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REFERENCE SHEET

CERTIFIED REFERENCE MATERIAL

IAEA-603 (calcite)

Stable Isotope Reference Material for $\delta^{13}C$ and $\delta^{18}O$

Assigned $\delta^{13}C$ and $\delta^{18}O$ values for the stable isotopic composition of IAEA-603 expressed in per mille (‰) on the respective VPDB scales [1]

	Assigned value[(1)]	Combined standard uncertainty[(2)] at 1σ-level
$\delta^{13}C$, ‰ on the VPDB $\delta^{13}C$ scale	+2.46	±0.01
$\delta^{18}O$, ‰ on the VPDB $\delta^{18}O$ scale	-2.37	±0.04

[(1)] The values and combined uncertainties were calculated from the raw data obtained by stable isotope mass-spectrometry directly vs remaining NBS19 material, including the necessary ^{17}O isotopic correction [1, 2], and the weighing uncertainties following [1, 3, 4]. Value and uncertainty for $\delta^{13}C$ provided here are rounded to two digits, values with the full three digit resolution are given in Table 2.

[(2)] The uncertainty is expressed as a combined standard uncertainty (1σ-level) using a coverage factor k = 1, and estimated in accordance with the JCGM 100:2008 'Evaluation of measurement data – Guide to the expression of uncertainty in measurement' [3] and ISO Guide 35 'Reference materials – General and statistical principles for certification [4].

Sample preparation

A slab of Carrara marble (which consists of the mineral *calcite*, the same marble material as used for preparation of IAEA-CO-1) had been obtained by IAEA in 1990 from Italy for preparation of the radiocarbon quality assurance material IAEA-C1 and later on of IAEA-CO-1. For the preparation of IAEA-603, eighty kilograms of the remaining coarsely crushed raw marble material were later on ground and sieved to collect about 15 kg of a grain size fraction between 200 to 500 μm, the same grain size fraction as for the former NBS19 material (as it was distributed by the IAEA). The selected grain size fraction was homogenized and split in 31 portions. Each portion was carefully washed using deionised water in order to remove fines, was oven-dried and the final product stored in 15 glass flasks. In order to prepare the first batch of IAEA-603 (approximately 2.8 kg), the content of 3 flasks was carefully re-mixed and divided into glass ampoules (each contains 0.5 g of calcite). The ampoules were flushed with argon and were flame-sealed. The first batch prepared consists of about 5000 ampoules; the remaining material (total of about 12 kg) was transferred to four flame-sealed glass containers for long term storage and later use.

Note-1: the prepared grain-size fraction of 200 to 500μm is represented by clumped smaller calcite crystals which reflect the material nature. The grains can be disaggregated by vigorous shaking (this does not affect the material suitability), therefore the grain size distribution was not characterised.
Note-2: ampoule-sealing aims to eliminate air/moisture exposure or contamination causing a potential alteration of isotopic composition during storage.

Homogeneity of the material:

A feasibility study was performed at GEOTOP - UQAM (Montreal, Canada) on the initial sieved material [5]. One sample each from the 31 portions prepared was taken and shipped to GEOTOP. This was done prior to the final re-mixing and ampouling at IAEA. At GEOTOP the material was further disaggregated and re-sieved into different grain size fractions; the 4 sub-fractions (below 90 μm, 90-125 μm, above 125 μm and bulk) were analysed at a sample size of about 120 μg each by reacting with H_3PO_4 (1.93 g/cm^3 density) at 90°C (Table 1). It was concluded that IAEA-603 is homogeneous at this sample size within the standard deviations observed (0.02 ‰ and 0.04 ‰ for $\delta^{13}C$ and $\delta^{18}O$ correspondingly, n=31 for each size fraction, see Table 1) and is suitable to be used as international reference material, including for use at small sample sizes down to 120 μg within these standard deviations.

Table 1. Results of the IAEA-603 feasibility study done at GEOTOP - UQAM (Montreal, Canada), [5].

Fraction/sub-fraction	**$\delta^{13}C$ of IAEA-603, ‰ on VPDB scale** [(1)]	**Number of measurements** (120 μg aliquots)	**$\delta^{18}O$ of IAEA-603, ‰ on VPDB scale** [(1)]	**Number of measurements** (120 μg aliquots)
Unseparated bulk	2.462±0.022	n=31	-2.392±0.041	n=31
> 125 μm	2.470±0.018	n=31	-2.380±0.034	n=31
90-125 μm	2.478±0.018	n=31	-2.359±0.039	n=31
< 90 μm	2.481±0.017	n=31	-2.360±0.023	n=31
Grand average	**2.473±0.020**	**N=124**	**-2.373±0.038**	**N=124**

[(1)] The uncertainties are given as standard deviations (1σ uncertainties), as reported by GEOTOP – UQAM [5]

A heterogeneity study on the final ampouled product (the first batch of 5000 ampoules) was done at the IAEA as follows:

- According to ISO Guide 35 [4], 52 ampoules were randomly selected from the 5000 ampoules produced;
- Material from ampoules was transferred into glass vials with plastic caps; at least 2 individual analyses were performed from each ampoule;

- Twenty aliquots (10-12 mg each) were run in parallel, by reacting with 2.5 ml H_3PO_4 (1.93 g/cm^3 density) at 25 ± 0.1 °C for 24 hrs. The CO_2 released from the 20 aliquots was cryo-dried at -80 °C and measured in a single analytical sequence in dual inlet mode by mass spectrometry.

This heterogeneity study at the IAEA resulted in a standard deviation of ±0.009 ‰ and ±0.035 ‰ (n=195 and 148) for $\delta^{13}C$ and $\delta^{18}O$, respectively, as obtained on the total data population and also on each analytical sequence (20 aliquots in parallel). The performance of the preparation system in use at the IAEA was found to be the main factor for the standard deviations achieved (in particular for $\delta^{18}O$). Thereafter it was concluded that (i) these standard deviations include both the analytical uncertainty and the uncertainty component due to inhomogeneity at the aliquot size used (10–12 mg); (ii) the material is homogenous within the variability observed, with the uncertainty component due to possible heterogeneity being equal or smaller than the standard deviation values observed.

Characterization study:

The characterization of IAEA-603 versus the remaining NBS19 performed by the IAEA laboratory on the final product (IAEA-603 in ampoules) was done under the same conditions (10-12 mg sample size) as the homogeneity study (see above), directly following the homogeneity study (results of the characterization study are given in Table 2). Altogether 38 individual determinations were performed on carbon and oxygen for both IAEA-603 and NBS19 in the characterization study. The total combined uncertainty of the values included the following individual uncertainty components:

- The uncertainty due to characterization vs NBS19 (conservatively taken as 95 % confidence interval);
- The uncertainty obtained by the heterogeneity study (see above);
- The analytical uncertainty – that was not distinguished from the potential inhomogeneity. Thereafter the standard deviation values observed during the heterogeneity study were taken as a combined term of the uncertainty due to inhomogeneity and analytical uncertainty;
- The uncertainty due to storage effects was not addressed. This component is expected to be negligible, because the material itself is stable and the storage in sealed ampules prevents from exchange with atmospheric moisture and atmospheric CO_2;
- Due to the close proximity of δ-values of IAEA-603 and NBS19 (this is very special case), no normalization to the secondary standard (LSVEC) was performed. The correction would be negligible anyway and its practical application could potentially induce artificial problems increasing the uncertainty unduly.

Table 2. Results of the IAEA-603 characterization at the IAEA.

	$\delta^{13}C$ of IAEA-603, ‰ on VPDB scale [(1)]	**N_1 / N_2 - number of measurements on IAEA-603 and on NBS19**	**$\delta^{18}O$ of IAEA-603, ‰ on VPDB scale** [(1)]	**N_1 / N_2 - number of measurements on IAEA-603 and on NBS19**
Characterization study (given are 95%-confidence intervals)	2.460±0.005	N_1=38 (10-12 mg) N_2=38 (10-12 mg)	-2.373±0.017	N_1=38 (10-12 mg) N_2=38 (10-12 mg)
Heterogeneity study (given are 1σ uncertainties)	±0.009	N_1 = 195	±0.035	N_1 = 148
Assigned values, including the material heterogeneity (1σ uncertainties)	2.460±0.010 [(2)]		-2.373±0.039 [(2)]	

[(1)] The values and combined standard uncertainties were calculated from the raw isotopic data obtained by the stable isotope mass-spectrometry including necessary ^{17}O isotopic correction [1, 2], and combining uncertainties following [1, 3, 4]; [(2)] the conservative approach is to combine the 95% confidence intervals (instead of 1σ uncertainties) of the characterization study and the standard deviation obtained from the heterogeneity study (observed on the total population, N=195 and 148) in squared form.

Validation:

For the purpose of validation, results obtained by 3 other expert stable isotope laboratories were taken into account:

- GEOTOP-UQAM, Montreal, Canada – results of the feasibility study performed on the material sampled before the final material re-mixing and ampouling [5], at a sample size of 120 μg and with further details as given in the section "Homogeneity of the material";
- USGS, Reston, USA - study performed on the material sampled before final re-mixing and ampouling, with a sample size of 20 mg;
- MPI-BGC, Jena, Germany – study performed on 5 ampoules selected from the final product, with analysed sample size of 40 mg.

Results obtained at these three laboratories (Table 3) were found to agree well within the uncertainties with the results of the characterization study performed at the IAEA (Table 2, 3rd row). Notably, the results obtained at GEOTOP demonstrated the material behaviour at the sample size of about 120 μg; and it also implied that the $\delta^{18}O$ uncertainty observed at the IAEA (Table 2) was limited by the analytical performance.

Table 3. Results obtained on the IAEA-603 material at all involved laboratories, given are 95%-confidence intervals.

Laboratory/ material:	**$\delta^{13}C$ of IAEA-603, ‰ on VPDB scale** [(1)]	**N_1 / N_2 - number of measurements on IAEA-603 and on NBS19**	**$\delta^{18}O$ of IAEA-603, ‰ on VPDB scale** [(1)]	**N_1 / N_2 - number of measurements on IAEA-603 and on NBS19**
GEOTOP, unseparated material sampled before the final material re-mixing and ampouling	2.462±0.008	N_1=31 (120 μg)	-2.392±0.015	N_1=31 (120 μg)
USGS, material sampled before final re-mixing and ampouling	2.464±0.018	N_1=8 /N_2=5	-2.381±0.030	N_1=3 / N_2=4
MPI-BGC, material of 5 ampoules selected from the final product	2.458±0.006	N_1=13 / N_2=15 (40 mg)	-2.364±0.037	N_1=13 / N_2=15 (40 mg)
IAEA, the characterization study on 5 ampoules selected from the final product (Table 2, 1st row) supported by the homogeneity study on 52 ampoules	2.460±0.005	N_1=38 (10-12 mg) N_2=38 (10-12 mg)	-2.373±0.017	N_1=38 (10-12 mg) N_2=38 (10-12 mg)

(1) The values and 95%-confidence intervals were calculated from the assessment of isotopic data provided by participating laboratories, as obtained by the stable isotope mass-spectrometry including necessary ^{17}O isotopic correction [1, 2] and combining uncertainties following [1, 3, 4].

Assignment of values and associated uncertainties:

The results obtained at the IAEA (Table 2, 3rd raw) were taken for the value assignment of reference material aliquots within similar size (10-12 mg), for the following reasons: (i) measurements at the IAEA were done on representative number of aliquots sampled from the final ampouled product, thus addressing both the heterogeneity and the characterization; (ii) the IAEA results were validated by data obtained at the other three laboratories; (iii) pooling data obtained on the large sample selection from the final product with the data obtained on limited number of aliquots, with difference sample size and/or on the material sampled before the final re-mixing was considered

inappropriate. Notably, pooling would not change the reference values assigned within the uncertainty.

Note: Regarding values to be assigned for aliquots at sample size around 120 μg and the acid reaction at high temperature (e.g. 70 °C as used by many modern automated techniques), the following has been concluded:

i. Based on the results obtained on unseparated material and sub-fractions sampled at 120 μg (Table 1), IAEA-603 is concluded to be suitable to be used as international reference material for such small sample size (see "Homogeneity of the material"). This is in particular important as the material itself may be gradually disintegrated into finer particles, e.g. by shaking (see "Sample preparation");

ii. The results obtained on 120 μg sample size of unseparated (bulk) IAEA-603 material (Table 1, 1st raw) and the results obtained on the 10-12 mg on sample size at the IAEA (Table 2, 3rd raw) are in good agreement within the uncertainties stated (in both cases 1σ values given). Thereafter, the property values and uncertainties assigned for the sample size of 10-12 mg can also be applied to the aliquots at the sample size around 120 μg.

iii. The reaction time for the carbonate-H_3PO_4 reaction has to be adjusted to ensure complete reaction of IAEA-603, accordingly to the method and analytical set-up in use. Notably, the carbonate-H_3PO_4 (1.93 g/sm^3) reaction at 25 °C as performed at the IAEA demonstrated that IAEA-603 reacts somewhat faster than NBS19.

IAEA certification procedure:

Based on the information provided, the IAEA Reference Material Certification Committee accepted the assigned values as presented on the front page and in Table 2. In case of future changes of the certified values and/or assigned uncertainty, a revised reference sheet will be made available on the IAEA reference product web page, and users will be informed in due time by adequate means.

Statement on metrological traceability of assigned values and uncertainties:

The property values and uncertainties assigned to the reference material IAEA-603 are traceable to the VPDB $\delta^{13}C$ and VPDB $\delta^{18}O$ scales, in agreement with the ^{17}O isotopic correction recommended by the IUPAC [1]**.** The agreement between results obtained at four laboratories by using different analytical instruments confirmed the absence of any significant bias and demonstrates commutability with different carbonate preparation methods in use.

Intended use:

The reference material IAEA-603 has been prepared with the aim to replace the reference material NBS19 (exhausted), with the intended use as following:

- to serve as the highest metrological reference material aimed for the practical realisation of the VPDB $\delta^{13}C$ and VPDB $\delta^{18}O$ scales (including realisation of the VPDB-CO_2 $\delta^{18}O$ sub-scale [1]), with the purpose to calibrate analytical systems at end-user laboratories;
- to serve as the highest level reference material aimed for the characterization of lower level reference materials (secondary reference materials and internal laboratory standards) for $\delta^{13}C$ and $\delta^{18}O$ on the VPDB $\delta^{13}C$ and VPDB $\delta^{18}O$ scales (including VPDB-CO_2 $\delta^{18}O$ sub-scale [1]);
- to be used as the basic reference material (close to zero $\delta^{13}C$) aimed for the 2-point $\delta^{13}C$ data-normalisation [6] on the VPDB $\delta^{13}C$ scale.

Instructions for use:

The reference material IAEA-603 is supplied as 0.5 g units in flame sealed glass ampoules. Ampoules should be stored at ambient temperature. Upon first use end-users are advised to transfer the material from the opened ampoule into the capped vial provided for further storage. Glass fragments potentially introduced by breaking the ampoule neither affect $\delta^{13}C$ and $\delta^{18}O$ of the CO_2 released from the material by acid nor $\delta^{13}C$ released during thermal decomposition. In order to prepare CO_2 gas for the purpose to calibrate analytical systems, material may be treated by H_3PO_4 digestion or decomposed by EA when aiming for $\delta^{13}C$ calibration only.

After first opening and transfer into storage vials, the material should be stored dry at ambient temperature. In order to prevent storage effects such as isotopic exchange with the atmospheric moisture and CO_2, vial storage in a desiccator is a must. Notably, ensuring long term isotopic integrity of the material after opening the ampoule is the responsibility of the user.

Issue and expiry date:

The issue date of IAEA-603 is August 2016. Based on experience with similar materials, the expiry date is set to July 2026, provided the original ampoules are stored undisturbed, in accordance with the instructions given in this reference sheet (see "Instructions for Use"). The IAEA is monitoring the long term stability of the material and customers will be informed in case of any observed change.

Limit of distribution:

The material is available for limited distribution by allowing the purchase of up to three units of the material within a three year's period per laboratory. This limit is set to keep this reference material available to as many laboratories as possible for an extended time period, and is therefore a measure to maximize the reference material usability.

Absolute isotopic abundances:

No absolute isotope abundance determinations were yet performed on this material. However, absolute isotope abundance values can be calculated according to the latest IUPAC recommendation (e.g. [1]) with the ^{17}O correction performed followed to [1, 2] from absolute isotope abundance values as reported for VPDB and adjusted by the delta-scale difference of IAEA-603 versus VPDB.

Legal disclaimer:

The IAEA makes no warranties, expressed or implied, with respect to the data contained in this reference sheet and shall not be liable for any damage that may result from the use of such data.

Compliance with ISO Guide 31:2015:

The content of this IAEA Reference Sheet is in compliance with the ISO Guide 31:2015: Reference materials – Contents of certificates, labels and accompanying documentation [7].

Citation of this reference sheet:

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Note:

Certified values and associated uncertainties as stated in this reference sheet may be updated if more information and/or improvements of analytical techniques become available. Users of this material should ensure that the reference sheet in their possession is current. The current version may be found in the IAEA's Reference Materials online catalogue:
http://nucleus.iaea.org/rpst/ReferenceProducts/ReferenceMaterials/Stable_Isotopes/index.htm

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