High-precision quadruple isotope dilution method for simultaneous determination of nitrite and nitrate in seawater by GCMS after derivatization with triethyloxonium tetrafluoroborate

Pagliano, E.; Meija, J.; Mester, Z.

Publisher's version / Version de l'éditeur:
https://doi.org/10.1016/j.aca.2014.03.018
Analytica Chimica Acta, 824, pp. 36-41, 2014

Published in Analytica Chimica Acta (2014) 824:36-41 [DOI: 10.1016/j.aca.2014.03.018]

High-precision quadruple isotope dilution method for simultaneous determination of nitrite and nitrate in seawater by GCMS after derivatization with triethyloxonium tetrafluoroborate

Enea Pagliano[#], Juris Meija, Zoltán Mester

National Research Council of Canada, 1200 Montreal Road, Ottawa, ON K1A 0R6, Canada

[#]corresponding author. *e*-mails: <enea.pagliano@nrc-cnrc.gc.ca> and <e.pagliano@libero.it>

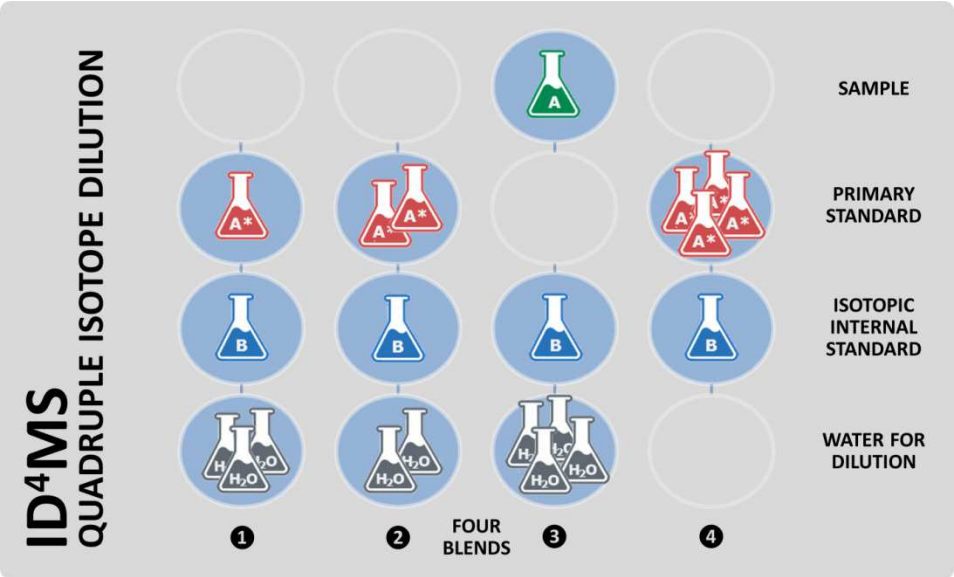
ABSTRACT

Quadruple isotope dilution mass spectrometry (ID^4MS) has been applied for simultaneous determination of nitrite and nitrate in seawater. ID^4MS allows for high-precision measurements and entails the use of isotopic internal standards (^{18}O-nitrite and ^{15}N-nitrate). We include a tutorial on ID^4MS outlining optimal experimental design which generates results with low uncertainties and obviates the need for direct (separate) evaluation of the procedural blank. Nitrite and nitrate detection was achieved using a headspace GCMS procedure based on single-step aqueous derivatization with triethyloxonium tetrafluoroborate at room temperature. In this paper the sample preparation was revised and fundamental aspects of this chemistry are presented. The proposed method has detection limits in the low parts-per-billion for both analytes, is reliable, precise, and has been validated using a seawater certified reference material (MOOS-2). Simplicity of the experimental design, low detection limits, and the use of quadruple isotope dilution makes the present method superior to the state-of-the-art for determination of nitrite and nitrate, and an ideal candidate for reference measurements of these analytes in seawater.

Keywords: quadruple isotope dilution, high-precision, nitrite and nitrate, seawater, triethyloxonium tetrafluoroborate derivatization, GCMS

Published in Analytica Chimica Acta (2014) 824:36-41 [DOI: 10.1016/j.aca.2014.03.018]

Graphic Abstract



1. INTRODUCTION

The precise and accurate determination of nitrite, nitrate and other nutrients in seawater is a mature topic in marine biology and the importance of this subject was recently highlighted by the Intergovernmental Panel on Climate Change (IPCC): "changes in nutrient concentrations can provide information on changes in the physical and biological processes that affect the carbon cycle and could potentially be used as indicators for large-scale changes in marine biology" [1].

In spite of the need for high-quality data for nutrients in seawater, most of the classical analytical chemistry methods for nitrite and nitrate are still based on spectrophotometric and electrochemical detection [2-3]. These approaches cannot deliver specificity and sensitivity and can be significantly affected by the seawater matrix. Furthermore, direct colorimetric assays are available for nitrite, but not for nitrate, which is routinely determined as nitrite after heterogeneous-phase reduction [4]. Analytical chemistry approaches for nitrite and nitrate in seawater are, therefore, limited when high-precision is demanded, and mass spectrometry should be preferred.

Mass spectrometry detection can address specificity and sensitivity and it allows for isotope dilution calibration [5]. Isotope dilution is widely recognized as one of the most accurate approach of modern quantitative analysis [6-7]. Since its introduction, however, isotope dilution has encountered many evolutions, including the recent formulation of the concept of quadruple isotope dilution (ID^4MS) [8]. ID^4MS achieves quantitation of the analyte by determination of the isotopic composition of four blends which are made by mixing the sample and the primary standard solution of the analyte with the isotopic internal standard. When an optimal experimental design (exact-matching) is adopted, ID^4MS provides results with high metrological quality [8]. In this paper ID^4MS has been applied for the first time to quantitate nitrite and nitrate in seawater. To facilitate the use of this new method, we also include a tutorial explanation of the ID^4MS along with an Excel spreadsheet.

The application of ID^4MS for the speciation of nitrite and nitrate depends on availability of mass spectrometric methodologies able to detect the analytes while avoiding any artifacts which may affect the

results [5]. Despite the use of ESIMS [9-10] and thermal ionization mass spectrometry [11] for such measurements, GCMS-based methods are more attractive due to the wide availability of the GCMS [12]. However, neither nitrite nor nitrate are volatile, therefore they cannot be subjected to gas chromatography without derivatization. In this vein, the nitration of an aromatic compound was first proposed for the conversion of nitrate into nitro-arenes [13]. This approach requires a strongly acidic reaction medium which can cause the unwanted conversion of nitrite to nitrate [14-15]. A modern alternative to nitration is alkylation with pentafluorobenzyl bromide (F_5BzBr). Alkylation with F_5BzBr was proposed for nitrite [16], and later extended to both nitrite and nitrate [17-18]. Despite the specificity and sensitivity of this method, sample preparation requires a non-aqueous reaction medium (acetone), elevated reaction temperature (50 °C for 1 h), and a toluene extraction after the evaporation of the reaction medium. To overcome the disadvantages of the F_5BzBr alkylation, we recently proposed an alternative carbon-based derivatization chemistry for nitrate and nitrite [19] based on use of triethyloxonium tetrafluoroborate, $Et_3O^+[BF_4]^-$ to convert nitrite and nitrate to ethyl nitrite and ethyl nitrate, respectively. Triethyloxonium tetrafluoroborate [20-22] is a water soluble reagent able to perform ethylation in aqueous media and at room temperature. Both EtONO and $EtONO_2$ are volatile and can be sampled in the headspace, allowing for their separation from the sample matrix before the GCMS analysis. This simple state-of-the-art GCMS method in combination with quadruple exact-matching isotope dilution calibration provide a robust approach for the speciation of nitrite and nitrate in seawater, producing high-precision metrological results.

2. MATERIALS AND METHODS

2.1 Reagents

Isotopically enriched nitrates, $K^{15}NO_3$ ($x(^{15}N)$ = 0.99 mol/mol) and $KN^{18}O_3$ ($x(^{18}O)$ = 0.75(5) mol/mol) were obtained from Cambridge Isotope Laboratories. A solution of ^{18}O-labeled nitrite was prepared by reduction of an aqueous solution of $KN^{18}O_3$ in a copper-cadmium column, as reported previously [19]. Triethyloxonium tetrafluoroborate, $Et_3O^+[BF_4]^-$, was obtained from Fluka (w > 0.97 g/g). Standard solutions of nitrite were prepared from high-purity $NaNO_2$ (Aldrich, trace metals basis; $w(NaNO_2)$ = 0.999 99 g/g) and standardized against sodium oxalate (NIST, RM 8040) by permanganometry using gravimetric titration [23]. Standard solutions of nitrate were prepared by dilution of the Nitrate Anion Standard Solution (NIST, SRM 3185). Ammonium hydroxide solution (Fluka, *Trace***SELECT**®; $w(NH_3)$ ≈ 0.25 g/g), sodium hydroxide solution (Fluka, *Trace***SELECT**®; w(NaOH) ≈ 0.3 g/g), sulfamic acid (Sigma-Aldrich, *ReagentPlus*®; $w(NH_2SO_3H) \geq 0.99$ g/g), and potassium permanganate (Baker & Adamson, ACS Reagent) were used. In addition, seawater certified reference material MOOS-2 (National Research Council Canada) and in-house low-nutrient seawater were also utilized.

2.2 Instruments

An Agilent 7000 series triple-quadrupole GCMS equipped with a CombiPAL autosampler (CTC Analytics, Switzerland) was used for all measurements. All preparations were performed gravimetrically by using an analytical balance Mattler Toledo XP205.

2.3 Safety considerations

Triethyloxonium tetrafluoroborate is a strong alkylating agent. However, its potential harm is limited by the fact that it is a nonvolatile salt which readily dissolves in water. Aqueous solutions of $Et_3O^+[BF_4]^-$ become acidic undergoing complete hydrolysis in few hours ($Et_3O^+ + 2H_2O \rightarrow EtOH + Et_2O + H_3O^+$). In

order to avoid exposure to the hydrolysis byproducts, it is recommended that this chemical be handled in a fumehood. The solid $Et_3O^+[BF_4]^-$ should be kept in a refrigerator at –20 °C temperature to limit its exposure to moisture.

2.4 Purification of the commercial triethyloxonium tetrafluoroborate

In order to reduce the reagent blank for nitrate, commercial $Et_3O^+[BF_4]^-$ reagent was purified in-house. One gram of the solid salt was transferred into a 10 mL glass vial which was sealed with a holed screw cap equipped with a teflon/silicon septum. The vial headspace was purged with nitrogen gas for two minutes and then heated at 80 °C for 15 min, followed by a 10 min purge at room temperature. This procedure was conducted in a vented fumehood and the exhaust fumes from the vial were neutralized with a water-filled U-trap. During this process, $Et_3O^+[BF_4]^-$ undergoes partial decomposition ($Et_3O^+[BF_4]^- \rightarrow Et_2O + EtF + BF_3$) [24]. Additionally, the nitrite and nitrate impurities, originally present in the commercial reagent, are converted in the corresponding volatile ethyl esters and subsequently removed from the headspace under nitrogen flush. This procedure contributes to a significant reduction of the reagent blank for nitrate ions. A picture of the experimental apparatus used for this operation is reported in the supplementary information (Figure S1).

2.5 Solutions of natural primary standard and isotopic internal standard

An aqueous solution of isotopically-labeled nitrite and nitrate (isotopic internal standard) was prepared in dilute sodium hydroxide (pH = 10) from aqueous ^{15}N-nitrate and ^{18}O-nitrite in order to reach final mass fractions of: $w(^{15}NO_3^-) \approx 13$ µg/g, and $w(N^{18}O_2^-) \approx 0.7$ µg/g. A primary standard solution of nitrite and nitrate was prepared from nitrite and nitrate of natural isotopic composition to reach mass fractions $w(NO_3^-) = 2.954$ µg/g, and $w(NO_2^-) = 0.3340$ µg/g. The above mass fractions of the isotopic internal standard and of the natural primary standard are ideal for the measurement of nitrite and nitrate in MOOS-2. General aspects of the preparation of these two standard solutions are discussed in section 3.1.

2.6 Preparation of the sample/standard blends

In order to perform a quadruple isotope dilution experiment, three calibration points are required. Such calibrators are prepared in 10 mL headspace vials suitable for the CombiPAL autosampler as follows:

1) Solution A*B-1: 0.75 mL of primary standard + 0.30 mL of isotopic internal standard + 2.25 mL of ultrapure water for dilution.
2) Solution A*B-2: 1.50 mL of primary standard + 0.30 mL of isotopic internal standard + 1.50 mL of ultrapure water.
3) Solution A*B-3: 3.00 mL of primary standard + 0.30 mL of isotopic internal standard.

The preparation of the sample solutions is done in a similar fashion:

4) Solution AB-1: 3.00 mL of sample (MOOS-2 seawater, for example) + 0.30 mL of isotopic internal standard + 3.00 mL of ultrapure water.

All four solutions (blends) can be prepared volumetrically but, for best results, it is important to measure the mass each solution added. For precise work, it is important that the ultrapure water used to prepare the blends and to build the primary standard solution comes from the same batch.

2.7 Derivatization of nitrite and nitrate

All sample/standard blends were pH adjusted by addition of 20 µL ammonium hydroxide solution followed by the addition of 1 mL freshly prepared aqueous $Et_3O^+[BF_4]^-$. The latter solution was prepared by dissolving 1 g $Et_3O^+[BF_4]^-$ in 10 mL of cold water (4 °C) and 500 µL ammonium hydroxide solution, $w(NH_3) \approx 0.25$ g/g. In such conditions, the reagent is not stable for a long time and must be added quickly to samples and standards. Once the derivatizing agent is added, the vials are sealed with holed screw-caps equipped with teflon/silicon septum for use with the CombiPAL and are kept at room temperature in the dark for at least 30 min in order to allow the ethylation of the analytes.

2.8 GCMS method

$Et_3O^+[BF_4]^-$ converts nitrite and nitrate into ethyl esters, EtONO and $EtONO_2$. These derivatives are stable and can be analyzed by GCMS. The vials are incubated at 35 °C for 3 min (agitator speed: 500 rpm; on-time: 5 s; off-time: 2 s). A 250 µL volume of headspace is sampled (gas-tight syringe held at 50 °C) and injected in the gas chromatograph. The syringe is cleaned by flushing it with helium for 5 min. The inlet liner (internal diameter of 1 mm) is hosted in an injector block hold at 100 °C and the injection is performed in pulsed-split mode (275 kPa for 1 min; spit ratio 7:1). Compounds are then separated on a mid-polarity column (DB-624; length: 60 m; stationary phase: 6%-cyanopropyl-phenyl-94%-dimethyl polysiloxane; 0.25 mm inner diameter; 1.40 µm coating) using the following temperature program: 8 min (at 35 °C), then 20 °C/min to 190 °C for 3.25 min for a total run time of 19 min (constant He flow rate: 0.9 mL/min). The temperature of the transfer line is 230 °C. Both ethyl nitrite and ethyl nitrate are readily ionized in negative chemical ionization mode (CH_4 reaction gas; CI gas flow: 40%; emission current: 50 µA; emission energy: –240 eV; source temperature: 150 °C; quad. 1 temperature: 150 °C; quad. 2 temperature: 150 °C). The acquisition is performed using selected ion monitoring and no collision energy is applied in the collision cell (He quench gas, 2.25 mL/min; N_2 collision gas, 1.5 mL/min). For ethyl nitrite, 47 Da, 45 Da, and 43 Da ions are monitored (100 ms dwell time), while for ethyl nitrate 47 Da and 46 Da ions are monitored (150 ms dwell time).

2.9 Derivatization chemistry

Triethyloxonium tetrafluoroborate has been recently proposed as analytical reagent for the derivatization of common inorganic anions, including nitrite and nitrate [19]:

$$NO_2^-(aq) + Et_3O^+(aq) \rightarrow EtONO(g) + Et_2O$$

$$NO_3^-(aq) + Et_3O^+(aq) \rightarrow EtONO_2(g) + Et_2O$$

$Et_3O^+[BF_4]^-$ is a powerful ethylating agent able to react in aqueous media at room temperature. This is a significant advantage over other reported derivatization techniques, such as silylation or alkylation with F_5BzBr.

Aqueous Et_3O^+ undergoes hydrolysis within a few hours, $Et_3O^+(aq) \rightarrow H^+(aq) + EtOH + Et_2O$, which renders aqueous solutions of $Et_3O^+[BF_4]^-$ acidic. An acidic environment, however, is undesirable for determination of nitrite and nitrate for two reasons.

1) *Protonation of nitrite.* In an acidic environment, nitrite exists in the form of its conjugate acid, $pK_a(HNO_2) = 3.4$, which undergoes oxidation to nitrate an aqueous media: $HNO_2(aq) + \frac{1}{2}O_2 \rightarrow NO_3^-(aq) + H^+(aq)$ [14-15].
2) *Oxygen exchange between nitrite and water*. Nitrous acid can be further protonated in acidic media to $H_2NO_2^+$ which undergoes fast exchange of oxygen with water: $^*H_2NO^*O^+ + 2H_2O \leftrightarrows H_2NOO^+ + 2\,^*H_2\,^*O$ [25]. This reaction is unwanted as it effectively scrambles the ^{18}O-nitrite, precluding application of isotope dilution [5].

Both of these unwanted processes can be avoided by working in an alkaline medium [19]. Sample pretreatment with aqueous ammonia in order to maintain a suitable alkalinity for the derivatization (pH from 9 to 10) has proven effective in thwarting both of these processes. We have found it is advantageous to buffer both the sample and $Et_3O^+[BF_4]^-$ solutions with ammonia, and not only the sample, as was done in our previous study [19]. Such an approach avoids any temporal/localized acidic conditions upon the addition of the acidic oxonium reagent to the sample. Albeit trivial, this modification in the measurement procedure significantly improves the precision of the nitrite measurement results. An alkaline reaction medium is recommended whenever nitrite is measured. However, when interest falls only on nitrate, this procedure can be modified by removing the nitrite ions with sulfamic acid ($NH_2SO_3H + HNO_2 \rightarrow H_2SO_4 + N_2 + H_2O$) [26] and carrying out the ethylation in acidic media. The hydrolysis of triethyloxonium in non-buffered solutions is slower and the derivatization yield is higher. Consequently, the detection limit

for nitrate is ca. two-fold improved by comparison to the alkaline approach. The analytical procedure for the determination of nitrate alone is detailed in the supplementary information.

3. RESULTS AND DISCUSSION

3.1 Quadruple isotope dilution

ID^{4}MS is a recent improvement of the classical isotope dilution concept [8]. Conventional single isotope dilution requires the measurement of a single blend, that of sample and isotopic standard. Additional information necessary for the model is gathered externally, including the isotopic composition of natural analyte (usually from the IUPAC tables), isotopic composition of isotopic standard (usually from the vendor), and concentration of isotopic standard (usually from the vendor; derived from the vendor's estimate of chemical purity). In contrast, a quadruple isotope dilution approach does not entail outside knowledge of the above quantities because they are embedded into the model through experimental design [8]. As a result, ID^{4}MS requires the measurement of four blends. For example, three blends of a primary natural standard solution with isotopic internal standard, and one blend of sample with isotopic internal standard. Considering that these four blends are measured in a short sequence, most potential biases in the isotope ratios (mass-bias drift, protonation/overlap between two fragment ions, or the contribution of ^{13}C or ^{2}H isotopes) – are common to all blends and, consequently, have minimal effect on the analytical results. An important feature of the quadruple isotope dilution method is that the knowledge of the chemical formula of the measured ions is not required as long as one can ascertain that the measured ions originate from the analyte.

The following experimental design – exact matching – has shown to provide the best measurement performance [8]:

1) The blend of sample and isotopic standard (AB) is prepared by adding as much of the isotopic standard required to reach an isotope ratio of AB which is approximately midway between the isotope ratios of pure natural analyte (A) and pure isotopic standard (B).

2) The exact-matching blend (A*B-2) of natural standard (A*) and isotopic standard (B) is prepared to mimic the blend AB. In other words, the final mass fraction composition of A*B-2 should be the same as in AB.
3) The two remaining bracketing blends (A*B-1 and A*B-3) are prepared as A*B-2. However, the final mass fraction of the analyte in A*B-1 and A*B-3 should be half and double that of A*B-2, respectively.

Note that the same amount of the isotopic standard (B) is used to prepare all four blends. Once all (four) blends are prepared, they can be subjected to GCMS analysis. For the quantitation of nitrate only, one ratio can be used (ratio of peak areas extracted at 46 over 47 Da), while for nitrite, two signal ratios can be used (ratio of peak areas extracted at 43 or 45 over 47 Da). The mathematical formulation of ID^{4}MS has been given elsewhere [8] and in this article we provide an Excel spreadsheet for the calculation of the results (see Figure 1 and supplementary information). The analyst, however, must be aware that ID^{4}MS is based on two assumptions. First, the isotopic composition of the analyte in the sample and in the primary standard is identical. Although the isotope ratios of oxygen and nitrogen vary by some 10% in seawater [27], this impacts the results of the exact-matching ID^{4}MS by approx. 0.1%. Second, the measured isotope amount ratios, r, are directly proportional to the actual amount ratios, R, i.e., $R = K \cdot r$ where K is the mass bias factor, and the value of K is the same for all four blends. In this method, the blends are sampled from the headspace and therefore matrix-induced effects are unlikely (blend AB contains seawater whereas blends A*B do not).

	A	B	C	D	E	F
1						
2	Quadruple isotope dilution, ID^{4}MS					
3						
4	*Calibration blends*		$m_{A^*(A^*B)}$/g	$m_{B(A^*B)}$/g	r_{A^*B}/(V/V)	w_{A^*}/(µg/g)
5		A*B-1	0.7486	0.3026	0.47004	
6		A*B-2	1.5001	0.3029	0.91010	2.9674
7		A*B-3	2.9903	0.3031	1.76723	
8						
9	*Sample blends*		$m_{A(AB)}$/g	$m_{B(AB)}$/g	r_{AB}/(V/V)	w_A/(µg/g)
10		AB-1	3.0791	0.3033	0.8322	**1.3189**
11		AB-2	3.0808	0.3027	0.8225	**1.2994**
12		AB-3	3.0829	0.3026	0.8390	**1.3254**
13		AB-4	3.0790	0.3034	0.8357	**1.3250**
14		AB-5	3.0991	0.3035	0.8379	**1.3207**

Figure 1. Excel spreadsheet for the ID^{4}MS model. The upper area describes the blends of the primary standard solution and isotopic standard (calibration blends A*B-1, A*B-2, and A*B-3) with the cell F5 reporting the mass fraction of the primary standard (A*). As an example, the mass of the A* solution in order to prepare the blend A*B-1 is given in C5, and the mass of the isotopic standard (B) to produce the blend A*B-1 is given in D5. The cell E5 is the measured isotope amount ratio for the blend A*B-1 (without any mass bias correction).
The lower area describes the blends of sample and isotopic standard (sample blends AB-1, AB-2, etc.) in a similar fashion. Finally, cells F10:F14 provide the mass fraction of the analyte in the sample.

3.2 Internalization of the procedural blank correction

Correction for the procedural blank is not trivial in higher-order isotope dilution. In double isotope dilution, for example, when blank contributes to blends AB and A*B, only a fraction of the analyte concentration in the blank is subtracted from the gross analyte concentration [28]. In addition, the concentration of the analyte in the blank is evaluated in a designated isotope dilution experiment. In this paper (see Section 2.5) we describe an experimental design which eliminates the need for blank subtraction and the need to perform designated isotope dilution measurements of the analyte levels in the blank. This proposed approach stems from the experimental design whereby all four blends are subjected to equal amounts of reagents and water. Consequently, since an equal amount of external contamination is introduced to all four blends, the obtained results are unaffected by the blank contributions. When the determination of trace amounts of nitrite/nitrate is carried in the presence of high blank, one can expect increased uncertainty, but not biased results. The proposed experimental design takes advantage of the quadruple isotope dilution method by making the blank correction an embedded feature of the method,

much like the determination of the isotopic composition of the isotopic standard. This implementation allows the analyst to avoid a direct evaluation (and subsequent subtraction) of the blank which is commonly done in trace analysis.

3.3 Analytical figures of merit

An example of chromatographic separation achieved for nitrite and nitrate is presented in Figure 2 for a sample of MOOS-2 seawater CRM. For this determination, a 1 mm ID liner was used because it allows for a sharper analytical peak for the early eluting ethyl nitrite. The derivate of nitrite (EtONO) elutes at 6.4 min and is well separated from the nitrate ($EtONO_2$ elutes at 13.5 min). Both ethyl nitrite and ethyl nitrate can be sampled from the headspace, which offers notably cleaner chromatography thus potentially enabling the analysis of complex matrices, such as blood or saliva. Both EtONO and $EtONO_2$ can be ionized in EI+, CI+ (methane), or CI– (methane) modes; however, the best results in terms of sensitivity are achieved in negative chemical ionization [19]. The CI– mass spectrum of ethyl nitrite shows two main signals corresponding to fragment ions containing oxygen, $^{12}CH_2=^{12}CH^{16}O^-$ (43 Da) and $^{12}CH_3–^{12}CH_2{}^{16}O^-$ (45 Da), and the ^{18}O- nitrite exhibits the expected +2 Da shift. The mass spectrum of ethyl nitrate is simpler with $^{14}N^{16}O_2^-$ (46 Da) as the only nitrogen-containing fragment and the ^{15}N-nitrate exhibits the expected +1 Da shift (Figure 3). The method produces instrumental detection limits in the low parts-per-billion for both analytes, Figure 2.

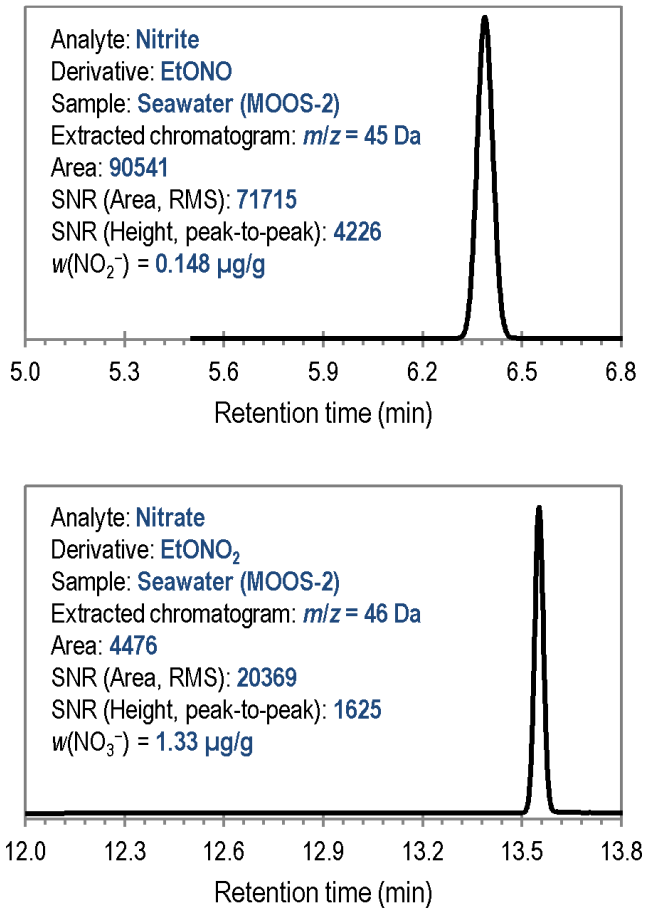


Figure 2. Chromatographic separation of nitrite and nitrate from a sample of MOOS-2 certified reference material for nutrients in seawater.

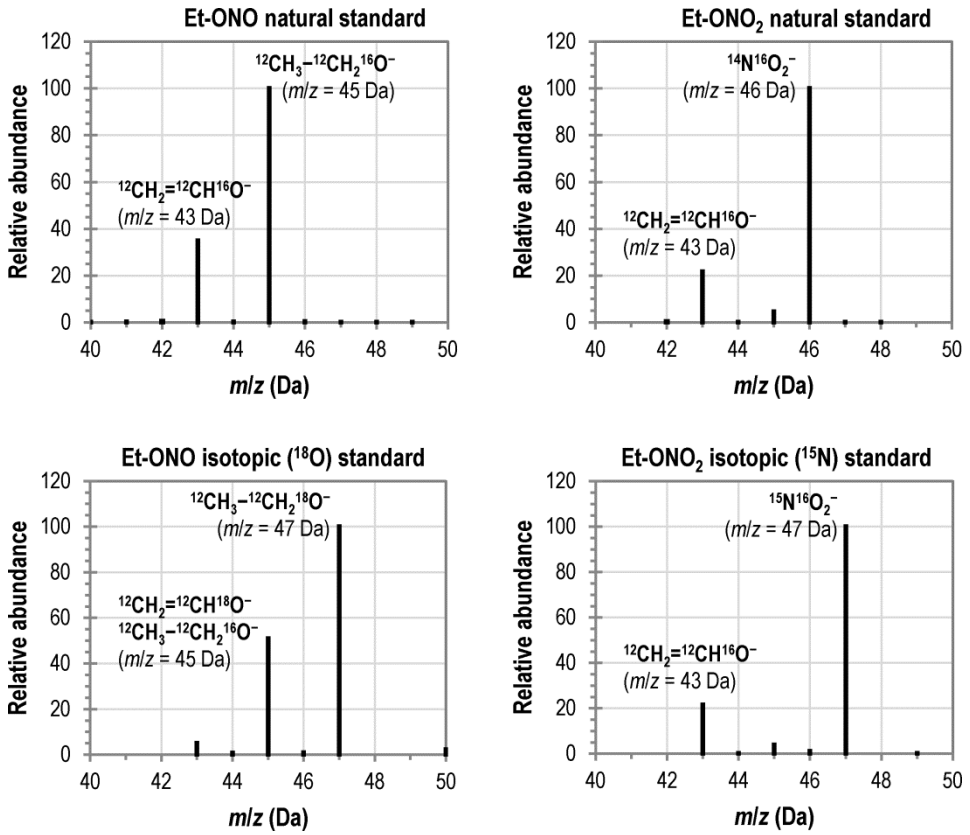


Figure 3. Negative chemical ionization (methane) mass spectra of: nitrite of natural isotopic composition (up-letf); isotopic ^{18}O-enriched nitrite (down-left); nitrate of natural isotopic composition (up-right); isotopic ^{15}N-enriched nitrate (down-right).

3.4 High-precision analysis of seawater

The method has been validated using a seawater certified reference material for nutrients (MOOS-2, National Research Council Canada). MOOS-2 was certified for nitrite and nitrate by comparing analytical results gathered using traditional colorimetric procedures and ion chromatography [29]. Table 1 summarizes the measurement results for nitrite and nitrate in MOOS-2 found using the method described herein. The isotope ratios used for the ID^{4}MS were obtained from the areas of the analytical peaks extracted at appropriate *m/z* values. For nitrite, the ID^{4}MS model was applied using two distinct isotope ratios, 43/47 and 45/47, both yielding identical results. Similar figures were obtained for nitrate, where the ID^{4}MS was applied using the ratio between the signals extracted at 46 and 47 Da (Table 1). As

evidenced from the results, sub-percent relative standard uncertainty is attained for both nitrite and nitrate, and all results are in good agreement with the certified reference values. To demonstrate the performance of this method at the low nitrite/nitrate levels, measurements of low-nutrient seawater were also performed (Table 2). Despite two-orders-of-magnitude lower levels of nitrite and nitrate in comparison to MOOS-2, it is possible to obtain results with 10 to 20% uncertainty (Table 2).

Table 1. Analysis of certified seawater reference material MOOS-2 [a]

Isotope ratio measured	$w(NO_2^-)$/(μg/g)	$w(NO_3^-)$/(μg/g)
43/47	0.1483 ± 0.0011 (u_r = 0.74%)	n/a
45/47	0.1481 ± 0.0009 (u_r = 0.63%)	n/a
46/47	n/a	1.3268 ± 0.0027 (u_r = 0.20%)
Certified quantity values	0.1480 ± 0.0040 (u_r = 2.7%)	1.301 ± 0.031 (u_r = 2.4%)

[a] All results are average of five (5) independent analyses (which include the sample preparation and the preparation of calibration blends). Uncertainty is given with a coverage factor k = 1.

Table 2. Analysis of low-nutrient seawater [a]

Sample	$w(NO_2^-)$/(ng/g)	$w(NO_3^-)$/(ng/g)
Batch 1	0.465 ± 0.070 (u_r = 15%)	10.9 ± 2.8 (u_r = 25%)
Batch 2	0.819 ± 0.061 (u_r = 7.5%)	6.9 ± 1.4 (u_r = 21%)

[a] All results are average of four (4) independent analyses (which include the sample preparation and the preparation of calibration blends). Uncertainty is given with a coverage factor k = 1.

4. CONCLUSION

The proposed method for the simultaneous determination of nitrite and nitrate offers an excellent combination of good experimental practice and state-of-the-art data analysis. An aqueous single-step derivatization allows for headspace GCMS detection of both analytes, thus providing an interference-free chromatography with detection limits in the low parts-per-billion range. In addition, quadruple isotope dilution exact-matching allows for extremely tight uncertainty of the measurement results and provides accurate blank compensation without the need for specific evaluation of the analyte levels in the blank. The resulting implementation is robust, precise, and reliable, and therefore could become a reference measurement procedure for nitrite and nitrate in seawater.

REFERENCES

[1] IPCC, 2007: *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change* [Solomon, S., D. Qin, M. Manning, Z. Chen, M. Marquis, K.B. Averyt, M. Tignor and H.L. Miller (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, chapter 5.4.4, pp 406-407.

[2] K. Grasshoff, M. Ehrhardt, K. Kremling, Method of Seawater Analysis, 2nd revised and extended ed., Verlag Chemie GmbH, Weinheim, 1983.

[3] A. Hioki, J.W. McLaren, Direct determination method of nitrate ions in seawater by UV-detection ion-chromatography with hydrochloric acid/sodium chloride eluent, AIST Bulletin of Metrology 7 (2008) 51-59.

[4] F. Nydahl, On the optimum conditions for the reduction of nitrate to nitrite by cadmium, Talanta 23 (1976) 349-357.

[5] J. Meija, Z. Mester, Paradigms in isotope dilution mass spectrometry for elemental speciation analysis, Anal. Chim. Acta. 607 (2008) 115-125.

[6] K.G. Heumann, Isotope dilution mass spectrometry (IDMS) of the elements, Mass Spectrom. Rev. 11 (1992) 41-67.

[7] E. Roth, Critical evaluation of the use and analysis of stable isotopes, Pure Appl. Chem. 69 (1997) 1753-1828.

[8] E. Pagliano, Z. Mester, J. Meija, Reduction of measurement uncertainty by experimental design in high-order (double, triple, and quadruple) isotope dilution mass spectrometry: application to GC-MS measurement of bromide, Anal. Bioanal. Chem. 405 (2013) 2879-2887.

[9] Y. Li, J.S. Whitaker, C.L. McCarty, Reversed-phase liquid chromatography/electrospray ionization/mass spectrometry with isotope dilution for the analysis of nitrate and nitrite in water, J. Chromatogr., A 1218 (2011) 476-483.

[10] A. Farhat, A.N. Dooley, F. Ahmada, Nitrite oxidation in ion chromatography-electrospray ionization-tandem mass spectrometry (IC-ESI-MS/MS), J. Mass. Spectrom. 46 (2011) 720-724.

[11] J.-C. Wolff, P.D.P. Taylor, P. De Bièvre, Traceable Values for Nitrate in Water Samples by Isotope Dilution Analysis Using a Small Thermionic Quadrupole Mass Spectrometer, Anal. Chem. 68, (1996) 3231-3237.

[12] S.M. Helmke, M.W. Duncan, Measurement of the NO metabolites, nitrite and nitrate, in human biological fluids by GC–MS, J. Chromatogr., B 851 (2007) 83-92.

[13] D.J. Glover, J.C. Hoffsommer, Gas chromatographic analysis of nitrate and nitrite ions in microgram quantities by conversion to nitrobenzene, J. Chromatogr. 94 (1974) 334-337.

[14] N.R. Dhar, S.P. Tandon, N.N. Biswas, A.K. Bhateacharya, Photo-oxidation of nitrite to nitrate, Nature 133 (1934) 213-214.

[15] P.K. Mudgal, S.P. Bansal, K.S. Gupta, Kinetics of atmospheric oxidation of nitrous acid by oxygen in aqueous medium, Atmos. Environ. 41 (2007) 4097-4105.

[16] H.-L. Wu, S.-H. Chen, S.-J. Lin, W.-R. Hwang, K. Funazo, M. Tanaka, T. Shono, Gas chromatographic determination of inorganic anions as pentafluorobenzyl derivatives, J. Chromatogr. 269 (1983) 183-190.

[17] D. Tsikas, Simultaneous derivatization and quantification of the nitric oxide metabolites nitrite and nitrate in biological fluids by gas chromatography/mass spectrometry, Anal. Chem. 72 (2000) 4064-4072.

[18] D. Tsikas, M.T. Suchy, A. Mitschke, B. Beckmann, F.M. Gutzki, Measurement of nitrite in urine by gas chromatography-mass spectrometry, Methods Mol. Biol. 844 (2012) 277-93.

[19] E. Pagliano, J. Meija, R.E. Sturgeon, Z. Mester, A. D'Ulivo, Negative chemical ionization GC/MS determination of nitrite and nitrate in seawater using exact matching double spike isotope dilution and derivatization with triethyloxonium tetrafluoroborate, Anal. Chem. 84 (2012) 2592-2596.

[20] H. Meerwein, E. Battenberg, H. Gold, E. Pfeil, G.J. Willfang, Über tertiäre Oxoniumsalze, II, Prakt. Chem. 154 (1939) 83-156.

[21] H. Perst, Oxonium Ions in Organic Chemistry, Verlag Chemie GmbH, Weinheim, 1971.

[22] G.A. Olah, K.K. Laali, Q. Wang, G.K. Surya Prakash, Onium Ions, Wiley, New York, 1998.

[23] A.I. Vogel, A Text-book of Quantitative Inorganic Analysis, 3rd ed, Longman, London, 1961.

[24] H. Meerwein, Triethyloxonium fluoroborate, Org. Synth. Coll. Vol. 5 (1973) 1080.

[25] J. Crugeiras, A. Ríos, H. Maskill, DFT and AIM study of the protonation of nitrous acid and the pK_a of nitrous acidium ion, J. Phys. Chem., A 115 (2011) 12357-12363.

[26] A.M. Hartley, R.I. Asai RI, Spectrophotometric determination of nitrate with 2,6-xylenol reagent, Anal. Chem. 35 (1963) 1207-1213.

[27] M.E. Wieser, T.B. Coplen, Atomic weights of the elements 2009 (IUPAC Technical Report), Pure Appl. Chem. 83 (2011) 359-396.

[28] L. Yang, R.E. Sturgeon, Blank correction considerations for isotope dilution and reverse isotope dilution calibration: Determination of methylmercury in fish tissue, J. Anal. At. Spectrom. 20 (2005) 724-729.

[29] http://www.nrc-cnrc.gc.ca/eng/solutions/advisory/crm/certificates/moos_2.html