Application Note

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A Direct Aqueous Injection Method for Contaminants in Drinking and Nonpotable Water

Analysis of acrylamide, haloacetic acids, and β-estradiol in water with an Agilent 6495D LC/TQ

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Abstract

A comprehensive liquid chromatography/triple quadrupole mass spectrometry (LC/TQ) method was developed for the quantitation of acrylamide, haloacetic acids (HAAs), and β-estradiol with the intention to accelerate and simplify routine laboratory water testing. Compound transitions and optimized parameters were applied in the analytical method. Method suitability was demonstrated using an Agilent 1290 Infinity II LC system coupled to an Agilent 6495D LC/TQ. The samples included drinking water, surface water, and ground water, using a 10 µL direct aqueous injection.

Method performance was evaluated based on instrument limit of detection (LOD), limit of quantification (LOQ), calibration curve linearity, recovery, and precision. The evaluation was done using calibration standards at varying levels for each compound; acrylamide down to 0.03 µg/L, HAAs down to 0.1 µg/L, and β-estradiol down to 1 ng/L. All of the analytes demonstrated linearity with $R^2 \geq 0.995$. Method precision was assessed using relative standard deviation (RSD). The RSDs for all compounds were within the limit of 12.5% and mean recoveries for all of the target analytes were within the limits of 75 to 125%.

Introduction

Recent regulations, such as EU 2020/218, have included the addition of new emerging contaminants in drinking water and raw waters, which supply water-treatment works. Some of these new contaminants to be analyzed include acrylamide, HAAs, and β-estradiol. However, acrylamide and HAAs can be difficult to analyze due to their high polarity and high interferences from hard water samples. Additionally, β-estradiol requires a very low level of detection (1 ng/L) compared to the other compounds (acrylamide 0.1 µg/L and HAAs 60 µg/L). The analysis uses a 1290 Infinity II LC coupled to a 6495D LC/TQ for analysis of water samples.

Experimental

Chemicals and reagents

LC/MS-grade solvents and analytical reagents were used for this study.

Standards and solutions

Ready-to-use and custom premixed individual standards were acquired where available. Neat compounds were sourced where custom mixes were not available.

Intermediate standard mixes were prepared from stock standards and used for the rest of the experiments. Working standards were diluted from the intermediate mixes and used for the preparation of prespiked calibration and QC samples.

No internal standards were used in this study, but are available if required.

Calibration standards were prepared in matrix water and ultrapure water. Serial dilutions were performed to prepare six calibration concentration levels. Calibration standards were prepared fresh and stored in a refrigerator at 3 °C if not used immediately.

Sample preparation

Samples were collected from three sources: river water, bore-hole water, and drinking water. Each sample had an addition of 0.2 M EDTA and formic acid to help with removal of free metal ions and retention of early-eluting compounds.

Samples were prepared by spiking with the relevant level of additive and transferred as 1 mL to an LC/MS amber vial. Sample bottles also contained some pretreatment with the addition of sodium thiosulphate to remove residual chlorine from drinking water samples.

Instrumentation

Chromatographic separation was performed using an Agilent InfinityLab Poroshell 120 Aq-C18, 3.0 × 150 mm, 2.7 µm (part number 693675-742) installed on the 1290 Infinity II LC system.

The individual modules of the 1290 Infinity II LC system included:

- Agilent 1290 Infinity II high-speed pump (G7120A)
- Agilent 1290 Infinity II autosampler (G7167B)
- Agilent 1290 Infinity II multicolumn thermostat (G7116B)

The 6495 LC/TQ with an Agilent Jet Stream (AJS) electrospray ion source was operated in dynamic multiple reaction monitoring (dMRM) mode. This mode allows more MRM transitions if future development is required for additional compounds. The LC/TQ autotune was performed in both unit and wide modes. All data acquisition and processing were performed using Agilent MassHunter software (version 12).

Results and discussion

For each compound, MRM transitions, collision energies, and ionization polarity were optimized using Agilent MassHunter Optimizer software. The two or three most abundant product ions per compound were selected automatically, where available. Depending on the fragmentation behavior of the individual compound, two or three MRM transitions were selected per compound. This selection was done to give confidence in identification and confirmation by the LC/TQ. The most abundant fragments were defined as primary transitions.

The chromatographic method was optimized using the Aq-C18 column, which resulted in good separation and distribution of compounds of interest. These compounds were analyzed within a 12-minute HPLC gradient. The flow rate offered effective desolvation of target ions using the AJS ion source.

The selected cycle time ensured that sufficient data points were collected across the chromatographic peaks for reproducible quantitation and conformation of results.

Verification of method performance and validation

The method performance criteria were assessed and a complete validation was based on linearity, method sensitivity, recovery, and precision. The three real matrix samples were spiked at blank, low, and high levels. Water matrices included drinking water, surface water, and ground water. All compounds met the requirements set including precision (< 12.5%), bias (< 25%), uncertainty of measurement (UoM) (< 60%), LOD, and LOQ.

Chromatography for all compounds showed excellent peak shape, retention, and sensitivity at these low levels in ultrapure water (Figure 2).

Table 1. Basic LC method setup.

Parameter	Value
Column	Agilent InfinityLab Poroshell Aq-C18, 3.0 × 150 mm, 2.7 µm
Flow Rate	0.4 mL/min
Column Oven Temperature	45 °C
Injection Volume	10 µL
Stop Time	12 min

Table 2. Compound transitions of each compound with MS parameters.

Compound Name	Precursor *m/z*	MS1 Resolution	Product *m/z*	MS2 Resolution	CE (V)	iFunnel Mode	Polarity
Acrylamide	72.1	Unit	55	Narrow	10	Fragile	Positive
Acrylamide	72.1	Unit	44	Wide	31	Fragile	Positive
MCAA	95	Unit	37	Unit	16	Fragile	Negative
MCAA	93	Unit	35	Unit	16	Fragile	Negative
DCAA	127	Unit	83	Unit	6	Fragile	Negative
DCAA	127	Unit	35	Unit	26	Fragile	Negative
MBAA	139	Unit	81	Unit	7	Fragile	Negative
MBAA	137	Unit	79	Unit	7	Fragile	Negative
DBAA	217	Unit	81	Unit	32	Fragile	Negative
DBAA	217	Unit	79	Unit	32	Fragile	Negative
TCAA	161	Wide	117	Wide	1	Fragile	Negative
TCAA	117	Wide	35	Wide	12	Fragile	Negative
17-β-Estradiol	271.2	Unit	183	Widest	47	Standard	Negative
17-β-Estradiol	271.2	Unit	145	Widest	45	Standard	Negative

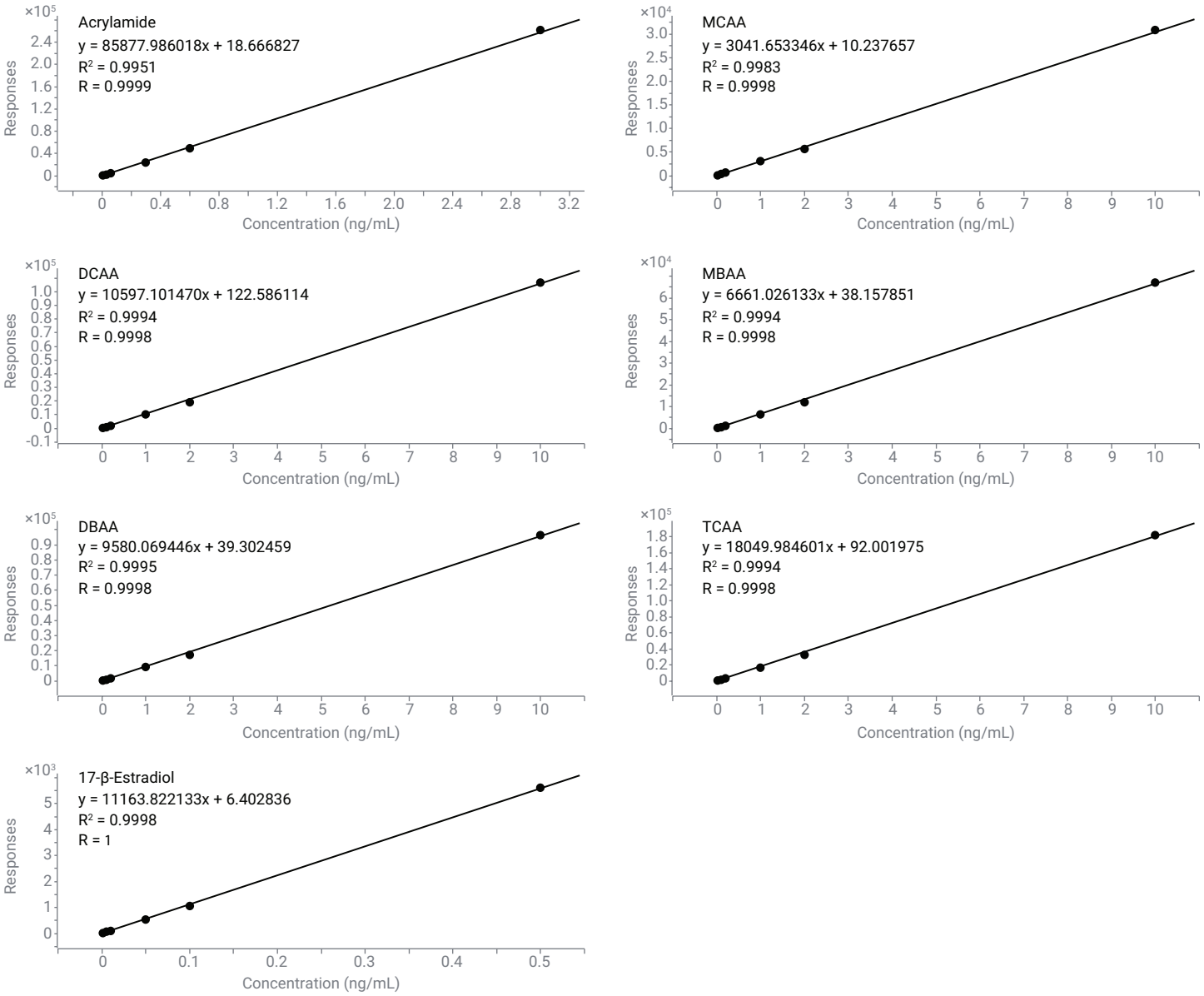


Figure 1. Various ranges of calibration for all compounds (µg/L): acrylamide 0.006 to 3 µg/L, HAAs 0.02 to 10 µg/L, and β-estradiol 0.001 to 0.5 µg/L.

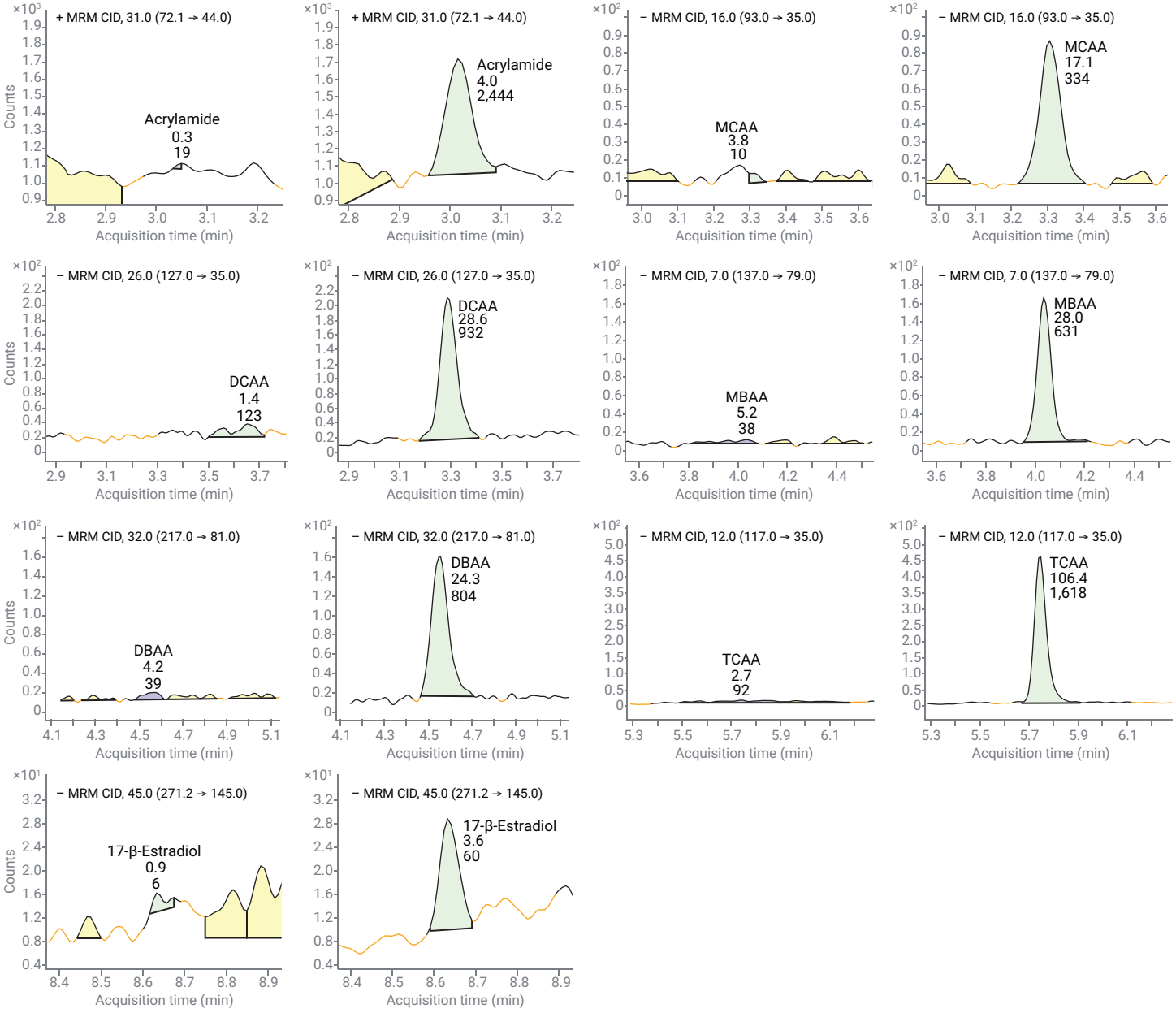
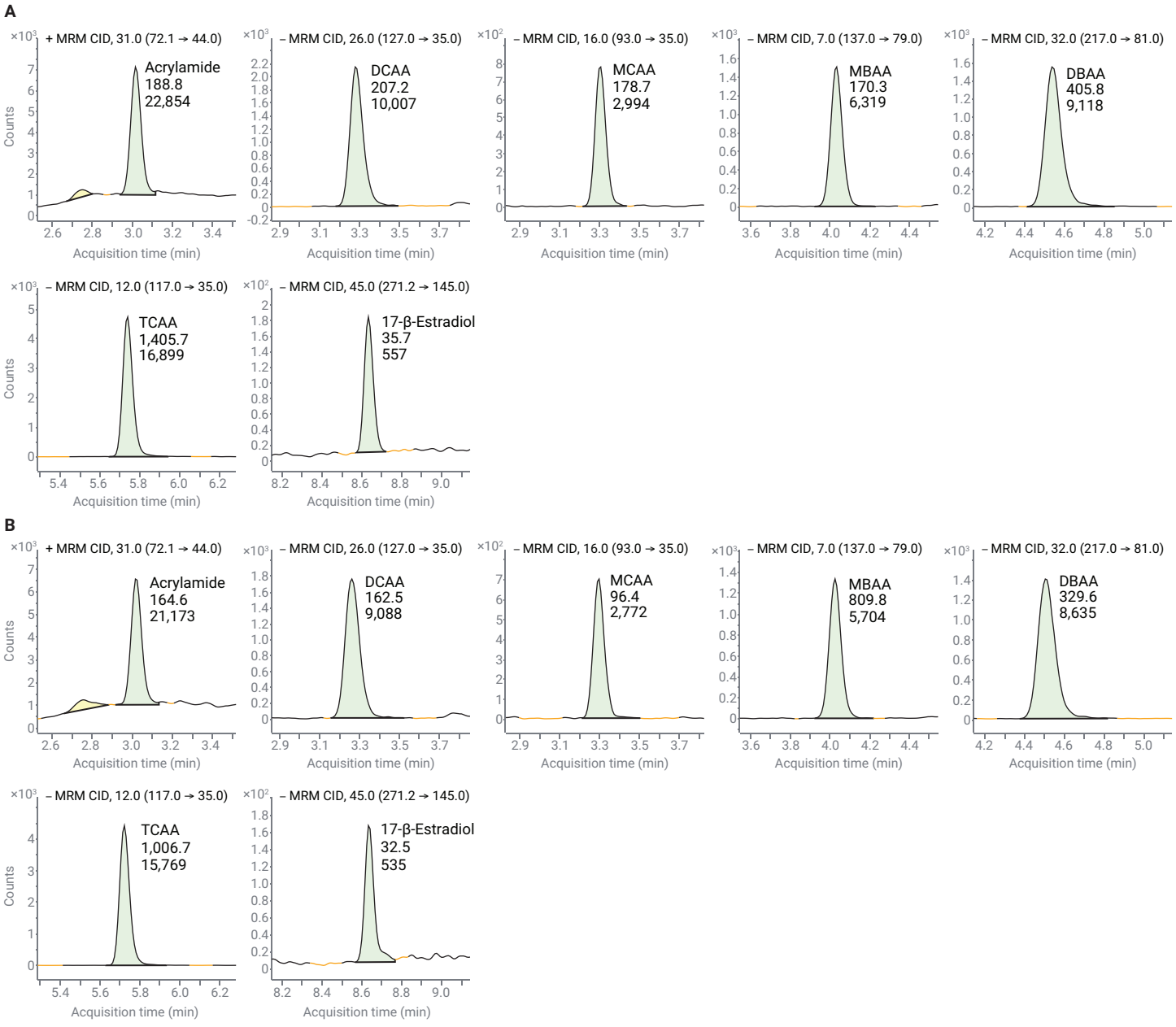


Figure 2. Chromatography of blank versus 0.03 µg/L for acrylamide, 0.1 µg/L for HAAs, and 0.005 µg/L for β-estradiol in ultrapure water. Signal-to-noise ratio and area are listed.

Chromatography for all compounds in all three water types showed excellent peak shape, retention, and sensitivity in all three real water samples at the levels spiked (Figure 3). Trichloroacetic acid (TCAA) shows a much higher result in the drinking water samples. This result is due to the presence of TCAA in the matrix. The blank drinking water contained approximately 17 µg/L of TCAA, and therefore, the 1 µg/L spike showed approximately 18 µg/L.



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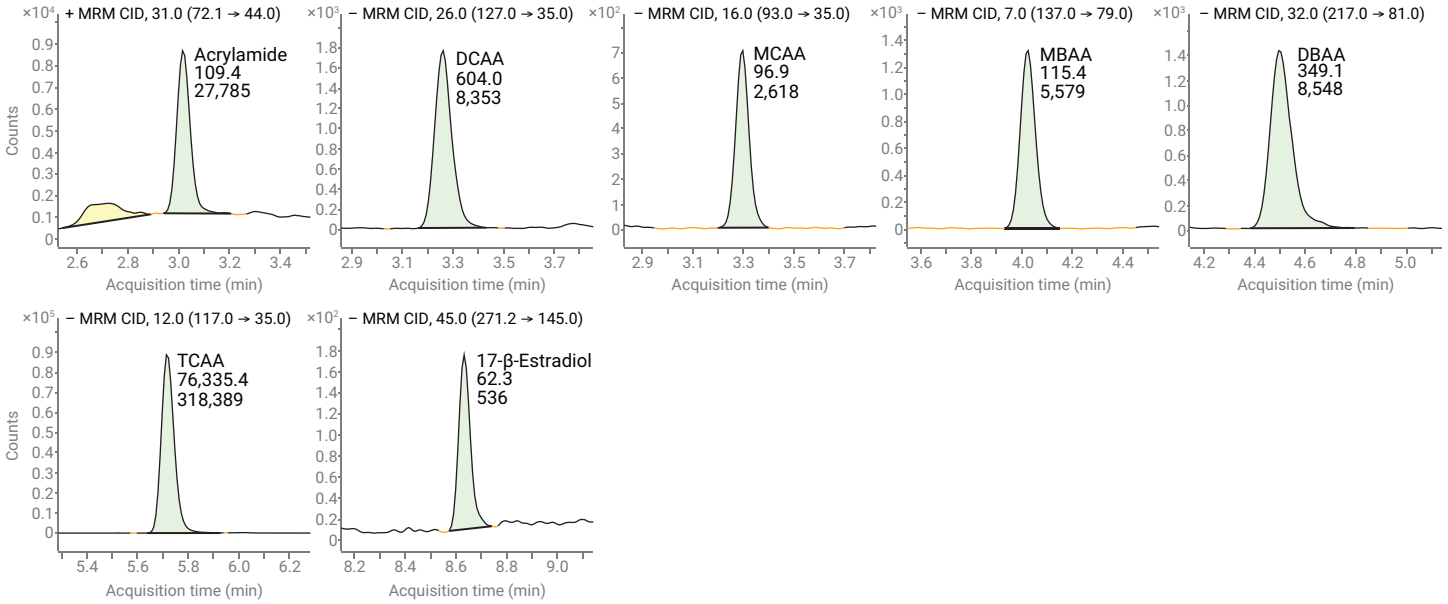


Figure 3. Chromatography at 0.3 µg/L for acrylamide, 1 µg/L for HAAs, and 0.05 µg/L for β-estradiol in real water samples: (A) ground water, (B) surface water, and (C) drinking water. Signal-to-noise ratio and area are listed.

Table 3 shows %RSD and method detection limits (MDL).

MDL calculation used:

$MDL_S = t_{(n-1,\,1-\alpha=0.99)} S_S$

Where:

t = 2.718

S = Standard deviation of calculated concentration (µg/L)

Table 3. Data for %RSD and MDL. Samples were spiked at 0.3 µg/L for acrylamide, 1 µg/L for HAAs, and 0.05 µg/L for β-estradiol.

		Acrylamide	DCAA	MCAA	MBAA	DBAA	TCAA	17-β-Estradiol
Ultra-Pure Water	Sample Number	12	12	12	12	12	12	12
	SD (Area)	537	166	99	78	106	129	11
	SD (Final Concentration)	0.0063	0.0157	0.0324	0.0117	0.0110	0.0071	0.0010
	%RSD (Area)	2.3	1.7	3.3	1.2	1.1	0.8	2.0
	%RSD (Final Concentration)	2.3	1.7	3.4	1.2	1.1	0.8	2.0
	MDL0.0170	0.0427	0.0880	0.0317	0.0299	0.0194	0.0027	
Ground Water	Sample Number	12	12	12	12	12	12	12
	SD (Area)	411	315	66	151	192	392	15
	SD (Final Concentration)	0.0048	0.0297	0.0218	0.0227	0.0201	0.0217	0.0013
	%RSD (Area)	1.8	3.5	2.5	2.6	2.2	2.4	2.8
	%RSD (Final Concentration)	1.8	3.6	2.5	2.6	2.2	2.5	2.8
	MDL	0.0130	0.0808	0.0592	0.0617	0.0545	0.0590	0.0036
Surface Water	Sample Number	12	12	12	12	12	12	12
	SD (Area)	622	207	85	83	148	246	14
	SD (Final Concentration)	0.0073	0.0196	0.0279	0.0125	0.0155	0.0136	0.0013
	%RSD (Area)	2.8	2.3	3.2	1.5	1.7	1.6	2.7
	%RSD (Final Concentration)	2.8	2.4	3.2	1.5	1.7	1.6	2.7
	MDL	0.0197	0.0532	0.0757	0.0340	0.0420	0.0371	0.0034
Drinking Water	Sample Number	12	12	12	12	12	12	12
	SD (Area)	425	120	71	63	149	1,237	17
	SD (Final Concentration)	0.0050	0.0113	0.0232	0.0094	0.0155	0.0686	0.0016
	%RSD (Area)	1.5	1.4	2.9	1.1	1.7	0.4	3.1
	%RSD (Final Concentration)	1.5	1.4	2.9	1.1	1.7	0.4	3.2
	MDL	0.0135	0.0307	0.0631	0.0256	0.0421	0.1863	0.0043

Note: TCAA in drinking water had high MDL due to its presence in the blank.

Conclusion

This application note describes a highly sensitive and reproducible method for the fast and reliable quantitation of the listed compounds in water by direct injection. The dMRM method was created and optimized using Agilent MassHunter software and allows the addition of more MRM transitions if future development is required for additional compounds.

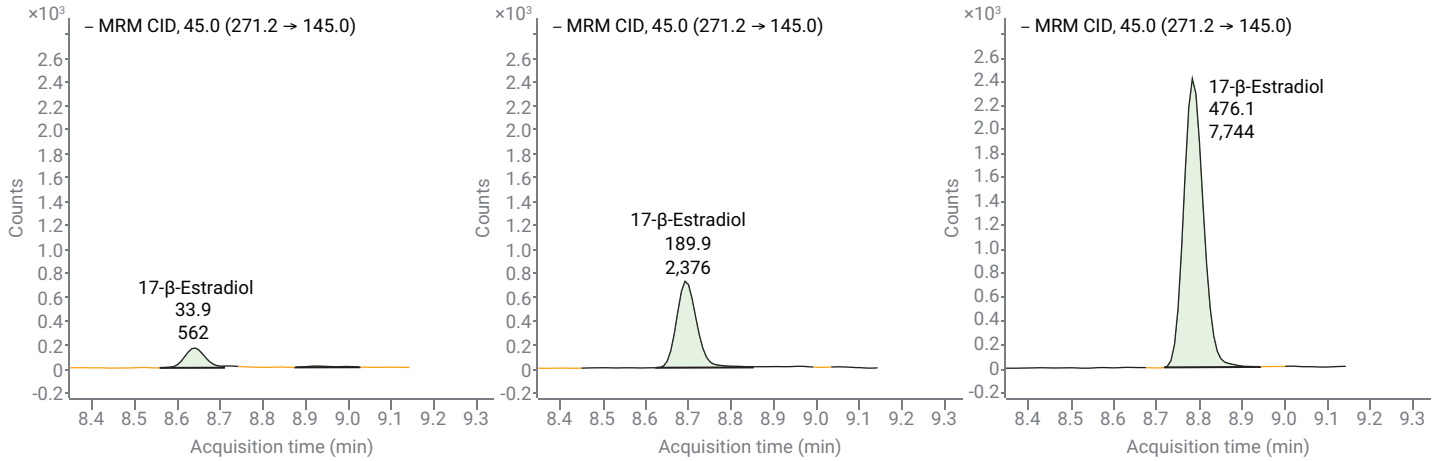
An Agilent 1290 Infinity II LC coupled to an Agilent 6495D LC/TQ was used for the analysis. The 12-minute LC gradient method using an Agilent Aq-C18 column offered good chromatographic separation and RT distribution of all targets. The LC/TQ data acquisition was in dMRM mode with fast polarity switching for the most efficient use of instrument cycle time. The method performance was verified based on requirements for calibration curve linearity, instrument LOD, recovery, and precision. The results demonstrate the ability of the quantitative analytical method for emerging contaminants in water by direct injection.

Further development

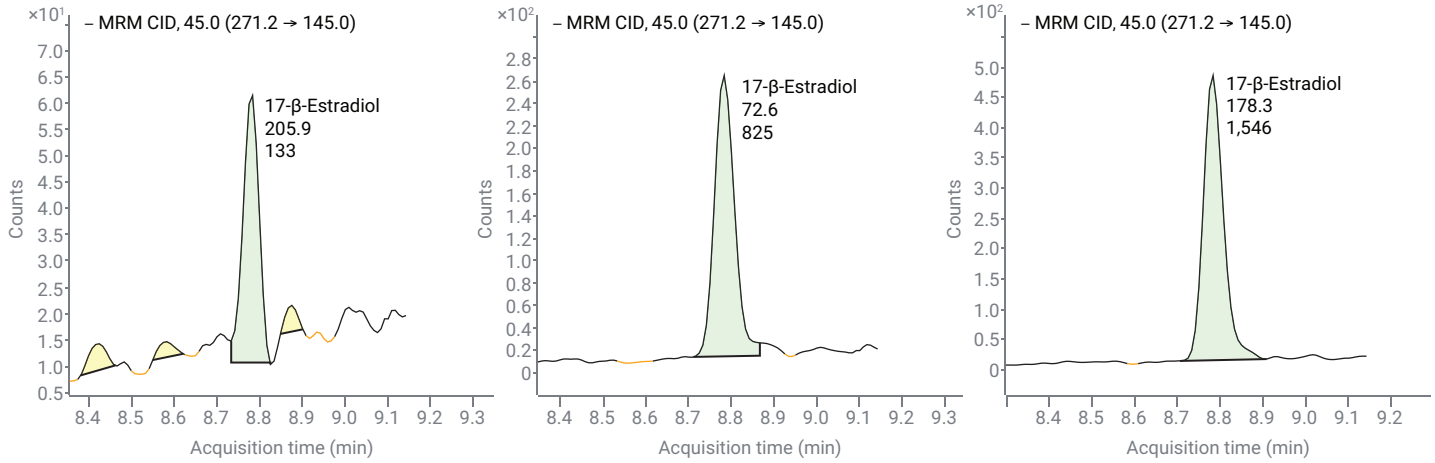
As part of the method development, larger injection volumes were also tested to see if lower limits of detection were required. The method described uses a 10 µL injection; however, 40 and 100 µL injections were also performed where more sensitivity was required. These larger injection volumes can help reach lower levels.

From the large volume injection data, injecting up to 100 µL for β-estradiol can give a 10x increase in sensitivity, allowing even lower limits of detection. For acrylamide and HAAs, a 40 µL injection was observed to give a 3 to 4x increase in sensitivity, without compromising the peak shape or signal-to-noise ratio (Figure 4).

β-Estradiol at 0.050 µg/L, with 10, 40, and 100 µL injections scaled to 100 µL injection



β-Estradiol at 0.001, 0.005, and 0.010 µg/L, with 100 µL injection



Acrylamide (0.3 µg/L) and HAAs (1 µg/L) 10 µL versus 40 µL injections, scaled to 40 µL injection

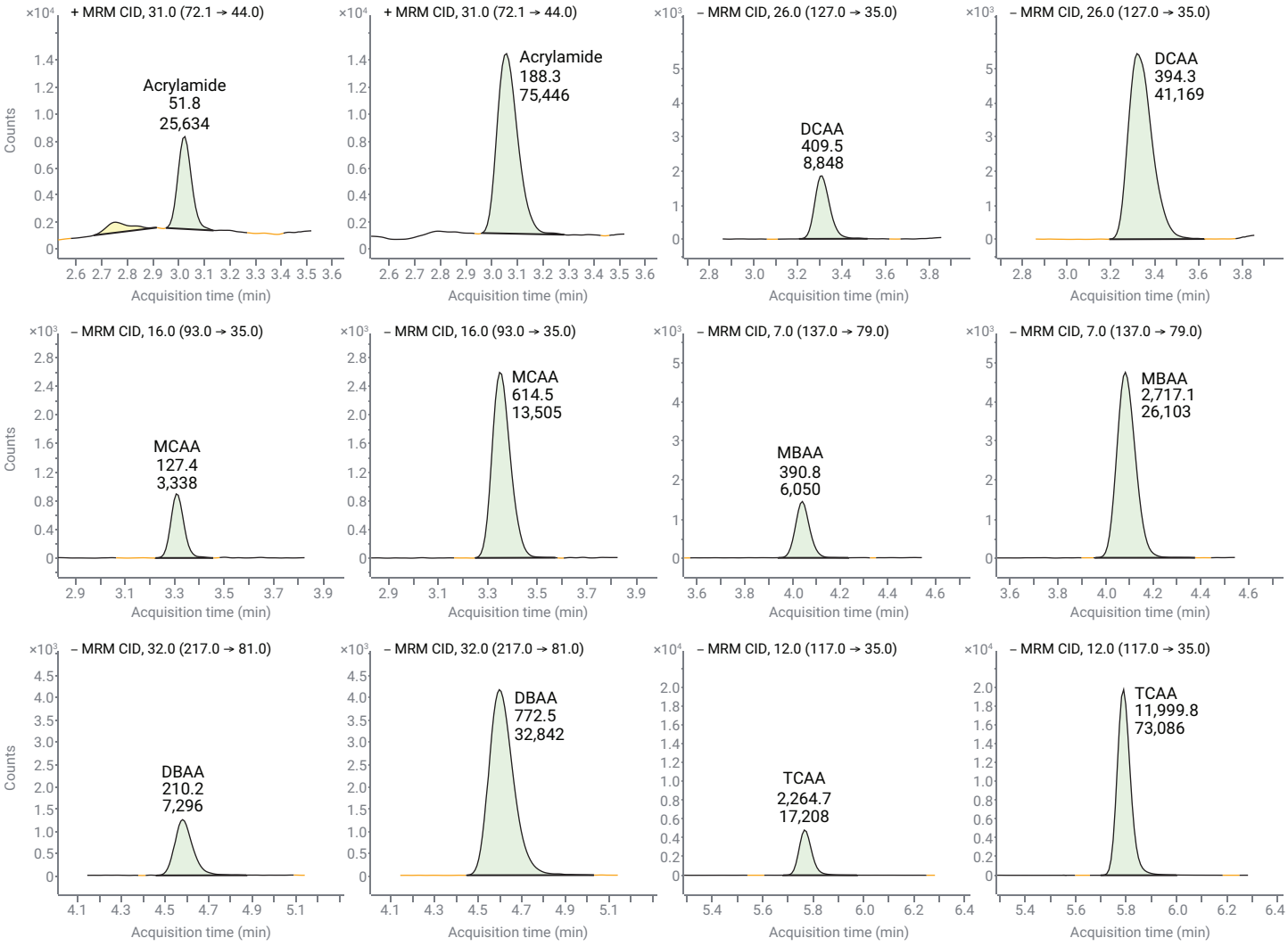


Figure 4. Larger injection volume data.

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