Certificate of Analysis

Standard Reference Material® 3285

Mixed Berry-Containing Solid Oral Dosage Form

This Standard Reference Material (SRM) is intended primarily for use in validating analytical methods for the determination of organic acids in solid oral dosage forms containing bilberries, blueberries, and cranberries and in similar matrices. This SRM can also be used for quality assurance when assigning values to in-house control materials. A unit of SRM 3285 consists of five packets, each containing approximately 2.5 g of powdered material.

The development of SRM 3285 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 organic acids 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]. Values were derived from the combination of results provided by NIST using two analytical methods. The certified values in this material are the equally weighted means of the individual sets of results; the associated uncertainties are expanded uncertainties at the 95 % level of confidence [2-4]. Values are reported on a dry-mass basis in mass fraction units [5].

Reference Values: Reference mass fraction values for additional organic acids and anions are provided in Table 2. Reference values for total antioxidant capacity are provided in Table 3. Reference values are noncertified values that are the best estimate of the true values based on available data; however, the values do not meet the NIST criteria for certification [1] and are 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.

Expiration of Certification: The certification of **SRM 3285** is valid, within the measurement uncertainty specified, until **30 November 2015**, provided the SRM is handled and stored in accordance with instructions given in this certificate (see "Warning and 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.

Support for the development of SRM 3285 was provided in part by NIH-ODS. Technical consultation was provided by J.M. Betz of the NIH-ODS.

The overall direction and coordination of the technical measurements leading to the certification of this SRM were performed by L.C. Sander, K.E. Sharpless, and S.A. Wise of the NIST Analytical Chemistry Division.

Acquisition of the material was performed by K.E. Sharpless of the NIST Analytical Chemistry Division.

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

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Certificate Issue Date: 24 February 2012
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Analytical measurements at NIST were performed by M.M. Phillips, B.J. Porter, and L.J. Wood of the NIST Analytical Chemistry Division. Results were also provided by analysts participating in an interlaboratory comparison exercise involving members of the Grocery Manufacturers Association Food Industry Analytical Chemists Committee (GMA FIACC), Washington, DC: Campbell Soup Company, Camden, NJ; Covance, Inc., Madison, WI; Eurofins Chemical Control, Cuneo, Italy; Eurofins S&S, Hanover, MD; General Mills, Golden Valley, MN; McCormick and Company, Hunt Valley, MD; National Center of Food Safety and Technology, Summit-Argo, IL; NSF International, Ann Arbor, MI; The Hershey Company Technical Center, Hershey, PA; and Welch's, Billerica, MA.

Support aspects involved in the issuance of this SRM were coordinated through the NIST Measurement Services Division.

WARNING AND INSTRUCTIONS FOR STORAGE AND USE

Warning: For laboratory use only. Not for human consumption.

Storage: The material should be stored at controlled room temperature (20 °C to 25 °C), in an unopened packet, until needed. The certification does not apply to contents of previously opened and stored packets as the stability of some analytes has not been investigated.

Use: Prior to removal of a test portion for analysis, the contents of a packet of material should be mixed thoroughly. For certified values to be valid, test portions of the powder equal to or greater than 0.1 g for organic acid analyses should be used. The stability of organic acids in previously opened and stored packets has not been investigated. Test portions should be analyzed as received and results converted to a dry-mass basis by determining moisture content (described below) on a separate test portion.

PREPARATION AND ANALYSIS(1)

Material Acquisition and Preparation: The materials used for production of SRM 3285 consisted of four commercially available products labeled as containing blueberry fruit, extract, and juice; cranberry juice; and bilberry extract. Contents of capsules were individually ground and sieved to 180 µm (80 mesh). The material was then shipped to High-Purity Standards (Charleston, SC), where it was blended, aliquotted, 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 3285 was irradiated (Neutron Products, Inc., Dickerson, MD) at an absorbed dose of 7.1 kGy to 8.9 kGy.

Analytical Approach for Determination of Organic Acids: Value assignment of the mass fractions of the organic acids in SRM 3285 was based on the combination of measurements from two different methods: isotope dilution liquid chromatography with mass spectrometric detection (ID-LC/MS) and ion chromatography with conductivity detection (IC-CD). Duplicate test portions of approximately 0.1 g were taken from each of six packets for analysis using each of the methods, and internal standards were added. Organic acids were extracted into water, and the solutions from four such successive extractions were combined.

For ID-LC/MS, an organic acid column was held at 40 °C. An aqueous mobile phase containing 0.5 % (volume fraction) formic acid was used under isocratic conditions. The flow rate was set to 0.5 mL/min and the sample injection volume was 10 µL. The mass spectrometer was operated in negative ion mode, with atmospheric pressure electrospray ionization (AP-ESI). Each organic acid was matched with a ^{13}C- or ^{2}H-labeled internal standard, and quantitation was based on response factors calculated from the relative peak areas and concentrations [6].

For analysis by IC-CD, a hydroxide-selective anion exchange column was held at 30 °C, and a flow rate of 1.5 mL/min was used for the separation. Ultrapure water was used for generation of a hydroxide gradient, and a current of 186 mA was applied for suppression of the background conductivity from the hydroxide mobile phase. Quantitation was based on relative peak areas with trifluoroacetic acid (TFA) as an internal standard [6].

Collaborating Laboratories' Analyses: The GMA FIACC laboratories were asked to use their usual methods to make single measurements on test portions taken from each of three packets of SRM 3285. The collaborating laboratories' data were used to assign reference values for antioxidant capacity. Three antioxidant methods were employed: oxygen radical absorbance capacity (ORAC), Folin-Ciocalteau's reagent (Folin-C), and 2,2-diphenyl-1-picrylhydraxyl (DPPH) as the reagent. The relative expanded uncertainty on the DPPH results was greater than 100 %, and a reference value was not assigned to SRM 3285 using this technique. This is indicative of disagreement among the small number of laboratories (3) who reported results using DPPH.

(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.

Determination of Moisture: Moisture content of SRM 3285 was determined at NIST (see "Warning and Instructions for Storage and Use") by (1) freeze-drying to constant mass over 7 d; (2) drying over magnesium perchlorate in a desiccator at room temperature for 21 d; and (3) drying for 2 h in a forced-air oven at 80 °C. Unweighted results obtained using all three techniques were averaged to determine a conversion factor of (0.9682 ± 0.0076) 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 (0.39 %) 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.

Homogeneity Assessment: The homogeneity of organic acid content was assessed at NIST by using the methods described above. An analysis of variance did not show inhomogeneity for the test portions analyzed; see (see "Warning and Instructions for Storage and Use").

Value Assignment: The equally weighted means from each set of data available were used to calculate the assigned values for organic acids. The median of laboratory means was used to calculate the reference values for antioxidant capacity.

Certified Mass Fraction Values for Organic Acids: Each certified mass fraction is an equally weighted mean of results provided by ID-LC/MS and IC-CD. The uncertainty provided with each value is an expanded uncertainty about the mean to cover the measurand with approximately 95 % confidence; it expresses both the observed difference between the results from the methods and their respective uncertainties, incorporating an uncertainty component for moisture correction, consistent with the ISO Guide and its Supplement 1 [2-4]. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the effects of the combined components of uncertainty, and k is a coverage factor corresponding to approximately 95 % confidence for each analyte.

Table 1. Certified Mass Fraction Values for Organic Acids in SRM 3285

	Mass Fraction (mg/g)			k
Malic Acid	22.83	±	0.53	2.00
Quinic Acid	24.87	±	0.42	2.00

Reference Mass Fraction Values for Organic Acids and Anions: Each reference mass fraction value is a mean of results provided by IC-CD, except for citric acid, which was determined by ID-LC/MS and IC-CD, and shikimic acid, which was determined by ID-LC/MS. The uncertainty provided with each value is an expanded uncertainty about the mean to cover the measurand with approximately 95 % confidence [2]. The uncertainty incorporates within-method uncertainty and a component related to moisture correction. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the effects of the combined components of uncertainty, and k is a coverage factor corresponding to approximately 95 % confidence for each analyte. The uncertainty provided for citric acid is an expanded uncertainty about the mean to cover the measurand with approximately 95 % confidence; it expresses both the observed difference between the results from the methods and their respective uncertainties, incorporating an uncertainty component for moisture correction, consistently with the ISO Guide and its Supplement 1 [2-4]. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the effects of the combined components of uncertainty, and k is a coverage factor corresponding to approximately 95 % confidence for each analyte.

Table 2. Reference Mass Fraction Values for Organic Acids and Anions in SRM 3285

	Mass Fraction (mg/g)			k
Citric Acid	60	±	14	2.00
Galacturonic Acid	2.82	±	0.07	2.16
Glycolic Acid	0.45	±	0.02	2.21
Isocitric Acid	0.23	±	0.01	2.20
Oxalic Acid	1.01	±	0.02	2.16
Phosphate	2.78	±	0.10	2.19
Shikimic Acid	1.01	±	0.05	2.19
Sulfate	0.42	±	0.05	2.22

Reference Values for Antioxidant Capacity: Each reference value is the median of the mean results provided by the GMA FIACC laboratories. The uncertainty provided with each value is an expanded uncertainty about the median to cover the measurand with approximately 95 % confidence, consistent with the ISO Guide [2]. The uncertainty incorporates within-method uncertainty and a component related to moisture correction. The expanded uncertainty is calculated as $U = ku_c$, where u_c is intended to represent, at the level of one standard deviation, the effects of the combined components of uncertainty, and k is a coverage factor corresponding to approximately 95 % confidence for each analyte.

Table 3. Reference Values for Total Antioxidant Capacity of SRM 3285

Method	Result	Units	k
ORAC	248 ± 22	micromoles Trolox equivalents per gram	2.57
Folin-C	20.1 ± 3.4	milligrams gallic acid per gram	2.36

REFERENCES

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[6] Phillips, M.M.; Case, R.J.; Rimmer, C.A.; Sharpless, K.E.; Wise, S.A.; Sander, L.C.; *Determination of Organic Acids in Vaccinium Berry Standard Reference Materials*; Anal. Bioanal. Chem., Vol. 398, pp. 425-434 (2010).

Certificate Revision History: 24 February 2012 (Added reference values for antioxidant capacity; editorial changes); 14 September 2010 (Original certificate issue date).