National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 1577c

Bovine Liver

Standard Reference Material (SRM) 1577c consists of liver tissue derived from healthy steers. The material was collected and prepared under strict protocols designed to preserve the original composition, and to minimize contamination. SRM 1577c is primarily intended for use in evaluating the accuracy of analytical methods for selected elements and 25-hydroxyvitamin D_3 in animal tissues and other biological materials. A unit of the SRM consists of one bottle containing 20 g of freeze-dried liver powder.

Certified Mass Fraction Values: Certified mass fraction values for elements are provided in Table 1. A certified mass fraction value for 25-hydroxyvitamin D_3 is provided in Table 2. The certified values for elements are based on results from either a primary analytical technique carried out at NIST, or the combined results from two or more chemically independent analytical techniques obtained at NIST and collaborating expert laboratories [1]. The certified value for 25-hydroxyvitamin D_3 is based on the combined results from NIST and collaborating expert laboratories. A NIST certified value is a value for which NIST has the highest confidence in its accuracy in that all known or suspected sources of bias have been investigated or taken into account [1].

Reference Values: Reference mass fraction values for additional elements are provided in Table 3. Reference values are non-certified values that are the best estimates of the true values. However, the values do not meet the 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 analytical methods [1].

Information Values: Information mass fraction values for additional elements are provided in Table 4. An information value is considered to be a value that will be of interest and use to the SRM user, but for which insufficient information is available to assess adequately the uncertainty associated with the value, or is a value derived from a limited number of analyses [1]. Information values cannot be used to establish metrological traceability.

Expiration of Certification: The certification of **SRM 1577c** is valid, within the measurement uncertainty specified, until **01 October 2028**, provided the SRM is handled and stored in accordance with the instructions given in this certificate (see "Instructions for Handling, Storage, and Use"). The certification is nullified if the SRM is damaged, contaminated, or otherwise modified.

Maintenance of SRM Certification: NIST will monitor this SRM over the period of its certification. If substantive technical changes occur that affect the certification before the expiration of this certificate, NIST will notify the purchaser. Registration (see attached sheet or register online) will facilitate notification.

Coordination of the investigations and technical measurements leading to the certification of elements was under the leadership of R. Zeisler, formerly of NIST. Coordination of the technical measurements leading to the certification of 25-hydroxyvitamin D_3 was performed by M.M. Phillips of the NIST Chemical Sciences Division and J. Roseland and K. Patterson of the US Department of Agriculture (USDA, Beltsville, MD).

Consultation on the statistical design of the experimental work and evaluation of the data was provided by S.D. Leigh, formerly of NIST, and J.H. Yen of the NIST Statistical Engineering Division.

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

Carlos A. Gonzalez, Chief
Chemical Sciences Division

Gaithersburg, MD 20899
Certificate Issue Date: 09 October 2018
Certificate Revision History on Last Page

Steven J. Choquette, Director
Office of Reference Materials

Collection and preparation of SRM 1577c were performed by M.P. Cronise and C.N. Fales of the NIST Office of Reference Materials and E.A. Mackey, R.O. Spatz, and R. Zeisler of the NIST Chemical Sciences Division. The bovine liver material was collected at Texas A&M University (College Station, TX) with the assistance of W.D. James of the Center for Chemical Characterization and Analysis and R.R. Riley of the Rosenthal Meat Science and Technology Center.

Technical measurements were performed by C.Q. Burdette, S.J. Christopher, R.R. Greenberg, S.E. Long, E.A. Mackey, K.E. Murphy, B.J. Porter, S.A. Rabb, R.O. Spatz, B.E. Tomlin, L.J. Wood, L.L. Yu, and R. Zeisler of the NIST Chemical Sciences Division. The following collaborating laboratories and analysts contributed to value assignment for elements: C. Xiao, B. Ni, and W. Tian of China Institute of Atomic Energy (Beijing, China); J. Che and L.-W. Hu of Massachusetts Institute of Technology, Nuclear Reactor Laboratory (Cambridge, MA); J. Kučera of Nuclear Physics Institute ASCR (Řež, Czech Republic); W.D. James of Department of Chemistry and R.J. Taylor of the College of Veterinary Medicine at Texas A&M University (College Station, TX); C.S. Nomura and P.V. Oliveira of University of São Paulo, Institute of Chemistry (São Paulo, Brazil); and J. Harnly and E. Greene of USDA Beltsville Agricultural Research Center, Human Nutrition Research Center (Beltsville, MD). The following collaborating laboratories participated in a USDA interlaboratory comparsion and contributed to value assignment for 25-hydroxyvitamin D_3 [2]: Covance Laboratories, Inc. (Madison, WI); Health Canada (Longueuil, QC, Canada); Heartland Laboratories (Ames, IA); Technical University of Denmark (Kongens Lyngby, Denmark).

Support for assignment of the value for 25-hydroxyvitamin D_3 was provided by National Institutes of Health, Office of Dietary Supplements (NIH-ODS). Technical consultation was provided by J.M. Betz (NIH-ODS).

NOTICE TO USERS: SRM 1577c IS INTENDED FOR RESEARCH USE; NOT FOR HUMAN CONSUMPTION.

INSTRUCTIONS FOR HANDLING, STORAGE, AND USE

Handling: This material was derived from healthy steers. These animals were inspected by a Veterinary Medical Officer and did not show signs of infectious, contagious, and/or communicable disease. Normal caution and care should be exercised during handling and use of the material. Users should be aware of sources of contamination. To avoid contamination a Class 100 clean-air environment is recommended.

Storage: The material should be stored in its original container at room temperature (10 °C to 30 °C). SRM 1577c should not be exposed to intense sources of radiation, including ultraviolet light from lamps or sunlight.

Use: Prior to removal of test portions for analysis, the contents of the bottle should be mixed. For elemental analysis, the recommended minimum sample size is 100 mg; see "Homogeneity Assessment" below. For determination of 25-hydroxyvitamin D_3, the recommended minimum sample size is 2 g. The mass fractions of elements in SRM 1577c are reported on a dry-mass basis. Desiccator drying over $CaSO_4$ (e.g., Drierite) to stable mass (approximately 10 days) is recommended prior to elemental analysis.

PREPARATION AND ANALYSIS[(1)]

Sample Collection and Preparation: The liver tissue was collected and processed under observation of principles for "true and representative" sampling as documented in the protocols for human and marine mammal tissues of the National Biomonitoring Specimen Bank [3]. The liver tissue was harvested from 31 steers that were slaughtered at Texas A&M University College of Veterinary Medicine. The supplier of this material has reported that this material was produced under sanitary conditions and was derived from clinically healthy animals. The animals were slaughtered for the purpose of teaching bovine anatomy and how to butcher. The meat from these animals was prepared for retail under the supervision of a State of Texas meat inspector to ascertain the health of the animals. The livers were excised whole, placed on a clean Teflon sheet, and inspected. Each liver was rinsed with HPLC-grade water to remove excess blood, bile, and any other extraneous material. The outer membrane and major blood vessels were removed with titanium blade knives, and the tissue was cut into portions of approximately 10 cm^3; 120 kg of fresh tissue was obtained from this process, frozen in clean Teflon bags, and then shipped to NIST. The tissue was thawed and homogenized at NIST with a food processor equipped with titanium blades. The resulting paste was poured into glass trays, frozen, and lyophilized. The dry material was blended again in the food processor before being jet-milled. The resulting fine powder was radiation sterilized and bottled.

[(1)]Certain commercial equipment, instruments or materials are identified in this certificate 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.

Homogeneity Assessment: The homogeneity of elements in SRM 1577c was assessed by analyzing test portions of approximately 100 mg with high-precision instrumental neutron activation analysis (INAA). Twelve bottles were randomly selected from the lot and two 100 mg test portions were taken for INAA from different locations in each bottle. The results for all elements reported by INAA (see Tables 1 and 3) did not reveal any significant components of uncertainty due to heterogeneity; therefore the recommended minimum sample size for elemental analysis is 100 mg. The values and uncertainties reported in this Certificate for elements are valid for a 100 mg minimum sample size. Analysis of smaller amounts may be subject to additional uncertainty due to heterogeneity.

Analyses with solid-sampling graphite furnace atomic absorption spectrometry (SS-GFASS) using test portions in the range of 20 µg to 70 µg showed homogeneity for distribution of Cd, Cu, Pb, and Zn within the uncertainty of the method. For 1 mg test portions, an uncertainty component from heterogeneity of 1 % to 2 % relative uncertainty was estimated from the experimental data for these elements.

The homogeneity of 25-hydroxyvitamin D_3 in SRM 1577c was assessed by analyzing test portions of 2 g to 3 g with isotope dilution (ID) with liquid chromatography (LC) and tandem mass spectrometry (MS/MS) detection. Analysis of variance at a 5 % significance level indicated homogeneity for distribution of 25-hydroxyvitamin D_3 within the uncertainty of the method.

Analytical Approach for Determination of Elements: All elements for which certified and reference values are provided were determined by using at least one of the following methods carried out at NIST: INAA, radiochemical neutron activation analysis (RNAA), prompt gamma activation analysis (PGAA), pre-concentration and pre-separation neutron activation analysis (PNAA), inductively coupled plasma mass spectrometry (ICP-MS), and ICP optical emission spectrometry (ICP-OES). NIST values for Cd, Pb, and Se were obtained by using isotope dilution (ID) ICP-MS, and for Hg by isotope dilution cold vapor (ID/CV) ICP-MS. The measurements were complemented by results provided by collaborating scientists from research laboratories using ICP-MS, ICP-OES, INAA, RNAA, and SS-GFAAS.

Analytical Approach for Determination of 25-Hydroxyvitamin D_3: The mass fraction of 25-hydroxyvitamin D_3 was measured in duplicate 2.0 g to 3.0 g test portions taken from each of ten bottles of SRM 1577c, and 25-hydroxyvitamin D_3-$^{13}C_5$ was added as an internal standard. Prior to extraction, the samples of SRM 1577c were incubated with lipase at 40 °C for 2 h to hydrolyze the fats. Ethanol containing butylated hydroxytoluene (BHT) and potassium carbonate was added to each sample, and the analytes and internal standards were extracted into hexane containing BHT by overnight stirring. The samples were centrifuged, the supernatants were decanted, and an additional aliquot of hexane containing BHT was added. Samples were extracted further by a combination of sonication and rotary mixing, then centrifuged, and the supernatants combined with those from the previous extraction. An additional cycle of sonication and rotary mixing was conducted, for a total of three extractions. The pooled organic layers were dried under nitrogen to approximately 40 mL and magnesium sulfate was added. Following vortex mixing and centrifugation, the organic layer was decanted and evaporated to dryness under nitrogen. The analytes were derivatized with 4-phenyl-1,2,4-triazoline-3,5-dione (PTAD) and reconstituted in a mixture of methanol and ethyl acetate for analysis by positive-ion mode LC-MS/MS. A gradient method with a water/methanol mobile phase and a pentafluorophenyl column were used for LC-MS/MS determination. 25-Hydroxyvitamin D_3+PTAD and 25-hydroxyvitamin D_3-$^{13}C_5$+PTAD were measured at transitions m/z 558 → m/z 298 and m/z 563 → m/z 298, respectively. Calibrants were prepared gravimetrically, at levels intended to approximate the level of the vitamin in the SRM following extraction. The purity of the neat 25-hydroxyvitamin D_3 calibrant material was determined at NIST using LC-absorbance, Karl Fischer titration, thermogravimetric analysis, and quantitative proton nuclear magnetic resonance spectroscopy. A single internal standard solution was used for the calibrants and samples.

Certified Values for Elements: Certified values were derived from the NIST analytical results and the results provided by collaborating laboratories. The uncertainty listed with each value is an expanded uncertainty, with coverage factor 2 (approximately 95 % confidence). The reporting follows the JCGM Guide [4-6]. The measurands are the total mass fraction of the elements listed in Table 1. The certified values are metrologically traceable to the SI-derived unit for mass fraction (expressed as percent, micrograms per kilogram, or milligrams per kilogram).

For each element, there is a NIST result with an uncertainty that is complete in terms of coverage of recognized sources of uncertainties. Except for the elements measured by a single NIST primary method, these results are combined with results with similarly complete uncertainties from collaborating laboratories, and in certain cases several results without complete uncertainties. The uncertainties of these results were augmented for probable bias on the basis of the differences among the results obtained by different methods [4].

Table 1. Certified Mass Fraction Values for Elements (Dry-Mass Basis) in SRM 1577c

Element	Mass Fraction (%)	Element	Mass Fraction (%)
Potassium (K)[A,a,d,F]	1.023 ± 0.064	Sulfur (S)[a,D,d,F]	0.749 ± 0.034
Sodium (Na)[A,a,d]	0.2033 ± 0.0064		
	Mass Fraction (mg/kg)		**Mass Fraction (µg/kg)**
Calcium (Ca)[A,a,D,d]	131 ± 10	Arsenic (As)[C]	19.6 ± 1.4
Cobalt (Co)[A,a,b]	0.300 ± 0.018	Cadmium (Cd)[C,E]	97.0[g] ± 1.4
Copper (Cu)[A,a,C,D,d]	275.2 ± 4.6	Chromium (Cr)[A]	53 ± 14
Iron (Fe)[A,a,D]	197.94 ± 0.65	Lead (Pb)[E]	62.8 ± 1.0
Magnesium (Mg)[A,a,D,d]	620 ± 42	Nickel (Ni)[B,b,c]	44.5 ± 9.2
Manganese (Mn)[A,a,b,D,d]	10.46 ± 0.47	Silver (Ag)[A,B,b,C]	5.9 ± 1.6
Molybdenum (Mo)[A,a,b,C,D,d]	3.30 ± 0.13	Strontium (Sr)[B,b,d]	95.3 ± 4.2
Selenium (Se)[A,E]	2.031 ± 0.045	Vanadium (V)[c,d,G]	8.17 ± 0.66
Zinc (Zn)[A,a,D,E,h]	181.1 ± 1.0		

NOTE: Analytical techniques used for assignment of certified values; capital letters indicate that the method was used by NIST.
[A,a] INAA
[B,b] ICP-MS
[C,c] RNAA
[D,d] ICP-OES
[E] ID ICP-MS
[F] PGAA
[G] Pre-concentration pre-separation PNAA
[h] SS-GFAAS
[g] Alternate statistical method [7].

Certified Value for 25-Hydroxyvitamin D_3: The certified value was derived from the NIST analytical results and the results provided by collaborating laboratories. The uncertainty provided with the value is an expanded uncertainty about the mean to cover the measurand with approximately 95 % confidence. The expanded uncertainty is calculated as $U = ku_c$, where the combined uncertainty u_c incorporates the observed difference between the results from the methods and their respective uncertainties, consistent with the JCGM Guide and with its Supplement 1, and k is a coverage factor corresponding to approximately 95 % confidence [6,8,9]. The measurand is the total mass fraction of 25-hydroxyvitamin D_3 listed in Table 2. The certified value is metrologically traceable to the SI-derived unit for mass fraction (expressed as nanograms per gram).

Table 2. Certified Mass Fraction Value for 25-Hydroxyvitamin D_3 in SRM 1577c

Element	Mass Fraction (ng/g)
25-Hydroxyvitamin D_3	13.7 ± 3.1

Reference Values: Reference values are based on results from one method carried out at NIST or at NIST and in several collaborating laboratories. The methods of combining the results of different methods from different laboratories were applied as above. These results do not fulfill the criteria for certification because they lack a full estimate of method bias. The reporting follows the JCGM Guide [6]. The measurands are the total mass fractions of the elements listed in Table 3 as determined by the methods indicated. The reference values are metrologically traceable to the SI-derived unit for mass fraction (expressed as percent, micrograms per kilogram, or milligrams per kilogram), as realized by the method(s) used.

Table 3. Reference Mass Fraction Values for Elements (Dry-Mass Basis) in SRM 1577c

Element	Mass Fraction (%)	Element	Mass Fraction (µg/kg)
Chlorine (Cl)[A,a]	0.287 ± 0.013	Antimony (Sb)[a,B]	3.13 ± 0.31
Hydrogen (H)[D]	7.35 ± 0.24	Cesium (Cs)[A,a]	21.7 ± 1.4
Nitrogen (N)[D]	10.30 ± 0.34	Mercury (Hg)[E]	5.36 ± 0.17
Phosphorus (P)[C,c]	1.175 ± 0.027		
	Mass Fraction (mg/kg)		
Rubidium (Rb)[A,a]	35.3 ± 1.1		

NOTE: Analytical techniques used for assignment of reference values; capital letters indicate that the method was used by NIST.
[A,a] INAA
[B] RNAA
[C,c] ICP-OES
[D] PGAA
[E] ID/CV ICP-MS

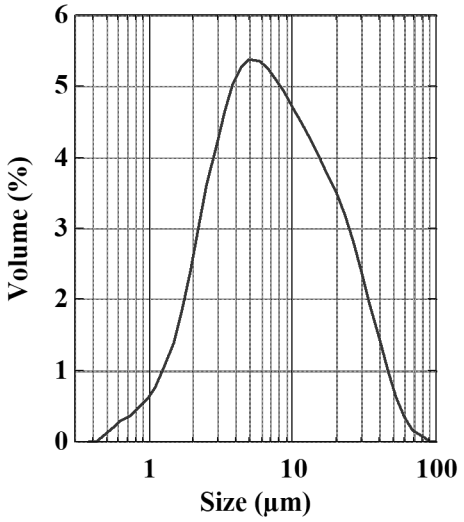
Information Values: Information values are given to assist users in the assays of two non-certified elements that may be of interest in method development and other investigations. These information values are based on results that did not allow complete assessment of all sources of uncertainty. The measurands are the total mass fractions of the elements listed in Table 4 as determined by the method indicated.

Table 4. Information Mass Fraction Values for Elements (Dry-Mass Basis) in SRM 1577c

Element	Mass Fraction
Lithium (Li)[a]	12 µg/kg
Silicon (Si)[a]	6 mg/kg

[a] ICP-OES by a laboratory collaborating with NIST

SUPPLEMENTAL INFORMATION



Particle size:
Figure 1. Particle size distributions in SRM 1577c determined in aqueous suspension via laser light scattering instrumentation (Malvern Mastersizer 2000). Calculated 10, 50, and 90 percentile particle sizes (percent volume of particles smaller than the value) for SRM 1577c are: $d_{0.1}$ = 2.31 µm, $d_{0.5}$ = 7.57 µm, $d_{0.9}$ = 28.5 µm. Uncertainties in these values are estimated at ± 10 % relative (2*s*).

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Certificate Revision History: 09 October 2018 (Addition of certified value for 25-hydroxyvitamin D_3; change of expiration date; editorial changes); 06 January 2016 (Editorial changes); 15 June 2009 (Original certificate date).

Users of this SRM should ensure that the Certificate of Analysis in their possession is current. This can be accomplished by contacting the SRM Program: telephone (301) 975-2200; fax (301) 948-3730; e-mail srminfo@nist.gov; or via the Internet at https://www.nist.gov/srm.