Negative chemical ionization GC/MS determination of nitrite and nitrate in seawater using exact matching double spike isotope dilution and derivatization with triethyloxonium tetrafluoroborate

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Negative chemical ionization GC–MS determination of nitrite and nitrate in seawater using exact matching double spike isotope dilution and derivatization with triethyloxonium tetrafluoroborate

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ABSTRACT

The alkylation of nitrite and nitrate by triethyloxonium tetrafluoroborate allows determination of their ethyl esters by headspace GC–MS. In the present study, significant improvement in analytical performance are achieved by using negative chemical ionization (CI–). Detection limits are improved for at least an order of magnitude than those achieved using electron impact ionization (EI). The derivatization procedure has been optimized and alkaline conditions are adopted to minimize the conversion of nitrite to nitrate (determined to be 0.07% at 100 mg/L NO_2^-) and to avoid the exchange of oxygen between the analytes and the solvent (water). Quantitation entails the use of isotopically enriched standards ($N^{18}O_2^-$ and $^{15}NO_3^-$), which also permits monitoring of potential conversion from nitrite to nitrate during the analysis (double spike isotope dilution).

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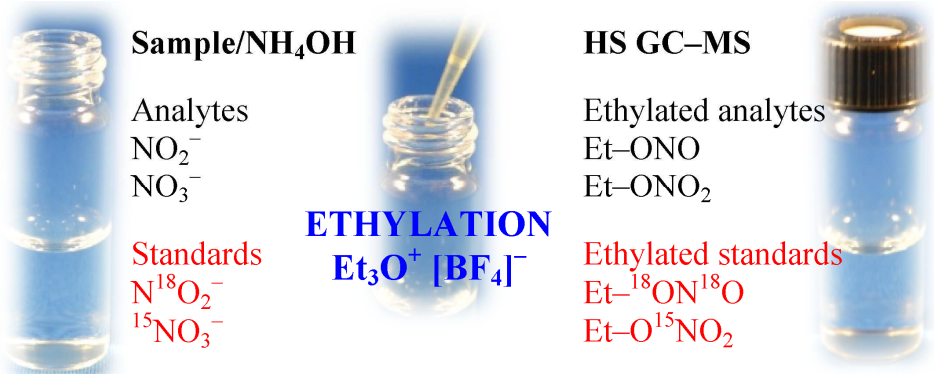
Graphic Abstract



INTRODUCTION

The determination of nitrite and nitrate in samples of different origin is a challenge to analytical chemistry and it is important from environmental and biological perspectives[1]. Moorcroft et al.[1] reviewed strategies for detection of these analytes, discussing advantages and limitations of the various methodologies. Jobgen et al.[2] focused their analysis on the determination of nitrite and nitrate in biological samples using HPLC, whereas Helmke et al.[3] discussed the application of GC–MS. In general, methods which entail the use of molecular spectroscopy or electrochemical detection have limited sensitivity and selectivity, and suffer from matrix effects. Furthermore, there are more methods for the direct detection of nitrite than nitrate and in many analytical protocols the determination of nitrate is achieved following a critical reduction to nitrite, usually by cadmium[4]. Nitrite and nitrate are nonvolatile anions and their determination by GC–MS can be achieved by derivatization in order to generate volatile species. The nitration of aromatic compounds has been used to convert nitrate[3]. This approach, however, does not work for nitrite and requires the use of strong acid conditions which may be critical if any nitrite is present because of its possible conversion to nitrate[5–6]. Another technique used with GC–MS entails alkylation with pentafluorobenzyl bromide (F_5BzBr)[7] to convert nitrite and nitrate to $F_5Bz–NO_2$ and $F_5Bz–ONO_2$, respectively. The pentafluorobenzyl derivates are suitable for negative chemical ionization GC–MS, but they are not been employed to perform headspace analysis likely for their low volatility. Despite the availability of low detection limits (sub-fmol, absolute) and the possibility of simultaneously determination of both analytes, the derivatization method requires organic solvents (acetone and toluene) and the subsequent injection of the organic extract which may contain sample matrix and the unreacted F_5BzBr. The use of reversed phase liquid chromatography coupled with electrospray ionization mass spectrometry has recently been used for the determination of nitrite and nitrate in water with detection limit of 1 and 12 μg N L^{-1} for nitrate and nitrite, respectively [8].

Alkylation of anions with triethyloxonium tetrafluoroborate has been proposed only recently[9–10] despite the well-established chemistry of $Et_3O^+[BF_4]^-$[11–12]. From a practical point of view, the use of triethyloxonium offers several advantages which make this analytical technique unique: (i) the alkylation can be performed in aqueous medium; and (ii) the alkylation entails the chemical vapor generation of simple inorganic anions (Cl^-, Br^-, I^-, CN^-, SCN^-, S^{2-}, NO_3^-, NO_2^-) to their volatile derivatives, potentially permitting headspace and SPME sampling with GC–MS detection. In general, the application of chemical vapor generation is always advantageous

because it is a separation technique which eliminates the introduction of sample matrix to the instrument thereby minimizing contamination of the device and reducing background levels[13].

An isotope dilution method based on alkylation by triethyloxonium for simultaneous determination of nitrite and nitrate by EI GC–MS has recently been discussed by the authors.[10] The drawbacks of the previous procedure are related to poor detection limits and the potential conversion of nitrite to nitrate during the analysis due to acid hydrolysis of the reactant. Here we propose an improved method which eliminates the above mentioned drawbacks: pretreatment of the sample with ammonium hydroxide maintains an alkaline pH during alkylation, avoiding the problem of conversion of nitrite to nitrate, and negative chemical ionization provides enhanced detection limits for both analytes.

EXPERIMENTAL SECTION

Reagents and materials. Isotopically labeled nitrate ($K^{15}NO_3$, $x(^{15}N)$ = 0.99 mol/mol; $KN^{18}O_3$, $x(^{18}O)$ = 0.70-0.80 mol/mol) were obtained from Cambridge Isotope Laboratories (Cambridge MA, USA). Aqueous solutions of these salts were prepared by dissolution in ultrapure water. Triethyloxonium tetrafluoroborate (Fluka; w > 0.97 g/g) alkylating solution was prepared by dissolving the x g of the solid salt in 1.5x g of ultrapure water. Since aqueous triethyloxonium salts undergo hydrolysis, the solution was prepared prior to sample derivatization. $Et_3O^+[BF_4^-]$ has to be handled in a fume hood avoiding the prolonged contact with the air moisture or in dry box and kept in freezer.

Standard solutions of nitrite and nitrate were prepared by dissolution/dilution of $NaNO_2$ (Aldrich; w = 0.99999 g/g) and Nitrate Anion Standard Solution (SRM 3185, NIST) with ultrapure water. All solutions were stored at 4 °C temperature. A 20% NH_4OH solution (Tamapure AA–100) was employed.

Preparation of $KN^{18}O_2$. The ^{18}O-labeled nitrite was prepared from a 15 mM aqueous solution of potassium (^{18}O)nitrate. To reduce nitrate to nitrite, a copper–cadmium reductor column was used through which 2 mL of nitrate solution was passed using a peristaltic pump. The effluent was collected in a vial and stored overnight with 0.5 g of Cu–Cd grains. After separation of the metal, the solution was diluted to 150 mL with NaOH (pH = 10) and used for isotope dilution experiments (150-200 μM $KN^{18}O_2$, $x(^{18}O)$ = 0.70-0.80 mol/mol in aqueous NaOH, pH = 10). The completeness of the nitrite/nitrate conversion was verified by GC–MS using the method described herein.

GC–MS methods. After derivatization to the corresponding ethyl ester, nitrite and nitrate were separated and detected by GC–MS (Hewlett-Packard 6890 gas chromatograph equipped with a Hewlett-Packard 5973 mass detector). The operating conditions are summarized in Table 1. A manual injection of 250 μL of sample vial headspace with a gas-tight syringe was performed for subsequent quantitation by CI–. Selected ion monitoring (SIM) mode was employed: m/z = 45, 47, 63, 65 for Et–ONO and m/z = 46, 47, 48, 50 for Et–ONO_2 (100 ms dwell time was used for each ion).

Table 1. GC–MS operating conditions

Gas chromatography		
Column	Model	J&W 122–1364 DB–624
	Length	60 m
	Mode	Constant flow (1 mL He/min)
Inlet	Mode	Pulsed spit
	Temperature	120 °C
	Split ratio	8:1
	Pulsed pressure	25.0 psi for 0.5 min
Oven	Isotherm	30 °C for 10 min
	Ramp	20 °C/min to 140 °C
	Isotherm	140 °C for 1.5 min
	Run time	17 min

Mass Spectrometry	**CI+**	**CI–**
Reaction gas	CH_4	CH_4
Transfer line temperature	250 °C	250 °C
Emission current	237.3 µA	49.4 µA
Electron energy	151.5 eV	114.4 eV
Ion source temperature	250 °C	150 °C
Quadrupole temperature	150 °C	106 °C
EMV	1388 V	2000 V

Sample preparation. A 2 mL volume of sample was introduced without any pretreatment into a 4 mL vial and spiked with 200 µL of (^{18}O)nitrite and 50 µL of 864 µM (^{15}N)nitrate. The amount of the spikes was chosen to provide an exact (1:1) match to the concentration of these analytes in a MOOS-2 sample for signals m/z = 45, 47 (nitrite) and m/z = 46, 47 (nitrate). After addition of 50 µL of 20% NH_4OH, the sample was derivatized by adding 100 µL triethyloxonium tetrafluoroborate solution. The vial was then sealed with a screw cap PTFE/silicone septum and kept in the dark at room temperature for at least 30 min. Headspace analysis was then performed by GC–MS.

RESULTS AND DISCUSSION

CI mass spectra. Triethyloxonium tetrafluoroborate can convert nitrite and nitrate to their ethyl esters through an O–alkylation of both analytes. The characterization and the EI mass spectra of these derivatives has been discussed earlier[10]. Both Et–ONO and Et–ONO_2 undergo chemical ionization by methane in positive (CI+) and negative (CI–) mode. In CI+ mass spectrum the molecular ion appears as the most intense for both analytes. However, due to poor sensitivity, the CI– mode was employed.

CI– mass spectra of nitrite and nitrate derivatives are reported in Fig. 1. Et–ONO (Fig. 1a) shows ions at *m/z* 43 (CH_2=CHO^-), *m/z* 45 (CH_3–CH_2O^-) and *m/z* 63, which is due to the formation of a H_2O-adduct with the ion at *m/z* 45 (during the experiment the recommended cartridge for moisture retention in the reagent gas was not used). The (^{18}O)nitrite mass spectrum is reported in Fig. 1c and the notable shift from *m/z* 43 to 45 and from 45 to 47 is the consequence of the ^{18}O isotope replacement. Since the CI– mass spectrum of ethyl nitrite does not show any nitrogen-containing fragments nitrogen labeling is not possible for nitrite. Et–ONO_2 (Fig. 1b) shows ions at *m/z* 46 (NO_2^-) and *m/z* 43 (CH_2=CHO^-). The ^{15}N–labeled compound (Fig. 1d) exhibits *m/z* 47 ($^{15}NO_2^-$) as the most abundant fragment. For isotope dilution quantitation purposes, the signal ratios 45/47 and 46/47 were used for nitrite and nitrate, respectively.

Double spike isotope dilution and analyte interconversion. Despite the numerous methods proposed for the simultaneous determination of nitrite and nitrate, the possibility of their interconversion is seldom considered[14–15]. As discussed previously[10], during the derivatization with triethyloxonium, no conversion of nitrate to nitrite occurs even at high nitrate concentration; however, oxidation of nitrite occurs (< 10%). In this work, attention has been given to minimize the conversion of nitrite to nitrate. This conversion is likely a consequence of the acid hydrolysis of the alkylating agent which promotes the formation of nitrous acid from the nitrite ion. The reaction of the nitrous acid with dissolved oxygen results in the production of nitrate[5–6]. In order to avoid this effect, alkylation was conducted in an alkaline medium (pH = 10) promoted by the pretreatment of the samples with NH_4OH solution. In alkaline environment we observed significant oxidation of nitrite only at high concentrations of nitrite. At 100 mg/L of nitrite, only 0.07% of the nitrite ions converted to nitrate and no conversion was detected below this mass fraction.

Despite these improvements, constant monitoring of potential interconversion is necessary to ensure a valid analytical procedure. This could be achieved by the use of double spiking isotope dilution[16, 17]. In this case, by spiking the sample with (^{18}O)nitrite and (^{15}N)nitrate it is possible to correct for the conversion from nitrite to nitrate by monitoring signals at *m/z* 48 and 50 in the mass spectrum of the ethyl nitrate. Conversion of $N^{18}O_2^-$ into nitrate (with O_2) yields $N^{18}O_2{}^{16}O^-$, whose CI– spectrum features ions $N^{18}O_2^-$ (*m/z* = 50) and $N^{18}O^{16}O^-$ (*m/z* = 48).

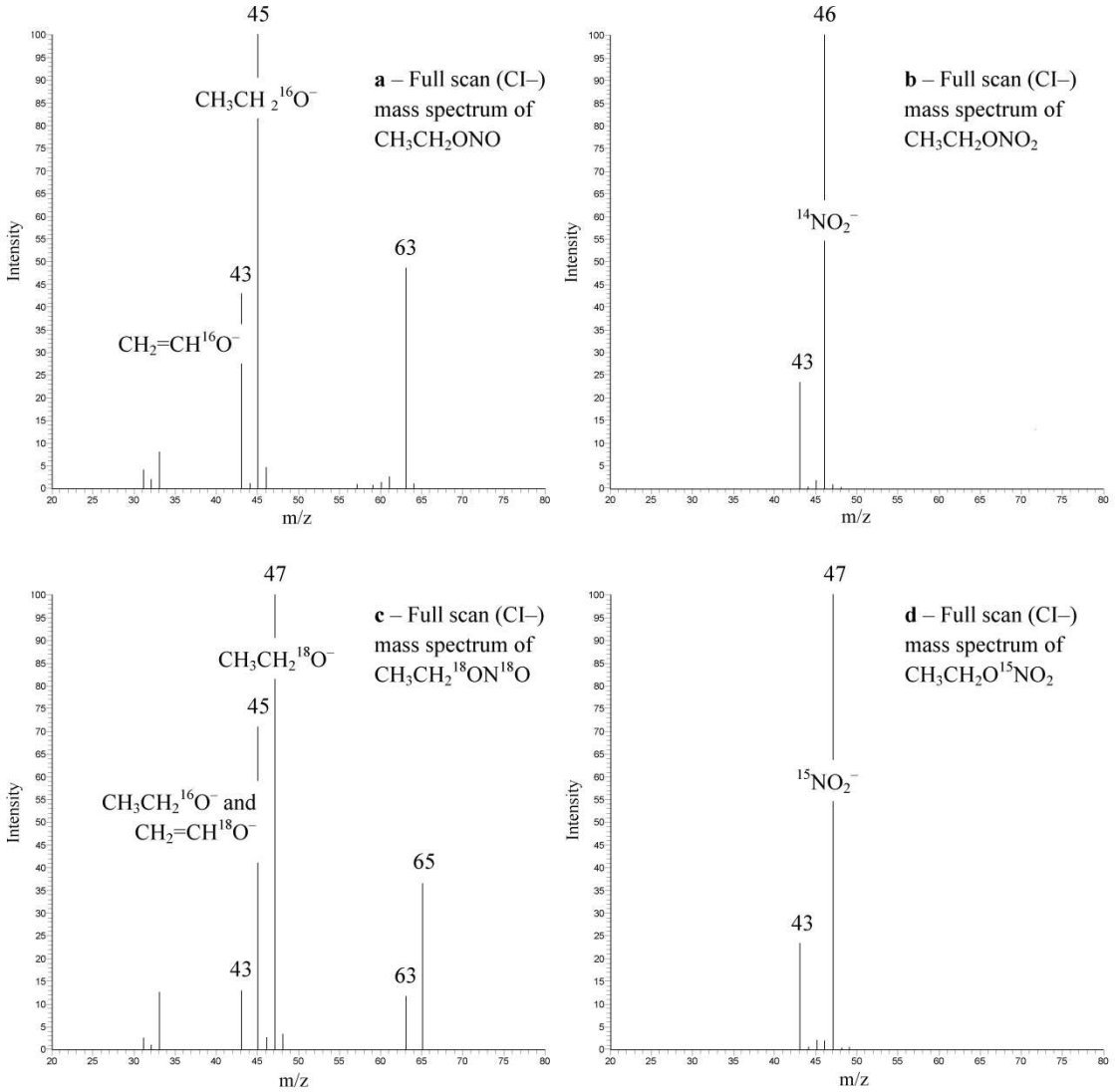


Fig. 1. Negative chemical ionization mass spectra. (a) Ethyl nitrite: *m/z* 43 $CH_2{=}CH^{16}O^-$, *m/z* 45 $CH_3{-}CH_2{}^{16}O^-$, *m/z* 63 $CH_3{-}CH_2{}^{16}O^-{\cdot}H_2O$; (b) ethyl nitrate: *m/z* 43 $CH_2{=}CH^{16}O^-$, *m/z* 46 $^{14}NO_2^-$; (c) ethyl (^{18}O)nitrite: *m/z* 43 $CH_3{=}CH_2{}^{16}O^-$, *m/z* 45 $CH_3{-}CH_2{}^{16}O^-$ and $CH_2{=}CH^{18}O^-$, *m/z* 47 $CH_3{-}CH_2{}^{18}O^-$, *m/z* 63 $CH_3{-}CH_2{}^{16}O^-{\cdot}H_2O$, *m/z* 65 $CH_3{-}CH_2{}^{18}O^-{\cdot}H_2O$; (d) ethyl (^{15}N)nitrate: *m/z* 43 $CH_3{=}CH_2{}^{16}O^-$, *m/z* 47 $^{15}NO_2^-$.

Oxygen scrambling. When an isotopically enriched standard is used for quantitation, it is crucial that any scrambling which can alter the isotope pattern of the enriched spike be avoided. From evidence reported by Klein et al.[18] for nitrate and from the experimental results obtained herein, the oxygen exchange/scrambling between nitrite, nitrate and water may occur. However, no oxygen scrambling occurs in alkaline or neutral conditions for either nitrite or nitrate. Hence, the working pH was chosen to be in the alkaline region (pH = 10) to enable the use of (^{18}O)nitrite for isotope dilution analysis. At the pH = 10, the mass spectrum arising from the (^{18}O)nitrite was constant during the time of the analysis.

Analysis of MOOS-2 CRM. Fig 2 shows the CI– GC–MS chromatogram obtained analyzing nitrite and nitrate in seawater (MOOS-2 CRM). An external calibration curve and an exact matching isotope dilution technique are proposed for quantification purposes. Mass ratios 45/47 and 46/47 plotted against nitrite and nitrate concentration yield R^2 of 0.9998 and 0.9999, respectively based on six calibration points (1-10 µM nitrite, 10-50 µM nitrate). Using external calibration, the analytical results for MOOS-2 CRM sample ($c(NO_2^-)$ = 3.34 ± 0.08 µM and $c(NO_3^-)$ = 22.0 ± 0.1 µM; four independent determinations) are in agreement with the certified property values ($c(NO_2^-)$ = 3.31 ± 0.18 µM and $c(NO_2^-) + c(NO_3^-)$ = 24.9 ± 1.0 µM). The reduction in systematic error can be attributed to the use of the exact matching isotope dilution approach[19]. Results for fourteen independent measurements of MOOS-2 are reported in Table 2 and achieved by the use of the following equation:

$$c_A = c_A^0 \cdot \frac{R_2 - R_N}{R_2 - R_E} \cdot \frac{R_1 - R_E}{R_1 - R_N} \qquad \text{(Eq. 1)}$$

where c_A is the concentration of the analyte in the sample, $c^0{}_A$ is the concentration of the reference analyte (primary standard); R_1 and R_2 are the isotope amount ratios (m/z 45/47 for nitrite and 46/47 for nitrate) arising from the spiked blend of the sample (MOOS-2) and of the spiked blend of the reference, respectively; R_N and R_E are the isotope amount ratios for the sample and the enriched spike, respectively. The isotope patterns of the analyte and the primary standard are identical. Note that there is no need for mass bias correction since all four isotope amount ratios R_1, R_2, R_E, and R_N in Eq.1 can be substituted with the corresponding measured isotope ratios r_1, r_2, r_E, and r_N. The above equation is equivalent to that reported in 1994 by Henrion[19]. Formally, the concentration of the analyte is written as a function of the concentration of a primary standard

(Eq. 1) and of an isotope amount ratios measured. In order to minimize the instrumental measurement biases, both the sample and the reference were spiked with the same amount of the enriched analyte in order to obtain matching amount ratios $R_1 = R_2 = 1$. To realize this, the concentration of the primary standard has to be the same as that of the analyte in the sample. When $R_1 = R_2 = 1$, Eq. 1 reduces to $c_A = c^0_A$ which permits significant reduction in the systematic errors arising from measurements. In addition, prior knowledge of the isotope patterns of spike and analyte is not necessary[19]. From a practical point of view, an initial estimate of the analyte concentration in the sample can be obtained by external calibration method. Subsequently, a primary standard solution is prepared at the same concentration as the analyte in the sample.

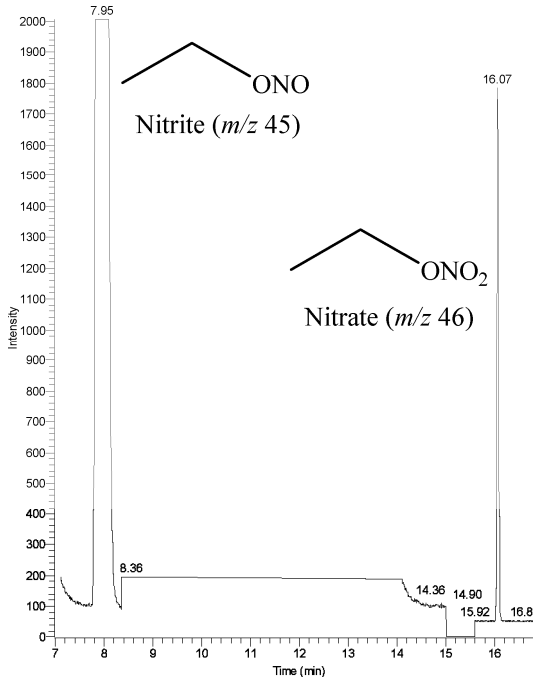


Fig. 2. HS CI– GC–MS chromatogram (SIM mode) of MOOS-2 CRM. The nitrite ion in the form of CH_3CH_2–ONO elutes at 7.95 min (*m/z* 45); The nitrate ion in the form of CH_3CH_2–ONO_2 elutes at 16.07 min (*m/z* 46).

Table 2. Exact matching isotope dilution determination of nitrite and nitrate in MOOS-2[a].

Nitrite				Nitrate			
r_1	r_2	c^0_A/µM	$c_{A,\ MOOS\text{-}2}$/ µM	r_1	r_2	c^0_A/µM	$c_{A,\ MOOS\text{-}2}$/ µM
0.9356			3.31	1.030			22.1
0.9543			3.48	1.028			22.1
0.9325	0.9371	3.32	3.28	1.035	1.007	21.6	22.3
0.9477			3.42	1.025			22.0
0.9255			3.22	1.022			22.0
1.086			3.48	1.003			21.7
1.073			3.38	1.022			22.1
1.068	1.064	3.32	3.35	1.029	0.9993	21.6	22.3
1.086			3.48	1.017			22.0
1.066			3.34	1.016			22.0
0.9565			3.41	1.021			21.9
0.9415	0.9596	3.43	3.29	1.021	1.027	22.0	21.9
0.9450			3.32	1.025			22.0
0.9606			3.44	1.028			22.0
		Mean	**3.37**			**Mean**	**22.0**
		u	**0.08**			***u***	**0.2**
		u_r	**2.5 %**			u_r	**0.7 %**

a. Certified property values: $c(NO_2^-)$ = 3.31 ± 0.18 µM and $c(NO_2^-) + c(NO_3^-)$ = 24.9 ± 1.0 µM.

Table 2 summarizes results for the measurements on MOOS-2 obtained by exact matching. For each measurement, the values of r_1 and r_2 and the concentration of the reference c^0_A are presented. The concentration of nitrite and nitrate found, $c(NO_2^-)$ = 3.37 ± 0.08 µM and $c(NO_3^-)$ = 22.0 ± 0.2 µM, are in good agreement with the certified property values, $c(NO_2^-)$ = 3.31 ± 0.18 µM and $c(NO_2^-) + c(NO_3^-)$ = 24.9 ± 1.0 µM, and exhibit a good precision for both nitrite and nitrate. Instrumental detection limits obtained from the standard solutions of the analytes in water are 150 ng/L for nitrite and 600 ng/L for nitrate. The estimation of the detection limits is done on the signal-to-noise ratio calculated on the standard deviation of the baseline (*i.e.* detection limit is the concentration which produce a signal-to-noise ratio of 3). Standard solutions of 10 µg/L NO_2^- and 50 µg/L NO_3^- produce signal-to-noise ratios of 211 and 327 respectively. In the present procedure, only 250 µL of headspace was used for analysis (*vs* 2 mL of headspace available). In order to further enhance the limits of detection, a purge-and-trap procedure operating at a temperature higher than the ambient should be beneficial.

CONCLUSIONS

The analytical method for the determination of nitrite and nitrate by GC–MS after derivatization to their ethyl esters[10] has been improved in terms of sensitivity and accuracy. Pretreatment of the sample with ammonium hydroxide to ensure alkaline conditions during the alkylation is crucial to avoid conversion of nitrite to nitrate and the negative chemical ionization allows detection limits improvements at least by one order of magnitude for nitrite and nitrate. The vapor generation methodology proposed here is based on exact matching isotope dilution and it is suitable for measurement with high precision and accuracy. This method allows traceability and is promising for application to samples of different origin.

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