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Methods and limitations of ‘clumped’ CO_2 isotope (Δ_{47}) analysis by gas-source isotope ratio mass spectrometry

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The geochemistry of multiply substituted isotopologues (‘clumped-isotope’ geochemistry) examines the abundances in natural materials of molecules, formula units or moieties that contain more than one rare isotope (e.g. $^{13}C^{18}O^{16}O$, $^{18}O^{18}O$, $^{15}N_2$, $^{13}C^{18}O^{16}O_2^{2-}$). Such species form the basis of carbonate clumped-isotope thermometry and undergo distinctive fractionations during a variety of natural processes, but initial reports have provided few details of their analysis. In this study, we present detailed data and arguments regarding the theoretical and practical limits of precision, methods of standardization, instrument linearity and related issues for clumped-isotope analysis by dual-inlet gas-source isotope ratio mass spectrometry (IRMS). We demonstrate long-term stability and subtenth per mil precision in 47/44 ratios for counting systems consisting of a Faraday cup registered through a 10^{12} Ω resistor on three Thermo-Finnigan 253 IRMS systems. Based on the analyses of heated CO_2 gases, which have a stochastic distribution of isotopes among possible isotopologues, we document and correct for (1) isotopic exchange among analyte CO_2 molecules and (2) subtle nonlinearity in the relationship between actual and measured 47/44 ratios. External precisions of ∼0.01‰ are routinely achieved for measurements of the mass-47 anomaly (a measure mostly of the abundance anomaly of ^{13}C-^{18}O bonds) and follow counting statistics. The present technical limit to precision intrinsic to our methods and instrumentation is ∼5 parts per million (ppm), whereas precisions of measurements of heterogeneous natural materials are more typically ∼10 ppm (both 1 s.e.). These correspond to errors in carbonate clumped-isotope thermometry of ±1.2 °C and ±2.4 °C, respectively.

Supporting information may be found in the online version of this article.

Keywords: carbonate thermometry; isotopologues; mass 47; clumped isotopes; precision

Introduction

The geochemistry of multiply substituted isotopologues – colloquially, ‘clumped-isotope’ geochemistry – examines the abundances in natural materials of molecules, formula units or moieties that contain more than one rare isotope; e.g. $^{13}C^{18}O^{16}O$, $^{18}O^{17}O$, $^{15}N_2$ or $^{13}C^{18}O^{16}O_2^{2-}$.[1] Such species participate in temperature-dependent homogeneous isotope exchange reactions that can be used as paleothermometers that rigorously constrain temperature based on the isotopic composition of one phase alone.[2] The most developed of these is the thermodynamically based carbonate clumped-isotope thermometer, which examines the extent to which ^{13}C and ^{18}O form bonds with each other in the lattices of carbonate minerals.[3,4] More generally, these species undergo distinctive fractionations during a variety of processes (diffusion, photolysis, irreversible reactions, etc.) and potentially provide unique independent constraints on the origins and budgets of natural materials. The best-developed applications of this sort concern the budget of atmospheric CO_2.[5–8]

This article describes the methods and limitations of analyses of multiply substituted isotopologues by gas-source isotope ratio mass spectrometry (IRMS), with particular focus on measurement of CO_2 isotopologues critical for carbonate clumped-isotope thermometry. Our subject has been addressed in the *Methods* sections and appendices of previous applied studies in this

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field,[6,9] but this is the first article to present detailed data and arguments regarding the theoretical and practical limits of precision, methods of standardization, instrument linearity and related analytical issues. We are motivated by three goals: to document the methods of analysis of multiply substituted isotopologues in greater detail than is typically possible in an applied paper; to guide others who might attempt measurements of this kind; and to illuminate the lessons these methods might provide for analogous attempts at exceptionally precise or sensitive mass spectrometric measurements. Although many details of this article are of interest only to other workers attempting carbonate thermometry, the data we present provide a more general demonstration of clumped-isotope geochemistry, and thus will guide future applied uses of multiply substituted isotopologues.

Mass Spectrometry of Multiply Substituted Isotopologues

The ability to perform high-precision analyses of molecular ions (e.g. CO_2^+ or O_2^+) is critical for clumped-isotope geochemistry. Clumped-isotope geochemistry provides unique constraints only when one can determine how a given population of rare isotopes is distributed among molecular species; any analysis that disproportionates the analyte into its constituent atoms will destroy that information. Assuming it is possible to avoid fragmentation of the analyte, analyses of naturally occurring multiply substituted isotopologues face two additional hurdles: First, such species typically constitute only 10^{-5} to 10^{-6} of the compound of interest, and in some cases 10^{-8} or less. This makes the clumped isotopic species easily the rarest analytical targets considered by stable isotope geochemistry. Furthermore, many of the processes of greatest interest produce relatively subtle isotopic variations. For example, the excess relative to a stochastic distribution of ^{13}C-^{18}O bonds in carbonates is less than 1‰ at room temperature equilibrium and varies by only ~0.005‰ per degree from 0 °C to 50 °C. Therefore, one must analyze abundance anomalies of multiply substituted isotopologues with precisions of 10^{-6} (thousandths of per mil), rivaling the best that have ever been achieved for any mass spectrometric measurement.

To date, all usefully precise measurements of multiply substituted isotopologues in natural materials have been made using a Thermo-Finnigan 253 gas-source IRMS. Such instruments are well suited to the task, as they typically analyze molecular ions and are equipped with an array of Faraday detectors, which permits relatively stable multicollection measurements. Introduction of samples using a dual-inlet and change-over valve allows rapid, repeated intercomparisons of a sample gas with a standard gas of nominally known isotopic composition, which enables one to correct for most instrumental biases and is key to achieving subtenth of per mil precision. Additionally, such instruments have mass resolving powers ($m/\Delta m \sim 200$) and abundance sensitivities sufficient to separate cleanly the relatively weak beams of many multiply substituted ions from the much stronger beams for isotopically normal and singly substituted ions.

However, several issues potentially affect measurements of multiply substituted isotopologues using gas-source IRMS systems. First, the mass resolution is insufficient to resolve the many interferences that one encounters when analyzing molecular ions. This is a particular concern for clumped-isotope measurements, where even a ppb-level contaminant that contributes to an analyzed ion beam (e.g. $^{12}C^{35}Cl$ on $^{13}C^{18}O^{16}O$) can lead to significant apparent changes in isotopic composition. In addition, the dynamically pumped electron bombardment sources used in such instruments typically yield only one ion per several hundred or more analyte molecules, making it difficult to achieve the desired low counting-statistics errors for analyses of rare isotopic species. As a consequence, we use counting systems consisting of a Faraday cup registered through a 10^{12} Ω resistor to measure ion beams of multiply substituted isotopologues. These have been used for the analysis of hydrogen deuterium (HD) but have not been shown previously to be suitable for analyses that demand subtenth per mil precision. Finally, electron bombardment sources fragment analyte molecules and some proportion of those fragments recombines to contribute to the population of detected ions, which may influence the measured proportion of isotopologues.

Experimental

Multiply substituted isotopologues of CO_2

Clumped-isotope analysis uses values of Δ_i to denote the excess of isotopologue i relative to the amount expected for the stochastic distribution of isotopes among isotopologues of a molecule. For the stochastic distribution, the abundance of an isotopologue is the product of the abundances of the isotopes it contains (e.g. $[^{13}C^{18}O^{16}O]^* = 2[^{13}C][^{18}O][^{16}O]$, where * denotes the random distribution, and 2 is a symmetry number[6]). The variable Δ_i defines the difference in abundances in terms of per mil deviation from the stochastic distribution:

$$\Delta_i = \left(\frac{R_i}{R_{i^*}} - 1\right) \cdot 1000 \tag{1}$$

where R_i is the abundance ratio of the isotopologue of interest relative to the isotopically normal isotopologue, and R_{i^*} is that ratio in a pool of molecules having the same bulk isotopic composition but a stochastic distribution of isotopologues.[2] Wang *et al.*[2] derived the relationship between Δ_i and the temperature-dependent equilibrium constant, K, for isotope exchange reactions involving one doubly substituted isotopologue. In the case of CO_2, for one such exchange reaction involving the doubly substituted isotopologue $^{13}C^{18}O^{16}O$ (i.e. $^{13}C^{16}O_2 + {}^{12}C^{18}O^{16}O \overset{K}{\leftrightharpoons} {}^{13}C^{18}O^{16}O + {}^{12}C^{16}O_2$), the ratio K/K^*, where K^* is the equilibrium constant for the stochastic distribution, is related to Δ_i values as follows:

$$-1000\ln(K/K^*) = \Delta_{^{13}C^{18}O^{16}O} - \Delta_{^{12}C^{18}O^{16}O} - \Delta_{^{13}C^{16}O_2} \tag{2}$$

$^{13}C^{18}O^{16}O$ makes up ~97% of mass-47 CO_2. Hence, even though existing sector mass spectrometers are unable to distinguish $^{13}C^{18}O^{16}O$ from its isobars, $^{12}C^{17}O^{18}O$ and $^{13}C^{17}O_2$, a measurement of the Δ_i value calculated as in Eqn (1), but including contributions from all isotopologues having a nominal cardinal mass of 47, should be strongly proportional to abundances of $^{13}C^{18}O^{16}O$.[2] Thus, from Eqns (1) and (2), the temperature-dependent mass-47 anomaly (Δ_{47}) describing the enrichment of $^{13}C^{18}O^{16}O$ in CO_2 relative to the stochastic distribution is defined as follows:

$$\Delta_{47} = \left[\left(\frac{R_{47}}{R_{47^*}} - 1\right) - \left(\frac{R_{46}}{R_{46^*}} - 1\right) - \left(\frac{R_{45}}{R_{45^*}} - 1\right)\right] \times 1000$$

which is calculated as

$$\Delta_{47} = \left[\frac{R_{47}}{2R_{13} \times R_{18} + 2R_{17} \times R_{18} + R_{13} \times (R_{17})^2} - \frac{R_{46}}{2R_{18} + 2R_{13} \times R_{17} + (R_{17})^2} - \frac{R_{45}}{R_{13} + 2R_{17}} + 1\right] \times 1000 \tag{3}$$

The numerator terms R_{47}, R_{46} and R_{45} in Eqn (3) are measured sample abundance ratios relative to mass 44, as derived from measured δ_{47}, δ_{46} and δ_{45} for the sample ('SA') referenced to a working gas ('WG') standard of nominally known isotopic composition $\{\delta_\iota = [(R_{i,SA}/R_{i,WG}) - 1] \times 1000\}$. The denominator terms are the calculated R_{47}, R_{46} and R_{45} that would occur in the sample if it had the stochastic distribution, which are based on the sample's measured δ_{47}, δ_{46} and δ_{45} values. As illustrated by the following example, one can calculate R_{47*}, R_{46*} and R_{45*} from R_{13}, R_{17} and R_{18} (the abundance ratios $^{13}C/^{12}C$, $^{17}O/^{16}O$ and $^{18}O/^{16}O$, respectively, for the sample) because R_{13} and R_{18} are derived from the measured R_{45} and R_{46} by assuming the stochastic distribution,[10,11] and R_{17} is derived from R_{18} assuming a specific mass-dependent fractionation between R_{17} and R_{18}. By definition, $[44]^* = [^{12}C][^{16}O][^{16}O]$ and $[45]^* = [^{13}C][^{16}O][^{16}O] + 2[^{12}C][^{16}O][^{17}O]$. Assuming the stochastic distribution, $[^{12}C] = 1/(1 + R_{13})$, $[^{13}C] = R_{13}/(1 + R_{13})$, $[^{16}O] = 1/(1 + R_{17} + R_{18})$ and $[^{17}O] = R_{17}/(1 + R_{17} + R_{18})$. Substituting these terms in $R_{45*} = [45]^*/[44]^*$, we find the ratio that would occur in the sample if it had the stochastic distribution: $R_{45*} = R_{13} + R_{17}$. Similarly, $R_{46*} = 2R_{18} + 2R_{13}R_{17} + (R_{17})^2$ and $R_{47*} = 2R_{13}R_{18} + 2R_{17}R_{18} + R_{13}(R_{17})^2$. Values of R_{48*} and R_{49*} are determined in an analogous fashion to R_{45*}, R_{46*} and R_{47*}, and can be used to calculate the mass-48 and mass-49 anomalies relative to the stochastic distribution:

$$\Delta_{48} = \left[\left(\frac{R_{48}}{R_{48*}} - 1\right) - 2\left(\frac{R_{46}}{R_{46*}} - 1\right)\right] \times 1000 \tag{4}$$

$$\Delta_{49} = \left[\left(\frac{R_{49}}{R_{49*}} - 1\right) - 2\left(\frac{R_{46}}{R_{46*}} - 1\right) - \left(\frac{R_{45}}{R_{45*}} - 1\right)\right] \times 1000 \tag{5}$$

A numerical demonstration of how to calculate Δ_{47}, Δ_{48} and Δ_{49} from mass spectrometer data is provided in MATLAB code format in the supporting information.

The fact that $\delta^{18}O$ and $\delta^{13}C$ values are calculated from measurements of R_{45} and R_{46} by assuming the stochastic distribution[10,11] has two noteworthy implications. First, the terms in Eqns (3)–(5) containing R_{45} and R_{46} in the numerator are equal to unity,[2] and thus do not contribute to the Δ_i values. Second, as pointed out by Eiler and Schauble,[5] Eqns (3)–(5) involve a circularity that might require an iterative calculation to circumvent. However, R_{47} is so small relative to R_{45} and R_{46} that variations in Δ_{47} are essentially independent of $\delta^{18}O$ and $\delta^{13}C$. Indeed, Δ_{47} measurements performed at Yale University (using a mass spectrometer identical to MS-I described below) of cylinder CO_2 samples representing a 40‰ variation in $\delta^{18}O$ and $\delta^{13}C$, but equilibrated at room temperature (namely, having the same temperature-dependent ^{13}C-^{18}O ordering) demonstrate that Δ_{47} is independent of $\delta^{18}O$ and $\delta^{13}C$ (Fig. s1 in the supporting information).

Carbonate clumped-isotope thermometry

The most developed application of clumped-isotope geochemistry to date is the carbonate clumped-isotope thermometer. If bonding among isotopes of carbon and oxygen in $CO_3{}^{2-}$ ionic groups in carbonate minerals were random, the abundance of each carbonate-ion isotopologue would be the stochastic/combinatory product of the abundances of the isotopes of which it is composed.[1] However, isotopologue abundances are not equal to a stochastic distribution in the carbonate crystal lattice; 'clumping' of heavy isotopes (^{13}C, ^{18}O) into bonds with each other is thermodynamically favored to an increasing degree as temperature decreases.[3,4] As a consequence, the extent to which a carbonate formed in thermodynamic equilibrium is enriched in ^{13}C-^{18}O bonds (relative to the amount expected for a stochastic distribution of isotopes) can provide a measure of the temperature of carbonate growth.

Currently, it is not possible to measure directly abundances of $CO_3{}^{2-}$ ionic groups in carbonates with the necessary precision to determine a sample's ^{13}C-^{18}O bond enrichment. Instead, this enrichment can be inferred by isotopic characterization of CO_2 produced by phosphoric acid digestion of carbonate, because the ^{13}C-^{18}O abundance anomaly in product CO_2 is proportional to the abundance anomaly in the carbonate mineral.[12] The Δ_{47} value of CO_2 extracted at 25 °C from inorganic, synthetic calcite grown at known temperatures between 0 °C and 50 °C is observed to vary as a function of the growth temperature according to the relation:[3]

$$\Delta_{47} = 0.0592 \times 10^6 \times T^{-2} - 0.02 \tag{6}$$

A least-squares linear regression that takes into account uncertainty in each measurement reported in Ghosh *et al.*[3] yields a slightly modified version of this relation, including 1σ uncertainties on the fit parameters (Fig. 1; see supporting information for details of the regression):

$$\Delta_{47} = (0.0605 \pm 0.0014) \times 10^6 \times T^{-2} - (0.031 \pm 0.016) \tag{7}$$

Note this is within uncertainty of Eqn (6). Measured Δ_{47} values for CO_2 extracted from natural surface and deep water dwelling

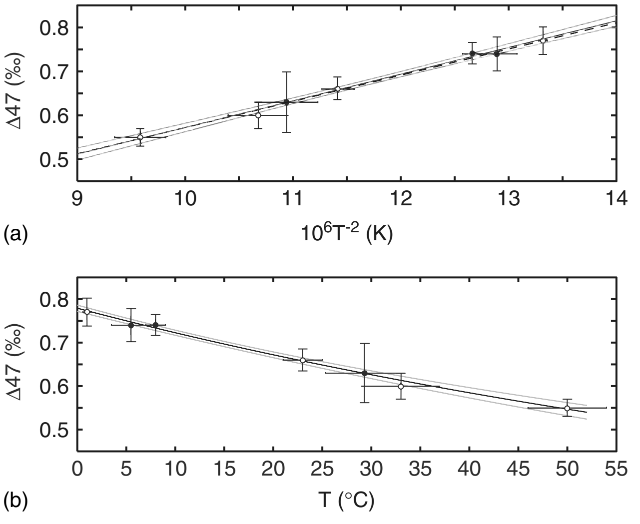


Figure 1. (a) The dashed line is the calibration line for mass-47 anomaly *versus* temperature of carbonate growth of Ghosh *et al.*[3] It is defined by a simple linear regression of Δ_{47} measurements on T^{-2} for synthetic carbonate data (white symbols with 2σ error bars) grown at known temperatures between 1 and 50 °C. The solid black line is defined by a least-squares linear regression of Δ_{47} on T^{-2}, weighted by the uncertainties in Δ_{47} and T.[13] Uncertainties in fit parameters (slope and intercept) are calculated as described in the supporting information, and 95% confidence limits (gray curves) are calculated by the method of Crow *et al.*[14] The dashed (Eqn (6)) and solid black (Eqn (7)) lines agree within uncertainty. (b) The same curve shown in (a) is plotted *versus* T instead of T^{-2}, with error hyperbole representing the 95% confidence limits shown in (a). Δ_{47} data for coral samples grown at known temperature[15] fall on the calibration line (black symbols with 2σ error bars).

corals grown at known temperatures between ∼5 °C and 30 °C[3,15] approximately conform to the trend defined by the synthetic calcite samples, as do results for many other natural carbonates of known or estimated growth temperatures.[1,16] Addition of these data does not significantly change temperature estimates (fit parameters are within 1σ of those listed in Eqn (7)). Hence, for consistency with all previous publications, carbonate growth temperature calculations in this article are performed using the published inorganic carbonate calibration line of Ghosh *et al*.[3] Although the relationship between growth temperature and Δ_{47} for fish otoliths is similar in slope to Eqns (6) and (7), it is offset to slightly lower (∼0.01–0.02‰) values of Δ_{47} at any given temperature.[17]

Potential sources of uncertainty in clumped-isotope analysis and temperature estimates

Several potential sources of uncertainty may be encountered when applying clumped-isotope thermometry and other isotopologue techniques to a particular sample. These include unconstrained isotopic fractionations during sample preparation and purification as well as random (i.e. shot noise) errors and any uncharacterized isotopic fractionations that might occur during mass spectrometry. Two mass spectrometric errors or uncertainties are particularly relevant to clumped-isotope analyses in general: (1) subtle but measurable nonlinearity in the relationship between actual and measured 47/44 ratios and (2) isotopic exchange among analyte CO_2 molecules associated either with fragmentation and recombination in the source or interaction with the walls of metal capillaries. As detailed below, these phenomena must be corrected for empirically based on analyses of heated CO_2 gases, which have a stochastic distribution of isotopes among all possible isotopologues (implying a value of $\Delta_{47} \equiv 0$‰[5,6]). Uncertainties in these empirical relationships contribute to overall analytical uncertainty. A final source of uncertainty relevant for clumped-isotope thermometry, in particular, is introduced by the empirical calibration of the thermometer itself (Eqn (6)). This uncertainty is systematic rather than random (i.e. it is shared by all unknown samples) and may differ for different classes of carbonates for unknown reasons, such as kinetic isotope effects[18,19] or crystal-structural controls on ^{13}C-^{18}O 'clumping'.[4]

Carbonate acid digestion, CO_2 extraction and purification

Carbon dioxide was extracted from 5 to 12 mg aliquots of carbonate powders by reaction with ∼2 ml anhydrous H_3PO_4 at 25 °C for 8–24 h following the methods of McCrea[20] and Swart *et al*.[21] After reaction, conventional vacuum cryogenic purification procedures were used to isolate product CO_2 using the glass vacuum apparatus described by Ghosh *et al*.[3] The cryogenically purified CO_2 was then entrained in He carrier gas flowing at a rate of 3 ml/min and passed through an Agilent Tech 6890N gas chromatograph (GC) column (Supelco-Q-PLOT column with 530 µm internal diameter, 30 m long) held at −10 °C. The GC was baked out at a temperature of 150 °C between samples and at 220 °C for 6 h once every 24 h. CO_2 exiting the column was cryogenically collected for 40 min in a glass trap immersed in liquid N_2. Following the GC step, the He carrier gas was removed while sample CO_2 remained condensed in the liquid N_2 trap. This procedure does not optimize CO_2 collection time (which could be reduced if a multiloop trap were introduced) but purifies CO_2 effectively without fractionating or losing sample gas (near 100% collection efficiency). Finally, conventional cryogenic purification procedures were repeated twice before transfer to the mass spectrometer.

Carbon dioxide for heated gas normalization measurements (described below) was prepared in quartz break seals and heated in a muffle furnace to 1000 °C for >2 h and rapidly quenched at room temperature. This procedure previously has been shown to yield CO_2 that closely approaches a stochastic distribution of isotopes among all possible isotopologues; i.e. its value of $\Delta_{47} \equiv 0$‰.[5] Heated gases were purified using conventional cryogenic procedures and GC processing in a manner identical to the preparation of samples before being transferred to the mass spectrometer.

Isotopic analysis

In addition to the Yale University instrument noted above, two dual-inlet gas-source Thermo-Finnigan 253 IRMS systems, denoted MS-I and MS-II, were used to measure the isotopic composition of CO_2 at the California Institute of Technology. The instruments were equipped with collection systems consisting of both the standard set of three Faraday cups registered through 3×10^8 Ω (for M/z 44), 3×10^{10} Ω (for M/z 45) and 10^{11} Ω (for M/z 46) resistors and three additional Faraday cups registered through 10^{12} Ω resistors (for M/z 47, 48 and 49), as described by Eiler and Schauble.[5] Sample sizes were typically approximately 50 µmol with resulting ion beam currents for M/z 44, 45, 46, 47, 48 and 49 of approximately 50 nA, 0.6 nA, 0.2 nA, 2 pA, 0.2 pA and 2 fA, respectively. The working reference gas was a CO_2 standard from Oztech ($\delta^{18}O_{VSMOW}$ = 24.96‰, $\delta^{13}C_{VPDB}$ = −3.60‰), which was standardized by comparison with CO_2 evolved from phosphoric acid reaction with NBS-19 standard. Values for $\delta^{13}C$ reported *versus* VPDB and $\delta^{18}O$ reported *versus* VSMOW were standardized by comparison with the working reference gas. The program Isodat 2.0 was used to calculate $\delta^{13}C$ and $\delta^{18}O$ from the observed isotopic abundances. For MS-I, isotopic compositions were typically measured for nine acquisitions of 10 cycles each (8 s integration time each for the reference gas and sample sides each cycle), with peak centering, background measurements and pressure balancing between the reference gas and sample sides made before each acquisition. Each acquisition requires 20–30 min, and the total analysis time per sample is typically 3–4 h. Measurements are typically made with an acceleration potential of *ca* 9.5 kV, an electron energy of ∼65–100 V, and with the 'sulfur window' (an aperture on the side of the ionization chamber used to control the pressure and residence time of gas in the source) closed. The same configuration was used for MS-II, except that MS-II measurements were made for eight acquisitions consisting of seven cycles (26 s integration time each), and the widths of the Faraday cups on MS-I and MS-II differ slightly. For both machines, the capillary aperture was adjusted to yield a 5 V signal for mass 44 for a bellow pressure of 50 mbar.

Comparison of sample gases to standards of known isotopic composition and state of ordering

The Δ_{47} value is defined as the difference between the measured R_{47} value of a sample and the R_{47} value one would have measured for a sample of that same bulk isotopic composition (i.e. the same $\delta^{13}C$ and $\delta^{18}O$ values) having the stochastic distribution of isotopologues. This requires that we compare a sample gas to a reference gas that has both a known bulk isotopic composition and a known state of ordering (i.e. its own Δ_{47} value must be independently known). This is challenging because the common reference materials one can use to establish a sample's bulk composition (e.g. CO_2 extracted from NBS-19 carbonate standard) do not have independently known Δ_{47} values. In addition, the procedure we use to create heated gases with nominally known Δ_{47} values (i.e. heating for two or more hours in a quartz tube to drive their composition to the stochastic distribution) can change their bulk isotopic compositions (primarily the $\delta^{18}O$ values).

Our solution to this problem is as follows: we establish the bulk isotopic composition of a working reference gas ('WG') by conventional means (i.e. comparison with recognized interlaboratory standards). Then, we analyze a heated gas ('HG') as a sample, using WG as the standard. We calculate the bulk isotopic composition of the heated gas as if both the HG and WG had the stochastic distribution (i.e. using normal ion correction algorithms). This assumption will introduce an error if the working gas has a Δ_{47} value very far from 0, but it is generally sound for common natural materials (i.e. anything other than synthetic materials that are mixtures of exceptionally isotopically enriched or depleted compounds) because the observed Δ_{47} range is small. We then calculate R_i/R_{i^*} values for observed masses ($i = 45, 46, 47$) as described above. From these, we compute Δ_{47} relative to the working gas for the HG using Eqn (3).

The process is repeated for the sample: after analyzing a sample ('SA') using the working gas as the standard, we calculate the bulk isotopic composition of the sample as if both the sample and WG had the stochastic distribution. We then calculate R_i/R_{i^*} values for the observed masses and compute the Δ_{47} for the sample using Eqn (3). Finally, we calculate the sample's mass-47 enrichment in excess of the stochastic distribution by subtracting the Δ_{47} for the heated gas from Δ_{47} for the sample: $\Delta_{47}[\text{SA } \textit{vs.} \text{ HG}] = \Delta_{47}[\text{SA } \textit{vs.} \text{ WG}] - \Delta_{47}[\text{HG } \textit{vs.} \text{ WG}]$. (Note that Δ_{48} and Δ_{49} are calculated analogously using Eqns (4) and (5).) This final step makes the approximation that Δ_{47} values can be added and subtracted linearly. Because Δ_{47} is not a linear function of isotope abundance, this is, strictly speaking, a convenient but incorrect approximation, but for the $\sim$1‰ range in Δ_{47} values of natural materials it adds no significant errors.

However, the mass spectrometry itself involves a nonlinearity between measured and real R_{47} values that can introduce significant errors in Δ_{47} values if it is not carefully documented and corrected.[16,17] We observe linear correlations between the Δ_{47} values of heated gases measured *versus* the working gas and their δ_{47} values measured *versus* that working gas. (We define $\delta_{47}[\text{HG } \textit{vs.} \text{ WG}] = (R_{47,HG}/R_{47,WG} - 1) \cdot 1000$, a convenient measure of bulk composition. Because Δ_{47} is a small number, δ_{47} is approximately equal to $(\delta_{45} + \delta_{46})$, and thus to $(\delta^{13}C + \delta^{18}O)$.) That is, if one analyzes two heated gases, one closely similar in δ_{47} to the working gas and a second that is substantially different in δ_{47}, two different $\Delta_{47}[\text{HG } \textit{vs.} \text{ WG}]$ values will be observed. Note that the working reference gas is the same in these two measurements, and all heated gases have nominal absolute Δ_{47} values of 0; hence, in the absence of any measurement artifact, the two measured Δ_{47} values should be indistinguishable. Thus, the observed relationship between Δ_{47} and δ_{47} for heated gases reflects a subtle nonlinearity in the relationship between actual R_{47} values and the measured intensity ratio between the mass-47 and mass-44 ion beams. Although we observe this nonlinearity in heated gas measurements for MS-I, MS-II and the Yale mass spectrometer, it differs in the three machines, varies gradually with time on any given instrument, and was not observed in initial studies using MS-I.[5] Hence, it may not be a universal phenomenon. It is not yet clear whether the ultimate cause of this nonlinearity comes from the performance of the detectors, the resistors through which ion currents are measured or some component of the source or analyzer. Nevertheless, a normalization based on the analysis of heated gases over a range of composition space is straightforward.

If the sample and heated gas have the same δ_{47}, then $\Delta_{47}[\text{SA } \textit{vs.} \text{ HG}] = \Delta_{47}[\text{SA } \textit{vs.} \text{ WG}] - \Delta_{47}[\text{HG } \textit{vs.} \text{ WG}]$. If the sample and heated gas differ in their δ_{47} values, then $\Delta_{47}[\text{HG } \textit{vs.} \text{ WG}]$ corresponding to $\delta_{47}[\text{SA } \textit{vs.} \text{ WG}]$ must be estimated by linear regression of measured $\delta_{47}[\text{HG } \textit{vs.} \text{ WG}]$ on $\Delta_{47}[\text{HG } \textit{vs.} \text{ WG}]$ for the time-period during which the sample was analyzed (see supporting information for details of the regression and error propagation). We find that the slope of the empirically determined line for heated gases in a plot of δ_{47} *versus* $\Delta_{47}[\text{HG } \textit{vs.} \text{ WG}]$ is stable over time scales of multiple weeks or months, but can in some cases change subsequent to cleaning the source and or replacing the tungsten filament or changing the ion source focusing – presumably reflecting a change in mass spectrometer performance that influences the nonlinearity between actual and observed R_{47} values (Fig. 2(a–c)).

In the months following our discovery of nonlinearity in Δ_{47} measurements, the intercepts of lines fit through data for heated gases in plots of δ_{47} *versus* Δ_{47} ('heated gas lines') were essentially invariant, even over large ranges in slope.[3] However, subsequent analyses have revealed measurable secular variations in the intercepts of heated gas lines (Fig. 2(d,e)). In order to establish the physical cause of this phenomenon, we conducted experiments in which we varied the residence time of gas in the source by opening and closing the sulfur window. This produced a large variation in the intercept of the heated gas line (Fig. 2(f)), consistent with the hypothesis that a long residence time or high gas pressure in the source promotes fragmentation/recombination reactions that 'scramble' isotopes among isotopologues, reducing the contrast in Δ_{47} between sample and standard gases. If so, then this artifact should be corrected for by multiplying the measured Δ_{47} values of unknowns by a factor that is proportional to the intercept of the heated gas line. In practice, we scale all data to a fixed intercept of the heated gas line, taken to be $\Delta_{47}[\text{HG } \textit{vs.} \text{ WG}] = -0.8453$‰ (i.e. the intercept of the heated gas line used for the calibration study of Ghosh *et al.*[3]). That is, the corrected $\Delta_{47}[\text{SA } \textit{vs.} \text{ HG}] = \Delta_{47}[\text{SA } \textit{vs.} \text{ HG}]_{\text{compressed}} \times (\Delta_{47}[\text{HG } \textit{vs.} \text{ WG}]_0/-0.8453)$, where $\Delta_{47}[\text{HG } \textit{vs.} \text{ WG}]_0$ is the heated gas line intercept for the period during which the sample was analyzed.

In principle, the best way to deal with isotopic 'scrambling' in the source would be to open the sulfur window, minimizing residence time in the source. However, the resulting loss of ion intensity dramatically degrades measurement precision. We conclude that the best practical approach to this artifact is a compromise: we accept and correct for a certain amount of isotopic scrambling in exchange for the high precision that comes with elevated source pressure. However, it is possible that other instruments or instrument tuning conditions might require a different approach – one obviously could not accept a condition that resulted in so much 'scrambling' that all gases are indistinguishable in Δ_{47}.

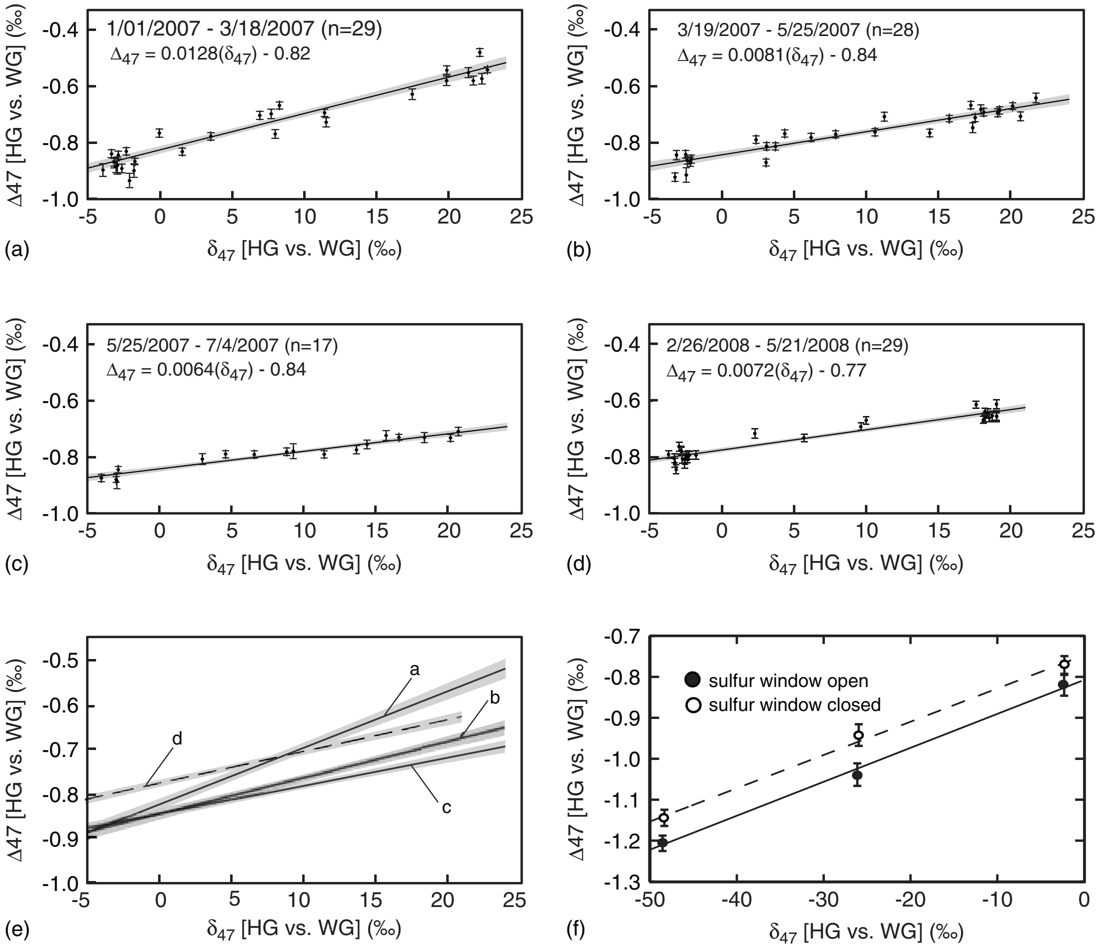


Figure 2. δ_{47}[HG *vs.* WG] and Δ_{47}[HG *vs.* WG] ('heated gas lines') are shown for different time-periods and mass spectrometer configurations. (a–d) The empirical relationship between δ_{47}[HG *vs.* WG] and Δ_{47}[HG *vs.* WG] is approximately linear for 16 V ^{44}V signal heated gas observations (*n*) from four distinct time intervals in 2007 and 2008. The small circles are individual analyses, and the vertical error bars represent 2 s.e. uncertainty in Δ_{47} measurements. The horizontal error bars are of the same magnitude as the vertical error bars. The solid lines represent the least-squares linear regression of data for each time-period, and the shaded gray region indicates the 95% confidence interval. (e) Best-fit lines and 95% confidence intervals (a–d) are superimposed to highlight temporal variations in the heated gas line. Heated gas lines (a), (b) and (c) have approximately the same intercept but different slopes. Heated gas line (d) has a significantly different intercept. (f) The mass-47 heated gas line is shown for different configurations of the sulfur window. The small circles are individual analyses, and the vertical error bars represent 2 s.e. uncertainty in Δ_{47} measurements.

Precision and Stability in Isotope Ratio Measurements

Internal precision

We compared measurements of Δ_{47} conducted using MS-I and MS-II (in continuous use since 2003 and 2008, respectively) to the predictions for Δ_{47} precision at the shot-noise limit (Fig. s2 in the supporting information). The observed precision for the two mass spectrometers approaches the shot-noise limit. Results for MS-I, in particular, indicate that the precision of Δ_{47} measurements can reach levels as low as 5 parts per million (ppm) (±0.005‰) for long ion-current integration times (3200 s, or approximately 24 h of mass spectrometer time) and large gas loads (16 V ^{44}V signal). For typical measurement protocol (16 V ^{44}V signal and ion-current integration times of 480–720 s, corresponding to six to nine acquisitions and approximately 2–4 h of mass spectrometer time), the shot-noise limit to precision is 0.013–0.016‰.

We integrated ion currents for up to 3200 s for gas loads corresponding to ^{44}V signals between approximately 4 and 32 V to examine the effect of sample size and of time-dependent fractionation of sample and/or standard gas reservoirs and/or changes in condition of the mass spectrometer on accuracy of the Δ_{47} measurements (Fig. s2). Isotope ratio observations for MS-II (700 s integration time, four acquisitions of seven 25 s cycles each) indicate that the precision in Δ_{47} measurements is at the shot-noise limit and improves with increased signal size. However, the MS-I data indicate that error climbs after crossing a threshold somewhere between a ^{44}V signal of ~21–30 V. These observations suggest that signals safely below this threshold – approximately 16 V, corresponding to ~8 mg of carbonate or 50 µmol CO_2 – are optimal for this instrument. For ion-current integrations of a 16 V ^{44}V signal, the running average Δ_{47} value stabilizes by ~320 s, but clearly drifts after approximately 1500 s, or 18–19 acquisitions of ten 8 s cycles each. We infer that on MS-I, precision can be optimized by integrating 16 V ^{44}V signals over periods in excess of 320 s (preferably 480–720 s, or six to nine acquisitions of ten 8 s cycles each), but does not continue to improve for integration times greater than approximately 1500 s.

Our conclusion that precisions of Δ_{47} measurements are primarily dominated by counting statistics applies to CO_2 from a variety of sources, including CO_2 generated by phosphoric acid digestion of carbonates. We compiled the internal precision (i.e. standard error) of isotopic measurements for 21 analyses of CO_2 from the NBS-19 carbonate standard and 172 analyses of different aliquots of 63 different carbonate sample unknowns performed under typical conditions from 2004 to 2008 (Fig. s3 in the supporting information). The average standard error for each Δ_{47} measurement (0.009 ± 0.003‰) is in agreement with the lower end of the shot-noise limit predicted for analyses of six to nine acquisitions (0.013–0.018‰). The average observed standard errors in δ^{18}O and δ^{13}C measurements of sample unknowns is roughly an order of magnitude better than that observed for Δ_{47}, although lower-precision outliers up to 0.005‰ exist. The internal precision in Δ_{47} measurements is poorly correlated with precision of either δ^{18}O or δ^{13}C for the standard and sample datasets, with Pearson's correlation coefficients (*r*) of 0.26–0.29 for NBS-19 and 0.07–0.09 for the samples. Internal precision in δ^{18}O and δ^{13}C are highly correlated with one another ($r = 0.78$–0.83), which is not surprising because unlike Δ_{47}, both δ^{18}O and δ^{13}C vary strongly with mass-dependent fractionation.[22]

Long-term signal stability

As long-term drifts can also affect measurement precision (i.e. in addition to shot noise), we examined the stability and accuracy of our IRMS system by using a modified form of the Allan variance technique, originally developed to characterize ultrastable oscillators.[23] Instead of using time as the independent variable, here we use individual IRMS acquisitions *x*:

$$\sigma^2_{Allan}(x) = \frac{1}{2(N-1)} \sum_i \left[\langle x \rangle_{i+1} - \langle x \rangle_i\right]^2 \tag{8}$$

Data are first binned into *N* bins (each consisting of one or more acquisitions), and the average of each bin, $\langle x \rangle_i$, is taken. The difference between adjacent bins is squared and then summed before being normalized to compute the Allan variance. For example, a data set with six elements [x_1, x_2, x_3, x_4, x_5, x_6] will produce three values of Allan variance corresponding to bins of one ([x_1], [x_2], [x_3], [x_4], [x_5], [x_6]), two ([x_1, x_2], [x_3, x_4], [x_5, x_6]) and three ([x_1, x_2, x_3] and [x_4, x_5, x_6]) elements each. The maximum bin size is therefore half the total number of elements in a data set.

Plotting Allan variance *versus* bin size, then, conveniently presents both noise- and drift-related effects on single plot. An example of the Δ_{47} Allan variance for a relatively long-duration analysis, consisting of 40 acquisitions of 80 s sample integration each (ten 8 s cycles), made on MS-I is shown in Fig. 3 (these are the same data depicted in Fig. s2(b–d)). An ideal statistical-noise-limited measurement, for which long-term drifts are negligible, will display a power–law relationship $\sigma^2 = Cx^\alpha$ in the Allan variance plot, where $\alpha = -0.5$, reflecting the Poisson statistics governing the precision of the overall measurement. In contrast, a measurement dominated by long-term, correlated random-walk drifts will have $\alpha = 0.5$. Consequently, the optimum number of measurements to average together in a given analysis (i.e. the optimum bin size) will occur near where the Allan variance reaches a minimum, i.e. where $d\alpha/dx \approx 0$. For our instrument, measurements seem to be dominated by statistical noise for $N < 10$ acquisitions, with only a small contribution from long-term drift. The variability of σ^2 appears to increase for measurements made in excess of nine acquisitions (Fig. 3), although we attribute this to the smaller number of bins with greater than nine acquisitions contributing to the Allan variance calculation. More precise values of the Allan variance with these numbers of acquisitions would require a more sustained analytical run by several factors in time. Nevertheless, the ideal precision that should be attainable on a single gas run for measurements that are clearly dominated by statistical noise (nine acquisitions, or 720 s counting time) at the optimal voltage (16 V) is 0.013‰.

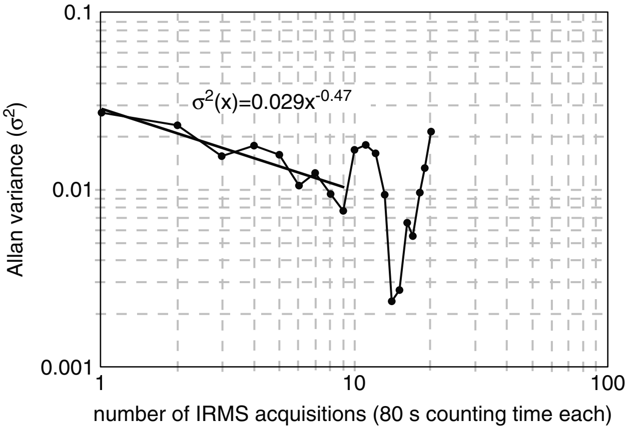


Figure 3. Allan variance plot of Δ_{47} over 40 IRMS acquisitions. A power–law regression is shown over the first nine acquisitions showing $\alpha = -0.47$, indicating that Poisson statistics, not long-term drifts, dominate the uncertainties in the average Δ_{47} in this regime.

External Precision and Error Propagation

Carbonate standards and sample unknowns

The most straightforward way to quantify external precision in Δ_{47} measurements is to determine the reproducibility (standard deviation) of independent analyses of the same material. We compiled the standard deviations in isotope measurements for 92 externally replicated samples (2–11 replicates each) analyzed on MS-I and found that the average external precision in Δ_{47} is 0.019±0.015‰, in agreement with the standard deviation of replicate analyses for the NBS-19 carbonate standard (0.022‰; Fig. s4 in the supporting information). The average external precision in Δ_{47} is approximately twice the average standard error we observe for each CO_2 gas made by acid digestion of carbonates. In addition, the standard deviation for replicate extractions varies over a wider range than does the standard error for each extraction. This suggests that substantial variance beyond counting statistics can be added to measurements of CO_2 from some carbonate samples due to some combination of sample heterogeneity and analytical artifacts such as uncontrolled fractionations or contaminants.

We suspect that sample heterogeneity causes the failure of many carbonate materials to achieve reproducibility in Δ_{47} comparable with counting statistics. An examination of measurements of δ^{18}O and δ^{13}C in the same gases (Fig. s4) reveals that standard deviations for replicate analyses of δ^{18}O and δ^{13}C for both sample unknowns and the NBS-19 standard are an order of magnitude larger than the average standard error we observe for each CO_2 gas made by acid digestion of carbonates (0.0014‰ for δ^{18}O and 0.0007‰ for δ^{13}C), well in excess of the limits imposed by counting statistics. Small errors in δ^{18}O and δ^{13}C that may be due to sample heterogeneity lead to small changes in calculated R_{47}/R_{47^*} values. Although this reduces the reproducibility of Δ_{47} measurements for samples that are heterogeneous in isotopic composition or otherwise affected by analytical artifacts, the variability in Δ_{47} is smaller than variability in δ^{18}O and δ^{13}C because changes in R_{47}/R_{47^*} are offset by changes in R_{46}/R_{46^*} and R_{45}/R_{45^*} (Eqn (3)).

Nevertheless, a large number of samples exhibit standard deviations for replicate analyses of unknowns that are much better than the average and comparable with the limits imposed by counting statistics: 49 of 92 samples exhibit standard deviations in Δ_{47} for replicate extractions of $\leq$0.010‰, and 23 are $\leq$0.005‰. These well-replicated samples are nearly twice as prevalent than would occur by random chance if the true analytical error were 0.019‰(the average for the entire population). We conclude that measurements of Δ_{47} in CO_2 extracted from chemically pure (i.e. free or organic matter, sulfides and other sources of contaminant gases) homogeneous carbonates have errors controlled by counting statistics and not significantly degraded by analytical artifacts.

A detailed example of analytical reproducibility for unknown natural samples

We examined replicate analyses of a suite of samples performed over a relatively long interval of time such that the stability of the stochastic reference frame is put to the test. The two biogenic carbonate samples (oyster 95123 and bivalve *anomia* sp. 95124) were analyzed previously by Spencer and Patchett[24] and Huntington *et al.*[25] and exhibit no obvious evidence of sample heterogeneity or contamination. Replicate Δ_{47} measurements of each sample incorporate uncertainties in the heated gas reference frame (Fig. 2), and potential analytical artifacts introduced by carbonate acid digestion, CO_2 extraction and purification procedures. The two samples experienced identical temperature conditions during growth and burial and have the same Δ_{47} value. Thus, reproducibility of their Δ_{47} measurements also should reflect any noise introduced by sample preparation (cleaning, sampling and powdering).

Figure 4 illustrates the use of heated gas data to normalize Δ_{47}[SA *vs.* WG] measurements of unknown samples to a common reference frame and to screen for contaminants. Divergent δ_{47} and Δ_{47}[SA *vs.* WG] results for independent analyses of 95123 and 95124 carried out during the three different time-periods (Fig. 4(a)) collapse to the same Δ_{47}[SA *vs.* HG] value within uncertainty (0.003–0.006‰, 1 s.e.) after the heated gas normalization is applied (Fig. 4(b,c)). Eiler and Schauble[5] found that mass-48 and

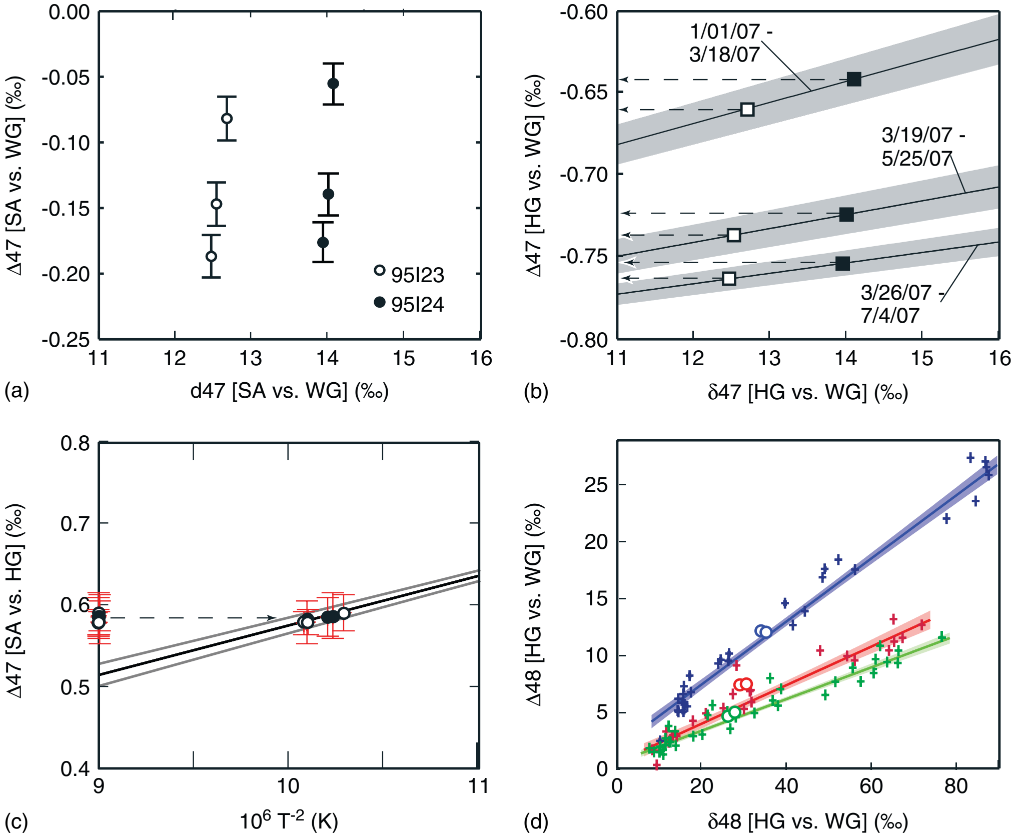


Figure 4. Isotope ratio and clumped-isotope thermometry results for two biogenic carbonates. (a) Δ_{47}[SA *vs.* WG] is plotted against δ_{47}[SA *vs.* WG] for three independent acid digestions each of sample 95123 (white circles with 2 s.e. error bars) and sample 95124 (black circles with 2 s.e. error bars). Each analysis represents 720 s of integration time of a 16 V ^{44}V signal. (b) Heated gas normalization for 95123 and 95124 measurements made over a 5-month period. The heated gas lines for each time-period are taken from Fig. 2, and are labeled with the appropriate range of dates. The dashed arrows illustrate how the best-fit Δ_{47}[HG *vs.* WG] value corresponding to the value of δ_{47}[SA *vs.* WG] from (a) for each analysis is found using the heated gas line for the time-period during which the analysis was performed. The white and black squares indicate the heated gas normalization factor for three analyses of sample 95123 and 95124, respectively. (c) After the heated gas normalization from (b) is applied (see text), the Δ_{47}[SA *vs.* HG] values for all six analyses are indistinguishable. The black line represents a zoomed-in portion of the temperature calibration line shown in Fig. 1(a). The dashed arrow illustrates how the measured Δ_{47} values are projected onto the calibration line to calculate temperature. (d) Mass-48 measurements (crosses) for the three heated gas lines shown in (b) define the relationship between δ_{48} and Δ_{48} expected for clean samples. The samples do not deviate significantly from the best-fit heated gas mass-48 lines (composed of results for clean CO_2 samples), suggesting that the samples are free of hydrocarbon, halocarbon and sulfur contamination. The symbols are larger than the uncertainties.

mass-49 signals can be sensitive indicators of the presence of hydrocarbons and chlorocarbons in spiked analyte CO_2, and Guo and Eiler[26] found evidence that sulfur-bearing contaminants can also lead to substantial interferences on masses 48 and 49 (presumably from species such as $^{32}S^{16}O^-$). However, we have observed a strong correlation between mass-49 signals and the pressure imbalance between the bellows that regulate the sample and working gas flow into the mass spectrometer source. Values of δ_{48} and Δ_{48} do not show this sensitivity to bellows pressure, suggesting that mass-48 measurements are most appropriate indicators of contaminant species; i.e. we can identify clearly contaminated samples by noting differences between their δ_{48}-Δ_{48} systematics and those exhibited by pure heated gases. As shown in Fig. 4(d), the mass-48 signals for all six analyses plot within the range of measured heated gas mass-48 values, suggesting that the samples are free of recognized contaminants. If Δ_{48}[SA *vs.* WG] for any analysis were significantly higher than Δ_{48}[HG *vs.* WG], this would provide evidence for impurities that lead to isobaric interferences within the mass range of CO_2; although contaminants clearly could interfere with mass 48 but not 47, we suggest that such evidence diminishes the reliability of a measurement.

Although their $\delta^{18}O$ values differ (Table 1), Δ_{47} values for 95I23 and 95I24 are indistinguishable. Given the shared history of these samples, we conclude that these six analyses reflect a population of measurements of samples that were indistinguishable in temperature of last equilibration, and thus provide a window into the internal and external precision of the technique. The Δ_{47}[SA *vs.* HG] results of all six analyses converge by approximately 40 cycles or four acquisitions (320 s integration time), and precision approximately follows counting statistics (Fig. 5). Average observed precision in Δ_{47} is 0.0227‰ (1 s.e.) at the level of a single acquisition (80 s integration time) and 0.0088‰ for each analysis (i.e. summing over all acquisitions run on that gas, for a total integration time of 720 s). Average external precision in Δ_{47} is 0.0046‰, after integrating data for all three extractions (i.e. a total integration time of 2160 s per sample). The weighted average Δ_{47} values for three independent analyses each of 95I23 and 95I24 are 0.583 ± 0.006‰ and 0.585 ± 0.003‰, and the external error is 0.0039‰ if results from both samples (six analyses) are combined.

Precision in absolute temperature estimates varies as a function of both the temperature of carbonate growth and the uncertainty in Δ_{47} (Fig. 6(a); supporting information). For a single analysis of a carbonate sample grown at 20 °C (typical ±0.010‰ precision in Δ_{47}), the uncertainty in absolute temperature is ±2.2 °C (1 s.e.). If three replicate measurements are made (typical ±0.005‰ precision in Δ_{47}), the temperature uncertainty is ∼1.4 °C (1 s.e.). Taking advantage of the large number of isotope ratio measurements (in our case, at least 90 cycles for each analysis), we can calculate the 95% confidence limit on temperature estimates by multiplying the standard error of the mean (1 s.e.) given by Fig. 6(a) by Student's *t*-factor (1.6).[27] Although we formally propagate uncertainties using the published calibration data,[3] we note that given the large number of additional data in agreement with the original calibration that have been generated,[1,16,28,29] actual contributions to temperature uncertainty from this calibration are likely to be very small.

The precision of temperature difference (ΔT) estimates is limited by the precision of the least-precise sample Δ_{47} measurement and the precision of the slope (i.e. temperature dependence) of the calibration data (Fig. 6(b)). If the absolute temperature estimates for samples A and B are 30.0 ± 2.5 °C and 20.0 ± 2.2 °C, respectively (1 s.e. in temperature for typical samples with ±0.010‰ precision in Δ_{47} from Fig. 4(a)), the temperature difference ($\Delta T = T_A - T_B$) would be 10.0 ± 3.3 °C (Fig. 4(b)), on the order of the root of the sum of the squared uncertainties in T_A and T_B. ΔT precision is slightly worse than the precision of absolute temperatures, but the error is relatively insensitive to the magnitude of the temperature difference Fig. 4(b).

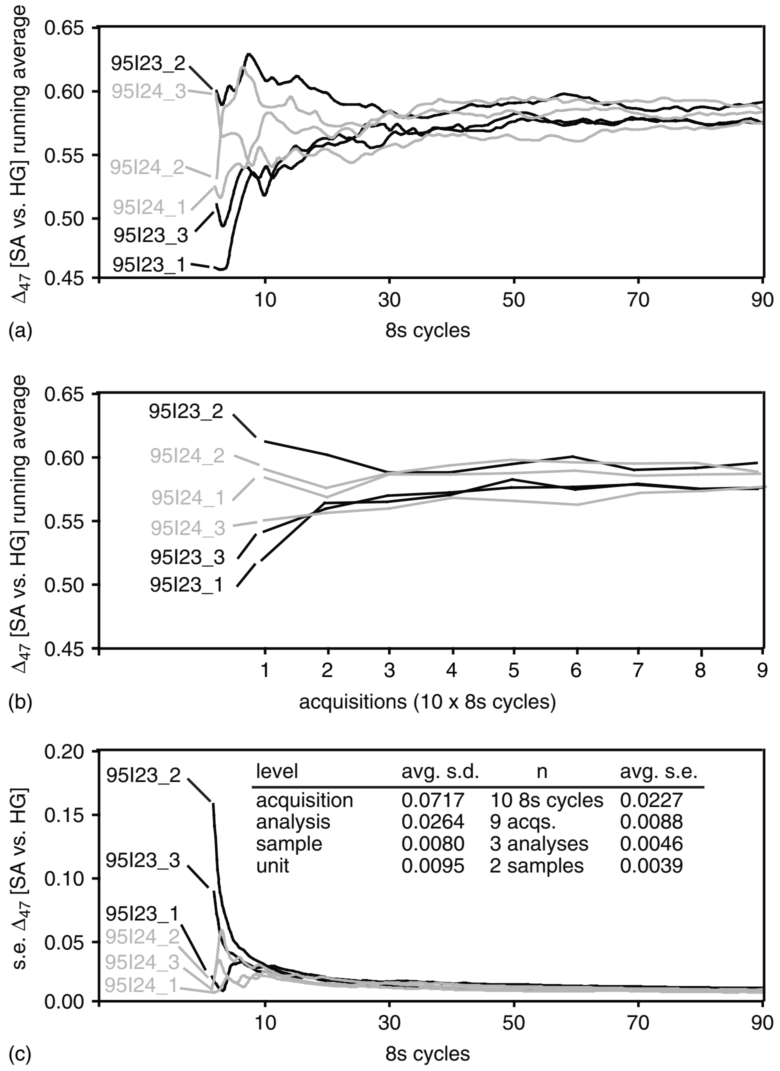
Discussion

Our analysis confirms that it is possible to measure very subtle variations – routinely with <0.1‰ precision – in the abundance of extremely rare species for clumped-isotope analysis using existing technology (Fig. s4). For clumped-isotope carbonate thermometry, precision of 1–2 °C can be achieved, depending primarily on the integration time of the analysis, and secondarily on the number of calibration data taken into consideration. These isotopologue

Table 1. Summary of carbon and oxygen isotopic data for two biogenic carbonate samples shown in Figs. s4 and 4. Heated gas-normalized Δ_{47} values are reported with uncertainties propagated formally as described in the supporting information. Note that the heated gas normalization contributes little uncertainty because of the large number of independent measurements used to define the best-fit line (Fig. 2)

Date (month/date/year)	$\delta^{13}C_{PDB}$ carb(‰)	$\Delta^{18}O_{SMOW}$ water (‰)	$\Delta_{47,sample}$ (‰)	1se$\Delta_{47,sample}$ (‰)	$\delta_{47,sample}$ (‰)	1se$\delta_{47,sample}$ (‰)	$\Delta_{47,HG}$ (‰)	Δ_{47} (‰)	1seΔ_{47} (‰)^a	1seΔ_{47} (‰)^b	T (°C)	1seT (°C)
Sample 95I23												
2/12/07	−1.84	0.872	−0.082	0.0083	12.687	0.0083	−0.661	0.579	0.0085	0.0095		
3/22/07	−1.86	0.396	−0.147	0.0081	12.552	0.0082	−0.742	0.594	0.0082	0.0095		
7/2/07	−1.86	1.041	−0.187	0.0081	12.482	0.0081	−0.764	0.577	0.0083	0.0103		
							Sample average:	*0.583*	*0.0056*		*40.4*	*2.0*
Sample 95I24												
2/12/07	−0.98	1.499	−0.056	0.0077	14.086	0.0078	−0.643	0.587	0.0079	0.0091		
3/24/07	−1.01	0.811	−0.140	0.0081	14.020	0.0079	−0.730	0.590	0.0082	0.0097		
7/2/07	−1.00	1.474	−0.176	0.0075	13.949	0.0076	−0.755	0.579	0.0078	0.0101		
							Sample average:	*0.585*	*0.0033*		*39.9*	*1.6*
							Formation average:	*0.584*	*0.0029*		*40.2*	*1.6*

[a] Without uncertainty from heated gas normalitation.
[b] Including uncertainty from heated gas normalitation.



level	avg. s.d.	n	avg. s.e.
acquisition	0.0717	10 8s cycles	0.0227
analysis	0.0264	9 acqs.	0.0088
sample	0.0080	3 analyses	0.0046
unit	0.0095	2 samples	0.0039

Figure 5. Summarized results for six independent analyses (three per sample) for the two biogenic carbonate samples shown in Fig. 4. (a) The lines show the running average of Δ_{47}[SA *vs.* HG] plotted *versus* the number of 8 s cycles averaged. The three analyses for sample 95I23 are shown in black, and the three analyses for sample 95I24 are shown in gray. (b) The running average is shown as in (a), but here the results are binned by acquisition (ten 8 s cycles). (c) 1 s.e. of Δ_{47}[SA *vs.* HG] measurements for the analysis shown in (a) and (b) *versus* cycle number. Inset table shows the average standard deviation (avg. s.d.) and standard error (avg. s.e.) in ‰, for each level of analysis of the two samples.

measurements approach the shot-noise limit of precision (Fig. s2), indicating that further improvements will require additional methods and/or instrument development that increase the intensity of analyzed ion beams or extend the practical period of analyses of each sample. Although our data compilation indicates that external precision for Δ_{47} analysis of relatively pure, apparently homogeneous carbonates follows counting statistics to the 0.005‰ level, many other materials fail to reach this level of external precision. In some cases, we can attribute poor precision to sample inhomogeneity, whereas in other cases the cause is not obvious.

Poor mass resolution is an outstanding limitation of the mass spectrometers we used that limits our ability to examine possible contaminants or other artifacts that may be to blame for poor external reproducibility in Δ_{47} measurements. Poor mass resolution results in an inability to resolve molecular ion interferences that may have a large impact on apparent isotopologue abundances. Nevertheless, it appears that comparing sample mass-48 anomalies with those of clean heated gases may provide a means to screen for certain contaminants that may affect the isotopologue signal of interest.

The most important limitation of current mass spectrometer technology is the trade-off between ion yield per molecule of analyte gas and the occurrence of fragmentation/recombination reactions in the source, or 'scrambling.' Our sulfur window experiments (Fig. 2(f)) illustrate that while the efficiency with which a dynamically pumped source ionizes analyte molecules can be improved by increasing the gas pressure, increased pressure increases scrambling. As scrambling influences the measured proportion of isotopologues by driving the analyte toward the stochastic distribution, the dynamically pumped source design fundamentally limits the number of ions that can be produced and counted in a given time interval.

Encouragingly, our analysis indicates reasonable stability and linearity at levels of thousandths of per mil of ion beams of interest collected in a Faraday cup registered through a 10^{12} Ω resistor (Figs. s1, 3). One consequence of stability is that we can integrate small ion currents over very long time-periods

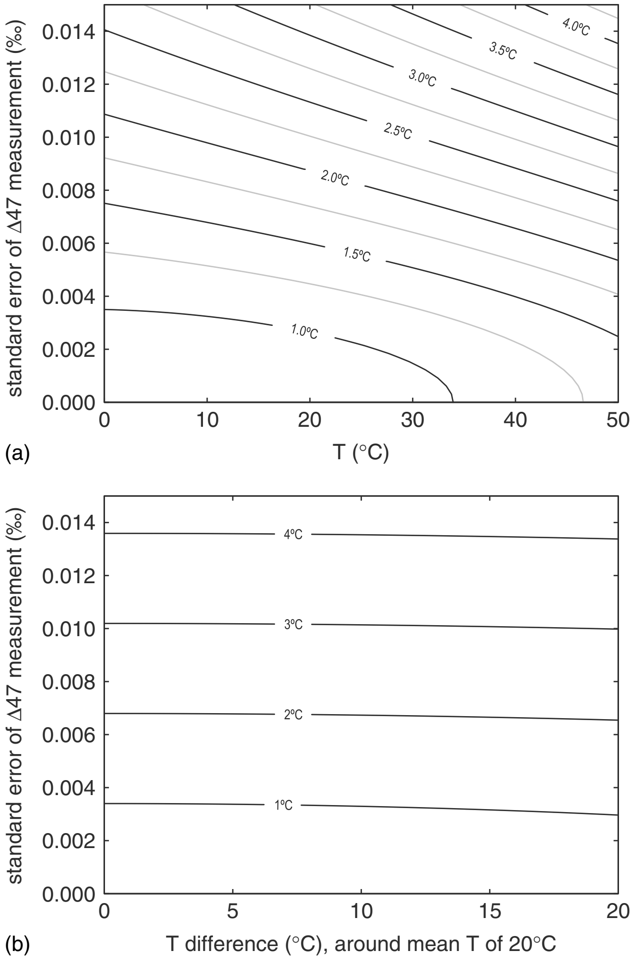


Figure 6. Formal error in temperature estimates and temperature differences (ΔT) deduced from Δ_{47}. (a) The contours represent σ_T, the formal standard error in absolute temperature estimates in °C, as a function of temperature and standard error of Δ_{47} measurements. For Δ_{47} measurement precision of ~0.01‰, the standard error in T is 2–3 °C. (b) The contours represent $\sigma\Delta_T$, the formal standard error in ΔT (estimated around an average of 20 °C) in°C, as a function of temperature and standard error of Δ_{47} measurements. For Δ_{47} measurement precision of ~0.01‰, the standard error in ΔT around an average of 20 °C is ~3 °C, regardless of the magnitude of the temperature difference.

to improve precision (Fig. 3). However, diminishing returns on precision with time and the possibility that the accuracy of isotopologue measurement may eventually decrease (Fig. s2) suggest that very long counting times (in excess of 720 s) may not be advantageous. Instead, machine time would be spent better on analysis of independently prepared replicates, which enable external precision to be characterized.

Perhaps most importantly, our examples highlight the dependence of precise clumped-isotope analysis on a heated gas reference frame. Unlike standard isotopic analyses, which are referenced to an arbitrary value (i.e. the $\delta^{18}O_{VSMOW}$ value), the heated gas reference for clumped isotopes has a unique physical meaning – by definition Δ_{47} of 0‰ means a stochastic distribution. The use of such an 'absolute' reference frame is as critical for practical considerations (i.e. removing the subtle nonlinearity observed in the relationship between actual R_{47} values and the measured intensity ratio between the mass-47 and 44 ion beams and removing the effects of scale compression due to isotopic 'scrambling' in the source) as it is elegant. We recommend analyzing a heated gas each day sample unknowns are analyzed and CO_2 extracted from a carbonate standard such as NBS-19 occasionally to establish the $\Delta_{47} = 0$‰ reference frame precisely and monitor possible changes in machine conditions.

Summary and Outlook

Our data compilation indicates that use of a Thermo-Finnigan MAT 253 dual-inlet gas-source IRMS routinely yields subtenth of a per mil precision in Δ_{47} measurements. For relatively pure, apparently homogeneous carbonates, precision follows counting statistics, and further improvement would require hardware modifications. To the best of our knowledge, our study provides the first detailed examination of the stability and linearity of a counting system consisting of a Faraday cup registered through a 10^{12} Ω resistor at levels of thousandths of per mil. We find the stability of response of this system to be sufficient for precise quantitative analyses, but careful standardization is required to correct for subtle nonlinearity. We outline a method by which heated CO_2 gases with a stochastic distribution of isotopes among all possible isotopologues ($\Delta_{47} \equiv 0$‰) are used to correct for (1) subtle nonlinearity in the relationship between actual and measured 47/44 ratios and (2) scale compression due to isotopic exchange among analyte CO_2 molecules in the source or capillaries.

Measurable improvements in the precision of isotopologue analyses may be possible with instrument and methods developments. Incremental advances in method development could include increasing the ion current by reducing the resistance through which the ion beams are registered or improving the extraction efficiency. We are not optimistic about the use of carrier gas introduction because its effects on exchange among analyte CO_2 molecules ('scrambling'), linearity, stability of ionization and extraction efficiency are unknown. Major advances in precision would require substantial hardware development to achieve higher mass resolution ($m/\Delta m \sim 10^3$), lower current ion counting, an improved ion-counting system, or potentially an improved vacuum system and source design. Until such improvements are realized, subtenth of a per mil precision in Δ_{47} remains at the limit of mass spectrometer measurements.

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Supporting information

Supporting information may be found in the online version of this article.

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