



Metrology for stable isotope reference materials: $^{13}\text{C}/^{12}\text{C}$ and $^{18}\text{O}/^{16}\text{O}$ isotope ratio value assignment of pure carbon dioxide gas samples on the Vienna PeeDee Belemnite- CO_2 scale using dual-inlet mass spectrometry

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Abstract

Isotope ratio measurements have been conducted on a series of isotopically distinct pure CO_2 gas samples using the technique of dual-inlet isotope ratio mass spectrometry (DI-IRMS). The influence of instrumental parameters, data normalization schemes on the metrological traceability and uncertainty of the sample isotope composition have been characterized. Traceability to the Vienna PeeDee Belemnite (VPDB)- CO_2 scale was realized using the pure CO_2 isotope reference materials (IRMs) 8562, 8563, and 8564. The uncertainty analyses include contributions associated with the values of IRMs and the repeatability and reproducibility of our measurements. Our DI-IRMS measurement system is demonstrated to have high long-term stability, approaching a precision of 0.001 parts-per-thousand for the 45/44 and 46/44 ion signal ratios. The single- and two-point normalization bias for the IRMs were found to be within their published standard uncertainty values. The values of $^{13}\text{C}/^{12}\text{C}$ and $^{18}\text{O}/^{16}\text{O}$ isotope ratios are expressed relative to VPDB- CO_2 using the $\delta^{13}\text{C}_{\text{VPDB-}\text{CO}_2}$ and $\delta^{18}\text{O}_{\text{VPDB-}\text{CO}_2}$ notation, respectively, in parts-per-thousand (‰ or *per mil*). For the samples, value assignments between (−25 to +2) ‰ and (−33 to −1) ‰ with nominal combined standard uncertainties of (0.05, 0.3) ‰ for $\delta^{13}\text{C}_{\text{VPDB-}\text{CO}_2}$ and $\delta^{18}\text{O}_{\text{VPDB-}\text{CO}_2}$, respectively were obtained. These samples are used as laboratory reference to provide anchor points for value assignment of isotope ratios (with VPDB traceability) to pure CO_2 samples. Additionally, they serve as potential parent isotopic source material required for the development of gravimetric based IRMs of CO_2 in CO_2 -free dry air in high pressure gas cylinder packages at desired abundance levels and isotopic composition values.

Keywords Isotope metrology · Isotope reference material · SI traceability · VPDB- CO_2 traceability · $\delta^{13}\text{C}$ · DI-IRMS

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Introduction

There is an ever increasing need to develop internationally available carbon dioxide gas stable isotope reference materials (IRMs) that provide measurement traceability to the System International (SI) and facilitate calibration of field deployable stable isotope ratio spectroscopic instrument [1–4] for atmospheric measurements leading to reliable measurements needed by policy makers.

In current practice [5], the isotopic ratios $^{13}\text{C}/^{12}\text{C}$ and $^{18}\text{O}/^{16}\text{O}$ of a carbon dioxide sample are expressed in parts per thousand difference (‰) relative to the Vienna PeeDee belemnite- CO_2 (VPDB- CO_2) reference isotope ratio. The sample and reference ratios give rise to an isotope delta value given as $\text{sample } \delta_{\text{VPDB}}^m \equiv \delta_{\text{sample,VPDB}}^m = \left(\frac{R_{\text{sample}}^m}{R_{\text{VPDB}}^m} - 1 \right)$.

The value of R_{sample}^m refers to the sample isotope number ratios $^{13}\text{C}/^{12}\text{C}$ and $^{18}\text{O}/^{16}\text{O}$ for $m = 13$ and 18 , respectively. As delta values are usually small they are expressed in parts-per-thousand (‰ or *per mil*). (The delta notation is also commonly applied to the number ratios $^{45}\text{CO}_2/^{44}\text{CO}_2$ and $^{46}\text{CO}_2/^{44}\text{CO}_2$, measured in isotope ratio mass spectrometry (IRMS) experiments.)

VPDB is a conceptual reference calcite (CaCO_3) artefact. The VPDB scale realization is achieved using the primary reference material NBS19 [a limestone(CaCO_3) artefact]. The isotope scale based on CO_2 derived from the conceptual calcite artifact, VPDB, using 100% phosphoric acid (H_3PO_4) at 25°C is called the VPDB- CO_2 scale. The VPDB- CO_2 scale is realized using the CO_2 gas obtained upon 100% phosphoric acid digestion at 25°C of the primary anchor of VPDB scale, NBS19 limestone mineral. The conversion [6, 7] between the VPDB and VPDB- CO_2 scale is obtained by applying the internationally-accepted isotopic fractionation factor for treatment of VPDB (calcite) at 25°C with 100% H_3PO_4 , $\alpha_{\text{CO}_2, \text{calcite}} [25^\circ\text{C}, 100\% \text{H}_3\text{PO}_4] = 1.01025$ [8] for $^{18}\text{O}/^{16}\text{O}$ and unity for $^{13}\text{C}/^{12}\text{C}$. (In order to establish the uncertainty in R_{VPDB}^{18} calculations a value of 0.0001 has been used [8, 9] for the $^{18}\text{O}/^{16}\text{O}$ $\alpha_{\text{CO}_2, \text{calcite}} [25^\circ\text{C}, 100\% \text{H}_3\text{PO}_4]$ fractionation factor.) The resulting relationship between the sample δ_{VPDB}^p and $\delta_{VPDB-\text{CO}_2}^p$ delta values for the VPDB and VPDB- CO_2 scales, respectively, such that $p = 13$ and 18 are

$$\delta_{VPDB-\text{CO}_2}^{13} = \delta_{VPDB}^{13} \text{ and}$$

$$\delta_{VPDB-\text{CO}_2}^{18} = (\delta_{VPDB}^{18} - 10.25) / 1.01025.$$

It is to be noted that unlike the sample $\delta^{18}\text{O}$ ($\equiv \delta^{18}$) value the $\delta^{13}\text{C}$ ($\equiv \delta^{13}$) value does not change in going from the VPDB to the VPDB- CO_2 scale. However, we refer our sample and reference CO_2 gas $\delta^{13}\text{C}$ values in the VPDB- CO_2 scale notation for consistency purposes. The isotope delta value of NBS19 relative to the VPDB scale is defined as $\delta^{13}\text{C}_{VPDB}$ ($\delta^{18}\text{O}_{VPDB}$) = $+1.95(-2.20) \text{‰}$ [10, 11]. For the VPDB- CO_2 scale this definition leads to $\delta^{13}\text{C}_{VPDB-\text{CO}_2}$ ($\delta^{18}\text{O}_{VPDB-\text{CO}_2}$) = $+1.95(-2.20) \text{‰}$ for NBS19- CO_2 . Here NBS19- CO_2 refers to the CO_2 released from reaction of the NBS19 mineral with 100% phosphoric acid at 25°C .

For the case of pure carbon dioxide gas three stable iRMs 8562, 8563 and 8564 are available with $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values spanning $(-3.76 \text{ to } -41.56) \text{‰}$ and $(-33.516 \text{ to } -10.087) \text{‰}$ on the VPDB- CO_2 scale, respectively [7, 12]. These pure CO_2 iRMs were prepared [7] at the National Institute of Standards and Technology (NIST) using CO_2 gas sources of paleomarine (CO_2 -Heavy), petrochemical (CO_2 -Light) and modern biomass (CO_2 -Biogenic) origin. These iRMs come in the form of small-sized (10 cm^3) borosilicate glass ampoules containing approximately 400 μmoles of pure CO_2 .

The iRMs 8562–8564 are used to value assign pure CO_2 gas standards on the VPDB- CO_2 isotope ratio scale.

In order to address the need for a unifying scale anchor for isotopes of CO_2 in air Max Planck Institute for Biogeochemistry, in its capacity as World Metrological Organization (WMO) Central Calibration Laboratory, developed the Jena reference air set (JRAS) [13]. The JRAS comprises of two CO_2 -Air reference materials. The source CO_2 gases are derived by reaction of well-characterized carbonates with concentrated phosphoric acid and mixed with CO_2 -free air to produce a gas mixture of CO_2 , N_2O , N_2 , Ar and O_2 at natural abundance levels. The two calcites used for JRAS development were chosen to have isotopic compositions close to NBS19 and atmospheric values. The reference set comprise of 5 L glass flasks filled close to 1.5 bar pressure. The isotope delta values for these reference materials are traceable to the VPDB- CO_2 scale via the CO_2 anchor, NBS19- CO_2 and are value assigned for each flask prepared within a batch. The JRAS batch variability is tightly controlled and reported [14] to have long-term production average(standard deviation) $\delta^{13}\text{C}_{VPDB-\text{CO}_2}$, $\delta^{18}\text{O}_{VPDB-\text{CO}_2}$ values of $+1.957(0.015)$, $-2.58(0.034)$ and $-4.373(0.019)$, $-8.92(0.048) \text{‰}$ for MAR-J1 and OMC-J1, respectively. A current infrastructural limitation of this approach is its low throughput, requiring two weeks to generate a reference air set [14]. An extension of the above approach, albeit with sample-specific treatment requirements, is the use of starting materials of known isotopic compositions for the stoichiometric extraction of CO_2 by chemical or physical means followed by gravimetric dilution with CO_2 -free dry air to attain desired amount abundance levels with isotopic compositions traceable to the VPDB- CO_2 scale.

To establish a global infrastructure of CO_2 and CO_2 -Air iRMs the adoption of methods commonly used in the preparation of SI traceable calibration gases provides a viable route for high volume production and distribution. In this regard, these methods which include gravimetric, static volumetric and dynamic volumetric preparation techniques have well established standardized protocols like ISO 6142, 6144 and 6145 [15–17] in place to enable delivery of standards with high quality and reliability. In the gravimetric method, using high precision weight balances, pure CO_2 gas, depleted in $^{13}\text{CO}_2$, can be mixed with varying amounts of pure $^{13}\text{CO}_2$ to generate ^{13}C isotopic composition over a wide range. The isotopically modified “parent” CO_2 is then diluted gravimetrically with CO_2 -free dry air to achieve the desired amount abundance at the chosen isotopic composition. This approach has been successfully used [1, 2, 4] for preparation of synthetic standard gas mixtures of CO_2 -Air with atmospheric ^{13}C composition, traceable to the VPDB- CO_2 scale.

Extending the gravimetric methodology, a step further, the mixing of commercially available isotopically pure $^{12}\text{CO}_2$ (12C, 99.99%) and $^{13}\text{CO}_2$ (13C, 99%, <2% 18O) source gases

at chosen ratios provides a pathway to develop CO_2 -Air mixtures of known amount abundance and known $^{13}\text{C}/^{12}\text{C}$ absolute isotope ratios, at precisions close to 0.1 ‰. However, a caveat of this method lies in the $^{18}\text{O}/^{16}\text{O}$ ratios being significantly altered due to the parent $^{13}\text{CO}_2$ (13C, 99%, <2% 18O) enriched 18O levels. Alternative methods to generate isotopically pure CO_2 [4, 18], involving combustion of isotopically pure natural gas, can allow both the $^{13}\text{C}/^{12}\text{C}$ absolute [18] isotope ratios to reach 0.1 ‰ precision and prevent the enrichment of the heavier oxygen isotopes in the product mixture. Furthermore, custom isotope purification runs on parent 12C source can allow the $^{13}\text{C}/^{12}\text{C}$ absolute isotope ratios close to 0.01 ‰ precision.

The availability of standards with known $^{13}\text{C}/^{12}\text{C}$ absolute ratio can facilitate instrument calibration and global comparison under the umbrella of SI traceability [19]. Such efforts will also enable to validate the $^{13}\text{C}/^{12}\text{C}$ absolute isotope ratios of VPDB traceable reference materials. In this regard several synthetic isotope mixtures based strategies have been demonstrated. Russe et al. (2004) [20] used volumetric, gravimetric mixing of isotopically enriched and depleted ^{13}C and ^{12}C carbon tetrafluoride (CF_4) gas to determine the SI value for $^{13}\text{C}/^{12}\text{C}$. Valkiers et al. (2007) [21] utilized synthetic isotopic mixtures of enriched and “natural” CO_2 gas, prepared at NPL (National Physical Laboratory, UK) and IRMM (Institute for Reference Materials and Measurements, Belgium), to obtain the SI traceable $^{13}\text{C}/^{12}\text{C}$ and $^{18}\text{O}/^{16}\text{O}$ values. In both instances measurements were performed on the specialized isotope ratio mass spectrometer, IRMM (Institute for Reference Materials and Measurements) Avogadro II amount comparator. In a recent study, Dunn et al. (2015) [22] used gravimetrically prepared synthetic mixtures from isotopically pure 13C-enriched ($>99\%$ $^{13}\text{C}_2\text{H}_5\text{NO}_2$) and 13C-depleted glycine ($>99.9\%$ $^{12}\text{C}_2\text{H}_5^{14}\text{NO}_2$), and developed a method on a commercially available elemental analyzer-isotope ratio mass spectrometer, to generate absolute carbon isotope ratio values traceable to the SI. Using their calibration strategy, the absolute carbon isotope ratio of the VPDB standard was found to be $11,115 \times 10^{-6}$ with an expanded uncertainty ($k=2$) of 27×10^{-6} . The values obtained by Dunn et al. were within (−5.8 and −10.8) ‰, when compared to the $^{13}\text{C}/^{12}\text{C}$ absolute isotope ratio obtained by Craig (1957, $11,237.2 \times 10^{-6}$) [10] and the currently accepted [23, 24] values of Chang & Li (1990, $11,180 \times 10^{-6}$) [25], respectively. The reader is referred to the work of Jan Kaiser [26] for a detailed comparison of historical $^{13}\text{C}/^{12}\text{C}$ absolute ratio values.

Yet another aspect concerning metrology is comparison of isotopic measurements across orthogonal techniques like IRMS and optical isotope ratio spectroscopy (OIRS) for common iRMs, ideally a SI traceable reference material. To set the data quality objective requirements for such comparisons the Global Atmosphere Watch (GAW) program of the WMO recommends [27] specific compatibility goals between isotope

measurements techniques for atmospheric monitoring. A pathway to producing gas-based reference materials in cylinder packages, as discussed above, not only addresses some of the challenges in comparing standards across orthogonal isotope ratio techniques but also provides a platform for attaining SI traceable $^{13}\text{C}/^{12}\text{C}$ measurements.

Furthermore, within a measurement methodology, like the ionization-based IRMS approach, there can be more than one strategy to make isotopic composition measurements. For example, a CO_2 -Air sample can be analyzed directly using a gas chromatographic (GC-IRMS) approach [28–31] against CO_2 -Air iRMs or indirectly using a dual-inlet (DI) method involving comparison of CO_2 extracted [32, 33] from the sample with pure CO_2 iRMs. Finally, for a given method, like DI-IRMS, factors like instrumental parameters, sample preparation, and sample-reference delivery can play a role in the final determination of the sample's isotopic composition.

It is clear from the above discussion that several aspects need to be considered for achieving high quality isotopic composition measurements of carbon dioxide. To summarize, these include availability of SI traceable reference materials, comparison of measurements across orthogonal techniques for common samples, comparison of sample preparation and delivery methods within a class of technique, and evaluation of metrological sensitivity factors for a chosen detection method. These components form the basis of our measurements towards the establishment of reliable isotope metrology for carbon dioxide reference standards.

In this paper, our focus is on the DI-IRMS technique, that provides the best (i.e. lowest) total uncertainty, to make measurements of a set of pure carbon dioxide samples of varying isotopic composition. Our goal is to establish VPDB traceability of these samples using available iRMs, namely 8562, 8563 and 8564. We have examined several instrumental factors, cross-contamination correction methods and data normalization schemes to assess their importance and contribution to the isotopic composition value assignment and its uncertainty.

Experimental setup and instrument optimization

Pure CO_2 samples were derived from commercial CO_2 from various isotopic sources. The nominal H_2O , N_2 , O_2 impurity levels were <10 , 20 , $10 \mu\text{mol mol}^{-1}$, respectively, as reported by the commercial supplier. The samples are designated as AL1, AL2, AL3 in this study. All samples were cryogenically purified and evacuated to remove oxygen and nitrogen impurities. In the case of AL1 an additional pre-extraction step to remove water using dry-ice ethanol slush (-78°C) was required to reduce isotopic data variability. The experimental setup consists of a commercial IRMS (Thermo, MAT 253) with an automated dual-inlet changeover switch manifold

for sample and reference CO₂ gas comparison. The dual-inlet changeover valve system was extended using a manual valve controlled multiport manifold to route different samples into the sample bellow “reservoir” for analysis. In addition to routing samples, dedicated ports connected to a helium gas cylinder and a pressure transducer were incorporated to purge and assess the vacuum level of the manifold prior to sample transfer. Manifold pumping was achieved using the vacuum line common to the dual-inlet manifold. Typical purge and pumping cycles produced a vacuum level of <0.005 mbar. The inlet capillaries leading the changeover manifold from the sample and reference bellows to the ion source were balanced via crimping to give an approximate 8000 mV signal at 30 mbar CO₂ bellows pressure for mass-to-charge (*m/z*) = 44 ion. Three pure CO₂ gas iRMs, 8562, 8564 and 8563, which are traceable to the international VPDB-CO₂ scale, were chosen to bracket the pure CO₂ sample measurements based on proximity of isotope delta values. The dual-inlet measurements comprised of sample-reference cycles of *m/z* = 44, 45 and 46 ion signals for the sample and the fixed working reference gas, WRG. A typical sequence used for a daily run is provided in Table 1. WRG vs WRG comparisons were used to assess the inlet capillary balance while ES (enriched sample) vs WRG (or iRM1 vs iRM2) were used to quantify the cross-contamination effect. In our experiments, the WRG used is a depleted CO₂ source derived from a coal burning power plant.

For each sequence run, multiple acquisitions were measured. The acquisition setting used for the reported isotope ratios involved 10 sample-reference cycles, 16 s integration time and 15 s idle time per cycle, resulting in a total sample integration time of 160 s per acquisition. Here idle time refers to the time spent “idle”, after the changeover valve switches from sample to standard capillary or vice-versa in the dual-inlet method, and integration time refers to the time spent thereafter for capturing the signal. Both these values were defined in the acquisition method. A typical acquisition run lasted 15 min. The internal precision (standard deviation) for

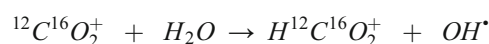
repeated acquisitions is found to be 0.003 and 0.006 ‰ for δ^{45} ($\equiv \delta^{45}\text{CO}_2$) and δ^{46} ($\equiv \delta^{46}\text{CO}_2$), and is consistent with shot-noise predictions (0.003, 0.005 ‰ over 160 s integration period per acquisition).

The variable ion source conductance (VISC) valve and filament emission current settings were set to 1 open turn and 1.5 mA, respectively. These conditions were used to optimize ion sensitivity (1006 molecules per ion for our settings), albeit with a small trade-off on the ion source residual gas clearance time.

Before admitting a new sample to the bellow, the manifold and sample bellow were purged with helium using pressure-vacuum cycles. A final manifold vacuum level of <0.005 mbar was achieved. To quantify the extent of cleaning achieved the sample bellow was next filled with helium to approximately 30 mbar pressure and the resulting ion signal at *m/z* = 44, 45, 46 measured. A level < 5 mV was routinely used as an indicator for the residual CO₂ removal from prior runs.

The dual-inlet sample-reference bellows were operated at pressures close to 30 mbar. The delta values exhibited a near linear dependence on bellows pressure. The isotope delta values varied by (0.033 and 0.086) ‰ for the δ^{45} , δ^{46} values, respectively across bellow pressures of (10 to 40) mbar. This corresponds to a net standard deviation of (0.013, 0.035) ‰ in the δ^{45} , δ^{46} values, respectively. By comparison, the standard deviation under typical operable conditions (fixed bellow pressure = 30 mbar) were (0.003 and 0.005) ‰, respectively for the δ^{45} , δ^{46} values. We also confirmed the presence of viscous flow conditions for the pressure range tested. The mean free path, $\lambda_m = \frac{RT}{\sqrt{2}\pi d^2 N_A P}$, (where *d* = molecular diameter, *N_A* = Avogadro Number and *P* is pressure) across the tested pressure range varied from (1×10^{-5} to 2.6×10^{-3}) m, and is (10 to 40) times smaller than the internal diameter of inlet capillaries. We therefore preclude the possibility of the fractionated gas, that is expanding into the ion source, from back diffusing into the bellow reservoir, under our experimental conditions. It is to be noted, that the principle of “Identical Treatment”, inherent to the dual-inlet balanced capillary measurement setup, ensures the fractionation effect, during gas expansion into the ion source, to cancel out in the sample to reference gas relative isotope ratio determination.

We have also estimated the shift in isotope delta value due to contribution of any water impurity to the *m/z* = 45 ion signal, occurring per ion-molecule reaction [34] per,



The contribution of water to *m/z* = 45 was estimated using observed peak centered ion currents for *m/z* = 18 and *m/z* = 44 ions. Using the relationship between fractional protonation and expected isotope ratio shift developed by Leckrone and Hayes [34] we expect a shift of <0.02 ‰ for $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ as a first approximation to this effect under our experimental conditions.

Table 1 Daily sequence of dual-inlet sample-reference bellow used for isotope measurements

Sequence #	Sample bellow	Reference bellow	Acquisitions
1	WRG	WRG	2
2	Sample1	WRG	3
3	iRM1	WRG	2
4	Sample2	WRG	3
5	iRM2	WRG	2
6	Sample3	WRG	3
7	iRM3	WRG	2
8	WRG	WRG	2
9	ES	WRG	2
10	WRG	WRG	2

Results & discussion

Allan deviation

To assess the presence of long-term drift contribution to measurement precision (in addition to shot-noise) we have used the Allan variance technique. For this analysis, long term (8 h) precision data was collected for WRG versus WRG sample-reference dual-inlet sequence.

An Allan deviation analysis of the δ^{45} and δ^{46} can be done [35] as a function of number of acquisitions by dividing the data into bins of size 1, 2, ..., up to $N/2$ or $(N-1)/2$ acquisitions, for even and odd N , respectively, where N = total number of acquisitions. A bin size of 1 would correspond to N elements (x_1, x_2, \dots, x_N). Bin size of 2 would correspond to $N/2$ or $(N-1)/2$ elements consisting of $(\langle x_1, x_2 \rangle, \langle x_3, x_4 \rangle, \dots, \langle x_{N/2-1}, x_{N/2} \rangle)$ when N is even. Here $\langle x_1, x_2 \rangle$ represents the first element for bin size = 2, constructed by taking average of two (bin size) adjacent elements. In a similar fashion elements of other bin sizes are constructed. The IRMS acquisitions acts as the independent variable in the Allan deviation equation. Pairwise differences are constructed at each bin size (number of acquisitions). This allows to use the standard Allan variance equation

$$\sigma_{Allan}^2(x) = \frac{1}{2(N-1)} \sum_{i=1}^{N-1} [\langle x \rangle_{i+1} - \langle x \rangle_i]^2 \quad (1)$$

to construct Allan deviation plots for δ^{45} and δ^{46} (Fig. 1). Acquisition settings of integration time = 8 s, idle time = 15 s, cycles = 20 were used resulting in a net integration time of 160 s per acquisition event. The Allan deviation is found to follow a power law given by a nearly Poisson distribution ($\propto x^{-0.5}$), and are given by $\sigma(x) = 0.0028x^{-0.47}$ and $\sigma(x) = 0.0049x^{-0.37}$ for δ^{45} and δ^{46} , respectively examined over the 16 acquisition bins. This indicates that the internal precision for the data presented in this work is dominated by statistical noise. In contrast, presence of any long-term drift during the 8 h test would be expected to follow a response closer to the $\sigma(x) \propto x^{+0.5}$ power law. The long-term stability over an 8 h test is found to approach 0.001 ‰ level precision for δ^{45} and δ^{46} . Suffice to say during the duration of a typical daily run there are no underlying long-term drift effects in our experimental design.

Cross contamination

The changeover between sample and standard capillary of the dual-inlet can lead to ion signal contamination and is referred to as the cross-contamination, η_p ($p = 45, 46$) effect. We have used three commonly [7, 36, 37] used methods to examine the cross-contamination level in our experiments. In the first method, the change in ion signal intensity for $m/z = 44, 45$

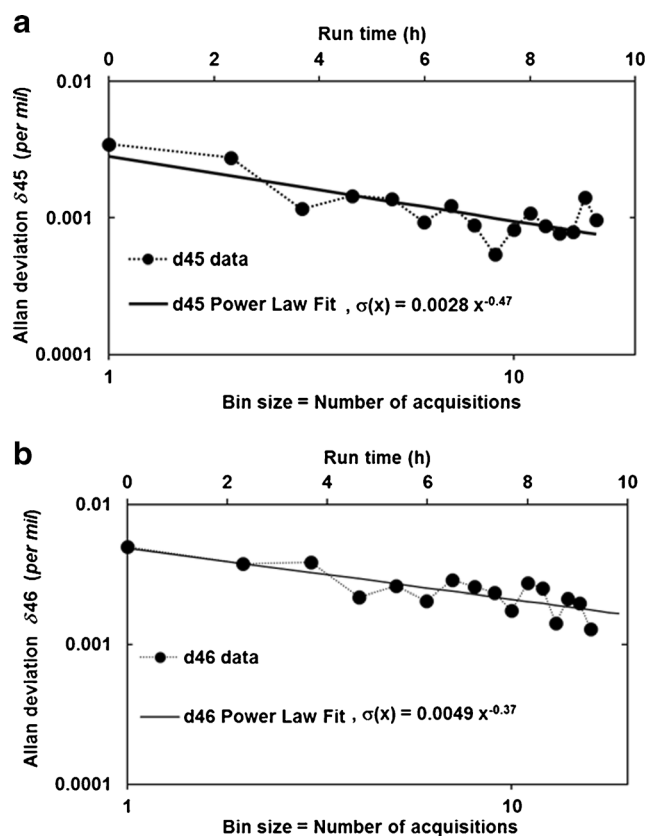
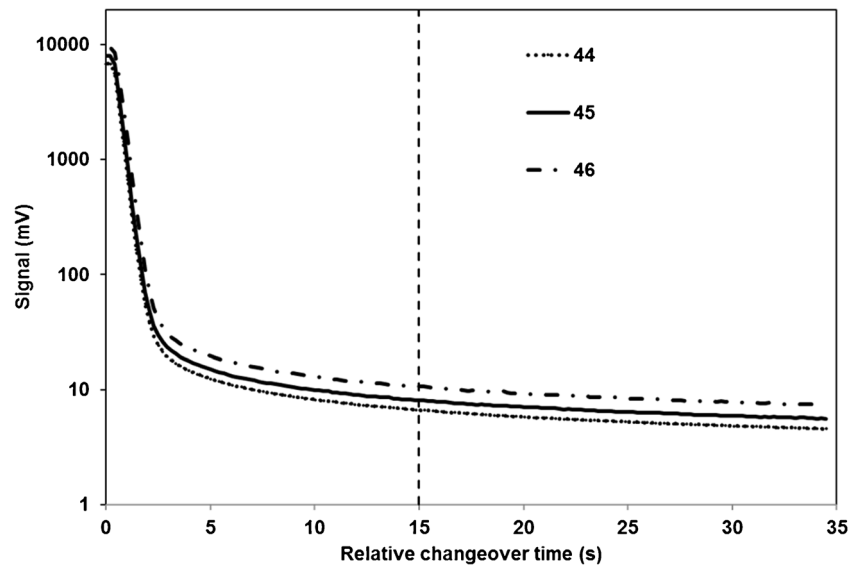


Fig. 1 Plot of Allan deviation versus bin size (or IRMS run time shown on secondary horizontal axis) in log-log scale for (a) δ^{45} (d45) and (b) δ^{46} (d46), respectively. Power law dependence fits give $\sigma(x) = 0.0028x^{-0.47}$ and $\sigma(x) = 0.0049x^{-0.37}$ for δ^{45} and δ^{46} , respectively. For each acquisition the cumulative sample integration time is 160 s

and 46 was recorded (Fig. 2) after manually switching off the sample flow to the ion source. This signal to time “change-over response” curve provides a rough measure of the residual crossover sample left in the ion source and is indicator [36] of cross-contamination. Signal for $m/z = 44, 45, 46$ were observed to decay to 0.3×10^{-3} fractional value relative to the starting levels (after background subtraction) within 15 s. This value is tabulated as the cross contamination coefficient, η_p , in Table 2. A hypothetical scale compression value of $2\eta_p\delta = 0.066 \text{ ‰}$ (see Eq. (8) later) for a $\delta = 30 \text{ ‰}$ is obtained at 15 s. The signal level achieved (data not shown) upon re-opening the sample valve at 15 s (idle time) is found to be within 2×10^{-3} of the levels prior to closing both sample and standard valves.

In addition to the “changeover response time” method, cross-contamination correction between the sample and reference line was examined using two additional approaches. One is derived from measuring the relative isotope delta values between iRMs of known isotopic composition. This pairwise method is best implemented between iRMs having a large delta value difference. In our experiments, we have tested this method using the iRMs, namely the NIST RMs pair 8562 versus 8563 [7, 12]. The other method involves measuring the perturbation caused by a highly enriched sample (ES)

Fig. 2 Sample-reference dual-inlet changeover switch response curve. Plot of ion signal measured in mV (log scale) versus relative changeover time in seconds (linear scale). Time = 0 is chosen to correspond to a switch from the state of sample side open (flow to IRMS source) and reference side to (vent) to the state of both valves closed. An estimated cross contamination of 0.3×10^{-3} at 15 s (marked by dashed vertical line) can be derived, see text for details



when measured against the working reference gas [37]. The cross-contamination term η_p for the two methods are described by Eqs. (2) and (3),

(i) Pairwise comparison of iRMs

$$\eta_p = \frac{\delta_{true}^p - \delta_{meas}^p}{\delta_{true}^p (2 + \delta_{meas}^p)} \quad (2)$$

where, $\delta_{true}^p, \delta_{meas}^p$ are the true and measured isotope delta values of iRM1 versus iRM2. The values of p are 45, 46 referring to the $^{45}\text{CO}_2/^{44}\text{CO}_2$, $^{46}\text{CO}_2/^{44}\text{CO}_2$ number ratios, respectively.

(ii) Enriched sample (ES) method

$$\eta_p = \Delta Z_{WRG}^p / (\Delta Z_{WRG}^p + \Delta Z_{ES}^p) \quad (3)$$

where ΔZ_{WRG}^p and ΔZ_{ES}^p refer [7] to the absolute difference in voltage ratio measurements of the WRG (on reference side) before and during the ES analyses and of the difference in voltage ratio of ES (sample side) relative to reference side, respectively. For our studies the nominal isotope delta values for the enriched sample, ES is (1060 and 1950) ‰ for $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ VPDB- CO_2 , respectively.

Table 2 Comparison of cross contamination methods. Standard deviation values are provided in brackets, where available

Method	η_{45}	η_{46}
Changeover response	0.30×10^{-3}	0.35×10^{-3}
Pairwise: iRMs 8562 vs 8563	1.5×10^{-3}	2.7×10^{-3}
ES	$1.50(0.01) \times 10^{-3}$	$4.5(0.4) \times 10^{-3}$

A knowledge of the cross contamination coefficient, η_p , allows for correction of the measured delta value, δ_{meas}^p .

The corrected isotope ratio delta value δ_{corr}^p is assigned by

$$\delta_{corr}^p = \delta_{meas}^p / (1 - 2\eta_p - \eta_p \delta_{meas}^p) \quad (4)$$

The cross contamination values for the three methods are provided in Table 2.

The “changeover response” approach is useful as it provides a quick diagnostic value for the scale of cross contamination present. However, it does not fully capture the slower component of sample memory effect [32] nor reflects the “idle” gas flow behavior following an acquisition switchover event. The cross-contamination coefficient values, η_{45} for the pairwise iRMs 8562 and 8563 and ES method methods are similar (Table 2). However, the η_{46} values vary by 1.8×10^{-3} between the two methods and might be related to the $\delta^{18}\text{O}$ equilibration time for the isotopically enriched sample ($\delta_{ES}^{18} = 1950\text{‰}$). During the course of our experimental work (60 days) the cross-contamination coefficients, as determined by the ES [37] method, were found to be nearly constant. Specifically, mean (standard deviation) values of $1.5(0.01) \times 10^{-3}$ and $4.5(0.4) \times 10^{-3}$ for η_{45} and η_{46} , respectively, were obtained. This provides a measure of the variability in sampling conditions over the experimental duration (60 d).

Using the cross contamination values reported in Table 2 for the three methods one can compute the hypothetical scale compression term, $2\eta_p \delta$ at $\delta = 30$ (typical delta range). This exercise suggests a mean (standard deviation) scale contraction of 0.07(0.04), 0.15(0.12) for δ^{45} and δ^{46} , respectively, across the three methods.

Finally, to make the cross contamination corrections to the isotope delta values reported in this work we have used the ES method in contrast to the pairwise method involving the RMs 8562 and 8563 samples. Though the pairwise method

involving RMs 8562 versus 8563 measurements give a truer representation of the isotope range observed in our experiment they are used sparingly, given their short supply. In a future study, the pairwise method will be further explored using reference pairs that have well characterized isotope delta values with differences mimicking typically examined isotopic range (30 to 40) ‰ in pure CO_2 samples.

Data normalization: Realization of VPDB scale for traceability

For the realization of the VPDB- CO_2 scale three normalization schemes [38–40] were applied to the sample and reference delta measured values, each being measured against the working reference gas (see Table 1). The reference samples were the three pure CO_2 gas iRMs 8562, 8563, and 8564. Briefly, for each of the normalization scheme the sample delta values, δ^{45} and δ^{46} , are first related to the VPDB- CO_2 scale given by Eqs. (5), (6) and (7) for the single-, two- and three-point normalization methods (the number of points refer to the number of reference materials used).

$$\delta_{sam,VPDB-CO_2}^p = \frac{(\delta_{sam,WRG}^m + 1)}{(\delta_{iRM,WRG}^m + 1)} - 1 \quad (5)$$

$$\delta_{sam,VPDB-CO_2}^p = \delta_{iRM1,VPDB-CO_2}^p + \left[\frac{\delta_{sam,WRG}^p - \delta_{iRM1,WRG}^p}{\delta_{iRM2,WRG}^p - \delta_{iRM1,WRG}^p} \right] \left[\delta_{iRM2,VPDB-CO_2}^p - \delta_{iRM1,VPDB-CO_2}^p \right] \quad (6)$$

$$\delta_{sam,VPDB-CO_2}^p = m \cdot \delta_{sam,WRG}^p + c \quad (7)$$

where $p = 45, 46$. $\delta_{sam,VPDB-CO_2}^p$, $\delta_{sam,WRG}^p$, $\delta_{iRM-VRG}^p$, $\delta_{iRM1-VRG}^p$, $\delta_{iRM2-VRG}^p$, refer to the normalized delta values for sample, iRM, iRM1, iRM2 relative to either WRG or VPDB- CO_2 . The values of m , c represent the slope and intercept for the linear fit to the three iRM data points ($\delta_{iRM,WRG}^p$, $\delta_{iRM,VPDB-CO_2}^p$) using the three-point normalization method. Here iRM corresponds to 8562, 8563 and 8564.

The $\delta_{iRM,VPDB-CO_2}^p$ values are calculated [40] from the known $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ value assignments for iRM [41] and the IAEA recommended parameters of Allison et al. (1995) for O17 correction, i.e. $R13(\text{VPDB}) = 0.0112372$, $R17(\text{VPDB}) = 0.000378866601$, $R18(\text{VPDB}) = 0.00206716068$ and $a = 0.5$ [42]. Here, $R13(\text{VPDB})$, $R17(\text{VPDB})$ and $R18(\text{VPDB})$ represent the abundance ratios $^{13}\text{C}/^{12}\text{C}$, $^{17}\text{O}/^{16}\text{O}$ and $^{18}\text{O}/^{16}\text{O}$ for the reference material VPDB [42]. The “ a ” term is the exponent relating the oxygen isotope ratios of the sample(sam) and oxygen pool (RM-O), $\frac{R17(sam)}{R18(sam)} = \left(\frac{R17(RM-O)}{R18(RM-O)} \right)^a$. The O17 correction parameter values have been kept consistent with those used [41] in

the value assignments of the NIST RMs 8562–8564. An adoption of the IUPAC recommended (2010) [23] O17 correction algorithm, i.e. $R13(\text{VPDB}) = 0.011180(28)$ and $R17(\text{VPDB-}\text{CO}_2) = 0.0003931(9)$, $R18(\text{VPDB-}\text{CO}_2) = 0.00208835$ and $a = 0.528$, resulted in comparable final $\delta^{13}\text{C}$ VPDB- CO_2 and $\delta^{18}\text{O}$ VPDB- CO_2 value assignments, within our reported combined standard uncertainty. These values are provided in the Electronic Supplementary Material (ESM, Table S1) for the sample AL1–AL3 single-point normalization case. For a complete review of the existing O17 correction algorithms the reader is referred to Kaiser’s [20] work.

The normalized values, given by Eqs. (5) to (7), are next used to numerically [40] compute the sample $\delta^{13}\text{C}$ VPDB- CO_2 and $\delta^{18}\text{O}$ VPDB- CO_2 . The results of the normalized $\delta^{13}\text{C}$, $\delta^{18}\text{O}$ VPDB- CO_2 scale values are tabulated in Table 3 and plotted in Fig. 3. The sample standard deviations represent measurement reproducibility over the duration (60 days) of the test.

For the case of single-point normalization, the iRM with delta value closest to the nominal sample $\delta^{13}\text{C}$ value for AL1, AL2 and $\delta^{18}\text{O}$ for AL2, AL3 is chosen for scaling according to Eq. (5) and listed in Table 3 normalization column. In order to have both the $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values scaled to the closest iRM, measurements relative to two iRMs for AL1 and AL3 are required. The calculated values are provided in the ESM (Table S2, the overall values obtained using this distinction are within the combined standard uncertainty of our measurements). For the implementation of the two- and three- point normalization schemes, per Eqs. (6) and (7) only samples bracketed by iRMs delta values are considered. In our study, this criterion is met by the AL3 $\delta^{13}\text{C}$ and AL2 $\delta^{18}\text{O}$ delta values, bracketed by the 8564–8563 and 8564–8562 iRM values, respectively (see Fig. 3). For both AL2 and AL3 the applicable normalization schemes results, tabulated in Table 3, are indistinguishable with their reported measurement standard uncertainties. This suggests that there is no detectable biasing between the normalization schemes for the isotopic CO_2 samples considered in this study.

As an additional check for the presence of any normalization related bias in our experimental method we have used the single- and two-point normalization schemes on our measurements of the NIST RMs and compared it to their known [41] values and uncertainties. In all cases (shown in Fig. 4) the deviations can be bound by the standard uncertainty of the known iRMs values [41]. Therefore, one can conclude that the normalization bias for the range of isotopic CO_2 samples and delta values used in this work is insignificant.

For AL1 sample, $\delta^{18}\text{O}$ single-point normalization was also carried out with respect to the iRM 8564 and compared to its normalized value relative to iRM 8562 (tabulated in Table 3). The two computed delta values, -1.21 (relative to iRM 8564) versus -1.12 (relative to iRM 8562), are found to be within the measurement standard uncertainty. It is to be noted from Fig. 3

Table 3 Comparison of sample $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values for different normalization schemes. The reference values of RMs 8562–8564 are also provided for comparison to experimental data

Sample ^a	$\delta^{13}\text{C}$ VPDB- CO_2 Mean (1 sd ^b)	$\delta^{18}\text{O}$ VPDB- CO_2 Mean (1 sd ^b)	Normalization iRMs
AL1	+1.85(0.05)	−1.12 (0.26)	RM 8562
AL2	−2.18 (0.06)	−15.98 (0.14)	RM 8562
AL2	n/a	−15.99 (0.12)	RM 8562 and RM 8564
AL2	n/a	−16.06 (0.15)	RM 8562, RM 8564 and RM 8563
AL3	−25.06 (0.01)	−33.53 (0.03)	RM 8563
AL3	−25.05 (0.01)	n/a	RM 8563 and RM 8564
AL3	−25.20 (0.23)	n/a	RM 8562, RM 8564 and RM 8563
RM 8562	−3.76 (0.04)	−18.49 (0.22)	NBS19- CO_2 [41]
RM 8563	−41.56 (0.05)	−33.52 (0.24)	NBS19- CO_2 [41]
RM 8564	−10.45 (0.04)	−10.09 (0.20)	NBS19- CO_2 [41]

^a For RM 8562–8564 published [41] value assignments and standard uncertainties are provided

^b For AL1–AL3 sd refers to the Type A standard uncertainty associated with our measurement

and Table 3 that for all the single-point normalization analyses considered in this work the sample to iRM isotope distance is largest for AL1 $\delta^{18}\text{O}$. Specifically, AL1 sample $\delta^{18}\text{O}$ difference is (10 and 20) ‰ relative to iRMs 8564 and 8562. Therefore, the AL1 $\delta^{18}\text{O}$ VPDB- CO_2 value assignment is going to be most sensitive to the accuracy of the reference material delta value and experimental cross contamination. As 8562 and 8564 are isotopically closest available pure CO_2 iRMs for linking AL1 to the VPDB- CO_2 scale our AL1 delta value assignments represent the best possible traceability.

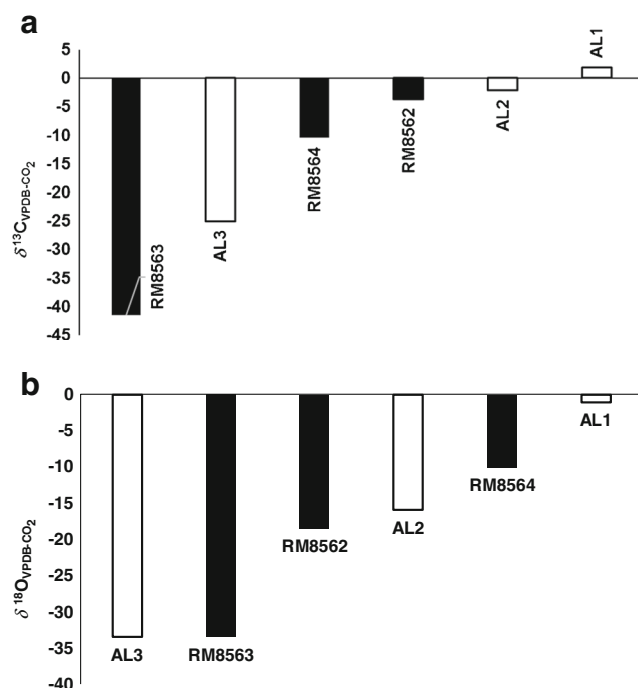


Fig. 3 Single-point normalized (a) $\delta^{13}\text{C}$ and (b) $\delta^{18}\text{O}$ of pure CO_2 samples (empty bar) traceable to VPDB- CO_2 scale. The VPDB- CO_2 values (solid bar) for iRMs 8562–8564 are provided for relative comparison

The overall measurement variation is found to be consistently higher for $\delta^{18}\text{O}$ determination when compared to the $\delta^{13}\text{C}$ values. The source of this observed deviation is attributed to sample non-uniformity, lower abundance, and $\delta^{18}\text{O}$ disequilibrium, and is largest for the isotopically heavier AL1 sample.

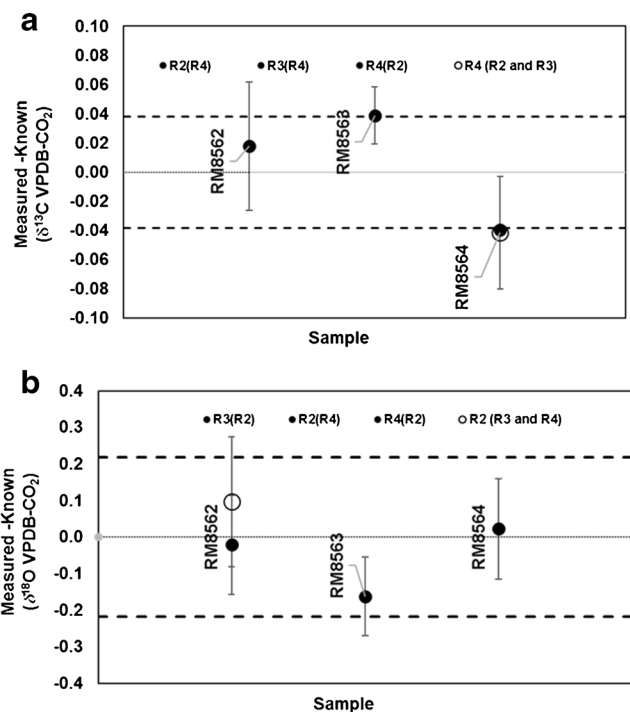


Fig. 4 Deviation of (a) $\delta^{13}\text{C}$ and (b) $\delta^{18}\text{O}$ VPDB- CO_2 from known (published) values of iRMs 8562–8564 as a function of single- (solid circle) and two- point (open circle) normalization. The references used for normalization are indicated in brackets. R2, R3 and 4 refer to iRMs 8562, 8563 and 8564, respectively. The horizontal dashed lines represent the mean value of the known standard uncertainties of iRMs. The error bars represent measurement standard uncertainty

We next examined the sensitivity of delta value correction to the cross contamination correction for each of the three normalization schemes.

The cross contamination correction, per Eq. (4), in the limit of $\delta_{\text{meas}}^p \ll 1$ and $\eta_p \ll 1$ (both identities being true in our study) reduces to

$$\delta_{\text{corr}}^p = \delta_{\text{meas}}^p (1 + 2\eta_p) \quad (8)$$

For a given cross contamination coefficient, η_p and reference material delta value, $\delta_{\text{iRM},\text{WRG}}^p$, the correction to the sample delta value, $\Delta\delta_{\text{sam},\text{VPDB}}^p(\eta_{p,\text{corr}}) = \delta_{\text{sam},\text{VPDB}}^p(\eta_p) - \delta_{\text{sam},\text{VPDB}}^p(\eta_p = 0)$, for single-point normalization is proportional to the magnitude of difference in δ values for sample and iRM. The complete expression for the difference in delta value due to cross contamination correction, is given by

$$\begin{aligned} \Delta\delta_{\text{sam},\text{VPDB}}^p(\eta_{p,\text{corr}}) &= \delta_{\text{sam},\text{VPDB}}^p(\eta_p) - \delta_{\text{sam},\text{VPDB}}^p(\eta_p = 0) \\ &= \frac{2\eta_p(\delta_{\text{sam},\text{WRG}}^p - \delta_{\text{iRM},\text{WRG}}^p)}{(1 + \delta_{\text{iRM},\text{WRG}}^p)(1 + \delta_{\text{iRM},\text{WRG}}^p + 2\eta_p\delta_{\text{iRM},\text{WRG}}^p)} \end{aligned} \quad (9)$$

The magnitude of delta correction for the set of samples and iRMs chosen in this experiment, is expected to be largest for AL3's $\delta^{13}\text{C}$ and AL1's $\delta^{18}\text{O}$ value based on sample and reference isotope delta value difference. A value of 0.05 (0.16) is obtained for the $\Delta\delta_{\text{sam},\text{VPDB}}^p(\eta_{p,\text{corr}})$ correction to the $\delta^{13}\text{C}$ ($\delta^{18}\text{O}$) values for the AL3(AL1) samples and is consistent with our results.

In the case of two-point normalization a similar treatment indicates $\Delta\delta_{\text{sam},\text{VPDB}}^p(\eta_{p,\text{corr}}) = 0$. Consequently, the $\Delta\delta_{\text{sam},\text{VPDB-CO}_2}^p(\eta_{p,\text{corr}})$ can be treated as independent of the cross-contamination correction, η_p for the two-point normalization case. This result is confirmed in our isotope delta value calculations. Specifically, the correction in isotope ratio due to cross-contamination was found to be 0.001(0.002) ‰ for the $\delta^{13}\text{C}$ ($\delta^{18}\text{O}$) correction for the AL3(AL1) case, and is therefore negligible.

Uncertainty budget

In arriving at the value assignment of our samples we have corrected for the cross-contamination bias, as discussed in the previous section. Furthermore, we evaluated the normalization bias between single- and two- point normalization schemes (see Fig. 4) to be within the reported standard

Table 4 Uncertainty budget for sample $\delta^{13}\text{C}_{\text{VPDB-CO}_2}$ ($\delta^{18}\text{O}_{\text{VPDB-CO}_2}$)

Sample	u_r Type A	u_{iRM} Type B	u_R Type A	u_η Type A
AL1	0.003 (0.006)	0.04 (0.2)	0.05 (0.3)	0.000 (0.013)
AL2	0.003 (0.006)	0.04 (0.2)	0.06 (0.1)	0.000 (0.002)
AL3	0.003 (0.006)	0.05 (0.2)	0.01 (0.03)	0.000 (0.012)

uncertainty of the iRMs used in this work. In this section we proceed to tabulate the single-point normalized value assignments along with their combined standard uncertainties for samples AL1-AL3.

To make the uncertainty calculation we consider the following sources: (1) Type B uncertainty of the VPDB- CO_2 primary anchor, NBS19- CO_2 , u_p , (2) Type A uncertainty introduced by the standard deviation of the cross-contamination correction factor in our measurements, u_η , (3) the iRM $\delta^{13}\text{C}$, $\delta^{18}\text{O}$ Type B uncertainties, u_{iRM} , (4) Type A IRMS measurement repeatability, u_r and (5) Type A isotope data reproducibility u_R for $\delta^{45}\text{CO}_2$ and $\delta^{46}\text{CO}_2$ values. The measurement standard uncertainties, u_r and u_R are represented as one standard deviation. Components (4) and (5) are taken to be the uncertainties of $\delta^{45}\text{CO}_2$ and $\delta^{46}\text{CO}_2$ instead of the uncertainty in the $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values derived from them. A Monte Carlo simulation (ESM, Fig. S1) of the uncertainty in the computed $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values from the measured $\delta^{45}\text{CO}_2$ and $\delta^{46}\text{CO}_2$ uncertainty values, assuming a normal distribution in $\delta^{45}\text{CO}_2$ and $\delta^{46}\text{CO}_2$, supports this assumption. The values of u_p is defined to be zero [5]. u_η is approximated using $u_\eta = 2\delta_{\text{sam},\text{WRG}}^{45,46}\Delta\eta_{45,46}$, where $\Delta\eta_{45,46}$ is the standard deviation in the ES cross-contamination factor recorded over the time span (60 d) of our experiments. The combined standard uncertainty for the sample $\delta^{13}\text{C}$, $\delta^{18}\text{O}$ VPDB- CO_2 values is therefore computed using contributions for the four components, (2)–(5). The combined uncertainty is reported as

$$u_{\text{sample}} = \sqrt{u_{\text{iRM}}^2 + u_r^2 + u_R^2 + u_\eta^2} \quad (10)$$

computed in accordance with the *Guide to the Expression of Uncertainty in Measurement* [43]. The uncertainty contributions are tabulated in Table 4. The measurement repeatability standard uncertainty, u_r , are (0.003 and 0.006) ‰ for δ^{45} and δ^{46} , respectively and found to be consistent with shot noise

Table 5 Sample $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ VPDB- CO_2 value assignments for samples studied along with their standard uncertainty in brackets

Sample	$\delta^{13}\text{C}$ VPDB- CO_2	$\delta^{18}\text{O}$ VPDB- CO_2
AL1	+1.85(0.06)	−1.12 (0.34)
AL2	−2.18 (0.07)	−15.98 (0.26)
AL3	−25.06 (0.05)	−33.53 (0.24)

predictions (Merritt and Hayes, 1994, [44]). The values for u_R are relatively larger and lie in the range (0.01 to 0.06) ‰, (0.1 to 0.3) ‰ for $\delta^{13}\text{C}_{\text{VPDB-CO}_2}$, $\delta^{18}\text{O}_{\text{VPDB-CO}_2}$, respectively. The iRM uncertainties [41] are of Type B, with standard uncertainty values in the (0.04 to 0.05) ‰, 0.2 ‰ range for $\delta^{13}\text{C}_{\text{VPDB-CO}_2}$, $\delta^{18}\text{O}_{\text{VPDB-CO}_2}$. These values are comparable to our measurement reproducibility uncertainties (u_R). A marginally higher uncertainty for AL1 $\delta^{18}\text{O}_{\text{VPDB-CO}_2}$ is attributed to sample behavior.

The values for the sample mean and the combined standard uncertainty are reported in Table 5. All values assignments represent single-point normalization scaling as listed in Table earlier.

Conclusion

Isotope ratio for three pure CO₂ samples, spanning $\delta^{13}\text{C}_{\text{VPDB-CO}_2}$, $\delta^{18}\text{O}_{\text{VPDB-CO}_2}$ from (−25 to +2) ‰, (−33 to −1) ‰ have been determined relative to the VPDB-CO₂ scale using iRM 8562–8564 traceability links. A comparison of one, two and three-point normalization reveals minute differences in value assignments and are equivalent within the reported measurement uncertainties. Contributions of cross-contamination were included in making value assignments and lead to 0.05 (0.08) ‰ correction of the $\delta^{13}\text{C}_{\text{VPDB-CO}_2}$ ($\delta^{18}\text{O}_{\text{VPDB-CO}_2}$) values for the AL3(AL1) samples. While single-point and three-point normalization is sensitive to cross-contamination, the two point is independent of this biasing component. The combined standard uncertainties are mostly governed by that of the iRMs and measurement reproducibility and in one case found sensitive to the oxygen $\delta^{18}\text{O}_{\text{VPDB-CO}_2}$ equilibration. Longer timescale data (6–12 month) will be evaluated to look at the trends in reproducibility.

These studies will be extended in the future to compare the isotopic composition of the pure CO₂ samples determined using DI-IRMS in this work with measurements on their derived CO₂-Air mixtures. The CO₂-Air mixtures will mimic natural abundance levels of CO₂ (400 μmol mol^{−1}) but have varying isotopic compositions consisting of CO₂ mixed in CO₂-free air high pressure gas cylinders. Isotope measurements for CO₂-Air gas cylinder will be achieved using the continuous flow gas chromatographic (GC-IRMS) as well as the online CO₂-Air cryogenic extraction coupled to dual-inlet (DI-IRMS) [32, 33, 45] method. Such comparisons will allow assessment of isotope composition measurements of a given pure CO₂ source across IRMS analysis methods that are dependent on CO₂-Air separation methods. As alluded to earlier our long- term goal is linked to attaining SI traceability and its comparability to the VPDB scale for carbon dioxide samples. This study investigates the metrology involved for establishing the gas isotope reference standards.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interests.

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