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Reston Stable Isotope Laboratory

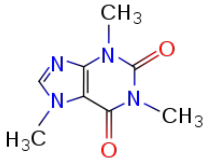
Report of Stable Isotopic Composition

Reference Materials USGS61, USGS62, and USGS63

(Hydrogen, Carbon, and Nitrogen Isotopes in Caffeine)

These reference materials (RMs) are intended for normalization of stable hydrogen (δ^2H), carbon (δ^{13}C) and nitrogen (δ^{15}N) measurements of unknown caffeines and similarly-behaving hydrogen-, carbon-, and nitrogen-bearing substances. 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 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 δ^2H 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 Peedee belemnite) on a scale normalized such that the δ^{13}C values of NBS 19 calcium carbonate and LSVEC lithium carbonate are +1.95 ‰ and –46.6 ‰, respectively [4]. Stable nitrogen isotopic compositions are expressed relative to atmospheric nitrogen, which is isotopically homogenous [5]. On this scale, the $\delta^{15}N_{AIR\text{-}N_2}$ value of USGS32 KNO_3 is +180 ‰ exactly. Stable hydrogen-, carbon-, and nitrogen-isotope delta values of USGS61, USGS62, and USGS63 caffeines with combined standard uncertainties are:

Reference	Structure	$\delta^2H_{VSMOW\text{-}SLAP}$	$\delta^{13}C_{VPDB\text{-}LSVEC}$	$\delta^{15}N_{AIR\text{-}N_2}$	Data source
USGS61		+96.9 ± 0.9 ‰	–35.05 ± 0.04 ‰	–2.87 ± 0.04 ‰	[6]
USGS62		–156.1 ± 2.1 ‰	–14.79 ± 0.04 ‰	+20.17 ± 0.06 ‰	[6]
USGS63		+174.5 ± 0.9 ‰	–1.17 ± 0.04 ‰	+37.83 ± 0.06 ‰	[6]

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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Expiration of Reference Value: The reference values for the isotopic compositions of USGS61, USGS62, and USGS63 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 [6]. Two kilograms of caffeine ($C_8H_{10}N_4O_2$, CAS # 58-08-2) from Alfa Aesar with a purity of 99.5 % (chromatographic purity >99.9 %) were mixed in an industrial-strength blender, sieved through a 20-mesh-metal sieve, and labeled caffeine USGS61. However, this caffeine was only moderately depleted in ^2H. It was not possible to find caffeine with a combined strong depletion in ^2H and ^{13}C because industrial decaffeination mixes coffees from different areas and yields isotopic ranges far smaller than those observed in natural caffeines [7,8]. Instead, these three caffeines form a sequence USGS61 – USGS62 – USGS63, where only the abundances of ^{13}C and ^{15}N are increasing, whereas the sequence for increasing ^2H abundance is USGS62 – USGS61 – USGS63. Isotopically fractionated caffeines for spiking included (i) caffeine-d3 (1-methyl-d3), ^2H fraction = 99.8 %, ICON, (ii) caffeine 3-methyl-^{13}C, ^{13}C fraction = 99 %, Cambridge Isotope Laboratories, Inc., and (iii) caffeine 1,3-$^{15}N_2$, ^{15}N fraction = 99 %, Cambridge Isotope Laboratories, Inc. Caffeine USGS62 contains 1.8 kg of ^2H-depleted caffeine from the Coffein Compagnie (Bremen, Germany) with added ^{13}C and ^{15}N-spiked caffeines. Caffeine USGS63 contains 900 g of caffeine from Alfa Aesar with added ^2H, ^{13}C, and ^{15}N-spiked caffeines. For caffeines USGS62 and USGS63 only small amounts of isotopic spikes were jointly dissolved in ultrapure water and added to powdered, water-wet regular caffeine. 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 20-mesh-metal sieve, and crushed in a stainless-steel, refrigerated shatter box. Homogeneity was demonstrated to be satisfactory at the 0.1-mg level by the RSIL. All caffeines were tested to confirm the absence of crystal water by heating pre-weighed aliquots in vacuum to 80 °C for at least 3 hr and re-weighing after cooling. No significant weight loss was detected. Users will receive aliquots of 0.5 g in glass vials as RMs for δ^2H, $\delta^{13}C$, and $\delta^{15}N$ normalization. Note that caffeine can have a strong physiological effect and express toxicity.

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. USGS61, USGS62, and USGS63 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 USGS61, along with USGS62 or USGS63 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. For δ^2H analysis by TC/EA, a chromium-packed reactor is required [9]. 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 hydrogen, carbon, and nitrogen 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 the δ^2H of SLAP = –428 ‰ exactly [3].
- The δ^{13}C values of all carbon-bearing substances be expressed relative to VPDB-LSVEC on a scale such that the δ^{13}C values of NBS 19 calcium carbonate and LSVEC lithium carbonate are +1.95 ‰ and –46.6 ‰, respectively [4], even though LSVEC is no longer recommended as a RM for δ^{13}C measurement [10].
- The δ^{15}N values of all nitrogen-bearing substances be expressed relative to atmospheric nitrogen [5].
- 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 (Peedee belemnite) be discontinued [11].

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