National Institute of Standards & Technology

# Certificate of Analysis

## Standard Reference Material® 3282

### Low-Calorie Cranberry Juice Cocktail

This Standard Reference Material (SRM) is intended primarily for use in validating analytical methods for the determination of organic acids and nutrient elements in cranberry juice cocktails and similar matrices. This SRM can also be used for quality assurance when assigning values to in-house control materials. A unit of SRM 3282 consists of five ampoules, each containing approximately 1.2 mL of material.

The development of SRM 3282 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:** Certified mass fraction values for organic acids and elements are provided in Tables 1 and 2, respectively. 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. The certified values were calculated as the mean of the mean values from NIST methods and the median of the mean results provided by collaborating laboratories, where appropriate. The associated uncertainties are expressed at an approximately 95 % level of confidence [2–4].

**Reference Mass Fraction Values:** Reference mass fraction values for additional organic acids, anions, elements, and sugars are provided in Tables 3 through 5. A NIST reference value is a noncertified value that is the best estimate of the true value 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. The reference mass fraction values were derived from results provided by NIST or collaborating laboratories. The associated uncertainties are expressed at an approximately 95 % level of confidence [2–4].

**Expiration of Certification:** The certification of **SRM 3282** is valid, within the measurement uncertainty specified, until **30 November 2025**, provided the SRM is handled and stored in accordance with the 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.

Overall direction and coordination of the technical measurements leading to the certification of this SRM were performed by L.C. Sander of the NIST Chemical Sciences Division, K.E. Sharpless of the NIST Special Programs Office, and S.A. Wise formerly of NIST.

Acquisition of the material was performed by K.E. Sharpless.

Support for the development of SRM 3282 was provided in part by NIH-ODS. Technical consultation was provided by J.M. Betz (NIH-ODS).

Carlos A. Gonzalez, Chief
Chemical Sciences Division

Gaithersburg, MD 20899
Certificate Issue Date: 07 October 2019
*Certificate Revision History on Last Page*

Steven J. Choquette, Director
Office of Reference Materials

Analytical measurements at NIST were performed by M.M. Phillips, M.A. Pichon, and L.J. Wood of the NIST Chemical Sciences Division.

Analyses for value assignment were also performed by the following laboratories participating in a Grocery Manufacturers Association (GMA, Washington, DC) Food Industry Analytical Chemists Committee (FIACC) interlaboratory comparison exercise: Campbell Soup Company (Camden, NJ); Covance, Inc. (Madison, WI); Eurofins Scientific, Inc. (Des Moines, IA); Hormel Foods Corporation (Austin, MN); Krueger Food Laboratories, (Cambridge, MA); The Coca-Cola Company (Apopka, FL); and The National Food Laboratory (Livermore, CA).

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

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

## NOTICE TO USERS

SRM 3282 IS INTENDED FOR RESEARCH USE; NOT FOR HUMAN CONSUMPTION.

## INSTRUCTIONS FOR HANDLING, STORAGE, AND USE

**Handling:** This SRM is contained in tip-sealed borosilicate glass ampoules with prescored stems. All appropriate safety precautions, including use of gloves during handling, should be taken.

**Opening an Ampoule:** When an ampoule is to be opened, that area of the stem where the prescored band is located should be carefully wiped with a clean, damp cloth and the body of the ampoule wrapped in absorbent material. Holding the ampoule steady and with thumb and forefinger grasping the stem, **minimal** thumb pressure should be applied to the stem to snap it. Correctly done, the stem should break easily where prescored. Use of a metal file to break the stem is **NOT** recommended.

**Storage:** The SRM should be stored under refrigeration (4 °C) in unopened ampoules.

**Use:** Prior to removal of a test portion, the contents of an ampoule should be mixed thoroughly. To relate analytical determinations to the certified values in this certificate, a test portion mass equal to or greater than 0.1 g for organic acid analyses and 1.4 g for element analyses should be used. The stability of organic acids and elements in opened ampoules has not been investigated. Results obtained in analyses should include their own estimates of uncertainty and can be compared to the listed values using procedures described in reference 5.

## SOURCE, PREPARATION AND ANALYSIS[(1)]

**Source and Preparation:** The low-calorie cranberry juice cocktail used for production of SRM 3282 was obtained from a commercial source. The material was ampouled under argon at NIST.

**Analytical Approach for Determination of Organic Acids:** Value assignment of the mass fractions of organic acids in SRM 3282 was based on the combination of results provided by NIST using liquid chromatography with absorbance detection (LC-absorbance) and ion chromatography with conductivity detection (IC-CD).

*NIST Analysis for Organic Acids using LC-absorbance*: Mass fractions of citric acid, malic acid, quinic acid, and shikimic acid were measured by LC-absorbance using duplicate test portions of approximately 0.1 g taken from each of six ampoules. Samples were diluted with a solution containing potassium phosphate monobasic and DL-dithiothreitol for stabilization prior to analysis. An isocratic separation using two organic acid columns connected in series and an aqueous mobile phase adjusted to pH 2.0 with concentrated hydrochloric acid was used at 43 °C. Organic acids were detected by absorbance at 210 nm. Quantification was based on an external calibration model [6].

*NIST Analysis for Organic Acids and Anions using IC-CD:* Mass fractions of citric acid, malic acid, quinic acid, galacturonic acid, glycolic acid, isocitric acid, oxalic acid, phosphate, and sulfate were measured by IC-CD using duplicate test portions of approximately 0.1 g taken from each of six ampoules. Samples were diluted with a solution containing potassium phosphate monobasic and DL-dithiothreitol for stabilization prior to analysis, and trifluoroacetic acid (TFA) was added as an internal standard. A hydroxide-selective anion exchange column was held at 30 °C, and

[(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.

ultrapure water was used for generation of the hydroxide gradient. A current of 186 mA was applied for suppression of the background conductivity from the hydroxide mobile phase for more sensitive detection of the organic acids [6].

**Analytical Approach for Determination of Elements:** Value assignment of the mass fractions of elements in SRM 3282 was based on the combination of measurements made at NIST using inductively coupled plasma optical emission spectrometry (ICP-OES) and by collaborating laboratories, where appropriate.

*NIST Analyses for Ca, Cu, Fe, K, Mg, Mn, Na, and Zn using ICP-OES:* Mass fractions of calcium, copper, iron, potassium, magnesium, manganese, sodium, and zinc were measured by ICP-OES in nominal 1.4 g test portions taken from each of six ampoules of SRM 3282. Test portions were digested in sealed vessels with nitric acid using a microwave digestion system. Quantification was based on the method of standard additions.

**Collaborating Laboratories' Analyses:** The GMA FIACC collaborating laboratories were asked to use their usual methods to make single measurements of elements and sugars on single test portions taken from each of two ampoules of SRM 3282.

**Homogeneity Assessment:** The homogeneity of organic acids and elements was assessed at NIST by using the methods described above. An analysis of variance at the 5 % significance level did not show statistically significant heterogeneity. All other analytes have been treated as though they are homogeneously distributed in the material although the homogeneity of the other analytes was not assessed.

**Value Assignment:** The equally weighted means from each set of data available were used to calculate the assigned values. For calculation of assigned values for analytes that were measured only by NIST, the mean of the mean values from NIST results were used. The collaborating laboratories reported the individual results for each of their analyses for a given analyte and the mean of each laboratory's results was then determined. For calculation of assigned values for analytes that were measured only by the collaborating laboratories, the median of the laboratory means was used. For analytes that were also measured by NIST, the mean of the individual sets of NIST data were averaged with the median of the individual collaborating laboratory means, as appropriate.

**Certified Mass Fraction Values for Organic Acids:** Each certified mass fraction value is the mean from the combination of the means of results from analyses at NIST by LC-absorbance and IC-CD. 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 is believed to lie 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 $U_{95\%}(x)/2$ [2-4]. The measurands are the total mass fractions of the organic acids as listed in Table 1. Metrological traceability is to the measurement unit (expressed as milligrams per gram) as realized through the purity determined for the primary chemical standards employed in the NIST methods.

Table 1. Certified Mass Fraction Values for Organic Acids in SRM 3282

| | Mass Fraction (mg/g) |
|---|---|
| Citric Acid | 3.221 ± 0.053 |
| Malic Acid | 2.133 ± 0.042 |
| Quinic Acid | 2.672 ± 0.048 |

**Certified Mass Fraction Values for Elements:** Each certified mass fraction value is the combined mean from the mean of results from analyses at NIST using ICP-OES and the median of the mean of results provided by collaborating laboratories. 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 is believed to lie 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 $U_{95\%}(x)/2$ [2-4]. The measurands are the total mass fraction of elements in protein powder as listed in Table 2 on a dry-mass basis. Metrological traceability is to the SI-derived unit for mass fraction (expressed as milligrams per kilogram).

Table 2. Certified Mass Fraction Values for Elements in SRM 3282

| | Mass Fraction (mg/kg) |
|---|---|
| Calcium (Ca) | 26.3 ± 1.6 |
| Copper (Cu) | 0.231 ± 0.061 |
| Magnesium (Mg) | 12.97 ± 0.84 |
| Manganese (Mn) | 0.493 ± 0.016 |
| Potassium (K) | 247 ± 12 |
| Sodium (Na) | 201 ± 20 |

**Reference Mass Fraction Values for Organic Acids and Anions:** Each reference mass fraction value is the mean result of NIST analyses. 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 is believed to lie within the interval $x \pm U_{95\%}(x)$ with about a 95 % confidence [2-4]. The measurands are the total mass fractions of each analyte listed in Table 3 as determined by the methods indicated. Metrological traceability is to mass fraction (expressed as milligrams per gram) as realized by the methods used.

Table 3. Reference Mass Fraction Values for Organic Acids and Anions in SRM 3282

| | Mass Fraction (mg/g) |
|---|---|
| Galacturonic Acid[(a)] | 0.3480 ± 0.0051 |
| Glycolic Acid[(a)] | 0.0463 ± 0.0021 |
| Isocitric Acid[(a)] | 0.0198 ± 0.0007 |
| Oxalic Acid[(a)] | 0.0963 ± 0.0026 |
| Phosphate[(a)] | 0.0266 ± 0.0006 |
| Shikimic Acid[(b)] | 0.051 ± 0.001 |
| Sulfate[(a)] | 0.0101 ± 0.0004 |

[(a)] NIST IC-CD
[(b)] NIST LC-absorbance

**Reference Mass Fraction Values for Elements:** Each reference mass fraction value is the mean result of NIST analyses by ICP-OES. Values are 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 about a 95 % confidence [2-4]. The measurands are the total mass fraction of each element listed in Table 4 as determined by the methods indicated. Metrological traceability is to the SI-derived unit for mass fraction (expressed as milligrams per kilogram), as realized by the method used.

Table 4. Reference Mass Fraction Values for Elements in SRM 3282

| | Mass Fraction (mg/kg) |
|---|---|
| Iron (Fe) | 0.54 ± 0.14 |
| Zinc (Zn) | 0.154 ± 0.061 |

**Reference Mass Fraction Values for Sugars:** Each reference mass fraction value is the median of the mean of results provided by collaborating laboratories. 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 is believed to lie within the interval $x \pm U_{95\%}(x)$ with about a 95 % confidence [2-4]. The measurands are the total mass fractions of each analyte listed in Table 5 as determined by the methods indicated. Metrological traceability is to mass fraction (expressed as grams per 100 grams) as realized by the methods used.

Table 5. Reference Mass Fraction Values for Sugars in SRM 3282

| | Mass Fraction (g/100 g) |
|---|---|
| Total Sugars | 2.862 ± 0.054 |
| Fructose | 2.08 ± 0.10 |
| Glucose | 0.850 ± 0.060 |

## 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; U.S. Government Printing Office: Washington, DC (2000); available at https://www.nist.gov/sites/default/files/documents/srm/SP260-136.PDF (accessed Oct 2019).

[4] 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 available at: https://www.bipm.org/utils/common/documents/jcgm/JCGM_100_2008_E.pdf (accessed Oct 2019); 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 https://www.nist.gov/pml/nist-technical-note-1297 (accessed Oct 2019).

[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 https://www.bipm.org/utils/common/documents/jcgm/JCGM_100_2008_E.pdf (accessed Oct 2019).

[4] Efron, B.; Tibshirani, R. J.; *An Introduction to the Bootstrap*, Chapman & Hall, London, UK (1993).

[5] Sharpless, K.E.; Lippa, K.A.; Duewer, D.L.; Rukhin, A.L.; *The ABCs of Using Standard Reference Materials in the Analysis of Foods and Dietary Supplements: A Practical Guide*; NIST Special Publication 260-181r1; U.S. Government Printing Office: Washington, DC (2014); available at https://www.nist.gov/sites/default/files/documents/srm/SP260-181r1-2.pdf (accessed Oct 2019).

[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:** 07 October 2019 (Removal of ascorbic acid reference value due to instability; discussion of uncertainty updated for all values, including removal of *k* values; editorial changes); 28 March 2016 (Change of expiration date; significant figures added to copper value; editorial changes); 14 September 2010 (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 https://www.nist.gov/srm.*