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Preparation of Synthetic Isotope Mixtures for the calibration of carbon and oxygen isotope ratio measurements (in carbon dioxide) to the SI

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Abstract

Different sets of synthetic isotope mixtures for the calibration of carbon and oxygen ion current ratio measurements obtained by mass spectrometry have been prepared by mixing carbon dioxide isotopically enriched in ^{18}O ($^{\text{nat}}$C^{18}O$_2$) and natural carbon dioxide $(CO_2)_{\text{nat}}$, and by mixing different natural CO_2 gases with slightly different carbon and oxygen isotopic compositions. These mixtures act as Primary Standards to the SI for carbon and oxygen isotope amount ratio measurements in CO_2. They will help to anchor existing carbon Isotope Reference Samples (i.e., NBS19, IAEA-CO-9) and therefore offer the basis for comparability of carbon (and oxygen) isotope measurement results, without any assumptive correction for the oxygen isotopes.

Through such ‘absolute’ isotope amount ratio measurements of carbon and oxygen on CO_2 produced from the Primary Standard for carbon to the VPDB-scale, NBS19_{CO_2}, calibrated by means of synthetic isotope mixtures, ‘absolute’ isotope amount carbon and oxygen ratios for the zero point of the VPDB conventional scale were calculated to be $R_{13/12} = (111{,}376 \pm 16) \times 10^{-7}$ and $R_{18/16} = (208{,}824 \pm 48) \times 10^{-8}$, respectively. This approach makes these values traceable to the derived SI unit mol/mol.

Keywords: CO_2; Isotope mass spectrometry; Synthetic isotope mixture; Gravimetry

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1. Introduction

Carbon isotope amount ratios play a significant role in many fields such as climate research and prediction [1], authentication in food and drinks [2] and in non-intrusive medical diagnosis [3].

The classical measurement method is to convert the carbon-containing compounds into CO_2 gas, and compare the carbon isotope amount ratio in the unknown sample to that in a known reference by (ultra-)high precision gas mass spectrometry on CO_2. However, when comparing CO_2 isotope measurement results, difficulties arise from the fact that the quantities measured are ion current ratios $J_{i/44} = I[^{i}(CO_2)^+]/I[^{44}(CO_2)^+]$ with $i = 45$ and 46 resulting from both sample and reference sample (e.g., PDB [4]). From those ratio-of-ratios of electric currents, the *differences* in carbon ratio values $J_{13/12(\text{sample})}/J_{13/12(\text{PS})}$ are calculated and reported as $\delta(^{13}\text{C})_{\text{VPDB}}$ values in the unit ‘per mil’ (‰):

$$\delta(^{13}\text{C})_{\text{VPDB}} = \left[\frac{R_{13/12(\text{sample})}}{R_{13/12(\text{PS})}} - 1\right] 1000$$

Abbreviations: VPDB, Vienna Pee Dee Belemnite; NBS19, Primary Reference Material; PS, Primary Standard; $R_{13/12}$, absolute amount ratio ^{13}C/^{12}C; $R_{18/16}$, absolute amount ratio ^{18}O/^{16}O; $R_{17/16}$, absolute amount ratio ^{17}O/^{16}O; $J_{45/44}$, ion current ratio $I(^{45}CO_2)/I(^{44}CO_2)$; $J_{46/44}$, ion current ratio $I(^{46}CO_2)/I(^{44}CO_2)$; $J_{47/44}$, ion current ratio $I(^{47}CO_2)/I(^{44}CO_2)$; SI, international System of Units; K, correction factor for systematic unknown effects in the mass spectrometer; $n(CO_2)$, amount CO_2; $R_{45/44}$, amount ratio $n(^{45}CO_2)/n(^{44}CO_2)$; $R_{46/44}$, amount ratio $n(^{46}CO_2)/n(^{44}CO_2)$; $R_{47/44}$, amount ratio $n(^{47}CO_2)/n(^{44}CO_2)$

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whereby $R_{13/12(\text{sample})}/R_{13/12(\text{PS})}$ is supposed to be identical to $J_{13/12(\text{sample})}/J_{13/12(\text{PS})}$.

For samples and reference samples measured on the same instrument at the same time and place, the correction factors K ($R_{13/12} = K_{13/12}J_{13/12}$) in nominator and denominator drop out; but this may not be the case exactly as the quantity intended to be measured (R) is different from the quantity subject to measurement (J).

To establish the 'degree-of-equivalence' of $\delta(^{13}\text{C})$ and $\delta(^{18}\text{O})$ measurement results amongst different laboratories, a reference sample is required that is proven to be sufficiently homogeneous and stable, with an internationally agreed value in the conventional delta carbon scale (NBS19 or a material that is linked via NBS19 to the conventional carbon delta scale), and with an isotopic composition of the sample close to the one in the reference sample. However, incomplete correction of measurement results obtained and making use of other isotope reference samples in the linking process of the delta measurement results to the conventional scale, currently prevent the establishment of the degree-of-equivalence of delta measurement results (between different laboratories) to be better than 0.01‰.[1]

And there are some more problems/limitations:

- For a correct realization to the VPDB-scale, the carbon isotope amount ratio of each Secondary Isotope Reference Sample used, should be linked to that of NBS19; however, for the currently available isotope reference samples (oil, PE foil, sugars or other organic materials) it is difficult to trace-back potential systematic isotope effects in the employed chemical conversion techniques to CO_2 [5–8], required for the measurement.
- The $\delta(^{13}\text{C})_{\text{VPDB}}$ value of NBS19 is assigned [6,8] to be +1.95‰ exactly (without any measurement uncertainty as is inherent to the concept of 'conventional scale').

  However, like any other material, the very use of NBS19 is also subject to measurement uncertainties including contributions from possible lack of homogeneity and long-term stability. Furthermore the material (limestone) needs to be treated with phosphoric acid to release CO_2 [5–7]; this conversion step introduces an uncertainty component (however good isotope labs are aware of this and report the measurement uncertainty of NBS19), which has to be entered into the measurement uncertainty budget when comparing carbon isotope measurements in CO_2 from NBS19 and in CO_2 obtained via other conversion techniques [8].
- Carbon isotope amount ratio measurements are usually performed with highest precision when using gas mass spectrometers with a dual inlet system; although all laboratories involved in an inter-laboratory comparison use very similar methods [8], the way in which the mass spectrometers are built (to measure ratio of ratios of ion currents) could prevent the detection of possible (small) systematic errors in the procedure, however the largest problems arise when such results are compared with results obtained by means of different techniques (C-IRMS, FTIR, Laser-ICPMS).
- Different ion correction algorithms [4,9–12] are currently used to calculate carbon isotope amount ratios $R_{13/12}$ from measured ion current ratios $J_{i/44}$ (with $i = 45$ and 46) on CO_2. Since $J_{45/44}$ is actually an ion current ratio, a correction must be applied for the ^{17}O contribution. The correction term to be subtracted from the ion current measured at mass 45 is equal to twice the ion current ratio $J_{17/16}$, assuming a complete stochastic isotope distribution.

  $$J_{13/12} = J_{45/44} - 2J_{17/16} \tag{1}$$

  $$J_{46/44} = 2J_{18/16} + \left(J_{17/16}\right)^2 + 2J_{13/12}J_{17/16} \tag{2}$$

  Assuming mass-dependent-fractionation processes, which is nearly valid for all *environmental and geochemical* (natural) applications, it can be safely assumed that ^{17}O variations are following the ^{18}O variations according to an approximate (mass-dependent) relation [9,10]: $\delta^{17}\text{O} \approx a\delta^{18}\text{O}$, which could be considered as a third equation, or expressed in an exponential relationship $J_{17S}/J_{17R} = (J_{18S}/J_{18R})^{0.5}$ and, therefore,

  $$J_{17S} = \frac{J_{17R}}{[J_{18R}]^{0.5}}[J_{18S}]^{0.5} = k[J_{18S}]^{0.5} \tag{3}$$

  The (four) algorithms commonly used [9–13], are not exactly identical. As a consequence artificial biases might appear in the comparison of carbon isotope amount ratios when applying these algorithms.

Meeting the above mentioned constraints, and at the same time guaranteeing long term metrological comparability of measured $\delta(^{13}\text{C})_{\text{VPDB}}$ values, can be achieved through calibrated measurements of the value embodied in the Reference Sample (NBS19) and of any future successors, by using Primary Standards (PS), in the metrological meaning of the term and as defined in the VIM which [35]. Such PS are 'realizations' of the SI units involved (in this specific case of the derived unit mol/mol) in the form of 'synthesized' isotope amount ratios with small full measurement uncertainties [14]. Such PS however will never be used as PRM, because the specific goal of the PS is to calibrate a particular instrument. If the values for the isotope amount ratios in these PS are shown to be traceable to the SI, metrological traceability of measurement results expressed on a conventional $\delta(^{13}\text{C})_{\text{VPDB}}$ can be established.

In this work a procedure is described to prepare ('synthesize') SI-traceable carbon and oxygen isotope amount ratios in CO_2. Following a gravimetric approach, a total of seven synthetic isotope mixtures have been prepared from CO_2 gases enriched in ^{18}O and with ^{13}C close to natural abundances. The ion current ratios $J_{i/44}$ (with $i = 45$–47) generated from the synthetic isotope mixtures, the starting materials, from CO_2 from NBS19 and from a high purity CO_2 gas from Air Liquide. In this way we wanted to obtain SI-traceable carbon and oxygen isotope amount ratios, enabling to calibrate measurements of the international carbon reference sample (NBS19) and thus establishing a direct link between the common 'VPDB-traceable' results and the SI.

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[1] W. Brand, personal communication (2007).

2. Gravimetrically prepared isotope mixtures: synthesizing isotope amount ratios

2.1. General considerations

When high purity CO_2 is weighed on a balance, the chemical amount n_{CO_2} is given by:

$$n_{CO_2} = \frac{m_{CO_2}(1-\varepsilon_{imp})}{M_{CO_2}} \tag{4}$$

where ε_{imp} is a small correction term for the (mass of) chemical impurities in the CO_2 gas. A stoichiometry term for gases (contrary to solids) can be neglected. As n_{CO_2} is not a function of other amounts (but only a function of molar mass M_{CO_2} and mass m_{CO_2}), this procedure is a '*primary measurement procedure*' [35], as the result is obtained by other means than the measurements for which it is intended to act as a calibrator. These methods are perhaps not the fastest, or not even the most convenient or flexible, but they have two important features which make them metrologically superior: the metrological traceability of the measurement results produced is visibly traceable to the SI measurement units (mol/mol), and they yield values with (very) small total combined uncertainties.

Gravimetry and the ensuring mass ratio is mostly used to produce mixtures of isotopes with isotope amount ratios having small combined uncertainties [15–19]. Materials of high isotope enrichment are used and their isotope ratio in a mixture X of such materials (A and B) is given by Eq. (5):

$$\frac{n(^iE,X)}{n(^jE,X)} = \frac{n(^iE,A)+n(^iE,B)}{n(^jE,A)+n(^jE,B)} = \frac{f_{i,A}n(E,A)+f_{i,B}n(E,B)}{f_{j,A}n(E,A)+f_{j,B}n(E,B)} \tag{5}$$

where f denotes the isotope abundance, for isotope ^iE, ^jE in sample A, e.g., $f_{i,A} = R_{i,A}/\Sigma R_{i,A}$. The amounts n can be expressed using Eq. (4). The starting materials A and B must be of high chemical purity, and the order of magnitude of the impurities known (as ε_{imp}).

For CO_2 mixtures, the imperfection prior to mixing is then carefully assessed: purity of the gas, isotope enrichment of the carbon and the oxygen, and – very important – isotopic equilibrium [20,21] in all materials. The systematic effects are measured, their uncertainties evaluated, and then combined with the uncertainty of the mass measurements (weighings). Depending on the target measurement uncertainty and on the amounts of the materials available, mixtures can be prepared to various final measurement uncertainties from various starting materials.

2.2. Theoretical considerations on the preparation of mixtures

The preparation of synthetic isotope mixtures in gaseous form is more difficult to achieve than when mixing solids. The full uncertainty is limited by the typically high tare mass of the gas ampoule (∼350 g) relative to the mass of gas (∼2 g). To decrease the measurement uncertainty of the gas amount, the amount of gas needs to be increased. High pressure however could increase the measurement uncertainties due to almost inevitable small leaks and potential flow disturbances and could make the handling of the high pressure containers unsafe, especially when heating is required to attain isotope equilibrium at $T = 500\,°C$ [20]. Furthermore, large amounts of isotopically enriched gas are expensive. It is concluded that the tare mass of the ampoules should be reduced as much as possible.

When targeting small total combined uncertainties by working at atmospheric pressure, volumetric mixing can be performed based on measurements of the pressure and of the volume of the parent gases for the mixture preparation. In volumetric mixing procedures, the amount of gas n is related to the volume V of the mixing vessel, the pressure p and the temperature T in the vessel following the equation $pV = nRT$, with R being the universal gas constant [22]. The behaviour of real gases however is better described by the empirical 'van der Waals'-equation (Eq. (6)) [23]:

$$\left(p + \frac{an^2}{V^2}\right)(V - nb) = nRT \tag{6}$$

The (an^2/V^2) term accounts for the attractive forces of the molecules and are responsible for a pressure increase, while the b-term accounts for repulsive forces which in turn decreases the volume available for the molecules. At the critical point of CO_2 ($T_c = 304.13\,K$) the terms a and b are related to the critical volume V_c ($0.094118\,m^3\,kmol^{-1}$) and pressure p_c (7.3773 MPa) as follows [22]: $V_c = 3b$ and $p_c = a/27b^2$. This results in a value for $a_{CO_2} = 0.1960\,Pa\,m^6\,mol^{-2}$ and for $b_{CO_2} = (3.1373) \times 10^{-5}\,m^3\,mol^{-1}$. When mixing gases at a pressure of 10^6 Pa (10 bar), this would result in a correction of 3.9×10^4 Pa (or 3.9% relative) of the pressure compared to the calculation via the ideal gas law ($(an^2/V^2) = nb = 0$) or the 'van der Waals' law reduced to the ideal gas law.

3. Preparation of the synthetic isotope mixtures

3.1. The starting materials

The starting materials used for the preparation of the mixtures are $^{nat}C^{18}O_2$ and $(CO_2)_{nat}$. The enriched gas was supplied by Chemotrade (Düsseldorf, D), with specifications as follows: '$C^{18}O_2$': "min 53.7 at.% ^{18}O", "Lot no. 498-285-652-C", initial amount: 22.28 L. The natural CO_2 gases are supplied by BOC Special Gases (N5.0), Guilford (UK) and by Air Liquide (N5.0), Liège (B) and Messer (N5.0), Krefeld (D).

An impurity check (including measurements of isobars at $m/e = 44–47$ by high resolution MS at $R = 8000$) on all these starting materials was performed before starting the experimental work (by means of the MAT 271 gas mass spectrometer). When the impurities are added together, their amount content was below 0.0001% (g/g) for all parent gases, which make their impact on the mixture calculations extremely small, but the effect was taken into account. The isobaric effects could be neglected.

Table 1
The CO_2 gases and their mass values used for the preparation of the four NPL mixtures

Cylinder ID	Contents	Mass of the $C^{18}O_2$ spike (g)	Mass of the $(CO_2)_{nat}$ (g)	Notes
#1	$C^{18}O_2$	3.11	0	Pure
#1B	$(CO_2)_{nat}$	0	>400	Pure
#6	$C^{18}O_2/(CO_2)_{nat}$	2.89004 (29)	20.38734 (78)	NPL CL10
#7	$C^{18}O_2/(CO_2)_{nat}$	2.88148 (29)	18.96005 (76)	NPL CL23
#8	$C^{18}O_2/(CO_2)_{nat}$	2.88115 (29)	21.80696 (76)	NPL CL24
#9	$C^{18}O_2/(CO_2)_{nat}$	2.89729 (29)	23.32092 (76)	NPL CL56

The stated uncertainties are total combined measurement uncertainties $U = ku_c$ ($k = 1$), calculated according to the GUM [14] guidelines.

3.2. Gravimetric mixing procedure

3.2.1. NPL mixtures

Two identical 500 mL stainless (electropolished) steel cylinders (Swagelok®) with identical valves (SS-4BK-Swagelok) were used for the weighing [24,25]. One cylinder was filled with air at atmospheric pressure and designated as the '*tare*' while the other was used for preparing the CO_2 mixture. The mixture cylinders were attached to a 1/4 in. stainless steel vacuum line (Dockweiler, electropolished), which was connected to a two-stage rotary fore-pump (pressure < 1 Pa) and a turbo-molecular pump (<10^{-5} Pa).

Before the first weighing cycle, the mixture cylinders were evacuated. Each cylinder was successively filled with natural CO_2 (BOC), then with $C^{18}O_2$ as for mixtures for the preparation of mixtures CL10, CL23, CL24 and CL56 (Table 1 and Fig. 1).

During each weighing cycle, each consisting of one filling of the cylinders with $(CO_2)_{nat}$ and one for $C^{18}O_2$, the differences in mass between the mixing cylinder and the tare cylinder were recorded five times using a single-pan mass comparator (Mettler-Toledo PR2004 with a capacity of 2.3 kg) following a conventional A-B-B-A substitution method [25]. The resolution of the Mettler balance was 0.1 mg with a weighing repeatability below 0.2 mg. The masses of both natural CO_2 and enriched $C^{18}O_2$ were calculated from results of the successive weighing cycles. The total combined GUM uncertainty ($U = ku_c$, with $k = 1$) for each weighing was calculated to be 0.5 mg. For the mixture preparation, the masses of the natural CO_2 gas were in the range of 19–23 g and of the enriched gas $C^{18}O_2$ added in the range of 3 g.

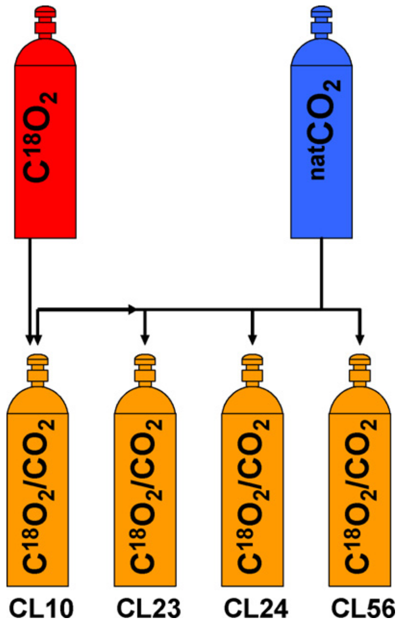


Fig. 1. A schematic presentation for the preparation of the 4 NPL mixtures CL10, CL23, CL24 and CL56, the amounts of which are given in Table 1.

3.2.2. IRMM mixtures

Besides the four NPL CO_2 mixtures, 3 more gravimetrical isotope mixtures were prepared at IRMM, in a similar way as at NPL [25].

For Mixture IRMM-1105-1 (Table 2), two NPL mixtures – CL24 (CO_2 enriched in ^{18}O, Table 1) and an enriched NPL $^{13}CO_2$ gas, CL01 (CO_2 enriched in ^{13}C but with natural oxygen isotopic composition) – were mixed again (Table 2 and Fig. 2). The benefit of such a mixture is that the ion current ratio $J_{47/44}$ on both starting materials (CL01 and CL24) can be measured with a measurement uncertainty of the same order as can be obtained on $J_{45/44}$ and $J_{46/44}$ (<10^{-4} relative). This is very important, as in solving the cubic equation (Eq. (9)), the mass uncertainty of $R_{45/44}$, $R_{46/44}$ and $R_{47/44}$ is equally distributed over the calculated carbon and the oxygen isotope amount ratios $R_{13/12}$, $R_{17/16}$ and $R_{18/16}$ for the parents and mixtures.

Mixture IRMM-1205-2 and IRMM-1205-3 (Table 3, Figs. 3 and 4) were prepared by mixing natural CO_2 (N5.0, Air Liquide) with slightly depleted carbon $\delta(^{13}C)_{VPDB} = -10‰$ and $-20‰$, respectively, in order to obtain mixtures which closely approach the natural carbon and oxygen isotopic composition of the unknown samples.

Table 2
Isotope mixture (IRMM Mix 1105-1) prepared at IRMM, by mixing NPL-CL01 ($^{13}CO_2$ enriched in ^{13}C and mixed in natural CO_2) and NPL-CL24 ($C^{18}O_2$ enriched in ^{18}O, mixed in natural CO_2) with their amounts used for the preparation of the mixture

Cylinder ID	#M1-11
Contents	$C^{18}O_2/^{13}CO_2$
Mass of CL01 (g)	0.34839 (25)
Mass of CL24 (g)	0.04042 (26)
Notes	IRMM Mix 1105-1

The stated uncertainties are combined measurement uncertainties $U = ku_c$ ($k = 1$), calculated according to the GUM [14] guidelines.

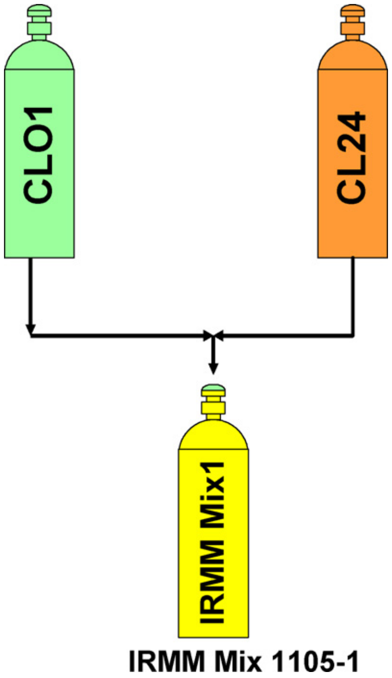


Fig. 2. Isotope mixture (IRMM Mix 1105-1) prepared at IRMM, by mixing NPL-CL01 ($^{13}CO_2$ enriched in ^{13}C which was mixed in natural CO_2) and NPL-CL24 ($C^{18}O_2$ enriched in ^{18}O, mixed in natural CO_2). The amounts of CO_2 used for MIX 1105-1 are given in Table 2.

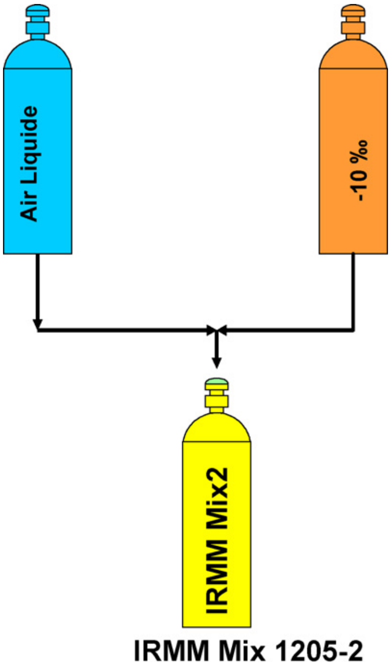


Fig. 3. Isotope mixture (IRMM Mix 1205-2) prepared at IRMM, by mixing natural CO_2 (N5.0, Air Liquide) with slightly depleted carbon ^{13}C, $\delta(^{13}C)_{VPDB} = -10‰$, in order to have a mixture which closely approach the carbon and oxygen isotopic composition of the unknown samples in the entire calibration process (Table 3).

Prior to the preparation of the IRMM mixtures, all mixing containers were first evacuated to a pressure of $<10^{-6}$ Pa and carefully checked for complete leak-tightness, by measuring the ion current ratio $I[^{28}(N_2)^+]/I[^{28}(N_2)^+]_{baseline}$. A steady ratio indicates the absence of leaks (or other effects such as adsorption and desorption) which would bias the results. Measurements have been done on an empty ampoule (internal surface ± 100 cm^2) which has been closed after it was connected to the pump. The residual gas spectrum was directly measured on the mass spectrometer: no intensity at $m/e = 44$ could be measured while the stability of the ion current at $m/e = 28$ was ≈1 fA.

All weighings were performed on a Mettler Toledo AX504 balance. It is specified to have a maximum load of 510 g, readability and repeatabilities of 0.1 mg, respectively, and a linearity of ±0.4 mg. The gas cylinders (50 mL, stainless steel) used for the preparation of these mixtures weighed approximately 380 g including the valve, whereas the mass of the gases varied from 0.35 to 3.8 g. The high tare of the gas ampoule thus limited the uncertainty of the weighings of the gases. Particularly with regard to moisture adsorbed on the outer ampoule surface, good repeatability conditions were not easy to realize. To minimise weighing errors, all weighings were performed relative to a reference container with similar shape and surface as the sample container. Both containers were exposed to exactly the same conditions prior to weighing, i.e., after cleaning the outer surface with ethanol (to remove any contamination, e.g., fingerprints) the containers were dried at 350 K for about 12 h and then placed next to the balance to equilibrate with ambient conditions. Already after about 3 h of conditioning in the weighing room, the mass of the container and reference container was constant within the readability of the balance (0.1 mg), whereas the repeatability of weighings of a single ampoule was of the same order. The handling of the ampoule (e.g., connecting and disconnecting from the mass spectrometer) could influence its tare weight. No small weight loss of the container however could be observed (abrasion of small metal pieces during screwing

Table 3
Isotope mixtures IRMM Mix 1205-2 and IRMM Mix 1205-3 prepared at IRMM, by mixing ^{13}C depleted CO_2 (respectively −10 and −20 δ‰ vs. VPDB) with natural CO_2

Cylinder ID	Contents	Mass of the spike (g)	Mass of the $^{nat}CO_2$ (g) Air Liquide	Notes
#IM-A	Slightly depleted CO_2 $\delta(^{13}C)_{VPDB} = -10‰$	23.12	–	Pure
#IM-B	Slightly depleted CO_2 $\delta(^{13}C)_{VPDB} = -20‰$	22.16	–	Pure
AL/N5.0	Natural CO_2	0	>3000 g	Pure
#IRMM-121	CO_2 $\delta(^{13}C)_{VPDB} = -10‰$ in $(CO_2)_{nat}$	0.3546 (3)	3.6602 (3)	IRMM Mix 1205-2
#IRMM-122	CO_2 $\delta(^{13}C)_{VPDB} = -20‰$ in $(CO_2)_{nat}$	2.0861 (3)	3.8170 (3)	IRMM Mix 1205-3

Their amounts used for the preparation are given with combined measurement uncertainties $U = ku_c$ ($k = 1$), evaluated according to the GUM [14] guidelines.

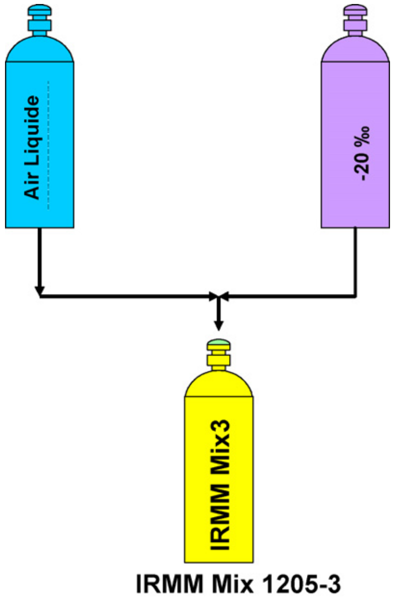


Fig. 4. Isotopic mixture (IRMM Mix 1205-3) prepared at IRMM, by mixing natural CO_2 (N5.0, Air Liquide) with slightly depleted carbon ^{13}C, carbon ^{13}C, $\delta(^{13}\mathrm{C})_{\mathrm{VPDB}} = -20‰$, in order to have a mixture which closely approach the carbon and oxygen isotopic composition of the unknown samples in the entire calibration process (Table 3).

were an inherent possibility; its uncertainty was estimated to be 0.2 mg).

3.3. The procedure for measuring the mixtures and the parent gases

The isotope amount ratio measurements were performed on IRMM's Avogadro II amount comparator [15,26–30], by measuring the ion current ratios $J_{i/44} = I[^{i}(\mathrm{CO}_2)^+]/I[^{44}(\mathrm{CO}_2)^+]$. The measurements were monitored sequentially using one Faraday cup and one SEM/Ion detector positioned for m/z = 44–47. Short term fluctuations were eliminated by symmetrically scanning [28,29] the four peaks. In the ideal case the measured ion current ratios J could be converted directly into isotope amount ratios R_{true}. The correction factors $K = R_{i/44}/J_{i/44}$ were expected to be unity (Eq. (7)), with $R_{i/44}$ being derived from the preparation of a synthetic mixture. Each measurement took about 2 h, and mean ion current ratios of the 5 successive measurements were obtained, with their associated standard measurement deviations (1 s).

The procedure used is very important when producing synthetic isotope mixtures. It must be reliable and transparent. Thus, different sources of measurement uncertainties can be easily identified in a step-by-step breakdown and, as a result, isotope amount ratios can be obtained with known and possibly smallest combined uncertainties.

Such an approach leads to the selection of particular types of methods and instrumentation which are different from what researchers use in 'differential' measurements. This is not surprising as the objectives in both approaches are different.

For absolute isotope amount ratio measurements the aim however is to 'maximise' biases in order to use them in their data treatment. In a generic way, this can be described by:

$$R_{\mathrm{true}} = \frac{n(^{i}E)}{n(^{j}E)} = J_{i,j}K = \frac{I(^{i}E^{+})}{I(^{j}E^{+})}K = \frac{I(^{i}E^{+})}{I(^{j}E^{+})}K_1K_2\cdots K_Z \quad (7)$$

The equation is an elaboration of Eq. (5). Firstly, it clarifies that the ratio of amounts (of isotopes) is not directly accessible, but only via measurements of ratios of ion currents. Secondly, the component by component investigation of possible systematic effects will result in a series of different K factors (Eq. (7)).

When aiming at highest metrological quality, gas source mass spectrometry is highly desirable because of its very good repeatability of the results. Additionally, the use of molecular flow sample gas inlet will result in a predictable mass discrimination, at the point of effusion of the gas in the spectrometer source, a property which has been exploited to the maximum in the re-determination of the Avogadro constant [15,19]. Applying this procedure, it is possible to identify and to quantify most of the K factors in Eq. (7), and just leaving small 'residual' factor K which can be determined by means of the prepared synthetic isotope mixtures.

Prior to starting the measurements however, complete equilibrium of the carbon and oxygen isotopes in CO_2 molecule amongst its 12 different isotopologues is a basic requirement for the calculation of the correct carbon and oxygen isotope amount ratios from measurements of the ion current ratios $J_{i/44} = I[^{i}(\mathrm{CO}_2)^+]/I[^{44}(\mathrm{CO}_2)^+]$ with i = 45–47. The degree, to which isotope distribution is achieved when mixing CO_2 molecules of different isotopic composition, is of key importance when measuring these ion current ratios on the mass spectrometer [20]. The gas mass spectrometric measurement procedure applied here and developed over the years in the re-determination of the Avogadro constant [15,26–30], creates the opportunities to do this. They are not accessible in commercial 'delta'-machines. Monitoring isotope fractionation during the measurements (by continuously looking for compatibility of measurement results with applying kinetic gas theory), including a correction for adverse or uncontrolled fractionation, provided crucial information about the isotopic equilibrium status of the sample [20]. When a statistical carbon and oxygen isotope distribution is not achieved within the CO_2 sample (mixture) prior the ion current ratio measurement, progressive equilibration will take place during the measurement, due to the high operating temperatures in the spectrometer (inlet vessel, ion source, ionization filament). An incompletely equilibrated sample will affect the behaviour of the molecular gas flow in the mass spectrometer, resulting in erroneous $J_{i/44}$ measurement results. In our case the equilibrium must be complete, otherwise uncontrolled equilibration will take place in the mass spectrometer and this will lead to wrong results [20]. Full isotopic equilibrium will result in compatibility with kinetic gas theory. Hence, it is of crucial importance that full isotopic equilibrium is achieved prior to the measurements. An 'in-flight' tool to verify this did not exist previously. Subsequently, it was found out, that it was even useful to quantify the degree of statistical isotope distribution between the carbon and oxygen isotopes in the gas

Table 4
Ion current ratios observed on the starting materials, the parent gases used for the preparation of the seven synthetic isotope mixtures

Materials	$J_{45/44}$	$J_{46/44}$	$J_{47/44}$
$C^{18}O_2$	0.559299 (21)	3.0179800 (50)	0.825768 (47)
Natural CO_2	0.01137143 (86)	0.0041752 (33)	0.000046850 (10)
CL01	0.1319122 (10)	0.01030932 (19)	0.00133822 (16)
CL24	0.0347982 (23)	0.1331486 (26)	0.00297582 (10)
Natural CO_2 Air Liquide	0.01172500 (51)	0.00412389 (64)	0.000046436 (11)
IM −10‰	0.01179256 (43)	0.0041599 (15)	0.000047351 (21)
IM −20‰	0.01158951 (21)	0.0040590 (11)	0.0000454981 (80)

Each measurement took about 2 h, and mean ion current ratios of the 5 successive measurements were obtained, with their associated standard deviations (1 s).

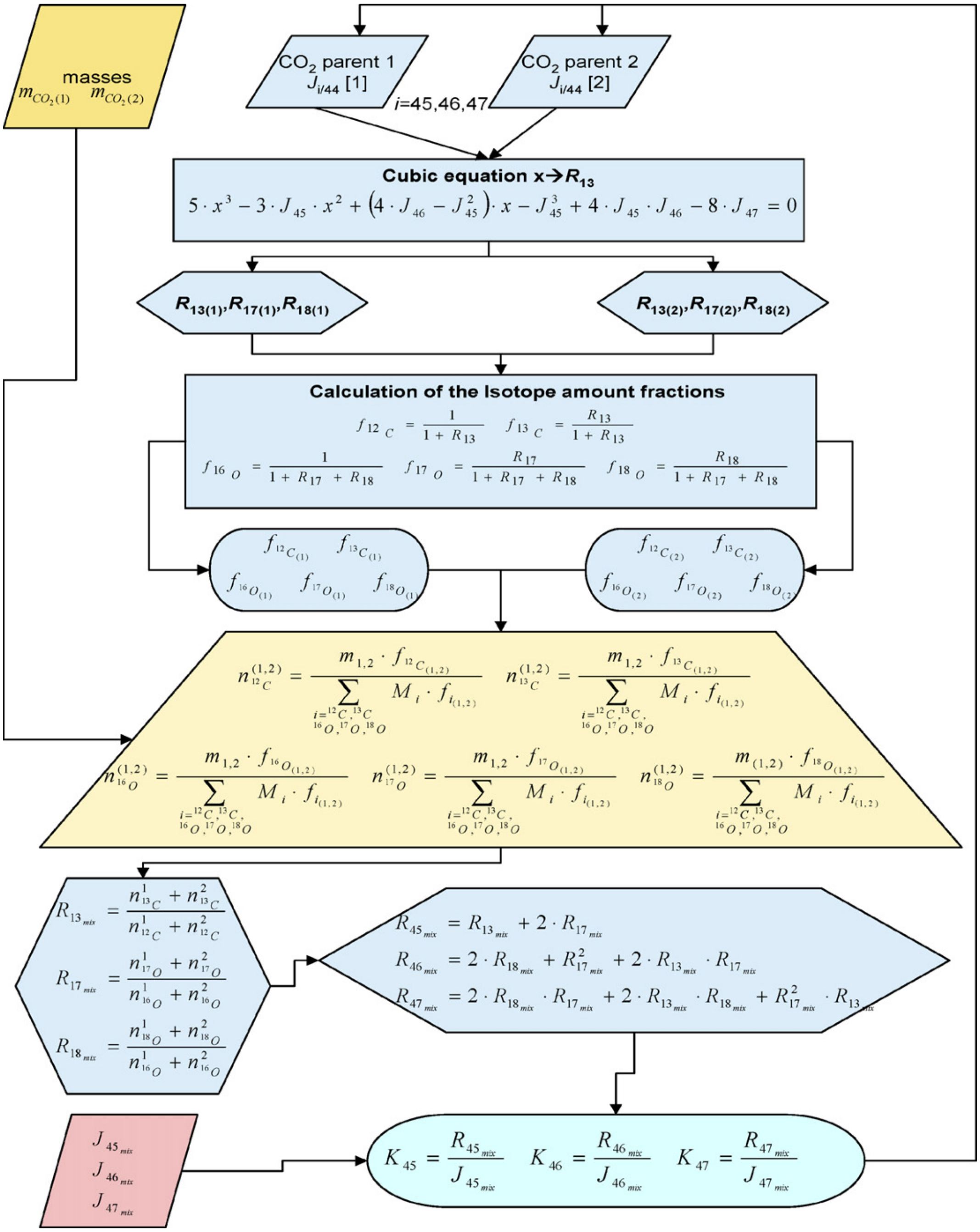


Fig. 5. The iterative procedure used to determine the absolute isotope amount ratios $R_{i/44}$ of all prepared synthetic isotope mixtures (exemplified for the mixtures CL in Table 1).

mixtures and in the starting materials [20], in the beginning of the measurement.

4. Results and discussion

4.1. Determination of the residual correction factors $K_{i/44}$ for conversion of $J_{i/44}$ to $R_{i/44}$ in CO_2 isotopic measurements

In order to determine the 'absolute' or 'true' isotope amount ratios $R_{i/44\,\mathrm{true}} = J_{i/44}K$ (with $i = 45$–47) of the seven isotope mixtures prepared (Tables 1–3), the absolute carbon and oxygen isotope amount ratios $R_{13/12}$, $R_{17/16}$ and $R_{18/16}$ of all starting materials (parent gases) need to be known. They are derived from the measured ion current ratios $J_{i/44}$ on the parent gases (Table 4). However to obtain the absolute values of the starting materials, the residual correction factors $K_{i/44}$, which are intended to be determined via the synthetic isotope mixtures, already need to be known. To achieve this, an iterative approach is chosen (Fig. 5).

For the initial step in the iterative process, the conversion factors $K_{i/44}$ were assumed to be one (exactly). Then, by comparing the measured ratios $J_{i/44}$ of the mixtures to the ones calculated (from the gravimetric mixing procedure), first estimates of $K_{i/44}$ were obtained. These preliminary correction factors in turn were applied to the ion current ratios $J_{i/44}$ of the starting materials (Table 4), and a new (slightly modified) set of $K_{i/44}$ values calculated. This iteration process was continued until no change in $K_{i/44}$ could be observed anymore. The iteration converged quickly and already after the fourth step the conversion factors stay constant (the variations on $K_{i/44}$ were below 0.01‰), and the iterative process could be stopped. The final correction factors $K_{i/44}$ obtained from the various mixtures are given in Tables 5–7, and visualised in Figs. 6–8.

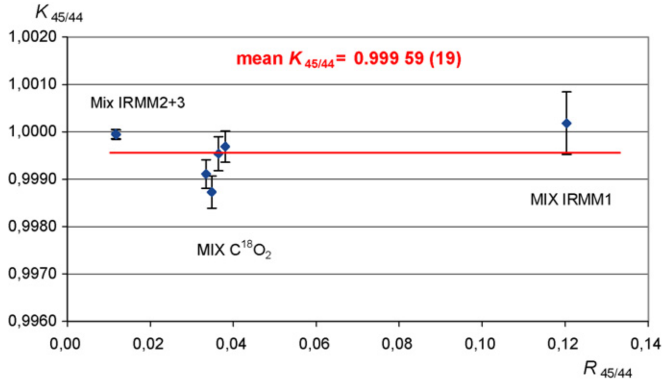


Fig. 6. The resulting conversion factors $K_{45/44} = R_{45/44}/J_{45/44}$ for the seven gravimetrically prepared isotope mixtures as calculated from the prepared isotope amount ratios $R_{45/44}$ and the measured ion current ratios $J_{45/44}$ ($U = ku_c$, $k = 1$).

The three average conversion factors for all seven gravimetrically prepared mixtures are close to unity: $K_{45/44} = 0.99959$ (19), $K_{46/44} = 0.99963$ (14) and $K_{47/44} = 0.99985$ (20), with repeatabilities stated as standard deviations of the mean. These experimental uncertainties are of the same order, in most cases

Table 5
Prepared isotope amount ratios $R_{45/44}$, measured ion current ratios $J_{45/44}$ and resulting conversion factors $K_{45/44} = R_{45/44}/J_{45/44}$ for the seven synthetic mixtures prepared gravimetrically from mixing enriched and natural CO_2, respectively

Synthetic mixtures	Prepared isotope amount ratio $R_{45/44}$	Measured ion current ratio $J_{45/44}$	Conversion factor $K_{45/44} = R_{45/44}/J_{45/44}$
CL10	0.036383 (12)	0.0364001 (30)	0.99953 (36)
CL23	0.038056 (11)	0.0380676 (18)	0.99970 (33)
CL24	0.034739 (11)	0.0347830 (23)	0.99874 (34)
CL56	0.033392 (10)	0.0334225 (13)	0.99909 (30)
MIX1	0.120344 (79)	0.1203225 (10)	1.00018 (66)
MIX2	0.0117315 (13)	0.01173227 (63)	0.99993 (10)
MIX3	0.0116776 (12)	0.01167821 (60)	0.999953 (96)
Mean $K_{45/44} = 0.99959$ (19)			

Uncertainties stated are evaluated according to GUM [14], values given in brackets are combined uncertainties u_c and apply to the last digits of the given value ($U = ku_c$, $k = 1$).

Table 6
Prepared isotope amount ratios $R_{46/44}$, measured ion current ratios $J_{46/44}$ and resulting conversion factors $K_{46/44} = R_{46/44}/J_{46/44}$ for the seven synthetic mixtures prepared gravimetrically from mixing enriched and natural CO_2, respectively

Synthetic mixtures	Prepared isotope amount ratio $R_{46/44}$	Measured ion current ratio $J_{46/44}$	Conversion factor $K_{46/44} = R_{46/44}/J_{46/44}$
CL10	0.141983 (27)	0.142021 (14)	0.99973 (16)
CL23	0.151455 (18)	0.1515554 (30)	0.99934 (12)
CL24	0.133047 (15)	0.1331482 (26)	0.99924 (11)
CL56	0.125658 (14)	0.1257588 (14)	0.99920 (11)
MIX1	0.022492 (52)	0.0224899 (80)	1.0001 (23)
MIX2	0.0041278 (14)	0.00412860 (98)	0.99981 (25)
MIX3	0.0041014 (12)	0.00410205 (11)	0.99984 (29)
Mean $K_{46/44} = 0.99963$ (14)			

Uncertainties stated are evaluated according to GUM [14], values given in brackets are combined standard uncertainties u_c and apply to the last digits of the given value ($U = ku_c$, $k = 1$).

Table 7
Prepared isotope amount ratios $R_{47/44}$, measured ion current ratios $J_{47/44}$ and resulting conversion factors $K_{47/44} = R_{47/44}/J_{47/44}$ for the seven synthetic mixtures prepared gravimetrically from mixing enriched and natural CO_2, respectively

Synthetic mixtures	Prepared isotope amount ratio $R_{47/44}$	Measured ion current ratio $J_{47/44}$	Conversion factor $K_{47/44} = R_{47/44}/J_{47/44}$
CL10	0.0032861 (32)	0.00328697 (63)	0.99974 (97)
CL23	0.0036211 (33)	0.00361998 (49)	1.00031 (91)
CL24	0.0029740 (24)	0.00297682 (10)	0.99905 (81)
CL56	0.0027253 (26)	0.00272660 (50)	0.99952 (94)
MIX1	0.0026235 (84)	0.00262168 (69)	1.0007 (32)
MIX2	0.000046519 (30)	0.000046523 (14)	0.99992 (56)
MIX3	0.000046117 (31)	0.000046131 (16)	0.99976 (57)
Mean $K_{47/44} = 0.99985$ (20)			

Uncertainties stated are evaluated according to GUM [14], values given in brackets are combined uncertainties u_c and apply to the last digits of the value ($U = ku_c$, $k = 1$).

even smaller, than the total combined uncertainties on the single mixtures as calculated from the mixing process, indicating that the uncertainty components (measurement of the isotope enrichment, weighings and impurities) were correctly taken into account.

Because of the small amount of gas which was available for the preparation of IRMM-Mix-1105-1, their weighing uncertainties become much more important than for the six other mixtures. Thus, relative combined uncertainties of 10^{-3} on the correction factors were obtained. For the other mixtures, these factors were about a factor of 10 better (10^{-4} rel.) (Figs. 6–8).

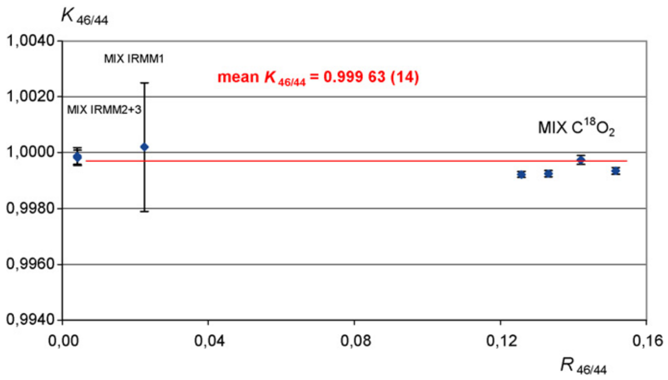


Fig. 7. The resulting conversion factors $K_{46/44} = R_{46/44}/J_{46/44}$ for the seven gravimetrically prepared isotope mixtures as calculated from the prepared isotope amount ratios $R_{46/44}$ and the measured ion current ratios $J_{46/44}$ ($U = ku_c$, $k = 1$).

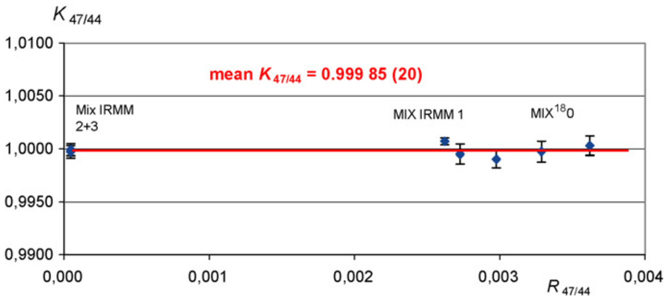


Fig. 8. The resulting conversion factors $K_{47/44} = R_{47/44}/J_{47/44}$ for the seven gravimetrically prepared isotope mixtures as calculated from the prepared isotope amount ratios $R_{47/44}$ and the measured ion current ratios $J_{47/44}$ ($U = ku_c$, $k = 1$).

The uncertainty contributions expressed in % for the different correction factors are given in Table 8 (exemplified for mixture CL10).

4.2. Absolute $R_{13/12}$, $R_{17/16}$ and $R_{18/16}$ values for NBS19

Via the conversion factors (Tables 5–7), the ion current ratios $J_{i/44}$ ($i = 45$–47) measured on NBS19-CO_2 could be converted into absolute isotope amount ratios $R_{13/12}$, $R_{17/16}$ and $R_{18/16}$, respectively, without any assumptive correction for oxygen (Eq. (8)). The results are presented in Table 9.

The uncertainties on $R_{13/12}$, $R_{17/16}$ and $R_{18/16}$ data (Tables 9 and 10) mainly stem from the measurement uncertainty of the ion current ratio $J_{47/44}$ measurements (10^{-3} rel.) on both gases. This uncertainty is entirely propagated to the amount ratio $R_{47/44}$ (Table 9), as the ratios $R_{13/12}$, $R_{17/16}$ and $R_{18/16}$ are

Table 8
Uncertainty contribution expressed in % to the different correction factors

Mixture CL10	$K_{45/44} = 0.99954$ (36), $uK_{45/44}$ (in %)	$K_{46/44} = 0.99973$ (16), $uK_{46/44}$ (in %)	$K_{47/44} = 0.99974$ (97), $uK_{47/44}$ (in %)
Starting material 1			
$J_{45/44}$–$(CO_2)_{nat}$	6.40	0.29	0.64
$J_{46/44}$–$(CO_2)_{nat}$	12.30	11.40	**25.79**
$J_{47/44}$–$(CO_2)_{nat}$	3.25	0.72	8.59
Starting material 2			
$J_{45/44}$–$C^{18}O_2$	6.99	1.55	8.57
$J_{46/44}$–$C^{18}O_2$	0.53	0.41	0.26
$J_{47/44}$–$C^{18}O_2$	22.00	0.45	**16.16**
Mixture			
$J_{45/44}$	**22.01**	<0.01	<0.01
$J_{46/44}$	<0.01	**37.14**	<0.01
$J_{47/44}$	<0.01	<0.01	**19.77**
Mass $(CO_2)_{nat}$	0.07	13.11	5.49
Mass $C^{18}O_2$	18.21	**35.02**	14.70
Impurities	<0.01	<0.01	<0.01
Nuclidic masses	<0.01	<0.01	<0.01

Uncertainties stated are evaluated according to GUM [14], values given in brackets are combined uncertainties u_c and apply to the last digits of the given value ($U = ku_c$, $k = 1$).

Table 9
Ion current ratios $J_{i/44}$ (with i = 45–47) measured on CO_2 gas obtained from: conversion of NBS19 carbonate via the H_3PO_4 procedure

	$NBS19\text{-}CO_2$
Measured ion current ratio	
$J_{45/44}$	0.01192449 (22)
$J_{46/44}$	0.00417748 (40)
$J_{47/44}$	0.000048097 (10)
Absolute amount ratio $R_{i/44} = KJ_{i/44}$	
$R_{45/44}$	0.0119196 (26)
$R_{46/44}$	0.00417593 (98)
$R_{47/44}$	0.000048090 (20)
Absolute amount ratio	
$R_{13/12}$	0.0111593 (16)
$R_{18/16}$	0.00208365 (48)
$R_{17/16}$	0.00038014 (48)

From the absolute $R_{i/44}$ the absolute carbon and oxygen isotope amount ratios $R_{13/12}, R_{17/16}$ and $R_{18/16}$ are calculated. Uncertainties stated are evaluated according to GUM [14], values given in brackets are combined uncertainties u_c and apply to the last digits of the given value ($U = ku_c, k = 1$). The uncertainty budgets are given in Tables 8 and 10.

calculated from the set of Eq. (8):

$$
\begin{aligned}
R_{45/44} &= R_{13/12} + 2R_{17/16}\\
R_{46/44} &= 2R_{18/16} + (R_{17/16})^2 + 2R_{13/12}R_{17/16}\\
R_{47/44} &= 2R_{13/12}R_{18/16} + 2R_{17/16}R_{18/16} + R_{13/12}(R_{17/16})^2
\end{aligned}
\tag{8}
$$

By re-arranging (Eq. (8)) to a cubic equation as a function of $R_{13/12}$ (Eq. (9)), besides $R_{13/12}$ also $R_{17/16}$ and $R_{18/16}$ can be calculated:

$$
\begin{aligned}
f(R_{13/12}) = {} & 5R_{13/12}^3 - 3R_{45/44}R_{13/12}^2\\
& + (4R_{46/44} - R_{45/44}^2)R_{13/12} - R_{45/44}^3\\
& + 4R_{45/44}R_{46/44} - 8R_{47/44}
\end{aligned}
\tag{9}
$$

Some reflections about the uncertainties of $R_{13/12}, R_{17/16}$ and $R_{18/16}$ need to be made, while they are biased by the isotope amount ratios $R_{45/44}$, $R_{46/44}$ and $R_{47/44}$. Each of their partial contribution to the final uncertainty of the ratios $R_{13/12}$, $R_{17/16}$ and $R_{18/16}$ can be calculated (Eq. (10)) with the help of the following matrix:

$$
\begin{Bmatrix} \delta R_{13/12}\\ \delta R_{17/16}\\ \delta R_{18/16} \end{Bmatrix}
=
\begin{pmatrix}
\left(\dfrac{\partial R_{13/12}}{\partial R_{45/44}}\right) & \left(\dfrac{\partial R_{13/12}}{\partial R_{46/44}}\right) & \left(\dfrac{\partial R_{13/12}}{\partial R_{47/44}}\right)\\
\left(\dfrac{\partial R_{17/16}}{\partial R_{45/44}}\right) & \left(\dfrac{\partial R_{17/16}}{\partial R_{46/44}}\right) & \left(\dfrac{\partial R_{17/16}}{\partial R_{47/44}}\right)\\
\left(\dfrac{\partial R_{18/16}}{\partial R_{45/44}}\right) & \left(\dfrac{\partial R_{18/16}}{\partial R_{46/44}}\right) & \left(\dfrac{\partial R_{18/16}}{\partial R_{47/44}}\right)
\end{pmatrix}
\times
\begin{Bmatrix} \delta R_{45/44}\\ \delta R_{46/44}\\ \delta R_{47/44} \end{Bmatrix}
\tag{10}
$$

In order to emphases the magnitude of these influences in the calculation of the isotope amount ratios from measured ion current ratios, such a calculation is exemplified below:

$$
\begin{Bmatrix} \delta R_{13/12}\\ \delta R_{17/16}\\ \delta R_{18/16} \end{Bmatrix}
=
\begin{pmatrix}
|-0.883| & |-5.250| & |464.748|\\
|0.941| & |2.625| & |-232.374|\\
|-0.0107| & |0.470| & |2.603|
\end{pmatrix}
\times
\begin{Bmatrix} \delta R_{45/44}\\ \delta R_{46/44}\\ \delta R_{47/44} \end{Bmatrix}
$$

This matrix (Eq. (10)) is however strongly "unbalanced", as the effect of the measurement uncertainty of $R_{47/44}$ to the isotope amount ratios $R_{13/12}$, $R_{17/16}$ and $R_{18/16}$ is much more dominant than of the other ratios $R_{45/44}$ and $R_{46/44}$, due to the small $R_{47/44}$ value in natural carbon dioxide samples. Although by applying Eq. (10) together with the repeatabilities obtained in Tables 9 and 10, the uncertainties of the isotope amount ratios $R_{13/12}$, $R_{17/16}$ and $R_{18/16}$ can be calculated.

$$
\delta R_k = \sqrt{\begin{aligned}&\left(\frac{\partial R_k}{\partial R_{45}}\right)^2_{R^m_{45}}(\delta R_{45})^2 + \left(\frac{\partial R_k}{\partial R_{46}}\right)^2_{R^m_{46}}(\delta R_{46})^2\\ &+ \left(\frac{\partial R_k}{\partial R_{47}}\right)^2_{R^m_{47}}(\delta R_{47})^2\end{aligned}}
\tag{11}
$$

Knowing the absolute carbon isotope amount ratio $R_{13/12\text{-NBS19}\,CO_2}$ (Table 9), and its assigned value relative to VPDB [6] or $\delta(^{13}C)_{NBS19\,CO_2\ \text{versus}\ VPDB\,CO_2}$, the absolute isotope amount ratio for PDB, $R_{13/12\text{-VPDB}\ \ CO2}$, can be calculated via Eq. (12):

$$
\delta(^{13}C)_{NBS19\,CO_2\ \text{versus}\ VPDB\,CO_2} = \left[\frac{R_{13/12\text{-NBS19}\,CO_2}}{R_{13/12\text{-VPDB}\,CO_2}} - 1\right]1000 = 1.95\ ‰
\tag{12}
$$

resulting in a value $R_{13/12\text{-VPDB}\,CO_2}$ = 0.0111376 (16). This carbon isotope amount ratio is lower than the value obtained by Craig [6] $R_{13/12\text{-VPDB}\,CO_2}$ = 0.011237 (30) or the value given by Chang and Chin [31] $R_{13/12\text{-VPDB}\,CO_2}$ = 0.0111949 (14), but higher than the isotope amount ratio obtained by Ruße et al. [32] which is $R_{13/12\text{-VPDB}\,CO_2}$ = 0.011101 (16) (Table 11).

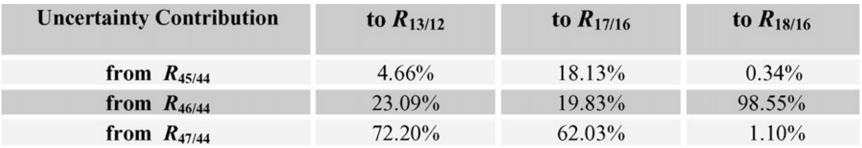
The $R_{13/12}$ value for VPDB-CO_2 given by Ruße [32], has been obtained by converting the ion current ratios

$$
J_{13/12} = \frac{I(^{13}CF_3)^+}{I(^{12}CF_3)^+}
$$

measured on NBS22, PEF1 and USGS24 after they were fluorinated via the F_2 route to CF_4 to isotope amount ratios $R_{13/12}$ by calibration against synthetic isotope mixtures of highly enriched $^iCF_4$ gases. This calibration procedure [32] is similar to the one used in this paper, although the chemical procedures and measurement techniques involved in both procedures are much different [33].

Via the isotope amount ratio $R_{18/16\text{-NBS19}\,CO_2}$ obtained on NBS19-CO_2 (Table 9), and its assigned value relative to VPDB-CO_2 [6] or $\delta(^{18}O)_{NBS19\,CO_2\ \text{versus}\ VPDB\,CO_2}$, the absolute isotope amount ratio for VPDB, $R_{18/16\text{-VPDB}\,CO_2}$, can be calculated via

Table 10
Uncertainty contribution from $R_{i/44}$ (with $i = 45, 46, 47$, expressed in %) to the amount ratios $R_{13/12}$, $R_{17/16}$ and $R_{18/16}$ applying Eqs. (8)–(10)



Uncertainty Contribution	to $R_{13/12}$	to $R_{17/16}$	to $R_{18/16}$
from $R_{45/44}$	4.66%	18.13%	0.34%
from $R_{46/44}$	23.09%	19.83%	98.55%
from $R_{47/44}$	72.20%	62.03%	1.10%

Table 11
The absolute carbon isotope amount ratio $R_{13/12\text{-VPDB}\,CO_2}$ *calculated* via NBS19-CO_2 (this paper) compared to all available literature values [32,6,31]

	Absolute carbon isotope amount ratio $R_{13/12\text{-VPDB}\,CO_2}$
This paper	0.0111376 (16)
Ruße et al. [32]	0.011101 (16)
Craig [6]	0.011237 (30)
Chang and Chin [31]	0.0111949 (14)

Values given in brackets are combined standard uncertainties u_c and apply to the last digits of the given value ($U = ku_c$, $k = 1$).

Eq. (13):

$$\delta(^{18}O)_{\text{NBS19}\,CO_2\ \text{versus VPDB}\,CO_2} = \left[\frac{R_{18/16\text{-NBS19}\,CO_2}}{R_{18/16\text{-VPDB}\,CO_2}} - 1\right]1000 = -2.20\ ‰ \qquad (13)$$

resulting in a $R_{18/16\text{-VPDB}\,CO_2} = 0.00208824$ (48). This value matches very well with the IAEA-recommended value [8,9] for $R_{18/16\text{-VPDB}\,CO_2} = 0.00208830$ (45).

Soon an IRMM-CO_2-RM [34] will be (commercially) available (via Messer, Germany) with absolute isotope amount ratios $R_{13/12}$, $R_{17/16}$ and $R_{18/16}$, and with $\delta(^{13}C)$ and $\delta(^{18}O)$ values linked via NBS19 to the conventional scale.

5. Conclusions

Seven gaseous isotope mixtures were prepared from isotopically enriched CO_2 and from natural CO_2 gas. The ratio values were 'synthesized' by preparing gravimetric gas mixtures and measuring them by an 'amount comparator' of proven linearity. These mixtures are realizations (embodiments) of the unit mol/mol used. They are therefore Primary Standard, suitable to calibrate carbon and oxygen ion current ratio measurements (e.g., by the "Avogadro measurement procedure") and lead to SI-traceable carbon *and* oxygen isotope amount ratio values, *independent* of any assumptive correction for the oxygen isotopes.

At the same time a start was made to realize long term metrological comparability of measured $\delta(^{13}C)_{\text{VPDB}}$ values in the conventional carbon scale by assigning SI-traceable isotope amount ratio values to the 'primary isotope reference sample' (NBS19) as well as to other carbon reference samples (NBS18, IAEA-CO-9, RM8562, etc.). Such measurements make it possible to reproduce the value on the $\delta(^{13}C)_{\text{VPDB}}$ scale by a completely independent measurement when the PS runs out of stock.

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