Stable carbon and nitrogen isotope analysis in aqueous samples – method development, validation and application

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“One can't understand everything at once, we can't begin with perfection all at once! In order to reach perfection one must begin by being ignorant of a great deal. And if we understand things too quickly, perhaps we shan't understand them thoroughly. I say that to you who have been able to understand so much already and… have failed to understand so much.”

Fjodor Michailowitsch Dostojewski

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Summary

Bulk and compound specific stable isotope analysis (BSIA and CSIA, respectively) of dissolved matter is of high interest in many scientific fields.

Traditional BSIA methods for carbon from aqueous solutions are time-consuming, laborious or involve the risk of isotope fractionation. No system able to analyze natural abundance stable nitrogen isotope composition of dissolved nitrogen directly (without offline sample preparation) has been reported so far. CSIA methods of dissolved carbon and nitrogen containing matter require either time consuming extraction and purification followed by elemental analysis isotope ratio mass spectrometry (EA/IRMS) or derivatization followed by gas chromatography IRMS (GC/IRMS). The only widely adopted direct method using high performance liquid chromatography IRMS (HPLC/IRMS) is suited for carbon only.

Based on these shortcomings the development and validation of analytical methods for accurate and sensitive carbon and nitrogen SIA from aqueous samples are the aims of this work.

A high-temperature combustion (HTC) system improves upon established methods. A novel total organic carbon (TOC) system, specially designed for SIA, was coupled to an isotope ratio mass spectrometer. The system was further modified to enable nitrogen BSIA. Finally, an interface for carbon and nitrogen CSIA via HPLC/IRMS was developed based on the previously developed concepts for BSIA.

Compounds resistant to oxidation, such as barbituric acid, melamine and humic acid, were analyzed with carbon recoveries of 100 ± 1% proving complete oxidation. Complete reduction of NO_x to N_2 was proven measuring different nitrogen containing species, such as nitrates, ammonium and caffeine without systematical errors. Trueness and precision of usually ≤0.5‰ were achieved for $\delta^{13}C$ and $\delta^{15}N$ CSIA, as well as BSIA. For $\delta^{13}C$ BSIA an integrated purge and trap technique and large volume injection system were used to achieve $LOQ_{SIA\ instr}$ of 0.2 mgC/L, considering an accuracy of ±0.5‰ as acceptable. In addition, the method was successfully applied to various real samples, such as river water samples and soil extracts. Further tests with caffeine solutions resulted in lower working limit values of 3.5 µgC for $\delta^{13}C$ CSIA and 20 µgN for $\delta^{15}N$ CSIA, considering an accuracy of ±0.5‰ as acceptable. Lower working limit of 1.5 µgN for $\delta^{15}N$ BSIA was achieved, considering an accuracy of ±1.0‰ as acceptable.

The novel HTC TOC analyzer coupled to an isotope ratio mass spectrometer represents a significant progress for $\delta^{13}C$ and $\delta^{15}N$ BSIA of dissolved matter. The development of a novel HPLC/IRMS interface resulted in the first system reported to be suitable for both $\delta^{13}C$ and $\delta^{15}N$ in direct CSIA of non-volatile compounds. Both may open up new possibilities in SIA-based research fields.

Zusammenfassung

Stabilisotopenanalytik von Kohlenstoff und Stickstoff in wässrigen Lösungen – Methodenentwicklung, -validierung und -anwendung.

Bulk-Stabilisotopenanalytik (BSIA) und substanzspezifische Stabilisotopenanalyse (CSIA) von wässrig gelösten Stoffen ist in vielen wissenschaftlichen Bereichen von großem Interesse.

Traditionelle BSIA-Methoden für Kohlenstoff in wässrigen Lösungen sind zeitaufwendig, mühsam oder beinhalten das Risiko der Isotopenfraktionierung. Ein System, welches die Stabile-Stickstoff-Isotopenzusammensetzung mit natürlicher Häufigkeit von gelösten Stickstoffverbindungen direkt (ohne Offline-Probenvorbereitung) analysieren kann, ist bisher in der Literatur nicht erwähnt.

CSIA-Methoden für gelöste Kohlen- und Stickstoffverbindungen benötigen entweder eine zeitraubende Extraktion und Aufreinigung, gefolgt von Elementaranalyse/Isotopenverhältnis-Massenspektrometrie (EA/IRMS) oder eine Derivatisierung, gefolgt von Gaschromatographie/IRMS (GC/IRMS). Die einzige weit verbreitete direkte Methode für Hochleistungsflüssigkeitschromatographie/IRMS (HPLC/IRMS) eignet sich nur für die Kohlenstoff CSIA.

Aufgrund dieser Defizite fokussiert sich diese Arbeit auf die Entwicklung und Validierung von Verfahren zur genauen und empfindlichen BSIA und CSIA des Kohlenstoffs und Stickstoffs in wässrigen Proben.

Ein Hochtemperaturverbrennungs-System (HTC-System) stellt eine Verbesserung gegenüber etablierten Methoden dar. Ein neuartiger und speziell für die BSIA entwickelter gesamter organischer Kohlenstoff-Analysator (TOC-Analysator), wurde mit einem Isotopenverhältnis-Massenspektrometer gekoppelt. Eine weitere Modifizierung des Systems ermöglicht die BSIA von Stickstoff. Schließlich erfolgte, unter Verwendung der zuvor gewonnenen Erkenntnisse, die Entwicklung eines Interfaces für Kohlenstoff- und Stickstoff-CSIA mittels HPLC/IRMS.

Schwer abbaubare Verbindungen, wie Barbitursäure, Melamin und Huminsäure, wurden mit den Kohlenstoff Wiederfindungsraten von 100 ± 1% analysiert um die Vollständigkeit der Oxidation zu belegen. Die vollständige Reduktion von NO_x zu N_2 wurde durch die erfolgreich durchgeführten Messungen (ohne systematischen Fehler) verschiedener stickstoffhaltiger Spezies, wie Nitraten, Ammonium und Koffein belegt. Richtigkeit und Präzision lagen in der

Regel bei ≤0,5 ‰ für $\delta^{13}C$ and $\delta^{15}N$ für die CSIA, ebenso wie für die BSIA. Für $\delta^{13}C$ BSIA wurde eine integrierte Purge and Trap Technik, sowie ein großvolumiges Einspritz-System verwendet um $LOQ_{SIA\ Instr}$ von 0,2 mgC/L zu erzielen (eine Genauigkeit von ±0,5 ‰ wurde hierfür als akzeptabel definiert). Außerdem wurde die Methode erfolgreich auf verschiedene reale Proben, wie Flusswasserproben und Bodenextrakte angewandt. Es wurde ein unterer Arbeitsbereich von 3,5 µgC für $\delta^{13}C$ CSIA und 20 µgN für $\delta^{15}N$ CSIA ermittelt (eine Genauigkeit von ±0,5 ‰ wurde hierfür als akzeptabel definiert). Es wurde ein unterer Arbeitsbereich von 1,5 µgN wurde für $\delta^{15}N$ BSIA erreicht (eine Genauigkeit von ±1,0 ‰ wurde hierfür als akzeptabel definiert).

Der neue, an einen IRMS-Detektor gekoppelte HTC TOC-Analysator, stellt einen bedeutenden Fortschritt für $\delta^{13}C$ und $\delta^{15}N$ BSIA von gelöster Materie dar. Die Entwicklung eines neuartigen HPLC/IRMS Interfaces führte zum ersten System, welches sich sowohl für direkte $\delta^{13}C$ als auch $\delta^{15}N$ CSIA von nichtflüchtigen Verbindungen als geeignet erwies. Beides könnte neue Möglichkeiten in SIA-basierten Forschungsfeldern erschließen.

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Chapter 1 General Introduction

1.1 Stable isotope analysis

1.1.1 Elements, isotopes and isotope fractionation

A trend in modern analytical chemistry is not only the identification and quantification of analytes but also the determination of their isotope composition, e.g., to infer sources or fate in the environment. Stable isotope analysis (SIA) quantifies this isotope composition and hence, provides additional and often unique means to allocate and distinguish sources of analytes as well as to identify and quantify transformation reactions.[1]

The term isotopes refers to nuclides having the same atomic number (same number of protons), but different mass numbers (different number of neutrons).[2] Object of this thesis are solely stable isotopes, i.e., those isotopes that do not undergo radioactive decay in contrast to radionuclides.

Up-to-date periodic tables[3] enclose for each element with two or more stable isotopes either an interval or a weighted average representing standard atomic weight (A_r(E)). The interval represents the span of A_r(E) values found on Earth. The weighted average is only applied if the interval is not assessed by the International Union of Pure and Applied Chemistry (IUPAC) yet.[4] This substantial change was implemented between publication of the IUPAC reports "Atomic weights of the elements 2007"[5] and "Atomic weights of the elements 2009"[6]. In the report 2007 carbon and nitrogen standard atomic weights (A_r(E)) are still given with 12.0107(8) and 14.0067(2) respectively. The number in parentheses following the last significant figure of A_r(E) represents the uncertainty. In the report 2009 A_r(C) and A_r(N) are given as an interval with [12.0096; 12.0116] and [14.006 43; 14.007 28] respectively. Calculations used in the report 2007 for standard atomic weight (weighted average) of an element are shown in Equation 1-1 and Equation 1-2.

$$A_\mathrm{r}(\mathrm{E}) = \sum[x(^i\mathrm{E}) \times A_\mathrm{r}(^i\mathrm{E})] \qquad \text{Equation 1-1}$$

$$A_\mathrm{r}(^i\mathrm{E}) = \frac{m_\mathrm{a}(^i\mathrm{E})}{\frac{1}{12}m_\mathrm{a}(^{12}\mathrm{C})} = \frac{m_\mathrm{a}(^i\mathrm{E})}{\mathrm{u}_{\mathrm{atom}\,m}} \qquad \text{Equation 1-2}$$

The notations in Equation 1-1 and Equation 1-2 are as follows: $A_r(^i\mathrm{E})$, the atomic weight of isotope ^{i}E; $x(^i\mathrm{E})$, the mole fraction of isotope ^{i}E; $u_{atom\ m}$, the unified atomic mass unit, ≈ $1.660540210 \times 10^{-27}$ kg; and $m_a(^i\mathrm{E})$, the atomic mass of isotope ^{i}E.

Using the example of A_r(N)[7]:

$$A_{\mathrm{r}}(\mathrm{N}) = 0.996337 \times 14.0030740074 + 0.003663 \times 15.000108973 = 14.0067$$

The mole fractions represent a natural abundance of corresponding isotopes as naturally found on the planet Earth and they can vary locally (variation of isotope composition), thus in Equation 1-1 averaged $x(^{i}\mathrm{E})$ values are used.

The $A_{\mathrm{r}}(\mathrm{E})$ refers to the expected atomic weight of an element in the environment of the Earth's crust and atmosphere (extraterrestrial materials are not included[6]) and thus represents the global distribution on Earth. The $A_{\mathrm{r}}(\mathrm{E})$ implement local variations[4] caused by fractionation processes occurring during physical or chemical reactions and can be used for, e.g., origin determination, investigation of reaction pathways etc.[8]

Carbon and nitrogen are in the focus of various disciplines in environmental biogeochemistry, life science, chemistry, food science and water resource management and therefore play a key role in SIA.[9,10] Figure 1-1 visualizes the range of natural variations for stable carbon and nitrogen isotopes on Earth, directly defining the $A_{\mathrm{r}}(\mathrm{E})$ intervals. For the quantitative description of stable isotope composition the delta notation was introduced by Harold Urey in the 1940s and used for the first time in a publication by McKinney et al. in 1950.[11,12] The following equation defines the $\delta^{h}\mathrm{E}$.[13]

$$\delta^{h}\mathrm{E}_{\mathrm{A,ref}} = \frac{R\left({}^{h}\mathrm{E}/\,{}^{l}\mathrm{E}\right)_{\mathrm{A}} - R\left({}^{h}\mathrm{E}/\,{}^{l}\mathrm{E}\right)_{\mathrm{ref}}}{R\left({}^{h}\mathrm{E}/\,{}^{l}\mathrm{E}\right)_{\mathrm{ref}}} \qquad \text{Equation 1-3}$$

where ${}^{h}\mathrm{E}/{}^{l}\mathrm{E}$ expresses the isotope ratio (R) of the heavy (${}^{h}\mathrm{E}$) to the light isotope (${}^{l}\mathrm{E}$) in a compound (analyte; $_{\mathrm{A}}$). $\delta^{h}\mathrm{E}$ values define the isotope composition converted to the international ratio scale ($_{\mathrm{ref}}$). $\delta^{13}\mathrm{C}$ values define carbon isotope compositions converted to the Vienna Pee Dee Belemnite (VPDB) scale. $\delta^{15}\mathrm{N}$ values define nitrogen isotope compositions converted to the AIR-N2 scale.[12,14]

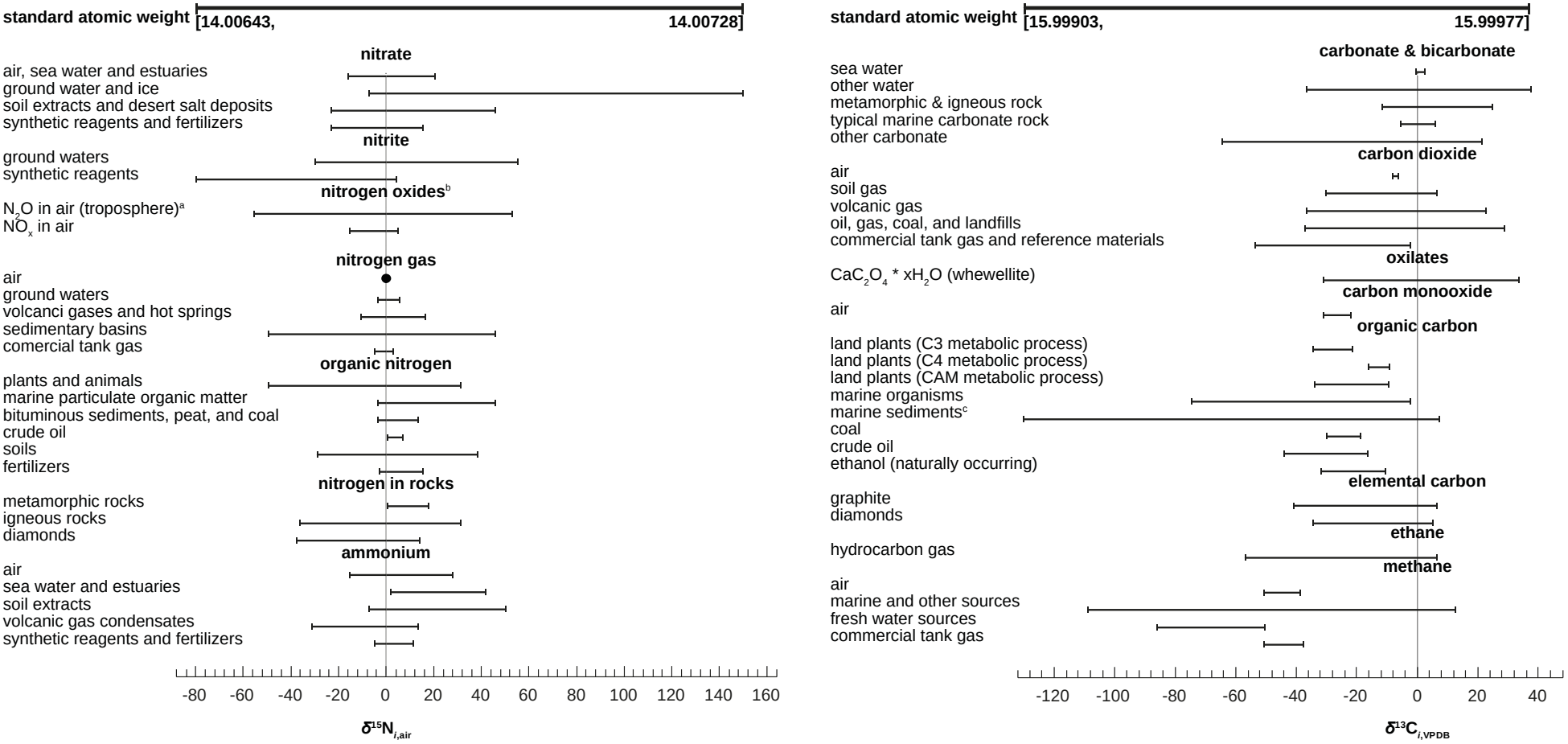

Figure 1-1 Natural variations of stable carbon (right) and nitrogen (left) isotope composition in selected materials. Isotope variations directly affect standard atomic weight interval. $\delta^{15}N$ and $\delta^{13}C$ express the isotope composition. Adapted from.[4,15,7] Notes: ([a]) N_2O in air (troposphere), sea and ground water; ([b]) NO_x from acid plant has an exceptional isotope composition with $\delta^{15}N$ of -150‰ ([c]) Marine sediments and compounds.

The δ-notation has three main advantages: Relative differences in isotope ratios can be determined far more precisely than absolute isotope ratios.[16] Additionally, it is more important to know the differences in isotope ratios between samples or compounds rather than absolute isotope ratios. The third advantage is that the low values are magnified for a better readability: the δ-notation eliminates the leading digits and makes handling of SIA results more convenient.[12,16]

Variations of isotope composition of an element occur as a result of isotope fractionation: the separation of isotopes of an element during naturally occurring processes as a result of the mass differences between their nuclei.[17] The isotope fractionation between two compounds (e.g., a substrate and its degradation product) can be expressed with the fractionation factor α[1] (see Equation 1-4).

$$\alpha = \frac{\mathrm{R}\left({}^{\mathrm{h}}\mathrm{E}/\ {}^{\mathrm{l}}\mathrm{E}\right)_{\mathrm{product}}}{\mathrm{R}\left({}^{\mathrm{h}}\mathrm{E}/\ {}^{\mathrm{l}}\mathrm{E}\right)_{\mathrm{reactant}}} = \frac{\delta^{h}\mathrm{E}_{\mathrm{product,ref}} + 1}{\delta^{h}\mathrm{E}_{\mathrm{reactant,ref}} + 1} \qquad \text{Equation 1-4}$$

The δ^hE -value of a product depends on the initial isotope composition of a reactant and on the extent of isotope fractionation during physical and chemical processes involved in the transformation of the reactant[18]. Exemplarily a reversible, nucleophilic aliphatic substitution leading to a halogen exchange in halomethanes is discussed (see Equation 1-5)[12].

$$^{13}\mathrm{CH_3I} + \mathrm{CH_3F} \leftrightarrow \mathrm{CH_3I} + {}^{13}\mathrm{CH_3F} \qquad \text{Equation 1-5}$$

Rearranging of Equation 1-4 results in:

$$\delta^{h}\mathrm{E}_{\mathrm{product,ref}} = \alpha \times \left(\delta^{h}\mathrm{E}_{\mathrm{reactant,ref}} + 1\right) - 1 \qquad \text{Equation 1-6}$$

With the corresponding α^{13}C(CH_3F/CH_3I) for the exemplary reaction, this results in:

$$\delta^{13}\mathrm{C}_{\mathrm{CH_3F,VPDB}} = 0.9703 \times \left(\delta^{13}\mathrm{C}_{\mathrm{CH_3I,VPDB}} + 1\right) - 1 \qquad \text{Equation 1-7}$$

A fractionation factor <1 implies a higher content of heavier isotope in the reactant (CH_3I) than in the product (CH_3F). In a closed system, if the δ-value for CH_3I is -10.3‰, the δ-value of CH_3F will amount to -39.7‰. Combining the fractionation factor with the mass balance equation a dependency of the CH_3F δ^{13}C value from its mass fraction ($f(CH_3F) = m(CH_3F)/m(CH_3I+CH_3F)$) can be modeled for a certain substrate isotope composition ($\delta^{13}C_{total}$) and temperature.[12]

In literature, to express isotope fractionation also the isotope enrichment factor ε (see Equation 1-8) and the 'isotope difference' $\Delta^h E_{product/reactant}$, a simple subtraction of the δ-value of the reactant from the δ-value of a product, are used.[12]

$$\varepsilon^h E_{product/reactant} = \alpha^h E_{product/reactant} - 1 \qquad \text{Equation 1-8}$$

Main physical isotope fractionation processes can be divided in those, which evolve during transport within a phase (e.g., diffusion) and those, which evolve during phase transfer between phases (e.g., evaporation). The isotope fractionation during phase transfer processes is generally small, but can be of relevance in multi-step processes. Chemical fractionation can occur during biotic and abiotic transformation processes when for identical chemical species, containing different isotopes, the reaction rates differ. Chemical fractionation occurs at the atomic level during the breaking and formation of bonds and is caused by the zero point energy differences.[17,19]

Independent of whether it is a phase transfer process or a chemical reaction the fractionation can be caused by a thermodynamic and a kinetic isotope effect (TIE and KIE, respectively). KIE is based on the fact that in most reactions molecules containing the light isotopes react faster than those containing heavy ones. Typical examples are evaporation in non-equilibrium systems or a carbon KIE for photosynthesis. TIE is also called equilibrium isotope effect and is the net sum of two opposing KIE that apply in an exchange reaction. The heavy isotope accumulates in a particular component of a system at equilibrium. A carbon TIE for CO_2 in a sealed headspace vial is an example.[20] According to Criss et al.[18] isotope disequilibrium at the Earth's surface is far more common than isotope equilibrium. Consequentially, KIEs play a decisive role for the final isotope composition of a compound.

1.1.2 Isotope ratio mass spectrometry – the SIA detector

Measurements of isotope ratios, especially at low enrichment or natural abundance, require such a high precision that it has resulted in a separate branch of mass spectrometer systems. The isotope ratio mass spectrometer is a highly precise multicollector mass spectrometer and is provided with a magnetic sector type ion optical system. Ionization in the ion source is realized either by highly sensitive thermal ionization or electron impact. The use of multiple Faraday collectors is a main requirement for the achievement of highly precise results because it allows a simultaneous collection of all relevant ion beams.[14] The elements of interest have to be converted into a gaseous form before introduction into the isotope ratio mass spectrometer (e.g., N_2 for $\delta^{15}N$ und CO_2 for $\delta^{13}C$ determination). The introduction of the gases

to the isotope ratio mass spectrometer is realized via an open split in case of continuous flow isotope ratio mass spectrometry (IRMS).[12]

Figure 1-2 illustrates the set-up of an isotope ratio mass spectrometer using the example of the system used within this thesis (IsoPrime100; Isoprime, Manchester, UK). A rectangular housing is placed under an ultra-high vacuum ($<10^{-8}$ mbar) by a turbomolecular pump located directly under the ion source and backed by an external rotary pump. Analyte gas within He (carrier gas) is introduced to the ion source via an open split connection (100 µm inner diameter (ID) fused silica placed into the 2 mm ID stainless steel tube connected to the exhaust of the inlet system, such as an elemental analyzer). Within the ion source (see Figure 1-3) analyte molecules collide with the electron beam emitted by a thorium coated iridium filament (cathode; thermal excitation at ~ 1800 °C) and accelerated by an electrostatic potential between the filament and the ion box (50 - 100 eV). Electrons follow a helical path through the source under influence of the magnetic field (two permanent source magnets) and electrons not involved in ionization are collected at the trap (anode). Analyte molecules react within the collision zone to positively charged ions (see Equation 1-9 to Equation 1-11). Ions are extracted out of the ion box by a lateral potential (-20 to 50 V) established by a repeller plate inside (back of the ion box, opposite the ion exit slit) and accelerated by an electrical potential (max. 5 kV). The ion beam leaving through the ion exit slit passes the ion optic, consisting of half plates, defining slit, z-plates and alpha plate before entering the flight tube of the housing. Half plates focus and steer the beam in the y-direction. The defining (source) slit defines the ion beam and collects any scattered ions by holding the defining slit plate at ground potential. Z-plates steer the ion beam in the z-plane. By analogy with half plates, z-plates steer by differential offset of the voltage references (±150 V). The alpha slit finally defines the maximum beam width prior to entry into the flight tube. The homogeneous magnetic field established by the electromagnet (1 - 5 A) spatially separates the ions according to their mass-to-charge ratio and thus produces defined ion beams towards the collector cups (Faraday cups). Deflection is thereby caused by the Lorentz force ($\vec{F}_{\mathrm{L}}$) and depends on the ion charge (q_{ion}), ion velocity ($\vec{v}_{\mathrm{ion}}$) and magnetic flux density vector ($\vec{B}$). Finally, the separated beams are detected within the collector array by Faraday cups (universal triple collector array for CNOS mode or four collector array, equipped with an electrostatic filter, for CHNOS mode). The signal for the rarer isotopes is amplified, e.g., for $^{13}CO_2$ (*m/*z: 45) by a factor of 100 relative to $^{12}CO_2$ (difference between feedback resistors). The amount of ions is represented by the ion current (*I*) reported in nA. Calculation of isotope

ratios, correction for known isobaric interferences (e.g. $C^{17}O^{16}O$ for $^{13}CO_2$), referencing to the reference gas and reporting of δ-values is completed by the software.[12,21]

For molecules (M), the ionization reactions by electron impact follow the relationship[12]:

$$M + e^- \rightarrow M^{+\bullet} + 2e^- \qquad \text{Equation 1-9}$$

Analyte molecules and generated molecular ions (positive radical ions) can undergo further reactions within the ion source, e.g., dissociate by further electron impact[12,22]:

$$M(ABC) + e^- \rightarrow AB^+ + C^\bullet + 2e^- \qquad \text{Equation 1-10}$$

$$M(ABC)^{+\bullet} + e^- \rightarrow AB^+ + C^\bullet + e^- \qquad \text{Equation 1-11}$$

The following examples clarify why these possible reactions need to be considered. In the presence of CO_2 in the ion source, those reactions cause formation of CO^+ (*m/z* 28) that causes isobaric interference with N_2^+ (*m/z* 28). Therefore CO_2 needs to be removed completely, e.g., via absorption in NaOH, adsorption on silica or freezing out using liquid nitrogen, prior to $\delta^{15}N$ measurements. Also for $\delta^{13}C$ measurements itself those reactions play an important role. Formation of ions depends on the partial pressure within the ion source and causes therefore an amount dependent non-linearity effect. This effect needs to be experimentally determined (quantified) using reference gas pulses and corrected for (for more details see Chapter 3).

Besides reactions caused by further electron impact, also intermolecular reactions need to be considered[23,24]:

$$M^+ + M(ABC) \rightarrow M(A)^+ + M(BC)^\bullet \qquad \text{Equation 1-12}$$

Water and methane for example can protonate the analyte molecules within the ion source possibly forming a product interfering with the heavier isotope containing molecule ($^{12}C^{16}O_2H^+$ with $^{13}CO_2$ and $^{14}N_2H^+$ with $^{14}N^{15}N^+$, respectively).[12,24] Therefore use of an appropriate drying agent and securing complete combustion are in IRMS.

In case of $\delta^{13}C$ isobaric interference of $^{13}C^{16}O_2$ with $^{12}C^{16}O^{17}O$ (both *m/z* 45) needs to be corrected for. This is done by monitoring *m/z* 46 and using a quasi-constant correlation between ^{17}O and ^{18}O (Craig correction; for more details see Chapter 3). In case of $\delta^{15}N$ isobaric interference of $^{14}N_2$ with $^{13}C^{16}O$ (both *m/z* 28) needs to be considered by ensuring CO absence. This can be accomplished by complete conversion to CO_2 that can be scavenged by, e.g. the NaOH trap.

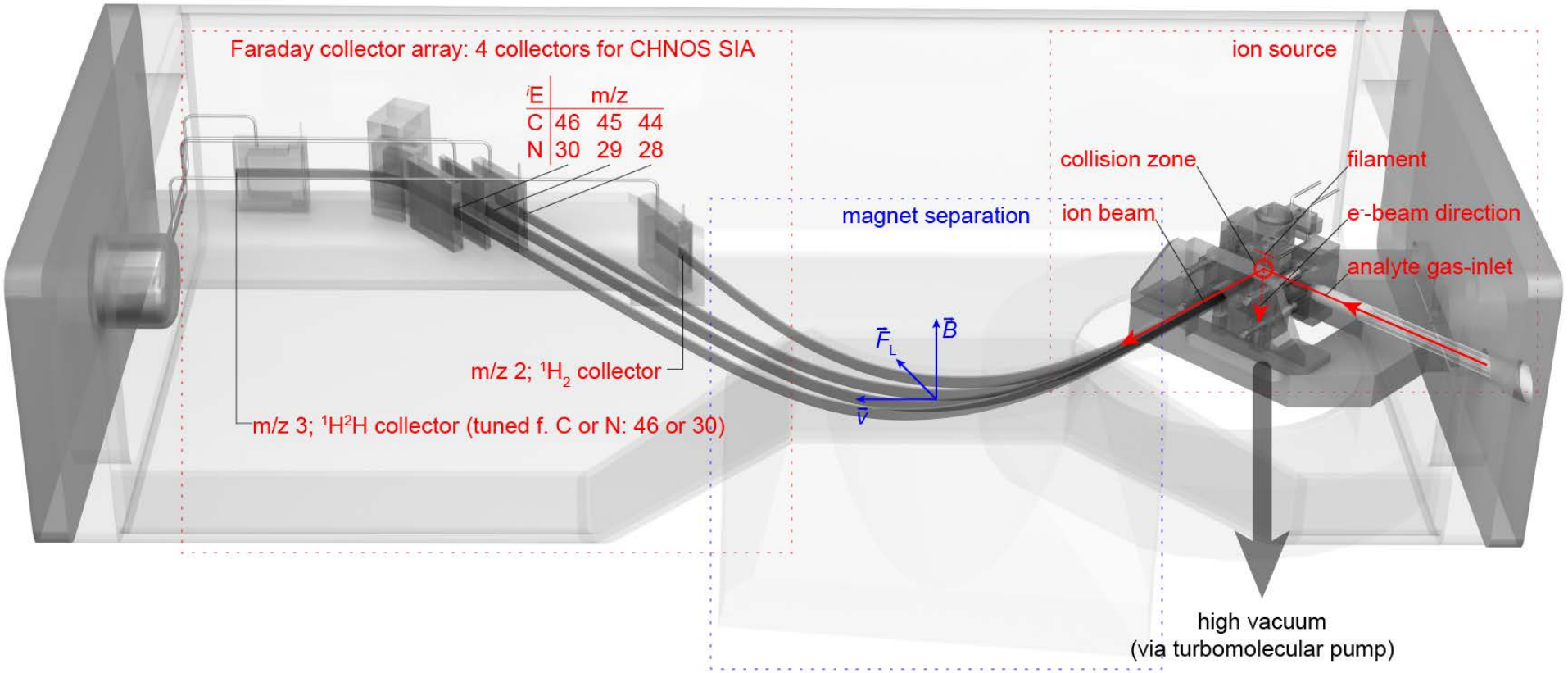

Figure 1-2 Functionality of an isotope ratio mass spectrometer (Background engineering drawing (grey) of the figure is reproduced by permission of Isoprime, Manchester, UK). Detailed ion source scheme is shown in Figure 1-3.

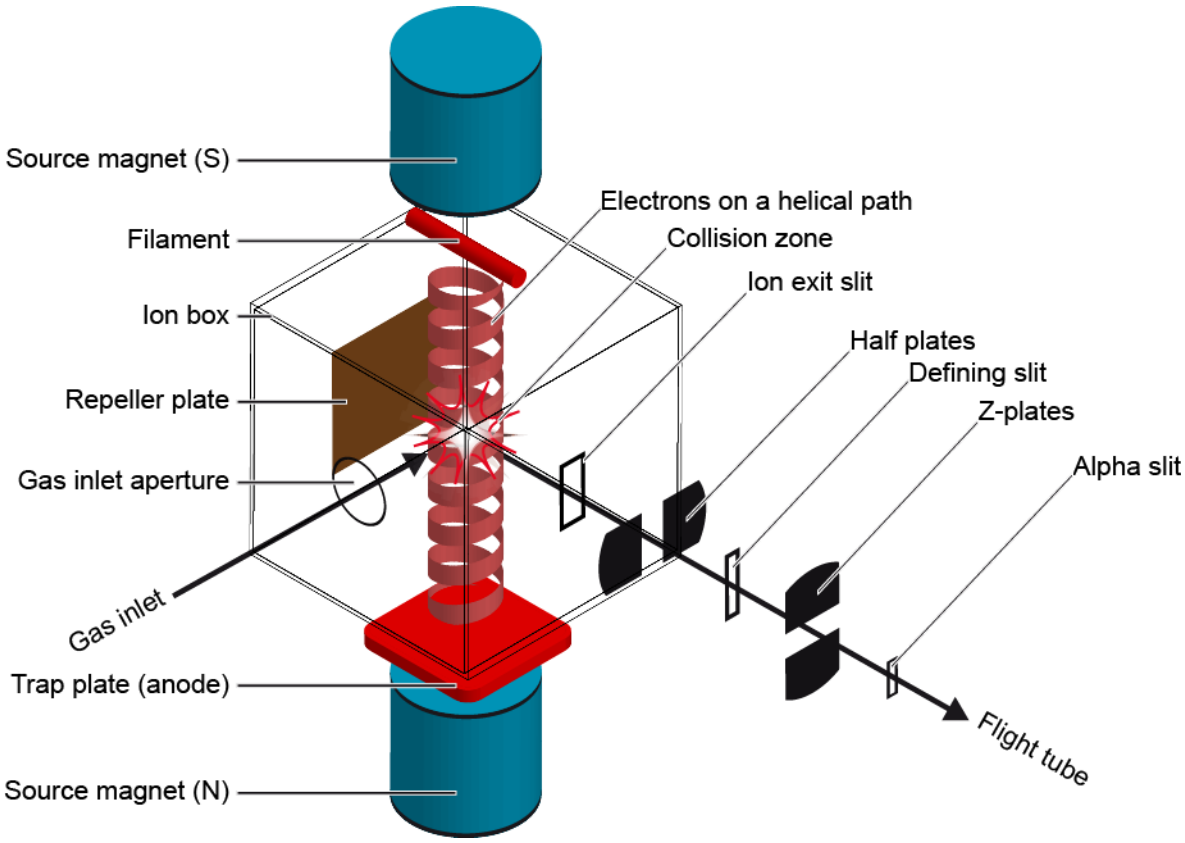

Figure 1-3 Ion source scheme. Note that the drawing is mirror-inverted in comparison to the real ion source shown in Figure 1-2. Electron entrance aperture and trap aperture (located on the upper and lower side of the ion box, respectively) are not shown.

Other common detectors for SIA are site-specific natural isotope fractionation nuclear magnetic resonance spectroscopy (SNIF-NMR), cavity ring-down laser absorption spectroscopy (CRLAS ≡ CRDS), Fourier transform infrared spectrometry (FTIR) and non-dispersive isotope-selective infrared spectrometry (NDIRS).[25–27] However, these methods are either not suited for natural isotope abundance measurements, only suited for pure liquids, not suited for coupling with chromatographic techniques or they cannot be used for N_2 SIA. IRMS on the other hand is in particular adapted to routine isotope analysis of light elements.[12,28] Thus, alternative detection methods have not been further considered and are beyond the scope of this thesis.

1.2 Carbon and nitrogen in environmental research

Carbon and nitrogen play a main role on our planet and beyond.[9,10,29,30] They are required for the existence of life and the biogeochemical cycle of C and N is indubitably an important aspect of the Earth system.[29] Therefore, both elements, in their different chemical forms, concentrations and isotope compositions, are in the focus of intensive research in science to understand the main influencing factors, such as temperature, humidity and reaction pathways, on the biogeochemical interactions on the planet Earth. Figure 1-4 systemizes the complex interactions in a very condensed form making clear the potential and reason for intensive investigation in various scientific disciplines and fields. Different sources of chemical species of C and N, their varying concentrations and isotope compositions and interactions through transport and transformation processes, e.g., between different Earth-atmosphere eco-systems makes the potential for various research fields obvious ranging from astronomy[30] via archaeology[31,32] to different fields of biogeochemistry.[9,10] Specific topics in these areas include investigations of chemical reaction pathways in environmental chemistry[33] as well as of solubility processes in physical chemistry[34].

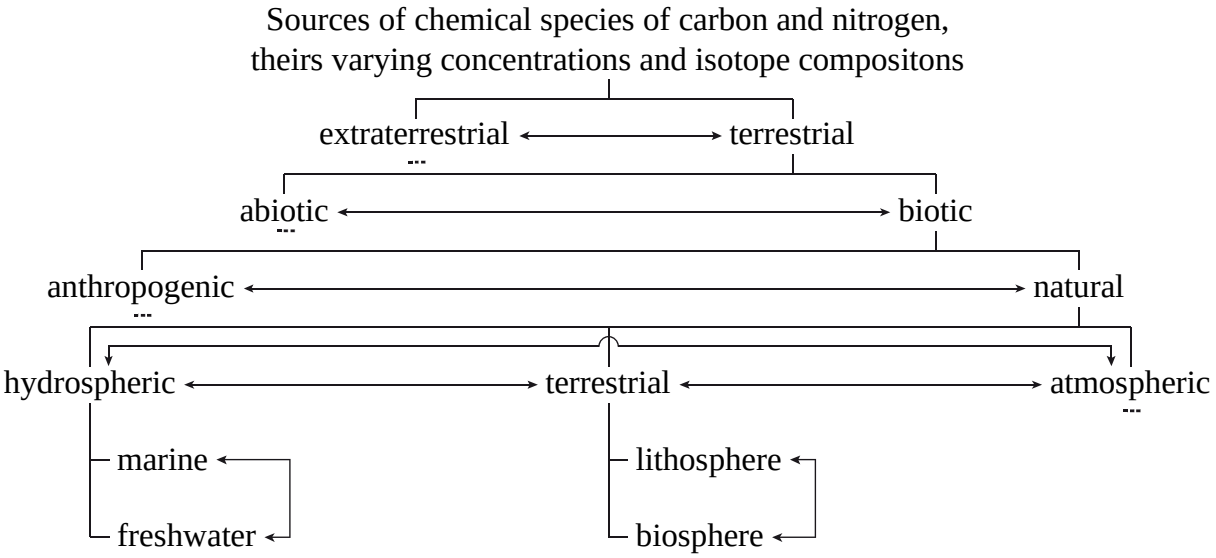

Figure 1-4 Different sources of chemical species of C and N – an overview. Ellipses indicate further subdivisions.

Carbon and nitrogen in aqueous samples imply some specific features.[35] In BSIA, in contrast to CSIA, determination of isotope composition in a sample refers, strictly speaking, to the entire set of species containing the concerned element – the isotope ratio of the bulk sample.[12] Deviating use of the term BSIA comes from the fractionation of the bulk, typically found in disciplines dealing with aqueous samples with dispersed carbon and nitrogen.[9,10,36] By previous filtration of the sample through 0.45 µm filter and acidification (pH <2) for

instance, the analyte is not total carbon (TC ≡ bulk), but its fraction dissolved organic carbon (DOC).[37] An overview of carbon and nitrogen species classification is given in Figure 1-5. To keep it simple and because the concerned SIA methods are still not compound specific the term BSIA will be extended (bulk and groups of compounds SIA) in this thesis.

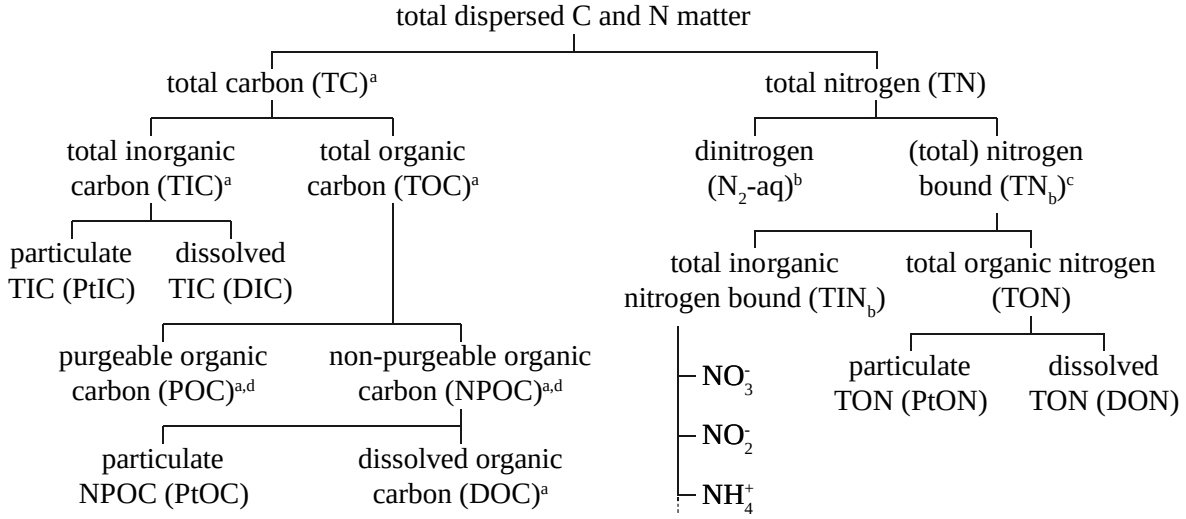

Figure 1-5 Classification of dispersed carbon and nitrogen matter; ([a]) defined by International Organization for Standardization (ISO) 8245[37]; ([b]) considering IUPAC recommendations[38]; ([c]) defined by ISO 12260[39]; In, e.g., soil-science studies TN_b measured in aqueous samples is often termed total dissolved nitrogen (TDN)[40,41] ([d]) volatile organic carbon (VOC) and non-volatile organic carbon (NVOC) are often incorrectly set equal with POC and NPOC respectively[42] and are not clearly distinguished within the norm.[37] A VOC is any organic compound having a boiling point ≤250 °C[43], thus comprised out of compounds such as benzene, toluene, cyclohexane and so one. Besides VOC, the purging process can remove further compounds by a continuous shift of equilibrium (Le Châtelier principle). Thus VOC is a part of POC.

Note that dissolved matter, in contrast to particulate matter, is defined in this classification operationally by passing a 0.45 µm filter pore size. Despite being generally accepted, this is in contrast to the fundamental definition in chemistry, where dispersion is a solution if the dissolved matter is <1 nm, a colloid if the dispersed matter lies between 1 nm – 1 µm and a suspension if the dispersed matter is >1 µm.[44]

Stable isotope data is used for a broad range of applications:

Variation in $\delta^{13}C$ values can be used to follow carbon flow through food webs as well as identifying sources of carbon contributing to soil organic matter or sediments.[9] Application

of ^{15}N-tracer techniques helps to investigate the nitrogen cycle in marine and fresh waters.[10] Stable carbon and nitrogen isotope composition can be used to investigate sources of pollution and pathways of transformation.[45] Stable isotope data is also used to define atomic weights.[4] Further examples can be found in corresponding sections.

1.3 Instrumental and methodological background for SIA in aqueous samples

While the isotope ratio mass spectrometer is considered to be the best suited detector for SIA, as addressed in this thesis, there is a large variety of sample preparation devices for special purposes. Classification of techniques considering the kind of sample introduced into the conversion interface (bulk or individual compound) is generally accepted and is illustrated in Figure 1-6. The illustration excludes position-specific stable isotope analysis (PSIA)[12], because it is out of the scope of this thesis.

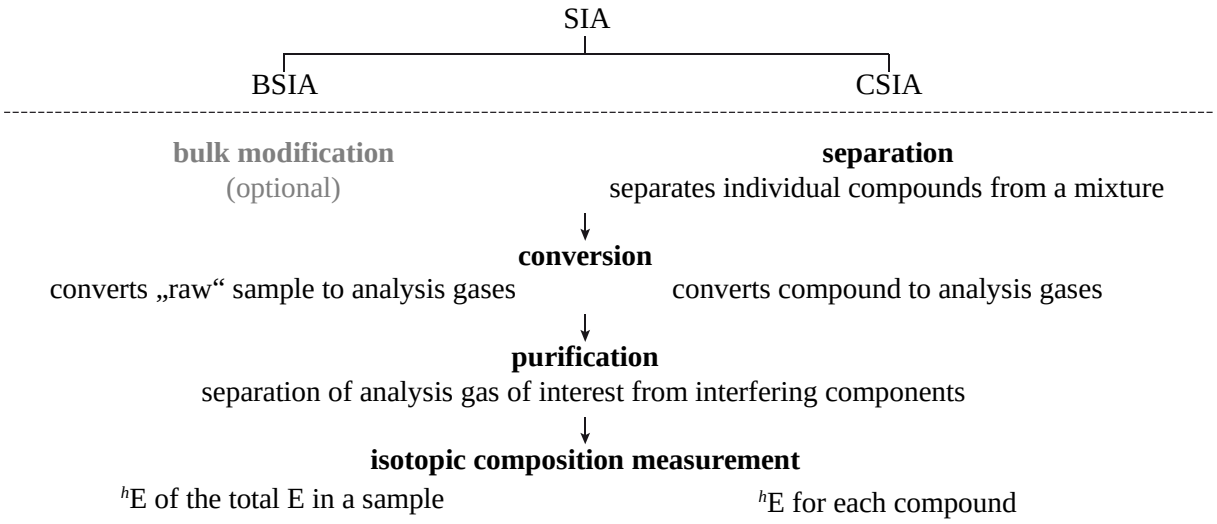

Figure 1-6 Classification of SIA techniques. Optional bulk modification is for example removal of total inorganic carbon (TIC) (via acidification and purging) prior to total organic carbon (TOC) SIA. Without this modification the measurement would relate to total carbon (TC) SIA. Also removal of the main matrix such as water (via lyophilisation) is a common bulk modification technique in BSIA.

Various analytical methods are available for stable carbon and nitrogen isotope analysis in aqueous samples.[9,10,46–49] A closer look at these methods, combined with the classification in Figure 1-6 reveals conversion and purification as a common central aspect of research and development in BSIA and CSIA methodology. Isotope ratio mass spectrometer follows as a standard detector for BSIA and CSIA and a separation technique is preceded in CSIA.

The various techniques can be classified into four main principles applied, whereby the first two are common for BSIA and CSIA:

1. Offline sample-preparation, such as lyophilization of the whole sample (BSIA) and extraction or purification of individual compounds from the sample (CSIA), followed by

elemental analyzer/isotope ratio mass spectrometry (EA/IRMS).[46,50,51] The interface design is shown in Figure 1-7.

The conversion of the analyte is performed in two steps. Combustion of the analyte to CO_2 and N_2 and NO_x is performed at high temperature (usually ≥650 °C) by oxygen. Combustion is often supported by a catalyst (e.g., Pt) and/or oxygen donor (e.g., CuO). The combustion temperature is adjusted to the working optimum of the chosen supportive material. NO_x is converted to N_2 on a reducer, such as Cu.

The conversion reaction (complete oxidation) for carbon can be expressed as following, shown exemplarily for a hydrocarbon:

$$C_xH_y + \left(x + \frac{y}{4}\right) O_2 \rightarrow \left(\frac{y}{2}\right) H_2O + xCO_2 \qquad \text{Equation 1-13}$$

The conversion reactions for nitrogen can be formulated in a simplified manner as follows (Equation 1-14 (oxidation) and Equation 1-17 (reduction)):

$$R{-}N_u + vO_2 \rightarrow wN_2 + xN_yO_z \qquad \text{Equation 1-14}$$

The yield of dinitrogen and nitrogen oxides depends strongly on combustion conditions (oxygen concentration, temperature and supportive material used) and concentration and species composition of nitrogen containing matter (e.g., nitrates, ammonium and various organic compounds). The main nitrogen oxide species is nitric oxide (NO). Nitrous oxide (N_2O) formation is insignificant at temperatures above ca. 600 °C[52]:

$$2\,N_2O \rightarrow 2N_2 + O_2 \qquad \text{Equation 1-15}$$

Nitrogen dioxide (NO_2) may be initially formed, but above temperatures of ca. 650 °C the equilibrium is shifted completely to the side of nitric oxide[52]:

$$2\,NO_2 \rightarrow 2NO + O_2 \qquad \text{Equation 1-16}$$

Reduction on cupper, typically used in elemental analysis since description by Dumas in 1833, can be formulated as:

$$2Cu + 2NO \rightarrow 2CuO + N_2 \qquad \text{Equation 1-17}$$

The purification system often consist of a dryer, such as a membrane dryer (Nafion™) or a chemical dryer (Sicapent®) and further filters or traps, such as a hydrogen halides and halogens trap. A separation unit (GC column or CO_2-focusing unit) is installed to separate CO_2 prior to N_2 measurements.

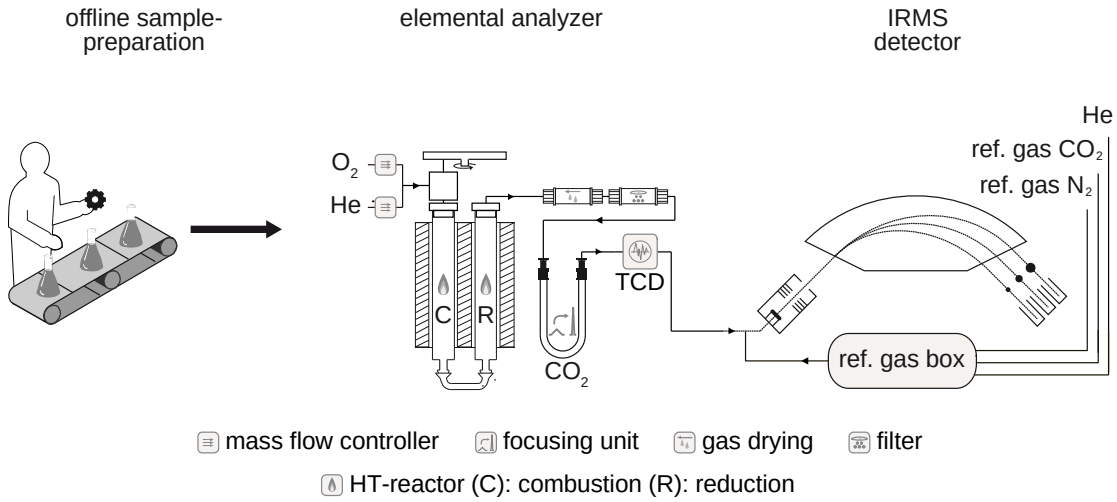

Figure 1-7 EA based technique. After the sample is brought into the solid form (offline sample-preparation), it is introduced using an autosampler into the high-temperature (HT) system. The combustion of the analyte is performed at high temperature (usually ≥600 °C) by oxygen and often supported by a catalyst and/or oxygen donor. Passing the reduction reactor, He as a carrier gas transports the analyte and other contents (matrix) to the purification system (often consisting of a dryer and a further filter). After the separation unit, analyses gases N_2 and CO_2, respectively, are directed by the He gas stream towards the optional concentration detector, such as thermal conductivity detector (TCD) and subsequently towards the open split connection of the isotope ratio mass spectrometer.

2. Wet chemical oxidation based techniques present the second principle. For BSIA a wet chemical oxidation based total organic carbon analyzer coupled to isotope ratio mass spectrometry (WCO TOC/IRMS) is used.[47,53] With respect to upstream separation equipment, CSIA can use the same principle, but with different dimensions and slightly different set-up of the instrumentation.[54,55] A typical interface design is shown in Figure 1-8 (Note that chemicals and radical generation techniques may differ).

So far, wet chemical oxidation (WCO) based systems are used for carbon SIA only. The most common oxidation reagent is sodium peroxodisulfate. The conversion of the analyte to CO_2 is performed mainly by sulfate radicals ($SO_4^{\bullet-}$; standard reduction potential $E°$ = 2.47) as main oxidative species, but also by peroxodisulfate ($S_2O_8^{2-}$; $E°$ = 2.01). Sulfate radicals are generated, e.g., thermally, by UV-photons or metal ions[12]:

$$S_2O_8^{2-} \xleftrightarrow{T,UV,metalions} 2SO_4^{\bullet-} \qquad \text{Equation 1-18}$$

The conversion reaction for carbon can be expressed as following, shown exemplarily for an average sum formula for carbohydrates CH_2O[12]:

$$4S_2O_8^{2-} + 2CH_2O + 2H_2O \rightarrow 8SO_4^{2-} + 2CO_2 + 8H^+ \qquad \text{Equation 1-19}$$

Formed CO_2 is subject to three processes: gas dissolution (Equation 1-20), carbonic acid formation (Equation 1-21) and carbonic acid equilibrium (Equation 1-22).

$$CO_2(g) \leftrightarrow CO_2(aq) \qquad \text{Equation 1-20}$$

$$CO_2(aq) + H_2O \leftrightarrow H_2CO_3(aq) \qquad \text{Equation 1-21}$$

$$H_2CO_3(aq) \leftrightarrow H^+(aq) + HCO_3^-(aq) \leftrightarrow 2H^+(aq) + CO_3^{2-}(aq) \qquad \text{Equation 1-22}$$

The buffer (pH ≤2) prevents bicarbonate and carbonate formation by shifting the carbonic acid equilibrium completely to the carbonic acid side. Purging out of the CO_2(g) shifts the gas dissolution equilibrium (Equation 1-20), which results in a corresponding shift of carbonic acid formation equilibrium (Equation 1-21).

WCO based systems for CSIA are adjusted with respect to continuous flow conditions (run-through reactor).[12]

Purification is analogous to that of HTC based EA systems described before. Purification in WCO based systems for CSIA is adjusted with respect to continuous flow conditions (additional membrane separation unit).[12]

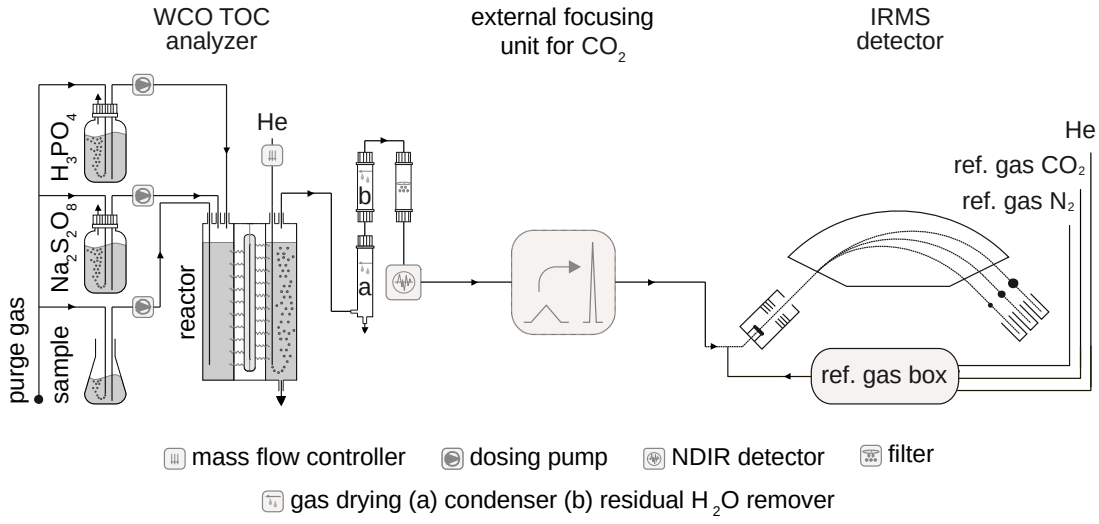

Figure 1-8 WCO TOC based technique. After the sample is introduced into the wet chemical oxidation (WCO) based system, oxidation reagents (e.g., sodium persulfate) and buffer solution are added. Highly reactive radicals are generated, e.g., by UV-photons. The formed

conversion product CO_2 is purged out by He and transported to the purification system (often consisting of a dryer and further filters). After the optional focusing unit (BSIA only), analysis gas CO_2 is directed towards the optional concentration detector, such as a nondispersive infrared (NDIR) detector, and subsequently towards the open split connection of the isotope ratio mass spectrometer.

3. High-temperature combustion TOC-analyzer coupled to an IRMS detector (HTC TOC/IRMS) was also utilized, but for BSIA only.[56,57] The main difference to the EA/IRMS methods is the design of the gas drying system suited to handle the large amount of water as a main matrix of the samples used. A typical interface design is shown in Figure 1-9.

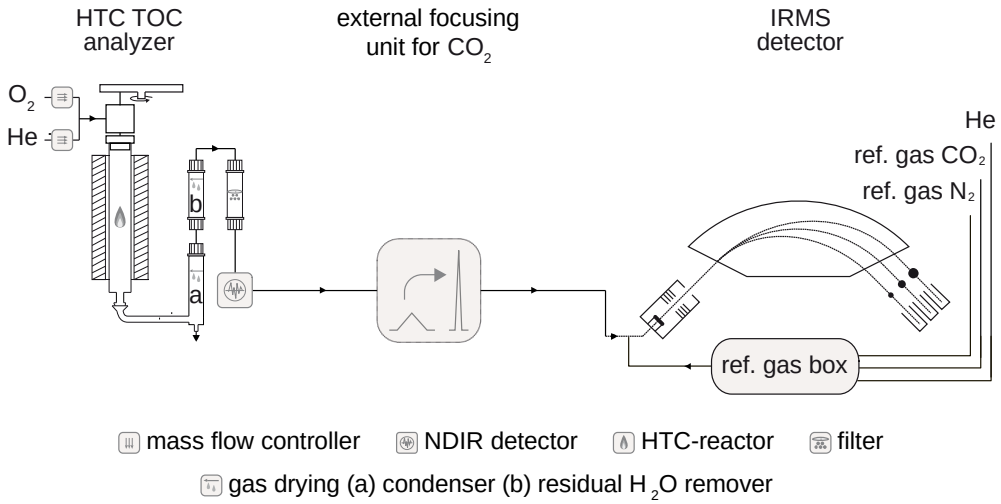

Figure 1-9 HTC TOC based technique. Aqueous samples are introduced using an autosampler into the high-temperature combustion TOC-Analyzer. The combustion of the analyte is performed at high temperature (usually ≥600 °C) by oxygen and often supported by a catalyst and/or oxygen donor. He as a carrier gas transports the analyte and other contents (matrix) to the purification system (often consisting of a condenser, dryer and further filters). After the separation unit, analyses gases N_2 and CO_2, respectively, are directed by the He gas stream towards the optional concentration detector, such as a NDIR detector and subsequently towards the open split connection of the isotope ratio mass spectrometer.

4. Derivatization followed by gas chromatography/isotope ratio mass spectrometry (GC/IRMS)[58] for CSIA of non-volatile compounds. The main difference of the interface to the EA are the dimensions of the reactors (HT combustion and reduction) and gas drying system to avoid peak broadening and suited to the continuous, but low gas flow used.

In addition to these four main principles for CSIA of dissolved (non-volatile) compounds the use of thermospray and a moving belt interface was described for the coupling of HPLC with IRMS.[59] Simultaneously, a chemical reaction interface (CRI) following HPLC separation was also combined with IRMS.[60] None of these technologies were further developed to a commercial instrument, though. In the case of the CRI the large signal from the reactant gas (O_2^+; *m/z* 32) spreads into the cup for the analyte gas ($N^{15}O^+$; *m/z* 31) preventing $\delta^{15}N$ measurement, while byproducts in the plasma (CO^+, NO_2^+ and $C_2H_5O^+$) led to incorrect $\delta^{13}C$ values.[60] In the case of the moving belt, no $\delta^{15}N$ SIA was ever reported and for $\delta^{13}C$ SIA the limitations include the limited capacity of the wire, depletion of semivolatile compounds with potential isotope fractionation and flow restriction[12].

The four main principles described are all applied, but have following limitations:

EA/IRMS BSIA and CSIA of non-volatile compounds need a very time-consuming and laborious offline sample preparation. It also involves a higher risk of contamination and fractionation. Possible fractionation must also be controlled in derivatization for subsequent GC/IRMS measurements. Both EA/IRMS and GC/IRMS CSIA also require additional corrections, which increase the uncertainty of the determined values.[50,61–65]

The WCO-based methods run the risk of carbon concentration underestimation as well as of isotope fractionation due to incomplete oxidation.[66] Incomplete oxidation is a well-known issue for seawater samples, in which sulfate radicals are scavenged by chloride ions, and are therefore no longer available for analyte oxidation.[67] Similarly, in soil science, compounds such as humic or fulvic acid that are resistant to oxidation are reported not to be completely oxidized by WCO[66] with the risk of compound-specific isotope fractionation. For samples with unknown composition such errors are non-systematic and cannot be corrected for in BSIA. WCO based interface for CSIA do not allow the measurement of $\delta^{15}N$ values. Furthermore, WCO-based systems for $\delta^{13}C$ CSIA suffer from the same problem as is common in BSIA, i.e. the risk of isotope fractionation due to incomplete oxidation.[66,34]

Many publications suggest HTC TOC analyzers as the most suitable device for DOC concentration measurements.[68] However, commercially available HTC-based systems are not optimized for SIA mainly because of their insufficient sensitivity.[56] For CSIA the principle was never applied.

Furthermore, a recent worldwide proficiency test[69] identified several common problems with reproducibility and consequently data validity for interpretation and comparability among institutes using different analytical techniques. Generally accepted or even standardized

operating procedures have been developed for many bulk and compound-specific stable isotope analyses.[11,23,70-73] However, the field of DOC SIA still shows a lack of standardized methods and approaches to account for all parameters required for accurate results such as the minimal required combustion temperature for complete mineralization or the handling of blanks.

In view of the limitations of the analytical techniques discussed above the aim of this thesis was to develop a novel HTC-based inlet system for $\delta^{13}C$ and $\delta^{15}N$ BSIA and an interface for $\delta^{13}C$ and $\delta^{15}N$ CSIA in aqueous samples and also to propose a data evaluation approach for DOC SIA.

1.4 References

[1] T. C. Schmidt, L. Zwank, M. Elsner, M. Berg, R. U. Meckenstock, S. B. Haderlein. Compound-specific stable isotope analysis of organic contaminants in natural environments: a critical review of the state of the art, prospects, and future challenges. Anal. Bioanal. Chem. 2004, 378, 283.

[2] IUPAC Gold Book. Isotopes. http://goldbook.iupac.org/I03331.html

[3] N. E. Holden, T. B. Coplen. ConfChem Conference on A Virtual Colloquium to Sustain and Celebrate IYC 2011 Initiatives in Global Chemical Education: The IUPAC Periodic Table of Isotopes for the Educational Community. J. Chem. Educ. 2013, 90, 1550.

[4] M. E. Wieser, N. Holden, T. B. Coplen, J. K. Bohlke, M. Berglund, W. A. Brand, P. De Bievre, M. Groning, R. D. Loss, J. Meija, T. Hirata, T. Prohaska, R. Schoenberg, G. O'Connor, T. Walczyk, S. Yoneda, X. Zhu. Atomic weights of the elements 2011 (IUPAC technical report). Pure Appl. Chem. 2013, 85, 1047.

[5] M. E. Wieser, M. Berglund. Atomic weights of the elements 2007 (IUPAC Technical Report). Pure Appl. Chem. 2009, 81, 2131.

[6] M. E. Wieser, T. B. Coplen. Atomic weights of the elements 2009 (IUPAC technical report). Pure Appl. Chem. 2011, 83, 359.

[7] T. B. Coplen, J. K. Bohlke, P. De Bievre, T. Ding, N. E. Holden, J. A. Hopple, H. R. Krouse, A. Lamberty, H. S. Peiser, K. Revesz, S. E. Rieder, K. J. R. Rosman, E. Roth, P. D. P. Taylor, R. D. Vocke, Y. K. Xiao. Isotope-abundance variations of selected elements (IUPAC technical report). Pure Appl. Chem. 2002, 74, 1987.

[8] R. Michener, K. Lajtha, Editors. Stable Isotopes in Ecology and Environmental Science. Blackwell Publishing, Oxford, 2007.

[9] D. C. Coleman, B. Fry. Carbon Isotope Techniques. Academic Press, San Diego, 1991.

[10] T. H. Blackburn, R. Knowles. Nitrogen Isotope Techniques. Academic Press, San Diego, 1993.

[11] C. R. McKinney, J. M. McCrea, S. Epstein, H. A. Allen, H. C. Urey. Improvements in mass spectrometers for the measurement of small differences in isotope abundance ratios. Rev. Sci. Instrum. 1950, 21, 724.

[12] M. A. Jochmann, T. C. Schmidt. Compound-Specific Stable Isotope Analysis. Royal Society of Chemistry, Cambridge, 2012.

[13] T. B. Coplen. Guidelines and recommended terms for expression of stable-isotope-ratio and gas-ratio measurement results. Rapid Commun. Mass Spectrom. 2011, 25, 2538.

[14] I. T. Platzner. Modern Isotope Ratio Mass Spectrometry. JohnWiley, Chichester, 1997.

[15] T. B. Coplen, J. A. Hopple, J. K. Bohlke, H. S. Peiser, S. E. Rieder, H. R. Krouse, K. J. R. Rosman, T. Ding, R. D. Vocke Jr., K. M. Revesz, A. Lamberty, P. Taylor, P. De Bievre. Compilation of minimum and maximum isotope ratios of selected elements in naturally occurring terrestrial materials and reagents. U.S. Geological Survey, Denver, 2002.

[16] J. T. Brenna, T. N. Corso, H. J. Tobias, R. J. Caimi. High-precision continuous-flow isotope ratio mass spectrometry. Mass Spectrom. Rev. 1998, 16, 227.

[17] J. Hoefs. Stable Isotope Geochemistry. Springer, Berlin, 2013.

[18] R. E. Criss. Principles of Stable Isotope Distribution. Oxford University Press, Oxford, 1999.

[19] C. M. Aelion, P. Höhener, D. Hunkeler, R. Aravena. Environmental Isotopes in Biodegradation and Bioremediation. CRC Press, Boca Raton, 2009.

[20] B. Fry. Stable Isotope Ecology. Springer, Berlin, 2007.

[21] Isoprime Users's Guide (Version 1.02). Isoprime, Manchester, 2012

[22] J. H. Gross, in Mass Spectrometry – A Textbook. (Eds: J. H. Gross). Springer, Berlin, 2011, pp. 21–66.

[23] A. L. Sessions, T. W. Burgoyne, J. M. Hayes. Correction of $H3^+$ contributions in hydrogen isotope ratio monitoring mass spectrometry. Anal. Chem. 2001, 73, 192.

[24] W. A. Brand, in Handbook of Stable Isotope Analytical Techniques, (Ed: P. A. de Groot). Elsevier, Amsterdam, 2004, pp. 835–857.

[25] F. K. Tittel, R. Lewicki, R. Lascola, S. McWhorter, in Trace Analysis of Specialty and Electronic Gases. (Eds: W. M. Geiger, M. W. Raynor). JohnWiley, Chichester, 2013, pp. 71–109.

[26] Z.-H. Du, Y.-Q. Zhai, J.-Y. Li, B. Hu. Techniques of on-line monitoring volatile organic compounds in ambient air with optical spectroscopy. Guangpuxue Yu Guangpu Fenxi 2009, 29, 3199.

[27] G. J. Martin, S. Akoka, M. L. Martin, in Modern Magnetic Resonance, Part 3. (Eds: G. A. Webb). Springer, Berlin, 2006, pp. 1629–1636.

[28] E. Roth. Critical evaluation of the use and analysis of stable isotopes. Pure Appl. Chem. 1997, 69, 1753.

[29] E. D. Andrulis. Theory of the origin, evolution, and nature of life. Life 2012, 2, 1.

[30] T. R. Ireland. Isotopic anomalies in extraterrestrial grains. J. R. Soc. West. Aust. 1996, 79, 43.

[31] K. J. Knudson, A. H. Peters, E. T. Cagigao. Paleodiet in the Paracas Necropolis of Wari Kayan: carbon and nitrogen isotope analysis of keratin samples from the south coast of Peru. J. Archaeol. Sci. 2015, 55, 231.

[32] N. Wang, Y. Hu, G. Song, C. Wang. Comparative analyses of amino acids and C, N stable isotopes between soluble collagen and insoluble collagen within archaeological bones. Disiji Yanjiu 2014, 34, 204.

[33] N. Zhang, S. Bashir, J. Qin, J. Schindelka, A. Fischer, I. Nijenhuis, H. Herrmann, L. Y. Wick, H. H. Richnow. Compound specific stable isotope analysis (CSIA) to characterize transformation mechanisms of α-hexachlorocyclohexane. J. Hazard. Mater. 2014, 280, 750.

[34] A. A. Chialvo, L. Vlcek. NO_3^- Coordination in Aqueous Solutions by $^{15}N/^{14}N$ and $^{18}O/^{nat}O$ Isotopic Substitution: What Can We Learn from Molecular Simulation?. J. Phys. Chem. B 2015, 119, 519.

[35] E. Federherr, C. Cerli, F. M. S. A. Kirkels, K. Kalbitz, H. J. Kupka, R. Dunsbach, L. Lange, T. C. Schmidt. A novel high-temperature combustion based system for stable isotope analysis of dissolved organic carbon in aqueous samples. I: development and validation. Rapid Commun. Mass Spectrom. 2014, 28, 2559.

[36] G. Förtsch. Handbuch Betrieblicher Gewässerschutz. Springer, Berlin, 2014.

[37] ISO 8245:1999. Water quality — Guidelines for the determination of total organic carbon (TOC) and dissolved organic carbon (DOC).

[38] T. Damhus, R. M. Hartshorn, A. T. Hutton. Nomenclature of Inorganic Chemistry: IUPAC Recommendations 2005. Royal Society of Chemistry, Cambridge, 2005.

[39] DIN EN 12260:2003-12. Water quality – Determination of nitrogen.

[40] R. Russow, J. Kupka, A. Goetz, B. Apelt. A New Approach to Determining the Content and ^{15}N Abundance of Total Dissolved Nitrogen in Aqueous Samples: TOC Analyzer-QMS Coupling. Isot. Environ. Health Stud. 2002, 38, 215.

[41] P. Lachouani, A. H. Frank, W. Wanek. A suite of sensitive chemical methods to determine the $\delta^{15}N$ of ammonium, nitrate and total dissolved N in soil extracts. Rapid Commun. Mass Spectrom. 2010, 24, 3615.

[42] ASTM D7573-09. Test Method for Total Carbon and Organic Carbon in Water by High Temperature Catalytic Combustion and Infrared Detection. 1999.

[43] EPA Technical Overview of Volatile Organic Compounds https://www.epa.gov/indoor-air-quality-iaq/technical-overview-volatile-organic-compounds.

[44] P. W. Atkins, J. De Paula. Physikalische Chemie. JohnWiley, Chichester, 2013.

[45] T. H. E. Heaton. Isotopic studies of nitrogen pollution in the hydrosphere and atmosphere: A review. Chem. Geol. 1986, 59, 87.

[46] H. Gandhi, T. N. Wiegner, P. H. Ostrom, L. A. Kaplan, N. E. Ostrom. Isotopic (^{13}C) analysis of dissolved organic carbon in stream water using an elemental analyzer coupled to a stable isotope ratio mass spectrometer. Rapid Commun. Mass Spectrom. 2004, 18, 903.

[47] C. L. Osburn, G. St-Jean. The use of wet chemical oxidation with high-amplification isotope ratio mass spectrometry (WCO-IRMS) to measure stable isotope values of dissolved organic carbon in seawater. Limnol. Oceanogr.: Methods 2007, 5, 296.

[48] R. J. Panetta, M. Ibrahim, Y. Gélinas. Coupling a High-Temperature Catalytic Oxidation Total Organic Carbon Analyzer to an Isotope Ratio Mass Spectrometer To Measure Natural-Abundance $\delta^{13}C$-Dissolved Organic Carbon in Marine and Freshwater Samples. Anal. Chem. 2008, 80, 5232.

[49] P. Albéric. Liquid chromatography/mass spectrometry stable isotope analysis of dissolved organic carbon in stream and soil waters. Rapid Commun. Mass Spectrom. 2011, 25, 3012.

[50] E. Richling, C. Hoehn, B. Weckerle, F. Heckel, P. Schreier. Authentication analysis of caffeine-containing foods via elemental analysis combustion/pyrolysis isotope ratio mass spectrometry (EA-C/P-IRMS). Eur. Food Res. Technol. 2003, 216, 544.

[51] B. Fry, S. Saupe, M. Hullar, B. J. Peterson. Platinumcatalyzed combustion of DOC in sealed tubes for stable isotopic analysis. Mar. Chem. 1993, 41, 187.

[52] C. Walz. NO_x-Minderung nach dem SCR-Verfahren: Untersuchungen zum Einfluss des NO_2-Anteils. 2000.

[53] S. Bouillon, M. Korntheuer, W. Baeyens, F. Dehairs. A new automated setup for stable isotope analysis of dissolved organic carbon. Limnol. Oceanogr.: Methods 2006, 4, 216.

[54] L. Zhang, D. M. Kujawinski, M. A. Jochmann, T. C. Schmidt. High-temperature reversed-phase liquid chromatography coupled to isotope ratio mass spectrometry: HT-RPLC coupled to IRMS. Rapid Commun. Mass Spectrom. 2011, 25, 2971.

[55] G. St-Jean. Automated quantitative and isotopic (^{13}C) analysis of dissolved inorganic carbon and dissolved organic carbon in continuous-flow using a total organic carbon analyser. Rapid Commun. Mass Spectrom. 2003, 17, 419.

[56] I. De Troyer, S. Bouillon, S. Barker, C. Perry, K. Coorevits, R. Merckx. Stable isotope analysis of dissolved organic carbon in soil solutions using a catalytic combustion total organic carbon analyzer-isotope ratio mass spectrometer with a cryofocusing interface. Rapid Commun. Mass Spectrom. 2010, 24, 365.

[57] S. Q. Lang, M. D. Lilley, J. I. Hedges. A method to measure the isotopic (^{13}C) composition of dissolved organic carbon using a high temperature combustion instrument. Mar. Chem. 2007, 103, 318.

[58] L. T. Corr, R. Berstan, R. P. Evershed. Optimization of derivatisation procedures for the determination of $\delta^{13}C$ values of amino acids by gas chromatography/combustion/isotope ratio mass spectrometry. Rapid Commun. Mass Spectrom. 2007, 21, 3759.

[59] W. A. Brand, P. Dobberstein. Isotope-ratio-monitoring liquid chromatography mass spectrometry (IRM-LCMS). First results from a moving wire interface system. Isot. Environ. Health Stud. 1996, 32, 275.

[60] Y. Teffera, J. J. Kusmierz, F. P. Abramson. Continuous-Flow Isotope Ratio Mass Spectrometry Using the Chemical Reaction Interface with Either Gas or Liquid Chromatographic Introduction. Anal. Chem. 1996, 68, 1888.

[61] D. M. Kujawinski, L. Zhang, T. C. Schmidt, M. A. Jochmann. When Other Separation Techniques Fail: Compound-Specific Carbon Isotope Ratio Analysis of Sulfonamide Containing Pharmaceuticals by High-Temperature-Liquid Chromatography-Isotope Ratio Mass Spectrometry. Anal. Chem. 2012, 84, 7656.

[62] L. Zhang, D. M. Kujawinski, E. Federherr, T. C. Schmidt, M. A. Jochmann. Caffeine in Your Drink: Natural or Synthetic?. Anal. Chem. 2012, 84, 2805.

[63] P. J. H. Dunn, N. V. Honch, R. P. Evershed. Comparison of liquid chromatography-isotope ratio mass spectrometry (LC/IRMS) and gas chromatography-combustion-isotope ratio mass spectrometry (GC/C/IRMS) for the determination of collagen amino acid $\delta^{13}C$ values for palaeodietary and palaeoecological reconstruction. Rapid Commun. Mass Spectrom. 2011, 25, 2995.

[64] L. Zhang, M. Thevis, T. Piper, M. A. Jochmann, J. B. Wolbert, D. M. Kujawinski, S. Wiese, T. Teutenberg, T. C. Schmidt. Carbon Isotope Ratio Analysis of Steroids by High-Temperature Liquid Chromatography-Isotope Ratio Mass Spectrometry. Anal. Chem. 2014, 86, 2297.

[65] J.-P. Godin, J. S. O. McCullagh. Review: Current applications and challenges for liquid chromatography coupled to isotope ratio mass spectrometry (LC/IRMS). Rapid Commun. Mass Spectrom. 2011, 25, 3019.

[66] K.Mopper, J.Qian, in Encyclopedia of Analytical Chemistry, (Ed.: R. A. Meyers). JohnWiley, Chichester, 2000. pp. 3532–3540.

[67] G. R. Aiken. Chloride interference in the analysis of dissolved organic carbon by the wet oxidation method. Environ. Sci. Technol. 1992, 26, 2435.

[68] P. J. Wangersky. Dissolved organic carbon methods: a critical review. Mar. Chem. 1993, 41, 61.

[69] R. van Geldern, M. P. Verma, M. C. Carvalho, F. Grassa, A. Delgado-Huertas, G. Monvoisin, J. A. C. Barth. Stable carbon isotope analysis of dissolved inorganic carbon (DIC) and dissolved organic carbon (DOC) in natural waters – Results from a worldwide

proficiency test: Carbon stable isotope proficiency test of DIC and DOC. Rapid Commun. Mass Spectrom. 2013, 27, 2099.

[70] AOAC 984.23-1988. Corn syrup and cane sugar in maple syrup. Carbon ratio mass spectrometric method.

[71] OIV AS312-07 2010. Method for the determination of the $^{13}C/^{12}C$ isotope ratio of glycerol in wines by gas chromatography combustion or high performance liquid chromatography coupled to isotope ratio mass spectrometry (GC-C-IRMS or HPLC-IRMS).

[72] OIV AS314-03 2005. Determination of the carbon isotope ratio $^{13}C/^{12}C$ of CO_2 in sparkling wines – method using isotope ratio mass spectrometry (IRMS).

[73] EEC/822/97. Determination of the isotopic ratio of oxygen of the water content in wines.

Chapter 2 Scope and Aim

The state of the art in stable isotope analysis (SIA) of aqueous samples described in Chapter 1 shows that aside from the already existing number of techniques and methods, there is still a lack of systems with the required performance for $\delta^{13}C$ and a lack of systems per sé for $\delta^{15}N$ determination. This is especially challenging for samples with natural isotope abundance.

The aim of this study was to increase understanding of the processes involved in the SIA of all dissolved forms of carbon and nitrogen and to subsequently develop suitable analytical instrumentation for both bulk and compound-specific stable isotope analysis (BSIA and CSIA) directly in aqueous solutions. For this purpose, four work packages were carried out as summarized in Figure 2-1.

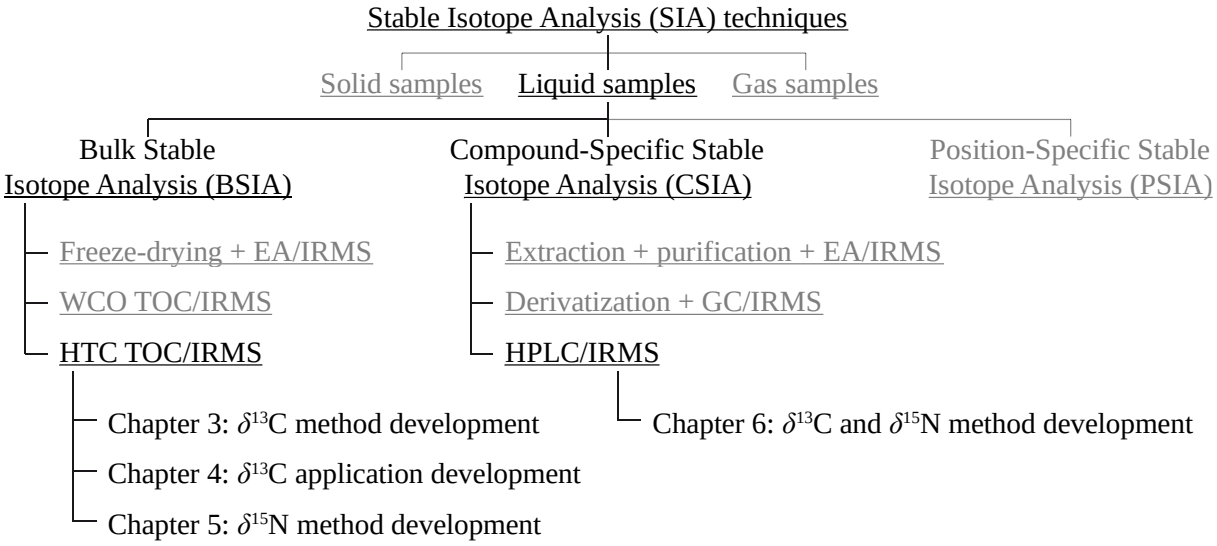

Figure 2-1 Overview of the contents of this thesis

Dissolved organic carbon (DOC) plays a key role in carbon cycle investigations and it is the focus of various disciplines in environmental biogeochemistry. Both the concentration and the stable isotope composition of DOC play an important role in carbon cycle studies, but traditional methods are either very time-consuming or involve the risk of isotope fractionation due to incomplete mineralization. Thus, a novel method suitable for SIA is needed. Chapter 3 comprises the detailed description of the development of an analytical system for accurate and sensitive DOC SIA (including its validation with standard solutions and simulated matrices) consisting in the coupling of a modified high-temperature combustion TOC analyzer with an isotope ratio mass spectrometer.

The results obtained with the newly developed system as described in Chapter 3 met the needed performance, thus confirmed the general suitability of the system, but further

validation and proper assessment of analytical performance with the real samples was still needed. Therefore, Chapter 4 aims at the validation of the system with a broad range of real samples. Soil extracts, river and seawater samples were analyzed and, to further prove the reproducibility of the developed method, a complete set of samples from an international round robin test were analyzed as well. Additionally, given the strong interest of the scientific community, the general suitability of the system for the determination of total inorganic carbon (TIC) SIA was tested.

Various disciplines in environmental biogeochemistry, such as oceanography and soil science are not only interested in $\delta^{13}C$ but even more so in the $\delta^{15}N$ determination directly in aqueous samples with natural isotope abundance. Because there is no suitable system available, the aim of Chapter 5 was to find a new analytical principle to overcome the limiting factors for the performance and, utilizing the novel principle, to develop a new system for SIA of total nitrogen bound (TNb), simultaneous to SIA of DOC.

All methods introduced from Chapter 3 to Chapter 5 are BSIA techniques for $\delta^{13}C$ and $\delta^{15}N$ determination in aqueous solutions but the interest often focuses on specific compounds within the aqueous samples, i.e., on CSIA especially of polar or ionic compounds. Current methods need several preparation steps, often including either laborious extraction or derivatization of the analyte or both. Wet chemical oxidation based HPLC/IRMS systems do not allow for $\delta^{15}N$ determination and also $\delta^{13}C$ measurements have limitations – potential bias due to incomplete mineralization. To avoid these limitations, a system for CSIA of non-volatile, polar and thermally labile compounds directly from aqueous solutions based on a HTC interface was conceived and is described in detail within Chapter 6.

Chapter 7 summarizes the main results of this study and depicts an outlook on the potential direction of future research and developments in the field of SIA in aqueous solutions.

Chapter 3 A novel high-temperature combustion based system for stable isotope analysis of dissolved organic carbon in aqueous samples - development and validation

Adapted from: E. Federherr, C. Cerli, F. M. S. A. Kirkels, K. Kalbitz, H. J. Kupka, R. Dunsbach, L. Lange and T. C. Schmidt; A novel high-temperature combustion based system for stable isotope analysis of dissolved organic carbon in aqueous samples. I: development and validation; Rapid Communications in Mass Spectrometry **2014**, 28, 2559-2573

3.1 Abstract

Rationale: Traditionally, dissolved organic carbon (DOC) stable isotope analysis (SIA) is performed using either offline sample-preparation followed by elemental analyzer/isotope ratio mass spectrometry (EA/IRMS) or a wet chemical oxidation (WCO)-based device coupled to an isotope ratio mass spectrometer. The first method is time-consuming and laborious. The second involves the risks of underestimation of DOC concentration and isotope fractionation due to incomplete oxidation. The development of an analytical method for accurate and sensitive DOC SIA is described in this study.

Methods: A high-temperature combustion (HTC) system improves upon traditional methods. A novel total organic carbon (TOC) system, specially designed for SIA, was coupled to an isotope ratio mass spectrometer. An integrated trap and flash technique (peak focusing), flexible injection volume (0.05 – 3 mL), favorable carrier gas flow, modified ash crucible, new design of combustion tube and optimized drying system were used to achieve the necessary performance.

Results: The system can reliably measure concentrations up to 1000 mgC/L. Compounds resistant to oxidation, such as barbituric acid, melamine and humic acid, were analyzed with recovery rates of 100 ± 1% proving complete oxidation. In this initial testing, the $\delta^{13}C$ values of these compounds were determined with precision and trueness of ≤0.2‰ even with 3.5% salinity. Further tests with samples with low DOC concentrations resulted in $LOQ_{SIA\ method}$ values of 0.5 mgC/L and 0.2 mgC/L for $LOQ_{SIA\ instr}$, considering an accuracy of ±0.5‰ as acceptable.

Conclusions: The novel HTC system coupled to an isotope ratio mass spectrometer resulted in significantly improved sensitivity. The system is suitable for salt-containing liquids and compounds that are resistant to oxidation, and it offers a large concentration range. A second paper (which follows this one in this issue) will present a more comprehensive assessment of the analytical performance with a broad set of solutions and real samples. This highly efficient TOC stable isotope analyzer will probably open up new possibilities in biogeochemical carbon cycle research.

3.2 Introduction

Dissolved organic carbon (DOC) plays a key role in carbon cycle investigations[1] and it is the focus of various disciplines in environmental biogeochemistry, such as oceanography[2] and soil science.[3,4] Both the concentration and the stable isotope composition of DOC play an important role in carbon cycle studies. Concentration measurements provide the possibility to balance the global as well as the local carbon cycle.[5–7] Stable isotope analyses (SIA) can provide very valuable additional information about the origin and transformation of organic matter.[8,9]

Analytical procedures for the determination of DOC concentration are well defined and understood[10] and for some applications even standardized.[11,12] Thus, the focus of this work is on the stable isotope analysis of dissolved organic carbon (DOC SIA). Various analytical methods are available for DOC SIA. Initially, it was carried out either by offline sample-preparation, such as lyophilization, followed by elemental analyzer/isotope ratio mass spectrometry (EA/IRMS)[13,14] or by a wet chemical oxidation total organic carbon analyzer coupled to isotope ratio mass spectrometry (WCO TOC/IRMS).[15,16] High-temperature combustion techniques were also utilized (HTC TOC/IRMS).[17,18] The most recent approaches include wet chemical oxidation flow injection analysis-IRMS (WCO FIA-IRMS),[19] the use of available interfaces for liquid chromatography/IRMS (LC/IRMS), and WCO TOC coupled to cavity ring-down spectroscopy (WCO TOC/CRDS).[20]

Table 3-1 gives an overview of the various analytical methods.

Offline sample-preparation is time-consuming and laborious.[13] The WCO-based methods run the risk of DOC concentration underestimation as well as of isotope fractionation due to incomplete oxidation.[10] Incomplete oxidation is a well- known issue for seawater samples, in which sulfate radicals are scavenged by chloride ions, and are therefore no longer available for analyte oxidation.[22] Similarly, in soil science, compounds such as humic or fulvic acid that are resistant to oxidation are reported not to be completely oxidized by WCO[10] with the risk of compound-specific isotope fractionation. For samples with unknown composition such errors are non-systematic and cannot be corrected for in bulk stable isotope analysis (BSIA). Many publications suggest HTC TOC analyzers as the most suitable device for DOC concentration measurements.[23] However, commercially available HTC-based systems are not optimized for SIA mainly because of their insufficient sensitivity[18] as a result of low injection volumes.

Analytical principle	Sample scope	Preparation	Insturmentation/ Measurement	Additional evaluation	Performance	Reference
WCO TOC-IRMS	Sea water, Shelf, Reference material	Filtration over 0.45 µm (DOC), acidification + sparging for IC removal	OI-Aurora 10330; Delta Plus HiPerTOC; Delta Plus	Blank correction (reagent blank)	SD typical 0.1-0.4‰ at 65-200 µmol C/L	(Bouillon *et al.* [a])[16] (Osburn and St-Jean[a])[15]
HTC TOC-IRMS	Sea water, Soil solution, Reference material	Filtration over 0.45 µm (DOC), acidification + sparging for IC removal	Thermalox; Sercon 20-20 cryo trap MQ 1001; Delta Plus	Blank correction (instrument + reagent blank)	SD typical 0.1-0.2‰ at 1-10 mg C/L SD typical 0.1-0.7‰ at 40-70 µmol C/L	(Troyer *et al.* [b])[17] (Lang, *et al.* [b])[18]
LC/IRMS	Soil water, bulk stream	Filtration over 0.45 µm (DOC), acidification + sparging for IC removal	Surveyor LC unit; Thermo Fisher LC-IsoLink and Delta V	Blank correction (reagent blank)	SD typical 0.3‰ at 1-10 mg C/L	(Alberic[c])[19]
EA/IRMS	Sea water	Filtration over 0.45 µm (DOC), acidification + sparging for IC removal, freeze-drying	Finnigan 251	Blank correction (reagent blank)	SD typical 0.01-0.04‰	(Fry *et al.* [d])[21]
WCO TOC-CRDS	River water, waste water	Filtration over 0.45 µm (DOC), acidification + sparging for IC removal	OI-Aurora 1030; Picarro G111-i	Blank correction (reagent blank)	SD typical 0.5‰ at 2-8 mg C/L	(Hartland *et al.* [e])[20]

WCO: wet chemical oxidation; HTC: high temperature combustion; LC: liquid chromatography; IC: inorganic carbon; EA: elemental analyzer; WCO/CRDS: wet chemical oxidation/cavity ringdown spectroscopy

[a] No CO_2 trapping, reduction of excess oxygen.

[b] Cryostatic CO_2 trapping by liquid N_2.

[c] Bulk analysis in FIA mode without separation column.

[d] Samples freeze-dried in the combustion tubes, home-built combustion system.

[e] Collection of CO_2 in a gas-tight bag.

Table 3-1 Current methods for determination of stable isotope composition of DOC.

Furthermore, a recent worldwide proficiency test[24] identified several common problems with reproducibility and consequently data validity for interpretation and comparability among institutes using different analytical techniques. The critical issues are not simply related to sample type and sampling procedure, but seem to be mostly method specific for DOC SIA. This includes sample-preparation, measurement and the evaluation of data.[25] In particular, the use of very different methods combined with the lack of a generally accepted strategy for data evaluation makes such comparisons very challenging. Generally accepted or even standardized operating procedures have been developed for many bulk and compound-specific stable isotope analyses (CSIA).[26-31] However, the field of DOC SIA still shows a lack of standardized methods and approaches to account for all parameters required for accurate results such as the minimal required combustion temperature for complete mineralization or the handling of blanks.

In view of the limitations of the analytical techniques discussed above we developed a novel HTC-based system for DOC SIA in challenging aqueous samples, and we propose a data evaluation approach. In this first manuscript we present the technical details of the instrument and the rationale for the proposed data processing, while, in the second one,[32] we focus on the assessment of the analytical performance based on a broad test with real samples.

3.3 Experimental

3.3.1 Chemicals and reagents

Reference materials IAEA-600 caffeine CAF1 ($\delta^{13}C_{VPDB}$ –27.771 ± 0.043‰), USGS-41 glutamic acid GLU1 ($\delta^{13}C_{VPDB}$ +37.626 ± 0.049‰) and IAEA-CH-6 sucrose SUC1 ($\delta^{13}C_{VPDB}$ –10.449 ± 0.033‰) were purchased from the International Atomic Energy Agency (Vienna, Austria). The internal laboratory standards were EAS-CIT1 citric acid (purchased from Sigma-Aldrich (Buchs, Switzerland)), EAS-CAS1 casein and EAS-GLU2 glutamic acid (in-house standards; Elementar Analysensysteme, Hanau, Germany). Benzoic acid BEN1 (≥99.5%) and humic acid HUM1 (technical grade, ash ≈ 20%) were purchased from Fluka (Buchs, Switzerland). Acetovanillone ACV1 (98%), caffeine CAF2 (≥99.0%) and melamine MEL1 (99%) were obtained from Aldrich (St. Louis, MO, USA). Citric acid CIT2 (≥99.5%), D-(+)-glucose monohydrate GLU3 (≥99.0%), barbituric acid BAR1 (≥99%), sodium chloride (≥99.5%) and hydrochloric acid (37%) were purchased from Merck (Darmstadt, Germany). Ultrapure, deionized water (UP water) produced by a Purelab Ultra system (MK2-Analytic, ELGA, High Wycombe, UK) was used for solution preparation. Helium 5.0 and oxygen 4.8 were purchased from Air Liquid (Oberhausen, Germany).

3.3.2 Instrumentation and methodology

The entire system consists of three parts: the TOC analyzer, the interface and the isotope ratio mass spectrometer (see Figure 3-1).

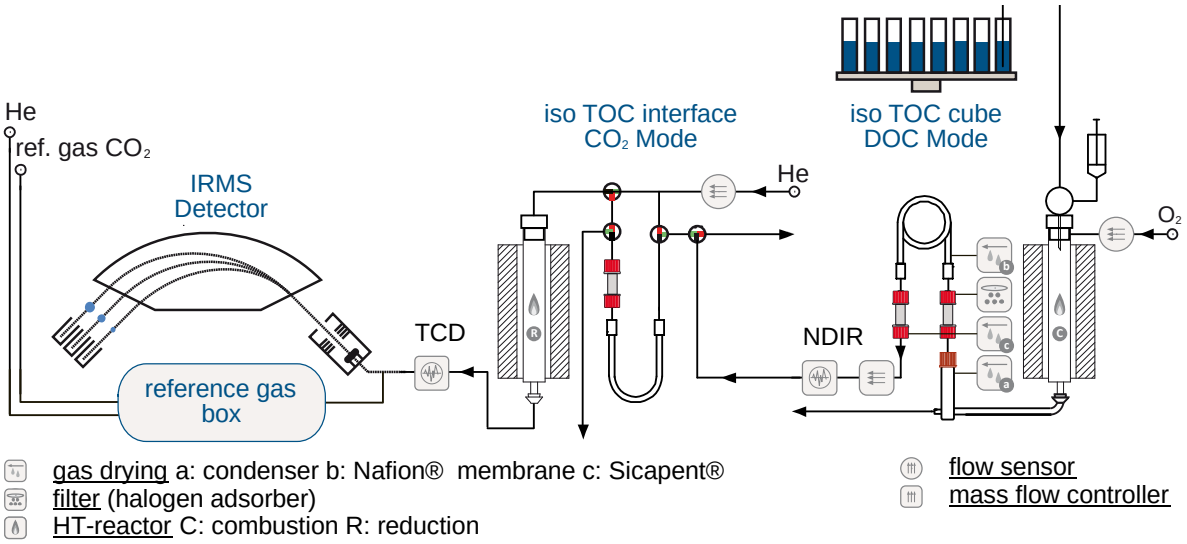

Figure 3-1 System setup for DOC SIA.

The TOC analyzer (iso TOC cube) is derived from the commercially available HTC-TOC analyzer vario TOC cube (Elementar Analysensysteme GmbH) which was modified and

adapted to meet the requirements for IRMS, namely, to improve the system sensitivity, to minimize instrumental background as well as the blank contribution, and to ensure the absence of isotope fractionation within the system.

Samples, filled in 40-mL borosilicate glass vials, are introduced using a 32-position autosampler into the combustion system by means of a 5-mL syringe and a multiway valve. The combustion is performed at 850 °C by oxygen and supported by a catalyst (Pt on ceramic carrier material). Water is removed in three steps: an air-cooled condenser, a counter-flow membrane dryer and a chemical dryer. Hydrogen halides and halogens are removed by silver wool. After the purification steps the carrier gas oxygen enters the nondispersive infrared (NDIR) detector for quantification of the evolved CO_2 (giving the DOC concentration of a pre-acidified sample).

The interface separates the CO_2 from O_2 allowing for focusing and gas exchange (replacement of the reaction gas oxygen by carrier gas helium). This part of the system was specifically developed for this application.

An IsoPrime100 (Isoprime Ltd, Manchester, UK) isotope ratio mass spectrometer was used to determine the stable isotope composition. No modifications were made to this instrument. A detailed description of all the instrument modifications and/or developments is given in the Method development section.

3.3.3 Nomenclature, evaluation and QA

Nomenclature

To express the variations of natural stable isotope abundance the widely applied 'delta-notation' is used. The $\delta^{13}C_{VPDB}$-value of an analyte (A) is described by Equation 3-1 as a relative difference between the isotope ratio (R) of an analyte ($R(^{13}C/^{12}C)_A$) and the isotope ratio defining an international reference scale, for carbon Vienna Pee Dee Belemnite ($R(^{13}C/^{12}C)_{VPDB}$):[26]

$$\delta^{13}C_{A,VPDB} = \frac{R\left(^{13}C/^{12}C\right)_A - R\left(^{13}C/^{12}C\right)_{VPDB}}{R\left(^{13}C/^{12}C\right)_{VPDB}} \qquad \text{Equation 3-1}$$

Please note that if no reference is mentioned, as exemplarily shown in Equation 3-2, the reported δ-values are related to the used, in-house reference gas (RG). That concerns all data before the final normalization to the VPDB scale.

$$\delta^{13}C_{\text{lin corr A}} \equiv \delta^{13}C_{\text{lin corr A,RG}} \qquad \text{Equation 3-2}$$

with $\delta^{13}C_{\text{lin corr A}}$ as a linearity corrected δ-value of an analyte A.

Evaluation

The description and rationale behind the chosen data evaluation strategy are extensively explained in the Results and Discussion section. Here only the finally applied equations are shown.

Non-linearity correction

Known and inevitable concentration-dependent fractionation occurs in the electron ionization (EI) source within the isotope ratio mass spectrometer inducing isotope ratio shifts. The isotope ratio linearity of the isotope ratio mass spectrometer ($L_{\text{R IRMS}}$) was quantified as the slope m_{lin} of a linear regression describing the δ-value as a function of corresponding ion current I.

$$m_{\text{lin}} = \frac{\sum_{k=1}^{n}\left(I_{\text{RG}_k} - \overline{I_{\text{RG}}}\right)\left(\delta^{13}C_{\text{RG},\overline{\text{RG}}_k} - \overline{\delta^{13}C_{\text{RG},\overline{\text{RG}}}}\right)}{\sum_{k=1}^{n}\left(I_{\text{RG}_k} - \overline{I_{\text{RG}}}\right)^2} \qquad \text{Equation 3-3}$$

$$\delta^{13}C_{\text{RG},\overline{\text{RG}}_k} = \frac{R\left({}^{13}C/{}^{12}C\right)_{\text{RG}_k} - R\left({}^{13}C/{}^{12}C\right)_{\overline{\text{RG}}}}{R\left({}^{13}C/{}^{12}C\right)_{\overline{\text{RG}}}} \qquad \text{Equation 3-4}$$

$L_{\text{R IRMS}}$ was monitored regularly, before and after each test series. All measured $\delta^{13}C$ raw data ($\delta^{13}C_{\text{meas A}}$) generated by the software IonVantage (Isoprime Ltd), and automatically corrected for ^{17}O-abundance and related to RG, were then linearity corrected to $\delta^{13}C_{\text{lin corr A}}$ as described by Brand[27] and expressed as shown in Equation 3-5:

$$\delta^{13}C_{\text{lin corr A}} = \delta^{13}C_{\text{meas A}} - m_{\text{lin}} \times (I_A - I_{\text{RG}}) \qquad \text{Equation 3-5}$$

with I_A as the ion current at the maximum of the peak for analyte A and I_{RG} the ion current of the reference gas peak pulse.

Note that the automatically created software report gives an absolute value of the slope m_{lin}. Therefore, m_{lin} was manually recalculated to account for its positive or negative sign in the calculation.

Blank corrections

An isotope mass balance (IMB) equation[26] was utilized for corrections. The amount of carbon is represented by the uncorrected area A from the integrated NDIR CO_2 peak of the TOC analyzer. The solution of the IMB equation for blank-'subtracted' δ-value $\delta^{13}C_{\Sigma\text{bl corr A}}$

results in Equation 3-6. The symbol Σ indicates that there is more than one possible blank contribution (water blank, instrumental blank, etc.) but, as will be shown in the Results and Discussion section, only the water blank needs to be considered (taking into account the concentration range of interest). Thus, for determination of the concentration as well as the δ-value of the blank, a direct determination by measuring the acidified water used for standards solution preparation (blanks) is possible:

$$\delta^{13}\mathrm{C}_{\Sigma\mathrm{bl\ corr\ A}} = \frac{\delta^{13}\mathrm{C}_{\mathrm{lin\ corr\ A}} \times A_{\mathrm{meas\ A}} - \sum_{k=1}^{n}(\delta^{13}\mathrm{C}_{\mathrm{bl}} \times A_{\mathrm{bl}})_k}{A_{\mathrm{meas}} - \sum_{k=1}^{n}(A_{\mathrm{bl}})_k} \qquad \text{Equation 3-6}$$

Two-point normalization

Finally, a referencing strategy to the VPDB scale was applied as recommended in the literature and described in Equation 3-7. Note that the equation for two-point normalization described in the literature[26] is mathematically a linear interpolation procedure and so equivalent to the two-point calibration equation used here. Thus, applied Equation 3-7 is valid:

$$\delta^{13}\mathrm{C}_{\mathrm{A},VPDB} = m_{\mathrm{norm}} \times \delta^{13}\mathrm{C}_{\Sigma\mathrm{bl\ corr\ A}} + b_{norm} \qquad \text{Equation 3-7}$$

$$m_{\mathrm{norm}} = \frac{\delta^{13}\mathrm{C}_{\mathrm{Std1,VPDB}} - \delta^{13}\mathrm{C}_{\mathrm{Std2,VPDB}}}{\delta^{13}\mathrm{C}_{\Sigma\mathrm{bl\ corr\ Std1,RG}} - \delta^{13}\mathrm{C}_{\Sigma\mathrm{bl\ corr\ Std2,RG}}} \qquad \text{Equation 3-8}$$

$$b_{\mathrm{norm}} = \overline{\delta^{13}\mathrm{C}_{\mathrm{Std,VPDB}}} - m_{\mathrm{norm}} \times \overline{\delta^{13}\mathrm{C}_{\Sigma\mathrm{bl\ corr\ Std,RG}}} \qquad \text{Equation 3-9}$$

Quality assurance

The developed method was tested (see Instrument testing section) with aqueous solutions based on the validation strategy described in DIN 17025[33] (modified for SIA by applying the recommendations of Jochmann and Schmidt[26]). In that way, the chosen referencing and quality assurance strategy ensures the metrological traceability[34] and the accuracy – sum of trueness and precision.[35–37] Uncertainty considerations within this work were adjusted according to the recommendation of CAC/GL 59–2006.[38] The standard uncertainty (u_{std}) is expressed as the standard deviation of replicate measurements and thus represents the uncertainty caused by the instrumentation. Error bars shown within this work represent the standard uncertainty (1σ). The combined uncertainty (u) estimation is discussed below in the Uncertainty and accuracy section.

3.4 Results and discussion

3.4.1 Method development

Injection and combustion system

A new ash crucible was designed to optimize protection of the quartz glass and catalyst from a high salt load without disturbing the gas flow. A crucible with slits at two different heights, as shown in Figure 3-2, led to the best peak shape results and thus to improved sensitivity and precision. A test solution of 1 mgC/L showed an improvement in instrument precision by nearly a factor of 2 (1.52 to 0.82% rel. SD).

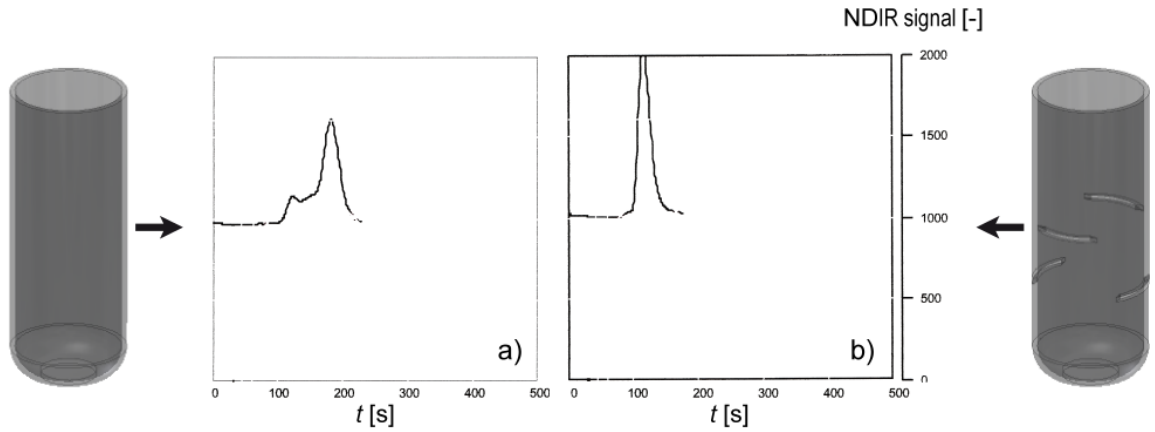

Figure 3-2 Improvement of the crucible to optimize flow conditions. Crucibles and corresponding peak shape before (a) and after (b) crucible optimization (slitted).

The bottom of the crucibles is filled with 1 cm quartz wool, which increases the area that the injected sample stream impinges on and avoids splash effects. Finally, salt residues are captured and thus excluded as an influence factor. North Sea water with ca 3.5% salt load was analyzed at a combustion temperature of 850 °C with an average precision of 0.073 mgC/L. Even brine solutions (1:1 diluted; 28% salinity) were analyzed successfully using an injection volume of 0.1 mL. The determined DOC concentration was 2.00 mgC/L with an uncertainty of ±0.07 mgC/L. Very high salt concentrations such as in brine solutions may adversely affect combustion efficiency, peak shape and in the worst case even cause carry over at the regular combustion temperature. Therefore, for the analysis of brine solutions the combustion tube temperature was reduced from 850 °C to 680 °C. In both cases around 100 measurements were conducted before the ash crucible was changed.

Furthermore, to avoid an influence of the septum on the magnitude and variability of the system blank the system was designed to be septa-free. Instead, the sample vials placed on the autosampler were covered by tin foil. Entrance to the combustion tube was achieved via a four-way valve. Thus, the pathway from the syringe is always connected to the system and injection is accomplished via switching of the valve position into the column. The tubing and multiway valve are rinsed with the sample. The rinse volume and number of rinse cycles are programmable: usually the rinse volume is about three times 1.5 mL. The total volume of the tubing and the multiway valve is 0.17 mL.

Water removal

Water removal is a crucial part of accurate NDIR measurements. Residual water in the system can lead to cross sensitivity through spectral interferences. This aspect is not only important for concentration measurements, but also for SIA, in order to conduct accurate stable isotope blank correction using mass balance equations. A low water background is essential for IRMS due to production of protonated species in the ion source which may interfere with the detection of ions containing heavy isotopes. The lowest water background was achieved using a three-step system. In the first step, the main amount of water is removed via an air-cooled condenser that can handle large water volumes of up to 3 mL per injection. The second step consists of a counter-flow membrane dryer (Nafion®; E. I. du Pont de Nemours and Co.,Wilmington, DE, USA) to remove the water passing through the condenser, lowering the concentration of water within the carrier gas further. In the third step a chemical desiccation with phosphorus pentoxide on a porous carrier material removes any residual water.

Sensitivity at low concentration and instrumentation blank

One of the main challenges in DOC SIA is to perform accurate measurements of samples with low DOC content. To achieve this it is necessary to increase the sensitivity of the system but at the same time also to exclude the sources of the instrumental blank within the TOC analyzer, such as carbon leaching out from the seals or CO_2 entering from the atmosphere.

Sensitivity

The relatively low sensitivity of a continuous flow isotope ratio mass spectrometer as a detector is one of the key issues. The sensitivity can mainly be improved by adjusting the trap current (200→600 μA). Major improvements can be achieved by introducing a sufficiently large amount of carbon into the system, i.e. by a large injection volume. Typical injection volumes in current systems are in the range of several hundred microliters.[17,39] In HTC systems the problems associated with injection of larger volumes are cooling down of the

reactor, partial condensation of the analyte containing vapor at colder upper parts of the combustion tube, and critical pressure peaks (causing sensor damage or leading to leakages between the connections). Lowering of the carrier gas (O_2) flow rate to 125 mL/min, the pre-pressure to 850 mbar and the injection speed to 100 µL/s, in combination with the improved reactor design, resulted in the injection performance necessary to introduce up to 3 mL of sample. By these changes the sensitivity was improved sufficiently to detect DOC concentrations below 0.2 mgC/L.

To further improve the sensitivity prior to the isotope ratio mass spectrometer measurement, the CO_2 peak is focused using an adsorption column filled with silica gel, without the need for liquid nitrogen often used for this purpose. In the interface, CO_2 is collected on the adsorption column, whereas the O_2 carrier gas passes the system without entering the isotope ratio mass spectrometer. When generation of the CO_2 peak in the TOC analyzer is complete, the adsorption column in the interface is resistance-heated, and CO_2 is released and transported with helium through a reduction tube into the isotope ratio mass spectrometer. The reduction tube, filled with Cu and heated to 600 °C, traps remaining traces of oxygen.

Sources of the instrumental blank

All materials that are in contact with the analyte or the carrier gas are potential sources of instrumental blank that can hamper the sensitivity of the method. Therefore, all the materials used were systematically reconsidered during the development of the system for DOC SIA.

All original plastic tubes were replaced by a partially fluorinated polymer (Elementar Analysensysteme GmbH) with a low CO_2 permeability (about ≈100 $cm^3mm/m^2atmday$), which reduced the permeability by a factor of 7 compared with the commonly used plastic tubing. Copper tubing, which has even better permeability characteristics, was adopted in the interface but could not be used in the TOC analyzer due to susceptibility to corrosion.

Thermo stable fluoroelastomer seals (Elementar Analysensysteme GmbH) were used at all critical passages such as hot zones, to avoid the substantial release of carbon found from standard seals that also became loose over time.

The filling initially used in the combustion tube was identified as the main source of the instrumental blank. Other than the catalysts all the parts used in the combustion tube (Figure 3-3(a)), i.e. the tube itself, the ash crucible and other filling materials (wool and chips), are made of quartz glass and therefore are not sources of the instrumental blank. We tested several catalysts, all made of platinum, on different types of ceramic carrier material. Most of

them showed a carbon leaching effect within the TOC instrument. Probably the pellets burst when coming in contact with the colder water vapor (see Figure 3-3(b)) because of the low thermal shock resistance of the carrier ceramics. The exposed ceramic was thus the source of the detected signal. Of all the materials tested, the EAS PtC04 platinum catalyst on ceramic (Elementar Analysensysteme GmbH) was the most suitable. The material itself is not blank-free but the signal is removed via washing during the usual conditioning phase.

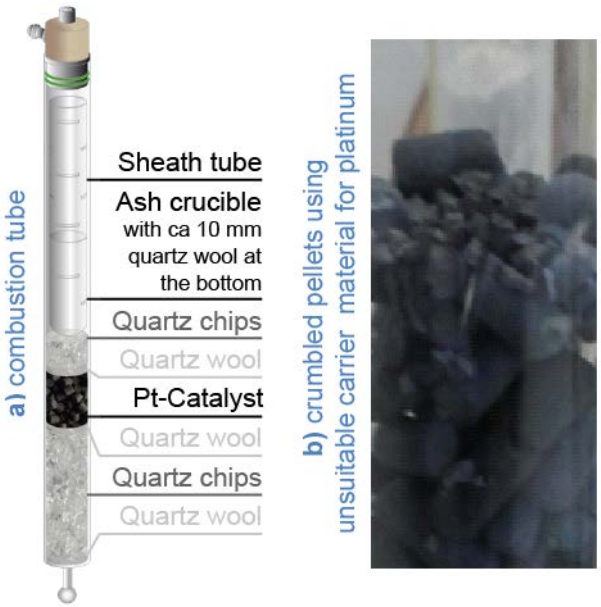

Figure 3-3 Combustion tube filling (a) and non-thermal shock resistant carrier material before optimization. The pellets are destroyed by the contact of the 850 °C hot catalyst with the colder water vapor during sample injection (b).

No detectable peak was observed when 'collecting' the potential background on the CO_2 adsorption unit by running the system without injection for the time that a measurement takes, including desorption at the end. This test checks for the possible contribution to the measured signals by CO_2 diffusing through the tubes, gas impurities or incompletely tight connections and valves. The results of additional experiments supporting the statement that there is no relevant instrumental blank can be found in Supplementary Figure S 3-5 (Supporting Information).

Final system

First tests were performed to roughly estimate the linear range as well as the sensitivity of the set up using sucrose solutions. Figure 3-4 shows the linear range and a good correlation between the signals of the TOC analyzer and the isotope ratio mass spectrometer. Figure 3-5 shows a typical run after the development was completed, as well as the capability referred to as the instrumental sensitivity.

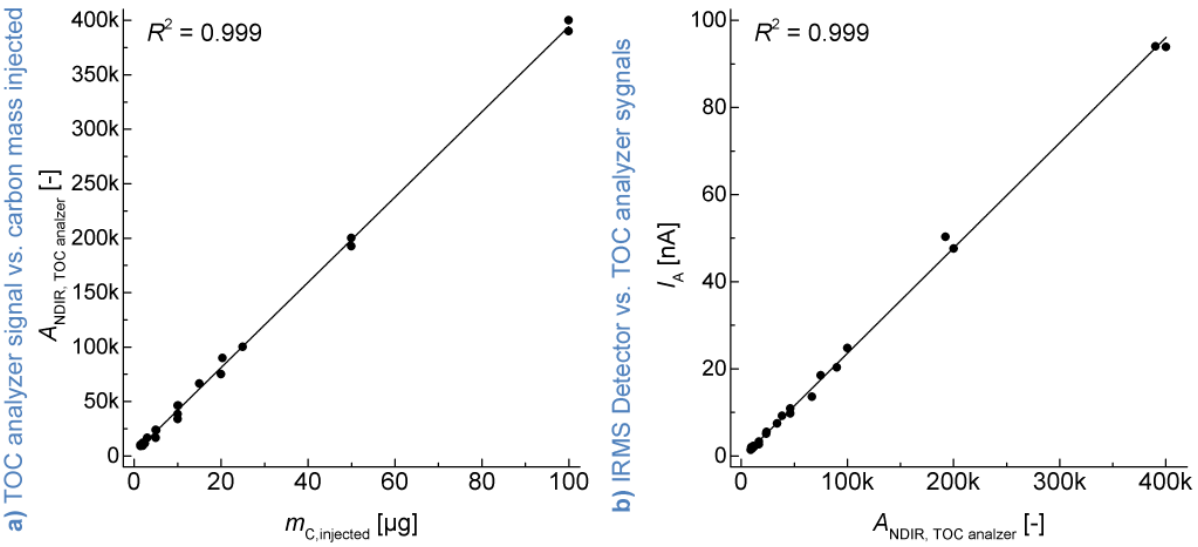

Figure 3-4 First test run (sucrose solutions, 1.5–100 µg C injected, 0.5–3 mg/L, 0.1–3 mL injection volume): correlation between injected mass ($m_{C, injected}$) and peak areas of the TOC analyzer ($A_{NDIR, TOC analyzer}$) (a) and correlation between isotope ratio mass spectrometer signals of coupled instruments (TOC analyzer and IRMS detector via an interface, I_A) and peak areas of the TOC analyzer ($A_{NDIR, TOC analyzer}$) (b).

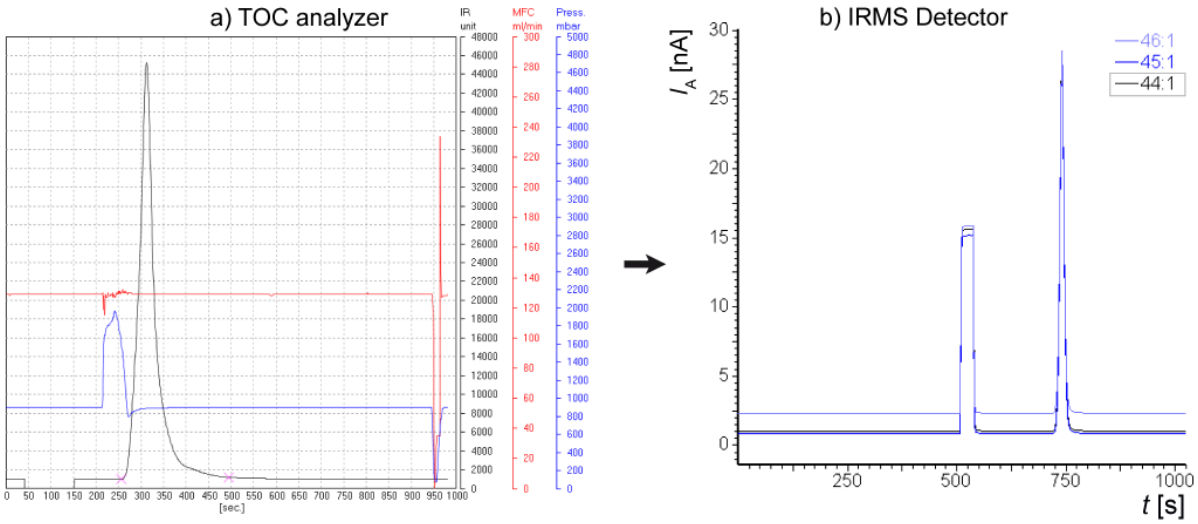

Figure 3-5 Typical progression of a DOC SIA run (5 mg/L sucrose solution, 3-mL injection volume). The TOC peak takes about 250 s (baseline to baseline) (a), whereas in the isotope ratio mass spectrometer the peak width is just 40 s (b). By this setting a TOC concentration as low as 0.2 mgC/L will produce an IRMS detector signal of >1 nA which can still be evaluated properly.

3.4.2 Instrument testing/validation with aqueous compound solutions

Carry-over (memory effects)

Carry-over was tested by measuring a sequence of samples with varying stable isotope composition. We measured each sample in four replicates in order to determine the number of replicates necessary to obtain reliable δ^{13}C values (SD ≤0.2‰). We chose a concentration of 10 mgC/L to avoid the water blank contribution.

Alternation of the δ-values of two sequential samples was expressed as a difference $\Delta(A_{n+1} - A_n)$ (Equation 3-10).

$$\Delta(A_{n+1} - A_n) = \delta^{13}C_{A_{n+1}} - \delta^{13}C_{A_n} \qquad \text{Equation 3-10}$$

A sequence within the test series is indicated by n in A_n. The δ-value of the first replicate for each sample was influenced by the stable isotope composition of the previous one. The magnitude depended on the δ^{13}C difference between the two subsequent samples. A $\Delta(A_{n+1} - A_n)$ value of, e.g., -61.95‰ led to a bias of 2‰ within the $\delta^{13}C_A$ value of the first replicate, while a $\Delta(A_{n+1} - A_n)$ value of 10.90‰ led to a bias of 0.25‰.

Adjusting the rinse settings did not result in an improvement, indicating that the whole pathway before the four-way valve is rinsed properly including the syringe. The carry-over volume calculated by a mass balance equation was within a constant range of 34 ± 9 µL. The precision of the syringe is by far smaller than this volume and could not be the source of such carry-over. The most probable source of the observed carry-over is the injection cannula which extends into the combustion tube and therefore cannot be rinsed during the preparation cycle.

Figure 3-6 shows a linear relationship between the δ^{13}C difference between two subsequent samples, $\Delta(A_{n+1} - A_n)$ and the bias of the first measured replicate of the second sample. Together with the determined transferred volume of 34 ± 9 µL this clearly indicates a systematic error. Unfortunately, correcting the first replicate with a range of ±9 µL would introduce too large contribution to the combined uncertainty; therefore, this cannot be used for correction.

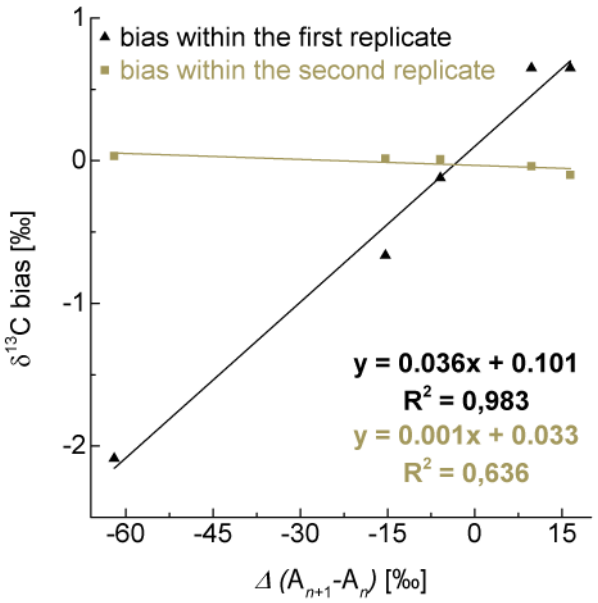

Figure 3-6 Correlation between δ^{13}C bias and δ^{13}C difference between two subsequent samples, with and without consideration of the first replicate (i.e. first injection). Supplementary Table S 3-1 (Supporting Information) shows the chosen sequence as well as results achieved in more details.

Exclusion of the first replicate value removes the systematic bias that falsely suggested a poor precision. Comparison of the precision with and without the first replicates shows a clear improvement, expressed as the standard uncertainty (1σ), from an average of ±0.38‰ (max. ±1.04‰) to 0.05‰ (max 0.09‰). Even the largest measured difference in the δ^{13}C value between two subsequent samples shows no significant influence on δ^{13}C values measured in the second injection of the second sample. The entire set of results achieved within this test series can be found in Supplementary Table S 3-1 (Supporting Information).

The carry-over cannot be avoided or corrected for. Therefore, the first value needs to be discarded and considered as a 'dummy' peak. This was done for all further test series and evaluations. Kirkels et al.[32] investigated further improvements of the precision by the use of additional replicates.

Precision

The precision obtained by averaged results of repeated measurements was ≤0.1‰ (u_{std}: SD ≡ 1σ; Supplementary Table S 3-1, Supporting Information). Even with a $\Delta(A_{n+1} - A_n)$ value of >50‰, u_{std} is equal to 0.04‰.

We roughly estimated the precision under reproducibility conditions by comparing measurements between two different instruments, run by different operators in different

laboratories (Institute for Biodiversity and Ecosystem Dynamics (IBED), University of Amsterdam, Amsterdam, The Netherlands). The reproducibility for the $\delta^{13}C$ value of the same DOC sample (compound solutions of HUM1) was ca 0.5‰. This value includes the precision of the instrument itself and differences in the sample-preparation, laboratory environment, etc. Inhomogeneity of the humic acid sample itself can also contribute to this number.

Linearity

The term linearity in SIA indicates that the measured isotope ratio is independent of the amount of analyte.[26] The isotope ratio mass spectrometer and the hyphenated instrumentation are two different, potential sources of non-linearity Equation 3-11:

$$L_{R\,\sum} = L_{R\,IRMS} + L_{R\,TOC} \qquad \text{Equation 3-11}$$

While the isotope ratio mass spectrometer linearity ($L_{R\,IRMS}$) influences both the reference gas and the analyte peak, the TOC linearity ($L_{R\,TOC}$) contributes only to the analyte peak. Either it needs to be demonstrated that the contributions are negligible or corrections are required (further general principles considered for corrections are given in the Supporting Information).

To test if there is an $L_{R\,TOC}$ effect, we measured different compounds (caffeine (CAF2), acetovanillone (ACF1) and citric acid (CIT2)) in various concentrations (25–160 mgC/L). Relatively high concentrations were used in order to avoid any influence of the water blanks. Different compounds were used to check if there are any compound-specific linearity effects.

The isotope ratio mass spectrometer linearity was corrected first using Equation 3-5. Figure 3-7 exemplifies for citric acid that after $L_{R\,IRMS}$ was corrected for, no additional nonlinearity effects remained (m_{lin} = 0.00 ‰/nA). This is also the case for all other tested compounds (see Supporting Information); thus, no compound-specific nonlinearities were observed. Correcting the systematic drift caused by nonlinearity of the isotope ratio mass spectrometer improves the u_{std} from ±0.22‰ to ±0.02‰. After the correction, even in cases where the data points still show a trend, the contribution of nonlinearity is negligible (max. m_{lin} = 0.001 ‰/nA). Since no significant $L_{R\,TOC}$ effects were observed, no additional linearity corrections are necessary. Note that if the peak height of the reference gas is adjusted to the peak height of the analyte $L_{R\,IRMS}$ does not need to be considered (Equation 3-5).

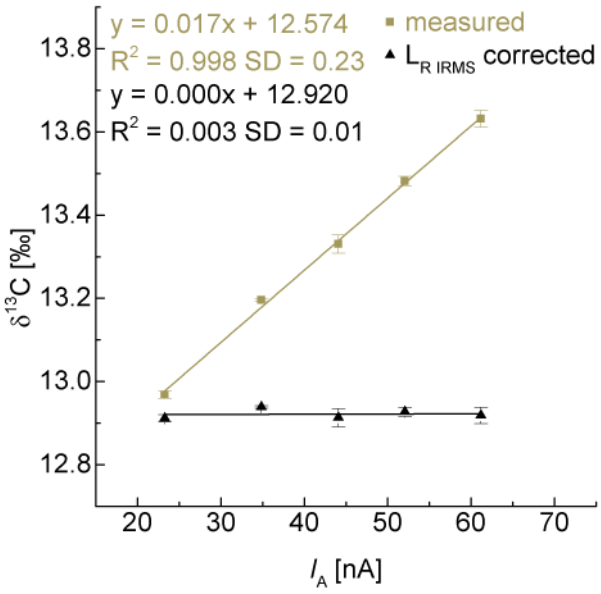

Figure 3-7 Correlation between $\delta^{13}C$ values and isotope ratio mass spectrometer detector signal (I_A) before (measured: brown line and squares) and after ($L_{R\ IRMS}$ corrected: black line and triangles) $L_{R\ IRMS}$ correction of citric acid (CIT2) solution (25–160 mgC/L).

If samples with very different DOC concentrations have to be analyzed, $L_{R\ IRMS}$ checks must be made at the beginning and at the end of each sample series in order to enable corrections for linearity deviations. Each correction will increase the combined uncertainty and needs to be assessed via error propagation. The assessment of the combined uncertainty is described in the Normalization and trueness section.

Blank correction

High concentration samples

Plausibility considerations suggest that at higher concentrations (≥10 mgC/L) the contribution has a low but still considerable influence on the final $\delta^{13}C$ value. Without correction a 10 mgC/L solution has a bias of 0.16‰ and a 5 mgC/L solution a bias of 0.31‰, assuming a difference of 15‰ between the sample and the blank (for details, see Supplementary Table S 3-2, Supporting Information).

Estimation of the blank carbon concentration was conducted via a standard addition method according to DIN 32 633.[40] The $\delta^{13}C$ value of the blank was measured by multiple 3-mL injections of pure water, incorporating both water and instrumentation as possible blank sources. As previously shown in the "Sources of the instrumental blank" section, the instrument contribution to the blank appears to be negligible. Mass balance Equation 3-6 was used to calculate the water blank.

After blank correction the uncertainty improved very little, indicating that the influence of the blank is minimal at high analyte (≥10 mgC/L) concentration (considering u_{std} ≤0.2‰ as good and ≤0.5‰ as acceptable). It should be noted that, especially at a very negative $\delta^{13}C$ value (CAF, confirmed by EA/IRMS) compared with the $\delta^{13}C_{\Sigma bl}$ values, such blank corrections still matter since a systematic bias was observed. The bias became distinct (Δ >0.2‰) at concentrations of 10 and 25 mgC/L (Figure 3-8(a)). The magnitude of this bias depends on the difference between the stable isotope composition of the analyte and that of the blank, and on the carbon concentrations of both. The improvement of u_{std} after the correction is on average 0.06‰ (from 0.09‰ to 0.03‰) with a maximum improvement of 0.14‰.

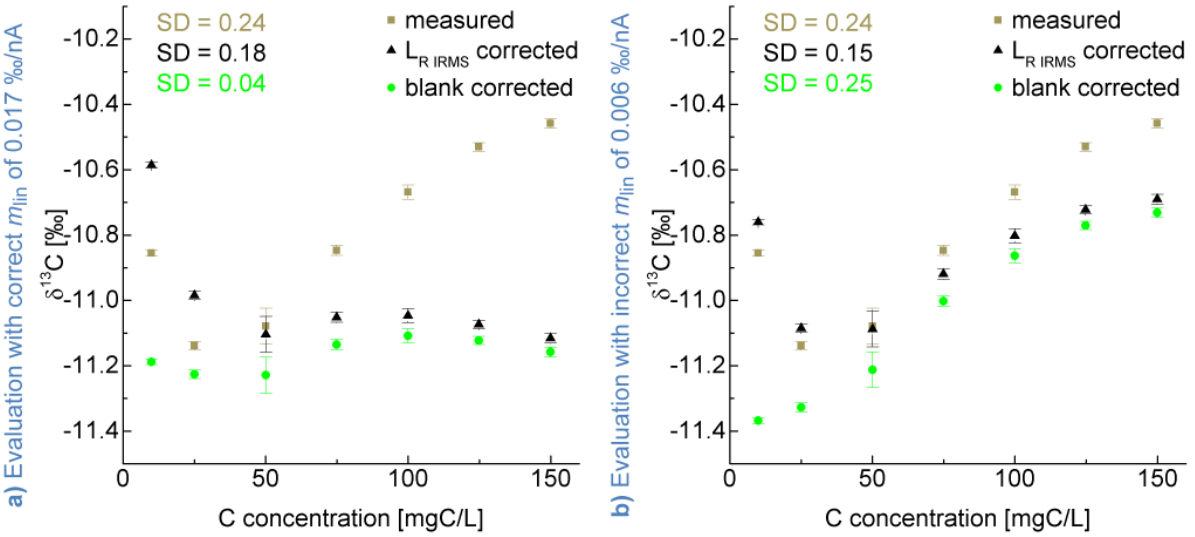

Figure 3-8 Evaluation inclusive blank correction (a). Correlation between $\delta^{13}C$ values and C concentration of caffeine (CAF2) solution (10–150 mgC/L) with proper (a) and with simulated incorrect linearity and blank corrections (b). Measured: brown squares, $L_{R\ IRMS}$ corrected: black triangles, blank corrected: green dots. In (b) an m_{lin} of 0.006 ‰/nA leads to better u_{std} of 0.15‰ after linearity correction and the blank correction seems to be false because it makes the u_{std} even worse (0.25 ‰). This demonstrates how the interdependence of blank and linearity correction can lead to misinterpretation and thus how important its proper investigation is.

A wrong estimation of the blank, e.g. 10% lower, would change the calculated $\delta^{13}C$-value of a 10 ppm caffeine solution by only 0.06‰. Therefore, a quick estimation of the blank can be used for high concentration samples, avoiding a laborious accurate determination. Note, however, that the stability of the blank over time needs to be ensured. Each wrongly estimated systematic deviation contributes to the combined uncertainty. Even when such uncertainty stays within an acceptable range, the contribution of several small biases can become

significant when not corrected for. Therefore, it is important also to blank correct the measurements of samples with higher DOC concentration.

Analyses of samples containing CAF2 showed how important an appropriate correction of linearity is. It is demonstrated by the comparison of the correlation between the $\delta^{13}C$ value and the C concentration with proper (Figure 3-8(a)) and with simulated incorrect linearity Figure 3-8(b). The results achieved with other substances can be found in Supplementary Figure S 3-3 and Figure S 3-4 (Supporting Information). Underestimation of its quantity would lead to false interpretation of the blank correction. The interaction of the linearity and the blanks makes a systematic and careful investigation necessary.

Samples with low DOC concentrations

Samples with low carbon content (≤10 mgC/L) differ fundamentally from those with high DOC concentrations. The impact of isotope ratio mass spectrometer nonlinearity is very low for such samples but the impact of the blank becomes highly significant (for explanation, see the Supporting Information). Thus, a proper investigation of the blank is of the utmost importance for SIA of DOC at low concentrations. At the same time this is also the most challenging part due to the required sensitivity and high relevance of possible contamination.

Caffeine, benzoic acid, citric acid and acetovanillone solutions (0.1, 0.2, 0.4, 0.6 and 1.2 mgC/L, all concentrations measured with 1, 2, and 3 mL injection volumes) were used as model compounds to investigate the instrumental performance in the low concentration range. As expected, for all the compounds an increasingly evident drift towards the blank value was found with decreasing concentration (Figure 3-9). The blank correction led to an improvement in the SD of the $\delta^{13}C$ values from 0.56‰ to 0.23‰. The poor precision of ±2.18‰ at 0.1 mgC/L indicates an instrumental limitation (avg. I_A = 0.31 nA). Still, also at concentrations above 0.1 mgC/L, outliers were observed. A view on the whole $\delta^{13}C$ dataset revealed an increase in variation with decreased concentration levels in a 'Horwitz trumpet'-like curve[41,42] (Figure 3-10). A concentration of 1.2 mgC/L showed a good repeatability (0.12‰), while concentrations of 0.4 and 0.6 mgC/L showed still acceptable repeatability (0.52 and 0.66‰). A concentration of 0.2 mgC/L showed poor precision (1.48‰).

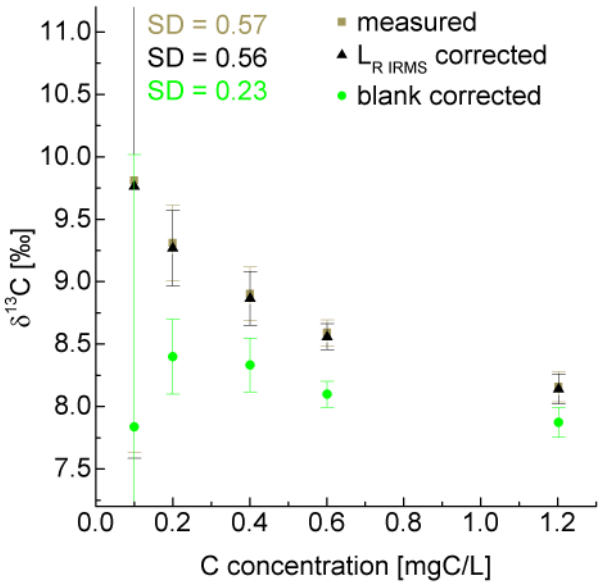

Figure 3-9 Correlation between δ^{13}C values and C concentration of acetovanillone (ACV1) solution (0.1–1.2 mgC/L) Measured: brown squares, $L_{R\ IRMS}$ corrected: black triangles, blank corrected: green dots. Blank correction corrects the values-drift towards the stable isotope composition of the blank (10.57‰) with decreasing concentration. SD from δ^{13}C values at all concentrations improves from 0.56‰ to 0.23‰ after the blank correction. Poor precision of ±2.18‰ (see error bars) at 0.1 mgC/L indicates instrumental limitation (avg. I_A = 0.31 nA).

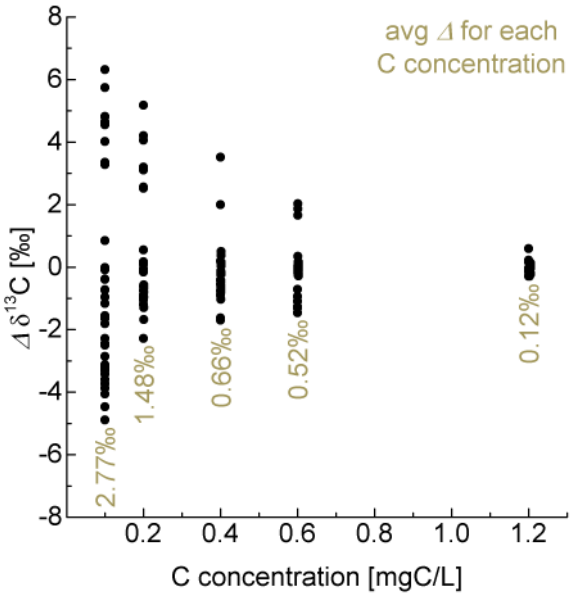

Figure 3-10 Deviation (Δ) of all single δ^{13}C-values from their respective true value (y axis) plotted against concentration (x axis). The scattering of the values represents the repeatability of stable isotope measurements at the corresponding concentration with the chosen method.

The following two facts confirm the assumption that the decrease in repeatability with decreased concentration is not an issue of poor isotope ratio mass spectrometer sensitivity. First, down to 200 µgC/L the sample peak heights of the isotope ratio mass spectrometer signals appear within the range where accurate values are generated (≥1 nA). Second, plotting of NDIR detector peak area (representing DOC concentration) versus progressive measurements (representing different concentrations, compounds and injection volumes) showed the same 'trumpet' progression of the variation in the low concentration range (Figure 3-11(a)) as with the stable isotope composition (Figure 3-10).

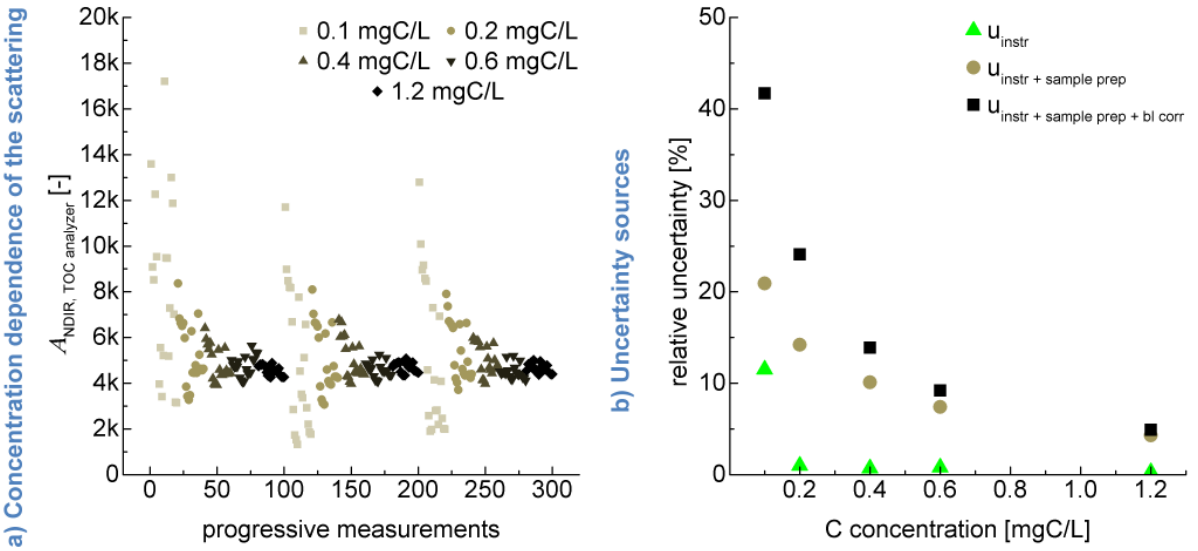

Figure 3-11 Carbon concentration values at lower carbon concentration range; (a) Investigation of the dependence of DOC concentration (y axis, NDIR Area from TOC analyzer) on its repeatability (x axis, number of measurements). The blank corrected NDIR areas are normalized to the injection volume of 1 mL and concentration of 1 mgC/L to enable comparability and they are plotted against measuring order (five replicates of each concentration: 0.1, 0.2, 0.4, 0.6 and 1.2 mgC/L with 4 different compounds each and using 3, 2 and 1 mL injection). (b) All estimated relative uncertainties (y axis) plotted against the DOC concentration (x axis). Instrumental caused uncertainty (u_{instr} = u_{std}, green triangles), combined uncertainty implementing sample-preparation caused error ($u_{instr\ +\ sample\ prep}$, brown dots) and combined uncertainty implementing also error caused by data evaluation ($u_{instr\ +\ sample\ prep\ +\ bl\ corr}$, blank squares).

As a consequence, the large scatter of the measured concentrations and of the stable isotope values at low DOC concentrations can potentially have two main sources: either the TOC instrument itself or the sample-preparation.

The average of standard deviations of replicate measurements from each vial was 1.2% (RSD) and this instrumental precision indicates that the TOC instrumentation is not a source of the observed variations at lower concentrations. In contrast, there is a substantial contribution from sample-preparation as represented by the scattering of the DOC concentrations measured between different vials with the same compound in the same concentration (11% RSD). Of course, any applied correction increases the uncertainty of the measurement following the principle of error propagation. In the case of low concentration samples, the contribution of the blank correction to the combined uncertainty is large due to the large ratio between the water blank and the analyte concentrations.

Our experiments confirmed these expectations, as shown in Table 3-2 and Figure 3-11(b), which show the series with 3-mL injection (for complete table, see the Supporting Information). Note that the high relative deviations (Figure 3-11(b)) are still small absolute ones. The instrumental limitation is about 0.2 mgC/L. The background noise of the instrument starts to show a significant influence on the measurements below 0.2 mgC/L. The graph shows clearly the significance of the contribution of the blank correction: the blank contribution to the uncertainty increases with decreasing sample concentration. At 0.1 mgC/L, where the blank and the concentration are in the same concentration range, it amounts to 20.8%.

Table 3-2 Different sources of uncertainty and their quantities for different concentrations (4 samples and 3 replicates each); Instrumental caused uncertainty ($u_{instr} = u_{std}$), combined uncertainty implementing sample-preparation caused error ($u_{instr + sample\ prep}$) and combined uncertainty implementing also evaluation caused error ($u_{instr + sample\ prep + bl\ corr}$); Note that the high relative deviations are still small absolute ones. 0.7% in brackets shows the average without 0.1 mgC/L sample justifying LOQ_{instr} of 0.2 mgC/L

C concentration [mgC/L]	rel. u_{instr} [%]	rel. $u_{instr + sample\ prep}$ [%]	rel. $u_{instr + sample\ prep + bl\ corr}$ [%]
0,1	*11,5*	20,9	41,7
0,2	1,0	14,2	24,1
0,4	0,7	10,1	13,9
0,6	0,8	7,4	9,2
1,2	0,3	4,3	4,9
avg	(0.7) *2.9*	11,4	18,8

The instrumental precision and sensitivity allow $\delta^{13}C$ values to be measured accurately (with u_{std} <0.2‰) down to a DOC concentration of 0.2 mgC/L. Minor contaminations and small absolute variations of the water blank have a large relative impact and magnify the uncertainty of the results in samples with low concentrations. Therefore, sample-preparation further limits the performance of the method in the low concentration range and appropriate handling of the samples and vials becomes crucial to significantly decreasing the uncertainty. The experimentally determined combined uncertainty results in a $LOQ_{SIA\ method}$ of ca 0.5 mgC/L, considering an accuracy of ±0.5‰ as acceptable and of 0.2 mgC/L for $LOQ_{SIA\ instr}$.

The entire set of results is summarized in Supplementary Table S 3-3 (Supporting Information) together with further details regarding the results of the DOC SIA of samples with low DOC concentrations.

Normalization and trueness

After blank correction the $\delta^{13}C_{\Sigma bl\ corr\ A}$ values were related to the in-house reference gas. Trueness quantifies the closeness of the agreement between the average value obtained from a series of test results and an accepted reference value.[36] To prove trueness, the measured values first have to be traced back to the VPDB scale. Therefore, to prove metrological traceability[34] and investigate trueness, all the blank corrected values were two-point normalized as described above.

No aqueous DOC reference materials exist for SIA. Therefore, solid reference substances were dissolved in ultrapure water to obtain a solution with defined carbon concentrations and $\delta^{13}C$ values. Enriched glutamic acid GLU1 with $\delta^{13}C_{A,VPDB}$ 37.626 ± 0.049 and caffeine CAF1 with $\delta^{13}C_{A,VPDB}$ –27.771 ± 0.043 were dissolved in pure water (10 mgC/L). This large Δ value was chosen on purpose to test if the normalization works within a large stable isotope composition range. The obtained stretching factors m_{norm} and b_{norm} were used to normalize all the measured $\delta^{13}C_{\Sigma bl\ corr\ A,RG}$ values to $\delta^{13}C_{A,VPDB}$ values.

A third reference material sucrose SUC1 with a $\delta^{13}C_{A,VPDB}$ value of –10.449 ± 0.033 was analyzed to test the trueness. The closeness of the agreement between the internationally accepted value and the obtained value of –10.40 ± 0.07‰, expressed as $\Delta_{trueness} = \delta^{13}C_{accepted\ A,VPDB} - \delta^{13}C_{obtained\ A,VPDB}$, of 0.05‰ indicated good trueness.

Due to the lack of certified reference material, three additional internal laboratory standards were used. Their traceability was ensured by referencing of the EA/IRMS $\delta^{13}C_A$ values to the reference materials and thus indirectly to the VPDB scale.[26] The obtained values were considered as true values for the investigation of trueness as $\Delta_{trueness}$.

The obtained $\Delta_{trueness}$ value (Table 3-3) was good (≤0.2‰). Only one value deviated more from the accepted value, but it was still within an acceptable range of ≤0.5‰. The coefficient of determination of the linear regression between the measured and true $\delta^{13}C$ values (r^2 = 0.994) also indicates good agreement.

Table 3-3 Trueness of the method expressed as difference between the true and measured value

Compound	true $\delta^{13}C$ [‰]	measured $\delta^{13}C$ [‰]	$\Delta_{meas-true}$ $\delta^{13}C$ [‰]
Sucrose (NIST 8542)	-10,47	-10.4 ± 0.09	0.07
Citric acid (working std.)	-16.00	-16.32 ± 0.05	0.32
Casein (working std.)	-22.40	-22.55 ± 0.02	0.15
Glutamic acid (working std.)	-26.77	-26.64 ± 0.05	0.13

Uncertainty and accuracy

Accuracy is described as "the closeness of the agreement between the result of a measurement and the true value of the measurand".[43] Accuracy is best assessed by the combined uncertainty estimated by error propagation.[26] The typical standard uncertainty (expressed as standard deviation) was ≤0.2‰ within the investigated concentration ranges. It represents mainly the instrumental precision. The combined standard uncertainty comprises several uncertainties and it increased to a value of up to 1.2‰. That uncertainty represents the accuracy of the complete method, from sample-preparation to the final measurement. It incorporates the poor accuracy of low concentration samples due to the water blank. Furthermore, the measurement of the water blank affects the uncertainty of the standard solution values and, through the normalization procedure, the uncertainty of the sample values. The very good precision achieved for replicated blank measurements from the same vial (avg. SD: 0.25‰) and the relatively poor precision for blanks measured from different vials (avg. SD: 1.31‰) with the same ultrapure water indicated vial cleanliness being the main contribution to the blank uncertainty. Pretreatment of the vials at 400 °C, after previous chemical cleaning with highly concentrated oxidizing acid, may improve accuracy in measuring low concentration samples.[44]

Oxidation efficiency and matrix effects

No component other than the analyte (matrix) should contribute to the result. Identification and assessment of typical matrix components were performed before testing the selectivity of

the method on the basis of model solutions (Table 3-4). DOC SIA is a bulk method and the composition of the samples is unknown. Thus, it is not possible to correct for compound-specific fractionation as a result of incomplete oxidation, and complete oxidation is therefore essential.

Barbituric acid, melamine and humic acid were analyzed as model compounds that are resistant to oxidation, and thus potentially affecting DOC and SI analyses.[23,45] We found a recovery rate of ≥99%, proving complete oxidation. This indicated the compound independence of the method. The precision with an average δ^{13}C SD of 0.13‰, and trueness with an average $\Delta\delta^{13}$C of 0.23‰, confirmed this conclusion. Kirkels et al.[32] showed similar values using natural DOC samples from terrestrial and aquatic environments.

High salt loads are another important challenge for the SIA of DOC.[10] The HTC method has to be used for samples with high salinity, such as seawater, as discussed in the introduction. We dissolved humic acid in a simplified model solution of 3.5% NaCl to investigate the influence of high salt load, e.g. possible adsorption or catalyst poisoning effects. High concentrations of the analyte (50 mg/L) were used to avoid problems related to low concentration (cf. the section, "Samples with low DOC concentrations"). We did not observe significant effects of salt, as indicated by an average δ^{13}C SD of 0.04‰ and trueness with an average $\Delta\delta^{13}$C of 0.02‰.

The analysis of additional seawater samples (98 injections; 3 mL each; 14 samples) showed an average δ^{13}C SD of 0.07‰. The standards showed an average δ^{13}C SD of 0.02‰ and an average $\Delta\delta^{13}$C of 0.03‰measured after 230 injections (90 river water, 40 ultrapure water, 40 standard solutions and 60 seawater).

In our validation, complete removal of the total inorganic carbon (TIC) appeared essential and thus efficient acidification and purging are crucial for accurate DOC measurements. Therefore, we had to increase the purging time for seawater. An additional 40 min resulted in a significant decrease in the systematic error from $\Delta\delta^{13}$C 1.18‰ to $\Delta\delta^{13}$C 0.08‰.

The conducted tests clearly indicated a general suitability of the developed system for DOC SIA of samples with a higher salt load. The preliminary tests that we carried out should be followed up by an intensive study with real seawater samples or brine solutions characterized by low carbon concentration.

Further issues related to matrix effects are discussed in the Supporting Information.

Table 3-4 Classification of potential problems and interferences related to DOC measurements in aqueous samples

Matrix	Interferences/ possible sources of isotopic discrimination (pID)	Problematic stage	typical issue?	Handling within developed system
Matrix components without TC				
water	CO_2 solubility in water (pID)	gas/liquid separation within the instrument	no (considered as low conc. blank issue)	Higher temperature of the water within the condenser (solubility decrease)
Non-carbon containing salt load	pID due to not complete mineralization	Reaction tube (Mineralisation): HTC: catalyst poisoning; glas affect; WCO: radical scavenging	yes[10]	HTC instead of WCO reactor; Salt trapping (ash finger)
Matrix molecular entities within TC				
particulate organic carbon	non-analyte carbon: within the instrument not distinguishable	sample preparation	no	separation via filtering (offline step)
total inorganic carbon	non-analyte carbon: within the instrument not distinguishable	sample preparation/ autosampler	critical stage: typical error source if counter measures insufficient	acidification and purging out in autosampler
volatile organic carbon	non-analyte carbon: within the instrument not distinguishable	sample preparation/ autosampler	no	outgasing while sampling and sample preparation (refilling etc.) and purging out in autosampler
Molecular form of DOC[a]				
attraction between atoms within the molecule (strength of the bond)	pID due to incomplete mineralization of persistent compounds	Reaction tube (mineralisation)	yes[10]	HTC instead of WCO reactor; Optimized conditions: Temp., catalyst and flow conditions (contact tims)

WCO: wet chemical oxidation; HTC: high temperature combustion;

[a]DOC measurements should not be influenced by the persistence of compounds or if they are present in colloidal form or truly dissolved.

3.5 Conclusions and outlook

A novel HTC-based TOC/IRMS system was developed and *inter alia* the standard uncertainty of ≤0.2‰and the $LOQ_{SIA\ instr}$ of 0.2 mgC/L confirm its suitability for accurate DOC SIA. The oxidation efficiency of the system is ≥99% and therefore an isotope fractionation related to limited oxidation of more resistant compounds is prevented. Compared with other methods our new approach improved the accuracy, especially at low DOC concentrations and in the presence of high salt loads, as well as for compounds that are resistant to oxidation. To the best of our knowledge, this novel system is the only HTC-based system which allows a 3-mL injection compared with a typical injection volume of <200 µL.[17,39,46] This improvement alone resulted in an increase in sensitivity by a factor of 15. With the developed system no laborious sample-preparation steps are necessary, such as time-consuming offline preconcentration steps (e.g. freeze-drying). This significantly reduces the possible sources of contamination.

This system also opens the possibility of a larger use of certified or internationally accepted reference materials (solution of low concentration with blank correction) to assure traceability and comparability among different laboratories. For the same reasons we proposed a method for data treatment but an internationally agreed method of validation still needs to be defined.

In summary, the described system offers a new and promising approach for the use of DOC SIA for routine analysis, also for the analysis of samples in difficult matrices without offline sample-preparation. The system was intensively optimized and validated with a broad set of real samples within the work by Kirkels et al.[32]

Additional supporting information may be found in section Supporting information.

3.6 References

[1] J. I. Hedges, in Biogeochemistry of Marine Dissolved Organic Matter, (Eds: D. A. Hansell, C. A. Carlson). Academic Press, London, 2002. pp. 1–33.

[2] B. J. Eadie, L. M. Jeffrey, W. M. Sackett. Some observations on the stable carbon isotope composition of dissolved and particulate organic carbon in the marine environment. Geochim. Cosmochim. Acta 1978, 42, 1265.

[3] R. Kindler, J. Siemens, K. Kaiser, D. C. Walmsley, C. Bernhofer, N. Buchmann, P. Cellier, W. Eugster, G. Gleixner, T. Grünwald, A. Heim, A. Ibrom, S. K. Jones, M. Jones, K. Klumpp,W. Kutsch, K. S. Larsen, S. Lehunger, B. Loubet, R. Mckenzie, E. Moors, B. Osborne, K. Pilegaard, C. Rebmann, M. Saunder, M. W. I. Schmidt, M Schrumpf, J. Seyfferth, U. Skiba, J. -F. Soussana, M. A. Sutton, C. Tefs, B. Vowinckel, M. J. Zeeman, M. Kaupenjohann. Dissolved carbon leaching from soil is a crucial component of the net ecosystem carbon balance. Global Change Biol. 2011, 17, 1167.

[4] K. Kalbitz, S. Solinger, J.-H. Park, B. Michalzik, E. Matzner. Controls on the dynamics of dissolved organic matter in soils: a review. Soil Sci. 2000, 165, 277.

[5] K. Mopper, E. T. Degens, in Scope 13 – The Global Carbon Cycle, (Eds: B. Bolin, E. T. Degens, S. Kempe, P. Ketner). Scientific Committee on Problems of The Environment, Paris, 1979, pp. 293–316.

[6] D. A. Hansell, C. A. Carlson. Marine dissolved organic matter and the carbon cycle. Oceanography 2001, 14, 41.

[7] R. A. Houghton. Balancing the Global Carbon Budget. Annu. Rev. Earth Planet. Sci. 2007, 35, 313.

[8] P. M. Williams. Stable carbon isotopes in the dissolved organic matter of the sea. Nature 1968, 219, 152.

[9] D. A. Nimick, C. H. Gammons, S. R. Parker. Diel biogeochemical processes and their effect on the aqueous chemistry of streams: A review. Chem. Geol. 2011, 283, 3.

[10] K.Mopper, J.Qian, in Encyclopedia of Analytical Chemistry, (Ed.: R. A. Meyers). JohnWiley, Chichester, 2000. pp. 3532–3540.

[11] ISO 8245:1999. Water quality – guidelines for the determination of total organic carbon (TOC) and dissolved organic carbon (DOC).

[12] ASTM D7573-09. Test Method for Total Carbon and Organic Carbon in Water by High Temperature Catalytic Combustion and Infrared Detection.

[13] B. Fry, S. Saupe, M. Hullar, B. J. Peterson. Platinumcatalyzed combustion of DOC in sealed tubes for stable isotopic analysis. Mar. Chem. 1993, 41, 187.

[14] H. Gandhi, T. N. Wiegner, P. H. Ostrom, L. A. Kaplan, N. E. Ostrom. Isotopic (^{13}C) analysis of dissolved organic carbon in stream water using an elemental analyzer coupled to a stable isotope ratio mass spectrometer. Rapid Commun. Mass Spectrom. 2004, 18, 903.

[15] C. L. Osburn, G. St-Jean. The use of wet chemical oxidation with high-amplification isotope ratio mass spectrometry (WCO-IRMS) to measure stable isotope values of dissolved organic carbon in seawater. Limnol. Oceanogr.: Methods 2007, 5, 296.

[16] S. Bouillon, M. Korntheuer, W. Baeyens, F. Dehairs. A new automated setup for stable isotope analysis of dissolved organic carbon. Limnol. Oceanogr.: Methods 2006, 4, 216.

[17] I. De Troyer, S. Bouillon, S. Barker, C. Perry, K. Coorevits, R. Merckx. Stable isotope analysis of dissolved organic carbon in soil solutions using a catalytic combustion total organic carbon analyzer-isotope ratio mass spectrometer with a cryofocusing interface. Rapid Commun. Mass Spectrom. 2010, 24, 365.

[18] S. Q. Lang, M. D. Lilley, J. I. Hedges. A method to measure the isotopic (^{13}C) composition of dissolved organic carbon using a high temperature combustion instrument. Mar. Chem. 2007, 103, 318.

[19] P. Albéric. Liquid chromatography/ mass spectrometry stable isotope analysis of dissolved organic carbon in stream and soil waters. Rapid Commun. Mass Spectrom. 2011, 25, 3012.

[20] A. Hartland, A. Baker, W. Timms, Y. Shutova, D. Yu. Measuring dissolved organic carbon $\delta^{13}C$ in freshwaters using total organic carbon cavity ring-down spectroscopy (TOC-CRDS). Environ. Chem. Lett. 2012, 10, 309.

[21] B. Fry, E. T. Peltzer, C. S. Hopkinson Jr, A. Nolin, L. Redmond. Analysis of marine DOC using a dry combustion method. Mar. Chem. 1996, 54, 191.

[22] G. R. Aiken. Chloride interference in the analysis of dissolved organic carbon by the wet oxidation method. Environ. Sci. Technol. 1992, 26, 2435.

[23] P. J. Wangersky. Dissolved organic carbon methods: a critical review. Mar. Chem. 1993, 41, 61.

[24] R. van Geldern, M. P. Verma, M. C. Carvalho, F. Grassa, A. Delgado-Huertas, G. Monvoisin, J. A. C. Barth. Stable carbon isotope analysis of dissolved inorganic carbon (DIC) and dissolved organic carbon (DOC) in natural waters – Results from a worldwide proficiency test: Carbon stable isotope proficiency test of DIC and DOC. Rapid Commun. Mass Spectrom. 2013, 27, 2099.

[25] G. Schwedt. Taschenatlas der Analytik. Wiley-VCH, Weinheim, 2007.

[26] M. A. Jochmann, T. C. Schmidt. Compound-Specific Stable Isotope Analysis. Royal Society of Chemistry, Cambridge, 2012.

[27] W. A. Brand, in Handbook of Stable Isotope Analytical Techniques, (Ed: P. A. de Groot). Elsevier, Amsterdam, 2004, pp. 835–857.

[28] AOAC 984.23-1988. Corn syrup and cane sugar in maple syrup. Carbon ratio mass spectrometric method.

[29] OIV AS312-07 2010. Method for the determination of the $^{13}C/^{12}C$ isotope ratio of glycerol in wines by gas chromatography combustion or high performance liquid chromatography coupled to isotope ratio mass spectrometry (GC-C-IRMS or HPLC-IRMS).

[30] OIV AS314-03 2005. Determination of the carbon isotope ratio $^{13}C/^{12}C$ of CO_2 in sparkling wines – method using isotope ratio mass spectrometry (IRMS).

[31] EEC/822/97. Determination of the isotopic ratio of oxygen of the water content in wines.

[32] F. M. S. A. Kirkels, C. Cerli, E. Federherr, J. Gao, K. Kalbitz. A novel high-temperature combustion based system for stable isotope analysis of dissolved organic carbon in aqueous samples. II: optimization and assessment of analytical performance. Rapid Commun. Mass Spectrom. 2014, 28, 2574.

[33] DIN EN ISO/IEC 17025:2005. General requirements for the competence of testing and calibration laboratories.

[34] P. De Bièvre, R. Dybkær, A. Fajgelj, D. B. Hibbert. Metrological traceability of measurement results in chemistry: concepts and implementation. Pure Appl. Chem. 2011, 83, 1873.

[35] M. Thompson, S. L. Ellison, R. Wood. Harmonized guidelines for single-laboratory validation of methods of analysis (IUPAC Technical Report). Pure Appl. Chem. 2002, 74, 835.

[36] M. Thompson, R. Wood. Harmonized guidelines for internal quality control in analytical chemistry laboratories. Nature 1995, 4, 4.

[37] A. Menditto, M. Patriarca, B. Magnusson. Understanding the meaning of accuracy, trueness and precision. Accredit. Qual. Assur. 2006, 12, 45.

[38] CAC/GL 59–2006: Guidelines on estimation of uncertainty of results.

[39] R. J. Panetta, M. Ibrahim, Y. Gélinas. Coupling a hightemperature catalytic oxidation total organic carbon analyzer to an isotope ratio mass spectrometer to measure natural-abundance δ^{13}C dissolved organic carbon in marine and freshwater samples. Anal. Chem. 2008, 80, 5232.

[40] DIN 32633:2013. Chemical analysis – methods of standard addition.

[41] M. Thompson. The amazing Horwitz function. AMC Technical Brief. 2004, No. 17.

42] P. Hall, B. Selinger. A statistical justification to relating interlaboratory coefficients of variation with concentration levels. Anal. Chem. 1989, 61, 1465.

[43] IUPAC Gold Book. Accuracy of measurement. http://goldbook.iupac.org/A00060.html.

[44] R. Otson, D. T. Williams, P. D. Bothwell, R. S. McCullough, R. A. Tate. Effects of sampling, shipping, and storage on total organic carbon levels in water samples. Bull. Environ. Contam. Toxicol. 1979, 23, 311.

[45] L. Zhang, D. M. Kujawinski, M. A. Jochmann, T. C. Schmidt. High-temperature reversed-phase liquid chromatography coupled to isotope ratio mass spectrometry. Rapid Commun. Mass Spectrom. 2011, 25, 2971.

[46] H. Gandhi, T. N. Wiegner, P. H. Ostrom, L. A. Kaplan, N. E. Ostrom. Isotopic (^{13}C) analysis of dissolved organic carbon in stream water using an elemental analyzer coupled to a stable isotope ratio mass spectrometer. Rapid Commun. Mass Spectrom. 2004, 18, 903.

3.7 Supporting information

Supporting information for section Carry-over (memory effects)

Testing protocol: 1 mL injection, 300 µL/s injection speed; 10 mgC/L; four replicates;

Table S 3-1 Sequence, single values of replicates and calculated parameters from the test series to investigate carry over.

sequence		$\delta^{13}C_{meas}$	$V_{carry\text{-}over}$	$\Delta(A_{n+1}\text{-}A_n)$	with 1st replicate	without 1st replicate
A_n	$replicate_i$	[‰]	[µl]	[‰]	$\delta^{13}C_{meas}\pm u_{std}$ [‰]	$\delta^{13}C_{meas}\pm u_{std}$ [‰]
GLU1	4	35,38				
CAF1	1	-25,00	35	-61,95	-26,57±1,04	-27.09±0.04
	2	-27,12				
	3	-27,10				
	4	-27,04				
SUC1	1	-11,30	41	16,44	-10,81±0,33	-10.65±0.09
	2	-10,55				
	3	-10,68				
	4	-10,72				
GLU2	1	-25,35	45	-15,36	-25.85±0.33	-26.01±0.04
	2	-26,00				
	3	-25,98				
	4	-26,06				
CIT1	1	-16,52	28	9,76	-16.32±0.14	-16.25±0.05
	2	-16,21				
	3	-16,30				
	4	-16,24				
CAS1	1	-22,03	21	-5,9	-22.12±0.06	-22.15±0.02
	2	-22,16				
	3	-22,16				
	4	-22,13				

Supporting information for section Linearity

General considerations/ principles to handle non-linearity issue: After $L_{R\ IRMS}$ was corrected only $L_{R\ TOC}$ remains to be quantified. $L_{R\ TOC}$ can be used for corrections only if the used system has no additional compound-specific fractionation due to, e.g. incomplete mineralization because the exact composition of DOC in real samples is unknown. If $L_{R\ TOC}$ correction is necessary (system dependent), it has to be applied after the blank correction.

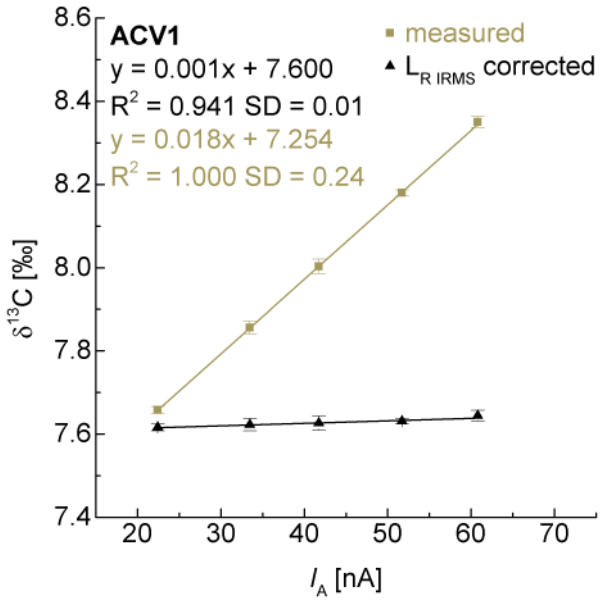

Figure S 3-1 $L_{R\ IRMS}$ correction using acetovanillone solution (25-160 mgC/L)

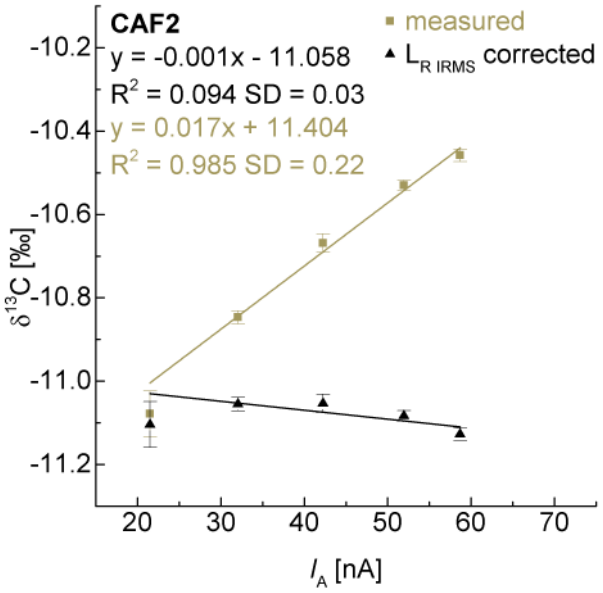

Figure S 3-2 $L_{R\ IRMS}$ correction using caffeine solution (25-160 mgC/L)

Supporting information for section Blank correction

Table S 3-2 Plausibility considerations a: Correction starts to matter (exceeding bias of 0.2 ‰); considering worst case: high blank and large delta difference: aqueous solution; b: Bias falsely considering no instrumental blank (exceeding bias of 0.5‰): real sample

a

bias	measured		Σblank		water blank			instr. Blank		sample		
$\delta^{13}C$ [‰]	$\delta^{13}C$ [‰]	m [µg]	$\delta^{13}C$ [‰]	m [µg]	$\delta^{13}C$ [‰]	V [mL]	ρ [µgC/mL]	$\delta^{13}C$ [‰]	m [µg]	$\delta^{13}C$ [‰]	V [mL]	ρ [µgC/mL]
0,03	-10,03	150,30	-26,00	0,30	-26	3,00	0,00	-26	0,30	-10	3,00	50
0,06	-10,06	75,30	-26,00	0,30	-26	3,00	0,01	-26	0,27	-10	3,00	25
0,16	-10,16	30,30	-26,00	0,30	-26	3,00	0,02	-26	0,24	-10	3,00	10
0,31	-10,31	15,30	-26,00	0,30	-26	3,00	0,04	-26	0,18	-10	3,00	**5**
1,45	-11,45	3,30	-26,00	0,30	-26	3,00	0,06	-26	0,12	-10	3,00	1
2,67	-12,67	1,80	-26,00	0,30	-26	3,00	0,08	-26	0,06	-10	3,00	0,5
8,00	-18,00	0,60	-26,00	0,30	-26	3,00	0,10	-26	0,00	-10	3,00	0,1
-0,16	-10,16	15,15	-26,00	0,15	-26	3,00	0,15	-26	-0,30	-10	3,00	5
-0,11	-10,11	15,10	-26,00	0,10	-26	3,00	0,10	-26	0,00	-10	3,00	5
-0,08	-10,08	15,08	-26,00	**0,08**	-26	3,00	0,08	-26	0,00	-10	3,00	5
-0,05	-10,05	15,05	-26,00	0,05	-26	3,00	0,05	-26	0,00	-10	3,00	5
-0,10	-10,10	15,08	-30,00	0,08	-26	3,00	0,08	-26	-0,17	-10	3,00	5
-0,07	-10,07	15,08	**-25,00**	0,08	-26	3,00	0,08	-26	0,00	-10	3,00	5
-0,05	-10,05	15,08	-20,00	0,08	-26	3,00	0,08	-26	0,00	-10	3,00	5
-0,02	-10,02	15,08	-15,00	0,08	-26	3,00	0,08	-26	0,00	-10	3,00	5

b

bias	measured		Σblank		water blank			instr. Blank		sample		
$\delta^{13}C$ [‰]	$\delta^{13}C$ [‰]	m [µg]	$\delta^{13}C$ [‰]	m [µg]	$\delta^{13}C$ [‰]	V [mL]	ρ [µgC/mL]	$\delta^{13}C$ [‰]	m [µg]	$\delta^{13}C$ [‰]	V [mL]	ρ [µgC/mL]
0,62	-10,62	3,12	-26,00	0,30	-26	3,00	0,060	-26	**0,120**	-10	3,00	1
0,31	-10,31	3,06	-26,00	0,30	-26	3,00	0,080	-26	0,060	-10	3,00	1
0,16	-10,16	3,03	-26,00	0,30	-26	3,00	0,090	-26	0,030	-10	3,00	1
0,00	-10,00	3,00	-26,00	0,30	-26	3,00	0,100	-26	0,000	-10	3,00	1

Supporting information for section High concentration samples (Blank correction)

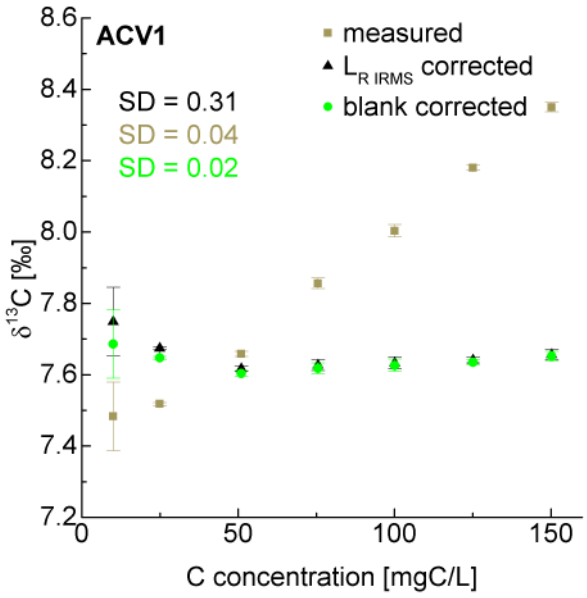

Figure S 3-3 Blank correction using acetovanillone solution (10-150 mgC/L)

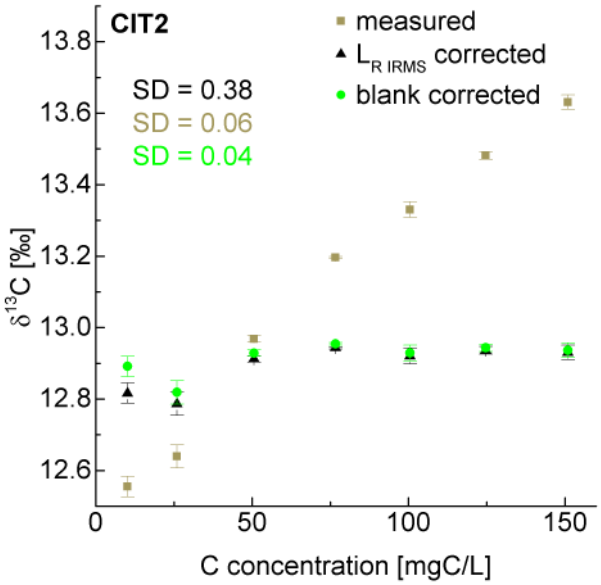

Figure S 3-4 Blank correction using citric acid solution (10-150 mgC/L)

Supporting information for section Samples with low DOC concentrations (Blank correction)

Table S 3-3 Different sources of uncertainty and their quantities for different concentrations (4 samples; 3 replicates each) and different injection volumes; Instrumental caused uncertainty ($u_{instr} = u_{std}$), combined uncertainty implementing sample-preparation caused error ($u_{instr + sample\ prep}$) and combined uncertainty implementing also evaluation caused error ($u_{instr + sample\ prep + bl\ corr}$); Note that the high relative deviations are still small absolute ones. 0.7% in brackets shows the average without 0.1 mgC/L sample justifying LOQ_{instr} of 0.2 mgC/L

C concentration [mgC/L]	rel. u_{instr} [%]	rel. $u_{instr + sample\ prep}$ [%]	rel. $u_{instr + sample\ prep + bl\ corr}$ [%]
	3 ml injection		
0,1	*11,5*	20,9	41,7
0,2	1,0	14,2	24,1
0,4	0,7	10,1	13,9
0,6	0,8	7,4	9,2
1,2	0,3	4,3	4,9
avg	(0.7) *2.9*	11,4	18,8
	2 ml injection		
0,1	3,1%	25,7%	68,8%
0,2	0,8%	16,2%	28,2%
0,4	1,8%	13,4%	18,0%
0,6	0,3%	3,7%	4,7%
1,2	0,4%	3,8%	4,3%
avg	1,3%	12,6%	24,8%
	1 ml injection		
0,1	2,5%	27,3%	72,7%
0,2	1,4%	12,8%	21,6%
0,4	0,9%	9,9%	13,5%
0,6	0,9%	2,3%	3,0%
1,2	0,7%	3,7%	4,2%
avg	1,3%	11,2%	23,0%

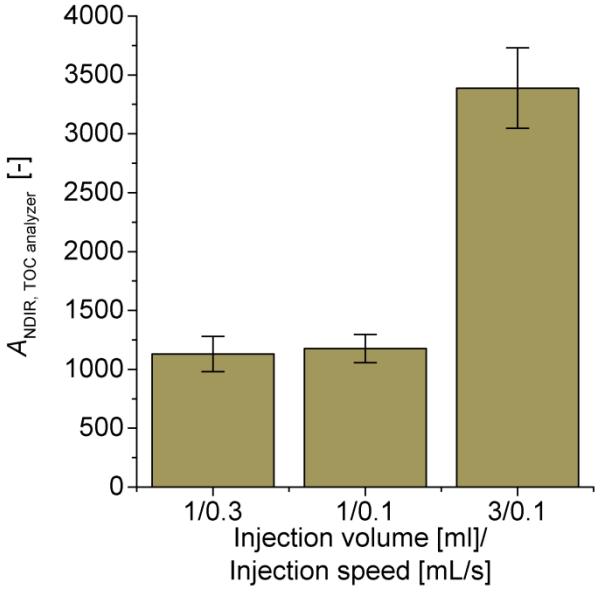

Figure S 3-5 The graph shows an additional indication for absence of considerable instrumental blank coming from e.g. washing out effect within the combustion tube. The amount of carbon, represented by the peak-area of NDIR, of the blank is independent of the amount or contact time (injection speed) of the water vapor. Varying injection volume and speed a constant NDIR peak area of 1129 ± 22 units per mL blank sample was found.

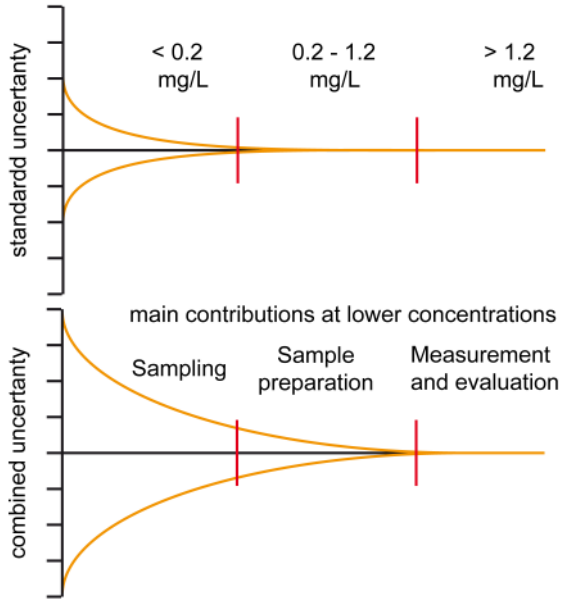

Figure S 3-6 Simplified visualization of uncertainty evolution; Instrument shows significant contribution below 0.2 mgC/L (observed u_{std} at 0.1 mgC/L > 2%); above 0.2 mgC/L a sample-preparation increases the uncertainty substantially and became a limiting factor (variations form vial to vial of the same sample); Generally to expect is even larger contribution to combined uncertainty coming from sampling itself.

The impact of IRMS non-linearity issue becomes very low: Sample with double concentration shows still a small absolute difference. Two times more concentrated sample (e.g. 0.8 mgC/L related to 0.4 mgC/L) shows still only 0.4 mgC/L absolute difference. With 1 mL injection the 0.4 µg difference correlates to ca. 0.4 nA signal. Taken typical IRMS non-linearity's in to account the error introduced without IRMS linearity correction will equal in ca. 0.008‰ bias.

On the other side the impact of blank with a small absolute value of e.g. 0.1 mgC/L becomes significant due to its addition to the sample. The small absolute difference (e.g. 0.1 mgC/L to 0.2 mgC/L sample) results in huge relative impact of ≈ 33%. Proper blank investigation becomes highly significant for DOC SIA for low concentration samples. Due to the needed sensitivity and high impact of possible contamination it becomes the most challenging part.

General consideration: note that the blank evaluation with aqueous solutions can differ strongly from real sample: Contribution for aqueous solutions of compounds can consist of both, water and instrumental blank, but for real samples it consists only of instrumental blank. For the determination of stable isotope composition of aqueous solutions these two contributions can be considered as one total blank using the same water and constant measurements conditions for the standards as well as samples within the run. Blank investigation and correction of aqueous solutions are also necessary for real sample measurements, because there are no liquid certified referencing materials available. Determination of stable isotope composition of real samples with only instrumental blank contribution needs discriminability between those two sources – its quantification - or negligibility prove of one of them. This is challenging due to the need of blank free water.

Testing protocol: Standard addition calibration method: 4 Standards for spiking + blank x 5 concentrations x 3 replicates each. Four compounds solutions were taken to investigate the instrumental performance in low concentration range: caffeine, benzoic acid, citric acid and acetovanillone with concentrations from 0.1 to 1.2 mgC/L and injection volumes of 1, 2 and 3 mL. The water blank concentration was estimated using standard addition calibration method (72 single measurements) and δ-value averaging 25 single water blanks results. Blank values of 143 ± 26 µgC/L and 10.57 ± 3.21‰ were achieved. Those values were utilized for blank correction of stable isotope composition of the samples.

Notes: Random variation caused errors can be narrowed through larger amount of replicates. This can be utilized for estimation of the blank δ-value. Since it was shown that right estimation is essential regular blank value monitoring can be recommended.

Supporting information for section Oxidation efficiency and matrix effects

Selectivity is per definition the ability of a method to determine accurately and specifically the analyte of interest in the presence of other components in a sample matrix under the stated conditions of the test.

Persistent compounds: Model substances were secondary standards; true value was determined by EA/IRMS as accepted procedure.

TIC: Acidification was done by HCl pa grade, because previous experiments have shown that ultra-high grade HCl contains a higher TOC background, most likely caused by the bottle material (plastics instead of glass). The sparging time for TIC removal is defined by the analysis of first replicate (ca. 15 min), which serves as “dummy” as described later on, but can be extended for higher TIC concentrations (>20mgC/L) combined with higher salt load.

In this work systematic preinvestigations of possible non-selectivity sources of real samples were conducted, with focus on instrumental/methodical limitations. Note that those indications do not replace investigation with real matrices using e.g. spiking methods/ internal standards and evaluating the recovery rates in case of concentration measurements or trueness of the, via mass balance equation calculated, δ-values of the spiking material in case of SIA.

Chapter 4 Uncertainty estimation and application of stable isotope analysis in dissolved carbon

4.1 Abstract

Rationale: The results obtained with the newly developed high-temperature combustion total organic carbon analyzer, interfaced with continuous flow isotope ratio mass spectrometry (HTC TOC/IRMS) system confirmed the general suitability of the system for $\delta^{13}C$ determination directly in aqueous solutions (Chapter 3), but proper assessment of analytical performance with real samples was still needed.

Methods: The analytical performance for determination of bulk dissolved organic carbon (DOC) $\delta^{13}C$ signatures was evaluated with realistic and challenging conditions, utilizing real sample measurements and round robin test participation. As part of the validation, an appropriate method to evaluate combined uncertainty of DOC stable isotope analysis (SIA) results was introduced. The total inorganic carbon (TIC) mode of the system was tested for $\delta^{13}C$ determination in TIC.

Results: Validation of the system with a broad range of real samples such as soil extracts and river and seawater samples was performed, and included proof of reproducibility of the developed method via participation in a round robin test. Good precision (standard deviation (SD) predominantly ≤0.15‰) and accuracy (coefficient of determination (R^2) 1.000 ± 0.001) were achieved for the DOC $\delta^{13}C$ analysis of a broad range of DOC solutions. Good reproducibility (predominantly ≤0.5‰) was shown in the context of international round robin testing for river and seawater samples. Furthermore, the general suitability of the system for the determination of total inorganic carbon (TIC) stable isotope analysis was tested. Precision of SD ≤0.2‰ was achieved for SIA of TIC, but further validation tests are required.

Conclusions: The novel HTC TOC/IRMS system enables reliable and rapid determination of $\delta^{13}C$ values in DOC, without laborious offline sample-preparation steps. Further investigations should focus on SIA of TIC. Thus, HTC TOC/IRMS may open new opportunities in DOC and potentially TIC research in aquatic and terrestrial environments.

4.2 Introduction

Bulk carbon concentration and $\delta^{13}C$ determination in aqueous samples is essential for carbon cycle investigation in aquatic and terrestrial systems and plays a key role in biogeochemical processes and ecosystem functioning. [1–4] Studies highlight the benefits of SIA to locate input sources and to understand, e.g., DOC cycling and involved transport and transformation processes.[5,6]

C_3 and C_4 vegetation have different photosynthetic pathways and have therefore naturally distinct isotope signatures. In soil science DOC SIA can be used to investigate the relative contribution of C_3 and C_4 vegetation to DOC percolating in soils.[7] In aquatic ecosystems, different source-specific $\delta^{13}C$ signatures can be used for food web studies.[8] Spatial and temporal variability in DOC stable isotope composition in freshwater systems and marine sites reflect changed dynamics and/or inputs.[7]

Carbonates were shown to play a main role in surface water carbon cycles.[9] Stable isotope composition in dissolved inorganic carbon can be used to investigate dissolution of sedimentary carbonates.[10] The general suitability of the introduced system for TIC SIA was therefore tested in this work.

Considering findings presented in Chapter 3 the system should be applicable in limnology, oceanography and soil science. However, further experimental proof of the suitability is needed. Additionally a proper evaluation of uncertainties is important to justify the data gained by DOC SIA. Participation in a round robin test, including fresh water and seawater samples and measuring soil science extracts was chosen for real sample application validation.

4.3 Experimental

4.3.1 Chemicals and reagents

Reference materials IAEA-600 caffeine CAF1 ($\delta^{13}C_{VPDB}$ –27.771 ± 0.043‰) and IAEA-CH-6 sucrose SUC1 ($\delta^{13}C_{VPDB}$ –10.449 ± 0.033‰) were purchased from the International Atomic Energy Agency (Vienna, Austria). The round robin test nine fresh water (fw i – fw ix), six sea water (sw i – sw vi) and one deep ocean water (dow) samples were delivered by Concordia University (Montreal, Canada). $LiCO_3$ was provided by University of Duisburg-Essen (Essen, Germany). Ultrapure, deionized water (UP water) produced by a Purelab Ultra system (MK2-Analytic, ELGA, High Wycombe, UK) was used for solution preparation. Helium 5.0 and oxygen 4.8 were purchased from Air Liquid (Oberhausen, Germany).

4.3.2 Instrumentation and methodology

The HTC TOC/IRMS system consists of three parts: iso TOC cube analyzer, iso TOC LCM focusing unit (Elementar Analysensysteme, Hanau, Germany) and IsoPrime100 isotope ratio mass spectrometer (Isoprime, Manchester, UK) (see Figure 3-1). Samples, filled in 40-mL borosilicate glass vials, are introduced using a 32-position autosampler into the combustion system by means of a 5-mL syringe and a multiway valve. The combustion is performed at 850 °C and is supported by a catalyst (Pt on ceramic carrier material). Water is removed in three steps: an air-cooled condenser, a counter-flow membrane dryer and a chemical dryer. Hydrogen halides and halogens are removed by silver wool. After the purification steps the carrier gas oxygen enters the nondispersive infrared (NDIR) detector for quantification of the evolved CO_2. The focusing unit separates the CO_2 from O_2 allowing for focusing. An IsoPrime100 (Isoprime Ltd, Manchester, UK) isotope ratio mass spectrometer was used to determine the stable isotope composition.

4.3.3 Nomenclature, evaluation and QA

Nomenclature

To express the variations of natural stable isotope abundance the widely applied ’delta-notation’ is used. The $\delta^{13}C_{VPDB}$-value of an analyte (A) is described by Equation 4-1 as a relative difference between the isotope ratio (R) of an analyte ($R(^{13}C/^{12}C)_A$) and the isotope ratio defining an international reference scale, for carbon Vienna Pee Dee Belemnite ($R(^{13}C/^{12}C)_{VPDB}$):[11]

$$\delta^{13}C_{A,VPDB} = \frac{R\left(^{13}C/^{12}C\right)_A - R\left(^{13}C/^{12}C\right)_{VPDB}}{R\left(^{13}C/^{12}C\right)_{VPDB}} \qquad \text{Equation 4-1}$$

Please note that if no reference is mentioned, as exemplarily shown in Equation 4-2, the reported δ-values are related to the used, in-house reference gas (RG). That concerns all data before the final normalization to the VPDB scale.

$$\delta^{13}\mathrm{C}_{\text{lin corr A}} \equiv \delta^{13}\mathrm{C}_{\text{lin corr A,RG}} \qquad \text{Equation 4-2}$$

with $\delta^{13}\mathrm{C}_{\text{lin corr A}}$ as a linearity corrected δ-value of an analyte A.

Evaluation

Non-linearity correction

The isotope ratio linearity of the isotope ratio mass spectrometer ($L_{\text{R IRMS}}$) was quantified as the slope m_{lin} of a linear regression describing the δ-value as a function of corresponding ion current I.

$$m_{\text{lin}} = \frac{\sum_{k=1}^{n}\left(I_{\text{RG}_k} - \overline{I_{\text{RG}}}\right)\left(\delta^{13}\mathrm{C}_{\text{RG},\overline{\text{RG}}_k} - \overline{\delta^{13}\mathrm{C}_{\text{RG},\overline{\text{RG}}}}\right)}{\sum_{k=1}^{n}\left(I_{\text{RG}_k} - \overline{I_{\text{RG}}}\right)^2} \qquad \text{Equation 4-3}$$

$$\delta^{13}\mathrm{C}_{\text{RG},\overline{\text{RG}}_k} = \frac{R\left({}^{13}\mathrm{C}/{}^{12}\mathrm{C}\right)_{\text{RG}_k} - R\left({}^{13}\mathrm{C}/{}^{12}\mathrm{C}\right)_{\overline{\text{RG}}}}{R\left({}^{13}\mathrm{C}/{}^{12}\mathrm{C}\right)_{\overline{\text{RG}}}} \qquad \text{Equation 4-4}$$

$L_{\text{R IRMS}}$ was monitored regularly, before and after each test series. All measured δ^{13}C raw data ($\delta^{13}\mathrm{C}_{\text{meas A}}$) generated by the software IonVantage (Isoprime Ltd), and automatically corrected for ^{17}O-abundance and related to RG, were then linearity corrected to $\delta^{13}\mathrm{C}_{\text{lin corr A}}$ as described by Brand[12] and expressed as shown in Equation 4-5:

$$\delta^{13}\mathrm{C}_{\text{lin corr A}} = \delta^{13}\mathrm{C}_{\text{meas A}} - m_{\text{lin}} \times (I_{\text{A}} - I_{\text{RG}}) \qquad \text{Equation 4-5}$$

with I_{A} as the ion current at the maximum of the peak for analyte A and I_{RG} the ion current of the reference gas peak pulse.

Blank corrections

An isotope mass balance (IMB) equation[11] was utilized for corrections. The amount of carbon is represented by the uncorrected area A from the integrated NDIR CO_2 peak of the TOC analyzer. The solution of the IMB equation for blank-'subtracted' δ-value $\delta^{13}\mathrm{C}_{\text{bl corr A}}$ results in Equation 4-6. For determination of the concentration as well as the δ-value of the blank, acidified water used for standards solution preparation (blanks) was used:

$$\delta^{13}\mathrm{C}_{\mathrm{bl\ corr\ A}} = \frac{\delta^{13}\mathrm{C}_{\mathrm{lin\ corr\ A}} \times A_{\mathrm{meas\ A}} - \delta^{13}\mathrm{C}_{\mathrm{bl}} \times A_{\mathrm{bl}}}{A_{\mathrm{meas}} - A_{\mathrm{bl}}} \quad \text{Equation 4-6}$$

Two-point normalization

Finally, a referencing strategy to the VPDB scale was applied as recommended in the literature and described in Equation 4-7.

$$\delta^{13}\mathrm{C}_{\mathrm{A,VPDB}} = m_{\mathrm{norm}} \times \delta^{13}\mathrm{C}_{\mathrm{bl\ corr\ A}} + b_{norm} \quad \text{Equation 4-7}$$

$$m_{\mathrm{norm}} = \frac{\delta^{13}\mathrm{C}_{\mathrm{Std1,VPDB}} - \delta^{13}\mathrm{C}_{\mathrm{Std2,VPDB}}}{\delta^{13}\mathrm{C}_{\mathrm{bl\ corr\ Std1,RG}} - \delta^{13}\mathrm{C}_{\mathrm{bl\ corr\ Std2,RG}}} \quad \text{Equation 4-8}$$

$$b_{\mathrm{norm}} = \overline{\delta^{13}\mathrm{C}_{\mathrm{Std,VPDB}}} - m_{\mathrm{norm}} \times \overline{\delta^{13}\mathrm{C}_{\mathrm{bl\ corr\ Std,RG}}} \quad \text{Equation 4-9}$$

with $\delta^{13}\mathrm{C}_{\mathrm{Std1,VPDB}}$ and $\delta^{13}\mathrm{C}_{\mathrm{Std2,VPDB}}$ as the accepted δ-values of the two standards used for normalization; and with $\delta^{13}\mathrm{C}_{\mathrm{bl\ corr\ Std1,RG}}$ and $\delta^{13}\mathrm{C}_{\mathrm{bl\ corr\ Std2,RG}}$ as the measured against reference gas and then blank corrected δ-values.

Quality assurance

The developed method was tested with aqueous solutions and real samples based on the validation strategy described in DIN 17025[13] (modified for SIA by applying the recommendations of Jochmann and Schmidt[11]). In that way, the chosen referencing and quality assurance strategy ensures the metrological traceability[14] and the accuracy – sum of trueness and precision.[15–17] The standard uncertainty (u_{std}) is expressed as the standard deviation of replicate measurements. A novel, SIA specific, way to asses combined uncertainty (u_{comb}) is discussed in the results section. Basic error propagation equations (see paragraph below) were modified to that end.

For addition (z = x + y + z…), addition of the absolute errors should be applied. For multiplication (z = x × y × z…), addition of the relative errors should be applied, using standard deviations following Equation 4-10.[18]

$$\frac{\Delta z}{z} = \sqrt{\left(\frac{\Delta x}{x}\right)^2 + \left(\frac{\Delta y}{y}\right)^2 + \cdots} \quad \text{Equation 4-10}$$

4.4 Results and discussion

4.4.1 Novel approach for uncertainty assessment in DOC SIA

Derivation and validation of the approach

On the basis of recommendations from the Comité International des Poids Mesures (CIPM) a concept of dealing with measurement uncertainty was introduced (see Figure 4-1).[19]

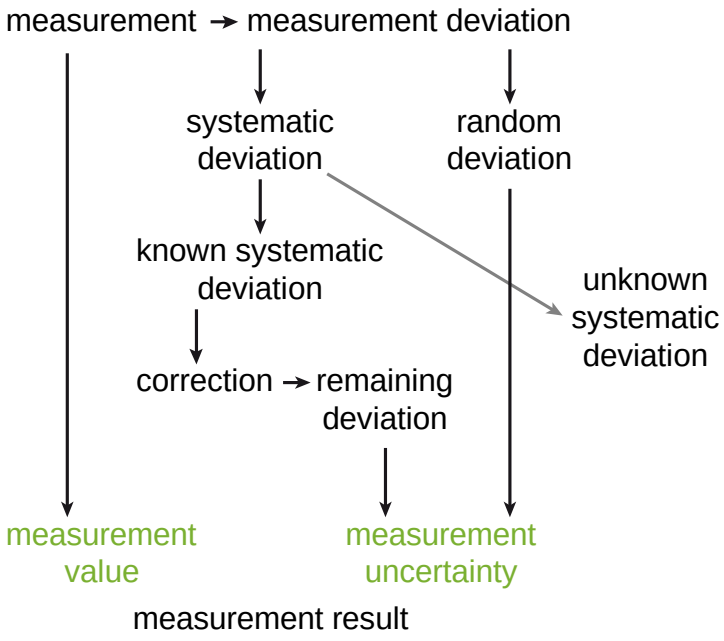

Figure 4-1 Concept for dealing with measurement uncertainty (redrawn from Neidhart et al.)[19]

The uncertainty assessment for DOC SIA suggested in this work, is based on a combination of this strategy and suggestions by Jochmann and Schmidt.[11,19] It takes into account that correction factors themselves carry an error, which can be seen as a remaining deviation after correction of an systematic deviation and, if significant, its contribution needs to be considered using error propagation.

The contribution of random deviation of replicate measurements and remaining deviation after the linearity correction is derived from corresponding Equation 4-5 via propagation of uncertainty.

$$u_{\text{std+ lin corr}} = \pm \sqrt{\left(SD_{\delta^{13}C_{\text{meas A}}}\right)^2 + \left(\sqrt{\left(\frac{SD_{m_{\text{lin}}}}{m_{\text{lin}}}\right)^2 + \left(\frac{\sqrt{\left(SD_{I_A}\right)^2 + \left(SD_{I_{RG}}\right)^2}}{\Delta_I}\right)^2} \times \Delta_{\delta_{\text{lin}}}\right)^2} \quad \text{Equation 4-11}$$

with $\Delta_{\delta\,\text{lin}}$ as the error caused by non-linearity effects (m_{lin}×(I_A-I_{RG}).

The contribution of remaining deviation after the blank correction is considered by derivation from corresponding Equation 4-12 via propagation of uncertainty (see Equation 4-15). Equation 4-12 is equal to Equation 4-6, which is recombined and simplified for better understanding.

$$\delta^{13}C_{\text{bl }corr\,A} = \delta^{13}C_{\text{lin corr A}} \times A_{\text{meas A}} \times \frac{1}{A_{\text{sample A}}} - \delta^{13}C_{\text{bl}} \times A_{\text{bl}} \times \frac{1}{A_{\text{sample A}}} \quad \text{Equation 4-12}$$

with $A_{\text{sample A}}$ as the blank corrected area value ($A_{\text{meas A}} - A_{\text{bl}}$). Note that the $\delta^{13}C_{\text{bl}}$ is also linearity corrected prior to further use.

For multiplication, addition of the relative errors should be applied (Equation 4-10). However it needs to be considered that δ-values are relative values. Exemplarily, measuring three replicates and receiving a SD of 0.2‰ and average values of 20‰, 1‰ or 0‰ the calculated relative SD would be 1%, 20% or no valid value (dividing by 0) and thus senseless. To work with ratios on the other hand is not straightforward. Therefore, the following solution is suggested in this study. Instead of an average δ-value, a value representing the dimension of all δ-values of a corresponding test series is used (Equation 4-13). The coefficient ½ of the range is derived empirically. More detailed explanation can be found after Equation 4-15 and in section 4.7.

$$½\Delta_\delta = ½ \times \left| \begin{matrix} \max\{(\delta^{13}C_{\text{bl corr A}})_1, \cdots, (\delta^{13}C_{\text{bl corr A}})_n\} - \\ \min\{(\delta^{13}C_{\text{bl corr A}})_1, \cdots, (\delta^{13}C_{\text{bl corr A}})_n\} \end{matrix} \right| \quad \text{Equation 4-13}$$

Intermediate state ($u_{i\,(\text{std+lin corr+bl corr})}$) of the final equation Equation 1-15 is shown in Equation 4-14.

$$u_{i\ (std+lin\ corr+bl\ corr)} = \pm\sqrt{u_{minuend}{}^2 + u_{subtrahend}{}^2}$$

$$\text{with } u_{minuend} = \sqrt{\left(\frac{u_{sl}}{½\Delta_\delta}\right)^2 + \left(\frac{SD_{A_{meas\ A}}}{A_{meas\ A}}\right)^2 + \left(\frac{u_{A_{sample\ A}}}{A_{sample\ A}}\right)^2} \times ½\Delta_\delta \times A_{meas\ A} \times \frac{1}{A_{sample\ A}}$$

$$\text{with } u_{subtrahend} = \sqrt{\left(\frac{u_{sl\ bl}}{½\Delta_\delta}\right)^2 + \left(\frac{SD_{A_{bl}}}{A_{bl}}\right)^2 + \left(\frac{u_{A_{sample\ A}}}{A_{sample\ A}}\right)^2} \times ½\Delta_\delta \times A_{bl} \times \frac{1}{A_{sample\ A}}$$

Equation 4-14

$$\text{with } A_{sample\ A} = A_{meas\ A} - A_{bl} \text{ and}$$

$$\text{with } u_{A_{sample\ A}} = \sqrt{\left(SD_{A_{meas\ A}}\right)^2 + \left(SD_{A_{bl}}\right)^2}$$

with u_{sl} as abbreviation for $u_{std+lin\ corr}$. $u_{sl\ bl}$ refers to the uncertainty in the blank (calculations are equal to u_{sl}).

The next step in the uncertainty estimation is introduced to account for the difference between the δ-value of the sample and that of the blank Equation 4-15. Meaning that if the difference between the δ-value of the sample and that of the blank is zero the blank correction contribution is zero and blank correction uncertainty equation gives u_{sl} as a result.

$$u_{std+lin\ corr+bl\ corr} = \pm (u_{std+lin\ corr} + (u_{i\ (std+lin\ corr+bl\ corr)} - u_{std+lin\ corr}) \times f_\Delta)$$

$$\text{with } f_\Delta = \frac{|\delta^{13}C_{bl\ corr\ A} - \delta^{13}C_{bl}|}{\max\{|\delta^{13}C_{bl\ corr\ A}|_1, \cdots, |\delta^{13}C_{bl\ corr\ A}|_n\}}$$

Equation 4-15

This concept was proven via the following test procedure:

Taking the large data set (436 single measurements) obtained within the round robin test a realistic worst case scenario could be modeled (WC-M). The WC-M was used to validate the approach suggested in this study (exemplarily demonstrated in Supporting information). It is proposed that the final achieved expanded uncertainty (U) should cover the maximal deviation observed via WC-M ($U \geq \max\{\Delta_{WC\text{-}M\ 1, \dots, \Delta_{WC\text{-}M\ n}}\}$). Expanded uncertainty is calculated by multiplication of the combined uncertainty with the coverage factor k ($U = u_{comb} \times k$). The value of coverage factor k is typically 2 or 3 corresponding to the confidence interval of U of 95.4% or 99.7%, respectively. With a k value of 2 the combined uncertainty should cover at least 50% of maximal deviation observed with the WC-M.

For the data achieved with the round robin test series the calculated $u_{std+lin\ corr+bl\ corr}$ covers 68% of the maximal deviation observed via WC-M. Therefore, the approach is assumed to be valid.

Note that the water blank correction and its contribution to combined uncertainty needs to be considered only for the standards prepared with this water (not for, e.g., river water samples).

Finally, the contribution of remaining deviation after two-point normalization is considered by derivation from corresponding Equation 4-7 to Equation 4-9 via propagation of uncertainty (see Equation 4-16 to Equation 4-18).

$$u_{m_{norm}} = \pm \sqrt{\left(\frac{\sqrt{u_{Std1,VPDB}^2 + u_{Std2,VPDB}^2}}{\Delta_{std,VPDB}}\right)^2 + \left(\frac{\sqrt{u_{Std1,RG}^2 + u_{Std2,RG}^2}}{\Delta_{std,RG}}\right)^2} \times m_{norm} \qquad \text{Equation 4-16}$$

with $\Delta_{std,VPDB}$ as $\delta^{13}C_{Std1,VPDB} - \delta^{13}C_{Std2,VPDB}$, $\Delta_{std,RG}$ as $\delta^{13}C_{Std1,RG} - \delta^{13}C_{Std2,RG}$, uncertainties in numerator are defined by the IAEA ($u_{Std1,VPDB}$, $u_{Std1,VPDB}$) or derived as described before $u_{std+lin\ corr+bl\ corr}$ ($u_{Std1,RG}$, $u_{Std1,RG}$).

$$u_{b_{norm}} = \pm \sqrt{avg_{u_{Std,VPDB}}^2 + \left(\sqrt{\left(\frac{u_{m_{norm}}}{m_{norm}}\right)^2 + \left(\frac{avg_{u_{Std,RG}}}{½\Delta_{Std,RG}}\right)^2} \times m_{norm} \times ½\Delta_{std,RG}\right)^2} \qquad \text{Equation 4-17}$$

with half range ($½\Delta_{Std,RG}$) as $|\delta^{13}C_{Std1,RG} - \delta^{13}C_{Std2,RG}|/2$, average $avg_{u(Std,VPDB)}$ as ($u_{Std1,VPDB}$ + $u_{Std2,VPDB}$)/2 and $avg_{u(Std,RG)}$ as ($u_{Std1,RG}$ + $u_{Std2,RG}$)/2.

The final combined uncertainty u_{comb} ($u_{std+lin\ corr+bl\ corr+norm}$) can then be formulated as follows.

$$u_{comb} = \pm \sqrt{\left(\sqrt{\left(\frac{u_{m_{norm}}}{m_{norm}}\right)^2 + \left(\frac{u_{slb}}{½\Delta_{Std,RG}}\right)^2} \times m_{norm} \times ½\Delta_{Std,RG}\right)^2 + u_{b_{norm}}^2} \qquad \text{Equation 4-18}$$

with u_{slb} as abbreviation for $u_{std+lin\ corr+bl\ corr}$.

Note that for standard and blank value determinations not only the average and standard deviations of one replicate set should be taken for each test series, but at least of three replicates sets, measured at the beginning, mid and end of the test series to be representative

for that test series. Use of mid replicate set (or sets) can be used to distinguish, e.g., a long term drift from a random deviation.

The combined uncertainty u_{comb} obtained as described before contains contributions from random deviation measuring replicates u_{std} as well as remaining deviations from linearity correction, blank correction and normalization. Combined uncertainty defines an interval having a level of confidence of approximately 68% (≡ ±1σ). Nevertheless, there may be unknown deviations (Figure 4-1) which can be considered by expanded uncertainty U obtained via multiplying of u_{comb} by a coverage factor (typically 2 or 3; confidence interval of 95% or 99.7%, respectively).

The combined uncertainty derived this way is representative for DOC SIA measurements, but exclude the contribution of potential error caused by sampling or further preparation steps. Depending on the focus it may be necessary to consider further contributions via error propagation, but a detailed description is out of scope in this work.

Application of the introduced approach to assess the uncertainties for a DOC SIA round robin test

The values of the data obtained from the international interlaboratory test (round robin test) by two participants working with the same instrumentation iso TOC cube HTC analyzer (Elementar Analysensysteme, Hanau, Germany) coupled to IsoPrime 100 isotope ratio mass spectrometer (Isoprime, Manchester UK) are shown in Table 4-1.

Table 4-1 Data set including uncertainties assessed for DOC SIA

	$\delta^{13}C_{VPDB}$ [‰] results participant[a]					$\delta^{13}C_{VPDB}$ [‰] results participant[b]					Δ_{a-b} [‰]
sample	$avg_{n=3}$	$\pm u_{std}$	$\pm u_{comb}$	$\pm U_{k=2\ (95\%)}$	$\pm U_{k=3\ (99.7\%)}$	$avg_{n=3}$	$\pm u_{std}$	$\pm u_{comb}$	$\pm U_{k=2\ (95\%)}$	$\pm U_{k=3\ (99.7\%)}$	
fw i	-27.58	0.03	0.33	0.66	1.00	-27.96	0.01	0.33	0.66	0.99	0.38
fw ii	-27.01	0.05	0.33	0.67	1.00	-27.29	0.05	0.33	0.67	1.00	0.28
fw iii	-28.33	0.03	0.33	0.66	0.99	-28.39	0.02	0.33	0.66	0.99	0.06
fw iv	-27.59	0.02	0.33	0.66	0.99	-27.70	0.02	0.33	0.66	0.99	0.11
fw v	-28.33	0.01	0.33	0.66	0.99	-28.40	0.02	0.33	0.66	0.99	0.07
fw vi	-24.30	0.02	0.33	0.66	0.99	-24.45	0.01	0.33	0.66	0.99	0.15
fw vii	-27.63	0.04	0.33	0.67	1.00	-27.59	0.03	0.33	0.66	1.00	-0.04
fw viii	-12.91	0.09	0.34	0.68	1.02	-14.81	0.77	0.83	1.65	2.48	1.90
fw ix	-16.94	0.17	0.37	0.74	1.11	-17.26	0.27	0.42	0.85	1.27	0.32
sw i	-16.45	0.30	0.44	0.88	0.99	-18.73	0.11	0.35	0.70	1.05	2.28
sw ii	-26.45	0.03	0.33	0.66	1.32	-26.30	0.03	0.33	0.66	1.00	-0.15
sw iii	-22.66	0.10	0.34	0.69	1.00	-23.25	0.08	0.34	0.68	1.02	0.59
sw iv	-24.43	0.04	0.33	0.67	1.03	-24.39	0.05	0.33	0.67	1.00	-0.04
sw v	-27.69	0.06	0.34	0.67	1.00	-27.36	0.05	0.33	0.67	1.00	-0.33
sw vi	-21.47	0.02	0.33	0.66	1.01	-20.83	0.26	0.42	0.84	1.26	-0.64
dow[c]	-23.19	0.02	0.33	0.66	0.99						
											$\Delta_{\lvert a-b \rvert}$ [‰]
avg		0.06	0.34	0.69	1.03		0.12	0.38	0.76	1.14	0.49
min		0.01	0.33	0.66	0.99		0.01	0.33	0.66	0.99	0.04
max		0.30	0.44	0.88	1.32		0.77	0.83	1.65	2.48	2.28

fw: fresh water sample; sw: seawater sample; dow: deep ocean water; Δ_{a-b} difference between delta values obtained by participant a and b;

[a]R&D, Elementar Group & Instrumental Analytical Chemistry, University of Duisburg-Essen; E. Federherr (responsible)

[b]Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam; C. Cerli (responsible)

[c]The ampules contained only ca. 20 - 25 ml sample instead of 40 ml. To ensure a sufficient sample volume for the measurement, samples of participants a and b were therfore combined.

Comparing data of the two participants show clearly that u_{std} is less suited to represent the deviations of the method than expanded uncertainty. Nine out of fifteen $\delta^{13}C_{VPDB}$ average deviations $\Delta_{a\text{-}b}$ are not covered by u_{std}. Requirement for covering is that the condition $u_a + u_b \geq \Delta_{a\text{-}b}$ is satisfied (Figure 4-2).

One sample is with $\Delta_{a\text{-}b} = 2.28$ obviously out of range. That is the only deviation not covered by $U_{k=3\ (99,7\%)}$. A closer look on the sequence shows that this sample was measured shortly after change of consumables (participant a). Conditioning before resuming measurement of real samples might have been insufficient.

Average $\Delta_{a\text{-}b}$ of ±0.49‰ confirms good reproducibility (≤0.5‰).

A significant contribution to u_{comb} is the uncertainty of the UP water NDIR detector peak area (±1295; corresponds to ±0.11 mgC/L) and $\delta^{13}C$ value (±1.35‰). Nevertheless, considering 10 mgC/L IAEA-CH6 standard solution, a relatively small concentration of the blank (ca. 0.35 mgC/L) results in a contribution to u_{comb} of the IAEA-CH6 $\delta^{13}C$ value of ±0.12‰, whereas skipping of the blank correction would lead to a systematic error of +0.57‰. Therefore blank correction was carried out. In the future, higher concentrated standards for normalization could be taken (e.g., 25 mgC/L) to minimize the contribution of the blank. On the other hand those standards would be less similar to the samples and therefore less representative. It is however out of scope in this work.

For the determination of standard values for normalization not only averages and standard deviations of one replicate set were taken (e.g., IAEA-CH6 $\delta^{13}C$ $SD_{n=3}$ ±0.03‰), but three replicate sets for each test series, measured at the beginning, mid and end of the test series to be representative for the test series (e.g., IAEA-CH6 $\delta^{13}C$ $SD_{n=9}$ ±0.22‰).

4.4.2 Round robin test and further real sample measurements

Table 4-2 Comparison of own results against expected values in round robin test

	$\delta^{13}C_{VPDB}$ [‰] results participant[a]		$\delta^{13}C_{VPDB}$ [‰][b]		Δ_{a-b} [‰]
sample	$avg_{n=3}$	$\pm U_{k=3\ (99.7\%)}$	expected	±uncertainty[c]	
fw i	-27.58	1.00	-28.16	1.00	0.58
fw ii	-27.01	1.00	-27.50	1.00	0.49
fw iii	-28.33	0.99	-28.16	1.00	-0.17
fw iv	-27.59	0.99	-27.50	1.00	-0.09
fw v	-28.33	0.99	-28.16	1.00	-0.17
fw vi	-24.30	0.99	-	-	-
fw vii	-27.63	1.00	-28.16	1.00	0.53
fw viii	-12.91	1.02	-	-	-
fw ix	-16.94	1.11	-	-	-
sw i	-16.45	0.99	-	-	-
sw ii	-26.45	1.32	-25.63	1.02	-0.82
sw iii	-22.66	1.00	-21.00	1.02	-1.66
sw iv	-24.43	1.03	-	-	-
sw v	-27.69	1.00	-26.35	1.02	-1.34
sw vi	-21.47	1.01	-	-	-
dow	-23.19	0.99	-21.00	1.50	-2.19
					$\Delta_{\lvert a-b \rvert}$ [‰]
avg		1.03			0.80
min		0.99			0.09
max		1.32			2.19

fw: fresh water sample; sw: seawater sample; dow: deep ocean water;

[a]R&D, Elementar Group & Instrumental Analytical Chemistry, University of Duisburg-Essen;

[b]expected values provided by round robin test organizer (no further details or sources were provided)

[c]no further details about the uncertaties of the values were provided by the round robin test organizer, therefore typical uncertainties reported in the literature were used (details can be found in text)

The comparison (principle is shown in Figure 4-2) shows that determined values are in agreement with the expected values. Since no uncertainty values were provided by the round robin test organizers, uncertainties typically found in DOC SIA were taken from literature for river water (Raymond et al.)[4], costal seawater (Raymond et. a)[4] and deep ocean water (Follett et al.)[20]. A further evaluation of the round robin test results was announced by the organizers but is not yet available.

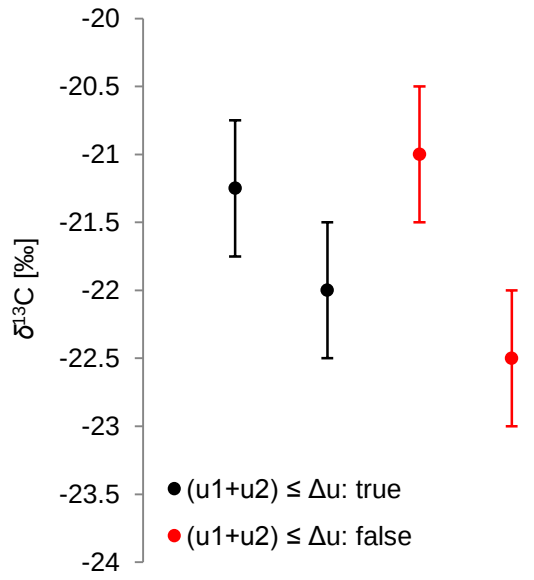

Figure 4-2 Visualization of principle to compare two values based on coverage through uncertainties

However, an exemplary evaluation and comparison of the results of all participants including the different methods used can already be shown in (Figure 4-3).

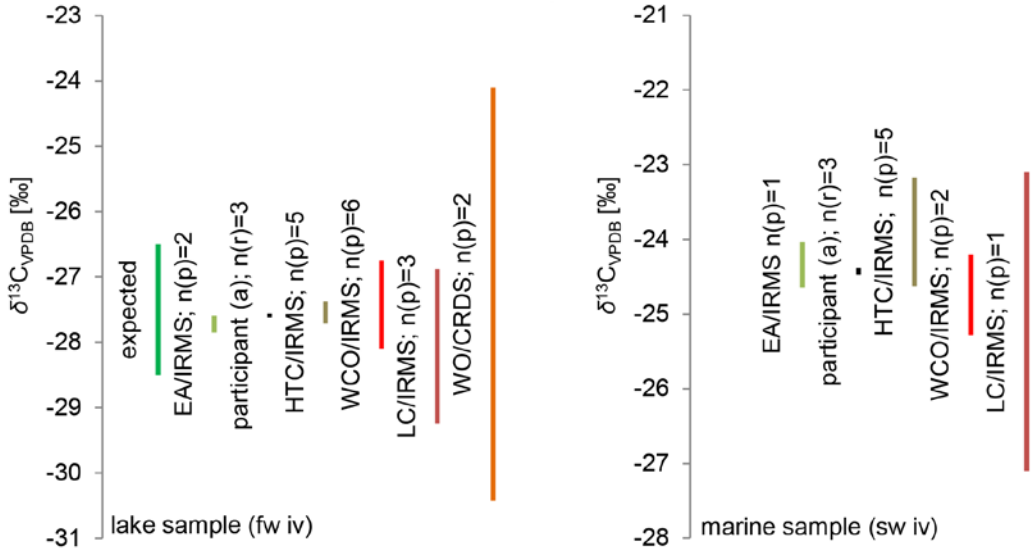

Figure 4-3 Exemplary results of the round robin test for a lake sample (left) und a marine sample (right). Average and standard deviation for n(p) different labs using the same method are shown (if n(p)>1). Average and standard deviation for n(r) replicate measurements of own results (participant (a)) and for cases were only one participant has used a certain method (n(p)=1) are shown. Wet oxidation coupled with cavity ring-down spectrometer (WO/CRDS) was not used for DOC measurements in sea water. Expected range is shown only for the lake sample (for sea water sample sw iv not available). For cases with more than one participant,

no standard deviations of replicate measurements achieved by different labs using the same method were provided.

Details, e.g., whether certain sample preparation methods such as desalinization using ion exchanger cartridge were used or not is not available but would be essential to justify the results. Nevertheless, it is clear that both, high temperature combustion and wet chemical oxidation based methods are possibly suited for DOC SIA, but standard deviations are predominantly larger in case of wet chemical oxidation based methods (WCO/IRMS, LC/IRMS and WO/CRDS). Again, further details are necessary to draw any robust conclusion.

Besides oceanography and limnology a direct measurement of DOC SIA is also of interest in soil science. Using different real samples, such as rice straw or black humus layer extracts, the suitability of the novel system as well as evaluation strategy for DOC SIA was clearly demonstrated:[7]

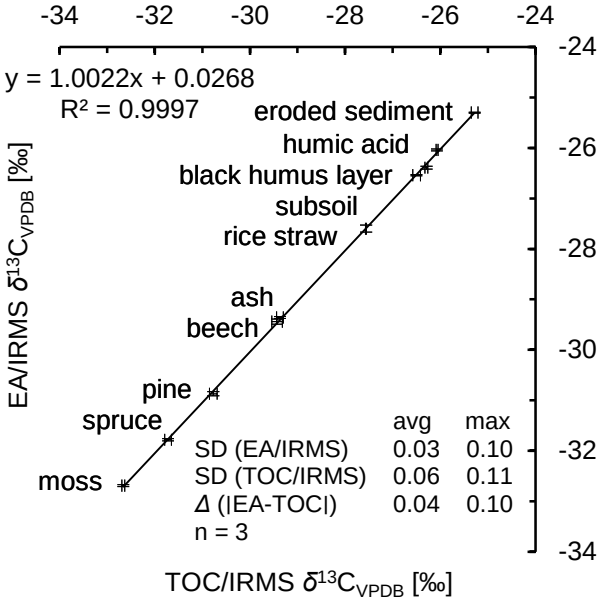

Figure 4-4 Excerpt from the data obtained with various natural DOC samples (redrafted from Kirkels et al.)[7]

The suitability of the HTC TOC/IRMS system was proven by comparison of the $\delta^{13}C$ values measured by HTC TOC/IRMS in aqueous, natural and complex DOC samples with those obtained via EA/IRMS measurements, where aliquots were freeze-dried prior to IRMS measurements. $\delta^{13}C$ values obtained by TOC/IRMS were all in good agreement with values obtained by EA/IRMS (R^2 = 0.9997). Trueness representing absolute differences of $\delta^{13}C$ values obtained with HTC TOC/IRMS and EA/IRMS were all ≤0.10‰. For both methods

similar precision of $\delta^{13}C$ values was achieved, with SD always ≤0.12 ‰. Obviously, there was no isotope fractionation observed for $\delta^{13}C$ analysis by HTC TOC/IRMS. Moreover, the high accuracy in terms of $\delta^{13}C$ values is in good accordance with the strongly linear relationship previously reported between HTC TOC/IRMS and EA/IRMS for a variety of chemical compounds (see Chapter 3).

Data gained with the novel HTC TOC/IRMS system demonstrate the suitability of the system for application in oceanography, limnology and soil since.[7]

4.4.3 Proof of principle of TIC SIA with the iso TOC cube system

A TIC mode instrumentation was implemented in iso TOC cube, derived from the commercially available HTC-TOC analyzer vario TOC cube (Elementar Analysensysteme GmbH). The HTC TOC system in TIC mode was coupled with the isotope ratio mass spectrometer using the focusing unit developed for DOC SIA. The final setup is shown in Figure 4-5.

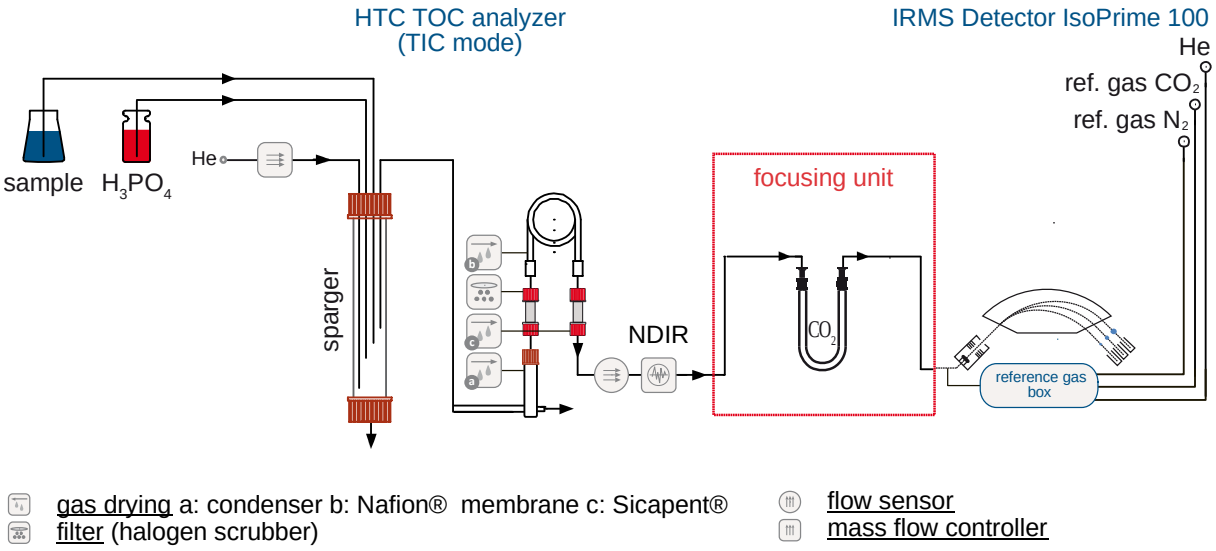

Figure 4-5 System setup for TIC SIA (simplified graph focusing on main principles). 0.05 to 3 mL of sample is injected in the sparger automatically via a syringe from an autosampler (not shown in the graph). Acid is added to the sample via an acid pump (not shown in the graph) to achieve a pH of ca. 2. Helium as a carrier gas is added via a mass flow controller, mixes the reactant with the sample in the reaction side in the sparger and provides the evaluated CO_2 to the purification system. The purification system consists of condenser, membrane dryer (Nafion®), chemical dryer (Sicapent®) and a halogen scrubber. After purification, the gas, passing the flow meter and NDIR detector enters the focusing unit with an adsorption column. Using high heating rates the CO_2 is rapidly focused desorbed and transported by helium gas towards the isotope ratio mass spectrometer for stable isotope composition measurement.

The system is fully automated and a first proof of principle for its general suitability for the determination of TIC SIA was carried out using five vials containing the same solution of 10 mgC/L $LiCO_3$. The precision obtained by averaged results of triplicate measurements with five sample vials, expressed as u_{std} (SD ≡ 1σ), was ≤0.20‰ ($u_{std,avg}$ = 0.10‰; $u_{std,max}$ = 0.19‰) for $\delta^{13}C$ values. However, further tests are necessary to investigate the performance, mainly LOQ and accuracy using standard solutions and suitability for real samples.

4.5 Conclusion and outlook

Good precision (standard deviation (SD) predominantly ≤0.15‰) and accuracy (coefficient of determination (R^2) 1.000 ± 0.001) were achieved for the $\delta^{13}C$ analysis of a broad diversity of DOC solutions. Good reproducibility (predominantly ≤0.5‰) was shown in the context of international round robin testing for river and seawater samples.

A novel strategy to evaluate combined uncertainty for DOC SIA was introduced and validated. Significance of the use of expanded uncertainty U, rather than standard uncertainty u_{std} to compare results was shown. Proper assessment of combined uncertainty is essential to obtain the expanded uncertainty. To ensure an international comparability of the data a standardized procedure would be necessary, which is not available to date.

A precision of SD ≤0.2‰ was achieved for fully automated SIA of TIC, but further validation tests are clearly required for that application.

4.6 References

[1] R. Kindler, J. Siemens, K. Kaiser, D. C. Walmsley, C. Bernhofer, N. Buchmann, P. Cellier, W. Eugster, G. Gleixner, T. Grünwald, A. Heim, A. Ibrom, S. K. Jones, M. Jones, K. Klumpp,W. Kutsch, K. S. Larsen, S. Lehunger, B. Loubet, R. Mckenzie, E. Moors, B. Osborne, K. Pilegaard, C. Rebmann, M. Saunder, M. W. I. Schmidt, M Schrumpf, J. Seyfferth, U. Skiba, J. -F. Soussana, M. A. Sutton, C. Tefs, B. Vowinckel, M. J. Zeeman, M. Kaupenjohann. Dissolved carbon leaching from soil is a crucial component of the net ecosystem carbon balance. Global Change Biol. 2011, 17, 1167.

[2] K. Kalbitz, S. Solinger, J.-H. Park, B. Michalzik, E. Matzner. Controls on the dynamics of dissolved organic matter in soils: a review. Soil Sci. 2000, 165, 277.

[3] J. E. Bauer, in Biogeochemistry of Marine Dissolved Organic Matter, (Ed: D. A. Hansell, C. A. Carison). Elsevier, Amsterdam, 2002, pp. 405-453.

[4] P. A. Raymond, J. E. Bauer. Use of ^{14}C and ^{13}C natural abundances for evaluating riverine, estuarine, and coastal DOC and POC sources and cycling: a review and synthesis. Org. Geochem. 2001, 32, 469.

[5] S. Steinbeiss, V. M. Temperton, G. Gleixner. Mechanisms of short-term soil carbon storage in experimental grasslands. Soil Biol. Biochem. 2008, 40, 2634.

[6] D. C. Coleman, B. Fry. Carbon Isotope Techniques. Academic Press, San Diego, 1991.

[7] M. S. A. K. Frédérique, C. Cerli, E. Federherr, K. Kalbitz. A novel high temperature combustion based system for stable isotope analysis of dissolved organic carbon in aqueous samples (II): optimization and assessment of analytical performance. Rapid Commun. Mass Spectrom. 2014, 28, 2574.

[8] T. A. Schlacher, R. M. Connolly. Land–Ocean Coupling of Carbon and Nitrogen Fluxes on Sandy Beaches. Ecosystems 2009, 12, 311.

[9] J. Barth. Influence of carbonates on the riverine carbon cycle in an anthropogenically dominated catchment basin: evidence from major elements and stable carbon isotopes in the Lagan River (N. Ireland). Chem. Geol. 2003, 200, 203.

[10] P. Schulte, R. van Geldern, H. Freitag, A. Karim, P. Negrel, E. Petelet-Giraud, A. Probst, J.-L. Probst, K. Telmer, J. Veizer, J. A. C. Barth. Applications of stable water and carbon isotopes in watershed research: Weathering, carbon cycling, and water balances. Earth-Sci. Rev. 2011, 109, 20.

[11] M. A. Jochmann, T. C. Schmidt. Compound-Specific Stable Isotope Analysis. Royal Society of Chemistry, Cambridge, 2012.

[12] W. A. Brand, in Handbook of Stable Isotope Analytical Techniques, (Ed: P. A. de Groot). Elsevier, Amsterdam, 2004, pp. 835–857.

[13] DIN EN ISO/IEC 17025:2005. General requirements for the competence of testing and calibration laboratories.

[14] P. De Bièvre, R. Dybkær, A. Fajgelj, D. B. Hibbert. Metrological traceability of measurement results in chemistry: concepts and implementation. Pure Appl. Chem. 2011, 83, 1873.

[15] M. Thompson, S. L. Ellison, R. Wood. Harmonized guidelines for single-laboratory validation of methods of analysis (IUPAC Technical Report). Pure Appl. Chem. 2002, 74, 835.

[16] M. Thompson, R. Wood. Harmonized guidelines for internal quality control in analytical chemistry laboratories. Nature 1995, 4, 4.

[17] A. Menditto, M. Patriarca, B. Magnusson. Understanding the meaning of accuracy, trueness and precision. Accredit. Qual. Assur. 2006, 12, 45.

[18] V. Lindberg. Uncertainties and Error Propagation. http://www.rit.edu/~w-uphysi/uncertainties/Uncertaintiespart2.html

[19] B. Neidhart, W. Wegscheider. Quality in Chemical Measurements: Training Concepts and Teaching Materials. Springer, Berlin, 2012.

[20] C. L. Follett, D. J. Repeta, D. H. Rothman, L. Xu, C. Santinelli. Hidden cycle of dissolved organic carbon in the deep ocean. Proc. Natl. Acad. Sci. U. S. A. 2014, 111, 16706.

4.7 Supporting information

Assessment of the uncertainty via a worst case model (WC-M)

A simple example is chosen to demonstrate the principle to assess the uncertainty (Table S 4-1). An area of a rectangle (A) is calculated using average lengths (l) of sides a and b. A standard error propagation approach (method i) is chosen to calculate the uncertainty of the area u(A_{ab}) out of lengths uncertainties u(l_a) and u(l_b). Using the same data set (lengths and their uncertainties) four worst case scenarios are modeled (WC-M) (four areas are calculated). These four scenarios are the four possible permutations with two variables a and b. Each of them can have a positive and a negative deviation: l_a + u(l_a) and l_b + u(l_b) (++), l_a + u(l_a) and l_b - u(l_b) (+-), l_a - u(l_a) and l_b + u(l_b) (-+), and l_a - u(l_a) and l_b - u(l_b) (--). In the next step, each of the calculated WC-M areas is subtracted from the area observed using average lengths. These subtractions results in four deviation values $\Delta(A_{ab})$. Taking the absolute values of deviations a maximum deviation is determined. Maximum deviation observed via WC-M is used for comparison with the uncertainty derived with the method i using coverage value ($A_{ab,avg}/A_{ab,WC\text{-}Mmax}$).

Table S 4-1 Demonstration of the principle used to assess uncertainty

method for uncertainty estimation[a] (i)						worst case model (WC-M)					
						scenario 1 (++)			scenario 2 (--)		
side	l [mm]	u(l) [mm]	rel. u(l) [mm]	A_{ab} [mm^2]	u(A_{AB}) [mm^2]	l [mm]	A_{AB} [mm^2]	$\Delta(A_{AB})$ [mm^2]	l [mm]	A_{AB} [mm^2]	$\Delta(A_{AB})$ [mm^2]
a	10.0	0.3	3.0%	50	1.80	10.30	52.53	2.53	9.70	47.53	2.47
b	5.0	0.1	2.0%			5.10			4.90		
						scenario 3 (+-)			scenario 4 (-+)		
						l [mm]	A_{AB} [mm^2]	$\Delta(A_{AB})$ [mm^2]	l [mm]	A_{AB} [mm^2]	$\Delta(A_{AB})$ [mm^2]
						10.30	50.47	0.47	9.70	49.47	0.53
						4.90			5.10		
						maximum deviation observed via WC-M:					2.53
						coverage of maximal deviation by uncertainty calculated via method (i):			1.80/2.53 =		71%

[a]estimated via error propagation

The empirical factor (½) was chosen to calculate the value representing the dimensions of the δ-value. This factor was validated under the consideration that the combined uncertainty should cover at least 50% of maximal deviation observed via WC-M. All samples of the test series were taken for validation. Coverage below 50% would mean underestimation of the uncertainty ($U_{k=2}$ would not cover the maximal deviation observed via WC-M).

Chapter 5 A novel tool for natural abundance stable nitrogen analysis in in aqueous samples

5.1 Abstract

Rationale: The bulk stable isotope analysis (BSIA) of dissolved matter (e.g. dissolved organic carbon, total nitrogen bound (TN_b), etc.) is of particular importance since this pool is a prime conduit in the cycling of N and C. Studying the two elemental pools is of importance, as the transformation and transport processes of N and C are inextricably linked in all biologically mediated systems. No system able to analyze natural abundance stable carbon and nitrogen isotope composition of dissolved nitrogen directly (without offline sample preparation) and simultaneously has been reported so far. Extension of the high temperature combustion (HTC) total organic carbon (TOC) analyzer, to the ability to measure TN_b stable nitrogen isotope composition is described in this study.

Methods: To extent the TOC analyzer to the ability to measure TN_b, modifications from the HTC high performance liquid chromatography/isotope ratio mass spectrometry (HPLC/IRMS) interface were implemented and expanded. Reduction reactor for conversion of NO_x to N_2 was implemented into the new developed system. Extension addresses mainly the development of the focusing unit for nitrogen and a degassing device for online separation of TNb from in the sample solved N_2 prior to injection.

Results: The proof of principle of the system with different compound solutions succeeded. In this initial testing, $\delta^{15}N_{AIR\text{-}N2}$ values of tested compounds were determined with precision and trueness of typically ≤0.5‰. Further tests aimed at the working range investigation. Good results ($U \leq 0.5‰$) could be achieved down to a TN_b concentration of 40 mgN/L and sufficient results ($U \leq 1.0‰$) down to 5 mgN/L. Additionally the development resulted in the first system reported to be suitable for simultaneous $\delta^{13}C$ and $\delta^{15}N$ direct BSIA of aqueous samples.

Conclusions: The expansion of a TOC analyzer, specially designed for coupling with isotope ratio mass spectrometry (IRMS) to the ability to measure TN_b resulted in the first system reported to be suitable for both $\delta^{13}C$ and $\delta^{15}N$ direct BSIA in aqueous samples. This system could open up new possibilities in SIA based research fields.

5.2 Introduction

The investigation of transformation and transport processes of carbon and nitrogen in ecosystems plays a vital role in understanding their biogeochemical dynamics.[1–4] Sources of dissolved organic matter (DOM) in soils are, e.g., the recent photosynthate and the leaching or further decomposition of microbially processed, older soil organic matter.[3] DOM sinks are among others mineralization, precipitation, adsorption, microbial immobilization, and transformation.[3] The stable isotope composition can be used as a marker of matter flow and to evaluate the direction and rate of those ecological processes.[4] Consequently, suitable and accurate online methods for stable isotope analysis (SIA) of carbon and nitrogen in aqueous samples such as soil extracts are required.[1,2] Over the last decade, there has been much effort to establish the routine analysis of $\delta^{13}C$ of dissolved organic carbon (DOC) through the coupling of total organic carbon analyzers with isotope ratio mass spectrometers (TOC/IRMS)[5–9]. The natural abundance bulk stable isotope analysis (BSIA) of $\delta^{15}N$ of total nitrogen bound (TN_b) still has large limitations and cannot be analyzed simultaneously with $\delta^{13}C$ directly from an aqueous sample. Low concentration of TNb makes elemental analysis coupled to isotope ratio mass spectrometry (EA/IRMS) laborious, time and sample consuming[10,11]. Russow et al.[12] introduced a first on-line measurement method for total dissolved nitrogen BSIA using the coupling of a high temperature combustion (HTC) based TOC analyzer and a quadrupole mass spectrometer. However, beside of other limitations such as a relatively high memory effect and a logarithmic non-linearity effect, this system was suitable only for samples enriched in the heavier isotope (^{15}N) because the observed detection limit was insufficient for natural abundance measurements. Indeed, the limiting factor of the system could have been the use of a quadrupole mass spectrometer instead of an isotope ratio mass spectrometer. However, the introduced HTC TOC analyzer inlet system was never coupled with an IRMS detector.

A first coupling of another HTC based TOC analyzer to isotope ratio mass spectrometer was carried out later for determining $\delta^{15}N$ in aqueous samples[13,14]. A first study of the system resulted in a SD of 2.8‰, which was still insufficient for natural abundance studies.[13] In a following study[14] the authors noted that the relatively high solubility of molecular nitrogen (N2-aq) in water remained a technical challenge and might be limiting. The SIA limit of quantification (LOQ_{SIA}) of 20 mgN/L and a corresponding precision for $\delta^{15}N$ with average SD of 0.8‰ and maximum SD of 1.8‰ for different compounds remained too high for routine measurement of natural abundance samples. Furthermore, a simultaneous $\delta^{13}C$ and $\delta^{15}N$ was neither reported with this system.

With respect to the shortcomings described, this study has the following aims: (i) an additional on-line separation step to separate N2-aq from TN_b prior to its SIA, (ii) a focusing unit for N_2, and (iii) a simultaneous $\delta^{13}C$, $\delta^{15}N$ SIA mode. To that end, we describe the further development of a high-temperature combustion (HTC) based TOC/IRMS system for the simultaneous determination of $\delta^{15}N$ in addition to $\delta^{13}C$ in aqueous solutions.

5.3 Experimental

5.3.1 Chemicals and reagents

The reference material IAEA-600 caffeine CAF1 (δ^{15}N 1.0 ± 0.2‰), USGS 41 glutamic acid (δ^{15}N 47.6 ± 0.2‰), USGS 25 ammonium sulfate (δ^{15}N -30.4 ± 0.4‰), USGS 26 glutamic acid (53.7 ± 0.4‰) and IAEA-N-2 ammonium sulfate (20.3 ± 0.2‰) were purchased from the International Atomic Energy Agency (Vienna, Austria). The internal laboratory standards were EAS-GLU1 glutamic acid, EAS-CAF2 caffeine, EAS-CAF3 caffeine, EAS-ACA1 acetanilide, EAS-GLU2 glutamic acid, EAS-SNO1 sodium nitrate and EAS-ANH1 ammonium chloride (in-house standards; Elementar Analysensysteme, Hanau, Germany). Ultrapure, deionized water (UP water) produced by a Milli-Q® system (Merck Millipore, Billerica, US) was used for solution preparation. Helium 4.6 was purchased from Air Liquide (Oberhausen, Germany) and used in combination with Helium purifier ExcelaSorb™ 27600-U (Supelco®; Sigma-Aldrich Group; Darmstadt, Germany). Oxygen 4.8 was purchased from Air Liquide (Oberhausen, Germany).

5.3.2 Instrumentation and methodology

HTC TOC/IRMS

The entire system consists of two parts: the modified TOC analyzer and the isotope ratio mass spectrometer. The, for SIA adapted TOC analyzer iso TOC cube (Elementar Analysensysteme GmbH) was used for further modifications, namely, (i) implementation of N_2 focusing unit to improve the system sensitivity, (ii) implementation of on-line degassing unit to minimize blank contribution, and (iii) a reduction tube. Reduction tube is implemented to ensure complete conversion of NO to N_2 and the absence of isotope fractionation within the system for δ^{15}N BSIA. An additional mass flow controller was implemented to dose oxygen gas accurately.

Samples, filled in 40-mL borosilicate glass vials, are introduced using a 32-position autosampler into the combustion system by means of a 5-mL syringe and a 5/4 multiway valve. Filling the syringe the sample is degassed automatically passing the vacuum/membrane degassing unit. The combustion is performed at 850 °C by oxygen and supported by a catalyst (Pt on ceramic carrier material). A reduction step over elemental copper at 500 °C is carried out to convert nitrogen oxides to N_2. Water is removed in three steps: an air-cooled condenser, a counterflow membrane dryer and a chemical dryer. Hydrogen halides and halogens are removed by silver wool. After the purification steps the carrier gas enters the nondispersive infrared (NDIR) detector. The sample is then directed to the interface

containing the aluminosilicate trap for $\delta^{13}C$ and the thermoelectric cooled zeolite trap for $\delta^{15}N$ (focusing units) prior to the IRMS analysis. The IRMS detector used is an Isoprime 100 (Isoprime, Ltd. Manchester, UK), without any modifications.

Newly developed units will be explained and described in Results and Discussion.

EA/IRMS

The $\delta^{13}C$ and $\delta^{15}N$ values of pure compounds were obtained via EA/IRMS measurements. Thereby, a vario ISOTOPE cube (Elementar Analysensysteme, Hanau, Germany) was coupled to visION (Isoprime, Manchester, UK). Around 0.5 mg of the sample is introduced using an autosampler into the combustion system. The combustion of the analyte to CO_2, N_2 and NO_x is performed at 950 °C by oxygen (60 s; 35 mL/min) and supported by a CuO. NO_x was reduced to N_2 on Cu at 600 °C. Water is removed by a chemical dryer (Sicapent®). Hydrogen halides and halogens are removed by silver wool. After the purification steps first N_2 and subsequent focused CO_2 are directed by the carrier gas helium (220 mL/min) towards the thermal conductivity detector and subsequently towards the open split connection of the isotope ratio mass spectrometer. The isotope ratio mass spectrometer was used to determine the stable isotope composition.

5.3.3 Nomenclature, evaluation and QA

In aqueous solutions the total dissolved nitrogen (TDN) consist of total nitrogen bound (TN_b) and dissolved molecular nitrogen. For the latter, we suggest the new, not yet established abbreviation N2-aq to avoid confusion.

To express the variations of natural stable isotope abundance the widely applied 'delta-notation' is used. The $\delta^{h}E_{A,\,ref}$-value of an analyte (A) is described by Equation 5-1 as a relative difference between the isotope ratio (R) of an analyte ($R(^{h}E/^{l}E)_A$) and the isotope ratio defining an international reference scale ($R(^{h}E/^{l}E)_{ref}$):

$$\delta^{h}E_{A,ref} = \frac{R\left(^{h}E/\,^{l}E\right)_A - R\left(^{h}E/\,^{l}E\right)_{ref}}{R\left(^{h}E/\,^{l}E\right)_{ref}} \qquad \text{Equation 5-1}$$

The international scale for carbon is Vienna Pee Dee Belemnite (VPDB) and for nitrogen AIR-N_2. The accepted ratio $R(^{13}C/^{12}C)_{VPDB}$ is $(11180.2\pm2.8)10^{-6}$. The accepted ratio $R(^{15}N/^{14}N)_{AIR\text{-}N2}$ is $(3678.2\pm1.5)10^{-6}$.[15] As all international scale defining reference materials in SIA also $\delta^{13}C_{VPDB,VPDB}$ and $\delta^{15}N_{AIR\text{-}N2,AIR\text{-}N2}$ have the value zero.[15]

Please note that if no reference is mentioned, as exemplarily shown in Equation 5-2, the reported δ-values are related to the used reference gas (RG). That concerns all data before the final normalization to the international reference scale.

$$\delta^{13}C_{\text{lin corr A}} \equiv \delta^{13}C_{\text{lin corr A,RG}} \qquad \text{Equation 5-2}$$

with $\delta^{13}C_{\text{lin corr A}}$ as a linearity corrected δ-value of an analyte A.

All measured $\delta^{13}C$ and $\delta^{15}N$ raw data ($\delta^{13}C_{\text{meas A}}$ and $\delta^{15}N_{\text{meas A}}$) generated by the software IonVantage (IsoPrime100 operating system) or IonOS (visION operating system) are automatically related to RG and in case of $\delta^{13}C$ values additionally corrected for ^{17}O-abundance. Then the values are linearity corrected to $\delta^{13}C_{\text{lin corr A}}$ and $\delta^{15}N_{\text{lin corr A}}$ and in case of EA/IRMS measurement blank corrected to $\delta^{13}C_{\text{bl corr A}}$ and $\delta^{15}N_{\text{bl corr A}}$. Finally, a referencing strategy to the international scale is applied using two-point normalization to $\delta^{13}C_{\text{A,VPDB}}$ and $\delta^{15}N_{\text{A,AIR-N2}}$.

The described evaluation strategy follows accepted recommendations in literature[15]. The standard uncertainty (u_{std}) is expressed as the standard deviation of replicate measurements and thus represents the uncertainty caused by the instrumentation. Error bars shown within this work represent the standard uncertainty (1σ).

5.4 Results and discussion

5.4.1 Instrumental development

In the focus of this work was the development and testing of a novel system for HTC TOC/IRMS δ^{15}N BSIA. A HTC based system rather than a WCO based system to enable measurement of δ^{15}N-values was selected based on previous findings[8]. Additionally, previous findings with a HTC based HPLC/IRMS-interface, such as the need of a reduction oven and additional mass flow controller were implemented.[16] To measure nitrogen stable isotope composition, all nitrogen species created within the oxidation reactor (combustion tube), mainly N_2 and NO need to be converted to N_2 completely.

A vacuum-membrane degassing unit was developed and installed in order to reduce the impact of dissolved molecular nitrogen.[17] The combination of a Teflon AF® membrane, sample drawing up speed of 5 µL/s and 50 mbar absolute in vacuum chamber showed the best performance regarding degassing efficiency. The degassing unit effectively (degassing efficiency 83%) removes N2-aq from the sample prior to combustion, decreasing its amount from 22 mgN/L to ≤4 mgN/L (Figure 5-1). This fully automated procedure improves and replaces the time consuming and laborious offline degassing procedure (degassing efficiency of the offline procedure 80%)[18]. The online removal of N2-aq also reduces the risk of contamination. Without degassing the equivalent of 22 mgN/L N2-aq would contribute to the TN_b signal using a focusing unit.

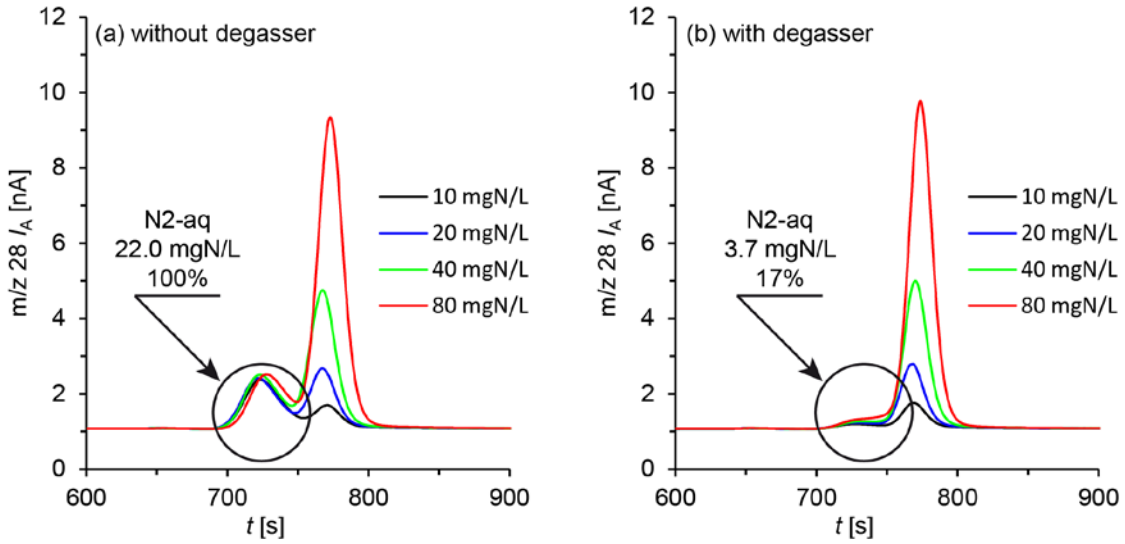

Figure 5-1 Test series with TN_b solutions (10 to 80 mgN/L as CAF2 solutions); Without (a) and with (b) membrane-based on-line degassing unit (b).

When measuring nitrogen stable isotope composition with IRMS, sensitivity is often a limiting factor, especially compared with determination of carbon isotope composition. Less nitrogen bound in organic matter compared with carbon is one reason, but also the lower isotope abundance of the heavier nitrogen compared to that of carbon (≈0.0107 for ^{13}C mole fraction; ≈0.00364 for ^{15}N mole fraction) as well as the need of two N atoms for one analyte molecule (N_2) make nitrogen SIA challenging. Therefore finding a way to concentrate the nitrogen peak was one of the goals of this work. The result was a focusing unit installed after conversion and purification of the carrier and analyte gas-mix stream (see Figure 5-2a and Figure 5-3). The promising adsorption material molecular sieve EAS-MS-10A-PF (compare Figure 5-2b i and ii), was used for further tests (see Instrument testing with aqueous standard solutions). The focusing performance depends on the analytical conditions, mainly the injection speed and volume, as well as on the sample composition and concentration and was between factor two and five (peak height comparison) using EAS-MS-10A-PF. Preliminary tests as well as proof of concept were conducted with EAS-MS-10A-PF, but further and parallel conducted experiments with adsorption materials resulted already in discovery of even more promising material (EAS-MS-5A-45/60M; see Figure 5-2b ii and iii). Its proper testing should be part of further system optimization.

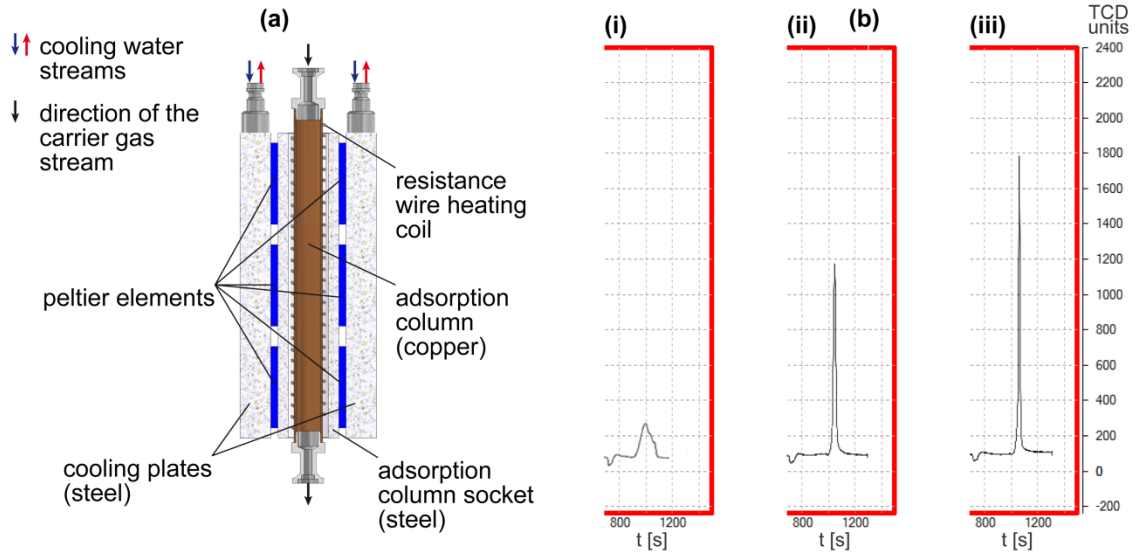

Figure 5-2 Focusing of nitrogen peak. Schematic few of the focusing unit (a). Focusing performance (174.6 mgN/L solution; adsorption temperature -38 °C; desorption temperature 100 °C; heating rate 3.4 °C/s; an EA analyzer was connected behind the focusing unit in order to record the TCD signal) (b): nitrogen peak without focusing (peak height 167 TCD units) (i); nitrogen peak using focusing unit with adsorption material EAS-MS-10A-PF (peak height 1064 TCD units) (ii); nitrogen peak using focusing unit with adsorption material EAS-MS-5A-45/60M (peak height 1690 TCD units) (iii).

The final set up of the HTC TOC/IRMS system with the possibility of direct TN_b SIA in aqueous solutions without any sample preparation is shown in Figure 5-3.

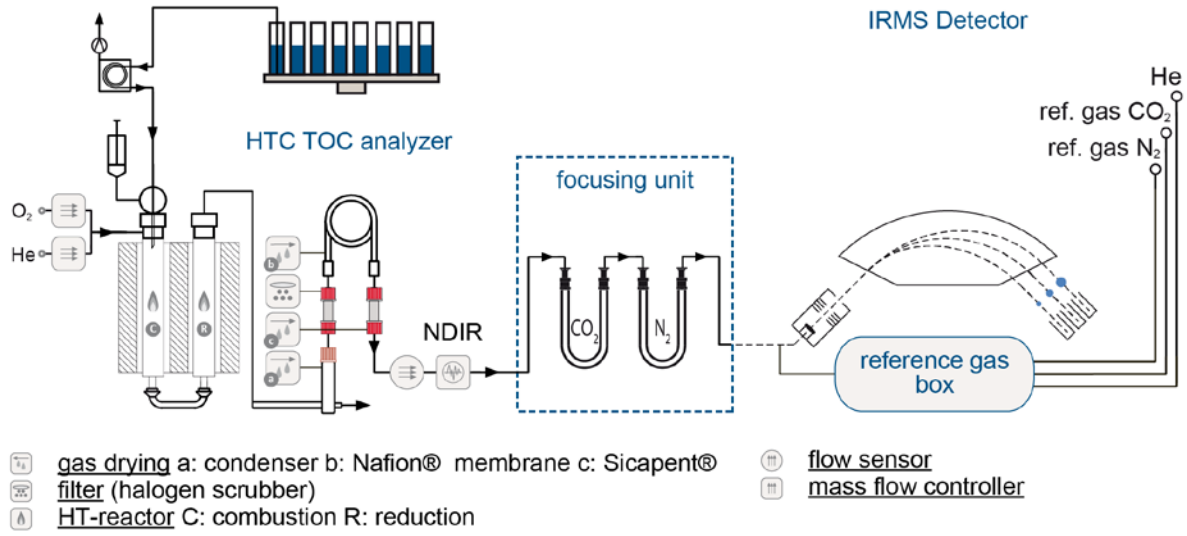

Figure 5-3 System setup for HTC TOC/IRMS BSIA.

5.4.2 Instrument testing with aqueous standard solutions

Carry-over (memory effects), drift and precision

The precision obtained by averaged results of quadruplicate measurements with standard solutions and expressed as u_{std} (SD≡1σ) was typically ≤0.15‰ ($u_{std,avg}$=0.13‰; $u_{std,max}$=0.22‰) for $\delta^{15}N$.

Carry-over was tested by measuring a sequence of samples with varying stable isotope composition (see Figure 5-4). Alternation of the delta values of two sequential samples $\Delta(A_{n+1} - A_n)$ up to 48‰ for $\delta^{15}N$ did not lead to any detectable carry over. The first replicate was not influenced by the sample before, proving absence of a significant bias caused by carry over . The averaged bias of ±0.07‰ is within the variation caused by respective precision. This performance could be achieved using a dummy peak injection implemented in the flushing sequence as shown in Figure 5-4. The achieved elimination of bias is an improvement compared to the previously reported HTC based systems.[12,13]

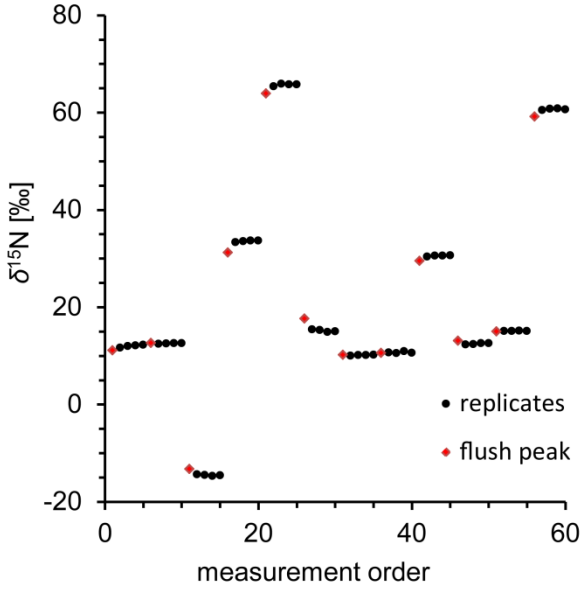

Figure 5-4 Test series to investigate the carry over effect. Shown data are referenced to the working gas only.

Trueness, accuracy and lower working limit estimation

Two-point normalization is suggested for the evaluation of data[15]. Either accepted international reference standards values or, due to the lack of further certified reference materials, the values obtained via EA/IRMS were considered as true values for the investigation of accuracy. Traceability was ensured by referencing of the EA/IRMS δ^{15}N values to the reference material and thus indirectly to the AIR-N_2 scale.

In order to cover the isotope range of interest, reference materials USGS 25 ($\delta^{15}N_{AIR\text{-}N2}$ -30.4 ± 0.4‰) was used as a first standard and USGS 26 ($\delta^{15}N_{AIR\text{-}N2}$ 53.7 ± 0.4‰) as a second standard for normalization for N BSIA.

Trueness is the difference between the true (either accepted value of an international reference material or, if that was not available, the value obtained via EA/IRMS) and measured (HTC TOC/IRMS) values. The obtained $\Delta_{trueness}$ values were typically ≤0.5‰ (average 0.5‰; maximum 0.85‰) for $\delta^{15}N_{AIR\text{-}N2}$ using the international standards. Using all compound solutions (incl. in-house standards) the average trueness was 0.5‰ and maximum 1.09‰. No compound specific effects were observed. The coefficient of determination of the linear regression between the measured and true values (R^2=0.9997) also indicates a good agreement (see Figure 5-5 and Table 5-1).

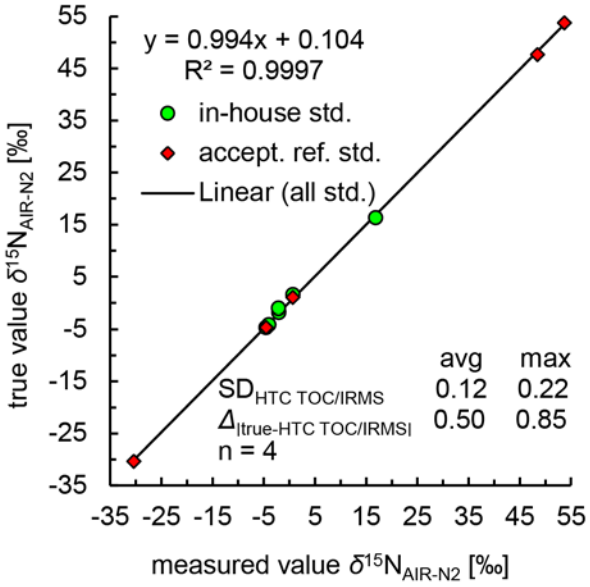

Figure 5-5 Least-squares linear regressions between true values and HTC TOC/IRMS measured values for nitrogen. Error bars represent the standard deviation of each sample. Error bars are typically smaller than symbol sizes. $\delta^{15}N$ values are referenced to AIR-N_2 scale.

Table 5-1 Trueness test with different species expressed as difference between true and measured value

Compound	true[a] $\delta^{15}N_{AIR-N2}$ accept. ± U [‰]	measured[b] $\delta^{15}N_{AIR-N2}$ avg ±SD [‰] (n = 3)	$\Delta_{\lvert meas-true \rvert}$ $\delta^{15}N_{AIR-N2}$ [‰]
glutamic acid (GLU1)	-4.66 ± 0.5*	-4.56 ± 0.08	0.10
caffeine (CAF2)	-1.76 ± 1.0*	-2.04 ± 0.04	0.28
acetanilide (ACA1)	1.52 ± 0.5*	0.71 ± 0.22	0.81
glutamic acid (GLU2)	-4.1 ± 0.5*	-3.97 ± 0.14	0.13
sodium nitrate (SNO1)	16.28 ± 0.5*	16.85 ± 0.10	0.57
ammonium chloride (ANH1)	-1.03 ± 1.0*	-2.12 ± 0.13	1.09
ammonium sulfate (IAEA-N-2)	20.3 ± 0.2**	20.00 ± 0.13	0.30
caffeine (IAEA-600)	1.0 ± 0.2**	0.65 ± 0.02	0.35
glutamic acid (USGS 41)	47.6 ± 0.2**	48.45 ± 0.12	0.85
ammonium sulfate (USGS 25)	-30.4 ± 0.4**	(cal[c]) ± 0.12	
glutamic acid (USGS 26)	53.7 ± 0.4**	(cal[c]) ± 0.19	
averaged values		0.12	0.50

[a] internationally accepted value if international reference material (*) and value obtained via EA/IRMS and traced back to AIR-N2 scale using international reference materials if in-house standard (**).

[b] via HTC TOC/IRMS obtained and subsequent normalized values

[c] international reference materials used for two-point normalization

Additionally, carbon NDIR detector peak areas were used in case of organic compounds to check the completeness of the combustion. An average rel. SD(A_{NDIR}) of 0.3% and max rel. SD(A_{NDIR}) of 0.4% and a correlation coefficient of the linear regression (A_{NDIR} vs. carbon concentration) of 0.999 demonstrate the efficiency of the combustion unit.

Considering the obtained trueness and precision, the accuracy can be quantified in a first approximation as ≤0.65‰.

Further tests aimed at the working range investigation. CAF2 solution and concentrations from 2.5 to 320 mgN/L (injection volume of 0.6 mL) were used to that end. Good (U ≤ 0.5‰) results could be achieved down to nitrogen concentration of 40 mgN/L and sufficient (U ≤ 1.0‰) down to 5 mgN/L. A concentration of 2.5 mgN/L could not be measured with the accepted accuracy (insufficient; U ≥ 1.0 ‰). As shown in Figure 5-6, with δ^{15}NAIR-N2 value -0.01‰ the 2.5 mgN/L of CAF2 solution was the first outside the accuracy range of 1.0‰ with Δ of -1.75‰. 5 mgN/L herewith marks the lower working limit.

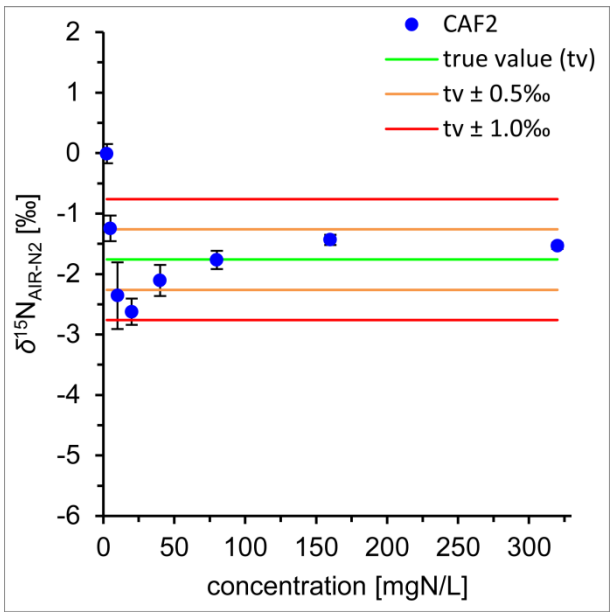

Figure 5-6 Working range investigation test series; orange lines mark upper and lower uncertainty interval defined as good (U ≤ 0.5‰) and indicate a nitrogen concentration of 40 mgN/L as the lowest concentration within that interval; red lines mark upper and lower uncertainty interval defined as sufficient (U ≤ 1.0‰) and indicate 5 mgN/L as the lowest concentration within that interval. Concentration of 2.5 mgN/L could not be measured with the accepted accuracy (insufficient; U ≥ 1.0 ‰).

Reported performance is achieved without any additional background correction. Further investigations of the background contribution were necessary in order to perform background corrections and it may improve the quality of the final results. That is however out of scope in this work. Shown data are only linear corrected and normalized as suggested in the literature[15]. Anyway background and blank correction cannot be applied at this stage, because there are different, not yet quantified and partially contrary effects. The remaining N2-aq is for example expected to be “heavier” compared to its original isotope composition. Together with N_2 evolved from TN_b it passes the condenser. Even the solubility is decreased by roughly factor of three (ca. 60 °C), restrained by dissolution nitrogen is expected to be heavier and thus lighter composition in the released gas stream is to expect. The course of the curve (Figure 5-6) probably reflects those exemplarily explained contrary effects. The effects need further investigation and together with removal of the condenser can lead to better understanding of the system and better performance regarding sensitivity. Removal of the condenser can be compensated by e.g. slower sample injection speed in combination with higher flow rate of drying gas on the membrane. This optimization step is promising due to water-selectivity of the used membrane.

The first proof of principle showed promising results. Still further optimization and measurements with real samples, containing different matrixes are required for a fully validated and optimized system.

5.4.3 Simultaneous $\delta^{13}C$ and $\delta^{15}N$ determination in aqueous solutions

Considering the special importance of simultaneous $\delta^{13}C$ and $\delta^{15}N$ determination in aqueous samples the system set-up (Figure 5-3) was developed to enable a simultaneous mode. In order to proof the principle of simultaneous $\delta^{13}C$, $\delta^{15}N$ SIA mode following tests were performed.

The HTC TOC/IRMS system in simultaneous mode, in particular the software sequence, was set up as following. The syringe injects the sample, which passes the degassing unit and enters the conversion and purification zone of the TOC analyzer. O_2 is automatically added to the carrier gas helium via a second mass flow controller for the time of the combustion. Required O_2 volume flow and dosage time needed were empirically derived during the preliminary tests and depend generally on the volume injected, concentration range of DOM in the sample and injection speed. The NDIR CO_2 cell detects the CO_2 peak start and integrates the peak. During CO_2 peak detection CO_2 is adsorbed in the CO_2 adsorption column and N_2 is adsorbed in the N_2 adsorption column within the focusing unit. N_2 is not detectable in the TOC analyzer. The NDIR NO cell is set up to control for the breakthrough of the NO during the test series (indicating a used up reduction tube). After the CO_2 peak end is detected, desorption of N_2 is initialized. Desorbed N_2 is directed to the isotope ratio mass spectrometer, where its isotope composition is determined. After the fixed desorption time has passed and therefore N_2 is desorbed completely, the N_2 column is automatically bypassed and desorption of CO_2 is initialized. Desorbed CO_2 is directed to the isotope ratio mass spectrometer, where its isotope composition is determined. After desorption of the CO_2 and completing of the run, the next injection is initialized.

First tests for simultaneous $\delta^{13}C$ and $\delta^{15}N$ determination with this system were successfully performed. Figure 5-7a shows a typical HTC TOC run in $\delta^{13}C$, $\delta^{15}N$ SIA mode used for subsequent IRMS meassurements. Figure 5-7b shows a typical HTC TOC/IRMS run in simultaneous mode. 1 mL injections of caffeine solutions (CAF3; 50 mgN/L) were used.

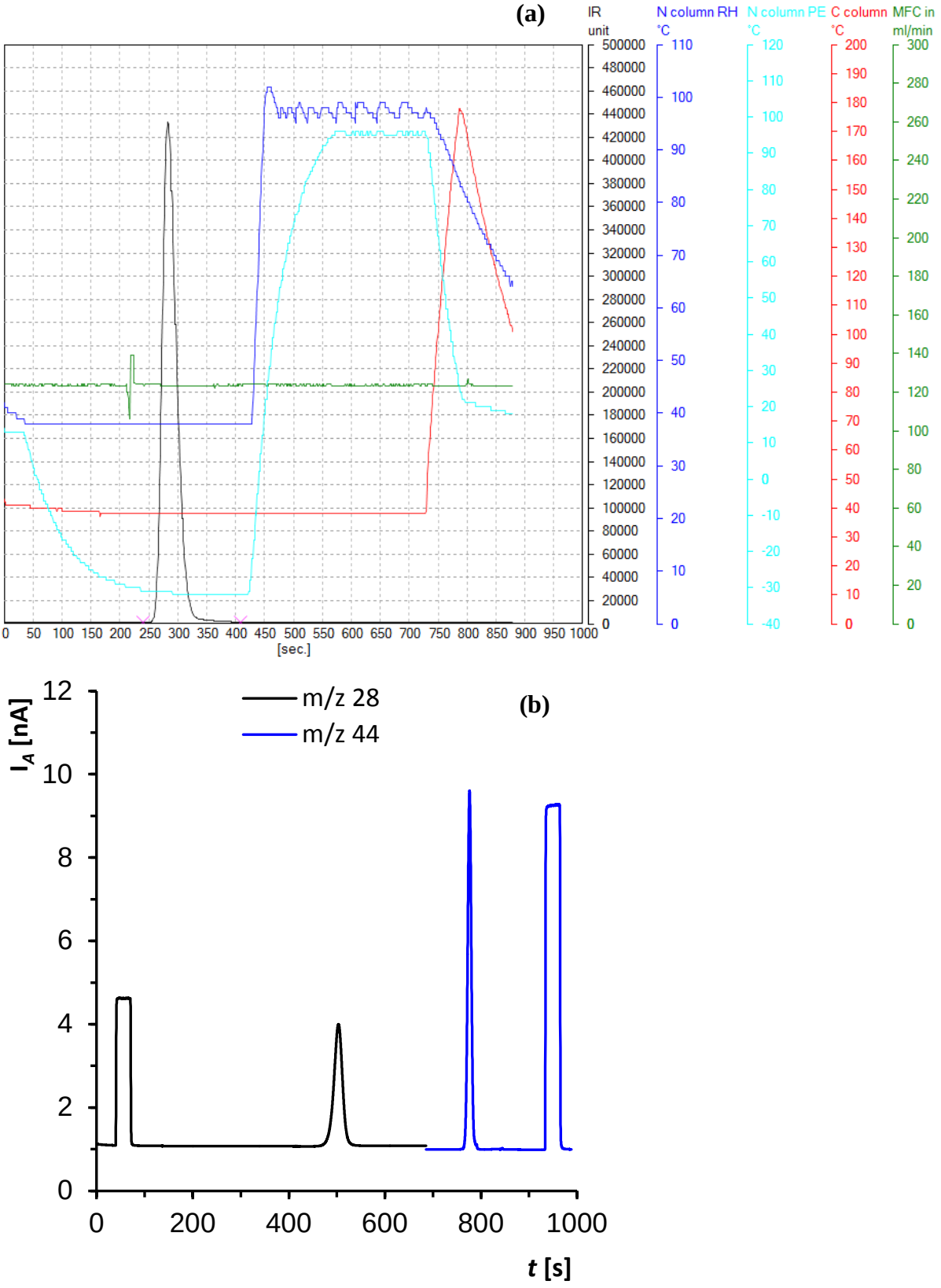

Figure 5-7 Typical HTC TOC/IRMS run in simultaneous $\delta^{13}C$, $\delta^{15}N$ SIA mode. (a) TOC run course; black line: NDIR cell CO_2 signal; dark blue line: temperature of the restriction heater (RH) of the N_2 adsorption column; light blue line: temperature of the peltier element (PE) of the N_2 adsorption column; red line: temperature of the restriction heater (RH) of the CO_2

adsorption column; green line: flow of the carrier gas helium controlled by the mass flow controller (MFC). (b) IRMS run course: black line: *m/z* 28 signal with the sample peak at ca. 500 s and the reference gas pulse at ca. 75 s; blue line: *m/z* 44 signal with the sample peak at ca. 780 s and the reference gas pulse at ca. 980 s.

A long measurement sequence with 189 single measurements (63 triplicates) conducted over ca. 52 h (ca 16.7 min per run) showed no significant drift over time and a good precision (δ^{13}C $SD_{n=189}$ = 0.07‰; δ^{15}N $SD_{n=189}$ = 0.42‰). Precision expressed as $SD_{n=3}$ of the 63 triplicates is for δ^{13}C values averaged 0.04‰ (maximum of 0.11‰; minimum of 0.00‰) and for δ^{15}N values averaged 0.12‰ (maximum of 0.44‰; minimum of 0.01‰). Three δ^{13}C and δ^{15}N outlier (single replicates; out of 189) were excluded from the long test series evaluation. Outliers were very obvious, with δ-value bias >1‰. Small air bubbles in the syringe might be a possible reason, but further investigations are necessary. The reduction lasts at least 189 measurements, which is threefold longer than in the system reported in 2007[14].

5.5 Conclusion and outlook

A novel high-temperature based TOC-system for direct bulk stable carbon isotope analysis out of aqueous samples was successfully extended to the possibility to measure also stable nitrogen isotope composition. Proof of principle demonstrated that nitrogen can be measured with precision and trueness of ≤0.5‰. Lower working limit of 5 mgN/L for δ^{15}N BSIA, was achieved with a caffeine solution considering an accuracy of ±1.0‰ as acceptable. No sample preparation is required and the system works fully automated. Main innovations are the automated degassing unit and zeolite based focusing unit.

Further work needs to focus on further validation of the system with real samples. Further investigation and quantification of the background as well as blank contribution for stable nitrogen isotope measurements are necessary. Further method development should focus on optimization e.g. by bypassing of the condenser and development of an automated blank and background correction software sequence. Also a high injection and its result on the performance regarding sensitivity should be further tested.

Finally it is the first carry-over free, on-line system for simultaneously measurements of dissolved carbon and nitrogen isotope composition in aqueous solutions.

In summary, the described system offers new possibilities for automated TN_b SIA directly from aqueous solutionsand in combination with simultaneous DOC SIA opens new opportunities for a wide range of stable isotope applications in, among others, soil science and limnology.

5.6 References

[1] T. H. Blackburn, R. Knowles. Nitrogen Isotope Techniques. Academic Press, San Diego, 1993.

[2] D. C. Coleman, B. Fry. Carbon Isotope Techniques. Academic Press, San Diego, 1991.

[3] W. H. McDowell. Dissolved organic matter in soils-future directions and unanswered questions. Geoderma 2003, 113, 179.

[4] A. V. Tiunov. Stable isotopes of carbon and nitrogen in soil ecological studies. *Biol. Bull. (N. Y., NY, U. S.)* 2007, 34, 395.

[5] C. L. Osburn, G. St-Jean. The use of wet chemical oxidation with high-amplification isotope ratio mass spectrometry (WCO-IRMS) to measure stable isotope values of dissolved organic carbon in seawater. Limnol. Oceanogr.: Methods 2007, 5, 296.

[6] S. Q. Lang, M. D. Lilley, J. I. Hedges. A method to measure the isotopic (^{13}C) composition of dissolved organic carbon using a high temperature combustion instrument. Mar. Chem. 2007, 103, 318.

[7] R. J. Panetta, M. Ibrahim, Y. Gélinas. Coupling a hightemperature catalytic oxidation total organic carbon analyzer to an isotope ratio mass spectrometer to measure natural-abundance δ^{13}C dissolved organic carbon in marine and freshwater samples. Anal. Chem. 2008, 80, 5232.

[8] E. Federherr, C. Cerli, F. M. S. A. Kirkels, K. Kalbitz, H. J. Kupka, R. Dunsbach, L. Lange, T. C. Schmidt. A novel high-temperature combustion based system for stable isotope analysis of dissolved organic carbon in aqueous samples. I: development and validation. Rapid Commun. Mass Spectrom. 2014, 28, 2559.

[9] M. S. A. K. Frédérique, C. Cerli, E. Federherr, K. Kalbitz. A novel high temperature combustion based system for stable isotope analysis of dissolved organic carbon in aqueous samples (II): optimization and assessment of analytical performance. Rapid Commun. Mass Spectrom. 2014, 28, 2574.

[10] S. D. Kelly, C. Stein, T. D. Jickells. Carbon and nitrogen isotopic analysis of atmospheric organic matter. *Atmos. Environ.* 2005, 39, 6007.

[11] F. C. Batista, A. C. Ravelo, J. Crusius, M. A. Casso, M. D. McCarthy. Compound specific amino acid δ^{15}N in marine sediments: A new approach for studies of the marine nitrogen cycle. Geochim. Cosmochim. Acta 2014, 142, 553.

[12] R. Russow, J. Kupka, A. Goetz, B. Apelt. A New Approach to Determining the Content and ^{15}N Abundance of Total Dissolved Nitrogen in Aqueous Samples: TOC Analyzer-QMS Coupling. Isot. Environ. Health Stud. 2002, 38, 215.

[13] D. Huygens, P. Boeckx, J. Vermeulen, X. D. Paepe, A. Park, S. Barker, C. Pullan, C. O. Van. Advances in coupling a commercial total organic carbon analyser with an isotope ratio mass spectrometer to determine the isotopic signal of the total dissolved nitrogen pool. Rapid Commun. Mass Spectrom. 2005, 19, 3232.

[14] D. Huygens, P. Boeckx, J. Vermeulen, X. De Paepe, A. Park, S. Barker, O. Van Cleemput. On-Line Technique to Determine the Isotopic Composition of Total Dissolved Nitrogen. Anal. Chem. (Washington, DC, U. S.) 2007, 79, 8644.

[15] M. A. Jochmann, T. C. Schmidt. Compound-Specific Stable Isotope Analysis. Royal Society of Chemistry, Cambridge, 2012.

[16] E. Federherr, S. Willach, N. Roos, L. Lange, K. Molt, T. C. Schmidt. A novel high-temperature combustion interface for compound-specific stable isotope analysis of carbon and nitrogen via high-performance liquid chromatography/isotope ratio mass spectrometry. Rapid Commun. Mass Spectrom. 2016, 30, 944.

[17] H.-P. Sieper, L. Lange, E. Federherr, H.-J. Kupka. Verfahren und Vorrichtung zur Analyse von Stickstoff (N) in einer Probe. 2015, 10 2014 002 266.

[18] M. Holtappels, G. Lavik, M. M. Jensen, M. M. M. Kuypers. ^{15}N-labeling experiments to dissect the contributions of heterotrophic denitrification and anammox to nitrogen removal in the OMZ waters of the ocean. Methods Enzymol. 2011, 486, 223.

Chapter 6 A novel high-temperature combustion interface for compound-specific stable isotope analysis of carbon and nitrogen via high-performance liquid chromatography/isotope ratio mass spectrometry

Adapted from: E. Federherr, S. Willach, N. Roos, L. Lange, K. Molt and T. C. Schmidt; A novel high-temperature combustion interface for compound-specific stable isotope analysis of carbon and nitrogen via high-performance liquid chromatography/isotope ratio mass spectrometry; Rapid Communications in Mass Spectrometry **2016**, 30, 944-952

6.1 Abstract

Rationale: In aqueous samples compound-specific stable isotope analysis (CSIA) plays an important role. No direct method (without sample preparation) for stable nitrogen isotope analysis (δ^{15}N SIA) of non-volatile compounds is known yet. The development of a novel HPLC/IRMS interface based on high-temperature combustion (HTC) for both δ^{13}C and δ^{15}N CSIA and its proof of principle are described in this study.

Methods: To hyphenate high-performance liquid chromatography (HPLC) with isotope ratio mass spectrometry (IRMS) a modified high-temperature combustion total organic carbon analyzer (HTC TOC) was used. A system to handle a continuously large amount of water (three-step drying system), favorable carrier and reaction gas mix and flow, an efficient high-temperature-based oxidation and subsequent reduction system and a collimated beam transfer system were the main requirements to achieve the necessary performance.

Results: The proof of principle with caffeine solutions of the system succeeded. In this initial testing, both δ^{13}C and δ^{15}N values of tested compounds were determined with precision and trueness of ≤0.5‰. Further tests resulted in lower working limit values of 3.5 µgC for δ^{13}C SIA and 20 µgN for δ^{15}N SIA, considering an accuracy of ±0.5‰as acceptable.

Conclusions: The development of a novel HPLC/IRMS interface resulted in the first system reported to be suitable for both δ^{13}C and δ^{15}N direct CSIA of non-volatile compounds. This highly efficient system will probably open up new possibilities in SIA-based research fields.

6.2 Introduction

Stable isotope analysis has proven to be a powerful tool in many research areas. In aqueous samples, in addition to bulk stable isotope analysis (BSIA), compound-specific stable isotope analysis (CSIA) also plays an important role and it has become an established tool in many application areas over the last two decades with large further potential.[1,2] Environmental[3] and forensic sciences[4–7] are prominent examples of such applications, utilizing naturally occurring fractionation processes during transport and transformation processes to, e.g., allocate contaminants or drugs sources. The broad range of involved application areas includes agriculture (e.g. biocides; glyphosate),[8] medicine (e.g. pharmaceuticals; sulphonamides),[9] the food industry (e.g. food components; caffeine),[10] archaeology (e.g. body tissue components; amino acids),[11] geology (soil components; e.g. amino sugars)[12] and sports (e.g. doping; steroids).[13] A number of these disciplines utilize standard analytical CSIA techniques. However, potential applications, such as the identification of contaminant sources where the determination of carbon isotope ratios is insufficient for an unequivocal result, are still often limited by the lack of optimal – simple and accurate – or even suitable methods. Therefore, further developments in analytical instrumentation and methods play an important role for progress in the mentioned areas.[3,4]

Various analytical approaches have been used for the CSIA of non-volatile, polar (water-soluble) compounds. CSIA was carried out either by offline sample preparation, such as extraction and purification of the analyte, followed by elemental analyzer/isotope ratio mass spectrometry (EA/IRMS),[14] or by derivatization followed by gas chromatography/isotope ratio mass spectrometry (GC/IRMS).[15] High-performance liquid chromatography (HPLC)/IRMS was the only direct method (i.e. without previous sample modification) also utilized.[1,16]

EA/IRMS CSIA of non-volatile compounds still dominates biogeochemical and ecological studies[16] although offline sample preparation is very time-consuming and laborious. It also involves a higher risk of contamination and fractionation. Possible fractionation must also be controlled in derivatization for subsequent GC/IRMS measurements. Both EA/IRMS and GC/IRMS CSIA also require additional corrections, which increase the uncertainty of the determined values.[9–11,13,16]

Based on these shortcomings of the described approaches for the CSIA of non-volatile, polar and thermally labile compounds, HPLC separation has become the method of choice,[16] and much effort has been aimed at the development of a suitable HPLC/IRMS interface. The main

problem results from the need to convert the analyte into the gas required for the IRMS analysis (CO_2 and NO or N_2 for C and N isotope ratio determination, respectively). In the 1990s, the use of thermospray and a moving belt interface led to the successful coupling of HPLC with IRMS.[17] Simultaneously, a chemical reaction interface (CRI) was also combined with IRMS.[18] None of these technologies were further developed to a commercial instrument, however. In the case of the CRI the large signal from the reactant gas (O_2^+; *m/z* 32) spreads into the cup for the analyte gas ($N^{15}O^+$; *m/z* 31) preventing $\delta^{15}N$ measurement, while byproducts in the plasma (CO^+, NO_2^+ and $C_2H_5O^+$) led to incorrect $\delta^{13}C$ values.[18] In the case of the moving belt, no $\delta^{15}N$ SIA was ever reported and for $\delta^{13}C$ SIA the limitations include the limited capacity of the wire, depletion of semivolatile compounds with potential isotope fractionation and flow restriction. One decade later the principle of wet chemical oxidation (WCO)-based total organic carbon (TOC) analyzers was utilized to couple IRMS with HPLC,[19] and two HPLC/IRMS interfaces based on this principle are commercially available, the LiquiFace™ (Elementar Analysensysteme, Hanau, Germany) and the LC-IsoLink™ (Thermo Fisher Scientific, Bremen, Germany), which enable online $\delta^{13}C$ CSIA following HPLC separations. However, these interfaces do not allow the measurement of $\delta^{15}N$ values. Furthermore, WCO-based systems for $\delta^{13}C$ CSIA suffer from the same problem as is common in BSIA, i.e. the risk of isotope fractionation due to incomplete oxidation.[10,20]

In view of the limitations of the existing instrumental approaches and taking into account the positive experience with the HTC TOC analyzer,[20,21] we have developed a novel high-temperature combustion (HTC)-based HPLC/IRMS system for $\delta^{13}C$ and $\delta^{15}N$ CSIA. In this manuscript, we present the technical details of the system and results of a first proof of principle study.

6.3 Experimental

6.3.1 Chemicals and reagents

The reference material IAEA-600 caffeine CAF1 was purchased from the International Atomic Energy Agency (IAEA, Vienna, Austria). Caffeine CAF2 (≥98.5%) and an internal laboratory standard EAS-ACA1 (p.a.) were purchased from Merck (Darmstadt, Germany). Caffeine standards CAF3 (ID C0751; ≥99.0%), CAF7 (ID C0750; Lot 061M0052V; ≥98.5%) and CAF8 (ID C0750; Lot 028 K0757; ≥98.5%) were purchased from Sigma-Aldrich (Buchs, Switzerland). Caffeine CAF4 (99.70%) was purchased from Alfa Aesar (Karlsruhe, Germany). Caffeine CAF5 was provided by the Institute for Biodiversity and Ecosystem Dynamics (in-house standard; University of Amsterdam, Amsterdam, The Netherlands). Caffeine CAF6 was purchased from NATECO2 (Wolnzach, Germany). Ultrapure, deionized water (UP water) produced by a Milli-Q® system (Merck Millipore, Billerica, MA, USA) was used for solution preparation. Helium 4.6 was purchased from Air Liquide (Oberhausen, Germany) and used in combination with helium purifier ExcelaSorb™ 27600-U (Supelco®; Sigma-Aldrich Group; Darmstadt, Germany). Oxygen 4.8 was purchased from Air Liquide.

6.3.2 Instrumentation and methodology

HPLC/IRMS

The entire system consists of three parts: the HPLC Infinity system (Agilent Technologies, Santa Clara, CA, USA), the HPLC/IRMS HTC interface (Elementar Analysensysteme) and the IsoPrime100 isotope ratio mass spectrometer (Isoprime, Manchester, UK) (see Figure 6-1).

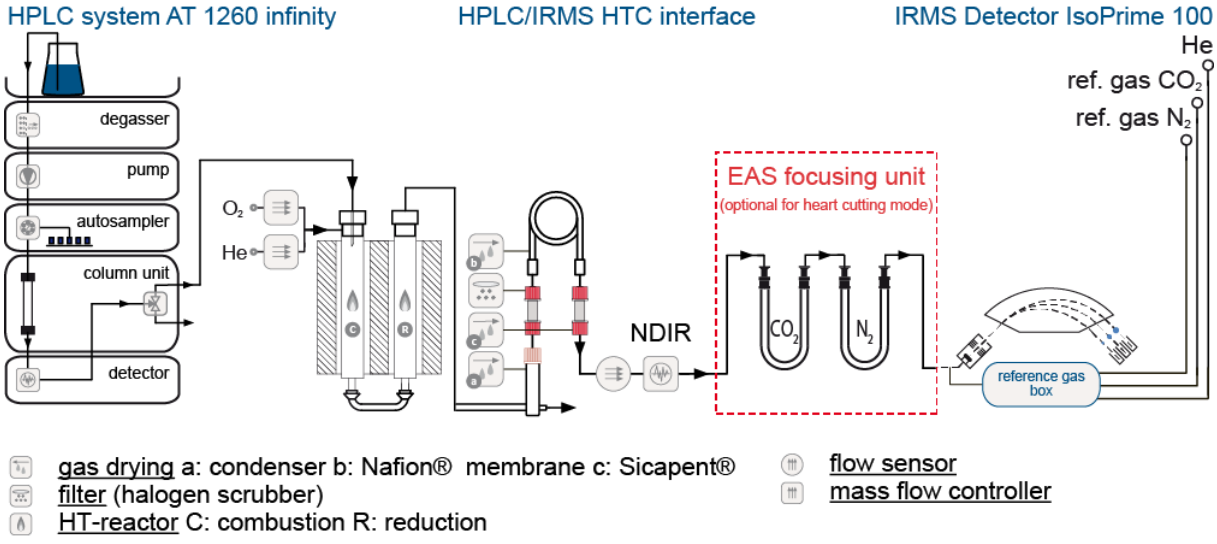

Figure 6-1 System setup for HPLC/IRMS CSIA

The Agilent HPLC system previously used with the LiquiFace WCO-based interface (Isoprime) was modified as follows. The column unit was replaced because of the need for a diverter valve controllable by the Agilent software for the heart-cut mode. The refractive index detector, which exhibited unsuitable pressure resistance, was replaced by an UV/Vis detector; the pressure resistance is required due to the back pressure caused by the transfer line capillary (i.d. ≤100 µm), installed after the HPLC detector and leading to the HTC interface.

The final HPLC system is modular and consists of a degasser unit (1260 degasser; G1322A), a pump unit (1260 iso pump; G1310B), an autosampler unit (1260 ALS; G1329B), a thermostatted column unit equipped with a diverter valve (1290 TCC; G1316C) and a multiple wavelength ultraviolet/visible spectroscopic (UV/Vis) detector unit, equipped with 10-mm cell path length flow cell with a pressure maximum of 120 bar (1260 MWD; G1365C). The column unit was equipped with a XBridge C_{18} guard column (2.1 × 10 mm, 3.5 µm; Waters, Eschborn, Germany) followed by a XBridge C_{18} reversed-phase column (2.1 × 100 mm, 3.5 µm, Waters). A mobile phase flow rate of 0.5 mL/min (UP water) was used.

In a previously reported HTC interface, oxygen was used as carrier and reaction gas.[20] Due to the need of a reduction tube (conversion of NO into N_2), the oxygen carrier gas had to be replaced by helium. The oxygen reaction gas could be added with the help of an additional mass flow controller. The unit to replace oxygen by helium installed between the iso TOC and isotope ratio mass spectrometer in the BSIA system[20] became redundant and it was removed. However, it turned out in preliminary experiments with caffeine that the addition of oxygen is not always necessary since water in combination with the platinum catalyst is sufficient as oxygen donor. The obtained δ-values were with 10 mL/min oxygen gas: $\delta^{13}C_{unc}$: 9.52 ± 0.03‰; C: 21.94 ± 0.16 mgC/L (rel. SD: 0.7%); and without oxygen gas: $\delta^{13}C_{unc}$: 9.47 ± 0.05‰; C: 22.54 ± 0.15 mgC/L (rel. SD: 0.7%). Therefore, all the caffeine results reported have been measured without addition of oxygen. However, a systematic investigation of oxygen gas demands requires further tests and is outside the scope of this work.

The final HPLC/IRMS HTC interface is derived from the commercially available SIA HTC-TOC analyzer iso TOC cube (Elementar Analysensysteme). The outflow from the HPLC system is either completely (continuous-flow (CF) mode) or partially (heart-cutting (HC) mode) introduced into the combustion system. The combustion within the interface takes place on the catalyst (Pt on ceramic carrier material) at 850 °C and can be supported by the addition of oxygen gas. The reduction is performed at 500 °C using reduced copper. Water is removed in three steps: an air-cooled condenser, a counter-flow membrane dryer and a

chemical dryer. Hydrogen halides and halogens are removed by silver wool placed between the condenser and the membrane dryer. After the purification steps the helium carrier gas with the analyte enters the nondispersive infrared (NDIR) detector and subsequently the open split of the mass spectrometer.

An IsoPrime100 isotope ratio mass spectrometer was used to determine the stable isotope composition. No modifications were made to this instrument.

A detailed description of the significant system modifications to the HTC interface and the HPLC system and/or developments is given in the Instrumental development section.

EA/IRMS

The $\delta^{13}C$ and $\delta^{15}N$ values of pure compounds were obtained via EA/IRMS measurements, where a vario ISOTOPE cube (Elementar Analysensysteme) was coupled to a visION isotope ratio mass spectrometer (Isoprime). Around 0.5 mg of the sample is introduced using an autosampler into the combustion system. The combustion of the analyte to CO_2 and N_2 and NO_x is performed at 950 °C by oxygen (60 s; 35 mL/min) and supported by CuO. NO_x is reduced to N_2 on Cu at 600 °C. Water is removed by a chemical dryer (Sicapent®). Hydrogen halides and halogens are removed by silver wool. After the purification steps and focusing of the CO_2 the helium carrier gas (220 mL/min) directs the analyte towards the thermal conductivity detector and subsequently towards the open split connection of the isotope ratio mass spectrometer.

6.3.3 Nomenclature, evaluation and QA

To express the variations of natural stable isotope abundance the widely applied 'delta-notation' is used. The $\delta^{h}E_{A,\,ref}$ value of an analyte (A) is described by Equation 6-1 as a relative difference between the isotope ratio (R) of an analyte ($R(^{h}E/^{l}E)_A$) and the isotope ratio defining an international reference scale ($R(^{h}E/^{l}E)_{ref}$):

$$\delta^{h}E_{A,ref} = \frac{R\left(^{h}E/\,^{l}E\right)_{A} - R\left(^{h}E/\,^{l}E\right)_{ref}}{R\left(^{h}E/\,^{l}E\right)_{ref}} \qquad \text{Equation 6-1}$$

The international scale for carbon is Vienna Pee Dee Belemnite (VPDB) and for nitrogen AIR-N_2. The accepted ratio $R(^{13}C/^{12}C)_{VPDB}$ is $(11180.2 \pm 2.8)10^{-6}$, and for $R(^{15}N/^{14}N)_{AIR\text{-}N2}$ is $(3678.2 \pm 1.5)10^{-6}$. As for all international scale defining reference materials in SIA, $\delta^{13}C_{VPDB,VPDB}$ and $\delta^{15}{}_{NAIR\text{-}N2,AIR\text{-}N2}$ have the value zero.[1]

Please note that if no reference is mentioned, as exemplarily shown in Equation 6-2, the reported δ-values are related to the used in-house reference gas (RG). That concerns all data before the final normalization to the international reference scale.

$$\delta^{13}C_{\text{lin corr A}} \equiv \delta^{13}C_{\text{lin corr A,RG}} \qquad \text{Equation 6-2}$$

with $\delta^{13}C_{\text{lin corr A}}$ as a linearity corrected δ-value of an analyte A.

All the measured $\delta^{13}C$ and $\delta^{15}N$ raw data ($\delta^{13}C_{\text{meas A}}$ and $\delta^{15}N_{\text{meas A}}$) generated by the software IonVantage (IsoPrime100 operating system) or IonOS (visION operating system) are automatically related to the RG and, in the case of $\delta^{13}C$ values, additionally corrected for ^{17}O-abundance. The values are then linearity corrected to $\delta^{13}C_{\text{lin corr A}}$ and $\delta^{15}N_{\text{lin corr A}}$ values and, in the case of EA/IRMS measurements, blank corrected to $\delta^{13}C_{\text{bl corr A}}$ and $\delta^{15}N_{\text{bl corr A}}$ values. Finally, a referencing strategy to the international scale was applied using two-point normalization to $\delta^{13}C_{\text{A,VPDB}}$ and $\delta^{15}N_{\text{A,AIR-N2}}$ values.

The described evaluation strategy follows accepted recommendations in the literature.[1] The chosen referencing and quality assurance strategy ensures the metrological traceability and accuracy. The standard uncertainty (u_{std}) is expressed as the standard deviation of replicate measurements and thus represents the uncertainty caused by the instrumentation. Error bars shown within this work represent the standard uncertainty (1σ).

The rationale behind the chosen data evaluation and QA strategy is further explained in the Results and Discussion section.

6.4 Results and discussion

6.4.1 Instrumental development

The focus of this work was the development and testing of the novel interface. Therefore, a known HPLC/IRMS method for caffeinewas used[10] in order to allowfor a comparison of results. The method was also chosen because it used a constant temperature of 80 °C thus avoiding the need for temperature gradients for which a special column ovenwould be required.[22]

A HTC-based system rather than a WCO-based system to enable measurement of both $\delta^{13}C$ and $\delta^{15}N$ values via HPLC/IRMS was selected based on previous findings.[20] The BSIA system (iso TOC)[20] can be seen as a precursor for the CSIA system that should allow its general advantage of complete mineralization to be transferred to CSIA(more details can be found above in the subsection headed HPLC/IRMS). To expand the BSIA system by online hyphenation to a separation technique required the following modifications.

The first modification refers to the transfer system. In BSIA the injection speeds of the autosampler syringe range typically from 100 to 300 µL/s. Connecting the HPLC effluent capillary to the corresponding injection cannula (i.d. 500 µm; wall thickness 250 µm) in the combustion tube led to droplet formation due to the relatively low flow rate of 0.5 mL/min (ca 8.3 µL/s). Therefore, the cannula was replaced by a fused silica capillary (i.d. 100 µm; wall thickness 130 µm) to exclude droplet formation. A clean cut (cutting edge has to be smooth and perpendicular to the capillary itself) of the capillary and a suitable carrier gas feed are essential to provide the jet spread-free injection. The finally used collimated beam transfer system (injection device) is shown in Figure 6-2.

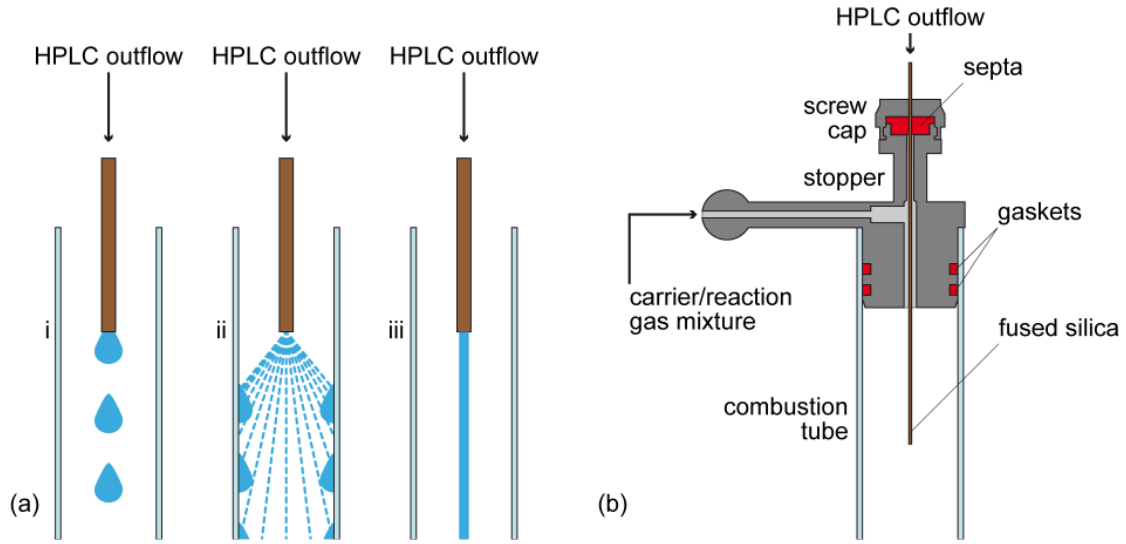

Figure 6-2 Transfer of the mobile phase into the combustion tube of the interface. (a) Injection beam related issues and solution: (i) Droplet formation leads to peak fission. (ii) Jet spread beam leads to condensation of fine droplets touching the colder part of the combustion tube of the interface and thus causes tailing and carry over. (iii) Solution: collimated beam injection (iii). (b) Schematic view of the collimated beam transfer system (injection device).

A further main modification of the reported BSIA system was the installation of an additional oven for the reduction tube. To measure the nitrogen stable isotope composition, all the nitrogen-containing species created within the oxidation reactor (combustion tube), mainly N_2 and NO, need to be converted into N_2 completely. It was shown that reduced copper fulfills the requirements at 500 °C: no nitric oxide could be detected with the nondispersive infrared detector (limit of detection 2 µgN absolute). The interface was tested by injecting 100 mgN/L solutions of different species (sodium nitrate, ammonium sulfate and acetanilide) directly into the interface (flow injection analysis mode). The obtained results indicated the absence of significant undesirable compound-specific effects since no systematic deviation from the certified or via EA/IRMS obtained values was observed ($\delta^{15}N_{AIR\text{-}N2}$ accuracy ≤0.5‰; average 0.34‰). Details in Table 6-1 prove the completeness of fractionation free conversion.

Table 6-1 Comparison of true and via HPLC/IRMS obtained δ-values for different species

Compound	true[a] $\delta^{15}N_{AIR-N2}$ [‰]	measured[b] $\delta^{15}N_{AIR-N2}$ avg ±SD [‰] (n = 3)	$\Delta_{\lvert meas-true \rvert}$ $\delta^{15}N_{AIR-N2}$ [‰]
glutamic acid (EAS-GLU)	-4.66 ± 0.5**	-4.51 ± 0.05	0.15
sodium nitrate (EAS-NIT)	16.28 ± 0.5**	16.87 ± 0.05	0.59
ammonium sulfate (IAEA-N-2)	20.30 ± 0.2*	20.03 ± 0.07	0.27
ammonium sulfate (USGS 25)	-30.40 ± 0.4*	(cal[c]) ± 0.08	
glutamic acid (USGS 26)	53.70 ± 0.4*	(cal[c]) ± 0.05	

[a] internationally accepted value if international reference material (*) and value obtained via EA/IRMS and traced back to AIR-N2 scale using international reference materials if in-house standard (**).

[b] via HTC interface coupled to IRMS obtained and subsequent normalized values; flow injection analysis mode

[c] international reference materials used for two-point normalization thus referred to as "cal"

6.4.2 Instrument testing with aqueous caffeine solutions

Initial validation

All the following measurements were conducted in HPLC/IRMS mode using an XBridge C_{18} column. The following conditions were used: standard continuous flow HPLC/IRMS mode; 17 µgC injected (2 µL; 89.2 mmolCAF/L; IRMS detector signal of ca 4.1 nA); 60 µgN injected (12 µL; 89.2 mmolCAF/L; IRMS detector signal of ca 2.4 nA).

Although the UV signal is not optimal due to overloading in order to ensure the required amount of caffeine for the IRMS, it can already be deduced from Figure 6-3 that any additional peak broadening caused by the HT interface after the UV detector is very small. Furthermore, no pronounced asymmetry effects caused by the interface could be observed. Figure 6-3 demonstrates the HTC interface performance regarding the peak shape.

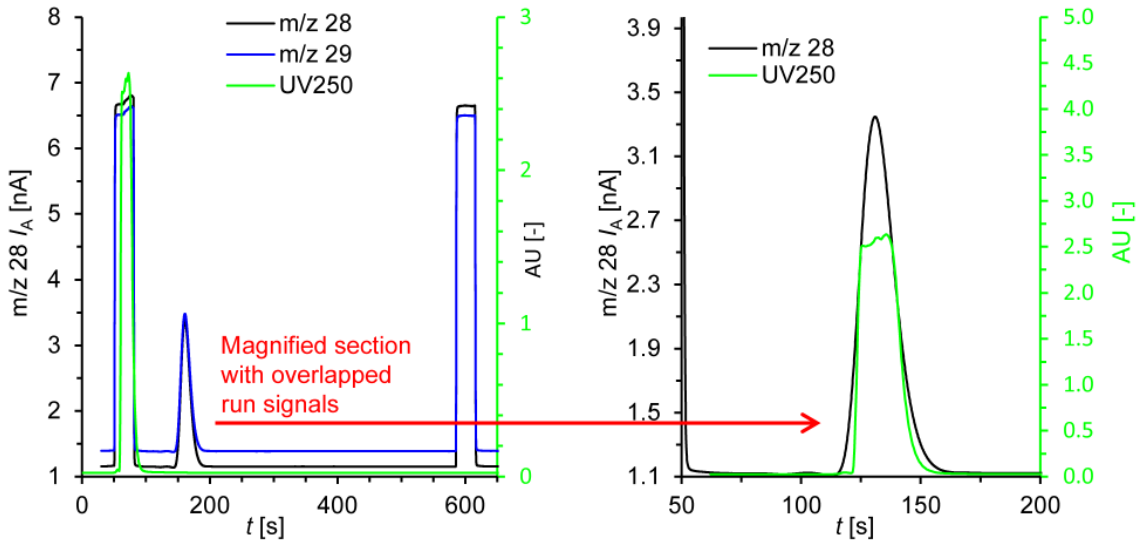

Figure 6-3 HPLC/IRMS run chosen to describe the typical HTC interface performance regarding the peak shape. Left: HPLC detector (UV250, green) and IRMS detector (*m/z* 28, black and *m/z* 29, blue) signal curves. UV250 scale range is represented by the secondary ordinate. Note that the two flat IRMS detector peaks at the beginning and end of the run are in-house reference gas pulses. The signal between them is the sample peak. The IRMS signal appears delayed relative to the HPLC sample peak due to the additional time needed to pass the interface volume. No significant fronting or tailing could be observed. Right: Shifting of the HPLC detector signal curve (UV250) on the time scale to overlap the sample peak with that of the IRMS detector signal curve (*m/z* 28). Magnification and the additional removal of the *m/z* 29 IRMS detector signal curve enable better observation of the significant lower peak part.

The precision obtained by averaged results of triplicate measurements with eight caffeine standards, expressed as u_{std} (SD ≡ 1σ), was typically ≤0.10‰ ($u_{std,avg}$ = 0.07‰; $u_{std,max}$ = 0.11‰) for $\delta^{13}C$ values and ≤0.15‰ ($u_{std,avg}$ = 0.11‰; $u_{std,max}$ = 0.19‰) for $\delta^{15}N$ values. No precision gain was observed conducting four replicates.

Carry over was tested by measuring a sequence of samples with varying stable isotope composition. Alternation of the δ-values of two sequential samples $\Delta(A_{n+1} - A_n)$ of maximal 20.56‰ for $\delta^{13}C$ and 10.71‰ for $\delta^{15}N$ did not lead to any detectable carry over. The result of the first replicate was not influenced by the sample before and the bias quantity of 0.001‰ for $\delta^{13}C$ values and 0.01‰ for $\delta^{15}N$ values lies within the variation caused by the respective precision.

A long measurement sequence with 60 replicates conducted over 19 h (ca 19 min per run) showed no drift over time and a SD of ≤0.1‰ for $\delta^{13}C$ values and ≤0.2‰ for $\delta^{15}N$ values.

Trueness, accuracy and lower working limit estimation

Two-point normalization is suggested for the evaluation of data,[1] but the only IAEA standard caffeine available is the used IAEA-600. Due to the lack of further certified reference materials, the values obtained via EA/IRMS were considered as true values for the investigation of accuracy. Traceability was ensured by referencing of the EA/IRMS $\delta^{13}C$ and $\delta^{15}N$ values to the reference material and thus indirectly to the VPDB and AIR-N2 scale, respectively.

In order to cover the isotope range of interest, reference material IAEA-600 ($\delta^{13}C_{VPDB}$ − 27.771 ± 0.043‰; $\delta^{15}N_{AIR\text{-}N2}$ 1.0 ± 0.2‰) was used as a first standard for both C and N CSIA and in-house standards CAF5 ($\delta^{13}C_{VPDB}$ –48.33 ± 0.02‰) for C and CAF4 ($\delta^{15}N_{AIR\text{-}N2}$ –9.67 ± 0.08‰) for N SIA in each case as a second standard for normalization.

Wet chemical oxidation (WCO)-based methods run the risk of concentration underestimation as well as of isotope fractionation due to incomplete oxidation, thus resulting in an offset (bias).[10] Contrary to a WCO-based interface, the HTC-based system presented does not show such an offset (bias). We found a recovery rate of ≥99%, proving complete conversion. In addition, the efficiency of the same combustion reactor was proven in previous work using barbituric acid, melamine and humic acid that are resistant to oxidation in a WCO-based method, and thus potentially affect the SIA. The mineralization was proven to be complete.[20]

Trueness is the difference between the true and measured (HPLC/IRMS) values. The obtained $\Delta_{trueness}$ values were typically ≤0.05‰ (average 0.02‰; maximum 0.15‰) for C CSIA and ≤0.20‰ (average 0.20‰; maximum 0.41‰) for N CSIA. The coefficient of determination of the linear regression between the measured and true values (R^2 = 0.9999 for carbon and R^2 = 0.9966 for nitrogen) also indicates a good agreement (see Figure 6-4). Caffeine solutions of 44.6 nmol/µL (4.3 µgC/µL and 2.5 µgN/µL) and injections of 16 µL were used for determination of trueness.

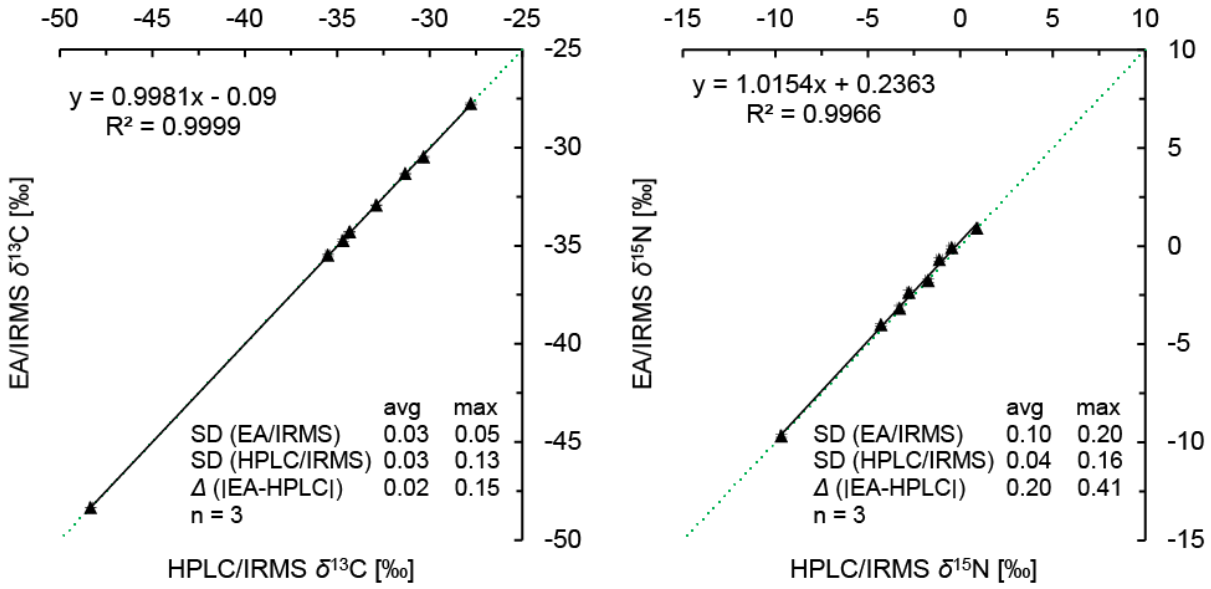

Figure 6-4 Least-squares linear regressions between EA/IRMS and HPLC/IRMS δ-values for carbon and nitrogen. The green dashed line (1:1 line) indicates the ideal estimation, and the solid line corresponds to the regression equation Line. Error bars represent the standard deviation of each sample. Left: δ^{13}C values are all referenced to the VPDB scale. Right: δ^{15}N values are referenced to the AIR-N2 scale.

Taking into account the trueness and the precision (described above), the accuracy can be quantified in a first approximation as <0.15‰ for δ^{13}C values and <0.35‰ for δ^{15}N values (see Table 6-2).[23]

Table 6-2 Accuracy (trueness and precision) estimation of δ-values

	precision average (estimation) [‰]	trueness average (estimation) [‰]	accuracy estimation[a] [‰]
$\delta^{13}C_{VPDB}$	0.07 (≤0.10)	0.02 (≤0.05)	≤0.15
$\delta^{15}N_{AIR-N2}$	0.11 (≤0.15)	0.20 (≤0.20)	≤0.35

[a] as a sum precision and trueness

The lower limits of the working range were determined to validate the minimal absolute amount of carbon and nitrogen which can still be measured with the agreed accuracy of ≤0.5‰. A 89.2 nmol/µL CAF3 solution (8.6 µgC/µL and 5.0 µgN/µL) and injections from 0.2 µL to 20 µL were used for both C and N CSIA via HPLC/IRMS. The corresponding

EA/IRMS values for CAF3 were for $\delta^{13}C_{VPDB}$ –31.32 ± 0.02‰ and for $\delta^{15}N_{AIR\text{-}N2}$ –3.28 ± 0.13‰.

As shown in Figure 6-5, with a $\delta^{13}C_{VPDB}$ value of -30.60‰, the 1.7 µgC injection of CAF3 solution was the first outside the accuracy range of 0.5 ‰ with Δ of 0.63‰. 3.5 µgC herewith marks the lower working limit. Within the range from 3.5 to 173 µgC the average u_{std} is 0.06‰ and relative standard deviation (RSD) for the IRMS detector peak area 1.4%.

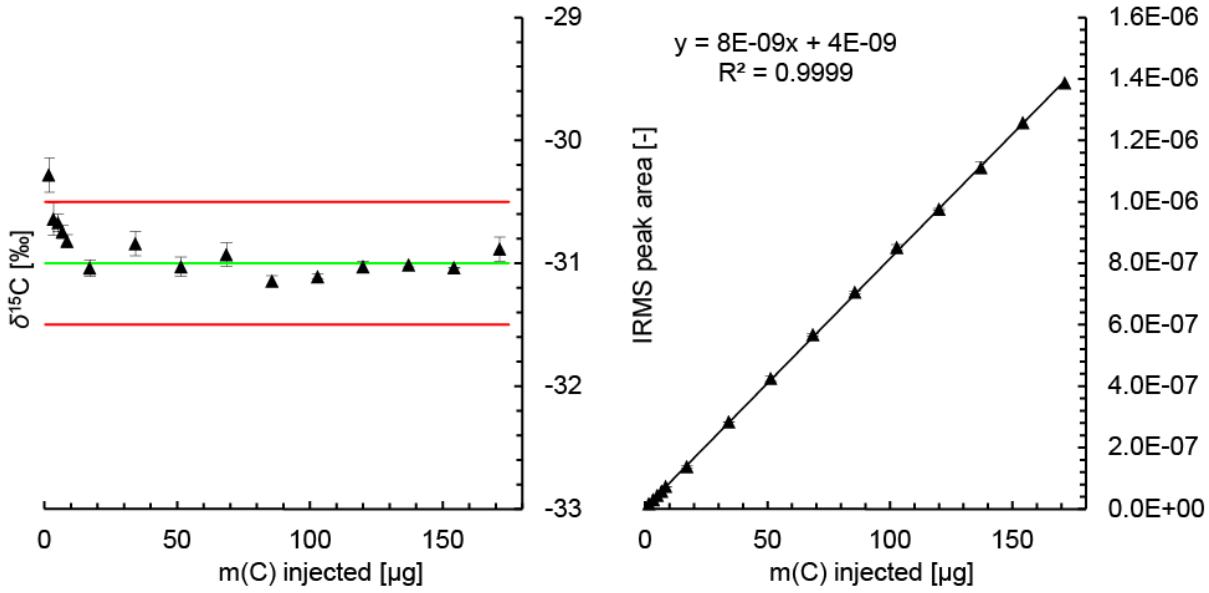

Figure 6-5 Lower working range estimation for C CSIA via HPLC/IRMS. Left: Correlation between $\delta^{13}C_{VPDB}$ values and C amount injected as CAF3 solution. The green line marks the $\delta^{13}C_{VPDB}$ reference value obtained via EA/ IRMS, considered as true. The red lines mark the agreed upper and lower limits for an accepted accuracy of 0.5‰. Right: Linear correlation between the IRMS detector peak area and C amount injected.

As shown in Figure 6-6, with a $\delta^{15}N_{AIR\text{-}N2}$ value of -4.49‰ the 10 µgN injection of CAF3 solution was the first outside the accuracy range of 0.5‰ with Δ of 1.21‰. 20 µgN herewith marks the lower working limit. Within the range from 20 to 100 µg the average u_{std} is 0.07‰ and RSD for the IRMS detector peak area 2.2%.

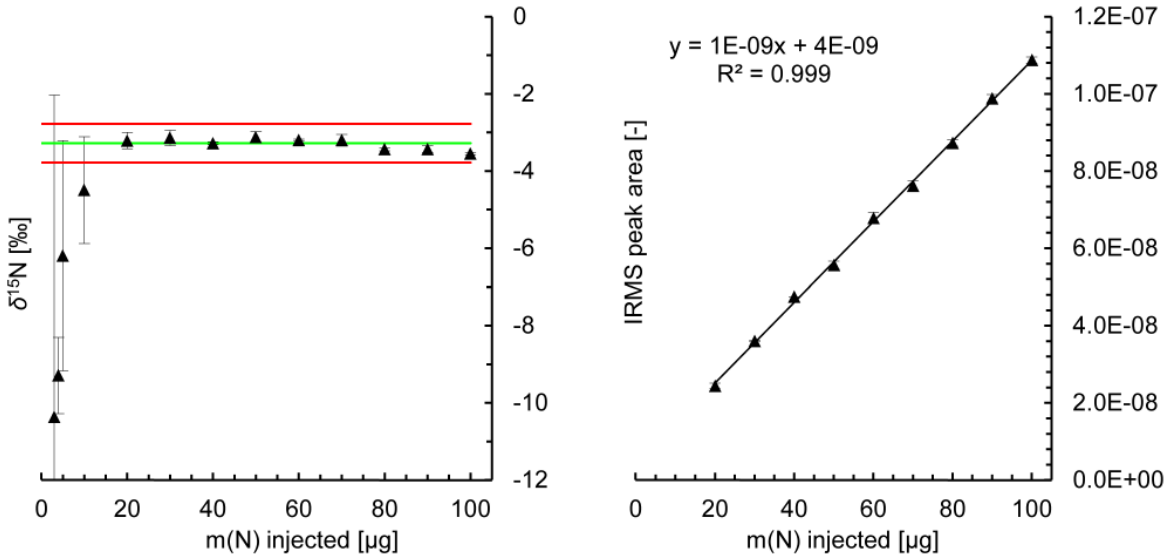

Figure 6-6 Lower working range estimation for N CSIA via HPLC/IRMS. Left: Correlation between $\delta^{15}N_{AIR\text{-}N2}$ values and N amount injected as CAF3 solution. The green line marks the $\delta^{15}N_{AIR\text{-}N2}$ reference value obtained via EA/IRMS, considered as true. The red lines mark the agreed upper and lower limits for an accepted accuracy of 0.5 ‰. Right: Linear correlation between the IRMS detector peak area and N amount injected.

Sensitivity can be expressed as the slope of the regression lines describing the correlation of IRMS detector peak area with the mass of analyte injected. The difference between N CSIA and C CSIA with regard to sensitivity is ca a factor of 8 (sensitivity for C 8.11×10^{-9} peak area units/µgC; sensitivity for N 1.04×10^{-9} peak area units/µgN). This reflects the expectations. A factor of 2 comes from the fact that one C atom yields one analyte molecule (CO_2), but two N atoms are needed for one analyte molecule (N_2). Another factor of 3 is explained by the lower isotope abundance of the heavier nitrogen than that of carbon (≈0.0107 for ^{13}C mole fraction; ≈0.00364 for ^{15}N mole fraction). The remaining factor of 1.3 is marginal and can appear from e.g. different background contributions within the system (there is 75.53% of N_2 in the air, but only 400 ppm CO_2). Keeping the trap current constant, and differences in ion source tuning can lead to marginal differences in sensitivity.

The first proof of principle showed very promising results. Further measurements with other compounds and real samples, containing different matrices, are required for a full validation of the system.

6.5 Exemplary application of C and N CSIA using the HTC interface

In an earlier paper[10] it was reported that $\delta^{13}C_{VPDB}$ data can be used to discriminate between natural and synthetic caffeine with $\delta^{13}C$ = -32‰ as threshold. This is in agreement with the data gained in this work with the exception that the horizontal border line in Figure 6-7(a) lies here at $\delta^{13}C$ = -31‰. The $\delta^{15}N_{AIR\text{-}N2}$ data gained in this study obviously are also suitable for such a discrimination, shown by the vertical line at $\delta^{15}N$ = -0.4‰ in Figure 6-7(a). However, in both cases, the samples which are nearest to the border lines have only a very small distance to these. Therefore, a bivariate approach using simultaneously both $\delta^{13}C_{VPDB}$ and $\delta^{15}N_{AIR\text{-}N2}$ values as discriminating variables was taken.[26,27] As a result of applying a Support Vector Machine (SVM),[28] Figure 6-7(b) shows the line partitioning the data into natural and synthetic samples. The partitioning was performed with the R[26]-package klaR[27] using the program 'partimat' together with the method 'svm' which applies the support vector machine SVM-Light.[28] Now the two types of caffeine are considerably more clearly distinguishable because the samples which are nearest to this border line have a considerably larger distance to this. Obviously, this bivariate approach makes the distinction between synthetic and natural caffeine more robust. There may be also a chance for further distinction, e.g. regarding geographical location, which is not possible with the aid of $\delta^{13}C_{VPDB}$ data alone.[29–31]

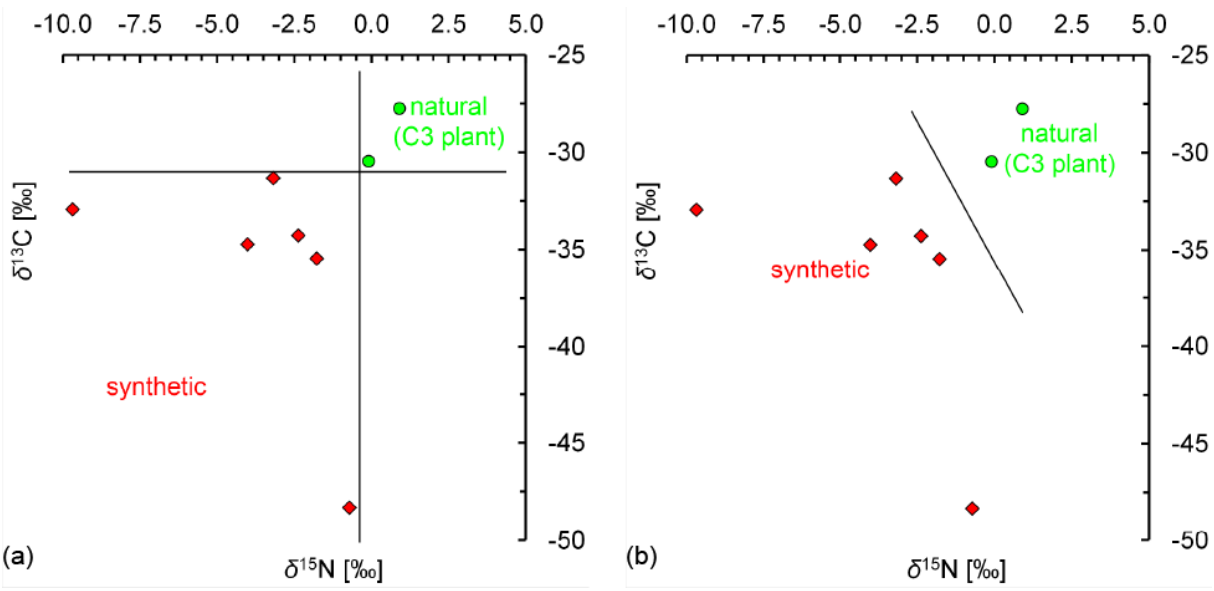

Figure 6-7 Two-dimensional graphs with $\delta^{13}C_{VPDB}$ plotted versus $\delta^{15}N_{AIR\text{-}N2}$ values measured by HPLC/IRMS. The two points in the upper right corner are natural caffeine samples CAF1 (IAEA-600[24,25]) and CAF6 (NATECO2) (green dots). The remaining six points are synthetic caffeine samples (red diamonds). The black lines display the classification borders. (a) Discrimination using either $\delta^{13}C$ or $\delta^{15}N$ values. (b) Bivariate discrimination using both $\delta^{13}C$ and $\delta^{15}N$ values.

6.6 Conclusions and outlook

A novel system for compound-specific stable isotope analysis of carbon and nitrogen via HPLC/IRMS was developed and its proof of principle demonstrated. Both carbon and nitrogen can be measured with precision and trueness of ≤0.5‰. Lower working limit values of 3.5 µgC for δ^{13}C CSIA and 20 µgN for δ^{15}N CSIA were achieved for caffeine, considering an accuracy of ±0.5 ‰ as acceptable. The novel interface is carry over free and without detectable compound-specific fractionation. No time-consuming sample preparation is required and the system works in a fully automated fashion.

Future work needs to focus on further validation of the system with real samples and in combination with other HPLC separation methods. Further method development should focus on optimization of the interface, e.g. by bypassing the condenser and miniaturization of the interface.

In summary, the described system offers the first possibility for δ^{15}N CSIA via HPLC/IRMS and together with C CSIA opens new opportunities for a wide range of stable isotope applications in, among others, environmental and forensic research.

6.7 References

[1] M. A. Jochmann, T. C. Schmidt. Compound-specific Stable Isotope Analysis. The Royal Society of Chemistry, Cambridge, 2012.

[2] J. McCullagh, J.-P. Godin, H. Schierbeek, T. Preston. Liquid Chromatography-Isotope Ratio Mass Spectrometry Users Meeting, November 23-24, 2010, University of Oxford, UK. Rapid Commun. Mass Spectrom. 2011, 25, 2969.

[3] T. C. Schmidt, L. Zwank, M. Elsner, M. Berg, R. U. Meckenstock, S. B. Haderlein. Compound-specific stable isotope analysis of organic contaminants in natural environments: a critical review of the state of the art, prospects, and future challenges. Anal. Bioanal. Chem. 2004, 378, 283.

[4] S. Benson, C. Lennard, P. Maynard, C. Roux. Forensic applications of isotope ratio mass spectrometry - A review. Forensic Sci. Int. 2006, 157, 1.

[5] N. NicDaeid, S. Jayamana, W. J. Kerr, W. Meier-Augenstein, H. F. Kemp. Influence of precursor solvent extraction on stable isotope signatures of methylamphetamine prepared from over-the-counter medicines using the Moscow and Hypophosphorous routes. Anal. Bioanal. Chem. 2013, 405, 2931.

[6] N. Gentile, R. T. W. Siegwolf, P. Esseiva, S. Doyle, K. Zollinger, O. Delemont. Isotope ratio mass spectrometry as a tool for source inference in forensic science: A critical review. Forensic Sci. Int. 2015, 251, 139.

[7] N. NicDaeid, W. Meier-Augenstein, H. F. Kemp, O. B. Sutcliffe. Using Isotopic Fractionation to Link Precursor to Product in the Synthesis of (±)-Mephedrone: A New Tool for Combating “Legal High” Drugs. Anal. Chem. 2012, 84, 8691.

[8] E. O. Mogusu, J. B. Wolbert, D. M. Kujawinski, M. A. Jochmann, M. Elsner. Dual element ($^{15}N/^{14}N$, $^{13}C/^{12}C$) isotope analysis of glyphosate and AMPA by derivatization-gas chromatography isotope ratio mass spectrometry (GC/IRMS) combined with LC/IRMS. Anal. Bioanal. Chem. 2015, 407, 5249.

[9] D. M. Kujawinski, L. Zhang, T. C. Schmidt, M. A. Jochmann. When Other Separation Techniques Fail: Compound-Specific Carbon Isotope Ratio Analysis of Sulfonamide Containing Pharmaceuticals by High-Temperature-Liquid Chromatography-Isotope Ratio Mass Spectrometry. Anal. Chem. 2012, 84, 7656.

[10] L. Zhang, D. M. Kujawinski, E. Federherr, T. C. Schmidt, M. A. Jochmann. Caffeine in Your Drink: Natural or Synthetic?. Anal. Chem. 2012, 84, 2805.

[11] P. J. H. Dunn, N. V. Honch, R. P. Evershed. Comparison of liquid chromatography-isotope ratio mass spectrometry (LC/IRMS) and gas chromatography-combustion-isotope ratio mass spectrometry (GC/C/IRMS) for the determination of collagen amino acid $\delta^{13}C$ values for palaeodietary and palaeoecological reconstruction. Rapid Commun. Mass Spectrom. 2011, 25, 2995.

[12] C. Decock, K. Denef, S. Bode, J. Six, P. Boeckx. Critical assessment of the applicability of gas chromatography-combustion-isotope ratio mass spectrometry to determine amino sugar dynamics in soil. Rapid Commun. Mass Spectrom. 2009, 23, 1201.

[13] L. Zhang, M. Thevis, T. Piper, M. A. Jochmann, J. B. Wolbert, D. M. Kujawinski, S. Wiese, T. Teutenberg, T. C. Schmidt. Carbon Isotope Ratio Analysis of Steroids by High-Temperature Liquid Chromatography-Isotope Ratio Mass Spectrometry. Anal. Chem. 2014, 86, 2297.

[14] E. Richling, C. Hoehn, B. Weckerle, F. Heckel, P. Schreier. Authentication analysis of caffeine-containing foods via elemental analysis combustion/pyrolysis isotope ratio mass spectrometry (EA-C/P-IRMS). Eur. Food Res. Technol. 2003, 216, 544.

[15] L. T. Corr, R. Berstan, R. P. Evershed. Optimization of derivatisation procedures for the determination of $\delta^{13}C$ values of amino acids by gas chromatography/combustion/isotope ratio mass spectrometry. Rapid Commun. Mass Spectrom. 2007, 21, 3759.

[16] J.-P. Godin, J. S. O. McCullagh. Review: Current applications and challenges for liquid chromatography coupled to isotope ratio mass spectrometry (LC/IRMS). Rapid Commun. Mass Spectrom. 2011, 25, 3019.

[17] W. A. Brand, P. Dobberstein. Isotope-ratio-monitoring liquid chromatography mass spectrometry (IRM-LCMS). First results from a moving wire interface system. Isot. Environ. Health Stud. 1996, 32, 275.

[18] Y. Teffera, J. J. Kusmierz, F. P. Abramson. Continuous-Flow Isotope Ratio Mass Spectrometry Using the Chemical Reaction Interface with Either Gas or Liquid Chromatographic Introduction. Anal. Chem. 1996, 68, 1888.

[19] G. St-Jean. Automated quantitative and isotopic (^{13}C) analysis of dissolved inorganic carbon and dissolved organic carbon in continuous-flow using a total organic carbon analyser. Rapid Commun. Mass Spectrom. 2003, 17, 419.

[20] E. Federherr, C. Cerli, F. M. S. A. Kirkels, K. Kalbitz, H. J. Kupka, R. Dunsbach, L. Lange, T. C. Schmidt. A novel high-temperature combustion based system for stable isotope analysis of dissolved organic carbon in aqueous samples. I: development and validation. Rapid Commun. Mass Spectrom. 2014, 28, 2559.

[21] M. S. A. K. Frédérique, C. Cerli, E. Federherr, K. Kalbitz. A novel high temperature combustion based system for stable isotope analysis of dissolved organic carbon in aqueous samples (II): optimization and assessment of analytical performance. Rapid Commun. Mass Spectrom. 2014, 28, 2574.

[22] M. Krummen, A. W. Hilkert, D. Juchelka, A. Duhr, H.-J. Schlüter, R. Pesch. A new concept for isotope ratio monitoring liquid chromatography/mass spectrometry. Rapid Commun. Mass Spectrom. 2004, 18, 2260.

[23] B. W. Wenclawiak, M. Koch, E. Hadjicostas. Quality Assurance in Analytical Chemistry. Springer, Berlin, 2004.

[24] T. B. Coplen, W. A. Brand, M. Gehre, M. Groning, H. A. J. Meijer, B. Toman, R. M. Verkouteren. New Guidelines for $\delta^{13}C$ Measurements. Anal. Chem. 2006, 78, 2439.

[25] M. Gehre. IAEA-600 natural caffeine; personal communication, November 9, 2015.

[26] R: A language and environment for statistical computing. (http://www.R-project.org/)

[27] C. Weihs, U. Ligges, K. Luebke, N. Raabe. klaR Analyzing German Business Cycles, in Data Analysis and Decision Support. Springer, Berlin, 2005.

[28] SVM light (http://svmlight.joachims.org/)

[29] J. Dunbar, A. T. Wilson. Determination of Geographic Origin of Caffeine by Stable Isotope Analysis. Anal. Chem. 1982, 54, 590.

[30] B. Weckerle, E. Richling, S. Heinrich, P. Schreier. Origin assessment of green coffee (Coffea arabica) by multi-element stable isotope analysis of caffeine. Anal. Bioanal. Chem. 2002, 374, 886.

[31] F. Serra, C. G. Guillou, F. Reniero, L. Ballarin, M. I. Cantagallo, M. Wieser, S. S. Iyer, K. Heberger, F. Vanhaecke. Determination of the geographical origin of green coffee by principal component analysis of carbon, nitrogen and boron stable isotope ratios. Rapid Commun. Mass Spectrom. 2005, 19, 2111.

Chapter 7 General conclusions and outlook

A trend in modern analytical chemistry is not only the identification and quantification of analytes but also the determination of their isotope composition, e.g., to infer sources or fate in the environment. In the work presented here stable isotope analysis (SIA) methods were developed to measure $\delta^{13}C$ and $\delta^{15}N$ directly from aqueous solutions. Overcoming some of the drawbacks, such as need of laborious sample preparation, this work resulted particularly in the first system reported to be suitable for both $\delta^{13}C$ and $\delta^{15}N$ direct compound specific stable isotope analysis (CSIA) of non-volatile compounds.

Dissolved organic carbon (DOC) plays a key role in carbon cycle investigations. A novel HTC-based TOC/IRMS system was developed specially for DOC SIA. Standard uncertainty of ≤0.2‰, the $LOQ_{SIA\ instr}$ of 0.2 mgC/L and the oxidation efficiency of ≥99% confirm its suitability for accurate DOC SIA and good analytical performance, in particular compared with alternative methods[1,2]. To the best of our knowledge, this novel system is the only HTC-based system that allows a 3-mL injection compared with a typical injection volume of <200 µL.[3] This improvement alone resulted in an increase in sensitivity by a factor of 15. With the developed system no laborious sample-preparation steps are necessary, such as time-consuming offline preconcentration steps (e.g. freeze-drying) as they are needed using EA/IRMS methods[2].

Good precision and accuracy were achieved for the $\delta^{13}C$ analysis of a broad diversity of DOC solutions. Good reproducibility (predominantly ≤0.5‰) was shown in the context of international round robin testing for river and seawater samples. The HTC TOC/IRMS system introduced in this thesis showed predominantly better performance compared to other methods, such as wet chemical oxidation based methods. Similar averaged results were achieved compared with other HTC TOC/IRMS methods. However, no proper comparison of the performance was possible within the scope of this work, because in most cases uncertainties achieved by each participant were not reported or, if reported, a notation how uncertainties were assessed was missing. This system opens the possibility of a larger use of certified or internationally accepted reference materials (solutions of low concentration) to assure traceability and comparability among different laboratories. For the same reasons we proposed a method for data treatment and evaluation of uncertainty, but an internationally agreed method of validation still needs to be defined.

$\delta^{15}N$ bulk stable isotope analysis (BSIA) in aqueous samples plays a significant role in many fields of environmental research. A novel high-temperature based TOC-system was

successfully extended to the possibility to measure also stable nitrogen isotope composition, resulting, to the best of our knowledge, in the first system reported to be suitable for simultaneous $\delta^{13}C$, $\delta^{15}N$ BSIA in aqueous samples. No sample preparation is required and the system works fully automated, in contrast to the established EA/IRMS based methods[4,5]. An automated degassing unit and a zeolite adsorber based focusing unit are main innovations. Complete conversion of nitrogen species to N_2 was demonstrated using a broad range of inorganic and organic nitrogen species. The system is carry over free and showed good analytical performance, in particular in comparison to the reported methods for $\delta^{15}N$ BSIA in aqueous samples.[6-8] Further work needs to focus on investigation and quantification of the background as well as blank contribution for stable nitrogen isotope measurements and on validation of the system with real samples. Further method development should focus on optimization e.g. by bypassing of the condenser and development of an automated blank and background correction procedure. The described system offers new possibilities for automated TN_b SIA directly from aqueous solutions and in combination with simultaneous DOC SIA opens new opportunities for a wide range of stable isotope applications in, among others, soil science and limnology.

In the future two different main directions could be followed for further development of BSIA methods of aqueous solutions. First, the existing system could be extended by flow injection analysis (FIA) to enable automated SIA, not only of total nitrogen bound, but also of its fractions, such as inorganic and organic nitrogen. Ammonium could be measured by alkalization and purging out of ammonia into the HTC TOC system coupled to the IRMS detector, then nitrate and nitrite could be reduced to ammonium before being treated as described before. The remaining solution should contain dissolved organic nitrogen (DON) only[9], which could then be than injected into the HTC TOC/IRMS system for DON SIA. A second direction would be the further development of the HTC TOC based system to a method suitable for sulfur BSIA in aqueous solutions, which is of high interest for environmental scientists. Adjustment of the combustion conditions, ensuring no cold places before water removal and removal of the condenser could be probably the steps to start with.

A direct method,without sample preparation, such as derivatisation needed in corresponding GC/IRMS methods[10], for stable nitrogen isotope analysis ($\delta^{15}N$ SIA) of non-volatile compounds was successfully developed within this work. Both carbon and nitrogen can be measured with precision and trueness of ≤0.5‰. Lower working limit values of 3.5 µgC for $\delta^{13}C$ CSIA and 20 µgN for $\delta^{15}N$ CSIA were achieved for caffeine, considering an accuracy of ±0.5 ‰ as acceptable. This performance is suitable for natural abundance CSIA of non-

volatile compounds, in particular compared to the alternative methods, such as derivatisation followed by GC/IRMS for $\delta^{13}C$ and $\delta^{15}N$ determinations, with found precision and trueness often exceeding 0.5‰.[11] The novel HTC based interface is carry over free and without detectable compound-specific fractionation. This is an advantage compared to the commercial wet chemical oxidation based systems, which are reported to suffer from the risk of isotope fractionation[12,13]. No time-consuming sample preparation is required and the system works in a fully automated fashion, which is a substantial advantage compared to the reported EA/IRMS based methods for CSIA of polar compounds.[14] The described system offers the first possibility for $\delta^{15}N$ CSIA via HPLC/IRMS and together with $\delta^{13}C$ CSIA opens new opportunities for a wide range of stable isotope applications in, among others, environmental and forensic research. Future work needs to focus on further validation of the system with real samples and in combination with other HPLC separation methods. Further method development should focus on optimization of the interface, e.g. by bypassing the condenser, implementing of a degassing unit and miniaturization of the interface.

In an earlier paper[12] it was reported that $\delta^{13}C_{VPDB}$ data can be used to discriminate between natural and synthetic caffeine. This is in agreement with the data gained in this work. The $\delta^{15}N_{AIR\text{-}N2}$ data gained in this study obviously are also suitable for such discrimination. However, in both cases, the samples which are nearest to the border lines have only a very small distance to these. A bivariate approach using simultaneously both $\delta^{13}C_{VPDB}$ and $\delta^{15}N_{AIR\text{-}N2}$ values as discriminating variables could successfully be taken to distinguish between those two types of caffeine considerably more clear. Obviously, this bivariate approach makes the distinction between synthetic and natural caffeine more robust and highlights the potential of multi-element CSIA of non-volatile substances. There may be also a chance for further distinction, e.g. regarding geographical location, which is not possible with the aid of $\delta^{13}C_{VPDB}$ data alone. Applications which require currently derivatization followed by GC/IRMS measurements due to the need of (additional) $\delta^{15}N$ value are of potential interest to be tested with the novel method, but in many cases suitable HT HPLC or IC separation methods have to be developed first since only a few ones are yet available[15,16].

Further directions could involve the development of a HTC based interface for $\delta^{34}S$ CSIA via HPLC/IRMS. The focus could be on a continuous flow system, using one element but all compounds properly separated in HPLC, or on a heart-cut system, using one compound but for simultaneous multi-element SIA (CNS SIA). Both approaches are of interest in different fields. For sure also a combination could be desirable, which could be realized via peak-parking or fraction collection. A second interesting direction is the development of

approaches to overcome the limitations in the eluent selection in HPLC/IRMS to water or water based inorganic buffers. Hereby again two different ways could be followed: a continuous flow mode with the interface capable to handle a certain amount of organic solvent (N or S only) or a heart-cut mode, where a replacement of the organic solvent by water or water buffer (solid-phase extraction based principle) or a thermal separation of organic solvent from the analyte prior to injection into the interface takes place. The thermal separation principle is analogous to that of the moving wire system[17] but without the need to handle continuous flow its limitations, regarding limited capacity, depletion of semivolatile compounds and flow restriction[11] can be avoided.

[1] C. L. Osburn, G. St-Jean. The use of wet chemical oxidation with high-amplification isotope ratio mass spectrometry (WCO-IRMS) to measure stable isotope values of dissolved organic carbon in seawater. Limnol. Oceanogr.: Methods 2007, 5, 296.

[2] B. Fry, E. T. Peltzer, C. S. Hopkinson Jr, A. Nolin, L. Redmond. Analysis of marine DOC using a dry combustion method. Mar. Chem. 1996, 54, 191.

[3] S. Q. Lang, M. D. Lilley, J. I. Hedges. A method to measure the isotopic (^{13}C) composition of dissolved organic carbon using a high temperature combustion instrument. Mar. Chem. 2007, 103, 318.

[4] S. D. Kelly, C. Stein, T. D. Jickells. Carbon and nitrogen isotopic analysis of atmospheric organic matter. *Atmos. Environ.* 2005, 39, 6007.

[5] F. C. Batista, A. C. Ravelo, J. Crusius, M. A. Casso, M. D. McCarthy. Compound specific amino acid δ^{15}N in marine sediments: A new approach for studies of the marine nitrogen cycle. Geochim. Cosmochim. Acta 2014, 142, 553.

[6] R. Russow, J. Kupka, A. Goetz, B. Apelt. A New Approach to Determining the Content and ^{15}N Abundance of Total Dissolved Nitrogen in Aqueous Samples: TOC Analyzer-QMS Coupling. Isot. Environ. Health Stud. 2002, 38, 215.

[7] D. Huygens, P. Boeckx, J. Vermeulen, X. D. Paepe, A. Park, S. Barker, C. Pullan, C. O. Van. Advances in coupling a commercial total organic carbon analyser with an isotope ratio mass spectrometer to determine the isotopic signal of the total dissolved nitrogen pool. Rapid Commun. Mass Spectrom. 2005, 19, 3232.

[8] D. Huygens, P. Boeckx, J. Vermeulen, X. De Paepe, A. Park, S. Barker, O. Van Cleemput. On-Line Technique to Determine the Isotopic Composition of Total Dissolved Nitrogen. Anal. Chem. (Washington, DC, U. S.) 2007, 79, 8644.

[9] R. P. Axler, J. E. Reuter. A Simple Method for Estimating the ^{15}N Content of Dissolved Organic Matter (DO^{15}N) in N-Cycling Studies. Can. J. Fish. Aquat. Sci. 1987, 44, 130.

[10] L. T. Corr, R. Berstan, R. P. Evershed. Optimization of derivatisation procedures for the determination of δ^{13}C values of amino acids by gas chromatography/combustion/isotope ratio mass spectrometry. Rapid Commun. Mass Spectrom. 2007, 21, 3759.

[11] M. A. Jochmann, T. C. Schmidt. Compound-Specific Stable Isotope Analysis. Royal Society of Chemistry, Cambridge, 2012.

[12] L. Zhang, D. M. Kujawinski, E. Federherr, T. C. Schmidt, M. A. Jochmann. Caffeine in Your Drink: Natural or Synthetic?. Anal. Chem. 2012, 84, 2805.

[13] E. Federherr, C. Cerli, F. M. S. A. Kirkels, K. Kalbitz, H. J. Kupka, R. Dunsbach, L. Lange, T. C. Schmidt. A novel high-temperature combustion based system for stable isotope analysis of dissolved organic carbon in aqueous samples. I: development and validation. Rapid Commun. Mass Spectrom. 2014, 28, 2559.

[14] J.-P. Godin, J. S. O. McCullagh. Review: Current applications and challenges for liquid chromatography coupled to isotope ratio mass spectrometry (LC/IRMS). Rapid Commun. Mass Spectrom. 2011, 25, 3019.

[15] D. M. Kujawinski, L. Zhang, T. C. Schmidt, M. A. Jochmann. When Other Separation Techniques Fail: Compound-Specific Carbon Isotope Ratio Analysis of Sulfonamide Containing Pharmaceuticals by High-Temperature-Liquid Chromatography-Isotope Ratio Mass Spectrometry. Anal. Chem. 2012, 84, 7656.

[16] D. M. Kujawinski, J. B. Wolbert, L. Zhang, M. A. Jochmann, D. Widory, N. Baran, T. C. Schmidt. Carbon isotope ratio measurements of glyphosate and AMPA by liquid chromatography coupled to isotope ratio mass spectrometry. Anal. Bioanal. Chem. 2013, 405, 2869.

[17] W. A. Brand, P. Dobberstein. Isotope-ratio-monitoring liquid chromatography mass spectrometry (IRM-LCMS). First results from a moving wire interface system. Isot. Environ. Health Stud. 1996, 32, 275.

Chapter 8 Appendix

8.1 List of abbreviations and symbols

$½\Delta_{\delta}$	half range δ-value [‰]
α	fractionation factor [-]
Δ	the difference of two values or the range of a set of values (context defined by suffix)
δ^{13}C SIA	stable carbon isotope analysis
δ^{15}N SIA	stable nitrogen isotope analysis
$\delta^{h}E_{A,\ ref}$	δ-value (expresses isotope composition) [‰]; E, chemical element symbol; *h*, mass of the less abundant (heavier) isotope; A, analyte; $_{ref}$, reference material
$\delta^{h}E_{bl\ corr\ A}$	for blank-'subtracted' δ-value of an analyte A [‰]
$\delta^{h}E_{lin\ corr\ A}$	linearity corrected δ –value of an analyte A [‰]
$\delta^{h}E_{meas\ A}$	measured raw δ –value of an analyte A [‰]
$\vec{v}_{ion}$	ion velocity [cm/s]
$x(^{i}E)$	the mole fraction of isotope ^{i}E [-]
A	analyte
A	peak area [-]
ACV	Acetovanillone
A_{bl}	blank peak area [-]
$A_{meas\ A}$	uncorrected peak area of an analyte A [-]
$A_r(E)$	standard atomic weight [-]
$A_r(^{i}E)$	atomic weight of isotope ^{i}E [-]
$A_{saple\ A}$	for blank-'subtracted' peak area of an analyte A ($A_{meas\ A}$ - A_{bl}) [-]
avg	average
$\vec{B}$	magnetic flux density vector [T]

BSIA	bulk stable isotope analysis
BAR	barbituric acid
BEN	benzoic acid
C_3 vegetation	plants that utilizes C_3 carbon fixation pathway
C_4 vegetation	plants that utilizes C_4 carbon fixation pathway
CAF	caffeine
CAS	casein
CF	continuous flow
CIPM	Comité International des Poids Mesures
CIT	citric acid
CRDS	cavity ring-down spectroscopy
CRI	chemical reaction interface
CSIA	compound-specific stable isotope analyses
DOC	dissolved organic carbon
DIC	dissolved inorganic carbon
DON	dissolved total organic nitrogen
dow	deep ocean water
EA	elemental analyzer
EA/IRMS	elemental analyzer/isotope ratio mass spectrometry
EI	electron ionization
FIA	flow injection analysis
$\vec{F}_{\mathrm{L}}$	Lorenz force [N]
fw	fresh water
GC/IRMS	gas chromatography/isotope ratio mass spectrometry

GLU	glutamic acid
HC	heart cutting
HPLC/IRMS	high performance liquid chromatography/isotope ratio mass spectrometry
HTC	high-temperature combustion
HTC TOC/IRMS	high-temperature combustion total organic carbon analyzer, interfaced with continuous flow isotope ratio mass spectrometer
HUM	humic acid
I	ion current [nA]
IC	inorganic carbon
ID	inner diameter
IMB	isotope mass balance
IAEA	international atomic energy agency
IRMS	isotope ratio mass spectrometry
ISO	International Organization for Standardization
IUPAC	International Union of Pure and Applied Chemistry
k	coverage factor
KIE	kinetic isotope effect
LC	liquid chromatography
LCM	low concentration module (focusing unit)
LOQ	limit of quantification [mg/L]
$L_{R\ IRMS}$	isotope ratio linearity of the isotope ratio mass spectrometer
M	molecule
$m_a(^iE)$	atomic mass of isotope ^iE [$u_{atom\ m}$]

MEL	melamine
m_{lin}	slope of the linear regression; for $L_{R\ IRMS}$ [‰/nA]
m/z	mass-to-charge ratio
n(p)	number of different labs (participants) used for average and SD calculations
n(r)	number of replicate measurements used for average and SD calculations
N2-aq	dissolved molecular nitrogen
NDIR detector	nondispersive infrared detector
NPOC	non-purgeable organic carbon
NVOC	non-volatile organic carbon
POC	purgeable organic carbon
PSIA	position-specific stable isotope analyses
PtIC	particulate total inorganic carbon
PtOC	particulate non-purgeable organic carbon
PtON	particulate total organic nitrogen
q_{ion}	ion charge [C]
R	isotope ratio [-]
R^2	coefficient of determination
RG	reference gas
RSD	relative standard deviation
SD	standard deviation
SD_R	reproducibility standard deviation
SIA	stable isotope analysis
SUC	sucrose

sw	sea water
TC	total carbon
TDN	total dissolved nitrogen
TIC	total inorganic carbon
TIN_b	total inorganic nitrogen bound
TIE	thermodynamic isotope effect
TN	total nitrogen
TOC	total organic carbon
TON	total organic nitrogen
TNb	total nitrogen bound
U	expanded uncertaity
$u_{atom\ m}$	unified atomic mass unit [$\approx 1.660540210 \times 10^{-27}$ kg]
u_{comb}	combined uncertainty = $u_{std + lin\ corr+ bl\ corr + norm}$
u_i	intermediate state uncertainty (context defined by suffix)
UP water	ultrapure, deionized water
u_{std}	standard uncertainty = SD = 1σ (confidence interval 68.3%)
$u_{std + lin\ corr}$	uncertainty containing standard uncertainty and contribution from linearity correction
$u_{std + lin\ corr + bl\ corr}$	uncertainty containing standard uncertainty and contribution from linearity correction and blanc correction = u_{slb} (abbreviation)
UV/Vis detector	ultraviolet/visible spectroscopic detector
USGS	United States geological survey
VOC	volatile organic carbon
VPDB	Vienna Pee Dee Belemnite

WC-M	worst case scenario model
WCO	wet chemical oxidation
WO	wet oxidation

8.2 List of Figures

8.3 List of supplementary Figures

8.4 List of Tables

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8.6 Curriculum vitae

Der Lebenslauf ist in der Online-Version aus Gründen des Datenschutzes nicht enthalten.

8.7 List of Publications

8.7.1 Manuscripts

1. **Eugen Federherr**, Sarah Willach, Natascha Roos, Lutz Lange, Karl Molt, Torsten C. Schmidt: A novel high temperature combustion interface for compound-specific stable isotope analysis of carbon and nitrogen via HPLC/IRMS Rapid Communications in Mass Spectrometry, 30 (7), (2016), 944-952 (**peer-reviewed** international journal)

2. **Eugen Federherr**, Chiara Cerli, Frédérique M. S. A. Kirkels, Karsten Kalbitz, Hans J. Kupka, Ralf Dunsbach, Lutz Lange, Torsten C. Schmidt Analyse der stabilen Isotopenzusammen-setzung des gelösten organischen Kohlenstoffs in wässrigen Proben Vom Wasser, 113 (2015), 56-59 (**non peer-reviewed** journal)

3. **Eugen Federherr**, Hans J. Kupka, Lutz Lange, Hans P. Sieper Verfahren und Vorrichtung zur Analyse von Stickstoff (N) in einer Probe DE102014002266 B3 & EP2919005 A1 (**patent**)

4. **Eugen Federherr**, Chiara Cerli, Hans J. Kupka, Almut Loos, Ralf Dunsbach, Lutz Lange Torsten C. Schmidt Dem Ursprung auf der Spur Laborpraxis, Dezember (2014) 52-54 (**non peer-reviewed** journal)

5. **Eugen Federherr**, Chiara Cerli, Frédérique M. S. A. Kirkels, Karsten Kalbitz, Hans J. Kupka, Ralf Dunsbach, Lutz Lange, Torsten C. Schmidt: A novel high-temperature combustion based system for stable isotope analysis of dissolved organic carbon in aqueous samples. I: development and validation Rapid Communications in Mass Spectrometry, 28 (23) (2014), 2559-2573 (**peer-reviewed** international journal)

6. Frédérique M. S. A. Kirkels, Chiara Cerli, **Eugen Federherr**, Jiajia Gao, Karsten Kalbitz: A novel high-temperature combustion based system for stable isotope analysis of dissolved organic carbon in aqueous samples. II: optimization and assessment of analytical performance Rapid Communications in Mass Spectrometry, 28 (23) (2014), 2574-2586 (**peer-reviewed** international journal)

7. Thomas Piper, Karoline Degenhardt, **Eugen Federherr**, Andreas Thomas, Mario Thevis, Martial Saugy: Effect of changes in the deuterium content of drinking water on the hydrogen isotope ratio of urinary steroids in the context of sports drug testing Analytical and Bioanalytical Chemistry, 405 (9) (2013), 2911-2921 (**peer-reviewed** international journal)

8. Lijun Zhang, Dorothea M. Kujawinski, **Eugen Federherr**, Torsten C. Schmidt, Maik A. Jochmann: Caffeine in Your Drink: Natural or Synthetic? Analytical Chemistry, 84 (6) (2012), 2805-2810 (**peer-reviewed** international journal)

8.7.2 Presentations (first author contributions only)

Scientific conferences

2015, Wasser (German water chemistry society - annual meeting), Stable isotope analysis (SIA) of carbon and nitrogen in waters and aqueous solutions, Schwerin (**Oral presentation**)

2014, **ASI** (German Association for Stable Isotope Research – annual meeting), Total Dissolved Matter Analyzer - novel tool for carbon and nitrogen stable isotope analyses in aqueous samples, München, **(Oral presentation)**

2014, BIOGEOMON (International Symposium on Ecosystem Behavior)**,** Novel tool for $\delta^{13}C$ and $\delta^{15}N$ determination in aqueous samples, Bayreuth, (**Poster**)

2013, ANAKON (analytical conference), A novel method for stable isotope analysis of dissolved organic carbon in the trace range in aqueous samples, Essen, (**Oral presentation**)

2012, **JESIUM** (Joint European Stable Isotope Users Group Meeting), Ultralow concentration TOC stable isotope measurements, Leipzig, (**Oral presentation**)

Scientific conferences (presented by a co-author)

2015, BASIS (Benelux Association of Stable Isotope Scientists – annual meeting) (**Oral presentation**); **2015, AIG-11** (Applied Isotope Geochemistry Conference) (**Oral presentation**); **2014, AGU** (American Geophysical Union Fall Meeting) (**Poster**)

8.8 Erklärung

Hiermit versichere ich, dass ich die vorliegende Arbeit mit dem Titel

„Stable carbon and nitrogen isotope analysis in aqueous samples – method development, validation and application"

selbst verfasst und keine außer den angegebenen Hilfsmitteln und Quellen benutzt habe, und dass die Arbeit in dieser oder ähnlicher Form noch bei keiner anderen Universität eingereicht wurde.

Essen, April 2016

Eugen Federherr