DIETARY SUPPLEMENTS

Certification of Elements in and Use of Standard Reference Material 3280 Multivitamin/Multielement Tablets

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Standard Reference Material 3280 Multivitamin/Multielement Tablets was issued by the National Institute of Standards and Technology in 2009, and has certified and reference mass fraction values for 13 vitamins, 26 elements, and two carotenoids. Elements were measured using two or more analytical methods at NIST with additional data contributed by collaborating laboratories. This reference material is expected to serve a dual purpose: to provide quality assurance in support of a database of dietary supplement products and to provide a means for analysts, dietary supplement manufacturers, and researchers to assess the appropriateness and validity of their analytical methods and the accuracy of their results.

More than half the U.S. population reported using dietary supplements between 2003 and 2006; 40% used multivitamin/multimineral products (1). It is important to consumers that these products contain what they are purported to contain and that they do not contain high levels of contaminants or adulterants. It is also important to public health agencies that contents of dietary supplements are accurately known because these agencies collect data on the population's consumption of dietary supplements based on interviews and product labels. If labels are inaccurate, estimates of nutrient intake and subsequent estimates of nutrient deficiencies and adequacies will also be inaccurate.

Standard Reference Material (SRM) 3280 Multivitamin/Multielement Tablets was issued by the National Institute of Standards and Technology (NIST) in 2009 and has certified and reference mass fraction values for 13 vitamins, 26 elements, and two carotenoids. Elements were measured using two or more analytical methods at NIST, with additional data contributed by collaborating laboratories. This SRM is expected to serve a dual purpose: to provide quality assurance in support of measurements made for a database of dietary supplement products and to provide a means for analysts, manufacturers, and researchers to validate and assess the accuracy of their analytical methods.

The U.S. Department of Agriculture (USDA), working with the National Institutes of Health, Office of Dietary Supplements (NIH/ODS) and other federal agencies, including the Centers for Disease Control and Prevention, National Center for Health Statistics (CDC/NCHS), has developed the Dietary Supplement Ingredient Database (DSID), in which levels of nutrients in dietary supplements are estimated (2–6). In conducting interviews for National Health and Nutrition Examination Surveys, CDC collects information about dietary supplement use (7). To relate this information to actual nutrient intake, information about the nutrient content of dietary supplements is needed, hence the generation of the DSID. The first version contains values for Ca, Cu, Fe, I, K, Mg, Mn, P, Se, and Zn, as well as vitamins C, B_6, B_{12}, thiamin, riboflavin, niacin, folic acid, and α-tocopherol. The DSID is modeled after USDA's National Food and Nutrient Analysis Program, in which the USDA provides qualified analytical laboratories with frequently consumed foods for analysis. Results are then assembled in the USDA National Nutrient Database for Standard Reference (8). To support the DSID project, NIST and NIH/ODS collaborated to develop SRM 3280 Multivitamin/Multielement Tablets for use as a QA tool during generation of data for inclusion in the DSID.

SRM 3280 can be used by analytical laboratories and dietary supplement manufacturers to support their compliance with the Dietary Supplement Health and Education Act (DSHEA) and current Good Manufacturing Practices (cGMPs). Congress passed DSHEA in 1994, allowing the U.S. Food and Drug Administration (FDA) to take action against unsafe dietary supplement products in the marketplace and requiring the products to be labeled accurately (9). DSHEA also required dietary supplement manufacturers to comply with cGMPs that have been established by FDA. Among other things, these cGMPs require that manufacturers establish specifications for

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identity, purity, strength, and composition of each ingredient, plus test for contaminants and adulterants. FDA is responsible for taking action against unsafe dietary supplement products in the marketplace. In 2003, the FDA published results for analysis of toxic elements (As, Cd, Pb, and Hg) in 95 dietary supplement products (10). In 2008, FDA published the Pb content in 324 commercially available women's and children's multivitamin/multielement products, most of which contained extremely low levels (11).

DSHEA requires that products are monitored during processing and at the end of manufacture to make sure specifications have been met. Manufacturers must follow processes for selecting and using appropriate analytical methods and reference materials. They must verify that methods are appropriate for their intended use, then use these methods to determine whether or not products meet specifications. Materials such as SRM 3280 can be used in this scheme to demonstrate that analytical methods are appropriate for a given analysis and to provide QA and metrological traceability.

The goal for the development of SRM 3280 was to assign mass fraction values for vitamins, carotenoids, and nutrient elements that might be present on a typical multivitamin/multielement supplement facts panel and to assign values for toxic elements that might be present as contaminants. The details of the certification of the vitamins and carotenoids have been previously described (12). This paper describes the assignment of mass fraction values for nutrient elements, toxic elements, and matrix constituents. Results from multiple analytical methods at NIST, including inductively coupled plasma-optical emission spectrometry (ICP-OES), ICP/MS, isotope dilution (ID)-ICP/MS, LC-ICP/MS, prompt-gamma activation analysis (PGAA), instrumental neutron activation analysis (INAA), radiochemical neutron activation analysis (RNAA), and X-ray fluorescence (XRF) spectrometry as well as results from collaborating laboratories, were used to assign certified and reference mass fractions for 26 elements.

Experimental

Certain commercial equipment, instruments, or materials are identified in this report to adequately specify the experimental procedures. Such identification does not imply recommendation or endorsement by NIST or any other governmental agency, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

Materials Description

A manufacturer of multivitamin/multielement tablets prepared a noncommercial batch of tablets according to its normal procedure. SRM 3280 is a direct-compression tablet formulation produced by blending a vitamin and a mineral pre-mix with the remaining bulk of the formulation, compression, and tablet film coating. Elements included in the pre-mix were Cr, I, Mo, Ni, Se, Sn, and V. Additional elements added were B, Ca, Cu, Fe, Mg, Mn, P, K, Si, Na, and Zn. A list of all the ingredients was previously published (12). The SRM is provided as whole tablets because of the potential for instability resulting from the various encapsulated ingredients coming into contact with each other had the tablets been ground.

Sample Preparation

Prior to removal of a test portion for analysis, batches of eight, 15, or 30 tablets were ground to obtain a powdered sample. A test portion for analysis was taken from the powdered material. NIST analysts used one of two methods to grind pellets to a powder prior to analysis: 30 tablets were ground in a Teflon disk mill, which involved shaking in an orbital pattern for 6 min, or batches of eight, 15, or 30 tablets were ground for 10 min using an automated mortar and pestle.

Analytical Methods

NIST made measurements by using ICP-OES, ICP/MS, LC-ICP/MS, PGAA, INAA, RNAA, and XRF spectrometry. The Grocery Manufacturers Association Food Industry Analytical Chemists Share Group laboratories used their usual methods (Table 1) to measure Ca, Cl, Cu, Fe, Mg, Mn, P, K, and Zn as well as vitamin B_{12}. USDA measured Ca, Cu, Fe, Mg, Mn, P, K, and Zn in whole tablets using ICP-OES (Table 2). Two participants of the European Committee for Standardization (CEN) Vitamin Working Group reported results for vitamin B_{12}.

(**a**) *ICP-OES*.—Cu, Mo, P, K, V, and Zn were measured by ICP-OES in 0.35 to 0.4 g powdered test portions taken from each of six to 10 bottles. Samples were digested in Teflon beakers in nitric, perchloric, and hydrofluoric acids. Heating above 200°C and long heating times were necessary to achieve complete digestion. Ni was measured in similarly sized test portions taken from each of six to 10 bottles; samples were digested in a microwave sample preparation system using nitric and hydrofluoric acids. Microwave digestion is recommended over open beaker hotplate digestion, if available. Quantitation for all ICP-OES analyses was based on the method of standard additions using In as an internal standard (IS) for Cu, Mo, Ni, and V, and Sc as an IS for P, K, and Zn.

(**b**) *ICP/MS*.—As, B, I, Ni, Se, and Sn were measured by ICP/MS in 0.3 to 0.45 g powdered test portions taken from each of six to 10 bottles. Samples for measurement of As, B, Ni, Se, and Sn were digested in nitric and hydrofluoric acids in a microwave sample preparation system. Solutions were transferred to Teflon beakers, then heated to near dryness to be sure no hydrofluoric acid remained. Samples for measurement of I were digested in a solution of sodium hydroxide and sodium sulfite. Quantitation was based on the method of standard additions using In as an IS for As and Se, Rh as an IS for B, Ni, and Sn, and Cs as an IS for I.

(**c**) *ID-ICP/MS*.—Cd and Pb were measured by ID-ICP/MS. Cd was measured in 0.25 g powdered test portions taken from each of 10 bottles. ^{111}Cd was added for isotope dilution quantitation. Samples were digested in nitric and hydrofluoric acids in open beakers. Following digestion, Mo and Sn, which interfere with Cd analysis, were removed by SPE (13). Pb was measured in 0.5 g powdered test portions taken from six bottles. ^{206}Pb was added for isotope dilution quantitation. Samples were digested in nitric acid in a microwave sample preparation system.

(**d**) *LC-ICP/MS*.—Vitamin B_{12} (cyanocobalamin) was measured by LC-ICP/MS in 4.5 g powdered test portions taken from each of 10 bottles. Vitamin B_{12} was extracted into water. A Cadenza CD-C18 column (4.6 × 250 mm id, 3 μm particle size; Imtakt USA, Philadelphia, PA) and the isocratic mobile

Table 1. Analytical methods used by collaborating laboratories reporting results for elements in SRM 3280

Lab	Ca	Cl	Cu	Fe	P	K	Mg	Mn	Zn
1	HNO_3, HCl; ICP			HNO_3, HCl; ICP	HNO_3, HCl; ICP	HNO_3, HCl; ICP	HNO_3, HCl; ICP		HNO_3, HCl; ICP
2	HCl; ICP		HCl; ICP	HCl; ICP	HCl; ICP	HCl; ICP	HCl; ICP	HCl; ICP	HCl; ICP
3	HNO_3; ICP	Titration	HNO_3; ICP	HNO_3; ICP	HNO_3; ICP	HNO_3; ICP	HNO_3; ICP	HNO_3; ICP	HNO_3; ICP
4	Dry ash HCl; FAAS		Dry ash HCl; FAAS	Dry ash HCl; FAAS	Dry ash HCl; colorimetry	Dry ash HCl; FAAS	Dry ash HCl; FAAS	Dry ash HCl; FAAS	Dry ash HCl; FAAS
5	Wet ash HNO_3, HCl; ICP	Potentiometric titration	Wet ash HNO_3, HCl; ICP	Wet ash HNO_3, HCl; ICP		Wet ash HNO_3, HCl; ICP	Wet ash HNO_3, HCl; ICP	Wet ash HNO_3, HCl; ICP	Wet ash HNO_3, HCl; ICP

[a] FAAS = flame atomic absorption spectrometry.

phase methanol–water (30 + 70, volume fractions) were used to separate vitamin B_{12} from other components, including elemental Co. A mixing tee was used to add a Ga IS solution to the eluent from the column prior to introduction into the ICP/MS instrument.

(**e**) *PGAA*.—B, Cl, Cu, Fe, K, and Ti were measured by using PGAA of individual disks prepared from 0.75 g subsamples of ground tablets. Disks were formed using a stainless steel die and hydraulic press. Standards were prepared by transferring a weighed portion of a solution containing a known amount of each element onto filter papers. Disks were formed from the dried filter papers. Samples, standards, and controls were packaged individually in clean polyethylene bags, and irradiated individually at a neutron fluence rate of $3.0 \times 10^8/cm^2$ s. The following γ-ray lines were used for quantitation: 477 keV line from ^{10}B (corrected for ^{10}B in the background and for ^{23}Na at 472 keV from the sample); 770 keV line from ^{39}K; 6111 keV line from ^{35}Cl; 341 and 1381 keV lines from ^{48}Ti; 278 keV line from ^{63}Cu; and 352 keV line from ^{56}Fe.

(**f**) *INAA*.—Ca, Co, Cr, Cu, Fe, I, La, Mg, Mn, Mo, Na, Sb, Se, V, and Zn were measured using INAA of individual disks prepared from 0.2 g subsamples of ground tablets taken from eight bottles. Standards were prepared by transferring a weighed portion of a solution containing a known amount of each element onto filter papers or from pure elements or compounds of known purity. For determination of short-lived nuclides (Ca, Cu, I, Mg, Mn, Na, and V), samples, standards, and controls were packaged individually in clean polyethylene bags and irradiated individually for 30 s at 20 mW. For determination of intermediate and long-lived nuclides (Co, Cr, Fe, La, Mo, Sb, Se, and Zn), samples, standards, and controls were irradiated for 3 h. Irradiation capsules were then inverted 180º, and materials were irradiated another 3 h. Short-lived nuclides were counted for 5 min after a 2 min decay, and again for 20 min following a 15 min decay. For the long irradiations, a first counting session began after a 5-day decay and lasted for 4 h. This was followed by a second session begun after a 25-day decay and lasting for 8 h.

(**g**) *RNAA*.—As was measured using RNAA of individual disks prepared from 0.2 g test portions taken from each of five bottles of the SRM. Disks were formed using a stainless steel die and hydraulic press. Standards were prepared by transferring a weighed portion of a solution containing a known amount of As onto filter papers. Disks were formed from the dried filter papers. Samples, standards, and controls were packaged individually in clean polyethylene bags and irradiated in one polyethylene irradiation vessel for 2 h at a neutron fluence rate of $1.0 \times 10^{14}/cm^2$ s. Samples and controls were combined with ^{77}As tracer and digested in nitric and perchloric acids. Following evaporation of the acids and dissolution of the residue in 1 mol/L nitric acid, As was sequestered on hydrated manganese dioxide resins, which were then counted. The 559 keV line from decay of ^{76}As was used for quantitation. The 239 keV line from decay of ^{77}As was evaluated for yield determination.

(**h**) *XRF spectrometry*.—XRF spectrometry was used for both homogeneity assessment and for quantitative determinations. Homogeneity testing measurements of Mg, Al, Si, P, S, Cl, K, Ca, Ti, Cr, Mn, Fe, Ni, Cu, Zn, Se, Sr, Mo, Sn, and I were made on briquettes pressed from ground tablets. From each bottle, two sets of eight tablets were selected. Each set of eight tablets was ground to a fine powder in an agate mortar and pestle, and the material was stored in a plastic vial with a screw-cap lid. One briquette was prepared from the contents of each vial by pressing 3.0 g of powder at 20 tons in a 31.5 mm steel die for 30 s. Each briquette was measured on one face, then flipped over and measured on the opposite face. The K-$L_{2,3}$ (Kα) fluorescent lines were used for all elements except I, for which the L_2-M_4 (Lβ) line was measured.

For quantitative determinations, two or three 3.0 g specimens of ground material were taken from each of six bottles of tablets. These specimens were prepared by borate fusion and cast as 40 mm diameter glass beads. Mg, Si, P, K, Ca, Cr, Mn, Fe, Sr, and Mo were successfully quantitated using a matrix-independent calibration approach based on calibration standards synthesized using high-purity compounds and NIST spectrometric solution SRMs (14). Quantitation of Al, Cl, Ti, Cu, and Zn failed. Cl present at high levels is somewhat volatile during borate fusion and causes reduction of some elements, in this case Cu and Zn, which are lost into the Pt crucible. Al and Ti results were not considered quantitative because these two elements are found exclusively in the wax-like tablet coating, which sticks strongly to the mortar and pestle and may cause segregation during grinding.

Collaborating Laboratories

Elements and vitamin B_{12} were measured as part of a Grocery Manufacturers Association (GMA) interlaboratory comparison exercise. Laboratories providing results for value assignment of element mass fractions were Campbell Soup Company, Camden, NJ; Covance, Madison, WI; General Mills, Inc., James Ford Bell Technical Center, Golden Valley, MN; Krueger Food Laboratories, Inc., Chelmsford, MA; and Novartis Nutrition Corp., St. Louis Park, MN. The analytical methods

Table 2. Evidence of tablet-to-tablet inhomogeneity in data provided by USDA

Sample	Ca[a]	Cu	Fe	Mg	Mn	K	P	Zn
Bottle 1, tablet 1	116	1.43	12.0	74.9	1.41	48.6	80.6	11.9
Bottle 1, tablet 2	98.0	1.16	10.5	61.5	1.23	45.9	81.6	9.45
Bottle 2, tablet 1	105	1.21	10.9	64.3	1.26	50.7	67.0	9.67
Bottle 2, tablet 2	108	1.35	11.4	67.9	1.43	49.9	71.0	9.87
Bottle 3, tablet 1	113	1.27	11.5	66.7	1.38	50.1	74.5	9.89
Bottle 3, tablet 2	105	1.29	11.2	65.1	1.34	47.1	73.1	9.84
Bottle 4, tablet 1	107	1.35	11.7	68.1	1.40	48.4	72.9	10.0
Bottle 4, tablet 2	108	1.30	11.5	69.3	1.38	45.6	75.3	9.85
Bottle 5, tablet 1	109	1.35	11.8	68.2	1.54	52.1	75.3	10.2
Bottle 5, tablet 2	110	1.38	11.9	70.3	1.47	54.1	75.4	10.1
Bottle 6, tablet 1	97.4	1.22	10.9	61.9	1.21	44.9	68.2	9.91
Bottle 6, tablet 2	114	1.52	12.3	74.5	1.56	46.2	80.5	10.7
Statistic								
Mean	107.5	1.32	11.5	67.7	1.38	48.6	74.6	10.12
SD	5.7	0.10	0.52	4.3	0.11	2.8	4.6	0.63
n	12	12	12	12	12	12	12	12
RSD, %	5.3	7.6	4.5	6.3	8.0	5.8	6.2	6.2
Minimum	97.4	1.16	10.5	61.5	1.21	44.9	67.0	9.45
Maximum	116	1.52	12.3	74.9	1.56	54.1	81.6	11.9
% Difference[b]	18	27	15	20	25	19	19	24
Certified value	110.7	1.40	12.35	67.8	1.44	53.1	75.7	10.15
Expanded uncertainty	5.3	0.17	0.91	4.0	0.11	7.0	3.2	0.81

[a] All data are mg/g.

[b] % difference = (range ÷ mean) × 100.

used are provided in Table 1; the laboratory numbers do not correspond to the order in which laboratories are listed above. Vitamins were also measured as part of a CEN interlaboratory comparison exercise. The following GMA and CEN laboratories reported results for vitamin B_{12} using microbiological methods: Danish Institute for Food and Veterinary Research, Søborg, Denmark; Swedish National Food Administration, Research and Development Department, Uppsala, Sweden; Covance; General Mills, Inc.; and Novartis.

Results and Discussion

The assignment of the certified values was based on the combination of results from at least two analytical methods at NIST and results from laboratories participating in an interlaboratory comparison exercise. Certified and reference mass fraction values for elements in SRM 3280 are provided in Table 3. Certified values are those for which NIST has the highest confidence in the accuracy of the data, in that all known or suspected sources of bias have been investigated or taken into account. Reference values are noncertified values that are the best estimate of the true values based on available data; estimates of measurement uncertainty may not include all sources of uncertainty or may reflect lack of statistical agreement among methods. The values provided in Table 3 are shown with an expanded uncertainty, *U*, calculated as:

$$U = ku_c$$

where u_c is the combined standard uncertainty and *k* is a coverage factor, similar to Student's *t*, chosen to provide an approximately 95% confidence interval for *U*. Information for relating this information to measurements obtained by an analyst using the SRM as a control material is provided in ref. 15.

Prior to making certification measurements, the homogeneity of the elements was assessed at NIST by using XRF spectrometry analysis. From each of 25 bottles, two sets of eight tablets were ground, and one briquette was pressed from 3.0 g of the ground material resulting from each set of eight tablets. Each briquette was measured twice, once on each side, for a total of four measurements/set of eight tablets. The analysis of variance results indicated that at the 95% confidence level and for each element, one to four bottles exhibited a statistically significant within-bottle variance among the four replicate measurements. The findings for bottle-to-bottle variance indicated that at the 95% confidence level, zero to four bottles exhibited a statistically significant difference from the rest of the population. The material failed the *F*-test for nearly every element; only Al, Cu, and Se passed the *F*-test and had *P* values that indicated homogeneous distribution within the material. All other elements exhibited significantly greater variability among bottles than within a bottle by factors ranging from 2.0 to 26.4. For comparison, the RSD of the data for each element can be taken into account. Nine elements (Al, Ca, Cu, Fe, P, S, Si, Sr, and Zn) had RSDs ≤2%. Another nine elements (Cl, Cr, I, K, Mg, Mn, Mo, Ni, and Se) had RSDs between 2 and 5%. Only Ti and Sn had RSDs >5%. Ti is found in the protective coating on the tablets and is of no concern for product quality analyses. Because the coating is waxy, it is prone to sticking on the ceramic parts of the mortar and pestle. Sn is the lowest mass fraction and least sensitive element measured in the XRF spectrometry test. Overall, it was concluded that sets of eight tablets may be insufficient test material because material heterogeneity is significant.

To assess the tablet-to-tablet variability, the USDA Food Composition Methods Development Laboratory analyzed two individual tablets from each of six bottles for an evaluation of between-tablet variability. Results are provided in Table 2. While the means of these 12 values agreed with the certified values, there can be a 15 to 25% difference among individual tablets. Consequently, recommendations for use state that 15 to 30 tablets from a bottle should be ground prior to removing a test portion for analysis.

SRM 3280 can be somewhat difficult to digest for analysis. It is the practice of analysts in some fields to filter out the undigested residue and analyze the clear solution. The dietary supplement industry might do this, for example, because the result obtained would be more representative of the concentration of elements that are bioavailable. However, from an analytical accuracy standpoint in which we want to establish the true concentration in the material, such results are considered biased. Two samples of powder were digested at a low temperature in nitric and hydrofluoric acids, and a residue remained. The residue was analyzed by XRF spectrometry and was found to contain Ca, Fe, Mg, P, K, and Ti. There were also lower levels of Al, Cl, Cr, Cu, Mn, Si, S, and Zn. For nutrient elements, product labels

Table 3. Certified (bold) and reference (normal typeface) mass fraction values, expanded uncertainties (*U*), coverage factors (*k*), individual method means, SDs, and RSDs of *n* measurements of elements in SRM 3280 used for value assignment. Values are reported on a dry-mass basis in mass fraction units of µg/g except as indicated in footnotes

Analyte	Certified and Reference values, µg/g	*k*	ICP/OES				ICP-MS[c]				PGAA				INAA				RNAA				XRF spectrometry				GMA mean
			Mean	SD	*n*	RSD, %	Mean	SD	*n*	RSD, %	Mean	SD	*n*	RSD, %	Mean	SD	*n*	RSD, %	Mean	SD	*n*	RSD, %	Mean	SD	*n*	RSD, %	
Antimony	0.159 ± 0.008	2.30													0.159	0.011	9	6.9									
Arsenic	**0.132 ± 0.044**	2.00					0.155	0.013	7	8.3									0.110	0.002	5	1.5					
Boron[a]	**0.141 ± 0.007**	2.36					0.137	0.007	6	4.9	0.145	0.004	10	2.4													
Cadmium[b]	**80.15 ± 0.86**	2.03					80.2	1.2	10	1.4																	
Calcium[a]	**110.7 ± 5.3**	2.45													111	3	8	2.6					113.93	0.24	15	0.2	107
Chloride[a]	**53.0 ± 2.3**	2.45									53.3	2.3	10	4.4													52.8
Chromium	**93.7 ± 2.7**	2. 06													0.091	0.002	9	2.0					0.0959	0.0009	15	0.9	
Cobalt	0.81 ± 0.01	2.22													0.813	0.017	9	2.1									
Copper[a]	**1.40 ± 0.17**	2.12	1.39	0.05	12	3.8					1.437	0.071	10	4.9	1.520	0.049	8	3.2									1.25
Iodine[a]	**0.1327 ± 0.0066**	2.23					0.13	0.01	6	8.6					0.134	0.010	8	7.6									
Iron[a]	**12.35 ± 0.91**	3.18									12.30	0.24	10	2.0	12.6	0.25	8	2.0					12.7	0.10	15	0.8	11.8
Lanthanum	0.70 ± 0.01	2.23													0.705	0.016	9	2.2									
Lead	**0.2727 ± 0.0024**	2.14					0.273	0.002	6	0.7																	
Magnesium[a]	**67.8 ± 4.0**	2.31													67.1	2.4	8	3.6					71.1	0.48	15	0.7	65.3
Maganese[a]	**1.44 ± 0.11**	2.57													1.491	0.027	8	1.8					1.486	0.020	15	1.3	1.35
Molybdenum	**70.7 ± 4.5**	2.57	68.9	4.1	11	6.0									69.4	7.0	8	10.0					73.9	1.99	15	2.7	
Nickel	**8.43 ± 0.30**	2.00	8.53	0.69	7	8.0	8.33	0.69	7	8.3																	
Phosphorus[a]	**75.7 ± 3.2**	2.16	73.4	1.3	12	1.8																	78.29	0.36	15	0.5	75.4
Potassium[a]	**53.1 ± 7.0**	2.02	51.5	2.1	12	4.1					59.2	2.6	10	4.4									47.2	0.64	15	1.4	54.6
Selenium	**17.42 ± 0.45**	2.00					17.3	1.3	7	7.3					17.6	1.1	7	6.1									
Silicon	2010 ± 10	2.00																					2006	10	15	0.5	
Sodium	330 ± 20	2.36													327	24	8	7.4									
Strontium	29.8 ± 0.2	2.00																					29.8	0.19	15	0.6	
Tin	11.1 ± 0.9	2.56					11.1	0.83	10	7.5																	
Titanium	5400 ± 300	2.25									5388	376	10	7.0													
Vanadium	8 ± 2	2.00	6.6	0.5	11	7.4									9.8	0.76	8	7.8									
Zinc[a]	**10.15 ± 0.81**	2.00	10.80	0.11	12	10									10.2	0.19	9	1.8									9.40

[a] mg/g.

[b] ng/g

[c] Lead and cadmium were measured by isotope dilution ICP/MS.

are considered accurate if analytical results are within 120% of the label value; therefore, for labeling purposes, it may not be critical for a manufacturer to account for the content of this residue. But for situations in which analytical data will be used to estimate intake or establish public health guidelines, for example, it is important to completely digest the material to obtain an unbiased result.

Individual elements often have their own analytical requirements, and some cannot be measured simultaneously even though it would be convenient. Pb analysis is given here as an example. In Exercise G of the Dietary Supplement Laboratory Quality Assurance Program (DSQAP), SRM 3280 was distributed to participants for the analysis of Pb (16). Pb usually goes into solution easily, but digestion with hydrochloric acid may form a $PbCl_2$ precipitate, making it difficult to get all of the Pb into solution. Another factor to remember when digesting SRM 3280 is that there is a high level of Cl (53.0 mg/g) in the material itself. If there is any precipitation at all, Pb will be lost. The solution may look clear, but all of the chlorine needs to be driven off to accurately determine Pb. This can be achieved by repeatedly taking a sample solution to near dryness using nitric acid. Digestion with nitric acid (alone or as a mixture with hydrofluoric acid) is recommended. Also of note in the DSQAP exercise, the analytical method that appeared to give the most difficulty was ICP-OES. Although many laboratories try to measure Pb by ICP-OES, the sensitivity for Pb is low, making it difficult to measure samples in which concentrations may be near the LOD. If using ICP-OES as the analytical method for determination of Pb, it is important to prepare sufficient procedural reagent blanks along with test samples to accurately determine LODs.

Another element that is prone to analytical difficulties is Cd. Cd occurs at low levels and is often measured using ICP/MS. However, the presence of In, Mo, Pd, Sn, or Zr can interfere with measurement of Cd. The formulation for SRM 3280 includes Mo and Sn. If these interferences are not removed, results will be biased. NIST has developed a cleanup procedure using a thiourea SPE cartridge, precipitation, and strong anion-exchange SPE prior to analysis by ID-ICP/MS as described by Murphy et al. (17). NIST considers ID-ICP/MS to be a primary method of analysis and allows certification based on data from this method alone (with confirmatory analysis using a second technique). Confirmatory analyses using collision cell ICP/MS and sector field ICP/MS gave consistent results.

Although INAA is often used for measurement of As, its determinations may suffer from interference or poor signal to background ratio at lower levels and in the presence of Na, Br, and P. For this reason, RNAA is used to eliminate the interferences and improve detection limits, as described by Paul (18). As was measured in SRM 3280 using both RNAA and ICP/MS. Measurement of As by ICP/MS yielded a value of 152.5 ± 6 μg/kg (mean ± U) compared with the RNAA value of 110 ± 2 μg/kg. The reason for the discrepancy remains unidentified. Although the samples appeared to dissolve completely as evidenced by a clear solution, it is possible that the nitric/perchloric acid dissolution used for RNAA, though effective on biological materials, may not have given complete dissolution of the multivitamin SRM, which contains a large amount of inorganic matter. The procedure for the ICP/MS determinations used a mixture of nitric and hydrofluoric acids with a microwave digestion. The addition of hydrofluoric acid to the digestion procedure used for RNAA did not correct the problem. Because the source of the bias could not be identified, the certified value was calculated as the equally weighted mean of the two sets of data, and the uncertainty is increased because of the bias.

Vitamin B_{12} is often measured using a microbiological assay because even fortified levels are below the LOD for most of the instrumental methods used in vitamin analyses. Surface plasmon resonance is able to measure vitamin B_{12}, but a specific instrument and vitamin B_{12} kit are required for this. Many of the water-soluble vitamins can be measured in a single chromatographic analysis, often with absorbance detection, but vitamin B_{12} levels are typically too low. LC with an MS or MS/MS detector may be sensitive enough, but without a stable isotope of vitamin B_{12} for use as an IS, results are expected to be unacceptably variable (12). Because vitamin B_{12} consists of a deprotonated corrinoid ring with a Co ion bound at its center, it is possible to use a very sensitive elemental analysis technique like ICP/MS to make this measurement, monitoring the mass of Co. The vitamin B_{12} must be chromatographically resolved from other water-soluble, Co-containing components in the SRM. Some Co is extracted during sample preparation (ultrasonication in water), but the extraction is not quantitative, and a mass balance cannot be performed to see whether the sum of the LC-ICP/MS results for elemental Co and vitamin B_{12} agrees with the result for total Co as determined by INAA. The result for vitamin B_{12} obtained by LC-ICP/MS (4.57 mg/kg, one SD = 0.52 mg/kg on a dry-mass basis) does agree with the value that was obtained by two groups of collaborating laboratories using microbiological methods (4.30 mg/kg with an SD of 1.75 mg/kg, and 5.47 mg/kg with an SD of 0.14 mg/kg, respectively). The certified value for vitamin B_{12} was calculated as an equally weighted combination of these three data sets, and is 4.8 ± 1.0 mg/kg, U, with k = 2.00.

I supplementation through iodized salt programs and dietary supplements has become quite important in combating I deficiency in world populations. I levels in supplements can often vary considerably from the declared content; therefore, the accurate measurement of I in supplement materials is extremely important. The measurement of I, especially at lower levels, can be challenging, and relatively poor results have been obtained in interlaboratory studies. I is often measured by an automated titrimetry method based on the classic Sandell-Kolthoff reaction. For the determination of low concentrations of I in food and supplement materials, sensitive and specific analytical methods such as ICP/MS and INAA are advantageous. Although ID-ICP/MS can be used for the determination of I, the addition of a ^{129}I radioactive isotopic spike requires special precautions. Alternatively, the use of ICP/MS with the method of standard additions, which was used here, is relatively straightforward. In both approaches, the challenge is to minimize the ICP/MS instrument background and sample-to-sample memory effects through the use of neutral to alkaline washout media. INAA determination of I is very sensitive. However, the activated isotope ^{128}I, which is measured at a γ-ray energy of 442.90 KeV, has an extremely short half-life (24.99 min). It is therefore challenging to implement the measurement process once the activated sample exits the reactor. In this procedure, two counting cycles were used to provide concordance of measurements. Measurement of I in SRM 3280 by standard additions ICP/MS yielded a value of 0.132 ± 0.012 mg/g (mean

± *U*) compared with the INAA value of 0.133 ± 0.007 mg/g. The certified value for I was calculated as an equally weighted combination of these two data sets, and is 0.1327 ± 0.0066 mg/g, *U*, with $k = 2.23$.

SRM 3280 joins a number of dietary supplement materials available from NIST. Many of the materials are botanicals with values assigned for active or marker compounds and toxic elements. Several materials are currently in preparation to address more elemental needs of the industry and analytical communities, including a Ca tablet and iodized salt.

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