National Institute of Standards and Technology
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Standard Reference Material® 3268

Kudzu (*Pueraria montana* var. *lobata*) Extract

CERTIFICATE OF ANALYSIS

Purpose: This Standard Reference Material (SRM) is intended primarily for evaluation of analytical methods for the determinations of isoflavones and elements in ground kudzu extract and similar matrices. SRM 3268 can be used for quality assurance when assigning values to in-house reference materials.

Description: A unit of SRM 3268 consists of five packets, each containing approximately 1 g of ground kudzu extract.

Certified Mass Fraction Values: 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 and variability have been taken into account [1].

Certified mass fraction values for elements in SRM 3268, reported on a dry-mass basis, are provided in Table 1. Analyses for value assignment were performed at NIST and collaborating laboratories. The measurands in Table 1 are total mass fractions for each analyte reported and metrological traceability is to the International System of Units (SI) derived unit for chemical mass fraction expressed as milligrams per kilogram [2].

Table 1. Certified Mass Fraction Values for Elements in SRM 3268

	Mass Fraction[(a)] (mg/kg)		
Arsenic (As)	0.849	±	0.083
Cadmium (Cd)	0.0821	±	0.0049
Lead (Pb)	1.16	±	0.16
Selenium (Se)	0.158	±	0.073

[(a)] 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 $U_{95\%}(x)/2$ [2–4].

Non-Certified Values: Non-certified values are provided in Appendix A.

Period of Validity: The certification of **SRM 3268** is valid, within the measurement uncertainty specified, until **01 August 2030**, provided the SRM is handled and stored in accordance with the instructions given in this certificate. The certification is nullified if the SRM is damaged, contaminated, or otherwise modified.

Maintenance of Certified Values: NIST will monitor this SRM to the end of the period of validity. 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.

Carlos A. Gonzalez, Chief
Chemical Sciences Division

Steven J. Choquette, Director
Office of Reference Materials

Safety: SRM 3268 IS INTENDED FOR RESEARCH USE; NOT FOR HUMAN CONSUMPTION.

Storage: SRM 3268 should be stored at controlled room temperature (20 °C to 25 °C) in the original unopened packet until required for use. For elemental analyses, the packet can be opened, test portions removed and analyzed, and then the packet resealed and used until the material reaches its expiration date. For isoflavone analyses, the packet can be opened, test portions removed and analyzed, and then the packet can be resealed with test portions able to be removed for analysis up to one week after initial opening of packet.

Use: The contents of the package should be thoroughly mixed 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, a test portion mass of 0.4 g should be used for arsenic, cadmium, lead, and selenium analyses and 10 mg should be used for isoflavone analyses. Test portions should be taken by gently tapping to remove the material needed from the SRM packet into a secondary container. Test portions should be analyzed as-received and results converted to a dry-mass basis. The moisture conversion factor given below (see "Determination of Moisture") can be used for the samples when using an unopened packet for the first time. If using a previously opened and resealed packet, moisture must be determined using one of the recommended techniques described below. Analytical results should include their own estimates of uncertainty and can be compared to the reference values using procedures described in reference [5].

Determination of Moisture: Moisture content of SRM 3268 was determined at NIST by (1) drying over magnesium perchlorate in a desiccator at room temperature for 28 d and (2) drying for 1 h in a forced-air oven at 80 °C. The means from both techniques were averaged to determine a dry-mass proportion of (0.9543 ± 0.0003) gram dry-mass per gram as-received mass; the uncertainty shown on this value is an expanded uncertainty to represent a 95 % level of confidence. A relative uncertainty component of 0.02 % obtained from the moisture measurements is incorporated in the uncertainties of the assigned values, reported on a dry-mass basis, that are provided in this report.

REFERENCES

[1] Beauchamp, C.R.; Camara, J.E.; Carney, J.; Choquette, S.J.; Cole, K.D.; DeRose, P.C.; Duewer, D.L.; Epstein, M.S.; Kline, M.C.; Lippa, K.A.; Lucon, E.; Phinney, K.W.; Polakoski, M.; Possolo, A.; Sharpless, K.E.; Sieber, J.R.; Toman, B.; Winchester, M.R.; Windover, D.; *Metrological Tools for the Reference Materials and Reference Instruments of the NIST Material Measurement Laboratory*; NIST Special Publication 260-136; U.S. Government Printing Office: Washington, DC (2020); available at https://nvlpubs.nist.gov/nistpubs/SpecialPublications/NIST.SP.260-136-2020.pdf (accessed Jul 2021).

[2] 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/special-publication-811 (accessed Jul 2021).

[3] 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 (JCGM) (2008); available at https://www.bipm.org/en/publications/guides (accessed Jul 2021); 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 2021).

[4] 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/publications/guides (accessed Jul 2021).

[5] Sharpless, K.E.; Lippa, K.A.; Duewer, 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://nvlpubs.nist.gov/nistpubs/SpecialPublications/NIST.SP.260-181.pdf (accessed Jul 2021).

[6] Rukhin, A.L.; Possolo, A.; *Laplace Random Effects Models for Interlaboratory Studies;* Computational Statistics and Data Analysis, Vol. 55, pp. 1815–1827 (2011).

[7] Efron, B.; Tibshirani, R.J.; *An Introduction to the Bootstrap*; Chapman & Hall: London UK (1993).

[8] Searle, S.R.; Casella, G.; McCulloch, C.E.; *Variance Components;* John Wiley: Hoboken, NJ (1992).

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, Maryland 20899-2300; telephone (301) 975-2200; e-mail srminfo@nist.gov; or the Internet at https://www.nist.gov/srm.

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APPENDIX A

Non-Certified Mass Fraction Values: NIST non-certified values do not meet the NIST criteria for certification [1] and 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.

Non-certified mass fraction values for analytes in SRM 3268, reported on a dry-mass basis, are provided in Table A1 and are the mean of results provided by NIST measurements. The measurands in Table A1 are total mass fractions for each analyte reported and metrological traceability is to the SI derived unit for mass fraction (expressed as milligrams per gram isoflavones) as realized by the methods used in its determination [2].

Table A1. Non-Certified Mass Fraction Values for Isoflavones in SRM 3268

	Mass Fraction[(a)] (mg/g)		
Daidzein	17.1	±	1.7
Daidzin	8.21	±	0.83
Puerarin	128	±	13

[(a)] Values are expressed as $x \pm U_{95\%}(x)$, where x is the non-certified value and $U_{95\%}(x)$ is the expanded uncertainty of the non-certified value. The method-specific value of the analyte lies within the interval $x \pm U_{95\%}(x)$ with 95 % confidence. To propagate this uncertainty, the non-certified value should be treated as a normally distributed random variable with mean x and standard deviation $U_{95\%}(x)/2$ [2–4].

Maintenance of Non-Certified Values: NIST will support the non-certified values in the tables below to the end of the period of validity stated on page 1. If substantive technical changes occur that affect the non-certified values during this period, NIST will update this document. Before making use of any of the values delivered by this material, users should obtain the most recent version of this documentation, available free of charge through the https://www.nist.gov/srm website. These values are for informational purposes only, therefore NIST will not support the maintenance of these values.

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APPENDIX B

RESPONSIBILITIES

Coordination: C.A. Rimmer and L.J. Wood of the NIST Chemical Sciences Division.

Analytical Measurements: C.A. Barber and L.J. Wood of the NIST Chemical Sciences Division and M.R. Ale, K.D. Chieh, and J.A. Lippert, formerly of NIST.

Analysts at the following laboratories performed measurements that contributed to the value assignment of elements in SRM 3268 as part of an interlaboratory comparison exercise coordinated by NIST: Advanced Botanical Consulting & Testing, Inc. (Tustin, CA); Advanced Laboratories Inc. (South Salt Lake City, UT); ALS Environmental (Salt Lake City, UT); Apex Analytical Laboratory (Tempe, AZ); Arizona Nutritional Supplements (Chandler, AZ); Brooks Applied Labs (Bothell, WA); Chemical Solutions LTD (Harrisburg, PA); Conagra Brands (Omaha, NE); CONCORZIO SANNIO TECH (TECNO BIOS SRL) (Apollosa, Benevento-Italy, Italy); First Source Laboratory Solutions LLP (Hyderabad, Telangana, India); HVL, LLC (Pittsburgh, PA); Intertek Champaign Laboratories (Champaign, IL); InvaPharma, Inc. (Ontario, Canada); ISURA (Burnaby, BC, Canada); IVC QC Lab (Mira Loma, CA); Nature's Way (Green Bay, WI); NOW Foods (Bloomingdale, IL); Red Rock Labs, LLC (Tempe, AZ); SGS Canada Inc. (Burnaby, BC, Canada); Tishcon Corp. (Salisbury, MD); Tishcon Corp. (Westbury, NY); US Food and Drug Admin CFSAN (MD) (College Park, MD); Weck Laboratories, Inc. (City of Industry, CA).

Statistical Analysis: J.H. Yen of the NIST Statistical Engineering Division.

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

Table B1. Methods Used for Value Assignment

Element	Method
Arsenic	NIST ICP-MS, Collaborating Laboratories (ICP-MS, ICP-OES)
Cadmium	NIST ICP-MS, Collaborating Laboratories (ICP-MS, ICP-OES, ID ICP-MS)
Lead	NIST ICP-MS, Collaborating Laboratories (ICP-MS, ICP-OES, ID ICP-MS)
Selenium	NIST ICP-MS, Collaborating Laboratories (ICP-MS, ICP-OES, ID ICP-MS)
Daidzein	NIST LC-MS
Daidzin	NIST LC-MS
Puerarin	NIST LC-MS, NIST LC-UV

ICP-MS	Inductively coupled plasma mass spectrometry
ICP-OES	Inductively coupled plasma optical emission spectrometry
ID ICP-MS	Isotope dilution inductively coupled plasma mass spectrometry
LC-MS	Liquid chromatology with mass spectrometry
LC-UV	Liquid chromatography with ultra-violet absorbance detection

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APPENDIX C

SOURCE, PREPARATION, AND ANALYSIS

Source and Preparation: Kudzu plant material was harvested in the growing region of West Virginia by a trained botanist. A portion of the material was retained to produce RM 8650 Ground Kudzu (*Pueraria montana* var. *lobata*) Rhizome and the remaining plant material was prepared into extract for production of SRM 3268 by a manufacturer of natural extracts. A quantity of 2.25 kg was received at NIST and transferred to High-Purity Standards (Charleston, SC) where it was blended, sieved, aliquoted, and heat-sealed inside nitrogen-flushed 4 mil polyethylene bags, which were then sealed inside nitrogen-flushed aluminized plastic bags along with two packets of silica gel each. After packaging, the material was irradiated by Neutron Products, Inc. (Dickerson, MD) by ^{60}Co to an absorbed dose of 6.8 kGy to 8.5 kGy.

Homogeneity Assessment: The homogeneity of elements and isoflavones was assessed at NIST where box information was available using the NIST methods and test portion sizes described below. An analysis of variance with a 5 % significance level and graphical analyses showed no evidence of statistically significant box effects for elements. A Type B relative uncertainty component of 5 % was added for the isoflavones values to account for possible inhomogeneities in the measurements that might occur with this class of analytes for this group of materials.

Value Assignment: For calculation of assigned values for analytes that were measured only by NIST, the mean of the mean values from NIST results were used. For calculation of assigned values for analytes that were measured only by the collaborating laboratories, the weighted median of the laboratory means was used [6]. For analytes that were also measured by NIST, the mean of the individual set of NIST data was averaged with the weighted median of the individual collaborating laboratory means, as appropriate.

Collaborating Laboratories' Analyses: The collaborating laboratories were asked to use their usual methods to make single measurements on test portions taken from each of three packets of SRM 3268. Because of the variability among data provided by laboratories participating in an interlaboratory comparison exercise, the weighted median of the individual laboratory means is used, and the uncertainty is estimated using a bootstrap procedure, both based on a Laplace random effects model [4,6,7].

Analytical Approach for Determination of Elements: Value assignment of the mass fractions of elements in SRM 3268 was based on NIST results using inductively coupled plasma mass spectrometry (ICP-MS) and collaborating laboratories where appropriate.

NIST Analysis for As, Cd, Pb, and Se Using ICP-MS: Mass fractions of arsenic, cadmium, and lead were determined by ICP-MS from duplicate, nominal 0.5 g test portions taken from each of eight packets of the SRM and from duplicate, nominal 0.5 g test portions taken each of four packets of the SRM for determination of selenium. Test portions were digested in sealed vessels with a HNO_3/HF mixture using a microwave digestion system. Quantification was based on the method of standard additions using calibration solutions prepared from the SRM 3100 series of single-element standard solutions.

Analytical Approach for Determination of Isoflavones: Value assignment of the mass fraction of puerarin in SRM 3268 was based on measurements provided by NIST using liquid chromatography with ultraviolet absorbance detection (LC/UV-absorbance). Value assignment of the mass fractions of daidzin and daidzein in SRM 3268 was based on measurements provided by NIST using liquid chromatography with mass spectrometry detection (LC-MS).

NIST Analyses for Isoflavones using LC/UV-Absorbance and LC-MS: The mass fraction of puerarin was measured by LC/UV-absorbance and daidzin and daidzein were measured by LC-MS in duplicate 10 mg test portions taken from each of ten packets of SRM 3268. Methanol/water (80/20 (v/v)) and an internal standard solution of 0.60 mL caffeine, 0.50 mL $^{13}C_6$-daidzin, and 0.60 mL $^{13}C_6$-daidzein was added to each test portion and mixed well and extracted using ultrasonication and centrifugation. The supernatant was saved and three additional extraction processes using only methanol/water (80/20 (v/v)) were repeated with the supernatant added to the previous portion. Sodium hydroxide was added to the supernatant to hydrolyze acetyl- and malonyl-glycosides to the corresponding glycosides. A gradient mobile phase was used to separate the isoflavones, and caffeine was monitored at 274 nm. Daidzin and $^{13}C_6$-daidzin were monitored at *m/z* 417 and *m/z* 423 respectively and daidzein and $^{13}C_6$-daidzein were monitored at *m/z* 255 and *m/z* 261. Four stock calibration solutions were prepared gravimetrically at levels intended to approximate the levels of the isoflavones in the SRM following extraction. The purity of the isoflavone calibrant materials was determined at NIST using quantitative proton nuclear magnetic resonance spectroscopy (qNMR).

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