National Institute of Standards and Technology
U.S. Department of Commerce

Date of Issue:
19 July 2021

Standard Reference Material® 3385
Asian Ginseng *(Panax ginseng)* Extract
CERTIFICATE OF ANALYSIS

Purpose: This Standard Reference Material (SRM) is intended primarily for evaluation of analytical methods for the determinations of ginsenosides and elements in ground Asian ginseng extract and similar matrices. SRM 3385 can be used for quality assurance when assigning values to in-house reference materials. A unit of SRM 3385 consists of five packets, each containing approximately 1 g of ground Asian ginseng 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 ginsenosides in SRM 3385, reported on an as-received 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 gram [2].

Table 1. Certified Mass Fraction Values for Ginsenosides in SRM 3385

	Mass Fraction[(a)] (mg/g)				Mass Fraction (mg/g)		
Ginsenoside Rb1	34.8	±	2.0	Ginsenoside Re	17.8	±	1.6
Ginsenoside Rb2	17.9	±	2.5	Ginsenoside Rf	2.94	±	0.64
Ginsenoside Rd	13.1	±	1.9	Ginsenoside Rg1	6.9	±	1.2

[(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].

Period of Validity: The certification of **SRM 3385** 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.

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

Additional Information: Additional information is provided in Appendices B and C.

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Steven J. Choquette, Director
Office of Reference Materials

Safety: SRM 3385 IS INTENDED FOR RESEARCH USE; NOT FOR HUMAN CONSUMPTION.

Storage and Handling: SRM 3385 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 until the material reaches its expiration date. For ginsenoside analyses, the packet can be opened for removal of test portions and resealed for one week after initial opening of packet.

Use: The contents of the packet 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 mass fraction values in this certificate, the test portion mass indicated in the description of the NIST analyses for each group of analytes (see "Appendix C: Source, Preparation, and Analysis" below) should be used: 0.03 g for ginsenosides and 0.5 g for arsenic, cadmium, and lead. Test portions should be taken by gently tapping to remove the material needed from the SRM packet into a secondary container. Analytical test results should include their own estimates of uncertainty and can be compared to the certified values using procedures described in reference [5].

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

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

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

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: Non-certified values are best estimates based on currently available information. However, they do not meet NISTs criteria for certification [1]. Non-certified values should not be used to establish metrological traceability to the International System of Units or other higher-order reference system.

Non-certified mass fraction values for analytes in SRM 3385, reported on an as-received basis, are provided in Table A1 and are the mean of results provided by NIST measurements.

Table A1. Non-Certified Mass Fraction Values for Elements and Ginsenoside Rc in SRM 3385

	Mass Fraction (mg/kg)				Mass Fraction (mg/g)		
Arsenic (As)	0.330	±	0.020	Ginsenoside Rc	13.64	±	0.51
Cadmium (Cd)	0.0223	±	0.0011				
Lead (Pb)	0.067	±	0.012				

[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 $U_{95\%}(x)/2$ [3,4].

Maintenance of Non-Certified Value: 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. 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.

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APPENDIX B

RESPONSIBILITIES

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

The development of SRM 3385 was a collaboration among the National Institute of Standards and Technology (NIST), the National Institutes of Health Office of Dietary Supplements (NIH-ODS), and the Food and Drug Administration Center for Drug Evaluation and Research (FDA CDER).

Analytical Measurements: H.V. Hayes and L.J. Wood of the NIST Chemical Sciences Division and M.R. Ale, formerly of NIST

Analysts at the following laboratories performed measurements that contributed to the value assignment of ginsenosides in SRM 3385 as part of an interlaboratory comparison exercise coordinated by NIST: Advanced Botanical Consulting & Testing, Inc. (Tustin, CA); Alkemists Laboratories (Garden Grove, CA); Analytical Resource Labs (Lehi, UT); BI Nutraceuticals (Sparks, NV); Eurofins Supplement Analysis Center (Petaluma, CA); Gaia Herbs Inc. (Brevard, NC); HVL, LLC (Pittsburg, PA); Intertek Champaign Laboratories (Champaign, IL); ISURA (Burnaby, BC, Canada); Natural Factors (Coquitlam, BC, Canada); Natural Remedies Private Limited (Bangalore, Karnataka India); Nature's Way (Green Bay, WI); NSF Authentechnologies (Petaluma, CA); Nutra Manufacturing (Greenville, SC); NOW Foods (Bloomingdale, IL); SGS Canada Inc. (Burnaby, BC, Canada); Silliker JR Laboratories ULC (Canada) (Burnaby, BC, Canada).

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 (As)	ICP-MS
Cadmium (Cd)	ICP-MS
Lead (Pb)	ICP-MS
Ginsenoside Rb1	LC-MS/MS, Collaborating Laboratories (LC-abs)
Ginsenoside Rb2	LC-MS/MS, Collaborating Laboratories (LC-abs)
Ginsenoside Rc	LC-MS/MS
Ginsenoside Rd	LC-MS/MS, Collaborating Laboratories (LC-abs)
Ginsenoside Re	LC-MS/MS, Collaborating Laboratories (LC-abs)
Ginsenoside Rf	LC-MS/MS, Collaborating Laboratories (LC-abs)
Ginsenoside Rg1	LC-MS/MS, Collaborating Laboratories (LC-abs)

ICP-MS	Inductively coupled plasma mass spectrometry
LC-MS/MS	Liquid chromatology with tandem mass spectrometry
LC-abs	Liquid chromatography with absorbance detection

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APPENDIX C

SOURCE, PREPARATION, AND ANALYSIS

Source and Preparation: Bulk material was prepared into extract for production of SRM 3385 by a manufacturer of natural extracts. The following quantity was received, 3 kg, 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 7.1 kGy to 8.5 kGy.

Homogeneity Assessment: The homogeneity of elements and ginsenosides was assessed at NIST using the methods and test portion sizes described above; analysis of variance with a 5 % significance level and graphical analyses showed no evidence of statistically significant box effects.

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. For analytes that were also measured by NIST, the means of the individual sets of NIST data were 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 3385. 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 based on a Laplace random effects model [8]. The uncertainty is estimated using a bootstrap procedure based on Laplace random effects model for the between-laboratory and within-laboratory effects [6–8].

Analytical Approach for Determination of Elements: Value assignment of the mass fractions of elements in SRM 3385 was based on NIST results using inductively coupled plasma mass spectrometry (ICP-MS).

NIST Analysis for As, Cd, and Pb 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 four packets of the SRM. 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 Ginsenosides: Value assignment of the mass fractions of ginsenosides Rb1, Rb2, Rc, Rd, Re, Rf, and Rg1 in SRM 3385 was based on measurements provided by NIST using liquid chromatography with tandem mass spectrometry (LC-MS/MS) and collaborating laboratories as appropriate.

NIST Analyses for Ginsenosides using LC-MS/MS: The mass fractions of ginsenosides Rb1, Rb2, Rc, Rd, Re, Rf, and Rg1 were measured by LC-MS/MS in 30 mg test portions taken from each of ten packets of SRM 3385. To each test portion 4-methylestradiol was added as an internal standard and ginsenosides were extracted in equal portions of 60 % methanol/water and 60 % methanol/0.4 M potassium hydroxide by end-over-end rotation for 20 min followed by ultrasonication for 60 min. After centrifugation for 10 min at 3000 rpm, ginsenosides in the sample extracts were separated using a C18 column and monitored by tandem mass spectrometry in negative ion mode. The 4-methylestradiol internal standard was monitored by tandem mass spectrometry in positive ion mode. Calibrants were prepared from SRM 3389 *Ginsenoside Calibration Solution* at levels intended to approximate the levels of the ginsenosides in the SRM following extraction. The purity of the neat calibrant materials used to prepare SRM 3389 was determined by NRC Canada using quantitative NMR (qNMR). A single internal standard solution was used for the calibrants and samples.

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