Isotope Dilution Mass Spectrometry for Highly Precise Determination of Dissolved Inorganic Carbon in Seawater Aiming at Climate Change Studies

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ABSTRACT: Dissolved inorganic carbon (DIC) is one of the most important parameters to be measured in seawaters for climate change studies. Its quantitative assessment requires analytical methodologies with overall uncertainties around 0.05% RSD for clear evaluation of temporal trends. Herein, two alternative Isotope Dilution Mass Spectrometry (IDMS) methodologies (on-line and species-specific) using an Isotope Ratio Mass Spectrometer (IRMS) and two calculation procedures for each methodology have been compared. As a result, a new method for the determination of DIC in seawaters, based on species-specific IDMS with Isotope Pattern Deconvolution calculation, was developed and validated. A ^{13}C-enriched bicarbonate tracer was added to the sample and, after equilibration and acidification, the isotope abundances at CO_2 masses 44, 45 and 46 were measured on an IRMS instrument. Notably, early spiking allows correcting for evaporations and/or adsorptions during sample preparation and storage and could be carried out immediately after sampling. Full uncertainty budgets were calculated taking into account all the factors involved in the determination (initial weighs, concentration and isotope abundances of standards and final IRMS measurements). Average DIC value obtained for CRM seawater agreed very well with the certified value. Propagated precision obtained ranged from 0.035 to 0.050% RSD for individual sample triplicates. Reproducibility, assessed by three independent experiments carried out in different working days, was excellent as well (-0.01% and 0.057%, error and full combined uncertainty, respectively). Additionally, the approach proposed improves on established methods by simplicity, higher throughput (15 min per sample) and lower volume requirements (10 mL).

Climate change studies, as a result of the anthropogenic CO_2 emissions, has gained a lot of momentum during the last few decades.[1,2] Around half of the CO_2 emitted after combustion of fossil fuels is located in the atmosphere while the rest is accumulated in the terrestrial biosphere and the oceans. The CO_2 concentration in the three compartments is closely related. Therefore, it is clear the need for high quality analytical strategies to assess and control the increase of the concentration of the dissolved inorganic carbon (DIC) in oceans as a result of the rising of the partial pressure of the atmospheric CO_2 (pCO_2).[3] Dissolved inorganic carbon (DIC) is defined as the sum of the concentrations of dissolved CO_2, carbonic acid, bicarbonate and carbonate. It is typically expressed as µmol C/kg of seawater, and ranges between 1800 and 2300 µmol C/kg in open seawaters. Since it has been estimated that the current acidification rate of oceans is about 1 µmol C/kg per year,[4] the trueness and precision required to monitor this small change is extremely demanding in analytical terms. Such requirement makes DIC determination one of the most challenging environmental analysis that the analytical chemist must face nowadays.

Very few analytical approaches have been proposed so far which fulfil such requirements. Most of them rely on the conversion of the different species into CO_2 after acidification and extraction into the gas phase, followed by highly precise and accurate measurement of the released CO_2. Nowadays, coulometric analysis is the technique of choice for DIC analysis, as it is able to provide routinely 0.05% RSD precision.[5] Limitations of this approach are the high sample volume requirements (0.3-1 L), the use of relatively toxic reagents (i.e. ethanolamine), and the need for a highly precise dispenser of the volume of water used. Additionally, the analysis protocol is quite complex which makes necessary a well-trained operator. Recently, a new coulometric system has been presented[6] able to provide comparable or even better precision while requiring greatly reduced operator intervention. A syringe pump equipped with a 12-port distribution valve is used to precisely dispense solutions into a gas stripper. In this alternative procedure, 23 mL of water samples are still necessary. Additionally, the instrumental set-up must be mounted and dismounted before and after sample analysis as it is necessary to prepare, clean and dry the coulometric cell and the humidity trap every day.

Other approaches rely on the use of non-dispersive infrared absorption (NDIR).[7,8] After acidifying and stripping, the released CO_2 is passed through a NDIR gas analyser. Accuracy is extremely dependent on the continuous correction of the sample analysis with a CRM at 2-min intervals. Precision attainable is as low as 0.05% RSD but critically depends on the constancy of the sample and carrier gas flows. Infrared measurements with cavity ring-down spectroscopy in combination with isotope dilution have been also tested.[9] Water sample and an isotopic ^{13}C-tracer ($NaH^{13}CO_3$) are mixed continuously and acidified. The $^{13}C/^{12}C$ ratio is measured on-line in the stripped CO_2 and used to compute DIC with precisions <0.09% RSD, limited by the ability to compute the sample to

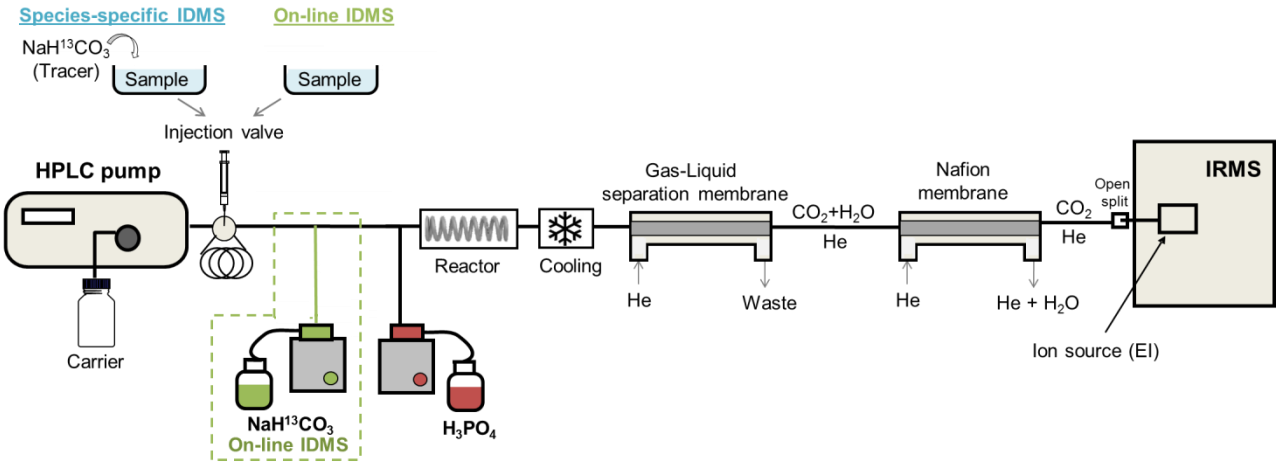

Figure 1. Alternative IDMS instrumental set-ups for on-line and species-specific IDMS procedures. Note on-line tracer flow (dotted line) is not used when performing species-specific IDMS.

isotopic tracer volumetric ratio. Such precision can be greatly enhanced by the use of deuterated water (D_2O) in the ^{13}C tracer solution, leading to a precision below 0.03%.[10] Although this is a very elegant solution, the use of simultaneous D and H isotope dilution makes the approach far more complex and expensive.

Mass spectrometry has been tested for DIC analysis in acidified seawater using a gas permeable membrane introduction mass spectrometer (MIMS) system.[11] The power of this approach is the possibility to use it as a portable sensor to build in-situ CO_2 profiles. Unfortunately, attainable precision is higher than 0.5% RSD, which limits significantly its application to climate change assessment. It is well known that precision can be enhanced significantly in mass spectrometry by the use of the isotope dilution concept.[12] In this context, we have recently developed a procedure for the universal detection and quantification of C-containing organic compounds in liquid phase based on on-line carbon isotope dilution mass spectrometry (IDMS). The sample is injected either into a constant carrier flow (FIA) or into a LC column and mixed on-line with a continuous flow of ^{13}C-tracer ($NaH^{13}CO_3$). On-line addition of phosphoric acid was employed to convert both DIC and tracer to $^{12}CO_2$ and $^{13}CO_2$, respectively, which were extracted from the aqueous phase using a gas-permeable membrane and brought to the regular quadrupole MS instrument.[13] Unfortunately, although precision obtained (>0.5% RSD) was adequate for the quantification of organic compounds, it did not meet the requirements to be applied to DIC measurements in seawater.

Herein, different alternative IDMS procedures using an Isotope Ratio Mass Spectrometer (IRMS) specifically designed for carbon isotope ratio measurement were tested for precise and accurate DIC measurements in seawater. Finally, a new IDMS-based method using species-specific approach and Isotope Pattern Deconvolution is proposed for high precision measurement of DIC (below 0.05% RSD) in seawater. This new procedure is simple to perform, requires low sample volumes (ca. 10 mL) and could be easily implemented in environmental laboratories focused on climate change studies.

EXPERIMENTAL SECTION

Reagents

Solid enriched $NaHCO_3$ (99% ^{13}C enrichment) was obtained as a high-purity chemical reagent (purity >98%) from Cambridge Isotope Laboratories (Andover, MA, U.S.A.). Solid phosphoric acid and solid natural pure $NaHCO_3$ were purchased from Sigma-Aldrich. Ultrapure water (18.2 MΩ cm) was obtained with a Milli-Q system (Millipore, Bedford, MA).

A certified reference material (CRM), consisting on a real seawater sample certified in dissolved inorganic carbon (2012.24 ± 0.28 µmol C/kg) was supplied by Andrew G. Dickson (Marine Physical Laboratory, Scripps Institution of Oceanography, University of California, San Diego, U.S.A.). It consists of natural seawater sterilized by a combination of filtration, ultra-violet radiation and addition of mercuric chloride.

Instrumentation

A Thermo Scientific (Bremen, Germany) LC−IRMS instrument consisting of an Accela 600 pump, an LC−Isolink interface and a Delta V Advantage sector field mass spectrometer was used. The LC-Isolink interface consists of two independent pumps for the acidification and oxidation reagents, a heated reactor, a cooler, a gas permeable membrane and a Nafion membrane for water removal, as shown in Figure 1.

Injection was made manually in a FIA system using a six-port valve and Milli-Q water as carrier. After acidification using 0.3 M of phosphoric acid, the CO_2 released was measured at masses 44, 45 and 46. For IDMS, the LC-IRMS was modified to attain equal cup amplification at masses 44 and 45, as described previously.[13] Such amplification can be selected by software so the original configuration of the instrument (100x amplification at mass 45) could still be used if required. Two instrumental set-ups (on-line IDMS and species-specific IDMS) were evaluated here and they are indicated in Figure 1. Injection volumes and flows used in each case are summarized in Table S-1 of the Supporting Information. For on-line IDMS, tracer was introduced prior the interface by means of a P-500 medium pressure piston pump (GE Healthcare, Chalfont St. Giles, U.K.) and a PEEK T-piece.

Procedures

Preparation of reagents, standards and samples

All reagent solutions and mobile phases were degassed under vacuum in an ultrasonic bath to eliminate the background

due to dissolved atmospheric CO_2. Carrier solutions, samples and standards were kept under Helium atmosphere in order to avoid contamination from atmospheric CO_2. The dissolution of solid standards was performed with degassed ultrapure water and was gently "stirred, not shaken" to prevent CO_2 incorporation from the atmosphere. Samples were injected directly without previous treatment, except when they were spiked with the tracer (species-specific IDMS).

Standard on-line double IDMS procedure

The instrumental set-up for this procedure is shown in Figure 1. The isotope ratio $^{12}C/^{13}C$ was measured as the signal ratio at masses 44/45 corresponding to the natural abundance $^{12}CO_2$ from the sample and the enriched $^{13}CO_2$, added on-line (dotted line, Figure 1), respectively. Thus, signal intensities at these m/z ratios were monitored continuously to build the isotope ratio FIAgram. Standard on-line isotope dilution equation was then applied to obtain the so-called "mass flow FIAgram".[14,15] Integration of the Gaussian peaks obtained in the mass flow FIAgram provided directly the amount of carbon eluted in each peak. A standard ($NaHCO_3$ of natural carbon isotope abundances and known concentration) was also analysed. In that way, peak areas ratio (analyte/standard) will be directly equal to the actual ratio of concentrations independently of the mass flow of spike and the loop size.[16] Thus, quantification of the amount of carbon under each peak can be determined just by calculating the areas of the standard and those corresponding to the samples in the mass flow FIAgram (see equation [S-6] in SI), after correcting for density differences between sample and standard.

On-line double IDMS procedure with flat-topped peaks

The instrumental set-up for this procedure is the same as for the standard on-line IDMS procedure (Figure 1) except for the use of a much larger injection loop of 800 µL. In this way, the isotope ratio measured at the top of the FIA peak is constant for a given time (flat-topped peaks) and the calculation of the mass flow FIAgram is not required anymore. Isotope ratios are computed as the average of the highly precise isotope ratios obtained in the stable apex of the flat-topped peaks corresponding both to the injection of the sample and the injection of a natural abundance standard (double IDMS). Precision is thus not limited anymore by the peak integration algorithm. Equation [1] provides directly the concentration of carbon C_s in the injected sample, being C_n the concentration of the $NaHCO_3$ natural abundance standard injected previously, d_n and d_s the densities of standard and sample, respectively, and R_{m1} and R_{m2} the isotope ratios $^{12}C/^{13}C$ measured for the sample and natural abundance standard, respectively. R_s and R_n are isotope ratio abundances in the sample and natural standard. A_s^{12} and A_n^{12} are isotope abundances of ^{12}C in the sample and standard, respectively. Finally, R_{t1} and R_{t2} correspond to the $^{12}C/^{13}C$ ratios measured in the background previous to the sample and standard peaks, respectively (tracer isotope ratios).

$$C_s = C_n \cdot \frac{d_n}{d_s} \cdot \frac{A_n^{12}}{A_s^{12}} \cdot \left(\frac{1 - R_{m2}R_n}{1 - R_{m1}R_s}\right) \cdot \left(\frac{R_{m1} - R_{t1}}{R_{m2} - R_{t2}}\right) \qquad [1]$$

If isotope abundances from the natural abundance standard and sample are considered identical, then equation [1] gets simpler:

$$C_s = C_n \cdot \frac{d_n}{d_s} \cdot \left(\frac{1 - R_{m2}R_n}{1 - R_{m1}R_n}\right) \cdot \left(\frac{R_{m1} - R_{t1}}{R_{m2} - R_{t2}}\right) \qquad [2]$$

Associated uncertainty can be then obtained directly from the propagation of the individual uncertainty of the factors involved in Eq. [1] and [2] using Kragten method.[17]

Species-specific double IDMS procedure

The instrumental set-up for this procedure is shown in Figure 1. In this case, the sample is spiked off-line with the isotopically enriched tracer and the mixture is injected in the FIA system. Under this configuration, the continuous flow of tracer is no longer required. We employed $NaH^{13}CO_3$ as the specific tracer for DIC. Seawater samples (ca. 10 mL) were spiked by weight with the corresponding amount of tracer in order to have an approximate isotope ratio $^{12}C/^{13}C$ of 1. A second mixture was performed in which a $NaHCO_3$ of natural abundance standard was also spiked with the tracer to perform a double IDMS experiment. This is the typical procedure used for elemental double IDMS analysis.[12]

Two alternative calculation procedures were evaluated. First, classical double IDMS equation[12] was employed using measured carbon isotope ratios ($^{12}C/^{13}C$) obtained from the signals measured at masses 44 and 45. The mathematical equation is as follows:

$$C_s = C_n \cdot \frac{m_n}{m_{t2}} \cdot \left(\frac{1 - R_{m2} \cdot R_n}{R_{m2} - R_t}\right) \cdot \frac{W_s}{W_n} \cdot \frac{A_n^{12}}{A_s^{12}} \cdot \frac{m_{t1}}{m_s} \cdot \left(\frac{R_{m1} - R_t}{1 - R_{m1} \cdot R_s}\right) \qquad [3]$$

where C_s and C_n are the concentrations in the sample and in the natural standard; m_n and m_s the masses taken from the natural standard and the sample when mixed in the two independent experiments (2 and 1, respectively) with the tracer; and m_{t2} and m_{t1} the masses of tracer mixed with the natural standard and sample, respectively. W_s and W_n are the atomic weights in the sample and natural standard; R_t is the isotope abundance ratio in the spike and R_{m2} and R_{m1} are the isotope abundance ratios measured in the mixture of the tracer with the standard and sample, respectively. Kragten procedure was again used in order to propagate the uncertainty.[17] Again, if isotope abundances from the natural standard and sample are considered identical, equation [3] simplifies to:

$$C_s = C_n \cdot \frac{m_n}{m_{t2}} \cdot \left(\frac{1 - R_{m2} \cdot R_n}{R_{m2} - R_t}\right) \cdot \frac{m_{t1}}{m_s} \cdot \left(\frac{R_{m1} - R_t}{1 - R_{m1} \cdot R_n}\right) \qquad [4]$$

The second calculation procedure employed was based on Isotope Pattern Deconvolution (IPD).[12] Instead of computing the carbon $^{12}C/^{13}C$ isotope ratios (R_{m2} and R_{m1}) from the 44 and 45 peak area ratios measured, we could directly use the 44, 45 and 46 peak areas obtained from independent analyses of the natural abundance standard, seawater samples and pure tracer and from mixtures of standard-tracer and sample-tracer to compute the molar fractions of the standard and tracer (x_n, x_{t2}) and sample and tracer (x_s, x_{t1}) in the corresponding mixtures using the Isotope Pattern Deconvolution approach.[12] The IPD equations are based on multiple linear regression and state that the molar fractions measured in the mixtures 1 and 2 are a linear combination of the isotope abundances of the pure components as indicated in equation [5] for experiment 2 (mixture of natural abundance standard and tracer) and equation [6] for experiment 1 (mixture of sample and tracer):

$$\begin{bmatrix} A_{m2}^{44} \\ A_{m2}^{45} \\ A_{m2}^{46} \end{bmatrix} = \begin{bmatrix} A_n^{44} & A_t^{44} \\ A_n^{45} & A_t^{45} \\ A_n^{46} & A_t^{46} \end{bmatrix} \times \begin{bmatrix} x_n \\ x_{t2} \end{bmatrix} + \begin{bmatrix} e_2^{44} \\ e_2^{45} \\ e_2^{46} \end{bmatrix} \qquad [5]$$

$$\begin{bmatrix} A_{m1}^{44} \\ A_{m1}^{45} \\ A_{m1}^{46} \end{bmatrix} = \begin{bmatrix} A_s^{44} & A_t^{44} \\ A_s^{45} & A_t^{45} \\ A_s^{46} & A_t^{46} \end{bmatrix} \times \begin{bmatrix} x_s \\ x_{t1} \end{bmatrix} + \begin{bmatrix} e_1^{44} \\ e_1^{45} \\ e_1^{46} \end{bmatrix} \quad [6]$$

where A_j^i terms correspond to the relative isotope abundances measured at mass i for the component j. As we have more equations than unknowns, the solutions to these equations (the molar fractions) are obtained by multiple linear regression. Finally, concentration in the sample (C_s) can be obtained as shown in equation [7] assuming that the atomic weights for carbon in the samples and natural abundance standard are the same:

$$C_s = C_n \cdot \frac{m_n}{m_{t2}} \cdot \frac{m_{t1}}{m_s} \cdot \left(\frac{x_s}{x_{t1}}\right) \cdot \left(\frac{x_{t2}}{x_n}\right) \quad [7]$$

RESULTS AND DISCUSSION

In this publication, two IDMS methodologies (on-line and species-specific) and two calculation procedures for each methodology have been compared for the precise and accurate determination of DIC in seawater. In all cases, uncertainty propagation has been carried out in order to take into account every uncertainty source from standards and sample preparation to final mass spectrometric analysis. Detailed description of the contribution of every parameter to the final uncertainty corresponding to each methodology is given as pie charts in Supporting Information (Figures S-1 and S-4 to S-7).

Determination of carbon isotope abundances and $^{12}C/^{13}C$ isotope ratios

Application of equations [1] to [4] for carbon IDMS requires the determination of the isotope composition of carbon both, for the natural abundance sample and standard, and for the enriched tracer. Natural isotopic abundances (sample and natural standard abundances) are usually taken directly from the IUPAC with their associated uncertainties.[12,18] Unfortunately, for the case of carbon these abundance uncertainties are high due to natural variability, and will be a large source of uncertainty in the calculated DIC concentrations. However, natural and spike isotopic abundances can be determined experimentally with adequate precision when using an IRMS instrument by direct FIA of the natural standard, seawater samples and tracer solutions. The measured peak areas at CO_2 masses 44 and 45 can be transformed into carbon abundances at masses 12 and 13 by taking into account the natural isotopic composition of oxygen.[18] Detailed deduction and equations are shown in Supporting Information (Eq. [S-1] to [S-5]).

Table 1. Isotope abundances (A) obtained by direct FIA-IRMS for $NaHCO_3$ natural standard, seawater CRM and $NaH^{13}CO_3$ tracer. IUPAC values are also included.

	A^{12}	A^{13}
$NaHCO_3$ natural standard	0.98880 ± 0.00001	0.01120 ± 0.00001
Seawater sample (CRM)	0.98893 ± 0.00002	0.01107 ± 0.00002
IUPAC range values[18]	[0.9884, 0.9904]	[0.0096, 0.0116]
$NaH^{13}CO_3$ tracer	0.03255 ± 0.00008	0.96745 ± 0.00008

The experimental carbon isotope abundances with the corresponding propagated uncertainties are given in Table 1 (n=6 replicates), together with the IUPAC range values.[18] As can be seen, the ^{12}C and ^{13}C natural abundances obtained using FIA-IRMS for the natural bicarbonate standard and the seawater sample are both within the range of the representative isotopic composition given by the IUPAC. However, lower associated uncertainties are obtained when the abundances are experimentally measured by direct FIA-IRMS, ranging from 0.001 to 0.003% and 0.09% to 0.27% RSD for the isotope abundances of ^{12}C and ^{13}C, respectively. It is worth mentioning that the ^{13}C isotope abundance obtained for seawater (0.01107 ± 0.00003) lies perfectly within the extremely low variation range typically described for seawater in the literature, 0.01104-0.01109.[19]

DIC determination in seawater using standard on-line IDMS

First on-line IDMS experiments for computation of DIC in seawater samples were carried out using a quadrupole mass spectrometer (QMS) as detector and following the procedure described.[13] The result obtained (2014 ± 16 µmol C/kg, n=5) was in good agreement with the certified value (2012.24 ± 0.28 µmol C/kg). Unfortunately, precision attained (0.8% RSD, not propagated) was far from the required values (< 0.05% RSD).

We wanted first to evaluate if precision could be improved enough simply by resorting to an IRMS instrument, which is specifically designed for carbon isotope ratio measurement in liquid phase, as shown in Figure 1.

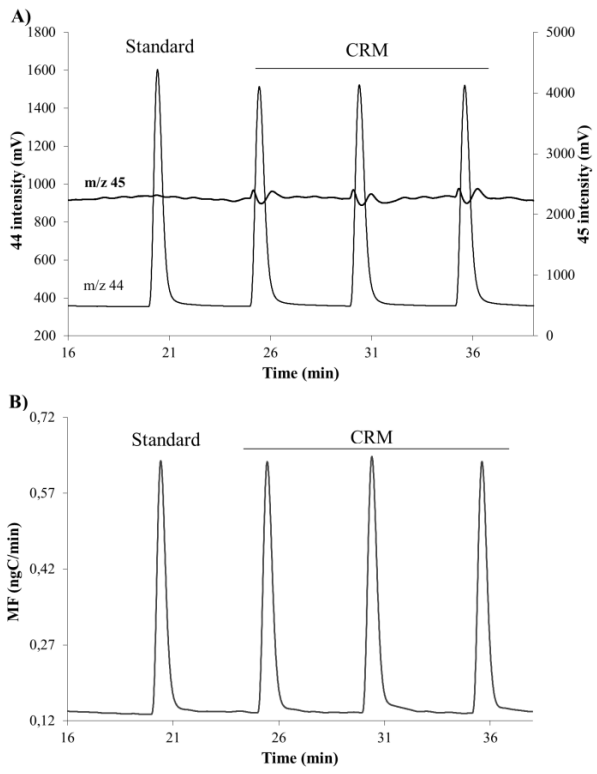

Figure 2. Intensity (A) and mass flow (B) FIAgrams for DIC determination in seawater CRM (n=3) using on-line IDMS and Gaussian peaks.

It is worth noting first it takes at least 25 min to get a stable signal for the background at m/z 44 and 45. One standard with

natural carbon isotope abundances ($NaHCO_3$, ca 2017.20 µmol C/kg) was injected every 3 undiluted seawater samples. DIC result obtained for n=9 and n=3 injections for CRM and standard, respectively was 2012 ± 11 µmol C/kg. Propagated precision obtained (0.57% RSD) was better than the one obtained using the regular QMS instrument but the precision was still far from the requirements. Individual contributions of each analytical parameter to the final propagated uncertainty are shown in Figure S-1. As can be seen, uncertainties corresponding to the peak area integrations accounted for 85% of the total uncertainty.

There are several reasons that could explain these poor precision results. First, the medium pressure piston pump used provided a slightly oscillating signal at m/z 45 which limited the precision (see Figure 2.A). Moreover, such instability is shown in the mass flow chromatogram obtained hampering, first, the detection of the contribution at m/z 45 corresponding to natural abundance of ^{13}C is as low as 1.1% and, second, it limited the integration of the mass flow FIAgram (see Figure 2.B) leading to poor peak area precision.

This effect is very significant in the case of the analysis the seawater CRM. A significant distortion of the signal at m/z 45 (see Figure 2.A) was clearly observed at the front and end of peak elution in comparison to the standard. As such distortion was only observed at signal 45 and when analyzing seawater samples, it was assumed that it came from the mixing of flows of different salt content. Note that the tracer solution was prepared by dissolving the enriched $NaH^{13}CO_3$ in pure water. As expected, this effect was also observed when NaCl was added to the standard $NaHCO_3$ solution (at 3.5% m/v), as shown in Figure S-2. Thus, this procedure was rejected and the use of a larger injection loop to obtain flat-topped peaks and avoid peak integration and calculation of the mass flow FIAgram was tested.

DIC determination in seawater using on-line IDMS and flat-topped peaks

We developed another on-line IDMS approach by increasing the sample volume from 10 µL to 800 µL. In this case, calibration of the isotopic tracer was carried out by means of a double IDMS experiment and the isotope ratios were measured point by point in the stable region of flat-topped peaks obtained instead of as area ratios of Gaussian peaks.

The combination of 800 µL loop and 240 µL/min carrier flow led to a 1 minute stable region where the 44/45 ratio could be precisely measured. This can be clearly seen in Figure 3, both for intensity (Figure 3.A) and isotope ratio (Figure 3.B) FIAgrams obtained for a bicarbonate standard (first peak on the FIAgrams). A stabilization time of 10 min was required in order to come back to the original background values prior to the next sample injection. It is worth mentioning that the choice of the tracer flow was limited by the volume of the pistons (10 mL) of the pump used. Furthermore, slightly different isotope enrichment for ^{13}C was observed in subsequent fillings with ^{13}C tracer of the piston pump likely due to isotopic exchange effects with traces of atmospheric CO_2 present in the headspace of the bottle where the tracer was stored. This problem will not affect the accuracy of the approach, as the isotopic enrichment for the tracer was computed each time the piston was filled. However, it prevented the use of replicates of the same sample using tracer solutions from different piston loads. In fact, stabilization time as high as 25 min was required after each piston load in order to get a completely stable background both at masses 44 and 45.

In order to evaluate the reproducibility of the on-line isotope ratio measurement, ten consecutive injections of the natural bicarbonate standard were carried out. Results are shown in Figure S-3. 44/45 isotope ratio precision for each individual flat-topped measurement of bicarbonate standard was excellent ranging from 0.01 to 0.04% RSD (average 0.02%). However, reproducibility between injections was significantly worse. Isotope ratio measurements drifted and were not stable until the fourth injection. It seems that the signal from the tracer was not fully stable until a certain time after the filling of the pump piston used. This was very significant for the first injection as shown in Figure S-3. Interestingly, after discarding the first three injections the reproducibility was as good as 0.075% RSD (n=7), close to the precision of the individual experiments.

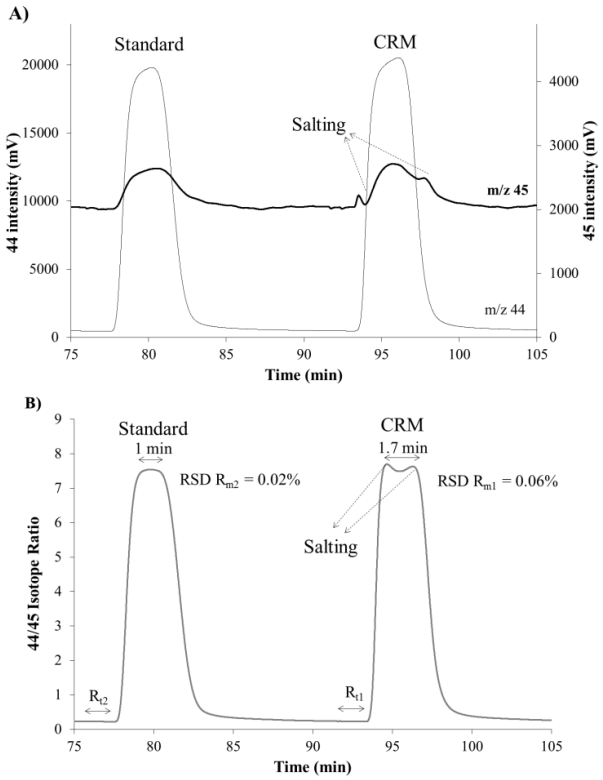

Figure 3. Intensity (A) and mass flow (B) FIAgrams for DIC determination in seawater CRM using on-line double IDMS and flat-topped peaks.

Analysis of seawater CRM was carried out as final evaluation of this second approach. As explained in detail in the procedures section, eq. [1] provides directly the concentration of carbon in the injected sample. The isotope ratio $^{12}C/^{13}C$ (44/45) was measured in the flat-topped peaks of each standard-sample analysis (R_{m2} and R_{m1}) and in the background previous to every standard and sample peaks (R_{t2} and R_{t1}) as shown in Figure 3.B. Four CRM analyses were carried out. Unfortunately, salt effects observed previously for the Gaussian peaks were apparent again in the seawater injection mostly at the beginning and end of the m/z 45 intensity peak (Figure 3.A) resulting in less stable and slightly broader (1.7 min) flat-topped peaks (Figure 3.B) in comparison to the standard ones. Finally, slightly worse 44/45 isotope ratio precision for the

seawater (0.03-0.06% RSD) in comparison to the standard (ca. 0.01-0.02% RSD) was obtained. Table 2 shows the quantitative results obtained for the four CRM analyses using carbon natural isotope abundances for standard and sample either obtained experimentally (Table 2.A and eq. [1]) or extracted from IUPAC (Table 2.B and eq. [2]). As mentioned before, propagated uncertainty is calculated using Kragten procedure. It can be seen that the first quantitative value must again be discarded. In fact, the second injection is also statistically different from the last 2 injections. It looks that, as previously observed for the first injections of the bicarbonate standard, complete signal equilibration from the tracer is not achieved until the second part of the piston (last 5 mL).

Table 2. DIC quantification results for the seawater CRM using on-line double IDMS flat-topped peaks and equations [1] and [2].

	Found (μmol C/kg)	Accuracy (%)	RSD (%)
Using Eq. [1]			
1	2032.60	1.0	0.083
2	1998.74	-0.7	0.064
3	2009.40	-0.14	0.058
4	2009.62	-0.13	0.052
Using Eq. [2]			
1	2034.91	1.1	0.081
2	2001.10	-0.6	0.061
3	2011.72	-0.03	0.055
4	2011.93	-0.02	0.048

Interestingly, accuracy of the last two independent experiments, where stability is observed, is excellent using both Eq. [1] and [2]. The individual uncertainties associated to such concentration values were as good as 0.058 and 0.052% RSD and 0.055 and 0.048% RSD for equations [1] and [2], respectively. Individual contribution of each parameter to the final uncertainty value using Kragten is given in the corresponding pie charts in Figures S-4 and S-5. As can be seen, the uncertainty associated to the isotope ratio measurement of the seawater (R_{m1}) is the limiting factor accounting for 70-80% of the total uncertainty in the first two analyses when tracer flow was not completely stable yet. Once the tracer flow was stable, the uncertainty budget was still limited by R_{m1} (around 50%) due to the salt effects but the contribution of the uncertainty associated to the natural standard became very important (around 30%). Of course, RSD is slightly higher when using equation [1] (see Table 2) because experimentally obtained uncertainties for sample and natural standard isotope abundances have to be considered in the calculation. Although accuracy is adequate using both equations, bias could be higher when using IUPAC values (Eq. [2]) if isotope abundances of seawater samples and standards are not almost identical. Therefore, DIC quantification using the isotope abundances of sample and standards is recommended.

Classical species-specific double IDMS method for DIC measurements in seawater

It is clear that the use of flat-topped peaks provides much better isotope ratio precision. However, stability of the on-line ^{13}C-enriched bicarbonate flow added and the need for separate standard and sample injections still limited the applicability of the on-line IDMS for high precise and highly accurate DIC determinations. One alternative could be to spike the seawater sample with the $NaH^{13}CO_3$ tracer and allow it to equilibrate before its injection to the IRMS (species-specific IDMS experiment). Therefore, the continuous flow of tracer is no longer required. A regular FIA system was applied in order to improve sample throughput, so isotope ratios would be computed again as peak area ratios. The instrumental configuration is shown in Figure 1.

The typical elemental double IDMS procedure[12] using equation [3] was applied. Tracer was mixed in two independent experiments with the natural abundance standard and the seawater CRM sample and the isotope ratios were computed as peak area ratios, R_{m2} (n=7) and R_{m1} (n=8), respectively. In this way, the only parameter required associated to the tracer is R_t, and it can be computed off-line beforehand. Again, we can also assume again that the ^{12}C isotope abundances are identical in the natural abundance standard and in the sample and then, Eq. [3] can be simplified further to Eq. [4].

The intensity FIAgram obtained for the CRM sample spiked with the ^{13}C tracer is shown in Figure 4. It is clear from this figure that the signal instabilities observed before for seawater analyses using on-line IDMS are not apparent anymore. It seems that the mixing and equilibration of sample and tracer prior the measurement step prevents the formation of the sample (salty matrix) - tracer (pure water matrix) interfaces at the beginning and end of the FIA peak which were responsible for the signal fluctuations. In fact, peak integration could be carried out without problems leading to much better and reproducible peak area ratio determinations. All replicates could be considered as valid as no clear trend was observed.

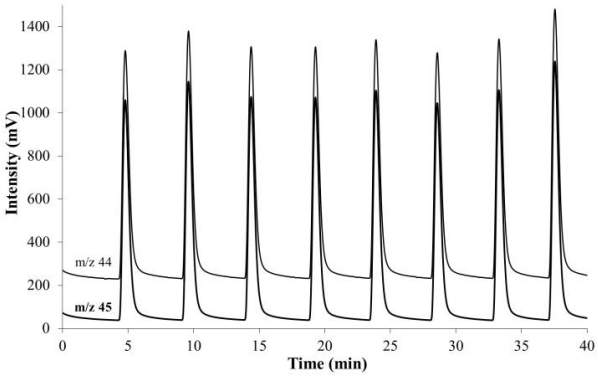

Figure 4. Intensity FIAgram (n=8) of seawater CRM spiked with $NaH^{13}CO_3$ for DIC quantification using species-specific isotope dilution.

DIC value obtained using Eq. [3] was 2010.78 ± 0.99 μmol C/kg (n=8). Accuracy obtained was excellent (error -0.071%). Precision obtained was as low as 0.049% RSD. The pie chart diagram, in which individual uncertainty contributions are shown, is given in Figure S-6.A. As can be seen, the isotope ratios measured in the mixtures (R_{m2} and R_{m1}) and the concentration of the natural standard accounted for almost 99.3% of the uncertainty (73.9, 18.0 and 7.4%, respectively). In this case only a small contribution (0.4%) to the uncertainty came from the terms related to the measurement of the natural isotope abundances in the standard and sample.

When using the simplified equation [4], the DIC value obtained was 2011.36 ± 0.98 µmol C/kg (n=8). Accuracy obtained was again excellent (error -0.044%), obtaining the same precision (0.049% RSD) as using Eq. [3]. Individual contributions to the uncertainty budget are given in Figure S-6.B and are also very similar to those obtained using the full Eq. [3]. As can be seen the isotope ratios measured in the mixtures (R_{m2} and R_{m1}) and the concentration of the natural standard (74.4, 18.1 and 7.5%, respectively) accounted for more than 99.9% of the combined uncertainty.

Note that the whole analysis was carried out within 40 min. Of course, the analysis of the standard and tracer mixture is just required once per working day. However, it is worth stressing that stability and robustness of the system would allow to carry out the quantification using a simple triplicate analysis (2010.99 ± 0.98 µmol C/kg), reducing sample throughput to just 15 min without affecting either accuracy (-0.062% error) or precision (0.049% RSD).

Reproducibility was evaluated analyzing the CRM in 3 independent measurements carried out in different working days. Only a triplicate of injections was performed for each individual analysis in order to simulate high throughput. Quantification results are shown in Table 3 and individual contributions to the uncertainty budget of the last two experiments are given in Figure S-6.C and S-6.D. As can be seen, R_{m1} and R_{m2} are again the individual factors that contribute most to the uncertainty. Results obtained were very reproducible, indicating the robustness of the method. In fact, average value obtained, 2012.34 ± 1.41 µmol C/kg, agreed well with the certified value (error -0.01%) and total combined uncertainty for the three experiments that takes into account both the individual uncertainties computed by Kragten and the uncertainty due to reproducibility increased only slightly to 0.070% RSD.

Table 3. DIC quantification results for the seawater CRM using species-specific IDMS (both classical and IPD calculations) obtained in three independent experiments.

	Found (µmol C/kg)	Accuracy (%)	Propagated uncertainty (RSD, %)
Using classical species-specific IDMS (Eq. [3])			
Exp. 1 (n=3)	2010.99	-0.06	0.049
Exp. 2 (n=3)	2012.80	+0.03	0.066
Exp. 3 (n=3)	2013.22	+0.05	0.067
Using species-specific IDMS and IPD (Eq. [8])			
Exp. 1 (n=3)	2011.10	-0.06	0.036
Exp. 2 (n=3)	2013.03	+0.04	0.047
Exp. 3 (n=3)	2013.46	+0.06	0.048

Species-specific double IDMS method for DIC measurements in seawater using IPD

Considering that most of the uncertainty is coming from the 12/13 isotope ratios obtained from the 44 and 45 signals, which in turn must be corrected by Oxygen contribution, we wanted to assess if we could use Isotope Pattern Deconvolution (IPD) for data treatment. In this way, as shown in Eq. [7], DIC concentration in the seawater sample is extracted from molar fractions in the corresponding samples, which are obtained directly from the experimental CO_2 peak areas measured at 44, 45 and 46 masses.

Obviously, absolute DIC values in the CRM, both using eight (2010.89 ± 0.72 µmol C/kg, error -0.067%) or three replicates (2011.10 ± 0.72 µmol C/kg, error -0.043%), were very similar to those obtained using classical data treatment. Interestingly, precision improved significantly from 0.049 to 0.036% RSD for both n=8 and n=3 data sets. Importantly this precision is now below the target 0.05% RSD and, to the best of our knowledge, at the same level of the lowest DIC precisions reported so far.[5,6,10] It is worth stressing here that such excellent precision is obtained in spite of the exhaustive uncertainty budget carried out, where every simple factor that could impact reproducibility was taken into account including weighs, standards and experimental measurements. As shown in Figure 5, 44 and 45 abundances for tracer-sample (16%) and tracer-standard (69%) mixtures turned out to be the most important contributions to the propagated uncertainty. Concentration of the natural standard contributes slightly as well (14%). An example of implementation of the Kragten spreadsheet procedure using the corresponding Excel file with the detailed computation of the total uncertainty budget using species-specific IDMS and IPD is given in the Supporting Information.[12,17]

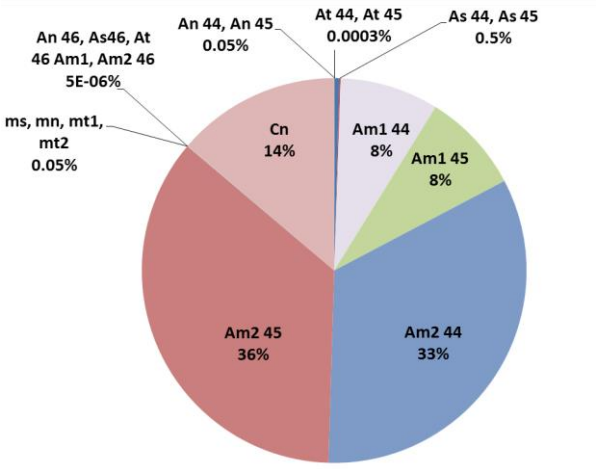

Figure 5. Uncertainty contributions pie chart using Isotope Pattern Deconvolution and Equation [7], experiment 1.

Reproducibility of the method proposed was again evaluated analyzing the CRM in 3 independent triplicate experiments carried out in different working days. Quantification results are shown in Table 3 and individual contributions to the uncertainty budget of the last 2 independent experiments are given in Figure S-7.A and S-7.B. Notably, every individual precision obtained for the three independent experiments (0.035-0.05% RSD) were below the precision requirements for DIC application to climate change studies. Experimental 44 and 45 abundances for tracer-sample and tracer-standard mixtures were again the most important contributions to the propagated uncertainty. In fact, as precision associated to the experimental measurements of the abundances is slightly worse in these cases, the constant contribution of the concentration of the natural standard to the individual propagated uncertainty became almost negligible. As can be seen, results were very reproducible, indicating the robustness of the method. In fact,

average value obtained, 2012.53 ± 1.15 µmol C/kg, agreed well with the certified value (error -0.01%) and its total combined uncertainty (taking into account both the individual uncertainties computed by Kragten and the uncertainty due to reproducibility) increased only slightly to 0.057% RSD.

CONCLUSIONS

This work evaluates two direct, accurate and precise methods based on the use of carbon isotope dilution and IRMS for Dissolved Inorganic Carbon determination in seawater aiming at meeting the extremely stringent requirements in terms of trueness and precision (below 0.05% RSD) demanded for its application in climate change studies. The method based on the concept of double on-line isotope dilution with continuous addition of the tracer ($NaH^{13}CO_3$) is highly precise for the measurement of carbon isotope ratios in the stable region of the flat-topped peaks obtained. However, drifts in the isotopic composition of the tracer and distortions produced by the salt matrix when mixing the tracer flow and the seawater sample prevent the application of this procedure to real samples. In contrast, methods based on the concept of double IDMS with species-specific spiking provided significantly better results as most of the above mentioned limiting factors related to the tracer flow addition and mixing are compensated. In spite of the exhaustive uncertainty budget computed, taking into account every factor playing a role in the analytical process (weighs, standard concentration, isotope abundances, final measurement), propagated precision obtained was still at the level of the lowest precisions ever published. Additionally, sources of error like evaporations or adsorptions that could take place during sample storage could be likely compensated after isotopic equilibration of the sample and tracer spiked, which could be performed immediately after sampling (even during the ocean survey campaign).

Interestingly, when Isotope Pattern Deconvolution was used for calculation, propagated uncertainty for 3 independent triplicate analyses was always below the target 0.05% RSD level. The approach turned out to be extremely robust and reproducible as demonstrated here by the low combined uncertainty obtained for an inter-day assessment (0.057% RSD). Furthermore, instrumental set-up is simpler since stable additional flow and controlled on-line mixing are not required anymore. Considerable progress has been gained with regard to sample throughput, which is as high as 15 min per sample triplicate. Notably, only regular injection loop washing is required from one sample to another. Sample requirement is much lower (10 mL of sample) as well; and could be easily reduced further to ca. 1 mL, with uncertainties only limited by the uncertainty of the balance, because sample loop used was as small as 10 µL. Taken together, all these features seem to suggest the potential of double IDMS using IRMS and species-specific spiking to become the method of choice for accurate and highly precise DIC determination in environmental laboratories focused on climate change.

ASSOCIATED CONTENT

Supporting Information

Additional Figures, Tables and Equations as list in the text (PDF).

Excel file with an example of implementation of the Kragten spreadsheet procedure for DIC uncertainty budget calculation using species-specific IDMS and IPD (excel file).

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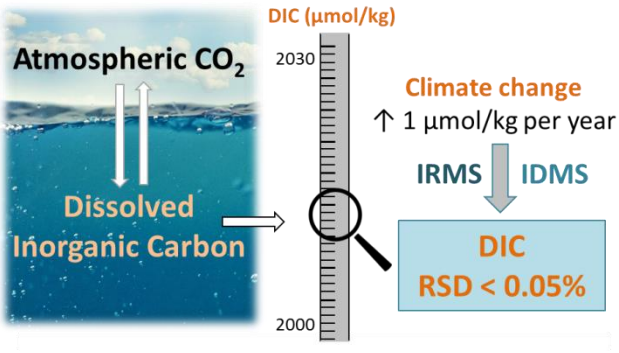
Atmospheric CO_2
Dissolved
Inorganic Carbon
DIC (µmol/kg)
2030
2000
Climate change
↑ 1 µmol/kg per year
IRMS
IDMS
DIC
RSD < 0.05%

For TOC only