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Reference materials selection for the stable carbon isotope analysis of dissolved carbon using a wet oxidation system

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ABSTRACT

Rationale: Wet chemical oxidation combined with isotope ratio mass spectrometry has become a routine technique for analyzing the stable carbon isotope composition of dissolved organic (DOC) and inorganic (DIC) carbon. Methodological inconsistencies between laboratories in using different reference materials lead to a discrepancy in results. We experimentally tested the precision and accuracy of the analysis of commonly available international reference materials and other chemicals potentially suitable for laboratory standards.

Methods: The solid international reference materials and other simple chemicals were used to prepare water solutions. A range of carbon concentrations was chosen to optimize tests for 1) precision and accuracy, 2) linearity, 3) detection limits, 4) memory effects, and 5) efficiency of DIC removal from a DOC/DIC mixtures. Samples were analyzed using an LC-IsoLink coupled with a Delta V Plus Isotope Ratio Mass Spectrometer (Thermo Fisher Scientific).

Results: The analytical setup had a negligible memory effect, good reproducibility (<0.21 ‰) and accuracy (maximum difference from the true values <0.35 ‰) for the analyzed organic compounds if approximately $\geq 9 \times 10^{-09}$ mole of dissolved carbon was injected into the system (~11 mg C L^{-1} if a 10-µL loop was used). Analyses of sodium bicarbonate or calcium carbonate solutions had a two-fold lower accuracy despite maintaining a high precision.

Conclusions: Water solutions of international reference materials such as L-glutamic acids (USGS40, USGS41), benzoic acid (IAEA-601) and sucrose (IAEA-CH-6), can be successfully used for direct normalization of results to the VPDB scale. By contrast, analyses of caffeine and urea returned very reproducible but highly inaccurate results and these materials are not recommended for standards.

Keywords: carbon; stable isotope; WCO; LC-IsoLink; reference material; IRMS

1. INTRODUCTION

Stable carbon isotope analyses (δ^{13}C values) of dissolved organic (DOC) and inorganic (DIC) carbon have over the last three decades become an important tool in biological, environmental, and pollution dispersal studies.[1-5] The δ^{13}C value of DIC is usually measured in bulk in natural or polluted water samples, whereas the δ^{13}C value of DOC can be measured either as bulk or at the specific compound levels in environmental water samples as well as in water extracts from soil, plant material, or food.[6,7] At present, these types of analyses are usually performed using continuous flow systems employing a wet chemical oxidation (WCO) technique combined with isotope ratio mass spectrometry (IRMS). These WCO-IRMS systems can be total organic carbon (TOC) analyzers[8-10] or instruments that couple high performance liquid chromatography (HPLC) with IRMS[11,12]. The concept of combining HPLC with IRMS was introduced by Thermo Fisher Scientific, and their LC-IsoLink system (Thermo Fisher Scientific, Bremen, Germany) has been available commercially since 2004 and is designed for the stable carbon isotope analysis of a broad spectrum of non-volatile compounds dissolved in water.[13]

Over the last decade, WCO analysis has become a routine analytical technique.[14-16] However, a unified world-wide laboratory protocol for selecting soluble international isotope reference materials has not yet been established, creating the potential for inconsistency in results obtained by different laboratories. One interlaboratory comparison study has confirmed a significant discrepancy in the results obtained for the same samples analyzed using different instruments, laboratory protocols, and normalization procedures.[14] Several laboratories still use the δ^{13}C value of high-pressure CO_2 gas cylinders for normalization of measured δ^{13}C values to the VPDB scale.[e.g.10,17] Normalization to the value of the gas reference is the simplest and easiest method to apply; however, in light of the findings of Paul et al[18] and Skrzypek[19], it also leads to the lowest accuracy among all known normalization methods and should be considered as inappropriate. Moreover, the accuracy of results normalized to a tank value depends on the δ^{13}C values of the reference gas and the sample and the relative difference between these two values.

This results directly from the design of the mathematical algorithm that summarizes the measured $\delta^{13}C$ value of the sample, the true $\delta^{13}C$ value of the tank gas and a multiplication component – the $\delta^{13}C$ value of the sample times the $\delta^{13}C$ value of the gas, divided by 1000.[18]

In this study, we experimentally tested the precision and accuracy of the analysis of commonly available international reference materials that can be processed using the WCO technique and the LC-IsoLink system. We also tested several simple chemical compounds that could be considered prospective laboratory standards for WCO instruments. We assessed the precision and accuracy of these reference material and laboratory standard analyses on LC-IsoLink by testing the instrument reproducibility, linearity, detection limits, and the memory effects of the instrument. Additionally, we also examined the preservation of the $\delta^{13}C$ signatures of the prepared solutions over an extended storage time. All these tests allowed the establishment of a practical recommendation for the selection of materials for use as potential laboratory standards. These results are not applicable to LC-IsoLink instrument but also to various other WCO instruments.

2. EXPERIMENTAL

The LC-IsoLink instrument has a variety of parameters that can be optimized to ensure proper oxidation and the desired separation of specific compounds from a sample (Figure 1).[20] Most important are the helium and reagent flow rates, reagent concentrations, size of sampling loops, temperature, and size of injected samples. The instrument can also be operated in two main preparations modes: 1) microEA mode for analyses of TC (Total Carbon) and 2) HPLC, when the liquid chromatographic column is used for organic compound separations. In this study, the primary focus was on the evaluation of chemical compounds that could potentially be used as laboratory reference materials for normalization of measured $\delta^{13}C$ values to the international VPDB isotope scale for this HPLC/IRMS system, but also for WCO-IRMS systems

in general. Therefore, we chose to prepare water solutions from relatively simple, readily soluble, and commonly available chemical compounds that can be also easily analyzed using an elemental analyzer for verification of the obtained $\delta^{13}C$ values. To avoid potential problems associated with chromatographic separations on the HPLC column, we chose to use single compound solutions prepared using Milli-Q quality degassed deionized (DI) water and to analyze them using the microEA mode.

Figure 1. Please insert here

2.1 Analytical setup

Our analytical setup consisted of commercially available devices: an LC-IsoLink instrument (Thermo Fisher Scientific, Bremen, Germany), coupled to a Delta V Plus isotope ratio mass spectrometer (Thermo Fisher Scientific, Bremen, Germany), a Surveyor MS Pump Plus (Thermo Fisher Scientific, San Jose, CA, USA), and an HTC PAL autosampler (MXY 04-01A, CTC Analytics, Zwingen, Switzerland). We optimized the instrument parameters to ensure full oxidation of each organic compound to avoid potential stable isotope fractionation that can occur during incomplete oxidation. The adjustment of the maximum oxidation power of the solution was monitored as the amount of excess oxygen introduced to the mass spectrometer (*m*/*z* 32; 50 V), following the guidelines from the instrument producer.[28] However, note that these highly oxidative conditions may significantly reduce the lifetime of the filament in the mass spectrometer. This problem can be reduced by using an oxygen scrubber online directly from the helium stream with the sample before it is introduced to the ion source of the mass spectrometer.[12,21]

For all analyses, the same concentrations of reagents were used: 1) oxidation reagent, peroxodisulfate 200 g L^{-1} (Sigma-Aldrich, St Louis, MO, USA; cat no. 71890-500G); 2) ortho-phosphoric acid 1.5 molar solution (Merck, Darmstadt, Germany; cat no 1.00565.0500). We kept

in mind our own future analyses of natural waters from arid environments that could have high concentrations of chloride ions; therefore, we did not use catalyzers such as $AgNO_3$ in our tests as this reagent reacts with Cl^- and could damage the wet oxidation reactor in the analytical system. The reagent solutions were prepared using degassed Milli-Q grade DI water (Millipore. Billerica, MA, USA) and kept in 1-L glass bottle purged with ultrahigh purity helium. The reference materials were stored in 40-mL sample vials with septa (Thermo Scientific, San Jose, CA, USA; Chromacol cat. no. 40 EPAVCS-PC) that had been prewashed with H_3PO_4 and DI water and dried at 110 °C. All samples were analyzed under the same conditions: reactor temperature 99.9 °C, helium purge pressure 1.4 bar, carrier gas pressure 1.8 bar, water flow rate 300 μL/min, acid and oxidation reagent flow rate 50 μL/min, loop 10 μL, sample injected volume 95 μL, pre-analysis wash three-times using the sample, post-analysis wash three-times using DI water.

A leak test to detect helium and reagent leaks was performed daily and the mass spectrometer was tuned manually to the highest signal of the reference gas (*m/z* 44) at the lowest linearity possible, adjusting parameters of the ion source and then using the autofocus function of the Isodat software (Thermo Fisher Scientific, Bremen, Germany).[28] The typical reproducibility during the on-off test using CO_2 gas from the high-pressure tank was <0.03±0.03 ‰ (standard deviation) and linearity <0.05 ‰/V. A pump stability test was performed to confirm the background stability. The typical background values for major diagnostic masses were as follow: *m/z* 18 = 12 V (cup 2, 3×10^{10}); *m/z* 32 = 10 V (cup 1, 3×10^{8}) *m/z* 40 = 9 V (cup 2, 3×10^{10}), *m/z* 44 = 1 V (cup 1, 3×10^{8}).

All stable isotope results are expressed as δ values, defined traditionally in parts per thousand (‰), as a relative difference between the isotope ratio of the sample and the reference.[22] All results were initially processed as measured raw data but after performing all experiments they were normalized following multi-point normalization[18] to the VPDB scale, based on water solutions of international reference materials (USGS40, USGS41, IAEA-CH-6,

IAEA-601) provided by the International Atomic Energy Agency (Vienna, Austria). Each reference material was analyzed twice with each batch of samples (in total, eight standards with each batch of samples). The used multi-point normalization procedure was as described by Skrzypek[19], and the values of international reference materials were as given by Coplen et al[23]. For some experiments, the measured δ values had to be reported and these are clearly stated in the text. The measured $\delta^{13}C$ results were provided from Isodat software (ver 2.82,) with $\delta^{13}C = 0$ ‰ assumed for high-pressure tank reference gas with an actual value around -5.5 ‰ on the VPDB scale.[19] Please note that one of the international standards (USGS41) is close to exhaustion and USGS released a replacement in 2016 , a new glutamic acid standard (USGS41a) with similar $\delta^{13}C$ values (currently available from USGS website only, isotopes.usgs.gov).[34] In our study we did not note any change in the $\delta^{13}C$ value of the USGS41 standard used.

2.2 Selected materials and preparation of solutions

In addition to the international reference materials, we selected eight commonly available chemical compounds as prospective laboratory standards, taking to account the chemical compositions of reference materials available from National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA), United States Geological Survey (USGS, Reston, VA, USA), and International Atomic Energy Agency (IAEA). All the materials selected for laboratory standards were analytical grade chemicals (Table 1). These chemicals selected for the preparation of DOC and DIC solutions for LC-IsoLink were analyzed first using EA techniques and normalized to VPDB against a set of international standards (NBS19, USGS24, NBS22, LSVEC), each replicated twice, as proposed by Skrzypek et al[24]. Currently, the anchors for VPDB are undergoing revision and NBS19 has been replaced by another carbonate IAEA-603, while the stability of LSVEC stable carbon isotope composition during long-term storage has been questioned.[29]

Table 1. Please insert here

The solid laboratory standards were used for the subsequent preparation of 70 individual vials containing solutions with known carbon concentrations for use over the duration of the experiments (see Table S1, supporting information). A range of carbon concentrations was chosen to optimize tests for 1) reproducibility, 2) linearity, 3) detection limits, 4) memory effects, and 5) efficiency of DIC removal from DOC/DIC mixture. Each solution of the laboratory standard was stored in the dark, in a fridge at 5 °C and analyzed within a week after preparation; in total, ~500 δ^{13}C results were obtained.

3. RESULTS AND DISCUSSION

3.1 Normalization of LC-Isolink δ^{13}C measured results to the VPDB scale

The stable carbon isotope composition is expressed on the VPDB scale originally defined by two primary anchors, reference materials NBS19 (limestone, 1.95 ‰) and LSVEC (lithium carbonate, -46.60 ‰).[23] However, preparation of water solutions from carbonates, in order to allow direct analyses on WCO instruments, can be impractical and lead to potential isotope fractionations. Consequently, the δ^{13}C value of DIC prepared from carbonate reference materials may not fully reflect the δ-value of these reference materials that would be obtained using other analytical methods, such as EA or GasBench. Moreover, the use of the DIC δ^{13}C value for the normalization of DOC does not fulfill the general recommendation of matching the general chemical composition of the standards and samples[26]. Hence, as the direct use of these two primary references was not considered as an option, we reviewed other available secondary reference materials[23].

Previous studies confirmed that the propagation of analytical uncertainties can be minimized when the selected reference materials embrace the whole range of δ-values observed in nature and when four standards are analyzed, each replicated at least twice[19,24]. Therefore, for our experiments, we used all available secondary reference materials that are organic compounds readily soluble in water. This allowed us to cover a wide range of $\delta^{13}C$ values: USGS40 (L-glutamic acid, -26.39 ‰), USGS41 (L-glutamic acid, +37.63 ‰), IAEA-CH-6 (sucrose, -10.45 ‰), IAEA-600 (caffeine, -27.77 ‰), and IAEA-601 (benzoic acid -28.81 ‰); IAEA-602 was discarded as it has a very similar value (-28.85 ‰) to IAEA-601.[23] All solutions of these selected reference materials were prepared to have the same concentration of carbon (between 26 and 27 mg C L^{-1}) to avoid potentially high uncertainty due to a linearity effect.

The relationship between the measured and the true $\delta^{13}C$ values of the analyzed reference materials was strong and highly significant if IAEA-600 (caffeine) was excluded. The results of the four reference materials formed a robust normalization line $\delta_{True}=1.016\times\delta_{Raw}$ - 5.586 (Figure 2). The IAEA-600 was a consistent outlier with high precision — the standard deviation of five replicates was 0.11 ‰ — but the results were not accurate. When the measured $\delta^{13}C$ value of IAEA-600 is normalized to VPDB by applying the normalization equation obtained using four other reference materials, its value will be -33.40 ‰. This is -5.91 ‰ more negative than the true value as calibrated by Coplen et al[23] using the EA technique.

Figure 2. Please insert here

The robustness of the normalization equation calculated using the four standards (IAEA-600 excluded) was further tested. The normalization equation was repetitively calculated using three of the four reference materials (any three of USGS40, USGS41, IAEA-CH-6, IAEA-601) with the fourth reference material used as a checking standard and its normalized value subtracted from the true value to calculate difference between the obtained and the expected $\delta^{13}C$

value. All possible combinations of reference materials were tested and the highest difference between the obtained value and the true was 0.40 ‰ when USGS41 was used as the checking standard. In this case, the checking standard lay far outside the range covered by the reference materials used for calculation the normalization equation (Figure 2). In all other cases, the difference between the $\delta^{13}C$ value of the normalized checking standard and the true value was ≤0.10 ‰. This confirms that the set of dissolved reference materials (USGS40, USGS41, IAEA-601, and IAEA-CH-6) can be successfully used on LC-IsoLink for direct normalization of unknown samples to the VPDB scale. By contrast, IAEA-600 returns incorrect results, perhaps due to incomplete oxidation and conversion to CO_2. Caffeine has a high nitrogen content and an aromatic structure, so it might be more difficult to fully oxidize during WCO. Incomplete conversion of carbon to CO_2 may lead to a stable isotope fractionation as presented here, and also for other types of analyses of nitrogen-rich chemical compounds.[35] Therefore, IAEA-600 should not be considered as a first choice for a reference material. This precaution needs to be considered, especially in studies such as that by Zhang et al[27], where WCO techniques are used to analyze drinks containing caffeine; in those cases, catalyzers that enhance oxidation need to be considered.

3.2 Analytical reproducibility and accuracy

The instrument reproducibility was tested using 21 water solutions of eight selected chemical compounds as prospective laboratory standards (Table 2; Table S1, supporting information). In total, 166 analyses were performed on different days. The instrument reproducibility was high and the overall precision was better than suggested by the manufacturer, at ~0.40 ‰[28]. The standard deviation calculated from replicated analyses of each chemical compound varied between 0.05 ‰ and 0.21 ‰, depending on the chemical compound and was, on average, ~0.15 ‰. The accuracy was assessed by comparison with the EA technique. It had a much lower precision and varied considerably depending on the type of chemical compounds. In general,

relatively simple chemical compounds had $\delta^{13}C$ values obtained with the LC-IsoLink that differed by not more than 0.35 ‰ from the values obtained with EA: acetanilide (-0.35 ‰), glutamic acid (-0.23 ‰), Na-L-glutamate (-0.18 ‰), and benzoic (-0.22 ‰). By contrast, compounds that were potentially more difficult to oxidize, such as those containing more nitrogen, had substantially different $\delta^{13}C$ values from these obtained with EA: urea differed by -6.15 ‰ and caffeine by -3.80 ‰ (Figure 3). These differences probably arose from incomplete oxidation, which may cause an isotope fractionation resulting in more negative $\delta^{13}C$ values of the CO_2 yielded during the wet chemical oxidation. A similar problem has been observed for caffeine IAEA-600 (see paragraph 3.1). Taking this complication into account, both urea and caffeine should be avoided as first choices for laboratory standards. In addition, the CO_2 yielded for analyzed complex organic compounds containing significant amount of nitrogen needs to be carefully assessed, as partial conversion may lead to significant stable isotope fractionation.

Table 2. Please insert here

Figure 3. Please insert here

Similarly, higher differences from the "true values" obtained with EA were noted for solutions of the carbonates, calcium carbonate (+0.38 ‰), and sodium bicarbonate (-0.49 ‰). We tested carbonates as potential laboratory standards because the original primary anchors for the VPDB scale are two carbonates, NBS19 and LSVEC.[23] However, carbonates are difficult to dissolve and the pH has to be lowered to at least 6 in order to make solutions in relatively short time. Even lowering the pH by one unit may result in degassing and partial loss of CO_2, which may result in an isotope fractionation and increase analytical uncertainty. Therefore, carbonates are also not good choices for laboratory standards for WCO.

3.3 Linearity and detection limit

The linearity effect is a decrease or increase in the δ-value with respect to the sample size and the amount of the CO_2 gas yielded and then introduced to the isotope ratio mass spectrometer.[30] Three major components can contribute to this effect: 1) the linearity of the ion source in the isotope ratio mass spectrometer, which may not be equally ionizing or focusing different portions of the gas; 2) the linearity of the preparation instrument (e.g., LC-IsoLink), which may not process different concentrations of carbon in the sample in exactly the same way or equally efficiently and 3) different contributions of the instrument background with respect to the sample versus background ratio (if the background has not been corrected). The linearity effect and the need for its correction can be avoided if all samples and standards are analyzed at the same signal level. However, this could create difficulties for the analysis of specific compounds when predicting carbon concentrations in samples, and correctly matching the signal from laboratory standards is challenging. Therefore, overall poor linearity can be critical for increasing analytical precision and accuracy if a mathematical correction is not applied.[30,31]

The linearity effect of the mass spectrometer was assessed daily based on eight measurements of CO_2 gas (1 to 8 V) from a high-pressure tank. This linearity was lower than 0.05 ‰ per 1 V change in the peak height. The combined linearity of the LC-IsoLink and mass spectrometer was evaluated by analyzing seven different solutions of the same chemicals (benzoic acid, glutamic acid, and sodium bicarbonate) in the range of carbon concentrations from 1.5 to 50 mg L^{-1}, which resulted in a CO_2 peak height between ~0.2 and ~8 V for *m/z* 44 (Figure 4). For this experiment, we selected three chemicals with significantly different $\delta^{13}C$ values: benzoic acid (-28.76 ‰), glutamic acid (-13.04 ‰), and sodium bicarbonate (-5.19 ‰) and each solution was analyzed 3–4 times (Figure 4). The reproducibility was better for solutions with higher carbon concentrations. For a solution of benzoic acid with a carbon concentration of ~2.11 mg C L^{-1} (0.30 V), the standard deviation of three replicates was 1.80 ‰, while for higher carbon concentrations of 5.80 to 54.22 mg L^{-1} (1.6-8.4 V), the replicates varied in a range below the expected analytical uncertainty of the instrument (0.11 to 0.23 ‰) and the $\delta^{13}C$ values did not differ from "the true value" (measured by EA) by more than -0.49 ‰. Similar observations

were made for glutamic acid. However, more variable $\delta^{13}C$ values (st. dev. 0.47 to 3.30 ‰) were observed for a wider range of solutions with low carbon concentrations (1.36 to 10.44 mg C L^{-1} 0.15 to 1.50 V). For samples with carbon concentrations above 10.44 mg C L^{-1}, the standard deviation was much lower (0.06-0.15 ‰) and the values were closer to the "true values", ranging from -0.25 to -0.74 ‰ in terms of differences. The non-linearity was negligible for samples with signals above 2 V (glutamic acid) and 1 V (benzoic acid) even without corrections (Figure 4). A similar observation has been made for sodium bicarbonate (Figure 4).

In summary, relatively low non-linearity effects characterize the LC-IsoLink instrument if the amount of carbon injected with the solution is above 9×10^{-09} mole. This reasonable minimum amount of carbon is an equivalent of ~11 mg C L^{-1} if a 10-µL injection load loop is used.

Figure 4. Please insert here

3.4 Memory effects

The memory effect is usually defined as a carryover occurring when the $\delta^{13}C$ value of an analyzed sample is compromised by the samples that were analyzed before it. This effect is particularly important when the difference in $\delta^{13}C$ values of subsequently analyzed samples is very large.[30] The memory effect usually occurs when traces of previous samples are not fully removed from the analytical system prior to the injection of a subsequent sample.

In our experiment, we used two chemical compounds with very contrasting $\delta^{13}C$ values on the VPDB scale: benzoic acid at -28.76 ‰ and L-glutamic acid (USGS41) at +37.63 ‰ (Table 1). The solutions of both chemicals contained the same concentration of carbon, at ~34 mg C L^{-1} (5.5-6.0 V for *m/z* 44). The syringe was washed as usual following the typical procedure used for the other analyses presented in this paper: three times with the sample itself prior to injection and

three times with DI water after sample injection. The solution of benzoic acid was analyzed four times, immediately followed by four analyses of L-glutamic acid. This cycle was repeated three times, for a total of 24 analyses. Despite very contrasting $\delta^{13}C$ values, no memory effect was observed (Figure 5), confirming negligible sample-to-sample carryover analyzed in the microEA mode at least for the tested chemical compounds. The $\delta^{13}C$ value of ANCA61 analyzed directly before and after the sequence of four injections of USG41 did not differ by more than 0.04 ‰. The signatures of USGS41 were more variable but still typical, as no memory effect trend of progressive increase in values was observed. The difference before and after injection of ANCA61 was 0.58 ‰ for the first cycle and 0.10 ‰ for the second cycle. The difference of 0.58 ‰ is respectively larger but still within the expected analytical uncertainty of 0.40 ‰ (one standard deviation).[28]

Figure 5. Please insert here

3.5 Carbonate removal from DOC/DIC mixtures

The $\delta^{13}C$ value for bulk organic carbon when samples are analyzed using microEA reflects the isotope signature of the total carbon in the sample, both organic (DOC) and inorganic (DIC). Consequently, degassing and efficient removal of DIC can be essential for accurate measurements of $\delta^{13}C$ values in DOC, particularly for natural samples that may be exposed to atmospheric CO_2 or carbonate dissolution and may contain high concentrations of DIC. Therefore, we tested the efficiency of a simple procedure for carbonate removal: 1) ~1 mL of sample was transferred to 1.5-mL glass vials (Grace-Davison, Melbourne, Australia; #98133) with septa (Grace-Davison; #98144); 2) one large drop (~0.02 mL) of 50% orthophosphoric acid (Merck cat no 1.00565.0500) was added and the vials were capped, shaken well, and left for 1 h at 23 °C for acid dissociation; 3) the vials were opened and purge/bubbled for more than 10 min using ultra high purity helium in a custom designed manifold with 0.3 μm fused silica capillaries

inserted to the bottom of the vials; and 4) after purging, the vials were capped and transferred to an autosampler tray. The vials used for purging are commonly used for samples analyzed using the CTC PAL autosampler with various instruments.[28,36]

In order to test the efficiency of this procedure, we prepared a solution using two components, DOC and DIC, with known and contrasting δ^{13}C values: glutamic acid (-13.27 ‰) and sodium bicarbonate (-5.68 ‰). Both reagents were dissolved in DI degassed water and then these solutions (glutamic acid 27.33 C mg L^{-1} and sodium bicarbonate 26.01 C mg L^{-1}) were mixed to give 51.01 % and 48.99 %, respectively, of the total dissolved carbon in the mixture. The theoretically calculated weighted mean δ^{13}C value, based on carbon concentrations and the δ^{13}C values of the mixed components (-9.55 ‰), was in good agreement with the δ^{13}C value obtained with the LC-IsoLink without sample mixture purging (-9.60±0.22 ‰) (Table 3).

The prepared mixture was transferred into five 1.5-mL vials and the DIC removal procedure was performed as described above. The δ^{13}C value of the remaining dissolved carbon in the vials should reflect the DOC signature only if the DIC removal was sufficient and should equal the values obtained for the solution of glutamic acid, at -13.27 ‰. Indeed, the obtained results did not differ significantly from the δ^{13}C value of glutamic acid and varied in the range of expected analytical uncertainty between -12.99±0.09 ‰ and -13.38±0.08 ‰, confirming very efficient removal of carbonates from the mixture, despite the high concentration of DIC in the sample (13.1 mg C L^{-1}) (Table 3).

Table 3. Please insert here

3.6 Storage effect on δ^{13}C values of reference material solutions

All solutions of the reference materials were prepared using degassed and filtered (0.21 μm pore size) water in acid prewashed 40-mL vials with septa (see methods 2.2) and stored in a

refrigerator at 5 °C. The solutions were subsampled and transferred to 1.5-mL autosampler vials, always using a sterile medical grade disposable syringe. The solutions were collected through the septa without opening the vials. However, despite careful handling of the reference material solutions, prolonged storage may have a negative impact on the preservation of the original $\delta^{13}C$ values of the dissolved chemical compounds. In order to test the preservation of the stable carbon isotope composition of the reference material solutions, we prepared water solutions of USGS40, USGS41, IAEA-601, and IAEA-CH-6 at an equal concentrations of carbon (26 and 27 mg C L^{-1}) to match the reference gas signal for *m/z* 44 ~8,000 mV. These solutions were subsampled to 1.5-mL vials for analyses and the remaining volume was left in the refrigerator at 5 °C (Table 4). After storage for one week and for one month, the solutions were reanalyzed and normalized against freshly prepared solutions of the same reference materials.

None of the $\delta^{13}C$ values obtained for solutions after one-week storage were significantly different from the "true" values described in Coplen et al[23]; the values did not differ by more than 0.07 ‰, which is far below the expected 0.40 ‰ analytical uncertainty of the instrument. The differences between the true values and those obtained for samples stored over a whole month varied with a maximum difference of 4.53 ‰ for USGS41 (Table 4). This suggests that storage of solutions over a one-week period is safe and that fresh solutions should be prepared weekly. One-month storage may lead to a change in the $\delta^{13}C$ value.

Table 4. Please insert here

4. CONCLUSIONS

The LC-IsoLink is characterized by a negligible memory effect and good reproducibility (<0.21 ‰) and accuracy (maximum difference from the true values <0.35 ‰) for simple organic

compounds. The accuracy was lower for bicarbonate laboratory standards (maximum difference from the true value 0.38 and 0.49 ‰) despite a high reproducibility (0.05 and 0.14 ‰). Some organic compounds, such as caffeine and urea, could not be accurately analyzed using the present instrumental setup and therefore should not be considered as suitable laboratory standards. A reasonable minimum amount of carbon that will ensure high precision and accuracy of the analyses is approximately 9×10^{-09} mole of dissolved carbon injected into the system; this is equivalent to ~11 mg C L^{-1} if a 10-μL loop is used. Dissolved secondary reference materials, such as USGS40, USGS41, IAEA-601, and IAEA-CH-6, can be successfully used for direct normalization of unknown samples to the VPDB scale. However, fresh solutions of reference materials should be used and storage for longer than one week should be avoided. DIC can be easily removed from samples prior the analyses in the microEA mode by purging the samples in 1.5-mL vials with helium at low pH.

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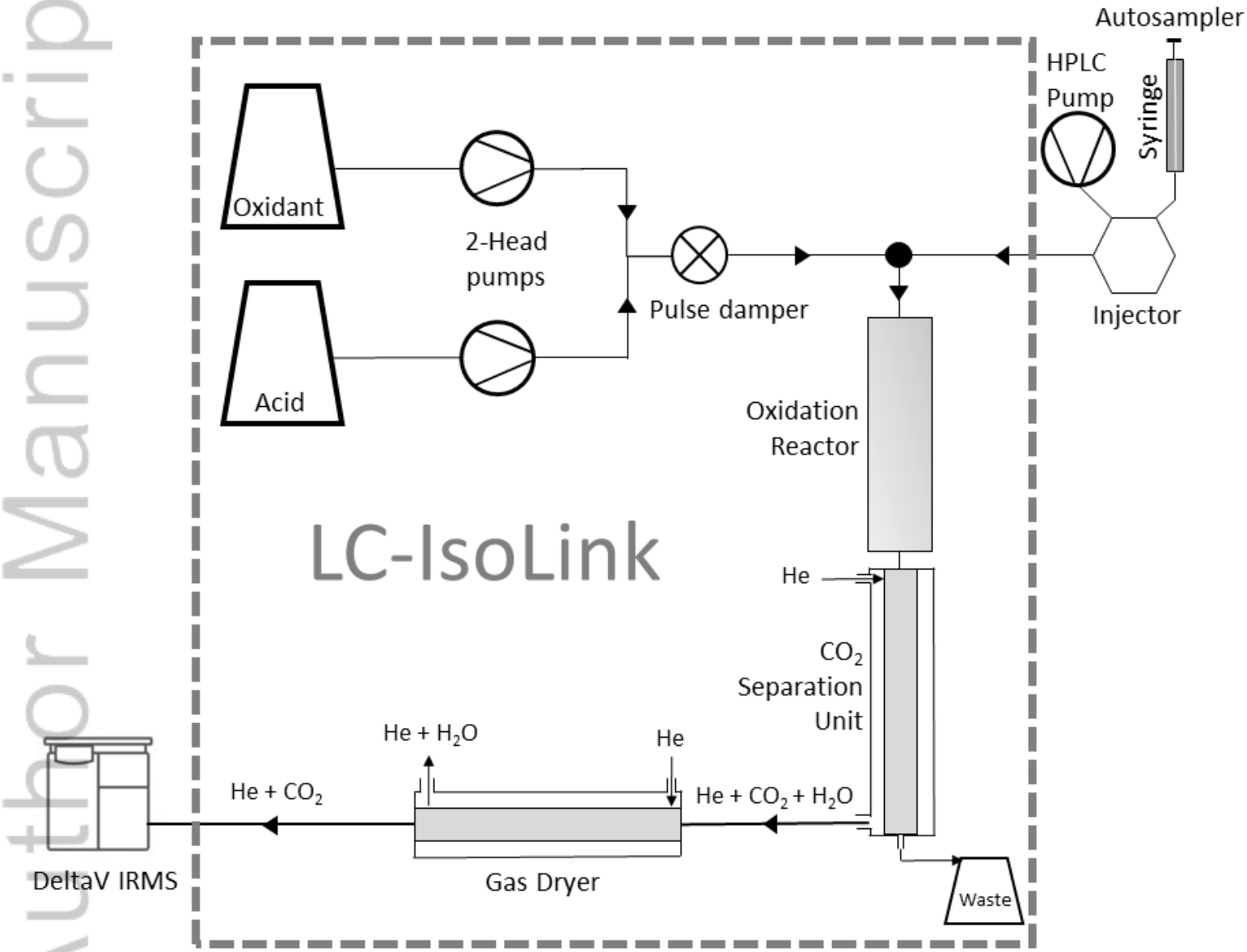

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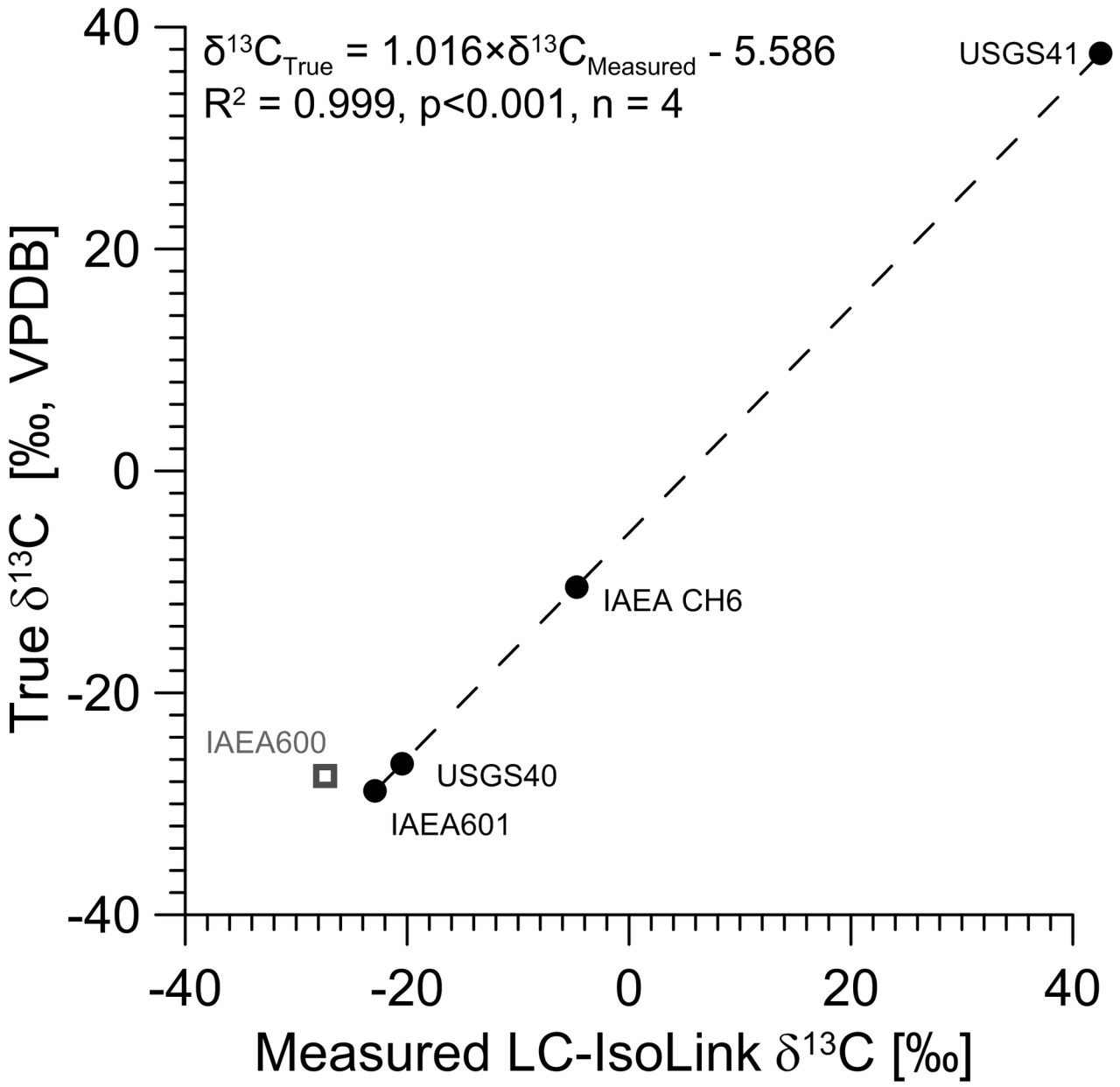

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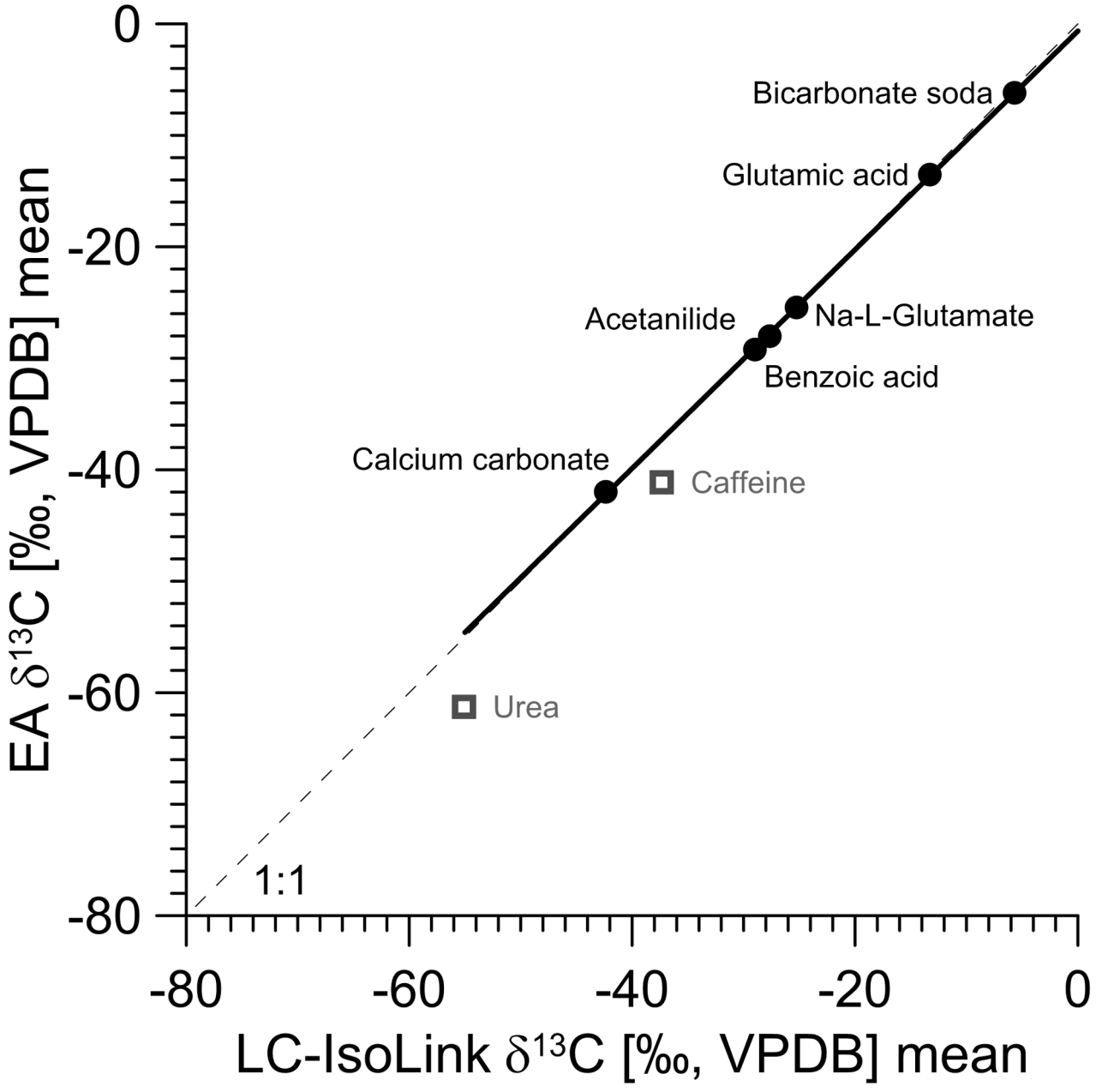

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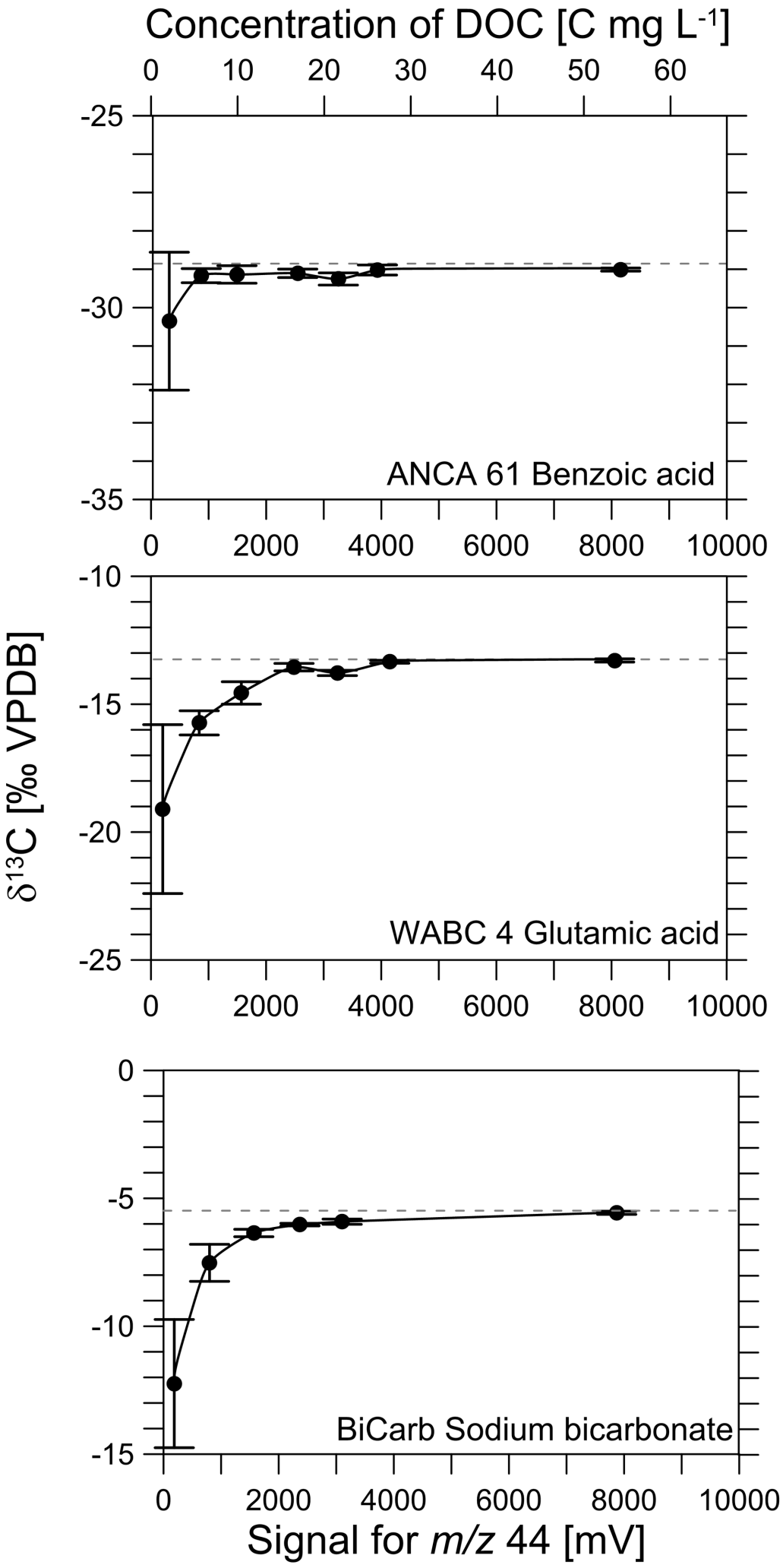

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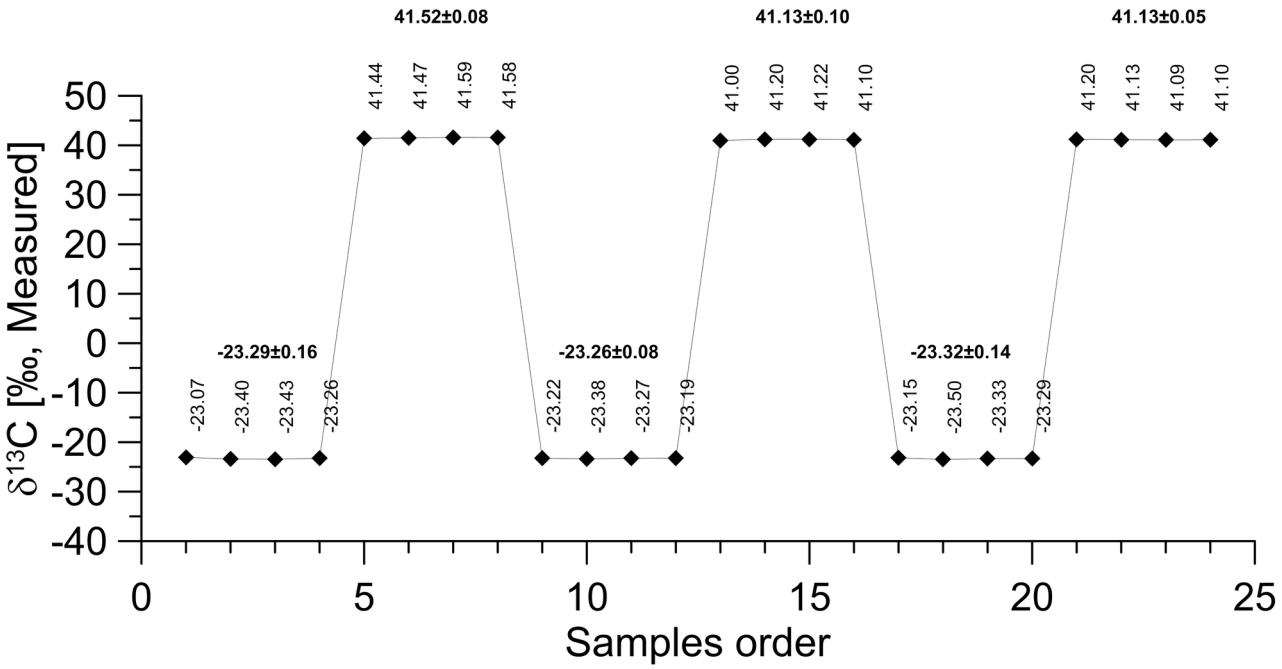

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