

# Standard Reference Material<sup>®</sup> 1869

## Infant/Adult Nutritional Formula II

(milk/whey/soy-based)

### CERTIFICATE OF ANALYSIS

**Purpose:** The certified values delivered by this Standard Reference Material (SRM) are intended for validating methods for determining elements, vitamins, choline, carnitine and cholesterol in infant and adult nutritional formulas and similar materials and for quality assurance when assigning values to in-house control materials.

**Description:** A unit of SRM 1869 consists of 10 packets, each containing approximately 10 g of material.

**Certified Values:** NIST certified values are traceable to the International System of Units (SI) derived unit of mass fraction, expressed as milligrams per kilogram or milligrams per gram. The values are reported on an as-received basis [1].

**Table 1. Certified Values for Various Measurands in SRM 1869**

	Mass Fraction <sup>(a)</sup> (mg/kg)		Mass Fraction <sup>(a)</sup> (mg/kg)
Calcium (Ca)	4560 ± 130	Ascorbic Acid (Vitamin C)	897 ± 43
Copper (Cu)	19.00 ± 0.38	Thiamine (Vitamin B <sub>1</sub> ) <sup>(b)</sup>	13.36 ± 0.32
Chlorine (Cl)	5130 ± 130	Riboflavin (Vitamin B <sub>2</sub> )	13.6 ± 1.5
Chromium (Cr)	0.859 ± 0.066	Niacinamide (Vitamin B <sub>3</sub> )	98.4 ± 2.2
Iodine (I)	1.28 ± 0.15	Total Vitamin B <sub>3</sub> as Niacinamide	99.5 ± 4.4
Iron (Fe)	164.7 ± 3.7	Pantothenic Acid (Vitamin B <sub>5</sub> )	64.9 ± 6.6
Magnesium (Mg)	947 ± 10	Pyridoxine (Vitamin B <sub>6</sub> ) <sup>(c)</sup>	13.09 ± 0.32
Manganese (Mn)	46.0 ± 1.6	Cyanocobalamin (Vitamin B <sub>12</sub> )	0.0447 ± 0.0049
Molybdenum (Mo)	1.612 ± 0.047	Biotin	1.89 ± 0.24
Phosphorus (P)	4186 ± 57	Total Choline <sup>(d)</sup>	1612 ± 64
Potassium (K)	7560 ± 110	Free Carnitine	103.5 ± 4.5
Selenium (Se)	0.806 ± 0.083		Mass Fraction <sup>(a)</sup> (mg/g)
Sodium (Na)	1877 ± 53		
Zinc (Zn)	144.0 ± 3.2	Cholesterol	0.1302 ± 0.0047

<sup>(a)</sup> Values are expressed as  $x \pm U_{95\%}(x)$ , where  $x$  is the certified value and  $U_{95\%}(x)$  is the expanded uncertainty of the certified value. The true value of the analyte lies within the interval  $x \pm U_{95\%}(x)$  with 95 % confidence. To propagate this uncertainty, treat the certified value as a normally distributed random variable with mean  $x$  and standard deviation  $U_{95\%}(x)/2$  [2–6].

<sup>(b)</sup> Vitamin B<sub>1</sub> is reported as thiamine ion (265.36 g/mol), not thiamine chloride or thiamine chloride hydrochloride.

<sup>(c)</sup> Vitamin B<sub>6</sub> is reported as pyridoxine (169.18 g/mol), not pyridoxine hydrochloride.

<sup>(d)</sup> Choline is reported as the choline ion (104.17 g/mol).

**Period of Validity:** The certified values delivered by **SRM 1869** are valid within the measurement uncertainty specified until **20 August 2032**. The certified values are nullified if the material is stored or used improperly, damaged, contaminated, or otherwise modified.

**Maintenance of Certified Values:** NIST will monitor this SRM over the period of its validity. If substantive technical changes occur that affect the certification, NIST will issue an amended certificate through the NIST SRM website (<https://www.nist.gov/srm>) and notify registered users. SRM users can register online from a link available on the NIST SRM website or fill out the user registration form that is supplied with the SRM. Registration will facilitate notification. Before making use of any of the values delivered by this material, users should verify they have the most recent version of this documentation, available through the NIST SRM website (<https://www.nist.gov/srm>).

**Non-Certified Values:** Non-certified values for vitamins, carotenoids, *myo*-inositol, fatty acids as free fatty acids, proximates, sugars, other nutrients, nucleotides, amino acids, taurine, and chlorate in SRM 1869 are provided in Appendix A.

**Additional Information:** Additional information is provided in Appendices B and C.

**Safety:** SRM 1869 is intended for research use only; not for human consumption.

**Storage:** The original unopened packets of SRM 1869 should be stored at  $-20\text{ }^{\circ}\text{C}$  or colder. For organic constituents, the certification only applies to the initial use and the same results are not guaranteed if the remaining powder is used at a later date. For inorganic constituents, an open packet can be reused until the material reaches its expiration date, provided that the open packet is resealed and stored at  $-20\text{ }^{\circ}\text{C}$  or lower.

**Use:** Before use, a packet should be allowed to warm to room temperature, and the contents of the packet should be mixed thoroughly by shaking the packet. Allow the contents to settle for one minute prior to opening to minimize the loss of fine particles. For certified values to be valid, test portion size should be based on descriptions of NIST methods in Appendix C. Results obtained should include their own estimates of uncertainty and can be compared to the certified values using procedures described in reference 7.

## REFERENCES

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**Certificate Revision History:** 10 October 2023 (Correction to protein non-certified value uncertainty; editorial changes); 23 December 2022 (Correction to zinc certified value); 30 November 2022 (Footnote added to Table A1; Appendix A tables renumbered; editorial changes); 07 November 2022 (Change of period of validity; updated format; editorial changes); 12 March 2021 (Vitamin B<sub>12</sub> reference value upgraded to certified value and moved to Table 2, chlorate reference value added to Table 9, fatty acids certified values converted to reference values to properly reflect traceability and moved to Table 5, solids, ash, and protein certified values converted to reference values to properly reflect traceability and moved to Table 6, reference values for carotenoids updated based on additional data from collaborating laboratories; additional method details added to Appendix B, table numbers updated; editorial changes), 16 August 2018 (Correction to tryptophan and tyrosine values in Table 9, editorial changes); 27 July 2018 (Original certificate date)

*Certain commercial equipment, instruments, or materials may be identified in this Certificate of Analysis to adequately specify the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.*

*Users of this SRM should ensure that the Certificate of Analysis in their possession is current. This can be accomplished by contacting the Office of Reference Materials 100 Bureau Drive, Stop 2300, Gaithersburg, MD 20899-2300; telephone (301) 975-2200; e-mail [srminfo@nist.gov](mailto:srminfo@nist.gov); or the Internet at <https://www.nist.gov/srm>.*

\* \* \* \* \* End of Certificate of Analysis \* \* \* \* \*

# APPENDIX A

**Non-Certified Values:** Non-certified values are suitable for use in method development, method harmonization, and process control but do not meet the NIST criteria for certification [1] nor provide metrological traceability to the International System of Units (SI) or other higher-order reference system. They are the best estimates of the true values based on available data. The values are provided with an uncertainty that may reflect only measurement reproducibility, may not include all sources of uncertainty, and/or may reflect a lack of sufficient statistical agreement among multiple analytical methods.

**Table A1. Non-Certified Values for Various Measurands in SRM 1869**

	Mass Fraction <sup>(a)</sup> (mg/kg)	
Niacin Equivalents <sup>(b)</sup>	136.3	± 3.1
Folic Acid	2.239	± 0.086
Free Choline <sup>(c)</sup>	1528	± 91
Total Carnitine	102.8	± 2.9
Retinol <sup>(d)</sup>	19.27	± 0.32
Retinyl Acetate	11.1	± 1.3
Retinyl Palmitate	17.1	± 2.9
Ergocalciferol (Vitamin D <sub>2</sub> )	0.1402	± 0.0073
Cholecalciferol (Vitamin D <sub>3</sub> )	0.1293	± 0.0031
α-Tocopherol (Free)	55.9	± 5.3
α-Tocopherol (Total)	217.2	± 6.2
α-Tocopheryl Acetate	174	± 17
β-Tocopherol	4.22	± 0.69
γ-Tocopherol	99.4	± 5.1
δ-Tocopherol	32.5	± 2.9
Phylloquinone (Vitamin K <sub>1</sub> )	1.22	± 0.18
<i>trans</i> -Vitamin K <sub>1</sub>	1.209	± 0.098
β-Carotene	1.02	± 0.20
Lutein	2.7	± 1.0
Lycopene	2.57	± 0.19
<i>myo</i> -Inositol	358	± 12
Adenosine Monophosphate	110.3	± 3.0
Cytidine Monophosphate	263	± 13
Inosine Monophosphate	146	± 15
Guanosine Monophosphate	147.0	± 4.9
Uridine Monophosphate	128.3	± 8.7
	Energy (kcal/100 g) <sup>(a,e)</sup>	
Calories	461.4	± 8.6

<sup>(a)</sup> Values are expressed as  $x \pm U_{95\%}(x)$ , where  $x$  is the estimated value and  $U_{95\%}(x)$  is the expanded uncertainty of the value. The method-specific value of the analyte lies within the interval  $x \pm U_{95\%}(x)$  with 95 % confidence.

<sup>(b)</sup> Results for niacin equivalents from collaborating laboratories were calculated by numerically combining reported values for niacinamide and tryptophan, using a conversion factor of 60 mg tryptophan = 1 niacin equivalent.

<sup>(c)</sup> Choline is reported as the choline ion (104.17 g/mol).

<sup>(d)</sup> Retinol was added to SRM 1869 as retinyl acetate and retinyl palmitate. This non-certified value is expressed as retinol equivalents obtained by experimental saponification of these esters or conversion through multiplication using the ratio of the relative molecular masses of retinol and each ester. The value represents total (cis + trans) retinol. No correction is made for differences in biological activity of the cis and trans forms.

<sup>(e)</sup> The non-certified value for calories is the median of lab mean caloric calculations from the interlaboratory comparison exercise. If the mean proximate values from Table A1 are used for calculation, with caloric equivalents of 9, 4, and 4 for fat (extracted), protein, and carbohydrate, respectively, the mean caloric content is 468.5 kcal/100 g.

**Table A2. Non-Certified Values for Various Measurands in SRM 1869**

	Mass Fraction <sup>(a)</sup> (g/100 g)
Alanine	0.539 ± 0.025
Arginine	0.571 ± 0.044
Aspartic Acid	1.300 ± 0.017
Cystine	0.1490 ± 0.0088
Glutamic Acid	2.936 ± 0.081
Glycine	0.3251 ± 0.0049
Histidine	0.3651 ± 0.0098
Isoleucine	0.778 ± 0.014
Leucine	1.394 ± 0.023
Lysine	1.184 ± 0.039
Methionine (Free)	0.131 ± 0.011
Methionine (Total)	0.474 ± 0.039
Phenylalanine	0.6823 ± 0.0071
Proline	1.260 ± 0.041
Serine	0.805 ± 0.034
Taurine	0.0372 ± 0.0032
Threonine	0.696 ± 0.013
Tryptophan	0.228 ± 0.024
Tyrosine	0.610 ± 0.082
Valine	0.861 ± 0.030
Ash	3.421 ± 0.069
Protein <sup>(b)</sup>	14.498 ± 0.083
Fat (extracted)	18.97 ± 0.19
Solids	96.63 ± 0.35
Carbohydrates	60.3 ± 1.3
Glucose	2.00 ± 0.45
Sucrose	27.88 ± 0.60
Free Maltose	2.64 ± 0.62
Lactose	0.52 ± 0.12
Total Fructans	2.12 ± 0.85
Total Sugars	32.5 ± 1.6
Chlorate	0.1053 ± 0.0058

<sup>(a)</sup>Values are expressed as  $x \pm U_{95\%}(x)$ , where  $x$  is the estimated value and  $U_{95\%}(x)$  is the expanded uncertainty of the value. The method-specific value of the analyte lies within the interval  $x \pm U_{95\%}(x)$  with 95 % confidence.

<sup>(b)</sup> Results for nitrogen were converted to protein using a factor of 6.38.

**Table A3. Non-Certified Mass Fraction Values for Values for Fatty Acids as Free Fatty Acids in SRM 1869<sup>(a)</sup>**

		Mass Fraction <sup>(a)</sup> (g/100 g)	
Hexanoic Acid (C6:0)	Caproic Acid	0.0082	± 0.0038
Octanoic Acid (C8:0)	Caprylic Acid	1.301	± 0.086
Decanoic Acid (C10:0)	Capric Acid	1.018	± 0.055
Dodecanoic Acid (C12:0)	Lauric Acid	0.0416	± 0.0061
Tetradecanoic Acid (C14:0)	Myristic Acid	0.085	± 0.012
Pentadecanoic Acid (C15:0)		0.0072	± 0.0022
Hexadecanoic Acid (C16:0)	Palmitic Acid	1.31	± 0.22
(Z)-9-Hexadecenoic Acid (C16:1 n-7)	Palmitoleic Acid	0.0196	± 0.0050
Heptadecanoic Acid (C17:0)	Margaric Acid	0.01102	± 0.00064
Octadecanoic Acid (C18:0)	Stearic Acid	0.49	± 0.11
(Z)-9-Octadecenoic Acid (C18:1 n-9)	Oleic Acid	6.6	± 1.3
(Z)-11-Octadecenoic Acid (C18:1 n-7)	Vaccenic Acid	0.151	± 0.034
Total Trans C18:1 Fatty Acids		0.0204	± 0.0040
(E,Z)-9,12-Octadecadienoic Acid (C18:2)		0.0157	± 0.0066
(Z,E)-9,12-Octadecadienoic Acid (C18:2)		0.0190	± 0.0058
(Z,Z)-9,12-Octadecadienoic Acid (C18:2 n-6)	Linoleic Acid	4.98	± 0.82
Total Trans C18:1 and C18:2 Fatty Acids		0.0520	± 0.0060
(Z,Z,Z)-9,12,15-Octadecatrienoic Acid (C18:3 n-3)	$\alpha$ -Linolenic Acid	0.520	± 0.077
Eicosanoic Acid (C20:0)	Arachidic Acid	0.051	± 0.019
Eicosadienoic Acid (C20:2)		0.0060	± 0.019
Eicosatrienoic Acid (C20:3 n-6)	Homo- $\gamma$ -Linolenic Acid	0.0100	± 0.0043
(Z,Z,Z,Z)-5,8,11,14-Eicosatetraenoic Acid (C20:4 n-6)	Arachidonic Acid	0.118	± 0.021
Docosanoic Acid (C22:0)	Behenic Acid	0.0529	± 0.0058
(Z,Z,Z,Z,Z,Z)-4,7,10,13,16,19-Docosahexaenoic Acid (C22:6 n-3)	DHA	0.123	± 0.014
Tetracosanoic Acid (C24:0)	Lignoceric Acid	0.025	± 0.011
(Z)-15-Tetracosenoic Acid (C24:1 n-9)	Nervonic Acid	0.01200	± 0.00085
Cis-Monounsaturated Fatty Acids		6.8	± 1.4
Cis-Polyunsaturated Fatty Acids		5.83	± 0.94
Saturated Fatty Acids		4.41	± 0.44
Omega-3 Fatty Acids		0.653	± 0.097
Omega-6 Fatty Acids		5.21	± 0.86
Total Trans Fatty Acids		0.074	± 0.023
Fat (as the sum of fatty acids as triglycerides)		17.9	± 2.8

<sup>(a)</sup> Values are expressed as  $x \pm U_{95\%}(x)$ , where  $x$  is the estimated value and  $U_{95\%}(x)$  is the expanded uncertainty of the value. The method-specific value of the analyte lies within the interval  $x \pm U_{95\%}(x)$  with 95 % confidence.

**Maintenance of Non-Certified Values:** NIST will monitor this material to the end of its period of validity. If substantive technical changes occur that affect the non-certified values during this period, NIST will update this Certificate of Analysis and notify registered users. SRM users can register online from a link available on the NIST SRM website or fill out the user registration form that is supplied with the SRM. Registration will facilitate notification. Before making use of any of the values delivered by this material, users should verify they have the most recent version of this documentation, available through the NIST SRM website (<https://www.nist.gov/srm>).

**Analytical Approach for Determination of Vitamins, Carotenoids, *myo*-Inositol, Nucleotides, Amino Acids, and Chlorate:** Value assignment of the mass fractions of vitamins, carotenoids, *myo*-inositol, nucleotides, amino acids, and chlorate in SRM 1869 was based on the combination of measurements by the material manufacturer and by collaborating laboratories, where available. Methods reported by collaborating laboratories are described in Appendix C.

**Analytical Approach for Determination of Proximates:** Value assignment of the mass fractions of solids and ash in SRM 1869 was based on the combination of measurements made by LACOMET using gravimetry, by the material manufacturer, and by collaborating laboratories, where available. Value assignment of the mass fraction of protein in SRM 1869 was based on the combination of measurements made by LACOMET using Kjeldahl, by the material manufacturer, and by collaborating laboratories, where available. Methods used by LACOMET and reported by collaborating laboratories are described in Appendix C.

**Analytical Approach for Determination of Fatty Acids:** Value assignment of the mass fractions of fatty acids in SRM 1869 was based on the combination of measurements made by NIST using gas chromatography (GC) with flame ionization detection (FID) by the material manufacturer, and by collaborating laboratories. Methods used by NIST and reported by collaborating laboratories are described in Appendix C. For fatty acids values containing NIST data, the uncertainty incorporates a component for possible inhomogeneity based on the NIST standard deviation. Value assignment of the mass fractions for hexanoic acid (C6:0); pentadecanoic Acid (C15:0); heptadecanoic acid (C17:0); total trans C18:1 fatty acids; (E,Z) 9,12 octadecadienoic acid (C18:2); (Z,E)-9,12-octadecadienoic acid (C18:2); eicosadienoic acid (C20:2), eicosatrienoic acid (C20:3 n-6); docosanoic acid (C22:0); (Z)-15-tetracosenoic acid (C24:1 n 9); and total trans fatty acids only included results from by the material manufacturer and by collaborating laboratories.

**Analyses by Collaborating Laboratories:** Collaborating laboratories were asked to use their usual methods to make measurements on single test portions taken from each of two or three packets of SRM 1869. Methods reported by collaborating laboratories are described in Table C2. The manufacturer of the material also provided data for most nutrients.

\*\*\*\*\* End of Appendix A \*\*\*\*\*

# APPENDIX B

## Contributors to the Development and Value Assignment of SRM 1869

### Coordination of Development, Production, Technical Measurements, and Maintenance

C.A. Barber (formerly of the NIST Chemical Sciences Division)  
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### Statistical Analysis

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### Collaborating Laboratories Contributing Data to Value Assignment through Grocery Manufacturers Association (GMA) Food Industry Analytical Chemists Committee (FIACC) Interlaboratory Study (ILS)

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ConAgra Foods Analytical Laboratory (Omaha, NE, USA)  
Del Monte Foods (Walnut Creek, CA, USA)  
Eurofins Frontier Global Sciences, Inc (Bothell, WA, USA)  
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Land O' Lakes (Arden Hills, MN, USA)  
MicroChem Silliker Pvt. Ltd. (Mumbai, India)  
Silliker Canada (Markham, ON, Canada)  
Silliker Sydney (Sydney, NSW, Australia)

### Collaborating Laboratories Contributing Data to Value Assignment through Multilaboratory Test (MLT) on Determination of Vitamin D in Infant Formulas and Adult Nutritionals

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## **Collaborating Laboratories Contributing Data to Value Assignment through NIST-Coordinated ILS**

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Arbro Pharmaceutical Pvt. Ltd. (New Delhi, India)  
AsureQuality Limited (Auckland, New Zealand)  
Balchem (Clearfield, UT, USA)  
BI Nutraceuticals (McCarren, NV, USA)  
Brooks Applied Labs (Bothell, WA, USA)  
CCC Eurofins Food testing (Heerenveen, Netherlands)  
Canadian Food Inspection Agency (Longueuil, QC, Canada)  
Chemical Solutions Ltd. (Harrisburg, PA, USA)  
Chinese Academy of Inspection Quarantine Comprehensive Test Center (Beijing, China)  
Covance Laboratories (Harrogate, United Kingdom; Madison, WI, USA; Singapore)  
Craft Technologies, Inc. (Wilson, NC, USA)  
DTS Food Laboratories (North Melbourne, VIC, Australia)  
DTU Food Laboratories (Søborg, Denmark)  
Dyad Labs (Salt Lake City, UT, USA)  
Eurofins Analytical Services India Pvt. Ltd. (Bangalore, Karnataka, India)  
Eurofins CLF Specialised Nutrition Testing Services GmbH (Friedrichsdorf, Germany)  
Eurofins Nutrition Analysis Center (Des Moines, IA, USA)  
Eurofins Steins (Vejen, Denmark)  
Eurofins WEJ Contaminants GmbH (Hamburg, Germany)  
US Food and Drug Administration Atlanta Center for Nutrient Analysis (Atlanta, GA, USA)  
First Source Laboratory Solutions LLP (Hyderabad, India)  
Fonterra Waitoa Nutritionals Laboratory (Waitoa, New Zealand)  
FrieslandCampina LQS (Leeuwarden, Netherlands)  
GAAS Analytical (Tucson, AZ, USA)  
Guangzhou Quality Supervision and Testing Institute (Guangzhou, China)  
HVL, LLC (Pittsburgh, PA, USA)  
Immunobiology, Nutrition and Toxicology Laboratory (Dhaka, Bangladesh)  
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Instituto Nacional de Tecnología Industrial – Lácteos (San Martín, Buenos Aires, Argentina)  
Instituto Nacional de Tecnología Industrial – Lácteos Rafaela (Buenos Aires, Argentina)  
Instituto Nacional de Tecnología Industrial – Toxicología y Nutrición (San Martín, Buenos Aires, Argentina)  
IonSense (Saugus, MA, USA)  
Krueger Food Laboratories, Inc. (Chelmsford, MA, USA)  
Kye Laboratory of Phytochemistry R&D, Hunan Normal University (Changsha, Hunan Province, China)  
Laboratorio Tecnológico del Uruguay (Montevideo, Uruguay)  
Land O'Lakes Analytical Laboratories (Arden Hills, MN, USA)  
Mead Johnson Nijmegen (Nijmegen, Gelderland, Netherlands)  
Mead Johnson Nutrition (Evansville, IN, USA; Zeeland, MI, USA)  
Mead Johnson Nutrition R&D Analytical Laboratory (Chonburi, Thailand)  
Mead Johnson Nutrition Quality Control Laboratory (Chonburi, Thailand)  
Mérieux NutriSciences (Crete, IL, USA)  
Midwest Laboratories (Omaha, NE, USA)  
Neutron (Modena, Italy)  
Nestlé Quality Assurance Center (Dublin, OH, USA; Shah Alam, Selangor, Malaysia)  
Nestlé Research Center (Lausanne, Switzerland)  
Perrigo Nutritionals (Georgia, VT, USA)  
SGS Canada Inc. (Burnaby, BC, Canada),  
Technical Center for Animal, Plant and Food Inspection & Quarantine, (Shanghai, China)  
Silliker Ibérica (Barcelona, Spain)  
SP Laboratorija (Bečej, Serbia)  
Swiss Vitamin Institute (Eapligen, Switzerland)  
Syngene International Limited (Bangalore, Karnataka, India)  
UL (Canton, MA, USA)  
US Food and Drug Administration (College Park, MD, USA)  
Weck Laboratories, Inc. (Industry, CA, USA)  
Wyeth Askeaton (Askeaton, Limerick, Ireland)  
Wyeth Nutritionals Pte Ltd (Singapore)

**Collaborating Laboratories Contributing Data to Value Assignment through MLT on Determination of Lutein,  $\beta$ -Carotene, and Lycopene in Infant Formulas and Adult Nutritionals**

AsureQuality Limited (Auckland, New Zealand)

CCIC Europe Food Test, formerly Ausnutria Hyproca Analytics BV (Lelystad, The Netherlands)

DSM Nutritional Products (Basel, Switzerland)

Eurofins, formerly Covance Laboratories (Madison, WI, USA)

Fonterra Co-operative Group Limited (Waitoa, New Zealand)

Laboratoire Aquanal (Pessac, France)

Laboratoire SCL de Strasbourg (Ilkkirch-Graffenstaden, France)

Livsmedelsverket (National Food Agency) (Uppsala, Sweden)

Mérieux NutriSciences Italia (Resana, TV, Italy)

Nestlé Research Center (Lausanne, Switzerland)

Perrigo Nutritionals (Georgia, VT, USA)

Triskelion (Zeist, The Netherlands)

Wyeth Nutrition (Limerick, Ireland)

\* \* \* \* \* End of Appendix B \* \* \* \* \*

# APPENDIX C

**Material Acquisition and Preparation:** The SRM is a soy, whey, and milk protein concentrate-based, hybrid infant/adult nutritional powder, prepared by a manufacturer of infant formula and adult nutritional products. A base liquid containing all constituents was conventionally heat processed, homogenized, and spray-dried. The product was packaged into single-use nitrogen-flushed pouches, each containing 10 g of powder. The material is stored at NIST at  $-20\text{ }^{\circ}\text{C}$  to enhance long-term stability.

**Analytical Approach for Determination of Elements:** Value assignment of the mass fractions of elements in SRM 1869 was based on the combination of measurements made by NIST using inductively coupled plasma optical emission spectrometry (ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS), by Laboratorio Costarricense de Metrología (LACOMET, Costa Rica) using flame atomic absorption spectrometry (FAAS), by the material manufacturer, and by collaborating laboratories, where available. Methods used by NIST and LACOMET and reported by collaborating laboratories are described in Appendix C.

**Analytical Approach for Determination of Vitamins, Choline, and Carnitine:** Value assignment of the mass fractions of vitamins, choline, and carnitine in SRM 1869 was based on the combination of measurements made by NIST using liquid chromatography with absorbance detection (LC-absorbance), isotope dilution liquid chromatography with tandem mass spectrometry (ID-LC-MS/MS), or LC-ICP-MS, by the material manufacturer, and by collaborating laboratories, where available. Methods used by NIST and reported by collaborating laboratories are described in Appendix C. The uncertainty for ascorbic acid incorporates a component for possible inhomogeneity based on the NIST standard deviation.

**Analytical Approach for Determination of Cholesterol:** Value assignment of the mass fraction of cholesterol in SRM 1869 was based on the combination of measurements made at NIST using ID-GC-MS, by the material manufacturer, and by collaborating laboratories. Methods used by NIST and reported by collaborating laboratories are described in Appendix C. The uncertainty incorporates a component for possible inhomogeneity based on the NIST standard deviation.

**Analyses by Collaborating Laboratories:** Collaborating laboratories were asked to use their usual methods to make measurements on single test portions taken from each of two or three packets of SRM 1869. Methods reported by collaborating laboratories are described in Table C2. The manufacturer of the material also provided data for most nutrients.

**Homogeneity Assessment:** The homogeneity of fatty acids, elements, vitamins, and cholesterol was assessed at NIST using the methods and test portion sizes described in Appendix C. Analysis of variance at a 95 % confidence level showed statistically significant heterogeneity in some cases, and the uncertainties for cholesterol, ascorbic acid, and fatty acids values containing NIST results incorporate an additional component for possible heterogeneity. Homogeneity of constituents measured solely by collaborating laboratories (e.g., proximates, amino acids) was not assessed, although the data were treated as though these analytes were homogeneously distributed.

## NIST Methods Used for Value Assignment of Elements

*NIST Analyses for Ca, Cu, Fe, Mg, Mn, Na, P, K, and Zn Using ICP-OES:* Mass fractions of calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc were measured by ICP-OES in duplicate 0.5 g test portions taken from each of 10 packets of SRM 1869. Samples were digested using a nitric acid/hydrofluoric acid mixture in a microwave oven. Quantitation was based on the method of standard additions using the SRM 3100 series single element standard solutions.

*NIST Analyses for Cl Using ICP-OES:* The mass fraction of chlorine was measured by ICP-OES in duplicate 0.3 g test portions taken from each of ten packets of SRM 1869. Samples were digested using nitric acid and hydrogen peroxide in the presence of silver nitrate in a microwave oven. Following digestion, samples were centrifuged and the precipitate was reconstituted in 20 % (volume fraction) ammonium hydroxide prior to analysis. Quantitation was based on the method of standard additions using SRM 3182 *Chloride Anion (Cl<sup>-</sup>) Standard Solution*.

*NIST Analyses for Cr, Mo, and Se Using ICP-MS:* Mass fractions of chromium, molybdenum, and selenium were measured by ICP-MS in duplicate 0.5 g test portions taken from each of six packets of SRM 1869. Samples were digested using nitric acid in a microwave oven. Quantitation was based on the method of standard additions using the SRM 3100 series single element standard solutions.

*NIST Analyses for I Using ICP-MS:* The mass fraction of iodine was measured by ICP-MS in duplicate 0.5 g test portions taken from each of six packets of SRM 1869. Iodine was extracted from the samples using ammonium

hydroxide. Quantitation was based on the method of standard additions using SRM 3180 *Iodide Anion (I<sup>-</sup>) Standard Solution*.

#### **NIST Methods Used for Value Assignment of Vitamins, Choline, and Carnitine**

*NIST Analyses for Ascorbic Acid Using LC-Absorbance:* The mass fraction of ascorbic acid was measured by LC-absorbance in two or more 2 g test portions from each of 10 packets of SRM 1869. Samples were dissolved in water and an internal standard, 4-pyridoxic acid, was added. Metaphosphoric acid was added as a stabilizing agent, and dithiothreitol was added to convert dihydroascorbic acid to total ascorbic acid. The ascorbic acid was extracted by room-temperature sonication, and following centrifugation, an aliquot of the supernatant was removed and filtered prior to analysis by LC-absorbance. Separations were performed on a C18 column using a gradient LC method with potassium phosphate (dibasic)/acetonitrile mobile phase. The separation was monitored using an absorbance detector at 243 nm for ascorbic acid and 260 nm for the internal standard. Calibrants were prepared gravimetrically, at levels intended to approximate the level of ascorbic acid in the SRM following extraction. The purity of the neat calibrant material was determined at NIST using LC-absorbance at 243 nm. A single internal standard solution was used for the calibrants and samples.

*NIST Analyses for Thiamine, Riboflavin, Niacinamide, Pantothenic Acid, Pyridoxine, and Biotin Using ID-LC-MS/MS:* Mass fractions of thiamine, riboflavin, niacinamide, pantothenic acid, pyridoxine, and biotin were measured by ID-LC-MS/MS in duplicate 1.0 g test portions taken from each of 10 packets of SRM 1869 using the internal standards listed in Table C1. The analytes and internal standards were extracted into ammonium acetate (adjusted to pH 2.6 with hydrochloric acid) by rotary mixing for 30 min. Samples were centrifuged and an aliquot of the supernatant was analyzed by positive-ion mode LC-MS/MS. A gradient method with an ammonium formate buffer/methanol mobile phase and a C18 column were used for LC-MS/MS determination of the vitamins. Calibrants were prepared gravimetrically, at levels intended to approximate the levels of the vitamins in the SRM following extraction. A single internal standard solution containing stable isotope labeled vitamins was used for the calibrants and samples. The purity of neat calibrant materials was determined at NIST using one or more of the following approaches: LC-absorbance, Karl Fischer titration, thermogravimetric analysis, differential scanning calorimetry, and quantitative proton nuclear magnetic resonance spectroscopy (<sup>1</sup>H-qNMR). The analyte and internal standard transitions monitored for ID-LC-MS/MS are listed in Table C1.

*NIST Analyses for Vitamin B<sub>12</sub> Using LC-ICP-MS:* The mass fraction of cyanocobalamin (vitamin B<sub>12</sub>) was measured by LC-ICP-MS in two 3.0 g test portions taken from each of 6 packets of SRM 1869 using elemental cobalt as an internal standard. The samples were digested using taka-diaxase and the analytes were converted to cyanocobalamin by heating with potassium cyanide, and cyanocobalamin was isolated from other sample matrix components using solid phase extraction prior to analysis by LC-ICP-MS. An isocratic method with an EDTA/methanol/water mobile phase and a C18 column was used for LC-ICP-MS determination with detection of Co at 59 u. Quantitation was based on the method of standard additions using neat calibrant materials with purity determined at NIST using LC-ICP-MS with traceability to SRM 3113 *Cobalt (Co) Standard Solution*.

*NIST Analyses for Total Choline and Free Carnitine Using ID-LC-MS/MS:* Mass fractions of choline and carnitine were measured by ID-LC-MS/MS in two 1.0 g test portions taken from each of 10 packets of SRM 1869 using <sup>2</sup>H<sub>9</sub>-choline chloride and <sup>2</sup>H<sub>9</sub>-carnitine hydrochloride as internal standards. The analytes and internal standards were extracted and hydrolyzed by microwave digestion into dilute hydrochloric acid for analysis by positive-ion mode LC-MS/MS. A gradient method with an ammonium formate/acetonitrile mobile phase and a mixed-mode C18 column were used for LC-MS/MS determination. Calibrants were prepared gravimetrically, at levels intended to approximate the levels of choline and carnitine in the SRM following extraction. The purity of neat calibrant materials was determined at NIST using <sup>1</sup>H-qNMR. A single internal standard solution was used for the calibrants and samples. The analyte and internal standard transitions monitored for ID-LC-MS/MS are listed in Table C1.

**Table C1. ID LC MS/MS Transitions Monitored for Vitamins, Choline, and Carnitine**

Compound	Precursor Ion → Product Ion ( <i>m/z</i> )	Product Ion ( <i>m/z</i> )	Internal Standard	IS Precursor Ion → IS Product Ion ( <i>m/z</i> )	IS Product Ion ( <i>m/z</i> )
Thiamine	266	42	<sup>13</sup> C <sub>4</sub> -Thiamine Chloride	270	42
		81			81
		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
Pantothenic Acid	220	41	Calcium <sup>13</sup> C <sub>3</sub> , <sup>15</sup> N-Pantothenate	224	41
		43			43
		72			76
		90			94
Pyridoxine	170	77	<sup>13</sup> C <sub>4</sub> -Pyridoxine Hydrochloride	174	81
		80			83
		134			138
		152			156
Biotin	245	97	<sup>2</sup> H <sub>2</sub> -Biotin	247	99
		227			229
Choline	105	58	<sup>2</sup> H <sub>9</sub> -Choline Chloride	113	66
		60			69
Carnitine	162	60	<sup>2</sup> H <sub>9</sub> -Carnitine Hydrochloride	171	69
		103			103

**NIST Methods Used for Value Assignment of Cholesterol**

*NIST Analyses for Cholesterol Using ID-GC-MS:* The mass fraction of cholesterol was measured using the ID-GC-MS method developed at NIST for serum cholesterol [8] and modified for the determination of cholesterol in food matrices using AOAC INTERNATIONAL Official Method 996.06 for hydrolysis [9]. Cholesterol was determined in triplicate 0.5 g test portions from each of nine packets of SRM 1869 using cholesterol-<sup>13</sup>C<sub>3</sub> as an internal standard. Cholesterol esters were hydrolyzed by refluxing in an alcohol-KOH solution for 1 h and cholesterol was extracted from the resulting solution using hexane. A portion of the hexane extract was evaporated to dryness and N,O-bis(trimethylsilyl)acetamide was added to convert cholesterol to its trimethylsilyl (TMS) derivative (cholesterol-TMS) for analysis by GC-MS in the electron ionization mode. The GC was equipped with a 30 m (5:95 phenyl/methyl polysiloxane [mole fraction]) non-polar fused silica column directly interfaced to the ion source. Cholesterol and cholesterol-TMS were monitored at *m/z* 458 and *m/z* 461, respectively. Four calibrants were used for quantitation, prepared gravimetrically from SRM 911c *Cholesterol*, at levels intended to approximate the level of cholesterol in the SRM following extraction. Calculations are based on linear regression of response factors for the calibrants.

### NIST Methods Used for Value Assignment of Fatty Acids

*NIST Analyses for Fatty Acids Using GC-FID:* Mass fractions of fatty acids were measured by GC-FID from single 0.3 g to 0.5 g test portions from each of 10 packets of SRM 1869 following *in situ* transesterification followed by fatty acid methyl ester extraction [10]. Samples were combined with pyrogallol, an internal standard solution containing tridecanoic acid triglyceride, and boiling stones in a glass test tube. Fatty acids were transesterified using methanolic sodium hydroxide under a nitrogen atmosphere at 90 °C for 10 min and boron trifluoride under a nitrogen atmosphere at 90 °C for 5 min. Fatty acid methyl esters were then extracted into hexane, facilitated by the addition of saturated aqueous sodium chloride, and transferred to autosampler vials containing sodium sulfate for analysis by GC-FID. GC-FID was performed using a 0.25 mm × 100 m poly(bis-cyanopropyl siloxane) fused silica capillary column. Five independently prepared calibrants were used for quantitation, prepared gravimetrically from SRM 2377 *Fatty Acid Methyl Esters in 2,2,4-Trimethylpentane*, at levels intended to approximate the levels of the fatty acids in the SRM following extraction. A single internal standard solution was used for the calibrants and samples. Calculations are based on linear regression of response factors for the calibrants.

### LACOMET Methods Used for Value Assignment

*LACOMET Analyses for Ca, K, Mg, and Na Using FAAS:* Mass fractions of calcium, magnesium, potassium, and sodium were measured by EC-FAAS [11] in seven 0.5 g test portions taken from a single packet of SRM 1869. Samples were digested using a nitric acid/hydrochloric acid mixture in a microwave oven. Quantitation was based on an external calibration using the SRM 3100 series single element standard solutions and pure salts.

*LACOMET Analyses for Solids Using Loss on Drying:* The mass fraction of solids was measured by loss on drying at 100 °C [12] in four 1.5 g test portions taken from a single packet of SRM 1869.

*LACOMET Analyses for Ash Using Gravimetry:* The mass fraction of ash was measured by gravimetry after calcination at 550 °C [13] in seven 5 g test portions taken from four packets of SRM 1869.

*LACOMET Analyses for Protein Using Kjeldahl:* The mass fraction of protein was measured as nitrogen by the Kjeldahl method using classic titration and potentiometric titration [14] in seven 1 g test portions taken from two packets of SRM 1869. The values obtained for nitrogen were converted to protein using a factor of 6.38 [15].

**Table C2. Methods Reported by Collaborating Laboratories Contributing Data to Value Assignment**

	Analyte(s)	Method	Official Method Number(s)
Fatty Acids	All	GC-FID	AOAC 996.06 AOAC 2012.13 GB 5413.27-2010 ISO 15884:2002/IDF 182 ISO 15884:2002/IDF 184
Elements	Ca, Cu, Fe, Mg, Mn, Na, P, K, Zn	Microwave digestion with ICP-OES, ICP-MS; open beaker digestion with ICP-OES; digestion with FAAS; ashing with ICP-OES; hot block digestion with FAAS, ICP-OES, ICP-MS; ICP-OES; FAAS; colorimetry	AOAC 984.27
			AOAC 985.01
			AOAC 999.10
			AOAC 2011.14
			AOAC 2011.19
			AOAC 2015.06
	Cl	Potentiometry; titration	AOAC 969.10 AOAC 971.27 AOAC 986.26 AOAC 2015.07 AOAC 2015.08
	Cr, Mo, Se	Microwave digestion with ICP-MS; open beaker digestion with ICP-MS; hot block digestion with ICP-OES, ICP-MS; ashing with ICP-OES; closed-vessel digestion with FAAS; hydride generation with ICP-OES	AOAC 986.15 AOAC 993.14 AOAC 2011.19 AOAC 2015.06
	I	Microwave digestion with ICP-MS; alkaline digestion with ICP-MS; absorption spectrophotometry, closed vessel digestion with ICP-MS, ion selective electrode	AOAC 2011.19 AOAC 2012.15 AOAC 2015.06

	<b>Analyte(s)</b>	<b>Method</b>	<b>Official Method Number(s)</b>
Vitamins	Ascorbic Acid	LC-absorbance; LC-fluorescence; LC with electrochemical detection; titrimetry	AOAC 967.21 AOAC 985.33 AOAC 2012.22 NFV 03 135:2011
	Thiamine	LC-fluorescence; digestion with LC-fluorescence or LC-absorbance; extraction with LC-fluorescence, LC-absorbance, or LC-MS/MS; hydrolysis with fluorescence detection	AOAC 942.23 AOAC 957.17 AOAC 986.27 EN 14122:2006
	Riboflavin	LC-fluorescence; digestion with LC-fluorescence or LC-absorbance; extraction with LC-fluorescence, LC-absorbance, or LC-MS/MS	EN 14122:2006 NF EN 14152
	Niacinamide	Microbiological assay; LC-absorbance; LC-MS; LC-MS/MS	--
	Pantothenic Acid	Microbiological assay; surface plasmon resonance spectroscopy; LC-absorbance; LC-MS; LC-MS/MS	AOAC 2012.16
	Pyridoxine	digestion with LC-fluorescence; enzyme treatment with LC-fluorescence; LC-MS; LC-MS/MS	AOAC 2004.07 EN 14164:2008
	Vitamin B <sub>12</sub>	Digestion with CN conversion, immunoaffinity cleanup, and LC-MS, LC-MS/MS, or LC-absorbance; extraction with CN conversion, immunoaffinity cleanup, and LC-absorbance; extraction with CN conversion and LC-MS, LC-MS/MS, or LC-absorbance; LC-fluorescence; LC-absorbance; microbiological assay; surface plasmon resonance spectroscopy	AOAC 952.20 AOAC 986.23 AOAC 997.05 AOAC 2011.10 AOAC 2014.02
	Biotin	LC-fluorescence, LC-MS/MS, microbiological assay; surface plasmon resonance spectroscopy	--
	Folic Acid	Double enzyme hydrolysis with LC-MS or LC-MS/MS; extraction with LC-MS, LC-MS/MS, LC-absorbance; microbiological assay; surface plasmon resonance spectroscopy	AOAC 944.12 AOAC 992.05 AOAC 2013.13
	Choline	(microwave) acid and enzyme digestion with LC-MS/MS; acid and enzyme digestion with colorimetric detection; extraction with LC-MS/MS; (microwave) acid digestion with ion chromatography with conductivity detection; acid digestions with LC with electrochemical detection; derivatization with fluorescence spectrophotometry	AOAC 999.14 AOAC 2012.18 AOAC 2012.20 AOAC 2014.04 AOAC 2015.10
	Carnitine	base digestion with LC-MS/MS; extraction with LC-MS/MS; (microwave) acid digestion with LC-MS/MS; acid digestion with absorbance spectrophotometry; enzyme treatment with colorimetry	AOAC 2012.17 AOAC 2014.04 AOAC 2015.10
	Vitamin A	Enzyme treatment with LC-fluorescence; enzyme treatment with LC-absorbance; extraction with LC-absorbance; extraction with LC-MS/MS; saponification with LC-fluorescence; saponification with LC-absorbance; saponification with LC-MS/MS	AOAC 992.04 AOAC 992.06 AOAC 2001.13 AOAC 2002.06 AOAC 2011.07 AOAC 2012.10/ISO 20633:2015 EN 12823-1:2009 ISO 12080-1:2009

Analyte(s)	Method	Official Method Number(s)	
Vitamin D	Extraction and derivatization with LC-MS/MS; extraction with LC-MS/MS; extraction with LC-absorbance; saponification and derivatization with LC-MS/MS; saponification with LC-MS/MS; saponification with LC-absorbance	AOAC 979.24	
		AOAC 980.26	
		AOAC 982.29	
		AOAC 992.26	
		AOAC 995.05	
		AOAC 2002.05	
		AOAC 2011.11	
		AOAC 2012.11	
		AOAC 2016.05	
		DIN EN ISO 12521:2009	
Vitamin E	Enzyme treatment with LC-fluorescence; enzyme treatment with LC-absorbance; extraction with LC-fluorescence; extraction with LC-MS/MS; saponification with LC-fluorescence; saponification with LC-absorbance; saponification with LC-MS/MS	AOAC 992.03	
		AOAC 2012.09	
		AOAC 2012.10/ISO 20633:2015	
		NF EN 12822:2014	
Vitamin K	Enzyme treatment with LC-fluorescence; enzyme treatment with LC-absorbance; extraction with LC-fluorescence; extraction with LC-MS/MS; saponification with LC-fluorescence; saponification with LC-MS/MS	AOAC 999.15	
		EN 14148:2003	
Carotenoids	Extraction with LC-absorbance; enzyme treatment with LC-absorbance; saponification with LC-absorbance	AOAC 2005.07	
		EN 12823-2:2000	
		NZTM 3, 17.11.7 2001	
		AOAC 2016.13	
<i>myo</i> -Inositol	Acid hydrolysis and enzyme digestion with LC with electrochemical detection; acid hydrolysis with GC-FID; derivatization with GC-MS; extraction with LC with electrochemical detection; extraction with LC-MS/MS; ion chromatography	AOAC 2011.18	
		AOAC 2012.12	
		GB 5413.25	
Proximates	Solids	Loss on drying in vacuum oven; loss on drying in forced-air oven; thermogravimetry; Karl Fischer titration	
		AOAC 927.05	
		AOAC 986.25	
		AOAC 990.20	
		IS 16072:2012	
			ISO 5537:2004/IDF 26:2004
	Ash	Mass loss after ignition in muffle furnace; thermogravimetry	AOAC 942.05
			AOAC 945.46
			AOAC 986.25
			BS 1743:1968
NMKL 173			
Protein	Nitrogen determination using Kjeldahl (factor of 6.25); nitrogen determination using Kjeldahl (factor of 6.38); nitrogen determination by combustion (factor of 6.25); nitrogen determination by combustion (factor of 6.38)	AOAC 955.04	
		AOAC 986.25	
		AOAC 991.20	
		AOAC 992.15	
		AOAC 997.09	
		ISO 14891:2002/IDF 185:2002	
		ISO 8968-1:2014/IDF 20:2014	
Cholesterol	GC-FID; GC-MS	AOAC 933.08	
		AOAC 970.50	
		AOAC 970.51	
		AOAC 994.10	
		AOCS Ce 12-16	
Carbohydrates	Calculation, [solids – (protein + fat (as the sum of fatty acids) + ash)]	AOAC 986.25	
Fat (extracted)	Roese-Gottlieb/Mojonnier/acid digestion with ether extraction; alkaline digestion with ether extraction	AOAC 945.48	
		AOAC 986.25	
		AOAC 989.05	
		DIN 10342 (1992-09)	
		ISO 1736/IDF 9:20	
		ISO 8262-1/IDF 124-1:2005	

	<b>Analyte(s)</b>	<b>Method</b>	<b>Official Method Number(s)</b>
	Calories	Calculation, [9×Fat + 4×Protein + 4×Carbohydrates]	--
Sugars	Glucose	LC with refractive index detection; LC with evaporative light-scattering detection	--
	Sucrose	LC with refractive index detection; LC with evaporative light-scattering detection	--
	Free Maltose	LC with refractive index detection	--
	Lactose	LC with refractive index detection; ion chromatography with amperometric detection; LC with amperometric detection	AOAC 997.08
	Total Fructans	Enzyme treatment with LC with amperometric detection; enzyme treatment with LC with refractive index detection; enzyme treatment with spectrophotometry	AOAC 997.08 AOAC 999.03 AOAC 2009.01 AOAC 2016.06/ISO 22579/IDF 241:2020
	Total Sugars	LC with refractive index detection; LC with evaporative light-scattering detection	
Amino Acids	All (except methionine, taurine, tryptophan)	Hydrolysis with derivatization and LC-MS or LC-MS/MS; derivatization with LC-fluorescence; hydrolysis with amino acid analyzer; hydrolysis and derivatization with LC-absorbance; hydrolysis with ion chromatography and electrochemical detection	AOAC 985.28 AOAC 994.12 AOAC 999.13 GB/T 5009.124-2003 ISO 13903:2005
	Methionine (free)	Extraction with ion chromatography and electrochemical detection; hydrolysis with LC-absorbance; LC with amino acid analyzer	AOAC 999.13 ISO 13903:2005
	Methionine (total)	Hydrolysis with ion chromatography and electrochemical detection; hydrolysis with LC-absorbance; LC with amino acid analyzer; hydrolysis with amino acid analyzer; derivatization with LC-fluorescence	AOAC 985.28 AOAC 994.12 AOAC 999.13 GB/T 5009.124-2003 ISO 13903:2005
	Taurine	Derivatization with LC-fluorescence; extraction and derivatization with ion chromatography and fluorescence detection; hydrolysis with amino acid analyzer; hydrolysis and derivatization with LC-absorbance; hydrolysis and derivatization with LC-fluorescence; derivatization with LC-absorbance; LC-absorbance	AOAC 985.28 AOAC 994.12 AOAC 997.05 AOAC 999.13 AOAC 2014.02 GB 5413.26-2010
	Tryptophan	Hydrolysis with amino acid analyzer; hydrolysis and derivatization with LC-fluorescence	AOAC 985.28 AOAC 994.12 AOAC 999.13
	Nucleotides	All	Extraction with LC-absorbance; solid-phase extraction with LC-absorbance; LC-MS/MS
Contaminants	Chlorate	Extraction with LC-MS/MS; solid-phase extraction with LC-MS/MS; extraction with IC-MS/MS	

\*\*\*\*\* End of Appendix C \*\*\*\*\*