Nota de aplicación

Determination of Oxyanions in Drinking Water by Direct Injection Using Liquid Chromatography-Tandem Quadrupole Mass Spectrometry

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Abstract

The purpose of this work is to demonstrate a direct injection method for the determination of chlorate, perchlorate, bromate and chlorite in drinking water that exceeds the requirements for the new EU Drinking Water Directive 2020. The method performance study was completed on an ACQUITY UPLC I-Class PLUS System with a Xevo TQ-XS using the Anionic Polar Pesticide Column. Method performance was assessed using 3 spike levels (20, 50, and 100 µg/L for chlorate and perchlorate, 2, 5, and 10 µg/L for bromate and 100, 250, and 500 µg/L for chlorite) with 22 replicates at each level split over 2 batches for each water type studied. The water types studied were mineral water, soft and hard drinking water. Residues of chlorate were detected in soft (54.3 µg/L) and hard (12.0 µg/L) water and quantified by standard addition, which are significantly lower than the parametric values listed by the EU Drinking Water Directive. Method performance for average trueness values across all water types and spike levels for perchlorate 96.2–100.3%, chlorate 98.1–100.5% (mineral water only), bromate 92.2–102.1% and chlorite 84.1–114.1%. RSDs were significantly below 10% except for chlorite where higher RSDs were observed for some water types. Retention time repeatability was recorded through all method performance batches and an additional repeatability study, where RSDs for all analytes across over 550 injections were less than 1%.

Benefits

- Robust and reproducible analytical method allowing for a simpler laboratory workflow with method performance that exceeds regulatory requirements
- Consistent method performance across all 6 method evaluation batches in the 3 different water types (mineral, soft, and hard drinking water)
- Simple sample handling before determination stage

Introduction

Bromate, chlorite, and chlorate are disinfection byproducts formed during the water treatment process. Chlorate and chlorite are formed during the disinfection process when using hyperchlorite, whereas bromate is formed as a byproduct from water ozonation. Perchlorate can be found in water either through natural or man-made sources such as run off from land, where natural fertilizers have been used. Perchlorate can also be formed during the degradation of sodium hypochlorite, during water disinfection, as well as being a byproduct of various industrial processes.[1] Perchlorate, chlorate, and chlorite are known to be hazardous to human health while bromate is classed as a possible human carcinogen.[1] Monitoring of these compounds in water is essential for public health.

A fast, efficient method for the determination of oxyanions in drinking water is important as we recognize and manage risks associated with chemical contamination in drinking water supplies. The World Health Organization (WHO) updated drinking water guidelines in 2017[2] and the EU are to set new standards in chemical contamination in an updated EU Drinking Water Directive.[3] The new Directive adds chlorite (with a new parametric value of 250 µg/L), whilst maintaining a requirement to monitor for chlorate (with an unchanged parametric value of 250 µg/L) and bromate (with an unchanged parametric value of 10 µg/L). The WHO has an additional guideline for perchlorate (with a level of 70 µg/L) and has a higher concentration of 700 µg/L for chlorate and chlorite. The US EPA includes bromate (MCL 0.01 mg/L) and chlorite (MCL 1 mg/L) in their National Primary Drinking Water Regulations.[4]

Common methods for the analysis of oxyanions require separation with ion chromatography using suppressed conductivity detection. Often to achieve the required detection limits, in some cases for bromate, post column derivatization (PCD)[5] is required, using UV detection. We have developed a fast, direct injection method with low injection volume utilizing the Anionic Polar Pesticide Column. This approach allows for rapid analysis of the 4 oxyanions within 5 minutes on a tandem quadrupole mass spectrometer which is becoming a popular tool for the analysis of these compounds. The benefit of this technology is that it gives reliable quantification of these analytes using multiple reaction monitoring (MRM), detecting all 4 oxyanions in drinking water without the need for PCD or specialized chromatography equipment.

Experimental

Sample Description

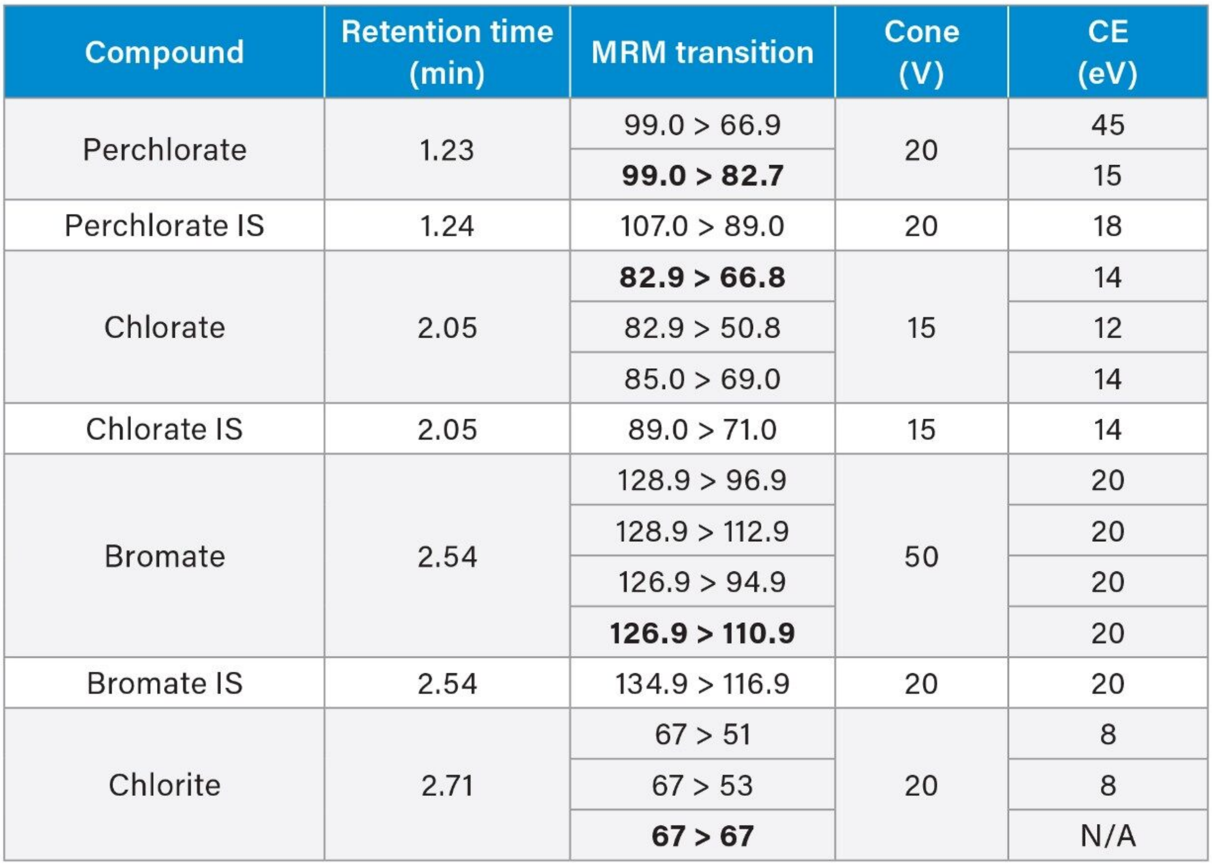
Several 1 liter samples of tap water from a soft water (Wilmslow, UK) and hard water (York, UK) area were collected and transported to the Applications laboratory (Wilmslow, UK). Several 1 liter samples of a generic mineral water were purchased in the UK for use as matrix blanks.

Method Conditions

Once the drinking water samples were received in the laboratory, they were split into aliquots of 50mL in plastic falcon tubes. The samples were then stored at 4 °C in a fridge until analysis.

Just before analysis, the sample was transferred to an LC vial, isotopically labelled internal standards were added ($Cl^{18}O_3$- and $Cl^{18}O_2$- at 100 µg/L in sample and $Br^{18}O_3$- at 10 µg/L in sample) for the determination of the oxyanions. Only chlorite was not internally calibrated as no suitable internal standard was identified for this analyte.

After the method development phase, a method performance study was conducted for mineral water, soft water, and hard water which consisted of 2 batches for each water type containing 33 spiked water samples: 11 spikes at a designated low, mid and high concentration; 2, 5, and 10 µg/L for bromate, 20, 50, and 100 µg/L for chlorate and perchlorate and 100, 250, and 500 µg/L for chlorite. The MRMs listed in Table 1 highlight the optimized transitions used for quantification and confirmation of the oxyanions in this application.



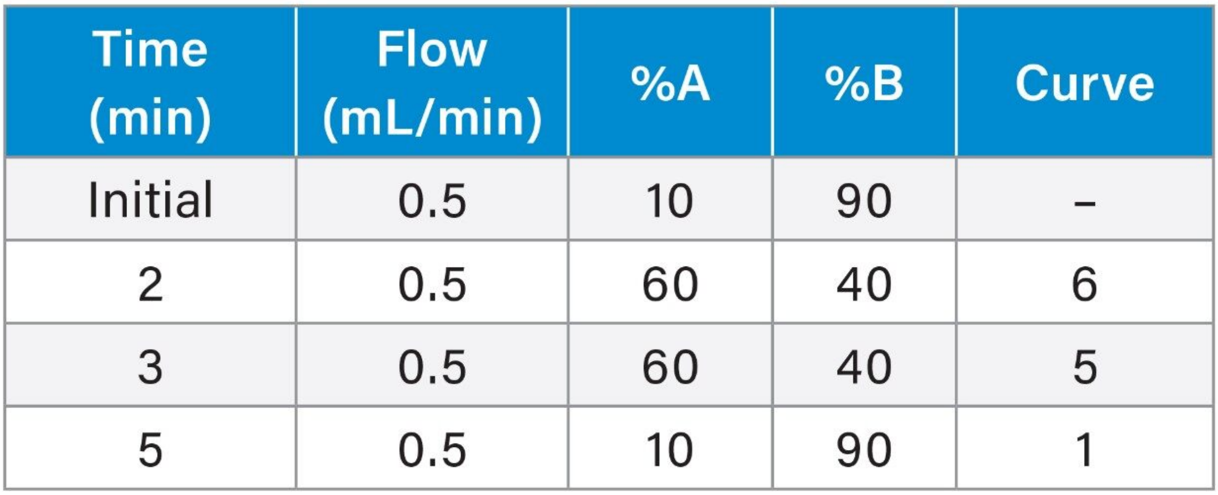
Compound	Retention time (min)	MRM transition	Cone (V)	CE (eV)
Perchlorate	1.23	99.0 > 66.9	20	45
		99.0 > 82.7		15
Perchlorate IS	1.24	107.0 > 89.0	20	18
Chlorate	2.05	**82.9 > 66.8**	15	14
		82.9 > 50.8		12
		85.0 > 69.0		14
Chlorate IS	2.05	89.0 > 71.0	15	14
Bromate	2.54	128.9 > 96.9	50	20
		128.9 > 112.9		20
		126.9 > 94.9		20
		126.9 > 110.9		20
Bromate IS	2.54	134.9 > 116.9	20	20
Chlorite	2.71	67 > 51	20	8
		67 > 53		8
		67 > 67		N/A

Table 1. MRM transitions, optimum dwell time was set automatically using the auto-dwell function. (quantitative transitions in bold).

LC Conditions

LC system:	ACQUITY UPLC I-Class PLUS System (FL)
Detection:	Tandem Quadruple MS
Vials:	Waters TruView LCMS Certified Clear Glass 12 x 32 mm, Screw Neck Vial (186005666CV)
Column(s):	Waters Anionic Polar Pesticide Column 5 µm, 2.1 x 50 mm (186009286)
Column temp.:	50 °C
Sample temp.:	10 °C
Injection volume:	5 µL
Flow rate:	0.5 mL/min
Mobile phase A:	Water with 0.9% formic acid and 50 mM ammonium formate (LC-MS grade)
Mobile phase B:	Acetonitrile with 0.9% formic acid (LC-MS grade)

Gradient:



Time (min)	Flow (mL/min)	%A	%B	Curve
Initial	0.5	10	90	–
2	0.5	60	40	6
3	0.5	60	40	5
5	0.5	10	90	1

MS Conditions

MS system:	Xevo TQ-XS
Ionization mode:	ESI-
Acquisition range:	MRM
Capillary voltage:	0.5 kV
Cone gas flow:	150 L/hr
Desolvation temperature:	400 °C
Desolvation gas glow:	800 L/hr
Source temperature:	150 °C

Data Management

Informatics:	MassLynx v4.2

Results and Discussion

The injection diluent was investigated during the method development phase of this project. We investigated the requirement to add acetonitrile to ensure acceptable peak shape for a HILIC Column. The results shown in Figure 1 confirm that there is no significant loss of peak integrity when injecting undiluted water compared to the standard that was composed of 50% acetonitrile. Injection volume was investigated to allow an increase in sensitivity if required. The results shown in Figure 2 confirm that there is no significant loss of peak integrity when injecting 1 µL compared to 5 µL of an undiluted water sample. For these initial investigations it was concluded that 5 µL of an undiluted water could be injected onto the Anionic Polar Pesticide Column without significant loss of peak integrity and this format was used for the method performance study to improve method limits of quantification.

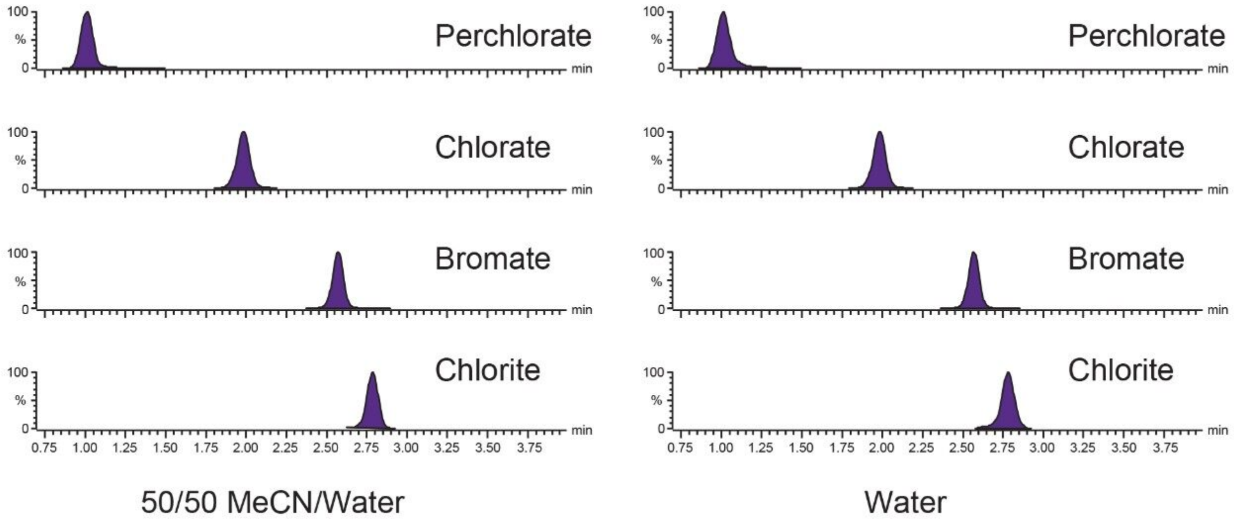


Figure 1. Comparison of peak shapes for chlorate, perchlorate, bromate, and chlorite in a calibration standard in 50/50 water/acetonitrile, compared with one injected in 100% water, 5 µL injection.

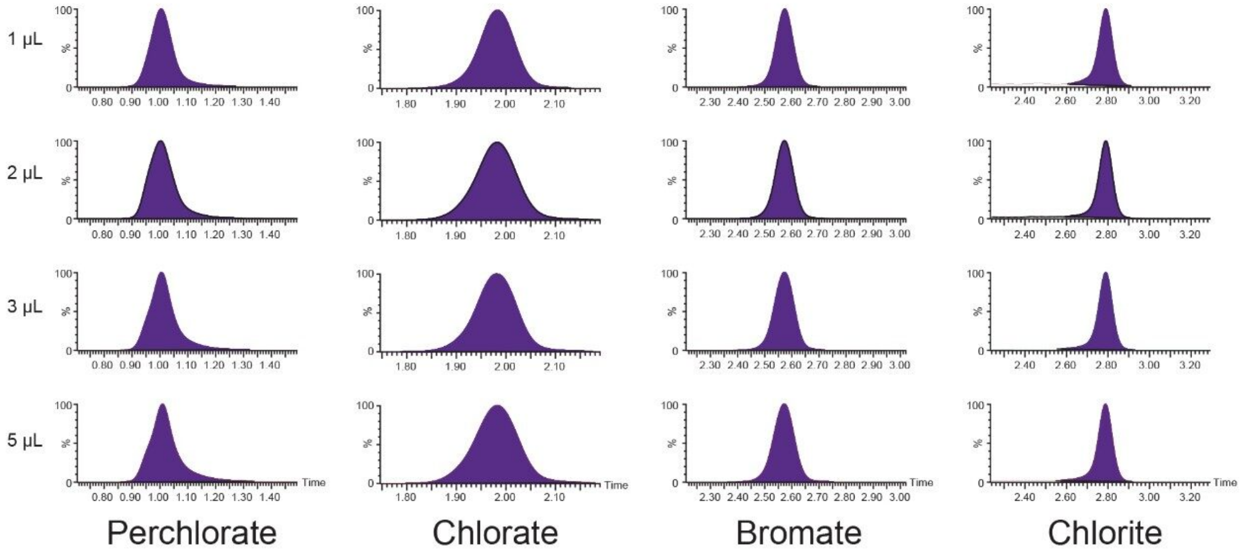


Figure 2. Injection volume assessment of calibration standard.

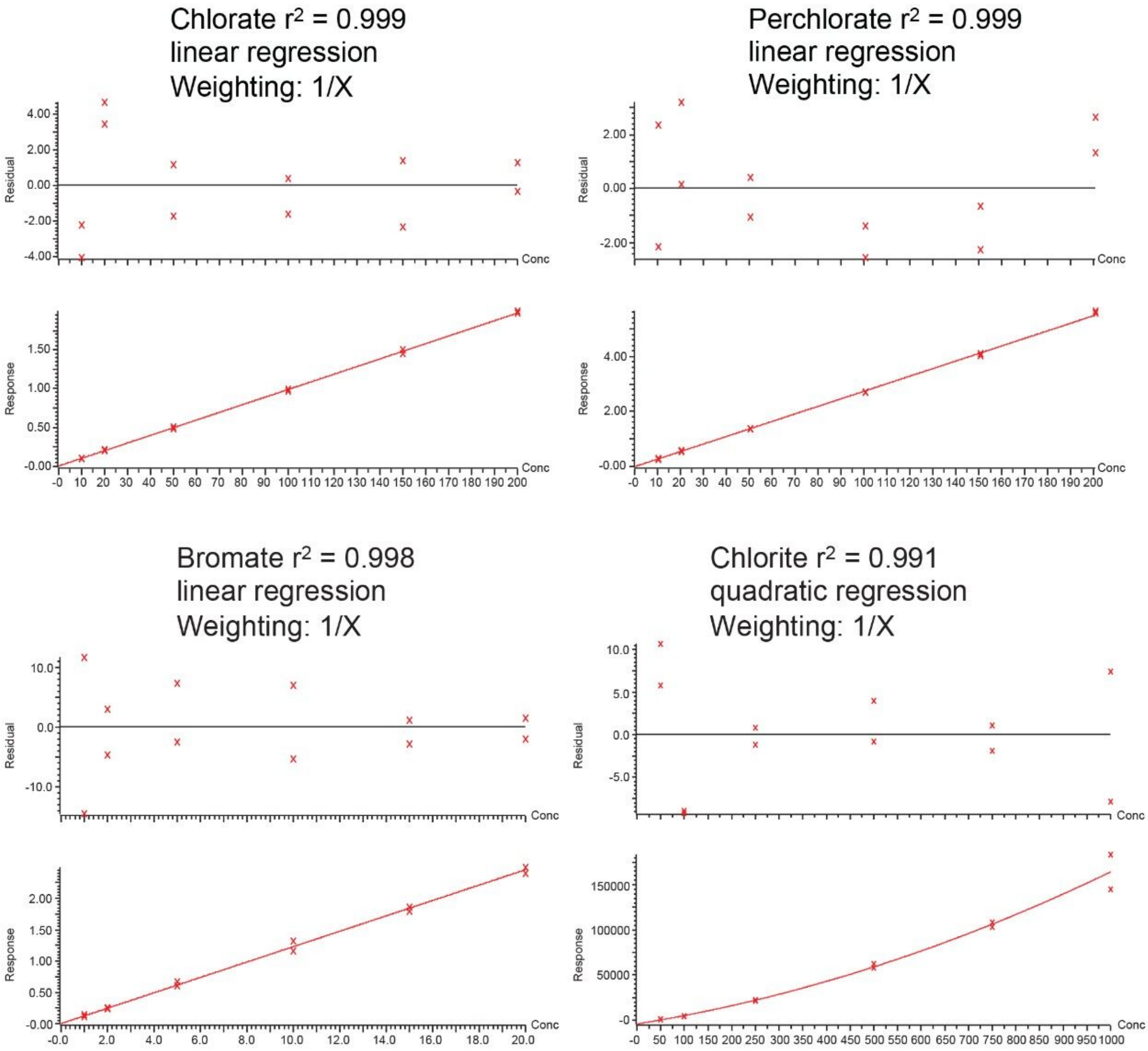
The drinking and mineral water samples collected were screened for the presence of oxyanions before the method performance study was started. No oxyanions were detected in mineral water; however, chlorate was detected in both the hard and soft water at levels significantly lower than the legal limits, but at concentrations that would interfere with the method performance study. The residue concentrations of chlorate were calculated by standard addition (n=6) and the results are included in the method performance data displayed in Table 2 and were significantly lower than the parametric limit.

Sample type		Perchlorate			Chlorate			Bromate			Chlorite		
	Spike conc (µg/L)	20	50	100	20	50	100	2	5	10	100	250	500
Mineral water	% Trueness	**99.6**	**99.5**	**100.3**	**98.1**	**98.9**	**100.5**	**92.2**	**100.1**	**100.5**	**104**	**99.5**	**96.8**
	%RSD	2.6	0.8	0.4	2.3	1.5	0.8	9.9	5.5	3.6	3.9	5.6	13.8
Soft water	% Trueness	**98.2**	**99.8**	**99.6**	**Residue in sample**			**101.8**	**101.3**	**100**	**92.7**	**108.7**	**114.1**
	%RSD	1.4	0.5	0.7	**54.3 µg/L, %RSD 0.9***			7.4	4.9	4.5	8.4	9.7	9.7
Hard water	% Trueness	**98.8**	**99.2**	**96.2**	**Residue in sample**			**101.4**	**102.7**	**98.7**	**84.1**	**89.2**	**85.3**
	%RSD	1.2	0.7	0.7	**12.0 µg/L, %RSD 1.7***			7.2	8.1	6.8	14	10.3	19.7

*Determination by standard addition (n=6)

Table 2. Method Performance Study Data – Note incurred residue of chlorate in both hard and soft water. Results for perchlorate, chlorate, and bromate internally standardised.

Method performance was assessed with 2 analytical batches per water type, where each batch was comprised of 33 spikes, with 11 at each designated low, mid, and high levels of each of the oxyanions. The results for chlorate were excluded for both the soft and hard water types due to the presence of chlorate residues in the samples. Mineral water was used to assess method performance for chlorate. Matrix matched calibration standards were used for all method performance study batches. Calibration solutions were prepared over the range 10–200 µg/L for perchlorate and chlorate, 1–20 µg/L for bromate and 100–1000 µg/L for chlorite. Figure 3 demonstrates expected calibration graphs from this approach. Coefficients of determination were 0.995 or greater, for chlorate, perchlorate, and bromate and 0.99 or better for chlorite. Residuals for perchlorate and chlorate were all under 10%, bromate were all under 12%, with chlorite 10% or under.



Chlorate $r^2 = 0.999$
linear regression
Weighting: 1/X

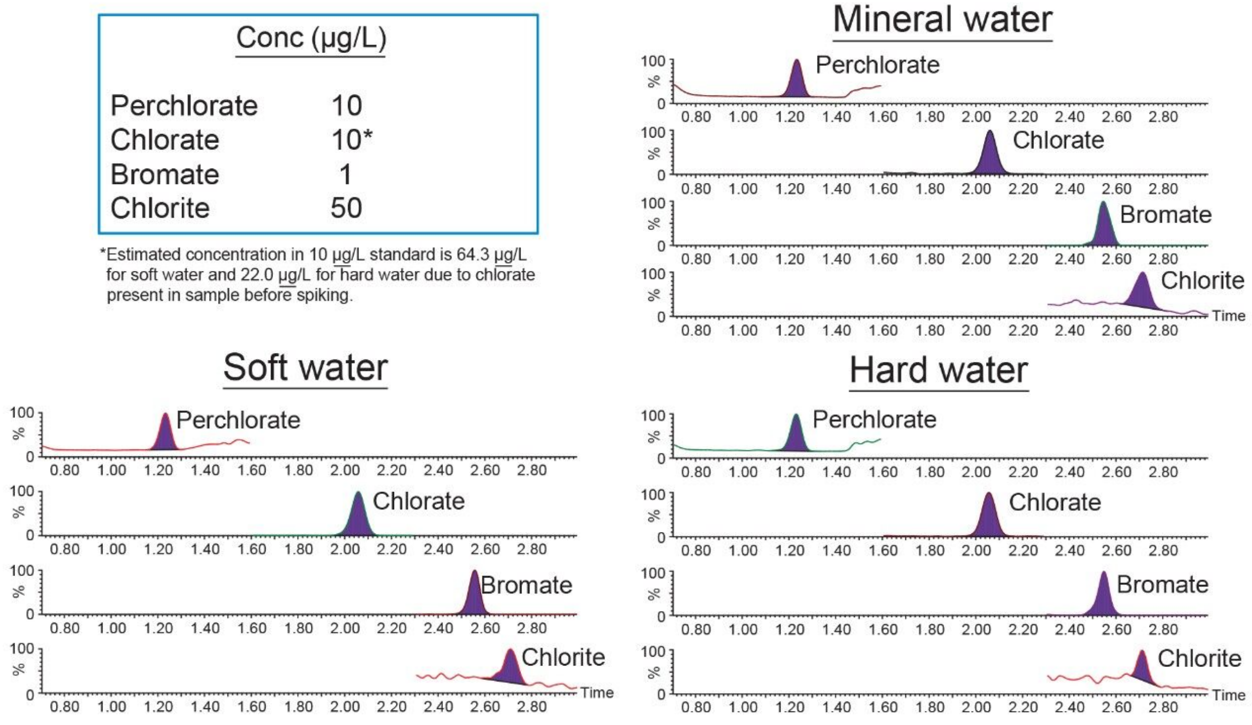
Perchlorate $r^2 = 0.999$
linear regression
Weighting: 1/X

Bromate $r^2 = 0.998$
linear regression
Weighting: 1/X

Chlorite $r^2 = 0.991$
quadratic regression
Weighting: 1/X

Figure 3. Mineral water calibrations for chlorate, perchlorate, bromate, and chlorate.

Chromatographic performance of the method was consistent throughout the method performance study. Figure 4 gives an example of the chromatography that was achieved for the lowest calibration standard from soft, hard, and mineral water calibration series. Across the three different types of water investigated there was no significant change in peak shape. Results from quantification of the spikes of the 6 method performance batches are displayed in Table 2. A consistent method performance for perchlorate and bromate was noted across all 6 batches and for chlorate in the mineral water batch. More variation for chlorite results was observed in hard water.



Conc (µg/L)	
Perchlorate	10
Chlorate	10*
Bromate	1
Chlorite	50

*Estimated concentration in 10 µg/L standard is 64.3 µg/L for soft water and 22.0 µg/L for hard water due to chlorate present in sample before spiking.

Figure 4. Lowest calibration standard chromatograms for quantitative transitions for soft, hard, and mineral drinking water.

Retention time stability is an important factor to investigate during a method performance study. To this end a repeatability study of 300 injections of an alternating soft, hard, and mineral water standards was run. All retention times across the 6 method performance batches and the retention time stability study were plotted and displayed in Figure 5. There is no significant change in retention time noted across the whole method performance study and repeatability study with all 4 oxyanions having RSDs under 1% across the whole study.

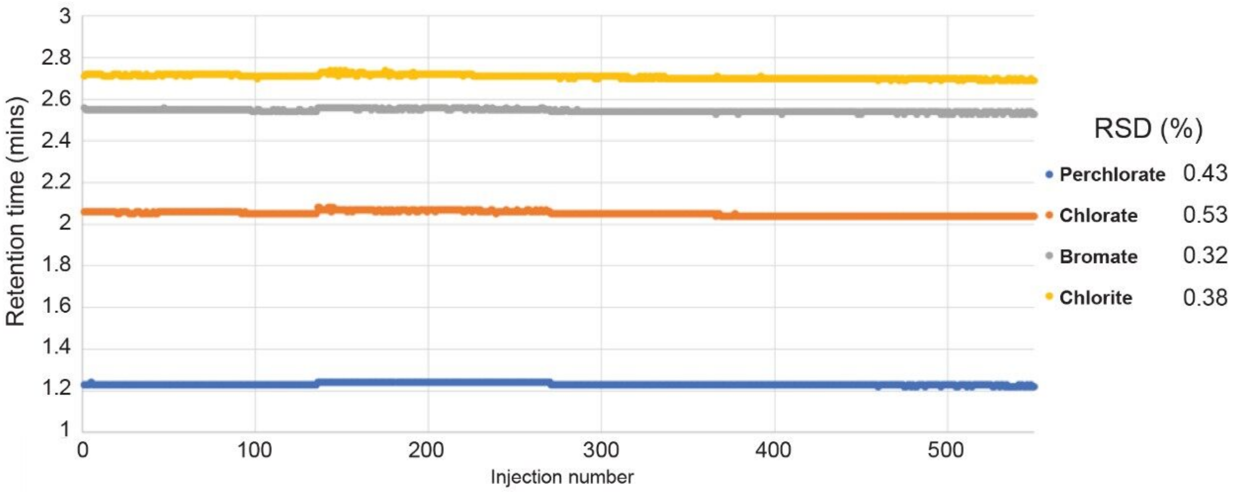


Figure 5. Retention time stability of oxyanion analysis over 550 injections across all 6 method evaluation batches and retention time stability study.

Conclusion

The method performance study data demonstrates a robust analytical method for the determination of oxyanions by direct injection of water samples onto an Anionic Polar Pesticide Column fitted in an ACQUITY UPLC I-Class PLUS System coupled to a Xevo TQ-XS. This application exceeds the new requirements in the EU Drinking Water Directive (EU DWD) coming into effect in late 2020[3], current WHO drinking water guidelines[2], and US EPA National Primary Drinking Water Regulations.[4] Limits of quantification for chlorate and perchlorate are 20 µg/L, for bromate 2 µg/L, and for chlorite 100 µg/L. For chlorate, this is 12.5 times lower than the proposed new EU DWD limit, for bromate 5 times lower and, for chlorite 2.5 times lower. Perchlorate is 2.5 times lower than the WHO recommended level. Retention time stability was proven over the course of the study with RSDs for all 4 oxyanions under 1% across all 6 method performance batches and retention time stability study. This short method time of 5 minutes with direct injection of samples allows for a high sample number throughput.

Scientists must validate the method in their own laboratories and demonstrate that the performance is fit for purpose and meets the needs of the relevant analytical control assurance system.

References

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4. US EPA National Drinking Primary Drinking Water Regulations, (accessed October 2020) https://www.epa.gov/ground-water-and-drinking-water/national-primary-drinking-water-regulations#six <https://www.epa.gov/ground-water-and-drinking-water/national-primary-drinking-water-regulations#six> .
5. Cordeior F, Schmitz F, Verbist I, Emteborg H, Charoud-Got J, Lpoex MC, Robouch P, Taylor P, de la Calle MB, *JRC Scientific and Technical Reports*, IMEP-25a: Determination of Bromate in Drinking Water.

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