National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 1570a

Trace Elements in Spinach Leaves

This Standard Reference Material (SRM) is intended primarily for use in evaluating the reliability of analytical methods for the determination of major, minor, and trace elements in botanical materials, agricultural food products, and materials of similar matrix. A unit of SRM 1570a consists of 60 g of finely powdered dried spinach leaves.

Certified Mass Fraction Values: Certified mass fraction values for selected constituent elements, reported on a dry-mass basis, 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 investigated or taken into account [1].

Reference Mass Fraction Values: Reference mass fraction values of constituent elements, reported on a dry-mass basis, are provided in Table 2. Reference values are noncertified values that are the best estimates of the true values based on available data; however, the values do not meet NIST criteria for certification [1] and are provided with associated uncertainties that may reflect only measurement reproducibility, may not include all sources of uncertainty, or may reflect a lack of sufficient statistical agreement among multiple analytical methods.

Information Mass Fraction Values: Information mass fraction values for additional constituent elements are provided in Table 3. A NIST information value is a value that may be of interest to the SRM user, but insufficient information is available to asses the uncertainty associated with the value, therefore no uncertainty is provided [1]. Values are reported on a dry-mass basis.

Expiration of Certification: The certification of **SRM 1570a** is valid, within the measurement uncertainty specified, until **31 August 2023**, provided the SRM is handled and stored in accordance with the 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 certified values before the expiration of this certificate, NIST will notify the purchaser. Registration (see attached sheet) will facilitate notification.

Coordination of analytical measurements for the characterization of this SRM was performed by D.A. Becker of the NIST Chemical Sciences Division. Revision of this certificate was coordinated by K.E. Murphy, D.J. O'Kelly, and L.J. Wood of the NIST Chemical Sciences Division.

Analytical measurements at NIST were performed by current and former staff at NIST, E.S. Beary, D.A. Becker, C.M. Beck II, M.S. Epstein, J.D. Fassett, K.M. Garrity, R.R. Greenberg, R.M. Lindstrom, E.A. Mackey, P. Morales, K.E. Murphy, P.J. Paulsen, B.J. Porter, T.A. Rush, R. Saraswati, J.M. Smeller, G.C. Turk, R.D. Vocke, Jr., R.L. Watters, Jr., and L.J. Wood.

Additional elemental analyses were performed by D.L. Anderson (Center for Food Safety and Applied Nutrition, U.S. Food and Drug Administration, College Park, MD), A.R. Byrne (Nuclear Chemistry Department, Jozef Stefan Institute, Ljubljana, Slovenia), and J. Kucera (Nuclear Physics Institute, Academy of Sciences of the Czech Republic, Rez, Czech Republic). Several elements were also measured in an International Atomic Energy Agency (IAEA, Vienna, Austria) interlaboratory comparison exercise.

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Statistical analysis of the experimental data was performed by W.F. Guthrie, S.B. Schiller, and L.M. Gill of the NIST Statistical Engineering Division.

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

INSTRUCTIONS FOR STORAGE AND USE

Storage: The material should be kept in its tightly closed original bottle and stored in the dark at a temperature between 10 °C and 30 °C. It should not be exposed to intense sources of radiation. Ideally, the bottle should be kept in a desiccator under the conditions indicated above. Spinach leaves have a tendency to rapidly bleach and to turn a tan or light brown color in the presence of visible light. By monitoring SRM 1570, the original SRM, it was determined that there is no evidence of any change in elemental mass fractions as a result of the color change.

Instructions for Use: The contents of a bottle should be thoroughly mixed by rotating and/or rolling before each use. Allow the contents to settle for 1 minute prior to opening to minimize the loss of fine dust particles. A minimum sample mass of 150 mg of the material, dried as described in the section (see "Instructions for Drying"), should be used to relate analytical determinations to the certified values on this certificate. In some cases, especially for volatile elements such as mercury, it is preferable to analyze samples from the bottle without drying, determine the moisture content on a separate sample from the same bottle taken at the same time, and convert the analytical results to a dry-mass basis.

Digestion procedures should be designed to avoid loss of volatile elements, such as arsenic and mercury. Digestion of the SRM in nitric and perchloric acids was found to be incomplete, with a small residue of siliceous material remaining. This residue must be considered an integral part of this SRM and should be dissolved with a small amount of hydrofluoric acid to obtain total dissolution. All certified values are based on the total dissolution.

Instructions for Drying: Samples of this SRM must be dried by one of the following two procedures in order for certified values to be valid:

1. Drying in a desiccator at room temperature (approximately 22 °C) for 120 h over fresh anhydrous magnesium perchlorate. The sample depth should not exceed 1 cm.

2. Freeze-drying for 24 h at a pressure of 13.3 Pa or lower and a shelf temperature of –5 °C or lower after having frozen the sample (not to exceed 1 cm in depth) at –40 °C or lower for at least 1 h. At the end of the 24 h period, samples should be placed immediately in a desiccator with fresh anhydrous magnesium perchlorate. Samples should be weighed after allowing a minimum of 4 h to establish temperature equilibrium.

Note: An approximate mass loss on drying of 3.5 % was observed for the measurements reported here. Vacuum drying at room temperature and oven drying at elevated temperatures have resulted in excessive mass losses and therefore are **NOT** recommended.

SOURCE, PREPARATION, AND ANALYSIS[(1)]

Source and Preparation of Material: The material (approximately 2270 kg) for this SRM was obtained from commercial supplier Oregon Freeze-Drying Corp. (Albany, OR). It consists of U.S. Grade A chopped frozen spinach. The material was thawed, placed in a ribbon mixer, thoroughly mixed, and blended. After mixing, the spinach was freeze-dried. The freeze-dried material was then ground in a stainless steel grinder and shipped to NIST. At NIST, the freeze-dried material was sieved through a polypropylene sieve having openings of 0.25 mm (equivalent to a U.S. Series 60 standard sieve). The sieved material was then jet milled and air classified to a particle size of approximately 75 μm (200 mesh). After mixing in a large blender, the spinach was irradiated with cobalt-60 radiation to a minimum absorbed dose of approximately 27.8 kGy for microbiological control and was 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.

Elemental Analysis: Value assignment of the mass fractions of the elements in SRM 1570a was based on the combination of measurements from two or more different analytical methods at NIST and collaborating laboratories. NIST and collaborating laboratories provided measurements by using colorimetry (COLOR), cold-vapor atomic absorption spectrometry (CVAAS), flow injection hydride generation atomic absorption spectrometry (FI-HGAAS), inductively coupled plasma optical emission spectrometry (ICP), isotope dilution inductively coupled plasma mass spectrometry (IDICPMS), isotope dilution thermal ionization mass spectrometry (IDTIMS), instrumental neutron activation analysis (INAA), laser-excited atomic fluorescence spectrometry (LEAFS), prompt gamma activation analysis (PGAA), and radiochemical neutron activation analysis (RNAA). Data from an IAEA interlaboratory comparison exercise were also used where available. A list of analytical methods used for measurement of each element is provided in Appendix A.

Homogeneity Assessment: Samples from randomly selected bottles of SRM 1570a were tested for homogeneity. No evidence of statistically significant inhomogeneity was observed.

Certified Mass Fraction Values: Certified mass fraction values are weighted means of results from two or more different analytical methods combined using the DerSimonian-Laird procedure [2]. The uncertainty in the certified mass fraction values was calculated according to the methods in Supplement 1 to the ISO/JCGM Guide [3] and the results are consistent with the methods given in the ISO/JCGM Guide [4]. The uncertainty of each certified value is expressed as $U = ku_c$. The quantity u_c is the combined standard uncertainty, which accounts for the combined effect of within-method uncertainty from all potential sources and any bias between methods at the level of one standard deviation. The coverage factor, k, is determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and a 95 % level of confidence for each analyte.

The certified values are reported on a dry-mass basis. For certified values to be valid, the material must be dried according to the instructions provided above. The measurand is the mass fraction of the element. The certified values are metrologically traceable to the SI unit of milligram per kilogram, expressed as percent.

Table 1. Certified Mass Fraction Values (Dry-Mass Basis) of Constituent Elements

Element	Mass Fraction (%)	Coverage Factor (k)
Calcium	1.526 ± 0.066	2.0299
Phosphorus	0.5187 ± 0.0067	1.9772
Potassium	2.900 ± 0.026	2.3226
Sodium	1.821 ± 0.023	1.9943

Element	Mass Fraction (mg/kg)	Coverage Factor (k)	Element	Mass Fraction (mg/kg)	Coverage Factor (k)
Aluminum	310 ± 15	2.0102	Mercury	0.0297 ± 0.0021	2.0492
Arsenic	0.068 ± 0.012	2.0561	Nickel	2.142 ± 0.058	1.9709
Boron	37.7 ± 1.2	2.0127	Selenium	0.1152 ± 0.0043	2.0371
Cadmium	2.876 ± 0.058	2.0464	Strontium	55.54 ± 0.50	2.5029
Cobalt	0.393 ± 0.030	2.0320	Thorium	0.0480 ± 0.0017	2.0053
Copper	12.22 ± 0.86	2.0488	Vanadium	0.568 ± 0.017	2.4938
Manganese	76.0 ± 1.2	1.9855	Zinc	82.3 ± 3.9	2.0136

Reference Mass Fraction Values: Each reference value, expressed as a mass fraction on a dry-mass basis, is an equally weighted mean of results provided by NIST and/or collaborating laboratories. The uncertainty in the reference mass fraction values is calculated as $U = ku_c$. The quantity u_c is the combined standard uncertainty calculated according to the ISO/JCGM Guide [3,4], which accounts for the combined effect of within-method uncertainty and for any bias between methods at the level of one standard deviation. The coverage factor, k, is determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and a 95 % level of confidence for each analyte.

These reference values are reported on a dry-mass basis. In order for these reference values to be valid, the material must be dried according to the instructions provided above. The measurand is the mass fraction of the element as determined by the method indicated. The reference values are metrologically traceable to the SI unit of milligram per kilogram, expressed as percent.

Table 2. Reference Mass Fraction Values (Dry-Mass Basis) of Constituent Elements

Element	Mass Fraction (%)
Nitrogen (Total)	6.06 ± 0.20

Element	Mass Fraction (mg/kg)	Element	Mass Fraction (mg/kg)
Europium	0.0055 ± 0.0010	Rubidium	12.7 ± 1.6
Scandium	0.0055 ± 0.0006	Uranium	0.155 ± 0.023

Information Mass Fraction Values: Each information value, expressed as a mass fraction on a dry-mass basis, is an equally weighted mean of results provided by NIST and/or collaborating laboratories. Insufficient information is available to asses the uncertainty associated with the value, therefore no uncertainty is provided.

Table 3. Information Mass Fraction Values (Dry-Mass Basis) of Constituent Elements

Element	Mass Fraction (%)
Magnesium	0.9
Sulfur	0.5

Element	Mass Fraction (mg/kg)
Lead	0.2

REFERENCE

[1] May, W.; Parris, R.; Beck, C.; Fassett, J.; Greenberg, R.; Guenther, F.; Kramer, G.; Wise, S.; Gills, T.; Colbert, J.; Gettings, R.; MacDonald, B.; *Definitions of Terms and Modes Used at NIST for Value-Assignment of Reference Materials for Chemical Measurements*; NIST Special Publication 260–136, U.S. Government Printing Office: Washington, DC (2000); available at http://www.nist.gov/srm/upload/SP260-136.PDF (accessed Feb 2014).

[2] DerSimonian, R; Laird, N.; *Meta-Analysis in Clinical Trials*; Controlled Clin. Trials, Vol. 7, pp. 177–188 (1986).

[3] JCGM 101:2008; *Evaluation of Measurement Data – Supplement 1 to the "Guide to the Expression of Uncertainty in Measurement" - Propagation of Distributions using a Monte Carlo Method*; JCGM (2008); available at http://www.bipm.org/utils/common/documents/jcgm/JCGM_101_2008_E.pdf (accessed Feb 2014).

[4] 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 http://www.bipm.org/utils/common/documents/jcgm/JCGM_100_2008_E.pdf (accessed Feb 2014); 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 http://www.nist.gov/pml/pubs/index.cfm (accessed Feb 2014).

Certificate Revision History: **25 February 2014** (Extension of certification period; updated certified values and uncertainties; removed reference and information values for proximates, calories, total dietary fiber, fatty acids, and nitrogen (organic and protein) due to instability of organic constituents; editorial changes); **08 October 2008** (Update of expiration date; editorial changes); **31 August 2001** (This technical revision reports the addition of reference and information values for proximates, calories, total dietary fiber, and fatty acids and a change from non-certified to reference and information values for several inorganic constituents); **15 July 1996** (Editorial changes); **20 October 1994** (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 http://www.nist.gov/srm.

APPENDIX A

Methods Used in Elemental Determinations

Element	Method Code	Element	Method Code
Aluminum (Al)	ICP INAA	Nitrogen (N)	PGAA
Arsenic (As)	FI-HGAAS RNAA	Phosphorus (P)	COLOR ICP
Boron (B)	IDICPMS PGAA	Potassium (K)	IDTIMS INAA
Cadmium (Cd)	IDICPMS PGAA RNAA	Rubidium (Rb)	IAEA INAA
Calcium (Ca)	IDTIMS INAA	Scandium (Sc)	IAEA INAA
Cobalt (Co)	INAA RNAA	Selenium (Se)	FI-HGAAS INAA RNAA
Copper (Cu)	ICP RNAA	Sodium (Na)	PGAA INAA
Europium (Eu)	IAEA INAA	Strontium (Sr)	IDTIMS INAA
Lead (Pb)	IAEA IDICPMS	Sulfur (S)	PGAA IAEA
Magnesium (Mg)	IDICPMS	Thorium (Th)	INAA RNAA
Manganese (Mn)	INAA LEAFS	Uranium (U)	RNAA
Mercury (Hg)	CVAAS RNAA	Vanadium (V)	IDTIMS INAA
Nickel (Ni)	IDICPMS RNAA	Zinc (Zn)	ICP INAA

Key:

COLOR:	Colorimetry
CVAAS:	Cold-vapor atomic absorption spectrometry
FI-HGAAS:	Flow injection hydride generation atomic absorption spectrometry
IAEA:	Various methods from an IAEA interlaboratory comparison exercise.
ICP:	Inductively coupled plasma optical emission spectrometry
IDICPMS:	Isotope dilution inductively coupled plasma mass spectrometry
IDTIMS:	Isotope dilution thermal ionization mass spectrometry
INAA:	Instrumental neutron activation analysis
LEAFS:	Laser-excited atomic fluorescence spectrometry
PGAA:	Prompt gamma activation analysis
RNAA:	Radiochemical neutron activation analysis