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Research article

Ultra-trace determination of oxyhalides in ozonated aquacultural marine waters by direct injection ion chromatography coupled with triple-quadrupole mass spectrometry

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ABSTRACT

A direct, robust, accurate and highly sensitive method for oxyhalide species in natural waters, including seawater, using suppressed ion chromatography coupled with mass spectrometry (IC-MS) is described. The method utilised a high capacity, high efficiency anion-exchange column (Dionex IonPac AS11-HC, 4 mm, 2 × 250 mm), with the separation achieved using an electrolytically generated potassium hydroxide gradient, delivered at 0.380 mL min^{-1}. Applying the method, detection limits for iodate, bromate, and chlorate in seawater after direct sample injection (20 μL injection volume, samples diluted 10-fold), were 11, 30 and 13 ng L^{-1} (ppt), respectively. Standard addition calibrations to ozonated seawater samples were linear, in all cases $R^2 > 0.999$ ($n = 10$), with intra-day repeatability of 3.7, 11.2 and 1.8 % RSD ($n = 10$) for a low-level standard mixture (0.30 μg L^{-1} of iodate, 0.15 μg L^{-1} of bromate, and 1.50 μg L^{-1} of chlorate). The method was applied to the analysis of seawater samples taken pre- and post-disinfection points within a recirculating aquacultural system. Iodate, bromate and chlorate were detected as the main oxyanionic disinfection by-products, demonstrating the practical utility of the new method as a valuable tool for monitoring changes to seawater composition following disinfection treatments.

1. Introduction

The process of natural water oxidative disinfection using species such as ozone (O_3) and chlorine dioxide (ClO_2), is becoming commonplace within the various aquaculture industries, specifically to address high levels of organic contaminants in incoming natural feed waters, and to eliminate (or deactivate) chlorine-resistant pathogens and microorganisms, particularly when used in conjunction with ultraviolet irradiation. Ozone in particular is a powerful oxidant and is variously applied to treat both influent and effluent streams, and in some cases as a continuous low-level quality control treatment during recirculating aquacultural systems (RAS) operations [1, 2].

However, the ozonation of natural waters is well known to produce a variety of oxyanionic disinfection by-products (DBPs), a number of which are of concern within the aquacultural industry for their known and suspected impact upon cultured marine organisms. The formation of bromate from the oxidation of bromide present in natural waters is one such case, which has received a great deal of attention over the past few decades, and remains of significant interest today [3, 4]. Indeed, this unintended by-product from the use of ozone disinfection is a rather widespread concern, with reports on its impact and determination across a range of areas, including food and beverages [5], drinking water [6], medical products and solutions [7], and treated waste waters [8], to name but a few. In a similar way, conversion of iodide to iodate following ozonation is also of significant interest to the aquaculture industry. In a previous study carried out within our research group [9], the complete conversion of iodide to iodate after ozonation of the seawater within a marine aquaculture farm was shown, which could cause undesirable iodide intake deficiencies in its fish population. It is widely reported that such deficiencies cause several preventable disorders, in particular those related with thyroid hormones [10]. For example, likely due to deficiencies in synthesis of thyroid hormones driven by deficiencies in water iodide concentrations, lower larvae survival and body size of certain fish, namely, Pacific threadfin (*Polydactylus sexlis*) has been observed [11].

Given the above concerns, it is very clear that sensitive and accurate analytical methods to monitor DBPs generated in seawater are essential and are necessary to better understand and control marine aquacultural

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environments. For the determination of oxyhalide and ozonation DBPs, traditional approaches have involved the use of ion chromatography (IC) with suppressed conductivity and/or post-column reaction with selective UV/Vis absorbance detection [12, 13]. An early paper comparing various approaches to the detection of bromate following its separation using IC was presented by Schminke and Seubert [14]. In this comparison study inductively coupled plasma mass spectrometry (ICP-MS) was one detection option applied to the detection of bromate (as Br), proving to be the most sensitive and rugged method at the time, although rather an expensive option.

When it comes to the determination of trace concentrations (e.g., low to sub-μg L^{-1}) of oxyhalides and other oxyanions in complex environmental sample matrices, which in the case of natural waters typically means samples of high salinity (e.g. seawater), both sensitivity and selectivity remain a significant challenge, particularly when using the standard traditional IC flow-through detectors (e.g. suppressed conductivity and/or UV/Vis absorbance detection). Efforts to overcome these limitations have typically involved some form of sample treatment, e.g. matrix ion removal by barium or silver ion cartridges [15], or more complex approaches, involving multidimensional separations and complex valving arrangements to help reduce matrix ion loadings on the analytical column [16]. However, without doubt some of the most impressive analytical results have been demonstrated when the selectivity of IC is coupled with the combined selectivity and sensitivity of mass spectrometry (MS). The advantages of coupling modern suppressed IC systems with MS are many fold. Modern IC is available with electrolytic eluent generation e.g. Thermo Fisher Scientific's 'Reagent-Free technology', which in itself is compatible with new generations of high-performance, high-capacity and high-efficiency 4 μm ion-exchange resins/columns. In addition, modern eluent suppressors provide low peak dispersion and act to deliver separated peaks within an MS-compatible ultrapure water effluent. Modern quantitative MS (e.g., triple quadrupole-based mass analysers) is now also routinely available, which provides high sensitivity and selectivity across the low mass range for small ions, delivering detection limits in the low ppt (ng L-1) range for target inorganic species. There have been several reviews on the combination of IC-MS published over recent years, where these advantages and the various applications of the coupled technique are discussed in greater detail [17, 18, 19].

Herein, we present a new highly sensitive and selective method for the identification and quantification of iodate, iodide, bromate, bromide, and chlorate, by direct injection of 10-fold diluted seawater into an IC-MS system. Merging the advantages of IC and tandem mass spectrometry, quantification of the target analytes was achieved at ultra-trace levels. To demonstrate the practicality and validity of the new method, the determination of targeted oxyanions in seawater samples collected from a recirculating aquacultural system, pre- and post-ozonation and UV treatment points was performed.

2. Materials and methods

2.1. Reagents and standards

Simulated seawater (SSW) was prepared by dissolving 24.08 g of sodium chloride (NaCl, AR, >99.7%) from Chem-Supply (Gillman, Australia), 9.03 g of sodium sulphate 10-hydrate ($Na_2SO_4{\cdot}10H_2O$, >99.0%) from BDH Laboratory Reagents (Poole, England), 0.69 g of potassium chloride (KCl, >99.5%) from Ajax Chemicals, 0.20 g of sodium bicarbonate ($NaHCO_3$, 99.7–100.3%) from Sigma-Aldrich (St. Louis, USA), 0.03 g of boric acid (H_3BO_3, >99.5%) from Sigma-Aldrich (St. Louis, USA) in 1008.7 g of DIW in accordance with the concentrations recommended within the method of Kester et al. [20], here with the exclusion of sodium fluoride, potassium bromide, magnesium chloride, calcium chloride and strontium chloride. Acetate, formate, bromate, bromide, chloride, chlorite, chlorate, nitrite, nitrate, sulfate, carbonate, phosphate, iodate, iodide and perchlorate stock solutions (1000 mg L^{-1}) were prepared by dissolving appropriate amounts of sodium acetate (NaOAc, >99%) from Ajax Chemicals (Australia), sodium formate (HCOONa, >99%) from Sigma-Aldrich (St. Louis, USA), sodium bromate ($NaBrO_3$, >99.5%) from Hopkin & Williams (London, England), sodium bromide (NaBr, ≥99.0%) from Sigma-Aldrich (St. Louis, USA), sodium chloride (NaCl, >99.7%) from Chem-Supply (Gillman, Australia), sodium chlorite 25% solution in water ($NaClO_2$) from Merck (Darmstadt, Germany), sodium chlorate ($NaClO_3$, ≥99.0%) from Sigma-Aldrich (St. Louis, USA), sodium nitrite ($NaNO_2$, AR) from Ajax Chemicals (Australia), sodium nitrate ($NaNO_3$, >98%) from BDH Laboratory Reagents (Poole, England), sodium sulfate 10-hydrate ($Na_2SO_4{\cdot}10H_2O$, >99.0%) from BDH Laboratory Reagents (Poole, England), sodium bicarbonate ($NaHCO_3$, 99.7–100.3%) from Sigma-Aldrich (St. Louis, USA), potassium phosphate tribasic (K_3PO4, >97%) from Acros Organics (Fair Lawn, USA), sodium iodate ($NaIO_3$, AR grade) from Hopkin & Williams (London, England), sodium iodide (NaI, ACS grade) from Sigma-Aldrich (St. Louis, USA), sodium perchlorate ($NaClO_4$, >98%) from Sigma-Aldrich (St. Louis, USA). The solutions were kept at 4 °C and stored in high-density polyethylene (HDPE) containers until use. SSW diluted 10 times (SSW-DF10) as sample matrix was used to prepare working and calibrated standards. Working standard solutions of 125 μg L^{-1} iodate, 125 μg L^{-1} bromate, 250 mg L^{-1} bromide, 125 μg L^{-1} chlorate, and 75 μg L^{-1} iodide were prepared by diluting certain amounts of stock solutions in SSW-DF10. Calibration standards between 0.015-80 μg L^{-1} iodate, 0.015–80 μg L^{-1} bromate, 0.03–160 mg L^{-1} bromide, 0.015–80 μg L^{-1} chlorate, and 0.009–48 μg L^{-1} iodide were prepared by appropriate dilution of the working standard solution of 5 anions in SSW-DF10. Working and calibration solutions were prepared daily.

2.2. Samples and sample treatment

Sampling was carried out by trained staff at 9 different sites within a recirculating aquaculture system for onshore crustacea production. Seawater samples were collected in duplicate (A and B) using 1 L high density polyethylene (HDPE) bottles, which were previously prepared following a rigorous cleaning protocol, consisting of soaking the bottles for 3 days in DIW, with rinsing every 24 h. A total of 18 samples was transported to the analytical laboratory on the same day and stored at 4 °*C prior* to analysis. All samples were filtered through a nylon syringe filter (pore size 0.45 μm) to eliminate solid particles and then diluted 10 times using DIW. Sample collection information is detailed within Table 1. Figure 1 shows the sampling points within a schematic of the RAS.

2.3. Instrumental

An ICS-5000+ Reagent-Free™ IC (RFIC™) system coupled to an Ion Max NG source operating in heated-ESI (H-ESI) mode and a TSQ Quantiva™ triple-stage quadrupole mass spectrometer was used throughout. Figure 2 shows a schematic of the IC-MS system used in this study. Following sample treatment, e.g., filtration and dilution, 20 μL of sample was injected using an AS-AP autosampler onto the coupled IonPac AS11-HC guard (4 μm, 2 × 50 mm) and analytical columns (4 μm, 2 × 250 mm). High purity KOH eluent from DIW was electrolytically generated by an eluent generator module using an EGC-KOH cartridge. An anion trap was installed after the eluent generator module to remove trace anionic contaminants from the eluent. Target analytes were separated at 45 °C using a KOH eluent, in gradient mode, at a flowrate of 0.380 mL min^{-1} within a total run time of 30 min, including a short cleaning step to elute strongly retained compounds, and an equilibration step before the subsequent injection. An anion self-regenerating suppressor 500e (AERS® 500e) was used in-line after the column which electrolytically converted the hydroxide eluent to water. The suppressor was regenerated in external mode using DIW at 0.4 mL min^{-1} delivered by an AXP pump. A conductivity detector (CD) was placed after the suppressor and before the mass spectrometer, to be used as secondary detector. Acetonitrile was

Table 1. Sample information data.

Sample number	Description
8	Raw seawater
9	Raw seawater +1st ozone treatment
1	Post foam fractionator-ozone treatment
2	Post 1st contact
3	Post 2nd contact
4	Post degasser
5	Post carbon filter
6	Culture tank-without UV treatment
7	Pre culture tank-after 10 min UV treatment

added using a second auxiliary pump at a flowrate of 0.12 mL min^{-1} into the IC eluent stream *via* a mixing tee immediately before the divert valve to assist in the ionisation at the ESI source. The MS operating conditions used for the quantification of DBPs, along with chromatographic parameters of the IC system, are summarised in Table 2 and Table 3. Detection was achieved in negative ESI and selected reaction monitoring (SRM) acquisition modes. The SRM mode is the key operating mode for target compound quantification with a triple quadrupole mass spectrometer, describing one single transition from a parent ion to a product ion and providing highly selective and sensitive detection. SRM transitions used for the detection of target analytes are detailed in Table 3. SRM acquisition mode provides better S/N ratios than full-scan acquisition mode due to a significant reduction in the background noise. As it can be observed in Table 3, as some anions do not fragment further, 'pseudo-SRM' transitions, where the precursor ion selected for Q1 was also monitored as the product ion for Q3, were used. SRM transitions, collision energy and RF lens voltages were optimised by direct infusion of individual standard solutions of 10 mg L^{-1} for each anion into the mass spectrometer using an automatic optimisation software tool. All instrumentation and columns were from Thermo Fisher Scientific (Sunnyvale, USA). Instrument control, data acquisition and processing were via Chromeleon® 7 Software, version 7.2.6 (10049).

2.4. Validation of the IC-MS method

The method was fully evaluated in terms of its analytical performance characteristics using standard solutions of iodate, iodide, bromate, bromide and chlorate. Chlorite was not included in this validation as it was not detected in a previous screening of the RAS samples. Standard solutions were prepared in DIW:SSW at 9:1 ratio to simulate diluted seawater matrix of real samples. Validation of the IC-MS method included the evaluation of matrix effects, carry-over, limit of detection (LOD), linearity and inter- and intra-day repeatability. Matrix effects were evaluated by comparing the response for a standard solution of the

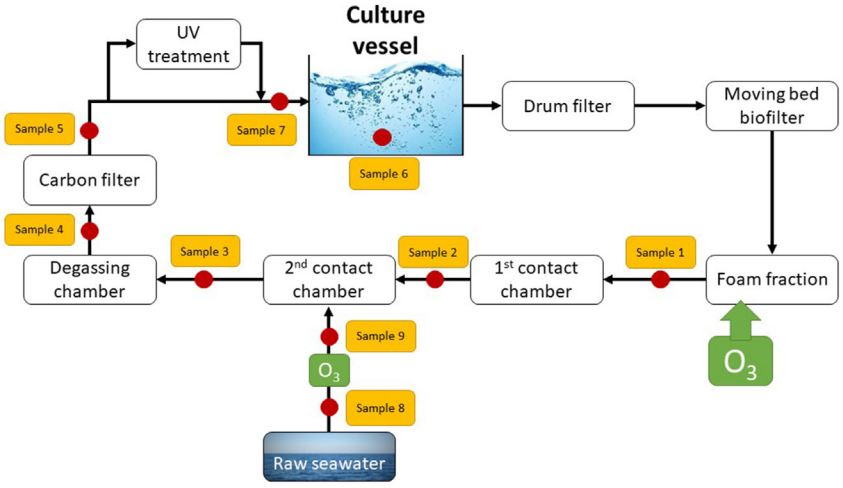

Figure 1. Sampling points within the recirculating aquacultural system (RAS).

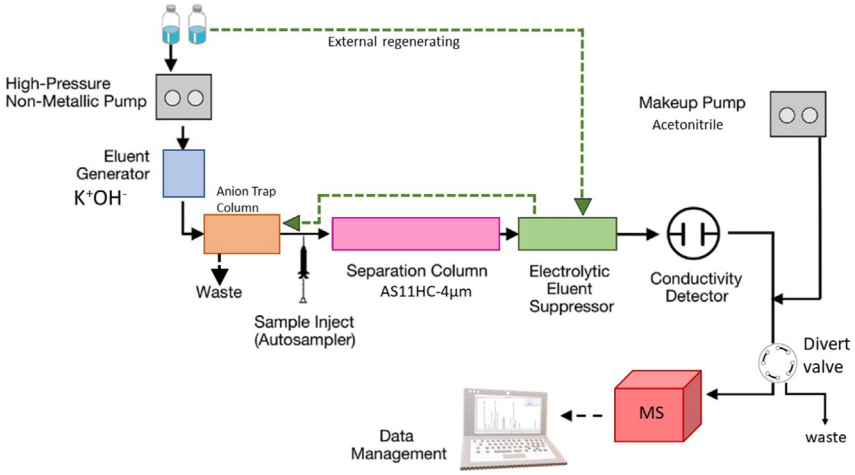

Figure 2. Schematic of the IC-MS system used in this study.

Table 2. IC instrumental parameters and settings.

Column:	IonPac AS11-HC (2 × 250 mm), 4 μm	
Guard:	IonPac AG11-HC (2 × 50 mm), 4 μm	
Temperature:	45 °C	
Injection volume:	20 μL	
Eluent:	Gradient of KOH:	
	Time (min)	KOH (mM)
	0	15
	12	15
	15	90
	20	90
	22	15
	30	15
Eluent flow rate:	0.380 mL min^{-1}	
Detector:	Conductivity	
Data collection rate:	5.0 Hz	
Cell temperature:	35 °C	
Suppressor:	AERS 500e (2 mm)	
External Water Mode flow rate:	0.400 mL min^{-1}	
Applied current:	43 mA	

Table 3. MS instrumental parameters and settings.

Make-up solvent			
Make-up solvent flow:			
Ion source Type:			
Ionisation mode:			
Spray voltage:			
Sheath gas:			
Aux gas:			
Sweep gas:			
Ion transfer tube temp:			
Vaporiser temp:			
Acquisition mode:			
Compound	Precursor (Q1) (m/z)	Product (Q3) (m/z)	Collision Energy (eV)
chloride	35	35	0
nitrite	46	46	0
nitrate	62	62	0
chlorite	67	67	0
bromide	79	79	0
chlorate	83	67	20
sulfate	97	79	24
phosphate	97	79	15
perchlorate	99	83	20
bromate	127	111	20
iodide	127	127	0
iodate	175	159	21
Q1 Resolution (FWHM)			
Q3 Resolution (FWHM)			
CID Gas (mTorr)			

target analytes prepared in DIW with that from a sample prepared in 10-times diluted SSW. Blank samples injected after the highest calibration standards and between samples were used to determine carry-over issues. LODs and LOQs were calculated as the concentration providing signal-to-noise ratios (S/N) equal to 3 and 10, respectively. Intra- and inter-day repeatability were determined over the same day and across two different days, respectively, from 10 replicates of a standard mixture containing 0.30 μg L^{-1} of iodate, 1.44 μg L^{-1} of iodide, 0.15 μg L^{-1} of bromate, 0.06 mg L^{-1} of bromide and 1.50 μg L^{-1} of chlorate.

3. Results and discussion

3.1. Optimisation of chromatographic separation

The IonPac™ AS11-HC-4μm column and guard were chosen as being optimal for this study as it is a column designed specifically to provide high resolution of a large number of inorganic anions and organic acid anions from complex samples in one gradient run using an hydroxide eluent system. This column is a high resolution (4 μm i.d. particle size)

and high capacity (72.5 μeq/column) anion-exchange column. The high resolution provides better separation and lower LODs due to better peak shape, whilst the high capacity allows injection of more concentrated samples or larger injection volumes without overloading the column. The main limitation of this column is that it can only be use with an IC capable of operating at 5000 psi or higher [21]. The standard working flow rate for this column and guard, 0.380 mL/min, which already generated around 3900 psi, was used throughout the optimisation process. Lower pressure could be obtained by reducing the flow rate, but that could deliver longer run times and poorer peak shapes.

In order to evaluate the selectivity of this column, a separation of 15 anions, namely, acetate, iodate, formate, chlorite, bromate, chloride, nitrite, bromide, nitrate, chlorate, carbonate, sulfate, phosphate, iodide, and perchlorate was optimised using various KOH gradients. Column capacity was tested by injecting up to 400 μL of the above anion mixture. Figure 3 shows the CD chromatogram obtained for a low-level standard mixture containing 50 μg L^{-1} of each anion prepared in DIW using the optimal KOH gradient (Gradient 1), which is shown as a dashed line in Figure 3. With extremely high concentrations of chloride and sulfate expected in real samples, optimisation of the chromatographic separation was focused on obtaining the best possible separation for all the probable anions present in the aqueous samples, especially between bromate and chloride, even at the expense of a slightly longer run time. It is important to note that to achieve this separation the optimal gradient started at very low KOH concentration (1mM) which may cause unwanted peak broadening, although this was compensated by the excellent efficiency provided by the 4 μm i.d. particle size of the stationary phase. It can be observed that although the injection volume was 200 times greater than the recommended injection volume for standard 2 mm i.d. columns, the high capacity of the AS11-HC-4μm column allowed for the injection of large volumes, whilst preserving peak shapes.

Anticipating the nature of the samples to be analysed, which were characterised by a high concentration of matrix anions, such as chloride and sulfate combined with an ultra-trace concentration of targeted ozonation by-products such as iodate, chlorate, chlorite or bromate, a compromise regarding dilution factor and injection volume was established. Injection volume was reduced to 20 μL and different dilution factors (DF) for a 20 μL injection volume size were tested, namely, 40, 25 and 10, finding that a DF of 10 was optimal to not overload the column with chloride, and to also allow the detection of the ultra-trace concentrations of targeted oxyhalides.

However, even after reducing injection volume and diluting seawater samples, when the optimised Gradient 1 was applied to the analysis of the same standard mixture prepared within DIW:SSW (9:1 v/v), bromate and chloride (the former now present in much higher concentration) were completely co-eluted. In addition, the peak widths for those analytes of interest eluting before chloride (e.g., iodate, chlorite and bromate) increased dramatically from sample self-elution, due to the high salt concentration. Therefore, although Gradient 1 is an excellent example of the separation achievable using the AS11-HC-4μm column, it was not directly applicable to the analysis of oxyhalides in diluted seawater samples. Assuming that no practical separation was indeed possible for bromate and chloride within the concentration ranges expected in seawater samples, the objective was to evaluate the extent of the matrix effect caused by the chloride concentration on the quantification of the target analytes, namely, bromide, bromate, iodide, iodate and chlorate, and to maximise the signal for these targeted compounds through the reduction of peak width generated by Gradient 1. Chlorite was taken out of the study at this point as it was not detected in any of the samples in pre-screening.

Consequently, different KOH gradients starting at higher concentrations than 1 mM to improve peak shape were evaluated. SRM traces for each anion and the values for peak width (at 50% high, min) were used for evaluation. It was found that peak width for iodate and bromate were reduced by 88 and 89 %, respectively, when using a gradient starting at 15 mM KOH (Gradient 2). For the targeted anions eluted after chloride (e.g., bromide, chlorate and iodide) the improvement in peak width was less significant being 26, 19 and 16 %, respectively. Nevertheless, by applying Gradient 2, bromate and chloride remained coeluted at the concentrations found in the real samples, but both peak width and run time were successfully reduced. To evaluate matrix effects, the response for a standard solution of the target analytes prepared in DIW and those prepared in 10-times diluted SSW were compared. Table 4 shows the absolute matrix effect, calculated as (Area in SSW/Area in DIW)*100, obtained for iodate, bromate, bromide, chlorate, and iodide. As can be observed, matrix effects were significant only for bromate, which suffered an approximately 3-times reduction in its response. However, as it will be detailed in the next section (*Validation of the IC-MS)*, bromate was still quantifiable at the ultra-trace concentrations expected in the seawater samples. Although the absolute matrix effect was less extensive for the rest of the targeted analytes, quantification of all target anions was carried out using the standard addition method, which is the calibration method commonly used to overcome this effect.

3.2. Validation of the IC-MS method

Validation of the IC-MS method included evaluation of matrix effects (already discussed in previous section), carry-over, limit of

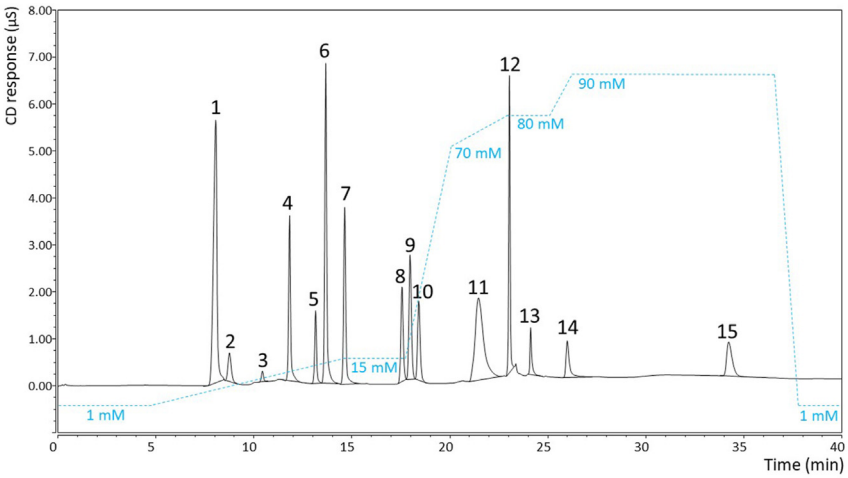

Figure 3. CD chromatogram obtained for the analysis of a standard mixture 15 anions, namely, acetate (1), iodate (2), formate (3), chlorite (4), bromate (5), chloride (6), nitrite (7), bromide (8), nitrate (9), chlorate (10), carbonate (11), sulfate (12), phosphate (13), iodide (14), and perchlorate (15), containing 50 μg L^{-1} of each anion. Injection volume 400 μL. Dash line: KOH gradient 1.

Table 4. Analytical Performance of the IC-MS method.

	Iodate	Bromate	Bromide	Chlorate	Iodide
Absolute matrix effect (%)[a]	109.1	36.0	100.3	94.1	70.6
LOD (μg L^{-1})[b]	0.011	0.030	0.425	0.013	0.064
LOQ (μg L^{-1})[b]	0.037	0.100	1.416	0.045	0.210
Calibration curve fit	linear	linear	polynomial, order 2	linear	linear
Regression Coefficient (R^2)	0.9994	0.9999	0.9990	0.9997	0.9996
Upper limit of calibration (μg L^{-1})	5	80	10000	80	24
Number of points	8	10	8	10	7
Repeatability intra-day (% RSD)[c]	3.65%	11.24%	3.09%	1.75%	2.44%
Repeatability inter-day (% RSD)[d]	4.74%	11.43%	5.55%	5.79%	4.97%

[a] Absolute matrix effect was calculated as (Area in SSW/Area in DIW)*100.
[b] LODs and LOQs were calculated as concentration providing a S/N $\geq$ 3 and S/N $\geq$ 10, respectively.
[c] This parameter was obtained by injection during the same day of 10 replicates of a standard mixture containing 0.30 μg $L^{-1}$of iodate, 0.15 μg L^{-1}, of bromate, 0.06 mg L^{-1}, of bromide, 1.50 μg L^{-1}, of chlorate and 1.44 μg L^{-1}, of iodide.
[d] This parameter was obtained by injection during two different days of 10 replicates of a standard mixture containing 0.30 μg L^{-1}, of iodate, 0.15 μg L^{-1}, of bromate, 0.06 mg L^{-1}, of bromide, 1.50 μg L^{-1}, of chlorate and 1.44 μg L^{-1}, of iodide.

detection (LOD), linearity and repeatability inter- and intra-day. Validation results are summarised in Table 4. To avoid carry-over issues, detected as the presence of chloride within blank samples (DIW) injected between seawater samples, an additional injection of a blank sample between seawater samples using a quick cleaning method, which reached a high KOH concentration (run time = 15 min), was applied. In this manner, no carry-over issues were identified for blank samples injected after the cleaning injection. LODs were similar or lower to those reported in the literature for the analysis of these anions in seawater samples by other methods [9, 22, 23, 24]. Upper limits of the calibration curves were set to evaluate the response linearity range for each targeted analyte. All analytes presented a calibration curve with a linear fit from the LODs to the upper limit tested, except for bromide which exhibited a calibration curve with a quadratic fit. All calibration curves showed regression coefficients (R^2) within the range 0.9990–0.9999. It can also be observed from Table 4 that both intra-day and inter-day repeatability were typically below 6%, based upon peak area. Only bromate gave values around 11%, resulting from its co-elution with chloride. Nevertheless, repeatability values of under 15% RSD are typically analytically acceptable at these concentration levels (μg L^{-1}) within a complex matrix.

Figure 4 shows the SRMs chromatograms for iodate, bromate, bromide, chlorate, and iodide obtained for the analysis of a standard mixture containing 1.25 μg L^{-1} of iodate, bromate and chlorate, 2.5 mg L^{-1} of bromide, and 0.75 μg L^{-1} of iodide using the new method developed.

3.3. Analysis of aquacultural seawater samples

Table 5 and Figure 5 summarise the results obtained for the analysis of the samples detailed in Table 1. Quantification was based on the standard addition method using calibration solutions prepared in SSW. Concentrations for each sample were calculated as an average of the duplicates, A and B, taken during the sampling. % RSD obtained for these duplicates was under 5% in most of the cases, although a higher deviation for some analytes was observed for samples 1A and 1B and for samples 6A and 6B. Nevertheless, those higher % RSD were always under 15% and the observed irreproducibility can be attributed to the fact that the RAS is a dynamic system and samples were taken at slightly different times.

Iodide was only detected in samples 8 and 9 at concentrations of 3.24 and 1.15 μg L^{-1}, respectively. The decrease from sample 8 (raw seawater) to sample 9 (after first ozonation treatment) can be due to oxidation of iodide to iodate by ozone. However, iodate concentration seems to be stable throughout all the RAS sample points, without displaying any significant variations. Although iodide concentration in raw sweater was below than the expected concentration [25], sample 8 was actually taken from the lower reaches of an estuarine environment at a depth of 15 m, where speciation typically favours iodate [26]. While it is known that low iodide waters can cause deficiencies in fish species [11], there is no evidence that low iodide marine waters have a negative effect on crustacean larval rearing.

Bromate concentration increased from the base level found in sample 8 with a concentration of 1.26 μg L^{-1}, up to 3.82 μg L^{-1} in sample 9, and in the range from 5.13 to 5.58 μg L^{-1} for the rest of the samples from different points within the RAS, demonstrating oxidation of bromide to bromate by ozone treatment. Bromate has been identified as a potential human carcinogen and is currently regulated at a maximum level within drinking water of 10 μg L^{-1} in US, China, Canada, EU, Japan and 20 μg L^{-1} in Australia [27]. Butler et al. [28] reviewed bromate ecotoxicity and summarised that lethal concentrations (LC_{50}) for bromate ranged from 31 mg L^{-1} to 2258 mg L^{-1}, depending on the species investigated, which included crustaceans, flatworms and juveniles of various fish species. Based upon those results, a cautionary ecotoxicity exposure safety value of 3.0 mg L^{-1} bromate in natural water sources, which is 10 times smaller than the LC_{50} observed for the most sensitive species, was recommended. Other studies mentioned by Butler et al. [28] observed persistent brain and spine diseases in fish eggs exposed to bromate and an increment in cell division in some species of marine phytoplankton when exposed to 13.6 mg L^{-1} of bromate. Nevertheless, bromate concentrations found in the samples analysed in the present study are far below from those values, including the proposed ecotoxicity exposure safety value of 3.0 mg L^{-1}.

Bromide was present at a steady concentration level throughout the RAS system, although sample 8 (raw seawater) and sample 3 (post-2nd contact chamber) showed higher concentrations than the rest of the samples. The bromide concentration found in raw seawater (70.22 mg L^{-1}) was within the normal concentration range reported in the literature (65–78 mg L^{-1}) [27].

As expected, chlorate was not found in raw seawater and was also not detected after the first ozonation treatment in sample 9. However, its concentration subsequently increased in all samples within the RAS, reaching a regular concentration level of around 100 μg L^{-1} for all samples, suggesting a clear production of chlorate by the second ozone treatment. However, according to the literature [29], chlorate appears to be non-toxic (LC_{50} is greater than 100 mg L^{-1}) to freshwater life. The lowest effect level, a 96-hour LC_{50} to a freshwater fish, (larval cherry salmon, *O. masou*) was 863 mg L^{-1} of chlorate. For marine life, the guideline is set to 5 μg L^{-1} to protect the most sensitive species, e.g., brown algae.

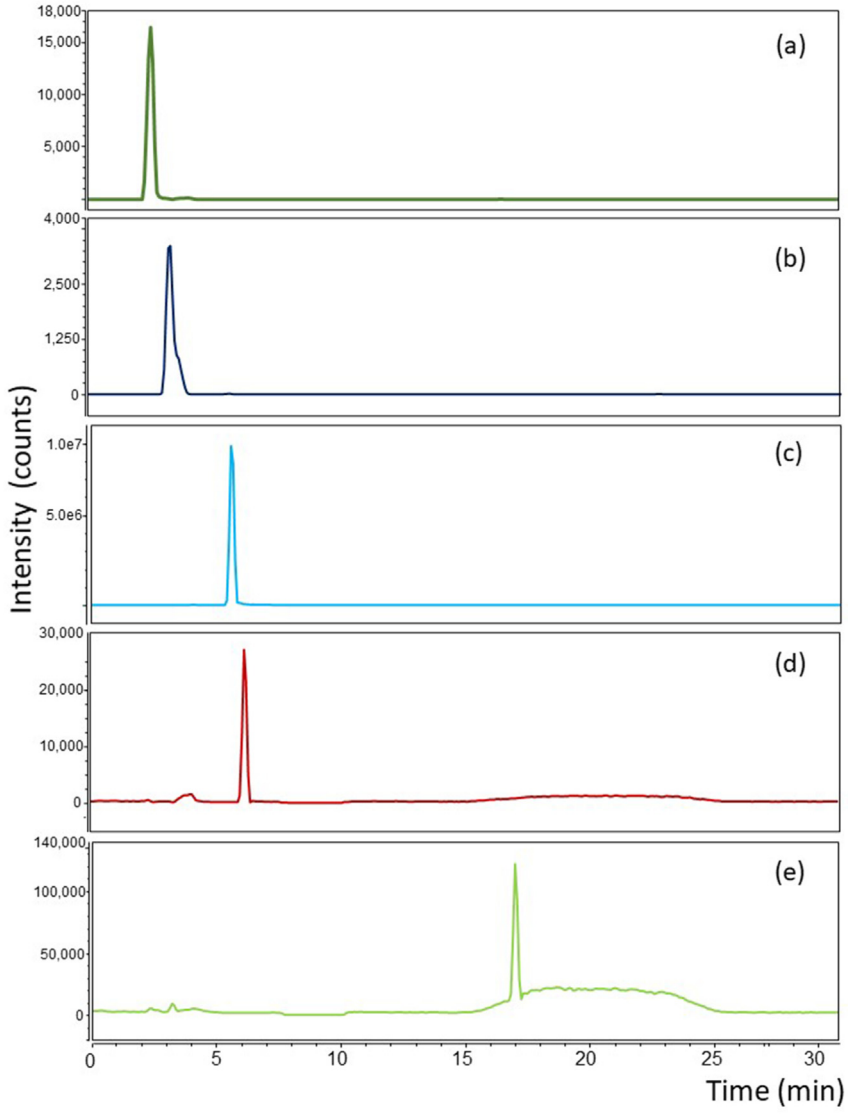

Figure 4. SRMs chromatograms for iodate (a), bromate (b), bromide (c), chlorate (d), and iodide (e) obtained for the analysis of a standard mixture containing 1.25 μg L^{-1} of iodate, bromate and chlorate, 2.5 mg L^{-1} of bromide, and 0.75 μg L^{-1} of iodide using the new method developed (instrumental conditions detailed in Tables 2 and 3).

Table 5. Iodate, bromate, bromide, chlorate and iodide concentration for the 9 samples analysed (Average ±SD, n = 2, duplicate A and B samples). SD: Standard deviation.

Sample	Concentration (μg L^{-1})				
	Iodate	Bromate	Bromide	Chlorate	Iodide
8	107.89 ± 2.13	1.26 ± 0.17	70.22 ± 1.00	n.d.	3.24 ± 0.32
9	113.49 ± 6.07	3.82 ± 0.28	69.66 ± 0.43	n.d.	1.15 ± 0.17
1	78.76 ± 19.79	5.58 ± 0.27	66.61 ± 2.40	109.35 ± 10.53	n.d.
2	99.23 ± 9.36	5.49 ± 0.41	64.41 ± 0.18	103.27 ± 2.37	n.d.
3	115.70 ± 4.35	5.49 ± 0.34	72.76 ± 1.35	100.91 ± 3.78	n.d.
4	114.41 ± 7.04	5.38 ± 0.19	68.30 ± 6.00	99.89 ± 1.94	n.d.
5	115.23 ± 6.27	5.13 ± 0.30	68.66 ± 0.71	95.55 ± 1.59	n.d.
6	100.95 ± 26.15	5.35 ± 0.60	66.07 ± 2.37	111.16 ± 3.99	n.d.
7	120.16 ± 12.65	5.49 ± 0.23	68.33 ± 6.00	98.72 ± 5.06	n.d.

n.d.: not detected.

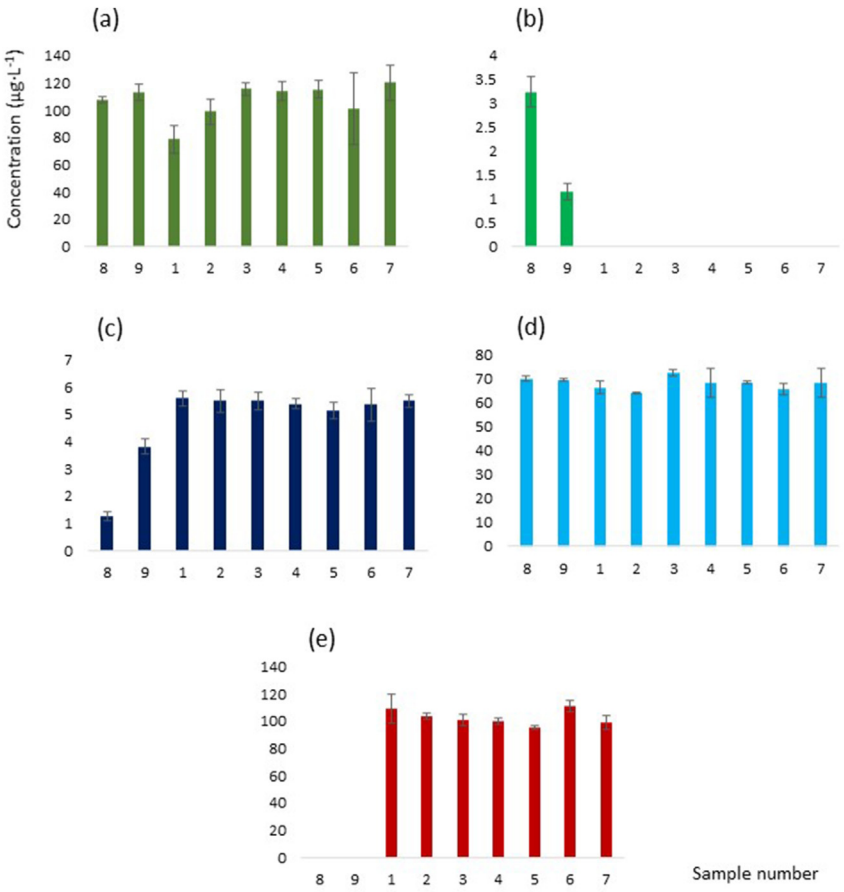

Figure 5. Iodate (a), iodide (b), bromate (c), bromide (d), and chlorate (e) concentration for the 9 samples analysed (Average ±SD, n = 2).

The application of ozone to seawater results in the production of numerous DBPs. The anions measured in this study were primarily the stable start (iodide and bromide) and end (iodate, bromate and chlorate) points of these reactions. Active disinfection is facilitated by intermediate species, including hypobromous acid/hypobromite ion ($HOBr/OBr^-$) [30]. The future quantification of these intermediate by-products will be of significant value for water treatment in mariculture.

4. Conclusion

A new IC-MS based method for the determination of iodate, bromate, chlorate, bromide, and iodide at ultra-trace levels in seawater samples has been developed. The proposed method owes its success to four key factors: (1) The high capacity of the chosen AS11-HC column allowed large-volume injections of seawater samples diluted just 10-fold. (2) The high resolution provided by the 4µm particle size packing of the AS11-HC column provided excellent peak shapes, even when using large injection volumes containing relatively high concentrations of matrix ions, which made possible the quantification of targeted analytes even at ultra-trace levels. (3) SRM acquisition mode, available using a triple quadrupole detector, provided the ultra-selective and sensitive detection required to quantify analytes such as bromate, present at low µg L^{-1} levels, even when they were co-eluted with species present at high concentration such as chloride. (4) Quantification by standard addition method to overcome matrix effects.

The method was applied for the analysis of various seawater samples from within a modern aquaculture facility wherein ozonation and UV treatment were used to disinfect the seawater circulating in the system. Results confirmed that iodide was completely transformed to iodate, with iodate, bromate and chlorate being the main ozonation by-products generated. The developed method has demonstrable practical utility as a valuable tool for monitoring and understanding water treatment protocols in aquaculture facilities, with a view to monitoring and avoiding undesirable iodide deficiencies and/or oxyhalides intake in fish populations.

Declarations

Author contribution statement

Estrella Sanz Rodriguez: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Shing Lam: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Gregory G. Smith: Contributed reagents, materials, analysis tools or data; Wrote the paper.

Paul R. Haddad: Analyzed and interpreted the data; Wrote the paper.

Brett Paull: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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