

United States Geological Survey
Reston Stable Isotope Laboratory

Report of Stable Isotopic Composition

Reference Materials USGS70, USGS71, and USGS72

(Hydrogen and Carbon Isotopes in Icosanoic Acid

Methyl Esters, C₂₀ FAMES)

These reference materials (RMs) are intended for normalization of stable hydrogen ($\delta^2\text{H}$) and carbon ($\delta^{13}\text{C}$) measurements of unknown icosanoic acid methyl esters ($\text{C}_{20}\text{H}_{39}\text{OOCH}_3$) and similarly-behaving hydrogen- and carbon-bearing substances. A unit of each RM consists of 100 mg of beads in a glass vial. There is no limit on distribution. These RMs were prepared by A. Schimmelmann (Indiana University, Bloomington, Indiana).

Recommended values: Stable hydrogen isotopic compositions are expressed herein as delta values [1] relative to VSMOW (Vienna Standard Mean Ocean Water) on a scale normalized such that the $\delta^2\text{H}$ value of SLAP (Standard Light Antarctic Precipitation) is -428‰ [2,3]. Stable carbon isotopic compositions are expressed herein as delta values relative to VPDB (Vienna Pee Dee belemnite) on a scale normalized such that the $\delta^{13}\text{C}$ values of NBS 19 calcium carbonate and LSVEC lithium carbonate are $+1.95\text{‰}$ and -46.6‰ , respectively [4]. Stable hydrogen- and carbon-isotope delta values of USGS70, USGS71, and USGS72 C₂₀ icosanoic acid methyl esters with combined standard uncertainties are:

Reference	$\delta^2\text{H}_{\text{VSMOW-SLAP}}$	$\delta^{13}\text{C}_{\text{VPDB-LSVEC}}$	Data source
USGS70	$-183.9 \pm 1.4\text{‰}$	$-30.53 \pm 0.04\text{‰}$	[5]
USGS71	$-4.9 \pm 1.0\text{‰}$	$-10.50 \pm 0.03\text{‰}$	[5]
USGS72	$+348.3 \pm 1.5\text{‰}$	$-1.54 \pm 0.03\text{‰}$	[5]

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).

Expiration of Reference Value: The reference values for the isotopic compositions of USGS70, USGS71, and USGS72 are valid until December 31, 2029, 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 [5]. These are also known as methyl eicosanoate or arachidic acid methyl ester ($C_{21}H_{42}O_2$, CAS # 1120-28-1). A 2-kg batch was custom-synthesized by Spectrum Chemical Mfg. Corp. (New Brunswick, New Jersey) with a purity of 99.9 %. A fraction of 800 g of the original material termed icosanoic acid methyl ester (USGS70) was melted to a homogeneous liquid and dripped through a glass capillary into liquid nitrogen, where it shock-solidified to ~2-mg waxy beads with uniform isotopic composition. The remaining original ester was subdivided into two aliquots for adding medium and high 2H and ^{13}C enrichments at the methyl group via transesterification with methan- 2H -ol (i.e. $^2H^1H_2COH$, CAS no. 4206-31-9; 2H fraction = 98 %, Aldrich) and methanol- ^{13}C (^{13}C fraction = 99 %, Aldrich). Each transesterified product was distilled under vacuum and only the center fractions were used to ultimately produce waxy beads as described above. Multi-gram amounts of beads of each type were flame-sealed into glass containers under argon for long-term storage. The three different icosanoic acid methyl esters have increasing δ^2H and $\delta^{13}C$ values in the order from USGS70, USGS71, to USGS72. Users will receive aliquots of 100 mg of beads in glass vials as RMs for δ^2H and $\delta^{13}C$ 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 100 mg of beads in a glass vial that is vacuum sealed in a plastic pouch. USGS70, USGS71, and USGS72 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, cool, and dry place is recommended. Exposure to water and high humidity may entail hydrolysis of the ester and isotope fractionation. Warming of the RMs and melting of the beads must be strictly avoided because slow recrystallization of a molten RM may entail isotopic fractionation across crystals. If an RM underwent accidental melting, the only safe way of continued use as an RM is subsampling from the isotopically homogeneous melt.

Instructions for Use: The user may either use a clean blade to cut individual beads to smaller size for EA applications, or dissolve weighed amounts of the RMs in clean solvents (e.g., dichloromethane, hexane) to generate stock solutions for injections into gas chromatographic interfaces.

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

- The δ^2H values of all hydrogen-bearing substances be expressed relative to VSMOW-SLAP on a scale where $\delta^2H_{SLAP} = -428 \text{ ‰}$ exactly or $\delta^2H_{SLAP2} = -427.5 \text{ ‰}$ [6].
- 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 $+1.95 \text{ ‰}$ and -46.6 ‰ , respectively [3,4].

- 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 SMOW and PDB (Pee Dee belemnite) be discontinued [7].

REFERENCES

- [1] Coplen, T. B., 2011, Guidelines and recommended terms for expression of stable-isotope-ratio and gas-ratio measurement results: *Rapid Communications in Mass Spectrometry*, v. 25, p. 2538–2560. <http://dx.doi.org/10.1002/rcm.5129>
- [2] Gonfiantini, R., 1978, Standards for stable isotope measurements in natural compounds: *Nature*, v. 271, p. 534–536. <http://dx.doi.org/10.1038/271534a0>
- [3] Coplen, T. B., 1994, Reporting of stable hydrogen, carbon, and oxygen isotopic abundances: *Pure and Applied Chemistry*, v. 66, p. 273–276. <http://dx.doi.org/10.1351/pac199466020273>
- [4] Coplen, T. B., Brand, W. A., Gehre, M., Gröning, M., Meijer, H. A. J., Toman, B., and Verkouteren, R. M., 2006, New guidelines for $\delta^{13}\text{C}$ measurements: *Analytical Chemistry*, v. 78, p. 2439–2441. <http://dx.doi.org/10.1021/ac052027c>
- [5] Schimmelmann, A., Qi, H., Coplen, T. B., Brand, W. A., Fong, J., Meier-Augenstein, W., Kemp, H. F., Toman, B., Ackermann, A., Assonov, S., Aerts-Bijma, A. T., Brejcha, R., Chikaraishi, Y., Darwish, T., Elsner, M., Gehre, M., Geilmann, H., Gröning, M., Hélie, J-F., Herrero-Martín, S., Meijer, H. A. J., Sauer, P. E., Sessions, A. L., and Werner, R. A., 2016, New organic reference materials for hydrogen, carbon, and nitrogen stable isotope-ratio measurements: caffeine, *n*-alkanes, fatty acid methyl esters, glycines, L-valines, polyethylenes, and oils, *Analytical Chemistry*, v. 88, p. 4294–4302. <http://dx.doi.org/10.1021/acs.analchem.5b04392> .
- [6] International Atomic Energy Agency (IAEA), Reference Sheet for International Measurement Standards, http://nucleus.iaea.org/rpst/Documents/VSMOW2_SLAP2.pdf (last accessed February 24, 2016).
- [7] Coplen, T. B., 1995, Discontinuance of SMOW and PDB: *Nature*, v. 375, p. 285. <http://dx.doi.org/10.1038/375285a0>