United States Geological Survey

Reston Stable Isotope Laboratory

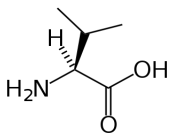
Report of Stable Isotopic Composition

Reference Materials USGS73, USGS74, and USGS75

(Carbon and Nitrogen Isotopes in L-Valine)

These reference materials (RMs) currently are intended for normalization of stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) measurements of unknown L-valines and similarly-behaving carbon- and nitrogen-bearing substances. At a future time, the stable hydrogen isotopic compositions ($\delta^{2}H$) of the non-exchangeable hydrogen in these RMs will be determined, but currently these values are not available. A unit of USGS73 consists of 0.5 g powdered RM. A unit of USGS74 and USGS75 consists of 0.1 g powdered RM. There is no limit on distribution. These RMs were prepared by A. Schimmelmann (Indiana University, Bloomington, Indiana).

Recommended values: Stable carbon isotopic compositions are expressed herein as delta values [1] relative to VPDB (Vienna Peedee belemnite) on a scale normalized such that the $\delta^{13}C$ values of NBS 19 calcium carbonate and LSVEC lithium carbonate are +1.95 ‰ and –46.6 ‰, respectively [2]. Stable nitrogen isotopic compositions are expressed relative to atmospheric nitrogen, which is isotopically homogenous [3]. On this scale, the $\delta^{15}N_{AIR\text{-}N_2}$ value of USGS32 KNO_3 is +180 ‰ exactly. Stable carbon- and nitrogen-isotope delta values of USGS73, USGS74, and USGS75 L-valines with combined standard uncertainties are:

Reference	Structure	$\delta^{13}C_{VPDB\text{-}LSVEC}$	$\delta^{15}N_{AIR\text{-}N_2}$	Data source
USGS73		–24.03 ± 0.04 ‰	–5.21 ± 0.05 ‰	[4]
USGS74		–9.30 ± 0.04 ‰	+30.19 ± 0.07 ‰	[4]
USGS75		+0.49 ± 0.07 ‰	+61.53 ± 0.14 ‰	[4]

Technical coordination for this RM was provided by Arndt Schimmelmann of Indiana University and Haiping Qi of the U.S. Geological Survey Reston Stable Isotope Laboratory (RSIL).

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September 11, 2026

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Expiration of Reference Value: The reference values for the isotopic compositions of USGS73, USGS74, and USGS75 are valid until December 31, 2036, provided these RMs are handled in accordance with the instructions given in this Report of Stable Isotopic Composition (see "Instructions for Use"). A reference value is nullified if the RM is damaged, contaminated, or otherwise modified.

Source of the RM: The following description is taken from Schimmelmann and others [4]. Four kilograms of L-valine ($C_5H_{11}NO_2$, CAS #72-18-4) were donated by the Amino GmbH (Frellstedt, Germany). The L-valine was mixed in an industrial-strength blender without adding any isotopic spikes, was sieved through a 40-mesh-metal sieve and was labeled USGS73. The two ^{2}H, ^{13}C, and ^{15}N-enriched L-valines, USGS74 and USGS75, were prepared from small additions of isotopically spiked L-valines to amounts of 2 and 1.6 kg of unspiked L-valine from Alfa-Aesar, respectively. The following spikes were used: (i) L-valine-3d1, ^{2}H fraction = 98 %, Cambridge Isotope Laboratories, Inc., (ii) L-valine-2-d1, ^{2}H fraction = 99 %, CDN Isotopes, (iii) L-valine-2-^{13}C, ^{13}C fraction ≥ 99 %, Isotech, (iv) L-valine-1-^{13}C, ^{13}C fraction = 99 %, Cambridge Isotope Laboratories, Inc., and (v) L-valine-^{15}N, ^{15}N fraction = 98 %, Aldrich. Unlike glycine, the lower solubility of L-valine made it impractical to dissolve kilogram amounts in ultrapure water. Instead, for L-valines USGS74 and USGS75, only the small amounts of isotopic spikes were jointly dissolved in ultrapure water and added to powdered, water-wet regular L-valine. Each paste was stirred for 5 hours and subsequently extruded as strings into liquid nitrogen. The frozen strings were freeze-dried, mixed in an industrial-strength blender, sieved through a 40-mesh-metal sieve, and crushed in a stainless-steel, refrigerated shatter box. Dry powder was finally tumbled for 12 weeks with stainless steel balls under nitrogen in rotating metal cans until the medium-enriched L-valine USGS74 was demonstrated to be sufficiently homogeneous at the 0.1 mg level. In spite of tumbling for 6 months, the highly enriched L-valine USGS75 only reached acceptable isotopic homogeneity at the 0.2 mg level. In order to approach bulk isotopic mean values, 3 g of the tumbled L-valine #3 (USGS75) were dissolved in ultrapure water, dripped into liquid nitrogen, and freeze-dried. Aliquots were sent to participating laboratories [4]. The purity of the L-valines was evaluated at JAMSTEC using ^{1}H NMR. The purity is 99.999 % for USGS73 from the Amino GmbH, 99.54 % for USGS74, and 99.50 % for USGS75. Variation in purity may reflect a combination of the different Alfa Aesar raw material batches, the added isotopic spikes, and the extended homogenization efforts. At 99.5 % purity, the magnitude of contamination remains limited. Bulk $\delta^{13}C$ and $\delta^{15}N$ values increase in the order from USGS73, USGS74, to USGS75. L-valines USGS74 and USGS75 feature contrasting ^{13}C-enrichments at different molecular sites (i.e. carbon atoms in positions1, 2, and 3). Future methods for site-specific carbon and hydrogen isotopic characterization may find value in the USGS74 ^{13}C-spike ratio 2:1 for C-molecular positions 2 and 1. In contrast, USGS75 has a ^{13}C-spike ratio of 1:2 for C-molecular positions 2 and 1. Aliquots of L-valines were flame-sealed under vacuum into round-bottom Pyrex® flasks. Users will receive aliquots of USGS73 of 0.5 g and of USGS74 and USGS75 of 0.1 g in glass vials as RMs for $\delta^{13}C$ and $\delta^{15}N$ normalization. Tumbled L-valine USGS75 has limited homogeneity below a sample size of 0.2 mg.

Maintenance of RM Report of Isotopic Composition: The U.S. Geological Survey RSIL will monitor these RMs and will notify the purchaser if substantive technical changes occur that affect their isotopic compositions.

Distribution and Stability: A distribution unit of USGS73 is available in amounts of 0.5 g in a glass vial that is vacuum sealed in a plastic pouch. Distribution units of USGS74 and USGS75 are available in amounts of 0.1 g in glass vials that are vacuum sealed in plastic pouches. USGS73, USGS74, and USGS75 are stable at normal room temperatures when stored under dry conditions. To minimize the potential for contamination, it is recommended that these RMs be stored in the container in which they were supplied. Storing in a dark and cool place is preferred.

Instructions for Use: Ideally, users may choose to use USGS73, along with USGS74 or USGS75 to make isotope-ratio-scale adjustments. A pair of these RMs (or all three) can be used at the beginning, the middle, and the end of the analysis sequence to enable satisfactory scale correction and correction of drift with time. These RMs can be interspersed among every 10–15 unknowns. To prevent these RMs from degrading over time, after they are opened, it is recommended that users always close the cap tightly after usage and store in a dry desiccator or in a refrigerator.

Reporting of stable-isotope-delta values: The following recommendations are provided for reporting stable carbon and nitrogen isotope-delta values. It is recommended that:

- The $\delta^{13}C$ values of all carbon-bearing substances be expressed relative to VPDB-LSVEC on a scale such that the $\delta^{13}C$ values of NBS 19 calcium carbonate and LSVEC lithium carbonate are +1.95 ‰ and –46.6 ‰, respectively [2], even though LSVEC is no longer recommended as a RM for $\delta^{13}C$ measurement [6].
- The $\delta^{15}N$ values of all nitrogen-bearing substances be expressed relative to atmospheric nitrogen [3].
- Authors report delta values of international distributed (secondary) isotopic reference materials as though they had been interspersed among and used for normalization of unknowns, as appropriate for the measurement method. In this manner, measurement results can be adjusted in the future as analytical methods improve and consensus values of internationally distributed isotopic reference materials change.
- Reporting of delta values relative to PDB (Peedee belemnite) be discontinued [7].

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