National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 3532

Calcium-Containing Solid Oral Dosage Form

This Standard Reference Material (SRM) is intended primarily for validation of methods for determining elements in a calcium dietary supplement and similar materials. This SRM can also be used for quality assurance when assigning values to in-house reference materials. A unit of SRM 3532 consists of 5 packets, each containing approximately 10 g of material.

The development of SRM 3532 was a collaboration between the National Institute of Standards and Technology (NIST) and the National Institutes of Health Office of Dietary Supplements (NIH-ODS).

Certified Mass Fraction Values: The certified mass fraction values of selected elements in SRM 3532, 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. Certified values were calculated as the mean of the mean values from NIST methods. The associated uncertainties are expressed at an approximately 95 % level of confidence [2-4].

Reference Mass Fraction Values: Reference mass fraction values, reported on a dry-mass basis, are provided for additional elements (Table 2). 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 associated uncertainties that may reflect only measurement reproducibility, may not include all sources of uncertainty, or may reflect a lack of sufficient statistical agreement among multiple analytical methods. The reference mass fraction values were derived from results reported by NIST. The associated uncertainties are expressed at an approximately 95 % level of confidence [2-4].

Expiration of Certification: The certification of **SRM 3532** is valid, within the measurement uncertainty specified, until **30 May 2019**, 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) will facilitate notification.

Coordination of the technical measurements leading to the certification of this SRM was performed by L.J. Wood of the NIST Chemical Sciences Division.

Support for the development of SRM 3532 was provided in part by NIH-ODS. Technical consultation was provided by J.M. Betz (NIH-ODS).

Analytical measurements at NIST were performed by A.F. Marlow, K.E. Murphy, D.J. O'Kelly, R.L. Paul, J.R. Sieber, T.W. Vetter, and L.J. Wood of the NIST Chemical Sciences Division.

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.

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Gaithersburg, MD 20899
Certificate Issue Date: 08 August 2014

Robert L. Watters, Jr., Director
Office of Reference Materials

NOTICE AND WARNING TO USERS

SRM 3532 IS INTENDED FOR LABORATORY USE ONLY, 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. For elemental analyses, the packet can be re-sealed, stored at controlled room temperature (20 °C to 25 °C), and test portions removed and analyzed until the material reaches its expiration date.

Use: Before use, the contents of the packet should be mixed thoroughly. Allow the contents to settle for one minute prior to opening to minimize the loss of fine particles. For certified values to be valid, minimum test portions of 0.3 g for Ca, Cu, Mg, Mn, and Zn; 0.35 g for Cd and Pb; and 0.7 g for B should be used (see descriptions of the NIST analyses below). Analysis results should include their own estimates of uncertainty and can be compared to the certified values using procedures described in reference 5. The moisture conversion factor can be used for the sample(s) when using an unopened packet for the first time. If using a previously opened and resealed packet, sample(s) need to be dried using one of the recommended techniques (See "Determination of Moisture").

SOURCE, PREPARATION, AND ANALYSIS[(1)]

Source and Preparation: The SRM is a calcium supplement powder. The material was ground at NIST to pass through a 180 µm (80-mesh) sieve. Three containers, each containing 10 kg of powdered calcium supplement, were blended and packaged by High-Purity Standards (Charleston, SC). The calcium supplement powder was heat-sealed in 10 g aliquots in 4 mil polyethylene bags then sealed inside nitrogen-flushed aluminized plastic bags along with two silica gel packets.

Determination of Moisture: Moisture content of SRM 3532 was determined at NIST by (1) freeze-drying to constant mass over 7 d; or (2) drying over magnesium perchlorate in a desiccator at room temperature for 49 d. The mean results obtained using both techniques were averaged to determine a conversion factor of (0.968 ± 0.012) gram dry mass per gram as-received mass, which was used to convert data from an as-received to a dry-mass basis; the uncertainty shown on this value is an expanded uncertainty. An uncertainty component for the conversion factor (0.61 %) obtained from the moisture measurements is incorporated in the uncertainties of the certified and reference values, reported on a dry-mass basis, that are provided in this certificate. Forced-air oven drying is not recommended for this SRM because of a direct correlation between amounts of material weighed to moisture loss.

Analytical Approach for Determination of Elements: Value assignment of the mass fractions of the elements in SRM 3532 was based on the combination of measurements from two different analytical methods at NIST, where appropriate. NIST provided measurements by using inductively coupled plasma optical emission spectrometry (ICP-OES), isotope dilution inductively coupled plasma mass spectrometry (ID ICP-MS), thermal neutron prompt gamma-ray activation analysis (PGAA), and wavelength dispersive X-ray fluorescence spectrometry (WDXRF).

NIST Analyses for Ca, Cu, Mg, Mn, and Zn Using ICP-OES: For the determination of calcium, copper, magnesium, manganese, and zinc by ICP-OES, duplicate 0.3 g test portions were taken from each of 10 packets of SRM 3532 and were digested in a nitric acid/hydrofluoric acid mixture using a microwave sample preparation system. Quantification was based on the method of standard additions.

NIST Analyses for Cd and Pb Using ID ICP-MS: For the determination of cadmium and lead by ID ICP-MS, duplicate nominal 0.35 g test portions were taken from each of six packets of SRM 3532. Samples were spiked with isotopically enriched ^{206}Pb and ^{111}Cd and were digested in nitric acid using a microwave sample preparation system. Quantification was based on the method of isotope dilution analysis. Prior to measurement, cadmium was isolated from the matrix using anion exchange chromatography. Methodologies developed for use of ID ICP-MS for measurement of cadmium in SRM 3532 have been described elsewhere [6].

NIST Analyses for B Using PGAA: For the determination of boron by PGAA, individual disks were prepared from 0.7 g test portions taken from each of six packets of SRM 3532. Samples and controls were pressed, pelleted, and packaged individually in clean Teflon bags. Samples and controls were irradiated individually from 0.5 h to 3 h.

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

Gamma-ray spectra up to 11 MeV were collected, and the boron gamma-ray signal at 478 keV was monitored and compared to that of a standard of known purity to determine the mass fraction of boron.

NIST Analyses for Ca, Cu, Mg, Mn, and Zn Using WDXRF: For the determination of calcium, copper, magnesium, manganese, and zinc by WDXRF, duplicate 2.75 g test portions were taken from each of 10 packets of SRM 3532. A borate fusion glass bead was prepared from each sample. The K-$L_{2,3}$ characteristic X-ray lines of all elements were used for quantification.

Homogeneity Assessment: The homogeneity of elements was assessed at NIST using the methods and test portion sizes described above. Analysis of the variance showed statistically significant heterogeneity in some cases, and the uncertainties for boron and lead incorporate an uncertainty component for possible heterogeneity.

Value Assignment: For analytes that were measured by NIST, the means of the individual sets of NIST data were averaged, as appropriate.

Certified Mass Fraction Values for Elements: Each certified mass fraction value is the mean from the combination of the mean of results from analyses by NIST. The uncertainty provided with each value is an expanded uncertainty about the mean to cover the measurand with an approximately 95 % level of confidence. The expanded uncertainty is calculated as $U = ku_c$, where u_c incorporates the observed difference between the results from the methods and their respective uncertainties, consistent with the ISO/JCGM Guide and k is a coverage factor corresponding to an approximately 95 % level of confidence [2–4]. The measurand in each case is the total mass fraction of each analyte. Metrological traceability in Table 1, is to the SI unit of mass expressed as milligrams per kilogram [7].

Table 1. Certified Mass Fraction Values for Elements in SRM 3532

	Mass Fraction (mg/kg)			Coverage Factor, k
Cadmium (Cd)[(a)]	0.097 9	±	0.001 2	2.0
Calcium (Ca)[(b,c)]	175 200	±	330 0	2.0
Copper (Cu)[(b,c)]	277.0	±	8.2	2.0
Magnesium (Mg)[(b,c)]	11 800	±	200	2.0
Manganese (Mn)[(b,c)]	543	±	12	2.0
Zinc (Zn)[(b,c)]	2 110	±	40	2.0

[(a)] NIST ID ICP-MS
[(b)]NIST ICP-OES
[(c)] NIST WDXRF

Reference Mass Fraction Values for Elements: Each reference mass fraction value is the mean result of analyses provided by NIST using a single analytical method. The uncertainty provided with each value is an expanded uncertainty about the mean to cover the measurand with an approximately 95 % level of confidence. The expanded uncertainty is calculated as $U = ku_c$, where u_c represents the combined uncertainty, consistent with the ISO/JCGM Guide and k is a coverage factor corresponding to an approximately 95 % level of confidence [2]. The uncertainties for boron and lead also incorporate an additional uncertainty component for possible inhomogeneity. The measurand is the mass fraction for each analyte listed, based on the method used for each analyte listed in Table 2. Metrological traceability is to the SI unit of mass expressed as milligrams per kilogram [7].

Table 2. Reference Mass Fraction Values for Elements in SRM 3532

	Mass Fraction (mg/kg)			Coverage Factor, k
Boron (B)[(a)]	89.9	±	3.7	2.4
Lead (Pb)[(b)]	0.225	±	0.033	2.2

[(a)] NIST PGAA
[(b)]NIST ID ICP-MS

REFERENCES

[1] May, W.; Parris, R.; Beck II, 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 (2000); available at http://www.nist.gov/srm/upload/SP260-136.PDF (accessed Aug 2014).

[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 http://www.bipm.org/utils/common/documents/jcgm/JCGM_100_2008_E.pdf (accessed Aug 2012); 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 http://www.nist.gov/pml/pubs/tn1297/index.cfm (accessed Aug 2014).

[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; Joint Committee for Guides in Metrology; International Bureau of Weights and Measures (BIPM), Sèvres, France (2008); available at http://www.bipm.org/utils/common/documents/jcgm/JCGM_101_2008_E.pdf (accessed Aug 2014).

[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] Murphy, K.E.; Vetter, T.W.; *Recognizing and Overcoming Analytical Error in the Use of ICP-MS for the Determination of Cadmium in Breakfast Cereal and Dietary Supplements*; Anal Bioanal Chem., Vol. 405, pp. 4579–4588 (2013).

[7] 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 http://www.nist.gov/pml/pubs/sp811/index.cfm (accessed Aug 2014).

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 http://www.nist.gov/srm.