

# Standard Reference Material<sup>®</sup> 1515

## Apple Leaves

### CERTIFICATE OF ANALYSIS

**Purpose:** The certified values delivered by this Standard Reference Material (SRM) are intended for use in validating analytical methods for the determination of major, minor, and trace elements in botanical materials, agricultural food products, and similar materials and for quality assurance when assigning values to in-house control materials.

**Description:** A unit of SRM 1515 consists of 50 grams of dried apple leaves of the Golden Delicious and Rome varieties.

**Certified Mass Fraction Values:** NIST certified values are traceable to the International System of Units (SI). The values are reported on a dry-mass basis [1].

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

Element	Mass Fraction <sup>(a)</sup> (mg/kg)			Element	Mass Fraction <sup>(a)</sup> (mg/kg)		
Aluminum (Al)	284.5	±	5.8	Mercury (Hg)	0.0432	±	0.0023
Boron (B)	27.6	±	2.8	Molybdenum (Mo)	0.095	±	0.011
Barium (Ba)	48.8	±	2.3	Nickel (Ni)	0.936	±	0.094
Cadmium (Cd)	0.0132	±	0.0015	Nitrogen (Total) (N)	22990	±	900
Calcium (Ca)	15250	±	100	Phosphorus (P)	1593	±	68
Chlorine (Cl)	582	±	15	Potassium (K)	16080	±	210
Copper (Cu)	5.69	±	0.13	Rubidium (Rb)	10.2	±	1.6
Iron (Fe)	82.7	±	2.6	Strontium (Sr)	25.1	±	1.1
Lead (Pb)	0.470	±	0.024	Vanadium (V)	0.254	±	0.027
Magnesium (Mg)	2710	±	120	Zinc (Zn)	12.45	±	0.43
Manganese (Mn)	54.1	±	1.1				

<sup>(a)</sup> Values are expressed as  $x \pm U_{95\%}(x)$ , where  $x$  is the certified value and  $U_{95\%}(x)$  is the expanded uncertainty of the certified value. The true value of the analyte lies within the interval  $x \pm U_{95\%}(x)$  with 95 % confidence. To propagate this uncertainty, treat the certified value as a normally distributed random variable with mean  $x$  and standard deviation  $U_{95\%}(x)/2$  [2–6]. Methods reported by NIST and collaborating laboratories are described in Appendix B.

**Non-Certified Values:** A non-certified value for sodium in SRM 1515 is provided in Appendix A.

**Additional Information:** Values of potential interest to users and additional information are provided in Appendix B [7].

**Period of Validity:** The certified values delivered by SRM 1515 are valid within the measurement uncertainty specified until **01 March 2027**. The certified values are nullified if the SRM is stored or used improperly, damaged, contaminated, or otherwise modified.

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*Certificate Revision History on Page 3*

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**Maintenance of Certified Values:** NIST will monitor this SRM over the period of its validity. If substantive technical changes occur that affect the certification, NIST will issue an amended certificate through the NIST SRM website (<https://www.nist.gov/srm>) and notify registered users. SRM users can register online from a link available on the NIST SRM website or fill out the user registration form that is supplied with the SRM. Registration will facilitate notification. Before making use of any of the values delivered by this material, users should verify they have the most recent version of this documentation, available through the NIST SRM website (<https://www.nist.gov/srm>).

**Safety:** SRM 1515 is intended for research use only.

**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 “Material Acquisition and Preparation” below, and Analytical Approach for Determination of Elements” in Appendix B) should be used. Test portions should be taken by gently tapping material needed from the SRM bottle. Do not remove test portions from the bottle using a spatula or other outside object. 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” below) 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.

**Material Acquisition 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 <sup>60</sup>Co to an estimated minimum absorbed dose of 27.8 kGy for microbial control.

**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.

**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 primary 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.

## REFERENCES

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- [2] DerSimonian, R; Laird, N.; *Meta-Analysis in Clinical Trials*; Controlled Clin. Trials, Vol. 7, pp. 177–188 (1986).
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- [4] Possolo, A.; *RandomEffects-Functions-2011-08-25a.R*, working file of R source code for fitting Gaussian random effects models (2011).
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- [6] 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 <https://www.bipm.org/en/committees/jc/jcgm/publications> (accessed Nov 2022).
- [7] Thompson, A.; Taylor, B.N.; *Guide for the Use of the International System of Units (SI)*; NIST Special Publication 811; U.S. Government Printing Office: Washington, DC (2008); available at <https://www.nist.gov/pml/pubs/sp811/index.cfm> (accessed Nov 2022).
- [8] Sharpless, K.E.; Lippa, K.A.; Diewer, D.L.; Rukhin, A.L.; *The ABCs of Using Standard Reference Materials in the Analysis of Foods and Dietary Supplements: A Practical Guide*; NIST Special Publication 260-181; U.S. Government Printing Office: Washington, DC (2014); available at <https://www.nist.gov/srm/upload/SP260-181.pdf> (accessed Nov 2022).

<p><b>Certificate Revision History:</b> 14 November 2022 (Correction to the certificate revision history; editorial changes); 07 October 2022 (Certified value for sodium converted to a non-certified value; updated format; editorial changes); 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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*Certain commercial equipment, instruments, or materials may be identified in this Certificate of Analysis 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.*

*Users of this SRM should ensure that the Certificate of Analysis in their possession is current. This can be accomplished by contacting the Office of Reference Materials 100 Bureau Drive, Stop 2300, Gaithersburg, MD 20899-2300; telephone (301) 975-2200; e-mail [srminfo@nist.gov](mailto:srminfo@nist.gov); or the Internet at <https://www.nist.gov/srm>.*

\* \* \* \* \* End of Certificate of Analysis \* \* \* \* \*

# APPENDIX A

**Non-Certified Values:** Non-certified values are suitable for use in method development, method harmonization, and process control but do not meet the NIST criteria for certification [1] nor provide metrological traceability to the International System of Units (SI) or other higher-order reference system. They are the best estimates of the true values based on available data. The values are provided with an uncertainty that may reflect only measurement reproducibility, may not include all sources of uncertainty, and/or may reflect a lack of sufficient statistical agreement among multiple analytical methods.

A non-certified mass fraction value for sodium is reported on a dry-mass basis and provided in Table A1. The non-certified mass fraction value is the mean result from analyses provided by NIST.

**Table A1. Non-Certified Mass Fraction Values for Sodium in SRM 1515**

	Mass Fraction (mg/kg)
Sodium (Na)	24.4 ± 2.1

**Maintenance of Non-Certified Values:** NIST will monitor this material to the end of its period of validity. If substantive technical changes occur that affect the non-certified values during this period, NIST will update this Appendix and notify registered users. SRM users can register online from a link available on the NIST SRM website or fill out the user registration form that is supplied with the SRM. Registration will facilitate notification. Before making use of any of the values delivered by this material, users should verify they have the most recent version of this documentation, available through the NIST SRM website (<https://www.nist.gov/srm>).

\*\*\*\*\* End of Appendix A \*\*\*\*\*

# APPENDIX B

**Table B1. Values of Potential Interest to Users in SRM 1515**

	Mass Fraction (mg/kg)
Nitrogen (N) <sup>(a)</sup>	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

<sup>(a)</sup>NIST KJEL. Although total nitrogen is certified, nitrogen determined by the Kjeldahl procedure is not.

## Additional Information

**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).

*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 HNO<sub>3</sub> and 5 mL H<sub>2</sub>SO<sub>4</sub> and heated gently for 2 h. Next, 5 mL of HClO<sub>4</sub> were added to each flask, and the solutions were heated to sulfuric acid fumes. Solutions were transferred to volumetric flasks where K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> 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 % HNO<sub>3</sub>. 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 HNO<sub>3</sub>/HF/HClO<sub>4</sub> 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 % HNO<sub>3</sub>. 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 <sup>135</sup>Ba, <sup>111</sup>Cd, <sup>97</sup>Mo, <sup>62</sup>Ni, and <sup>86</sup>Sr and then wet ashed with a HNO<sub>3</sub>/HF/HClO<sub>4</sub> acid mixture in Teflon beakers. For boron, samples were spiked with isotopically enriched <sup>10</sup>B and then were dissolved in quartz flasks with reflux condensers using a HNO<sub>3</sub>/H<sub>2</sub>O<sub>2</sub> 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 <sup>42</sup>Ca, <sup>206</sup>Pb, <sup>41</sup>K and <sup>26</sup>Mg and then wet ashed with a HNO<sub>3</sub>/HF/HClO<sub>4</sub> 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, 14<sup>th</sup> 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 HNO<sub>3</sub>/HF/HClO<sub>4</sub> 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 HNO<sub>3</sub>/HF/HClO<sub>4</sub> acid mixture. Samples were heated, the acid reduced, and samples were brought up to volume in 1 mol/L HNO<sub>3</sub>. 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 HNO<sub>3</sub>, 2 g HF, and 10 g HClO<sub>4</sub> and heated for 12 h. Additional HF and HClO<sub>4</sub> 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 HNO<sub>3</sub> using the method of standard additions. Zinc was determined in 0.25 mol/L CH<sub>3</sub>COOH + 0.25 mol/L NH<sub>4</sub>CHOO buffer, pH 4.7, using a 3-point calibration curve.

**Table B2. NIST Methods Used for Elemental Determinations for Certified Values**

Element	NIST Analytical Methods
Aluminum (Al)	ICP-OES, INAA
Boron (B)	ID ICP-MS
Barium (Ba)	INAA, ID ICP-MS
Cadmium (Cd)	RNAA, ID ICP-MS
Calcium (Ca)	INAA, ID TIMS
Chlorine (Cl)	INAA
Copper (Cu)	RNAA, POL
Iron (Fe)	INAA, DCP-OES
Lead (Pb)	ID TIMS
Magnesium (Mg)	INAA, ID TIMS
Manganese (Mn)	INAA, LEIS
Mercury (Hg)	CVAAS
Molybdenum (Mo)	RNAA, ID ICP-MS
Nickel (Ni)	ID ICP-MS, LEIS
Nitrogen (Total) (N)	KJEL
Phosphorus (P)	ICP-OES, DCP-OES
Potassium (K)	INAA, ID TIMS
Rubidium (Rb)	INAA, FAES
Strontium (Sr)	ID ICP-MS
Vanadium (V)	INAA
Zinc (Zn)	INAA, POL

CVASS	Cold-vapor atomic absorption spectrometry
DCP-OES	Direct current plasma optical emission spectrometry
FAES	Flame atomic emission spectrometry
HGAAS	Hydride generation atomic absorption spectrometry
ICP-OES	Inductively coupled plasma optical emission spectrometry
ID ICP-MS	Isotope dilution inductively coupled plasma mass spectrometry
ID TIMS	Isotope dilution thermal ionization mass spectrometry
INAA	Instrumental neutron activation analysis
KJEL	Kjeldahl nitrogen determination
LEIS	Laser-enhanced ionization spectrometry
PGAA	Prompt gamma-ray activation analysis
POL	Polarography
RNAA	Radiochemical neutron activation analysis
WDXRF	Wavelength dispersive X-ray fluorescence spectrometry

**Table B3. Collaborating Laboratory Methods Used for Elemental Determinations for Certified Values**

<u>Element</u>	<u>Reported Analytical Methods Included</u>
Boron (B)	PGAA
Chlorine (Cl)	PGAA
Mercury (Hg)	RNAA, CVAAS
Molybdenum (Mo)	RNAA
Nitrogen (Total) (N)	PGAA
Strontium (Sr)	INAA
Vanadium (V)	RNAA

CVASS	Cold-vapor atomic absorption spectrometry
INAA	Instrumental neutron activation analysis
PGAA	Prompt gamma-ray activation analysis
RNAA	Radiochemical neutron activation analysis

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\*\*\*\*\* End of Appendix B \*\*\*\*\*