



# National Institute of Standards & Technology

## Certificate of Analysis

### Standard Reference Material<sup>®</sup> 3286

#### Organic Acids Calibration Solution

This Standard Reference Material (SRM) consists of a solution of organic acids (citric acid, malic acid, quinic acid, shikimic acid, and tartaric acid) in water. SRM 3286 is intended primarily for use in calibration of instruments and techniques used for the determination of these organic acids. A unit of SRM 3286 consists of five 2 mL ampoules, each containing approximately 1.2 mL of the organic acids solution.

Development of SRM 3286 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 values for organic acids presented in Table 1 are based on the masses used in the gravimetric preparation of the solution and from the analytical results determined using liquid chromatography with ultraviolet absorbance detection (LC-UV). 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 are provided in mass fraction units [2].

**Reference Mass Fraction Value:** A reference mass fraction value for fumaric acid is provided in Table 2. A reference value is a non-certified 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 an associated uncertainty 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 3286** is valid, within the measurement uncertainty specified, until **27 January 2022**, 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.

Support for the development of SRM 3286 was provided in part by NIH ODS. Technical consultation was provided by J.M. Betz (NIH ODS).

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

Preparation of the SRM solution and analytical measurements were performed by M.M. Phillips of the NIST Chemical Sciences Division.

Statistical consultation 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.

Carlos A. Gonzalez, Chief  
Chemical Sciences Division

Gaithersburg, MD 20899  
Certificate Issue Date: 24 February 2016  
*Certificate Revision History on Last Page*

Steven J. Choquette, Acting Director  
Office of Reference Materials

## INSTRUCTIONS FOR HANDLING, STORAGE, AND USE

**Handling:** This SRM is an acidic solution contained in tip-sealed borosilicate glass ampoules with pre-scored stems. Therefore, all appropriate safety precautions, including use of gloves during handling, should be taken. Unopened ampoules should be stored under normal laboratory conditions in an upright position inside the original container supplied by NIST.

**Storage:** Sealed ampoules, as received, should be stored in the dark at or below room temperature.

**Use:** Prior to removal of a test portion for analysis, the contents of an ampoule should be mixed thoroughly. Test portions for use should be withdrawn immediately after opening the ampoules, and should be processed or diluted without delay for the certified mass fraction to be valid within the stated uncertainty. The stability of organic acids in opened ampoules has not been investigated.

## PREPARATION AND ANALYSIS<sup>(1)</sup>

**Solution Preparation:** The solution was prepared gravimetrically at NIST from water and primary standards for citric acid, malic acid, quinic acid, shikimic acid, and tartaric acid obtained from Sigma (St. Louis, MO). The solution was mixed overnight (18 h) and aliquoted into 2 mL amber glass ampoules that had been purged with argon prior to addition of the solution. The ampoules were then flame-sealed. The masses of the primary standards and the total mass of the solution were used to calculate the gravimetric mass fractions. The mass fractions were adjusted for the purity estimates of the primary standards, which were determined using LC-UV, LC with evaporative light-scattering detection, differential scanning calorimetry, and Karl Fischer titration.

**Analytical Approach:** Aliquots from 10 ampoules, selected using a stratified random sampling scheme, were analyzed in duplicate using LC-UV at 210 nm. Four independently prepared calibration solutions consisting of citric acid, malic acid, quinic acid, shikimic acid, and tartaric acid were gravimetrically prepared and chromatographically analyzed. Four independently prepared calibration solutions containing fumaric acid were also gravimetrically prepared and chromatographically analyzed. (Fumaric acid is present in the calibration solutions because it was an impurity in the malic acid that was used in preparation of this SRM.) Quantitation of all organic acids was based on an external calibration model. A representative chromatogram and the separation conditions are presented in Figure 1.

**Homogeneity Assessment:** The homogeneity of organic acid mass fractions was assessed at NIST by using the analytical approach described above. An analysis of variance did not show inhomogeneity for the 0.1 g test portions analyzed.

**Value Assignment:** For calculation of assigned values, the mean of the available sets of data were averaged. Certified values are means of mass fractions determined by gravimetric preparation and chromatographic measurements. The reference value is the mean of mass fractions determined by chromatographic measurements only.

Each certified value is a mean of mass fractions determined by gravimetric preparation and LC-UV measurements. 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 two methods and their respective uncertainties, consistent with the ISO/JCGM Guide and with its Supplement 1 [3–5]. The expanded uncertainty is calculated as  $U = ku_c$ , where  $u_c$  is the combined uncertainty, and  $k = 2$  is the coverage factor corresponding to approximately 95 % confidence. The measurand is the total mass fraction of each organic acid in Table 1 and the value is metrologically traceable to the SI derived unit for mass fraction (expressed as milligrams per gram).

Table 1. Certified Mass Fraction Values for Organic Acids in SRM 3286

	Mass Fraction (mg/g)		
Citric Acid	4.925	±	0.054
Malic Acid	4.877	±	0.053
Quinic Acid	4.762	±	0.054
Shikimic Acid	0.482	±	0.005
Tartaric Acid	4.979	±	0.053

<sup>(1)</sup>Certain commercial equipment 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.

The reference mass fraction value is the mean of results provided by LC-UV. The uncertainty provided with the value is an expanded uncertainty about the mean to cover the measurand with approximately 95 % confidence; it expresses within-method uncertainty, consistent with the ISO/JCGM Guide [3]. The expanded uncertainty is calculated as  $U = ku_c$ , where  $u_c$  is the combined uncertainty, and  $k = 2.1$  is the coverage factor corresponding to approximately 95 % confidence. The measurand is the mass fraction value of fumaric acid as determined by LC-UV and the value is metrologically traceable to the SI derived unit for mass fraction (expressed as milligrams per gram).

Table 2. Reference Mass Fraction Values for Organic Acids in SRM 3286

	Mass Fraction (mg/g)
Fumaric Acid	0.0675 ± 0.0008

An LC-UV chromatogram of SRM 3286 Organic Acids Calibration Solution is shown in the figure below.

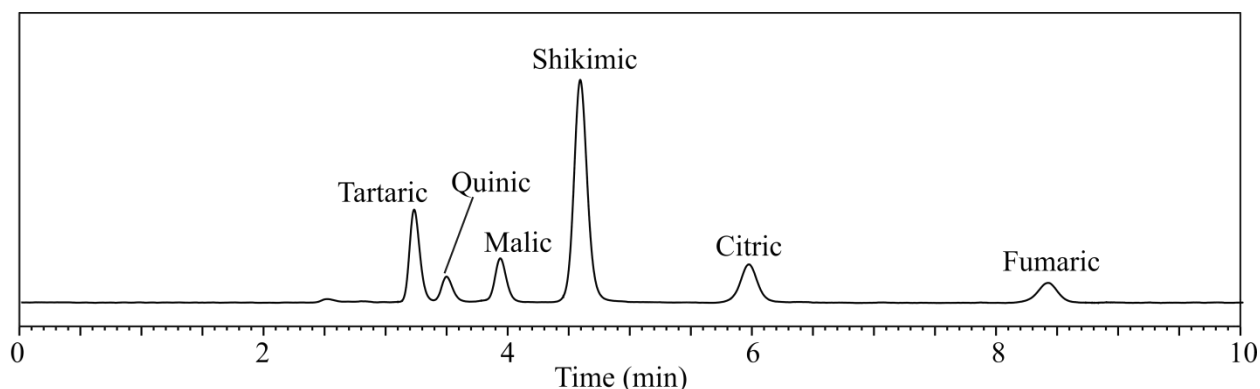


Figure 1. The chromatogram was obtained using an Allure Organic Acids column (Restek Corporation, Bellefonte, PA) with dimensions of 250 mm × 4.6 mm ID and containing 5 µm diameter particles with an isocratic mobile phase of 25 mmol/L potassium phosphate monobasic at pH 2.0 and UV absorbance detection at 210 nm.

## REFERENCES

- [1] May, W.; Parris, R.; Beck, C.; Fassett, J.; Greenberg, R.; Guenther, F.; Kramer, G.; Wise, S.; Gills, T.; Colbert, J.; Gettings, R.; MacDonald, B.; *Definitions 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 <http://www.nist.gov/srm/publications.cfm> (accessed Feb 2016).
- [2] 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/index.cfm/> (accessed Feb 2016).
- [3] 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 (JCGM) (2008); available at [http://www.bipm.org/utis/common/documents/jcgm/JCGM\\_100\\_2008\\_E.pdf](http://www.bipm.org/utis/common/documents/jcgm/JCGM_100_2008_E.pdf) (accessed Feb 2016); 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/index.cfm> (accessed Feb 2016).
- [4] JCGM 101:2008; *Evaluation of measurement data – Supplement 1 to the Guide to the Expression of Uncertainty in Measurement – Propagation of Distributions Using a Monte Carlo Method*; Joint Committee for Guides in Metrology (JCGM) (2008); available at [http://www.bipm.org/utis/common/documents/jcgm/JCGM\\_101\\_2008\\_E.pdf](http://www.bipm.org/utis/common/documents/jcgm/JCGM_101_2008_E.pdf) (accessed Feb 2016).
- [5] Efron, B.; Tibshirani, R.J.; *An Introduction to the Bootstrap*; Chapman & Hall, London, UK (1993).

**Certificate Revision History:** 24 February 2016 (Change of expiration date; editorial changes); 01 September 2015 (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](mailto:srminfo@nist.gov); or via the Internet at <http://www.nist.gov/srm>.