United States Geological Survey

Reston Stable Isotope Laboratory

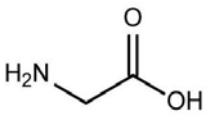
Report of Stable Isotopic Composition

Reference Materials USGS64, USGS65, and USGS66

(Carbon and Nitrogen Isotopes in Glycine)

These reference materials (RMs) currently are intended for normalization of stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) measurements of unknown glycines 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 consists of 0.5 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 USGS64, USGS65, and USGS66 glycines with combined standard uncertainties are:

Reference	Structure	$\delta^{13}C_{VPDB\text{-}LSVEC}$	$\delta^{15}N_{AIR\text{-}N_2}$	Data source
USGS64		–40.81 ± 0.04	+1.76 ± 0.06	[4]
USGS65		–20.29 ± 0.04	+20.68 ± 0.06	[4]
USGS66		–0.67 ± 0.04	+40.83 ± 0.06	[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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Source of the RM: The following description is taken from Schimmelmann and others [4]. Ten kilograms of glycine were purchased from Acros Organics ($C_2H_5NO_2$, CAS # 56-40-6, purity ≥99 %). Three kilograms were dissolved in ultrapure water and the homogeneous solution dripped through a glass capillary into liquid nitrogen where drops were flash frozen. Freeze-drying these pellets then produced fine-grained glycine (USGS64) with uniform isotopic composition. The two glycines enriched in ^{2}H, ^{13}C, and ^{15}N (USGS65 and USGS66) were prepared by small additions of isotopically spiked glycines to the unspiked original glycine from Acros Organics. The following spikes were purchased from ICON: (i) glycine-2,2-d2, ^{2}H fraction = 98 %, (ii) glycine-1-^{13}C, ^{13}C fraction = 99 %, (iii) glycine-2-^{13}C, ^{13}C fraction = 99 %, and (iv) glycine-^{15}N, ^{15}N fraction = 99 %. The homogeneous solutions of glycines USGS65 and USGS66 in ultrapure water were dripped into liquid nitrogen and freeze-dried as described above. Aliquots of glycines were flame-sealed under vacuum into multiple round-bottom Pyrex® flasks. ^{1}H NMR analyses at JAMSTEC indicated that >99.99 % of total hydrogen in all three RMs is in glycine. Bulk $\delta^{13}C$ and $\delta^{15}N$ values increase in the order from USGS64, USGS65, to USGS66. Glycines USGS65 and USGS66 feature contrasting ^{13}C-enrichments at molecular sites 1 and 2, namely in an atom ratio 1:2 in USGS65 and 2:1 in USGS66 for future use in site-specific carbon isotopic measurements. Users will receive aliquots of 0.5 g in glass vials as RMs for $\delta^{13}C$ and $\delta^{15}N$ normalization.

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 is available in amounts of 0.5 g in a glass vial that is vacuum sealed in a plastic pouch. USGS64, USGS65, and USGS66 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 USGS64, along with USGS65 or USGS66 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 [2,5].
- 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 [6].

REFERENCES

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