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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 µg L^{-1}. The limit of detection was 0.04 µg NO_2^- L^{-1} and 0.03 µg 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 μg 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 μg BrO_3^- 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, ≥99.8%), and,

121   sodium nitrate and sodium nitrite of ReagentPlus® grade (assay purity ≥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 μm 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

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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 × 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 µL min^{-1}. The temperature of the analysis was controlled in an oven at 25 ºC. The sample

151   injection volume was 5 µL. A column with polar stationary phase Water Acquity® BEH Amide

152   column (50 mm × 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^{TM} 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 $(^{81}BrO_3)^-$ and m/z 127.0 $(^{79}BrO_3^-)$, respectively. Capillary voltage 3.2 kV; source

163   temperature, 120ºC; desolvation temperature, 300ºC; 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 BrO_3^-, NO_3^- and NO_2^- 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.

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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 BrO_3^-, NO_3^- and NO_2^- in

179 metropolitan and bottled waters. In order to give the same weight to every value (BrO_3^- 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}), BrO_3^- 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 µL 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 µL 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 μL 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 mm × 2.1

212 mm i.d., 1.7 μm particle size, with a mobile phase consisting of methanol/water (75:25, v/v) in

213 isocratic elution at flow rate 200 μL 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 μL 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 ºC), desolvation temperature (250–450 ºC) and

226 desolvation gas (300–700 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 µg L^{-1} and was found to be lineal; 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 µg 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 µg L^{-1} and for NO$_3^-$ and BO$_3^-$ was

237 0.03 µg 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 µg 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 µg L^{-1} and 12 µg 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 5.83-13.45 µg L^{-1}; for

264   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

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 µg 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 µg L^{-1} [6, 28]. In contrast, the levels

274   of NO_3^- were all below the EPA limit of 10 mg L^{-1} (P 0.05) [4]. However, 92% of the samples

275   had NO_2^- levels above the EPA MCL for NO_2^- (1 mg L^{-1}, P 0.05) [4] despite all of them

276   complying with the Gulf standards (3 mg/L NO_2^-)[30].

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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% where

279   above the EPA limit for NO_2^- (P 0.05) [4] or entirely over the Gulf standards for bottled water

280   (0.2 mg $L^{-1}NO_2^-$) [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 comprenhensive study which

284   monitored 145 wells in Makkah City [33] and also contrasts with the low detection rate of NO_2^-

285   in the assessmnet 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 $NO_3^- > 2.6$ 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 $NO_3^- > 45$ 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

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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 µg 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 µg/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.

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374   REFERENCES

375   [1] M. W. Le Chevallier, Au. Kwok-Keung, Inactivation (disinfection) process, in: M. W. Le

376   Chevallier, Au. Kwok-Keung (Eds.), Water treatment and pathogen control: process

377   efficiency in achieving safe drinking water, World Health Organization titles with IWA

378   Publishing, London, UK, 2004, pp 41-65. ISBN 92 4 156255 2, ISBN 1 84339 069 8

379   [2] World Health Organization, Guidelines for drinking-water quality, forth ed., WHO

380   Switzerland, 2011. ISBN 978 92 4 154815 1.

381   [3] E. Brown, A. Colling, D. Park, J. Phillips, D. Rothery, J. Wright, Seawater: its composition,

382   properties and behavior, G. Berman editor, second ed., The Open University in Association

383   with Pergamon, Oxord, UK, 1995.

384   [4] U.S. Environmental Protection Agency (US EPA) National Primary Drinking Water

385   Regulations, EPA 816-F-09-004, 2009. Available from:

386   http://water.epa.gov/drink/contaminants/upload/mcl-2.pdf (accessed September 2015).

387   [5] D. Kim, G. L. Amy, T. Karanil, Disinfection by-product formation during seawater

388   desalination: A review, Water Res. 81 (2015) 343-355.

389   [6] I.H. Alsohaimi, Z. A. Alothman, M.R. Khan, M.A. Abdalla, R. Busquets, A.K. Alomary,

390   Determination of bromate in drinking water by ultraperformance liquid chromatography–

391   tandem mass spectrometry, J.Sep. Sci. 35 (2012) 2538–2543.

17

392   [7] A. B. DeAngelo, M. H.George, S. R. Kilburn, T. M. Moore, D. C. Wolf, Carcinogenicity of

393   potassium bromate administered in the drinking water to male B6C3F1 mice and F344/N

394   rats, Toxicol. Pathol. 26 (1998) 587-594.

395   [8] IARC Monograph on the Evaluation of the Carcinogenic Risk of Chemicals to Humans,

396   IARC, Lyon, France, 31 (1998) 481.

397   [9] World Health Organization, Guidelines for drinking-water quality, Vol.1. Recommendations.

398   Third ed. WHO Switzerland, 2008. ISBN 978 92 4 154761 1.

399   [10] U.S. Environmental Protection Agency (US EPA) Nitrates and nitrites. TEACH Chemical

400   Summary, 2006.

401   [11] U.S. Environmental Protection Agency (US EPA), Basic Information about nitrate in

402   drinking Water. 2015. [On-line] Available from:

403   http://water.epa.gov/drink/contaminants/basicinformation/nitrate.cfm, Accessed September

404   2015.

405   [12] L.W. Canter, Nitrates in Groundwater, CRC Press, Boca Raton, Florida. ISBN

406   9780873715690, 1996.

407   [13] Council of the European Communities, Implementation of Council Directive 91/676/EEC

408   concerning the protection of waters against pollution caused by nitrates from agricultural

409   sources. Synthesis from year 2000 Member States reports, Luxembourg, 2002, ISBN 92-

410   894-4103-8.   Available   from: http://ec.europa.eu/environment/water/water-

411   nitrates/pdf/91_676_eec_en.pdf. (accessed September 2015).

412   [14] M.K. Ji, Y.T. Ahn, M. A. Khan, R.A.I. Abou-Shanab, Y. Cho, J. Y. Choi, Y. J. Kim, H.

413   Song, B. H. Jeon, Removal of nitrate and ammonium ions from livestock wastewater by

414   hybrid systems composed of zero-valent iron and adsorbents, Environ. Technol. 32 (2011)

415   1851-1857.

416   [15] C.S. Bruning-Fann, J.B. Kaneene, The effects of nitrate, nitrite and N-nitroso compounds on

417   human health: a review, Vet. Hum. Toxicol. 35 (1993) 521–538.

418   [16] A.A. Avery, Infantile methemoglobinemia: re-examining the role of drinking water nitrates.

419   EHP107 (1999) 583–586.

420   [17] J. Sanchez-Echaniz, J. Benito-Fernandez, S. Mintegui-Raso, Methemoglobinemia and

421   consumption of vegetables in infants. Pediatrics 107(2001) 1024-1028.

422   [18] D. M. Klurfeld, Nitrite and Nitrate in Cancer in: N.S., Bryan, J. Loscalzo (Eds.), Nitrite and

423   nitrate in human health and disease, Springer, Humana Press, New York, 2011, pp 263-

424   278.

425   [19] IBWA, International Bottled Water Association 2015. Bottled water code of Practice.

426   IBWA Code of Practice Monitoring Requirements. Appendix 2, Alexandria. [on-line]

427   Available

428   from:http://www.bottledwater.org/public/IBWA%20MODEL%20CODE%202015%20Rev

429   %2002%202015.pdf. (Accessed September 2015)

430   [20] Council of the European Communities, Directive of the European Parliament and of the

431   Council on the Quality of Water Intended for Human Consumption (98/83/EC), 1998.

432   [21] US EPA, Methods for the Determination of Inorganic Substances in Environmental

433   Samples, EPA/600/R-93/100, Cincinnati, US, 1993.

434   [22] US EPA, Methods for the Determination of Organic and Inorganic Compounds in Drinking

435   Water, Volume 1,EPA/815-R-00-014, Cincinnati, US, 2000.

[23] M.B. Amran, M.D. Lakkis, F. Lagarde, M. J. F. Leroy, J. F. Lopez-Sanchez, G. Rauret, Separation of bromide, bromate, iodide, iodate, nitrite, nitrate and selenite anions by capillary zone electrophoresis, Fresenius J Anal Chem. 345 (1993) 420-423.

[24] 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.

[25] 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 wàter, J. Chromatogr. A 1218 (2011) 476–483.

[26] W. Ren, S. Mura, J.M.K. Irudayaraj, Modified graphene oxide sensors for ultra-sensitive detection of nitrate ions in water. Talanta 143 (2015) 234–239.

[27] W. Lawal, J. Gandhi, C. Zhang, Direct injection, simple and robust analysis of trace-level bromate and bromide in drinking water by IC with suppressed conductivity detection, J. Chromatogr. Sci. 48 (2010) 537–543.

[28] A. A. Othman, S. A. Al–Ansi, M. A. Al–Tufail, Determination of Bromate in Bottled Drinking Water from Saudi Arabian Markets by HPLC/ICP-MS, Anal. Lett. 43 (2010) 886–891.

[29] S. Zhang, Y. Shao, J. Liu, I.A. Aksay, Y. Lin, Graphene–polypyrrole nanocomposite as a highly efficient and low cost electrically switched ion exchanger for removing ClO_4^- from wastewater. ACS Appl. Mater. Interfaces 3 (2011) 3633–3637.

[30] Gulf Cooperation Council GSO 149, Unbottled drinking water, 1-12, 2008.

[31] Gulf Cooperation Council GSO 1025, Bottled Drinking Water, 1-10, 2008.

458 [32] A.M. Al-Omran., S.E. El-Maghraby, A.A. Aly, M.I. Al-Wabel, Z.A.Al-Asmari, M.E.

459   Nadeem, Quality assessment of various bottled waters marketed, Environ Monit Assess.

460   185 (2013) 51-59.

461 [33] N.H. Khdary, A.E. Gasim, M.E. Muriani, A.A. Alshehrie, Modeling Distribution of

462   Selective Ions in Urban and Rural Areas Using Geographical Information System, J. Water

463   Resour. Prot. 7 (2015) 516–529.

464 [34] D. Bertoldi, L. Bontempo, R. Larcher, G. Nicolini, S. Voerkelius, G.D. Lorenz, H.

465   Ueckermann, H. Froeschl, M.J. Baxter, J. Hoogewerff, P. Brereton, Survey of the chemical

466   composition of 571 European bottled mineral wàters, J. Food Compos. Anal. 24 (2011)

467   376–385.

468 [35] M.N.A. El-Din, I.M. Madany, A. Al-Tayaran, A.H. Al-Jubair, A. Gomaa,.Quality of water

469   from some wells in Saudi Arabia, Water, air soil Pollut. 66 (1993) 135–143.

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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°1 in Table 3). The chromatographic conditions were isocratic (75% methanol in water

475 (v/v)) at a flow rate of 200 µL min^{-1}, 25 °C. The column used was an Acquity® BEH C$_{18}$ (50 mm

476 × 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

489   is provided.

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509 Table 1. Quality parameters obtained with the optimised UPLC-ESI/MS method.

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Analyte	LOD ($\mu g\ L^{-1}$)	LOQ ($\mu g\ L^{-1}$)	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
$^{81}BrO_3^-$	0.029	0.092	1.4	2.7
$^{79}BrO_3^-$	0.040	0.12	1.6	3.1

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519

520 Table 2. Concentrations of NO_2^-, NO_3^- and $^{81}BrO_3^-$ 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^-		$^{81}BrO_3^-$	
		(mg L^{-1}) ± SD	R (%)	(mg L^{-1}) ± SD	R (%)	($\mu g\ L^{-1}$) ± SD	R (%)
Jeddah (1)	Desalinated + well water	1.37±0.04	95	4.11±0.03	95	5.83±0.03	96
Jeddah (2)	Desalinated + well water	1.41±0.04	97	4.08±0.03	97	6.35±0.02	97
Jeddah (3)	Desalinated water	1.35±0.04	96	4.35±0.03	97	9.31±0.01	97
Jeddah (4)	Desalinated water	0.89±0.05	95	2.02±0.04	94	13.45±0.01	98
Dammam (5)	Desalinated + well water	1.32±0.04	98	2.41±0.04	97	8.67± 0.02	98
Dammam (6)	Desalinated + well water	1.49±0.04	95	4.61±0.03	97	9.78±0.01	97

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		NO$_2^-$ (mg L^{-1})		NO$_3^-$ (mg L^{-1})		BrO$_3^-$ (µg L^{-1})	
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 [a]Treated 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 NO$_2^-$, NO$_3^-$ and ^{81}BrO$_3^-$ 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

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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 BrO_3^-, NO_3^- and NO_2^- in 1.5 min by UPLC-MS

538 • Monitoring of BrO_3^-, NO_3^- and NO_2^- in Saudi Arabia drinking water

539 • PCA analysis discriminates among types of metropolitan and bottled water
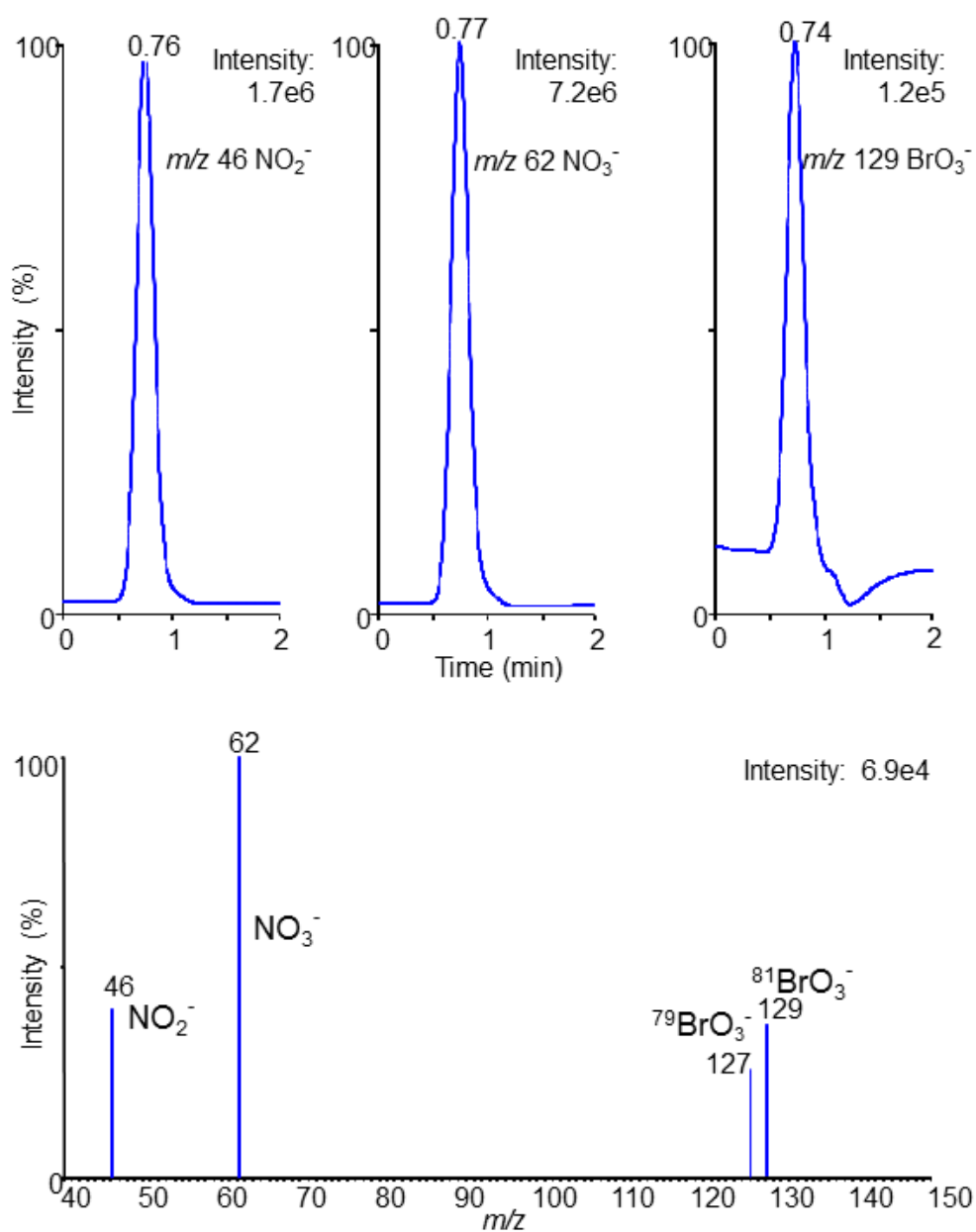
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Figure 1. UPLC-ESI/MS chromatograms and spectra of NO_2^-, BrO_3^-, NO_3^- in bottled water (sample n°1 in Table 3). The chromatographic conditions were isocratic (75% methanol in water (v/v)) at a flow rate of 200 μL min^{-1}, 25 °C. The column used was an Acquity® BEH C_{18} (50 mm × 2.1 mm i.d., 1.7 μm particle size).
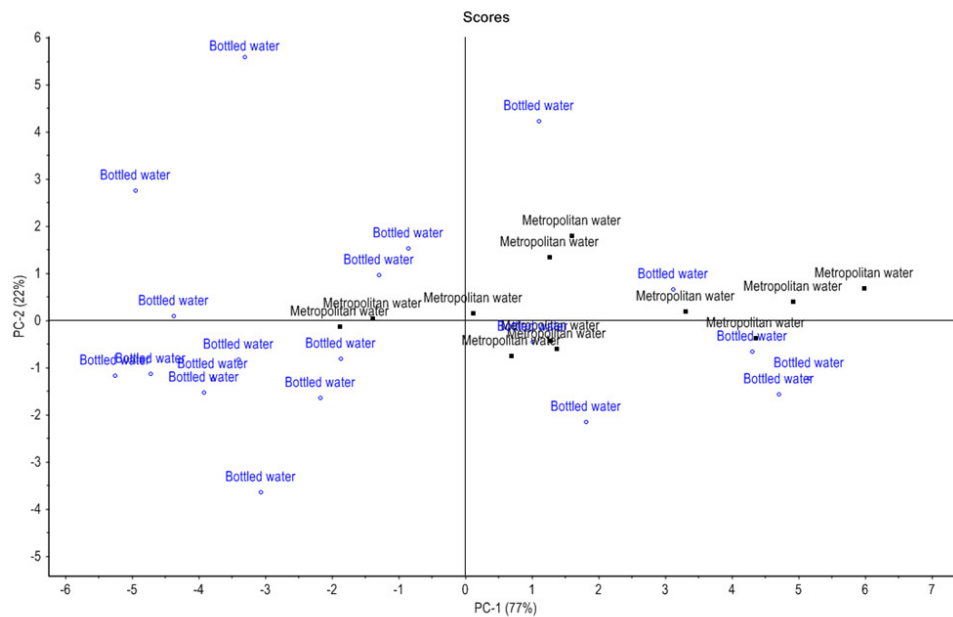
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Figure 2. PCA scores plot obtained from metropolitan and bottled waters as a function of BrO_3^-, NO_3^- and NO_2^- concentrations.
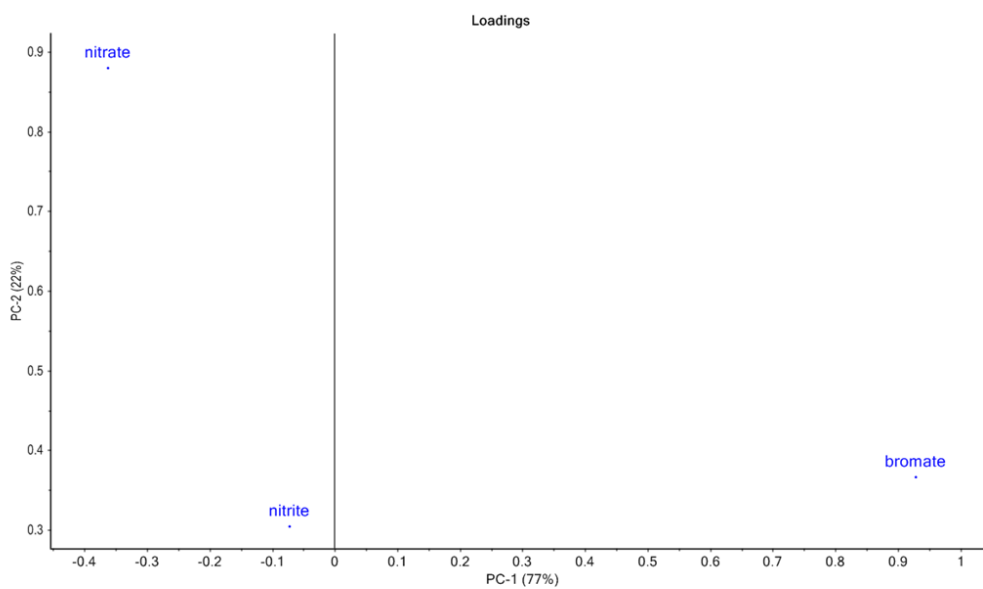
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Figure 3. PCA loading plot showing the contribution of the variables in the model

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