



National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material[®] 1515

Apple Leaves

This Standard Reference Material (SRM) is intended primarily for use in validating analytical methods for the determination of major, minor, and trace elements in botanical materials, agricultural food products, and similar materials. This SRM can be used for quality assurance when assigning values to in-house control materials. A unit of SRM 1515 consists of 50 grams of dried apple leaves of the Golden Delicious and Rome varieties.

Certified Mass Fraction Values: Certified mass fraction values for elements in SRM 1515, 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]. Analyses for value assignment were performed by NIST and collaborating laboratories. Certified values were calculated using the results from a single definitive method or the weighted means of results from two or more different analytical methods combined using the DerSimonian-Laird procedure [2-4]. The associated uncertainties are expressed at an approximately 95 % level of confidence [5-6].

Information Mass Fraction Values: Information mass fraction values for several elements are provided in Table 2 as additional information on the composition of the material. A NIST information value is a value that may be of interest to the SRM user, but insufficient information is available to assess the uncertainty associated with the value, and therefore no uncertainty is provided [1]. Values are reported on a dry-mass basis in mass fraction units [7]. Information values cannot be used to establish metrological traceability.

Expiration of Certification: The certification of **SRM 1515** is valid, within the measurement uncertainty specified, until **01 March 2027**, 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 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 leading to the certification of this SRM was performed by D.A. Becker of the former NIST Inorganic Analytical Research Division. Revision of this certificate was coordinated by L.J. Wood of the NIST Chemical Sciences Division.

Analytical measurements at NIST were performed by I.L. Barnes, E.S. Beary, D.A. Becker, D.S. Braverman, C.A. Clements, M.S. Epstein, R.R. Greenberg, L.B. Jassie, H.M. Kingston, J.R. Moody, T.J. Murphy, P.J. Paulsen, P.A. Pella, K.W. Pratt, T.A. Rush, G. Sleater, S.F. Stone, G.C. Turk, T.W. Vetter, R.D. Vocke, L.J. Wood, and X. Zhen of the NIST Chemical Sciences Division.

Statistical analysis was provided by W.F. Guthrie and S.B. Schiller of the NIST Statistical Engineering Division.

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

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Analyses for value assignment were also performed by D.L. Anderson (Center for Food Safety and Applied Nutrition, U.S. Food and Drug Administration, College Park, MD), A.R. Byrne and B. Smodis (Jozef Stefan Institute, Ljubljana, Yugoslavia), J. Kucera (Nuclear Physics Institute, Rez, Czechoslovakia), and N. Miller-Ihli, (Nutrient Composition Laboratory, U.S. Department of Agriculture, Beltsville, MD).

NOTICE AND WARNING TO USERS:

SRM 1515 IS INTENDED FOR RESEARCH USE ONLY.

INSTRUCTIONS FOR STORAGE AND USE

Storage: The SRM should be stored in the dark at controlled room temperature (20 °C to 25 °C) in its original unopened bottle until required for use. The SRM should not be exposed to intense sources of radiation. The bottle can be resealed and test portions removed and analyzed until the material reaches its expiration date.

Use: The contents of the bottle should be thoroughly mixed by rotating and/or rolling the bottle before each use. Allow the contents to settle for one minute prior to opening to minimize the loss of fine particles. To relate analytical determinations to the certified values in this certificate, the test portion mass indicated in the description of the NIST analyses for each group of analytes (see “Source, Preparation, and Analysis” below) should be used. Test portions should be taken by gently tapping material needed from the SRM bottle. Results in this certificate are reported on a dry-mass basis; sample(s) need to be dried using one of the recommended techniques (see “Determination of Moisture”) to compare results to certified values found in this certificate. Results obtained in analyses should include their own estimates of uncertainty and can be compared to the certified values using procedures described in reference 8.

Determination of Moisture: Moisture content of SRM 1515 was determined at NIST by (1) drying over fresh anhydrous magnesium perchlorate in a desiccator at room temperature for 120 h (sample depth should not exceed 1 cm) and (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 were placed immediately in a desiccator with fresh anhydrous magnesium perchlorate. Samples were weighed after allowing a minimum of 4 h to establish temperature equilibrium. It is preferable to analyze volatile elements such as mercury from the bottle without drying, determine the moisture content on a separate sample from the same bottle, and correct the analytical results to a dry-mass basis.

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: The plant material for this SRM is mid-shoot leaves, collected from equal amounts of Golden Delicious and Rome varieties, picked from orchards at the Horticultural Research Farm at Rock Spring, PA, and from orchards adjacent to the Fruit Research Laboratory at Biglerville, PA. The material for this SRM was collected and prepared under the direction of C.B Smith, College of Agriculture, The Pennsylvania State University, University Park, PA. Fungicide and insecticide sprays were controlled to minimize heavy metal contamination. The leaves were dried and ground in a stainless-steel mill to pass a 1 mm screen. At NIST, the ground leaves were jet milled and air classified to a particle size of approximately 75 µm (200 mesh). Prior to bottling, the material was irradiated by ⁶⁰Co to an estimated minimum absorbed dose of 27.8 kGy for microbial control.

Analytical Approach for Determination of Elements: Value assignment of the mass fractions of elements in SRM 1515 was based on measurements provided by NIST and collaborating laboratories, where appropriate. NIST provided measurements by using cold-vapor atomic absorption spectrometry (CVAAS), direct current plasma optical emission spectrometry (DCP-OES), flame atomic emission spectrometry (FAES); inductively coupled plasma optical emission spectrometry (ICP-OES), isotope dilution inductively coupled plasma mass spectrometry (ID ICP-MS), isotope dilution thermal ionization mass spectrometry (ID TIMS), instrumental neutron activation analysis (INAA), Kjeldahl nitrogen determination (KJEL), laser-enhanced ionization spectrometry (LEIS), polarography (POL), and radiochemical neutron activation analysis (RNAA).

⁽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.

NIST Analyses for Hg by CVAAS: The mass fraction of mercury was determined by CVAAS from duplicate, nominal 2 g test portions taken from three bottles of SRM 1515. Samples were placed in digestion flasks with 10 mL HNO₃ and 5 mL H₂SO₄ and heated gently for 2 h. Next, 5 mL of HClO₄ were added to each flask, and the solutions were heated to sulfuric acid fumes. Solutions were transferred to volumetric flasks where K₂Cr₂O₇ was added until each solution turned yellow. Solutions were diluted to 100 mL. Quantification was based on a five-point calibration curve.

NIST Analyses for Fe, Na, P, and Rb by DCP-OES or FAES: Mass fractions of iron and phosphorus were determined by DCP-OES. Mass fractions of rubidium and sodium were determined by FAES. For each technique, duplicate, nominal 1 g test portions were taken from three bottles of SRM 1515. Samples were mixed with 1 g lithium metaborate in a platinum crucible, placed in a muffle furnace, allowed to reach a temperature of 1000 °C, and held for 1 h. When cool, the crucibles were placed in a beaker and shaken overnight in 5 % HNO₃. Solutions were diluted to 100 mL. Quantification was based on the method of standard additions using the SRM 3100 series single element standard solutions.

NIST Analyses for Al and P by ICP-OES: Mass fractions of aluminum and phosphorus were determined by ICP-OES from duplicate, nominal 1 g test portions taken from three bottles of SRM 1515. Samples for P analyses were placed in Teflon beakers and wet ashed with a HNO₃/HF/HClO₄ acid mixture. Samples for Al analyses were mixed with 1 g lithium metaborate in a platinum crucible, placed in a muffle furnace, allowed to reach a temperature of 1000 °C, and held for 1 h. When cool, the crucibles were placed in a beaker and shaken overnight in 5 % HNO₃. Solutions were diluted to 100 mL. Quantification for Al and P was based on the method of standard additions using the SRM 3100 series single element standard solutions.

NIST Analyses for B, Ba, Cd, Mo, Ni, and Sr by ID ICP-MS: Mass fractions of boron, barium, molybdenum, nickel, and strontium were determined by ID ICP-MS from duplicate, nominal 1.0 g test portions taken from each of three bottles of SRM 1515. Single 2.0 g test portions were taken from each of six bottles for cadmium measurements. Samples were spiked with isotopically enriched ¹³⁵Ba, ¹¹¹Cd, ⁹⁷Mo, ⁶²Ni, and ⁸⁶Sr and then wet ashed with a HNO₃/HF/HClO₄ acid mixture in Teflon beakers. For boron, samples were spiked with isotopically enriched ¹⁰B and then were dissolved in quartz flasks with reflux condensers using a HNO₃/H₂O₂ mixture. The digests were placed on either a cation or an anion exchange column to selectively remove the elements of interest. The isotopic ratios were measured by ICP-MS using standard operating conditions. Quantification for the above analyses was based on the method of spiked calibrations using precisely weighed high purity metals of known origin.

NIST Analyses for Ca, Pb, Mg, and K by ID TIMS: Mass fractions of calcium, lead, magnesium, and potassium were determined by ID TIMS from duplicate, nominal 1.0 g test portions taken from each of three bottles of SRM 1515. Samples were spiked with isotopically enriched ⁴²Ca, ²⁰⁶Pb, ⁴¹K and ²⁶Mg and then wet ashed with a HNO₃/HF/HClO₄ acid mixture in Teflon beakers. The digests were placed on either an anion or cation exchange column to selectively remove the elements of interest. Thermal ionization was performed using a triple filament procedure and internal normalization of measured ratios. Quantification for the above analyses was based on the method of spiked calibrations using precisely weighed high purity metals of known origin.

NIST Analyses for N by KJEL: The mass fraction of nitrogen was determined by KJEL from single, nominal 2 g test portions taken from six bottles of SRM 1515. Sample preparation was based on the AOAC method 2.057, Improved Method for Nitrate-Free Samples, 14th edition.

NIST Analyses for Mn and Ni by LEIS: Mass fractions of manganese and nickel were determined by LEIS from duplicate, nominal 0.5 g test portions taken from each of three bottles of SRM 1515. Samples were digested in a microwave sample preparation system with subsequent hot plate digestion using a HNO₃/HF/HClO₄ acid mixture. Single wavelength excitation was performed at 279.83 nm for manganese and 300.25 nm for nickel. Quantification was based on the method of standard additions using SRM 3132 *Manganese (Mn) Standard Solution* and high purity metals of known origin.

NIST Analyses for Al, Ba, Ca, Cl, Fe, K, Mg, Mn, Na, Rb, V, and Zn by INAA: Mass fractions of aluminum, barium, calcium, chlorine, iron, potassium, magnesium, manganese, rubidium, sodium, vanadium, and zinc in SRM 1515 were determined by INAA from single, nominal 0.1 g test portions taken from each of 15 bottles of SRM 1515. Samples, controls, and standards were pressed into cylindrical pellets, packaged individually in clean polyethylene bags, and irradiated for 60 s. For determination of aluminum and vanadium, radioactive decay was counted for 6 min after a decay of 2 min. For determination of calcium and magnesium, radioactive decay was counted for 12 min after a decay of 9 min. For determination of chlorine, radioactive decay was counted for 30 min after a decay of 22 min. For determination of barium, manganese, sodium, and potassium, radioactive decay was counted for 5 h to 8 h after a decay of 60 min. For determination of iron, rubidium, and zinc, radioactive decay was counted for at least 3 d after a

decay of 60 d to 120 d. Quantification was based on pure elements or compounds on compressed filter papers and foil fluence monitors.

NIST Analyses for Cd, Cu, and Mo by RNAA: Mass fractions of cadmium, copper, and molybdenum were determined by RNAA from duplicate, nominal 0.15 g test portions taken from each of three bottles of SRM 1515. Samples were placed in quartz vessels and irradiated for 2 h at 19 MW. After several days, samples were transferred to Teflon vessels using a HNO₃/HF/HClO₄ acid mixture. Samples were heated, the acid reduced, and samples were brought up to volume in 1 mol/L HNO₃. Samples were passed through a column and fractions separated. Fractions were counted after a 24 h decay for Cd and Cu and after a 48 h decay for Mo. Quantification was based on irradiated and un-irradiated standard solution carriers added to sample materials.

NIST Analyses for Cu and Zn by POL: Mass fractions of copper and zinc in SRM 1515 were determined by polarography from duplicate, nominal 0.7 g test portions taken from each of three bottles of SRM 1515. Samples were weighed into PFA pressure vessels with 5 g HNO₃, 2 g HF, and 10 g HClO₄ and heated for 12 h. Additional HF and HClO₄ were added, and the vessels were heated for 30 h. Determinations were performed by differential pulse polarography using a PAR 384B electrode stand. Copper was determined in 0.5 mol/L HNO₃ using the method of standard additions. Zinc was determined in 0.25 mol/L CH₃COOH + 0.25 mol/L NH₄CHOO buffer, pH 4.7, using a 3-point calibration curve.

Homogeneity Assessment: Samples from randomly selected bottles of SRM 1515 were tested for homogeneity by INAA. No evidence of statistically significant inhomogeneity was observed.

Value Assignment: Calculations of assigned values are based on results from a single definitive method or on weighted means of multiple assessments of the mass fraction of each element made using different chemical methods or in different laboratories. Weighted means were calculated using the DerSimonian-Laird procedure [2], which weights the results from each method in proportion to the inverse of the square of its uncertainty. The weights include uncertainty contributions from both between- and within-method dispersion of values.

Certified Mass Fraction Values for Elements: Each certified mass fraction value is the combined mean from the means of NIST data and the results provided by collaborating laboratories, where appropriate. The uncertainty provided with each 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 u_c incorporates the observed difference between the results from the methods and their respective uncertainties and an uncertainty component for moisture correction, consistent with the JCGM Guide and with its Supplement 1, k is a coverage factor corresponding to approximately 95 % confidence used to control the level of confidence, and u_c is intended to represent, at the level of one standard deviation, the combined effect of all between- and within-method components of uncertainty [5,6]. The measurand is the total mass fraction for each element, on a dry-mass basis, listed in Table 1. Metrological traceability is to the SI derived unit for mass fraction (expressed as milligrams per kilogram).

Table 1. Certified Mass Fraction Values for Elements in SRM 1515

| | Mass Fraction (mg/kg) | | | Coverage Factor, k |
|---------------------------------------|--------------------------|---|--------|----------------------|
| Aluminum (Al) ^(a,b) | 284.5 | ± | 5.8 | 2.00 |
| Boron (B) ^(c,d) | 27.6 | ± | 2.8 | 2.02 |
| Barium (Ba) ^(b,c) | 48.8 | ± | 2.3 | 1.95 |
| Cadmium (Cd) ^(c,e) | 0.0132 | ± | 0.0015 | 2.04 |
| Calcium (Ca) ^(b,f) | 15250 | ± | 100 | 2.03 |
| Chlorine (Cl) ^(b,d) | 582 | ± | 15 | 2.01 |
| Copper (Cu) ^(e,g) | 5.69 | ± | 0.13 | 1.95 |
| Iron (Fe) ^(b,h) | 82.7 | ± | 2.6 | 1.99 |
| Lead (Pb) ^(f) | 0.470 | ± | 0.024 | 3.35 |
| Magnesium (Mg) ^(b,f) | 2710 | ± | 120 | 2.03 |
| Manganese (Mn) ^(b,i) | 54.1 | ± | 1.1 | 2.02 |
| Mercury (Hg) ^(j,k) | 0.0432 | ± | 0.0023 | 2.05 |
| Molybdenum (Mo) ^(c,e,l) | 0.095 | ± | 0.011 | 2.02 |
| Nickel (Ni) ^(c,i) | 0.936 | ± | 0.094 | 2.03 |
| Nitrogen (Total) (N) ^(d,m) | 22990 | ± | 900 | 2.07 |
| Phosphorus (P) ^(a,h) | 1593 | ± | 68 | 2.04 |
| Potassium (K) ^(b,f) | 16080 | ± | 210 | 1.99 |
| Rubidium (Rb) ^(b,n) | 10.2 | ± | 1.6 | 1.98 |
| Sodium (Na) ^(b,n) | 24.4 | ± | 2.1 | 2.03 |
| Strontium (Sr) ^(c,o) | 25.1 | ± | 1.1 | 2.48 |
| Vanadium (V) ^(b,l) | 0.254 | ± | 0.027 | 2.01 |
| Zinc (Zn) ^(b,g) | 12.45 | ± | 0.43 | 1.97 |

^(a) NIST ICP-OES

^(b) NIST INAA

^(c) NIST ID ICP-MS

^(d) Collaborating laboratories. Reported methods included PGAA.

^(e) NIST RNAA

^(f) NIST ID TIMS

^(g) NIST POL

^(h) NIST DCP-OES

⁽ⁱ⁾ NIST LEIS

^(j) NIST CVAAS

^(k) Collaborating laboratories. Reported methods included RNAA and CVAAS.

^(l) Collaborating laboratories. Reported methods included RNAA.

^(m) NIST KJEL

⁽ⁿ⁾ NIST FAES

^(o) Collaborating laboratories. Reported methods included INAA.

Information Mass Fraction Values for Elements: Each information mass fraction value is the mean result from analyses provided by NIST. No uncertainty is provided because there is insufficient information available for its assessment. Information values cannot be used to establish metrological traceability.

Table 2. Information Mass Fraction Values for Elements in SRM 1515

| | Mass Fraction (mg/kg) |
|-----------------------------|--------------------------|
| Nitrogen (N) ^(a) | 23100 |
| Sulfur (S) | 1800 |
| Antimony (Sb) | 0.013 |
| Bromine (Br) | 1.8 |
| Cerium (Ce) | 3 |
| Chromium (Cr) | 0.3 |
| Cobalt (Co) | 0.09 |
| Europium (Eu) | 0.2 |
| Gadolinium (Gd) | 3 |
| Gold (Au) | 0.001 |
| Iodine (I) | 0.3 |
| Lanthanum (La) | 20 |
| Neodymium (Nd) | 17 |
| Samarium (Sm) | 3 |
| Scandium (Sc) | 0.03 |
| Terbium (Tb) | 0.4 |
| Thorium (Th) | 0.03 |
| Tungsten (W) | 0.007 |
| Uranium (U) | 0.006 |
| Ytterbium (Yb) | 0.3 |

^(a) NIST KJEL. Although total nitrogen is certified, nitrogen determined by the Kjeldahl procedure is not.

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| <p>Certificate Revision History: 29 January 2019 (Corrected Ytterbium symbol; editorial changes); 21 April 2017 (Certified values brought into compliance with the NIST uncertainty policy; removed values for As and Se; updated instructions for use; editorial changes); 22 January 1993 (Editorial changes); 02 July 1991 (Original certificate date).</p> |
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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>.