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Determination of Haloacetic Acids and Acrylamide 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 acrylamide and haloacetic acids 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 with a Xevo TQ-S micro using a reversed-phase LC method. Method performance was assessed using 3 spike levels (2, 4, and 30 µg/L) for 9 haloacetic acids and acylamide (0.02, 0.04, and 0.08 µg/L) with 22 replicates at each level in mineral water preserved with ammonium chloride additive. All average recoveries were between 97 to 102% with RSDs all less than 8% for analytes at each spike level. This was achieved using a short LC-MS/MS run time of under 8 minutes and a 10 µL injection. Repeatability was tested with 270 injections of a 2 µg/L haloacetic acids and 0.1 µg/L acrylamide mineral water calibration standard with no user intervention. Results showed RSDs less than 10% for peak area response and RSDs less than 1% for retention time for all analytes tested. From the method performance study, limits of quantification (LOQ) for individual haloacetic acids was 0.5 µg/L and 0.02 µg/L for acrylamide based on the lowest calibration standard. These LOQs exceed the requirements of the new EU Drinking Water Directive of 60 µg/L for the sum of MCAA, DCAA, TCAA, MBAA, and DBAA and 0.1 µg/L for acrylamide.

Benefits

- Application covers both existing requirements for acrylamide plus newly added haloacetic acids therefore increasing laboratory efficiency
- Exceeds requirements in new EU Drinking Water Directive 2020
- Rapid reversed phase LC-MS/MS method eliminating the need for sample pre-concentration, derivatization or specialized ion chromatography equipment

Introduction

The increased focus in the haloacetic acids is derived from their risk as potential human carcinogens[1] and are formed during the water disinfection process in the presence of organic material. Acrylamide is a recognized human carcinogen[2] and can be present in drinking water through contamination from grouting in the water network (it is present in grouting agents) and in flocculants for the clarification of potable water.

A fast, efficient method for the determination of haloacetic acids and acrylamide contaminants in drinking water is important as we recognize and manage risks associated with chemical contaminant in drinking water supplies. The World Health Organization (WHO) has updated guidelines in 2017[3] and the EU are to set new standards in chemical contamination in an updated EU Drinking Water Directive.[4] The new Directive includes MCAA, DCAA, TCAA, MBAA, and DBAA (with a parametric value of 60 µg/L) plus identifies that continued monitoring of acrylamide is still required (with a parametric value of 0.1 µg/L). The US EPA includes 5 haloacetic acids (HAA MCL = 60 µg/L) and acrylamide (zero tolerance) in their National Primary Drinking Water Regulations.[5]

Common methods for the analysis of haloacetic acids require either sample preparation or derivatization with determination by GC-ECD, GC-MS(/MS) or by direct injection onto an Ion Chromatography-MS(/MS) System.[6, 7, 8] We have developed a direct injection method with low injection volume utilizing Reversed Phase Liquid Chromatography coupled to a Tandem Quadrupole Mass Spectrometer that includes both haloacetic acids and acrylamide. This approach reduces the need for either sample preparation and/or derivatization and decreases risk of potential analyte losses associated with each. The method also eliminates the use of HILIC with the associated long column re-equilibration times and removes the need for the addition of ion chromatography separation systems.

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 50 mL with 5 mg of ammonium chloride added as a preservative.[1,7] The samples were then stored at 4 °C in a fridge until analysis.

Just prior to analysis, 50 µL of formic acid was added to the 50 mL sample. An aliquot of the resulting mix was then transferred to a 2 mL LC vial where isotopically labelled internal standard was then added (acrylamide d3) for the determination of acrylamide. The haloacetic acids were calibrated externally and acrylamide-d3 was used as an internal standard for the acrylamide only.

After the method development phase, a method performance study was conducted which consisted of 2 batches containing 33 spiked mineral water samples: 11 spikes at a designated low, mid and high concentration (2, 4, and 30 µg/L) for the haloacetic acids and (0.02, 0.04, and 0.08 µg/L) for acrylamide. A matrix effect assessment was conducted in the first batch by running calibration standards in both LC-MS grade water and mineral water.

LC Conditions

LC system:	ACQUITY UPLC I-Class PLUS
Vials:	TruView LCMS Certified Clear Glass 12 x 32 mm, Screw Neck Vial (p/n: 186005666CV)
Column(s):	ACQUITY UPLC HSS C_{18} SB 1.8 µm, 2.1 x 100 mm (p/n: 186004119)
Column temp.:	30 °C
Sample temp.:	10 °C
Injection volume:	10 µL
Flow rate:	0.4 mL/min
Mobile phase A:	Water with 0.05% acetic acid (LC-MS grade)
Mobile phase B:	Methanol with 0.05% acetic acid (LC-MS grade)

Gradient

Time (min)	Flow (mL/min)	%A	%B	Curve
Initial	0.4	99	1	6
3.5	0.4	95	5	6
4.0	0.4	10	90	6
5.0	0.4	10	90	6
6.5	0.4	99	1	6
7.5	0.4	99	1	6

MS Conditions

MS System:	Xevo TQ-S micro
Ionization mode:	ESI+ for acrylamide, ESI- for HAAs
Acquisition range:	MRM
Capillary voltage:	0.5 kV for both polarities
Cone gas flow:	50 L/hr
Desolvation temp.:	600 °C
Desolvation gas flow:	1000 L/hr
Source temp.:	150 °C

Data Management

Informatics:	MassLynx v4.2

Results and Discussion

The MRMs listed in Table 1 highlight the optimized transitions used for quantification and confirmation of the haloacetic acids and acrylamide in this application. The protonated parent ion was identified for acrylamide and deprotonated parent ions were identified for MCAA, DCAA, MBAA, BCAA, and DBAA. For TCAA, BDCAA, CDBAA, TBAA $(M\text{-}COOH)^-$ was identified as a suitable parent ion fragment for use. From the mass spectra produced from infusion experiments, stable in-source fragments were identified and taken forward to generate fragment ions. The results presented in this application note highlights that this is a valid approach for these haloacetic acids based on the results presented and is a common approach reported in literature for the haloacetic acids.[1,6]

Analyte	Retention time (min)	Polarity	Adduct	MRM	Cone (V)	CE (eV)
Acrylamide	1.35	ESI+	$[M+H]^+$	**72.1>55.0**	20	8
				72.1>26.9		12
Acrylamide d3	1.34	ESI+	$[M+H]^+$	**75.0>58.1**	20	8
Dichlororacetic acid (DCAA)	0.84	ESI-	$[M-H]^-$	**126.9>82.9**	2	8
				128.9>84.9		10
Monochloroacetic acid (MCAA)	0.86	ESI-	$[M-H]^-$	**93.0>49.0**	2	12
				93.0>35.0		6
Bromochloroacetic acid (BCAA)	0.90	ESI-	$[M-H]^-$	**172.9>128.9**	2	10
				170.9>126.9		10
Monobromoacetic acid (MBAA)	0.97	ESI-	$[M-H]^-$	**138.9>80.9**	2	10
				136.9>78.9		10
Dibromoacetic acid (DBAA)	0.98	ESI-	$[M-H]^-$	**214.9>170.8**	2	11
				218.9>174.8		11
Trichloroacetic acid (TCAA)	1.39	ESI-	$[M-COOH]^-$	**118.9>34.7**	10	8
				116.9>34.7		8
Dibromchloroacetic acid (BDCAA)	1.56	ESI-	$[M-COOH]^-$	**160.8>78.9**	10	8
				162.9>78.9		8
Chlorodibromoacetic acid (CDBAA)	1.79	ESI-	$[M-COOH]^-$	**208.8>80.8**	10	12
				206.8>78.8		12
Tribromoacetic acid (TBAA)	2.06	ESI-	$[M-COOH]^-$	**250.8>78.8**	20	12
				252.8>80.8	15	12

Table 1. MRM transitions of the analytes and respective isotopically labelled acrylamide analog (quantitative transitions in bold).

The drinking and mineral water samples collected were screened for the presence of haloacetic acids and acrylamide before the method performance study was started. Acrylamide was not detected in any of the water samples. No haloacetic acids were detected in the mineral water. For the soft water only TBAA was not detected, with the remaining 8 haloacetic acids quantified by standard addition found to be present with concentrations ranging from 0.33 to 11.1 µg/L. Hard water only tested positive for DBAA at 0.44 µg/L with quantification by standard addition. As the levels detected in both the soft and hard drinking water samples would affect the method performance study, the mineral water was selected as a representative sample type. To simulate sample collection by water companies, ammonium chloride (preservative) was added to all samples (and calibration standards) with formic acid being added just before analysis to reflect current practice.

The effect of matrix was investigated on the response of the analytes. Table 2 highlights the difference in results obtained when quantifying against “solvent” standards (in this case LC-MS grade water) and matrix standards (mineral water). Therefore, matrix matching the calibration standards to the type of water is an important

operational aspect to implement with this approach.

Name	Matrix effects
Acrylamide	120%
MCAA	64%
TCAA	102%
DCAA	64%
MBAA	79%
BDCAA	97%
BCAA	76%
CDBAA	104%
DBAA	86%
TBAA	102%

Table 2. Matrix effects, response in LC-MS grade water vs mineral water.

Method performance was assessed over 2 analytical batches, where each batch was comprised of 11 spikes at designated low, mid, and high levels for each of the haloacetic acids (2, 4, and 30 µg/L respectively) and acrylamide (0.02, 0.04, and 0.08 µg/L respectively). Mineral water, treated as described in the experimental section, was used throughout the method performance study. Matrix matched calibration standards in mineral water were prepared over the range of 0.5–80 µg/L for the haloacetic acids and 0.02–0.8 µg/L for acrylamide. Figure 1 demonstrates expected calibration graphs from this approach. All coefficients of determination were greater than 0.996 and residuals from the calibration graphs were all lower than 15%, Table 3 shows calibration performance observed. Results from the method performance batches are presented in Table 4 which highlights the method performance as suitable for the quantification of the haloacetic acids and acrylamide in drinking water. An example of the chromatography achieved at low spike level for all compounds in the mineral water spikes (verses the standard addition in hard water) is displayed in Figure 2. From the method performance study, limits of quantification (LOQ) for individual haloacetic acids were 0.5 µg/L and 0.02 µg/L for acrylamide based on the lowest calibration level injected. A sensitivity study conducted before the method performance study highlighted detection of several HAAs at concentrations of 0.05 µg/L. These LOQs exceed the requirements of the new EU Drinking Water Directive (5) of 60 µg/L for the sum of MCAA, DCAA, TCAA, MBAA, and DBAA and 0.1 µg/L for acrylamide.

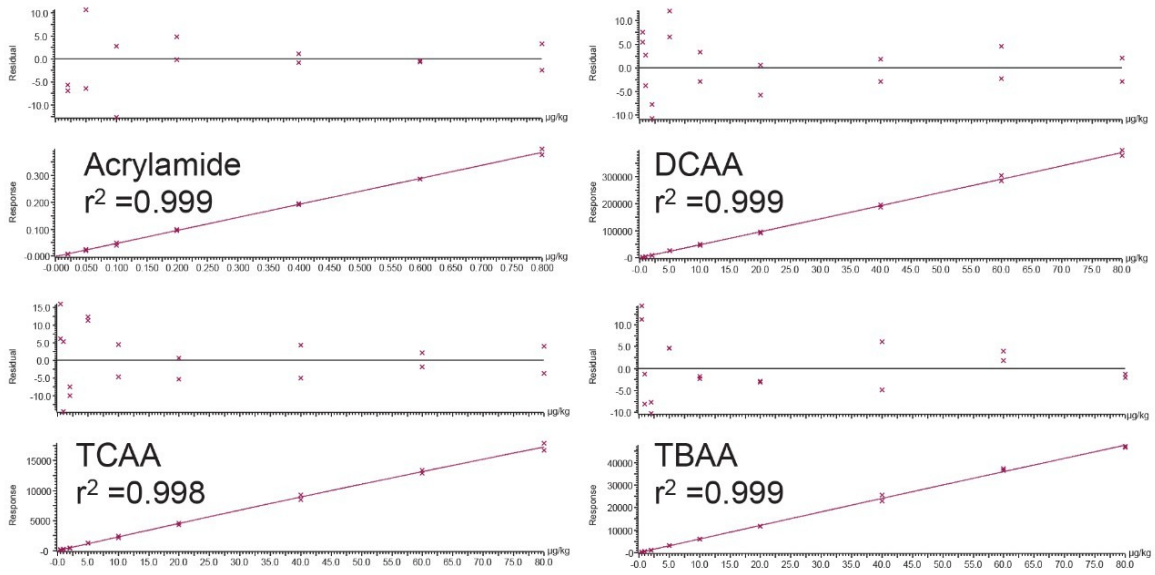

Figure 1. Typical calibration graphs for acrylamide, DCAA, TCAA, and TBAA in mineral water with preservatives.

Name	Quantification transition	Coeff. of determination	Residual	Range
Acrylamide	72 .1>55.0	0.999	<15%	0.02–0.80 ppb
MCAA	93.0>35.0	0.999	<15%	0.5–80 ppb
TCAA	118.9>34.7	0.998	<15%	0.5–80 ppb
DCAA	126.9>82.9	0.999	<15%	0.5–80 ppb
MBAA	138.9>80.9	0.999	<15%	0.5–80 ppb
BDCAA	160.8>78.9	0.997	<15%	0.5–80 ppb
BCAA	172.9>128.9	0.999	<15%	0.5–80 ppb
CDBAA	208.8>80.8	0.998	<15%	0.5–80 ppb
DBAA	214.9>170.8	0.999	<15%	0.5–80 ppb
TBAA	250.8>78.8	0.999	<15%	0.5–80 ppb

Table 3. Typical calibration parameters achieved for mineral water calibration standards from the validation batches.

Compound	Spike level		
	Low – trueness (%RSD)	Mid – trueness (%RSD)	High – trueness (%RSD)
Acrylamide	100 (7.3)	97 (3.1)	99 (3.1)
MCAA	97 (5.8)	99 (4.5)	98 (5.4)
TCAA	101 (5.3)	104 (4.2)	101 (4.1)
DCAA	96 (3.5)	100 (2.9)	100 (3.1)
MBAA	98 (4.7)	101 (5.3)	101 (4.2)
BDCAA	102 (6.0)	105 (2.7)	102 (2.8)
BCAA	97 (3.8)	100 (3.8)	100 (3.5)
CDBAA	100 (5.1)	102 (4.5)	99 (4.8)
DBAA	98 (4.7)	100 (3.6)	98 (1.7)
TBAA	102 (4.6)	105 (3.3)	100 (3.3)

Table 4. Method accuracy parameters obtained for the validation data on mineral water. Concentrations for low, mid and high spikes are 2, 4, and 30 µg/L for the haloacetic acids and 0.02, 0.04, and 0.08 µg/L for acrylamide (n=22 for each level).

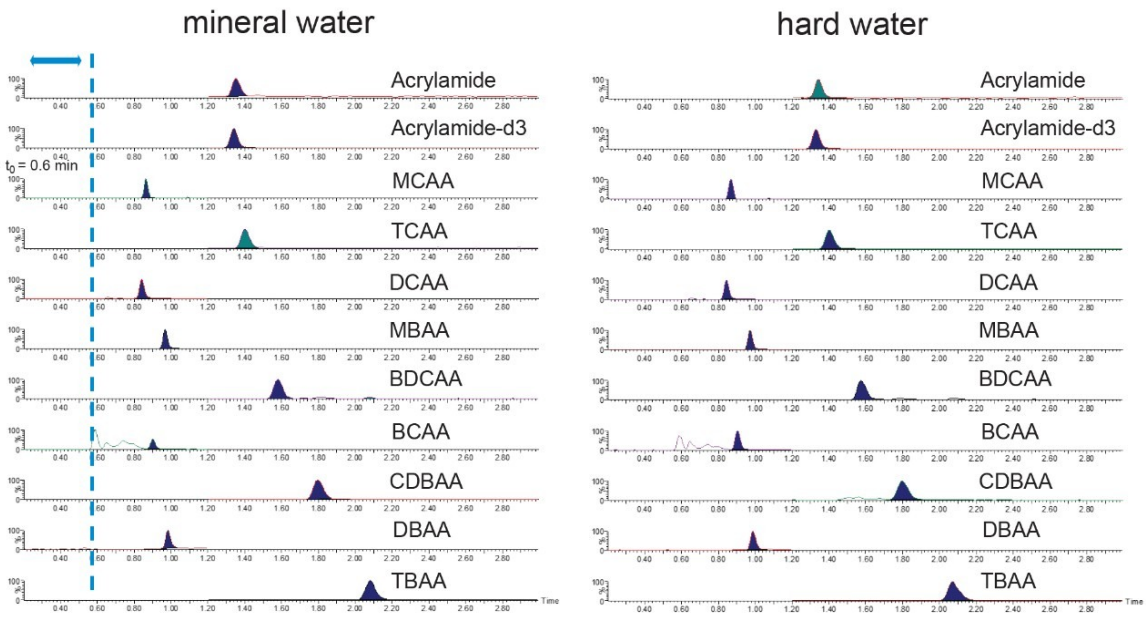

Figure 2. Chromatographs showing the quantitative transitions for haloacetic acids at 2 µg/L and Acrylamide at 0.1 µg/L spiked into mineral water and hard water samples.

Response and retention time stability are important factors to investigate during a method verification process. To this end a repeatability study of 270 injections of a mineral water matrix standard at 2 µg/L haloacetic acids and 0.1 µg/L acrylamide were run continuously on the method presented. Peak area response variation across all

analytes was below 10% RSD, with retention time stability less than 0.7% RSD for all analytes. These results are detailed in Table 5 with the retention time plot for selected analytes presented in Figure 3.

	Acrylamide	MCAA	TCAA	DCAA	MBAA	BDCAA	BCAA	CDBAA	DBAA	TBAA
% RSD (peak area)	7.2	6.1	8.2	7.4	6.2	8.3	4.7	4.9	4.4	4.5
% RSD (RT)	0.4	0.3	0.5	0.3	0.3	0.6	0.4	0.6	0.4	0.6

Table 5. Repeatability of 270 injections of mineral water matrix standard, haloacetic acids at 2 µg/L and acrylamide at 0.1 µg/L. This was a set of continuous injections without any intervention by the operator.

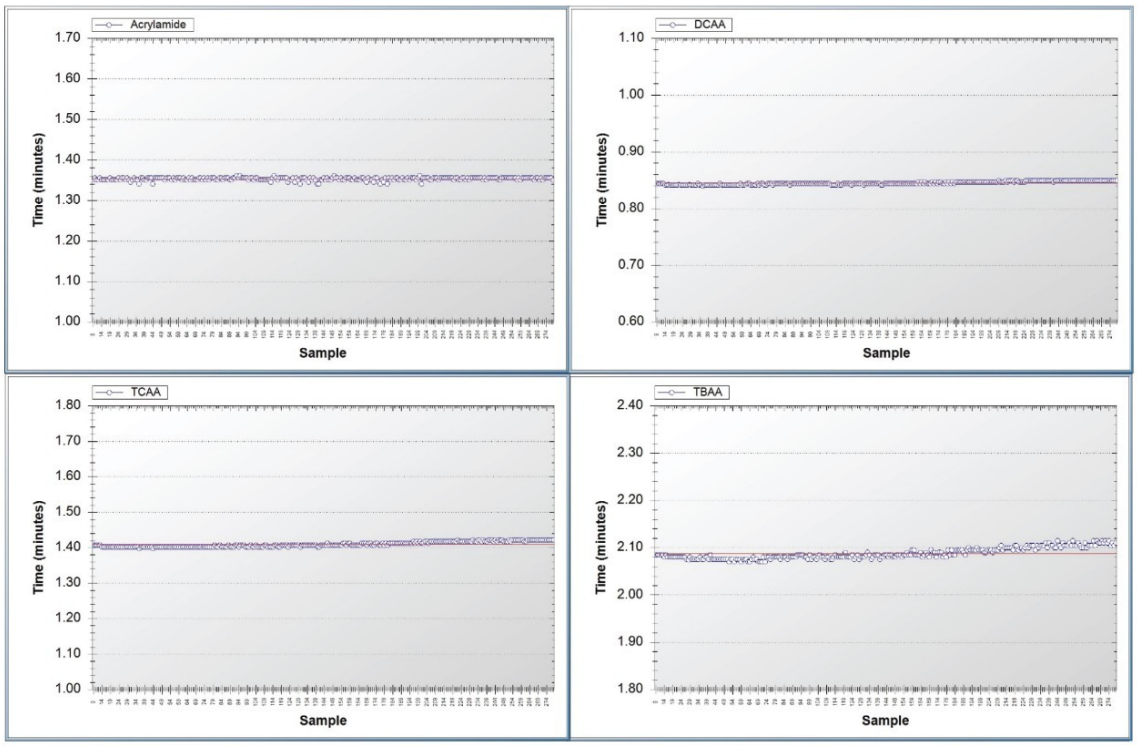

Figure 3. Retention time stability of acrylamide, DCAA, TCAA, and TBAA in 270 injections of mineral water matrix standard, haloacetic acids at 2 ug/L and acrylamide at 0.1 ug/L. This was a set of continuous injections without any intervention by the operator.

Conclusion

The method performance study data demonstrates that the method for the determination of haloacetic acids and acrylamide by direct injection of water samples onto an ACQUITY UPLC I-Class PLUS coupled to a Xevo TQ-S micro exceeds new requirements in the EU Drinking Water Directive coming into effect in late 2020.[4] Limits of

quantification for all 9 haloacetic acids was 0.5 µg/L where the directive states the legal limit is 60 µg/L for the sum of MCAA, DCAA, TCAA, MBAA, and DBAA and 0.1 µg/L for acrylamide. The combination of acrylamide into the method increases the scope of the method and improves laboratory efficiency. By using a reversed phase LC approach, potential problems when using HILIC separations have been removed allowing a direct injection approach to be taken. In combination of using a standard UPLC-MS/MS system, this analysis removes the need for specialist LC or IC equipment and method robustness over 270 injections and demonstrates the ability of the method to continually meet required reporting levels over the course of at least 2 days without user intervention. 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.

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