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# Quantitative analysis of some inorganic anions (nitrate and nitrite) in metropolitan and bottled water samples using ultra-performance liquid chromatography/electrospray ionization mass spectrometry

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**ABSTRACT**

*N*-Nitroso compounds have been recognized as potent carcinogens and are produced endogenously from drinking water and nutritional supply of nitrate and nitrite. Thus, the screening of nitrate and nitrite has become essential to certify the quality and protection of drinking water. In the present study, a new, rapid and precise technique based on ultra-performance liquid chromatography/electrospray ionization mass spectrometry (UPLC-ESI/MS) has been optimized for the analysis of nitrate and nitrite in drinking water. The nitrate and nitrite were separated using reversed-phase Acquity UPLC™ BEH $C_{18}$ (50 mm × 2.1 mm i.d., 1.7 µm particle size) analytical column at optimum isocratic mobile phase compositions (water/methanol, 25/75, v/v). The established technique was linear ($R^2$ > 0.999) over the working concentration values from 0.010 to 10 mg $L^{-1}$, the run-to-run and day-to-day precisions were <4% ($n$ = 5) in terms of relative standard deviation (RSD, %), when examining a nitrate and nitrite standard mixture of concentration 0.05 mg $L^{-1}$. Nitrate and nitrite detection limits were found to be 0.03 µg $L^{-1}$ and 0.04 µg $L^{-1}$, respectively. The proposed UPLC-ESI/MS technique has been employed effectively for determination of nitrate and nitrite in metropolitan and bottled water samples that have already used in Saudi Arabia. Ten metropolitan and twenty bottled water samples have been examined and result was found in the range of 0.35–9.02 mg $L^{-1}$ for both anions. The recovery rates of nitrate and nitrite were higher than 99% in all of the analyzed water samples.

*Keywords:* Nitrate; Nitrite; Metropolitan water; Bottled water; Ultra-performance liquid chromatography/electrospray ionization mass spectrometry

## 1. Introduction

Drinking water must be sufficiently safe for human consumption or should have a minimal threat of instantaneous or enduring harm [1]. Nitrate and nitrite are naturally occurring ions created by the oxidation of nitrogen in the presence of microbes [2]. They are prevalent in the environment, food, and industrial and physiological systems [3]. The major sources of nitrate and nitrite are wastewater, agricultural activities, discharges from industrial processes, motor vehicles and erosion of natural deposits [3,4]. Nitrates and nitrites are usually considered to be harmful compounds because they can be fatal to humans [5]. Neoplasmic illnesses in humans are linked to *N*-nitroso compounds, several of which have carcinogenic properties in

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experimental animals [6,7]. Excess amounts of nitrate and nitrite in drinking water can lead to potential health threats, together with the risk of methemoglobinemia in infants, which is directly related to the transformation of nitrates into nitrites in the human body [8,9]. These health concerns necessitate highly advanced techniques for the precise screening of nitrate and nitrite concentrations in drinking water. Many countries and environmental regulatory bodies have set the acceptable limits for nitrate and nitrite in drinking water [10]. The United States Environmental Protection Agency (US EPA) and International Bottled Water Association have set the maximum contaminant levels for nitrate (10 mg $L^{-1}$) and nitrite (1 mg $L^{-1}$) in drinking water [11,12]. In 2008, Saudi Arabia was designated as the highest consumer of bottled water at 25.2 gallon per capita [13]. This high consumption revealed that bottled water is considered to be the safest source of drinking water in Saudi Arabia.

In recent years, a number of analytical methods e.g., flow injection analysis [14,15], ion chromatography-UV/DAD [16,17], capillary electrophoresis-UV [18,19], liquid chromatography-UV/chemiluminescence [20,21], and liquid chromatography/electrospray ionization mass spectrometry have been proposed for the analysis of nitrate and nitrite in water samples of varying complexity [22]. The restrictions of the above conventional techniques are analysis time, selectivity and occasionally sensitivity. To overcome these drawbacks, a variety of sensitive and fast techniques based on UPLC-ESI/MS have been reported. For example, ultra-performance liquid chromatography/tandem mass spectrometry has been used for the analysis of bromate content in drinking water [23], UPLC-ESI/MS has been used for the determination of bromate content in non-alcoholic beer [24], and UPLC-ESI/MS has been used for the determination of nitrate content in drinking water [25]. These analytical techniques have been established for the detection of single inorganic compounds either in drinking water or non-alcoholic beer. Owing to the presence of nitrate and nitrite in drinking water and their serious toxicological effects, the development of a fast and sensitive analytical method for the screening of such potentially hazardous contaminants in metropolitan and bottled water is much needed.

In the present study, a simple, fast and sensitive UPLC-ESI/MS method has been developed for the analysis of nitrate and nitrite in metropolitan and commercially available bottled water. The obtained data offers the performance of the proposed method and the amounts of nitrate and nitrite to which the inhabitants is exposed globally.

## 2. Experimental

### 2.1. Chemicals and materials

All solvents and chemicals used in this study were of analytical or HPLC grade, obtained from Merck (Darmstadt, Germany). Sodium nitrate and sodium nitrite of *ReagentPlus*® grade (assay purity ≥ 99.0%) were obtained from Sigma-Aldrich (Steinheim, Germany). Ultrapure water was purified by means of Milli–Q water purification system (Millipore Corporation, Bedford, USA). Stock standard solutions of nitrate and nitrite at concentration level 200 mg $L^{-1}$ were prepared in ultrapure Milli-Q water and utilized for further dilutions processes. Standard mixtures of the nitrate and nitrite at different concentrations were prepared by weight to establish the range of linearity and standard addition in all systems. Standard solutions and water samples were filtered through a 0.22 µm PTFE syringe filter (Macherey-Nagel GmbH, Düren, Germany) before being injected into the UPLC system.

### 2.2. Sample preparation and quantitative analysis

Metropolitan water was obtained from different locations using clear glass bottles (200 mL), supplied by the Saudi Arabian nationalized company Saline Water Conversion Corporation. Bottled water (non-carbonated) from various trademarks was purchased from hypermarket in Riyadh, Saudi Arabia. Metropolitan and bottled samples were refrigerated at 4°C and analyzed in maximum of two days to evade any microbial contamination. In addition, quality control and blank samples were also analyzed in each batch to make certain that contamination of water samples did not arise and detection sensitivity of the target analytes was stable throughout the analysis. The quantification of the target analytes was performed by standard addition procedure consisting of samples spiked with nitrate and nitrite at three (50%, 200% and 500%) levels and a non-spiked sample (duplicate). Recoveries were obtained from the slope obtained when demonstrating the relationship between the added quantity of nitrate and nitrite, and the found quantity.

### 2.3. UPLC-ESI/MS instrumentation and functioning parameters

Chromatographic separation of nitrate and nitrite was carried out using a Waters Acquity® UPLC system (Milford USA) with an Acquity® BEH $C_{18}$ column (50 mm × 2.1 mm i.d., 1.7 µm particle size) (Waters, Milford, USA) column. A pre-column, VanGuard™ BEH $C_{18}$ 1.7 µm was used to protect the analytical column during the analysis. The most favourable chromatographic separation of nitrate and nitrite was obtained using mobile phase water/methanol, (25/75, v/v) at an isocratic flow rate of 200 µL $min^{-1}$. The temperature of column was set to 25°C and the total UPLC run time was 2 min. The injection volume of the sample was 5 µL.

The detection of nitrate and nitrite was performed on triple quadrupole mass spectrometer, Quattro Premier™ (Micromass, Milford, USA) equipped with an electrospray ionization source (Z–spray) coupled with an Acquity® UPLC system. The instrument was operated in negative ionization mode. The data acquisition was carried out in selected ion recording (SIR) mode which monitors the ions *m/z* 62 for nitrate and *m/z* 46 for nitrite. Factors affecting the ion transmission parameters were optimized by infusing a standard mixture of sodium nitrate and sodium nitrite solution at 10 mg $L^{-1}$. The optimized working conditions were as follows: cone voltage, 50 V; capillary voltage, 3.2 kV; source temperature, 120°C; desolvation temperature, 300°C; cone gas flow rate, 60 L $h^{-1}$; desolvation gas flow rate, 600 L $h^{-1}$. Nitrogen (99.99% purity), generated with a Peak Scientific nitrogen generator, model NM30LA (Inchinann, United Kingdom), was used as cone gas. Argon (99.99%

purity), obtained from Speciality Gas Centre (Jeddah, Saudi Arabia), was used as collision gas. The vacuum for the mass spectrometer was provided with a rotary pump, Oerlikon, model SOGEVAC SV40 BI (Paris, France). The SIR parameters used with the MS system have been given in Table 1. The processing and data acquisition were performed using MassLynx V4.1 software.

## 3. Results and discussion

### 3.1. Optimisation of UPLC conditions

At the present time, UPLC has been recognized to be an advanced separation method which improves the chromatographic performance of traditional liquid chromatography in terms of sensitivity, analysis speediness and resolution [26,27]. Previously, we have individually optimized the UPLC methods for the determination of bromate and nitrate in different types of matrices [23–25]. Therefore, the advancement of a high throughput method for the separation of nitrate and nitrite using UPLC is essential. The most important goal of the chromatographic technique was to attain separation and determination of nitrate and nitrite with a short run time including sensitivity. At first, the isocratic UPLC conditions were optimised for nitrate and nitrite. The isocratic flow was chosen as an approach in order to evade the time consumed in conditioning the column after each run and distinct of gradient flow programs. Preliminary experiments were carried out on hydrophobic stationary phases $C_8$, $C_{18}$ columns. In addition, the Hydrophilic Interaction Chromatography (HILIC) was also investigated by a column with stationary phase of amide groups. The mobile phase for instance methanol, acetonitrile and water at different proportions were optimised at flow rate between 100 µL $min^{-1}$ and 500 µL $min^{-1}$. The consequence of the addition of organic modifier formic acid (0.1%–1%) in the mobile phase was also investigated. The poor peak shape and longer retention times of nitrate and nitrite were obtained with $C_8$ and HILIC column. The addition of formic acid as an organic modifier in the mobile phase did not advance the peak shape distinct similar to that was obtained in the previous analysis of bromate concerning with a reversed-phase column [23] and the neutral pH of mobile phase produces the Gaussian peaks. The optimal chromatographic separation was accomplished on an Acquity® BEH $C_{18}$ column with dimension 50 mm × 2.1 mm i.d., 1.7 µm particle size, using a mobile phase consisting of water/methanol (25/75, v/v)in isocratic elution at flow rate 200 µL $min^{-1}$. Comparatively, low flow rate was found to be most favourable for the analysis of nitrate and nitrite. At low flow rate, ionic evaporation and efficient desolvation in the electrospray ionization source were favourable giving rise the adequate defining of a chromatographic peak ample as a minimum 15 scan points in their analysis. From these parameters, the elution time for nitrate and nitrite was less than 1 min. The column dead volume was 0.2 min which confirmed that the low interaction between hydrophobic stationary phase and polar anion. At the best possible chromatographic conditions, Fig. 1 demonstrates the elution of nitrate and nitrite standards mixture with Acquity® BEH $C_{18}$ column.

Table 1
SIR parameters used with the MS method

| Analyte | Molecular formula | MS parameters* | |
|---|---|---|---|
| | | Molecular ion, *m/z* | Cone voltage, V |
| Nitrate | $NO_3^-$ | 62.20 | 40 |
| Nitrite | $NO_2^-$ | 46.20 | 46 |

*Dwell time was 0.025 s in both cases.

### 3.2. Optimisation of the ESI-MS conditions

To desolvate effectively the mixture of organic and aqueous mobile phase, and to provide utmost target compounds response in the determination of water samples, the electrospray ionization mass spectra were primarily optimized by infusing mixture of nitrate and nitrite standard. Full scan mode was used to choose the most abundant ion from nitrate and nitrite while SIR was applied for its detection with superior sensitivity. As illustrated in Fig. 1, the resulting two ions correspond to nitrate (*m/z* 62) and nitrite (*m/z* 46). The electrospray ionization conditioned related with the desolvation and transmission of the analyte ions for instance, cone voltage (10–100 V), capillary voltage (2.0–4.5 kV), source temperature (80–150°C), desolvation temperature (250–450°C) and desolvation gas (300–700 L $h^{-1}$) were studied. The ESI/MS parameters that offered the best sensitivity were provided in UPLC-ESI/MS instrumentation and functioning parameters section.

### 3.3. Method validation

#### 3.3.1. Linearity

The performance of the optimised UPLC-ESI/MS system was evaluated. To illustrate the linearity of the method for nitrate and nitrite, the standard solutions of concentrations varying from 0.01 to 10 mg $L^{-1}$ were injected. The correlation co-efficient values were calculated for nitrate and nitrites and found to be greater than 0.997 and 0.999, respectively, which showed the excellent relationship between the analyte amount and peak area.

#### 3.3.2. Limits of detection and quantification

The limit of detection (LOD) and limit of quantification (LOQ) were achieved by means of signal to noise approach. The obtained LOD (signal-to-noise ratio, 3:1) of nitrate and nitrite were 0.03 µg $L^{-1}$ and 0.04 µg $L^{-1}$, respectively, whereas LOQ (signal-to-noise ratio, 10:1) of nitrate and nitrite were found to be 0.1 µg $L^{-1}$ and 0.15 µg $L^{-1}$, respectively. The LOD and LOQ values were determined by determining five replicates of a blank sample (Milli–Q water) spiked with sodium nitrate and sodium nitrite at low amounts. The blank sample (Milli–Q water) was analysed without spiking and found to be free from nitrate and nitrite.

#### 3.3.3. Precision

UPLC-ESI/MS method precision was assessed by injecting five different concentrations of the system appropriateness solution and measured the relative standard deviation (RSD, %) for each analyte. The run-to-run precision was

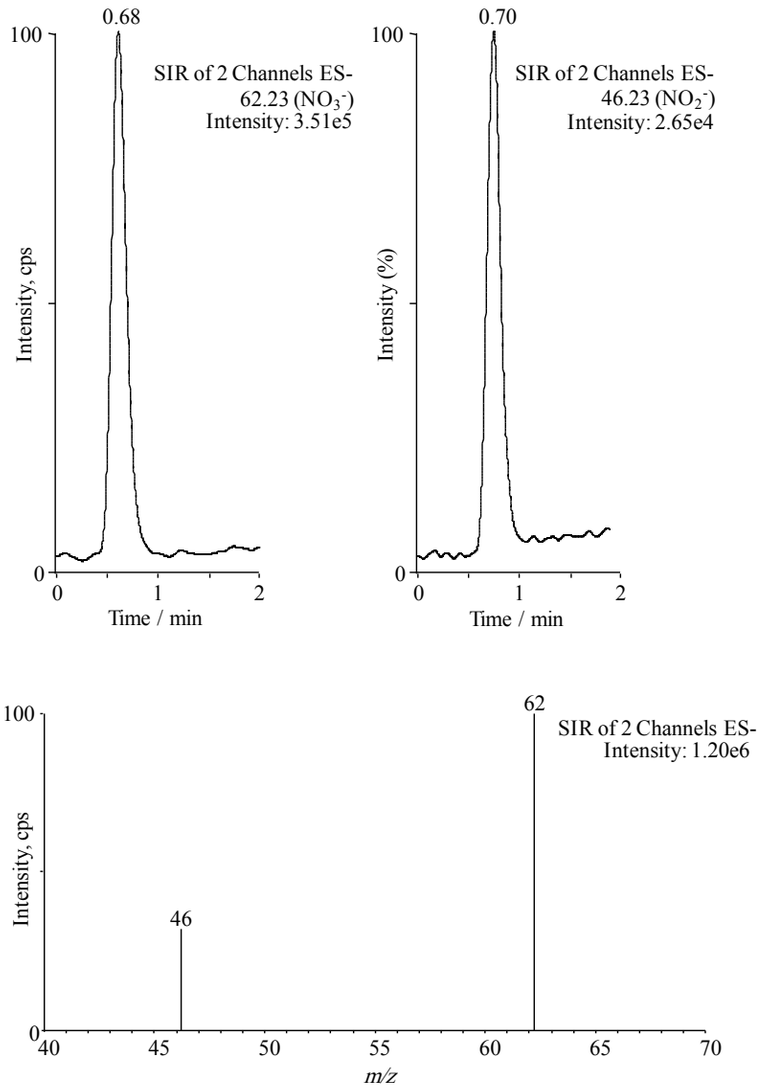


Fig. 1. UPLC-ESI/MS chromatograms of nitrate and nitrite standard mixture (0.5 mg $L^{-1}$), and spectrum *m/z* values 62 (nitrate) and 46 (nitrite).

approximated from five replicate injections of nitrate and nitrite standard solutions (0.05 mg $L^{-1}$) on the same day. Day-to-day precision was determined by five replicate injections of the aforesaid standard solutions along three successive days. These determinations were determined on the basis of the estimation of RSD values of the peak area. The run-to-run precision for nitrate and nitrite were obtained between 2.01% and 1.52%, respectively, while the day-to-day precision for nitrate and nitrite were found between 3.20% and 2.64%, respectively. The obtained precision by the proposed UPLC-ESI/MS technique for the determination of low amounts of nitrate and nitrite was acceptable.

### *3.3.4. Accuracy*

Recovery rates were performed to authenticate the accuracy of the proposed UPLC-ESI/MS technique. The standard addition quantification method has been evaluated to verify the effect of the sample matrix on the signal-to-noise ratio of nitrate and nitrite. A total of thirty independent metropolitan and bottled drinking water samples were examined and the recovery rates for nitrate and nitrite were obtained between 95% and 99% (Tables 2 and 3). The low matrix effect has been observed and can be in part because of the Z-configuration of the ionization source, which not permitted the access of neutral analytes in the MS system and chromatographic parameters. These outcomes specify that the matrix does not amend the target analyte signal in such type of water samples and that external calibration can be applied as quantification method.

The optimized UPLC-ESI/MS technique deals with an important issue such as symmetrical peak shape with no tailing. Even though, the efficient separation is not definitely required in MS detection, nevertheless, it gives

additional improvement in selectivity and sensitivity of the quantitative and qualitative analysis [28].

#### 3.3.5. UPLC–ESI/MS versus conventional techniques

Inorganic ions usually have greater retention with ion chromatography (IC) and/or HILIC than with the reversed phase liquid chromatography [29,30]. The IC and HILIC phases have hydrophilic moieties; IC interrelate with anions by ion-exchange process and measures amounts of ionic species by separating them based on their interaction with a resin [29]. The HILIC offers partition and electrostatic interaction, and the separation method is derived from the differential supply of the injected compound between the organic mobile phase and water enhanced layer adsorbed onto the hydrophilic stationary phase [30]. Nevertheless, revered phase merely retains nitrate and nitrite through hydrophobic interaction. In previous studies, many authors have separated various anions, among them nitrate and nitrite that have revealed higher retention and separation time as high as eight minutes [31,32]. Nevertheless, a common drawback of IC is that the mobile phase typically contains a non-volatile salt leading to not well-suited with mass spectrometric detection system. Furthermore, the mixed mode phases with hydrophilic interaction, ion-exchange and reversed-phase were incompatible parameters for mass spectrometric detection system [33]. A variety of analytical methods for the quantification of nitrate and nitrite in water/food have been developed for instance capillary electrophoresis in determination of nitrate and nitrite in water. Study showed that the detection limits of 0.010 mg $L^{-1}$ for nitrates and 0.003 mg $L^{-1}$ for nitrites with analysis time of about 8 min [18]. Another method based reversed phase high performance liquid chromatography/diode array detector used to quantify nitrate and nitrite in ham, the detection limits were 0.019 and 0.050 mg $kg^{-1}$, respectively, with analysis time of about 5 min [34]. Recently, a reversed-phase liquid chromatography-electrospray ionization/mass spectrometry method has been applied for the analysis of nitrate and nitrite in water, the detection limits were 1 µg $L^{-1}$ and 12 µg $L^{-1}$, respectively, with analysis time of 12 min [22]. Relatively, these conventional methods have limited sensitivity and selectivity even higher analysis time. In present work an UPLC-ESI/MS method has been optimized to achieve a fast analysis of nitrate and nitrite in isocratic eluent mode, with the total run time of 2 min and the high selectivity and sensitivity offered by the MS detector that lead to attain a detection limit of 0.03 µg $L^{-1}$ and 0.04 µg $L^{-1}$ with a very insignificant matrix effect. These outcomes meet the sensitivity needed for the quantification of nitrate and nitrite in drinking water as recognized by the EPA [11].

### 3.5. Application to water sample analysis

The proposed UPLC-ESI/MS technique was applied to the analysis of nitrate and nitrite in several water samples. Three replicates of each water sample were used to assess the mean amounts of nitrate and nitrite. From the obtained results, the quantification of nitrate and nitrite was not affected by the investigated water sample matrices. Therefore, sample pre-treatment before UPLC-ESI/MS analysis was not necessary in comparative to the traditional techniques since sample pre-treatment steps would raise the solvent use, analysis time, inconsistency and significant losses of analysed compounds and therefore sensitivity. Ten metropolitan water samples were achieved from various locations of Saudi Arabia and were sanitized using hypochlorite. The obtained results are demonstrated in Table 2. Amounts of nitrate and nitrite determined in metropolitan water samples were between 0.75 mg $L^{-1}$ and 4.52 mg $L^{-1}$ with more than 99% recovery rates. The nitrate concentrations were lower as established by the EPA [11]. However, the nitrite concentrations were little bit higher as regulated by EPA in drinking water [11]. Nitrate and nitrite were also analysed in commercial bottled drinking water which were sterilized with ozone. The achieved nitrate and nitrite concentrations were between 0.46 mg $L^{-1}$ and 9.02 mg $L^{-1}$ displaying recover rates between 95% and 99%, and amounts claimed on label have been presented in Table 3. As an example, Fig. 2 illustrates the UPLC-ESI/MS chromatograms of nitrate and nitrite and their related SIR spectra of bottled water sample (11). Relatively, the amounts of nitrate and nitrite in bottled water were found at higher concentrations. The nitrate

Table 2
Amounts of nitrate, nitrite, and recovery rates in metropolitan water samples analyzed with UPLC-ESI/MS method

| Metropolitan water* | Water source | Nitrate[b]/mg $L^{-1}$ | Recovery, % | Nitrite[b]/mg $L^{-1}$ | Recovery, % |
|---|---|---|---|---|---|
| Sample 1 | Desalinated + well water | 3.83 ± 0.03 | 97 | 1.21 ± 0.04 | 97 |
| Sample 2 | Desalinated water | 4.12 ± 0.03 | 99 | 1.27 ± 0.04 | 98 |
| Sample 3 | Desalinated + well water | 3.95 ± 0.03 | 98 | 1.23 ± 0.04 | 98 |
| Sample 4 | Desalinated water | 1.73 ± 0.04 | 95 | 0.80 ± 0.05 | 95 |
| Sample 5 | Desalinated water | 1.98 ± 0.04 | 97 | 0.91 ± 0.05 | 96 |
| Sample 6 | Desalinated + well water | 2.22 ± 0.04 | 98 | 1.10 ± 0.05 | 97 |
| Sample 7 | Desalinated water | 3.45 ± 0.03 | 99 | 1.15 ± 0.05 | 96 |
| Sample 8 | Desalinated + well water | 4.52 ± 0.03 | 99 | 1.35 ± 0.04 | 99 |
| Sample 9 | Desalinated water | 1.45 ± 0.04 | 97 | 0.75 ± 0.06 | 98 |
| Sample 10 | Desalinated + well water | 2.33 ± 0.03 | 96 | 1.12 ± 0.05 | 97 |

*Water samples were pre-treated with hypochlorite and collected from various places; b. Mean ± standard deviation, n = 3.

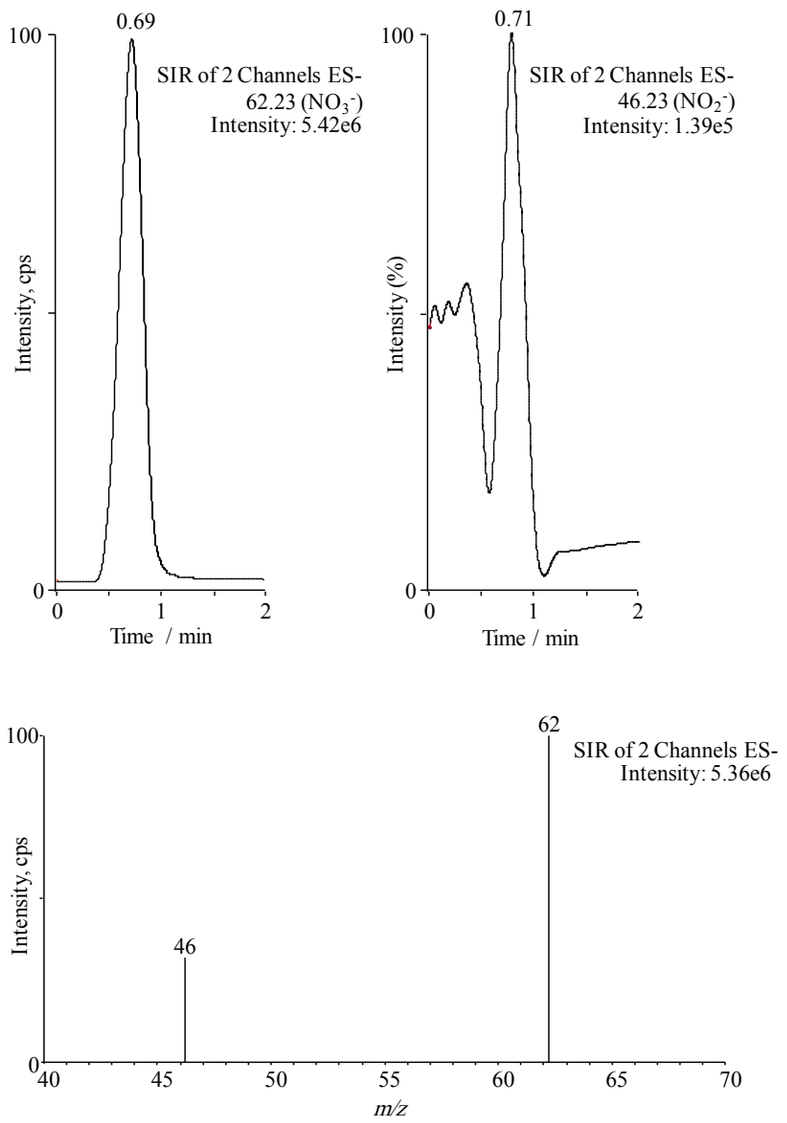


Fig. 2. UPLC-ESI/MS chromatograms of nitrate and nitrite in bottled water sample 11, and spectrum *m/z* values 62 (nitrate) and 46 (nitrite).

concentrations were below the concentrations established by EPA [11], and nitrite concentrations in some samples were greater than the levels regulated by EPA [11]. The analysis of blanks samples (Milli-Q water) were carried out repeatedly which signified that cross contamination by nitrate and nitrite throughout the analysis. These outcomes are the data source on the occurrence of nitrate and nitrite in metropolitan and bottled water in Saudi Arabia.

## 4. Conclusions

A method based on UPLC–ESI/MS has been optimized for the analysis of nitrate and nitrite drinking water. The proposed method has demonstrated to be speedy with two minutes analysis time, sensitive with a detection limit of nitrate and nitrite were 0.03 μg $L^{-1}$ and 0.04 μg $L^{-1}$, respectively, and accurate with run-to-run precision up to 2.01% and day-to-day precision up to 3.20%. The high sensitivity of the method and nonappearance of matrix effects observed in the water sample analysis have made possible to analyse nitrate and nitrite, and provide benefits over traditional methods. In addition, without sample pre-treatment prior to analysis made possibility to quantify nitrate and nitrite with external calibration. The performance of method as well as the outcomes found in the determination of metropolitan and bottled drinking water samples make promising to offer a new methodology based on reversed-phase UPLC-ESI/MS for its routine analysis in drinking water samples. The obtained data could be used to evaluate the human intake of nitrate and nitrite in Saudi Arabia, and thus to improve the water quality and safety.

Table 3
Levels of nitrate, nitrite, and recovery rates in bottled water samples obtained with UPLC-ESI/MS method

| Bottled water[a] | Water source | Nitrate[b]/ mg $L^{-1}$ | Nitrate claimed on bottle label/ mg $L^{-1}$ | Recovery, % | Nitrite[b]/mg $L^{-1}$ | Nitrite claimed on bottle label/ mg $L^{-1}$ | Recovery, % |
|---|---|---|---|---|---|---|---|
| Sample 1 | Well water | 4.76 ± 0.02 | 4.0 | 96 | 1.34 ± 0.03 | – | 97 |
| Sample 2 | – | 0.35 ± 0.04 | <0.1 | 97 | 0.55 ± 0.05 | – | 96 |
| Sample 3 | Well water | 2.68 ± 0.02 | 2.6 | 95 | 0.87 ± 0.05 | – | 98 |
| Sample 4 | Well water | 3.51 ± 0.01 | 3.7 | 97 | 1.37 ± 0.03 | – | 99 |
| Sample 5 | – | 0.41 ± 0.02 | <0.1 | 98 | 0.64 ± 0.05 | – | 96 |
| Sample 6 | Well water | 1.03 ± 0.03 | <1.0 | 96 | 0.47 ± 0.05 | – | 98 |
| Sample 7 | – | 3.27 ± 0.02 | – | 98 | 1.16 ± 0.03 | – | 99 |
| Sample 8 | Well water | 4.52 ± 0.02 | 4.00 | 98 | 0.46 ± 0.05 | – | 98 |
| Sample 9 | – | 8.73 ± 0.01 | 5.0 | 98 | 2.79 ± 0.02 | – | 97 |
| Sample 10 | – | 1.51 ± 0.03 | <1 | 97 | 0.56 ± 0.05 | – | 98 |
| Sample 11 | Well water | 6.93 ± 0.01 | 3.0 | 96 | 3.46 ± 0.02 | – | 96 |
| Sample 12 | Well water | 9.02 ± 0.01 | 7.0 | 95 | 3.01 ± 0.02 | – | 97 |
| Sample 13 | – | 3.81 ± 0.02 | 3.08 | 98 | 1.33 ± 0.02 | – | 98 |
| Sample 14 | – | 2.72 ± 0.02 | 2.0 | 97 | 1.25 ± 0.02 | – | 98 |
| Sample 15 | Well water | 4.87 ± 0.02 | 3.0 | 98 | 1.35 ± 0.02 | – | 98 |
| Sample 16 | – | 1.42 ± 0.03 | <0.1 | 99 | 0.62 ± 0.05 | – | 97 |
| Sample 17 | – | 2.75 ± 0.02 | 2.0 | 98 | 1.52 ± 0.03 | – | 99 |
| Sample 18 | Well water | 3.89 ± 0.02 | 3.0 | 99 | 1.22 ± 0.03 | – | 98 |
| Sample 19 | Well water | 4.79 ± 0.02 | 5.0 | 99 | 1.31 ± 0.03 | – | 97 |
| Sample 20 | Well water | 2.86 ± 0.02 | 3.5 | 98 | 1.10 ± 0.03 | – | 98 |

*Bottled water were sterilized with ozone before packaging; – not defined; b. Mean ± standard deviation, n = 3.

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**References**

[1] G. Alison, "Healthy living-Water", BBC Health, Retrieved 2007-02-19.
[2] Health Canada, Guidelines for Canadian Drinking Water Quality: Guideline Technical Document—Nitrate and Nitrite. Water and Air Quality Bureau, Healthy Environments and Consumer Safety Branch, Health Canada, Ottawa, Ontario, (2013) (Catalogue No H144-13/2-2013E PDF).
[3] M.J. Moorcroft, J. David, R.G. Compton, Detection and determination of nitrate and nitrite: A review, Talanta, 54 (2001) 785–803.
[4] R. Michalski, I. Kurzyca, Determination of nitrogen species (nitrate, nitrite and ammonia ions) in environmental samples by ion chromatography, P. J. Environ. Stud., 15 (2006) 5–18.
[5] M.I. Helaleh, T. Korenaga, Ion chromatographic method for simultaneous determination of nitrate and nitrite in human saliva, J. Chromatogr. B, 744 (2000) 433–437.
[6] S.S. Mirvish, Formation of N-nitroso compounds: chemistry, kinetics, and in vivo occurrence, Toxicol. App. Pharmacol., 31 (1975) 325–351.
[7] K.P. Cantor, Drinking water and cancer, Cancer Causes Control, 8 (1997) 292–308.
[8] L.B. Dusdieker, J.P. Getchell, T.M. Iiarakos, W.J. Hausler, C.I. Dungy, Nitrate in baby foods: adding to the nitrate mosaic, Arch. Pediatr. Adolesc. Med., 148 (1994) 490–494.
[9] J. Sanchez-Echaniz, J. Benito-Fernandez, S. Mintegui-Raso, Methemoglobinemia and consumption of vegetables in infants, Pediatrics, 107 (2001) 1024–1028.
[10] EC, European Commission Council Directive 98/83/EC on the quality of water intended for human consumption, 1998.
[11] EPA, Environmental Protection Agency, National Primary Drinking Water Regulations, 2009, EPA 816-F-09-004, http://www.epa.gov/ogwdw/consumer/pdf/mcl.pdf.
[12] IBWA, International Bottled Water Association, Code of practice. Revised version, (2005) 1-30, http://www.bottledwater.org/public/pdf/IBWA05ModelCodeMar2.pdf.
[13] J.G. Rodwan, Confronting challenges. U.S. and International Bottled Water Developments and Statistics, (2008) 18.
[14] L. Monser, S. Sadok, G.M. Greenway, I. Shah, R.F. Uglow, A simple simultaneous flow injection method based on phosphomolybdenum chemistry for nitrate and nitrite determinations in water and fish samples, Talanta, 57 (2002) 511–518.
[15] F.R.P. Rocha, B.F. Reis, A flow system exploiting multicommutation for speciation of inorganic nitrogen in waters, Anal. Chim. Acta, 409 (2002) 227–235.
[16] J.S. Fritz, D.T. Gjerde, Wiley-VCH; Weinheim, (2000) 247–260.
[17] P. Niedzielski, I. Kurzyca, J. Siepak, A new tool for inorganic nitrogen speciation study: Simultaneous determination of ammonium ion, nitrite and nitrate by ion chromatography with post-column ammonium derivatization by Nessler reagent and diode-array detection in rain water samples, Anal. Chim. Acta, 577 (2006) 220–224.
[18] E. Martínková, T. Křížek, P. Coufal, Determination of nitrites and nitrates in drinking water using capillary electrophoresis, Chem. Pap., 68 (2014) 1008–1014.
[19] A. Padarauskas, V. Paliulionyte, B. Pranaityte, Single-run capillary electrophoretic determination of inorganic nitrogen species in rainwater, Anal. Chem., 73 (2001) 267–271.

[20] R.G. Gerritse, Rapid simultaneous determination of nitrate and nitrite by high-performance liquid chromatography using ultraviofet detection, J. Chromatogr. A, 171 (1979) 527–529.
[21] H. Kodamatani, S. Yamazaki, S. Keiitsu, T. Tomiyasu, Y. Komatsu, Selective determination method for measurement of nitrite and nitrate in water samples using high-performance liquid chromatography with post-column photochemical reaction and chemiluminescence detection, J. Chromatogr. A, 1216 (2009) 3163–3167.
[22] 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.
[23] I.H. Alsohaimi, Z.A. Alothman, M.R. Khan, M.A. Abdalla, R. Busquets, A.K. Alomary, Determination of bromate in drinking water by ultraperformance liquid chromatography-tandem mass spectrometry, J. Sep. Sci., 35 (2012) 2538–2543.
[24] M.R. Khan, Z.A. Alothman, N.J. Alqahtani, I.H. Alsohaimi, M. Naushad, Quantitative analysis of bromate in non-alcoholic beer using ultra performance liquid chromatography-electrospray ionization mass spectrometry, Anal. Methods, 6 (2014) 4038–4045.
[25] M.R. Khan, Z.A. Alothman, M.A. Khan, R. Busquets, I.H. Alsohaimi, An ultra performance liquid chromatography-electrospray ionization-mass spectrometry method for the rapid analysis of nitrate in drinking water, Anal. Methods, 5 (2013) 1225–1230.
[26] R. Plumb, J. C. Perez, J. Granger, I. Beattie, K. Joncour, A. Wright, Ultra-performance liquid chromatography coupled to quadrupole-orthogonal time-of flight mass spectrometry, Rapid Commun. Mass Spectrom., 18 (2004) 2331–2337.
[27] M.E. Swartz, UPLC™: An Introduction and Review, J. Liq. Chromatogr. Relat. Technol., 28 (2005) 1253–1263.
[28] L. Novakova, A. Vildova, J.P. Mateus, T. Gonçalves, P. Solich, Development and application of UHPLC–MS/MS method for the determination of phenolic compounds in chamomile flowers and chamomile tea extracts, Talanta, 82 (2010) 1271–1280.
[29] J. Weiss, T. Weiss, Handbook of Ion Chromatography, 3rd ed.; John Wiley and Sons Inc.; New York, (2005) pp 931, ISBN: 3-527-28701-9.
[30] P. Hemström, K. Irgum, Hydrophilic interaction chromatography, J. Sep. Sci., 29 (2006) 1784–1821.
[31] D. Connolly, B. Paull, Rapid determination of nitrate and nitrite in drinking water samples using ion-interaction liquid chromatography, Anal. Chim. Acta, 441 (2001) 53–62.
[32] Y. Zuo, C. Wang, T. Van, Simultaneous determination of nitrite and nitrate in dew, rain, snow and lake water samples by ion-pair high-performance liquid chromatography, Talanta, 70 (2006) 281–285.
[33] H. Qiu, A.K. Mallik, M. Takafuji, S. Jiang, H. Ihara, New poly(ionic liquid)-grafted silica multi-mode stationary phase for anion-exchange/reversed-phase/hydrophilic interaction liquid chromatography, Analyst, 137 (2012) 2553–2555.
[34] I.M.P.L.V.O. Ferreira, S. Silva, Quantification of residual nitrite and nitrate in ham by reverse-phase high performance liquid chromatography/diode array detector, Talanta, 74 (2008) 1598–1602.