National Institute of Standards and Technology
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Standard Reference Material® 2389a
Amino Acids in 0.1 mol/L Hydrochloric Acid
CERTIFICATE OF ANALYSIS

Purpose: The certified values delivered by this Standard Reference Material (SRM) are intended primarily for use in calibration of chromatographic instrumentation for the determination of amino acids.

Description: SRM 2389a is a solution of amino acids in a 0.1 mol/L aqueous solution of hydrochloric acid. A unit of SRM 2389a consists of five 2 mL ampoules, each containing approximately 1.2 mL of the solution under argon.

Certified Values: The certified concentrations and estimated uncertainties for the 16 amino acids provided in Table 1 are based on the results obtained from the gravimetric preparation of the solutions and from the analytical results determined using liquid chromatography-tandem mass spectrometry (LC-MS/MS). 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]. These values are traceable to the International System of Units (SI). Values are provided in mass fraction units (milligrams per gram), and, for user convenience, in amount-of-substance concentrations (millimoles per liter) [2]. The amount-of-substance concentrations were calculated from the mass fraction values using the density of the solution determined at 20 °C (g/mL) and the relative molecular masses of each amino acid. An allowance for the change in density over the range 18 °C to 23 °C is included in the uncertainty.

Additional Information: Preparation and analysis methods for SRM 2389a are provided in Appendix A.

Table 1. Certified Values for Amino Acids in SRM 2389a

Amino Acid	Mass Fraction[(a)] (mg/g)	Concentration[(b)] (mmol/L)	Amino Acid	Mass Fraction[(a)] (mg/g)	Concentration[(b)] (mmol/L)
Alanine	0.223 ± 0.007	2.50 ± 0.07	Lysine	0.353 ± 0.024	2.41 ± 0.17
Arginine	0.436 ± 0.012	2.51 ± 0.07	Methionine	0.373 ± 0.011	2.51 ± 0.07
Aspartic acid	0.333 ± 0.010	2.50 ± 0.08	Phenylalanine	0.421 ± 0.014	2.55 ± 0.09
Cystine	0.295 ± 0.013	1.23 ± 0.06	Proline	0.282 ± 0.013	2.46 ± 0.11
Glycine	0.189 ± 0.005	2.52 ± 0.07	Serine	0.256 ± 0.012	2.44 ± 0.11
Histidine	0.390 ± 0.011	2.52 ± 0.07	Threonine	0.296 ± 0.009	2.49 ± 0.07
Isoleucine	0.320 ± 0.015	2.44 ± 0.11	Tyrosine	0.459 ± 0.014	2.54 ± 0.08
Leucine	0.319 ± 0.014	2.44 ± 0.11	Valine	0.293 ± 0.012	2.51 ± 0.10

[(a)] The results are expressed as the certified value ± the expanded uncertainty. Each result is the average of the gravimetric and the LC-MS/MS means. 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 uncertainty components for purity correction and gravimetry, consistently with the ISO/JCGM Guide and with its Supplement 1 [3–5]. The certified values are metrologically traceable to the International System of Units (SI)-derived unit for mass fraction (expressed as milligrams per gram).

[(b)] The amount-of-substance concentrations (mmol/L) were obtained by multiplying the certified values in mass fraction units by the density of the SRM solution at 20 °C and dividing by the relative molecular masses of each of the compounds. These concentrations are for use in the temperature range of 18 °C to 23 °C, and an allowance for the change in density over this temperature range is included in the Type B components of uncertainty.

Period of Validity: The certified values delivered by **SRM 2389a** are valid within the measurement uncertainty specified until **01 January 2029**. The certified values are nullified if the material is stored or used improperly, damaged, contaminated, or otherwise modified.

Michael J. Tarlov, Chief
Biomolecular Measurement Division

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Office of Reference Materials

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Maintenance of Certified Values: NIST will monitor this SRM over the period of its validity. If substantive technical changes occur that affect the certification, NIST will issue an amended certificate through the NIST SRM website (https://www.nist.gov/srm) and notify registered users. SRM users can register online from a link available on the NIST SRM website or fill out the user registration form that is supplied with the SRM. Registration will facilitate notification. Before making use of any of the values delivered by this material, users should verify they have the most recent version of this documentation, available through the NIST SRM website (https://www.nist.gov/srm).

Safety: SRM 2389a is intended for research use. This material contains 0.1 mol/L hydrochloric acid and should be handled with care. Use proper disposal methods.

Storage: Sealed ampoules, as received, should be stored in the dark at approximately 4 °C.

Use: Prior to removal of the test portion for analysis, the contents of an ampoule of material should be allowed to warm to room temperature (18 °C to 23 °C). Test portions for use should be withdrawn immediately after opening the ampoules and should be processed or diluted without delay for the certified concentration to be valid within the stated uncertainty. The certified concentration values listed in Table 1 apply only to aliquots removed at 18 °C to 23 °C.

REFERENCES

[1] Beauchamp, C.R.; Camara, J.E.; Carney, J.; Choquette, S.J.; Cole, K.D.; DeRose, P.C.; Duewer, D.L.; Epstein, M.S.; Kline, M.C.; Lippa, K.A.; Lucon, E.; Molloy, J.; Nelson, M.A.; Phinney, K.W.; Polakoski, M.; Possolo, A.; Sander, L.C.; Schiel, J.E.; Sharpless, K.E.; Toman, B.; Winchester, M.R.; Windover, D.; *Metrological Tools for the Reference Materials and Reference Instruments of the NIST Material Measurement Laboratory*; NIST Special Publication 260-136, 2021 edition; National Institute of Standards and Technology, Gaithersburg, MD (2021); available at https://nvlpubs.nist.gov/nistpubs/SpecialPublications/NIST.SP.260-136-2021.pdf (accessed Sep 2023).

[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 https://www.nist.gov/pml/special-publication-811 (accessed Sep 2023).

[3] JCGM 100:2008; *Evaluation of Measurement Data — Guide to the Expression of Uncertainty in Measurement* (ISO GUM 1995 with Minor Corrections); Joint Committee for Guides in Metrology (2008); available at https://www.bipm.org/en/publications/guides (accessed Sep 2023); 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 Sep 2023).

[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 https://www.bipm.org/en/publications/guides (accessed Sep 2023).

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

Certificate Revision History: 29 September 2023 (Change of period of validity; updated format; editorial changes); **02 June 2021** (Editorial changes); **17 June 2019** (Removed certified value for glutamic acid; editorial changes); **06 December 2018** (Change of expiration date; editorial changes); **01 July 2010** (Original certificate date).

Certain commercial equipment, instruments, or materials may be identified in this Certificate of Analysis 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.

Users of this SRM should ensure that the Certificate of Analysis in their possession is current. This can be accomplished by contacting the Office of Reference Materials 100 Bureau Drive, Stop 2300, Gaithersburg, MD 20899-2300; telephone (301) 975-2200; e-mail srminfo@nist.gov; or the Internet at https://www.nist.gov/srm.

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APPENDIX A

Preparation of Material: All chemicals used in the preparation of this SRM were of the highest purity available and were obtained from a commercial source. The amino acid solution was prepared by weighing the individual amino acids, concentrated HCl, and water and mixing until the amino acids were completely dissolved. The total mass of this solution was measured. The concentration of each amino acid was calculated using the measured density of the 0.1 mol/L HCl solution at 20 °C. Corrections were made to the calculated amino acid concentrations based on purity as determined by LC with absorbance detection with confirmatory data provided by the manufacturer. The purity of each amino acid was also evaluated by elemental analysis at Galbraith Laboratories (Knoxville, TN). Moisture content for all amino acids was determined using Karl Fischer titration at Galbraith Laboratories and for lysine at NIST. Arginine and lysine contained measurable levels of water and values were corrected; correction for moisture was not necessary for the remaining amino acids.

The bulk solution was dispensed in 1.2 mL aliquots into argon-flushed 2 mL amber ampoules that were then flame sealed and stored in numbered boxes at ≈ 4 °C.

LC-MS/MS Measurements: Three aliquots from four randomly selected ampoules were analyzed in duplicate by LC-MS/MS on a SIELC (Prospect Heights, IL) Primesep 100 mixed-mode LC column. Chromatographic separation was performed using microflow liquid chromatography coupled in-line to a triple quadrupole mass spectrometer equipped with a standard source. Data were acquired through multiple reaction monitoring of amino acid precursor-to-product ion transitions. Unique isotopically-labeled internal standards were used for quantification of each amino acid. Internal standards and calibrants were prepared gravimetrically in 0.1 mol/L HCl as stock solutions and were further diluted gravimetrically to working concentrations. Internal standards were mixed gravimetrically with calibrants and were measured quantitatively by LC-MS/MS to determine the calibration curves. The same internal standard solution was mixed with aliquots of SRM 2389a and was analyzed in an identical manner. Mass fractions of the amino acids in SRM 2389a were determined by interpolation of integrated peak area ratios through calibration curves created from multiple reaction monitoring analysis of the calibrants.

Preparation of the SRM solution was performed in the NIST Chemical Sciences Division by L.T. Sniegoski and M.J. Welch, formerly of NIST. Analytical measurements were performed by M.S. Lowenthal of the NIST Bioanalytical Science Group, B.E. Lang of the NIST Chemical Sciences Division, and B.S. Benford formerly of NIST.

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