



# Certificate of Analysis

## Standard Reference Material<sup>®</sup> 2384

### Baking Chocolate

This Standard Reference Material (SRM) is intended primarily for use in validating methods for determining proximates, fatty acids, calories, vitamins, elements, catechins, caffeine, and theobromine in baking chocolate and similar matrices. This SRM can also be used for quality assurance when assigning values to in-house reference materials. The SRM is baking chocolate prepared from 100 % cocoa beans by a manufacturer of baking chocolate and consists of a single production lot. A unit of SRM 2384 consists of five 91 g (3.2 oz) individually wrapped bars of baking chocolate.

**Certified Mass Fraction Values:** Certified mass fraction values of fat, fatty acids, elements, caffeine, theobromine, and catechins in SRM 2384 are provided in Tables 2 through 4. 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 were calculated as the mean of the mean values from NIST methods and the median or the mean of the mean measurements made by collaborating laboratories, where appropriate. The associated uncertainties are expressed at an approximately 95 % level of confidence [2,3]. 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, calories, total dietary fiber, vitamins, and other analytes are provided in Tables 5 through 8. 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 2384** is valid, within the measurement uncertainty specified, until **31 December 2019**, provided the SRM is handled and stored in accordance with 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 this 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 Grocery Manufacturers Association (GMA, Washington, DC).

Analytical measurements at NIST were performed by K.D. Chieh, K.E. Murphy, B.C. Nelson, M.M. Phillips, C.S. Phinney, B.J. Porter, K.E. Sharpless, J.R. Sieber, J.B. Thomas, 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 GMA Food Industry Analytical Chemists Committee (FIACC) interlaboratory comparison exercise: Covance, Inc., Madison, WI; General Mills, Inc., Minneapolis, MN; Hormel Foods Corporation, Austin, MN; Kraft Foods, Glenview, IL; Nabisco, Inc., East Hanover, NJ; Nestlé USA, Dublin, OH; Novartis Nutrition Corporation, St. Louis Park, MN; Pillsbury, St. Paul, MN; Ralston Purina Company, St. Louis, MO; U.S. Department of Agriculture, Food Composition Laboratory, Beltsville, MD; and Woodson-Tenent Laboratories, Memphis, TN. Data for extractable fat was also provided by Dionex Corporation, Salt Lake City, UT, USA. Additional laboratories providing measurements for value assignment of catechins included: European Commission - DG Joint Research Centre, Ispra, Italy; Antioxidant Research Group, King's College, London, UK; M&M/Mars, Inc., Hackettstown, NJ; and U.S. Department of Agriculture, Little Rock, AR.

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

**NOTICE TO USERS:** SRM 2384 IS INTENDED FOR LABORATORY USE ONLY, NOT FOR HUMAN CONSUMPTION.

### INSTRUCTIONS FOR STORAGE AND USE

**Storage:** Until required for use, the baking chocolate should be stored under refrigeration at approximately 4 °C (39.2 °F).

**Use:** Test portions for analysis may be melted or grated as described in AOAC Official Method 970.20 [5]. The following masses used for NIST analyses should be used as the minimum sample size to ensure valid results: 11 g for water-soluble vitamins; 1 g for fat, fatty acids, catechins, caffeine, theobromine, and theophylline; and 0.5 g for elements.

### SOURCE, PREPARATION, AND ANALYSIS

**Source and Preparation:** SRM 2384 is baking chocolate prepared from 100 % cocoa beans and taken from a single production lot.

**NIST Analyses for Fat:** Two sets of seven samples of chocolate were prepared for analysis by pressurized-fluid extraction. One-gram portions of grated chocolate were extracted into petroleum ether. Extracts were evaporated under nitrogen and then dried at 100 °C to constant mass, per AOAC Official Method 963.15, Fat in Cocoa Products [5].

**NIST Analyses for Fatty Acids:** Mass fractions of fatty acids were measured by gas chromatography with flame-ionization detection (GC-FID) in three sets of five samples of chocolate over a three-day period. Using pressurized-fluid extraction, fat was extracted into petroleum ether from approximately 1 g samples of grated chocolate. Methyl nonadecanoate 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). FAMES were extracted into hexane and injected for analysis by GC-FID.

**NIST Analyses for Ca, Cu, Fe, K, Mg, Mn, Na, P, and Zn:** Mass fractions of calcium, copper, iron, potassium, magnesium, manganese, sodium, phosphorus, and zinc were measured by inductively-coupled plasma optical emission spectroscopy (ICP-OES) in six bars of chocolate on two or more days. A quarter of each bar was melted in individual beakers and single or duplicate 0.5 g portions were taken from each bar and digested in a microwave sample preparation system using nitric acid. Digests were transferred to plastic bottles and diluted with the appropriate volume of 1.5 % (volume fraction) nitric acid. Quantitation was based on the method of standard additions. The ICP-OES result for sodium was confirmed using X-ray fluorescence (XRF) spectroscopy.

**NIST Analyses for Cd and Pb:** Mass fractions of cadmium and lead were measured by inductively-coupled plasma mass spectrometry (ICP-MS) in six bars of chocolate. Quantitation was based on the method of isotope dilution analysis. Two 5 g squares of each bar were melted in individual beakers and duplicate 1.0 g portions were taken, spiked with isotopically enriched <sup>206</sup>Pb and <sup>111</sup>Cd, and digested in a microwave sample preparation system using nitric acid. Digests were transferred to plastic bottles and diluted with the appropriate volume of 2 % (volume fraction) nitric acid. Lead was measured by ICP-MS in standard mode, whereas cadmium was measured in collision cell/kinetic energy discrimination mode.

**NIST Analyses for Water-Soluble Vitamins:** Mass fractions of thiamine, riboflavin, niacinamide, niacin, pantothenic acid, pyridoxamine, and pyridoxine were measured by isotope dilution liquid chromatography with tandem mass spectrometry (ID-LC-MS/MS) in duplicate 11 g test portions taken from each of 10 bars on a single day. The analytes and internal standards were extracted into dilute ammonium acetate at pH 2.6 by heating at 35 °C for 90 min with constant stirring. The samples were centrifuged and the aqueous layer decanted into a clean vessel. The remaining solid and fat materials were reextracted with fresh solvent by heating at 35 °C for 90 min with constant stirring. The process was repeated for a total of 3 extraction cycles. An aliquot of the combined 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. A single internal standard solution was used for the calibrants and samples.

Table 1. 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
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

**NIST Analyses for Caffeine, Theobromine, and Theophylline:** Mass fractions of caffeine, theobromine, and theophylline were measured by LC-absorbance in single 1 g test portions taken from each of eight bars of chocolate over an eight-day period. The chocolate was melted, an internal standard (β-hydroxyethyltheophylline) was added, and fat was removed from the sample via four successive extractions into hexane. The defatted chocolate was dried under a stream of nitrogen. Water was added, and the sample was placed in an ultrasonic bath and then centrifuged. The supernatant was filtered twice. Samples were injected onto a C18 column and analytes were eluted using an isocratic mixture of acetonitrile, water, and acetic acid. Absorbance was measured at 274 nm.

**NIST Analyses for Catechins:** Mass fractions of catechin and epicatechin were measured by LC/MS in single 250 mg test portions taken from eight bars of chocolate on a single day. Approximately 1 g of chocolate was combined with an internal standard solution (tryptophan methyl ester hydrochloride), and the chocolate was melted. Fat was removed from the sample via three successive extractions into hexane. The defatted chocolate was dried under a stream of nitrogen. The dried powder was stirred, and approximately 250 mg were removed. Catechins from this aliquot were extracted into two portions of methanol via ultrasonication. The supernatants were filtered and combined. The extract was diluted with water. Samples were injected onto a C18 column, and analytes were eluted using a gradient of water and acetonitrile, both of which contained trifluoroacetic acid. Analytes were measured by LC/MS with electrospray ionization using *m/z* of 291 for the catechins and *m/z* of 219 for the internal standard.

**Analyses by Collaborating Laboratories:** Data from additional sources were used for certification of this material, including an interlaboratory comparison exercise organized by the GMA FIACC and four laboratories participating in an exercise in which catechins were measured. Not every laboratory measured every analyte, and some data were excluded as outliers. The GMA FIACC laboratories were asked to use AOAC methods or their equivalent, to make single measurements from each of two bars, and to report the analytical method that was used. The other laboratories were asked to use their usual methods to make single measurements of catechins in each of four bars.

**Homogeneity Assessment:** The homogeneity of elements, caffeine, theobromine, theophylline, fatty acids, water-soluble vitamins, and catechins was assessed at NIST using the methods described above. A small but statistically significant heterogeneity was found for cadmium, calcium, lead, manganese, and the water-soluble vitamins; an inhomogeneity component has been included in the expanded uncertainties for these analytes. All other analytes, including those for which homogeneity was not assessed, have been treated as homogeneous.

**Value Assignment:** The collaborating laboratories reported the individual results for two to eight 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 the collaborating laboratory means was determined. In the case of total vitamin B<sub>3</sub>, the median of the collaborating laboratory means was determined. For analytes that were also measured by NIST, the median or mean of the individual collaborating laboratory means and the mean of the individual sets of NIST data were averaged, as appropriate. For analytes that were only measured by NIST, the mean of the individual sets of NIST data were averaged, as appropriate.

**Certified Mass Fraction Values for Fat and 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 the fatty acids in baking chocolate. The certified values are metrologically traceable to the SI unit of mass, expressed as grams per 100 grams.

Table 2. Certified Mass Fraction Values for Fat and Fatty Acids as Free Fatty Acids in SRM 2384

	Mass Fraction (g/100 g)	Coverage Factor, $k$
Fat (Extractable)	51.4 ± 1.1	2.23
Fat (as the sum of fatty acids as triglycerides)	50.3 ± 1.1	2.31

  

	Common Name	Mass Fraction (g/100 g)	Coverage Factor, $k$
Tetradecanoic Acid (C14:0)	Myristic Acid	0.076 ± 0.005	2.31
Hexadecanoic Acid (C16:0)	Palmitic Acid	12.44 ± 0.26	2.26
(Z)-9-Hexadecenoic Acid (C16:1 n-7)	Palmitoleic Acid	0.127 ± 0.007	2.45
Octadecanoic Acid (C18:0)	Stearic Acid	17.24 ± 0.38	2.31
(Z)-9-Octadecenoic Acid (C18:1 n-9)	Oleic Acid	15.73 ± 0.35	2.26
(Z)-11-Octadecenoic Acid (C18:1 n-7)	Vaccenic Acid	0.172 ± 0.017	3.18
(Z,Z)-9,12-Octadecadienoic Acid (C18:2 n-6)	Linoleic Acid	1.458 ± 0.046	2.45
(Z,Z,Z)-9,12,15-Octadecatrienoic Acid (C18:3 n-3)	$\alpha$ -Linolenic Acid	0.093 ± 0.006	2.37
Eicosanoic Acid (C20:0)	Arachidic Acid	0.501 ± 0.012	2.37
Docosanoic Acid (C22:0)	Behenic Acid	0.088 ± 0.006	2.78
Tetracosanoic Acid (C24:0)	Lignoceric Acid	0.050 ± 0.002	2.45

**Certified Mass Fraction Values for Elements:** Each certified mass fraction value is the combined mean from the mean of results from analyses by NIST and the median or mean 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. 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 with its Supplement 1, and  $k$  is a coverage factor corresponding to approximately 95 % confidence [2,6-7]. The uncertainties for cadmium, calcium, lead, and manganese also incorporate an additional uncertainty component for possible inhomogeneity. The measurands are the mass fractions of elements in baking chocolate. The certified values are metrologically traceable to the SI unit of mass, expressed as milligrams per kilogram.

Table 3. Certified Mass Fraction Values for Elements in SRM 2384

	Mass Fraction (mg/kg)	Coverage Factor, $k$
Cadmium (Cd) <sup>(a)</sup>	0.0734 ± 0.0077	2.45
Calcium (Ca) <sup>(b,c)</sup>	840 ± 74	2.31
Copper (Cu) <sup>(b,c)</sup>	23.9 ± 1.0	2.00
Iron (Fe) <sup>(b,c)</sup>	132 ± 11	2.23
Lead (Pb) <sup>(a)</sup>	0.0357 ± 0.0046	2.20
Magnesium (Mg) <sup>(b,c)</sup>	2610 ± 120	2.00
Manganese (Mn) <sup>(b,c)</sup>	20.8 ± 1.3	2.00
Phosphorus (P) <sup>(b,c)</sup>	3330 ± 210	2.00
Potassium (K) <sup>(b,c)</sup>	8650 ± 400	2.00
Zinc (Zn) <sup>(b,c)</sup>	37.6 ± 1.9	2.00

<sup>(a)</sup> ICP-MS

<sup>(b)</sup> ICP-OES

<sup>(c)</sup> Collaborating laboratories

**Certified Mass Fraction Values for Additional Analytes:** Each certified mass fraction value is the weighted 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 [2–4]. The measurands are the mass fractions of the analytes in baking chocolate. The certified values are metrologically traceable to the SI unit of mass, expressed as milligrams per kilogram.

Table 4. Certified Mass Fraction Values for Additional Analytes in SRM 2384

	Mass Fraction (mg/kg)	Coverage Factor, $k$
Caffeine	1060 ± 50	2.23
Theobromine	11600 ± 1100	2.40
Catechin	245 ± 51	2.45
Epicatechin	1220 ± 240	2.37
Catechin monomers <sup>(a)</sup>	1490 ± 220	2.16

<sup>(a)</sup> Sum of catechin and epicatechin. Sum was determined mathematically and analytically.

**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 [2,3]. The measurands are the mass fractions of proximates and caloric content in baking chocolate and were determined by the collaborating laboratories and the methods they used. The reference values for the 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 5. Reference Values for Proximates and Calories in SRM 2384

	Mass Fraction (g/100 g)	Coverage Factor, $k$
Solids	98.37 ± 0.35	2.26
Ash	2.78 ± 0.11	2.26
Protein <sup>(a)</sup>	13.18 ± 0.46	2.26
Carbohydrates	32.4 ± 1.9	2.26
Total Dietary Fiber	14.5 ± 3.0	2.57
	Energy (kcal per 100 g)	Coverage Factor, $k$
Calories <sup>(b)</sup>	631.0 ± 9.3	2.31

<sup>(a)</sup> A factor of 6.25 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 635 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, and  $k$  is a coverage factor corresponding to approximately 95 % confidence [2,3]. The measurands are the mass fractions of free fatty acids in baking chocolate as determined by the methods indicated above. The results are metrologically traceable to the SI unit of mass, expressed as grams per 100 grams.

Table 6. Reference Mass Fraction Values for Fatty Acids as Free Fatty Acids in SRM 2384

	Common Name	Mass Fraction (g/100 g)	Coverage Factor, $k$
Dodecanoic Acid (C12:0)	Lauric Acid	0.021 ± 0.004	2.45
Pentadecanoic Acid (C15:0)		0.017 ± 0.003	2.78
Heptadecanoic Acid (C17:0)	Margaric Acid	0.110 ± 0.006	2.36
9-Eicosenoic Acid (C20:1)	Gadoleic Acid	0.022 ± 0.004	3.18

**Reference Mass Fraction Values for Vitamins:** Each reference mass fraction value is the mean from the combination of the mean results from NIST and the median of the mean of results provided by collaborating laboratories, where appropriate. The uncertainty provided is an expanded uncertainty about the mean 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 with its Supplement 1, and  $k$  is a coverage factor corresponding to approximately 95 % confidence [2,6–7]. The uncertainties also incorporate an additional uncertainty component for possible inhomogeneity. The measurands are the mass fractions of selected vitamins in baking chocolate as determined by the methods indicated above and noted in the footnotes. The results are metrologically traceable to the SI unit of mass, expressed as milligrams per kilogram.

Table 7. Reference Mass Fraction Values for Vitamins in SRM 2384

	Mass Fraction (mg/kg)	Coverage Factor, $k$
Thiamine (Vitamin B <sub>1</sub> ) <sup>(a,b,c)</sup>	1.59 ± 0.27	2.09
Riboflavin (Vitamin B <sub>2</sub> ) <sup>(a,c)</sup>	2.58 ± 0.36	2.09
Niacinamide (Vitamin B <sub>3</sub> ) <sup>(a,c)</sup>	1.35 ± 0.16	2.09
Niacin (Vitamin B <sub>3</sub> ) <sup>(a,c)</sup>	10.9 ± 1.8	2.09
Total Vitamin B <sub>3</sub> as Niacinamide <sup>(a,d)</sup>	11.6 ± 2.0	2.00
Pantothenic Acid <sup>(a,c)</sup>	3.19 ± 0.51	2.09
Pyridoxamine (Vitamin B <sub>6</sub> ) <sup>(a,c,e)</sup>	0.102 ± 0.017	2.09
Pyridoxine (Vitamin B <sub>6</sub> ) <sup>(a,c,f)</sup>	0.129 ± 0.020	2.09
Total Vitamin B <sub>6</sub> as Pyridoxine <sup>(a,g)</sup>	0.231 ± 0.034	2.09

<sup>(a)</sup> NIST ID-LC/MS/MS

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

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

<sup>(d)</sup> Collaborating laboratories

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

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

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

**Reference Mass Fraction Values for Additional Analytes:** Each reference value is the weighted mean of results provided by NIST or 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 [2,3]. The measurands are the mass fractions of sodium, theophylline, and total procyanidins in baking chocolate as determined by the method indicated. The results are metrologically traceable to the SI unit of mass, expressed as milligrams per kilogram.

Table 8. Reference Values for Additional Analytes in SRM 2384

	Mass Fraction (mg/kg)	Coverage Factor, $k$
Sodium (Na) <sup>(a)</sup>	49 ± 17	2.00
Theophylline <sup>(b)</sup>	151 ± 3	2.36
Total Procyanidins <sup>(c,d)</sup>	10300 ± 1100	2.00

<sup>(a)</sup> NIST ICP-OES

<sup>(b)</sup> NIST LC-Absorbance

<sup>(c)</sup> Collaborating laboratories

<sup>(d)</sup> “Total procyanidins” represents the sum of catechin, epicatechin, and the dimer through the decamer of the procyanidin oligomers.

## REFERENCES

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**Certificate Revision History:** 05 February 2015 (Addition of certified values for Cd and Pb; update of reference values for Na, riboflavin and niacin; addition of reference values for vitamins; change of reference values for elements (Cu, Mg, Mn, P, K, and Zn) to certified values; removal of reference values for tocopherols; editorial changes); 25 September 2014 (Extension of certification period; removed acrylamide reference value in Table 6; editorial changes); 19 July 2013 (Removed specific rotation notation from catechin and epicatechin; editorial changes) 27 January 2009 (This revision reflects an extension in the expiration date); 23 January 2007 (Editorial changes); 28 September 2004 (This revision reflections the addition of a reference value for acrylamide); 04 June 2003 [(This revision reflects correction in Table 1) certified values for (+)-catechin, (-)-epicatechin, catechin monomers; 2) reference values for theophylline and total procyanidins; and 3) text of Appendix C-Other Analytes. Units for caffeine and theobromine given in mg/kg (were g/kg)]; 22 March 2002 (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; e-mail [srminfo@nist.gov](mailto:srminfo@nist.gov); or via the Internet at <http://www.nist.gov/srm>.*