

Author comments, Anonymous referee #1

I thank referee 1 for perceptive and constructive comments and make the following responses:

Major comment:

The author recommends using the “direct calibration by isotopologues amounts” due to its apparent advantages (no pressure dependence, the need of only one reference gas in different “dilutions”) as opposed to the calibration via different reference gases with different δ -value (“Calibration by delta values”). I think this recommendation can only be done if the targeted precision in δ -value is not exceeding the precision of the absolute concentrations of the reference gas and its “dilutions”. For example, the precision of CO₂ absolute concentration in reference gases from international reference labs is around 0.07 ppm¹. The same type of labs can provide the $\delta_{13}\text{C}(\text{CO}_2)$ in such gases using IRMS with a precision in the 0.02‰ range or better, while this δ -value is relative to a material with a specific isotopic composition (reference material) and therefore independent of the absolute concentration of CO₂ in that gas. The 0.02‰ in $\delta_{13}\text{C}(\text{CO}_2)$ corresponds to a precision in absolute concentration of about 0.008 ppm (for a 400 ppm concentration gas), so about one order of magnitude better than the absolute concentration actually can be known (in absolute, SI-traceable fashion).

As far I understand the recommended calibration approach, it ultimately depends on the precision of the absolute CO₂ concentration of the used gases. Since the current limit of these values is around 0.07 ppm, the corresponding precision in $\delta_{13}\text{C}(\text{CO}_2)$ cannot be better than about 0.2‰ (exceptions exist if the measured value is very close to the used reference gas in the calibration). If the user dilutes its reference gas in a self-made experiment, it's very likely that the dilution process will introduce additional uncertainty making the in $\delta_{13}\text{C}(\text{CO}_2)$ precision (and obviously also the precision of the absolute CO₂ concentration) worse.

This disadvantage of the “direct calibration by isotopologues amounts” has to be addressed in the manuscript and the suggestion has to be adapted accordingly. A 0.2‰ in $\delta_{13}\text{C}(\text{CO}_2)$ is one order of magnitude above the GAW recommendations for which reason the proposed calibration method seems not applicable for instruments applied in the context of GAW. Some of the references used in the manuscript as an example of the alternative “Calibration by delta values” are used in the GAW context with corresponding precision targets. So the context of these references has to be reconsidered.

Response:

This is a valid comment, also raised by referee 2, but highlights some misunderstandings which clearly should be clarified.

Firstly there is the minor misconception that the reference standards for absolute isotopologue calibration are obtained by dilution of a high-level standard. This is not usually the case, the reference gases are normally obtained as whole air mixtures individually calibrated by a certified GAW laboratory (such as NOAA, ICOS or Gaslab) with a range of CO₂ mole fractions but in a matrix of other gases (N₂, O₂, Ar, CH₄, CO, N₂O) which are typical of atmospheric composition. Thus there is no loss of accuracy due to an in-lab dilution process. If standards are prepared in the reference gas supply lab over a range of mole fractions using the same CO₂ source gas the delta values will be the same for all standards over the range of mole fractions – this was the case in Flores et al. Such standards are easier to prepare but suitable only for isotopologue-based calibration.

The referee points out that for reference standards with absolute mole fractions known to 0.07 ppm (0.18‰), the best possible absolute accuracy for $\delta^{13}\text{C}$ would be $\sim 0.2\%$. However this is only true if the individual isotopologue mole fractions are each individually known to 0.18‰ with independent errors (the uncertainty in $\delta^{13}\text{C}$ would actually be $\sqrt{2}$ greater, or 0.25‰ because there are two measurements in the calculation of delta). In practice the calibration lab provides a total CO_2 amount fraction (accuracy say 0.18‰) and $\delta^{13}\text{C}$ value (accuracy say 0.02‰). Following the calculations in this paper, the 636/626 ratio is as accurately known as the reference $\delta^{13}\text{C}$ value provided (not the total CO_2 accuracy), and the individual isotopologue values as accurately as the total CO_2 accuracy. The accuracy of the isotope ratio provided by the calibration lab is preserved through the isotopic calculations and the calibration process.

Now consider the optical measurement, whether FTIR or laser-based. Using the 1-min Allan deviation figures from Griffith et al (2012) as an example, we take a repeatability for CO_2 isotopologues of 0.02 ppm (0.05‰) and for $\delta^{13}\text{C}$ we take 0.07‰ (which is approximately $0.05 \cdot \sqrt{2}$ ‰). For laser instruments the figures are similar. If errors in retrieving the individual isotopologue amounts from the spectra were to some degree correlated, the $\delta^{13}\text{C}$ repeatability would be better. (This may be more the case for laser instruments analysing single lines than for FTIR analysing whole spectral bands, as pointed out in a specific comment by this referee). Thus the *repeatability* of the spectral $\delta^{13}\text{C}$ measurement in this example is of the order of 0.07‰, but its *accuracy* is determined by the calibration against reference standards, which is governed by the reference $\delta^{13}\text{C}$ values and may be better (ie the measurement of delta may be more accurate than it is precise). The ultimately reported accuracy of the sample delta value will be limited by the largest source of error, the isotopologue measurement repeatabilities, and thus of the order of 0.07‰. It is not determined by the absolute accuracy of the total CO_2 amounts in the reference gases. This accuracy is still larger than the GAW-GGMT requirement, but is the current state of the art for optical measurements.

Finally, it is important to recognise that both the absolute and the ratio calibration methods take a ratio of 636/626 to determine ^{13}r and $\delta^{13}\text{C}$ and thus both take advantage of the accuracy of the isotope ratio calibration of the reference gases. There is no equivalent in optical measurements to IRMS, where the instrumentation primarily measures a ratio of ion counts, but not individual amounts. In this sense both methods are equally precise. The difference between the absolute and ratio calibration of optical measurements lies in whether the calibrated or uncalibrated 636/626 ratio is used. In the former case (ratio of calibrated 636 and 626) the calibrated $\delta^{13}\text{C}$ is calculated directly from the calibrated isotopologue amounts, but in the latter (ratio calibration) a range of $\delta^{13}\text{C}$ in reference gases is required, and there is an unavoidable concentration dependence (Eq. 15) which must be characterised. The form of eq 15 suggests a 3-parameter fit to characterise this concentration dependence, but the coefficients β and γ are not actually constants so the parameterisation is only approximate, and the values of β and γ are related to the instrument calibration and should be in principle re-determined with every calibration. In my FTIR experience, the residuals in fitting data to Eq 15 may result in residuals as large as 0.5‰. Other instruments may take a different approach to determining this concentration dependence, but it is likely to add error to the determined delta values, and can never reduce the error.

To address these comments I have extended the discussion of ratio calibration in section 4.2 to include a figure illustrating the concentration dependence through application of Eq. 15 and the potential errors using real FTIR data, and I have added a new section 4.3 which is a condensed form of the response given above.

Added to 4.2

describe the concentration dependence. The linear term becomes relatively more important than the inverse term at high CO₂ mole fractions where the inverse CO₂ term becomes small and any quadratic contribution to the calibration equation leading to the linear term becomes large.

Figure 1 illustrates this concentration dependence with a typical $\delta^{13}\text{C}$ vs CO₂ dependence for an FTIR analyser similar to that used in the example of section Error! Reference source not found. below. The dependence was determined by continuous flow measurements of a single CO₂-spiked air tank while the CO₂ content was gradually reduced by passing a fraction of the flow through Ascarite. The measured $\delta^{13}\text{C}$ vs CO₂ data are fitted to Eq Error! Reference source not found. with fitted parameters $\beta = -1227 \text{ ‰ ppm}$ and $\gamma = 0.0054 \text{ ‰ ppm}^{-1}$, corresponding to CO₂ - dependent corrections of up to 5‰ over the CO₂ range 400-1000 ppm. The residuals of the fit illustrate potential errors from the modelled behaviour of up to $\pm 0.3\%$. Uncertainties in calibrating the CO₂ concentration dependence can lead to significant errors in Keeling-type analyses over a wide range of total CO₂ amounts even if the isotopologue calibration non-linearity is very small (Pang et al., 2016; Wen et al., 2013).

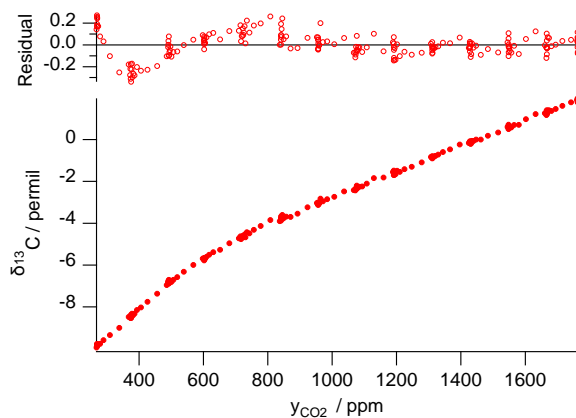


Figure 1. Example of $\delta^{13}\text{C}$ dependence on CO₂ mole fraction for a Spectronus FTIR analyser. The measured data are fitted with a function of form of Eq. Error! Reference source not found. with fitted parameters $\beta = -1227 \text{ ‰ ppm}$ and $\gamma = 0.0054 \text{ ‰ ppm}^{-1}$.

Added 4.3

Comments on the accuracy of optical isotopologue and ratio calibration

As an example assume a calibration laboratory provides calibrated reference gases with an absolute accuracy of 0.05 ppm for total CO₂ amount (0.12‰ in 400 ppm CO₂) and 0.02‰ for $\delta^{13}\text{C}$ measured by IRMS. The isotope ratio is thus more accurately determined than the total amount fraction for the reference gases. Now take as a practical measurement repeatability for optical analysers 0.02 ppm

(0.05‰) for total CO₂ amount and 0.07‰ for $\delta^{13}\text{C}$ (e.g. Griffith et al. (2012), laser instruments similar). The absolute accuracy for the calibrated optical measurement of total CO₂ is limited by the reference gas amount fraction, but the more accurately known reference ¹³r or 626/636 ratio is carried through the calibration calculations and this accuracy is preserved when measured isotopologue amounts are ratioed. The accuracy of measured ¹³r or $\delta^{13}\text{C}$ is thus limited by the optical measurement (0.07‰) which is less precise than the IRMS-provided reference accuracy (0.02‰). This does not currently meet GAW requirements for clean background air measurements (WMO-GAW, 2016), but is nevertheless useful for continuous analysis of air in non-baseline scenarios such as urban air or agricultural flux measurements.

This reasoning applies to both isotopologue and ratio calibration schemes, which both benefit from the higher accuracy in the isotopologue ratios than in absolute isotopologue amounts. The principle differences between the isotopologue and ratio calibration schemes are twofold:

- The isotopologue scheme does not require calibration gases spanning a range of delta values, it is sufficient to span the range of total amount fractions of interest. This simplifies the preparation of reference gases for calibration laboratories.
- The ratio scheme has an unavoidable CO₂ concentration dependence which must be characterised and leads potentially to a loss of accuracy, as shown in section **Error! Reference source not found.** This complicates the calibration procedure for optical analysers.

Errors are discussed further in section 6.

Specific comments

Clumped isotopes are mentioned a few times in the manuscript, but there is no example of it. It would be nice to have an example for the uneducated reader. In general, one could consider to extend table 2 with all 18 (or 12 distinct) possible isotopologues as an overview and list its abundances (as much as known) as an overview.

There is a confusion (from both referees) here between multiply-substituted isotopologues (isotopologues with more than one minor isotope) and clumped isotopes (referring to multiply-substituted isotopologues where the relative amounts of the isotopes are not statistical, eg where two isotopes are both enriched together above their bulk statistical abundances). To introduce clumped isotopes more clearly, as requested, I have amended the text at first usage in section 2:

Using CO₂ as an example, considering the stable isotopes ¹²C, ¹³C, ¹⁶O, ¹⁷O and ¹⁸O, there are eighteen possible isotopologues (2 x 3 x 3 isotopic possibilities). ¹⁴C is a negligible proportion of total carbon for these purposes and is neglected. Only twelve of these eighteen possibilities are distinct due to symmetry. Assuming the substitution of each isotope at each position in the molecule follows its bulk statistical abundance (i.e. no clumping, see section **Error! Reference source not found.**), only four independent quantities are required to fully define the total amount and full isotopic composition of CO₂. These quantities may equivalently be the total CO₂ amount and three isotopic ratios ¹³r, ¹⁷r and ¹⁸r (or delta values δ¹³C, δ¹⁷O and δ¹⁸O), or the amounts of four individual isotopologues with each isotope substituted, most conveniently ¹⁶O¹²C¹⁶O, ¹⁶O¹³C¹⁶O, ¹⁶O¹²C¹⁷O and ¹⁶O¹²C¹⁸O. Once these are known, the abundances of all multiply-substituted isotopologues can be calculated.

I have also expanded and clarified this description of “clumping” in section 6:

The relative amounts of multiply-substituted minor isotopologues are assumed to be in statistical relative abundance, i.e. there is no isotope clumping. Clumping refers to the case where the enrichment (or depletion) of two or more isotopes in a multiply-substituted isotopologue are correlated rather than each following their statistical amounts independently. Clumping effects are normally much less than 1‰, and according to **Error! Reference source not found.** therefore insignificant.

-Page 4, line 2 and following formulas: you shorten from n(¹²C) to ¹²C. I recommend to not do this because it complicates the comparison with the previous formulas and the shortening is only used in the following formulas (as much I can see).

Done:

$$\begin{aligned}
{}^{12}x &= \frac{n({}^{12}\text{C})}{n({}^{12}\text{C}) + n({}^{13}\text{C})} = \frac{1}{(1 + {}^{13}r)} \\
{}^{13}x &= \frac{n({}^{13}\text{C})}{n({}^{12}\text{C}) + n({}^{13}\text{C})} = \frac{{}^{13}r}{(1 + {}^{13}r)} \\
{}^{16}x &= \frac{n({}^{16}\text{O})}{n({}^{16}\text{O}) + n({}^{17}\text{O}) + n({}^{18}\text{O})} = \frac{1}{(1 + {}^{17}r + {}^{18}r)} \\
{}^{17}x &= \frac{n({}^{17}\text{O})}{n({}^{16}\text{O}) + n({}^{17}\text{O}) + n({}^{18}\text{O})} = \frac{{}^{17}r}{(1 + {}^{17}r + {}^{18}r)} \\
{}^{18}x &= \frac{n({}^{18}\text{O})}{n({}^{16}\text{O}) + n({}^{17}\text{O}) + n({}^{18}\text{O})} = \frac{{}^{18}r}{(1 + {}^{17}r + {}^{18}r)}
\end{aligned}$$

-Page 4, line 11-16: This section is not very clear to me. You only take 4 distinct isotopologues into consideration, but R_{sum} seems to be the sum of all 18 (according to the current text). Or how do I have to understand this? R_{sum} seems to be a rather abstract parameter, or does it have any easy- understandable meaning?

R_{sum} is analogous to a partition sum over all possible energy levels of a molecule and provides a convenient normalising factor when calculating relative isotopologue abundances (cf relative populations in the case of energy levels). In practice it is most easily thought of as the ratio of the total amount of CO_2 in a sample to the that of the major isotopologue. For example if $R_{sum}=1.01$, $1/R_{sum} = 0.99$ and 99% of the sample is 626. I have expanded the wording in this paragraph as follows to try and make this clearer:

The labels 626, 636, 628, 627 are the common isotopic shorthand used in spectroscopy and the Hitran database. The sum of all isotopologue abundances x over all 18 isotopologues is equal to unity. R_{sum} is a sum of 18 products of isotope ratios, one corresponding to each of the 18 possible isotopologues of CO_2 . R_{sum} conveniently accounts for all possible isotopologues in calculations of abundances, providing a normalising factor somewhat analogous to a partition sum over all energy levels of a molecule. From Eq. **Error! Reference source not found.**, $x_{626} = 1/R_{sum}$ i.e. $1/R_{sum}$ is the fractional abundance of the major isotopologue and $R_{sum} - 1 \approx 1 - x_{626}$ is that fraction of the sample that is made up of all the minor isotopologues. Equivalently from Eq.

Error! Reference source not found. and the following paragraph it can be seen that R_{sum} is the ratio of the total amount of CO_2 to that of the major isotopologue in a sample.

Abundances of the major and three singly-substituted isotopologues and R_{sum} values for standard reference scales are given in **Error! Reference source not found.** Abundances of the multiply-substituted isotopologues can be calculated following the examples of Eq.

Error! Reference source not found. They are also listed for Hitran isotope ratios on the Hitran website <https://www.cfa.harvard.edu/hitran/molecules.html>.

-Page 7, line 35 and following: IRMSs can also be used to measure absolute abundances of the single isotopologues, but the measurement is in general noisy and very difficult to calibrate. This “absolute” approach has been used in the beginning of mass spectrometry before it has been realized that a relative measurement with IRMS is much more precise than an absolute measurement³. This is also the case for isotopologue-specific optical systems. The noise in a line ratio is much smaller than the noise of the individual lines because there is a lot of correlated/technical noise on two simultaneously measured lines which cancels out in a ratio (given that the instrument can measure the targeted lines simultaneously in real-time as possible e.g. with a direct absorption spectrometer). It’s not due to the nature of IRMS that the community has converged to go for relative δ -scales, but rather due to the more fundamental analytical advantage of measuring relative values with respect to a reference material as opposed to absolute measurements.

This point has been addressed in the response to this referee’s major comment on the absolute vs ratio calibration. Some sources of noise are common to the retrieval of amounts from two isotopologue absorption lines or bands, and these may ratio out, but fundamentally the spectrum is analysed for each isotopologue independently and then the results are ratioed. Technically this is also true of the ion counts in IRMS, but as the referee points out the absolute ion counts are very noisy, more so in IRMS than in retrievals from spectra.

Author response to anonymous referee #2

I thank referee 2 for his/her perceptive and constructive comments and make the following responses:

General comment

My one general comment would be that although the approach taken by the author does indeed side-step the concentration dependence arising if one calibrates using a ratio-based approach, IRMS methods arose because of the precision advantage arising when measuring a ratio. It is certainly still the case that the precision with which any of the isotopologue-specific optical instruments can measure the minor isotopologues is limited 0.1 ppm (generously), so this will impact the precision with which one can determine a delta value from these instruments via this otherwise straightforward approach. As such, there remain advantages in working with the ratio even with instruments that 'naturally' lend themselves to this approach. Some acknowledgement of this ought to be made in the text.

Please see detailed response to the same issue raised by referee 1, I have addressed both together.

Specific comments

P 3, line 12: Clumped isotopes are mentioned, but not described, but as I see great value in this manuscript for students, I think it would be helpful to extend the parentheses to something like: (i.e. clumped isotopes, is the term for isotopologues carrying two (or more) of the heavy, rare isotopes.)

Please see detailed response to referee 1 for the same issue.

p 9, line11: 2018 should read 2017.

Corrected.

p 9. line 25: I think case 7 needs a little more explanation to be clear to readers.

I have expanded case 7 as follows:

1) is a hypothetical standard with the isotopic composition of VPDB-CO₂. Examples include typical clean air (case 2), synthetic air synthesised with ¹³C-depleted CO₂ with $\delta^{13}\text{C} = -35\text{‰}$ (case 3), realistic errors of 2‰ in $\delta^{18}\text{O}$ and $\delta^{17}\text{O}$ (cases 4,5), and using isotope ratios assumed by Hitran rather than VPDB-CO₂ (case 6). Case 7 simulates the result if only singly-substituted isotopologues are included in the sum and all doubly-substituted minor isotopologues are ignored. Other cases can be assessed

Additional author response to offline comments:

I have received two short email comments outside of the AMT discussion forum, both of which pointed out small typographical errors.

I thank Kentaro Ishijima, JMA, for pointing out a missing factor of 2 in the last 2 equations of (4) and (8) which was not carried through correctly. This is a typographical error, it does not have any impact on subsequent calculations. The correct equations are:

$$\begin{aligned}x_{626} &= {}^{16}x \cdot {}^{12}x \cdot {}^{16}x = \frac{1}{R_{sum}} \\x_{636} &= {}^{16}x \cdot {}^{13}x \cdot {}^{16}x = \frac{{}^{13}r}{R_{sum}} \\x_{627} &= 2 \cdot {}^{16}x \cdot {}^{12}x \cdot {}^{17}x = \frac{2 \cdot {}^{17}r}{R_{sum}} \\x_{628} &= 2 \cdot {}^{16}x \cdot {}^{12}x \cdot {}^{18}x = \frac{2 \cdot {}^{18}r}{R_{sum}}\end{aligned}\tag{1}$$

$$\begin{aligned}y_{626} &= y_{CO2} \cdot x_{626} = y_{CO2} / R_{sum} \\y_{636} &= y_{CO2} \cdot x_{636} = y_{CO2} \cdot {}^{13}r / R_{sum} \\y_{627} &= y_{CO2} \cdot x_{627} = y_{CO2} \cdot 2 \cdot {}^{17}r / R_{sum} \\y_{628} &= y_{CO2} \cdot x_{628} = y_{CO2} \cdot 2 \cdot {}^{18}r / R_{sum}\end{aligned}\tag{2}$$

I also thank Jessica Connolly, NPL, for pointing out an erroneous extra 0 in the isotopic abundances of ¹⁵N in Table 1. Isotopic figures quoted by Hitran have varied over time in the significant figures quoted which may affect the last significant figure in Table 1. The quoted isotope ratios in Figure 1 are back-calculated from the isotopologue abundances given on the Hitran website.

Table 1. Standard isotope ratios for relevant reference scales used in atmospheric trace gas analysis. ¹(Werner and Brand, 2001), ²(Brand et al., 2010), ³(Bievre et al., 1984), ⁴<https://www.cfa.harvard.edu/hitran/molecules.html>

Element	Ratio	VPDB ¹	VPDB-CO ₂ ²	Hitran ^{3,4}	VSMOW ¹	air N ₂ ¹
C	¹³ r = ¹³ C/ ¹² C	0.0111802	0.0111802	0.0112374		
O	¹⁸ r = ¹⁸ O/ ¹⁶ O	0.0020672	0.00208835	0.0020052	0.00200518	
O	¹⁷ r = ¹⁷ O/ ¹⁶ O	0.000386	0.0003931	0.0003729		
N	¹⁵ r = ¹⁵ N/ ¹⁴ N			0.00367		0.0036782

H

$^2r =$
 $^2\text{H}/^1\text{H}$

0.000156

0.00015575

Calibration of isotopologue-specific optical trace gas analysers: A practical guide

David W. T. Griffith¹,

¹ Centre for Atmospheric Chemistry, University of Wollongong, Australia

5

Correspondence to: David Griffith (Griffith@uow.edu.au)

Abstract

The isotopic composition of atmospheric trace gases such as CO₂ and CH₄ provides a valuable tracer for the sources and sinks that contribute to atmospheric trace gas budgets. In the past, isotopic composition has typically been measured with high
10 precision and accuracy by Isotope Ratio Mass Spectrometry (IRMS) offline and separately from real-time or flask-based measurements of concentrations or mole fractions. In recent years, development of infrared optical spectroscopic techniques based on laser and Fourier Transform Infrared spectroscopy has provided high precision measurements of the concentrations of one or more individual isotopologues of atmospheric trace gas species in continuous field and laboratory measurements, thus providing both concentration and isotopic measurements simultaneously. Several approaches have been taken to the
15 calibration of optical isotopologue-specific analysers to derive both total trace gas amounts and isotopic ratios, converging into two different approaches: calibration via the individual isotopologues as measured by the optical device, and calibration via isotope ratios, analogous to IRMS.

This paper sets out a practical guide to the calculations required to perform calibrations of isotopologue-specific optical
20 analysers, applicable to both laser or broadband FTIR spectroscopy. Equations to calculate the relevant isotopic and total concentration quantities without approximation are presented, together with worked numerical examples from actual measurements. Potential errors which may occur when all required isotopic information is not available, or is approximated, are assessed. Fortunately, in most such realistic cases, the errors incurred are acceptably small and within the compatibility limits specified by the World Meteorological Organisation – Global Atmosphere Watch. Isotopologue-based and ratio-based
25 calibration schemes are compared. Calibration based on individual isotopologues is simpler because the analysers fundamentally measure amounts of individual isotopologues, not ratios. Isotopologue calibration does not require a range of isotopic ratios in the reference standards used for the calibration, only a range of concentrations or mole fractions covering the target range. Ratio-based calibration leads to concentration dependence which must also be characterised.

30

1 Introduction

Until recently, measurements of the amounts of CO₂ and other trace gases in the atmosphere and in calibration gas standards within the Global Atmosphere Watch - Greenhouse Gas Monitoring Techniques (GAW-GGMT) community were mostly made by analytical techniques which do not discriminate between isotopic variants of the target gases. Manometry and gravimetry enable the calibration of gas mixtures to be traceable to SI units of pressure, volume, mass and temperature, but measure only the total amounts of the target trace gas, without taking into account differences in isotopic composition. Gas chromatography is also commonly used both in atmospheric measurements and in the propagation of standards, but is also blind to the isotopic composition of the target gas and measures only total amounts.

Non-dispersive infrared analysers (NDIRs) have been used for many years as an instrument of choice for atmospheric trace gas monitoring. NDIR is an optical technique based on infrared absorption by the target trace gas, and like any optical/spectroscopic instrument, NDIR instruments have a different response to different isotopologues of the target species because different isotopologues have different absorption spectra. Earlier NDIR instruments such as URAS, UNOR, Siemens and APC employed microphone detectors filled with the target trace gas that responded selectively to the absorption of infrared radiation by the target gas in the sample (Griffith, 1982). The NDIR instrument response depends in a complex and non-linear way on the isotopic composition of the target gas and on the carrier gas. The more recent Licor instruments replaced the microphonic detector with an optical semiconductor detector which relies on a broad bandpass filter to restrict the wavelength range from the source to that absorbed by the target gas, for example for CO₂ around 4.3 μm. Optical NDIR detectors also respond differently to the different isotopologues of the target gas because the bandpass filter does not cover the entire absorption range of the trace gas, and because different isotopologues have different absorption strengths and sensitivities. NDIR instruments thus have an ill-defined sensitivity to isotopic variability which must be empirically quantified for the most precise atmospheric measurements (Lee et al., 2006; Tohjima et al., 2009).

Most recently, laser and Fourier Transform Infrared (FTIR) based optical infrared analysers have taken on a major role in atmospheric trace gas measurements for many gases, especially the dominant greenhouse gases CO₂ and CH₄. These instruments are based on infrared absorption by single absorption lines or bands of specific isotopologues, which are only a proxy for the total amount of the target trace gas. If the isotopic composition of the trace gas is invariant, such analysis provides a valid measure of the total amount of the gas after calibration, but it has long been recognised that isotopic differences between the calibration gases and the samples measured lead to variations in the total trace gas amounts deduced from a single isotopologue measurement that are significant relative to GAW compatibility goals (Loh et al., 2011). Several studies have addressed isotopic calibration (e.g. Esler et al., 2000; Bowling et al., 2003; Griffis et al., 2005; Mohn et al., 2008; Loh et al., 2011; Tuzson et al., 2011; Griffith et al., 2012; Wehr et al., 2013; Wen et al., 2013; Rella et al., 2015; Vardag et al., 2015; Pang et al., 2016; Flores et al., 2017; Tans et al., 2017; Braden-Behrens et al., 2017) and compared calibration approaches (Wen et al., 2013), but until recently most studies made some level of approximation in dealing with the calculations required to properly include the contributions of all possible isotopologues of the target species in the calculation scheme. Most recently Griffith et al (2012), Flores et al. (2017) and Tans et al. (2017) have published isotopic calibration strategies which are equivalent and which correctly and completely account for the full isotopic composition of the target gas (CO₂ in these studies, but applicable in principle to any species).

Established calibration laboratories using mass spectrometry as the primary method for isotopic analysis normally provide calibration standards which specify total amount and isotopic ratios of a trace gases in an air matrix, such as CO₂, δ¹³C and δ¹⁸O, while optical analysers fundamentally determine individual isotopologue amounts of isotopologues such as ¹⁶O¹²C¹⁶O, ¹⁶O¹³C¹⁶O and ¹⁶O¹²C¹⁸O. Here we present a practical guide to the calculations required to rigorously but simply convert

between the two equivalent descriptions and to derive isotope-specific calibrations for optical analysers. The calculations described here are equivalent to those described by Wehr et al. (2013), Flores et al. (2017) and Tans et al. (2017). The motivation for this technical note is thus threefold:

- to show that the complete and correct treatment of isotopic composition in calibration calculations is straightforward and that there is no need to invoke some approximations often made in earlier analyses,
- to provide a practical guide to isotope-specific calibration calculations, and
- to assess the potential errors when all isotopic information is not available and approximations or assumptions must be made.

2 Calculation of isotopic quantities

Using CO₂ as an example, considering the stable isotopes ¹²C, ¹³C, ¹⁶O, ¹⁷O and ¹⁸O, there are eighteen possible isotopologues (2 x 3 x 3 isotopic possibilities). ¹⁴C is a negligible proportion of total carbon for these purposes and is neglected. Only twelve of these eighteen possibilities are distinct due to symmetry. Assuming the substitution of each isotope at each position in the molecule follows its bulk statistical abundance (i.e. no clumping, see section 6), only four independent quantities are required to fully define the total amount and full isotopic composition of CO₂. These quantities may equivalently be the total CO₂ amount and three isotopic ratios ¹³r, ¹⁷r and ¹⁸r (or delta values δ¹³C, δ¹⁷O and δ¹⁸O), or the amounts of four individual isotopologues with each isotope substituted, most conveniently ¹⁶O¹²C¹⁶O, ¹⁶O¹³C¹⁶O, ¹⁶O¹²C¹⁷O and ¹⁶O¹²C¹⁸O. Once these are known, the abundances of all multiply-substituted isotopologues can be calculated.

The most fundamental quantity defining isotopic composition for each element is the *isotope ratio* of the minor to the major isotope

$$\begin{aligned} {}^{13}r &= \frac{n({}^{13}\text{C})}{n({}^{12}\text{C})} \\ {}^{17}r &= \frac{n({}^{17}\text{O})}{n({}^{16}\text{O})} \\ {}^{18}r &= \frac{n({}^{18}\text{O})}{n({}^{16}\text{O})} \end{aligned} \tag{1}$$

where for example $n({}^{13}\text{C})$ is the amount of ¹³C in a sample (number of moles or atoms). Isotope ratios for standard or reference materials are assigned by the isotope metrology community, (e.g. Allison et al., 1995; Brand et al., 2010; Werner and Brand, 2001).

Isotope ratios are commonly expressed as delta values relative to a standard or reference material

$$\begin{aligned} \delta^{13}\text{C} &= \left(\frac{{}^{13}r}{{}^{13}r_{ref}} - 1 \right) \\ \delta^{17}\text{O} &= \left(\frac{{}^{17}r}{{}^{17}r_{ref}} - 1 \right) \\ \delta^{18}\text{O} &= \left(\frac{{}^{18}r}{{}^{18}r_{ref}} - 1 \right) \end{aligned} \tag{2}$$

(Following the recommendation of Coplen (2011) and to simplify equations, the factor 1000 ‰ is not included in the definition of δ .) For the relevant reference scales commonly used in atmospheric analysis, the reference isotope ratios are given in Table 1.

For each isotope of an element, the *isotopic abundance* or *isotopic fraction* is the fraction of that isotope relative to all isotopes in a sample

$$\begin{aligned}
 {}^{12}x &= \frac{n({}^{12}\text{C})}{n({}^{12}\text{C}) + n({}^{13}\text{C})} = \frac{1}{(1 + {}^{13}r)} \\
 {}^{13}x &= \frac{n({}^{13}\text{C})}{n({}^{12}\text{C}) + n({}^{13}\text{C})} = \frac{{}^{13}r}{(1 + {}^{13}r)} \\
 {}^{16}x &= \frac{n({}^{16}\text{O})}{n({}^{16}\text{O}) + n({}^{17}\text{O}) + n({}^{18}\text{O})} = \frac{1}{(1 + {}^{17}r + {}^{18}r)} \\
 {}^{17}x &= \frac{n({}^{17}\text{O})}{n({}^{16}\text{O}) + n({}^{17}\text{O}) + n({}^{18}\text{O})} = \frac{{}^{17}r}{(1 + {}^{17}r + {}^{18}r)} \\
 {}^{18}x &= \frac{n({}^{18}\text{O})}{n({}^{16}\text{O}) + n({}^{17}\text{O}) + n({}^{18}\text{O})} = \frac{{}^{18}r}{(1 + {}^{17}r + {}^{18}r)}
 \end{aligned}$$

(3)

Note that these are fractional abundances such that ${}^{12}x + {}^{13}x = 1$ and ${}^{16}x + {}^{17}x + {}^{18}x = 1$.

10 Similarly, for a molecule the *isotopologue abundances* or *isotopologue fractions* are defined – for example for CO₂ the isotopologue abundances for ¹²C¹⁶O₂ (626), ¹³C¹⁶O₂ (636), ¹²C¹⁶O¹⁸O (628) and ¹²C¹⁶O¹⁷O (627) are:

$$\begin{aligned}
 x_{626} &= {}^{16}x \cdot {}^{12}x \cdot {}^{16}x = \frac{1}{R_{sum}} \\
 x_{636} &= {}^{16}x \cdot {}^{13}x \cdot {}^{16}x = \frac{{}^{13}r}{R_{sum}} \\
 x_{627} &= 2 \cdot {}^{16}x \cdot {}^{12}x \cdot {}^{17}x = \frac{2 \cdot {}^{17}r}{R_{sum}} \\
 x_{628} &= 2 \cdot {}^{16}x \cdot {}^{12}x \cdot {}^{18}x = \frac{2 \cdot {}^{18}r}{R_{sum}}
 \end{aligned}
 \tag{4}$$

where

$$R_{sum} = (1 + {}^{13}r) \cdot (1 + {}^{17}r + {}^{18}r)^2 \tag{5}$$

15 The labels 626, 636, 628, 627 are the common isotopic shorthand used in spectroscopy and the Hitran database. The sum of all isotopologue abundances x over all 18 isotopologues is equal to unity. R_{sum} is a sum of 18 products of isotope ratios, one corresponding to each of the 18 possible isotopologues of CO₂. R_{sum} conveniently accounts for all possible isotopologues in calculations of abundances, providing a normalising factor somewhat analogous to a partition sum over all energy levels of a molecule. From Eq. (4), $x_{626} = 1/R_{sum}$ i.e. $1/R_{sum}$ is the fractional abundance of the major isotopologue and

20 $R_{sum} - 1 \approx 1 - x_{626}$ is that fraction of the sample that is made up of all the minor isotopologues. Equivalently, from Eq. (10) and the following paragraph it can be seen that R_{sum} is the ratio of the total amount of CO₂ to that of the major isotopologue in a sample.

Abundances of the major and three singly-substituted isotopologues and R_{sum} values for standard reference scales are given in Table 2. Abundances of the multiply-substituted isotopologues can be calculated following the examples of Eq. (4). They are also listed for Hitran isotope ratios on the Hitran website <https://www.cfa.harvard.edu/hitran/molecules.html>.

- 5 For a calibration or reference gas, $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ are usually provided by calibration laboratories, and $\delta^{17}\text{O}$ can normally be deduced from $\delta^{18}\text{O}$ assuming mass dependent fractionation of oxygen isotopes with negligible error (Brand et al., 2010):

$$\begin{aligned} {}^{17}r / {}^{17}r_{ref} &= ({}^{18}r / {}^{18}r_{ref})^{0.528} \\ \text{or} & \\ \delta^{17}\text{O} &= 0.528 \cdot \delta^{18}\text{O} \end{aligned} \quad (6)$$

- 10 The mass dependent fractionation assumption is discussed below in section 6. The isotope ratios ${}^{13}r$, ${}^{17}r$ and ${}^{18}r$ for a sample can be thus be calculated from inverting equations (2)

$$\begin{aligned} {}^{13}r &= (1 + \delta^{13}\text{C}) \cdot {}^{13}r_{ref} \\ {}^{17}r &= (1 + \delta^{17}\text{O}) \cdot {}^{17}r_{ref} \\ {}^{18}r &= (1 + \delta^{18}\text{O}) \cdot {}^{18}r_{ref} \end{aligned} \quad (7)$$

thence R_{sum} can be calculated from (5) for any sample or reference gas.

- 15 If the total mole fraction of CO_2 in a sample of air, y_{CO_2} , is also known (for example, for a certified calibration gas), the individual isotopologue amounts or mole fractions can be calculated from

$$\begin{aligned} y_{626} &= y_{\text{CO}_2} \cdot x_{626} = y_{\text{CO}_2} / R_{sum} \\ y_{636} &= y_{\text{CO}_2} \cdot x_{636} = y_{\text{CO}_2} \cdot {}^{13}r / R_{sum} \\ y_{627} &= y_{\text{CO}_2} \cdot x_{627} = y_{\text{CO}_2} \cdot 2 \cdot {}^{17}r / R_{sum} \\ y_{628} &= y_{\text{CO}_2} \cdot x_{628} = y_{\text{CO}_2} \cdot 2 \cdot {}^{18}r / R_{sum} \end{aligned} \quad (8)$$

(Following the recommendation of the IUPAC Gold book (McNaught and Wilkinson, 2014) and usage by Tans et al. (2017), the symbol y is used here for mole fraction (more formally amount fraction) of a trace gas or isotopologue in air, to distinguish from x , the isotope or isotopologue fractional abundance.)

- 20 Conversely, if a set of calibrated isotopologue mole fractions $\{y_{626}, y_{636}, y_{628}, y_{627}\}$ in a sample are measured with an isotopologue-specific analyser, the total CO_2 mole fraction y_{CO_2} and isotope ratios or delta values can be calculated. The isotope ratios are derived directly from the isotopologue amounts,:

$$\begin{aligned} {}^{13}r &= y_{636} / y_{626} \\ {}^{18}r &= 0.5 \cdot y_{628} / y_{626} \\ {}^{17}r &= ({}^{18}r / {}^{18}r_{ref})^{0.528} \cdot {}^{17}r_{ref} \end{aligned} \quad (9)$$

then delta values are calculated from (2) and R_{sum} from (5). The total CO_2 mole fraction is then calculated from (8):

- 25
$$y_{\text{CO}_2} = y_{626} \cdot R_{sum} \quad (10)$$

The key quantity in these calculations is R_{sum} , which correctly and completely accounts for all possible isotopologues of the molecule at their actual isotopic abundances. Note that to correctly calculate the amount of *any* isotopologue in a sample, *all* isotope ratios should be known to calculate R_{sum} exactly. Errors incurred when this requirement is relaxed are discussed and quantified in section 5.

3 Normalised isotopologue mole fractions

In the Hitran database, tabulated line strengths are normalised by the natural abundance of the relevant isotopologue; the reference isotopologue natural abundances assumed in Hitran are listed in Table 2. Retrievals from spectra based on Hitran line parameters thus provide scaled or normalised mole fractions of isotopologues which are referenced to the isotopic scales assumed by Hitran. For some purposes it may be convenient to work with these normalised mole fractions directly rather than to convert them to absolute mole fractions as in section 2 because the reference isotopologue abundances are inherently included in the normalised amounts. In terms of normalised mole fractions, equations (8) become:

$$\begin{aligned}
 y'_{626} &= \frac{y_{626}}{x_{626,ref}} = y_{CO_2} \cdot \frac{R_{sum,ref}}{R_{sum}} = y_{CO_2} / X_{sum} \\
 y'_{636} &= \frac{y_{636}}{x_{636,ref}} = y_{CO_2} \cdot \frac{{}^{13}r}{{}^{13}r_{ref}} \cdot \frac{R_{sum,ref}}{R_{sum}} = y_{CO_2} \cdot (1 + \delta^{13}C) / X_{sum} \\
 y'_{627} &= \frac{y_{627}}{x_{627,ref}} = y_{CO_2} \cdot \frac{{}^{17}r}{{}^{17}r_{ref}} \cdot \frac{R_{sum,ref}}{R_{sum}} = y_{CO_2} \cdot (1 + \delta^{17}O) / X_{sum} \\
 y'_{628} &= \frac{y_{628}}{x_{628,ref}} = y_{CO_2} \cdot \frac{{}^{18}r}{{}^{18}r_{ref}} \cdot \frac{R_{sum,ref}}{R_{sum}} = y_{CO_2} \cdot (1 + \delta^{18}O) / X_{sum}
 \end{aligned} \tag{11}$$

where r_{ref} and $R_{sum,ref}$ refer to the reference scales listed in Table 1 and Table 2 and $X_{sum} = R_{sum} / R_{sum,ref} = R_{sum} \cdot x_{626,ref}$.

Equations (11) allow normalised mole fractions to be calculated from total CO₂ mole fraction and δ values on any reference scale for which r_{ref} and $R_{sum,ref}$ are known.

Calculation of δ values from normalised isotopologue mole fractions is analogous to Eq. (9) and (10):

$$\begin{aligned}
 \delta^{13}C &= \frac{y'_{636}}{y'_{626}} - 1 \\
 \delta^{18}O &= \frac{y'_{628}}{y'_{626}} - 1 \\
 \delta^{17}O &= 0.528 \cdot \delta^{18}O
 \end{aligned} \tag{12}$$

and the total CO₂ mole fraction is

$$y_{CO_2} = y'_{626} \cdot \frac{R_{sum}}{R_{sum,ref}} = y'_{626} \cdot X_{sum} \tag{13}$$

The normalised mole fractions have the convenient property that they are all equal to the total CO₂ mole fraction in a sample if all isotopes are in natural abundance in the reference scale (i.e. Eq. (11) with $\delta = 0$, $R_{sum} = R_{sum,ref}$ and $X_{sum} = 1$). Hitran natural abundances are based on a superseded definition of VPDB isotope ratio for carbon and SMOW for oxygen, while for atmospheric CO₂ the isotopic scale of choice is VPDB-CO₂, which is based on VPDB for both carbon and oxygen, and may be adjusted over time as scales are re-determined. To convert normalised mole fractions retrieved directly from spectra (Hitran scale) to the VPDB-CO₂ scale, each normalised mole fraction can be multiplied by $x_{ref,Hitran} / x_{ref,VPDB}$. The reference isotopologue abundances and rescaling factors are listed in Table 2.

4 Calibration and measurement procedures – step by step

Calibration of an isotopologue-specific analyser can in principle be carried out in two ways, calibrating on either the individual isotopologue amounts or on the derived isotope ratios or delta values. Both methods have been used in the published work to

date. The former is more fundamental because optical methods actually measure individual isotopologue amounts, not ratios. Ratio or delta-based calibration leads to the additional complication of concentration dependence in the calibration. A step by step method for direct isotopologue calibration is presented in section 4.1 based on the equations of section 2, ratio or delta calibration is discussed in section 4.2, and the two methods are compared in section 4.3.

5

4.1 Direct calibration by isotopologue amounts

The steps described here are consistent with those recently-published by Flores et al. (2017) and Tans et al. (2017). Griffith et al. (2012) previously described the same methods but used a minor approximation in accounting for the sum of all multiply-substituted isotopologues in the calculation of R_{sum} in Eq (5) or X_{sum} in Eq (11).

10 There are two parts to the calibration and unknown measurement procedure: (1) determination of the reference isotopologue amounts and the calibration equation for each isotopologue in a calibration gas, and (2) measurement of the isotopologue amounts in an unknown sample and calculation of its total trace gas amount and delta quantities. As above CO₂ is used as an example, but the procedures apply in principle to any molecule.

15 Calibration

1. From reference standard tank data provided by the calibration laboratory {CO₂, δ¹³C, δ¹⁸O, (δ¹⁷O)}, calculate isotope ratios ¹³r, ¹⁸r, ¹⁷r and R_{sum} for each standard (Eq. (7) then Eq. (5)).

2. Calculate the calibrated amount of each isotopologue $y_{626}, y_{636}, y_{628}$ in each standard (Eq (8)).

3. Measure uncalibrated analyser responses or raw isotopologue amounts of each standard

20 $y_{626,meas}, y_{636,meas}, y_{628,meas}$ with the analyser.

4. Derive the calibration equation for each isotopologue, for example for a linear calibration

$$y_{626,meas} = a_{626} \cdot y_{626} + b_{626} \quad (14)$$

Sample measurement

25 1. Measure the sample with the analyser and determine the analyser responses or raw isotopologue amounts.

2. Apply the inverted calibration determined in 4. above for each isotopologue to determine calibrated isotopologue amounts.

3. Calculate ¹³r, ¹⁸r, ¹⁷r and R_{sum} from calibrated isotopologue amounts (Eq. (9))

4. Calculate δ¹³C and δ¹⁸O on the desired reference isotope scale (Eq. (2) or (12)).

30 5. Calculate total CO₂

$$y_{CO_2} = y_{626} \cdot R_{sum} \quad (\text{Eq. (10)})$$

With this scheme, for complete calibration of the analyser the total CO₂ amount, δ¹³C and δ¹⁸O should be known for each reference standard, and each isotopologue should be measured by the analyser (or a combination of analysers). δ¹⁷O can be calculated with sufficient accuracy from δ¹⁸O. Calibration gases may but do not need to span a range of delta values, they need only span the range of amounts of each isotopologue covered by the range of samples to be measured (Bowling et al., 2003). Flores et al. (2017) demonstrated isotopic calibration of CO₂ in which all standards were synthesised from the same CO₂ source gas and all had the same δ¹³C and δ¹⁸O values.

4.2 Calibration by delta values

Spectroscopic analysers fundamentally determine the amounts of individual isotopologues, and the isotopologue-based analysis as described in the preceding section is the natural choice as a basis for calibration. Historically however, isotope ratio mass spectrometry (IRMS) has been the method of choice for isotopic analysis because many sources of noise cancel in calculating the ratio. Traditional IRMS calibration schemes are based on standards over a range of isotope ratios or delta values directly rather than on isotopologue amounts. Ratio or delta calibration schemes have thus, perhaps inevitably, flowed through to optical techniques. Ratio calibration schemes use calibration standards which cover a range of delta values and derive calibration equations analogous to Eq. (14) directly in terms of delta values rather than isotopologue amounts. The raw measured delta values are calculated from the uncalibrated isotopologue amounts. However, as shown in the following, this method inevitably leads to a concentration dependence of the calibration equations which must be characterised as part of (and that significantly complicates) the calibration procedure.

Several groups have reported on ratio calibration schemes and the consequent concentration dependence (e.g. Griffith et al., 2012; Wen et al., 2013; Rella et al., 2015; Pang et al., 2016; Braden-Behrens et al., 2017; Flores et al., 2017). The concentration dependence inevitably follows if the actual calibration relationships between measured and true amounts of individual isotopologues (section 4.1, Eq. (14)) have a non-zero y-intercept or an additional non-linear term. Griffith et al. (2012, Eq. 14) showed that a non-zero intercept in the calibration equations leads to an approximate inverse concentration dependence of measured $\delta^{13}\text{C}$. Extending that to include a quadratic term in the calibration equation representing non-linearity adds an approximately linear term to the concentration dependence, which can then be described by a combination of an inverse and linear dependence on y_{CO_2} :

$$\delta^{13}\text{C}_{\text{meas}} = \alpha \cdot \delta^{13}\text{C}_{\text{true}} + (\alpha - 1) + \frac{\beta}{y_{\text{CO}_2}} + \gamma \cdot y_{\text{CO}_2} \quad (15)$$

where $\delta^{13}\text{C}_{\text{meas}}$ is calculated from the raw measured isotopologue amounts. For a perfectly linear calibration equation (14) with $b_{626} = b_{636} = 0$ both β and γ are zero, $\alpha = a_{636} / a_{626}$ and Eq. (15) represents a simple concentration-independent scale shift of $(\alpha-1)$ in the δ scale. β is a function of the intercept terms b_{626} and b_{636} , and γ becomes non-zero if non-zero quadratic terms are added to the calibration equations. The inverse and linear y_{CO_2} dependences are not exact because the coefficients β and γ contains terms dependent on $\delta^{13}\text{C}$ and there are weak cross-terms, but together they provide a useful model to describe the concentration dependence. The linear term becomes relatively more important than the inverse term at high CO_2 mole fractions where the inverse CO_2 term becomes small and any quadratic contribution to the calibration equation leading to the linear term becomes large.

Figure 1 illustrates this concentration dependence with a typical $\delta^{13}\text{C}$ vs CO_2 dependence for an FTIR analyser similar to that used in the example of section 5 below. The dependence was determined by continuous flow measurements of a single CO_2 -spiked air tank while the CO_2 content was gradually reduced by passing a fraction of the flow through Ascarite. The measured $\delta^{13}\text{C}$ vs CO_2 data are fitted to Eq (15) with fitted parameters $\beta = -1227 \text{‰ ppm}$ and $\gamma = 0.0054 \text{‰ ppm}^{-1}$, corresponding to CO_2 - dependent corrections of up to 5‰ over the CO_2 range 400-1000 ppm. The residuals of the fit illustrate potential errors from the modelled behaviour of up to $\pm 0.3\text{‰}$. Uncertainties in calibrating the CO_2 concentration dependence can lead to significant errors in Keeling-type analyses over a wide range of total CO_2 amounts even if the isotopologue calibration non-linearity is very small (Pang et al., 2016; Wen et al., 2013).

The concentration dependence is a function of the isotopologue calibration coefficients, and thus in principle for best accuracy it should be re-determined for every calibration, complicating the calibration procedure. The Thermo-Fisher Delta Ray isotope analyser, for example, takes this approach in a prescribed sequence of measurements using several reference standards;

however Braden-Behrens et al. (2017) and Flores et al. (2017) found this procedure not to be sufficiently accurate or stable and invoked separate a posteriori calibration schemes. Rella et al. (2015; Picarro, 2017) similarly describe a calibration procedure for Picarro analysers to take concentration dependence into account.

4.3 Comments on the accuracy of optical isotopologue and ratio calibration

5 As an example assume a calibration laboratory provides calibrated reference gases with an absolute accuracy of 0.05 ppm for total CO₂ amount (0.12‰ in 400 ppm CO₂) and 0.02‰ for δ¹³C measured by IRMS. The isotope ratio is thus more accurately determined than the total amount fraction for the reference gases. Now take as a practical measurement repeatability for optical analysers 0.02 ppm (0.05‰) for total CO₂ amount and 0.07‰ for δ¹³C (e.g. Griffith et al. (2012), laser instruments are similar). The absolute accuracy for the calibrated optical measurement of total CO₂ is limited by the reference gas amount fraction, but
10 the more accurately known reference ¹³r or 626/636 ratio is carried through the calibration calculations and this accuracy is preserved when retrieved isotopologue amounts are ratioed. The accuracy of measured ¹³r or δ¹³C is thus limited by the optical measurement (0.07‰) which is less precise than the IRMS-provided reference accuracy (0.02‰). This does not currently meet GAW requirements for clean background air measurements (WMO-GAW, 2016), but is nevertheless useful for continuous analysis of air in non-baseline scenarios such as urban air or agricultural flux measurements.

15 This reasoning applies to both isotopologue and ratio calibration schemes, which both benefit from the higher accuracy and precision in the isotopologue ratios than in absolute isotopologue amounts. The principle differences between the isotopologue and ratio calibration schemes are twofold:

- The isotopologue scheme does not require calibration gases spanning a range of delta values, it is sufficient to span the range of total amount fractions of interest. This simplifies the preparation of reference gases for calibration laboratories.
- The ratio scheme has an unavoidable CO₂ concentration dependence which must be characterised and leads potentially to a loss of accuracy, as shown in section 4.2. This complicates the calibration procedure for optical analysers.

Errors are discussed further in section 6.

25 5 Tutorial: a practical worked example

This section presents a worked example of the calibration of an optical analyser using reference gases of given total CO₂ mole fraction, δ¹³C and δ¹⁸O, followed by measurements of air to which this calibration is applied. The data are derived from an Ecotech Spectronus FTIR analyser which measures three isotopologues of CO₂ (626, 636, 628) in the calibration gases and in the sampled air. The calculations follow section 4.1.

30 Calibration

The calibration data were collected in the laboratory at the University of Wollongong on 27 Sept 2017. Four reference tanks were sourced from CSIRO GASLAB, with total CO₂ mole fraction, δ¹³C and δ¹⁸O provided on the current WMO reference scales (WMO X2007 scale for total CO₂, VPDB-CO₂ for δ¹³C and δ¹⁸O). For each calibration tank, ¹³r, ¹⁸r, ¹⁷r, R_{sum}, and
35 reference isotopologue mole fractions are calculated from equations (7), (5) and (8). The four reference gases were measured in the analyser, and raw measured values of the isotopologue mole fractions corrected to dry air and for small spectroscopic cross-sensitivities to pressure, temperature and water vapour as described by Griffith et al. (2012). A two-parameter linear regression (slope and intercept) of measured against reference mole fractions for each isotopologue provides the linear calibration coefficients *a* and *b* for the analyser, Eq. (14). The worked data are presented in Table 3 and calibration plots shown
40 in Figure 2.

Sample air measurements

Figure 3 shows an example of one day of calibrated 1-minute measurements from the same FTIR analyser collected at a rural site in SE Australia on 23 and 24 Jan 2018. Table 4 illustrates the worked calibration of the raw data at four times of differing CO₂ amounts and isotopic fractionations. The linear calibration of 27 Sept 2017 described above has been applied to the measured data without further correction. The calculations follow section 4.1 to determine y_{CO_2} , $\delta^{13}C$ and $\delta^{18}O$ for each 1-minute measurement. Figure 4 shows an example of a Keeling plot derived from the data of Figure 3, with an intercept -24.5‰ typical of the dominant plants in this agricultural area.

6 Assessment of potential errors

Table 5 shows examples of actual isotopologue amounts for samples with total CO₂ = 400 ppm and a range of isotopic compositions. The table includes R_{sum} values calculated for each sample. The potential error incurred in calculating the total CO₂ amount from a spectroscopic measurement of y_{626} via Eq. (10) if the different isotopic composition between sample and reference gases is not taken into account is shown in the rightmost column – it is the difference from 400 ppm of the total CO₂ calculated from Eq. (10) taking the reference value $R_{sum,ref}$ (case 1) instead of the correct value on the same line R_{sum} . This simulates the effect of ignoring the difference in isotopic composition between reference and sample. The reference case (case 1) is a hypothetical standard with the isotopic composition of VPDB-CO₂. Examples include typical clean air (case 2), synthetic air synthesised with ¹³C-depleted CO₂ with $\delta^{13}C = -35‰$ (case 3), realistic errors of 2‰ in $\delta^{18}O$ and $\delta^{17}O$ (cases 4,5), and using isotope ratios assumed by Hitran rather than VPDB-CO₂ (case 6). Case 7 simulates the result if only singly-substituted isotopologues are included in the sum and all doubly-substituted minor isotopologues are ignored. Other cases can be assessed following the equations of section 2. Potential errors are fortunately small relative to GAW compatibility goals for realistic isotopic variations of a few per mil around clean air values. However the potential for significant errors (> 0.1 ppm) exists for reference gas mixtures or samples with ¹³C-depleted CO₂ as is often the case for synthetic mixtures or for samples with added CO₂ derived from plant or fossil fuel sources.

These potential errors in computation of delta values should also be viewed in the context of experimental measurement errors. Flores et al. (2017) formally evaluated the uncertainty budget for their particular FTIR measurements of $\delta^{13}C$ in CO₂ and found a standard uncertainty of 0.09‰, of comparable magnitude to the largest potential computational approximation errors. The measurement uncertainty was dominated by uncertainty in assigned reference mole fractions for the reference standards rather than the spectroscopic measurement uncertainty.

30

Three assumptions, previously mentioned and summarised here, have negligible impact on the calculations of section 2 and Table 5:

- ¹⁴C, with an isotopic abundance of < 1 ppt is ignored in all calculations.
- The relative amounts of multiply-substituted minor isotopologues are assumed to be in statistical relative abundance, i.e. there is no isotope clumping. Clumping refers to the case where the enrichment (or depletion) of two or more isotopes in a multiply-substituted isotopologue are correlated rather than each following their statistical amounts independently. Clumping effects are normally much less than 1‰, and according to Table 5 therefore insignificant.
- ¹⁷r and $\delta^{17}O$ are calculated from ¹⁸r and $\delta^{18}O$ (Eq. (6)) assuming mass dependent fractionation. Thermodynamic and kinetic fractionation processes are mass-dependent and account for most fractionation mechanisms in nature. Mass-independent fractionation typically occurs in quantum processes such as photolysis and can cause small

40

deviations from mass dependence. These deviations are also typically < 1‰ (e.g. Miller et al., 2002) and thus also negligible for the purposes of this work.

7 Other molecules

Similar considerations apply to other molecular species, see Table 6. For CH₄, ¹³CH₄ measurements are commonly made using laser analysers such as that of Picarro (Rella et al., 2015), and isotopic reference gases are available. An analysis similar to that in section 6 and Table 5 shows that for 2000 ppb CH₄ in air, an error of 10‰ in the assumed value of δ¹³C leads to an error of 0.2 ppb in the calculated total CH₄ mole fraction, and for a -35‰ error the total CH₄ error is 0.7ppb. A 100‰ error in δ²H leads to an error in total CH₄ of only 0.1 ppb.

For N₂O there is the additional complication of the isotopomers ¹⁵N¹⁴N¹⁶O and ¹⁴N¹⁵N¹⁶O for which standard reference gases are not available, and for which measurement technologies are currently less advanced. The general magnitude of potential errors will be similar to those of CO₂. For CO, reference gases are available, but current optical techniques are not able to resolve isotopic variations with sufficient accuracy at the typical low total mole fractions in air.

8 Calibration of commercially-available analysers

Several commercial manufacturers offer isotopologue-specific optical analysers based on laser (Campbell, Picarro, Los Gatos Research, Aerodyne, Thermo Fisher Delta Ray) or FTIR (Ecotech Spectronus) spectroscopy that analyse sampled air for one or more specific isotopologues. These instruments report results in a variety of ways, as isotopologue mole fractions and/or as total mole fractions and isotopic delta values, both calibrated and uncalibrated. In most cases the scheme by which total mole fractions and delta values are calculated from the raw measured data is not fully described, although some details are available in user manuals and published works. In most cases some level of approximation is used in accounting for the full molecular isotopic composition when converting between isotopologue amounts and total amounts and delta values. As shown in section 6, these approximations are fortunately in most cases acceptably small, but it is nevertheless recommended that they be assessed and documented if the full computation scheme is not used or measurement and calibration data for all isotopologues are not available.

25

GAW Greenhouse Gas Measurement Techniques reports since 2011 (WMO-GAW, 2012) recommend that the computational scheme for isotopic quantities derived from all commercial and non-commercial analysers be published and fully transparent to the user to avoid the potential for biases and inaccuracies stemming from different calibration and calculation schemes. Potential errors and calibration biases due to inconsistent isotopic calculations and the empirical determination of concentration dependences can be avoided if only the raw output isotopologue amounts from the analyser(s) are used and calibrated and isotopic quantities are calculated a posteriori following consistent calculation schemes such as those described here and in Flores et al. (2017) and Tans et al. (2017).

30

9 Summary, discussion and conclusions

Optical trace gas analysers based on laser or FTIR spectroscopy measure the concentrations or mole fractions of individual isotopologues of a trace gas rather than the total amount of all possible isotopologues of the target gas. This leads to potential calibration inaccuracies in relating the individual isotopologue measurements made by the analyser to the more usual quantities of total amount and isotopic ratios or delta values. This paper reviews previous studies addressing isotopic calibration of optical analysers and presents a practical guide to the calculations required to completely and rigorously account for the isotopic

35

composition of a trace gas when determining its total concentration with an isotopologue-specific optical analyser. Although most previous work has made some level of approximation in accounting for the full isotopic composition, this paper shows that such approximations are not required and save little effort - the complete calculations are relatively straightforward. The approach described here is consistent with those of Flores et al. (2017) and Tans et al. (2017); for CO₂ for example, the measurement of either three isotopologues (¹²C¹⁶O₂, ¹³C¹⁶O₂, ¹²C¹⁶O¹⁸O), or total CO₂ and two delta values (δ¹³C, δ¹⁸O) is necessary and sufficient to specify the complete isotopic composition with sufficient accuracy to meet GAW compatibility goals. Calculations to interconvert between these equivalent specifications of composition accurately are described.

Potential errors which may arise when making sometimes-unavoidable approximations in the calculations are assessed and in most cases fortunately found to be small, and often negligible. However significant errors can arise when the isotopic composition of an air sample is very different from that used to calibrate the analyser. Two common cases where this may occur in practice are in the production of synthetic reference standards using highly depleted ¹³C in CO₂, and in environmental studies such as soil chambers where high levels of ¹³C-depleted CO₂ are analysed with an analyser calibrated around clean atmospheric ¹³C levels.

Provided the appropriate calibration standards are available, this paper recommends that the calibration of optical analysers be carried out via direct measurement of the amounts of individual isotopologues, from which the total trace gas amount and isotopic composition can then be calculated completely and accurately. It recommends against ratio or delta-based calibration because this approach leads inevitably to concentration dependences in the calibration that must be characterised. Direct isotopologue calibration avoids concentration dependence and requires only reference standards spanning the range of concentrations to be measured and of known isotopic composition. There is no requirement for the reference gases to span the range of expected delta values, they can all be produced from the same source of trace gas and all have the same isotopic composition.

10 Acknowledgements

I would like to thank the GAW-GGMT community for many discussions on this topic, and especially Edgar Flores, Joelle Viallon, Camille Yver-Kwok and Grant Forster who provided comments on the manuscript and checked the calculations.

11 References

- Allison, C., Francey, R., and Meijer, H.: Recommendations for the reporting of stable isotope measurements for carbon and oxygen in CO₂ gas, reference and Intercomparison Materials for stable isotopes of light element, IAEA-TECDO, 155-162, 1995., IAEA, Vienna, 155-162, 1995.
- Bievre, P. D., Holden, N. E., and Barnes, I. L.: Isotopic Abundances and Atomic Weights of the Elements, *J.Phys.Chem.Ref.Data*, 809-891, 1984.
- Bowling, D. R., Sargent, S. D., Tanner, B. D., and Ehleringer, J. R.: Tunable diode laser absorption spectroscopy for stable isotope studies of ecosystem-atmosphere CO₂ exchange, *Agric. For. Meteorol.*, 118, 1-19, [https://doi.org/10.1016/S0168-1923\(03\)00074-1](https://doi.org/10.1016/S0168-1923(03)00074-1), 2003.
- Braden-Behrens, J., Yan, Y., and Knohl, A.: A new instrument for stable isotope measurements of ¹³C and ¹⁸O in CO₂ – instrument performance and ecological application of the Delta Ray IRIS analyzer, *Atmos. Meas. Tech.*, 10, 4537-4560, 10.5194/amt-10-4537-2017, 2017.
- Brand, W. A., Assonov, S. S., and Coplen, T. B.: Correction for the ¹⁷O interference in del(¹³C) measurements when analyzing CO₂ with a stable isotope mass spectrometry (IUPAC Technical Report), *Pure Appl. Chem.*, 82, 1719-1733, 2010.
- Coplen, T. B.: Guidelines and recommended terms for expression of stableisotope-ratio and gas-ratio measurement results, *Rapid Commun. Mass Spectrom.*, 25, 2538–2560, 10.1002/rcm.5129, 2011.
- Esler, M. B., Griffith, D. W. T., Wilson, S. R., and Steele, L. P.: Precision trace gas analysis by FT-IR spectroscopy 2. The ¹³C/¹²C isotope ratio of CO₂, *Analytical Chemistry*, 72, 216-221, 2000.

- Flores, E., Viallon, J., Moussay, P., Griffith, D. W. T., and Wielgosz, R. I.: Calibration Strategies for FT-IR and Other Isotope Ratio Infrared Spectrometer Instruments for Accurate $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ Measurements of CO_2 in Air, *Analytical Chemistry*, 89, 3648-3655, 10.1021/acs.analchem.6b05063, 2017.
- Griffis, T. J., Lee, X., Baker, J. M., Sargent, S. D., and King, J. Y.: Feasibility of quantifying ecosystem-atmosphere $\text{C}^{18}\text{O}^{16}\text{O}$ exchange using laser spectroscopy and the flux-gradient method, *Agric. For. Meteorol.*, 135, 44-60, 2005.
- Griffith, D. W. T.: Calculations of carrier gas effects in non-dispersive infrared analysers I. Theory, *Tellus*, 34, 376-384, 1982.
- Griffith, D. W. T., Deutscher, N. M., Caldow, C., Kettlewell, G., Riggensbach, M., and Hammer, S.: A Fourier transform infrared trace gas and isotope analyser for atmospheric applications, *Atmos. Meas. Tech.*, 5, 2481-2498, 10.5194/amt-5-2481-2012, 2012.
- Lee, J.-Y., Yoo, H.-S., Marti, K., Moon, D. M., Lee, J. B., and Kim, J. S.: Effect of carbon isotopic variations on measured CO_2 abundances in reference gas mixtures, *J. Geophys. Res.*, 111, doi:10.1029/2005JD006551, 2006.
- Loh, Z. M., Steele, L. P., Krummel, P. B., Schoot, M. v. d., Etheridge, D. M., Spencer, D. A., and Francey, R. J.: Linking Isotopologue Specific Measurements of CO_2 to the Existing International Mole Fraction Scale, 15th WMO/IAEA Meeting of Experts on Carbon Dioxide, Other Greenhouse Gases and Related Tracers Measurement Techniques (WMO/GAW report no. 194), Jena, Germany, August 2009, 2011.
- McNaught, A. D., and Wilkinson, A.: IUPAC Compendium of Chemical Technology - the Gold Book, IUPAC, 2014.
- Miller, M. F., Franchi, I. A., Thiemens, M. H., Jackson, T. L., Brack, A., Kurat, G., and Pillinger, C. T.: Mass-independent fractionation of oxygen isotopes during thermal decomposition of carbonates, *PNAS*, 99, 10988-10993, 10.1073/pnas.172378499, 2002.
- Mohn, J., Zeeman, M. J., Werner, R. A., Eugster, W., and Emmenegger, L.: Continuous field measurements of $\delta^{13}\text{C}$ - CO_2 and trace gases by FTIR spectroscopy, *Isotopes in Environmental and Health Studies*, 44, 241-251, 2008.
- Pang, J., Wen, X., Sun, X., and Huang, K.: Intercomparison of two cavity ring-down spectroscopy analyzers for atmospheric $^{13}\text{CO}_2$ / $^{12}\text{CO}_2$ measurement, *Atmos. Meas. Tech.*, 9, 3879-3891, 10.5194/amt-9-3879-2016, 2016.
- Picarro: Calibration guide for Picarro Analyzers, 2017.
- Rella, C. W., Hoffnagle, J., He, Y., and Tajima, S.: Local- and regional-scale measurements of CH_4 , $^{13}\text{CH}_4$, and C_2H_6 in the Uintah Basin using a mobile stable isotope analyzer, *Atmos. Meas. Tech.*, 8, 4539-4559, 10.5194/amt-8-4539-2015, 2015.
- Rothman, L. S., Jacquemart, D., Barbe, A., Benner, D. C., Birk, M., Brown, L. R., Carleer, M. R., Chackerian, J., Chance, K., Dana, V., Devi, V. M., Flaud, J.-M., Gamache, R. R., Goldman, A., Hartmann, J.-M., Jucks, K. W., Maki, A. G., Mandin, J.-Y., Massie, S. T., Orphali, J., Perrin, A., Rinsland, C. P., Smith, M. A. H., Tennyson, J., Tolchenov, R. N., Toth, R. A., Auwera, J. V., Varanasi, P., and Wagner, G.: The HITRAN 2004 molecular spectroscopic database, *J. Quant. Spectry.Rad. Tfer*, 96, 139-204, 2005.
- Tans, P. P., Crotwell, A. M., and Thoning, K. W.: Abundances of isotopologues and calibration of CO_2 greenhouse gas measurements, *Atmos. Meas. Tech.*, 10, 2669-2685, 10.5194/amt-10-2669-2017, 2017.
- Tohjima, Y., Katsumata, K., Morino, I., Mukai, H., Machida, T., Akama, I., Amari, T., and Tsunogai, U.: Theoretical and experimental evaluation of the isotope effect of NDIR analyzer on atmospheric CO_2 measurement, *J. Geophys. Res.*, 114, doi:10.1029/2009JD011734, 2009.
- Tuzson, B., Henne, S., Brunner, D., Steinbacher, M., Mohn, J., Buchmann, B., and Emmenegger, L.: Continuous isotopic composition measurements of tropospheric CO_2 at Jungfraujoch (3580 m a.s.l.), Switzerland: real-time observation of regional pollution events, *Atmos. Chem. Phys.*, 11, 1685-1696, 10.5194/acp-11-1685-2011, 2011.
- Vardag, S. N., Hammer, S., Sabasch, M., Griffith, D. W. T., and Levin, I.: First continuous measurements of ^{18}O - CO_2 in air with a Fourier transform infrared spectrometer, *Atmos. Meas. Tech.*, 8, 579-592, 10.5194/amt-8-579-2015, 2015.
- Wehr, R., Munger, J. W., Nelson, D. D., McManus, J. B., Zahniser, M. S., Wofsy, S. C., and Saleska, S. R.: Long-term eddy covariance measurements of the isotopic composition of the ecosystem-atmosphere exchange of CO_2 in a temperate forest, *Agric. For. Meteorol.*, 181, 69-84, 2013.
- Wen, X. F., Meng, Y., Zhang, X. Y., Sun, X. M., and Lee, X.: Evaluating calibration strategies for isotope ratio infrared spectroscopy for atmospheric $^{13}\text{CO}_2$ / $^{12}\text{CO}_2$ measurement, *Atmos. Meas. Tech.*, 6, 1491-1501, 10.5194/amt-6-1491-2013, 2013.
- Werner, R. A., and Brand, W. A.: Referencing strategies and techniques in stable isotope ratio analysis, *Rapid Commun. Mass Spectrom.*, 15, 501-519, 2001.
- WMO-GAW: GAW report No. 206. 16th WMO/IAEA Meeting on Carbon Dioxide, Other Greenhouse Gases, and Related Measurement Techniques (GGMT-2011, Wellington, NZ, Oct 2011), 2012.
- WMO-GAW: GAW report 229. 18th WMO/IAEA meeting on carbon dioxide, other greenhouse gases and related 74 measurement techniques (GGMT-2015). WMO, 2016.

Tables

5 **Table 1. Standard isotope ratios for relevant reference scales used in atmospheric trace gas analysis.** ¹(Werner and Brand, 2001), ²(Brand et al., 2010), ³(Bievre et al., 1984), ⁴ <https://www.cfa.harvard.edu/hitran/molecules.html>

Element	Ratio	VPDB ¹	VPDB-CO ₂ ²	Hitran ^{3,4}	VSMOW ¹	air N ₂ ¹
C	$^{13}\text{r} = ^{13}\text{C}/^{12}\text{C}$	0.0111802	0.0111802	0.0112374		
O	$^{18}\text{r} = ^{18}\text{O}/^{16}\text{O}$	0.0020672	0.00208835	0.0020052	0.00200518	
O	$^{17}\text{r} = ^{17}\text{O}/^{16}\text{O}$	0.000386	0.0003931	0.0003729		
N	$^{15}\text{r} = ^{15}\text{N}/^{14}\text{N}$			0.00367		0.0036782
H	$^2\text{r} = ^2\text{H}/^1\text{H}$			0.000156	0.00015575	

10

15 **Table 2. Isotopologue fractional abundances and isotopic sums for the VPDB-CO₂ and Hitran scales and conversion factors.** Abundances are taken from ¹Rothman et al (2005) and ²<https://www.cfa.harvard.edu/hitran/molecules.html> for Hitran and ³ Brand et al. (2010) for VPDB-CO₂. The Brand et al. values supersede earlier values given by Allison et al. (1995).

Isotopologue	Notation	Abundance ^{1,2}	Abundance ³	Rescaling factor (Hitran / VPDB-CO ₂)
		x_{HITRAN}	$x_{\text{VPDB-CO}_2}$	
$^{16}\text{O}^{12}\text{C}^{16}\text{O}$	626	0.98420	0.984054	1.000150
$^{16}\text{O}^{13}\text{C}^{16}\text{O}$	636	0.01106	0.0110019	1.005280
$^{16}\text{O}^{12}\text{C}^{18}\text{O}$	628	0.0039471	0.00411009	0.960319
$^{16}\text{O}^{12}\text{C}^{17}\text{O}$	627	0.000734	0.00077366	0.948734
R_{sum}	-	1.016205	1.016053	0.9998505

20

Table 3. Worked data for calibration of an FTIR analyser using four reference standards (a) using actual mole fractions of all isotopologues, and (b) using normalised mole fractions on the VPDB-CO₂ scale. ¹⁷r and δ¹⁷O values were not directly determined and are not included in the table – they are derived from ¹⁸r and δ¹⁸O following equation (6).

(a) Tank	y _{CO2} ppm	δ ¹³ C ‰	δ ¹⁸ O ‰	¹³ r	¹⁸ r	R _{sum}	y ₆₂₆ ppm	y ₆₃₆ ppm	y ₆₂₈ ppm	
	Calibration tank data						Reference mole fractions			
CB11138	396.74	-8.38	0.30	0.011087	0.002089	1.016112	390.45	4.3287	1.6313	
CB11483	452.06	-8.19	-2.11	0.011089	0.002084	1.016103	444.90	4.9333	1.8543	
CA06845	416.06	-10.69	-2.71	0.011061	0.002083	1.016072	409.48	4.5291	1.7056	
CB09950	392.91	-8.38	-0.20	0.011087	0.002088	1.016110	386.68	4.2870	1.6147	
							Measured mole fractions			
CB11138							426.50	4.9011	1.8942	
CB11483							486.46	5.5937	2.1768	
CA06845							447.49	5.1310	1.9891	
CB09950							422.33	4.8533	1.8731	
							Calibration coefficients			
							Slope a	1.10146	1.14563	1.26747
							Intercept b	-3.56	-0.0579	-0.1733

5

(b) Tank	y _{CO2} ppm	δ ¹³ C ‰	δ ¹⁸ O ‰	¹³ r	¹⁸ r	X _{sum}	y' ₆₂₆ ppm	y' ₆₃₆ ppm	y' ₆₂₈ ppm	
	Calibration tank data						Reference normalised mole fractions			
CB11138	396.74	-8.38	0.30	0.011087	0.002089	0.999909	396.78	393.45	396.90	
CB11483	452.06	-8.19	-2.11	0.011089	0.002084	0.999900	452.11	448.40	451.15	
CA06845	416.06	-10.69	-2.71	0.011061	0.002083	0.999869	416.11	411.67	414.99	
CB09950	392.91	-8.38	-0.20	0.011087	0.002088	0.999906	392.95	389.66	392.87	
							Measured normalised mole fractions			
CB11138							433.414	445.48	460.87	
CB11483							494.342	508.43	529.62	
CA06845							454.742	466.38	483.96	
CB09950							429.172	441.13	455.74	
							Calibration coefficients			
							Slope a	1.10146	1.14563	1.26748
							Intercept b	-3.62	-5.27	-42.16

10

Table 4. Worked calibration of sample data in Figure 3 at four times with varying CO₂ mole fractions. Columns 2-4 contain the raw measured isotopologue mole fractions corrected to dry air, columns 5-7 contain the calibrated dry air mole fractions after applying the coefficients from Table 3, columns 8-10 are the isotopic ratios and R_{sum} for each sample, and columns 11-13 contain the final calibrated total CO₂, $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$.

5

Time	y_{626,meas}	y_{636,meas}	y_{628,meas}	y_{626,cal}	y_{636,cal}	y_{628,cal}	¹³r	¹⁸r	R_{sum}	y_{CO2}	δ¹³C	δ¹⁸O
23/24	ppm	ppm	ppm	ppm	ppm	ppm				ppm	‰	‰
Jan												
18:00	433.79	4.9845	1.9325	397.07	4.4015	1.6614	0.011085	0.002092	1.016117	403.47	-8.53	1.76
00:00	492.97	5.6550	2.2211	450.80	4.9867	1.8891	0.011062	0.002095	1.016102	458.05	-10.56	3.31
06:00	541.01	6.2000	2.4531	494.41	5.4624	2.0722	0.011048	0.002096	1.016088	502.37	-11.80	3.46
12:00	433.37	4.9800	1.9309	396.69	4.3975	1.6601	0.011086	0.002092	1.016119	403.08	-8.47	1.97

10

Table 5. Actual isotopologue amounts and R_{sum} values in 400 ppm total CO₂ for various isotopic compositions. The last column lists errors in calculating total CO₂ if different isotopic composition between reference (calibration) and sample measurements are not accounted for. See text for details of the various cases.

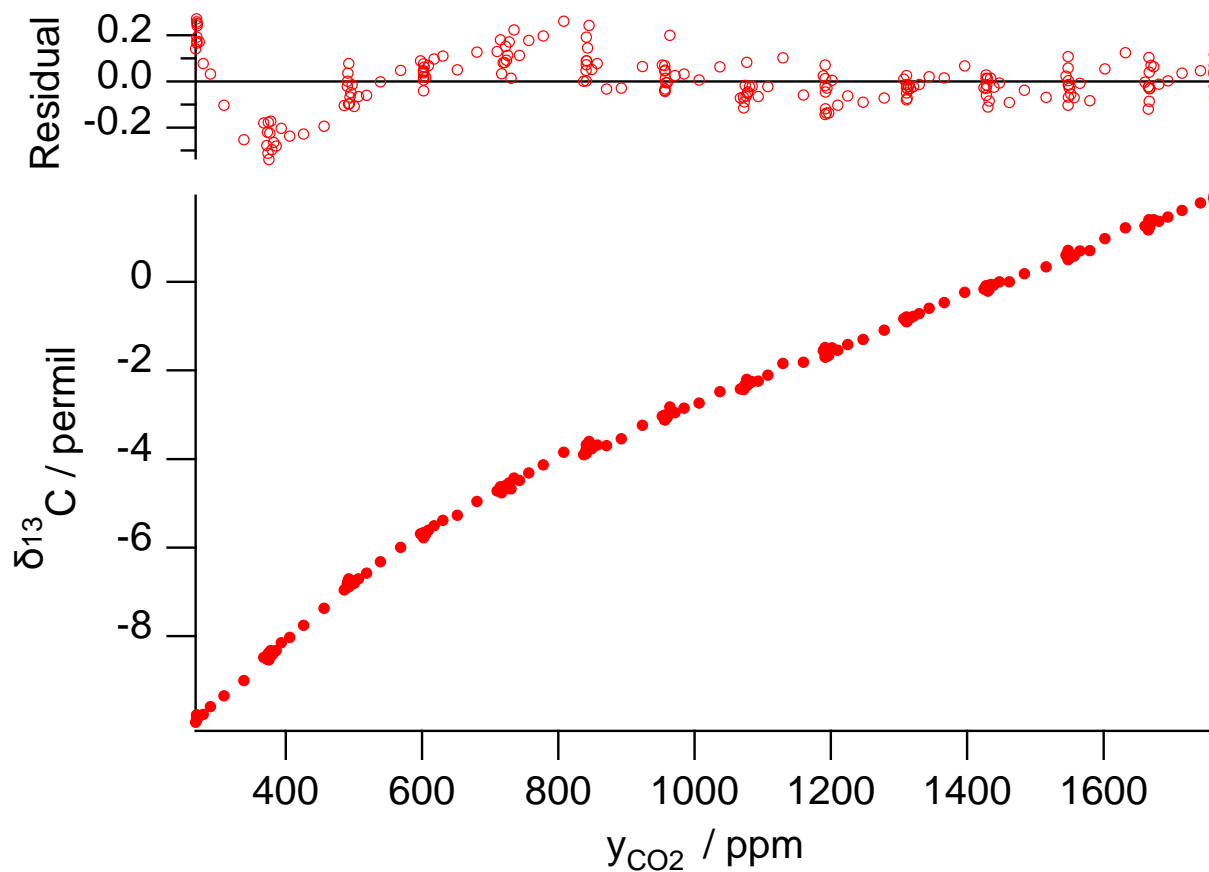
Case	y_{CO2}	δ¹³C	δ¹⁸O	δ¹⁷O	R_{sum}	y₆₂₆	y₆₃₆	y₆₂₈	y_{CO2}
	ppm	‰	‰	‰		ppm	ppm	ppm	error
									ppm
1	400	0	0	0	1.01620	393.62	4.4077	1.6440	0.000
2	400	-8	0	0	1.01611	393.66	4.3660	1.6442	0.035
3	400	-35	0	0	1.01581	393.77	4.2484	1.6447	0.155
4	400	0	2	0	1.01621	393.62	4.4007	1.6473	-0.003
5	400	0	0	2	1.01621	393.62	4.4007	1.6440	-0.001
6	400	5.13	-39.82	-51.4	1.01605	393.68	4.4240	1.5788	0.060
7	400	0	0	0	1.01614	393.65	4.4010	1.6441	0.024

15

Table 6. Details of isotopologues of common atmospheric species.

Species	Stable isotopes	No. isotopocules Total (distinct)	No. independent quantities to specify isotopic composition	R_{sum}
CO ₂	¹² C, ¹³ C	18 (12)	4	$(1+^{13}\text{r}).(1+^{17}\text{r}+^{18}\text{r})^2$
	¹⁶ O, ¹⁷ O, ¹⁸ O			
CH ₄	¹² C, ¹³ C	32 (10)	3	$(1+^{13}\text{r}).(1+^2\text{r})^4$
	¹ H, ² H			
N ₂ O	¹⁴ N, ¹⁵ N	12 (12)	4	$(1+^{15}\text{r})^2.(1+^{17}\text{r}+^{18}\text{r})$
	¹⁶ O, ¹⁷ O, ¹⁸ O			
CO	¹² C, ¹³ C	6 (6)	4	$(1+^{13}\text{r}).(1+^{17}\text{r}+^{18}\text{r})$
	¹⁶ O, ¹⁷ O, ¹⁸ O			
H ₂ O	¹ H, ² H	12 (9)	4	$(1+^2\text{r})^2.(1+^{17}\text{r}+^{18}\text{r})$
	¹⁶ O, ¹⁷ O, ¹⁸ O			

Figures and Figure captions



5

Figure 1. Example of $\delta^{13}\text{C}$ dependence on CO_2 mole fraction for a Spectronus FTIR analyser. The measured data are fitted with a function of form of Eq. (15) with fitted parameters $\beta = -1227 \text{ ‰ ppm}$ and $\gamma = 0.0054 \text{ ‰ ppm}^{-1}$.

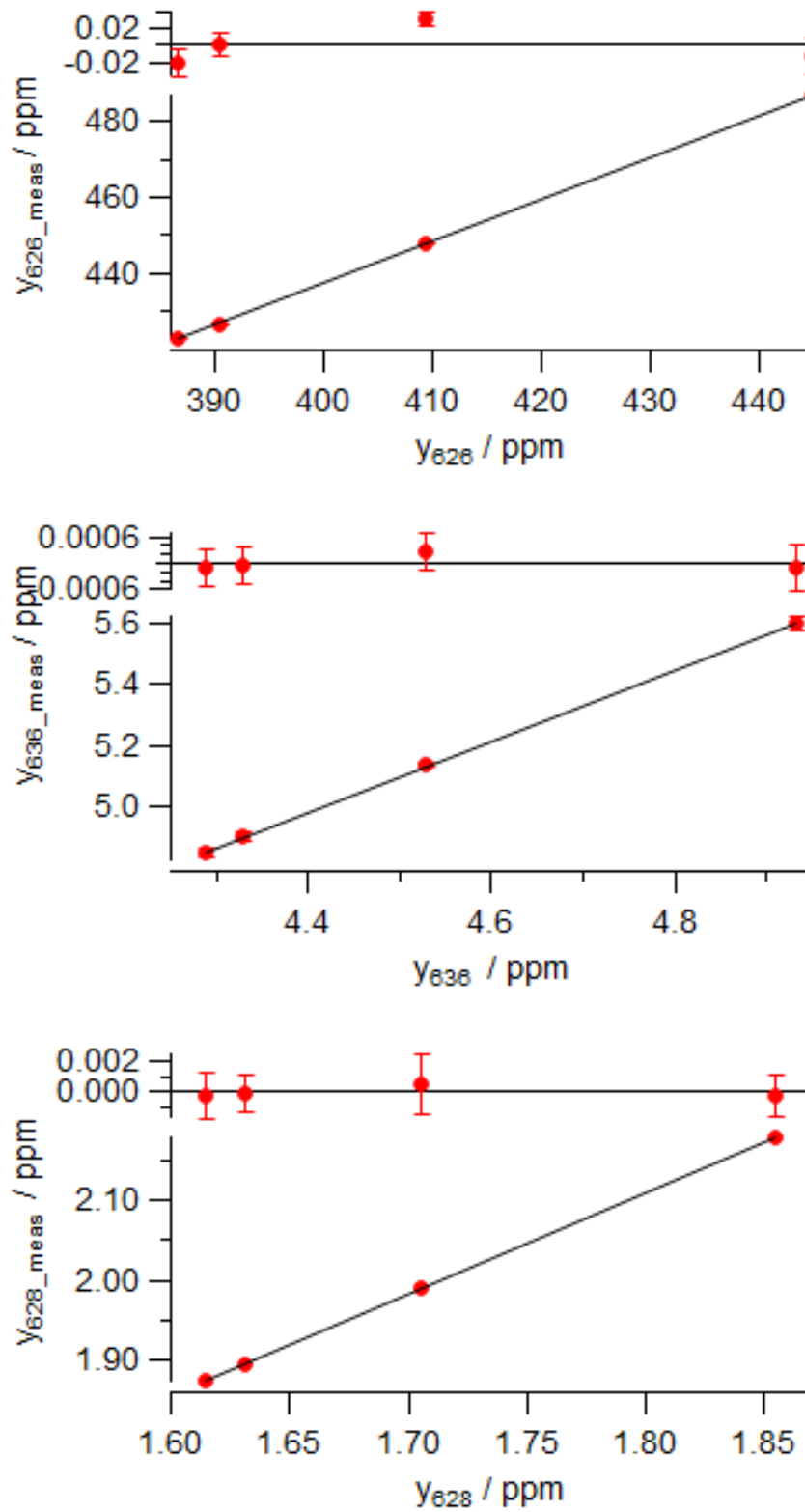
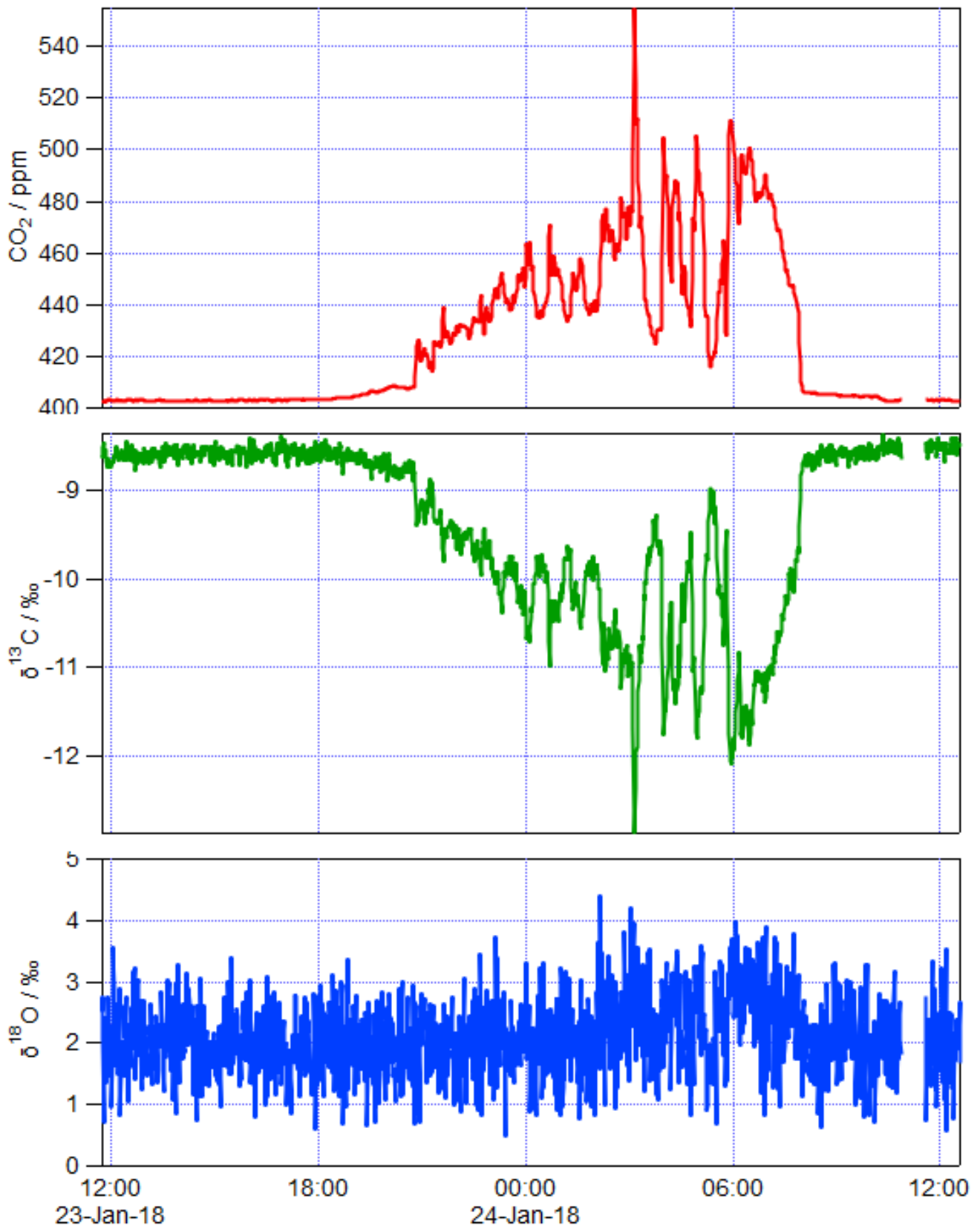
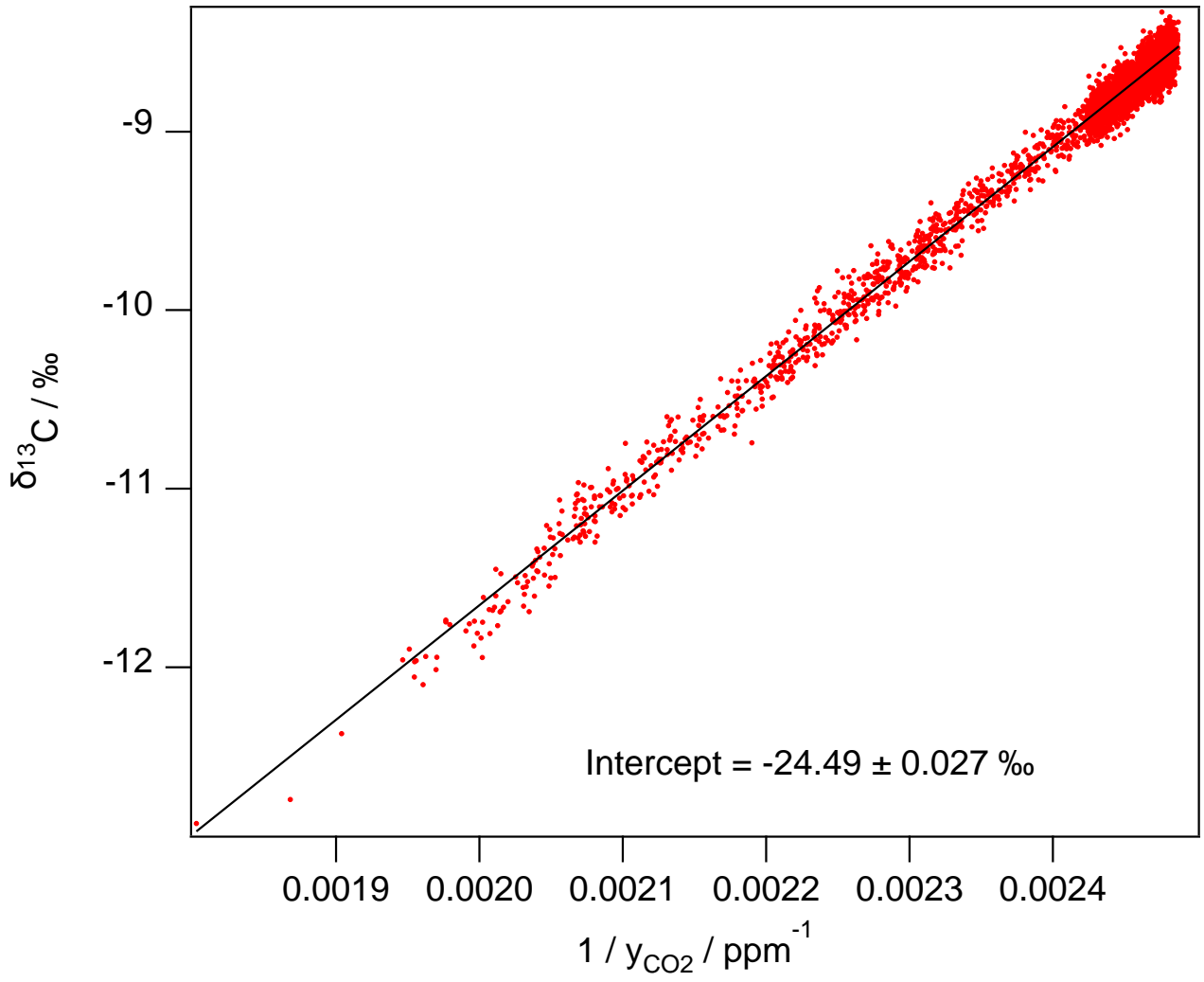


Figure 2. Calibration plots for three CO₂ isotopologues.



5

Figure 3. Calibrated total CO₂, δ¹³C and δ¹⁸O of sampled air on 23-24 Jan 2018 at a rural site in SE Australia. Air was sampled continuously, the displayed data are 1 minute averages.



5

Figure 4. Keeling plot of data shown in Figure 2.