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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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1 Method for the fast determination of bromate, nitrate and nitrite by Ultra Performance Liquid
2 Chromatography–Mass Spectrometry and their monitoring in Saudi Arabian drinking water with
3 chemometric data treatment.

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28 ABSTRACT

29 A rapid, sensitive and precise method for the determination of bromate (BrO_3^-), nitrate (NO_3^-)
30 and nitrite (NO_2^-) in drinking water was developed with Ultra performance Liquid
31 Chromatography–Mass Spectrometry (UPLC-ESI/MS). The elution of BrO_3^- , NO_3^- and NO_2^-
32 was attained in less than two minutes in a reverse phase column. Quality parameters of the
33 method were established; run-to-run and day-to-day precisions were $<3\%$ when analysing
34 standards at $10 \mu\text{g L}^{-1}$. The limit of detection was $0.04 \mu\text{g NO}_2^- \text{L}^{-1}$ and $0.03 \mu\text{g L}^{-1}$ for both
35 NO_3^- and BrO_3^- . The developed UPLC-ESI/MS method was used to quantify these anions in
36 metropolitan water from Saudi Arabia (Jeddah, Dammam and Riyadh areas) and commercial
37 bottled water (from well or unknown source) after mere filtration steps. The quantified levels of
38 NO_3^- were not found to pose a risk. In contrast, BrO_3^- was found above the maximum
39 contaminant level established by the US Environmental Protection Agency in 25 and 33% of the
40 bottled and metropolitan waters, respectively. NO_2^- was found at higher concentrations than the
41 aforementioned limits in 70 and 92% of the bottled and metropolitan water samples,
42 respectively. Therefore, remediation measures or improvements in the disinfection treatments are
43 required. The concentrations of BrO_3^- , NO_3^- and NO_2^- were mapped with Principal Component
44 analysis (PCA), which differentiated metropolitan water from bottled water through the
45 concentrations of BrO_3^- and NO_3^- mainly. Furthermore, it was possible to discriminate between
46 well water; blend of well water and desalinated water; and desalinated water. The point or source
47 (region) was found to not be distinctive.

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51 KEYWORDS: bromate; nitrate; nitrite; UPLC-MS; Saudi Arabia; drinking water

52
53 INTRODUCTION

54
55 Water disinfection is an important step to ensure that water can be consumed safely.
56 Typical disinfection methods to destroy pathogens include treatment with chemical reactive
57 agents (i.e chlorine, chlorine dioxide, chloramines, ozone, and potassium permanganate) or
58 physical means (i.e irradiation with UV or nanofiltration) [1]. Side effect of some these
59 treatments are the generation of disinfection by-products (DBPs) when disinfection agents react
60 with substance already present naturally in the water to be treated. The risks to health of DBPs is
61 considered to be low compared to the exposure to pathogens [2]. Despite that non-chemical
62 disinfection methods would avoid DBPs, small doses of chlorine or mono-chloramine are added
63 to water to ensure its safety after distribution [2].

64 Bromide, which is a majority element in seawater (i.e 67 mg/L) [3], is present in water
65 used in the production of metropolitan drinking water in Saudi Arabia. Bromate (BrO_3^-) is a
66 DBP that can be generated from the ozonation of naturally occurring bromide present in such
67 source water [2,4]. Therefore, desalinated seawater can lead to high levels of BrO_3^- due to
68 remaining levels of its precursor before the oxidising treatment [5]. In a previous study carried
69 out by the authors, BrO_3^- was found between 8-75 $\mu\text{g L}^{-1}$ in desalinated water [6]. BrO_3^- was
70 found to be carcinogenic in animals which revealed the need to control this substance in drinking
71 water [7]. In 1998, the International Agency of Research Cancer (IARC) listed the BrO_3^- in
72 Group 2B (possibly carcinogenic to humans) [8]. Thereafter, the World Health Organization
73 (WHO) and US Environmental Protection Agency (EPA) set up provisional guideline value and

74 a maximum contaminant level (MCL), respectively, at $10 \mu\text{g BrO}_3^- \text{L}^{-1}$ in drinking water [2,4,9]
75 and the public health goal at “zero” [4].

76 Nitrate (NO_3^-) and nitrite (NO_2^-) have a different origin than BrO_3^- in drinking water. In
77 nature, both NO_3^- and NO_2^- derive from the nitrogen cycle in plants and soils; they originate
78 from the microbial digestion of nitrogen rich sources such as plant tissues, faeces or nitrogen-
79 based fertilisers [10-11]. Both NO_3^- and NO_2^- are highly soluble species that can consequently
80 leach to surface and ground water [12-14]. This has detrimental effects on biodiversity as well as
81 on human beings; for instance; it can cause eutrophication [2, 10]; methemoglobinemia disease
82 in infants [15-18] and they have been associated with increased incidence of cancer [10]. Many
83 environmental regulatory organizations have set the guidelines for NO_3^- and NO_2^- in drinking
84 water. The EPA has set the Maximum Contaminant Level (MCL) for NO_3^- at 10 mg L^{-1} in
85 drinking water [4], which coincides with the standard of quality in bottled water set by the
86 International Bottled Water Association (IBWA) [19]. In contrast the WHO and European
87 Commission set up the guideline level at 50 mg L^{-1} [2, 20]. For NO_2^- , the EPA and IBWA has
88 set the MCL and standard of quality, respectively, at 1 mg L^{-1} in drinking water [4,19]; the
89 current WHO guideline value is 3 mg L^{-1} [2] but the limit in Europe is more restrictive: 0.5 mg
90 L^{-1} [20].

91 Monitoring studies are necessary to know the level of exposure to BrO_3^- , NO_3^- and NO_2^-
92 and identify potential hotspots. Many analytical procedures have been developed to assess the
93 levels of BrO_3^- , NO_3^- and NO_2^- in drinking water. Ion chromatography is recognized as the
94 method with best analytical achievability for the determination of BrO_3^- by WHO [2]; ion
95 chromatography-conductivity detection method has been extensively applied for the analysis of
96 such types of compounds in water matrices [21-22]. The low running cost of capillary zone

97 electrophoresis and typical high separation efficacy makes it also an attractive technique for the
98 analyses of anions in drinking water [23]. Improvements in stationary phases that lead to the
99 development of Ultra Performance Liquid Chromatography (UPLC) implied gains in analysis
100 throughput and sensitivity compared to earlier methods. UPLC, in combination with the superior
101 confirmatory capability of mass spectrometry (MS), made UPLC-MS a technique of choice.
102 Earlier works by our group showed the potential of UPLC-MS for the individual analysis of
103 BrO_3^- and NO_3^- [6, 24], however ion suppression made not possible the simultaneous analysis
104 of such similar anions. A method suitable for the fast determination BrO_3^- , NO_3^- and NO_2^- has
105 been developed in this work. The developed method will be applied to quantify the potential
106 hazardous species in metropolitan and bottled water samples from the Kingdom of Saudi Arabia.
107 The levels of the study anions will be examined with Principal Component Analysis (PCA), for
108 the first time to our knowledge, to establish whether BrO_3^- , NO_3^- and NO_2^- can be used to
109 identify the type of sample (desalinated water; well water; desalinated and ground water) from
110 different Saudi regions and possible counterfeit bottled water.

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118 2. Materials and methods

119 2.1 Materials. All solvents and chemicals used in this study were of HPLC or analytical grade,
120 obtained from Merck (Darmstadt, Germany). Potassium bromate (ACS reagent, $\geq 99.8\%$), and,
121 sodium nitrate and sodium nitrite of ReagentPlus[®] grade (assay purity $\geq 99.0\%$) were obtained
122 from Sigma-Aldrich (Steinheim, Germany). Ultrapure water was purified by means of Milli-Q
123 water purification system (Millipore Corporation, Bedford, USA). Stock standard solutions of
124 BrO_3^- , NO_3^- and NO_2^- at concentration level 500 mg L^{-1} were prepared in ultrapure Milli-Q
125 water and used for further dilutions. Standard mixtures of the nitrate and nitrite were prepared by
126 weight. Standard solutions and water samples were filtered through a $0.22 \mu\text{m}$ PTFE syringe
127 filter (Macherey-Nagel GmbH, Düren, Germany) before being injected into the UPLC system.

128
129 2.2 Sample preparation and quantitative analysis. Metropolitan water was obtained from
130 different locations using clear glass bottles (500 mL) supplied by the Saudi Arabian nationalised
131 company Saline Water Conversion Corporation. Bottled water (non-carbonated), from various
132 trademarks, was purchased from hypermarket in Riyadh, Saudi Arabia. These bottled water
133 samples had been treated with ozone. Metropolitan and bottled samples were stored in
134 refrigerator at 4°C and analysed within two days to avoid microbial growth. Blank (ultrapure
135 water) and quality control samples were analysed in each batch to ascertain that contamination of
136 water samples did not arise and detection sensitivity of the target analytes was stable throughout
137 the analysis. The quantification was carried out by external calibration in triplicate and the
138 quantification of the recovery rates was carried out with standard addition method consisting of
139 samples spiked with BrO_3^- , NO_3^- and NO_2^- at four (50, 200, 400 and 500%) levels in addition to
140 non-spiked samples (duplicate). Recovery rates were obtained from the slope obtained when

141 plotting the correlation between the added amount of bromate, nitrate and nitrite, and the amount
142 found.

143

144 2.3 UPLC-ESI/MS analysis. The chromatographic analysis of BrO_3^- , NO_3^- and NO_2^- was
145 carried out using a Waters Acquity® UPLC system (Milford USA) with an Acquity® BEH C_{18}
146 column (50 mm \times 2.1 mm i.d., 1.7 μm particle size) (Waters, Milford, USA) column. A pre-
147 column, VanGuard™ BEH C_{18} 1.7 μm was used to protect the analytical column during the
148 analysis. The optimal chromatographic conditions for the analysis of BrO_3^- , NO_3^- and NO_2^- was
149 obtained using isocratic elution mode consisting of 75% methanol in water (v/v) at a flow rate of
150 200 $\mu\text{L min}^{-1}$. The temperature of the analysis was controlled in an oven at 25 °C. The sample
151 injection volume was 5 μL . A column with polar stationary phase Water Acquity® BEH Amide
152 column (50 mm \times 2.1 mm i.d., 1.7 μm particle size) was also tested.

153 The detection of BrO_3^- , NO_3^- and NO_2^- was performed on Quattro Premier™ triple quadrupole
154 mass spectrometer (Micromass, Milford, USA) with an electrospray ionization source (Z-spray)
155 coupled with an Acquity® UPLC system. The instrument was operated in negative ionization
156 mode. The data acquisition in full scan mode (m/z 40-200) was used to select the most abundant
157 ions from each analyte. Selected Ion Recording (SIR) was applied for their detection with higher
158 sensitivity. Dwell time was 0.025 s and the total scan cycle was 1 s. Factors affecting the ion
159 transmission parameters were optimized by infusing a standard mixture of sodium nitrate,
160 potassium bromate and sodium nitrite solution at 10 mg L^{-1} . The optimized working parameters
161 were as follows. Cone voltage: 40; 46; 40; 38 V for m/z 46.2 (NO_3^-), m/z 62.2 (NO_2^-), m/z
162 129.0 ($^{81}\text{BrO}_3^-$) and m/z 127.0 ($^{79}\text{BrO}_3^-$), respectively. Capillary voltage 3.2 kV; source
163 temperature, 120°C; desolvation temperature, 300°C; cone gas flow rate, 60 L h^{-1} ; desolvation

164 gas flow rate, 600 L h⁻¹. Nitrogen (99.99% purity), produced with a Peak Scientific nitrogen
165 generator model NM30LA (Inchinann, United Kingdom), was used as cone gas. Argon (99.99%
166 purity), obtained from Speciality Gas Centre (Jeddah, Saudi Arabia), was used as collision gas.
167 The primary vacuum for the mass spectrometer was provided with an Oerlikon rotary pump,
168 model SOGEVAC SV40 BI (Paris, France). The data acquisition and processing were performed
169 using MassLynx V4.1 software.

170 Quality parameters of the optimised UPLC-MS method were assessed. Linearity was measured
171 between 0.1 and 1000 µg·L⁻¹; limits of detection (LOD) were established at a signal-to-noise
172 ratio of 3; Run-to-run precision was estimated from six replicate injections of a mixture of
173 BrO₃⁻, NO₃⁻ and NO₂⁻ standard at 0.05 µg·mL⁻¹ in the same day, and day-to-day precision was
174 measured from six replicate injections of the same standard along three consecutive days.

175

176 2.4 Chemometric analysis.

177 The Unscrambler® X version 10.3 (CAMO Software AS., Norway) was used to build a Principal
178 Components Analyses (PCA) model from the concentration of BrO₃⁻, NO₃⁻ and NO₂⁻ in
179 metropolitan and bottled waters. In order to give the same weight to every value (BrO₃⁻ was
180 found at µg·L⁻¹ level in contrast with the other two analytes, which were present at levels of
181 mg·L⁻¹), BrO₃⁻ concentrations were given a weight of 1000 times higher than its quantified
182 values. Validation of PCA models relied on the leave-one-out cross-validation, in which each
183 particular sample was predicted by using the remaining samples as standards for building the
184 calibration model.

185

186 3. Results and discussion

187 3.1 Optimisation of UPLC conditions. The separation of BrO_3^- , NO_3^- and NO_2^- with reverse
188 phase columns (C_8 , C_{18}) was challenging because the interaction of the 3 anions with the
189 stationary phase was weak and very similar among them. Their behaviour was dominated by
190 their net negative charge, which becomes stabilised by solvation in the mobile phase. Higher
191 level of retention was intended when using Hydrophilic Interaction Chromatography (HILIC)
192 with amide groups in the stationary phase. Mobile phase compositions, constituted with
193 methanol/acetonitrile in water at different proportions (0-100%), were tested at flow rate
194 between 100 and 500 $\mu\text{L min}^{-1}$. The addition of formic acid (0.1 – 1%) in the mobile phase was
195 also investigated as a way to shield the charge of the analytes or displace the equilibria towards
196 nitrous acid (case of NO_2^-) and achieve higher retention for the neutral species. All the
197 conditions studied lead to very limited or no separation among BrO_3^- , NO_3^- and NO_2^- . The
198 HILIC column (with mobile phase methanol: water 50:50, v/v) made possible to achieve slightly
199 higher retention for nitrite and nitrate (retention time 0.80 and 0.82 min, respectively), however
200 the symmetry factor of the peaks led to limited peak height and sensitivity and for that reason
201 that column was discarded. In terms of retention and separation in reversed phase
202 chromatography, the percentage of organic solvent in the mobile phase did not cause a great
203 effect on the retention of NO_3^- and NO_2^- . BrO_3^- presented the lowest retention of the three and
204 its signal was affected by the composition of the mobile phase. In a previous work, a mobile
205 phase of water with 0.1% formic acid in water, at 200 $\mu\text{L min}^{-1}$, led to a retention time of 0.4
206 min. Despite that the added acid reduced peak tailing, the peak asymmetry factor for BrO_3^-
207 (measured at 10% of the peak height) was 1.1 [6]. In this work, a mobile phase of 75% methanol
208 in water, in absence of formic acid, led to higher retention (0.7 minute) and improved peak

209 symmetry (tailing factor: 1.0) at $200 \mu\text{L min}^{-1}$, being the dead volume 0.1 min at these
210 conditions. Peak symmetry and separation from the dead volume was poorer with a C_8 column
211 when compared to a C_{18} . The latter, an Acquity[®] BEH C_{18} column with dimension $50 \text{ mm} \times 2.1$
212 mm i.d., $1.7 \mu\text{m}$ particle size, with a mobile phase consisting of methanol/water (75:25, v/v) in
213 isocratic elution at flow rate $200 \mu\text{L min}^{-1}$, was adopted as the optimal conditions which lead to
214 the chromatogram shown in Figure 1. The chromatographic peaks from the analytes were not
215 resolved but their co-elution was not found to affect their detection. Acetonitrile was not found
216 advantageous with respect to the results achieved with methanol. Comparatively, low flow rates,
217 which favoured ionic evaporation and efficient desolvation in the electrospray ionization source,
218 were found to be advantageous for the analysis of the anions. At $200 \mu\text{L min}^{-1}$, chromatographic
219 peaks presented peak width that could be defined with a minimum of 15 scan points and analysis
220 time of just 1 min.

221

222 3.2 Optimisation of the MS conditions and UPLC-MS quality parameters.

223 The electrospray (ESI) ionization conditions related with desolvation, ionic evaporation and
224 transmission of the analyte ions were investigated: cone voltage (10–100 V), capillary voltage
225 (2.0–4.5 kV), source temperature (80–150 °C), desolvation temperature (250–450 °C) and
226 desolvation gas ($300\text{--}700 \text{ L h}^{-1}$). The ESI/MS parameters that offered the best sensitivity are
227 provided in section 2.1. Temperatures and source gases in the higher range were found to
228 provide higher sensitivity because these favoured ionic evaporation. However, the high
229 percentage of organic solvent in the mobile phase (75%) and relative low flow rate prevented
230 needing extreme settings for optimal sensitivity.

231 The relationship between the concentration of the analytes and their response was assessed across the
232 range 0.1-1000 $\mu\text{g L}^{-1}$ and was found to be linear; calibration curves with $r^2 < 0.999$ were obtained
233 in all cases and t correlation test confirmed the correlation (P 0.05). The working range was
234 limited to 0.1-100 $\mu\text{g L}^{-1}$ given that the concentrations of the analytes were expected in that
235 range. Quality parameters obtained under optimal conditions are given in Table 1. The
236 instrumental limit of detection (S/N=3) for NO_2^- was 0.04 $\mu\text{g L}^{-1}$ and for NO_3^- and BO_3^- was
237 0.03 $\mu\text{g L}^{-1}$. Run-to-run and day-to-day precisions were $< 3\%$ (n = 6) for the three anions when
238 analyzing a standard mixture solutions at 10 $\mu\text{g L}^{-1}$. The sensitivity achieved for NO_3^- did not
239 decrease when compared to its individual analysis with UPLC-MS [24]. It improved previous
240 LC-MS method using reversed phase chromatography where LODs for nitrate and nitrite were 1
241 $\mu\text{g L}^{-1}$ and 12 $\mu\text{g L}^{-1}$ [25], respectively. The sensitivity of the developed method was, however,
242 about 10 times poorer than a non-routine method recently developed based on spectroscopic
243 detection assisted by graphene oxide modified with amino groups and gold nanoparticles [26].
244 Furthermore, the detection of bromate improved by 10 times previous results with UPLC-MS
245 [6]. This improvement can be attributed to better ionic evaporation achieved with the higher
246 proportion of organic solvent in the mobile phase. The sensitivity reached was also superior to
247 the levels achieved with completely different approaches: ionic chromatography-conductivity
248 detection; liquid chromatography-inductively coupled plasma/ mass spectrometry detection;
249 capillary electrophoresis and mass spectrometry detection by 2-25 times [27-29]. An analysis
250 time of just 1.5 minutes for BrO_3^- , NO_3^- and NO_2^- , without need of column preconditioning due
251 to isocratic conditions, made the optimised method as one the fastest and most sensitive methods
252 available to the best of our knowledge.

253

254 3.3 Determination of BrO_3^- , NO_3^- and NO_2^- in bottled and metropolitan samples from Saudi
255 Arabia.

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

263 In metropolitan water, the concentration range found for BrO_3^- was $5.83\text{-}13.45 \mu\text{g L}^{-1}$; for
264 NO_2^- , $0.83\text{-}1.57 \text{mg L}^{-1}$; and for NO_3^- , $1.65\text{-}4.61 \text{mg L}^{-1}$ (quantification shown in Table 2). The
265 higher levels of BrO_3^- were found in desalinated water which had not been blended with well
266 water. This is because well water may dilute the species (Br^-) that would subsequently be
267 oxidised to BrO_3^- . In contrast, desalinated water contained among the lowest levels NO_3^- and
268 NO_2^- compared to well water, which may have received higher level of leachates from the
269 degradation of nitrogen rich sources.

270 Among the relatively low number of metropolitan water samples analysed and given in Table 2,
271 33% contained BrO_3^- levels above the $10 \mu\text{g L}^{-1}$ target by the WHO, US EPA and Gulf
272 standards for metropolitan water in Saudi Arabia (P 0.05) [4,30]. In previous works >70% of a
273 limited number of samples analysed were higher than the $10 \mu\text{g L}^{-1}$ [6, 28]. In contrast, the levels
274 of NO_3^- were all below the EPA limit of 10mg L^{-1} (P 0.05) [4]. However, 92% of the samples
275 had NO_2^- levels above the EPA MCL for NO_2^- (1mg L^{-1} , P 0.05) [4] despite all of them
276 complying with the Gulf standards (3mg/L NO_2^-)[30].

277 Among the bottled water samples, 25% presented higher BrO_3^- concentration than the WHO and
278 EPA targets (P 0.05) [2,4]; all the samples were below the MCL for NO_3^- [4]; and 70% were
279 above the EPA limit for NO_2^- (P 0.05) [4] or entirely over the Gulf standards for bottled water
280 ($0.2 \text{ mg L}^{-1}\text{NO}_2^-$) [31]. Previous work carried out in Saudi Arabia showed a broad range of
281 concentration for these compounds: 8% [32]; 17% [6]; and 60 % of the samples had higher
282 BrO_3^- [28] than the EPA MCL. The levels of NO_2^- detected in this research are about two time
283 the highest level of nitrite detected (0.38 mg L^{-1}) in a recent comprehensive study which
284 monitored 145 wells in Makkah City [33] and also contrasts with the low detection rate of NO_2^-
285 in the assessment of 571 European bottled mineral waters (< 8% had levels above 0.01 mg L^{-1})
286 [34]. Another important difference with these European samples is that whereas 37% of the
287 samples had $\text{NO}_3^- > 2.6 \text{ mg L}^{-1}$ [34], only 15% of the samples in our equivalent study (Table 3)
288 reached such high level. Oxidation conditions prior bottling water and ammonia-related levels
289 before oxidation can be the origin of such differences. Our data shows that NO_3^- is at non
290 problematic levels; this is in agreement with other recent studies in Saudi Arabia found that all
291 the samples tested were below the EPA goal [24] while others showed that it is still of concern
292 since 20% of the samples presented higher NO_3^- than the EPA limits [32]. A study where 388
293 wells of 6 regions of the Kingdom of Saudi Arabia were investigated, much higher
294 concentrations of nitrate were found: 8% of the wells had $\text{NO}_3^- > 45 \text{ mg L}^{-1}$ [35]. Our study
295 shows both NO_2^- and BrO_3^- are problematic in both metropolitan and bottled water. The levels
296 of these toxicants in bottled water are especially relevant because this type of water is typically
297 used to prepare infant formulas. Remediation measures to decrease Br^- and nitrogenated
298 compounds in water sources; optimising the disinfection conditions currently applied and
299 selecting those (concentration, pH, duration) that would make possible effective disinfection,

300 reducing the formation of BrO_3^- and increase the oxidation of NO_2^- to NO_3^- (keeping both
301 below the regulated levels); or blending types of water to lower the levels of these potential
302 toxicants are recommendable.

303

304 3.4 Mapping bottle and metropolitan waters with PCA model built from BrO_3^- , NO_3^- and NO_2^-
305 levels in samples from Saudi Arabia.

306 A PCA model was built from the quantified BrO_3^- , NO_3^- and NO_2^- levels in metropolitan and
307 bottled water samples given in Tables 2 and 3. The data was represented using axis, PC, which
308 better explain the variation among the data. The scores plot (Figure 2), which classifies the
309 samples based on the concentration of the anions, revealed patterns that were related with the
310 sample characteristics. The samples were mainly distributed along PC1 (which explains 77%) of
311 the variation. Metropolitan water appeared distributed mainly in the first 2 quarters of the plot,
312 whereas bottled water appeared mainly in the second half of the plot. However, there were some
313 bottled water samples appearing in the region where metropolitan water predominated. It could
314 be hypothesised that these bottled samples contained metropolitan water.

315 Figure 3 shows the distribution of the variables (loading plot). When correlating the scores plot
316 (Figure 2) with the loading plot (Figure 3), it can be observed that BrO_3^- , which is located at one
317 extreme of PC1, was the variable causing the main differentiation among samples along PC1. In
318 contrast, NO_3^- is the variable responsible for the distribution of the samples along PC2.
319 Therefore metropolitan samples were mainly described by the levels of BrO_3^- (which is in
320 agreement with higher level of this ion in desalinated water. Bottled water (mainly from well
321 water and thus with input of nitrogenated species from organic matter degradation) appeared
322 mostly distributed along PC2. A more detailed PC model giving details of the type of water and

323 its origin is shown in Figure 4. Metropolitan water from the same geographical region were not
324 grouped, however those samples from desalinated water were distinctively separated from
325 samples containing both desalinated water and well water, and from well water only, along PC1.
326 Bottled water samples number 12, 13, 16 and 20 did not have their origin in their label.
327 According to our model, these samples could be bottled metropolitan water. The bottled water
328 samples number 2, 4 and 6 appeared in the interface between metropolitan water and bottled well
329 water. These appeared labelled as well water, however their levels of BrO_3^- ($>8 \mu\text{g L}^{-1}$) were in
330 the higher range of their type (Table 3). Based on the position of these samples in the PCA
331 model, it could be hypothesized that these bottled water samples could contain a blend of well
332 water and desalinated water.

333

334 4. Conclusions

- 335 • The UPLC-ESI/MS method developed for the determination of BrO_3^- , NO_3^- and NO_2^- ,
336 with an analysis time of just 1.5 min; high sensitivity 0.03-0.04 $\mu\text{g/L}$; high precision
337 ($<3\%$) and recoveries ($>94\%$) is advantageous for monitoring drinking water.
- 338 • The analysis of BrO_3^- , NO_3^- and NO_2^- in 20 bottled water samples and in 12
339 metropolitan water samples from different sites in the Kingdom of Saudi Arabia showed
340 that the levels of BrO_3^- and NO_2^- were in many cases above recommended levels.
341 Specifically,
342 the concentration of BrO_3^- was found higher than the US EPA MCL in 25 and 33% of
343 the bottled and metropolitan waters, respectively. The levels of NO_2^- were higher than
344 the US EPA MCL in 70 and 92% of the bottled and metropolitan water samples,
345 respectively, and all samples were below the EPA limits for NO_3^- . These results indicate

346 that remediation measures/disinfection conditions need to be further optimised with view
347 to the DBPs generated.

- 348 • A PCA model using BrO_3^- , NO_3^- and NO_2^- concentrations showed capacity to
349 discriminate between desalinated water; well water; and desalinated water blended with
350 well water in Saudi Arabia. The level of bromate was the main variable making possible
351 the distinction among drinking water samples. NO_3^- had less influence in mapping the
352 samples and mainly described the bottled drinking waters. The geographical sampling
353 site was not useful to classify the metropolitan drinking water samples.

354

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471 Figure captions

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473 Figure 1. UPLC-ESI/MS chromatograms and spectra of NO_2^- , BrO_3^- , NO_3^- in bottled water
474 (sample n^o 1 in Table 3). The chromatographic conditions were isocratic (75% methanol in water
475 (v/v)) at a flow rate of $200 \mu\text{L min}^{-1}$, 25°C . The column used was an Acquity® BEH C_{18} (50 mm
476 \times 2.1 mm i.d., 1.7 μm particle size).

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478 Figure 2. PCA scores plot obtained from metropolitan and bottled waters as a function of BrO_3^- ,
479 NO_3^- and NO_2^- concentrations.

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481 Figure 3. PCA loading plot showing the contribution of the variables in the model.

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

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Analyte	LOD ($\mu\text{g L}^{-1}$)	LOQ ($\mu\text{g L}^{-1}$)	Run-to-run precision (n=6), RSD (%)	Day-to-day precision (n=6), RSD (%)
NO_3^-	0.030	0.092	1.3	2.6
NO_2^-	0.039	0.12	1.5	2.9
$^{81}\text{BrO}_3^-$	0.029	0.092	1.4	2.7
$^{79}\text{BrO}_3^-$	0.040	0.12	1.6	3.1

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519520 Table 2. Concentrations of NO_2^- , NO_3^- and $^{81}\text{BrO}_3^-$ in metropolitan water samples and
521 recoveries (R) obtained in their determination with UPLC-ESI/MS

Metropolitan water (sampleno)*	Water source	NO_2^-		NO_3^-		$^{81}\text{BrO}_3^-$	
		(mg L^{-1}) \pm SD	R (%)	(mg L^{-1}) \pm SD	R (%)	($\mu\text{g L}^{-1}$) \pm SD	R (%)
Jeddah (1)	Desalinated + well water	1.37 \pm 0.04	95	4.11 \pm 0.03	95	5.83 \pm 0.03	96
Jeddah (2)	Desalinated + well water	1.41 \pm 0.04	97	4.08 \pm 0.03	97	6.35 \pm 0.02	97
Jeddah (3)	Desalinated water	1.35 \pm 0.04	96	4.35 \pm 0.03	97	9.31 \pm 0.01	97
Jeddah (4)	Desalinated water	0.89 \pm 0.05	95	2.02 \pm 0.04	94	13.45 \pm 0.01	98
Dammam (5)	Desalinated + well water	1.32 \pm 0.04	98	2.41 \pm 0.04	97	8.67 \pm 0.02	98
Dammam (6)	Desalinated + well water	1.49 \pm 0.04	95	4.61 \pm 0.03	97	9.78 \pm 0.01	97

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

522 ^aTreated with hypochlorite disinfectant and obtained from different locations

523 ^b R: Recovery

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

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529 Table 3. Concentrations of NO₂⁻, NO₃⁻ and ⁸¹BrO₃⁻ in bottled water samples and recoveries

530 obtained in their determination with UPLC-ESI/MS

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Bottled water (number)	Water source	NO ₂ ⁻ (mg L ⁻¹)		NO ₃ ⁻ (mg L ⁻¹)		BrO ₃ ⁻ (μg L ⁻¹)	
		mean± SD	R ^b (%)	mean± SD	R (%)	mean± SD	R (%)
1	Well water	1.59 ±0.04	99	4.89 ±0.03	98	6.79 ± 0.02	99
2	Well water	0.89 ±0.05	98	2.85 ±0.03	97	8.41 ± 0.02	98
3	Well water	1.52 ±0.04	96	3.96 ±0.03	98	4.15 ± 0.03	97

4	Well water	0.56 ±0.05	98	0.97 ± 0.05	99	8.52 ± 0.02	96
5	Well water	0.58 ±0.05	99	4.65 ±0.03	99	2.35 ± 0.04	99
6	Well water	3.46 ±0.02	99	6.54 ±0.02	98	10.16 ± 0.01	98
7	Well water	3.22 ±0.03	98	9.65 ±0.01	96	6.60 ± 0.02	99
8	Well water	1.62 ±0.04	99	5.21 ±0.02	99	3.60 ± 0.03	99
9	Well water	1.36 ±0.04	98	3.45 ±0.03	97	5.59 ± 0.03	99
10	Well water	1.45 ±0.04	98	5.32 ±0.03	99	7.42 ± 0.02	99
11	Well water	1.32 ±0.04	99	3.78 ±0.03	97	3.65 ± 0.03	99
12	– ^a	0.63 ±0.05	98	0.41 ±0.05	99	11.42 ± 0.01	98
13	–	0.72 ± 0.05	99	0.53 ±0.05	98	11.96 ± 0.01	98
14	–	1.43 ±0.04	99	4.22 ±0.03	98	2.82 ± 0.04	98
15	–	2.84 ±0.03	99	7.65 ±0.02	99	4.02 ± 0.03	98
16	–	0.64 ±0.05	98	1.45 ±0.04	99	11.40 ± 0.01	99
17	–	1.42 ±0.04	99	3.52 ±0.03	97	3.41 ± 0.03	99
18	–	1.65 ±0.04	99	2.65 ±0.03	97	4.97 ± 0.03	99
19	–	0.78 ±0.05	97	1.33 ± 0.04	99	3.42 ± 0.03	98
20	–	1.88 ±0.44	99	2.80 ± 0.03	98	10.74 ± 0.01	98

532 ^a Unknown source of water

533 ^b R: Recovery

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

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536 HIGHLIGHTS:

- 537 • Analysis of BrO₃[–], NO₃[–] and NO₂[–] in 1.5 min by UPLC-MS
- 538 • Monitoring of BrO₃[–], NO₃[–] and NO₂[–] in Saudi Arabia drinking water
- 539 • PCA analysis discriminates among types of metropolitan and bottled water

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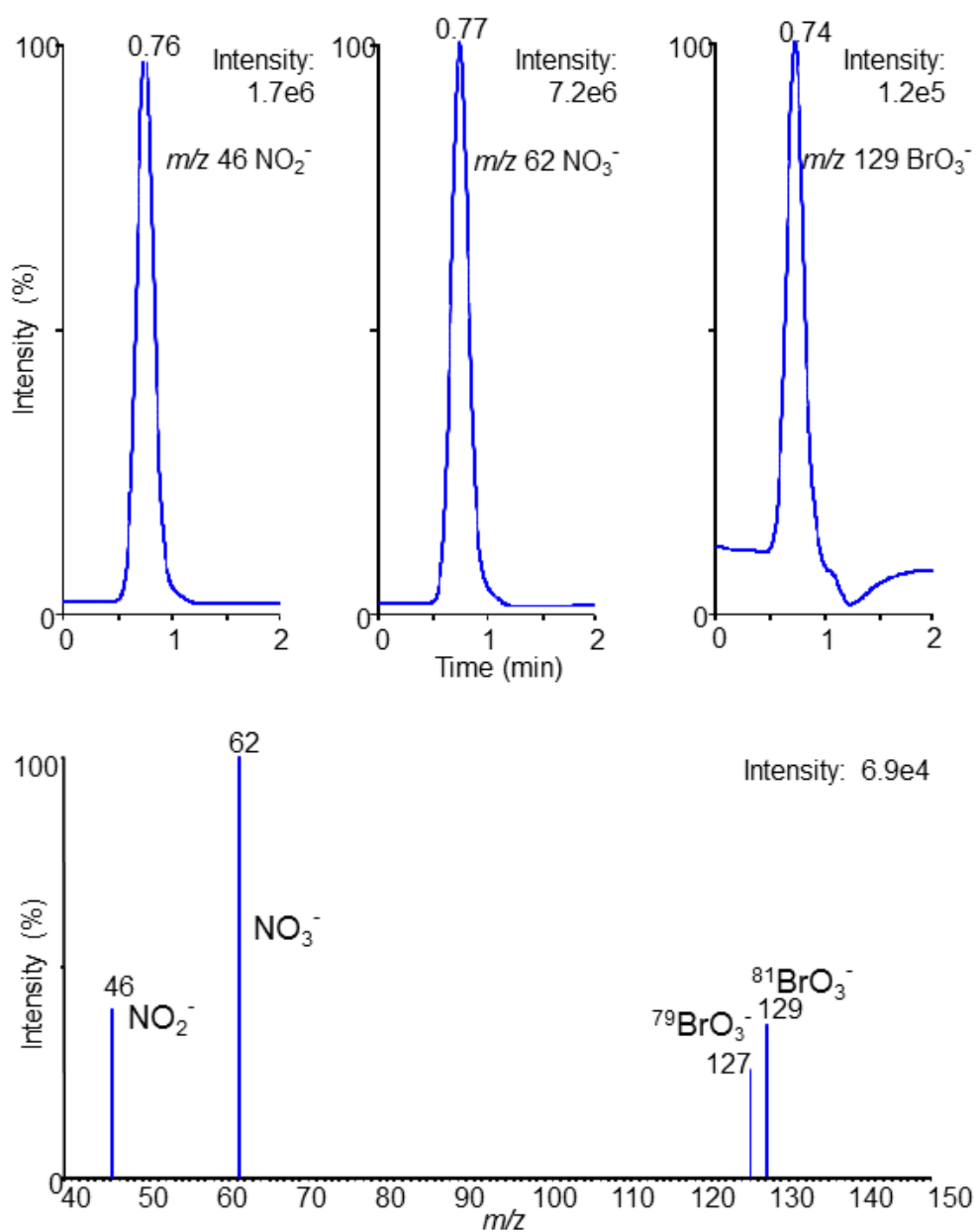


Figure 1. UPLC-ESI/MS chromatograms and spectra of NO_2^- , BrO_3^- , NO_3^- in bottled water (sample n°1 in Table 3). The chromatographic conditions were isocratic (75% methanol in water (v/v)) at a flow rate of $200 \mu\text{L min}^{-1}$, 25°C . The column used was an Acquity® BEH C_{18} (50 mm \times 2.1 mm i.d., $1.7 \mu\text{m}$ particle size).

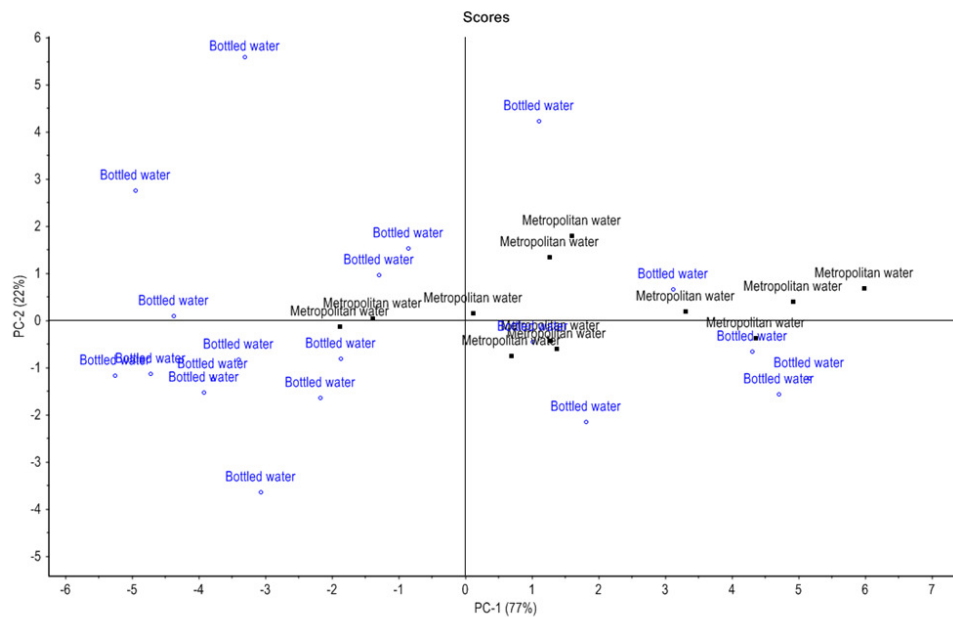


Figure 2. PCA scores plot obtained from metropolitan and bottled waters as a function of BrO_3^- , NO_3^- and NO_2^- concentrations.

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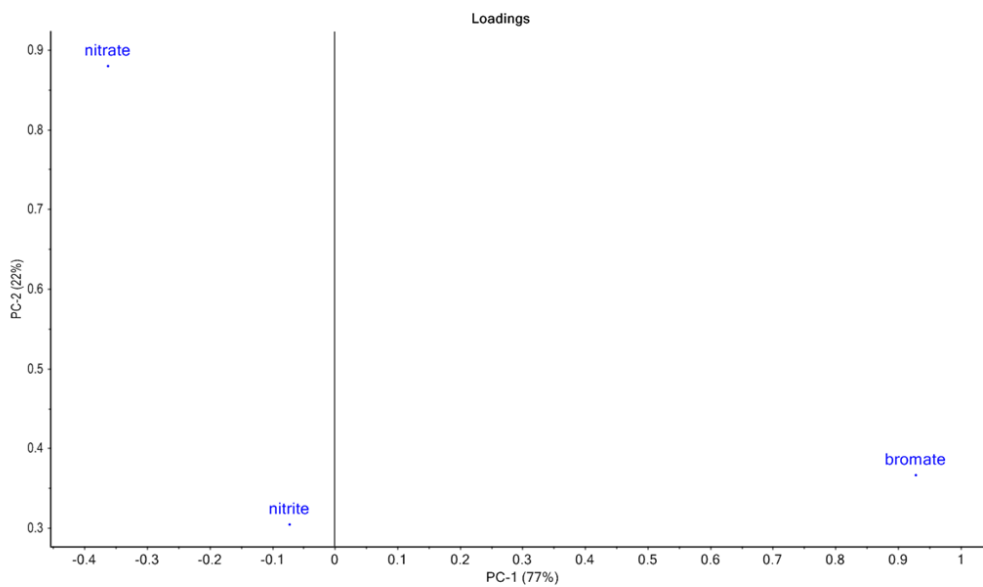


Figure 3. PCA loading plot showing the contribution of the variables in the model

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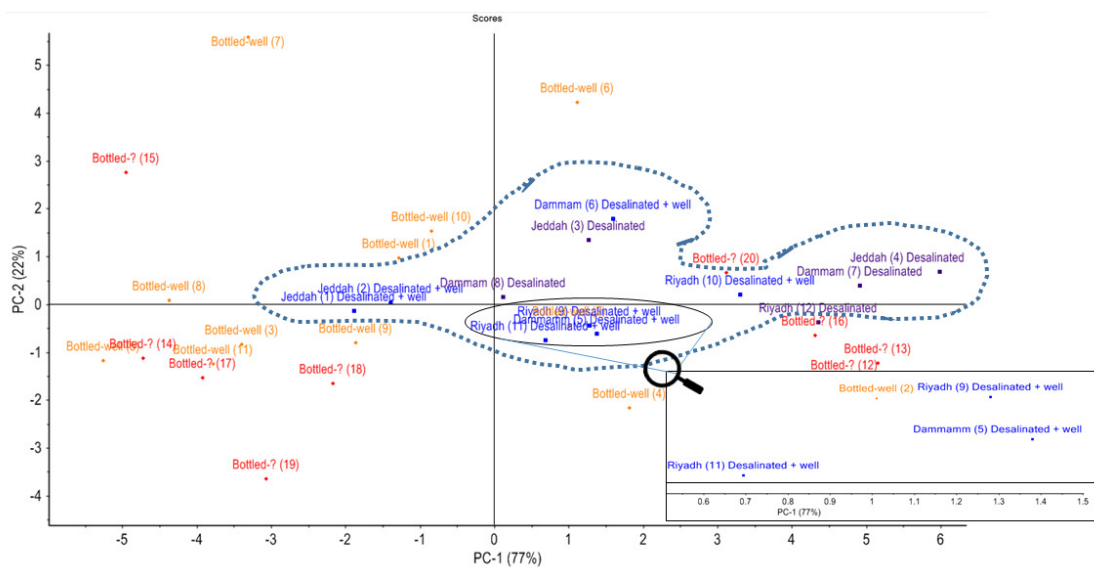


Figure 4. Scores plot showing the distribution of metropolitan and bottled water samples. The metropolitan water sampling site and type of water (desalinated; well; blend of well and desalinated water; and unknown (?)) appear indicated in the sample name. The number in brackets corresponds to the sample 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.

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