National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material 3299

Ground Turmeric (*Curcuma longa* L.) Rhizome

This Standard Reference Material (SRM) is intended primarily for use in validating analytical methods for the determination of curcuminoids and elements in ground turmeric (*Curcuma longa* L.) rhizomes and similar matrices. SRM 3299 can also be used for quality assurance when assigning values to in-house control materials. A unit of SRM 3299 consists of five pouches, each containing approximately 3 g of turmeric rhizome.

The development of SRM 3299 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).

Certified Mass Fraction Values: Certified mass fraction values of curcuminoids and elements in SRM 3299, reported on a dry-mass basis, are provided in Tables 1 and 2. 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 as the unweighted means of the mean values from NIST methods and the weighted median of the collaborating laboratories means, where appropriate. The associated uncertainties are expressed at an approximately 95 % level of confidence [2–4].

Expiration of Certification: The certification of **SRM 3299** is valid, within the measurement uncertainty specified, until **30 June 2029**, 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 C.A. Rimmer and L.J. Wood of the NIST Chemical Sciences Division.

Support for the development of SRM 3299 was provided in part by NIH-ODS and FDA CDER. Technical consultation was provided by J.M. Betz (NIH-ODS) and A. Nguyen Pho (FDA CDER). Acquisition of the material was coordinated by A. Nguyen Pho of FDA CDER and K.E. Sharpless of the NIST Special Programs Office.

Analytical measurements at NIST were performed by M.R. Ale, C.A. Barber, R. Oflaz, H. Simon, and L.J. Wood of the NIST Chemical Sciences Division.

Statistical analysis was provided by J.H. Yen of the NIST Statistical Engineering Division.

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Gaithersburg, MD 20899
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Steven J. Choquette, Director
Office of Reference Materials

Analysts at the following laboratories performed measurements that contributed to the value assignment of curcuminoids and elements in SRM 3299 as part of an interlaboratory comparison exercise coordinated by NIST: Advanced Botanical Consulting & Testing, Inc. (Tustin, CA); Alkemists Laboratories (Costa Mesa, CA); Arizona Nutritional Supplements (Chandler, AZ); Atlas Bioscience Labs (Tucson, AZ); BI Nutraceuticals (McCarran, CA); Brighton Laboratory (Henderson, NV); British Columbia Institute of Technology (Burnaby, BC, Canada); Canadian Food Inspection Agency (Longueuil, Canada); Craft Technologies, Inc (Wilson, NC); Eurofins Supplement Analysis Center (Petaluma, CA); ISURA (Burnaby, Canada); Nard Naturex SA France (Avignon, France); Natural Remedies Private Limited (Bangalore, India); NOW Foods (Bloomingdale, IL); Oregon's Wild Harvest (Redmond, OR); Shree Dhootapapeshwar Ltd (Mumbai, India); Silliker JR Laboratories ULC (Burnaby, Canada); SORA Labs (Forsyth, MO); Tishcon Corp (Salisbury, MD).

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

NOTICE AND WARNING TO USERS: SRM 3299 IS INTENDED FOR RESEARCH USE; NOT FOR HUMAN CONSUMPTION.

INSTRUCTIONS FOR STORAGE AND USE

Storage: The SRM 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, resealed, and stored at room temperature; test portions can be removed and analyzed until the material reaches its expiration date. The stability of curcuminoids in previously opened packets has not been investigated, and therefore the certification only applies to the initial use and the same results are not guaranteed if the remaining powder is used at a later date.

Use: Before use, the contents of a packet of material should be mixed thoroughly. To relate analytical determinations to the certified values in this Certificate of Analysis, the following masses used for NIST analyses should be used as the minimum sample size to ensure valid results: 0.5 g for elements (see "Source and Preparation" below), and 0.1 g for curcuminoids. 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 sample(s) 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 certified values using procedures described in reference 5.

Determination of Moisture: Moisture content of SRM 3299 was determined at NIST by (1) freeze-drying to constant mass over 7 days; (2) drying over magnesium perchlorate in a desiccator at room temperature for 28 d; and (3) drying for 2 h in a forced-air oven at 80 °C. The means from all three techniques were averaged to determine a dry-mass proportion of (0.9146 ± 0.0060) 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. The conversion factor used to convert data from an as-received to a dry-mass basis is the inverse of the dry-mass proportion, i.e. as-received data were divided by 0.9146 to determine dry-mass results. A relative uncertainty component of 0.3 % for the conversion factor obtained from the moisture measurements is incorporated in the uncertainties of the certified values, reported on a dry-mass basis, that are provided in this certificate.

SOURCE, PREPARATION AND ANALYSIS[(1)]

Source and Preparation: The material for production of SRM 3299 is a turmeric rhizome powder. The material was received as nominally 180 μm (80 mesh) particle size and was packaged without additional grinding. The extract was transferred to High-Purity Standards (Charleston, SC) where it was blended, 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. Following packaging, SRM 3299 was irradiated (Neutron Products, Inc., Dickerson, MD) to an absorbed dose of 8.4 kGy to 10.7 kGy.

Analytical Approach for Determination of Curcuminoids: Value assignment of the mass fractions of bisdemethoxycurcumin, desmethoxycurcumin, and curcumin in SRM 3299 was based on the combination of measurements provided by NIST using liquid chromatography with absorbance detection (LC-absorbance) and by

[(1)]Certain commercial instruments, materials, or processes 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 instruments, materials, or processes identified are necessarily the best available for the purpose.

data provided by collaborating laboratories using LC-absorbance and LC with fluorescence detection (LC-fluorescence).

NIST Analyses for Curcuminoids using LC-Absorbance: The mass fractions of bisdemethoxycurcumin, desmethoxycurcumin, and curcumin were measured by LC-absorbance in duplicate 100 mg test portions taken from each of ten packets of SRM 3299. Phenol was added to each test portions as an internal standard and curcuminoids were extracted in methanol by end-over-end rotation for 15 min followed by ultrasonication for 15 min. After centrifugation for 10 min at 3000 rpm, curcuminoids in the sample extracts were separated using a mixed-mode C18-phenyl column and monitored by absorbance at 425 nm. The phenol internal standard was monitored by absorbance at 270 nm. Calibrants were prepared gravimetrically at levels intended to approximate the levels of the curcuminoids in the SRM following extraction. The purity of the neat calibrant materials was determined at NIST using LC-absorbance at 210 nm, 254 nm, and 425 nm. A single internal standard solution was used for the calibrants and samples.

Analytical Approach for Determination of Elements: Value assignment of the mass fractions of elements in SRM 3299 was based on the combination of results from NIST and collaborating laboratories. NIST provided measurements by 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 six packets of the SRM. Test portions were digested in sealed vessels with a HNO_3/HF mixture using a microwave digestion system. Quantitation was based on the method of standard additions using calibration solutions prepared from the SRM 3100 series of single-element standard solutions.

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 3299. 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 [6,7].

Homogeneity Assessment: The homogeneity of curcuminoids and elements was assessed at NIST using the methods and test portion sizes described above; analysis of variance with 5 % significance showed possible inhomogeneity for bisdemethoxycurcumin. The uncertainty for bisdemethoxycurcumin incorporates a component for possible inhomogeneity based on the standard deviation.

Certified Mass Fraction Values for Curcuminoids: Each certified mass fraction value, reported on a dry-mass basis, is the combined mean from each set of analyses by NIST and the median of the means of results provided by collaborating laboratories. 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 is believed to lie within the interval $x \pm U_{95\%}(x)$ with 95 % confidence. To propagate this uncertainty, the certified value should be treated as a normally distributed random variable with mean x and standard deviation $U_{95\%}(x)/2$ [2–4]. The measurand is the total mass fraction for each curcuminoid listed in Table 1 on a dry-mass basis. Metrological traceability is to the SI-derived unit for mass fraction (expressed as milligrams per gram).

Table 1. Certified Mass Fraction Values for Curcuminoids in SRM 3299

	Mass Fraction (mg/g)
Bisdemethoxycurcumin [a,b,c]	3.66 ± 0.23
Desmethoxycurcumin [a,b,c]	3.79 ± 0.37
Curcumin [a,c]	12.17 ± 0.56

[a] NIST LC-absorbance
[b] Collaborating Laboratories LC-fluorescence
[c] Collaborating Laboratories LC-absorbance

Certified Mass Fraction Values for Elements: Each certified mass fraction value, reported on a dry-mass basis, is the combined mean from each set of analyses by NIST using ICP-MS and the median of the means of results provided by collaborating laboratories using ICP-MS. 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 is believed to lie within the interval $x \pm U_{95\%}(x)$ with 95 % confidence. To propagate this uncertainty, the certified value should be treated as a normally distributed random variable with mean x and standard deviation $U_{95\%}(x)/2$ [2–4]. The measurand is the total mass fraction for each element listed in Table 2 on a dry-mass basis. Metrological traceability is to the SI-derived unit for mass fraction (expressed as milligrams per kilogram).

Table 2. Certified Mass Fraction Values for Elements in SRM 3299

	Mass Fraction (mg/kg)
Arsenic (As)	0.332 ± 0.042
Cadmium (Cd)	1.73 ± 0.24
Lead (Pb)	1.20 ± 0.11

REFERENCES

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[3] JCGM 101:2008; *Evaluation of Measurement Data – Supplement 1 to the Guide to Expression of Uncertainty in Measurement - Propagation of Distributions Using a Monte Carlo Method*; JCGM (2008); available at https://www.bipm.org/utils/common/documents/jcgm/JCGM_101_2008_E.pdf (accessed Sep 2019).

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[5] Sharpless, K.E.; Duewer, D.L.; *Standard Reference Materials for Analysis of Dietary Supplements*; J. AOAC Int., Vol. 91, pp. 1298–1302 (2008).

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

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.