National Institute of Standards & Technology

Report of Investigation

Reference Material 8573

L-glutamic Acid USGS40

(Light Carbon and Nitrogen Isotopes in L-glutamic Acid)

This Reference Material (RM) is intended to aid in normalizing isotope-amount ratio data as well as developing and validating methods for measuring the relative differences in carbon (C) and nitrogen (N) isotope-amount ratios in biological materials. It should be used in conjunction with RM 8574 (Heavy Carbon and Nitrogen Isotopes in L-glutamic acid).

L-glutamic acid is a chemically stable compound with a C/N mole ratio of 5, similar to many natural biological substances, such as blood and animal tissue. The equivalent name for this RM, as used by the International Atomic Energy Agency (IAEA) and the U.S. Geological Survey (USGS), is USGS40 and is listed in column 2 of Table 1. A unit of RM 8573 consists of one bottle containing approximately 1 g of L-glutamic acid.

Table 1. Reference Values and Expanded Uncertainties for the Relative C and N Isotope-Amount Ratios of RM 8573

RM Number	Name	$\delta^{13}C_{VPDB}$ (a,b) × 10^3	$\delta^{15}N_{AIR}$ (a) × 10^3
RM 8573	USGS40	–26.39 ± 0.09 ‰	–4.52 ± 0.12 ‰

(a) The $\delta^{13}C$ and $\delta^{15}N$ values are expressed as the mean ± the expanded uncertainty. The expanded uncertainty in these values is equal to $U = ku_c$, where u_c is the combined standard uncertainty calculated according to the ISO and NIST Guides [1] and k is the coverage factor. The value of u_c is intended to represent, at the level of one standard deviation, the combined effect of the uncertainties in the reference value. Uncertainty in the bias of the method is not included. Here u_c is given by the standard uncertainty of the mean of the values. The coverage factor k is the Student's t-value for the 95 % confidence interval with three degrees of freedom.

(b) Normalized to $\delta^{13}C_{VPDB}$ of RM 8545 (L-SVEC) = –46.6 ‰.

Expiration Date of Reference Material: This reference material is valid, within the measurement uncertainty specified, until **31 December 2020,** provided the RM is handled in accordance with instructions given in this Report of Investigation (see "Instructions for Use"). These reference values are nullified if the RM is damaged, contaminated, or modified.

Maintenance of this Report: NIST will monitor this RM. If substantive technical changes occur that affect the value assignment before the expiration of this report, NIST will notify the purchaser. Registration (see attached sheet) will facilitate notification.

The coordination of the technical measurements was under the direction of T.B. Coplen of the U.S. Geological Survey, W.A. Brand of the Max-Planck-Institute for Biogeochemistry, Jena, Germany, and M. Gröning of the International Atomic Energy Agency, Vienna, Austria.

This Report of Investigation was prepared by T.B. Coplen and R.D. Vocke, Jr.

The technical aspects involved in the issuance of this RM were coordinated through the NIST Analytical Chemistry Division by R.D. Vocke, Jr.

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Preparation of the bulk material and the analytical measurements leading to the δ^{15}N reference value were performed by H. Qi, T.B. Coplen, and J.K. Böhlke of the U.S. Geological Survey and W.A. Brand and H. Geilmann of the Max-Planck-Institute for Biogeochemistry, Jena, Germany [2]. Analytical measurements leading to the δ^{13}C reference value were performed by H. Qi and T.B. Coplen of the U.S. Geological Survey, W.A. Brand and H. Geilmann of the Max-Planck-Institute for Biogeochemistry, Jena, Germany, M. Gehre and U. Guenther of the UFZ Umweltforschungszentrum Leipzig-Halle GmbH, Leipzig, Germany, and H.A.J. Meijer, and A. Aerts of the Centrum voor Isotopen Onderzoek, Rijksuniversiteit Groningen, Groningen, Netherlands. Isotopic homogenization and bottling of material was performed by M.P. Cronise and C.N. Fales of the NIST Measurement Services Division.

Consultation on evaluation of uncertainties was provided by R.M. Verkouteren of the NIST Surface and Microanalysis Science Division and B. Toman of the NIST Statistical Engineering Division.

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

Reference Difference in Isotope-Amount Ratio Values: The differences in measured isotope-amount ratios of stable carbon isotopes $[n(^{13}\mathrm{C})/n(^{12}\mathrm{C})]$ are reported as δ^{13}C values, where $\delta^{13}\mathrm{C} = ([n_{\mathrm{sample}}(^{13}\mathrm{C})/n_{\mathrm{sample}}(^{12}\mathrm{C})] - [n_{\mathrm{VPDB}}(^{13}\mathrm{C})/n_{\mathrm{VPDB}}(^{12}\mathrm{C})])/\ [n_{\mathrm{VPDB}}(^{13}\mathrm{C})/n_{\mathrm{VPDB}}(^{12}\mathrm{C})]$. VPDB refers to the Vienna PDB scale, which is determined by assigning a δ^{13}C value of +1.95 ‰ to RM 8544 (NBS 19) [3]. The reported δ^{13}C result for the L-glutamic acid sample has been normalized to yield a consensus value of –46.6 ‰ for RM 8545.

The differences in measured nitrogen isotope-amount ratios $[n(^{15}\mathrm{N})/n(^{14}\mathrm{N})]$ are reported as δ^{15}N values: $\delta^{15}\mathrm{N} = ([n_{\mathrm{sample}}(^{15}\mathrm{N})/n_{\mathrm{sample}}(^{14}\mathrm{N})] - [n_{\mathrm{AIR}}(^{15}\mathrm{N})/n_{\mathrm{AIR}}(^{14}\mathrm{N})])/[n_{\mathrm{AIR}}(^{15}\mathrm{N})/n_{\mathrm{AIR}}(^{14}\mathrm{N})]$. AIR refers to tropospheric N_2, for which $n_{\mathrm{AIR}}(^{15}\mathrm{N})/n_{\mathrm{AIR}}(^{14}\mathrm{N}) = 0.003677$ [4]. The reported δ^{15}N results for nitrate samples have been normalized to yield a value of +180 ‰ for RM 8558 (USGS32) and a value of +0.43 ‰ for RM 8547 (IAEA-N-1). The recommended values for δ^{13}C and δ^{15}N for RM 8573 listed in Table 1, together with their expanded uncertainties, are based on the continuous flow isotope-ratio mass spectrometry method [5], implemented by Qi *et al.* [2] and Coplen *et al.* [6]. A reference value is a non-certified value that is the best estimate of the true value; however, the value may reflect only the measurement precision and may not include all sources of uncertainty.

INSTRUCTIONS FOR USE

Distribution: The distribution of RM 8573 (USGS40) is limited to one unit per three year period of time. One unit of RM 8573 can provide two thousand 500 µg sample analyses using continuous flow isotope-ratio mass spectrometry. With this method, users are advised to intersperse it routinely with sample unknowns.

Handling: Users are advised to prepare L-glutamic acid reference materials for daily laboratory use using the methods of Qi *et al.* [2] and to calibrate those materials with this RM and RM 8574.

Storage and Stability: L-glutamic acid is stable at ambient temperature and has no specific storage requirements.

Use: Following the analytical method below, users can make L-glutamic acid reference materials with a variety of isotopic compositions for local laboratory use. Users are cautioned to use only L-glutamic acid and not mixtures of L-glutamic acid and D-glutamic acid. If both materials are put into solution together, they exsolve upon precipitation, giving rise to a substantial isotopic inhomogeneity within the precipitate.

Normalization: The δ^{13}C value is expressed as parts per thousand (‰) difference from the Vienna PDB (VPDB) isotope scale, which is determined by assigning a δ^{13}C value of +1.95 ‰ to RM 8544 (NBS 19) [3]. Following the recommendations of the International Union of Pure and Applied Chemistry (IUPAC) and the International Atomic Energy Agency (IAEA), the L-glutamic acid RM was normalized by using a consensus value of –46.6 ‰ for RM 8545 [6]. RM 8545 was assigned a δ^{13}C consensus value of –46.6 ‰ based on high accuracy dual-inlet mass spectrometry measurements (optimized to minimize memory and isotopic fractionation) by Ghosh *et al.*[7], who determined a value of –46.607 ‰ ± 0.057 ‰ (u_c), and by Verkouteren and Klinedinst [8] who calculated a dual-inlet value of –46.57 ‰ ± 0.13 ‰ (u_c) based on the relationship between δ^{17}O and δ^{18}O of 0.528 [9] and a $n(^{17}\mathrm{O})/n(^{16}\mathrm{O})$ ratio in VSMOW reference water of 0.000386913 [10]. Generalized equations for normalization are presented by Coplen [11] and a database program for assisting with normalization is available [12]. Users are advised to report measured or assumed values for reference materials when reporting isotopic data for sample unknowns.

The $\delta^{15}N$ value is expressed as parts per thousand (‰) differences from tropospheric N_2, which is isotopically homogeneous [13]. Many investigators normalize their $\delta^{15}N$ results by assigning a value of +180 ‰ to RM 8558 [14] and a value of +0.43 ‰ to RM 8547 [2].

PREPARATION AND ANALYSIS[1]

Preparation: Twelve 1-kg bottles of L-glutamic acid ($C_5H_9NO_4$) with a chemical purity of 99+ % were purchased from Aldrich Chemical Co (catalog number 12,843-0). This material was used to prepare RM 8573. The material was first assessed for isotopic homogeneity by collecting 6 aliquots from each bottle, 2 from the top, 2 from the center, and 2 from the bottom. The 1-σ standard deviation for $\delta^{13}C$ measurements was ±0.06 ‰ and that for $\delta^{15}N$ measurements was ±0.05 ‰ for aliquots that weighed 760 µg. Subsequently, eleven 1 kg bottles of this L-glutamic acid were sieved through a 70-mesh (<210 µm) sieve. The material with grain sizes greater than 210 µm was discarded. The remainder was homogenized by tumbling for 10 h and then split into 500 g fractions using a stainless-steel splitting device and bottled.

Analytical Methods: Off-line carbon isotopic analyses were performed on CO_2 gas following sealed tube combustion procedures [15] and reported by Qi *et al.* [2]. Aliquots of RM 8573, RM 8574, and RM 8539 (approximately 23 µmol C per aliquot) were weighed into silver capsules. The capsules were placed in quartz tubes that were loaded with about 6 g CuO wire. The tubes were evacuated, sealed, and baked at 850 °C for 4 h. After cooling, gas from each tube was introduced into a vacuum line, and the CO_2 was extracted and purified. The CO_2 was analyzed on a DuPont isotope-ratio mass spectrometer [16]. The data were expressed relative to the VPDB scale by adopting a $\delta^{13}C$ value of +1.95‰ for RM 8544 [3] and normalizing to a $\delta^{13}C$ value of –46.48 ‰ for RM 8545 [17].

Off-line nitrogen isotopic analyses were performed on N_2-gas samples following the low-blank, sealed-tube combustion procedure of Böhlke *et al.* [18]. Aliquots of RM 8573 and RM 8574 (approximately 23 µmol N per aliquot) were loaded into quartz glass tubes with 1.5 g of Cu + Cu_2O mixture and 0.3 g of pre-baked CaO. The tubes were evacuated, sealed, baked at 850 °C for 2 h to produce N_2, and cooled at 40 °C/h to ambient temperature (modified from Kendall and Grim [19]). The N_2 gas was analyzed using a Finnigan MAT 251 isotope-ratio mass spectrometer. The new reference materials were analyzed along with aliquots of the international reference materials RM 8547 (ammonium sulfate, assigned $\delta^{15}N$ = +0.43 ‰ [14]) and RM 8558 (potassium nitrate, assigned $\delta^{15}N$ = +180 ‰ [14]), which were used to normalize the results. The average deviation from the mean for individual analyses of various reference materials was ~0.05‰ after normalizing. Aliquots of RM 8548 (ammonium sulfate), RM 8550 (ammonium sulfate), and RM 8551 (ammonium sulfate) also were analyzed in the same sample sets.

At the U.S. Geological Survey, Reston, Virginia, the new reference material and international reference materials were weighed into tin capsules (Costech 3.5 × 5 mm) and introduced sequentially into an elemental analyzer (Carlo Erba, NA2500) for on-line combustion continuous flow isotope-ratio mass spectrometry. Individual samples were combusted and converted into CO_2 and N_2 at an oxidation furnace temperature of 1020 °C and a reduction furnace temperature of 650 °C. CO_2 and N_2 were transferred with 99.999 % He carrier gas at flow rate of 95 mL/min and were separated by a GC column (Costech 051082) maintained at a temperature of 50 °C. The gases then were transferred to a Finnigan Conflo II interface and into a Finnigan Delta Plus isotope-ratio mass spectrometer. The mass spectrometers used for continuous flow measurements in Reston and in Jena are equipped with ion sources that reduce effects of sputtering and inter-peak memory and thus minimize scale contraction [20].

For on-line $\delta^{15}N$ analyses only, 3 aliquots of each sample containing 72 ± 1 µg N were analyzed sequentially in the order RM 8547, RM 8573, RM 8548, RM 8574, RM 8558, RM 8551, and RM 8550. This order was repeated once. A blank consisting of an empty 3.5 × 5 mm tin capsule was analyzed before, during, and after the sequence. Blank correction was applied to all on-line results. The $\delta^{15}N$ value used for blank correction was assumed to be 0 ‰; it generally is so small that it is difficult to quantify its nitrogen isotopic composition.

For on-line $\delta^{13}C$ analyses only, 3 aliquots of each sample containing 310 ± 4 µg C were analyzed sequentially in the order RM 8539, RM 8573, RM 8574, RM 8544, RM 8545, and IAEA-CO-1, followed by a repeat of this order a second time. Thus, in a complete sequence, 6 aliquots of each sample were analyzed. Because calibrations against RM 8544 and RM 8545 may have unknown biases resulting from different behavior of organic and inorganic material in the on-line combustion system, three sets of experiments were performed: (1) All samples (sample amount = ~ 310 µg carbon) were analyzed with an oxidation furnace temperature of 1020 °C, a reduction furnace temperature of 650 °C, and a GC

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[1] Certain commercial equipment, instruments, or materials are identified in this certificate in order 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.

temperature of 50 °C, and with He dilution; (2) Same as above, except the oxidation furnace temperature was increased to 1050 °C; (3) The amount of sample was reduced to 82 µg carbon with an oxidation furnace temperature of 1020 °C and with low He dilution. Blank correction was applied to all the observed on-line results. The δ^{13}C value of the blank was assumed to be -25.0 ‰. The value generally was so small that it is difficult to quantify.

At the Max-Planck-Institute in Jena, the measurement procedures for both δ^{13}C and δ^{15}N on-line analysis were only slightly different from U.S. Geological Survey procedures in Reston, VA. The samples were wrapped in tin capsules from IVA, Viersen (Germany), and combusted in a CE 1100 elemental analyzer with a He carrier gas flow rate of 85 mL/min. The combustion temperature was set to 1020 °C and the reduction furnace was kept at 650 °C. The Porapak PQ 3.5 m GC column (80/100 mesh) was maintained at 40 °C. The product gases were transferred to the mass spectrometer (Finnigan MAT Delta^{+}XL) via a ConFlo III interface [21], operated in dilution mode for δ^{13}C and in undiluted mode for δ^{15}N analysis. For measurement of the RM 8573 and RM 8574 glutamic acid reference materials, analysis sequences included between 2 and 4 aliquots of local quality control materials, blanks, and reference materials RM 8544, RM 8539, RM 8545, and IAEA-CO-1 for δ^{13}C. The international reference materials included in the δ^{15}N analysis sequence were RM 8547, RM 8548, RM 8558, RM 8551, and RM 8550. Details of data handling, blank correction and scaling can be found in Werner and Brand [22].

Following the methods of Qi *et al.* [2], calibration of stable carbon isotopic reference materials was organized by M. Gröning of the International Atomic Energy Agency. Four laboratories (Centrum voor Isotopen Onderzoek, Groningen, Netherlands; Max-Planck-Institute for Biogeochemistry, Jena, Germany; UFZ Leipzig-Halle, Leipzig, Germany; U.S. Geological Survey, Reston, Virginia) performed analytical measurements [6]. Participants at NIST headed the task to estimate consensus means and uncertainties using the Bayesian method [6].

Homogeneity: The δ^{13}C and δ^{15}N homogeneities of this RM, for sample amounts as small as 100 µg, were assessed by taking the mean of the standard deviation of the measurements and were determined to be better than 0.13 ‰ and 0.13 ‰, respectively [2].

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