APPLICATION NOTE

Liquid Chromatography/ Mass Spectrometry

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Analysis of Challenging Polar Contaminants in Water by LC/MS/MS with Direct Injection

Introduction

The Water Framework Directive, and its subsequent daughter directives at the EU level,[1] indicate environmental quality standards for water,[2] and define more than 30 priority substances as significant risk to humans. These substances include heavy metals, pesticides, herbicides, pharmaceutical and personal care products (PPCPs), per- and polyfluoroalkyl substances (PFASs), and other industrial pollutants.[3]

The limits established under the minimum requirements for parametric values used to assess the quality of water are at low µg/L (ppb) levels, which require highly sensitive methods. In addition to the sensitivity, these contaminants encompass a wide variety of compounds and chemical classes which present a further analytical challenge for water scientists.

Liquid chromatography coupled to tandem mass spectrometry (LC/MS/MS) has been demonstrated to be the technology of choice when specificity and sensitivity are required. Indeed, several standard methods based on LC/MS/MS have been established to assess water contaminants. However, owing to the diversity of compounds, different methods are typically required. Organic contaminants such as PPCPs metfomin and dimethylsulfamide, or PFAS' and their metabolites, can be chromatographically separated using reversed phase chemistry. However, polar compounds like glyphosate, bromate and chlorate require different chemistry, such as ion exchange, for a reliable separation and determination.

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Therefore, in this study, different methods for the separation, detection and quantitation of complex polar contaminants by UHPLC/MS/MS in water matrices are demonstrated. Two columns were employed using three separate analytical methods to successfully quantify metabolites such as trifluoracetic acid, the antidiabetic metformin, and various inorganic contaminants, such as chlorate and bromate.

Experimental

Hardware and Software

Chromatographic separation and subsequent detection were carried out using the PerkinElmer LX50 ultra-high-performance liquid chromatograph (UHPLC) and the QSight® 400 series triple quadrupole tandem mass spectrometer, respectively. All instrument control, analysis and data processing were performed using the Simplicity™ 3Q software platform.

Solvents, Standards, and Sample Preparation

LC/MS grade acetonitrile (ACN), water and formic acid used for the analysis and were obtained from Carl Roth (Karlruhe, Germany). Each authentic standard was purchased from Merck KGaA (Darmstadt, Germany), with the exception of dimethysulfamide, which was purchased from TCI Deutschland GmbH (Eschborn, Germany). The stock solutions were kept in the refrigerator until usage. All stock standards were initially combined to make an intermediate stock solution, which was subsequently used for the preparation of calibration standards. Several water sources were used, including tap water and untreated water.

Method Parameters

The LC methods and MS parameters are presented in Tables 1 and 2, respectively. Table 2 is further divided into Table 2a, MRM transitions with their respective optimized voltages, and Table 2b, MS source parameters. The MRM transitions, collision energies (CE), entrance voltages (EV) and collision cell lens 2 (CCL2) for each analyte were detected and optimized by direct infusion of the standards. MS source conditions, such as drying, nebulizer gas flow, and temperature settings were optimized by flow injection analysis (FIA).

Table 1. LC Parameters for the Three Different Methods

Metformin and DMS

Step	Time (min)	Flow Rate (mL/min)	% A	% B	Curve
1	Initial	0.6	70	30	
2	1.5	0.6	90	30	Linear
3	2.2	0.6	90	90	Linear
4	2.3	0.6	5	90	Linear
5	3	0.6	5	95	Linear
6	3.2	0.6	70	98	Linear
7	4	0.6	70	98	Linear
Mobile Phase A	0.1 % Formic Acid in Water				
Mobile Phase B	0.1 % Formic Acid in Acetonitrile				
Column Oven Temperature	40 °C				
Auto Sampler Temperature	20 °C				
Injection Volume	100 µL				

TFA

Step	Time (min)	Flow Rate (mL/min)	% A	% B	Curve
1	Initial	0.6	100	0	
2	1.5	0.6	100	0	Linear
3	1.7	0.6	2	98	Linear
4	3	0.6	2	98	Linear
5	3.2	0.6	100	0	Linear
6	5	0.6	100	0	Linear
Mobile Phase A	0.05 % Formic Acid in Water				
Mobile Phase B	0.05 % Formic Acid in Acetonitrile				
Column Oven Temperature	40 °C				
Auto Sampler Temperature	20 °C				
Injection Volume	10 µL				

Chlorate, Bromate, Bromide Glyphosate and AMPA

Step	Time (min)	Flow Rate (mL/min)	% A	% B	Curve
1	Initial	0.6	85	30	
2	10	0.6	85	30	Linear
Mobile Phase A	0.05 % Formic Acid in Water				
Mobile Phase B	0.05 % Formic Acid in Acetonitrile				
Column Oven Temperature	40 °C				
Auto Sampler Temperature	20 °C				
Injection Volume	100 µL				

Table 2. MS Method Parameters for the Three Methods (a) MRM Parameters.

Compound Name	Polarity	Precursor Ion	Product Ion	CE	EV	CCL2
Metformin	Positive	130	60	-18	6	-44
Metformin-2	Positive	130	113	-18	18	-36
Dimethylsulfamide	Positive	125	108	-14	10	-40
Dimethylsulfamide-2	Positive	125	44	-41	19	-48
TFA	Negative	112.8	68.9	16	-4	40
Chlorate	Negative	82.9	67	27	-41	36
Chlorate-2	Negative	84.9	69.1	26	-35	36
Bromate	Negative	126.8	111	31	-37	56
Bromate-2	Negative	126.8	94.9	45	-37	45
Bromide	Negative	80.7	80.7	50	-2	64
Bromide-2	Negative	78.7	78.7	100	-2	52
AMPA	Negative	110	63	29	-24	52
AMPA-2	Negative	110	79	33	-24	40
Glyphosate	Negative	168	63	38	-20	44
Glyphosate-2	Negative	168	81	22	-16	56

(b) Source parameters based on different methods:

Metformin and DMS

Parameter	Setting Value
Ionization Mode	ESI Positive
Drying Gas Setting (arbitrary units)	100
HSID Temperature (°C)	320 °C
Nebulizer Gas Setting	250
Electrospray Voltage (V)	4000
Source Temperature (°C)	350 °C

TFA

Parameter	Setting Value
Ionization Mode	ESI Negative
Drying Gas Setting	100
HSID Temperature (°C)	150 °C
Nebulizer Gas Setting	220
Electrospray Voltage (V)	-4000
Source Temperature (°C)	300 °C

Chlorate, Bromate, Bromide Glyphosate and AMPA

Parameter	Setting Value
Ionization Mode	ESI Negative
Drying Gas Setting	100
HSID Temperature (°C)	320 °C
Nebulizer Gas Setting	250
Electrospray Voltage (V)	-4000
Source Temperature (°C)	350 °C

Results and Discussion

For improved separation and sensitivity, various analytical columns were tested with different combinations of mobile phases. Following the evaluation, two columns using three separate analytical methods, based on various criteria (retention, thermal stability, etc.), were established for the quantification of these polar contaminants.

Metformin and Dimethylsulfamide

Metformin and dimethylsulfamide (DMS) are emerging as high-profile compounds, as they are becoming ever more present in the environment, especially our wastewater. As metformin is the most commonly prescribed antidiabetic drug, and the fact that it is unchanged in the human body, it is quite prevalent in wastewater.[4] Moreover, DMS is a metabolite of the fungicides dichlofluanid and tolylfluanid. As the use of these fungicides is quite common, especially in Germany, it makes the monitoring of DMS crucial for the water supply. DMS has also been shown to be the starting material for the formation of the carcinogenic compound nitrosamine (NDMA) during the ozonation process during water treatment.

As these contaminants should be monitored in the water supply, an analytical method was developed showcasing the linearity of metformin and DMS over two orders of magnitude (10 - 500 ng/L), with regression coefficients (r^2) ≥ 0.995. Figure 1 shows the calibration curves for the two compounds in the range of 10 - 500 ng/L, and an example of a chromatogram at 50 ng/L is shown in Figure 2. These figures illustrate the overlay of the two chemicals' quantifier fragments.

To demonstrate that the PerkinElmer QSight 420 is capable of detecting and quantifying these polar contaminants, various untreated wastewater and drinking water samples were tested, as shown in Table 4. Metformin was present in both untreated water samples, as well as DMS in one of the samples.

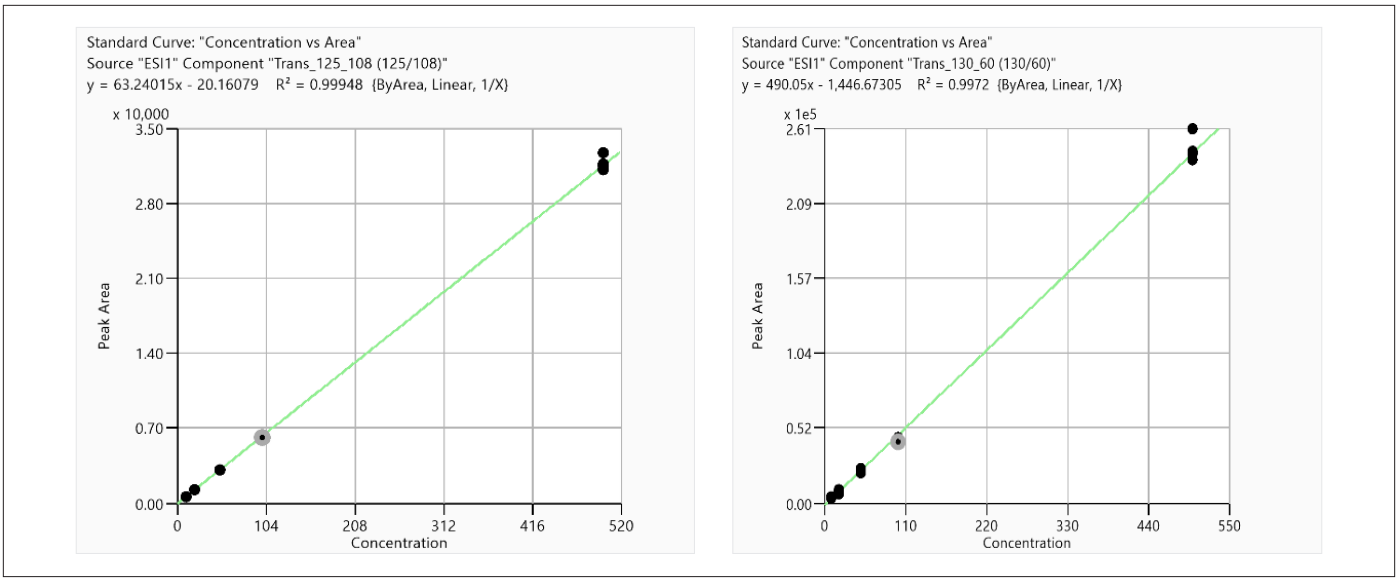

Figure 1. Calibration curves for metformin and dimethylsulfamide (10 - 500 ng/L).

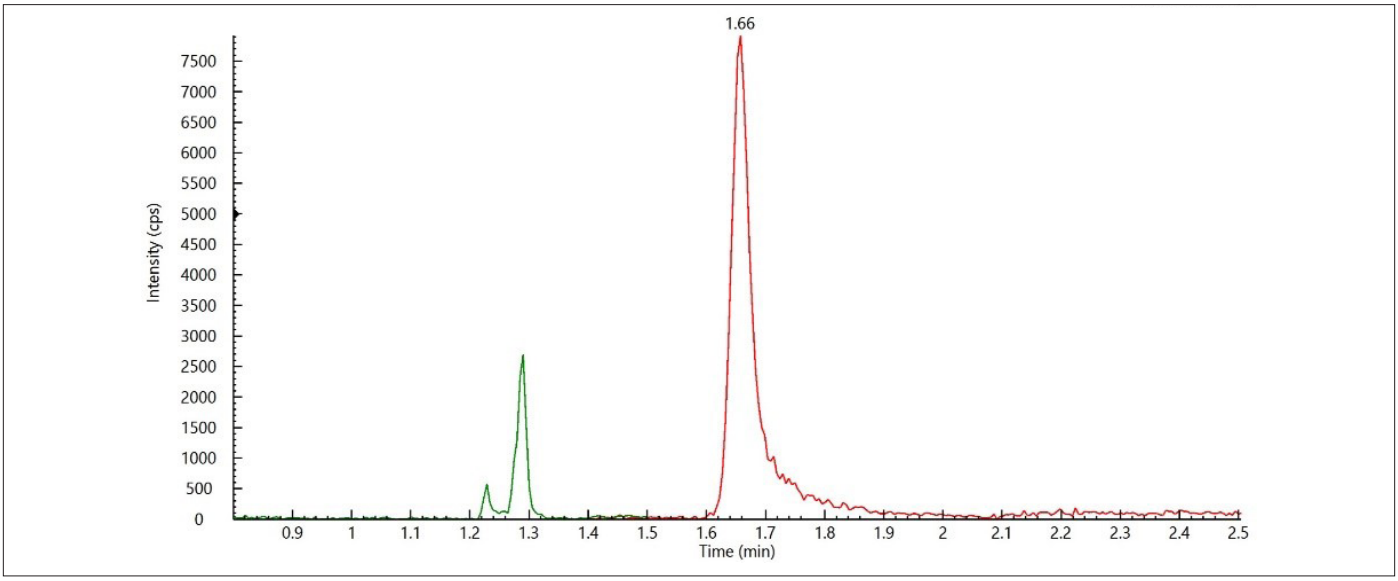

Figure 2. Overlay of quantifier fragments for dimethylsulfamide (in green) and metformin (in red) at 50 ng/L.

Table 4. Results of samples of untreated and treated (drinking) water along with their respective %RSDs.

Sample	Substance	Calculated Amount (ng/L)	%RSD (n=4)
Untreated Water-I	Metformin/	187	0.9
	Dimethylsulfamide	16.9	2.6
Untreated Water-II	Metformin/	187	0.9
	Dimethylsulfamide	16.9	2.6
Drinking Water-I	Metformin/	187	0.9
	Dimethylsulfamide	16.9	2.6
Drinking Water-II	Metformin/	187	0.9
	Dimethylsulfamide	16.9	2.6

Trifluoroacetic Acid

Trifluoroacetic acid, also known as TFA, is emerging as a contaminant of concern throughout the EU and its member states, owing to the discovery that it is a prevalent contaminant in the environment. The source of TFA in the environment has been linked to the breakdown product of various pesticides, as well as industrial chemicals including hydrochlorofluorocarbons and hydrofluorocarbons, making TFA quite ubiquitous in the environment and the water supply.[5]

To demonstrate that TFA can be measured sufficiently with the QSight, the linearity is considered. As shown in Figure 3, the linearity ranging from 100 - 1500 ng/L resulted in a regression coefficient

(r^2) ≥ 0.995. Figure 4 depicts an overlay of the chromatograms for TFA at concentrations of 100, 500 and 1500 ng/L. The untreated and drinking water samples were then measured to test the amount of TFA present, as shown in Table 5. Important to note, is that TFA is not yet covered by any EU regulation, however, as more attention is being drawn to its continuous presence in different matrices from the food to the water supply, it will likely be implemented into new regulations soon.[5] Furthermore, work is ongoing to develop a combined method for the detection of TFA with other haloacetic acids (HAAs) currently included under EU regulations. PerkinElmer has demonstrated, in a previously published application, the capabilities of the QSight for the analysis these compounds, thus combining these methods would expand on the overall compound profile for these emerging contaminants.[6]

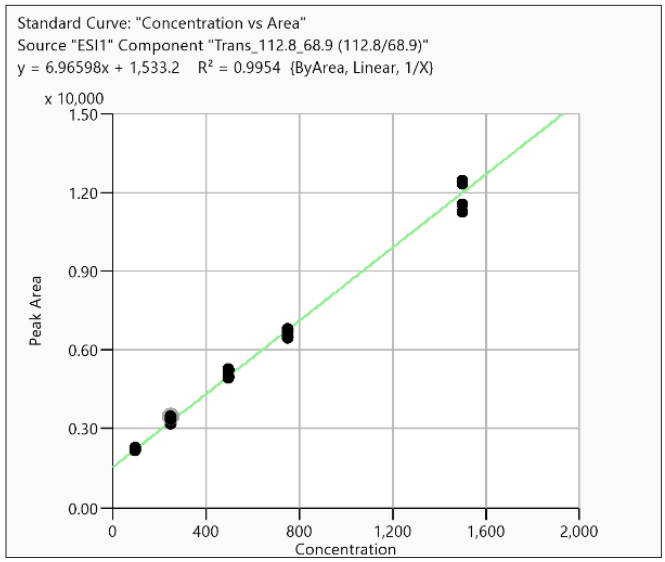

Figure 3. Calibration curve for TFA (100 - 1500 ng/L).

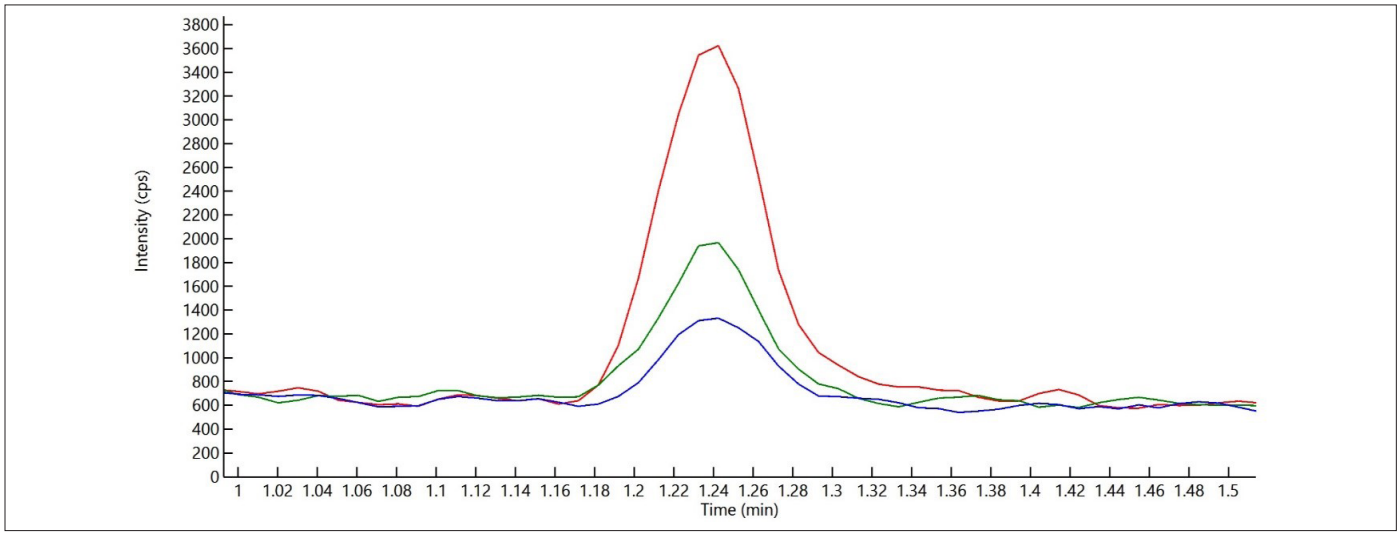

Figure 4. Overlay of chromatograms for TFA at concentrations of 100 ng/L (blue) 500 ng/L (green) and 1500 ng/L (red).

Table 4. Results of samples of untreated and treated (drinking) water along with their respective %RSDs.

Sample	Substance	Calculated Amount (ng/L)	%RSD (n=3)
Untreated Water-I	TFA	250	3.5
Untreated Water-II	TFA	143	4.7
Drinking Water-I	TFA	238	5.9
Drinking Water-II	TFA	113	3.9

Chlorate, Bromate, Bromide Glyphosate and AMPA

An analytical method covering chlorate, bromate, bromide, glyphosate and AMPA was successfully developed for these very difficult polar contaminants utilizing an ion exchange column. Linearity could be obtained for each compound, with a dynamic range of 2-3 magnitudes and regression coefficients (r^2) ≥ 0.995, as shown in Figure 5. Moreover, with this column, adequate separation could be obtained, as shown in Figure 6.

As chlorate and bromate are covered in the new EU water regulation, bromide was included as an interesting add-on to the analysis. As bromide is usually known as an ICP-MS component, good results could be shown that prove it can also be determined using the QSight triple quadrupole system. Moreover, as chlorate is present in the water supply owing to its use during the water treatment process, bromate is also formed likely from the ozonation during the water treatment process.[7] This is in contrast to glyphosate and AMPA, as detailed above, which are major contaminants from agriculture. However, in bringing these very polar compounds together in one method, it enables the user to cover a wider range of metabolites in one run. The results for untreated and drinking water samples are shown in Table 6.

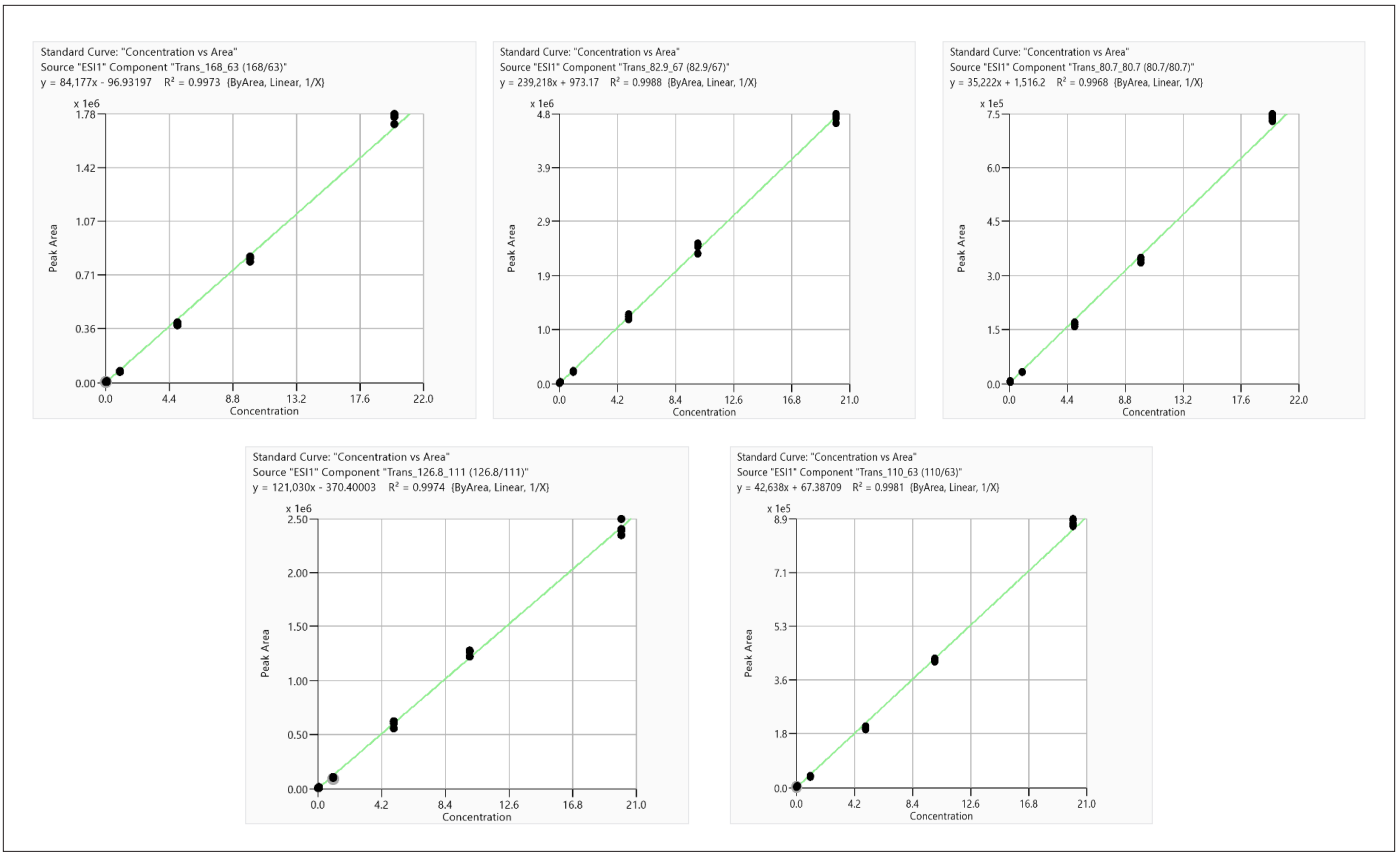

Figure 5. Calibration curves for chlorate (0.05 - 20 µg/L), bromate (0.05 - 20 µg/L), bromide (0.1 - 20 µg/L), glyphosate (0.01 - 20 µg/L) and AMPA (0.01 - 20 µg/L).

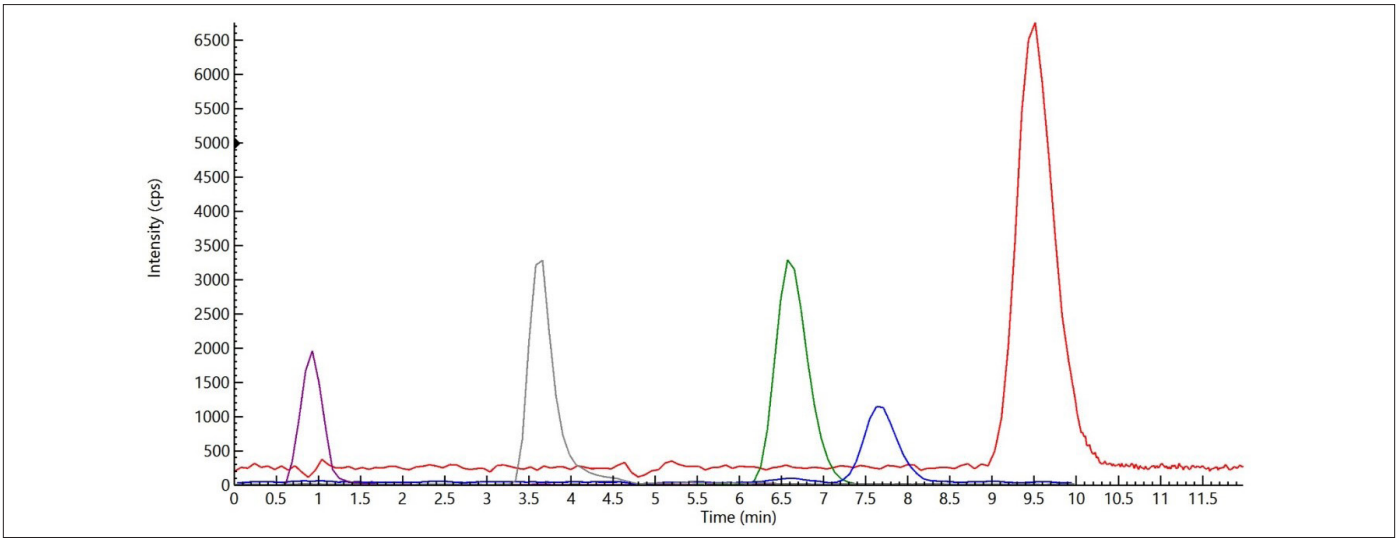

Figure 6. Overlay of AMPA (purple), glyphosate (grey), bromate (green), bromide (blue) and chlorate (red) at 0.5 µg/L.

Conclusion

Three analytical methods, using just two HPLC columns, were developed to measure several of the most difficult polar contaminants in the global water supply. Whether analyzing untreated or drinking water, the coupling of PerkinElmer's LX50 UHPLC to the QSight 420 proved to be an efficient and effective solution in tackling these tough compounds. Furthermore, this work demonstrated that it is not necessary to use special chromatographic separation techniques. such as ion chromatography, which add additional hardware and cost to the system. Owing to the superb sensitivity of the QSight 400 series, it was additionally not required to employ pre-concentration steps like SPE or online-SPE into the methodology, saving further time and cost.

Not only was the sensitivity more than adequate when compared to current regulations, the reproducibility of the results also demonstrated the robustness of these methods for use in a water laboratory (Table 7). With regulations becoming stricter and stricter each year, having methods covering a wide range of compounds is a must in every lab's toolbox.

Table 6. Results of samples of untreated and treated (drinking) water along with their respective %RSDs.

Sample	Substance	Calculated Amount (ng/L)	%RSD (n=3)
Untreated Water-I	Chlorate	0.13	3.1
	Bromate	ND	-
	Bromide	2.33	1.3
	AMPA	<10	-
	Glyphosate	0.017	6.9
Untreated Water-II	Chlorate	0.31	3.6
	Bromate	ND	-
	Bromide	8.6	4.9
	AMPA	<10	-
	Glyphosate	0.024	4.2
Drinking Water-I	Chlorate	0.33	4.8
	Bromate	0.76	3.3
	Bromide	4.1	0.8
	AMPA	0.017	3.3
	Glyphosate	0.034	1.7
Drinking Water-II	Chlorate	0.33	2.6
	Bromate	0.58	7.6
	Bromide	3.3	3.5
	AMPA	0.016	6.3
	Glyphosate	0.038	4

Table 7. Action level summary table.

S. No.	Name	QSight 420	%CV (n=3)	EU Action Level
1	Metformin	10 ng/L	1.7	100 ng/L*
2	Dimethylsulfamide	10 ng/L	1.3	100 ng/L
3	TFA	100 ng/L	2.3	100 ng/L*
4	Chlorate	50 ng/L	4	250 µg/L
5	Bromate	50 ng/L	4.6	10 µg/L
6	Bromide	100 ng/L	6.1	10 µg/L*
7	AMPA	10 ng/L	6.4	100 ng/L
8	Glyphosate	10 ng/L	3.0	100 ng/L

**Not yet covered, estimated levels to be introduced.*

References

1. COUNCIL DIRECTIVE 98/83/EC of 3 November 1998 on the quality of water intended for human consumption [Internet]. [cited 2019 Sep 30]. Available from: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:31998L0083&from=EN.
2. DIRECTIVE 2008/105/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 16 December 2008 on environmental quality standards in the field of water policy, amending and subsequently repealing Council Directives 82/176/EEC, 83/513/EEC, 84/156/EEC, 84/491/EEC, 86/280/EEC and amending Directive 2000/60/EC of the European Parliament and of the Council [Internet]. [cited 2020 Feb 2]. Available from: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32008L0105&from=EN.
3. DIRECTIVE 2017/0332 (COD) OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL on the quality of water intended for human consumption (recast) [Internet]. [cited 2020 Feb 1]. Available from: https://eur-lex.europa.eu/resource.html?uri=cellar:8c5065b2-074f-11e8-b8f5-01aa75ed71a1.0016.02/DOC_1&format=PDF.
4. Study_report_public_consultation_pharmaceuticals_environment.pdf [Internet]. [cited 2020 Feb 3]. Available from: https://ec.europa.eu/info/sites/info/files/study_report_public_consultation_pharmaceuticals_environment.pdf.
5. EURL-SRM – Residue Findings Report Difluoroacetic acid (DFA), Trifluoroacetic acid (TFA) [Internet]. Available from: https://www.eurl-pesticides.eu/userfiles/file/eurlsrm/eurlsrm_residue-observation_tfa-dfa.pdf.
6. Analysis of Haloacetic Acids in Drinking Water- Application Note [Internet]. [cited 2020 Feb 23]. Available from: https://www.perkinelmer.com/lab-solutions/resources/docs/APP_Analysis_of_HaloaceticAcids_in_Drinking_Water_014446_01.pdf.
7. Bromate in Drinking-water Background document for development of WHO Guidelines for Drinking-water Quality [Internet]. [cited 2020 Feb 3]. Available from: https://www.who.int/water_sanitation_health/dwq/chemicals/bromate260505.pdf.

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