National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 1566b

Oyster Tissue

This Standard Reference Material (SRM) is intended primarily for use in evaluating analytical methods and instruments used for the determination of the mass fraction values of selected elements and proximates in marine bivalve tissue, foods, or similar materials. A unit of SRM 1566b contains approximately 25 g of freeze-dried oyster tissue.

Certified Mass Fraction Values of Constituent Elements: Certified values for elements and for methylmercury are provided in Table 1. 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 fully investigated or taken into account [1].

Reference Mass Fraction Values: Reference values for elemental mass fractions are provided in Table 2. Reference values for selected proximates, nitrogen, and total dietary fiber are provided in Table 3. Reference values are noncertified values that are the best estimates of the true values; however, the values do not meet NIST criteria for certification [1]. Such values 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.

Expiration of Certification: The certification of **SRM 1566b** is valid, within the measurement uncertainty specified, until **01 June 2030**, provided the SRM is handled and stored in accordance with instructions given in this certificate (see "Instructions for 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 technical measurements was performed by R.R. Greenberg, D.J. O'Kelly, S.A. Rabb K.E. Sharpless and S.A. Wise of the NIST Chemical Sciences Division.

Preparation of the oyster tissue material was performed by B.J. Porter of the NIST Chemical Sciences Division and M.P. Cronise, C.N. Fales, and D.G. Friend of the NIST Office of Reference Materials.

Statistical analysis of the data was performed by N.A. Heckert, H.-K. Liu, J.H. Yen, L.M. Gill, and M.S. Levenson of the NIST Statistical Engineering Division.

The NIST analysts and collaborating laboratories that participated in the characterization of this SRM are listed in Appendix A.

Support aspects involved with the issuance 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: 02 August 2019
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Steven J. Choquette, Director
Office of Reference Materials

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NOTICE AND WARNINGS TO USERS

SRM 1566B IS INTENDED FOR RESEARCH USE; NOT FOR HUMAN CONSUMPTION.

INSTRUCTIONS FOR STORAGE AND USE

Storage: The material should be kept in its original bottle, tightly closed, and stored in a desiccator over magnesium perchlorate, $Mg(ClO_4)_2$, at temperatures between 10 °C to 30 °C. It should **NOT** be exposed to intense sources of radiation, including ultraviolet light or sunlight.

Use: A minimum sample mass of 250 mg of material is necessary for values in this certificate to be valid within stated uncertainties. This amount of material should be on a dry-mass basis (see "Instructions for Drying"). The contents of the bottle should be shaken well before each use, and the bottle should be closed tightly **immediately** after use and stored as described above.

Instructions for Drying: Prior to removal of test portions for elemental analysis, the contents of the bottle should be thoroughly mixed. Before the mass determination, samples of SRM 1566b must be dried to constant mass by one of the following procedures:

1. Drying at room temperature for *at least* 5 d over $Mg(ClO_4)_2$ in a desiccator.
2. Vacuum drying at room temperature for *at least* 24 h at a pressure of approximately 30 Pa (0.2 mm Hg) using a cold trap.
3. Freeze drying for *at least* 5 d at a pressure of approximately 30 Pa (0.2 mm Hg).

The analyst should ascertain that the material has indeed reached constant mass. Although the above procedures have been generally sufficient, in a few instances the time needed to reach constant mass was longer than listed above. If the constituents of interest are volatile, a separate test portion of the oyster tissue should be removed from the bottle at the time of analysis and dried to determine the mass fraction values on a dry-mass basis.

SOURCE, PREPARATION, AND ANALYSIS[(1)]

Source and Preparation of Material: The oysters (*Crassostrea virginica*, an American Eastern oyster) used for the preparation of SRM 1566b were purchased from Bon Secour Fisheries, Inc. (Bon Secour, AL). The oysters were collected from the Gulf of Mexico, shucked, rinsed twice to remove sediment and shells, packed and sealed in polyethylene bags, and frozen. The frozen oysters and fluids were shipped in coolers containing dry ice to NIST. At NIST, the oysters and fluids were ground in a Robot-Coupe Vertical Cutter Mixer that was equipped with a stainless steel bowl and titanium blades. The oyster tissue was blended for 100 s into a slurry; approximately 5 kg of slurry was poured into each of 40 specially cleaned aluminum trays outfitted with temperature probes, and frozen at –20 °C. The trays were taken to a large freeze-drying facility at the Frederick Cancer Research and Development Center, Natural Products Group in Frederick, MD. The freeze-dryer's initial temperature was –45 °C and gradually increased to a temperature of 10 °C over a period of five days. The freeze-dried material was stored at –20 °C, then broken into smaller pieces, blended in the Robot-Coupe Mixer, jet milled, and homogenized in a V-blender for 30 min to 40 min. The material was radiation sterilized (^{60}Co) at Neutron Products, Inc. (Dickerson, MD), for approximately 5 h to an absorbed dose of 30 kGy and then aliquoted into amber bottles.

Description of Calculations Used in Certified Value Assignment

Sulfur: The certified mass fraction value of sulfur is the result of a single NIST method, thermal ionization mass spectrometry (TIMS), with confirmation by a second NIST method, prompt gamma activation analysis (PGAA). Its uncertainty is expressed as an expanded uncertainty, U, and is calculated as $U = ku_c$. The quantity u_c is the combined standard uncertainty, calculated according to the JCGM Guide [2], that accounts for the combined components of uncertainty for the method at one standard deviation. The coverage factor, k, is determined from the Student's t-distribution corresponding to five degrees of freedom and a 95 % confidence. Because of heterogeneity, the uncertainty associated with sulfur takes the form of a prediction interval.

Methylmercury: The results for methylmercury are expressed as milligrams per kilogram as mercury. The certified value is the mean of results from four different laboratory analyses of SRM 1566b using four different analytical methods. The expanded uncertainty in the certified value is equal to $U = ku_c$ where u_c is the combined standard uncertainty calculated according to the JCGM Guide [2] and k is the coverage factor. The value u_c is intended to represent, at the level of one standard deviation, the combined effect of all the uncertainties in the certified value.

[(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.

Here, u_c is given by the standard error of the mean of the four analyses. The coverage factor, k, is determined from the Student's t-distribution corresponding to three degrees of freedom and 95 % confidence.

All Other Elements: All other certified mass fraction values are weighted means of results from two or more analytical methods. For these certified values, the uncertainty is calculated as $U = ku_c + B$. The quantity u_c is the combined standard uncertainty calculated according to the JCGM Guide [2], which accounts for the combined effect of the within variance for all methods at one standard deviation. The coverage factor, k, is determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and 95 % confidence for each element. The term B is a bias adjustment for the difference between methods, which is the maximum difference between the certified value and the method means [3]. Because of heterogeneity, the uncertainty associated with calcium and thorium takes the form of a prediction interval.

Table 1. Certified Mass Fraction Values for Elements and Methylmercury[a]

Element	Mass Fraction (%)	Element	Mass Fraction (%)
Calcium (Ca)[b,c]	0.0838 ± 0.0020	Potassium (K)[c,d,e]	0.652 ± 0.009
Chlorine (Cl)[c,d]	0.514 ± 0.010	Sodium (Na)[c,f]	0.3297 ± 0.0053
Magnesium (Mg)[b,c]	0.1085 ± 0.0023	Sulfur (S)[d,g]	0.689 ± 0.014

Element	Mass Fraction (mg/kg)	Element	Mass Fraction (mg/kg)
Aluminum (Al)[c,h]	197.2 ± 6.0	Mercury (total) (Hg)[i,j]	0.0371 ± 0.0013
Arsenic (As)[c,k]	7.65 ± 0.65	Methylmercury (as mercury)[l,m,n,o]	0.0132 ± 0.0007
Cadmium (Cd)[p,q]	2.48 ± 0.08	Nickel (Ni)[e,k,p]	1.04 ± 0.09
Cobalt (Co)[c,p]	0.371 ± 0.009	Rubidium (Rb)[c,p]	3.26 ± 0.14
Copper (Cu)[c,p,q]	71.6 ± 1.6	Selenium (Se)[c,k]	2.06 ± 0.15
Iron (Fe)[c,e,r]	205.8 ± 6.8	Silver (Ag)[c,p]	0.666 ± 0.009
Lead (Pb)[b,p]	0.308 ± 0.009	Thorium (Th)[b,c]	0.0367 ± 0.0043
Manganese (Mn)[c,p]	18.5 ± 0.2	Vanadium (V)[c,p]	0.577 ± 0.023
		Zinc (Zn)[c,e]	1 424 ± 46

[a] The measurands are the mass fractions of the elements listed in Table 1. Metrological traceability is to the SI derived unit for mass fraction (expressed on a dry-mass basis as a percent or milligram per kilogram).

Analytical Methods:

[b] Isotope dilution inductively coupled plasma mass spectrometry (ID-ICP-MS) at NIST
[c] Neutron activation analysis (instrumental) (INAA) at NIST
[d] PGAA at NIST; confirmation method for S measurement only.
[e] ID-ICP-MS at National Research Council Canada (NRCC)
[f] Flame atomic emission spectrometry at NIST
[g] ID-TIMS at NIST
[h] ICP-MS at NRCC
[i] Cold vapor atomic absorption spectrometry (CVAA) at NIST
[j] Neutron activation analysis (radiochemical) (RNAA) at Jožef Stefan Institute (Ljubljana, Slovenia)
[k] Electrothermal atomic absorption spectrometry (AAS) at NRCC
[l] Gas chromatography with atomic emission detection (GC-AED) at NIST
[m] Gas chromatography with atomic fluorescence (GC-AFS) detection at Jožef Stefan Institute
[n] CVAA at Jožef Stefan Institute
[o] CVAA at Research Centre Jülich, (Jülich, Germany)
[p] ICP-MS at NIST
[q] RNAA at NIST
[r] Inductively coupled plasma optical emission spectrometry (ICP-OES) at NRCC

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Table 2. Reference Mass Fraction Values for Elements[a]

Element	Mass Fraction (%)
Nitrogen (N)[b]	7.6 ± 0.4

Element	Mass Fraction (mg/kg)	Element	Mass Fraction (mg/kg)
Antimony (Sb)[c]	0.011 ± 0.002	Strontium (Sr)[f]	6.8 ± 0.2
Barium (Ba)[d]	8.6 ± 0.3	Tin (Sn)[d]	0.031 ± 0.008
Boron (B)[b]	4.5 ± 1.9	Titanium (Ti)[g]	12.24 ± 0.39
Gold (Au)[e,f]	0.0106 ± 0.0028	Uranium (U)[d]	0.2550 ± 0.0014
Hydrogen (H)[b]	7.2 ± 0.4		

[a] The measurands are the mass fractions of the elements listed in Table 2 as determined by the indicated method or methods. Metrological traceability is to the SI derived unit for mass fraction (expressed on a dry-mass basis as a percent or milligram per kilogram), as realized by the method used.

Analytical Methods:
[b] PGAA at NIST
[c] RNAA at NIST
[d] ID-ICP-MS at NRCC
[e] INAA at NIST
[f] ICP-MS at NIST
[g] ICP-OES at NIST

Reference Mass Fraction Values of Selected Proximates, Nitrogen, and Total Dietary Fiber: Each reference value, expressed as a mass fraction of the material on an as-received or dry-mass basis, for a stated measurand is an equally weighted mean of results from the collaborating laboratories listed in Appendix A. Each of four laboratories analyzed one portion from each of three bottles of SRM 1566b using their routine methods. Determinations were performed on the material "as-received," with conversion of results to a dry-mass basis using moisture values determined by the four laboratories on separate subsamples taken from each of the three bottles. The uncertainty in the reference values is expressed as an expanded uncertainty, *U*, at the 95 % level of confidence, and is calculated according to the method described in the JCGM Guide [2]. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the combined effect of between-laboratory and within-laboratory components of uncertainty. The coverage factor, *k*, is determined from the Student's *t*-distribution corresponding to the appropriate associated degrees of freedom and 95 % confidence for each analyte. Analytical methodology information is provided in Table 4. The measurands are the constituents listed in Table 3 as determined by the methods used by the laboratories. Metrological traceability is to the SI derived unit for mass fraction (expressed as-received and dry-mass basis as percent), as realized by the method used.

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Table 3. Reference Mass Fraction Values of Selected Proximates, Nitrogen, and Total Dietary Fiber

Constituent	Mass Fraction as-received[a] (%)	Mass Fraction dry-mass basis[a] (%)
Moisture	4.6 ± 3.6	0 (by definition)
Solids	95.4 ± 3.6	100 (by definition)
Ash	3.87 ± 0.09	4.05 ± 0.15
Protein[b]	42.6 ± 1.3	44.7 ± 2.6
Protein Nitrogen[b]	6.82 ± 0.20	7.16 ± 0.42
Total Dietary Fiber	6.5 ± 1.6	6.8 ± 1.4

[a] Note that SRM 1566b was originally certified in 2001 and, at that time, reference and information values were provided for fatty acids. Those reference and information values are no longer valid as these compounds are not stable in this matrix for more than 10 y under these storage conditions.
[b] The protein mass fraction value was calculated from the nitrogen values reported by the laboratories using a conversion factor of 6.25. The value for protein is the mean of the individual protein calculations. If the mean nitrogen values above are used for calculation, the mean protein mass fraction values are 42.6 % and 44.7 % on as-received and dry-mass basis, respectively.

Table 4. Analytical Methods Used by Collaborating Laboratories for the Determination of Proximates, Nitrogen, and Total Dietary Fiber in SRM 1566b

Ash	mass after ignition in a muffle furnace
Moisture	mass loss after drying in a vacuum oven (3 laboratories) or forced-air oven (1 laboratory)
Protein nitrogen	Dumas (1 laboratory); modified Dumas (1 laboratory); Kjeldahl (2 laboratories)
Protein	calculated from nitrogen using a factor of 6.25
Solids	calculated; (sample mass – moisture)
Total dietary fiber	enzymatic gravimetry

REFERENCES

[1] May, W.; Parris, R.; Beck II, C.; Fassett, J.; Greenberg, R.; Guenther, F.; Kramer, G.; Wise, S.; Gills, T.; Colbert, J.; Gettings, R.; MacDonald, B.; *Definition of Terms and Modes Used at NIST for Value-Assignment of Reference Materials for Chemical Measurements*; NIST Special Publication 260-136 (2000); available at https://www.nist.gov/sites/default/files/documents/srm/SP260-136.PDF (accessed Jul 2019).

[2] JCGM 100:2008; *Evaluation of Measurement Data — Guide to the Expression of Uncertainty in Measurement* (GUM 1995 with Minor Corrections); Joint Committee for Guides in Metrology (2008); available at https://www.bipm.org/utils/common/documents/jcgm/JCGM_100_2008_E.pdf (accessed Jul 2019); see also Taylor, B.N.; Kuyatt, C.E.; *Guidelines for Evaluating and Expressing the Uncertainty of NIST Measurement Results*; NIST Technical Note 1297; U.S. Government Printing Office: Washington, DC (1994); available at https://www.nist.gov/pml/nist-technical-note-1297 (accessed Jul 2019).

[3] Schiller, S.; Eberhardt, K.; *Combining Data from Independent Chemical Analysis Methods*; Spectrochimica Acta, Vol. 46B, No. 12, pp. 1607–1613 (1991).

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Certificate Revision History: 02 August 2019 (Change of expiration date; editorial changes); 26 June 2017 (Corrected units for gold; editorial changes); 18 November 2015 (Added reference values for gold and titanium in Table 2; editorial changes); 07 February 2011 (Corrected the definition of ash in Table 4); 09 July 2010 (Removed reference and information concentration values of fatty acids and removed reference concentration values of caloric content, carbohydrates, and fat along with the corresponding analytical methods; changed the expiration date; editorial changes); 17 January 2001 (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.

Appendix A

NIST Analysts, Chemical Sciences Division

B. Buehler	J.L. Mann
T.A. Butler	J.L. Molloy
R. Demiralp	K.E. Murphy
M.S. Epstein	S.A. Rabb
M.E. Howard	M.S. Rearick
W.R. Kelly	S. Tutschku
R.M. Lindstrom	R.D. Vocke, Jr.
S.E. Long	R.P. Watson
E.A. Mackey	L.L. Yu

Collaborating Laboratories and Analysts for Elemental Determinations

Analytical Chemistry Division, National Research Council Canada (Ottawa, Canada): V. Boyko, J. Lam, S. Willie, and L. Yang
Jožef Stefan Institute, (Ljubljana, Slovenia): H. Akagi, V. Fajon, M. Horvat, K. Jereb, and M. Logar
Research Centre Jülich (Jülich, Germany): H. Emons and K. May

Collaborating Laboratories for Selected Proximates, Nitrogen, and Total Dietary Fiber Determinations

Covance Laboratories (Madison, WI)
Lancaster Laboratories (Lancaster, PA)
Medallion Laboratories (Minneapolis, MN)
Southern Testing and Research Laboratories (Wilson, NC)

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