National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 2383

Baby Food Composite

Standard Reference Material (SRM) 2383 is intended primarily for use in validating methods for determining proximates, calories, vitamins, and minerals in food matrices. This SRM can also be used for quality assurance when assigning values to in-house control materials. The baby food composite is a mixture of ingredients used in the preparation of commercially available baby foods. A unit of SRM 2383 consists of four jars, each containing approximately 70 g of material.

Certified Concentration Values: Certified concentration values of selected fat-soluble vitamins and carotenoids in SRM 2383 are provided in Table 1. Analyses for value assignment were performed by NIST and collaborating laboratories. Certified values were derived from a combiniation of these results. All assigned values are the equally weighted means of the measurements made by laboratories reporting results for a given analyte; the associated uncertainties are expressed at the 95 % level of confidence [1,2]. Values are reported on an as-received (not dry mass) basis in mass fraction units [3].

Reference Concentration Values: Reference concentration values for additional vitamins and carotenoids, proximates, cholesterol, calories, minerals, and trace elements are provided in Tables 2, 3, 4, and 5. Most of these reference concentrations were derived from results reported by collaborating laboratories; fat-soluble vitamins and carotenoids were measured by NIST and collaborating laboratories. The reference values are noncertified values that do not meet NIST criteria for certification and are provided with associated uncertainties that may reflect only measurement precision, may not include all sources of uncertainty, or may reflect a lack of sufficient statistical agreement among multiple methods. Explanations in support of each reference value are given as a note in each table.

Information Concentration Values: Information concentration values for additional analytes are provided in Table 6. These are noncertified values with no uncertainties reported as there is insufficient information to make an assessment of the uncertainties. The information values are given to provide additional characterization of the material.

Expiration of Certification: The certification of this SRM lot is valid until **31 August 2002**, within the measurement uncertainties specified, provided the SRM is handled and stored in accordance with the instructions given in this certificate. However, the certification is invalid if the SRM is damaged, contaminated, or 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. Return of the attached registration card will facilitate notification.

The support aspects involved in the preparation, certification, and issuance of this SRM were coordinated through the Standard Reference Materials Program by J.C. Colbert and G.V. Iyengar.

Gaithersburg, MD 20899
Certificate Issue Date: 23 December 1997

Thomas E. Gills, Chief
Standard Reference Materials Program

Coordination of the technical measurements leading to the certification of this SRM was performed by K.E. Sharpless and S.A. Wise of the NIST Analytical Chemistry Division and E.R. Elkins of the National Food Processors Association, Washington, DC.

Analytical measurements at NIST were performed by M. Arce-Osuna (Guest Scientist), J. Brown Thomas, S.A. Margolis, B.J. Porter, and K.E. Sharpless of the NIST Analytical Chemistry Division.

Statistical analysis was provided by L.M. Gill of the NIST Statistical Engineering Division.

NOTICE AND WARNING TO USERS

Storage: The SRM should be stored under refrigeration at a temperature between 2 °C and 8 °C in the dark, in the original sealed jars. The certification does not apply to contents of previously opened jars as the stability of all analytes has not been investigated.

Warning: For laboratory use only. Not for human consumption.

Instructions for Use: Before use, the contents of the jar should be mixed by thorough stirring. A minimum sample size of 1 g should be used for any analytical determination of fat-soluble vitamins and carotenoids in this material; a minimum sample size of 2.5 g should be used for determination of minerals and trace elements.

PREPARATION AND ANALYSIS

Preparation: SRM 2383 is a mixture of foods and was prepared by the Gerber[1] Products Company, Fremont, MI by combining the following ingredients (in order of decreasing mass): orange juice, infant formula, corn, rice flour, creamed spinach, carrots, papaya juice, tomato paste, beef, macaroni, wheat flour, non-fat milk, Romano cheese, soya protein, onion powder, green pepper, celery oil, and oregano oil. The infant formula added contained non-fat milk, lactose, corn oil, coconut oil, retinyl palmitate, vitamin D_3, *dl*-α-tocopheryl acetate, phylloquinone, thiamine hydrochloride, riboflavin, pyridoxine hydrochloride, vitamin B_{12}, niacinamide, folic acid, calcium pantothenate, biotin, ascorbic acid, choline chloride, inositol, zinc sulfate, manganese sulfate, cupric sulfate, and taurine; the creamed spinach contained spinach, non-fat milk, rice flour, oat flour, and onion. Beef and carrots were ground prior to weighing. The carrots, beef, macaroni, green peppers, and corn were transferred to a mixing cooker and were precooked for 15 min at 99 °C. This mixture was then passed through a 1.02 mm (0.040 in) finisher screen. The remaining ingredients were slurried and combined with the precooked ingredients, and the mixture was passed through a 0.84 mm (0.033 in) finisher screen to remove lumps and corn kernel husks. The mixture was poured into a mixing kettle and was heated to 90 °C. While the mixer continued to operate, the mixture was pumped into jars that held 70 g each. The jars were heated in retorts for 38 min (121 °C) at 207 kPa (30 psi). The material was stored in the dark at room temperature for three months and then refrigerated at 4 °C.

Analytical Approach: Analyses were performed by NIST and by collaborating laboratories. Carotenoids, retinol, retinyl palmitate, and the tocopherols were measured by NIST using the three sample preparation procedures and three liquid chromatographic (LC) methods described in the next section. Collaborating laboratories analyzed the material as part of two interlaboratory comparison exercises. In an interlaboratory comparison exercise organized by the National Food Processors Association's (NFPA) Food Industry Analytical Chemists Subcommittee (FIACS), laboratories were asked to analyze the material for proximates, vitamins, minerals, and trace elements using AOAC methods or their equivalents. In an interlaboratory comparison exercise involving participants in the NIST/National Cancer Institute (NCI) Micronutrients Measurement Quality Assurance Program (QA Program), laboratories were asked to analyze the material for carotenoids, retinol, retinyl palmitate, and/or carotenoids using their usual methods of analysis.

[1]Certain commercial materials and equipment are identified 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 dentified are the best available for the purpose.

NIST ANALYSES FOR CAROTENOIDS AND FAT-SOLUBLE VITAMINS

Calibration: The maintenance of pure carotenoid compounds for detector calibration is difficult, therefore detector responses for the carotenoids and fat-soluble vitamins measured by NIST were calibrated against solutions whose concentrations were determined spectrophotometrically using Beer's Law [4]. NIST concentration calculations were based on the following absorptivities and wavelengths: retinol in ethanol - 1843 dL/g•cm at 325 nm; retinyl palmitate in ethanol - 975 dL/g•cm at 325 nm; lutein in ethanol - 2550 dL/g•cm at 445 nm; zeaxanthin in ethanol - 2540 dL/g•cm at 450 nm; β-cryptoxanthin in ethanol - 2356 dL/g•cm at 452 nm; *trans*-lycopene in hexane - 3450 dL/g•cm at 472 nm; *trans*-α-carotene in hexane - 2800 dL/g•cm at 444 nm; *trans*-β-carotene in hexane - 2592 dL/g•cm at 452 nm; 9-*cis*-β-carotene in hexane - 2550 dL/g•cm at 445 nm; 13-*cis*-β-carotene in hexane - 2090 dL/g•cm at 443 nm; 15-*cis*-β-carotene in hexane - 1820 dL/g•cm at 447 nm; δ-tocopherol in ethanol - 91.2 dL/g•cm at 297 nm; γ-tocopherol in ethanol - 91.4 dL/g•cm at 298 nm; and α-tocopherol in ethanol - 75.8 dL/g•cm at 292 nm.

Sample Preparation 1: Retinol or retinyl palmitate, tocopherols, and carotenoids were measured by LC in duplicate test portions from six jars of SRM 2383 over a twelve-day period. Extracted test portions were analyzed using an intermediate C_{18} column [5] on all twelve days; extracts were analyzed using a polymeric C_{30} column [5] on six of these days and using a polymeric C_{18} column [5] on the other six. The extracts were saponified on six of the days, and these saponified test portions were analyzed using an intermediate C_{18} column on all six days, a polymeric C_{30} column on three of the days, and a polymeric C_{18} column on the other three days.

Extraction: Approximately 2.5 g of the baby food composite were combined with calcium carbonate, an ethanolic internal standard solution, tetrahydrofuran (THF), and methanol, and the mixture was homogenized for 1 min. This mixture was then vacuum filtered, and aqueous sodium chloride solution was added to the filtrate. The analytes were extracted into a mixture of diethyl ether and petroleum ether. This solution was washed with water, and the organic phase was then evaporated to approximately 0.5 mL under nitrogen. Ethanol was added to yield a total volume of approximately 3.5 mL, and portions of the resultant solution were injected into the appropriate LC systems or carried through the saponification procedure described below.

Saponification: Three mL of the extract described above were combined with methanolic pyrogallol and aqueous potassium hydroxide solutions. This mixture was allowed to sit at room temperature for 30 min, and ascorbic acid was then added. The analytes were extracted into a mixture of diethyl ether and petroleum ether, which was then washed with water. The organic phase was removed and was evaporated under nitrogen. The residue was redissolved in ethanol.

Sample Preparation 2: Retinol, tocopherols, and carotenoids were measured in individual test portions from six jars of the composite over a twelve-day period. Extracts were analyzed using an intermediate C_{18} column on all twelve days and using a polymeric C_{30} column on six of the days. Approximately 1 g of the baby food composite was combined with an ethanolic internal standard solution, THF, and methanol. The mixture was homogenized for approximately 45 s, and the beaker containing the mixture was placed in a 40 °C water bath. Methanolic potassium hydroxide solution was added, and the mixture was saponified for 30 min. Ascorbic acid was then added to neutralize any remaining potassium hydroxide. Aqueous sodium chloride solution was added, and the analytes were extracted into a mixture of hexane and diethyl ether. The organic phase was washed with water, and the organic solvents were evaporated under nitrogen. The residue was redissolved in ethanol.

Chromatographic Analysis:

Intermediate C_{18} Column: Retinol, retinyl palmitate, δ-tocopherol, γ-tocopherol, α-tocopherol, lutein, zeaxanthin, β-cryptoxanthin, *trans*-lycopene, total lycopene, *trans*-α-carotene, total α-carotene, *trans*-β-carotene, 9-*cis*-β-carotene, 13- + 15-*cis*-β-carotene, and total β-carotene were measured using an intermediate C_{18} analytical column and a gradient consisting of acetonitrile, methanol, and ethyl acetate [4,6]. A programmable UV/visible absorbance detector with a tungsten lamp was used for measurement of the retinoids and the carotenoids. Retinol/retinyl palmitate and the carotenoids were monitored at 325 nm and 450 nm, respectively. A fluorescence spectrometer was used to measure the tocopherols using an excitation wavelength of 295 nm and an emission wavelength of 335 nm. Signals from both detectors were recorded simultaneously.

Polymeric C_{30} Column: Measurement of zeaxanthin, β-cryptoxanthin, total lycopene, *trans*-α-carotene, *trans*-β-carotene, 9-*cis*-β-carotene, 13-*cis*-β-carotene, and 15-*cis*-β-carotene was performed using a NIST-engineered polymeric C_{30} carotenoid column and a gradient consisting of methanol, water, and methyl *tert*-butyl ether [7,8]. (A peak co-eluted with lutein, therefore this carotenoid was not measured on the C_{30} column.) A programmable UV/visible absorbance detector with a tungsten lamp was used for measurement of the carotenoids at 450 nm.
Polymeric C_{18} Column: Retinol, lutein, zeaxanthin, β-cryptoxanthin, total α-carotene, *trans*-β-carotene, and total β-carotene were measured using a polymeric C_{18} column and a gradient of methanol, *n*-butanol, and water [4]. UV/visible absorbance detection using a deuterium lamp was used for determination of retinol at 325 nm and the carotenoids at 452 nm.

Collaborating Laboratories' Analyses: Data from two additional sources were used for certification of this material: an interlaboratory comparison exercise organized by the NFPA FIACS (17 participating laboratories) and an interlaboratory comparison exercise organized by the NIST/NCI QA Program (16 participating laboratories).

The NFPA laboratories (Appendix A) were asked to use AOAC methods or their equivalents and to make single measurements from each of four jars. The laboratories listed in Appendix A analyzed SRM 1846, Infant Formula [9,10] for quality assurance. Three criteria were used for excluding data from the calculation of assigned values: (1) If a laboratory's results for SRM 2383 disagreed with other laboratories' results and that laboratory obtained results outside of the 95 % confidence interval for a given analyte in the control material, the laboratory's results for the analyte were not used for value assignment for SRM 2383. (2) Data were not used for value assignment for analytes for which a laboratory reported no results for the control material. (3) Data were not used for value assignment if their mean was beyond three standard deviations from the mean for SRM 2383. A summary of the methodological information and the number of laboratories using a particular analytical technique are provided in Appendix B.

NIST/NCI QA Program laboratories (Appendix C) were asked to measure retinol/retinyl palmitate, tocopherols, and carotenoids in three test portions taken from one jar. Results were discarded if the Studentized deleted residual was significant at the 0.01 level. A summary of the methodological information and the number of laboratories using a particular analytical technique are provided in Appendix D.

Homogeneity Assessment: The homogeneity of retinol, tocopherols, and carotenoids in 1 g and 2.5 g samples was assessed at NIST using the methods described. No statistically significant heterogeneity was found for these analytes, and data for all analytes have been treated as homogeneous although the homogeneity of the other analytes was not assessed.

Value Assignment: The laboratories listed in Appendices A and C reported the individual results for each of their analyses for a given analyte. The mean of each laboratory's results was then determined. For calculation of assigned values for analytes that were measured only in the NFPA FIACS interlaboratory comparison exercise, each of the laboratory means was weighted equally. For analytes that were measured by NFPA FIACS laboratories, QA Program laboratories, and NIST, the grand mean of the individual NFPA laboratory means was equally weighted with the grand mean of the laboratory means from the QA Program analyses and means from the individual sets of NIST data. For analytes that were measured by QA Program laboratories and NIST, the grand mean of the QA Program laboratory means was equally weighted with the means from the individual sets of NIST data.

Results for retinol/retinyl palmitate, the tocopherols, and the carotenoids, for which concentrations can vary based on the type of sample preparation employed, were divided as follows: data for saponified samples were used for value assignment of retinol, α-tocopherol, lutein, zeaxanthin, and β-cryptoxanthin concentrations; data for unsaponified samples were used for value assignment of retinyl palmitate, and "free" (unesterified) α-tocopherol, lutein, zeaxanthin, and β-cryptoxanthin concentrations; and data for saponified and unsaponified samples were combined for δ-tocopherol, γ-tocopherol, lycopene, α-carotene, and β-carotene concentrations.

Table 1. Certified Concentrations (Mass Fractions) for Selected Fat-Soluble Vitamins and Carotenoids[a]

Analyte	Mass Fraction (in mg/kg)
Trans-Retinol[b]	0.80 ± 0.15
δ-Tocopherol[c]	1.51 ± 0.43
γ-Tocopherol[c,d]	5.51 ± 0.93
α-Tocopherol[b]	25.0 ± 3.3
Lutein (includes esters)[b]	1.16 ± 0.33
Zeaxanthin (includes esters)[b]	0.86 ± 0.14
β-Cryptoxanthin (includes esters)[b]	1.38 ± 0.31
Total α-Carotene[c,e]	0.83 ± 0.16
Total β-Carotene[c,e]	3.12 ± 0.63

[a] Each certified concentration value, expressed as a mass fraction of the material (as received), is an equally weighted mean from the combination of results from analyses by NIST and laboratories listed in Appendices A and C. The uncertainty in the certified concentration is calculated as $U = ku_c + B$. The quantity u_c is the combined standard uncertainty, calculated according to the ISO guide [1], and accounts for the combined effect of the within variance for all participating laboratories at one standard deviation. The coverage factor, k, is determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and 95 % confidence level for each analyte. B is a bias adjustment for the difference between methods, which is the maximum difference between the certified value and method means [2]. Analytical methodology information, including the number of laboratories whose data were used for value assignment, is provided in Appendices B and D.

[b] Concentration in saponified samples.

[c] Concentration in saponified and unsaponified samples.

[d] Concentration may include β-tocopherol.

[e] Concentration is the sum of *cis* and *trans* isomers.

Table 2. Reference Concentrations (Mass Fractions) for Selected Fat-Soluble Vitamins and Carotenoids[a]

NOTE: These concentrations are provided as reference values because concentrations may vary depending on the sample preparation procedure (i.e., isomerization or de-esterification may result during sample preparation).

Analyte	Mass Fraction (in mg/kg)
Retinyl Palmitate[b]	1.45 ± 0.28
α-Tocopherol (free)[c]	10.1 ± 2.2
Lutein (free)[c]	0.75 ± 0.35
Zeaxanthin (free)[c]	0.46 ± 0.10
β-Cryptoxanthin (free)[c]	0.47 ± 0.12
Trans-Lycopene[d]	6.3 ± 1.2
Total Lycopene[d,e]	7.0 ± 1.5
Trans-α-Carotene[d]	0.85 ± 0.24
Trans-β-Carotene[d]	2.40 ± 0.80
9-*Cis*-β-Carotene[d]	0.42 ± 0.14
13-*Cis*-β-Carotene[d]	0.297 ± 0.027
15-*Cis*-β-Carotene[d]	0.158 ± 0.049
13- + 15-*Cis*-β-Carotene[d]	0.321 ± 0.071

[a] Each reference concentration value, expressed as a mass fraction of the material (as received), is an equally weighted mean from the combination of results from analyses by NIST and laboratories listed in Appendices A and C. The uncertainty in the reference concentration is calculated as $U = ku_c + B$. The quantity u_c is the combined standard uncertainty, calculated according to the ISO guide [1], and accounts for the combined effect of the within variance for all participating laboratories at one standard deviation. The coverage factor, k, is determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and 95 % confidence level for each analyte. B is a bias adjustment for the difference between methods, which is the maximum difference between the reference value and method means [2]. Analytical methodology information, including the number of laboratories whose data were used for value assignment, is provided in Appendices B and D.

[b] The reference concentration value for retinyl palmitate is based on the retinol concentrations reported by participating laboratories (retinolconcentrations were multiplied by the ratio of the molecular weights of retinyl palmitate and retinol) and the determination of retinyl palmitate using one NIST method.

[c] Concentration in unsaponified samples.

[d] Concentration in saponified and unsaponified samples.
[e] Concentration is the sum of *cis* and *trans* isomers.

Table 3. Reference Concentrations (Mass Fractions) for Proximates, Calories, and Cholesterol[a]

NOTE: These concentrations are provided as reference values because the results have not been confirmed by an independent analytical technique as required for certification and/or analyses have not been performed at NIST. These reference values should be useful for comparison with results obtained using similar procedures (i.e., AOAC methods).

Analyte	Mass fraction
Solids[b]	(37.19 ± 0.46) %
Ash	(1.090 ± 0.044) %
Fat	(4.67 ± 0.26) %
Protein	(3.89 ± 0.17) %
Carbohydrates	(27.49 ± 0.65) %
Cholesterol	(22.6 ± 4.0) µg/g
Calories[c]	(166.5 ± 3.5) kcal/100 g

[a] Each reference concentration value, expressed as a mass fraction of the material (as received), for proximates, cholesterol, and calories, is an equally weighted mean of results from an interlaboratory comparison exercise among the laboratories listed in Appendix A. The uncertainty in the reference value is expressed as an expanded uncertainty, U, at the 95 % level of confidence, and is calculated according to the method described in the ISO guide [1]. 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 and within-laboratory 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 % level confidence for each analyte. Analytical methodology information, including the number of laboratories whose data were used for value assignment, is provided in Appendix B.

[b] The reference concentration value for solids was determined using AOAC methods for moisture determination. A moisture determination performed at NIST using lyophilization gave a result of 38.56 % ± 0.09 % solids. This uncertainty is expressed as an expanded uncertainty, U, at the 95 % level of confidence, and is calculated according to the method described in the ISO guide [1]. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the measurement error. The coverage factor, k, is determined from the Student's t-distribution corresponding to seven degrees of freedom and 95 % confidence.

[c] Note that the value for calories is the mean of individual caloric calculations from the NFPA round robin exercise. If the mean proximate values above are used for calculation, with caloric equivalents of 9, 4, and 4 for fat, protein, and carbohydrate, respectively, the mean caloric content is 167.5 kcal/100 g.

Table 4. Reference Concentrations (Mass Fractions) for Selected Water-Soluble Vitamins[a]

NOTE: These concentrations are provided as reference values because the results have not been confirmed by an independent analytical technique as required for certification and/or analyses have not been performed at NIST.

Analyte	Mass Fraction (in mg/kg)
Vitamin B_1	1.15 ± 0.19
Vitamin B_2	2.70 ± 0.38
Vitamin B_6	1.51 ± 0.22
Vitamin B_{12}	0.0044 ± 0.0019
Niacin	18.1 ± 2.2
Pantothenic Acid	3.7 ± 1.4
Biotin	0.054 ± 0.012

[a] Each reference concentration value, expressed as a mass fraction of the material (as received), for selected water-soluble vitamins, is an equally weighted mean of results from an interlaboratory comparison exercise among the laboratories listed in Appendix A. The uncertainty in the reference values is expressed as an expanded uncertainty, U, at the 95 % level of confidence, and is calculated according to the method described in the ISO guide [1]. 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 and within-laboratory 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 level for each analyte. Analytical methodology information, including the number of laboratories whose data were used for value assignment, is provided in Appendix B.

Table 5. Reference Concentrations (Mass Fractions) for Minerals and Trace Elements[a]

NOTE: These concentrations are provided as reference values because the results have not been confirmed by an independent analytical technique as required for certification and/or analyses have not been performed at NIST.

Analyte	Mass Fraction (in mg/kg)
Calcium	853 ± 28
Chloride	890 ± 15
Copper	1.42 ± 0.12
Iron	8.44 ± 0.44
Magnesium	248.3 ± 5.2
Manganese	1.39 ± 0.11
Phosphorus	948 ± 33
Potassium	3600 ±100
Sodium	390 ± 28
Zinc	10.46 ± 0.27

[a] Each reference concentration value, expressed as a mass fraction of the material (as received), for selected minerals and trace elements is an equally weighted mean of results from an interlaboratory comparison exercise among the laboratories listed in Appendix A. The uncertainty in the reference value is expressed as an expanded uncertainty, U, at the 95 % level of confidence, and is calculated according to the method described in the ISO guide [1]. 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 and within-laboratory 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 level for each analyte. Analytical methodology information, including the number of laboratories whose data were used for value assignment, is provided in Appendix B.

Table 6. Information Concentrations (Mass Fractions) for Additional Vitamins and Trace Elements, and for Fats, Fiber, and Sugars[a]

NOTE: These concentrations are provided as information values only because the disagreement among the methods was greater than expected for reference values or because results were reported by a limited number of laboratories. The data for these information values are not of sufficient quality or quantity to adequately assign uncertainties.

Vitamins	Mass Fraction (in mg/kg)	Fats, Fiber, and Sugars	Mass Fraction (in %)
Vitamin D	0.014	Monounsaturated fat	1.5
Folic acid	0.15	Polyunsaturated fat	0.74
Choline (ion)	250	Saturated fat	1.8
Inositol	1500	Total dietary fiber	0.50
Vitamin C[b]		Fructose	4.1
		Glucose	3.8
		Lactose	7.8
Trace Elements		Sucrose	2.6
Iodine	0.35		
Molybdenum	0.065		
Selenium	0.026		

[a] Information values are the equally weighted means of results obtained by the laboratories listed in Appendix A reported on an "as received" basis. Analytical methodology information, including the number of laboratories whose data were used for value assignment, is provided in Appendix B.

[b] No value has been assigned for the vitamin C concentration; measurements at NIST indicated that vitamin C is not stable in the material.

REFERENCES

[1] *Guide to the Expression of Uncertainty in Measurement,* ISBN 92-67-10188-9, 1st Ed. ISO, Geneva, Switzerland, (1993): see also Taylor, B.N. and Kuyatt, C.E., "Guidelines for Evaluating and Expressing the Uncertainty of NIST Measurement Results," NIST Technical Note 1297, U.S. Government Printing Office, Washington DC, (1994).

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[3] Taylor, B.N., "Guide for the Use of the International System of Units (SI)," NIST Special Publication 811, 1995 Ed., (April 1994).

[4] Brown Thomas, J., Kline, M.C., Schiller, S.B., Ellerbe, P.M., Sniegoski, L.T., Duewer, D.L., and Sharpless, K.E., "Certification of Fat-soluble Vitamins, Carotenoids, and Cholesterol in Human Serum: Standard Reference Material 968b," Fresenius J. Anal. Chem. **356**, pp. 1-9, (1996).

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[6] Epler, K.S., Ziegler, R.G., and Craft, N.E., "Liquid Chromatographic Method for the Determination of Carotenoids, Retinoids, and Tocopherols in Human Serum and in Food," J. Chromatogr. **619**, pp. 37-48, (1993).

[7] Sander, L.C., Epler Sharpless, K., Craft, N.E., and Wise, S.A., "Development of Engineered Stationary Phases for the Separation of Carotenoid Isomers," Anal. Chem. **66**, pp. 1667-1674, (1994).

[8] Sharpless, K.E., Brown Thomas, J., Sander, L.C., and Wise, S.A., "Liquid Chromatographic Determination of Carotenoids in Human Serum Using an Engineered C_{30} and a C_{18} Stationary Phase," J. Chromatogr. B **678**, pp. 187-195, (1996).

[9] Certificate of Analysis, Standard Reference Material (SRM) 1846, Infant Formula, National Institute of Standards and Technology, Gaithersburg, MD, (8 May 1996).

[10] Sharpless, K.E., Schiller, S.B., Margolis, S.A., Brown Thomas, J., Iyengar, G.V., Colbert, J.C., Gills, T.E., Wise, S.A., Tanner, J.T., and Wolf, W.R., "Certification of Nutrients in Standard Reference Material 1846: Infant Formula," J. AOAC Intl. **80**, pp. 611-621, (1997).

APPENDIX A

Analysts at the laboratories listed below performed measurements that contributed to the value assignment of proximates, vitamins, minerals, and/or trace elements in SRM 2383, Baby Food Composite.

Beech-Nut Nutrition Corporation, Canajoharie, NY
Campbell Soup Company, Camden, NJ
Campbell's R&D, Farmington, AR
The Dial Corporation, Scottsdale, AZ
General Mills, Inc., Minneapolis, MN
Gerber Products Company, Fremont, MI
Grand Metropolitan Pillsbury, Minneapolis, MN
Hormel Foods Corporation, Austin, MN
Kraft USA, Glenview, IL
Lancaster Laboratories, Lancaster, PA
Nabisco, Inc., East Hanover, NJ
Nestlé Food Corporation, Dublin, OH
Ralston Purina Company, St. Louis, MO
Sandoz Nutrition Technical Center, St. Louis Park, MN
Tree Top, Inc., Selah, WA
Department of Chemistry, University of Massachusetts, Amherst, MA[a]
U.S. Department of Agriculture, Beltsville, MD
Woodson-Tenent Laboratories, Memphis, TN

[a] Not a participant in the NFPA FIACS interlaboratory comparison exercise.

APPENDIX B

The methodological information reported by the collaborating laboratories listed in Appendix A whose results were used for value assignment is summarized below. The number of laboratories using a particular method is provided in parentheses.

Proximates, Cholesterol, Calories, Nitrogen, Fiber, and Sugars

Solids	Moisture determined by weight loss after oven-drying: Forced-air oven (1) Vacuum oven (10)
Ash	Weight loss after ignition in muffle furnace (13) Thermogravimetric analysis (1)
Fat	Mojonnier (2) Acid digestion, ether extraction (5) Chloroform/methanol extraction (2) Fatty acid quantitation by gas chromatography (4) "Soxtech" (1)
Monounsaturated Fat	Gas chromatography (2)
Polyunsaturated Fat	Gas chromatography (1)
Saturated Fat	Gas chromatography (2)
Nitrogen	Kjeldahl (6) Thermal conductivity (1) Pyrolysis, gas chromatography (1) Pyrolysis, thermal conductivity (3) Autoanalyzer (2)
Protein	Calculated; a factor of 6.25 was used to calculate protein from nitrogen results
Carbohydrates	Calculated; carbohydrate = solids - (protein + fat + ash)
Cholesterol	Gas chromatography (10)
Calories	Calculated; calories = 9(fat) + 4(protein) + 4(carbohydrate)
Total Dietary Fiber	Enzymatic - gravimetry (1)
Sugars	Liquid chromatography - refractive index detection (1)

Fat-Soluble Vitamins

Vitamin A	Saponification - reversed-phase liquid chromatography - absorbance detection (RPLC; 4) Extraction - saponification - normal-phase liquid chromatography - absorbance detection (NPLC; 4) Extraction - saponification - NPLC - fluorescence detection (1) Extraction - saponification - RPLC - absorbance detection (2)
β-Carotene	Saponification - NPLC - absorbance detection (2) Saponification - RPLC - absorbance detection (1) Extraction - saponification - RPLC - absorbance detection (3) Extraction - RPLC - absorbance detection (1)
Vitamin D	Saponification - NPLC - absorbance detection (2) Saponification - RPLC - absorbance detection (2)
Vitamin E	Saponification - RPLC - absorbance detection (1) Saponification - RPLC - fluorescence detection (3) Saponification - NPLC - absorbance detection (1) Saponification - NPLC - fluorescence detection (2)

Water-Soluble Vitamins

Vitamin B_1	Microbiological (2) Digestion - fluorescence detection (5) Extraction - RPLC - fluorescence detection (1) Extraction - ion pairing chromatography - fluorescence detection (1)
Vitamin B_2	Microbiological (1) Digestion - fluorescence detection (6) Extraction - RPLC - fluorescence detection (3)
Vitamin B_6	Microbiological (4)
Vitamin B_{12}	Microbiological (6)
Niacin	Microbiological (6) Acid digestion - absorption spectrophotometry (2) Extraction - RPLC - fluorescence detection (1)
Folic acid	Microbiological (4)
Pantothenic acid	Microbiological (4)
Biotin	Microbiological (6)
Choline	Acid digestion - absorption spectrophotometry (1) Acid digestion - electrochemical detection (1) Microbiological (1)
Inositol	Microbiological (2)

Minerals and Trace Elements

Calcium	Flame atomic absorption spectrometry (6) Inductively coupled plasma atomic emission spectrometry (9) Direct current plasma atomic emission spectrometry (1)
Chloride	Colorimetric titration (5) Electrochemical titration (3)
Copper	Flame atomic absorption spectrometry (5) Inductively coupled plasma atomic emission spectrometry (6) Direct current plasma atomic emission spectrometry (1) Inductively coupled plasma mass spectrometry (1)
Iodine	Colorimetric titration (1) Gas chromatography - electron capture detector (1) Ion-selective electrode (1) Inductively coupled plasma mass spectrometry (1)
Iron	Flame atomic absorption spectrometry (4) Inductively coupled plasma atomic emission spectrometry (7) Direct current plasma atomic emission spectrometry (1) Inductively coupled plasma mass spectrometry (1)
Magnesium	Flame atomic absorption spectrometry (5) Inductively coupled plasma atomic emission spectrometry (8) Direct current plasma atomic emission spectrometry (1) Inductively coupled plasma mass spectrometry (1)
Manganese	Flame atomic absorption spectrometry (4) Inductively coupled plasma atomic emission spectrometry (6) Inductively coupled plasma mass spectrometry (1)
Molybdenum	Inductively coupled plasma mass spectrometry (1)
Phosphorus	Absorption spectrophotometry (4) Inductively coupled plasma atomic emission spectrometry (8) Inductively coupled plasma mass spectrometry (1)
Potassium	Flame atomic absorption spectrometry (4) Flame atomic emission spectrometry (1) Inductively coupled plasma atomic emission spectrometry (9)
Selenium	Inductively coupled plasma mass spectrometry (1)
Sodium	Flame atomic absorption spectrometry (4) Flame atomic emission spectrometry (1) Inductively coupled plasma atomic emission spectrometry (8) Direct current plasma atomic emission spectrometry (1) Inductively coupled plasma mass spectrometry (1)
Zinc	Flame atomic absorption spectrometry (4) Inductively coupled plasma atomic emission spectrometry (7) Direct current plasma atomic emission spectrometry (1) Inductively coupled plasma mass spectrometry (1)

APPENDIX C

Analysts at the institutions listed below performed measurements that contributed to the value assignment of retinol/retinyl palmitate, tocopherols, and/or carotenoids in SRM 2383.

Beltsville Human Nutrition Research Center, U.S. Department of Agriculture, Beltsville, MD
Biochemistry and Nutrition Laboratory, ICDDR, Dhaka, Bangladesh
Cancer Research Center of Hawaii, University of Hawaii at Manoa, Honolulu, HI
Craft Technologies, Wilson, NC
Department of Animal and Nutritional Sciences, University of New Hampshire, Durham, NH
Department of Biomedical Sciences, University of Ulster, Coleraine, Northern Ireland
Department of Human Nutrition and Dietetics, University of Illinois at Chicago, Chicago, IL
Department of Nutritional Sciences, University of Illinois, Urbana, IL
Human Nutrition Unit, National Institute of Nutrition, Rome, Italy
Institut Pasteur de Lyon, Lyon, France
Institut Suisse des Vitamines, Lausanne, Switzerland
Loyola University Medical Center, Maywood, IL
M.D. Anderson Cancer Center, Houston, TX
Servicio de Nutricion, Clinica Puerta de Hierro, Madrid, Spain
TNO Nutrition and Food Research, Zeist, The Netherlands
Vitamins and Fine Chemicals Division, Hoffmann-LaRoche, Basel, Switzerland

APPENDIX D

The methodological information reported by the laboratories listed in Appendix C whose results were used for value assignment is summarized below. The number of laboratories using a particular method is provided in parentheses.

trans-Retinol	Saponification - reversed-phase liquid chromatography - absorbance detection (RPLC; 19 + NIST)
Retinyl Palmitate	Extraction - RPLC - absorbance detection (9 + NIST)
δ-Tocopherol	Saponification - RPLC - absorbance detection (5) Saponification - RPLC - fluorescence detection (5 + NIST) Extraction - RPLC - absorbance detection (5)
γ-Tocopherol	Saponification - RPLC - absorbance detection (10) Saponification - RPLC - fluorescence detection (5 + NIST) Extraction - RPLC - absorbance detection (10)
α-Tocopherol	Saponification - RPLC - absorbance detection (13) Saponification - RPLC - fluorescence detection (5 + NIST) Extraction - RPLC - absorbance detection (11)
Lutein	Extraction - RPLC - absorbance detection (11 + NIST) Saponification - RPLC - absorbance detection (12 + NIST)
Zeaxanthin	Extraction - RPLC - absorbance detection (8 + NIST) Saponification - RPLC - absorbance detection (12 + NIST)
β-Cryptoxanthin	Extraction - RPLC - absorbance detection (12 + NIST) Saponification - RPLC - absorbance detection (12 + NIST)
trans-Lycopene	Extraction - RPLC - absorbance detection (9 + NIST) Saponification - RPLC - absorbance detection (7 + NIST)
Total Lycopene	Extraction - RPLC - absorbance detection (12 + NIST) Saponification - RPLC - absorbance detection (15 + NIST)
trans-α-Carotene	Extraction - RPLC - absorbance detection (5 + NIST) Saponification - RPLC - absorbance detection (3 + NIST)
Total α-Carotene	Extraction - RPLC - absorbance detection (13 + NIST) Saponification - RPLC - absorbance detection (12 + NIST)
trans-β-Carotene	Extraction - RPLC - absorbance detection (11 + NIST) Saponification - RPLC - absorbance detection (10 + NIST)
Total β-Carotene	Extraction - RPLC - absorbance detection (15 + NIST) Saponification - RPLC - absorbance detection (15 + NIST)