

Use of Quadrupole Mass Spectrometers for the Automatic Online Determination of Isotope Ratios of the Stable Isotopes Carbon-13 and Nitrogen-15

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ABSTRACT

Quadrupole mass spectrometers are highly sensitive mass spectrometers with a large dynamic measuring range. They come in a compact, robust design and are relatively simple to use and maintain. Moreover, they are also fairly inexpensive. Therefore, when it comes to the online determination of the isotope abundance of light elements from isotopically enriched samples using a continuous flow mode of operation (as demonstrated by the examples for ^{13}C and ^{15}N), quadrupole mass spectrometers make a worthwhile alternative to standard magnetic mass spectrometers.

1. INTRODUCTION

Stable isotopes of the light elements hydrogen, carbon, nitrogen and sulphur are a very efficient methodological and analytical tool for performing diverse research tasks in chemistry, biochemistry/medicine, agricultural and environmental science. One particularly useful area comprises the isotopes of the important bioelements ^{13}C and ^{15}N in both their natural isotope variations and their isotopically enriched form as tracers in order to track the fate of an element, a species or a phase/component in a complex system.

$^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$ isotope ratios are determined by using isotope ratio mass spectrometers. In the past, these were usually magnetic sector field instruments. Nowadays, these magnetic mass spectrometers feature very high precision of better than 1‰, including when connected online to peripheral devices for automatic sample preparation and dosage.

However, in many cases such a high level of precision is unnecessary. A case in point is isotopically enriched samples of biological origin. Since relative biological scattering is usually $> 25\%$, a measuring precision of $\leq 3\%$ RSD (relative single standard deviation) would clearly suffice. This level of precision can easily be achieved with quadrupole mass spectrometers (QMS), too, making them suitable for the isotope determination of such samples.

In addition, quadrupole mass spectrometers have a number of advantages over magnetic instruments:

- robust, compact design
- easy to use
- cheaper to buy and run
- larger measuring dynamic range

- quasi-parallel measurement of numerous different individual masses
- higher measuring sensitivity, meaning less of the substance is consumed

The latter three characteristics in particular make QMSs especially suitable for online analysis. For example, QMSs have been used in previous GC-MS couplings for the ¹⁵N analysis of amino acids and the ¹³C analysis of fatty acids [1, 2]. Below, tried and tested online applications of modern QMSs for the isotope analysis of ¹³C und ¹⁵N are described.

2. FUNDAMENTALS

2.1 Stable Isotope Methodology

Stable isotopes of an element are nuclides of it that vary in terms of their mass, the reason being that although they have the same atomic number, they contain different numbers of neutrons. The following stable isotopes of the main bioelements are known:

hydrogen: ¹H = 99.985 at.%; ²H (deuterium – D) = 0.015 at.%

carbon: ¹²C = 98.892 at.%; ¹³C = 1.108 at.%

nitrogen: ¹⁴N = 99.634 at.%; ¹⁵N = 0.366 at.%

The proportion of each isotope within an element's overall set of isotopes is frequently referred to as the atomic abundance **a**, expressed in atom-% (at.%). It is defined as follows:

$$a_1 \text{ (in at.\%)} = n_1 / (n_1 + n_2 + n_i) \times 100 \quad \text{Eq. 1a}$$

n: number of atoms

For example, for nitrogen the formula is:

$$a \text{ (in at.\%)} = n_{15} / (n_{14} + n_{15}) \times 100 \quad \text{Eq. 1b}$$

Isotopes of the same element have more or less the same chemical characteristics. This assumption is the basis of all tracer techniques using stable isotopes as tracers. The term 'isotope tracer' is used if the abundance of an isotope within the isotope mixture of an element differs measurably from the natural abundance (see above). Since just the deviation from the natural abundance is of interest for tracer techniques, the excess abundance is usually used:

$$a_{\text{exc.}} = a - a^0 \quad \text{Eq. 2a}$$

In the case of nitrogen:

$$a_{\text{exc.}} \text{ (in at.\%)} = a - 0.366 \quad \text{Eq. 2b}$$

a^0 : natural abundance in aerial nitrogen = 0.366 at.%

Natural variations in isotope abundance (i.e. deviations occurring naturally) can also be used as tracers. However, as these deviations are very low, excess abundance is expressed using δ notation in ‰, e.g. for nitrogen the $\delta^{15}\text{N}$ value:

$$\delta^{15}\text{N (in ‰)} = ((a - a_{\text{st}})/a_{\text{st}}) \times 1000 \quad \text{Eq. 3}$$

The standard used is the natural ^{15}N abundance of aerial nitrogen, which is a constant 0.366 at.‰.

The main measuring technique used for isotope abundance is mass spectrometry. Mass spectrometry requires the elements to be measured to be in gaseous form (usually the molecular form N_2 for nitrogen, and CO_2 for carbon). The abundance **a** is obtained for each isotope mass measured of an element depending on the number of atoms or molecules. The isotope ratio **R** is calculated from the measured signals **I**:

$$R_2 = I_2/I_1 \quad \text{Eq. 4a}$$

Being a diatomic molecule, nitrogen forms the following masses:

$$^{14}\text{N}_2 = 28, \quad ^{14}\text{N}^{15}\text{N} = 29, \quad ^{15}\text{N}_2 = 30.$$

Hence this results in the following isotope ratios:

$$R_{29} = I_{29}/I_{28} \quad \text{and} \quad R_{30} = I_{30}/I_{28} \quad \text{Eqs. 4b and c}$$

The isotope ratio is necessary to calculate the ^{15}N abundance as follows:

$$a \text{ (in at.‰)} = R_{29} + 2R_{30}/(1+R_{29} + R_{30}) \times 100 \quad \text{Eq. 5}$$

Since the ^{15}N atoms in the N_2 molecule are randomly distributed, Eq. 5 can be simplified as follows:

$$a \text{ (in at.‰)} = R_{29}/2 + R_{29} \times 100 \quad \text{Eq. 6}$$

This equation is normally used in mass spectrometer software to calculate the ^{15}N abundance **a**.

For natural ^{15}N abundance the equation 3 can be simplified through substitution of the abundance **a** by the isotope ratio **R**:

$$\delta^{15}\text{N (in ‰)} = (R_s - R_o)/R_o \times 1000 \quad \text{Eq. 7}$$

R_s – $^{15}\text{N}/^{14}\text{N}$ ratio of sample

R_o – $^{15}\text{N}/^{14}\text{N}$ ratio of atmospheric N_2

2.2 Design of quadrupole mass spectrometers and how they work

There are many types of mass spectrometer systems, which differ regarding the technology used. Quadrupole mass spectrometers are nowadays the instruments most commonly used because they are very powerful, compact and robust. Their basic design and how they work are shown in Fig. 1.

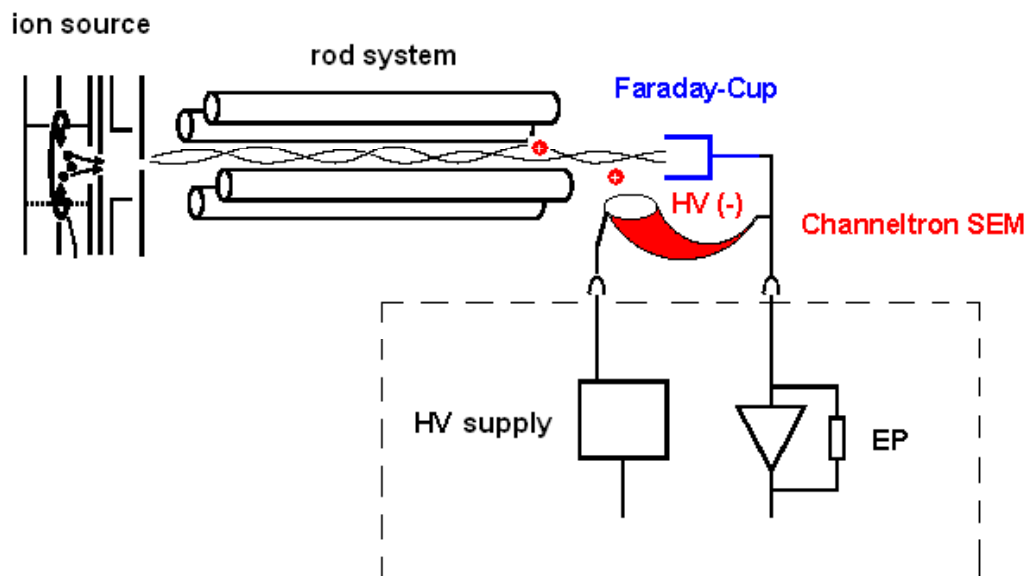


Fig. 1: Diagram showing how a quadrupole mass spectrometer works

To carry out mass separation, quadrupole instruments use a high-frequency electrical alternating field superimposed by a DC voltage. Depending on the DC voltage at the quadrupole, only one certain mass can pass through the rod system and reach the detector. A quadrupole mass spectrometer works as a mass filter, i.e. only one mass can be detected at a time. In order to capture the complete mass spectrum of a mixture that needs to be analysed, the entire range of masses has to be scanned. However, in isotope analysis only a small number of masses of the isotopes concerned (sometimes just two) need to be measured. The quasi-parallel recording of the individual isotope masses (**Selected Ion Monitoring/SIM** or **Multiple Ion Detection/MID**) can be achieved by rapidly switching electronically from one mass to another. This measuring technique is considerably more sensitive than scanning, since a longer measuring time is available per mass number. Faraday cups and secondary electron multipliers (Channeltron-SEM) are used as detectors.

Quadrupole mass spectrometers enable rapid measurements to be taken in a matter of milliseconds and feature high measuring dynamics ranging from the ppb to the percentage range. Depending on the purpose in mind, these systems can be differently configured for online analysis, making them good value for money.

EXAMPLE APPLICATIONS

3.1 The continuous flow principle for online coupling of mass spectrometers

Fig. 2 shows the principle of the continuous flow technique. Since only gases can be introduced into the ion source of the mass spectrometer, assuming the sample is not in gaseous form (upper flow), the nitrogen and carbon it contains must be converted using a suitable method (e.g. combustion) into a

gaseous N or C compound (lower flow). Following any further separation required (e.g. GC) and gas drying, this can then be conducted into the QMS's ion source. Since the ion source, which is in a high vacuum, can only consume very small gas inflows ($\ll 1\text{ml/min}$), gas splitting (open split) is required ahead of the ion source.

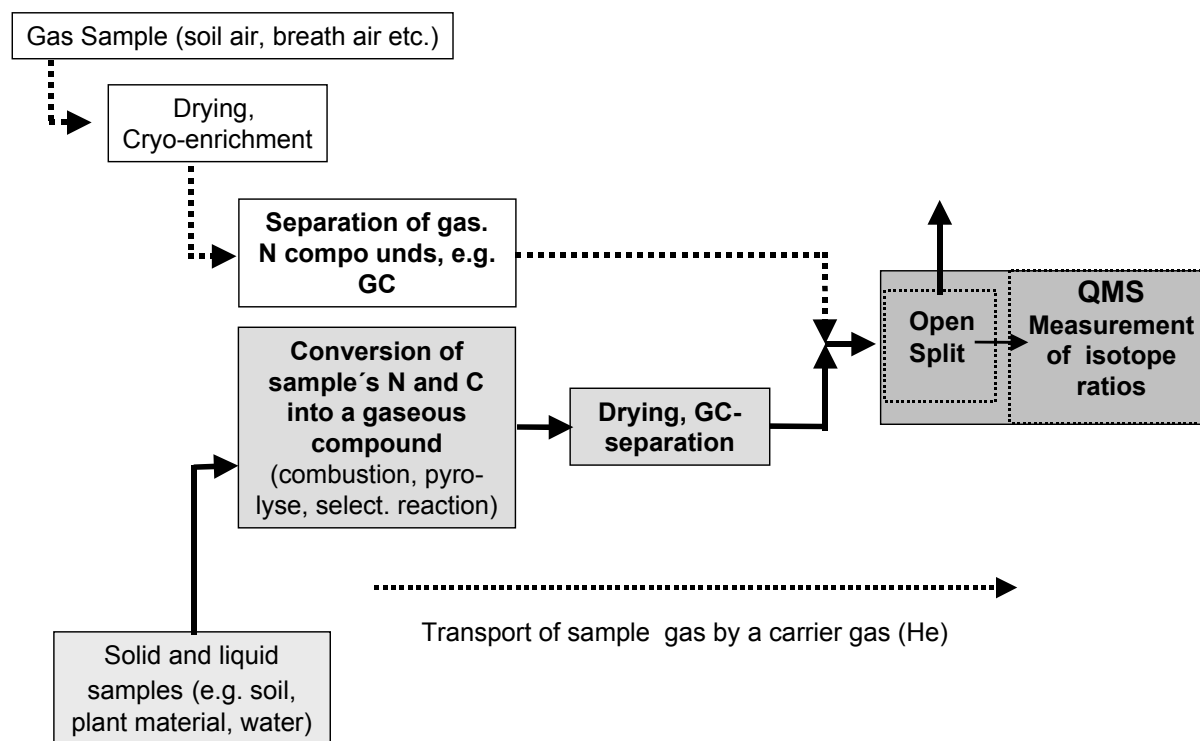


Fig. 2: Principle of the continuous flow approach

3.2 Automatic and simultaneous determination of total carbon and ^{13}C or total nitrogen and ^{15}N

Determining the isotopic abundances of ^{13}C or ^{15}N in the total carbon or nitrogen is a constantly recurring analytical task when these stable isotopes are used in biological systems. This can now be done very successfully in a routine manner by connecting an elemental analyser (EA) to a suitable mass spectrometer, such as a QMS. The QMS is connected to a suitable EA via a simple open split and an inlet system to reduce the EA's outlet pressure on the internal pressure of the ion source. For example, a Vario EL EA (Elementar Analysensysteme Hanau, Germany) was successfully connected in this manner to an MS Cube QMS (Balzers AG, Liechtenstein) to determine the ^{13}C and ^{15}N abundances in soil and plant samples [3]. The level of precision which can be achieved using this combination is $\leq 0.2\%$ RSD for ^{15}N with N quantities per measurement of $\geq 100\ \mu\text{g}$, which corresponds to a detection limit of 0.003 at.-% excess (or 8‰ expressed in δ notation). As far as ^{13}C is concerned, the level of precision is $\leq 0.05\%$ RSD, which results in a detection limit of 0.002 at.-% excess (2‰ in δ notation) [4].

Using the QMS ESD 100 (InProcess Instruments Bremen, Germany) achieved comparable precision with the dual advantages of lower substance consumption and reduced maintenance.

3.3 Automatic determination of the content and isotopic composition of dissolved total carbon and nitrogen in aqueous samples using the online coupling of a TOC analyser with a quadrupole mass spectrometer

The standard method used for the ^{15}N determination of the total dissolved nitrogen (TDN) and total dissolved organic carbon (DOC/TOC) in aqueous samples (leachate, soil extracts, urine, etc) is wet chemical oxidation (Kjeldahl, persulphate) followed by the isotope measurement of the isolated ammonium salts or carbonate in an EA-MS coupling (see 3.2). However, this manual technique is very laborious and time-consuming. Using the aqueous samples directly in the EA-MS coupling is impracticable owing to the relatively high sample volume required of > 0.5 ml.

One way of solving this problem is to connect a TOC analyser featuring the catalytic combustion of the dissolved carbon and nitrogen to form CO_2 and NO online to a QMS. The highTOC TOC analyser (Elementar Analysensysteme Hanau, Germany) is especially suitable for this purpose since it almost completely meets the conditions for QMS coupling, or at least does so following a few minor modifications:

- a) The carrier gas must be absolutely free of nitrogen. Therefore, a He/O_2 mixture is used as carrier gas instead of synthetic air.
- b) The O_2 in the carrier gas must be kept to a minimum. Therefore, the gas inlet on the highTOC is divided into a He and an O_2 branch, so that the O_2 content in the gas mixture can be controlled via an additional MFC.
- c) The CO_2 and NO formed during oxidation must leave the analyser without being chemically altered or diluted with the carrier gas.

^{13}C is measured from the CO_2 molecule (molecular numbers = 44, 45, 46) and ^{15}N from NO (mn = 30, 31). ^{15}N - NO measurement is the harder of the two, requiring a QMS with high NO sensitivity and low NO background (mn = 30). The ESD 100 and especially the ISD 400 quadrupole mass spectrometers (both built by InProcess Instruments, Bremen) proved suitable. Despite the high sensitivity of this QMS, the NO formed still has to be extracted from the He/O_2 carrier gas flow of the highTOC by means of 'trap and flush' before being introduced into the QMS. As well as removing the otherwise disruptive oxygen, this also enables a better shape and concentration of the NO peak. With the exception of very low C levels, this step is unnecessary for CO_2 measurement. Fig. 3 contains a diagram of a highTOC coupled to an ESD 100 as tested.

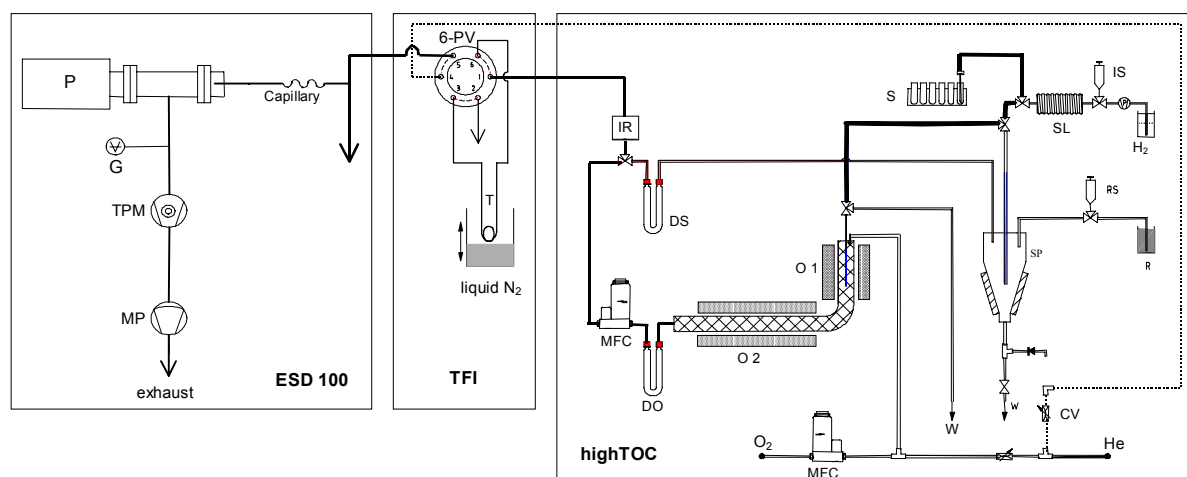


Fig. 3: Diagram of the online couple of the highTOC analyser with the ESD 100 QMS via the TFI trap and flush interface (the O1–O2 oven line used of the highTOC is accentuated)

Main items: DO – gas dryer; IR – IR detector for quantity measurement; IS – injection syringe for sample; MFC – mass flow controller; MP – membrane pump; O1 – dynamic oven for pre-combustion; O2 – oven for catalytic afterburning; S – sampler; SL – sample loop; T – cryotrap; TMP – turbo molecular pump; W – wastewater; 6-PV – 6-port valve

The analytical parameters achieved with this arrangement were as follows

- ^{15}N from NO [4]: Max. dosable sample volume: 3 ml
Limit of detection: $2\ \mu\text{g N}$, corresponding to $0.7\ \text{mg N/l}$
Limit of quantitation for ^{15}N : 0.1 at.-% excess at a dosage of $50\ \mu\text{g N}$
High quantities of N result in lower ^{15}N limits of quantitation
Time required for 1 measurement: about 15 min
- ^{13}C from CO_2 [5]: Max. dosable sample volume: 3 ml
Limit of detection: $5\ \mu\text{g C}$, corresponding to $1.7\ \text{mg C/l}$
Limit of quantitation for ^{13}C : 0.01 at.-% excess at a dosage of $20\ \mu\text{g C}$
Higher quantities of C result in low ^{13}C limits of quantitation
Time required for 1 measurement: about 7 min (excluding trap and flush)

3.4 Reaction Continuous Flow QMS System (SPINMAS)

As explained in 3.1, to achieve the continuous flow regime, the element being measured in the sample needs to be turned into a gaseous form so that it can be transported by the carrier gas to the mass spectrometer's ion source for isotope measurement. As well as combustion (see above), selective chemical reactions are also suitable.

As far as the ^{15}N analysis of inorganic N compounds (e.g. ammonium, nitrite and nitrate) in aqueous samples is concerned, a method based on this principle was developed in which the nitrogen is converted into either N_2 (from ammonium) or NO (from nitrite and nitrate), and is then transported by He into the

QMS for ^{15}N measurement [6]. The high measuring sensitivity required for nitrite, which is usually present in very low concentrations ($\mu\text{g N/l}$), calls for mass spectrometers with very high NO sensitivity. As explained above, this requirement is met by the ESD 100, while the ISD 400 is ideal.

To automate the entire analysis method, a special sample preparation device known as SPIN (Sample Preparation for Inorganic Nitrogen 15) was developed in which the analyte undergoes chemical conversion into gaseous N compounds under PC control [7]. The diagram in Fig. 4 shows how this SPIN unit is connected to the ISD 400 QMS.

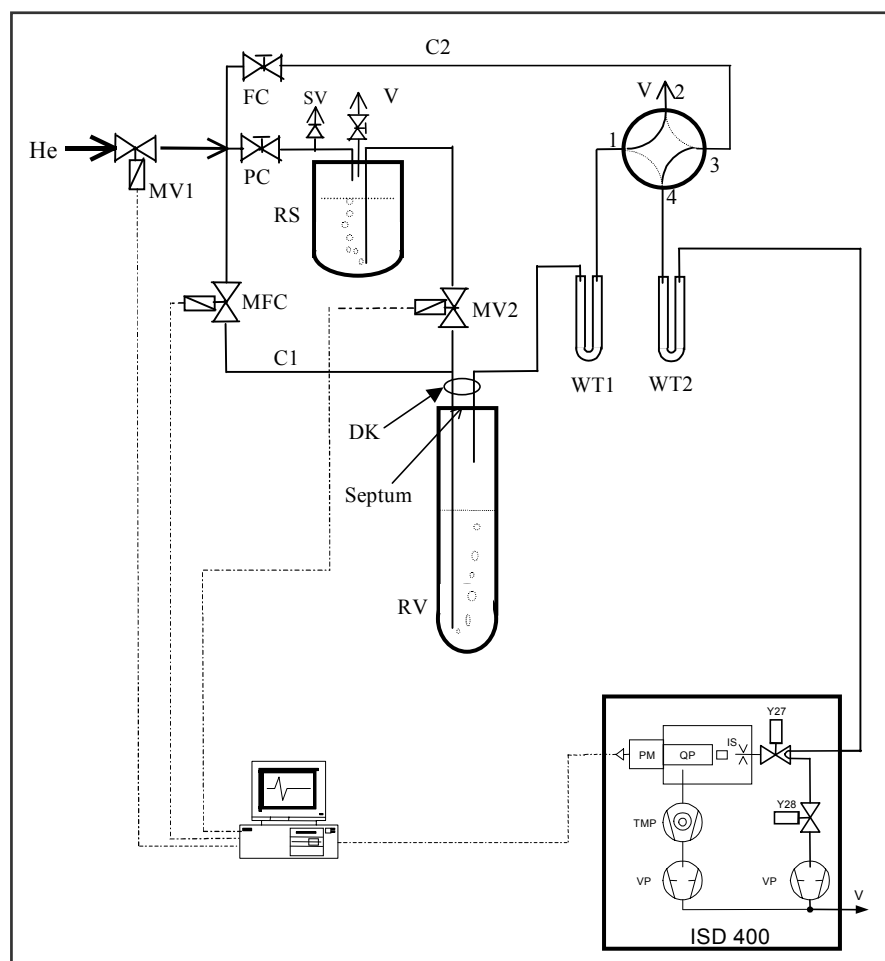


Fig. 4: Diagram of the SPINMAS systems

C1 – flow of carrier gas; C2 – bypass flow; MV1 – magnetic valve; MV2 – rotary valve; MFC – mass flow controller for He; 4PV – 4-port valve; PC – pressure controller; FC – flow controller; RS – reagent store; RV – reaction vessel, also sample vials in the sampler; DK – coaxial double hollow needle; SV – safety valve; WT – water traps

The heart of the SPIN unit is the reaction vessel (RV). It consists of a 20 ml headspace vial with a septum and a screw top, which can accommodate up to 10ml of the sample. The vial is placed inside a heated autosampler (e.g. the GILSON 222 XL Liquid Handler) for automatic sample conversion and dosing. An MV2 rotary valve is used to inject the reagent from the RS into the RV through a coaxial double hollow needle, which also provides gas connection via the four-port valve (4PV) to the ISD 400. The complex

process comprising chemical sample conversion, dosing and ^{15}N measurement by mass spectrometer as well as the evaluation of the measurements is controlled by a PC running special software. It takes about 8 minutes to measure a single sample. The individual methods tried out so far are listed in Tab. 1 along with the analytical parameters.

Tab. 1: ^{15}N determination of inorganic N compounds in aqueous samples using SPINMAS: methods and parameters

N-compound	converted to	with reagent	detection limit ng N	quantitation limit at	
				≥ 1 at.% $\mu\text{g N}$	n. a. $\mu\text{g N}$
Ammonium	N_2	NaClO/NaOH	1000	5	20
Nitrite	NO	$\text{NaI}/\text{H}_3\text{PO}_4$	10	0,1	1
Nitrate	NO	VCl_3/HCl	100	1	5
Hydroxyl-amine	N_2O	$\text{Ce}(\text{SO}_4)_2/\text{H}_2\text{SO}_4$	200	2	10

The SPIN unit could also be used to determine ^{13}C in total inorganic carbon (TIC) and total organic carbon (TOC) via the CO_2 released by acid and wet oxidation using persulphate in accordance with the method published by St-Jean [8].

3.5 Gas chromatograph/quadrupole MS couplings

Couplings with gas chromatographs are the classical domain of quadrupole mass spectrometry. They are mainly used for organic analytical chemistry, although these GC-QMS instruments were also used early on (before the introduction of GC combustion/IRMS for precision isotope analysis) for ^{15}N and ^{13}C analysis (see Section 1 [1, 2]). Therefore, suffice it to mention here that specially configured GC-QMS couplings remain advantageous for the ^{13}C and ^{15}N analysis of inorganic gases such as CO, CO_2 , N_2 , NO and N_2O . In particular, when used in combination with the above-mentioned NO-sensitive QMS, a very sensitive and selective method was developed for the ^{15}N determination of nitrogen oxide (NO) in trace range [9]. This method was for example used for the first ever direct in vivo detection of the formation of NO from arginine in the human body by the oral administration of L-[Guanidino- $^{15}\text{N}_2$]arginine and measuring the ^{15}N -NO in the breath [10].

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