National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 3279

Chromium Dietary Supplement

This Standard Reference Material (SRM) is intended for use in the evaluation of chemical methods of analysis for chromium and vanadium to support chemical exposure and safety compliance measurements associated with testing oral dietary supplements. A unit of SRM 3279 consists of five pouches, each containing six grams of powdered material, individually sealed in nitrogen-flushed Mylar® bags.

The development of SRM 3279 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 Value: A certified mass fraction value for chromium (Cr), reported on a dry-mass basis, is 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]. The certified value for Cr was calculated as a variance weighted mean, based on the mean value obtained using inductively coupled plasma mass spectrometry (ICP-MS) calibrated with the method of standard additions at NIST [2], and the weighted mean of individual results provided by the collaborating laboratories listed in Appendix A.

Reference Value Mass Fraction: A reference mass fraction value for vanadium (V), reported on a dry-mass basis, is provided 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, such values do not meet the NIST criteria for certification and are provided with an uncertainty where all known or suspected sources of bias have not been fully investigated [1]. The reference value for V is based on the mean value obtained using a single NIST method (ICP-MS) calibrated with the method of standard additions.

Expiration of Certification: The certification of **SRM 3279** is valid, within the measurement uncertainty specified, until **01 April 2029**, provided the SRM is handled and stored in accordance with instructions given in this certificate (see "Instructions for Handling, 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 certification of this SRM was provided by S.J. Christopher and L.J. Wood of the NIST Chemical Sciences Division.

Analytical measurements were performed by S.J. Christopher and W.C. Davis of the NIST Chemical Sciences Division.

Statistical consultation and analyses were performed by C.R. Hagwood of the NIST Statistical Engineering Division.

Material production was coordinated by A.J. Moors, R.S. Pugh, J. Rhoderick, J.M. Ness, D. Peterson, and S.E. Long of the NIST Chemical Sciences Division.

Material acquisition was coordinated by K.E. Sharpless of the NIST Chemical Sciences Division.

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Certificate Issue Date: 10 October 2019

Steven J. Choquette, Director
Office of Reference Materials

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

Analysts at many collaborating laboratories (Appendix A) analyzed SRM 3279 as part of an interlaboratory comparison exercise coordinated by NIST.

INSTRUCTIONS FOR HANDLING, STORAGE, AND USE

NOTICE TO USERS: SRM 3279 IS INTENDED FOR RESEARCH USE; NOT FOR HUMAN OR ANIMAL CONSUMPTION.

Handling: The material may contain constituents of unknown toxicity, and the fine powder may aerosolize, presenting an inhalation or dermal irritation risk. Caution and care should be exercised during SRM handling and use.

Storage: The SRM should be stored in its original packaging under normal laboratory conditions and kept away from sunlight and intense sources of heat or radiation.

Use: Prior to removal of test portions for analysis, the packet should be positioned horizontally and mixed thoroughly by shaking in the horizontal plane for approximately 1 minute. The packet should be allowed to rest briefly after shaking (approximately 1 minute) to allow for suspended particles to settle, after which the packet should be opened slowly to minimize aerosolizing material contents. A minimum test portion mass of 0.25 g was used for total chromium and vanadium mass fraction determinations. NIST cannot guarantee the suitability of using smaller test portions for total mass fraction determinations. Test portions should be analyzed as received and results converted to a dry-mass basis. The moisture conversion factor given below (see "Moisture Determination") 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 the recommended technique.

MATERIAL SOURCE, PREPARATION, AND HOMOGENEITY TESTING[(1)]

Material Source and Preparation: The source material for SRM 3279 was derived from two commercially available chromium dietary supplements listing chromium in the chemical forms of chromium picolinate and chromium polynicotinate. Approximately 4 kg of material was blended and homogenized into a fine powder using a Teflon® disk mill. The homogenized material was passed through a US No. 45 mesh (0.354 mm) sieve, pooled, and further blended for final production.

Homogeneity Testing: Material homogeneity was assessed before and after final packaging of SRM 3279 using acid-assisted sample decomposition followed by inductively coupled plasma optical emission spectroscopy (ICP-OES). The mass fraction relative standard deviations for six samples drawn from across the entire SRM 3279 production lot were under 1 % for chromium and under 2 % for vanadium. Interlaboratory comparison data for chromium were used as further verification of material homogeneity. Sample test portions of 0.25 g indicated no material heterogeneity during the value assignment of total Cr and V using ICP-MS.

MOISTURE DETERMINATION

Moisture Determination: Eight nominal 1 g samples of SRM 3279 were dried in a gravity convection oven for 2 h (set point = 110 °C) to drive off residual moisture. Dried samples were stored in glass weighing containers in a vacuum desiccator containing calcium sulfate to facilitate sample cooling prior to weighing. A mean dry mass/wet mass conversion factor of 0.92466 ± 0.00020 (1 standard deviation) was used to establish the dry mass fraction values reported in Tables 1 and 2.

MASS FRACTION VALUES

Certified Mass Fraction Value for Chromium: The certified mass fraction value given in Table 1 is the combined variance weighted mean of the mean result from the set of analyses by NIST and the weighted mean of results provided by collaborating laboratories. The value is 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

[(1)] Certain commercial instruments, materials, or processes are identified in this report to adequately specify the experimental procedures. 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.

$x \pm U_{95\%}(x)$ with 95 % confidence. To propagate this uncertainty, treat the certified value as a normally distributed random variable with mean x and standard deviation $U_{95\%}(x)/2$ [3, 4]. The measurand is the total mass fraction for Cr on a dry-mass basis. Metrological traceability is to the derived SI unit for mass fraction, as realized through the purity determined for the primary chemical standards employed in the NIST chromium calibrator solutions of SRM 3112a *Chromium (Cr) Standard Solution* [5].

Table 1. Certified Mass Fraction Value for Chromium

Chromium (Cr)	1299 mg/kg ± 15 mg/kg

Reference Mass Fraction Value for Vanadium: The reference mass fraction value given in Table 2 is the mean result from the set of analyses by NIST. The value is expressed as $x \pm U_{95\%}(x)$, where x is the reference value and $U_{95\%}(x)$ is the expanded uncertainty of the value. The method-specific value of the analyte is believed to lie within the interval $x \pm U_{95\%}(x)$ with 95 % confidence. To propagate this uncertainty, treat the reference value as a normally distributed random variable with mean x and standard deviation $U_{95\%}(x)/2$ [3, 4]. The measurand is the total mass fraction for V on a dry-mass basis. Metrological traceability is to the derived SI unit for mass fraction, as realized through the purity determined for the primary chemical standards employed in the NIST vanadium calibrator solutions of SRM 3165 *Vanadium (V) Standard Solution* [6].

Table 2. Reference Mass Fraction Value for Vanadium

Vanadium (V)	2032 mg/kg ± 37 mg/kg

SUPPLEMENTAL INFORMATION

Supplemental Information: Information is provided below on the chemical speciation of chromium in SRM 3279. The chromium in SRM 3279 is derived from chromium (III) picolinate and chromium (III) nicotinate (Fig. 1), based on the label contents of the SRM source materials.

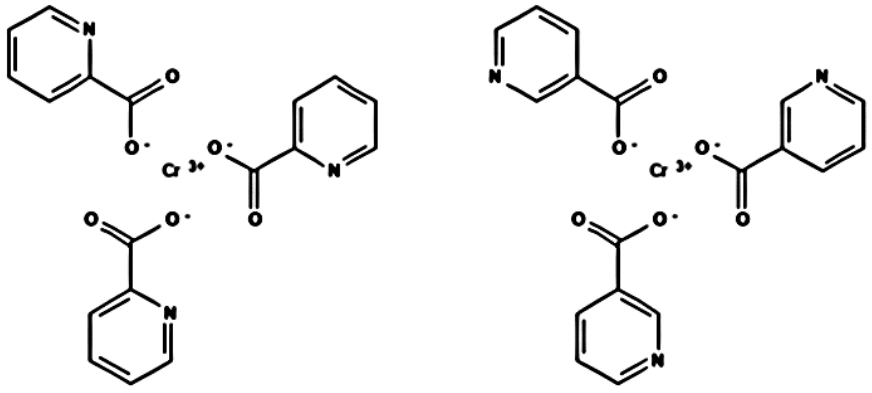

Figure 1. Chromium (III) Picolinate (left) and Chromium (III) Nicotinate (right).

Often dietary supplements are tested for the presence of toxic hexavalent chromium, Cr (VI), which is difficult to measure accurately. This material is expected to be low in native Cr (VI) species, based on the Cr speciation of the parent compounds comprising the SRM. At the time of measurement at NIST, the extractable Cr (VI) mass fraction was observed to be less than an estimated limit of detection (LOD) of 16 micrograms per kilogram. The method used to determine the LOD value for extractable Cr (VI) is summarized below. Analytical methods, reaction conditions and certain material substrates can lead to redox transformation of Cr species during laboratory sample preparation. The estimated LOD value for extractable Cr (VI) may be of use when validating methods of analytical testing of dietary supplements for product safety, and for assistance with uncovering matrix-dependent or sample preparation method-dependent factors that could lead to interconversion of Cr (III and VI) species in the laboratory.

Method Description: Extractable Cr (VI) was determined for SRM 3279 using a microwave-assisted extraction method similar to one that has been documented to minimize the transformation of Cr species during chemical extraction [7]. Chemical extraction was accomplished in 35 mL glass vessels, using a Discover SP microwave system (CEM, Matthews, NC). The extraction solvent was aqueous 50 mmol/L ethylenediaminetetraacetic acid (EDTA) solution (prepared from disodium EDTA salt, Sigma-Aldrich, St. Louis, Mo) adjusted to a pH of 10. The pH of the EDTA solution was adjusted through dropwise addition of high purity Optima™ grade (Thermo Fisher Scientific, Waltham, MA) ammonium hydroxide (30 % w/w) while monitoring pH changes with a commercial pH meter. Six test portions of SRM 3279 (one nominal 50 mg test portion from each of six individual SRM packets) were combined with varying amounts of Cr (VI) ranging from 0 µg to 25 µg and extracted in 5 mL of extractant solution using the microwave program in Table 3.

Table 3. Microwave Extraction Program used for Cr Speciation Experiments

Stage	Temperature (°C)	Ramp Time (min)	Hold Time (min)	Pressure (psi)	Power (W)	Air Cooling	Stirring
1	90	1	5	250	200	On	Hi
2	25	0	10	250	0	On	Hi
3	110	1	5	250	200	On	Hi

Samples were stirred with a Teflon® coated stir bar during microwave irradiation to increase the Cr extraction efficiency. This microwave extraction methodology has been shown to be effective in minimizing Cr species interconversion reactions during microwave extraction of solid samples [7]. The resultant sample extracts were diluted gravimetrically 1:10 with water to match the mobile phase (5 mmol/L EDTA, pH 10) prior to chromatographic analysis. Chromatographic analysis was conducted using a Dionex (Sunnyvale, CA) ICS-3000 ion chromatography system coupled to an Agilent (Santa Clara, CA) 8800 QQQ-ICP-MS. Chromatography settings are tabulated in Table 4.

Table 4. Chromatographic System Program Settings and Column Parameters for Cr Speciation Experiments

Column	Dimensions	Injection Volume (µL)	Run Time (s)	Flow Rate (mL/min)	Separation Type	Mobile Phase	Mobile Phase pH
Dionex AS9-HC	2 mm x 250 mm	10	1140	0.250	Isocratic	5 mM EDTA	10

Cr interferences (mainly $^{40}Ar^{12}C^{+}$) were mitigated by using the oxygen mass shift mode of the QQQ-ICP-MS to measure $^{52}Cr^{+}$ as the molecular oxide ($^{52}Cr^{16}O^{+}$).

Initial method development studies utilized Cr picolinate, ^{53}Cr (III) and Cr (VI) standards (with and without subjection to microwave extraction) to identify chemical species through retention time matching and to track the chemical conversion of species. The chemical extraction data for standards showed minimal interspecies conversion. However, when Cr (VI) was microwave extracted in the presence of 50 mg of SRM 3279, some of the Cr (VI) was converted to Cr (III). This is likely due to the electron-rich Cr picolinate/polynicotinate matrix substrate donating electrons to Cr (VI). A series of chromatograms is presented in Figure 2 for microwave extraction of varying levels of added Cr (VI) while holding the mass of SRM 3279 constant. For determination of an extractable Cr (VI) value, the matrix-induced species interconversion required the accounting of three peaks: Cr picolinate/nicotinate, designated as Cr (Nic), Cr (III) and Cr (VI). The zero-addition standard level chromatogram (see Figure 2) indicated that there was a negligible amount of native extractable Cr (VI) in SRM 3279. The Cr (Nic) and Cr (III) peak areas increased as a function of added Cr (VI). Stacked integrated peak areas from the chromatograms were used to construct a total extractable Cr calibration curve. The calibration curve was then used to estimate a Hubaux-Vos limit of detection value [8, 9] for total extractable chromium, using a 99 % confidence level. The total extractable mass fraction of Cr (VI) was subsequently estimated as a percentage of the total extractable Cr, using the quotient of the peak area of Cr (VI) to total peak area, at the zero-addition standard level.

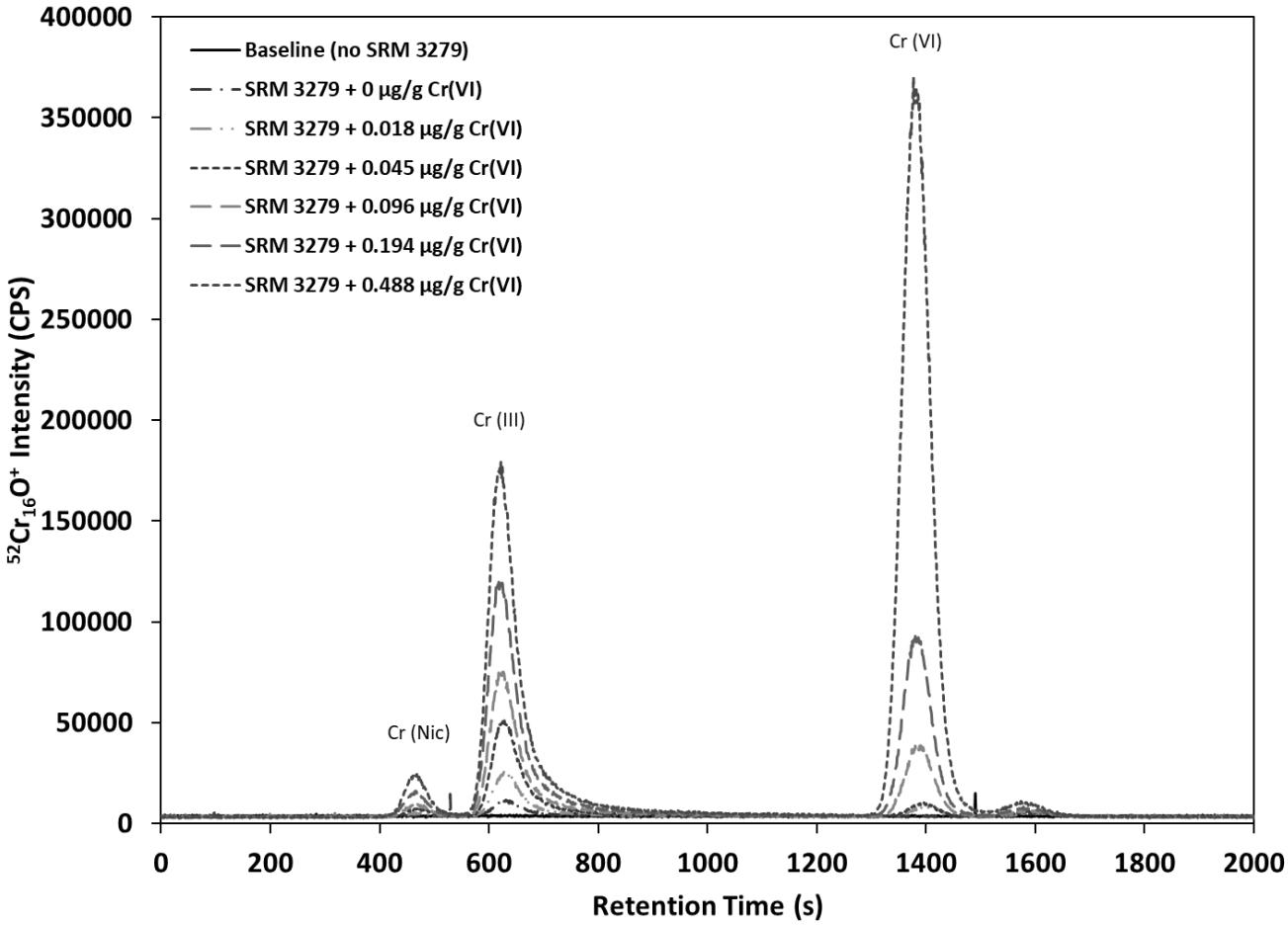

Figure 2. Chromatographic Series for Six (50 mg) Samples of SRM 3279 Spiked with Varying Levels of Cr (VI) and Subjected to Microwave Extraction at pH = 10. The peaks are designated as chromium nicotinate, Cr (Nic), and chromium Cr (III) or Cr (VI). Intensity data are shown as counts per second (CPS).

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[6] SRM 3165; *Vanadium (V) Standard Solution* National Institute of Standards and Technology; U.S. Department of Commerce: Gaithersburg, MD (05 July 2017) available at https://www-s.nist.gov/srmors/view_detail.cfm?srm=3165 (accessed Oct 2019).

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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; fax (301) 948-3730; e-mail srminfo@nist.gov; or via the Internet at https://www.nist.gov/srm.

Appendix A

NIST Interlaboratory Comparison List of Participating Laboratories

ABCO Laboratories, Inc., Fairfield, CA
Access Business Group (Nutrilite), Buena Park, CA
Advanced Botanical Consulting & Testing, Inc., Tustin, CA
Advanced Laboratories Inc, South Salt Lake City, UT
ALS Environmental, Salt Lake City, UT
Amway, Ada, MI
Apex Analytical Laboratory, Tempe, AZ
Arizona Nutritional Supplements, Chandler, AZ
Atlas Bioscience Labs, Tucson, AZ
Brooks Applied Labs, Bothell, WA
California Department of Public Health, Food and Drug Laboratory, Richmond, CA
Chemical Solutions LTD, Harrisburg, PA
Covance Food Solutions, Boulder, CO
Eurofins - Nutrition Analysis Center, Des Moines, IA
Eurofins Frontier Global Sciences, Inc, Bothell, WA
Eurofins Steins Laboratorium, Vejen, Denmark
Exova Inc., Santa Fe Springs, CA
Herbalife, Torrance, CA
HVL, LLC, Pittsburgh, PA
ISURA, Burnaby, BC, Canada
Intertek Champaign Laboratories, Champaign, IL
McCoy & McCoy Laboratories, Madisonville, KY
Natural Factors, Coquitlam, BC, Canada
Natural Remedies Private Limited, Bangalore, Karnataka, India
Nature's Way, Green Bay, WI
NOW Foods, Bloomingdale, IL
NSF International, Ann Arbor, MI
Nutra Manufacturing, Greenville, SC
Pure Essence Labs, Las Vegas, NV
SGS Canada Inc, Burnaby, BC, Canada
Silliker JR Laboratories ULC (Canada), Burnaby, BC, Canada
Standard Process Inc, Palmyra, WI
Sustainable Labs, Inc., Ogden, UT
Tishcon Corp, Salisbury, MD
US Food and Drug Administration CFSAN (GA), Atlanta, GA
US Food and Drug Administration CFSAN (MD), College Park, MD
Underwriters Laboratories, Canton, MA
VMI Nutrition, Salt Lake City, UT