled Florunner (primarily) peanuts were received from several suppliers and were roasted. The skins were removed from the roasted peanuts, and discolored peanuts were discarded. The roasted peanuts were then ground, and the remaining ingredients were added. After mixing, the peanut butter was further ground to a fine particle size, air was removed, and the peanut butter was cooled and packed in colorless polyethyl tetraethylene (PETE) jars with white screw caps and foil liners.

**NIST Analyses for Fat:** The mass fraction of gravimetric fat was measured from one set of three samples of peanut butter. One-gram portions of peanut butter were mixed with diatomaceous earth. The mixture was then briefly chilled at  $4\text{ }^{\circ}\text{C}$  to improve handling. The fat was then extracted from the mixture by pressurized fluid extraction (PFE) using hexane:acetone (4:1 volume fraction). Extracts were evaporated under nitrogen and then dried at  $100\text{ }^{\circ}\text{C}$  to constant mass.

**NIST Analyses for Fatty Acids:** Mass fractions of twelve fatty acids were measured by gas chromatography with flame ionization detection (GC-FID) in two sets of six samples of peanut butter prepared on two different days. The fat was extracted from approximately 1 g samples of peanut butter by PFE using a mixture of hexane:acetone (4:1 volume fraction). Methyl nonadecanoate (C19:0 fatty acid methyl ester [FAME]) was used as an internal standard. A two-step process employing methanolic sodium hydroxide and boron trifluoride was used to convert the fatty acids to their methyl esters. FAMES were extracted into hexane and analyzed by GC-FID.

**NIST Analyses for Elements:** Mass fractions of calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc were measured by inductively coupled plasma optical emission spectrometry (ICP-OES) in eight jars of peanut butter. Two 0.5 g portions were taken from each jar and digested in a nitric, perchloric, and hydrofluoric acid mixture. Because of the high fat content, the samples were predigested on a hotplate before digestion in a microwave oven. Digests were transferred to plastic bottles and diluted with the appropriate volume of 1.5 % (volume fraction) nitric acid. To correct for matrix effects caused by differences between samples and calibrants, the method of standard additions was used; spikes were added to one aliquot prepared from each 0.5 g test portion. Four measurements using ICP-OES were made and averaged for each sample and each spiked solution. Results were corrected for spike recoveries.

**NIST Analyses for  $\delta$ -Tocopherol and  $\gamma$ - (plus  $\beta$ -) Tocopherol:** Mass fractions of  $\delta$ -tocopherol and  $\gamma$ - (plus  $\beta$ -) tocopherol were measured using liquid chromatography with absorbance (LC-Abs) and fluorescence (LC-FL) detection in test portions taken from six jars of peanut butter over a seven-day period. The peanut butter may contain  $\beta$ -tocopherol, but the chromatographic system described below is incapable of resolving  $\beta$ - and  $\gamma$ -tocopherol. Samples of approximately 5 g to 7 g were homogenized and saponified using potassium hydroxide. Analytes were extracted into a mixture of diethyl ether and hexane, which was subsequently evaporated, and the analytes were redissolved in a mixture of ethanol and ethyl acetate. Samples were analyzed by LC using a C18 column using a gradient of acetonitrile, methanol, and ethyl acetate [5] and with absorbance monitored at 450 nm for measurement of *trans*- $\beta$ -apo-10'-carotenal oxime (the internal standard) and fluorescence excitation at 295 nm and monitored at an emission wavelength of 335 nm for quantitation of the tocopherols. Calibrants of  $\delta$ - and  $\gamma$ -tocopherol were prepared gravimetrically, at levels intended to approximate the levels of the vitamins in the SRM following extraction, and the concentrations were assigned spectrophotometrically. A single internal standard solution was used for the calibrants and samples.

**NIST Analyses for  $\alpha$ -Tocopherol:** Mass fractions of  $\alpha$ -tocopherol were measured using liquid chromatography with fluorescence (LC-FL) detection in test portions taken from six jars of peanut butter on a single day. Samples of approximately 5 g were homogenized and saponified using potassium hydroxide. Analytes were extracted into a mixture of ethyl acetate and hexane using rotary mixing and sonication, and the supernatants from five extractions were combined. The solvent from the combined supernatant was subsequently evaporated, and the analytes were redissolved in a mixture of ethanol and ethyl acetate. Samples were analyzed by LC using a C18 column using an isocratic flow of 99:1 methanol:water and with fluorescence excitation at 295 nm and monitored at an emission wavelength of 330 nm for quantitation of  $\alpha$ -tocopherol, using tocol as an internal standard. Calibrants were prepared gravimetrically, at levels intended to approximate the levels of the vitamins in the SRM following extraction, and the concentrations were assigned spectrophotometrically. A single internal standard solution was used for the calibrants and samples.

**NIST Analyses for Water-Soluble Vitamins:** Mass fractions of thiamine, riboflavin, niacinamide, niacin, pantothenic acid, pyridoxal, pyridoxamine, and pyridoxine were measured by isotope dilution liquid chromatography with tandem mass spectrometry (ID-LC-MS/MS) in duplicate 10 g test portions taken from each of 10 jars of peanut butter on a single day. The analytes and internal standards were extracted into dilute ammonium acetate at pH 2.6 by sonication for 2 h. The samples were centrifuged and an aliquot of the supernatant was filtered before 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. A single internal standard solution was used for the calibrants and samples. The analyte ions and internal standard ions monitored for ID-LC-MS/MS are listed in Table 10.

**Analyses by Collaborating Laboratories:** Data from additional sources was used for certification of this material, including an interlaboratory comparison exercise organized by the NFPA FIACS, four laboratories participating in an exercise in which only aflatoxins were measured, and 15 laboratories participating in an exercise organized by the JIFSAN Acrylamide Working Group in which acrylamide was measured. Not every laboratory measured every analyte. The NFPA FIACS laboratories listed were asked to use AOAC methods or their equivalent, to make single measurements from each of two jars, and to report the analytical method that was used. The laboratories measuring aflatoxins were asked to use their usual methods to make single measurements in each of three jars. The JIFSAN laboratories were asked to use their usual methods to make duplicate measurements of acrylamide in a single jar.

**Homogeneity Assessment:** The homogeneity of calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, zinc, fatty acids, tocopherols, and vitamins was assessed at NIST using the methods described above. A small but statistically significant heterogeneity was found for some analytes, and an inhomogeneity component of approximately 5.6 % has been incorporated in the uncertainty for alpha-tocopherol and an inhomogeneity component of approximately 1 % has been incorporated in the uncertainty for all other analytes.

**Value Assignment:** The collaborating laboratories reported values for two to twelve 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 mean of laboratory means was determined. For analytes that were also measured by NIST, this mean of the individual collaborating laboratory means and the means of the individual sets of NIST data were averaged.

**Certified Mass Fraction Values for Fat and Selected Fatty Acids as Free Fatty Acids:** Each certified mass fraction value is the weighted mean of results from NIST and the mean of results provided by collaborating laboratories. The uncertainty provided with each value is an expanded uncertainty about the mean to cover the measurand with approximately 95 % confidence. The expanded uncertainty is calculated as  $U = ku_c$ , where  $u_c$  incorporates the observed difference between the results from the methods and their respective uncertainties, consistent with the ISO/JCGM Guide, and  $k$  is a coverage factor corresponding to approximately 95 % confidence [2–3]. The measurands are the mass fractions of selected fatty acids in peanut butter. The certified values are metrologically traceable to the SI unit of mass, expressed as grams per 100 grams.

Table 1. Certified Mass Fraction Values for Fat and Selected Fatty Acids as Free Fatty Acids in SRM 2387

	Mass Fraction (g/100 g)	Coverage Factor, $k$
Fat (Extractable)	51.6 ± 1.4	2.57
Fat (as the sum of fatty acids as triglycerides)	49.8 ± 1.9	2.20
Saturated Fatty Acids	10.4 ± 0.2	2.04
Monounsaturated Fatty Acids	24.4 ± 0.9	2.20
Polyunsaturated Fatty Acids	13.2 ± 0.4	2.16

  

	Common Name	Mass Fraction (g/100 g)	Coverage Factor, $k$
Tetradecanoic Acid (C14:0)	Myristic Acid	0.024 ± 0.002	2.20
Hexadecanoic Acid (C16:0)	Palmitic Acid	4.94 ± 0.15	2.16
(Z)-9-Hexadecenoic Acid (C16:1 n-7)	Palmitoleic Acid	0.044 ± 0.010	2.36
Octadecanoic Acid (C18:0)	Stearic Acid	2.13 ± 0.08	2.16
(Z)-9-Octadecenoic Acid (C18:1 n-9)	Oleic Acid	23.38 ± 0.90	2.20
(Z)-11-Octadecenoic Acid (C18:1 n-7)	Vaccenic Acid	0.255 ± 0.016	2.45
(Z,Z)-9,12-Octadecadienoic Acid (C18:2 n-6)	Linoleic Acid	13.15 ± 0.41	2.16
(Z,Z,Z)-9,12,15-Octadecatrienoic Acid (C18:3 n-3)	$\alpha$ -Linolenic Acid	0.030 ± 0.001	2.18
Eicosanoic Acid (C20:0)	Arachidic Acid	0.710 ± 0.029	2.20
(Z)-11-Eicosenoic Acid (C20:1 n-9)	Gondoic Acid	0.643 ± 0.031	2.26
Docosanoic Acid (C22:0)	Behenic Acid	1.81 ± 0.08	2.12
Tetracosanoic Acid (C24:0)	Lignoceric Acid	0.781 ± 0.044	2.23

**Certified Mass Fraction Values for Selected Elements:** Each certified mass fraction value is the combined mean from the mean of results from analyses by NIST and the mean of results provided by collaborating laboratories. The uncertainty provided with each value is an expanded uncertainty about the mean to cover the measurand with approximately 95 % confidence. The expanded uncertainty is calculated as  $U = ku_c$ , where  $u_c$  incorporates the observed difference between the results from the methods and their respective uncertainties consistent with the ISO/JCGM Guide, and  $k$  is a coverage factor corresponding to approximately 95 % confidence [2–3]. The measurands are the mass fractions of selected elements in peanut butter. The certified values are metrologically traceable to the SI unit of mass, expressed as milligrams per kilogram.

Table 2. Certified Mass Fraction Values for Selected Elements in SRM 2387

	Mass Fraction (mg/kg)	Coverage Factor, $k$
Calcium (Ca)	411 ± 18	2.23
Copper (Cu)	4.93 ± 0.15	2.14
Iron (Fe)	16.4 ± 0.8	2.08
Magnesium (Mg)	1680 ± 70	2.10
Manganese (Mn)	16.0 ± 0.6	2.14
Phosphorus (P)	3378 ± 92	2.26
Potassium (K)	6070 ± 200	2.16
Sodium (Na)	4890 ± 140	2.23
Zinc (Zn)	26.3 ± 1.1	2.20

**Certified Mass Fraction Values for Tocopherols:** Each certified mass fraction value is the combined mean from the mean of results from analyses by NIST and the mean of results provided by collaborating laboratories. The uncertainty provided with each value is an expanded uncertainty about the mean to cover the measurand with approximately 95 % confidence. The expanded uncertainty is calculated as  $U = ku_c$ , where  $u_c$  incorporates the observed difference between the results from the methods and their respective uncertainties consistent with the ISO/JCGM Guide, and  $k$  is a coverage factor corresponding to approximately 95 % confidence [2–3]. The measurands are the mass fractions of tocopherols in peanut butter. The certified values are metrologically traceable to the SI unit of mass, expressed as milligrams per kilogram, through the molar absorptivities of the compounds.

Table 3. Certified Mass Fraction Values for Tocopherols in SRM 2387

	Mass Fraction (mg/kg)	Coverage Factor, $k$
$\delta$ -Tocopherol	10 ± 3	2.57
$\gamma$ - + $\beta$ -Tocopherol	100 ± 19	2.57

**Reference Mass Fraction Values for Proximates and Calories:** Each reference mass fraction value is the weighted mean of results provided by collaborating laboratories. The uncertainty provided is an expanded uncertainty about the mean to cover the measurand with approximately 95 % confidence. The expanded uncertainty is calculated as  $U = ku_c$ , where  $u_c$  represents the combined uncertainty consistent with the ISO/JCGM Guide, and  $k$  is a coverage factor corresponding to approximately 95 % confidence [2]. The measurands are the mass fractions of selected proximates and caloric content in peanut butter as determined by the collaborating laboratories and the methods they used. The reference values for selected proximates are metrologically traceable to the SI unit of mass, expressed as grams per 100 grams. The reference value for caloric content is metrologically traceable to the SI unit of energy, expressed as kilocalories per 100 grams.

Table 4. Reference Values for Proximates and Calories in SRM 2387

	Mass Fraction (g/100 g)	Coverage Factor, $k$
Solids	99.2 ± 2.1	2.09
Ash	3.10 ± 0.10	2.04
Protein <sup>(a)</sup>	22.2 ± 0.5	2.05
Carbohydrates	25.0 ± 1.8	2.14
Total Dietary Fiber	5.57 ± 0.42	2.78
	Energy (kcal per 100 g)	Coverage Factor, $k$
Calories <sup>(b)</sup>	629 ± 15	2.06

<sup>(a)</sup> A factor of 5.46 was used to convert nitrogen results to protein.

<sup>(b)</sup> 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 as triglycerides), protein, and carbohydrate, respectively, the mean caloric content is 637 kcal per 100 grams.

**Reference Mass Fraction Values for Fatty Acids as Free Fatty Acids:** Each reference mass fraction value is the weighted mean of results provided by collaborating laboratories. The uncertainty provided with each value is an expanded uncertainty about the mean to cover the measurand with approximately 95 % confidence. The expanded uncertainty is calculated as  $U = ku_c$ , where  $u_c$  is 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 free fatty acids in peanut butter as determined by the collaborating laboratories and the methods they used. The reference values are metrologically traceable to the SI unit of mass, expressed as grams per 100 grams.

Table 5. Reference Mass Fraction Values for Fatty Acids as Free Fatty Acids in SRM 2387

	Common Name	Mass Fraction (g/100 g)	Coverage Factor, $k$
Heptadecanoic Acid (C17:0)	Margaric Acid	0.048 ± 0.001	2.05
Heptadecenoic Acid (C17:1)		0.033 ± 0.006	2.78
Eicosadienoic Acid (C20:2)		0.016 ± 0.007	3.18
(Z,Z,Z,Z)-5,8,11,14-Eicosatetraenoic Acid (C20:4 n-6)	Arachidonic Acid	0.024 ± 0.015	4.30
(Z)-13-Docosenoic Acid (C22:1 n-9)	Erucic Acid	0.054 ± 0.012	2.57

**Reference Mass Fraction Values for Amino Acids:** Each reference mass fraction value is the weighted mean of results provided by collaborating laboratories. The uncertainty provided with each value is an expanded uncertainty about the mean to cover the measurand with approximately 95 % confidence. The expanded uncertainty is calculated as  $U = ku_c$ , where  $u_c$  is 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 peanut butter as determined by the collaborating laboratories and the methods they used. The reference values are metrologically traceable to the SI unit of mass, expressed as grams per 100 grams.

Table 6. Reference Mass Fraction Values for Amino Acids in SRM 2387

	Mass Fraction (g/100 g)	Coverage Factor, $k$
Alanine	0.93 ± 0.10	2.45
Arginine	2.65 ± 0.31	2.45
Aspartic Acid	2.83 ± 0.19	2.31
Cystine	0.27 ± 0.01	2.31
Glutamic Acid	4.69 ± 0.26	2.26
Glycine	1.41 ± 0.12	2.36
Histidine	0.55 ± 0.06	2.45
Isoleucine	0.77 ± 0.07	2.45
Leucine	1.56 ± 0.09	2.26
Lysine	0.78 ± 0.08	2.45
Methionine	0.21 ± 0.04	2.57
Phenylalanine	1.21 ± 0.08	2.31
Proline	0.96 ± 0.08	2.36
Serine	1.16 ± 0.09	2.36
Threonine	0.54 ± 0.08	2.45
Tryptophan	0.21 ± 0.06	2.78
Tyrosine	0.81 ± 0.14	2.57
Valine	0.94 ± 0.09	2.45

**Reference Mass Fraction Value for  $\alpha$ -Tocopherol:** The reference mass fraction value is the combined mean from the mean of results from analyses by NIST and the mean of results provided by a collaborating laboratory. 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  $u_c$  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 [2,6–7]. The uncertainty also incorporates an additional uncertainty component for possible inhomogeneity. The measurand is the mass fraction of  $\alpha$ -tocopherol in peanut butter as determined by the collaborating laboratories and the methods they used. The reference value is metrologically traceable to the SI unit of mass, expressed as milligrams per kilogram, based on the molar absorptivity of the compound.

Table 7. Reference Mass Fraction Value for  $\alpha$ -Tocopherol in SRM 2387

	Mass Fraction (mg/kg)	Coverage Factor, $k$
$\alpha$ -Tocopherol	73.7 ± 8.4	2.0

**Reference Mass Fraction Values for Selected Vitamins:** Each reference mass fraction value is the mean of the results obtained by NIST using ID-LC-MS/MS. The uncertainty provided is an expanded uncertainty about the mean to cover the measurand with approximately 95 % confidence. The expanded uncertainty is calculated as  $U = ku_c$ , where  $u_c$  represents the combined uncertainty consistent with the ISO/JCGM Guide, and  $k$  is a coverage factor corresponding to approximately 95 % confidence [2]. The uncertainties also incorporate an additional uncertainty component for possible inhomogeneity. The measurands are the mass fractions of selected vitamins in peanut butter as determined by the single method indicated. The reference values are metrologically traceable to the SI unit of mass, expressed as milligrams per kilogram.

Table 8. Reference Mass Fraction Values for Selected Vitamins in SRM 2387

	Mass Fraction (mg/kg)	Coverage Factor, $k$
Thiamine (Vitamin B <sub>1</sub> ) <sup>(a,b)</sup>	0.563 ± 0.012	2.00
Riboflavin (Vitamin B <sub>2</sub> ) <sup>(b)</sup>	0.179 ± 0.012	2.07
Niacinamide (Vitamin B <sub>3</sub> ) <sup>(b)</sup>	3.006 ± 0.068	2.00
Niacin (Vitamin B <sub>3</sub> ) <sup>(b)</sup>	38.50 ± 0.97	2.00
Total Vitamin B <sub>3</sub> as Niacinamide <sup>(c)</sup>	41.2 ± 1.0	2.00
Pantothenic Acid <sup>(b)</sup>	8.53 ± 0.18	2.00
Pyridoxamine (Vitamin B <sub>6</sub> ) <sup>(b,d)</sup>	0.0852 ± 0.0036	2.04
Pyridoxal (Vitamin B <sub>6</sub> ) <sup>(b,e)</sup>	0.0976 ± 0.0026	2.00
Pyridoxine (Vitamin B <sub>6</sub> ) <sup>(b,f)</sup>	0.1171 ± 0.0049	2.04
Total Vitamin B <sub>6</sub> as Pyridoxine <sup>(g)</sup>	0.3006 ± 0.0087	2.00

<sup>(a)</sup> Reported as thiamine ion (relative molecular mass of 265.36 g/mol), not chloride or chloride hydrochloride.

<sup>(b)</sup> This value represents the free (unbound) form of the vitamin.

<sup>(c)</sup> NIST measured niacin and niacinamide individually, and niacin was mathematically converted to niacinamide by multiplication by the ratio of the relative molecular masses.

<sup>(d)</sup> Reported as pyridoxamine (relative molecular mass of 168.19 g/mol), not as pyridoxamine dihydrochloride.

<sup>(e)</sup> Reported as pyridoxal (relative molecular mass of 167.16 g/mol), not as pyridoxal hydrochloride.

<sup>(f)</sup> Reported as pyridoxine (relative molecular mass of 169.18 g/mol), not as pyridoxine hydrochloride.

<sup>(g)</sup> NIST measured pyridoxamine, pyridoxal, and pyridoxine individually, and pyridoxamine and pyridoxal were mathematically converted to pyridoxine by multiplication by the ratio of the relative molecular masses.

**Reference Mass Fraction Values for Selected Additional Analytes:** Each reference mass fraction value is the weighted mean of results provided by collaborating laboratories. The uncertainty provided is an expanded uncertainty about the mean to cover the measurand with approximately 95 % confidence. The expanded uncertainty is calculated as  $U = ku_c$ , where  $u_c$  represents the combined uncertainty consistent with the ISO/JCGM Guide, and  $k$  is a coverage factor corresponding to approximately 95 % confidence [2]. The measurands are the mass fractions of aflatoxins and acrylamide in peanut butter as determined by the collaborating laboratories and the methods they used. The reference values are metrologically traceable to the SI unit of mass, expressed as nanograms per gram.

Table 9. Reference Values for Selected Additional Analytes in SRM 2387

	Mass Fraction (ng/g)	Coverage Factor, $k$
Acrylamide	87.0 ± 7.8	2.07
Aflatoxin B1	4.2 ± 0.9	2.57
Aflatoxin B2	0.7 ± 0.3	3.18
Total Aflatoxins <sup>(a)</sup>	5.0 ± 0.5	2.57

<sup>(a)</sup> The reference value for total aflatoxins is the mean of the laboratory means of the sum of aflatoxins B1 and B2.

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

Compound	Precursor Ion ( <i>m/z</i> )	→ Product Ion ( <i>m/z</i> )	Internal Standard	IS Precursor Ion ( <i>m/z</i> )	→ IS Product Ion ( <i>m/z</i> )
Thiamine	266	42	<sup>13</sup> C <sub>3</sub> -Thiamine	269	42
		123			123
Riboflavin	377	43	<sup>13</sup> C <sub>4</sub> , <sup>15</sup> N <sub>2</sub> -Riboflavin	383	43
		172			175
		198			202
		243			249
Niacinamide	123	53	<sup>2</sup> H <sub>4</sub> -Niacinamide	127	56
		78			81
		80			84
Niacin	124	52	<sup>2</sup> H <sub>4</sub> -Niacin	128	53
		53			56
		78			81
		80			84
Pantothenic Acid	220	41	<sup>13</sup> C <sub>3</sub> , <sup>15</sup> N-Pantothenic Acid	224	41
		43			43
		72			76
		90			94
Pyridoxal	168	41	<sup>2</sup> H <sub>3</sub> -Pyridoxal	171	43
		67			70
		94			97
		150			153
Pyridoxamine	169	77	<sup>2</sup> H <sub>3</sub> -Pyridoxamine	172	79
		134			136
		152			155
Pyridoxine	170	77	<sup>13</sup> C <sub>4</sub> -Pyridoxine	174	81
		80			83
		134			138
		152			156

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**Certificate Revision History:** **21 July 2015** (Addition of water-soluble vitamin values; removal of certified value for  $\alpha$ -tocopherol; addition of reference value for  $\alpha$ -tocopherol; editorial changes); **23 September 2014** (Extension of certificate period; editorial changes); **30 September 2009** (Extension of certificate period); **12 January 2007** (This revision reflects an editorial change); **29 September 2004** (This revision reflects the addition of a reference value for acrylamide); **14 March 2003** (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; fax (301) 948-3730, email [srminfo@nist.gov](mailto:srminfo@nist.gov); or via the Internet at <http://www.nist.gov/srm>.*