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 cholecalciferol (vitamin D_3) and 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 five 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 cholecalciferol (vitamin D_3) and additional elements in 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 October 2021**, 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 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 C.Q. Burdette, A.F. Marlow, K.E. Murphy, R. Oflaz, 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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Certificate Revision History on Last Page

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

NOTICE AND WARNING TO USERS

SRM 3532 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. 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. For cholecalciferol (vitamin D_3) analyses, the packet can be re-sealed, stored at controlled room temperature (20 °C to 25 °C), and test portions removed and analyzed for up to one week after initial opening.

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 and reference values to be valid, minimum test portions of 1 g for cholecalciferol (vitamin D_3); 0.3 g for Al, Ca, Cu, Mg, Mn, V, 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). Analytical results include their own estimates of uncertainty and may be compared to the certified and reference 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: SRM 3532 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 Cholecalciferol: Value assignment of the mass fractions of cholecalciferol (vitamin D_3) in SRM 3532 was measured at NIST using liquid chromatography with atmospheric pressure chemical ionization tandem mass spectrometry (LC-APCI-MS/MS). Duplicate 1 g to 2 g test portions of powder from each of 10 packets were accurately weighed into 50 mL polyethylene centrifuge tubes and an internal standard solution containing vitamin D_3-d_3 was added. A 10 % EDTA solution was added and samples were sonicated to dissolve the gel matrix that encapsulates the vitamin. Analytes were extracted into hexane by sonication and rotational agitation for 30 min each. After centrifuging for 10 min, the hexane portion was removed. Five additional extractions were performed using sonication and rotational agitation. The supernatants for the individual test portions were combined, were evaporated under nitrogen and resuspended in approximately 1.0 mL of ethanol. Samples were prepared under subdued lighting. Separations were performed on a C_{18} column with an isocratic mobile phase of methanol/acetonitrile (40:60, volume fraction). The transitions at m/z 385 → m/z 259 and at m/z 388 → m/z 370 were monitored for vitamins D_3 and D_3-d_3, respectively. Calibrants were prepared gravimetrically, at levels intended to approximate the levels of the vitamins in the SRM. Two years after the first set of measurements were made for cholecalciferol (vitamin D_3) in SRM 3532 this procedure was repeated using duplicate 1 g to 2 g test portions of powder from each of 6 packets to ensure that the analyte was stable over the long term.

[(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.

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), instrumental neutron activation analysis (INAA), 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. 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 Al, Cu, Mn, and V Using INAA*:** Mass fractions of aluminum, copper, manganese, and vanadium were measured by INAA in duplicate 0.03 g test portions taken from each of six packets of SRM 3532. Powders were pressed into cylindrical pellets, and samples, standards, and controls were packaged individually in clean polyethylene bags. Samples, standards, and controls were irradiated together in the pneumatic tube RT-2 of the NIST reactor at a reactor power of 20 MW for 30 s. For the determination of aluminum, copper, manganese, and vanadium, samples were counted for 5 min after a decay of 5 min.

***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 measured by NIST, the mean of the mean values from NIST results were used, as appropriate.

Certified Mass Fraction Values for Selected Elements: Each certified mass fraction value is the combined mean from the mean of results from analyses provided 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 and an uncertainty component for moisture correction, consistent with the ISO/JCGM Guide, and k is a coverage factor corresponding to an approximately 95 % level of confidence [2–4]. The measurands are the total mass fractions of selected elements in Table 1. Metrological traceability is to the SI derived unit for mass fraction (expressed as milligrams per kilogram) [7].

Table 1. Certified Mass Fraction Values for Selected 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	±	3 300	2.0
Copper (Cu)[b,c,d]	280.7	±	7.6	2.0
Magnesium (Mg)[b,c]	11 800	±	200	2.0
Manganese (Mn)[b,c,d]	532	±	18	2.0
Zinc (Zn)[b,c]	2 110	±	40	2.0

[a] NIST ID ICP-MS
[b] NIST ICP-OES
[c] NIST WDXRF
[d] NIST INAA

Reference Mass Fraction Values for Selected Analytes: 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, incorporating an uncertainty component for moisture correction, 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 measurands are the mass fractions of the selected analytes in calcium-containing solid oral dosage form as determined by the methods indicated in Table 2. Metrological traceability is to the SI derived unit for mass fraction (expressed as milligrams per kilogram) [7].

Table 2. Reference Mass Fraction Values for Selected Analytes in SRM 3532

	Mass Fraction (mg/kg)			Coverage Factor, k
Cholecalciferol (Vitamin D_3)[a]	1.310	±	0.033	2.0
Aluminum (Al)[b]	202.4	±	5.3	2.1
Boron (B)[c]	89.9	±	3.7	2.4
Lead (Pb)[d]	0.225	±	0.033	2.2
Vanadium (V)[b]	0.487	±	0.068	2.2

[a] NIST LC-APCI-MS/MS
[b] NIST INAA
[c] NIST PGAA
[d] NIST ID ICP-MS

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Certificate Revision History: 18 November 2015 (Reference values for cholecalciferol (vitamin D_3) added; editorial changes); 13 February 2015 (Reference values for Al and V added; certified values updated for Cu and Mn; editorial changes); 08 August 2014 (Original certificate date).

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.