National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 3300

Curcumin Extract of Turmeric (*Curcuma longa* L.) Rhizome

This Standard Reference Material (SRM) is intended primarily for use in validating analytical methods for the determination of curcuminoids in curcumin extracts of turmeric (*Curcuma longa* L.) rhizomes and similar matrices. SRM 3300 can also be used for quality assurance when assigning values to in-house control materials. A unit of SRM 3300 consists of five packets, each containing approximately 1 g of a curcumin extract of a turmeric rhizome.

The development of SRM 3300 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 in SRM 3300, reported on an as-received 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 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 3300** is valid, within the measurement uncertainty specified, until **30 September 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 3300 was provided in part by NIH-ODS. 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 H. Simon formerly of NIST.

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

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

Carlos A. Gonzalez, Chief
Chemical Sciences Division

Gaithersburg, MD 20899
Certificate Issue Date: 20 April 2020

Steven J. Choquette, Director
Office of Reference Materials

Analysts at the following laboratories performed measurements that contributed to the value assignment of curcuminoids in SRM 3300 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); 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).

NOTICE AND WARNING TO USERS: SRM 3300 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 curcuminoid analyses, the packet can be opened and resealed; test portions can be removed for up to seven (7) days following the initial opening of the packet.

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, a minimum samples size of 10 mg should be used to ensure valid results for the determination of curcuminoids. Analytical results should include their own estimates of uncertainty and can be compared to the certified values using procedures described in reference 5.

SOURCE, PREPARATION, AND ANALYSIS[(1)]

Source and Preparation: The material for production of SRM 3300 is a curcuminoid extract of turmeric rhizome powder prepared from the same stock rhizome as SRM 3299. 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 3300 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 3300 was based on the combination of measurements provided by NIST using liquid chromatography with absorbance detection (LC-absorbance) and by 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 10 mg test portions taken from each of ten packets of SRM 3300. Phenol was added to each test portion 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.

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 3300. 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].

[(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.

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

Certified Mass Fraction Values for Curcuminoids: Each certified mass fraction value, reported on an as-received 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 an as-received basis. Metrological traceability is to the measurement unit as realized through the purity determined for the primary chemical standards employed in the NIST methods.

Table 1. Certified Mass Fraction Values for Curcuminoids in SRM 3300

	Mass Fraction (mg/g)		
Bisdemethoxycurcumin [a,b,c]	18.06	±	0.75
Desmethoxycurcumin [a,b,c]	115.4	±	5.5
Curcumin [a,c]	815	±	25

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

REFERENCES

[1] May, W.; Parris, R.; Beck, C.; Fassett, J.; Greenberg, R.; Guenther, F.; Kramer, G.; Wise, S.; Gills, T.; Colbert, J.; Gettings, R.; MacDonald, B.; *Definition of Terms and Modes Used at NIST for Value-Assignment of Reference Materials for Chemical Measurements*; NIST Special Publication 260-136; U.S. Government Printing Office: Washington, DC (2000); available at https://www.nist.gov/system/files/documents/srm/SP260-136.PDF (accessed Apr 2020).

[2] 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 (2008); available at https://www.bipm.org/utils/common/documents/jcgm/JCGM_100_2008_E.pdf (accessed Apr 2020); 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 Apr 2020).

[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 Apr 2020).

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

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

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

[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.