National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material 3245

Ephedra Dietary Supplement Suite

Standard Reference Material (SRM) 3245 is a suite of five ephedra-related dietary supplement SRMs: SRM 3240 *Ephedra sinica* Stapf Aerial Parts, SRM 3241 *Ephedra sinica* Stapf Native Extract, SRM 3242 *Ephedra sinica* Stapf Commercial Extract, SRM 3243 Ephedra-Containing Solid Oral Dosage Form, and SRM 3244 Ephedra-Containing Protein Powder. These SRMs are intended primarily for use in validating analytical methods for the determination of ephedrine alkaloids and toxic elements in *Ephedra sinica* and ephedra-containing matrices. These SRMs can also be used for quality assurance when assigning values to in-house control materials. A unit of SRM 3245 consists of two bottles of each of the five ephedra-containing SRMs: 5 g in each bottle of SRM 3240; 1.2 g in each bottle of SRM 3241; 1.2 g in each bottle of SRM 3242; 2.5 g in each bottle of SRM 3243; 12 g in each bottle of SRM 3244. See the Certificate of Analysis for each SRM for additional details (http://www.nist.gov/srm).

Certified Concentration Values: 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 accounted for [1]. Certified concentration values of selected ephedrine alkaloids, synephrine, caffeine, and toxic elements in each of the five SRMs contained in SRM 3245 are provided in Tables 1 and 3. Certified concentration values are also provided for additional elements and water-soluble vitamins in SRM 3244 in Tables 4 and 5. Certified values were derived from the combination of results provided by NIST and collaborating laboratories. The certified values in this material are the equally weighted means of the individual sets of NIST results and the means of the individual sets of measurements made by collaborating laboratories; the associated uncertainties are expanded uncertainties at the 95 % level of confidence [2,3]. Values are reported on a dry-mass basis in mass fraction units [4].

Reference Concentration Values: 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 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. Reference concentration values for additional ephedrine alkaloids and elements are provided in Tables 2 and 4. Reference concentration values are provided in Tables 5 through 8 for selected water-soluble vitamins, alkaloids, proximates, fatty acids, and caloric content in SRM 3244.

Information Concentration Values: Information values are considered to be values that will be of interest to the SRM user; however, either insufficient information is available to assess the uncertainty or the uncertainty is relatively large. Information values are generally reported with no associated uncertainty. Information concentration values for two ephedrine alkaloids in SRM 3244 are provided in Table 2.

Expiration of Value Assignment: The value assignment of this SRM is valid until **31 March 2014**, within the measurement uncertainties specified, provided the SRM is handled and stored in accordance with the instructions given in this certificate. Value assignment is nullified if the SRM is damaged, contaminated, or modified.

Maintenance of SRM Value Assignment: NIST will monitor this SRM over the period of its value assignment. If substantive technical changes occur that affect the value assignment before the expiration of this certificate, NIST will notify the purchaser. Registration (see attached sheet) will facilitate notification.

Coordination of the technical measurements leading to the certification of this SRM was performed by L.C. Sander, K.E. Sharpless, and S.A. Wise of the NIST Analytical Chemistry Division.

Stephen A. Wise, Chief
Analytical Chemistry Division

Gaithersburg, MD 20899
Certificate Issue Date: 19 June 2006

Robert L. Watters, Jr., Chief
Measurement Services Division

The development of SRM 3245 was a collaboration among the National Institute of Standards and Technology (NIST); the National Institutes of Health (NIH), Office of Dietary Supplements (ODS); and the Food and Drug Administration (FDA), Center for Food Safety and Applied Nutrition (CFSAN) and FDA, Center for Drug Evaluation and Research (CDER).

Acquisition and preparation of the ephedra materials was coordinated by A. NguyenPho of FDA CDER and K.E. Sharpless of the NIST Analytical Chemistry Division. Material grinding and sieving, when necessary, was performed by B.J. Porter of the NIST Analytical Chemistry Division.

Analytical measurements at NIST were performed by T.A. Butler, T. Ihara, S.E. Long, E.A. Mackey, K.E. Murphy, K.W. Phinney, B.J. Porter, L.C. Sander, M.B. Satterfield, R.D. Vocke, and L.J. Wood of the NIST Analytical Chemistry Division. Analyses for value assignment were also performed by C. Fraser, G. Gardner, J.W. Lam, M. McCooeye, C. Scriver, and L. Yang of the National Research Council Canada (Ottawa, ON); D.L. Anderson, J. Cheng, M.L. Gay, and W. Mindak at the FDA CFSAN (College Park, MD); S. Mitvalsky and M. Roman at ChromaDex, Inc. (Clearwater, FL); and laboratories participating in an interlaboratory comparison exercise organized by the Food Products Association's (FPA's; formerly the National Food Processors Association) Food Industry Analytical Chemists Subcommittee (FIACS). The FPA FIACS interlaboratory comparison exercise was coordinated by K.E. Sharpless of the NIST Analytical Chemistry Division and D.W. Howell of the FPA (Washington, DC).

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

Support for the development of SRM 3245 was provided in part by NIH ODS, FDA CFSAN, and FDA CDER. Technical consultation from these agencies was provided by J. Betz (NIH ODS), A. NguyenPho (CDER), and G. Ziobro (CFSAN).

The support aspects involved with the issuance of this SRM were coordinated through the NIST Measurement Services Division.

NOTICE AND WARNING TO USERS

Storage: The materials should be stored at controlled room temperature (20 °C to 25 °C) in their unopened bottles until required for use.

WARNING: FOR LABORATORY USE ONLY. NOT FOR HUMAN CONSUMPTION.

INSTRUCTION FOR USE

Prior to removal of a test portion for analysis, the contents of a bottle of material should be mixed thoroughly. The concentrations of constituents in SRMs 3240 through 3244 are reported on a dry-mass basis. A separate subsample of the SRM should be removed from its bottle at the time of analysis and dried to determine the concentration based on dry mass. (See "NIST Determination of Moisture," below, for drying conditions.) Test portions used for NIST analyses described below were 0.15 g to 5 g for ephedrine alkaloids, 0.2 g to 0.3 g for arsenic and mercury, 1 g for cadmium and lead, and 0.5 g to 1 g for elements of nutritional interest, depending on the specific SRM. See the Certificate of Analysis for each individual SRM within the ephedra SRM suite for details. Certificates are available at http://www.nist.gov/srm.

Note 1: SRMs 3241 and 3242 extracts were originally packaged as powders; however, over time the extracts may become single solid pellets or several solid pieces. For hardened samples, an appropriate portion should be removed and subdivided using a knife. The certified and reference values for composition provided in Tables 1 through 4 are valid independent of the sample consistency.

Note 2: In accordance with the requirements of Title 21 Code of Federal Regulations Sections 1309 and 1310 (21 CFR 1309 and 1310), NIST is registered with the U.S. Drug Enforcement Agency (DEA) to distribute SRMs 3241 and 3242; the extract materials are classified as List 1 chemicals. Analytical laboratories are not required to register with the DEA to purchase and handle these materials (21 CFR 1309.21). SRM 3240 is a natural ephedra plant material and is therefore exempt from requirements of 21 CFR 1310. SRMs 3243 and 3244 are also exempt from requirements of 21 CFR 1310; concentrations of ephedrine alkaloids in these materials are less than those specified in section 1310.12 (c) of the regulation, thereby exempting these materials. (For more information, see http://www.deadiversion.usdoj.gov.)

PREPARATION AND ANALYSIS[1]

Material Acquisition and Preparation

SRM 3240, SRM 3241, SRM 3242: A single year's harvest of *Ephedra sinica* was acquired from a single field in China in 2002 from Jinke Group USA, Inc. (Diamond Bar, CA) through Modern Nutrition and Biotech (Appleton, WI). The crop was examined by a Chinese taxonomist (Xian-Chun Zhang, Institute of Botany, Chinese Academy of Sciences, Beijing, China) who verified its identity; representative herbarium specimens were collected at the time of flower and shipped with the dried botanical following harvest in the same year. The herbarium sheets were deposited at FDA's herbarium (CFSAN, College Park, MD; FDA Accession No. 1221) and the Missouri Botanical Garden (St. Louis, MO; Herbarium Sheet Number 5827116; http://www.mobot.org/MOBOT/research/diversity/herbarium/compendium_model.aspx?id=3; click on *Ephedra sinica* Specimen 1). While still in China, the plant material (aerial parts) was dried, powdered, sieved to 177 µm (80 mesh), and sterilized using a 6 kGy dose of ^{60}Co. Approximately 100 kg of the dried powdered plant material was shipped to NIST and processed as the powdered botanical raw material (SRM 3240). The remainder of the biomass was extracted in China with hot water under pressure, and the resulting extract was used in the production of the "native extract" (SRM 3241) and the "commercial extract" (SRM 3242). A portion of the water extract was filtered, concentrated, and spray dried to produce the native extract. A second portion of the water extract was filtered, concentrated, and then fortified with ephedrine to yield nominally 8 % total ephedrine alkaloids prior to spray drying to produce the commercial extract. Approximately 15 kg of each of the extracts were shipped to NIST. The native plant material (SRM 3240) and extract materials (SRM 3241 and SRM 3242) were subsequently transferred to ChromaDex, Inc. (Santa Ana, CA) where they were individually blended and bottled under nitrogen in amber high-density polyethylene bottles with polypropylene screw caps. After bottling, the materials were irradiated by ^{60}Co to an absorbed dose of 12.8 kGy to 15.4 kGy at Neutron Products (Dickerson, MD).

SRM 3243, SRM 3244: SRM 3243 Ephedra-Containing Solid Oral Dosage Form was prepared from several different commercially available products (both tablets and capsules) that were purchased in the marketplace. The products were intentionally purchased from multiple vendors to obtain material from different production lots. The tablets and the contents of the capsules were ground using a Teflon disc mill at room temperature, and the powdered material was then sieved to 177 µm (80 mesh). SRM 3244 Ephedra-Containing Protein Powder was prepared from several brands of commercially available products that were purchased in the marketplace. The products were intentionally purchased from multiple vendors to obtain material from different production lots. These materials were primarily milk-based products, although some egg protein was present. Individual amino acids, flavorings, botanicals (including *E. sinica*), vitamins, and elements were among the other ingredients in the products that were combined. Materials for products of SRM 3243 and SRM 3244 were shipped to Sun-Ten (Irvine, CA) where the products were blended for 20 min to uniformity using a V-blender. Following blending, the materials for SRM 3243 and SRM 3244 were transferred to ChromaDex, Inc. (Santa Ana, CA), where they were bottled under nitrogen in amber high-density polyethylene bottles with polypropylene screw caps. After bottling, the materials were irradiated by ^{60}Co to an absorbed dose of 12.5 kGy to 15.7 kGy at Neutron Products (Dickerson, MD).

Analytical Approach for Determination of Ephedrine Alkaloids, Synephrine, Caffeine, and Vitamins

Value assignment of the concentrations of the ephedrine alkaloids in the Ephedra Dietary Supplement Suite was based on the combination of measurements from different analytical methods at NIST and at three collaborating laboratories. NIST provided measurements by using a combination of two sample extraction procedures and three liquid chromatography (LC) methods with different detection (i.e., ultraviolet absorbance spectrometry [UV], mass spectrometry [MS], tandem mass spectrometry [MS/MS]) and capillary electrophoresis (CE) [5]. Results for ephedrine alkaloids were provided by three collaborating laboratories: National Research Council Canada (NRCC), FDA, and ChromaDex. NRCC provided results from three analytical methods: LC/UV, LC/MS/MS, and high-field asymmetric waveform ion mobility spectrometry (FAIMS). FAIMS is a mass spectrometry technique that provided results without using a chromatographic separation [6]. FDA results were based on LC/MS/MS [7], and ChromaDex results were based on LC/UV [8]. The analytical methods used for the ephedrine alkaloids by NIST and the collaborating laboratories are described briefly in the Certificate of Analysis for each of the SRMs in the Ephedra Dietary Supplement Suite. A more detailed discussion of the analytical methods for the ephedrine alkaloids is reported by Sander et al. [9]. Chromatograms from the LC/UV, LC/MS, LC/MS/MS, and CE methods for each material in the Ephedra Dietary Supplement Suite are also provided in the Certificate of Analysis for each individual SRM.

[1]Certain commercial equipment, instruments, or materials are identified in this report to specify adequately 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.

Value assignment of concentrations of synephrine in SRM 3243 and caffeine in SRM 3243 and SRM 3244 was based on the combination of measurements from different analytical methods at NIST and at two collaborating laboratories. Synephrine was determined at NIST by using LC/MS/MS and LC/MS, at FDA by using LC/MS/MS, and at ChromaDex by using LC/UV. Caffeine was determined at NIST by using LC/UV [10] and LC/MS/MS and at Chromadex by using LC/UV. Value assignment of concentrations of theobromine and theophylline in SRM 3244 was based on measurements at NIST by using LC/UV.

Proximates (protein, carbohydrate, etc.), individual fatty acids, amino acids, water-soluble vitamins, and elements of nutritional interest were determined in SRM 3244 as part of an FPA FIACS interlaboratory comparison exercise. (See the Certificate of Analysis for SRM 3244 for the participating laboratories and analytical methods used.) Niacin and pyridoxine hydrochloride (vitamin B_6) were also determined at NIST by using LC/UV.

Analytical Approach for Determination of Elements: The elements of primary interest for the Ephedra Dietary Supplement Suite were the potentially toxic contaminants arsenic, cadmium, lead, and mercury. Value assignment of the concentrations of these elements was based on the combination of measurements at NIST using a single analytical method and results from one or two collaborating laboratories (NRCC and FDA). At NIST instrumental neutron activation analysis (INAA) was used for the determination of arsenic; isotope dilution inductively coupled plasma mass spectrometry (ID ICP-MS) was used for determination of cadmium and lead [11]; and cold vapor (CV) ID ICP-MS was used for determination of mercury [12]. For all NIST measurements, botanical-matrix SRMs with certified values for the elements of interest were analyzed concurrently as control samples. NRCC used ID ICP-MS for the determination of cadmium and lead and hydride generation graphite furnace atomic absorption spectrometry (HG GFAAS) for the determination of arsenic. FDA provided results for arsenic, cadmium, lead, and mercury using ICP-MS. FDA also provided results using prompt gamma activation analysis (PGAA) for the concentrations of additional elements including boron, calcium, carbon, chlorine, gadolinium, hydrogen, iron, magnesium, nitrogen, phosphorus, potassium, samarium, silicon, sodium, sulfur, and zinc [13].

For assignment of concentration values for elements of nutritional interest in SRM 3244, three sets of results from NIST and collaborating laboratories were used. Elements of nutritional interest were determined as part of an FPA FIACS interlaboratory comparison exercise (see the Certificate of Analysis for SRM 3244 for the participating laboratories). Nutritive elements were determined by NIST using inductively coupled plasma optical emission spectrometry (ICP-OES). Nutritive elements were also determined by FDA using prompt gamma activation analysis (PGAA). The analytical methods for NIST and the collaborating laboratories are described briefly in the Certificates of Analysis for each of the SRMs in the Ephedra Dietary Supplement Suite.

NIST Determination of Moisture: Moisture content of each of the SRMs within the Ephedra Dietary Supplement Suite was determined by (1) freeze-drying to constant mass (over 7 days for SRM 3240, SRM 3243, SRM 3244; over 11 days for SRM 3241, SRM 3242); (2) drying over magnesium perchlorate in a desiccator at room temperature (5 days for SRM 3240, SRM 3244; 17 days for SRM 3241, SRM 3242; 55 days for SRM 3243) and (3) drying in a forced-air oven at 85 °C for 4 h. Unweighted results obtained using all three techniques were averaged to determine a conversion factor (gram dry mass per gram as-received mass) for each of the five SRMs: SRM 3240 (0.9548), SRM 3241 (0.9570), SRM 3242 (0.9574), SRM 3243 (0.9537), and SRM 3244 (0.9643), which were used to convert NIST data from an as-received to a dry-mass basis. Collaborating laboratories converted their data to a dry-mass basis using their own moisture determinations. A variability-in-moisture component is included in the uncertainties of both the certified and reference values, reported on a dry-mass basis, that are provided in this Certificate of Analysis.

Homogeneity Assessment: The homogeneity of ephedrine alkaloids was assessed at NIST by using the LC/UV method employed for value assignment. An analysis of variance using measurements for ephedrine did not show inhomogeneity for the sample amounts used for SRMs 3241 through 3244 (0.15 g to 1.0 g). There was no trend in ephedrine data across bottles for 1 g test portions of SRM 3240, therefore the data were treated as though the alkaloids were homogeneously distributed in the material. Other measurands were treated as though they were homogeneously distributed, although homogeneity was not assessed, with the exception of SRM 3244 for which the homogeneity of selected elements was assessed by using the ICP-OES method. An analysis of variance using ICP-OES measurements of calcium, copper, iron, potassium, magnesium, manganese, sodium, phosphorus, and zinc did not show inhomogeneity for a 0.5 g sample of SRM 3244. Because inhomogeneity appeared to be significant relative to other sources of uncertainty for cadmium in SRM 3244, a prediction interval was used to establish the uncertainty on this certified value.

Value Assignment: The equally weighted means from each set of data were used to calculate the assigned values. In cases where NIST made measurements, the NIST means were averaged with the individual data set means provided by the collaborating laboratories to obtain the assigned value. Results from collaborating laboratories were considered individual data sets except for the FPA FIACS interlaboratory comparison exercise, in which case an exercise mean was calculated from each of the laboratory means; this exercise mean, the NIST mean, and the mean of data from other collaborating laboratories (where available) were weighted equally to calculate the assigned values. In cases where NIST did not make measurements, the mean of the data set means became the assigned value.

Supplemental Information: In addition to the analyses of the material described above, further characterization of SRM 3240 was provided using microscopy. See the Certificate of Analysis for SRM 3240 for details (http://www.nist.gov/srm).

Table 1. Certified Concentration Values (mg/g) for Ephedrine Alkaloids, Synephrine, and Caffeine in SRM 3245[(a)]

Analyte	SRM 3240	SRM 3241	SRM 3242	SRM 3243	SRM 3244
Ephedrine	11.31 ± 0.76	28.86 ± 1.17	78.1 ± 2.3	11.21 ± 0.42	0.242 ± 0.038
Methylephedrine	1.18 ± 0.14	2.61 ± 0.51	2.77 ± 0.57	0.323 ± 0.031	0.0075 ± 0.0024
Pseudoephedrine	3.53 ± 0.26	10.74 ± 1.11	9.27 ± 0.94	2.81 ± 0.11	0.0361 ± 0.0086
Total Ephedrine Alkaloids	17.0 ± 1.2	43.3 ± 2.7	91.2 ± 2.0	14.78 ± 0.54	0.296 ± 0.067
Synephrine[(b)]				0.54 ± 0.19	
Caffeine[(b)]				76.5 ± 4.1	2.99 ± 0.54

[(a)] Each certified concentration value, expressed as a mass fraction on a dry-mass basis, is an equally weighted mean of results from eight or nine analytical methods carried out at NIST and at collaborating laboratories. The uncertainty in the certified value, calculated according to the method described in the ISO Guide [2,3], is expressed as an expanded uncertainty, U. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the combined effect of between-laboratory, within-laboratory, and drying components of uncertainty. The coverage factor (k) is determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and approximately 95 % confidence for each analyte.

[(b)] For synephrine and caffeine, certified concentration values were based on four and three analytical methods, respectively.

Table 2. Reference Concentration Values (mg/g) for Ephedrine Alkaloids in SRM 3245[(a)]

Analyte	SRM 3240	SRM 3241	SRM 3242	SRM 3243	SRM 3244
Methylpseudo-ephedrine	0.046 ± 0.015	0.11 ± 0.09	0.124 ± 0.044	0.020 ± 0.011	0.00028 ± 0.00011
Norephedrine	0.44 ± 0.09	0.48 ± 0.20	0.57 ± 0.18	0.160 ± 0.026	0.0030[(b)]
Norpseudoephedrine	0.65 ± 0.14	0.44 ± 0.17	0.40 ± 0.16	0.186 ± 0.029	0.0034[(b)]

[(a)] Each reference concentration value, expressed as a mass fraction on a dry-mass basis, is an equally weighted mean of the results from three to eight analytical methods carried out at NIST and at collaborating laboratories. The uncertainty in the reference value, calculated according to the method described in the ISO Guide [2,3], is expressed as an expanded uncertainty, U. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the combined effect of between-laboratory, within-laboratory, and drying components of uncertainty. The coverage factor (k) is determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and approximately 95 % confidence for each analyte.

[(b)] Information value only.

Table 3. Certified and Reference Concentration Values (mg/g) for Toxic Elements in SRM 3245[a]

Element	SRM 3240	SRM 3241	SRM 3242	SRM 3243	SRM 3244
As	0.265 ± 0.016	1.285 ± 0.081	1.030 ± 0.033	0.554 ± 0.018	(0.0196 ± 0.0027)[b]
Cd	0.0906 ± 0.0039	0.0587 ± 0.0036	0.0538 ± 0.0032	0.1218 ± 0.0033	0.01266 ± 0.00069[c]
Pb[d]		0.241 ± 0.012	0.362 ± 0.014	0.692 ± 0.056	0.0270 ± 0.0027
Hg	0.0167 ± 0.0005	0.00383 ± 0.00029	0.00418 ± 0.00042	0.00900 ± 0.00044[e]	0.000253 ± 0.000033[e]

[a] Values in parentheses are reference concentration values; all other values are certified concentration values. A certified concentration value, expressed as a mass fraction on a dry-mass basis, is an equally weighted mean of the results from NIST and collaborating laboratories, except where noted. The uncertainty in the certified value, calculated according to the method described in the ISO Guide [2,3], is expressed as an expanded uncertainty, U. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the combined effect of between-laboratory, within-laboratory, and drying components of uncertainty. The coverage factor (k) is determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and approximately 95 % confidence for each analyte.

[b] This reference concentration value, expressed as a mass fraction on a dry-mass basis, is the equally weighted mean of results provided by a collaborating laboratory. The uncertainty in the reference value, calculated according to the method described in the ISO Guide [2,3], is expressed as an expanded uncertainty, U. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the combined effect of within-laboratory and drying components of uncertainty. The coverage factor, k, is determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and 95 % confidence for this analyte.

[c] The certified concentration value for cadmium in SRM 3244, expressed as a mass fraction on a dry-mass basis, is the equally weighted mean of results from one analytical method (ID ICP-MS) at NIST. The uncertainty in the certified value, calculated according to the method described in the ISO Guide [2,3], is expressed as an expanded uncertainty, U. Because of concerns about possible inhomogenity of cadmium, a prediction interval is used to represent the expanded uncertainty. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the combined effect of within-laboratory, drying, and possible inhomogeneity components of uncertainty. The coverage factor, k, is determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and 95 % confidence.

[d] A concentration value is not assigned for lead in SRM 3240. Analyses by collaborating laboratories showed that lead was not homogeneously distributed in this material, with concentrations ranging from 1.3 mg/kg to 16 mg/kg.

[e] This certified concentration value for mercury, expressed as a mass fraction on a dry-mass basis, is the equally weighted mean of results from one analytical method (CV ID ICP-MS) at NIST. The uncertainty in the certified value, calculated according to the method described in the ISO Guide [2,3], is expressed as an expanded uncertainty, U. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the combined effect of within-laboratory and drying components of uncertainty. The coverage factor, k, is determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and 95 % confidence.

Table 4. Certified and Reference Concentration Values (%) for Selected Elements in SRM 3245[a,b]

Element	SRM 3240	SRM 3241	SRM 3242	SRM 3243	SRM 3244
C	(45.0 ± 1.1)	(41.3 ± 1.5)	(41.5 ± 1.1)	(38.5 ± 1.1)	(44.7 ± 1.5)
Ca	(2.69 ± 0.08)	(0.845 ± 0.050)	(0.742 ± 0.087)	(1.03 ± 0.05)	1.328 ± 0.090
Cl	(0.460 ± 0.012)	(1.83 ± 0.05)	(2.75 ± 0.06)	(1.07 ± 0.03)	(0.0800 ± 0.0048)
H	(5.51 ± 0.13)	(5.59 ± 0.19)	(6.06 ± 0.14)	(5.32 ± 0.10)	(6.13 ± 0.06)
K	(0.547 ± 0.012)	(3.08 ± 0.09)	(2.46 ± 0.05)	(1.39 ± 0.03)	1.60 ± 0.18
Mg	(0.338 ± 0.041)	(0.719 ± 0.060)		(4.80 ± 0.14)	0.310 ± 0.012
N	(1.58 ± 0.08)	(3.20 ± 0.18)	(2.88 ± 0.07)	(4.45 ± 0.21)	
Na		(0.248 ± 0.028)	(0.244 ± 0.028)	(0.196 ± 0.014)	0.091 ± 0.010
P				(0.68 ± 0.10)	1.220 ± 0.088
S	(0.177 ± 0.005)	(0.385 ± 0.017)	(0.325 ± 0.011)	(0.263 ± 0.010)	(0.650 ± 0.010)
Si	(0.360 ± 0.023)	(0.248 ± 0.030)	(0.278 ± 0.037)	(1.62 ± 0.03)	(0.499 ± 0.022)
Zn				(0.325 ± 0.031)	0.01264 ± 0.00077

Certified and Reference Concentration Values (mg/kg)

B	(13.0 ± 0.4)	(62.2 ± 1.8)	(52.5 ± 1.1)	(70.6 ± 1.4)	(3.56 ± 0.13)
Cr				(63.4 ± 1.3)	
Cu					10.2 ± 1.0
Gd	(0.085 ± 0.016)			(0.133 ± 0.007)	
Fe	(457 ± 67)	(900 ± 100)	(870 ± 230)	(760 ± 160)	(107 ± 15)
Mn					30.0 ± 1.4
Sm	(0.097 ± 0.015)			(0.132 ± 0.009)	

[a] Values in parentheses are reference concentration values; all other values are certified concentration values. Each reference concentration value, expressed as a mass fraction on a dry-mass basis, is the equally weighted mean of results provided by one collaborating laboratory using PGAA or, in the case of Cr in SRM 3243, NIST's ICP-OES analysis. The uncertainty in the reference value, calculated according to the method described in the ISO Guide [2,3], is expressed as an expanded uncertainty, U. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the combined effect of within-laboratory and drying components of uncertainty. The coverage factor (k) is determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and approximately 95 % confidence for each analyte.

[b] Each certified concentration value, expressed as a mass fraction on a dry-mass basis, is the equally weighted mean of results from NIST and collaborating laboratories (see the Certificate of Analysis for SRM 3244 for a list of collaborating laboratories and analytical methods used). The uncertainty in the certified values, calculated according to the method described in the ISO Guide [2,3], is expressed as an expanded uncertainty, U. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the combined effect of between-laboratory, within-laboratory, and drying components of uncertainty. The coverage factor (k) is determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and approximately 95 % confidence for each analyte.

Table 5. Certified and Reference Concentration Values for Alkaloids and Water-Soluble Vitamins in SRM 3244[a]

Analyte	Mass Fraction (mg/kg)
Vitamin B_6	34.1 ± 2.2[b]
Niacin	304 ± 10[b]
Theobromine	(762 ± 26)[c]
Theophylline	(80 ± 3)[c]

[a] Values in parentheses are reference concentration values; all other values are certified concentration values.

[b] Each certified concentration value, expressed as a mass fraction on a dry-mass basis, is the equally weighted mean of results from NIST and the mean of results provided by the FPA FIACS laboratories (see the Certificate of Analysis for SRM 3244 for a list of collaborating laboratories and analytical methods used). The uncertainty in the certified values, calculated according to the method described in the ISO Guide [2,3], is expressed as an expanded uncertainty, U. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the combined effect of between-laboratory, within-laboratory, and drying components of uncertainty. The coverage factor (k) is determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and approximately 95 % confidence for each analyte.

[c] The reference concentration value, expressed as a mass fraction on a dry-mass basis, is the equally weighted mean of results from one analytical method (LC/UV) at NIST. The uncertainty in the reference values, calculated according to the method described in the ISO Guide [2,3], is expressed as an expanded uncertainty, U. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the combined effect of within-laboratory and drying components of uncertainty. The coverage factor, k, is determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and 95 % confidence for each analyte.

Table 6. Reference Concentration Values for Selected Water-Soluble Vitamins in SRM 3244[a]

Analyte	Mass Fraction (mg/kg)
Vitamin C	890 ± 100
Vitamin B_1[b]	20.5 ± 3.6
Vitamin B_2	29.9 ± 2.3
Vitamin B_{12}	0.107 ± 0.017
Pantothenic Acid	172 ± 33
Biotin	4.36 ± 0.38
Folic Acid	5.4 ± 1.2
Choline Ion	1500 ± 600
Inositol	1550 ± 450

[a] Each reference concentration value, expressed as a mass fraction on a dry-mass basis, is the mean of results provided by the collaborating FPA FIACS laboratories (see the Certificate of Analysis for SRM 3244 for a list of collaborating laboratories and analytical methods used). The uncertainty in the reference values, calculated according to the method described in the ISO Guide [2,3], is expressed as an expanded uncertainty, U. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the combined effect of between-laboratory, within-laboratory, and drying components of uncertainty. The coverage factor, k, is determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and 95 % confidence for each analyte.

[b] Thiamine, *not* thiamine hydrochloride

Table 7. Reference Values for Proximates, Selected Fatty Acids (As Triglycerides), and Caloric Content in SRM 3244[a]

Analyte	Mass Fraction (%)
Solids	96.4 ± 1.2
Ash	9.11 ± 0.36
Protein	66.1 ± 1.3
Fat[b]	1.41 ± 0.18
Carbohydrate (by difference)	20.0 ± 4.9
Dodecanoic Acid (C12:0) (Lauric Acid)	0.021 ± 0.005
Tetradecanoic Acid (C14:0) (Myristic Acid)	0.075 ± 0.008
Hexadecanoic Acid (C16:0) (Palmitic Acid)	0.375 ± 0.040
Octadecanoic Acid (C18:0) (Stearic Acid)	0.253 ± 0.025
(Z)-9-Octadecenoic Acid (C18:1 n-9) (Oleic Acid)	0.342 ± 0.042
(Z,Z)-9,12-Octadecadienoic Acid (C18:2 n-6) (Linoleic Acid)	0.192 ± 0.009
(Z,Z,Z)-9,12,15-Octadecatrienoic Acid (C18:3 n-3) (Linolenic Acid)	0.024 ± 0.002
Calories[c]	(366.5 ± 9.6) kcal/100 g

[a] Each reference concentration value, expressed as a mass fraction on a dry-mass basis, is the mean of results provided by the FPA FIACS laboratories (see the Certificate of Analysis for SRM 3244 for a list of collaborating laboratories and analytical methods used). The uncertainty in the reference values, calculated according to the method described in the ISO Guide [2,3], is expressed as an expanded uncertainty, U. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the combined effect of between-laboratory, within-laboratory, and drying components of uncertainty. The coverage factor, k, is determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and 95 % confidence for each analyte.

[b] Based on fat as the sum of the fatty acids.

[c] The value for caloric content is the mean of individual caloric calculations from the collaborating laboratories (see the Certificate of Analysis for SRM 3244 for a list of collaborating laboratories and analytical methods used). If the proximate values above are used for calculation, with caloric equivalents of 9, 4, and 4 for fat (as the sum of the fatty acids), protein, and carbohydrate, respectively, the mean caloric content is 357 kcal/100 g.

Table 8. Reference Concentration Values for Amino Acids in SRM 3244[(a)]

Analyte	Mass Fraction (%)		
Alanine	2.12	±	0.96
Arginine	2.26	±	0.52
Aspartic Acid	5.29	±	0.28
Cystine	0.48	±	0.14
Glutamic Acid	14.3	±	2.1
Glycine	1.23	±	0.13
Histidine	1.73	±	0.17
Isoleucine	3.00	±	0.61
Leucine	6.16	±	0.88
Lysine	4.78	±	0.77
Methionine	1.71	±	0.28
Phenylalanine	3.48	±	0.50
Proline	6.64	±	0.73
Serine	3.80	±	0.35
Threonine	2.76	±	0.54
Tryptophan	0.84	±	0.29
Tyrosine	3.16	±	0.71
Valine	3.67	±	0.98

[(a)] Each reference concentration value, expressed as a mass fraction on a dry-mass basis, is the mean of results provided by the FPA FIACS laboratories (see the Certificate of Analysis for SRM 3244 for a list of collaborating laboratories and analytical methods used). The uncertainty in the reference values, calculated according to the method described in the ISO Guide [2,3], is expressed as an expanded uncertainty, U. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the combined effect of between-laboratory, within-laboratory, and drying components of uncertainty. The coverage factor, k, is determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and 95 % confidence for each analyte.

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