Combined $^{13}$C–D and D–D clumping in methane: Methods and preliminary results

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Abstract

The stable isotopic composition of methane (e.g., $\delta^D$ and $\delta^{13}$C values) is often used as a tracer for its sources and sinks. Conventional $\delta^D$ and $\delta^{13}$C measurements represent the average isotope ratios of all ten isotopologues of methane, though they are effectively controlled by the relative abundances of the three most abundant species: $^{12}$CH$_4$, $^{13}$CH$_4$, and $^{12}$CH$_3$D. The precise relative abundances of the other seven isotopologues remains largely unexplored because these species contain multiple rare isotopes and are thus rare themselves. These multiply substituted (or ‘clumped’) isotopologues each have their own distinctive chemical and physical properties, which could provide additional constraints on the geochemistry of methane. This work focuses on quantifying the abundances of two rare isotopologues, $^{13}$CH$_3$D and $^{12}$CH$_2$D$_2$, of methane in order to assess their utility as a window into methane’s geochemistry. Specifically, we seek to assess whether clumped isotope distributions might be useful to quantify the temperature at which methane formed and/or equilibrated. To this end, we report the first highly precise combined measurements of the relative abundances of $^{13}$CH$_3$D and $^{12}$CH$_2$D$_2$ at natural abundances (i.e., unlabeled) via the high-resolution magnetic-sector mass spectrometry of intact methane. We calibrate the use of these measurements as a geothermometer using both theory and experiment, and apply this geothermometer to representative natural samples. The method yields accurate average (i.e., bulk) isotopic ratios based on comparison with conventional techniques. We demonstrate the accuracy and precision of measurements of $^{13}$CH$_3$D and $^{12}$CH$_2$D$_2$ through analyses of methane driven to high temperature (>200 °C) equilibrium in the laboratory. Application of this thermometer to natural samples yields apparent temperatures consistent with their known formation environments and appears to distinguish between biogenic and thermogenic methane.

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1. INTRODUCTION

Understanding the processes that control the generation, abundance, and fate of methane (CH$_4$) across the atmosphere, oceans, and shallow crust is critical for both our economic and environmental future. For example, methane is a key constituent of natural gases used for energy (Hamak and Sigler, 1991), a naturally and anthropogenically emitted greenhouse gas (Wuebbles and Hayhoe, 2002), and an important chemical component of geothermal systems (Welhan, 1988). Additionally it is hypothesized to play a role in controlling past climate states (Kennett et al., 2003) and is an important carbon source and product of microbial metabolisms (Valentine, 2011). The stable isotopic building...
blocks of methane molecules, $^{12}$C, $^{13}$C, H ($^1$H), and D ($^2$H), as well as radioactive T ($^3$H) and $^{14}$C, serve as quantitative tracers of various processes involving methane in the environment. Different formation, transport, and removal processes of methane often impart distinct isotopic fractionations. As a result, the stable isotopes of methane can be used to reconstruct and quantify those processes. As an example, methane formed from methanogenic organisms sometimes has a distinct isotopic signature as compared to methane formed from the higher temperature, abiogenic breakdown of organic matter (Whiticar, 1999). Studies of the stable isotopes of methane have contributed to a better understanding of its biogeochemical cycling in many environments including modern air (Stevens and Rust, 1982), ancient air preserved in ice (Craig et al., 1988), gas deposits (Schoell, 1980), and sediment (Whiticar et al., 1986).

The stable isotopic composition of methane is typically quantified through the isotopic ratios $^{13}$C/$^{12}$C and D/H, which are reported through $\delta^{13}$C and $\delta$D values (see footnote 1). These ratios quantify the mean composition of all isotopologues of methane. Though ten different isotopologues of methane contribute to these ratios (Table 1), their precise relative contributions are not directly measured using conventional mass spectrometry. Instead, all isotopologues are converted to a common molecular form (CO$_2$ or H$_2$), which destroys all information about how isotopes are partitioned (ordered) amongst the ten isotopologues.

Molecules containing more than one rare isotope are referred to as ‘multiply substituted isotopologues’ or, equivalently, ‘clumped isotopologues’ (Eiler, 2007, 2013). Clumped isotopologues are of interest as their chemical and physical properties are distinct from singly substituted (one rare isotope) and unsubstituted (no rare isotopes) isotopologues, which control the average isotopic distributions. For example, at equilibrium, there is, usually, an excess of clumped isotopologues compared to a random distribution of isotopes among all isotopologues. The magnitude of this excess is a function of temperature. Consequently, a measurement that constrains the abundances of unsubstituted, singly substituted, and at least one clumped isotopologue can serve as a geothermometer (Wang et al., 2004). Additionally, non-equilibrium, i.e., kinetic processes often distinctively fractionate clumped isotopologues. Accordingly, measurements of clumped isotopologue abundances can quantify and give insight into kinetic processes (Daéron et al., 2011). Clumped isotopologues have been studied previously in atmospheric CO$_2$ (Eiler and Schauble, 2004; Affek and Eiler, 2006; Affek et al., 2007), CO$_2$ released from carbonate bearing minerals via digestion in phosphoric acid (Eiler, 2007, 2011; Ghosh et al., 2006) and in atmospheric O$_2$ (Yeung et al., 2012). However, sufficiently precise studies of clumped isotopologues of hydrocarbons at natural abundances have not previously been realized because of the inherent difficulty in distinguishing $^{13}$C from D substitutions.

$^1 \delta = (R/R_{rd} - 1) \times 1000$ where $^{13}\text{R} = [^{13}\text{C}]/[^{12}\text{C}]$, $^{13}\text{R} = [\text{D}]/[\text{H}]$, and ‘std’ denotes the standard to which all measurements are referenced. For this paper all carbon measurements are referenced to VPDB and all hydrogen measurements to VSMOW.

Studies of isotopic clumping in methane could have a variety of geochemical and geobiological applications. For example, if methane forms in internal isotopic equilibrium, excesses of clumped isotopologues would yield formation temperatures that allow for unambiguous designations of high-temperature thermogenic vs. low-temperature biogenic sources, which is not always possible using just the stable isotopes of methane (Valentine et al., 2004). Abiogenic sources of methane, such as methane produced via serpentinization or in hydrothermal systems (Etiope and Lollar, 2013) could also yield potentially distinctive temperatures (Fig. 1). If, instead, methane does not form in or preserve internal isotopic equilibrium due to kinetic fractionations associated with transport or irreversible reactions, its distribution of clumped isotopologues could serve as a fingerprint for these processes, much as $^{14}$C is used to distinguish fossil from recent sources of methane where $^{13}$C and D alone are insufficient (Wahlen et al., 1989).

Kinetic isotope effects associated with atmospheric photochemical oxidation of methane are likely to be particularly important applications of this kind. The atmospheric lifetimes of multiply substituted isotopologues are predicted to be longer than their singly and unsubstituted counterparts, such that methane in air may develop an excess in the abundance of $^{13}$CH$_2$D, $^{12}$CH$_3$D$_2$, and other clumped species relative to a random distribution of isotopologues. Although quantitative estimates for this enrichment have only been made for $^{13}$CH$_3$D and $^{12}$CD$_4$ (Kaye and Jackson, 1990), all seven multiply substituted isotopologues could exhibit distinctive photochemical signatures. Thus, abundances of clumped isotopologues of methane in modern or fossil air could reflect changes in atmospheric sink processes (e.g., OH$^-$ concentrations). Clearly, some processes could reflect both equilibrium and kinetic processes. Additionally, in some cases, equilibrium and kinetic effects could result in similar clumped isotopic compositions. At present, because so little is understood about multiply substituted isotopologues of methane in nature, we do not attempt to address these possible complexities. Rather, we focus here on the equilibrium effects that will be the basis

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Table 1

<table>
<thead>
<tr>
<th>Isotopologue Proportional relative abundance of CH$_4$</th>
<th>Exact mass (amu)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{12}$CH$_4$</td>
<td>9.88 x 10$^{-3}$</td>
</tr>
<tr>
<td>$^{13}$CH$_4$</td>
<td>1.11 x 10$^{-2}$</td>
</tr>
<tr>
<td>$^{12}$CH$_3$D</td>
<td>6.16 x 10$^{-4}$</td>
</tr>
<tr>
<td>$^{13}$CH$_3$D</td>
<td>6.92 x 10$^{-6}$</td>
</tr>
<tr>
<td>$^{12}$CH$_2$D$_2$</td>
<td>1.44 x 10$^{-7}$</td>
</tr>
<tr>
<td>$^{13}$CH$_2$D$_2$</td>
<td>1.62 x 10$^{-9}$</td>
</tr>
<tr>
<td>$^{12}$CHD$_3$</td>
<td>1.49 x 10$^{-11}$</td>
</tr>
<tr>
<td>$^{13}$CHD$_3$</td>
<td>1.68 x 10$^{-13}$</td>
</tr>
<tr>
<td>$^{12}$CD$_4$</td>
<td>5.82 x 10$^{-16}$</td>
</tr>
<tr>
<td>$^{13}$CD$_4$</td>
<td>6.54 x 10$^{-18}$</td>
</tr>
</tbody>
</table>

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* Assumes that isotopes are randomly distributed throughout all isotopologues and that $^{13}$C = 0$^\circ$ and D = 0$^\circ$ (relative to VPDB and VSMOW respectively).
of any methane clumped isotope thermometer and that will serve as a reference frame for recognizing and interpreting non-equilibrium isotope effects.

Critical to any of these possible applications is the ability to measure the relative abundances of clumped isotopologues of methane precisely and accurately. A few attempts have been made to measure clumped isotopologues of methane in natural materials. Mroz et al. (1989) used a quadrupole mass spectrometer to measure the abundance of mass-20 methane isotopologues ($^{13}$CHD$_3$ and $^{12}$CD$_4$) in the troposphere. They found that tropospheric mass-20 methane is 500 fold ($\approx 500,000$) enriched compared to a random distribution of isotopes, which is two orders of magnitude larger than model predictions (Kaye and Jackson, 1990). Mroz et al. (1989) attributed this extreme enrichment to atmospheric loss processes, but this result has not been tested or reproduced using different samples and techniques – it could be correct, but it also might reflect release of $^{12}$CD$_4$ in atmospheric circulation experiments or an analytical error. Additionally, two published attempts have been made to measure $^{13}$CH$_3$D abundances through infrared spectroscopy (Ma et al., 2008; Tsuji et al., 2012). Only Tsuji et al. (2012) measured methane with a natural abundance of isotopes. Their precision is $\approx 20\%$ (1σ), which is several times ($\approx 2.5\times$) larger than the expected range for equilibrium processes between 0 and 1000 ºC (see below). If errors improve or, as suggested by Mroz et al. (1989), signals are large, then spectroscopy might become an attractive method to measure clumped isotopes of methane (Ono et al., 2013).

We present here a new mass spectrometric technique to directly measure the stable isotopologues of methane throughout the introduction of methane gas directly to the ion source of an isotope-ratio mass spectrometer (IRMS) instead of first converting it to H$_2$ or CO$_2$, as is commonly done. This paper describes the methodology, accuracy, precision, and experimental reproducibility, including all stages of sample preparation and handling. Of our technique, calibrates the relevant clumped isotope thermometer isotope exchange reaction theoretically and experimentally, and reports preliminary measurements of experimentally produced and natural methane. Preliminary reports of this work were presented by Stolper et al. (2012, 2013).

2. THEORY AND NOMENCLATURE OF MULTIPLY SUBSTITUTED (CLUMPED) ISOTOPOLOGUES

A detailed review of the theory and nomenclature of clumped isotope geochemistry is presented in Eiler (2007, 2011, 2013) and Wang et al. (2004). We briefly review the critical concepts of this material here. Generally, most stable isotopic studies assume that isotopes are distributed randomly amongst all isotopologues. For example, this assumption requires the abundance of $^{12}$CH$_2$D as a fraction of all methane molecules to equal $4[^{12}\text{C}[\text{H}]^3[^{1}]\text{D}]$, where $[^{12}\text{C}]$ is the fractional abundance of $^{12}$C among all carbon atoms, $[^{1}]\text{D}$ is the fractional abundance of D among all hydrogen atoms, and the prefactor of 4 is needed to account for the 4 symmetrically equivalent configurations of D in this isotopologue. Although this assumption is at the foundation of nearly all gas-source isotopic measurements of H, C, N, O, and S (excluding SF$_6$), it is not precisely true except at infinite temperature (Urey and Rittenberg, 1933), or per-
happens through unknown non-equilibrium processes that can randomize isotopic distributions. Instead, for systems at equilibrium, isotopes are generally distributed such that isotopologues with two or more heavy (usually rare) isotopes have a higher concentration compared to a random distribution of isotopes amongst all isotopologues (Wang et al., 2004). For example, for the reaction:

$$^{13}\text{CH}_3\text{D} + ^{12}\text{CH}_4 \rightarrow ^{13}\text{CH}_4 + ^{12}\text{CH}_3\text{D} \tag{1}$$

the left side of the equation, which contains the clumped species $^{13}\text{CH}_3\text{D}$, is thermodynamically favored over the right side at finite temperatures. This preference largely occurs due to subtle differences in vibrational energies between isotopologues, which make the left side of the reaction $^{13}\text{CH}_3\text{D} + ^{12}\text{CH}_4$ lower in total zero point energy than the right ($^{13}\text{CH}_4 + ^{12}\text{CH}_3\text{D}$). The temperature dependence of such reactions arises due to entropic contributions to the total energy, which increasingly favor disorder at higher temperatures. Disorder is maximized when all isotopes are randomly distributed amongst all isotopologues. For equilibrated systems, this maximization occurs at infinite temperature. For a given average isotopic composition ($\delta^{13}\text{C}$ and $\delta^{18}\text{D}$), this results in $^{13}\text{CH}_3\text{D}$ being more abundant at equilibrium at low temperatures and less abundant at high temperatures.

Measurements of stable isotope compositions are generally reported relative to a reference frame based on the known or assumed composition of a reference material such as VSMOW for oxygen isotopes. For clumped isotope geochemistry, measurements are reported as deviations from a random state in which the isotopes are randomly distributed amongst all isotopologues. We report the enrichment or depletion of multiply substituted species compared to the random state using the symbol $\Delta_i$ where $'i'$ indicates the reaction or reactions of interest. For the case of Eq. (1), we write:

$$\Delta_i = \left(\frac{R_{\text{enh}} - 1}{R_{\text{enh}} - 1} - \frac{R_{\text{depl}} - 1}{R_{\text{depl}} - 1}\right) \times 1000 \tag{2}$$

(Wang et al., 2004).

In Eq. (2), $R = \left(\frac{R}{\text{enh}}\right)^{12}\text{CH}_4$ where brackets denote fractional concentrations and the * denotes the random state. Note that at natural isotopic abundances, $\Delta_i$ is overwhelmingly dominated by the first term; i.e., the fractional concentrations of $^{13}\text{CH}_4$ and $^{12}\text{CH}_3\text{D}$ generally do not deviate significantly from their abundances at a random isotopic distribution. For example, if the concentration of $^{13}\text{CH}_4$ is $10^{\text{ppm}}$ enriched over its abundance at a random isotopic distribution, there are 70 ppm 'extra' $^{13}\text{CH}_3\text{D}$ molecules in the system. These extra molecules dominantly come at the expense of $^{12}\text{CH}_4$ and $^{12}\text{CH}_3\text{D}$ molecules and translate into deficits of $0.01^{\text{ppm}}$ for the concentrations of $^{13}\text{CH}_4$ and $0.1^{\text{ppm}}$ for $^{12}\text{CH}_3\text{D}$ compared to a random isotopic distribution. These depletions are over two orders of magnitude smaller than the enrichment in the concentration of $^{13}\text{CH}_3\text{D}$ and thus can be ignored for processes with $\Delta_i$ values less than $\approx \pm 10^{\text{ppm}}$, which is the expected range for most equilibrium processes. Consequently, positive values of $\Delta_i$ occur when there is more $^{13}\text{CH}_3\text{D}$ relative to $^{12}\text{CH}_4$ in the sample as compared to a random isotopic distribution and negative values when there is less $^{13}\text{CH}_3\text{D}$ relative to $^{12}\text{CH}_4$ as compared to a random isotopic distribution. $\Delta_i$ is related to the equilibrium constant for Eq. (1), $K_{\text{eq}}$, through the following equation:

$$\Delta_i \approx -1000 \ln \left(\frac{K_{\text{eq}}}{K_{\text{eq}}}\right) \tag{3}$$

(Wang et al., 2004).

As explained below, the measurement of isotopic clumping in methane that we present here combines the ion currents of $^{13}\text{CH}_3\text{D}$ and $^{12}\text{CH}_3\text{D}_2$, which are the only two mass-18 doubly substituted species. For this reason, we introduce an additional nomenclature:

$$\Delta_{18} = \left(\frac{18R - 1}{18R - 1}\right) \times 1000, \quad \tag{4}$$

where $^{18}R = \left(\frac{13^{12}\text{CH}_3\text{D} + 12^{12}\text{CH}_3\text{D}_2}{12^{12}\text{CH}_4}\right)$. Eq. (4) is exactly analogous to clumped isotope nomenclature for measurements of CO$_2$ at mass 47, which include all three of the multiply substituted species at mass 47 and assumes the unsubstituted and singly substituted species are randomly distributed. A second homogeneous exchange reaction in addition to Eq. (1) influences the $\Delta_{18}$ value defined in (4):

$$^{12}\text{CH}_3\text{D}_2 + ^{12}\text{CH}_4 \rightarrow 2^{12}\text{CH}_3\text{D}. \tag{5}$$

Fig. 1 presents model predictions of the combined equilibrium constants for Eqs. (1) and (5) using the theory of Bigeleisen and Mayer (1947) and Urey (1947), with vibrational frequencies calculated by Bottinga (1969). The total range of values for equilibrated systems from 0 to 1000 K is $\approx 7^{\circ}$ with a temperature sensitivity at 25 $^\circ$C of $\approx 30^{\circ}$C/1000. We consider a reasonable target for the precision of initial measurements to be $\pm 10^{\circ}$ at 25 $^\circ$C, which translates into an uncertainty in $\Delta_{18}$ (or, almost equivalently, $\Delta_i$) of $\pm 0.3^{\circ}$. This level of precision would allow for the differentiation of most thermogenic gases from biogenic or low-temperature abiogenic gases if they form in and preserve isotopic equilibrium.

The fact that we combine signals from $^{13}\text{CH}_3\text{D}$ and $^{12}\text{CH}_3\text{D}_2$ leads to potential ambiguities in the interpretation of $\Delta_{18}$ values. At equilibrium, this combination has no meaningful effect as both $\Delta_i$ and $\Delta_i$ are temperature dependent, and thus $\Delta_{18}$ values are a well-defined function of temperature (Fig. 1). However, in methane that has been subjected to kinetic fractionations or mixing without re-equilibration, this approach obscures any information that might be recorded by distinctive differences between $\Delta_i$ and $\Delta_i$. Except in extraordinary circumstances, $\Delta_{18}$ values will closely approximate $\Delta_i$ values because $^{12}\text{CH}_3\text{D}_2$ is $\approx 50$ times less abundant than $^{13}\text{CH}_3\text{D}$ at natural isotopic abundances (Table 1). Thus, if $\Delta_i$ changes by $1^{\circ}$ without any change in $\Delta_i$, $\Delta_{18}$ will change by $0.02^{\circ}$, which is $\approx 10^{\circ}$ of our analytical uncertainty. In order for $\Delta_{18}$ to be measurably influenced by differential fractionations of $^{13}\text{CH}_3\text{D}_2$ vs. $^{12}\text{CH}_3\text{D}$,
\[ \Delta_{\text{CH}_2D}, \text{ must change by at least } \sim 10-15\% \text{, more than expected based on the } \Delta_{\text{CH}_2D} \text{ value. Processes that might lead to such effects include photochemical enrichments or depletions of } ^{12}\text{CH}_2\text{D}_2 \text{ or mixtures of end members that differ in } \delta D \text{ by } \sim 250^{\circ}\text{ or more. In these cases, interpretations of } \Delta_{18} \text{ measurements would be aided if accompanied by an additional constraint, such as a measurement of } ^{12}\text{CH}_3\text{D} \text{ alone, such that the abundance of } ^{12}\text{CH}_2\text{D}_2 \text{ could be calculated by difference. This measurement is possible with the instrument we use and methods broadly similar to those we present; however, a full exploration of this alternative approach is beyond the scope of this paper.} \]

### 3. STABLE ISOTOPE MEASUREMENTS

Conventional mass spectrometric analyses of the average (bulk) isotopic composition of methane first convert methane to either CO$_2$ for carbon isotopes (Craig, 1953) or H$_2$ for hydrogen isotopes (Bigeleisen et al., 1952). This conversion is necessary because most modern gas-source IRMS can only mass resolve species that differ by one or more amu (mass resolutions typically ~200), and thus cannot distinguish between $^{13}$CH$_4$ and $^{12}$CH$_3$D$^+$ or interfering isotobyes like $^{17}$O$^+$ or $^{16}$OH$^+$, which are produced mostly from fragmentation of H$_2$O. These interfering isotobyes are always present in the background of mass spectrometers. Similar issues with isotobic interferencies would be encountered in any attempt to measure the clumped iso-toyses of methane at mass 18, $^{13}$CH$_3$D$^+$ and $^{12}$CH$_2$D$_2$$^+$, using a conventional, low-resolution (mass resolution of ~200) gas-source IRMS. H$_2$$^{16}$O$^+$ is the most problematic as its abundance in any IRMS is generally ~5-fold larger than that of $^{13}$CH$_3$D$^+$ and $^{12}$CH$_2$D$_2$$^+$. The conventional response to these challenges – conversion of methane to other gases having simpler mass spectra – cannot be applied to the measurement of clumped isotobyes because the proportional distribution of $^{13}$C and D among the various isotobyes of CH$_4$ is destroyed on conversion along with the original molecules.

Our approach to this problem is the direct mass spectro-metric analysis of methane using a high-resolution multi-collector mass spectrometer. Our measurements make use of a new instrument, the Thermo Scientific IRMS-253 Ultra (or ‘Ultra’) housed in the Division of Geological and Planetary Sciences at Caltech. The design and capabilities of this instrument are described in Eiler et al. (2013). Significantly, for the purposes of the work presented here, the Ultra routinely achieves mass resolving powers between 20,000 and 25,000, which is sufficient to separate methane isotobyes from each other and from contaminating isotobyes like H$_2$O$^+$, OH$^+$, NH$_3^+$, etc. We use this instrument to examine the species $^{12}$CH$_4^+$, $^{13}$CH$_4^+$, $^{12}$CH$_3$D$^+$, $^{12}$CH$_2$D$_2^+$, and $^{12}$CH$_2$D$_3^+$. We have found that the most precise and rapid analyses are made when some isotobyes are collected along with isotobic hydrogen adducts (H-adducts) such as $^{12}$CH$_3$H$^+$ and fragments such as $^{13}$CH$_4^+$ and $^{12}$CH$_3$D$^+$. These isotobyes are then corrected for during data processing rather than attempting to mass resolve them. Importantly, as previously discussed, we measure $^{13}$CH$_3$D$^+$ and $^{13}$CH$_2$D$_2^+$ together. However, we demonstrate below that the direct analysis of $^{13}$CH$_3$D$^+$ without $^{13}$CH$_2$D$_2^+$, $^{13}$CH$_2$D$_3^+$, and $^{12}$CH$_2$D$_3^+$ can be made with a precision of ±0.43% similar to the internal precision of our measurements of $\Delta_{18}$ (±0.27–0.29) and thus that the $^{12}$CH$_2$D$_2$ abundance could be calculated by difference between the two measurements. We do not develop this alternative approach in any further detail here, preferring to leave it for future studies that focus on photochemical fractiona-ions of methane, as opposed to the smaller equilibrium thermodynamic effects that motivate this study. We first de-scribe how measurements are made at mass 17 to derive bulk $\delta^{13}$C and $\delta$D values and then discuss measurements at mass 18. The species present in our measurements of masses 16, 17, and 18 and their relative abundances are given in Tables A1 and A2.

#### 3.1. Mass 17

The mass spectrum of methane at mass 17 as measured in the Ultra visibly contains (as read on a Faraday cup through a 10$^{12}$Ω amplifier), in order from lower to higher mass, $^{16}$OH$^+$ (produced from the fragmentation of H$_2$$^{18}$O), $^{14}$NH$_3^+$, $^{13}$CH$_3$D$^+$, $^{12}$CH$_3$D$^+$, and $^{12}$CH$_2$D$_2^+$ (produced by the addition of an H to $^{12}$CH$_2$D$^+$, which we term an H-adduct; Fig. 2A). Additionally, $^{17}$O$^+$, $^{14}$NH$_3^+$, and fragments of heavier methane isotobyes (e.g., $^{13}$CH$_2$D$^+$) must also be present, but at a level we cannot detect on a Faraday cup read through a 10$^{12}$Ω amplifier. $^{14}$NH$_3^+$ is not a contaminant from our samples, but is instead present in the instrument background at all times. $^{16}$OH$^+$ is fully separated from all methane ions, despite our use of relatively wide collectors (1.3 mm) to permit flat-topped peaks. $^{14}$NH$_3^+$ is formally mass resolved from methane ions, but overlaps the collector aperture for much of the peak width of the methane isotobyes, resulting in a shoulder on the high-mass side of the major composite peak that is fully resolved from $^{14}$NH$_3^+$ (Fig. 2A). As explained below, we measure on this high-mass shoulder of the methane peak (>17.045 amu). Consequently the only species contributing to the measured mass-17 ion current are $^{12}$CH$_3$D$^+$, $^{12}$CH$_2$D$_2^+$, $^{12}$CH$_2$D$_3^+$, and the fragments, $^{12}$CH$_3$D$^+$ and $^{12}$CH$_2$D$_2^+$. Because both fragments are present at low abundances (<0.05% of the signal for both measurements described below) and likely contribute roughly equally to both the sample and the standard gases, we can safely ignore them. Their presence may need to be trea-ted explicitly in the study of gases that exhibit photochemical or other fractionations of many thousands of per mil through additional corrections.

The conceptually simplest approach to determining the $\delta^{13}$C and $\delta$D values for a sample would be to measure the ion-current ratios for $^{12}$CH$_3$D$^+$/$^{12}$CH$_4^+$ and $^{13}$CH$_3$D$^+$/ $^{13}$CH$_4^+$ separately and with no isotobic interferencies on either species. However, as is apparent in Fig. 2, this approach is not possible without employing a much narrower collector width because $^{12}$CH$_3$D$^+$ is situated between $^{13}$CH$_3$H$^+$ and $^{12}$CH$_4^+$ and $^{13}$CH$_4^+$ is between $^{14}$NH$_3^+$ and $^{12}$CH$_3$D$^+$. 

Any collector narrow enough to permit resolution of all of these species would likely fail to achieve flat peak tops and thus stable ion currents. For this reason, we opted to retain the wide collectors used to make the scan in Fig. 2 and to constrain the relative abundances of the species of interest through corrections applied to measurements that combine two or more species.

Our approach is to constrain the $\delta^{13}$C and $\delta$D values of a sample by measuring the ratios, ($^{13}$CH$_3^+$ + $^{13}$CH$_5^+$)/(12CH$_3^+$ + $^{12}$CH$_5^+$) and ($^{12}$CH$_3^+$ + $^{13}$CH$_5^+$)/(13CH$_3^+$ + 13CH$_5^+$ + 12CH$_3^+$ + 13CH$_4^+$). The mass-16 measurement includes ~1% contributions from the fragments 13CH$_5^+$ and 12CH$_3^+$, which we correct for during data processing (Appendix A). We remove the H-adduct contribution from both the sample and standard using the independently calibrated dependence of the 13CH$_5^+$/(13CH$_4^+$ + 13CH$_5^+$ + 12CH$_3^+$) ratio on the intensity of the mass-16 ion beam, and then solve for the 13CH$_5^+$/12CH$_4^+$ and 13CH$_4^+$/12CH$_3^+$ ratios (Appendix A). We chose not to make any measurements that include 14NH$_3^+$ as a background species because it can drift in intensity over the course of a measurement making it difficult to accurately subtract from both the sample and standard measurements. We assume these ratios fully constrain $\delta^{13}$C and $\delta$D; i.e., excesses or deficits in the amount of D and 13C contained in the clumped isotopologues compared to a random distribution of isotopes are too small to change the fractional abundances of 13CH$_4$ and 12CH$_3$D measurably beyond their random isotopic distributions. This approach may need to be re-evaluated in cases where samples have large enrichments in multiply substituted species (e.g., $\geq 10\%$).

Our approach to correct for the presence of 12CH$_3^+$ in our measurements is analogous to that used in conventional $\delta$D measurements of hydrogen gas where H$_3^+$ (the H-adduct of H$_2^+$) contributions must be subtracted from the mass-3 ion current to recover the true HD signal (Friedman, 1953). We assume, as is similarly done for hydrogen isotope measurements of H$_2$, that $^{12}$CH$_5^+$ forms via the ion-neutral reaction:

$^{12}$CH$_3$ + $^{12}$CH$_4$ + $^{12}$CH$_5$ + $^{12}$CH$_7$.

(6)

We will assume that this reaction includes both second and third order reactions. Generally, for H$_3^+$ formation, this equation is assumed to only include a second order reaction. However, we have experimentally verified, as will be developed below, that a third order reaction is needed to describe the formation of 13CH$_5^+$ as a function of 13CH$_5^+$ pressure in the source. This combined third and second order dependence is written as

$^{12}$CH$_3$ + $^{12}$CH$_4$ + $^{12}$CH$_5$ + $^{12}$CH$_7$.

(7)

where $k_1$ and $k_2$ are constants. The current registered by a Faraday cup at mass 17 ($i_{17,\text{total}}$) is made up of two or three distinct contributors depending on the measurement being made, $i_{17,\text{total}} = i_{17,\text{CH}_4} + i_{17,\text{CH}_3}$ and/or $i_{17,\text{CH}_5}$ and $i_{17,\text{CH}_7}$, and is given by

$i_{17,\text{total}} = i_{17,\text{CH}_5} + i_{17,\text{CH}_3}$

(8a)

and

$i_{17,\text{total}} = i_{17,\text{CH}_5} + i_{17,\text{CH}_3} + i_{17,\text{CH}_7}$.

(8b)

Rewriting Eq. (7) in terms of ion currents gives $i_{17,\text{total}} = k_1(i_{16})^3 + k_2(i_{16})^2$, where $i_{16}$ is the total current at mass 16. This is not strictly correct as our measurement of $i_{16}$ includes 12CH$_4^+$, which makes up 99% of the signal, and the fragments, 13CH$_5^+$ + 12CH$_3$D$^+$, which together make up 1% percent of the signal. The correction needed to account for the presence of 13CH$_5^+$ and 12CH$_3$D$^+$ at mass 16 is presented in Appendix A. However, it is a small correction, changing $\Delta_{18}$ by $<0.02\%$ compared to not making the correc-
is proportional to $^{12}$CH$_5$, we substitute Eq. (6) into (8) giving

$$i_{17,\text{total}} = iv_{\text{CH}_4D} + k_1(i_{16})^3 + k_2(i_{16})^2$$

(9a)

and

$$i_{17,\text{total}} = iv_{\text{CH}_4} + iv_{\text{CH}_3D} + k_1(i_{16})^3 + k_2(i_{16})^2.$$  

(9b)

Division of Eq. (9) by $i_{16}$ gives:

$$\frac{i_{17,\text{total}}}{i_{16}} = \frac{iv_{\text{CH}_4D}}{i_{16}} + k_1(i_{16})^2 + k_2(i_{16}).$$  

(10a)

and

$$\frac{i_{17,\text{total}}}{i_{16}} = \frac{iv_{\text{CH}_4} + iv_{\text{CH}_3D}}{i_{16}} + k_1(i_{16})^2 + k_2(i_{16}).$$  

(10b)

Eqs. (10a) and (10b) are equations for a line (called an ‘adduct line’) where $iv_{\text{CH}_4D}/i_{16}$ and $(iv_{\text{CH}_4}+iv_{\text{CH}_3D})/i_{16}$ are both the intercepts, and importantly, the ratios of interest. The constants, $k_1$ and $k_2$, define the curvature of the line. We use the constants to remove the adduct contribution from all measured ratios at a given current of mass 16.

We determine the value of $k_1$ and $k_2$ daily by measuring the $i_{17,\text{total}}/i_{16}$ ratio as a function of $i_{16}$ (Fig. 3) – this is done for two separate cases, one in which the ion current for mass 17 includes $^{13}$CH$_4^+$, $^{12}$CH$_3^+$, and $^{13}$CH$_2^+$ and one that includes only $^{12}$CH$_3^+$ and $^{12}$CH$_2^+$. We vary $i_{16}$ by manipulating the pressure of gas being delivered to the source via expansion and contraction of the bellows reservoir. A quadratic fit of these data yields the values of $k_1$ and $k_2$, which are then used to correct all subsequent measurements for $^{13}$CH$_3^+$ contributions.

As discussed above, adduct lines for H$_3^+$ contributions are generally treated as linear as opposed to quadratic (Sessions et al., 2001) and, thus, the reaction in Eq. (7) for hydrogen isotope measurements of H$_2$ is considered to be second order. However, the relationship between $i_{17,\text{total}}/i_{16}$ shown in Fig. 3 is demonstrably curved rather than linear, which is why we have assumed that Eq. (7) includes both a third order and second order reaction pathway. We note, though, that the presence of curvature in adduct lines is also found in hydrogen isotope measurements of H$_2$ (Friedman, 1953) and thus could be included in those measurements as well. Though we do not know precisely what causes the curvature in the adduct line, some physical causes include: (1) $^{13}$CH$_3^+$ forms via the combined third and second order reactions as presented in Eq. (7). (2) $^{12}$CH$_2^+$ abundances in the source are affected by pressure dependent reactions with $^{12}$CH$_3$ that form larger hydrocarbons in the source such as C$_2$H$_6^+$ (Munson and Field, 1966). And (3) the abundance of $^{12}$CH$_4^+$ is lower due to increased fragmentation of $^{12}$CH$_4$ and $^{12}$CH$_2^+$ at lower source pressures (Friedman, 1953). Regardless, for our purposes, the critical observation is that $^{12}$CH$_2^+$ has a defined and stable dependence on $^{12}$CH$_4$ pressures in the source and thus can be modeled and removed from the measurement. We now proceed to show that this relationship yields accurate δD and δ$^{13}$C measurements when compared to conventional mass spectrometric techniques.

To establish the accuracy of our measurements, we compared the δD and δ$^{13}$C values of samples measured using both the Ultra and established combustion/reduction techniques. We compared four methane gases with differing δD values and thirteen samples with differing δ$^{13}$C values (Fig. 4; Tables 2 and 3). Conventional, offline δD measurements were made at Indiana University through combustion of CH$_4$ to CO$_2$ and H$_2$O followed by reduction of H$_2$O to H$_2$ with uranium following Schimmelmann et al. (1999). Adherence to the principle of equal treatment of sample and standard and the use of two-point calibrations along isotopic axes anchored to VPDB and VSMOW was achieved by using the primary international standards, NBS 19, L-SVEC, VSMOW and SLAP (the latter two waters had been reduced to H$_2$ with uranium at 800 °C).

One methane sample is used as the internal standard for the Ultra, leaving only three independent comparisons between the two techniques. All samples are shown on the VSMOW scale. In order to establish our accuracy, we linearly regressed δD values of samples measured using the offline uranium-reduction method (y-axis; Fig. 4A) against our measured δD values from the Ultra from the same samples (x-axis). These regressions are weighted regressions and incorporate the error in the conventional measurements, which vary from sample to sample. The slope of the line is 1.00 ± 0.023 (1 standard deviation, $\sigma$) and the intercept is $-1.35 ± 0.29$ ($\sigma$). The best fit line is thus indistinguishable within 1 standard deviation from a line with a slope of 1 that passes through the origin.

Conventional δ$^{13}$C values were measured at Petrobras’s research laboratories, the Power Environmental Energy Research Institute (PEERI), or at Indiana University. At Petrobras and PEERI, measurements were made by introducing a sample to a gas chromatograph coupled to an online combustion furnace with helium as the carrier...
gas, combusted to CO$_2$ and measured in continuous flow mode on a mass spectrometer. At Indiana University, samples were processed as above and the CO$_2$ measured in dual-inlet mode on a mass spectrometer. Two of these three labs (PEERI and Indiana University) analyzed our internal reference standard, letting us evaluate the ability of our approach to recover differences in $\delta^{13}$C based on direct comparisons to common standards; in these cases, we report the measured difference between a given sample and this standard. Our internal standard was not analyzed by Petrobras’s lab, raising the possibility that their measurements do not conform to a common reference frame with the others. To account for this, we compare our $\delta^{13}$C values to those measured at Petrobras through comparison to the sample closest to the average $\delta^{13}$C value of those samples. In other words, we treat this sample as the internal standard for this data set (only). This treatment is needed to compare the data, but implicitly requires that the standard is identical for both techniques. Thus, in our regression, we must enforce an intercept of zero. Regressing the values generated with conventional techniques (Fig. 4B; $y$-axis) against those derived on the Ultra (Fig. 4B; $x$-axis) with an intercept of zero results in a line with a slope of 0.93 ± 0.074 (1$\sigma$). This slope is statistically indistinguishable from a 1:1 line. This regression is again a weighted regression and includes the errors from the conventional measurement, which vary depending on the technique. Overall, the bulk isotopic ratios measured by Ultra and conventional techniques are within error of each other and we conclude that the Ultra
d

![Fig. 4](image-url)

Fig. 4. Comparison of bulk isotopic measurements made on the Ultra and through conventional techniques for $\delta$D (Fig. 4A) and $\delta^{13}$C measurements (Fig. 4B). Conventional $\delta$D measurements were made through conversion of CH$_4$ to H$_2$ while conventional $\delta^{13}$C measurements were made through conversion of CH$_4$ to CO$_2$. For 4b no intercept is given as the fit was forced through the origin – see text for details.

d

<table>
<thead>
<tr>
<th>$\delta^{13}$C offline ($^\circ_{\text{VPDB}}$) ± ($^\circ_{\text{VPDB}}$)</th>
<th>$\delta^{13}$C Ultra ($^\circ_{\text{VPDB}}$) ± ($^\circ_{\text{VPDB}}$)</th>
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<tr>
<td>$-5.22^A$</td>
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<tr>
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<td>$30.81^C$</td>
<td>$0.35$</td>
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</table>

* On the VSMOW scale for $\delta$D and VPDB scale for $\delta^{13}$C.

A indicates measurements made at Petrobras, ‘B’ PEERI, and ‘C’ Indiana University.

The reference standard is the sample measured with the closest $\delta^{13}$C to the average of the entire suite for samples supplied by Petrobras. All other samples are referenced relative to our working reference gas.

1$\sigma$ error.
is capable of making measurements that are on the same isotopic scale as conventional δ13C and δD measurements of methane.

### 3.2. Mass 18

The mass-18 mass spectrum (measured on an electron multiplier) visibly contains H16O+, 13CH3D+, and 14NH4+ (Fig. 2B; note we do not display H16O− due to its large size, ~100,000 counts per second at high resolution). Additionally, though not visible at the plotted scale, 12CH2D2+ and 12CH1D3+ are present and are significant factors in the interpretation of our measurements. H16O+, D16O+, 15NH3+, 14NH3+ and fragments of heavier methane isotologues such as, 13CH2D+ must also be present but are either fully mass resolved or sufficiently low in abundance (e.g., 13CH2D+ <0.01%) as to be negligible factors. Ions derived from isotologues of water and ammonia are fully resolved from methane ions at the medium-resolution setting of the instrument (mass resolving power of 16,000–17,000; 16 μm entrance slit). Thus our measurement of the mass-18 ion current includes signals from the 13CH2D+ fragment, which we ignore, the isotologues of interest, 12CH2D+ and 13CH2D3+; the H-adducts, 13CH5+ and 12CH5+.

13CH2D+ can be mass-resolved from 13CH5+ and 12CH2D2+ at the highest-resolution setting of the instrument (mass resolving power of 20,000 using a 5 μm entrance slit) as demonstrated in Fig. 2B. We have performed measurements of the 13CH2D+/13CH5+ (12CH2D2+/12CH5+) ratio, which is a direct constraint on 13CH2D abundance when accompanied by the measurements of mass-17 species described above, with an internal precision as good as ±0.43‰ (1σ) and similar to the counting statistics limit for that measurement of 0.35‰. However, there are several advantages to measuring at the high-mass side of the peak in Fig. 2B (near mass 18.053), where the ion currents of 13CH2D+ and 12CH2D2+ are combined along with the adducts, 12CHD2+ and 13CH3+. First, this approach minimizes potential contributions from 12NH3+ or 14NH3+ that are not clearly visible in Fig. 2B but must be present, based on observations at mass 17 (Fig. 2A). Second, it minimizes potential contributions from the ‘tailing’ of the water peak. And third, it can be made at the medium-resolution setting of the Ultra, which provides a threefold increase in transmission as compared to the high-resolution setting and thus allows for shorter integration times. For these reasons, we include in the mass-18 ion current, 13CH2D+, 13CH5+, 12CH2D2+, and 12CH2D+. We correct for the contributions from adducts at mass 18 in a manner similar to that for mass-17 species. The details of this correction are provided in Appendix B.

We make measurements of mass-18 methane isotologues using a secondary electron multiplier with typical signal intensities of ~20,000 counts per second. A dead time of 40 ns is applied to this amplifier. Consequently, a precision of ±0.3‰ for Δ18 measurements requires integration times of ~1200 s for both the sample and standard such that their counting statistics errors, added in quadrature, sum to 0.3‰.

### 4. ANALYTICAL PROCEDURES

We outline here how each measurement is made on a daily basis. This material is presented in greater detail than is common in published reports of isotopic measurements because this is a new method. All measurements are made in reference to our laboratory’s standard ‘working’ reference gas (‘wg’) taken from a high purity (~99.999%) gas cylinder purchased from Air Liquide America. The reference gas’s δD and δ13C values were calibrated at Indiana University as described above. The procedures outlined below are also provided in a schematic flow chart (Fig. A3) in the appendix.

#### 4.1. Measurement of 12CH3D+/ (13CH4+ + 13CH5+ + 13CH2D7+)

The Ultra’s detector array is first configured to measure the ratio, (13CH4+/13CH5+ + 13CH2D7+), which is a key constraint on the δD value of the sample. We quantify this measurement, after removing the contribution of 13CH5+ to the measured signal using an adduct line (see above), with δ and R notations similar to δD and R. Specifically, the ratio, 12CH3D+/12CH4+ + 12CH3D+ in the sample vs. in the standard working reference gas (‘wg’), is expressed via the symbol δ12CH3D, where δ12CH3D = [12CH3Dample/12CH3Dwg] × 1000 and 12CH3D = 12CH3Dample/12CH4ample + 12CH5ample + 12CH2D7ample. The denominator of 12CH3D is the mass-16 signal and is dominated by 12CH4+ (99%) but also contains contributions from the fragments, 13CH3+ and 12CH2D+. Because 12CH3D includes contributions from both 12CH2D+ and 12CH4+ (via the fragment 13CH5+), we need a second, independent measurement of 13CH4 vs. 12CH3D to fully constrain the 12CH3D/12CH4 abundance and thus calculate the δD value. This additional measurement is described in Section 4.2. However, to first order, a sample’s δD value and precision are controlled by its δ12CH3D value and precision. We correct for the methane fragments, 13CH3+ and 12CH2D+, in the denominator of 12CH3D during data processing (see Appendix A).

Mass-16 species are collected in a Faraday cup registered through a feedback electrometer employing a nominal 1010 Ω feedback resistor (hereafter 1010 Ω amplifier) on one of the Ultra’s moveable collector trolleys. The sum of 12CH4+ + 12CH5+ at mass ~17.05 is collected in a Faraday cup registered through 1012 amplifier on the central, fixed collector position. The mass spectrometer is set to high resolution (~20,000–25,000 mass resolving power) by using a 5 μm entrance slit in order to generate a flat shoulder in the mass spectrum at ~17.05 amu (Fig. 2). At the beginning of an analytical session, typically once per day, the H-adduct correction is calibrated using the working reference gas on this feature with, generally, 6 points that span ~0.7–1.9 V on mass 16 (Fig. A1). The intercept of this calibration measured for the working reference gas is the 13CH3DRefpwk value referred to below. We will show that variations in 13CH3DRefpwk values control small (~1‰, relative) compressions or expansions of the scale of measured
$^{12}\text{CH}_3\text{D}$ values within and between analytical sessions. $^{12}\text{CH}_3\text{D}R_{\text{wg, int}}$ is the primary variable we examine when we calibrate a uniform reference frame for reporting absolute $^{12}\text{CH}_3\text{D}$ values and, thus, absolute δD values.

Measurements are organized into ‘blocks’ referred to as acquisitions. We start each acquisition by manually balancing the sample and standard bellows such that the ion current at mass 16 for both is 1.3 V, which corresponds to ~100 mV at mass 17. Next, the background signal for each mass is measured manually off-peak at ~16.3 and 17.3 amu with the standard gas flowing into the source. The sample and standard gases are then measured in 10 cycles composed of 16 s integration times and 15 s idle times. Finally, background signals are measured again before, averaged with the initial background, and subtracted from the measured voltages. We usually perform 7 acquisitions at which point the standard error of all of the acquisitions approaches 0.10–0.12%. Measurement uncertainty approaches and follows the combined Johnson noise (John and Adkins, 2010) and counting statistics limit (Merritt and Hayes, 1994; Fig. A2A).

4.2. Measurement of $(^{13}\text{CH}_4^+ + ^{12}\text{CH}_2\text{D}^+)/($^{13}\text{CH}_4^+ + ^{12}\text{CH}_2\text{D}^+ + ^{12}\text{CH}_2\text{D}^2+)$

After measuring the $^{12}\text{CH}_2\text{D}^+/($^{12}\text{CH}_4^+ + ^{12}\text{CH}_2\text{D}^+ + ^{12}\text{CH}_2\text{D}^2+)$ ratio, we replace the high-resolution entrance slit with the 16 μm medium-resolution entrance slit, achieving ~16,000–17,000 mass resolving power, and make a measurement that simultaneously collects ions at mass 16 (as above), the sum of $^{13}\text{CH}_4^+ + ^{12}\text{CH}_2\text{D}^+$ at mass 17 (near ~17.045 amu in Fig. 2A), and the sum of $^{13}\text{CH}_4^+ + ^{12}\text{CH}_2\text{D}^+ + ^{13}\text{CH}_3^+ + ^{12}\text{CH}_2\text{D}^+$ at mass 18 (near ~18.053 amu in Fig. 2B). The measurements are again quantified using δ and R values. For this mass-17 measurement, we first subtract the contribution of $^{12}\text{CH}_2\text{D}^+$ from the measured signal using an adduct line (see above). We then quantify ratio of $(^{13}\text{CH}_4^+ + ^{12}\text{CH}_2\text{D}^+)/(^{13}\text{CH}_4^+ + ^{12}\text{CH}_2\text{D}^+ + ^{12}\text{CH}_2\text{D}^2+)$ for the sample vs. the standard working reference gas with the symbol $^{12}\text{CH}_3\text{D}R_{\text{sample}}/^{13}\text{CH}_4R_{\text{sample}}$, where $^{12}\text{CH}_3\text{D}R_{\text{sample}}/^{13}\text{CH}_4R_{\text{sample}} = (^{12}\text{CH}_3\text{D} + ^{13}\text{CH}_4R_{\text{sample}})/(^{12}\text{CH}_3\text{D} + ^{13}\text{CH}_4R_{\text{sample}})$. This ratio is dominated by the abundance of $^{13}\text{CH}_4$ and provides the first order control on the δ$^{13}$C value and precision of a sample – however the δ$^{12}$C is required to actually calculate the δ$^{13}$C value (see below and Appendix A). The presence of the fragments in the measurement of mass 16 is corrected for during data processing (Appendix A).

We quantify and represent the mass-18 measurements similarly. First the contribution of the adducts $^{13}\text{CH}_3^+$ and $^{12}\text{CH}_4\text{D}^+$, to the mass-18 signal are subtracted using an adduct line (Appendix B). We then quantify the ratio of $(^{13}\text{CH}_3^+ + ^{12}\text{CH}_2\text{D}^+)/(^{13}\text{CH}_3^+ + ^{13}\text{CH}_4^+ + ^{12}\text{CH}_2\text{D}^+)$ in the sample vs. in the standard working reference gas with the symbol $^{12}\text{CH}_3\text{D}R_{\text{sample}}/^{18}\text{H}_2\text{O}R_{\text{sample}}$, where $^{12}\text{CH}_3\text{D}R_{\text{sample}}/^{18}\text{H}_2\text{O}R_{\text{sample}} = (^{12}\text{CH}_3\text{D} + ^{18}\text{H}_2\text{O}R_{\text{sample}})/(^{12}\text{CH}_3\text{D} + ^{18}\text{H}_2\text{O}R_{\text{sample}})$. The precision of a δ$^{18}$ measurement controls the ultimate precision of a sample’s Δ$^{18}$ value.

Masses 16 and 17 are collected in the same Faraday cups registered through the same amplifiers as above. Mass 18 is measured on a secondary electron multiplier on a moveable collector trolley. The H-adduct corrections are calibrated on the working reference gas with 6 points from ~3–7 V on mass 16 before the measurements are made. The intercept of this adduct line calibration for the mass-18 measurements is the $^{18}\text{H}_2\text{O}R_{\text{wg, int}}$ or H 2 value referred to below. We will show that variations in $^{18}\text{H}_2\text{O}R_{\text{wg, int}}$ values control small (~1%, relative) compressions or expansions of the scale of measured δ$^{18}$ values within and between analytical sessions. $^{18}\text{H}_2\text{O}R_{\text{wg, int}}$ is the primary variable we examine when we calibrate a uniform reference frame for reporting absolute δD values.

Measurements are again organized into acquisition blocks. First the source pressures are balanced using the bellows as above such that the signal at mass 16 for both the sample and standard registers 4 V, which results in mass-17 signals of ~4.5 V and mass-18 signals of ~20,000 counts per second (cps). Background signals are manually measured at ~16.3, 17.3, and 18.3 amu with the standard gas flowing to take into account the presence of scattered ions, which range from ~300–700 cps at mass 18. We measure the background of the standard gas before and after each acquisition to determine an average and subtract that number from both the sample and standard measurements. Acquisition parameters are the same as above. We measure until the internal precision of δ$^{18}$ approaches 0.25‰ (~15 acquisitions). Measurement errors for δ$^{13}\text{CD}_{3+}\text{D}^+\text{C}$ approach 0.005‰. Both the δ$^{13}\text{CD}_{3+}\text{D}^+\text{C}$ and δ$^{18}$ measurements follow counting statistics (Fig. A2B and C).

With measured values for $^{12}\text{CH}_3\text{D}, ^{12}\text{CH}_3\text{D}^+\text{C}$, and δ$^{18}$ the values and precisions for δD, δ$^{13}$C, and Δ$^{18}$ measurements can be calculated for a sample. The equations required for these calculations are provided in Appendix A. Generally, the internal precision achieved for δD measurements is 0.10–0.12‰ for δ$^{13}$C is 0.005‰, and for Δ$^{18}$ is 0.27–0.29‰. Additionally, unless otherwise noted, all Δ$^{18}$ values are reported relative to a reference frame in which the Δ$^{18}$ value of our laboratory’s working reference gas is assumed to be zero; i.e., $^{18}\text{H}_2\text{O}R_{\text{wg}} = 0$ where ‘wg’ refers to the laboratory’s working reference gas. In Section 7 of this paper we develop a universal reference frame based on the analyses of gases equilibrated at known temperatures, demonstrating that a $^{18}\text{H}_2\text{O}R_{\text{wg}}$ value of 0 corresponds to a Δ$^{18}$ value relative to random isotopic distribution of +2.981‰.

5. SAMPLE MANIPULATION

Dual-inlet stable isotopic measurements generally require relatively pure (~99%) samples. As methane is never (to the authors’ knowledge) the direct analytic for mass-spectrometric stable isotopic measurements, samples are generally purified from contaminants only after being converted to either CO2 or H2. We, however, require pure methane samples. To that end, we developed techniques for its concentration, purification and storage without isotopic fractionation. If methane must be separated from
other components of a natural gas sample, such as other hydrocarbons or $\text{H}_3$, methane is first frozen into a helium-cooled cryostat (CTI-Cryogenics and Janis Research Co.) set to 20 K. Residual gas is pumped away and the cold trap closed. The cold trap is warmed to 40 K, allowed to equilibrate for 2 min, and then the sample is exposed to vacuum for 2–3 min. Methane has a low vapor pressure at 40 K (~1 mPa) and thus little is lost. The cold trap is again closed and then warmed to 45 K, allowed to equilibrate for 2 min, and finally the sample is exposed to vacuum for 2–4 min. Methane has a larger vapor pressure at 45 K (25 mPa), but trial experiments demonstrate that pumping for 4 min at this temperature causes a negligible isotopic fractionation (see below). The cold trap is then warmed to 70 K, allowed to equilibrate for 2 min and then the sample is released from the trap (leaving more condensable compounds behind), condensed onto molecular sieves in a Pyrex® break seal (see below), and sealed with a natural gas torch. Gases removed at 20 K include helium and hydrogen. Oxygen and nitrogen are removed at 40 and 45 K. Ethane and larger hydrocarbons are retained at 70 K. Purified methane samples are stored in Pyrex® break-seals. To condense methane into the break-seals, type 5A molecular sieves (1/16 inch; EM) are used. Sieves are preheated under vacuum using a natural gas torch until baseline pressures are reached (typically <10 mPa). Samples are then condensed onto the sieve by cooling the break-seals in liquid nitrogen. Samples are released from the sieves by heating tubes for ~2 h using a heat gun set to 150 °C. Samples are then introduced into the bellows of the mass spectrometer with the sieve being continually heated. After the break-seal tube is cracked and gas has expanded into the bellows, the bellows are cycled from 100% to 30% open 25 times to ensure the methane is well mixed (Yeung et al., 2012).

We conducted a series of tests to ensure that our sample handling does not affect the measurements. Samples were measured directly from a bottle of pure gas (i.e., without vacuum line manipulation or condensation into a break seal), frozen onto a sieve and released into the mass spectrometer’s bellows without heating above room temperature, frozen onto a sieve and released with heating (i.e., our normal break-seal procedure), frozen into the 20 K trap and pumped for 2 min at both 20 and 40 K, then 4 min at 45 K, and then transferred to the mass spectrometer using our usual break-seal procedure as described above. The results of these experiments are presented in Table 4. Use of the sieve and heating of the sample during its release from the sieve does subtly shift the measured $\delta^{13}$C value by 0.1‰ and $\delta$D value by 0.5‰. Importantly for our purposes, though, no significant shift is observed in $\Delta_{18,\text{lg}}$ values (within 0.05‰; Table 4). We repeated this comparison of gas directly from a bottle vs. that released from a sieve and found offsets of 0.04‰ in $\delta$D (identical within error), 0.08‰ in $\delta^{13}$C, and 0.18‰ in $\Delta_{18}$ (identical within error). Consequently, it appears the $\Delta_{18}$ values are not disturbed within error through use of the sieve, and any fractionations of bulk isotopic compositions are subtle. We conclude that storage and retrieval of samples on and from molecular sieves preserves the $\Delta_{18}$ signature with minor changes to the bulk isotopes.

Freezing samples into the helium-cooled cryostat, pumping, then freezing onto a molecular sieve and releasing with heating does not change the bulk isotopic or $\Delta_{18,\text{lg}}$ values within the 1‰ error of the measurements as compared to not pumping but still freezing onto a sieve and releasing with heating. Failure to heat samples while releasing from the molecular sieves changes $\delta^{13}$C by $-1.4$‰, $\delta$D by $-1.2$‰, and $\Delta_{18,\text{lg}}$ by $0.49$‰ demonstrating the need to heat gases to fully release them with minimal fractionation. This may reflect a sorption isotope effect associated with the retention of a fraction of methane on the molecular sieve at room temperature.

### Table 4

Experiments involving sample handling. The sample marked ‘bottle’ is a sample stored as a gas and expanded into the mass spectrometer. ‘Sieve’ denotes a sample that was first frozen onto a molecular sieve and stored in a break seal. ‘Preheated’ represents a sample that was heated with a heat gun prior to introduction to the mass spectrometer. Finally ‘pumped on’ is a sample that was exposed to vacuum at a variety of temperatures before being frozen onto a sieve. See text for full details. All samples were taken from the same reservoir before manipulation.

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<th>± (‰)</th>
<th>$\delta$D (‰)</th>
<th>± (‰)</th>
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</table>

### 6. EXPERIMENTAL REPRODUCIBILITY

The internal precision associated with mass spectrometric acquisitions (described above) determines the minimum possible error of a measurement. However, experimental uncertainties associated with analyses of unknown samples may be larger due to random or systematic errors during purification, gas handling, changes in accuracy in the mass spectrometric standardization scheme, or other unknown factors. This is a particular concern for clumped isotope analyses of methane because we seek to achieve errors in $\Delta_{18}$ of $<0.3$‰ – which is better than the analytical uncertainties in conventional measurements of $\delta$D of methane and other organic compounds that have typical external precisions of $\sim1.8$‰, 1‰ (Dai et al., 2012). Consequently, we must establish our external reproducibility both within a single analytical session and over the course of multiple, separate sessions in order to fully document the real precision of our measurements. Note that the complexities we explore in this section are second order phenomena; our measurements of $\delta^{13}$C and $\delta$D are accurate to within the precision of conventional techniques without the corrections discussed here.
We demonstrate below that the key factors that control our reproducibility both within and between analytical sessions are small variations in the $^{12}\text{CH}_3^3\text{D}$ and $^{18}\text{R}_{\text{wg,int}}$ values – the intercepts of the adduct lines generated each day (see above). We show that measurements made days or even many months apart can be mutually standardized to within very narrow limits by normalizing the measurements based on differences in their respective $R_{\text{wg}}$ values. We explain this procedure by first demonstrating experimental reproducibility within a single measurement session (generally 1–3 weeks), and then apply the same concept to the problem of external reproducibility between sessions months apart.

6.1. Intrasession reproducibility

Within a single analytical session (~1–3 weeks), we notice that on replicate analysis, a sample’s measured $\delta^{13}\text{CH}_3^3\text{D}$ value and $\delta^{18}$ value are linear functions of the intercepts of the adduct lines. $^{12}\text{CH}_3^3\text{D}_{\text{wg,int}}$ and $^{18}\text{R}_{\text{wg,int}}$, used to correct those samples for H-adducts (Fig. 5). Importantly, the slopes of the lines in Fig. 5 imply that measured $\delta^{13}\text{CH}_3^3\text{D}$ and $\delta^{18}$ values decrease in direct proportion to increases in the $R_{\text{wg,int}}$ value; i.e., if $R_{\text{wg,int}}$ increases by 1% relative, the $\delta$ value decreases by 1% relative. This relationship is seen in samples with compositions varying by over 150‰ in $\delta^D$ and 29‰ in $\delta^{13}\text{C}$.

This effect is a form of scale compression (or expansion), which is observed in many measurements of stable isotope ratios such as in $\delta^D$ measurements of H2 and clumped isotope measurements of CO2 and O2. Two potential causes of this compression/expansion for measurements within a single analytical session are: (1) the intrinsic errors on the constants derived for the adduct lines cause inaccurate projections to zero pressure in the source. This would result in the observed effect because steeper adduct lines would cause smaller $R_{\text{wg,int}}$ values, and, thus, larger relative differences between the sample and standard. And (2) sessions differ from one another in the intensity of background counts from scattered ions (e.g., from OH+ and/or H2O+) that have been inaccurately accounted for in our background correction. This might occur if the measurements of backgrounds off peak do not accurately reflect the contributions of these signals on peak. The effects in question are too subtle (often <0.1 mV) for us to definitively prove by inspection of peak shape scans or other raw data. However, we suspect that the first of these two explanations, i.e., errors in the adduct line, is the dominant cause, because the actual errors of the slope and intercept generally overlap with the mean value for that week, implying that, in general, the parameters derived for a given day for an adduct line are statistically indistinguishable from lines produced on different days during that session. Additionally, it is unclear how a contaminant would cause the robust relationship seen in the plots of $^{13}\text{CH}_3^3\text{D}$ and $^{18}\text{R}_{\text{wg,int}}$ vs. $\delta^{13}\text{C}$ values (Fig. 5).

Regardless, we correct for this artifact by normalizing the data generated in each session to a constant value for $^{13}\text{CH}_3^3\text{D}_{\text{wg}}$. We perform this normalization by multiplying the $\delta^{13}\text{CH}_3^3\text{D}$ for each sample cycle of each acquisition by the average $^{13}\text{CH}_3^3\text{D}_{\text{wg}}$ for that cycle and then dividing by a constant assumed value of $^{12}\text{CH}_3^3\text{D}_{\text{wg}}$ that is used in all measurements in that analytical session. This procedure reduces the external precision of replicate analyses made in the same session to near the level of internal precision of each sample analysis. For the example in Fig. 5A, before correction, the standard deviation of the five $\delta^{13}\text{CH}_3^3\text{D}$ measurements is 1.14‰. This is an unacceptably high standard deviation because, no matter how precise a $\delta^{18}$ value can be achieved, the external precision for a $\delta^D$ will be ~1.14‰.

**Fig. 5.** Dependence of the $\delta$ value of a sample on the $R_{\text{wg,int}}$ value of the adduct line (the intercept) generated for each individual measurement using the working reference gas. In both 5a and b, all measurements have been made on aliquots of the same sample over multiple days. The linear dependence of the measured $\delta$ on the $R_{\text{wg,int}}$ is likely created due to slight inaccuracies in the adduct lines used to remove the contribution of $^{13}\text{CH}_2^2\text{D}^+$ from the measurement of $\delta^{13}\text{CH}_3^3\text{D}$ and $^{13}\text{C}_5^5\text{D}^+$ from the measurement of $\delta^{18}$. Correction to a constant $^{12}\text{CH}_3^3\text{D}_{\text{wg}}$ and $^{18}\text{R}_{\text{wg}}$ for each measurement in an analytical session (generally 1–3 weeks) can be made such that the internal and external precisions of the measurements overlap and these dependencies disappear. See text for a full explanation. (A) Dependence of $\delta^{13}\text{CH}_3^3\text{D}$ on $^{13}\text{CH}_3^3\text{D}_{\text{wg,int}}$. (B) Dependence $\delta^{18}$ on $^{18}\text{R}_{\text{wg,int}}$. 

Consequently, the external precision for $\Delta R$ will be at least 1.14%, which translates into a precision of $\pm 40^\circ C$ at 25°C. Application of the correction outlined above reduces the standard deviation of $\delta^{13}CH_2D$ to 0.18‰. In this example, the constant $^{13}CH_2D^R_{\text{avg}}$ used for the correction is the average $^{13}CH_2D^R_{\text{avg}}$ from all adduct lines measured through February 2013. With the exclusion of 1 measurement, the standard deviation of the four remaining samples is 0.08‰. This case is representative; generally external corrections for a single session for $\delta^{13}CH_2D$ range from 0.07 to 0.18‰—similar, but sometimes slightly larger than the internal precision of 0.1–0.12‰. No analogous correction is needed for measurements of $\delta^{28}CH_3D$–$^{28}CH_4$, which has internal precisions, generally, of 0.005–0.006‰ and an external precision of $\pm 0.020‰$, i.e., any increase in error due to this artifact is not important to the overall precision of $\Delta R$. A clear question is what is the correct $^{13}CH_2D^R_{\text{avg}}$ value that samples in an analytical session should be corrected to and does that value vary across analytical sessions. We address this question in more rigorous terms in the next section related to intersession reproducibility.

We interpret the correlation between $\delta^{18}$ and $^{18}R_{\text{avg,int}}$ in a single analytical session of a sample measured multiple times (Fig. 5B) to be caused for the same reasons given for the relationship between $\delta^{13}CH_2D$ and $^{13}CH_2D^R$ above. Again, the errors of the slopes and intercepts for a given day generally overlap in error with the average values for the whole session. For the example in Fig. 5B, internal precisions of the $\delta^{18}$ measurements range from 0.3 to 0.4‰ with an average internal precision of 0.37‰ (we had not yet decreased our internal errors to 0.25‰, when the measurements represented in Fig. 5B were made). However, the standard deviation of the measurements, 0.77‰, is larger than the internal precision. If, however, we correct, as above, each cycle within an acquisition to a constant $^{18}R_{\text{avg}}$ value and correct all samples using the same $^{18}R_{\text{avg}}$ (in this case the average $^{18}R_{\text{avg,int}}$ for all adduct lines from that measurement session), the standard deviation of the $\delta^{18}$ values contracts from 0.77 to 0.33‰, similar to the zavgerage internal precision of 0.37‰ for these samples. Again, as before, it is not obvious what value to use for $^{18}R_{\text{avg}}$ when making this correction; we establish this in the next section.

6.2. Intersession reproducibility

The corrections described above work well for short time-scales (weeks) for both $\delta^{13}CH_4D$ and $\delta^{18}$. However over many month time scales, during which there are filament changes, source bakes and venting of the mass spectrometer, external precision degrades when correcting all samples from different analytical sessions with the same $^{13}CH_2D^R_{\text{avg}}$ and $^{18}R_{\text{avg}}$ values. For $\delta^{13}CH_2D$ measurements, differences up to $\sim 1%$ between sessions are seen for samples $\sim 200‰$ different in $\delta^{13}CH_2D$ than the working reference gas, with smaller differences for samples that are more similar to the standard in bulk composition. For $\delta^{18}$ measurements, deviations up to $1%$ are seen between sessions for $\delta^{18}$ values $\sim 100‰$ different from the working reference gas, with smaller deviations if samples are more similar to the standard in $\delta^{18}$.

We examined whether these intersession variations are caused by changes in the instrumental mass bias, i.e., changes in the measured $^{13}CH_2D^R_{\text{avg}}$ value that arise from differences in fractionation in the ion source, detector efficiency, or other time-varying instrument properties. If so, it should be possible to correct for such artifacts by adopting a reference frame in which we assume a constant difference in $\delta$ value between two or more standards, which can be analyzed in each session and serve as a basis for ‘stretching’ or ‘compressing’ measured data to a constant long-term reference frame. Our approach here draws on a common practice in conventional $\delta D$ measurements (Hagemann et al., 1970), as well as many other measurements that attempt to observe relative differences between samples with high long-term precision. Here, we perform such a correction by adopting an assumed value for the difference in $\delta$ values between two standards (our working reference gas and a second reference material), and then test the success of our scheme by examining a third known material, which is analyzed and corrected as if it were an unknown sample.

For example, in a session run in September 2012, the average $\delta^{13}CH_2D$ of a standard, ‘standard 1,’ was 227.97 ± 0.07‰ ($n = 3, 1\sigma$) and in February 2013 was 226.92 ± 0.12‰ ($n = 1, 1\sigma$). ‘Standard 2’ had a $\delta^{13}CH_2D$ of 56.41 ± 0.18‰ ($n = 5, 1\sigma$) in September 2012, and 56.12 ± 0.11‰ ($n = 3, 1\sigma$) in February 2013, where $n$ is the number of measurements. During the February session, we analyzed a variety of gases having independently known $\delta D$ values. We varied the $^{13}CH_2D^R_{\text{avg}}$ value to which all measurements are corrected for this session to find the value that best fits all measured $\delta D$ values to those already established by conventional analyses standardized to the VSMOW–SLAP scale. This yielded a value for $^{13}CH_2D^R_{\text{avg}}$ that is indistinguishable from the average measured intercept value of all adduct lines over all sessions up to that point, i.e., our average value for $^{13}CH_2D^R_{\text{avg,int}}$, yields a ‘scale’ for $\delta D$ values that is indistinguishable from the VSMOW–SLAP scale.

Next, we rescaled our September 2012 data to the February 2013 session. To do this, we chose an $^{13}CH_2D^R_{\text{avg}}$ for the September 2012 session that forces the average $\delta^{13}CH_2D$ value for standard 1 to be 226.92‰ (again, relative to our working reference gas). Importantly, this $^{13}CH_2D^R_{\text{avg}}$ value was chosen only by using standard 1. After applying this $^{13}CH_2D^R_{\text{avg}}$ value to the measurement of standard 2 in September 2012, the average $\delta^{13}CH_2D$ value for standard 2 becomes 56.15‰, indistinguishable from the value for February 2013 (56.12‰). Thus, even subtle inter-session variations in our $\delta D$ scale can be removed through this procedure. We have tested this approach over the course of 1 year on standard 2 using standard 1 for the normalization. Doing so yields a long-term external precision for $\delta D$ of 0.11‰ (1σ) on 10 total measurements.

One implication of the success of this calibration of long-term accuracy is that the methods we present can be used to establish the $\delta D$ values of methane samples with external precisions approximately an order of magnitude smaller than previous $\delta D$ measurements of methane within a reference frame that asserts some axiomatic values of two or more methane standards. This could be useful for the
study of hydrogen isotope variations in methane sources that are complex mixtures of sources but vary little in δD (e.g., the atmosphere). Generally, we find it best to anchor this long-term calibration of the accuracy of δD using standards with large absolute values of δ18CH3D (100–200‰ greater than the working reference gas intralab standard) as they are more sensitive to scale compressions. This is the same reason that VSMOW and SLAP are used to calibrate scale compressions of conventional δD measurements.

We use a similar approach as above to correct for session-to-session variations in δ18 values of samples. However, unlike for δD measurements, there are no available gases with known δ18 values making it difficult to assign correct δ18 values to our standards as was done for the δ18CH3D measurements above. Our solution to this problem is patterned after that taken to calibrate previously established clumped isotope measurements of CO2 and O2, which report data relative to a ‘stochastic reference frame’ (i.e., a state in which all isotopes are distributed randomly among all isotopologues). The accepted method for establishing this reference frame is to equilibrate gases at known temperatures and then assume that they have taken on the theoretically predicted equilibrium constants for reactions such as Eqs. (1) and (5) at those temperatures. When these standards are equilibrated at very high temperatures, this is sometimes referred to as the ‘heated gas reference frame’ (Eiler and Schauble, 2004; Yeung et al., 2012). Note that an explicit assumption of these calibration methods is that statistical thermodynamic methods for calculating equilibrium constants for reactions such as Eqs. (1) and (5) are accurate. Although we cannot externally establish whether these models are correct, this assumption has allowed multiple labs to share the same reference frame for clumped isotope measurements of CO2 and thus have comparable measurements (Dennis et al., 2011).

One feature of such reference frames is that they can be experimentally generated by heating or otherwise equilibrating gases over a range of bulk isotopic compositions. This capacity has been crucial for permitting clumped isotope measurements of CO2 and O2 to match, or interpolate to composition-match samples and standards as closely as possible to minimize all analytical artifacts.

7. CALIBRATION OF A METHANE CLUMPED ISOTOPE THERMOMETER

There are no previously demonstrated methods to internally equilibrate the isotopes of methane, which is needed to ensure accuracy and calibrate a clumped isotope thermometer. CO2 can be equilibrated at high temperatures through heating in quartz glass at 1000 °C for 2 h (Eiler and Schauble, 2004). However, heating of CH4 at 1000 °C results in decomposition to H2 and C with 50% loss of methane within 4 h (Sackett, 1995). Additionally, even at these temperatures, it is not known if the sample will equilibrate its hydrogen and carbon isotopes either internally or with the graphite and hydrogen generated. Therefore, in order to generate samples with known clumped isotope compositions, we exposed methane gas to nickel catalysts at temperatures between 200–500 °C. A similar method was used by Horibe and Craig (1995) to equilibrate the hydrogen isotopes of H2 and CH4 and by Horita (2001) to equilibrate the carbon isotopes of CO2 and CH4. We performed two experiments to examine whether these established procedures drive methane to an internal equilibrium. First we
performed a bracketing experiment in which samples with varying bulk isotopic and starting \( \Delta^{18} \) values were all heated to 500 °C to ensure samples all converge to the same \( \Delta^{18} \) value at the same temperature. Second, we performed a series of heating experiments from 200-500 °C to see whether \( \Delta^{18} \) values have the same temperature dependence as predicted by theory.

For all heating experiments, we condensed samples in Pyrex\textsuperscript{®} tubes on 5A molecular sieves along with a nickel catalyst (65 wt.% nickel dispersed on a silica/alumina support; 190 m\textperthousand;g; Sigma Aldrich). Samples were heated for the given time periods (Table 5) in a box furnace, directly adjacent to a calibrated thermocouple in the furnace. Additionally, the catalyst was packed at the tip of the tube and held in place with glass wool to minimize the importance of any thermal gradients that exist in the box furnace. We quenched samples in ~1 min to room temperature with a flow of air from a fan. Samples were then recovered, purified and analyzed following the procedures above. Sample purification is necessary as methane always forms \( \text{H}_2 \) due to catalytic decomposition. We observe this \( \text{H}_2 \) as a measurable residual vapor pressure in the vacuum line at 20 K. Generally, ~1/2 of the starting methane converts to \( \text{H}_2 \) over the course of our heating experiments at 500 °C, with less at lower temperatures.

To perform a bracketing experiment, we first created samples of methane that differed markedly in \( \Delta^{18} \) from both our intralaboratory reference gas and expected equilibrium compositions by mixing aliquots of our intralaboratory reference gas with small amounts of synthetically enriched methane comprised of 99% \(^{12}\text{CH}_3\text{D} \) (Sigma Aldrich). This spike caused \( \delta^D \) values to increase and \( \Delta^{18} \) values to decrease relative to the references gas but changed the \( \delta^{13} \text{C} \) by <1‰ (Table 5 and Fig. 6). Mixing alters \( \Delta^{18} \) values due to the curvature of the random reference frame (\(^{18}\text{R}^\prime\)) as a function of isotopic composition – full explanations of this effect can be found in Eiler and Schauble (2004) and Eiler (2007, 2013). Mixtures were exposed to nickel catalysts at 500 °C for 3–4 days, following the procedure outlined above.

The isotopic compositions of gases before and after heating are presented in Table 5 and Fig. 6. The horizontal axis of Fig. 6 tracks the overall bulk composition of the gas with higher \( \delta^{18} \) values signifying an increase in \( \delta^D \) and/or \( \delta^{13} \text{C} \). For these experiments, \( \delta^{18} \) mostly tracks the increase in \( \delta^D \) from the addition of the labeled \(^{12}\text{CH}_3\text{D} \) to the experiment. Note that after heating the residual methane is lower in both \( \delta^D \) and \( \delta^{13} \text{C} \) values compared to their starting compositions. These changes are surprising because any kinetic isotope effects associated with methane decomposition and the exchange equilibrium between methane and generated \( \text{H}_2 \) should serve to enrich the residual methane in heavy stable isotopes. Though we do not know precisely the reason for the lowering of \( \delta^D \) and \( \delta^{13} \text{C} \) values of methane over the course of the experiments, a potential cause could be interactions between methane in the gas phase and that sorbed on the nickel catalyst or partially dehydrogenated methyl groups (e.g., Ni–\( \text{CH}_3 \), Ni–\( \text{CH}_2 \), etc.). If these species were to be enriched in \( D \) and \( ^{13} \text{C} \) at equilibrium, then the bulk methane would be depleted. Exchange with water vapor or other unknown species contained on the glass wall could also result in isotopic changes. Regardless, as long as internal equilibrium is reached, the precise cause of the depletion in bulk composition is unimportant.

The data presented in Table 5 and Fig. 6 demonstrate two key aspects of the measurements: (1) heating of samples with different starting \( \Delta^{18} \) values – both above and below the measured final value – generate the same final \( \Delta^{18,\text{wg}} \) value; and (2) for samples heated to the same temperature, there appears to be no dependence of \( \Delta^{18} \) on bulk isotopic composition, as reflected by the slope of the \( \delta^{18} \) vs. \( \Delta^{18} \) line in Fig. 6, which is 0.0003 ± 0.0006 (1σ) and thus indistinguishable from zero. Thus, for samples equilibrated to the same clumped composition, our measurement of \( \Delta^{18} \) of methane is, within the error of the measurement, independent of differences in bulk isotopic composition (i.e., \( \delta^{13} \text{C} \) and \( \delta^D \) values). This is distinct from measurements of \( \Delta^{47} \) of \( \text{CO}_2 \), which typically have slopes of 0.01–0.02 in analogous dimensions (Huntington et al., 2009) and \( \Delta^{18} \) measurements of \( \text{O}_2 \) which have slopes of ~0.06. This difference may reflect the fact that our measurements make use of a background correction that takes into account scattered or secondary ions, whereas conventional clumped isotope measurements of \( \text{CO}_2 \) do not (although clumped \( \text{O}_2 \) measurements do attempt to take this into account); scattered secondary ions have been suggested as the cause of the dependence of uncorrected \( \Delta^{47} \) values on bulk isotopic compositions for measurements of clumped isotopes in \( \text{CO}_2 \) (He et al., 2012; Bernasconi et al., 2013).

A potential issue with the experiments above is that the results are dependent on the \(^{12}\text{CH}_3\text{D}^{\text{R}}_{\text{wg}}\) and \(^{13}\text{R}_{\text{wg}}\) values used to normalize the data. If different numbers were chosen, then the line in Fig. 6 could have a slope. However, the \(^{12}\text{CH}_3\text{D}^{\text{R}}_{\text{wg}}\) value used to correct the measurements was

<table>
<thead>
<tr>
<th>Sample</th>
<th>( \delta^{13} \text{C} ) (‰)</th>
<th>± (‰)</th>
<th>( \delta^D ) (‰)</th>
<th>± (‰)</th>
<th>( \Delta^{18} ) (‰)</th>
<th>± (‰)</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1 unheated</td>
<td>-42.857</td>
<td>0.006</td>
<td>-175.528</td>
<td>0.110</td>
<td>-0.021</td>
<td>0.286</td>
</tr>
<tr>
<td>#1 heated</td>
<td>-48.083</td>
<td>0.005</td>
<td>-199.778</td>
<td>0.110</td>
<td>-1.794</td>
<td>0.281</td>
</tr>
<tr>
<td>#2 unheated</td>
<td>-42.997</td>
<td>0.005</td>
<td>-83.431</td>
<td>0.118</td>
<td>-0.450</td>
<td>0.262</td>
</tr>
<tr>
<td>#2 heated</td>
<td>-47.493</td>
<td>0.005</td>
<td>-127.165</td>
<td>0.099</td>
<td>-1.855</td>
<td>0.290</td>
</tr>
<tr>
<td>#3 unheated</td>
<td>-42.569</td>
<td>0.005</td>
<td>11.673</td>
<td>0.122</td>
<td>-1.088</td>
<td>0.308</td>
</tr>
<tr>
<td>#3 heated</td>
<td>-46.711</td>
<td>0.007</td>
<td>-46.647</td>
<td>0.150</td>
<td>-1.979</td>
<td>0.370</td>
</tr>
<tr>
<td>#4 unheated</td>
<td>-42.093</td>
<td>0.005</td>
<td>546.611</td>
<td>0.123</td>
<td>-5.838</td>
<td>0.341</td>
</tr>
<tr>
<td>#4 heated</td>
<td>-44.476</td>
<td>0.005</td>
<td>252.659</td>
<td>0.116</td>
<td>-1.678</td>
<td>0.299</td>
</tr>
</tbody>
</table>

Table 5
Bracketing experiment where samples were heated in the presence of a nickel catalyst at 500 °C. ‘Heated’ marks a sample from a larger reservoir that was not heated. ‘Heated’ denotes a sample from a larger reservoir (same reservoir as the ‘unheated’ sample) that was heated.
chosen independent of the clumped isotope measurements in Fig. 6 – instead it is used only to correct our measurements so that they adhere to the VSMOW–SLAP scale. The $^{18}$R$_{wg}$ value used to correct the measurements in Fig. 6 is the average value of the intercept for all mass-18 vs. 16 adduct lines made during that analytical session ($^{18}$R$_{wg,int}$). This demonstrates that our choice in value for $^{18}$R$_{wg}$ to correct all samples is the session average for that value and independent of the results of the heating experiments. Consequently, we suggest that we are justified in our choices of $^{12}$CH$_3$D$^{18}$R$_{wg}$ and $^{18}$R$_{wg}$ used for the corrections independent of the bracketing experiment in Fig. 6. This experiment has been repeated three additional times, all in different sessions, though over smaller isotopic ranges in $\delta^{18}$ of 80%. Measurements of samples with an average $\delta^{18}$ value of $-11.8 \pm 2.3_{\text{w.o.}}$ (n = 3; 1σ) have $\Delta_{18}$ values of $-2.10 \pm 0.22_{\text{w.o.}}$ (1σ). Measurements of samples with an average $\delta^{18}$ value of $69.4 \pm 8.4_{\text{w.o.}}$ (n = 3; 1σ) have $\Delta_{18}$ values of $-2.06 \pm 0.25_{\text{w.o.}}$ (1σ) and thus have $\Delta_{18}$ values that are indistinguishable statistically from the heated gases with lower $\delta^{18}$ values. Thus, use of the correction scheme outlined above not only causes standards to give reproducible values across months of time, but also allows for a stable, external, and experimentally derived heated gas reference frame.

We also internally equilibrated methane at temperatures varying from 200 to 500 °C in order to experimentally calibrate the methane clumped isotope thermometer. These data were generated in two separate sets of experiments. One set of experiments was evaluated before we fully understood the importance of 2-h long heating during extraction of methane from molecular sieves. The second set of experiments included this heating step. We corrected the samples that were not heated during removal from the molecular sieves by subtracting 0.49% from the $\Delta_{18, wg}$ values of measurements – i.e., the measured offset between samples heated during recovery from molecular sieve and those recovered without heating (see above). Though exclusion of these data does not significantly change our calibration, we include all data here, both for completeness and because it illustrates the apparent consistency of the fractionation on the molecular sieve.

Table 6 presents the compiled results of heating experiments between 200 and 500 °C (along with the heating durations). The samples show a clear trend of decreasing $\Delta_{18}$ with increasing temperature, as expected from theory (Fig. 1). In order to directly compare the experimental data

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Time (days)</th>
<th>$\Delta_{18}$ (‰)</th>
<th>± (‰)</th>
</tr>
</thead>
<tbody>
<tr>
<td>200</td>
<td>1</td>
<td>$-0.603$</td>
<td>$0.258$</td>
</tr>
<tr>
<td>300</td>
<td>3.8</td>
<td>$-1.242$</td>
<td>$0.270$</td>
</tr>
<tr>
<td>300</td>
<td>0.9</td>
<td>$-1.254$</td>
<td>$0.296$</td>
</tr>
<tr>
<td>400</td>
<td>3.8</td>
<td>$-2.033$</td>
<td>$0.272$</td>
</tr>
<tr>
<td>400</td>
<td>0.2</td>
<td>$-1.676$</td>
<td>$0.244$</td>
</tr>
<tr>
<td>500</td>
<td>4</td>
<td>$-2.079$</td>
<td>$0.283$</td>
</tr>
<tr>
<td>500</td>
<td>3.9</td>
<td>$-1.979$</td>
<td>$0.370$</td>
</tr>
<tr>
<td>500</td>
<td>3</td>
<td>$-1.794$</td>
<td>$0.281$</td>
</tr>
<tr>
<td>500</td>
<td>3</td>
<td>$-1.678$</td>
<td>$0.299$</td>
</tr>
<tr>
<td>500</td>
<td>2.9</td>
<td>$-1.855$</td>
<td>$0.290$</td>
</tr>
<tr>
<td>500</td>
<td>2</td>
<td>$-2.364$</td>
<td>$0.222$</td>
</tr>
<tr>
<td>500</td>
<td>2</td>
<td>$-1.7669$</td>
<td>$0.242$</td>
</tr>
<tr>
<td>500</td>
<td>2</td>
<td>$-1.941$</td>
<td>$0.248$</td>
</tr>
<tr>
<td>500</td>
<td>2</td>
<td>$-2.225$</td>
<td>$0.271$</td>
</tr>
<tr>
<td>500</td>
<td>2</td>
<td>$-2.002$</td>
<td>$0.256$</td>
</tr>
<tr>
<td>500</td>
<td>2</td>
<td>$-2.188$</td>
<td>$0.242$</td>
</tr>
</tbody>
</table>

* Denotes correction by subtracting 0.49% from account for sieve fractionation artifact.
† Not used in calibration as this sample is $\sim500$‰ different in $\delta^{18}$ from typical samples. As such if there are unknown scale compressions or analytical artifacts over such ranges, this sample would be the most susceptible.
to the theoretical model, we must account for the fact that our working reference gas standard (i.e., intralaboratory reference gas) is highly unlikely to have a \( \Delta_{18} \) value of zero; i.e., our measurements, as presented so far, reflect differences between gases equilibrated at known temperatures and this gas. When the average measured \( \Delta_{18} \) value at each temperature is compared to the expected theoretical value (Fig. 7A), the data define a linear trend with a slope of 1 and an x intercept of 2.981 \( \pm 0.015 \)\(^{\circ}\) (1\(\sigma\)). This line is found through a weighted regression of the measured \( \Delta_{18,\text{wg}} \) values (incorporating their error) against the theoretical \( \Delta_{18} \) values with the requirement that the slope equal 1. Assuming that the there are no intrinsic errors in the theoretical line, this implies that the intralaboratory reference gas has a ‘true’ \( \Delta_{18} \) value of +2.981\(^{\circ}\) relative to a random isotopic distribution.

A question is whether or not the plot of measured vs. expected \( \Delta_{18} \) values (Fig. 7A) defines a line of exactly slope 1 or follows some other slope, which might be evidence for either an analytical artifact such as fragmentation/recombination reactions in the ion source that tend to diminish differences in \( \Delta_{18} \) between samples (Huntington et al., 2009; Dennis et al., 2011) or inaccuracies in the modeled dependence of \( \Delta_{18} \) on temperature (Fig. 1). However, a weighted least squares regression of the data results in a slope of 0.86 \( \pm 0.09 \) (1\(\sigma\)), which could indicate a scale ‘compression’ of \( \Delta_{18} \) values as previously observed for clumped isotope measurements of CO\(_2\) (Dennis et al., 2011), by deeper exploration of the effects of instrument components and methods on measured differences in \( \Delta_{18} \) values, and/or by more advanced statistical mechanical calculations of the equilibrium constants for Eqs. (1) and (5).

In the absence of any statistically significant evidence for scale compression or other artifacts, we assume here that our measurements follow the same functional form as the theoretically predicted dependence of \( \Delta_{18} \) on temperature (i.e., we assume our measurements are 1:1 with the theoretical values), with a constant offset equal to the \( \Delta_{18} \) value of our intralaboratory standard, which we take to be +2.981\(^{\circ}\). This set of assumptions lets us create a \( \Delta_{18,\text{wg}} \) vs. temperature (in Kelvin) calibration where the dependent variable is \( 10^6/T^2 \) (Fig. 7B), which results in the following equation:

\[
\Delta_{18,\text{wg}} = -0.0117 \left( \frac{10^6}{T} \right)^2 + 0.708 \left( \frac{10^6}{T^2} \right) - 3.318. \tag{11}
\]

Alternatively, one could simply add +2.981\(^{\circ}\) to \( \Delta_{18} \) values measured vs. our working reference gas and compare the

Fig. 7. Heating experiments from 200–500 °C. Samples were heated with a nickel catalyst for varying amounts of time (Table 5). Each point represents an average of experiments if multiple samples were measured. (A) Comparison of measured values vs. theoretically expected values for equilibrium. A 1:1 line has been fit through the data. Data are offset from the origin as all samples are measured relative to a working reference gas, which has a real, but unknown \( \Delta_{18} \) value. If this line is used to find that value, the standard has a \( \Delta_{18} \) value of \( \sim 2.981^{\circ}\) or 170 °C. (B) Experimentally derived \( \Delta_{18,\text{wg}} \) vs. temperature calibration. 2.981\(^{\circ}\) has been subtracted from the theoretical line to account for the difference between the theoretical and working gas reference frames.
resulting value to the theoretically predicted relationship in Fig. 1.

8. INITIAL ANALYSES OF NATURAL METHANE

A critical question for clumped isotope measurements of methane is whether or not $\delta^{18}D$ values reflect formational temperatures and thus equilibrium processes or if they are entirely controlled by kinetics. Additionally, once a gas forms (in isotopic equilibrium or not), it is not known if the sample will preserve its formational $\delta^{18}D$ during transport and storage. The working reference gas used in our laboratory is a commercially supplied sample that we believe to be a natural thermogenic gas based on its $\delta^{13}C$ and $\delta^{18}D$ values ($-42.88$ and $-175.5\%$), which fall in the thermogenic gas field of Whiticar (1999). When the composition of this gas is compared with gases equilibrated in the laboratory, its apparent clumped isotope temperature is $170 \pm 1°C$—roughly in the middle of the range generally inferred for formation of thermogenic methane (Killops and Killops, 2005). This could be a coincidence but suggests natural gases may record their temperatures of formation and preserve that record through extraction, storage, and handling in the laboratory.

Much of the methane produced on earth and released to the atmosphere is generated by methanogens at temperatures from $0$ to $100°C$. It is of interest to establish if this other end member process produces methane distinct in $\delta^{18}D$ from thermogenic gases and indicative of low temperatures. If so, one could apply methane clumped isotope thermometry to recognize biogenic methane and perhaps even characterize the specific environments in which it is formed.

We performed a preliminary test of this possibility by measuring a gas of known biological origin (Fig. 8). This sample was produced by a pure culture of *Methanothermobacter marburgensis*, grown in an $H_2/CO_2$ (80:20) atmosphere following Boone et al. (1989) at $65°C$ until only methane remained. Samples were purified on the vacuum extraction line as explained above and corrected using standards run at the time for both $\delta^{12}CH_3D$ and $\delta^{18}D$ as explained above. Additionally, this sample was run before we routinely heated molecular sieves during release of methane, and so a correction for the isotope effect associated with partial retention of methane was applied (i.e., we subtracted $0.49\%$ from the measured $\delta^{18}D$ value, as explained above). This gives a $\Delta^{18}w_g$ value of $2.05 \pm 0.29\%$ or $5.03 \pm 0.29$ relative to a stochastic reference frame. Based on the temperature calibration above, this yields a temperature of $63 \pm 11°C$ (1σ), within error of its known growth temperature of $65°C$. This result, interpreted in the most straightforward manner, indicates that this methanogen generates methane with a clumped isotopic composition that is within error of the equilibrium value for this growth temperature. This result may have implications for our understanding of methane’s biogeochemistry and opens the possibility of identifying the source of methane in a given environment as a function of formation temperature, such as methane from psychrophiles vs. thermophiles vs. thermogenic sources. Furthermore, this technique may help to elucidate biochemical mechanisms in biogenic methane formation. For example, it is currently unknown whether methanogens that use acetate or methyl groups from larger organic molecules will form methane that is similar or distinct in clumped isotopic compositions from methanogens that metabolize $H_2$ and $CO_2$—these questions represent clear targets for future studies.

Fig. 8. Samples measured compared to their calculated $\Delta^{18}w_g$-based temperatures. Ranges include 1 standard error of the measurement. As seen, the thermogenic reference gas gives a high but reasonable temperature of $170°C$ while the biogenic sample from a methanogen grown at $65°C$ yields a temperature of $63°C$. 

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We present data for only two natural methane samples here, and more detailed studies are clearly required to develop confident interpretations of the clumped isotope compositions of natural methane. Additionally, an experimental calibration of this thermometer needs to be extended to lower temperatures, perhaps using specialized catalysts to promote equilibration in the range 0–100 °C. Nevertheless, these initial results suggest that the clumped isotope composition of natural methane can reflect its temperature of formation, can be preserved through later storage, extraction and processing, and distinguishes between high-temperature and low-temperature natural sources by amounts that are a large multiple (~10×) of analytical precision.

9. CONCLUSIONS

We have presented a method to measure the combined ratio of $^{13}$CH$_3$D$^+$ and $^{13}$CH$_2$D$^+$ to $^{13}$CH$_4^+$, relative to its random distribution, through mass spectrometry. We have documented the accuracy of this technique through comparison to established methods for the determination of δD and δ$^{13}$C values and through measurements of gases that have been driven to equilibrium with respect to the abundances of their multiply substituted isotopologues by heating in the presence of a catalyst. We have additionally generated a calibration of the equilibrium-based thermometer between 200 and 500 °C. Our working reference gas, which appears to be of thermogenic origin, gives a geologically reasonable temperature (170 °C), while a biogenic gas gives a lower temperatures appropriate to its growth conditions (63 °C). This suggests that methane may form through mechanisms in nature where its isotopes are internally equilibrated. If so, then clumped isotopes of methane may prove useful for understanding the physics and chemistry of methane formation, for characterizing the sources of natural methane, and perhaps for identifying the location where it formed.

ACKNOWLEDGEMENTS

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APPENDIX A. CALCULATION OF δ$^{13}$C, δD, AND Δ$_{18}$

Finding the δ$^{13}$C, δD, and Δ$_{18}$ values from measurements of mass-17/16 and mass-18/16 ion-current ratios must be done through iteration due to the inclusion of fragments at mass 16. Measurements of mass 16 include measurable quantities of $^{13}$CH$_3^+$ and $^{13}$CH$_2$D$^+$. These fragments must be accounted for as they are approximately 1% of the mass-16 signal, with $^{13}$CH$_3^+$ representing 95% of the fragments. A measurement can be made without the fragments (as the resolution is sufficient to resolve them out), but we find it methodologically easier to correct for the fragments at the end of the measurement.

We correct for the fragments by first measuring a ‘fragmentation’ rate. This is the amount of fragment produced for a given amount of $^{13}$CH$_4$ in the source. We do this by measuring the ion-current ratio of mass-15/16 via peak hopping. Mass 15 includes $^{13}$CH$_3^+$ and mass 16 $^{13}$CH$_4^+$ only. These fragmentation measurements are made at the same source pressure as the actual isotope ratio measurements. Generally, the mass-15/16 ion-current ratio is ~0.8, with a range seen over a year of measurements of about ±0.02. We assume that this fragmentation rate (F) is independent of isotopologues and is identical for $^{13}$CH$_4$, $^{13}$CH$_3$, and $^{13}$CH$_2$D. We can then generate a non-linear system of equations to describe our bulk isotopic measurements where:

\[ ^{13}\text{CH}_3^+ R = \frac{[^{13}\text{CH}_3]^D}{[^{12}\text{CH}_4] + F([^{13}\text{CH}_4] + 0.75[^{12}\text{CH}_3]^D)} \]  

and

\[ ^{13}\text{CH}_2^+ ^{13}\text{CH}_4^+ R = \frac{[^{13}\text{CH}_2]^D + [^{13}\text{CH}_4]}{[^{12}\text{CH}_4] + F([^{13}\text{CH}_4] + 0.75[^{12}\text{CH}_3]^D)} \]

where F is the fragmentation rate (usually ~0.8). If we assume that the isotopes are randomly distributed we can write these as:

\[ ^{13}\text{CH}_3^+ R = \frac{4[^{12}\text{C}] [^{13}\text{H}]^4 [^{13}\text{D}]}{[^{12}\text{C}] [^{13}\text{H}]^4 + F([^{12}\text{C}] [^{13}\text{H}]^4 + 3[^{12}\text{C}] [^{13}\text{H}]^2 [^{13}\text{D}])} \]  

and

\[ ^{13}\text{CH}_2^+ ^{13}\text{CH}_4^+ R = \frac{4[^{12}\text{C}] [^{13}\text{H}]^4 [^{13}\text{D}]}{[^{12}\text{C}] [^{13}\text{H}]^4 + F([^{12}\text{C}] [^{13}\text{H}]^4 + 3[^{12}\text{C}] [^{13}\text{H}]^2 [^{13}\text{D}])} \]

These can be written as

\[ ^{13}\text{CH}_3^+ R = \frac{4^D R}{1 + F(13R + 3^D R)} \]  

and

\[ ^{13}\text{CH}_2^+ ^{13}\text{CH}_4^+ R = \frac{4^D R + 13 R}{1 + F(13 R + 3^D R)} \]

These equations are solved iteratively in Matlab™ to find the $^{13}$R and D$^R$ values of the sample. As a note, we have implicitly removed the standard (which is in the denominator of all original measurements) through multiplication of its known isotopologue concentrations for a random distribution.

To calculate Δ$_{18}$ we first assume that our working reference gas is randomly distributed. This is necessary as the
the error and $\Delta R_{\text{adduct}}$ measurements are made in what is known as the ‘working gas reference frame.’

**APPENDIX B. ADDUCT LINE FOR MASS 18**

We assume that the adducts at mass 18 form through the following reactions:

$$12\text{CH}_4 + 13\text{CH}_4^+ \rightarrow 12\text{CH}_3 + 13\text{CH}_5^+$$  \hspace{1cm} (1a)

and

$$12\text{CH}_4 + 13\text{CH}_3\text{D}^+ \rightarrow 12\text{CH}_3 + 12\text{CH}_3\text{D}^+.$$  \hspace{1cm} (1b)

We assume the following kinetics for adduct formation based on these equations:

$$13\text{CH}_5^+ = k_{11,1}(12\text{CH}_4)(13\text{CH}_4)^2 + k_{13,2}(12\text{CH}_4) \times (13\text{CH}_4)$$  \hspace{1cm} (2a)

and

$$12\text{CH}_3\text{D}^+ = k_{D,1}(12\text{CH}_4)(12\text{CH}_3\text{D})^2 + k_{D,2}(12\text{CH}_4) \times (12\text{CH}_3\text{D}).$$  \hspace{1cm} (2b)

In these equations, the $k$ terms are constants. We can relate the amount of current we measure, $i_{18,\text{total}}$, to the current containing $13\text{CH}_3\text{D}^+$ and $12\text{CH}_3\text{D}_2^+$, $i_{18,\text{isotopes}}$, and the current from the adducts, $i_{18,\text{adducts}}$, via the following relation:

$$i_{18,\text{total}} = i_{18,\text{isotopes}} + i_{18,\text{adduct}}.$$  \hspace{1cm} (3)

Substitution of (2a) and (2b) into (3) and the assumption that the fragments at mass 16 can be corrected for later and that the rate constants are identical gives

$$i_{18,\text{total}} = i_{18,\text{isotopes}} + k_1(i_{16})(i_{17})^2 + k_2(i_{16})(i_{17}).$$  \hspace{1cm} (4)

where $i_{17} = i_{17,\text{CH}_4} + i_{17,\text{CH}_3\text{D}}$. Division of (4) by $i_{16}$ gives

$$\frac{i_{18,\text{total}}}{i_{16}} = \frac{i_{18,\text{isotopes}}}{i_{16}} + k_1(i_{17})^2 + k_2(i_{17}),$$  \hspace{1cm} (5)

which is the equation of line in which the curvature is defined by $k_1$ and $k_2$ and the intercept, which is the measurement of interest, is $i_{18,\text{isotopes}}/i_{16}$. $i_{17}$ does not include the adduct at mass 17 ($13\text{CH}_3^+$). Thus to find $i_{17}$ we generate, adducts lines at both mass 17 and mass 18 where the mass-17 adduct line includes $13\text{CH}_3^+$, $12\text{CH}_3\text{D}^+$, and $12\text{CH}_3\text{D}_2^+$. This line is used to both correct the mass 17-measurement for bulk isotope calculations and to have an adductless current for mass 17 that can be used to make the mass-18 adduct line. An example mass-18 adduct line is given in Fig. A1. As in the case of the mass-17 adduct line, the mass-18 adduct line is best fit with a quadratic line. The assumption that the rate constants for reactions involving $13\text{CH}_4$ and $12\text{CH}_3\text{D}$ are the same is likely incorrect. However, as the adduct is dominantly $13\text{CH}_3$ ($>98\%$), the error from not knowing the precise value for the $12\text{CH}_3\text{D}$ rate constant is diminished. However, at large $\delta D$ differences, this could be an issue. We demonstrate this not to be the case in the bracketing experiment described in the text where $\Delta_{18}$ is not a function of $\delta D$.

**APPENDIX FIGURES**

![Fig. A1. Demonstration of the dependence of the mass-18/mass-16 ion-current ratio ($i_{18,\text{total}}/i_{16}$) on the mass-17 ion current ($i_{17}$). In this example, $i_{16}$ includes $12\text{CH}_4^+ + 13\text{CH}_4^+ + 12\text{CH}_3\text{D}^+$, $i_{17}$ includes $13\text{CH}_3^+ + 12\text{CH}_3\text{D}^+$, and $i_{18}$ includes $13\text{CH}_3\text{D}^+ + 12\text{CH}_3\text{D}_2^+ + 13\text{CH}_3^+ + 12\text{CH}_3\text{D}_2^+$. The line describing the dependence of $i_{18,\text{total}}/i_{16}$ on $i_{17}$ is known as an adduct line. The formula for the adduct line is found by fitting the data with a quadratic function. The fitted parameters are used to remove the contribution of $13\text{CH}_3^+ + 12\text{CH}_3\text{D}^+$ from all measurements.](image-url)
Fig. A2. Demonstration that all measurements follow their theoretical statistical limits in precision. (A) Measurement of $\delta^{12}$CH$_3$D where mass 17 includes $^{13}$CH$_3$D$^+$. Measurements are shown after the H-adducts have been removed. Samples approach the combined Johnson noise and counting statistics limit of 0.1–0.12% at the end of the measurement. (B) Measurement of $\delta^{12}$CH$_3$D$^+$ where mass 17 includes $^{12}$CH$_3$D$^+$ and $^{13}$CH$_4$+. Measurements have been corrected for the presence of H-adducts and approach the theoretical counting statistics limit of 0.005% at the end of the measurement. (C) Measurement of $\delta^{18}$, which at mass 18 includes $^{18}$CH$_3$D$^+$ and $^{12}$CH$_2$D$_2$+. H-adducts have been corrected for and samples approach the counting statistics limit of 0.25% at the end of the measurement.

Fig. A3. Flow chart representation of the analytical procedures involved in making a measurement. Abbreviations: mass resolving power (MRP); secondary electron multiplier (SEM). (A) Measurement of $\delta^{12}$CH$_3$D. (B) Simultaneous measurement of $\delta^{12}$CH$_3$D$^+$, $^{13}$CH$_4$+, $^{12}$CH$_2$D$_2$+, $^{18}$CH$_3$D$^+$, and $\delta^{18}$. 

A: $^{12}$CH$_3$D/mass-16 ($^{12}$CH$_3$D$^+$) measurement

resolution

set instrument to high resolution (20,000-25,000 MRP)

pressure balance

adjust mass 16 ion current of sample and standard to 1.3 V

background measurements

measure background with gas flowing at masses 16.3 and 17.3 amu

repeat 7 times

B: Combined ($^{12}$CH$_4$+$^{12}$CH$_3$D$^+$)mass-16 (12CH$_3$D$^+$-13CH$_4$) and ($^{12}$CH$_3$D$^+$+$^{14}$CH$_3$D$^+$)mass-16 ($\delta^{18}$) measurements

resolution

set instrument to medium resolution (16,000-17,000 MRP)

pressure balance

adjust mass 16 ion current of sample and standard to 4 V

background measurements

measure background with gas flowing at masses 16.3, 17.3, and 18.3 amu

repeat 15 times

measurement error for $^{12}$CH$_3$D$^+$/mass 16

measurement error for $^{12}$CH$_3$D$^+$/mass 16

measurement error for $^{13}$CH$_4$/mass 16
### APPENDIX TABLES

#### Table A1
Species present in the mass-17 to mass-16 measurement (Δδ13CH2D) used to constrain the 12CH3D/12CH4 ratio. This measurement is made at the high-resolution setting of the instrument (20,000–25,000 mass resolving power). In bold are the major methane peaks at a given mass. Abundances are given relative to the major methane peak at the given mass. Species <1 * 10⁻⁴ of the major methane peak are not shown.

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<th>Species (Mass 17)</th>
<th>Mass (amu)</th>
<th>Relative abundance</th>
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<td>17.003</td>
<td>0.03</td>
<td>Resolved</td>
</tr>
<tr>
<td>14NH3</td>
<td>17.027</td>
<td>2.39 * 10⁻³</td>
<td>Resolved</td>
</tr>
<tr>
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<td>17.038</td>
<td>0.055</td>
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</tr>
<tr>
<td>12CH5</td>
<td>17.039</td>
<td>5.98 * 10⁻³</td>
<td>Corrected</td>
</tr>
</tbody>
</table>

#### Table A2
Species present in the mass-17 to mass-16 measurement (Δδ13CH2D, Δδ13CH3) used to constrain the (12CH3D + 13CH4)/12CH4 ratio and in the mass-18 to mass-16 measurement (Δδ18) used to constrain the (13CH3D + 12CH2D2)/12CH4 ratio. The mass-16 species are identical to those given in Table A1 and are not shown. This measurement is made at the medium-resolution setting of the instrument (16,000–17,000 mass resolving power). In bold are the major methane peaks at a given mass. Abundances are given relative to the major methane peak at the given mass. Species <1 * 10⁻⁴ of the major methane peak are not shown.

<table>
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#### REFERENCES


