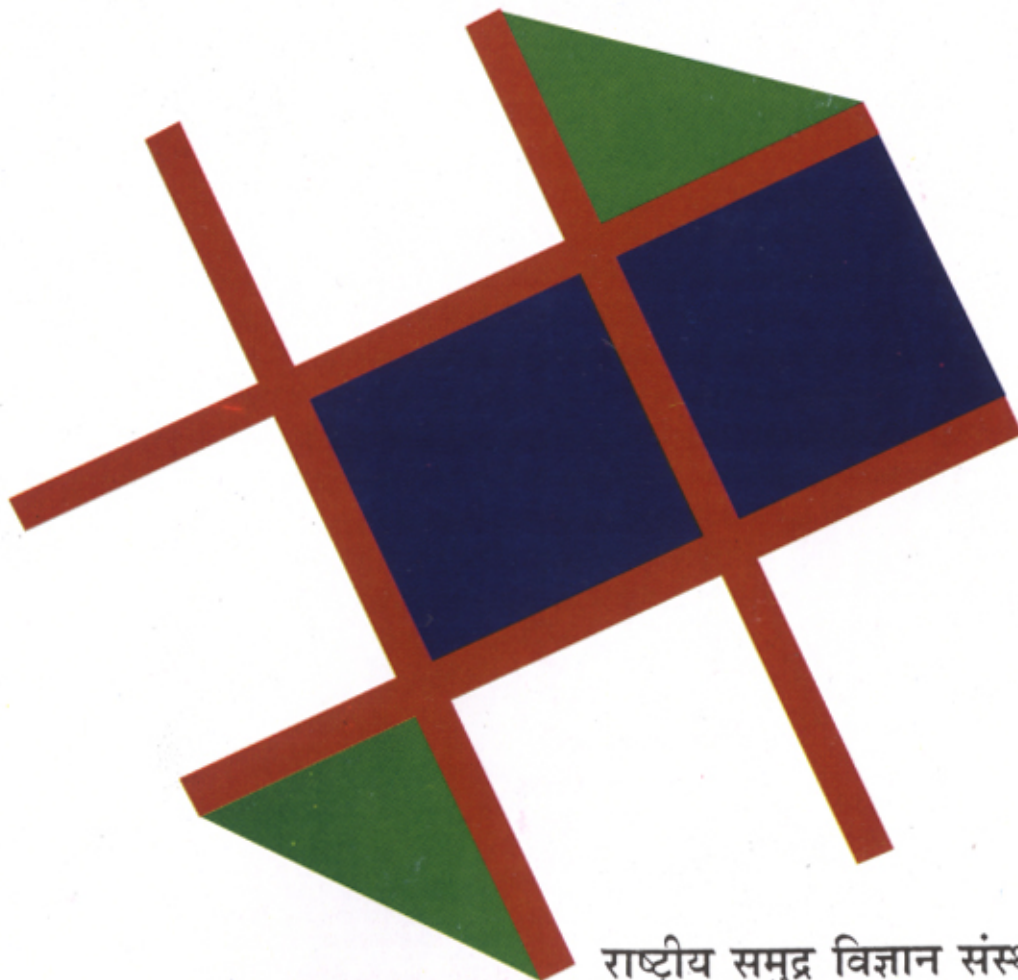


NIO/TR - 02 /2008

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Delta V Plus Stable Isotope Ratio Mass Spectrometer via
different preparatory systems



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राष्ट्रीय समुद्र विज्ञान संस्थान



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Measurement of C and N isotopes of geological samples using *Delta V Plus* Stable Isotope Ratio Mass Spectrometer via different preparatory systems

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Keywords: Stable isotopes, Delta, IRMS, Continuous flow mode, Dual inlet, Tube Cracker device

Abstract

Stable isotope studies have proven their unique potential in unraveling several key biogeochemical processes occurring on solid earth as well as in world oceans. In addition, measurements of stable isotopes of lighter elements viz. C, N, O have provided several hitherto insights to fundamental natural processes capable of impacting global climate ranging from seasonal to orbital timescales. Understanding a 'biogeochemical' process using isotopic tracers is profoundly dependent on quality of isotopic data generated using stable isotope ratio mass spectrometer (IRMS). Exact determination of accuracies and precisions achievable in a given laboratory condition greatly contribute correct interpretation of the isotopic data. We present here standardization of newly acquired IRMS (*Delta V Plus; Thermo*) dedicated for measurements of C & N isotopes in a variety of geological solid samples. Results were calibrated against each other using different preparatory systems such as *on-line* combustion of organic matter in *Elemental Analyzer* (EA) coupled with IRMS in a continuous mode and off-line preparation of CO₂ gas samples and analyzing them on standard *dual-inlet* system. We also present short and long term variability data of an *in-house* laboratory standard developed for routine C and N isotope analyses of various types of geological samples containing organic matter in order to assess stabilities/ reproducibility of the IRMS in different time windows.

1. Introduction

Stable isotopes of C and N of sedimentary organic matter (OM) ($\delta^{13}\text{C}_{\text{org}}$ and $\delta^{15}\text{N}$) are widely used as proxies for decoding and tracking biogeochemical processes occurring in coastal zones such as OM synthesis, nutrient utilization, denitrification, and accumulation of organic carbon in sediments (Sweeney & Kaplan, 1980; Altabet, 2005; Naqvi, 2006). Changes in $\delta^{15}\text{N}$ of sediments from open oceans also reveal variations in productivity and nutrient levels in the water column (Altabet and Francois, 1994). In addition, sedimentary $\delta^{15}\text{N}$ has been trusted as a reliable proxy for tracking past subsurface denitrification variability in major oceanic oxygen deficient zones (ODZs) and have revealed intimate coupling of this oceanic bio-mediated phenomenon to the global climate on orbital to centennial timescales (Altabet et al., 1995, 2002; Ganeshram et al., 1995; 2000; Suthoff et al., 2001; Agnihotri et al., 2006). Isotope signatures of sedimentary N are affected more by biogeochemical processes relative to that with C isotopes. For example, easily degradable organic compounds like amino acids and carbohydrates or marine OM generally are enriched in ^{15}N and ^{13}C relative to compounds like lignin, lipids or terrestrial OM, resulting in an overall lowering of isotopic compositions of these elements (Lange de et al., 1994, Prahl et al., 1997, and Böttcher et al., 1998). In addition, apparent increases in $\delta^{15}\text{N}$ values in marine sediments have been interpreted as a consequence of bacterial metabolism or ingestion by higher trophic level organisms (Sigman et al., 1999). Thus, simultaneous measured (in same aliquot) $\delta^{13}\text{C}_{\text{org}}$ and $\delta^{15}\text{N}$ of particulate organic matter provides a unique and very useful handle in order to characterize source(s) and state of OM remineralization, especially in complex environments such as estuaries and coastal zones.

Thus, simultaneous extraction and purification of CO_2 and N_2 from a geological sample containing OM is required for their isotopes $\delta^{13}\text{C}_{\text{org}}$ and $\delta^{15}\text{N}$ measurements. These measurements were initiated with off-line preparation of analyte i.e. combustion of sedimentary OM with copper oxide (CuO) in presence of silver (Ag) at higher temperature ($\sim 850^\circ\text{C}$) followed by extraction and purification of CO_2 and N_2 on glass vacuum line. Extracted CO_2 and N_2 were then analyzed on dual inlet system of IRMS for their isotopes (Agnihotri, 2002). Such off-line protocols were compounded with several limitations such as

(i) larger amounts (~100-200mg) required for low C and N containing samples (ii) extraction and purification on glass vacuum lines was very laborious and time consuming work leading to very low sample throughputs. In recent years, usage of elemental analyzer coupled with IRMS in continuous flow mode came up with several fold advantages over off-line methods in terms of greater accuracy/ precision with much lower sample amounts (~10-25 mg) as well as high sample throughputs. We present here results of standardization and details of developed protocols for measurements of C & N isotopes of variety of geological samples using different preparatory systems of *Delta V Plus* Stable Isotope Ratio Mass Spectrometer.

2. Instrument

Due to multifaceted potential applications of N isotopes in tracking biogeochemical changes occurring in marine as well as fresh waters systems, our group in chemical Oceanography at NIO procured a new stable isotope ratio mass spectrometer (*Delta V Plus*, Thermo®; simply 'Delta' henceforth). Delta is equipped with multiport- tubecracker device, conventional dual-inlet and three external ports for connecting different preparatory systems such as Elemental Analyzer (EA), Gas Bench and separate pyrolysis unit (if needed) in continuous flow mode. The Delta has universal three cup collectors (Faraday cups) for detection of resolved ion beams. Instead of conventional flight tube, Delta is equipped with a monolithic analyzer which has some additional advantages such as wide magnet pole gap giving a superior sensitivity, intrinsic alignment of all ion optics, signal amplifiers and digitizers integrated into the analyzer block. The magnetic deflection radius of Delta is 191mm providing larger mass dispersion than its older versions. 0.75 Tesla electromagnet and 12-bit digital mass selector helps in quick peak jumps between different masses especially needed for simultaneous analysis of N₂ and CO₂ isotopes. Universal three cup collectors are used to collect the ion beams of lighter element (C, N, O and S) isotopes. For online combustion of sedimentary OM or other solid geological samples containing OM and extracting CO₂ and N₂, Delta was interfaced with an EA (EURO 3000, Eurovector®) in continuous flow mode during its installation in early 2007. Basic schematic of Delta coupled with EA in continuous flow mode is shown in Figure 1.

3. Methodology

Solid geological samples (sediment, particulate matter etc.) are decalcified with 1N HCl and dried for measurements of C_{org} and N contents, C/N (wt/wt) ratio and their isotopic ratios ($\delta^{13}\text{C}_{\text{org}}$ and $\delta^{15}\text{N}$). δ notations for C and N isotopes are described in equation 1 (section 3.1). Adequate amounts are weighed (5-50mg, depending upon their C and N content) and wrapped in tin cups. Packed tin cups are dropped into the combustion furnace (at 1050 °C) of the Elemental Analyzer. Flash combustion takes place in the presence of high purity (6-grade) oxygen (O₂). The evolved gases are passed through reduction furnace (at 670°C) filled with activated metallic copper that reduces the oxides of nitrogen (NO_x) to N₂. Dry helium of 5.5grade is used as carrier gas for sample introduction. Gas streams are then passed through a water-trap filled with magnesium perchlorate (MgClO₄) ensuring removal of moisture and separated using gas chromatograph (GC) before being introduced to open split of the *Delta* IRMS (Figure 1). Separation of N₂ and CO₂ for modern marine organic matter containing samples was optimized at 50°C oven temperature of GC. The $\delta^{13}\text{C}_{\text{org}}$ and $\delta^{15}\text{N}$ are measured together with C_{org} and N contents and the C/N ratio using Delta interfaced with EA in a continuous flow mode.

3.1 Isotopic notations of C and N

$\delta^{13}\text{C}_{\text{org}}$ and $\delta^{15}\text{N}$ are defined as -

$$\delta^{13}\text{C}_{\text{org}} \text{ and } \delta^{15}\text{N} = ((R_{\text{sample}} - R_{\text{standard}}) / R_{\text{standard}}) \times 1000 \text{ -----(1)}$$

where $R = {}^{13}\text{C}/{}^{12}\text{C}$ and ${}^{15}\text{N}/{}^{14}\text{N}$

$\delta^{13}\text{C}$ values of all the carbon containing substances are expressed with respect to *V-PDB* (Vienna Peedee Belemnite). Likewise, $\delta^{15}\text{N}$ values are expressed with respect to atmospheric N₂.

3.2 Internal Precisions of N₂ and CO₂ references on Delta

Internal precisions of N₂ and CO₂ on Delta were checked by repeated pulse injection analyses of reference gas (usually termed as Std On/Off run). For a set of 100 runs, containing 10 pulses for each gas for 20 seconds, reproducibility (internal precision) of $\delta^{15}\text{N}$ is better than

0.07% (percentage error on the mean). Likewise internal precision is better than 0.02% for $\delta^{13}\text{C}$.

The figures 2 a & b show typical standard on/off runs for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}_{\text{org}}$ respectively.

3.3 Calibration of reference tanks

Calibrations for C_{org} and N contents and their isotopes $\delta^{15}\text{N}$ and $\delta^{13}\text{C}_{\text{org}}$ were carried out using a well standardized laboratory standard (ϵ -Amino-n-Caproic acid, $\text{C}_6\text{H}_{15}\text{NO}_2$; *ACA henceforth*) supplied by School for Marine Sciences and Technology (SMAST), University of Massachusetts-Dartmouth, USA (Higginson and Altabet, 2004). Reported values of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ are 4.6‰ and -25.3‰ respectively, well suiting for the typical ranges observed in marine sedimentary OM (4–12‰ for $\delta^{15}\text{N}$ and -19– 27‰ $\delta^{13}\text{C}$). Reference tanks for N and C isotopes were made of pure N_2 (5.5grade, 99.9995%) and CO_2 (4.5grade; 99.995%). Several aliquots (n=10) of solid ACA (<1mg) were combusted on EA and analyzed on Delta analyzer. Using reported δ values of ACA, isotopic compositions of reference tanks were determined. In order to check accuracy of reference tank calibrations, five different international isotopic standards were analyzed against the calibrated reference tanks and results were compared with their reported isotopic compositions (Table 1; Figure 3). Except IAEA N-1, measured $\delta^{15}\text{N}$ of all international standards showed excellent match with their reported values corroborating accuracy of our reference tanks. Likewise, different carbon containing international isotopic standards were also analyzed in order to check accuracy of CO_2 reference tank. Results are presented in Table 2 and Figure 4. It is evident from Figure 3; $\delta^{15}\text{N}$ reproducibility is better than 0.2‰, typical overall uncertainty reported on $\delta^{15}\text{N}$ data of marine geological samples (Altabet et al., 1999). Likewise, typical overall $\delta^{13}\text{C}$ reproducibility is better than 0.2‰ (based on sedimentary *in-house* standard; shown later in section 3.6).

3.4 Size effect on N and C isotopes

After successfully calibrating reference tanks of CO_2 and N_2 and establishing measurement procedures on Delta, it is very important to investigate response of varying amounts of analyte (N_2 and CO_2 in our case) on their isotopic measurements. For this, ACA

standard being easily soluble in deionized water, serves the purpose much better. A solution containing 40 $\mu\text{mole N /ml}$ was made stoichiometrically. Appropriate amounts of this dissolved solution were dispensed on pre-combusted GF/F filter and dried overnight in a laminar flow. Dried filters were then packed in tin cups; making them ready for combustion in EA. ACAs containing N contents ranging from 1 to 6 μmole shows a conspicuous size effect (Figure 5c) as far as isotopic measurements are concerned, despite the fact ionization of N_2 in Delta of analyte having varying N contents is quite linear (Figure 5a). Slope of the curve showing size effect on $\delta^{15}\text{N}$ is thus exponential in nature (Figure 5c). Therefore, final $\delta^{15}\text{N}$ of geological samples having varying amount of N has to be corrected. We used logarithmic equation as shown in Figure 5a. Important to mention that, this ACA calibration for ‘size effect’ has to be done on daily basis as column characteristics of EA also play an important role in determining the slope of the curve. As stoichiometric C/N ratio of ACA is 6, same set of ACAs yield C contents ranging from 6 to 36 μmole . Similar to the case of N, ionization of CO_2 from analyte having varying C contents seems to be quite linear (Figure 5b). Noticeable difference with $\delta^{15}\text{N}$, $\delta^{13}\text{C}$ do not show any significant size effect (Figure 5d). Hence, it is evident that there is definite size effect on $\delta^{15}\text{N}$ while $\delta^{13}\text{C}$ measurements do not show any significant size effect (Figure 5c,d). This fact also translates into ultimate achievable precision on $\delta^{13}\text{C}$ which is relatively better than that of $\delta^{15}\text{N}$ for a given material containing organic matter.

A typical chromatogram of N and C analysis of ACA-2 (ACA filter containing 2 μmole of N and 12 μmole of C) on Delta is shown in Figure 6. Sample N_2 gas pulse is followed by pulse of reference N_2 gas. Sample CO_2 pulse is followed by that of standard (reference) CO_2 . Clear separation of two analytes (N_2 & CO_2) (Figure 6) is achieved by tuning the GC temperature (50°C in our case).

3.5 Long term reproducibility of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ on Delta

ACA internal isotopic standard supplied by SMAST-UMASS is being used as regular laboratory standard as primary standard against which standards with specific matrix viz. Gulf of California (GOC) sedimentary standard or our *in-house* sedimentary standard COD are used as secondary control standards. Here we are presenting long term reproducibility of the

ACA-2 to check whether any long term drift is there present in Delta (Figure 7). Long term reproducibility plots of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ of ACA show that most of the measurements fall within the precision limit of the instrument and there is no noticeable drift present in the instrument measurement on long term (monthly) basis.

3.6 Preparation of *in-house* sedimentary standard

For estimating overall analytical precisions of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ measurements of sedimentary organic matter (including sample preparation and analysis), a large quantity of coastal marine sediment was collected off Goa (water depth ~27 m; $15^{\circ}31.127'$, $73^{\circ}45.642'$). A chunk of ~2 kg aliquot was dried in oven (at ~65°C), powdered using a ball-mill grinder then agate mortar, decalcified using 1M HCl and washed thoroughly with deionized water and dried again. Dried powder was well-homogenized for using it as an *in-house* laboratory sedimentary standard (COD; henceforth). Several aliquots of COD were analyzed against ACA calibrations to check overall uncertainties involved in $\delta^{13}\text{C}_{\text{org}}$ and $\delta^{15}\text{N}$ measurements. Achievable precision with this sedimentary standard also was found to be better than ~0.2‰ (Figure 8). This indicates flash combustion of OM in combustion column of EA at 1050°C is apparently free from any sample matrix effect for most of the samples containing recent OM. Usage of COD along with a set of ACAs ensures smooth functioning of combustion and reduction columns of EA and thus provide additional control on a given batch of analyses. For most of our biogeochemical investigations, COD serves the purpose of internal laboratory standard. The long term reproducibility of the COD standard is as shown in the Figure 9.

3.7 Multiport- tube-cracker device – Dual inlet measurements

For batch analysis of externally extracted sample gas enclosed in sealed glass tubes, ten tube-crackers are provided which are to be attached on multi-port system of dual-inlet inlet of Delta. Thus, ten sample tubes can be fixed in these tube-crackers and could be cracked individually by pneumatically actuated valves through software. Automated multi-port tube-cracker device brings some advantages over the conventional tube-cracking methods (e.g. with magnet) such as it is less time consuming and could be done unattended reducing manual errors. Different international standards were externally extracted (for CO_2 gas) for its

isotopic measurements on dual-inlet of Delta. Extracted CO₂ samples were analyzed using automated/ multi-port tube-cracker system attached to *Delta* (Figure10). Externally extracted sealed CO₂ samples of international standards were supplied by Dr. Koushik Dutta from AMS Laboratory, Indian Institute of Physics, Bhubaneswar. Same international standards were also run as solid samples on Delta coupled with EA on continuous flow mode (Figure 4.) Results obtained by both the methods matched closely, boasting the confidence of CF-IRMS in terms of accuracy.

4. Concluding remarks

Reported isotopic data of several international and *in-house* laboratory standards produced by Delta clearly establishes accuracy and precision achievable for C and N isotopic analyses are well within 0.2‰ for a variety of geological samples. On-line production of analyte gases viz. CO₂ and N₂ by Elemental Analyzer coupled with IRMS in continuous flow mode is able to yield accuracy as good as achievable on externally (off-line) extracted analytes run on conventional dual-inlet system. Long-term running of laboratory /reference standards on Delta do not show any systematic trends and stability for both C and N isotopes is close to practical precision achievable on these isotopic data ($\leq 0.2\text{‰}$).

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Table 1: Comparison of *reported versus measured $\delta^{15}N$ values of the international standards against our calibrated reference tanks.*

Intl stds. (no. of aliquots)	Reported $\delta^{15}N\text{‰}$	Measured $\delta^{15}N\text{‰}$
ACA (2)	4.6	4.837, 4.706
IAEA-N2 (2)	20.3	20.324, 20.444
IAEA-N1(2)	0.4	0.807, 0.815
Tyrocene-(2)	10	10.152, 10.144
GOC-(2)	10.5	10.658, 10.563

Table 2: Comparison of *reported versus measured $\delta^{13}C$ values of the international standards against our calibrated reference tanks.*

Intl stds.	Reported $\delta^{13}C\text{‰}$	Measured $\delta^{13}C\text{‰}$
ANU Sucrose	-10.8 ±0.47	-10.35
NBS Ox-II	-17.8 ±0.40	-17.39
NBS Ox-I	-19 ±0.5	-19.05
IAEA_C-1	2.42 ±0.4	2.47
ACA Umass_Std	-25.3	-25.60

Table 3: Comparison of *$\delta^{13}C$ values of the externally extracted CO_2 of international standards, measured on dual-inlet of Delta using tube-cracker device with those obtained by on-line produced gas (CO_2) by EA and measured on continuous-flow mode.*

Intl stds.	Reported $\delta^{13}C\text{‰}$	Measured on Dual-inlet $\delta^{13}C\text{‰}$	Measured on EA $\delta^{13}C\text{‰}$
ANU Sucrose	-10.8 ±0.47	-10.19	-10.35
NBS Ox-II	-17.8 ±0.40	-17.29	-17.39
NBS Ox-I	-19 ±0.5	-18.97	-19.05
IAEA_C-1	2.42 ±0.4	2.63	2.47

Figure 1: Basic schematic of IRMS interfaced with EA in continuous flow mode.

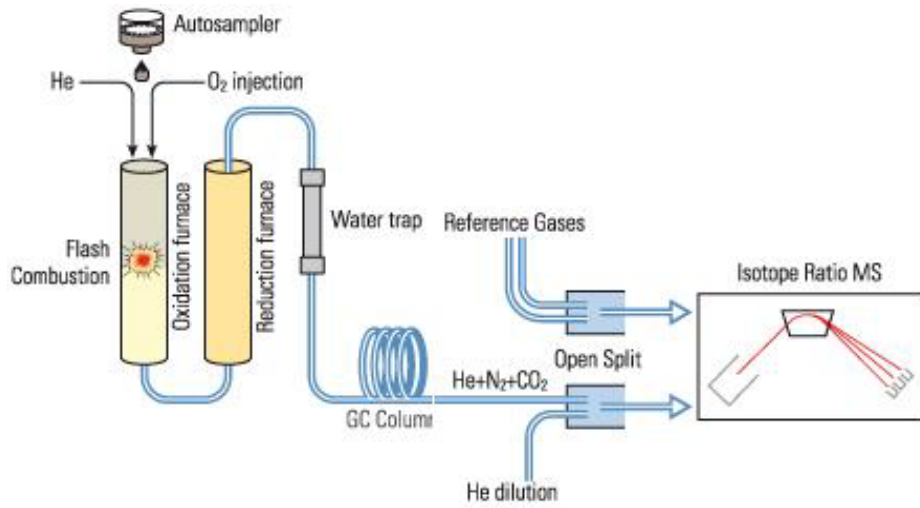


Figure 2: Standard On/Off runs of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ showing internal precision of Delta.

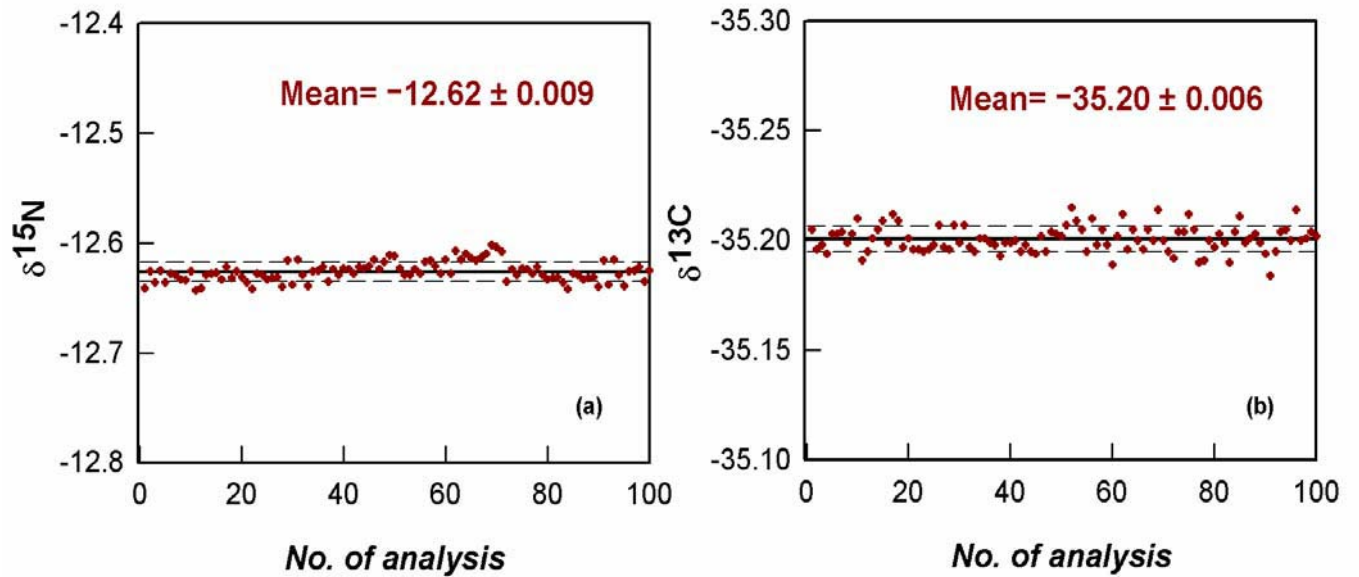


Figure 3: 5 international standards run via EA coupled to Delta V Plus mass-spectrometer in continuous flow mode. CO₂ and N₂ reference tank were calibrated using ACA.

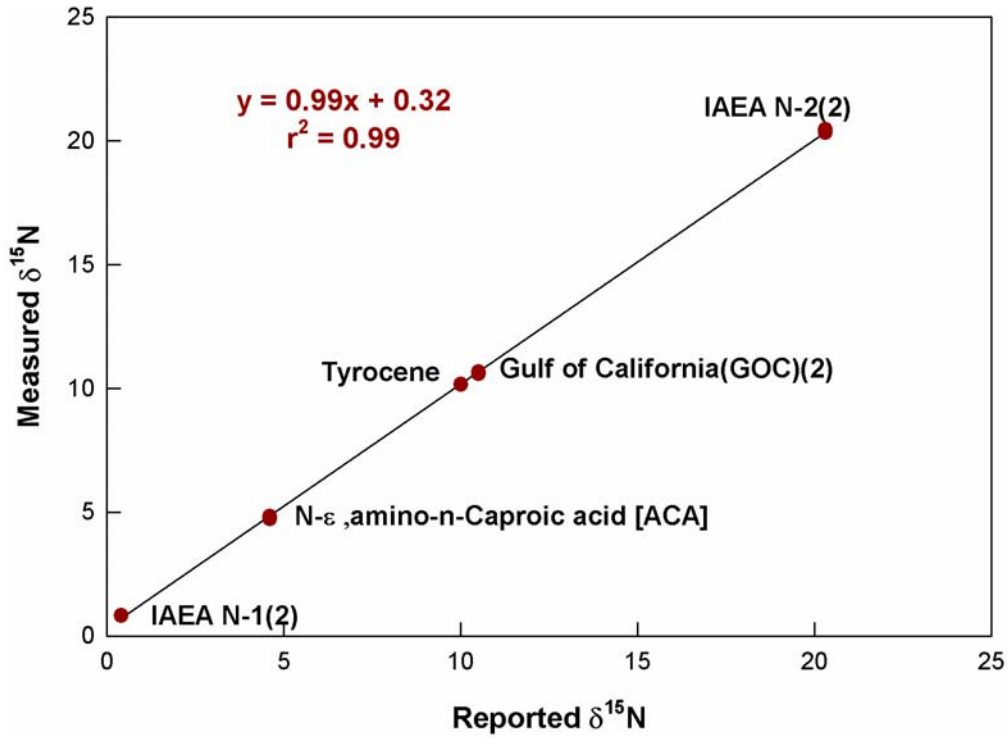


Figure 4: CO₂ produced from different international standards by Continuous flow analyzed using Delta V plus. Sample gases were introduced to mass-spec by Elemental Analyzer

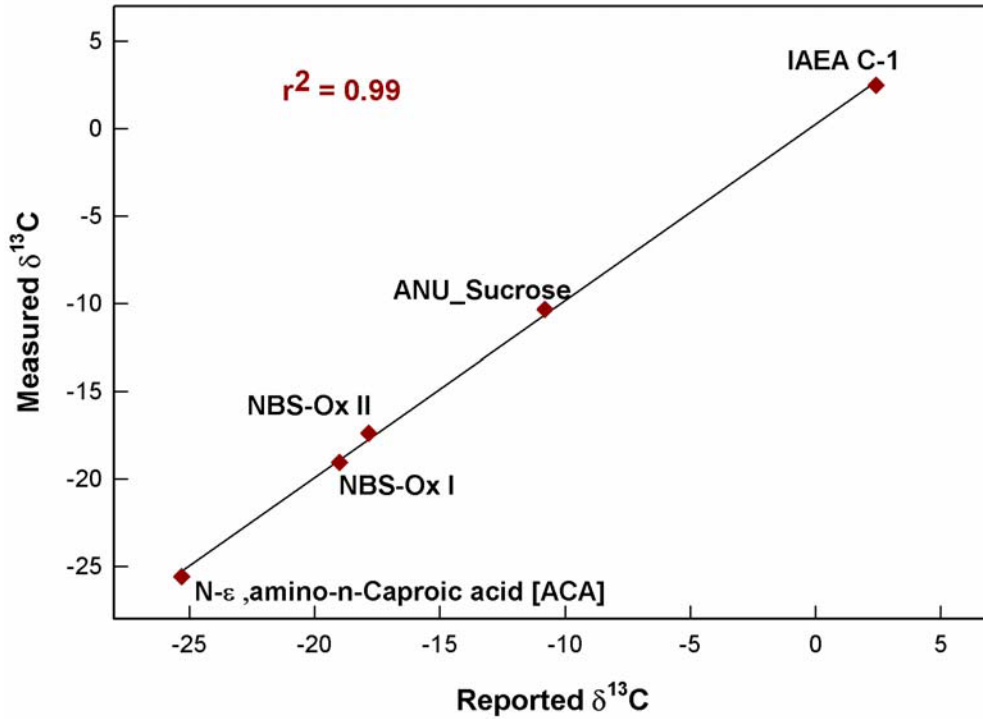


Figure 5: Observed size effects on N, C content and, $\delta^{15}\text{N}$ & $\delta^{13}\text{C}$ measurements.

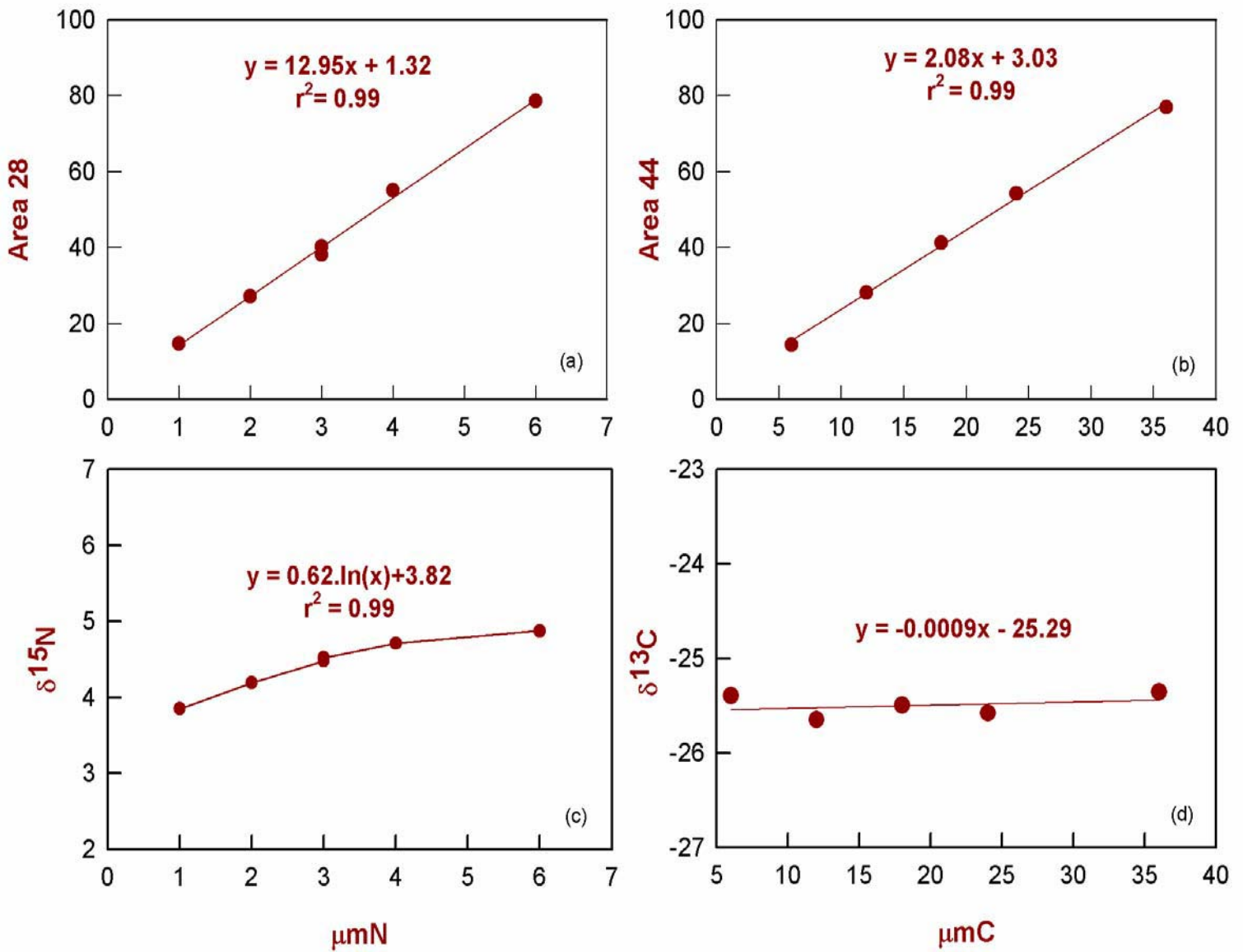


Figure 6: Typical chromatogram of N_2 and CO_2 extracted from ACA-2 on Delta IRMS.

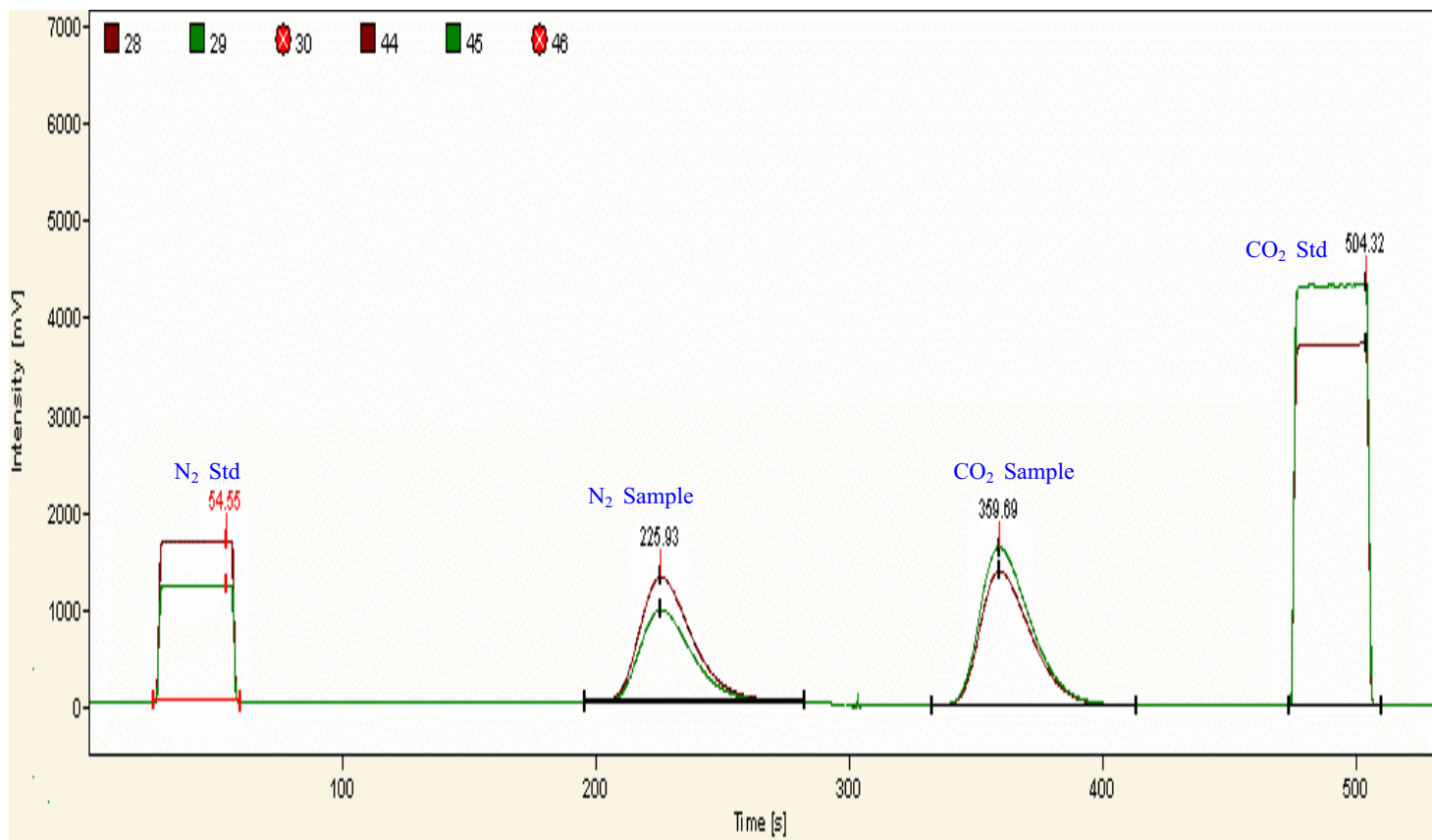


Figure 7: Long term reproducibility of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ on Delta using ACA-2. Solid line depicts the mean value whereas broken lines represent 1σ uncertainty.

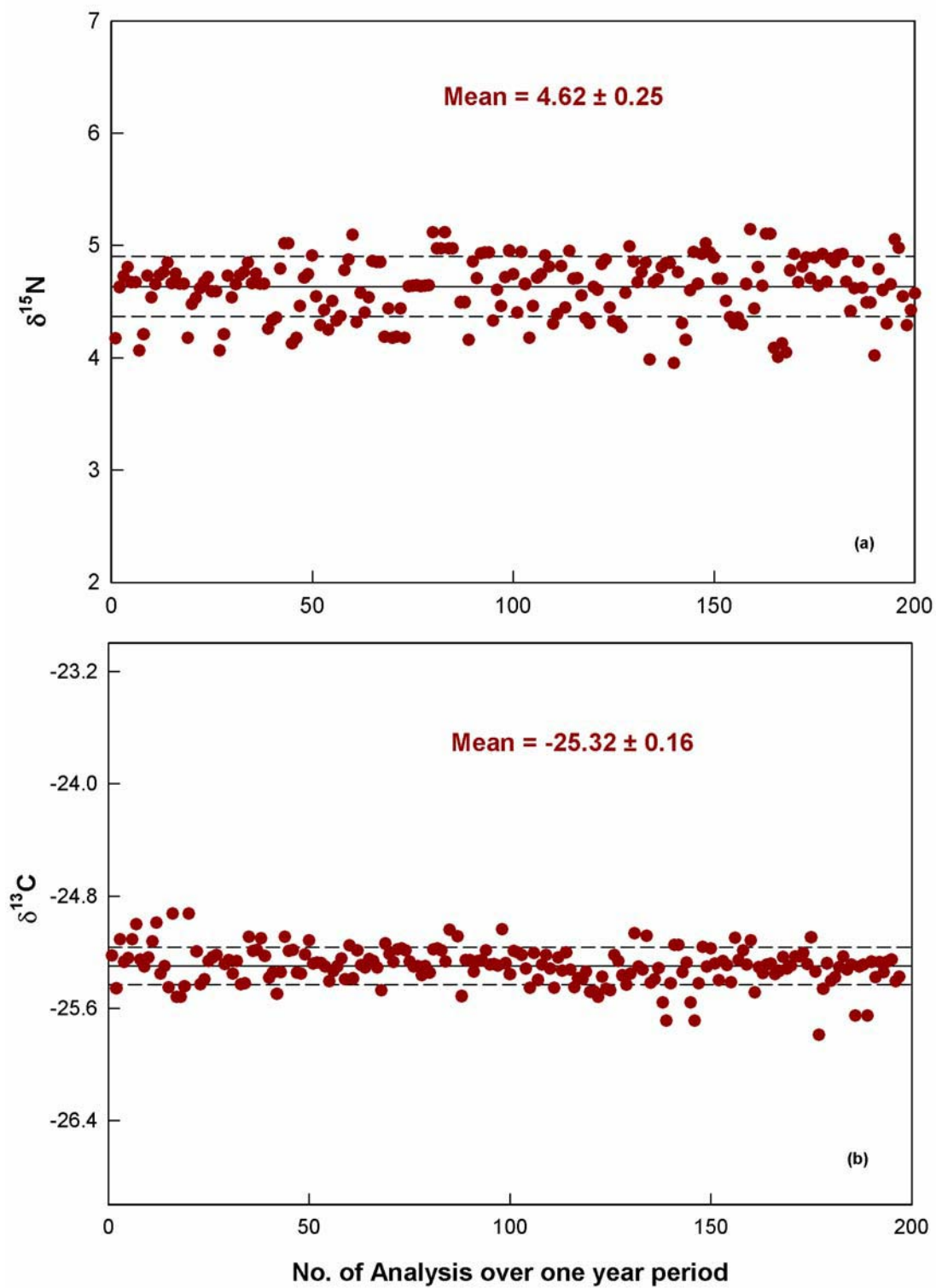


Figure 8: Internal sedimentary standard-COD $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ calibration. Solid line denotes arithmetic mean while broken lines represent 1σ standard deviations.

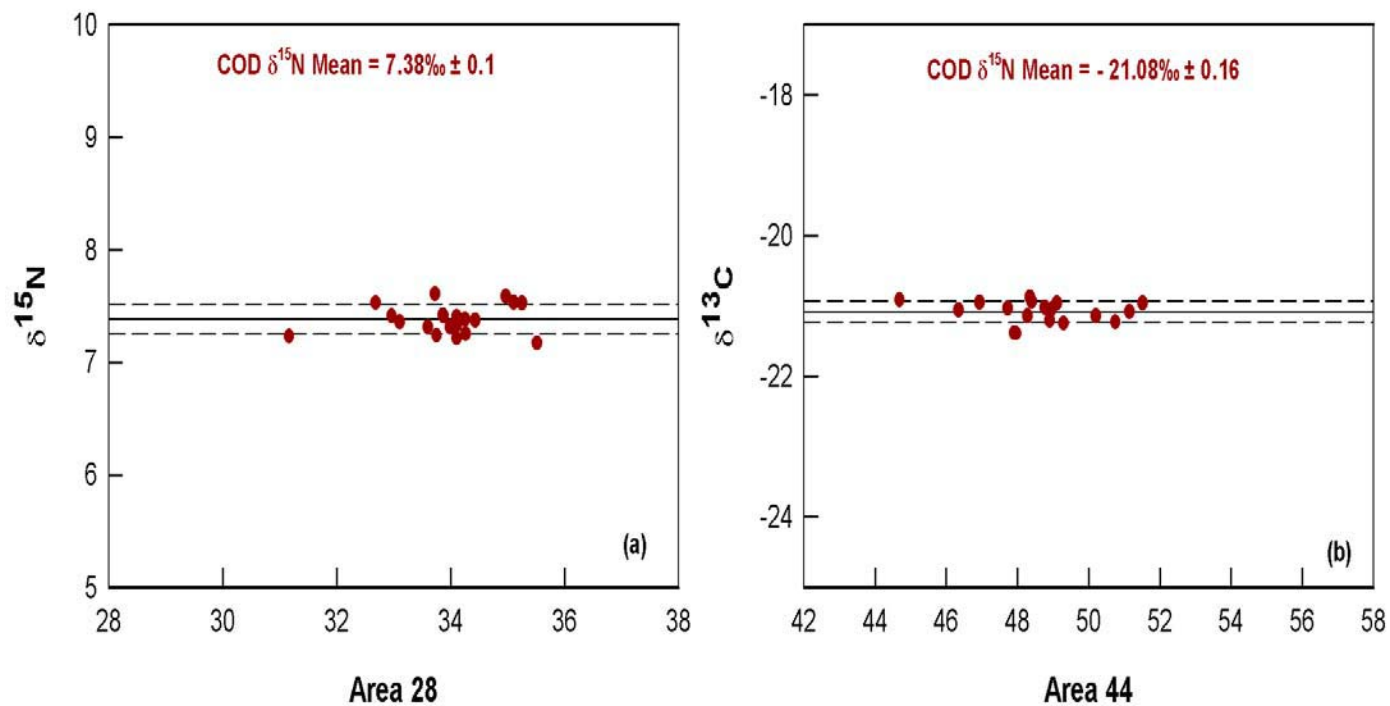


Figure 9: Long term reproducibility of in-house sedimentary standard (COD).

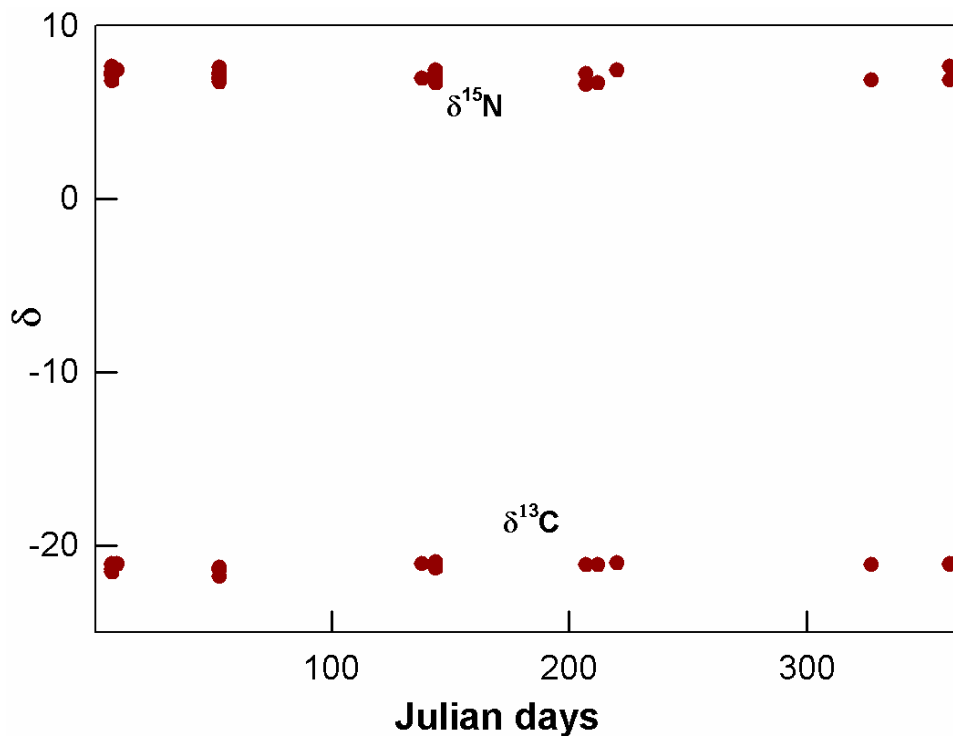


Figure 10: Externally extracted different international standards analyzed using automated multi-port tube-cracker IRMS.

