Method for the fast determination of bromate, nitrate and nitrite by ultra performance liquid chromatography–mass spectrometry and their monitoring in saudi arabian drinking water with chemometric data treatment

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ABSTRACT

A rapid, sensitive and precise method for the determination of bromate (BrO₃⁻), nitrate (NO₃⁻) and nitrite (NO₂⁻) in drinking water was developed with Ultra performance Liquid Chromatography–Mass Spectrometry (UPLC-ESI/MS). The elution of BrO₃⁻, NO₃⁻ and NO₂⁻ was attained in less than two minutes in a reverse phase column. Quality parameters of the method were established; run-to-run and day-to-day precisions were <3% when analysing standards at 10 µg L⁻¹. The limit of detection was 0.04 µg NO₂⁻ L⁻¹ and 0.03 µg L⁻¹ for both NO₃⁻ and BrO₃⁻. The developed UPLC-ESI/MS method was used to quantify these anions in metropolitan water from Saudi Arabia (Jeddah, Dammam and Riyadh areas) and commercial bottled water (from well or unknown source) after mere filtration steps. The quantified levels of NO₃⁻ were not found to pose a risk. In contrast, BrO₃⁻ was found above the maximum contaminant level established by the US Environmental Protection Agency in 25 and 33% of the bottled and metropolitan waters, respectively. NO₂⁻ was found at higher concentrations than the aforementioned limits in 70 and 92% of the bottled and metropolitan water samples, respectively. Therefore, remediation measures or improvements in the disinfection treatments are required. The concentrations of BrO₃⁻, NO₃⁻ and NO₂⁻ were mapped with Principal Component analysis (PCA), which differentiated metropolitan water from bottled water through the concentrations of BrO₃⁻ and NO₃⁻ mainly. Furthermore, it was possible to discriminate between well water; blend of well water and desalinated water; and desalinated water. The point or source (region) was found to not be distinctive.
INTRODUCTION

Water disinfection is an important step to ensure that water can be consumed safely. Typical disinfection methods to destroy pathogens include treatment with chemical reactive agents (i.e. chlorine, chlorine dioxide, chloramines, ozone, and potassium permanganate) or physical means (i.e. irradiation with UV or nanofiltration) [1]. Side effect of some these treatments are the generation of disinfection by-products (DBPs) when disinfection agents react with substance already present naturally in the water to be treated. The risks to health of DBPs is considered to be low compared to the exposure to pathogens [2]. Despite that non-chemical disinfection methods would avoid DBPs, small doses of chlorine or mono-chloramine are added to water to ensure its safety after distribution [2].

Bromide, which is a majority element in seawater (i.e. 67 mg/L) [3], is present in water used in the production of metropolitan drinking water in Saudi Arabia. Bromate (BrO$_3^-$) is a DBP that can be generated from the ozonation of naturally occurring bromide present in such source water [2,4]. Therefore, desalinated seawater can lead to high levels of BrO$_3^-$ due to remaining levels of its precursor before the oxidising treatment [5]. In a previous study carried out by the authors, BrO$_3^-$ was found between 8-75 µg L$^{-1}$ in desalinated water [6]. BrO$_3^-$ was found to be carcinogenic in animals which revealed the need to control this substance in drinking water [7]. In 1998, the International Agency of Research Cancer (IARC) listed the BrO$_3^-$ in Group 2B (possibly carcinogenic to humans) [8]. Thereafter, the World Health Organization (WHO) and US Environmental Protection Agency (EPA) set up provisional guideline value and
a maximum contaminant level (MCL), respectively, at 10 μg BrO₃⁻ L⁻¹ in drinking water [2,4,9] and the public health goal at “zero” [4].

Nitrate (NO₃⁻) and nitrite (NO₂⁻) have a different origin than BrO₃⁻ in drinking water. In nature, both NO₃⁻ and NO₂⁻ derive from the nitrogen cycle in plants and soils; they originate from the microbial digestion of nitrogen rich sources such as plant tissues, faeces or nitrogen-based fertilisers [10-11]. Both NO₃⁻ and NO₂⁻ are highly soluble species that can consequently leach to surface and ground water [12-14]. This has detrimental effects on biodiversity as well as on human beings; for instance; it can cause eutrophication [2, 10]; methemoglobinemia disease in infants [15-18] and they have been associated with increased incidence of cancer [10]. Many environmental regulatory organizations have set the guidelines for NO₃⁻ and NO₂⁻ in drinking water. The EPA has set the Maximum Contaminant Level (MCL) for NO₃⁻ at 10 mg L⁻¹ in drinking water [4], which coincides with the standard of quality in bottled water set by the International Bottled Water Association (IBWA) [19]. In contrast the WHO and European Commission set up the guideline level at 50 mg L⁻¹ [2, 20]. For NO₂⁻, the EPA and IBWA has set the MCL and standard of quality, respectively, at 1 mg L⁻¹ in drinking water [4,19]; the current WHO guideline value is 3 mg L⁻¹ [2] but the limit in Europe is more restrictive: 0.5 mg L⁻¹ [20].

Monitoring studies are necessary to know the level of exposure to BrO₃⁻, NO₃⁻ and NO₂⁻ and identify potential hotspots. Many analytical procedures have been developed to assess the levels of BrO₃⁻, NO₃⁻ and NO₂⁻ in drinking water. Ion chromatography is recognized as the method with best analytical achievable for the determination of BrO₃⁻ by WHO [2]; ion chromatography-conductivity detection method has been extensively applied for the analysis of such types of compounds in water matrices [21-22]. The low running cost of capillary zone
electrophoresis and typical high separation efficacy makes it also an attractive technique for the analyses of anions in drinking water [23]. Improvements in stationary phases that lead to the development of Ultra Performance Liquid Chromatography (UPLC) implied gains in analysis throughput and sensitivity compared to earlier methods. UPLC, in combination with the superior confirmatory capability of mass spectrometry (MS), made UPLC-MS a technique of choice. Earlier works by our group showed the potential of UPLC-MS for the individual analysis of BrO$_3^-$ and NO$_3^-$ [6, 24], however ion suppression made not possible the simultaneous analysis of such similar anions. A method suitable for the fast determination BrO$_3^-$, NO$_3^-$ and NO$_2^-$ has been developed in this work. The developed method will be applied to quantify the potential hazardous species in metropolitan and bottled water samples from the Kingdom of Saudi Arabia. The levels of the study anions will be examined with Principal Component Analysis (PCA), for the first time to our knowledge, to establish whether BrO$_3^-$, NO$_3^-$ and NO$_2^-$ can be used to identify the type of sample (desalinated water; well water; desalinated and ground water) from different Saudi regions and possible counterfeit bottled water.

2. Materials and methods
2.1 Materials. All solvents and chemicals used in this study were of HPLC or analytical grade, obtained from Merck (Darmstadt, Germany). Potassium bromate (ACS reagent, ≥99.8%), and, 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 BrO$_3^-$, NO$_3^-$ and NO$_2^-$ at concentration level 500 mg L$^{-1}$ were prepared in ultrapure Milli-Q water and used for further dilutions. Standard mixtures of the nitrate and nitrite were prepared by weight. 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 (500 mL) supplied by the Saudi Arabian nationalised company Saline Water Conversion Corporation. Bottled water (non–carbonated), from various trademarks, was purchased from hypermarket in Riyadh, Saudi Arabia. These bottled water samples had been treated with ozone. Metropolitan and bottled samples were stored in refrigerator at 4°C and analysed within two days to avoid microbial growth. Blank (ultrapure water) and quality control samples were analysed in each batch to ascertain that contamination of water samples did not arise and detection sensitivity of the target analytes was stable throughout the analysis. The quantification was carried out by external calibration in triplicate and the quantification of the recovery rates was carried out with standard addition method consisting of samples spiked with BrO$_3^-$, NO$_3^-$ and NO$_2^-$ at four (50, 200, 400 and 500%) levels in addition to non-spiked samples (duplicate). Recovery rates were obtained from the slope obtained when
plotting the correlation between the added amount of bromate, nitrate and nitrite, and the amount found.

2.3 UPLC-ESI/MS analysis. The chromatographic analysis of $\text{BrO}_3^-$, $\text{NO}_3^-$ and $\text{NO}_2^-$ 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 optimal chromatographic conditions for the analysis of $\text{BrO}_3^-$, $\text{NO}_3^-$ and $\text{NO}_2^-$ was obtained using isocratic elution mode consisting of 75% methanol in water (v/v) at a flow rate of 200 µL min$^{-1}$. The temperature of the analysis was controlled in an oven at 25 ºC. The sample injection volume was 5 µL. A column with polar stationary phase Water Acquity® BEH Amide column (50 mm × 2.1 mm i.d., 1.7 µm particle size) was also tested.

The detection of $\text{BrO}_3^-$, $\text{NO}_3^-$ and $\text{NO}_2^-$ was performed on Quattro Premier™ triple quadrupole mass spectrometer (Micromass, Milford, USA) with an electrospray ionization source (Z–spray) coupled with an Acquity® UPLC system. The instrument was operated in negative ionization mode. The data acquisition in full scan mode (m/z 40-200) was used to select the most abundant ions from each analyte. Selected Ion Recording (SIR) was applied for their detection with higher sensitivity. Dwell time was 0.025 s and the total scan cycle was 1 s. Factors affecting the ion transmission parameters were optimized by infusing a standard mixture of sodium nitrate, potassium bromate and sodium nitrite solution at 10 mg L$^{-1}$. The optimized working parameters were as follows. Cone voltage: 40; 46; 40; 38 V for m/z 46.2 ($\text{NO}_3^-$), m/z 62.2 ($\text{NO}_2^-$), m/z 129.0 ($^{81}\text{BrO}_3^-$) and m/z 127.0 ($^{79}\text{BrO}_3^-$), respectively. Capillary voltage 3.2 kV; source temperature, 120ºC; desolvation temperature, 300ºC; cone gas flow rate, 60 L h$^{-1}$; desolvation
gas flow rate, 600 L h\(^{-1}\). Nitrogen (99.99% purity), produced 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 primary vacuum for the mass spectrometer was provided with an Oerlikon rotary pump, model SOGEVAC SV40 BI (Paris, France). The data acquisition and processing were performed using MassLynx V4.1 software.

Quality parameters of the optimised UPLC-MS method were assessed. Linearity was measured between 0.1 and 1000 µg·L\(^{-1}\); limits of detection (LOD) were established at a signal-to-noise ratio of 3; Run–to–run precision was estimated from six replicate injections of a mixture of BrO\(_3^−\), NO\(_3^−\) and NO\(_2^−\) standard at 0.05 µg·mL\(^{-1}\) in the same day, and day–to–day precision was measured from six replicate injections of the same standard along three consecutive days.

2.4 Chemometric analysis.

The Unscrambler® X version 10.3 (CAMO Software AS., Norway) was used to build a Principal Components Analyses (PCA) model from the concentration of BrO\(_3^−\), NO\(_3^−\) and NO\(_2^−\) in metropolitan and bottled waters. In order to give the same weight to every value (BrO\(_3^−\) was found at µg·L\(^{-1}\) level in contrast with the other two analytes, which were present at levels of mg·L\(^{-1}\)), BrO\(_3^−\) concentrations were given a weight of 1000 times higher than its quantified values. Validation of PCA models relied on the leave-one-out cross-validation, in which each particular sample was predicted by using the remaining samples as standards for building the calibration model.
3. Results and discussion

3.1 Optimisation of UPLC conditions. The separation of \( \text{BrO}_3^- \), \( \text{NO}_3^- \) and \( \text{NO}_2^- \) with reverse phase columns (C\(_8\), C\(_{18}\)) was challenging because the interaction of the 3 anions with the stationary phase was weak and very similar among them. Their behaviour was dominated by their net negative charge, which becomes stabilised by solvation in the mobile phase. Higher level of retention was intended when using Hydrophilic Interaction Chromatography (HILIC) with amide groups in the stationary phase. Mobile phase compositions, constituted with methanol/acetonitrile in water at different proportions (0-100%), were tested at flow rate between 100 and 500 µL min\(^{-1}\). The addition of formic acid (0.1 – 1%) in the mobile phase was also investigated as a way to shield the charge of the analytes or displace the equilibria towards nitrous acid (case of \( \text{NO}_2^- \)) and achieve higher retention for the neutral species. All the conditions studied lead to very limited or no separation among \( \text{BrO}_3^- \), \( \text{NO}_3^- \) and \( \text{NO}_2^- \). The HILIC column (with mobile phase methanol: water 50:50, v/v) made possible to achieve slightly higher retention for nitrite and nitrate (retention time 0.80 and 0.82 min, respectively), however the symmetry factor of the peaks led to limited peak height and sensitivity and for that reason that column was discarded. In terms of retention and separation in reversed phase chromatography, the percentage of organic solvent in the mobile phase did not cause a great effect on the retention of \( \text{NO}_3^- \) and \( \text{NO}_2^- \). \( \text{BrO}_3^- \) presented the lowest retention of the three and its signal was affected by the composition of the mobile phase. In a previous work, a mobile phase of water with 0.1% formic acid in water, at 200 µL min\(^{-1}\), led to a retention time of 0.4 min. Despite that the added acid reduced peak tailing, the peak asymmetry factor for \( \text{BrO}_3^- \) (measured at 10% of the peak height) was 1.1 [6]. In this work, a mobile phase of 75% methanol in water, in absence of formic acid, led to higher retention (0.7 minute) and improved peak
symmetry (tailing factor: 1.0) at 200 μL min⁻¹, being the dead volume 0.1 min at these conditions. Peak symmetry and separation from the dead volume was poorer with a C₈ column when compared to a C₁₈. The latter, an Acquity® BEH C₁₈ column with dimension 50 mm × 2.1 mm i.d., 1.7 μm particle size, with a mobile phase consisting of methanol/water (75:25, v/v) in isocratic elution at flow rate 200 μL min⁻¹, was adopted as the optimal conditions which lead to the chromatogram shown in Figure 1. The chromatographic peaks from the analytes were not resolved but their co-elution was not found to affect their detection. Acetonitrile was not found advantageous with respect to the results achieved with methanol. Comparatively, low flow rates, which favoured ionic evaporation and efficient desolvation in the electrospray ionization source, were found to be advantageous for the analysis of the anions. At 200 μL min⁻¹, chromatographic peaks presented peak width that could be defined with a minimum of 15 scan points and analysis time of just 1 min.

3.2 Optimisation of the MS conditions and UPLC-MS quality parameters.

The electrospray (ESI) ionization conditions related with desolvation, ionic evaporation and transmission of the analyte ions were investigated: 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). The ESI/MS parameters that offered the best sensitivity are provided in section 2.1. Temperatures and source gases in the higher range were found to provide higher sensitivity because these favoured ionic evaporation. However, the high percentage of organic solvent in the mobile phase (75%) and relative low flow rate prevented needing extreme settings for optimal sensitivity.
The relationship between the concentration of the analytes and their response was assessed across the range 0.1-1000 µg L\(^{-1}\) and was found to be lineal; calibration curves with \(r^2 < 0.999\) were obtained in all cases and \(t\) correlation test confirmed the correlation (\(P 0.05\)). The working range was limited to 0.1-100 µg L\(^{-1}\) given that the concentrations of the analytes were expected in that range. Quality parameters obtained under optimal conditions are given in Table 1. The instrumental limit of detection (S/N=3) for NO\(_2^-\) was 0.04 µg L\(^{-1}\) and for NO\(_3^-\) and BO\(_3^-\) was 0.03 µg L\(^{-1}\). Run-to-run and day-to-day precisions were <3% (\(n = 6\)) for the three anions when analyzing a standard mixture solutions at 10 µg L\(^{-1}\). The sensitivity achieved for NO\(_3^-\) did not decrease when compared to its individual analysis with UPLC-MS [24]. It improved previous LC-MS method using reversed phase chromatography where LODs for nitrate and nitrite were 1 µg L\(^{-1}\) and 12 µg L\(^{-1}\) [25], respectively. The sensitivity of the developed method was, however, about 10 times poorer than a non-routine method recently developed based on spectroscopic detection assisted by graphene oxide modified with amino groups and gold nanoparticles [26]. Furthermore, the detection of bromate improved by 10 times previous results with UPLC-MS [6]. This improvement can be attributed to better ionic evaporation achieved with the higher proportion of organic solvent in the mobile phase. The sensitivity reached was also superior to the levels achieved with completely different approaches: ionic chromatography-conductivity detection; liquid chromatography-inductively coupled plasma/ mass spectrometry detection; capillary electrophoresis and mass spectrometry detection by 2-25 times [27-29]. An analysis time of just 1.5 minutes for BrO\(_3^-\), NO\(_3^-\) and NO\(_2^-\), without need of column preconditioning due to isocratic conditions, made the optimised method as one the fastest and most sensitive methods available to the best of our knowledge.
3.3 Determination of BrO$_3^-$, NO$_3^-$ and NO$_2^-$ in bottled and metropolitan samples from Saudi Arabia.

Reporting the concentration of the potentially hazardous anions in drinking water is important to identify improvements needed in the production of drinking water. A total of 32 samples of different origin from the Kingdom of Saudi Arabia were analysed by external calibration and standard addition. High recovery rates (94-99\%) were found for the 3 analytes in both metropolitan and bottled waters (Table 2 and 3), which indicates that ion suppression in the ESI or losses in the filtration step were marginal. Therefore, external calibration is an appropriate way to carry out the quantification.

In metropolitan water, the concentration range found for BrO$_3^-$ was 5.83-13.45 µg L$^{-1}$; for NO$_2^-$, 0.83-1.57 mg L$^{-1}$; and for NO$_3^-$, 1.65-4.61 NO$_3^-$ (quantification shown in Table 2). The higher levels of BrO$_3^-$ were found in desalinated water which had not been blended with well water. This is because well water may dilute the species (Br$^-$) that would subsequently be oxidised to BrO$_3^-$. In contrast, desalinated water contained among the lowest levels NO$_3^-$ and NO$_2^-$ compared to well water, which may have received higher level of leachates from the degradation of nitrogen rich sources.

Among the relatively low number of metropolitan water samples analysed and given in Table 2, 33\% contained BrO$_3^-$ levels above the 10 µg L$^{-1}$ target by the WHO, US EPA and Gulf standards for metropolitan water in Saudi Arabia (P 0.05) [4,30]. In previous works >70\% of a limited number of samples analysed were higher than the 10 µg L$^{-1}$ [6, 28]. In contrast, the levels of NO$_3^-$ were all below the EPA limit of 10 mg L$^{-1}$ (P 0.05) [4]. However, 92\% of the samples had NO$_2^-$ levels above the EPA MCL for NO$_2^-$ (1 mg L$^{-1}$, P 0.05) [4] despite all of them complying with the Gulf standards (3 mg/L NO$_2^-$)[30].
Among the bottled water samples, 25% presented higher BrO$_3^-$ concentration than the WHO and EPA targets (P 0.05) [2,4]; all the samples were below the MCL for NO$_3^-$ [4]; and 70% where above the EPA limit for NO$_2^-$ (P 0.05) [4] or entirely over the Gulf standards for bottled water (0.2 mg L$^{-1}$NO$_2^-$) [31]. Previous work carried out in Saudi Arabia showed a broad range of concentration for these compounds: 8% [32]; 17% [6]; and 60% of the samples had higher BrO$_3^-$ [28] than the EPA MCL. The levels of NO$_2^-$ detected in this research are about two time the highest level of nitrite detected (0.38 mg L$^{-1}$) in a recent comprehensive study which monitored 145 wells in Makkah City [33] and also contrasts with the low detection rate of NO$_2^-$ in the assessment of 571 European bottled mineral waters (< 8% had levels above 0.01 mg L$^{-1}$) [34]. Another important difference with these European samples is that whereas 37% of the samples had NO$_3^-$ > 2.6 mg L$^{-1}$ [34], only 15% of the samples in our equivalent study (Table 3) reached such high level. Oxidation conditions prior bottling water and ammonia-related levels before oxidation can be the origin of such differences. Our data shows that NO$_3^-$ is at non problematic levels; this is in agreement with other recent studies in Saudi Arabia found that all the samples tested were below the EPA goal [24] while others showed that it is still of concern since 20% of the samples presented higher NO$_3^-$ than the EPA limits [32]. A study where 388 wells of 6 regions of the Kingdom of Saudi Arabia were investigated, much higher concentrations of nitrate were found: 8% of the wells had NO$_3^-$ > 45 mg L$^{-1}$ [35]. Our study shows both NO$_2^-$ and BrO$_3^-$ are problematic in both metropolitan and bottled water. The levels of these toxicants in bottled water are especially relevant because this type of water is typically used to prepare infant formulas. Remediation measures to decrease Br$^-$ and nitrogenated compounds in water sources; optimising the disinfection conditions currently applied and selecting those (concentration, pH, duration) that would make possible effective disinfection,
reducing the formation of BrO$_3^-$ and increase the oxidation of NO$_2^-$ to NO$_3^-$ (keeping both below the regulated levels); or blending types of water to lower the levels of these potential toxicants are recommendable.

3.4 Mapping bottle and metropolitan waters with PCA model built from BrO$_3^-$, NO$_3^-$ and NO$_2^-$ levels in samples from Saudi Arabia.

A PCA model was built from the quantified BrO$_3^-$, NO$_3^-$ and NO$_2^-$ levels in metropolitan and bottled water samples given in Tables 2 and 3. The data was represented using axis, PC, which better explain the variation among the data. The scores plot (Figure 2), which classifies the samples based on the concentration of the anions, revealed patterns that were related with the sample characteristics. The samples were mainly distributed along PC1 (which explains 77%) of the variation. Metropolitan water appeared distributed mainly in the first 2 quarters of the plot, whereas bottled water appeared mainly in the second half of the plot. However, there were some bottled water samples appearing in the region where metropolitan water predominated. It could be hypothesised that these bottled samples contained metropolitan water.

Figure 3 shows the distribution of the variables (loading plot). When correlating the scores plot (Figure 2) with the loading plot (Figure 3), it can be observed that BrO$_3^-$, which is located at one extreme of PC1, was the variable causing the main differentiation among samples along PC1. In contrast, NO$_3^-$ is the variable responsible for the distribution of the samples along PC2. Therefore metropolitan samples were mainly described by the levels of BrO$_3^-$ (which is in agreement with higher level of this ion in desalinated water. Bottled water (mainly from well water and thus with input of nitrogenated species from organic matter degradation) appeared mostly distributed along PC2. A more detailed PC model giving details of the type of water and
its origin is shown in Figure 4. Metropolitan water from the same geographical region were not
grouped, however those samples from desalinated water were distinctively separated from
samples containing both desalinated water and well water, and from well water only, along PC1.

Bottled water samples number 12, 13, 16 and 20 did not have their origin in their label.
According to our model, these samples could be bottled metropolitan water. The bottled water
samples number 2, 4 and 6 appeared in the interface between metropolitan water and bottled well
water. These appeared labelled as well water, however their levels of \( \text{BrO}_3^- \) (>8 µg L\(^{-1}\)) were in
the higher range of their type (Table 3). Based on the position of these samples in the PCA
model, it could be hypothesized that these bottled water samples could contain a blend of well
water and desalinated water.

4. Conclusions

- The UPLC-ESI/MS method developed for the determination of \( \text{BrO}_3^- \), \( \text{NO}_3^- \) and \( \text{NO}_2^- \),
  with an analysis time of just 1.5 min; high sensitivity 0.03-0.04 µg/L; high precision
  (<3%) and recoveries (>94%) is advantageous for monitoring drinking water.

- The analysis of \( \text{BrO}_3^- \), \( \text{NO}_3^- \) and \( \text{NO}_2^- \) in 20 bottled water samples and in 12
  metropolitan water samples from different sites in the Kingdom of Saudi Arabia showed
  that the levels of \( \text{BrO}_3^- \) and \( \text{NO}_2^- \) were in many cases above recommended levels.
  Specifically,
  the concentration of \( \text{BrO}_3^- \) was found higher than the US EPA MCL in 25 and 33% of
  the bottled and metropolitan waters, respectively. The levels of \( \text{NO}_2^- \) were higher than
  the US EPA MCL in 70 and 92% of the bottled and metropolitan water samples,
  respectively, and all samples were below the EPA limits for \( \text{NO}_3^- \). These results indicate
that remediation measures/disinfection conditions need to be further optimised with view to the DBPs generated.

- A PCA model using BrO₃⁻, NO₃⁻ and NO₂⁻ concentrations showed capacity to discriminate between desalinated water; well water; and desalinated water blended with well water in Saudi Arabia. The level of bromate was the main variable making possible the distinction among drinking water samples. NO₃⁻ had less influence in mapping the samples and mainly described the bottled drinking waters. The geographical sampling site was not useful to classify the metropolitan drinking water samples.

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Determination of bromate in drinking water by ultraperformance liquid chromatography–


Figure captions

Figure 1. UPLC-ESI/MS chromatograms and spectra of NO$_2^-$, BrO$_3^-$, NO$_3^-$ in bottled water (sample n°1 in Table 3). The chromatographic conditions were isocratic (75% methanol in water (v/v)) at a flow rate of 200 µL min$^{-1}$, 25 °C. The column used was an Acquity® BEH C$_{18}$ (50 mm × 2.1 mm i.d., 1.7 µm particle size).

Figure 2. PCA scores plot obtained from metropolitan and bottled waters as a function of BrO$_3^-$, NO$_3^-$ and NO$_2^-$ concentrations.

Figure 3. PCA loading plot showing the contribution of the variables in the model.
Figure 4. Scores plot showing the distribution of metropolitan and bottled water samples. The metropolitan water sampling site and type of water (desalinated; well; blend of well and desalinated water; and unknown (?)) appear indicated in the sample name. The number in brackets corresponds to the sample number listed in Tables 2 and 3. The region of the plot comprising metropolitan water samples has been circled. A zoom into closely distributed samples is provided.
Table 1. Quality parameters obtained with the optimised UPLC-ESI/MS method.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>LOD (µg L(^{-1}))</th>
<th>LOQ (µg L(^{-1}))</th>
<th>Run-to-run precision (n=6), RSD (%)</th>
<th>Day-to-day precision (n=6), RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\text{NO}_3^-)</td>
<td>0.030</td>
<td>0.092</td>
<td>1.3</td>
<td>2.6</td>
</tr>
<tr>
<td>(\text{NO}_2^-)</td>
<td>0.039</td>
<td>0.12</td>
<td>1.5</td>
<td>2.9</td>
</tr>
<tr>
<td>(^{81}\text{BrO}_3^-)</td>
<td>0.029</td>
<td>0.092</td>
<td>1.4</td>
<td>2.7</td>
</tr>
<tr>
<td>(^{79}\text{BrO}_3^-)</td>
<td>0.040</td>
<td>0.12</td>
<td>1.6</td>
<td>3.1</td>
</tr>
</tbody>
</table>

Table 2. Concentrations of \(\text{NO}_2^-\), \(\text{NO}_3^-\) and \(^{81}\text{BrO}_3^-\) in metropolitan water samples and recoveries (R) obtained in their determination with UPLC-ESI/MS

<table>
<thead>
<tr>
<th>Metropolitan water (sample(^n))</th>
<th>Water source</th>
<th>(\text{NO}_2^-) (mg L(^{-1})) ± R (%)</th>
<th>(\text{NO}_3^-) (mg L(^{-1})) ± R (%)</th>
<th>(^{81}\text{BrO}_3^-) (µg L(^{-1})) ± R (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jeddah (1)</td>
<td>Desalinated + well water</td>
<td>1.37±0.04 95</td>
<td>4.11±0.03 95</td>
<td>5.83±0.03 96</td>
</tr>
<tr>
<td>Jeddah (2)</td>
<td>Desalinated + well water</td>
<td>1.41±0.04 97</td>
<td>4.08±0.03 97</td>
<td>6.35±0.02 97</td>
</tr>
<tr>
<td>Jeddah (3)</td>
<td>Desalinated water</td>
<td>1.35±0.04 96</td>
<td>4.35±0.03 97</td>
<td>9.31±0.01 97</td>
</tr>
<tr>
<td>Jeddah (4)</td>
<td>Desalinated water</td>
<td>0.89±0.05 95</td>
<td>2.02±0.04 94</td>
<td>13.45±0.01 98</td>
</tr>
<tr>
<td>Dammam (5)</td>
<td>Desalinated + well water</td>
<td>1.32±0.04 98</td>
<td>2.41±0.04 97</td>
<td>8.67±0.02 98</td>
</tr>
<tr>
<td>Dammam (6)</td>
<td>Desalinated + well water</td>
<td>1.49±0.04 95</td>
<td>4.61±0.03 97</td>
<td>9.78±0.01 97</td>
</tr>
<tr>
<td>Bottled water (number)</td>
<td>Water source</td>
<td>NO$_2^-$ (mg L$^{-1}$)</td>
<td>mean± SD</td>
<td>R$^b$ (%)</td>
</tr>
<tr>
<td>----------------------</td>
<td>-------------</td>
<td>------------------------</td>
<td>----------</td>
<td>----------</td>
</tr>
<tr>
<td>1</td>
<td>Well water</td>
<td>1.59 ±0.04</td>
<td>99</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Well water</td>
<td>0.89 ±0.05</td>
<td>98</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Well water</td>
<td>1.52 ±0.04</td>
<td>96</td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Concentrations of NO$_2^-$, NO$_3^-$ and BrO$_3^-$ in bottled water samples and recoveries obtained in their determination with UPLC-ESI/MS.

$^a$Treated with hypochlorite disinfectant and obtained from different locations

$^b$ R: Recovery

$^c$ SD = standard deviation (n = 3)

Dammam (7) Desalinated water 0.97±0.05 95 2.14±0.04 96 12.35±0.01 94

Dammam (8) Desalinated water 1.29±0.04 94 3.65±0.03 96 7.80±0.02 98

Riyadh (9) Desalinated + well water 1.57±0.04 98 2.53±0.03 95 8.63±0.02 96

Riyadh (10) Desalinated + well water 1.32±0.04 95 2.45±0.03 95 10.76±0.01 95

Riyadh (11) Desalinated + well water 1.33±0.04 96 2.53±0.03 97 7.98±0.02 98

Riyadh (12) Desalinated water 0.83±0.05 97 1.65±0.04 96 11.54±0.01 98

Dammam (7) Desalinated water 0.97±0.05 95 2.14±0.04 96 12.35±0.01 94

Dammam (8) Desalinated water 1.29±0.04 94 3.65±0.03 96 7.80±0.02 98

Riyadh (9) Desalinated + well water 1.57±0.04 98 2.53±0.03 95 8.63±0.02 96

Riyadh (10) Desalinated + well water 1.32±0.04 95 2.45±0.03 95 10.76±0.01 95

Riyadh (11) Desalinated + well water 1.33±0.04 96 2.53±0.03 97 7.98±0.02 98

Riyadh (12) Desalinated water 0.83±0.05 97 1.65±0.04 96 11.54±0.01 98

Riyadh (9) Desalinated + well water 1.57±0.04 98 2.53±0.03 95 8.63±0.02 96

Riyadh (10) Desalinated + well water 1.32±0.04 95 2.45±0.03 95 10.76±0.01 95

Riyadh (11) Desalinated + well water 1.33±0.04 96 2.53±0.03 97 7.98±0.02 98

Riyadh (12) Desalinated water 0.83±0.05 97 1.65±0.04 96 11.54±0.01 98

Riyadh (9) Desalinated + well water 1.57±0.04 98 2.53±0.03 95 8.63±0.02 96

Riyadh (10) Desalinated + well water 1.32±0.04 95 2.45±0.03 95 10.76±0.01 95

Riyadh (11) Desalinated + well water 1.33±0.04 96 2.53±0.03 97 7.98±0.02 98

Riyadh (12) Desalinated water 0.83±0.05 97 1.65±0.04 96 11.54±0.01 98

Riyadh (9) Desalinated + well water 1.57±0.04 98 2.53±0.03 95 8.63±0.02 96

Riyadh (10) Desalinated + well water 1.32±0.04 95 2.45±0.03 95 10.76±0.01 95

Riyadh (11) Desalinated + well water 1.33±0.04 96 2.53±0.03 97 7.98±0.02 98

Riyadh (12) Desalinated water 0.83±0.05 97 1.65±0.04 96 11.54±0.01 98
<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>0.56 ±0.05</td>
<td>98</td>
<td>0.97 ± 0.05</td>
<td>99</td>
<td>8.52 ± 0.02</td>
</tr>
<tr>
<td>5</td>
<td>0.58 ±0.05</td>
<td>99</td>
<td>4.65 ±0.03</td>
<td>99</td>
<td>2.35 ± 0.04</td>
</tr>
<tr>
<td>6</td>
<td>3.46 ±0.02</td>
<td>99</td>
<td>6.54 ±0.02</td>
<td>98</td>
<td>10.16 ±0.01</td>
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<tr>
<td>7</td>
<td>3.22 ±0.03</td>
<td>98</td>
<td>9.65 ±0.01</td>
<td>96</td>
<td>6.60 ± 0.02</td>
</tr>
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<td>8</td>
<td>1.62 ±0.04</td>
<td>99</td>
<td>5.21 ±0.02</td>
<td>99</td>
<td>3.60 ± 0.03</td>
</tr>
<tr>
<td>9</td>
<td>1.36 ±0.04</td>
<td>98</td>
<td>3.45 ±0.03</td>
<td>97</td>
<td>5.59 ± 0.03</td>
</tr>
<tr>
<td>10</td>
<td>1.45 ±0.04</td>
<td>98</td>
<td>5.32 ±0.03</td>
<td>99</td>
<td>7.42 ± 0.02</td>
</tr>
<tr>
<td>11</td>
<td>1.32 ±0.04</td>
<td>99</td>
<td>3.78 ±0.03</td>
<td>97</td>
<td>3.65 ± 0.03</td>
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<tr>
<td>12</td>
<td>–</td>
<td>98</td>
<td>0.41 ±0.05</td>
<td>99</td>
<td>11.42 ±0.01</td>
</tr>
<tr>
<td>13</td>
<td>–</td>
<td>99</td>
<td>0.53 ±0.05</td>
<td>98</td>
<td>11.96 ±0.01</td>
</tr>
<tr>
<td>14</td>
<td>–</td>
<td>99</td>
<td>4.22 ±0.03</td>
<td>98</td>
<td>2.82 ± 0.04</td>
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<tr>
<td>15</td>
<td>–</td>
<td>99</td>
<td>7.65 ±0.02</td>
<td>99</td>
<td>4.02 ± 0.03</td>
</tr>
<tr>
<td>16</td>
<td>–</td>
<td>98</td>
<td>1.45 ±0.04</td>
<td>99</td>
<td>11.40 ±0.01</td>
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<tr>
<td>17</td>
<td>–</td>
<td>99</td>
<td>3.52 ±0.03</td>
<td>97</td>
<td>3.41 ± 0.03</td>
</tr>
<tr>
<td>18</td>
<td>–</td>
<td>99</td>
<td>2.65 ±0.03</td>
<td>97</td>
<td>4.97 ± 0.03</td>
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<tr>
<td>19</td>
<td>–</td>
<td>97</td>
<td>1.33 ±0.04</td>
<td>99</td>
<td>3.42 ± 0.03</td>
</tr>
<tr>
<td>20</td>
<td>–</td>
<td>99</td>
<td>2.80 ±0.03</td>
<td>98</td>
<td>10.74 ±0.01</td>
</tr>
</tbody>
</table>

\^a Unknown source of water  
\^b R: Recovery  
\^c SD = standard deviation (n = 3)

**HIGHLIGHTS:**

- Analysis of \( \text{BrO}_3^- \), \( \text{NO}_3^- \) and \( \text{NO}_2^- \) in 1.5 min by UPLC-MS
- Monitoring of \( \text{BrO}_3^- \), \( \text{NO}_3^- \) and \( \text{NO}_2^- \) in Saudi Arabia drinking water
- PCA analysis discriminates among types of metropolitan and bottled water
Figure 1. UPLC-ESI/MS chromatograms and spectra of NO$_2^-$, BrO$_3^-$, NO$_3^-$ in bottled water (sample n°1 in Table 3). The chromatographic conditions were isocratic (75% methanol in water (v/v)) at a flow rate of 200 µL min$^{-1}$, 25 ºC. The column used was an Acquity® BEH C$_{18}$ (50 mm × 2.1 mm i.d., 1.7 µm particle size).
Figure 2. PCA scores plot obtained from metropolitan and bottled waters as a function of $\text{BrO}_3^-$, $\text{NO}_3^-$ and $\text{NO}_2^-$ concentrations.

Figure 3. PCA loading plot showing the contribution of the variables in the model.
Figure 4. Scores plot showing the distribution of metropolitan and bottled water samples. The metropolitan water sampling site and type of water (desalinated, well, blend of well and desalinated water, and unknown (?)) appear indicated in the sample name. The number in brackets corresponds to the sample number in Tables 2 and 3. The region of the plot comprising metropolitan water samples has been circled. A zoom into closely distributed samples is provided.
Graphical Abstract (for review)