APPLICATION NOTE

Liquid Chromatography/ Mass Spectrometry

AUTHORS

Derek J. Mattern
PerkinElmer, Inc., (LAS) Germany GmbH

Roberto Bozic
PerkinElmer, Inc., Italy

LC/MS/MS: Improved Throughput and Flexibility for Compliant Drinking Water Analysis with Dual Source Capability

Introduction

Acrylamide has gained notoriety over the past years as it can cause certain cancers.[1] Polyacrylamides are often used as flocculants in the water clarification process and can lead to the leaching of the monomer acrylamide into our drinking water systems.[2] Therefore, governing bodies have implemented various directives to monitor acrylamide in drinking water. This also applies in the European Union where the water framework directive and subsequent daughter directives have defined many priority substances, including acrylamide.[3] Compounds chlorate, perchlorate and N,N-dimethylsulfamide (DMS) in water samples are also analyzed by many drinking water laboratories as chlorate and perchlorate are common byproducts in the purification process and DMS being a metabolite of known herbicides that can end up in drinking water. In this application note we demonstrate the benefits of the dual source capability of the PerkinElmer QSight™ Triple Quadrupole LC/MS/MS for a sensitive determination of organic contaminants in water samples that require different ionization techniques. Also, we demonstrate that using the QSight LC/MS/MS dual source can provide a simpler, faster, and more sensitive method for contaminants in water with method improvements compliant to current European regulations.

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Experimental

Hardware and Software

The PerkinElmer QSight LX50 ultra high-performance liquid chromatograph (UHPLC) and the QSight 400 Series LC/MS/MS was used for chromatographic separation and subsequent detection, respectively. For diverting the flow between the ESI and APCI probes, a Rheodyne diverter valve was implemented. The instrument was completely controlled using the PerkinElmer Simplicity™ 3Q Software Platform.

The PerkinElmer QSight LC/MS/MS have a unique two stand-alone ionization sources configuration (Figure 1). The system permits maximum flexibility of using either electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI) without manual intervention and use of tools.[4] The Simplicity 3Q Software controls source electronics and inlet flow switching. This permit to switch sources by simply scheduling it within the LC/MS/MS method. During method development, analysts would benefit to have the possibility to easily select the best ionization technique to apply for each molecule after chromatographic separation. Noteworthy, implementing different ionization modes in the same separation run permit an advanced multiplexing level of the LC-MS/MS method.

Figure 1: Picture of the source area of the QSight LC/MS/MS where two separate ionization probes are shown.

Solvents, Standards, and Sample Preparation

LC/MS grade methanol, water, acetic acid, and formic acid were used for the analysis and were obtained from Carl Roth (Karlsruhe, Germany). Authentic standards were purchased from Merck KGaA, Darmstadt, Germany, except for N,N-Dimethylsulfamide (DMS), which was purchased from TCI Chemicals (Germany) Water samples were obtained from drinking water locations around the Frankfurt am Main metropolitan area.

Method Parameters

The LC methods and MS parameters are presented in Tables 1, 2, 3, 4 and 5. For optimization of the MRM transitions, collision energies (CE), entrance voltages (EV) and collision cell lens 2 (CCL2) each analyte was detected and optimized by direct infusion of the standards. Flow injection analysis (FIA) was used to optimize the MS source conditions such as drying and nebulizer gas flow and temperature settings. Figure 2 also gives a quick look into the software and how the MS method is setup for switching between APCI and ESI.

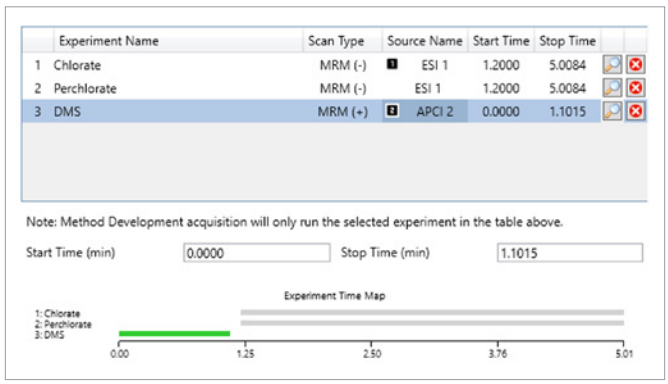

Figure 2: View from the Simplicity 3Q Software Platform of how the MS method is setup. DMS is first measured with APCI from 0 - 1.1 minutes. Then chlorate and perchlorate are measured from 1.2-5 minutes.

Table 1: LC parameters for the acrylamide method.

Step	Time (min)	Flow Rate (mL/min)	% A	% B	Curve
1	Initial	0.5	99	1	
2	1.0	0.5	99	1	Linear
3	5.0	0.5	10	90	Linear
4	7.0	0.5	10	90	Linear
5	7.2	0.5	99	1	Linear
6	9.0	0.5	99	1	Linear

Mobile Phase A	0.1 % Formic acid in Water
Mobile Phase B	0.1 % Formic acid in Methanol
Column Oven Temperature	40 °C
Auto Sampler Temperature	20 °C
Injection Volume	100 µL
Column	Porous graphitic carbon 5 µm, 100 x 2.1 mm

Table 2: LC parameters the chlorate, perchlorate and DMS method.

Step	Time (min)	Flow Rate (mL/min)	% A	% B	Curve
1	Initial	0.6	100	0	
2	4.0	0.6	100	0	Linear
3	4.2	0.6	0	100	Linear
4	6.0	0.6	0	100	Linear
5	6.2	0.6	100	0	Linear
6	8.0	0.6	100	0	Linear

Mobile Phase A	1 % Acetic acid in Water
Mobile Phase B	Methanol
Column Oven Temperature	40 °C
Auto Sampler Temperature	20 °C
Injection Volume	100 µL
Column	PFP 2.7 µm, 100 x 2.1 mm

Table 3: QSight LC/MS/MS MRM parameters.

Compound	Component Type	RT (min)	Q1	Q2	EV	CCL2	CC
Acrylamide	Quantifier	1	72	55	12	-28	-18
	Qualifier		72	27	16	-32	-33
	IS		75	58	25	-48	-14
Chlorate	Quantifier	2	82.9	66.9	-2	32	29
	Qualifier		84.9	68.9	-3	44	30
	IS		88.9	71	-29	40	28
Perchlorate	Quantifier	3	99	83	-37	44	34
	Qualifier		99	67	-37	44	49
	IS		106.9	88.9	-38	76	39
DMS	Quantifier	4	125	108	22	-32	-13
	Qualifier		125	44	14	-48	-45
			131	114	6	-60	-13

Table 4: MS source parameters for acrylamide.

Acrylamide	
Parameter	Setting Value
Ionization Mode	APCI Positive
Drying Gas Setting	100
HSID Temperature (°C)	200
Nebulizer Gas Setting	150
Corona Discharge Pos	3
Source Temperature (°C)	200

Table 5: MS source parameters for DMS, chlorate and perchlorate.

DMS APCI Settings	
Parameter	Setting Value
Ionization Mode	APCI Positive
Drying Gas Setting	100
HSID Temperature (°C)	320
Nebulizer Gas Setting	250
Corona Discharge Pos	3
Source Temperature (°C)	350
Chlorate/Perchlorate ESI Settings	
Parameter	Setting Value
Ionization Mode	ESI Negative
Drying Gas Setting	100
HSID Temperature (°C)	320
Nebulizer Gas Setting	250
Electrospray Voltage (V)	-4000
Source Temperature (°C)	300

Results and Discussion

Acrylamide in APCI Mode

Acrylamide is a known contaminant of drinking water. It is important to develop a robust and reliable method to detect this compound in water. For analysis of acrylamide in drinking water, two separate methods were setup using ESI and APCI (Figure 3). Because of the true dual source design of the QSight LC/MS/MS, it allowed for easy and automatic switching between ESI and APCI. This not only gave us flexibility, but also allowed us to run different methods without having to switch anything manually on the instrument. As shown in Figure 3, when measuring acrylamide with ESI, there was a very strong matrix effect, but when running the same sample with APCI the matrix effect was significantly reduced. Moreover, the sensitivity was not compromised as lower ppt levels could still be achieved with APCI even if acrylamide is a polar compound.

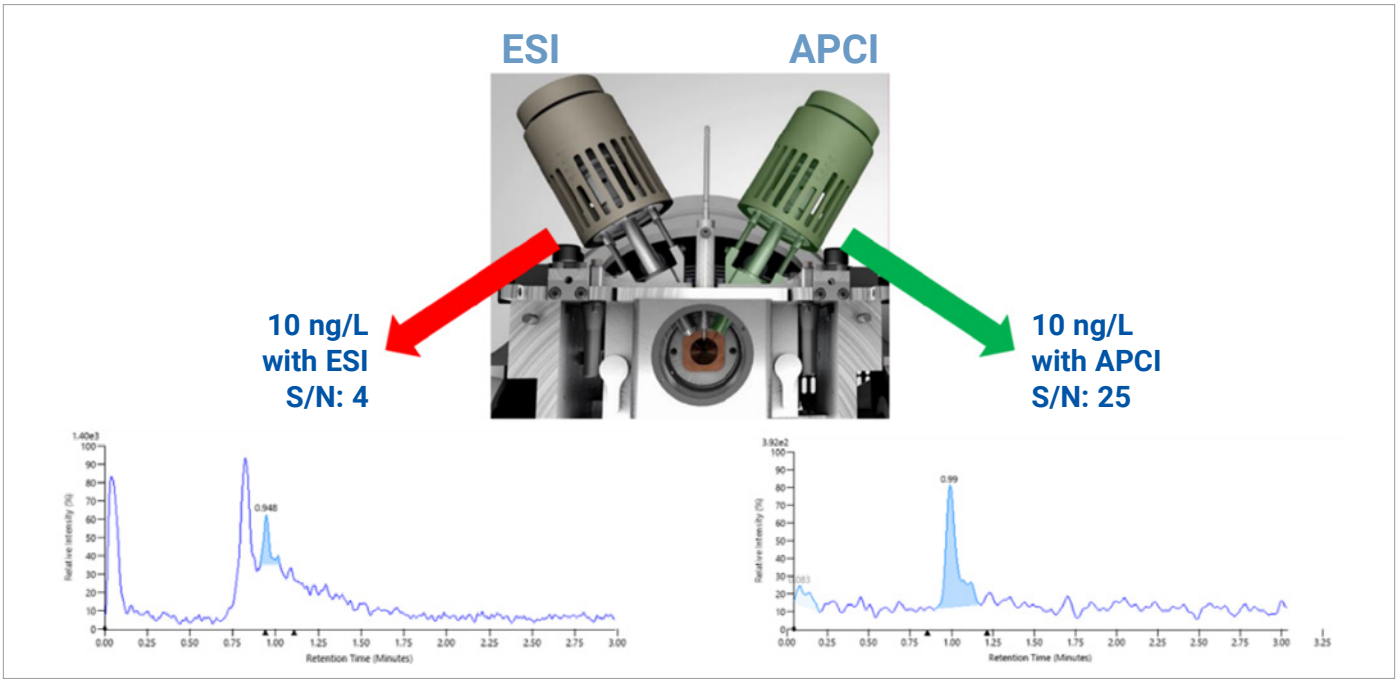

Figure 3: Chromatograms of acrylamide quantifier fragment at 10 ng/L with ESI (left) and APCI (right).

To test the method and show the robustness of measuring acrylamide with APCI, a calibration curve with five injections per calibration point was run as seen in Figure 4. Figure 4A shows an overview of various concentrations of acrylamide with the blank also having a clean spectrum. Moreover, the calibration curve in Figure 4B shows excellent linearity (R^2 > 0.99) at levels well below 0.1 µg/L even lower than the stringent regulation limits (5). Furthermore, to show the robustness of the method, table 6 showcases the accuracy and precision of this method. All spiked calibration points had accuracy of <10 % and RSD of <8 %.

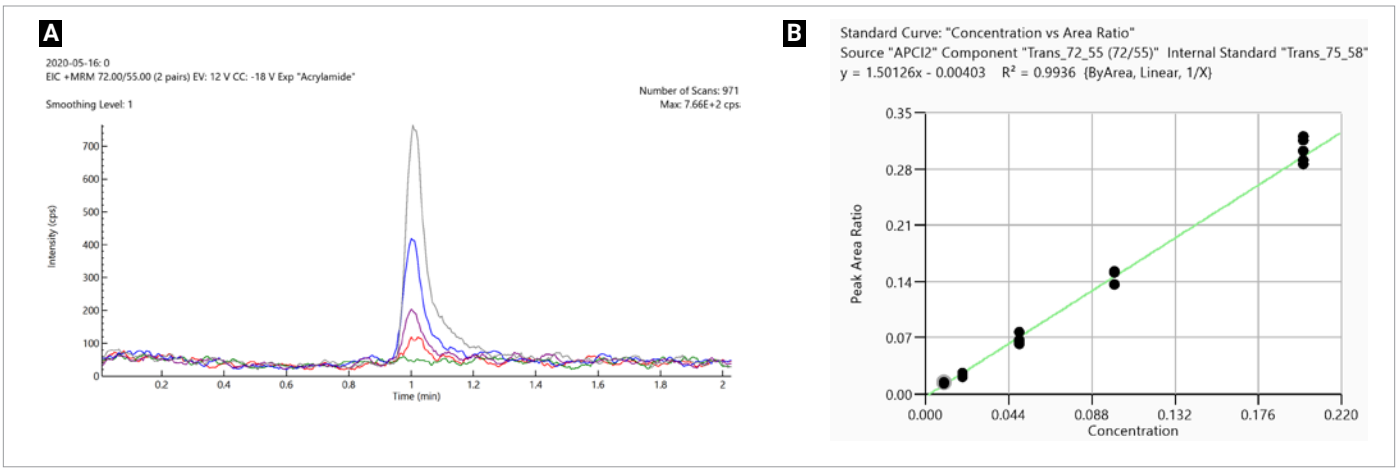

Figure 4: Overlay of acrylamide quantifier fragment at different matrix-matched calibration levels, green= 0 µg/L, red= 0.01 µg/L, purple= 0.02 µg/L, blue 0.05 µg/L and grey= 0.1 µg/L. B. Calibration curve for acrylamide (0.01-0.2 µg/L) with an R^2 value of 0.9936 with five replicates per calibration standard.

Table 6: The average calculated concentration, accuracy % and RSD % data (n=5) for all five calibration points for acrylamide.

Acrylamide	Spiked Concentration (µg/L)	Avg. Calculated Concentration	Accuracy %	RSD %
Calibration 1	0.01	0.011	110	6.1
Calibration 2	0.02	0.019	95	6.9
Calibration 3	0.05	0.047	94	8
Calibration 4	0.1	0.098	98	7.7
Calibration 5	0.2	0.205	102	4.8

Drinking water samples were also tested to see if any traces of acrylamide could be found. As seen in Figure 5, one sample showed that 0.02 µg/L could be detected. As with the calibration standards, samples were also injected five times and this sample had a % RSD of 5.8 %, showing that even at low levels of acrylamide, the method was robust and reproducible.

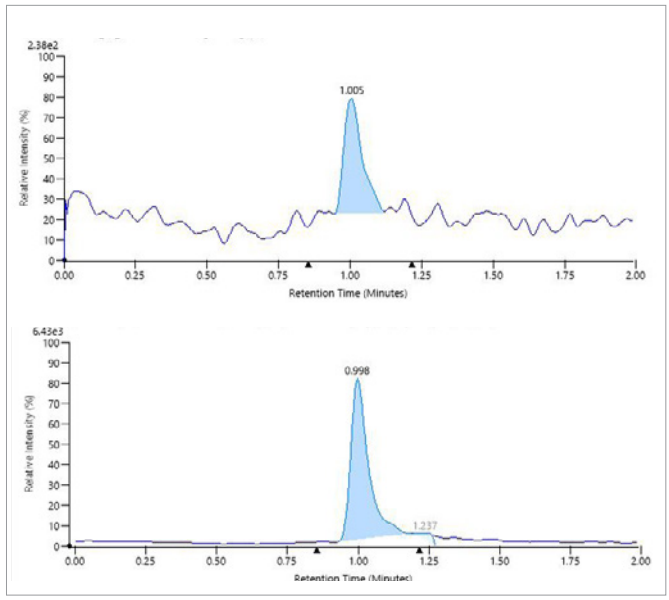

Figure 5: Chromatogram of a drinking water sample with a positive find of acrylamide at 0.02 µg/L, left pane is the quantifier fragment and right pane is the isotopically labelled internal standard. The % RSD (n=5) was 5.8 %.

Source Switching Within the Same Method

In the second example, we aim to demonstrate the true analytical benefits of QSight LC/MS/MS dual source, presenting a method that includes ionization switches between ESI and APCI in the same run. In this example we showcase DMS, a metabolite of frequently used fungicides, dichlofluanid and tolylfluanid ionized in APCI positive mode, and the common disinfectant products chlorate and perchlorate ionized in ESI negative mode. For switching we simply divert the LC flow from APCI to ESI with a diverter valve and schedule this in both the LC and MS methods. As shown in Figure 6, the chromatograms demonstrate typical peak shape and levels as low as 0.01 µg/L can be reached for these extremely polar compounds.

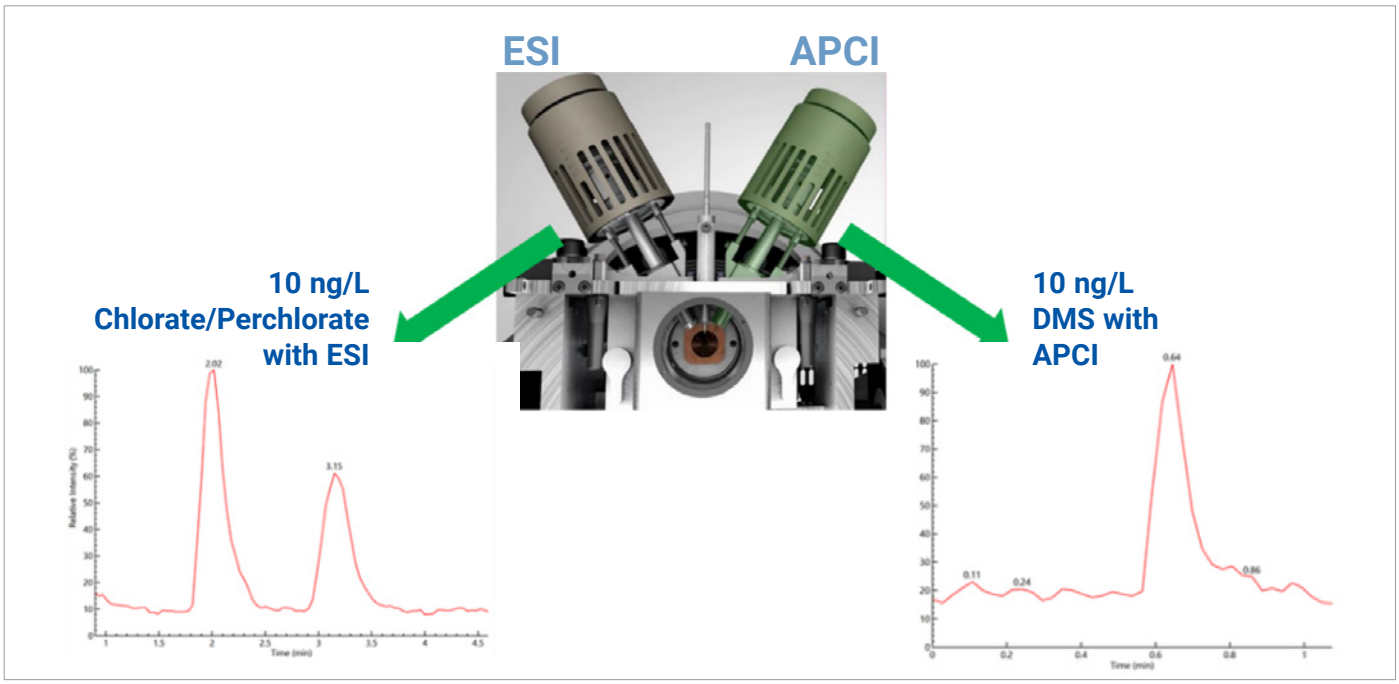

Figure 6: Shows that switching between ESI negative mode and APCI positive mode is possible in a single run without losing key sensitivity of the metabolites. On the left is a chromatogram of chlorate and perchlorate in ESI negative mode and on the right is DMS in APCI positive mode.

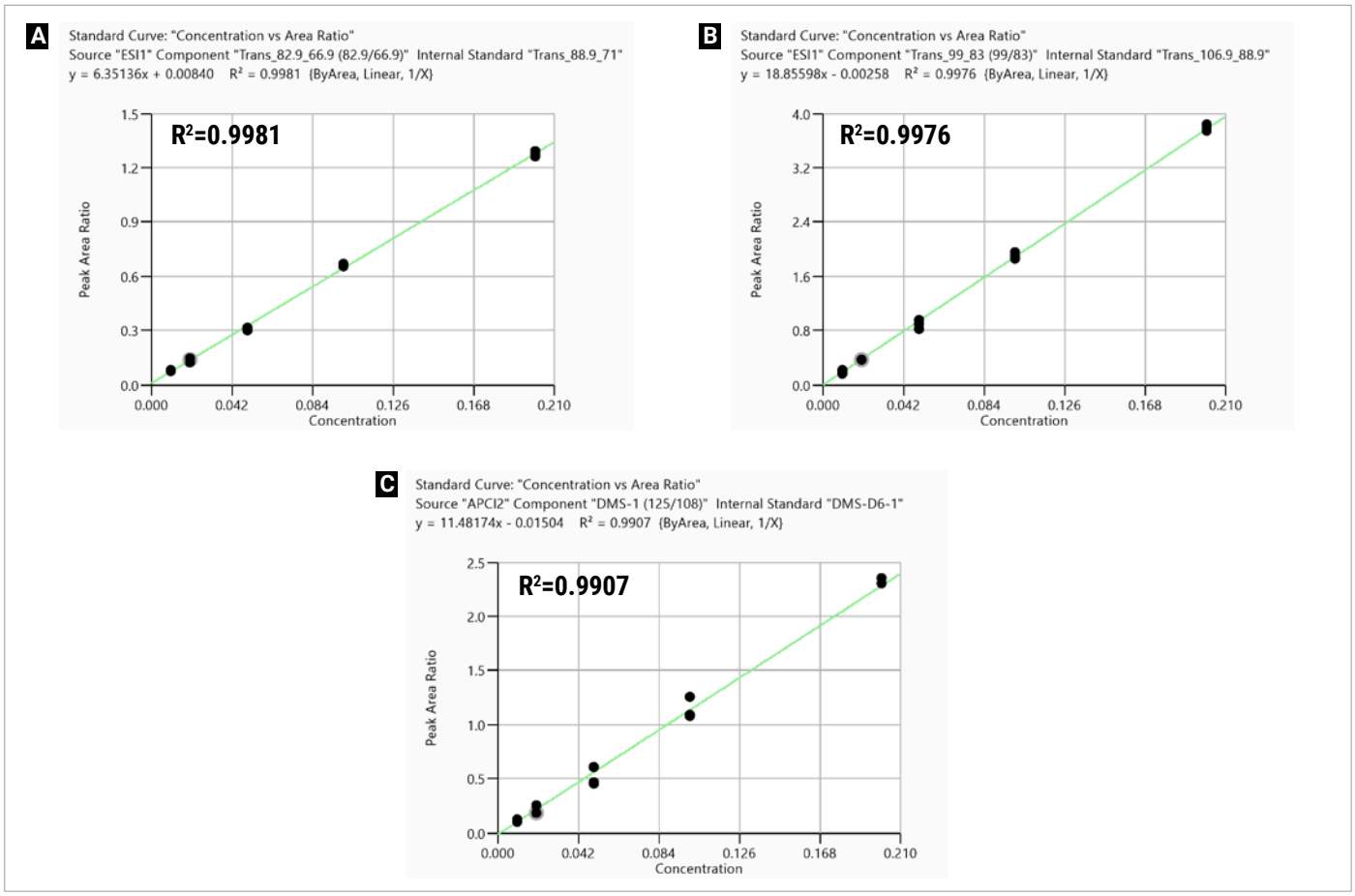

Figure 7: Calibration curves for the 3 metabolites in concentration ranges of 0.01-0.2 µg/L with an R^2 values all > 0.99 for A. chlorate, B. perchlorate and C. DMS.

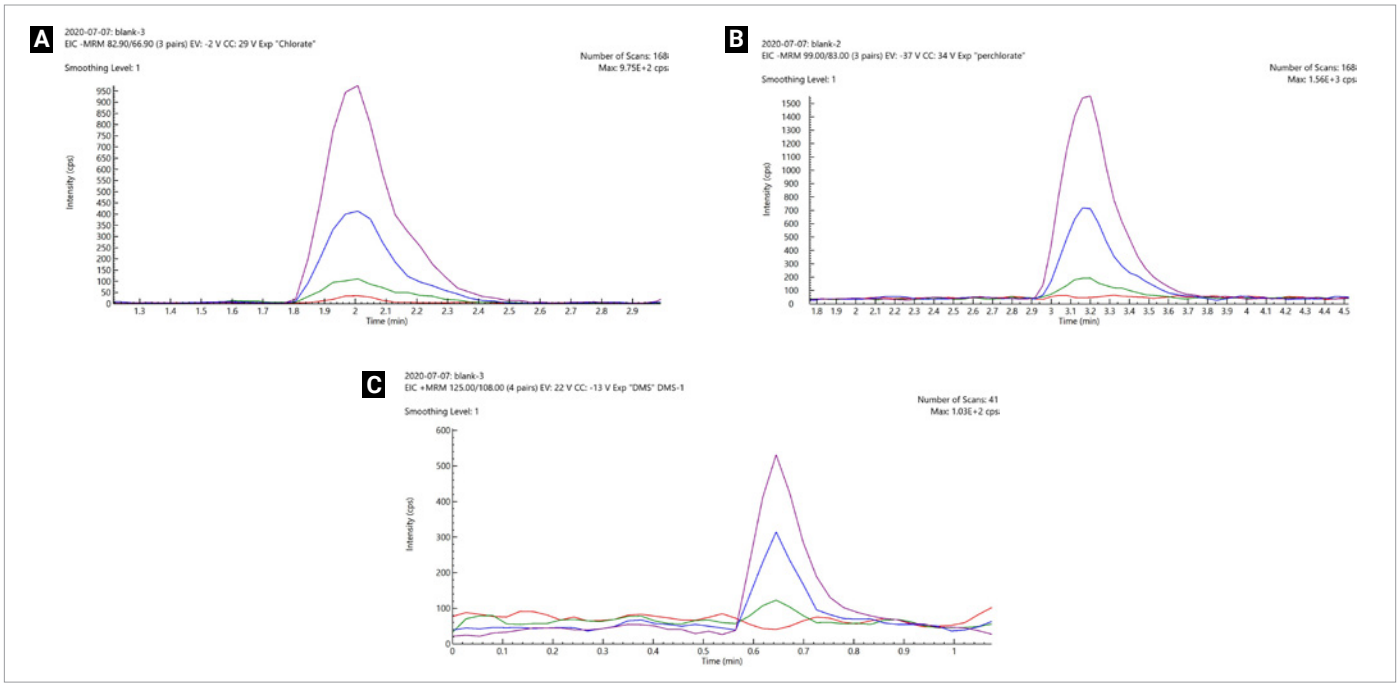

Figure 8: Overlay of the 3 metabolites quantifier fragments at different calibration levels, red= 0 µg/L, green= 0.01 µg/L, blue 0.05 µg/L and purple= 0.1 µg/L for A. chlorate, B. perchlorate and C. DMS.

Table 7: Shows the average calculated concentration, accuracy % and RSD % data (n=3) of II five calibration points for DMS, chlorate and perchlorate.

Compound	Calibration Point	Spiked Concentration (µg/L)	Avg. Calculated Concentration	Accuracy %	% RSD
DMS	Calibration 1	0.01	0.011	110	10
	Calibration 2	0.02	0.019	95	3.7
	Calibration 3	0.05	0.045	91	3.7
	Calibration 4	0.1	0.100	100	5.6
	Calibration 5	0.2	0.204	102	2.5
Chlorate	Calibration 1	0.01	0.010	103	4.6
	Calibration 2	0.02	0.020	98	4.7
	Calibration 3	0.05	0.047	94	2.9
	Calibration 4	0.1	0.103	103	0.3
	Calibration 5	0.2	0.200	100	0.1
Perchlorate	Calibration 1	0.01	0.011	110	9.4
	Calibration 2	0.02	0.020	98	3.2
	Calibration 3	0.05	0.048	95	5.1
	Calibration 4	0.1	0.101	101	1.5
	Calibration 5	0.2	0.201	101	0.7

To test the linearity and robustness of the method, a calibration curve was run in triplicates in a concentration range of 0.01 - 0.2 µg/L. The results exhibited excellent linearity as shown in Figure 7 with all R^2 of each compound, > 0.99. Moreover, in Figure 8, overlays of different concentrations of the metabolites show the peak shape and stable retention times of the metabolites even if there is a switch between sources. Additionally, DMS, a metabolite that does not retain well on most standard columns because of its extreme polarity, is no longer affected by the matrix.[6] To further demonstrate how robust the method performs Table 7 illustrates the accuracy and precision of this method. All calibration points had accuracy of <10 % and RSD of <10 %.

Besides demonstrating the possibility of switching ionization techniques in the same method, we could also show that sensitivity was not hindered during the process. All metabolites could be measured down to extremely low levels, 0.01 µg/L, which is well below the EU regulations. According to the EU, chlorate has a MRL of 0.25 mg/L and DMS a fungicide, 0.1 µg/L.[5] Thus, illustrating that switching between APCI and ESI in the same method does not hinder the end results and allows you to easily reach regulatory limits.

Conclusion

Two analytical methods showcasing the benefits of having a true dual source on a LC/MS/MS system were presented in the context of determination of organic contaminants in drinking water. The high sensitivity, at low ppb levels, achieved with these methods is well below the limits set forth by the European Union for these contaminants in water. The QSight triple quadrupole LC-MS/MS system gives the user maximum flexibility to test compounds using different ionization techniques to achieve improved results. Interestingly, we have found that the signal intensity of acrylamide depends on the ionization technique used. The examples presented here were not typical APCI nonpolar compounds, but because ESI showed a drastic matrix effect even in drinking water, APCI proved to be the best choice. Not only was the sensitivity more than adequate when looking at current regulations, the reproducibility of the results also demonstrated the robustness of these methods for use in a water testing laboratory. With regulations becoming stricter over the years, having the convenience of automatically and easily switching between ionization techniques can be a great advantage when tackling difficult compounds

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PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

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