Determination of Acrylamide in Raw and Drinking Waters

Application

Environmental

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Abstract

A liquid chromatography/mass spectrometry (LC/MS) method is developed for the fast, high throughput quantitation of acrylamide in drinking water using online sample extraction coupled with the Agilent liquid chromatography/mass selective detector time-of-flight (LC/MSD TOF). The limit of quantitation of 0.25 µg/L meets the World Health Organization guideline for the maximum safe level concentration of acrylamide in drinking water at 0.5 µg/L. In addition to sensitivity, justifying the advantage of using time-of-flight mass spectrometry, is the demonstrated specificity with mass accuracy of 6.4 ppm and reproducibility with standard deviations to three or more decimal places.

Introduction

The acrylamide monomer (see Figure 1) is a component of polyacrylamides, used in the water industry as a coagulant, and particularly in drinking water treatments to aid water clarification [1]. However, the acrylamide monomer is a well-known neurotoxin and genotoxic carcinogen, so its presence in drinking water could have a negative impact on human health.

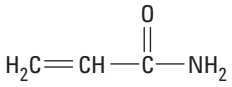

Figure 1. Acrylamide structure.

Classical analytical methods, gas chromatography-electron capture detection (GC-ECD) or liquid chromatography-ultraviolet absorbance detection (LC-UV), need bromination and solid phase extraction (SPE) clean-up procedures, respectively. But the problem in both cases is that the current reachable limit of detection (LOD) and limit of quantitation (LOQ) are far from the 0.5 µg/L requested in the World Health Organization (WHO) guideline [2]. All the methods, currently in use, can only report a high LOQ, above 1 µg/L, which is two times higher than what is expected.

Consequently, a suitable method measuring low levels of acrylamide contaminant in drinking water is required in environmental laboratories. This application note describes such a method, coupling online extraction with a liquid chromatograph/time-of-flight mass spectrometer (LC/TOF MS) for fast, high throughput quantitation of acrylamide. Along with accurate mass analysis for empirical formula determination, reproducibility (%RSD) to at least three decimal places justifies the advantage of using the Agilent LC/MSD TOF in terms of both sensitivity and specificity.

The method described here involves online, C18 pre-column extraction of the analyte followed by a C18 chromatographic separation for identification and quantification. The determined LOQ is 0.25 µg/L, which is four times lower than all that was done before. Furthermore, the precolumn extraction and LC/MS combination eliminates the time-consuming steps of SPE and derivatization prior to analysis. The investigations concerning method validation and results on Drinking Water Treatment Plants (DWTPs) are included.

Materials and Methods

Chemicals and Standards

Analytical solvents, HPLC water, and acetonitrile are acquired from J. T. Baker (Atlantic Labo, Eysines, France). Methanol was obtained from Merk (Fontenay sous Bois, France) and the formic acid (95%–97%) came from Sigma-Aldrich (Saint Quentin Fallaviers, France).

Pure standards of acrylamide (99.4%) and acrylamide-2,3,3-d_3 (99%) are purchased from C. I. L. Cluzeau (Sainte Foy la Grande, France).

Water standards are prepared by dilution of stock solutions of 100 mg/L acrylamide or acrylamide-d3 in methanol. These solutions are then stored in amber glass at –18 °C for 1 month.

Samples

Prior to the analysis, samples are stored at 4 °C. Water samples are filtered using Whatman GD/X 0.45-µm filters for the high purity water and 0.2-µm filters for both the high purity and drinking waters. Then, 1 ng/mL of acrylamide-d_3 is added for the quantification.

Online extraction and LC/TOFMS analytical conditions are shown in Tables 1 and 2 respectively.

Table 1. Optimized Pre-column Extraction

Apparatus	Agilent 1100 series binary pump, autosampler, 6-port switching valve, and column oven.
Extraction column	ZORBAX SB-C18, 4.6 mm × 12.5 mm × 5 µm
Pre-column temperature	20 °C
Injection volume	400 µL
Loading flow	0.5 mL/min
Loading solvent	100% HPLC Water
Back flush start	0.6 min (via the analytical column)
Back flush stop	3.2 min (via the waste)

After the sample is loaded, the valve is switched to the analytical column and the acrylamide is eluted with the conditions given in Table 2.

Table 2. Optimized Analytical Conditions

HPLC separation	Agilent 1100: binary pump 2, column oven
Column	ZORBAX SB-C18, 2.1 mm × 150 mm × 5 µm
Column temperature	40 °C
Analytical flow	0.5 mL/min
Elution solvents	A: 97% HPLC water (0.01% formic acid) B: 3% CH_3CN (0.01% formic acid)
Back flush	Off
Analysis start	0.6 min
Analysis end	3.2 min
Analytical detection	Agilent MSD TOF
Source	ESI
Ion polarity	Positive
Drying gas flow	12 mL/min
Drying gas temperature	300 °C
Nebulizer pressure	50 psi
Fragmentor	150 V
Octopole RF	100 V
Mass range	50 to 1000 *m/z*
Reference ions	121.05087 and 922.00979

This protocol (online, pre-column extraction procedure) is applied to both real samples and HPLC water standards, and is illustrated in the sample flow diagrams of Figures 2 and 3.

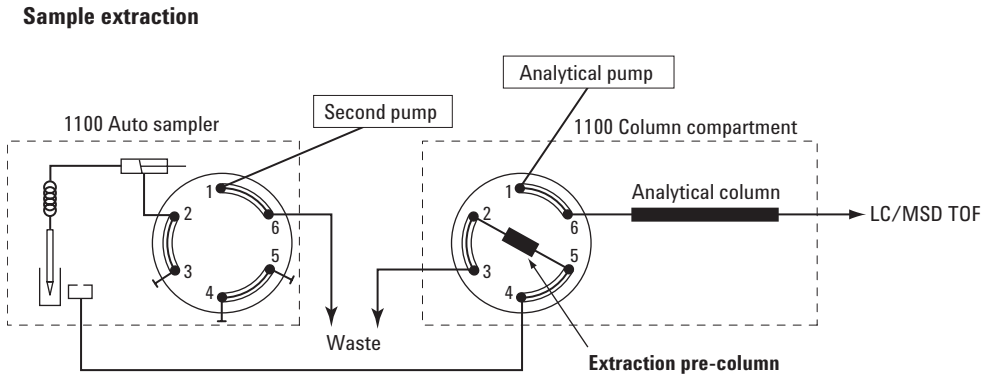

Figure 2. Sample extraction using pre-column.

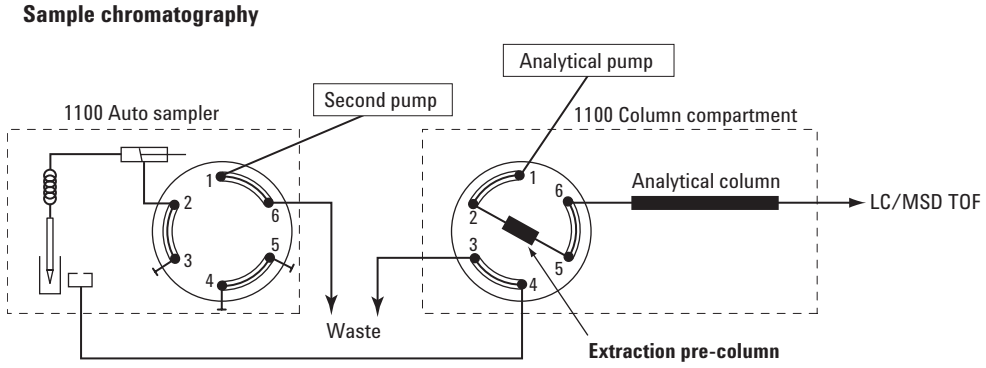

Figure 3. Sample chromatography using the analytical column.

Results and Discussion

Specificity of clean standards

Table 3 shows the coeluting retention times (RTs) and the difference between the theoretical and experimental isotopic masses for acrylamide.

Table 3. Isotopic Masses for Acrylamide

Acrylamide	RT, min	Theoretical mono-isotopic mass	Average quant ion	Mass accuracy amu	Mass error ppm
Native	1.93	72.0443	72.0454	0.0011	14
Deuterated	1.93	75.0632	75.0647	0.0015	19.7

It is worth noting that the average ion mass of 72.0454, even with a mass window of 20 ppm, gives only one possible empirical formula, C_3H_6NO. Since smaller molecules have fewer possible empirical formulae, the requirement for mass accuracy is less stringent as compared to molecules of higher mass. Using the ChemIndex database of 77,000 compounds, the only explainable match is with acrylamide (C_3H_5NO), where there are five hydrogens in the neutral molecule and six in the protonated ion. See Figure 4.

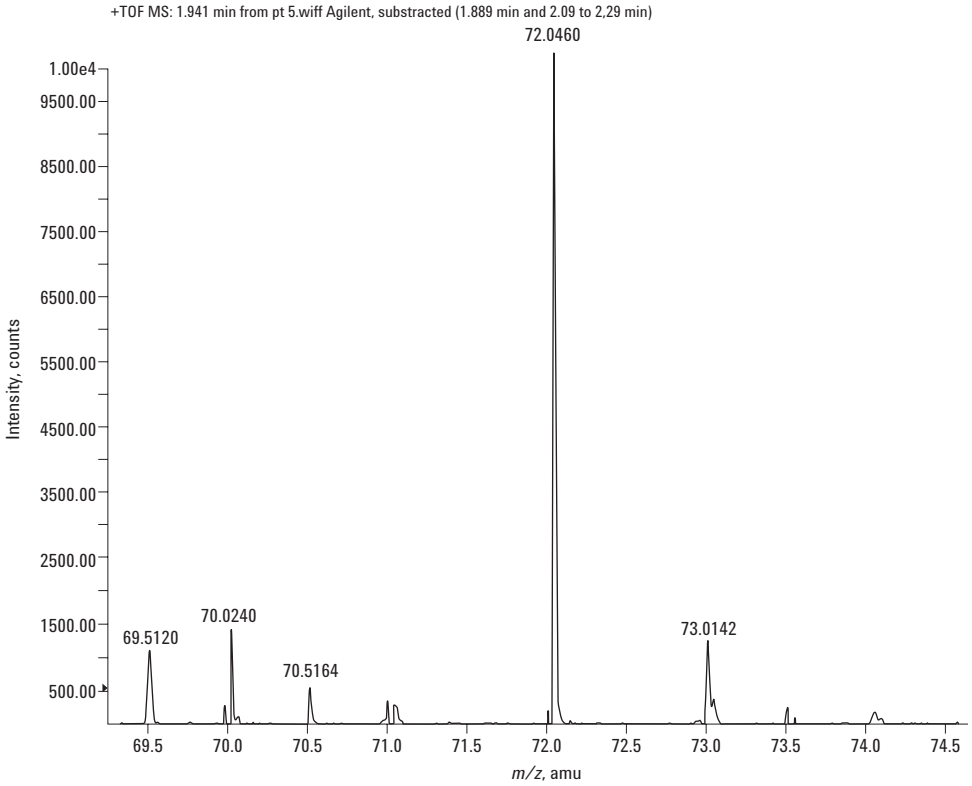

Figure 4. Example of an acrylamide mono-isotopic molecular ion mass of $(M+H)^+$ at 72.0460 from a single injection.

Figures 5 and 6 show individual extracted ion chromatograms (EICs) for acrylamide and acrylamide-d_3, respectively. The contribution of each ion is exclusively attributed to the original compound.

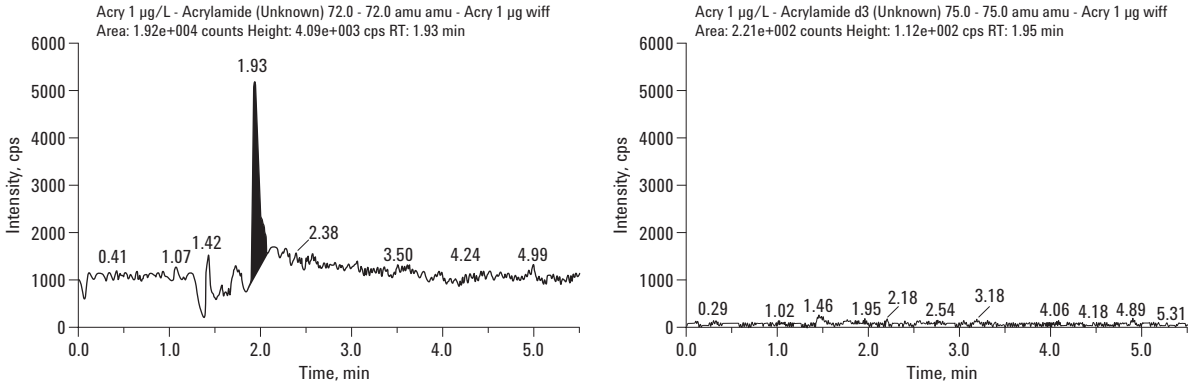

Figure 5. Extracted ion profile of acrylamide at 1 µg/L.

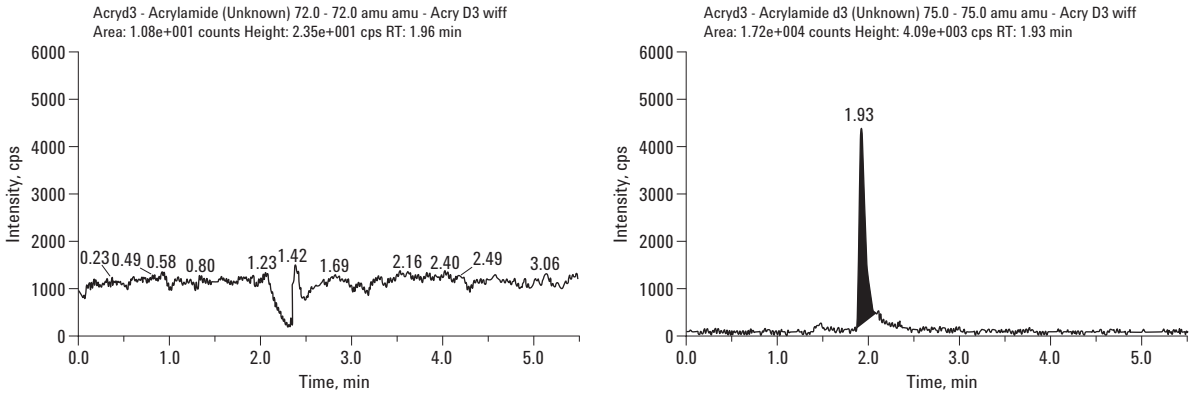

Figure 6. Extracted ion profile of acrylamide-d_3 at 1 µg/L.

Linearity and LOQ

Figure 7 shows the internal calibration curve of acrylamide between 0.25 and 3 µg/L

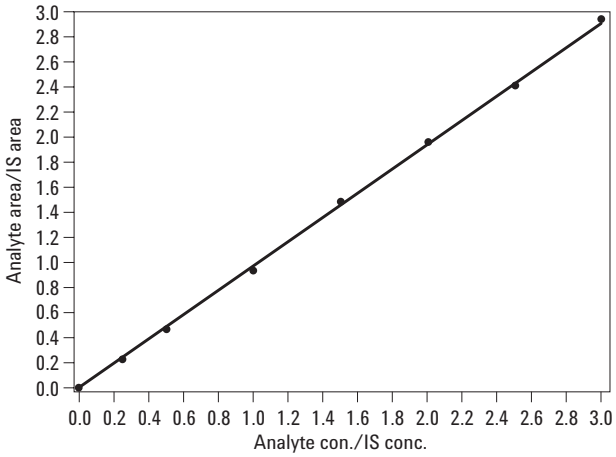

Figure 7. Linearity plot for acrylamide/acrylamide-d_3 response.

These results confirm good linearity using HPLC water and the acceptable LOQ at 0.25 µg/L

Statistical results shown in Tables 4 and 5, validate the analytical method.

Table 4. Repeatability of Calibration Curves, 0.25–3.00 µg/L

Calibration Curves	R^2	Slope	%RSD on EI*-d_3 area
1	0.9980	0.98	5.5
2	0.9998	1.02	9.4
3	0.9998	1.06	11.9
4	0.9998	0.88	6.0
5	0.9994	0.84	5.5

*EI extracted ion

Table 5. Relative Error on Values

Concentration µg/L (n = 5)	%RSD
0.25	4.3
0.50	9.2
1.00	5.7
1.50	4.3
2.00	3.4
2.50	2.2
3.00	2.5

Accurate Mass Stability

From a sequence, we observe good mass assignment stability according to the low SD (<10% to the fourth decimal place). Moreover, the relative error, in comparing theoretical and experimental mass assignments to the third decimal place, is about ±0.0023 for the acrylamide standard and ±0.0027 for it deuterated analog. See Table 6.

Table 6. Standard Deviation (SD) on Accurate Masses

Sequence (no. of samples)	SD on accurate mass of acrylamide	SD on accurate mass of acrylamide-d3
Seq 1 (30)	0.00050 (n = 24)	0.00050 (n = 29)
Seq 2 (30)	0.00046 (n = 23)	0.00040 (n = 29)
Seq 3 (53)	0.00055 (n = 43)	0.00030 (n = 53)
Seq 4 (53)	0.00072 (n = 30)	0.00036 (n = 53)

Sample Specificity and Reproducibility

Sample Specificity and reproducibility data are summarized in Table 7.

Table 7. Specificity and Reproducibility in Three Different DWTPs

	Theoretical spiked value 0.25 µg/L		Theoretical spiked value 0.5 µg/L	
Sample*	**Mean /%RSD**	**%Recovery**	**Mean /%RSD**	**%Recovery**
RW1 (n=4)	0.22/11.7	88	0.47/3.4	94
RW2 (n=5)	0.26/10.5	104	0.45/1.8	90
RW3 (n=3)	0.20/17.0	80	0.52/2.8	104
DW1 (n=4)	0.28/22.5	112	0.48/16.7	96
DW2 (n=5)	0.23/12.9	92	0.43/8.6	86
DW3 (n=3)	0.24/13.0	96	0.50/8.3	100

* RW Raw water (HPLC grade); DW Drinking water

The values obtained for recoveries and RSD are satisfactory.

Figure 8 shows drinking water and Figure 9 shows raw water both spiked with 0.25 µg/L of acrylamide and 1 µg/L of acrylamide-d_3.

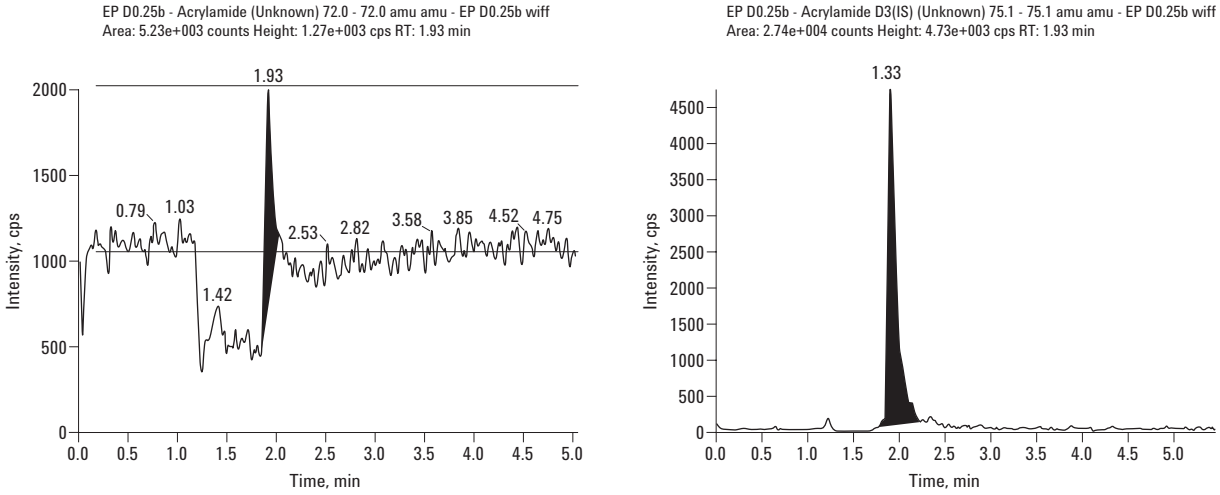

Figure 8. Drinking water spiked with 0.25 µg/L of acrylamide and 1 µg/L of acrylamide-d_3.

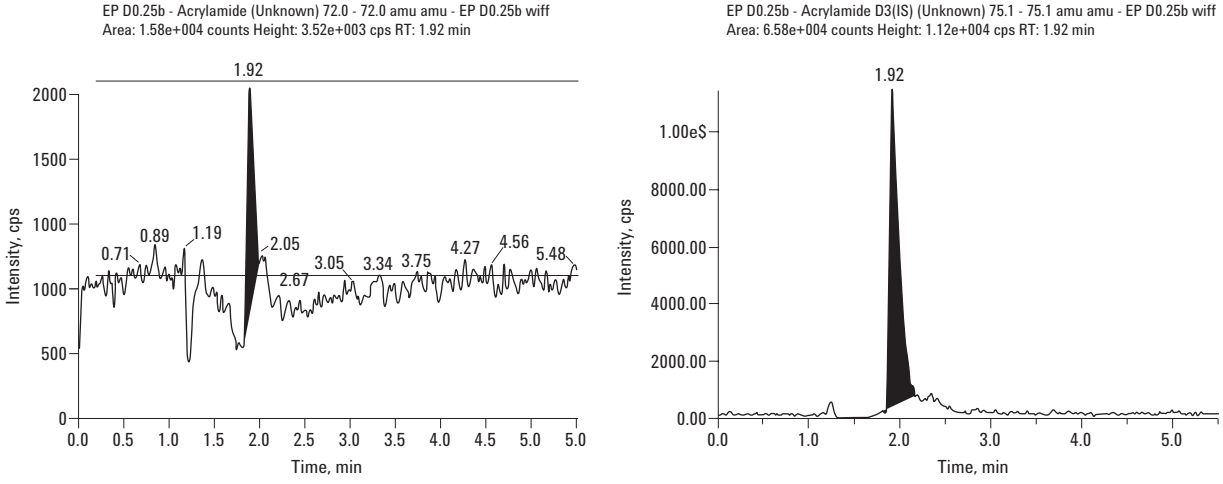

Figure 9. Raw water (HPLC grade) spiked with 0.25 µg/L of acrylamide and 1 µg/L of acrylamide-d_3.

Conclusion

The objective of this work was to develop a method for trace level determinations of acrylamide in water from DWTPs. Statistical results demonstrate that the time-of-flight mass spectrometer (TOF MS) is an appropriate mass detector for this application. The sensitivity permits one to reach an LOQ of 0.25 µg/L and the specificity permits unequivocal identification of acrylamide in surface, groundwater and drinking water samples. Coupled with this analytical technique, the online extraction column-switching method reduces analysis time.

References

1. J.T. Novak and J.H. O'Brien, (1975), *J. Water Pollut. Control Fed.*, **47**, 2397.
2. Guidelines for drinking-water quality, 2nd ed., vol. 1. Recommendations, Geneva, WHO, 1993, p.72.

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