National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 3398

Ginger (*Zingiber officinale*) Rhizome

This Standard Reference Material (SRM) is intended primarily for use in evaluating analytical methods for the determination of gingerols, shogaols, and trace elements in ground ginger (*Zingiber officinale*) rhizomes and similar matrices. SRM 3398 can also be used for quality assurance when assigning values to in-house control materials. A unit of SRM 3398 consists of five packets, each containing approximately 1.6 g of ground ginger rhizome.

The development of SRM 3398 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 trace elements in SRM 3398, reported on a dry-mass 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 [2–5].

Reference Mass Fraction Values: Reference mass fraction values of gingerols, shogaols, and arsenic species in SRM 3398, reported on a dry-mass basis, are provided in Tables 2 and 3. A NIST reference value is a noncertified value that is the best estimate of the true value based on available data; however, the value does not meet the NIST criteria for certification [1] and is provided with an associated uncertainty that may not include all sources of uncertainty. The reference values in this material are the means of measurements provided by NIST using one technique, and the associated uncertainty values represent a 95 % level of confidence [2–5].

Expiration of Certification: The certification of **SRM 3398** is valid, within the measurement uncertainty specified, until **01 January 2030**, 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 and W. Koshute of the Grocery Manufacturers Association (GMA, Washington, DC).

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Analytical measurements at NIST were performed by S.H. Coskun, C.A. Rimmer, L.J. Wood, and L.L. Yu of the NIST Chemical Sciences Division, and J.F. Browning, K.D. Chieh, S.E. Long, R. Oflaz, and S.L. Whitehead formerly of NIST.

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

Analyses for value assignment were also provided by analysts participating in a GMA Food Industry Analytical Chemists (FIAC) Share Group interlaboratory comparison exercise: Con Agra Foods (Omaha, NE); Covance (Asia) Pte. Ltd. (The Synergy, Singapore); Covance Laboratories (Battle Creek, MI); Covance Laboratories (Greenfield, IN); Del Monte Foods (Walnut Creek, CA); Eurofins - Nutrition Analysis Center (Des Moines, IA); Eurofins Frontier Global Sciences, Inc. (Bothell, WA); Eurofins Steins Vitamin Competence Center (Vejen, Denmark); Eurofins WEJ Contaminants GmbH (Hamburg, Germany); Krueger Food Labs (Chelmsford, MA); Land O'Lakes (Arden Hills, MN); Mérieux Nutrisciences Brasil (Sao Paulo, Brazil); Mérieux Nutrisciences China (Beijing, China); Microchem Silliker (Mumbai, India); NSF International (Ann Arbor, MI); Silliker Canada Co. (Markham, ON, Canada); The Coca-Cola Company (Shanghai, China).

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

NOTICE AND WARNING TO USERS: SRM 3398 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, test portions removed and analyzed, and then resealed until the material reaches its expiration date. For gingerol and shogaol analyses, the packet can be opened and resealed, and 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, the following masses used for NIST analyses should be used as the minimum sample size to ensure valid results: 0.5 g for arsenic, arsenic species, lead, gingerols, and shogaols; 0.25 g for mercury (see "Source and Preparation" below). 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 reference values using procedures described in reference 6.

Determination of Moisture: Moisture content of SRM 3398 was determined at NIST by (1) drying over magnesium perchlorate in a desiccator at room temperature for 21 d, (2) drying for 3 h in a forced-air oven at 90 °C, and (3) freeze-drying for 7 d. The means from all techniques were averaged to determine a dry-mass proportion of (0.9295 ± 0.0054) 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 0.3 % 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.

SOURCE, PREPARATION AND ANALYSIS[(1)]

Source and Preparation: The material for production of SRM 3398 was obtained through Modern Nutrition and Biotech (Ridgefield, CT) and sourced from Guizhou Province, China. The material was received as nominally 250 µm (60 mesh) particle size and was packaged without additional grinding. The material 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

[(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.

two packets of silica gel each. Following packaging, SRM 3398 was irradiated (Neutron Products, Inc., Dickerson, MD) to an absorbed dose of 6.3 kGy to 8.4 kGy.

Analytical Approach for Determination of Gingerols and Shogaols: Value assignment of the mass fractions of 6-gingerol, 8-gingerol, 10-gingerol, 6-shogaol, 8-shogaol, and 10-shogaol in SRM 3398 was based on measurements provided by NIST using liquid chromatography with UV absorbance (LC-UV-abs).

NIST Analyses for Gingerols and Shogaols using LC-UV-abs: The mass fractions of 6-gingerol, 8-gingerol, 10-gingerol, 6-shogaol, 8-shogaol, and 10-shogaol were measured by LC-UV-abs in duplicate 0.5 g test portions taken from each of ten packets of SRM 3398. Formononetin was added to each test portion as an internal standard and gingerols and shogaols were extracted in 5 mL of methanol by ultrasonication for 60 min followed by 15 min of centrifugation. After centrifugation, the supernatant was separated, and the above process was repeated using fresh methanol then the supernates were combined for analysis. LC separation followed by UV absorption was used as the detection method with analytes being monitored at the 281 nm wavelength and the internal standard being monitored at the 310 nm wavelength. Calibrants were prepared from vender supplied solutions at levels intended to approximate the levels of the analytes in the SRM following extraction. The purity of the neat calibrant materials used to prepare SRM 3398 was determine by the manufacturer and evaluated at NIST by LC-UV-abs. 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 3398 was based on NIST results using inductively coupled plasma mass spectrometry (ICP-MS), liquid chromatography ICP-MS (LC-ICP-MS), isotope dilution cold vapor ICP-MS (ID CV-ICP-MS) and collaborating laboratories where appropriate.

NIST Analysis for As and Pb Using ICP-MS: Mass fractions of arsenic and lead were determined by ICP-MS from duplicate, nominal 0.5 g test portions taken from each of ten 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.

NIST Analysis for Hg Using ID CV-ICP-MS: The mass fraction of mercury was determined by ID CV-ICP-MS from single, nominal 0.25 g test portions taken from each of six packets of the SRM. Test portions were digested in nitric acid in sealed vessels using a microwave digestion system. Measurements were made using cold-vapor mercury generation coupled with ICP-MS. The ^{201}Hg and ^{202}Hg isotopes were monitored for a duration of 60 s in a pulse counting Time-Resolved-Analysis mode (TRA) and the ^{201}Hg/^{202}Hg ratios were calculated to determine the amount of Hg in the sample. Quantification was based on reverse isotope dilution using a ^{201}Hg stock solution and SRM 1641d *Mercury in Water*.

Determination of Arsenic Species: Value assignment of the mass fractions of arsenic species in SRM 3398 was based on measurements at NIST using liquid chromatography followed by online ICP-MS (LC-ICP-MS).

NOTE: THE EXTRACTION PROCEDURE BELOW MUST BE FOLLOWED TO RELATE THE MEASURED VALUES TO THOSE IN THE CERTIFICATE.

Extraction procedure: Single or duplicate test portions of 0.5 g from 9 packets were transferred into 15 mL polypropylene test tubes, into which 10 mL of water was added. The contents were vortexed at 40 Hz for 1 min and then allowed to stand on the bench for 16 h at ambient temperature. The contents were vortexed at 40 Hz for 30 s and centrifuged at 3600 g_n for 30 min. The supernatant was measured for arsenous acid (AsIII) and arsenic acid (AsV). Method validation was accomplished using SRM 2669 *Arsenic Species in Frozen Human Urine, Level II* and SRM 3669 *Arsenic Species in Frozen Human Urine (Elevated Levels)*.

LC-ICP-MS method: From the above extraction procedure, arsenic species were measured using LC coupled with ICP-MS operated in the dynamic reaction mode using O_2 as the reaction gas. Arsenic was measured as AsO^+ at 91 u.

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 3398. 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 based on a Laplace random effects model for the between-lab and within-lab effects [1−5].

Homogeneity Assessment: The homogeneity of gingerols, shogaols, and elements was assessed at NIST using the methods and test portion sizes described above, based on analysis of variance (ANOVA) with 5 % significance. The ANOVA results for AsIII and AsV indicated that some inhomogeneity may be present. Therefore, the uncertainty values associated with their assigned mass fraction values incorporate a component for possible inhomogeneity based on the standard deviation of the measurements.

Certified Mass Fraction Values for Elements: The certified mass fraction values for arsenic and for lead are each the combined mean from the mean result from the NIST analysis using ICP-MS and the weighted median of the means of results provided by collaborating laboratories, where appropriate. The certified mass fraction value for mercury is the mean result from the NIST analysis using ID CV-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 lies 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 $U_{95\%}(x)/2$ [2,3]. The measurands are the total mass fractions of elements in ground ginger as listed in Table 1 on a dry-mass basis. Metrological traceability is to the SI measurement unit for chemical mass fraction, expressed as milligrams per kilogram.

Table 1. Certified Mass Fraction Values for Elements in SRM 3398

	Mass Fraction (mg/kg)
Arsenic (As)	49.6 ± 2.6
Lead (Pb)	1.445 ± 0.055
Mercury (Hg)	0.0522 ± 0.0017

Reference Mass Fraction Values for Gingerols and Shogaols: Each reference mass fraction value is the mean result from the NIST analysis using LC-UV-Abs. Values are expressed as $x \pm U_{95\%}(x)$, where x is the estimated value and $U_{95\%}(x)$ is the expanded uncertainty of the value. The method-specific value of the analyte lies within the interval $x \pm U_{95\%}(x)$ with 95 % confidence [2,3]. The measurands are the total mass fraction of each gingerol or shogaol listed in Table 2, on a dry-mass basis. Metrological traceability is to the SI derived unit for mass fraction (expressed as milligrams per gram), as realized by the methods used.

Table 2. Reference Mass Fraction Values for Gingerols and Shogaols in SRM 3398

	Mass Fraction (mg/g)
6-Gingerol	3.919 ± 0.059
8-Gingerol	0.618 ± 0.013
10-Gingerol	0.894 ± 0.018
6-Shogaol	2.706 ± 0.043
8-Shogaol	0.734 ± 0.021
10-Shogaol	1.233 ± 0.021

Reference Mass Fraction Value for Arsenic Species: The reference mass fraction values for arsenous acid (AsIII), arsenic acid (AsV), and inorganic arsenic (iAs) are the mean results from the NIST analysis using LC-ICP-MS. Values are expressed as $x \pm U_{95\%}(x)$, where x is the estimated value and $U_{95\%}(x)$ is the expanded uncertainty of the value. The method-specific value of the analyte lies within the interval $x \pm U_{95\%}(x)$ with 95 % confidence [2,3]. The measurand is the total mass fraction of the analyte listed in Table 3, on a dry-mass basis, and is metrologically traceable to the SI derived unit for chemical mass fraction, expressed as milligrams per kilogram, as realized by the measurement processes and standards employed by NIST [7].

Table 3. Reference Mass Fraction Values for Arsenic Species in SRM 3398

	Mass Fraction (mg/kg)
Arsenous Acid (AsIII)	3.4 ± 1.5
Arsenic Acid (AsV)	1.31 ± 0.94
Inorganic Arsenic (iAs)	4.72 ± 0.19

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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; e-mail srminfo@nist.gov; or via the Internet at https://www.nist.gov/srm.