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Determination of acrylamide in drinking water by large-volume direct injection and ion-exclusion chromatography–mass spectrometry

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Abstract

Acrylamide, a known neurotoxin and putative human carcinogen, has been included among the substances to be monitored in drinking water according to the European Union Directive 98/83 on potable water. This paper reports a new method based on the combination of ion-exclusion chromatographic separation and MS detection. Samples of drinking water have been directly injected in the microbore ICE-AS1 column and detected in the selected-ion monitoring mode by a single quadrupole system with electrospray ionization. Chromatographic conditions, such as eluent composition and flow rate, have been optimized by a central composite design experiment. Statistical analysis of data showed that the amount of acetonitrile fraction in the eluent mixture, composed by acetonitrile and formic acid solution, is the variable that most influences retention of the acrylamide peak. After optimization of MS detection parameters, this method has been validated for spiked drinking water samples. The effect of large-volume injection (up to 500 μl) has been also explored. Linearity was evaluated from 0.5 to 5 $\mu g\,l^{-1}$. Repeatability, expressed as R.S.D., was 16 and 12% at 0.5 and 1 $\mu g\,l^{-1}$, respectively. The limit of detection was 0.20 ppb with 500 μl injection volume.

Keywords: Water analysis; Large-volume injection; Acrylamide

1. Introduction

The International Agency for Research on Cancer (IARC) stated that acrylamide could be classified as "probably carcinogenic to humans" (Group 2A) [1] and the World Health Organization (WHO) guideline value associated with a lifetime cancer risk of 10^{-5} is 0.5 $\mu g\,l^{-1}$ in drinking water [2]. A risk assessment evaluation is being prepared by the European Union (EU) [3] to evaluate the risks to human health and the environment because this compound is produced or imported into the EU in volumes above 10×10^3 kg per year. This document will provide all the available information about production, use, fate, and toxicological properties. The main source of acrylamide to drinking water is the release of residual monomer from polyacrylamide coagulants used as a clarifier in the raw water treatment. In general the maximum authorized dose of polymer is 1 $mg\,l^{-1}$. At a monomer content of 0.05%, this dose corresponds to a maximum theoretical concentration of 0.5 $\mu g\,l^{-1}$ of the monomer in water. Practical concentrations in treated water may be lower by a factor of two to three [2]. Polyacrylamides are also used as grouting agents in the construction of drinking water reservoirs and wells, and can be discharged in land and water by plastics and dyes industries.

The US Environment Protection Agency (EPA) set the maximum contaminant level (MCL) goals for acrylamide at zero and requires the water supplier to show that when acrylamide is added to water, the amount of uncoagulated acrylamide is less than 0.5 $\mu g\,l^{-1}$ [4]. Acrylamide has also been regulated in EU countries by the EU 98/83 Drinking Water Directive that stated a minimum quality requirement of 0.1 $\mu g\,l^{-1}$ for water intended for human consumption [5]. Searching for such sub-ppb levels on a regular basis requires methods that are both highly sensitive and non-time-consuming. Determination of acrylamide in environmental water has been carried out by direct injection and reversed-phase HPLC–UV with a limit of detection (LOD) of 5 $\mu g\,l^{-1}$ [6] and by solid-phase extraction (SPE), extraction with activated carbon filter and GC–MS analysis [7]. The latter method proved very sensitive (MDL =

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0.02 μg l^{-1}), but needed the extraction of 500 ml of water. Direct injection (200 μl) in a reversed-phase HPLC–UV system is also recommended by EPA Method 8316 [8]. This method claimed an MDL of 10 μg l^{-1}, which is not sufficient for drinking water monitoring. The introduction of a high-capacity ion-exclusion column, proposed in an European Standard [9] and the application of the same column in microbore version with MS detection in food analysis [10] suggested that we test this type of column for acrylamide determination in drinking water using large-volume injection and MS detection. The aim of this work is to optimize the chromatographic conditions by a chemometric approach and validate the method for drinking water analysis.

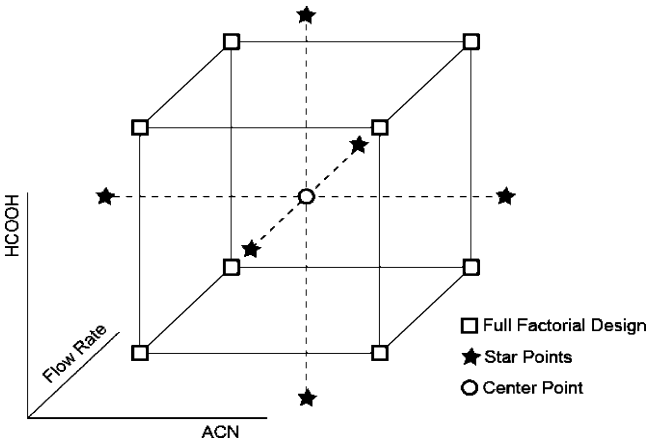

Fig. 1. Representation of a CCD with three factors on five levels. The length of the arm of the star is $\alpha = 1.68$.

2. Experimental

2.1. Materials and chemicals

Acrylamide standard (purity >99% electrophoresis grade) and formic acid (96% ACS reagent-grade) were purchased from Aldrich (Steinheim, Germany). Acetonitrile (ACN) of HPLC gradient-grade was purchased from Merck (Darmstadt, Germany). Water for chromatography was purified (18 MΩ cm^{-1} quality) by a Milli-Q system (Millipore, Bedford, MA, USA).

2.2. Chromatographic separation

Chromatographic analyses were performed on a DX-600 ion chromatograph (Dionex, Sunnyvale, CA, USA) equipped with a GS50 gradient pump, an MSQ single quadrupole mass spectrometer, and an AS50 autosampler. A 250 mm × 4 mm i.d. IonPac ICE-AS1 (Dionex) microbore column (7.5 μm cross-linked poly(styrene–divinylbenzene) functionalised with sulphonate functional groups) was used with acetonitrile–formic acid eluents. All measurements were made at 30 °C and all samples were filtered through 0.45 μm filters. Dionex Chromeleon 6.40 chromatography software controlled data collection and the operation of all components in the system.

2.3. MS Detection

MS detection was carried out by a single stage quadrupole detector (Thermo Finnigan MSQ, Dionex). The MS was operated in the positive electrospray ionization (ESI+) mode at 3.0 kV. Probe temperature was set at 350 °C, cone voltage was 50 V.

The protonated molecular ion $[M + H]^+$ of acrylamide has been detected at a mass-to-charge ratio (*m*/*z*) 72 and dwell time 1 s. Fragment ion at *m*/*z* 55 has been used for peak identity confirmation.

2.4. Experimental design approach and statistical analysis

Response surface methodology (RSM) was used to study the simultaneous effects of three experimental factors (two eluent components and flow rate) on the chromatographic performance of acrylamide.

A central composite rotatable design (CCD) was chosen to describe the relationship between the analytical conditions (independent variables), the retention factor *k* and the acrylamide response (response variables). The aim of this work was to identify the analytical conditions suitable for both maximizing the acrylamide response and minimizing the retention time. Five levels of each factor were chosen according to CCD, as shown in the Table 1, to describe second-order effects (curvature). The CCD is composed of a full factorial 2^k design to which is added star and center points according to central composite rotatable design. The total number of experiments with *k* factors is: $N = 2^k + 2k + c$. In Fig. 1, the first term is related to full factorial design, the second to the star points and the third to the center points. From the repetition of the center point, the experimental variance at the center of the domain can be estimated. For a three factors central composite rotatable

Table 1
Factors and levels tested (coded values in parentheses) for the central composite rotatable design

Factors	Lowest (−1.68)	Low-level (−1)	Neutral (0)	High-level (+1)	Highest-level (+1.68)
Acetonitrile (%)	10	18	30	42	50
Formic acid (mM)	1	2	5	8	10
Flow rate (μl min^{-1})	100	130	180	220	250

design, 23 experiments are necessary. The order of the experiments was completely random.

Experimental design and statistical analysis were performed using Statgraphics Plus 5.1 for Windows (Manugistics, Rockville, MD).

3. Results and discussion

Previous works [9,10] suggested that the use of an ion exclusion column instead of a classical reversed-phase column can have the advantage to separate acrylamide by potential interferences by exploiting the multiple retention mechanism of the stationary phase. Furthermore, the high-capacity characteristic allows large-volume injection to overcome sensitivity limitations. A microbore version of this column, which is more prone to coupling with electrospray ionization system, has been used to gain further sensitivity.

Optimization of the chromatographic conditions was carried out by a CCD (see Section 2) with the aim to investigate the effect of the eluent composition and the flow rate on the retention factor k and the peak height of acrylamide. The responses were fitted by a multiple regression equation, including second-order and interaction terms. For each experimental factor, the regression variance was partitioned into linear (A, B, C), quadratic (A^2, B^2, C^2) and cross-product components (AB, AC, BC) to determine the suitability of the second-order polynomial function and the relative significance of each components, as shown in Table 2. The significance of the equation parameters was assessed by F-test. Table 2 shows coefficients of the regression model for k and the peak height. Fig. 2 shows the main effects of the factors on the chromatographic responses. Three-dimensional response surfaces show the effect of two independent variables on a given response, at a constant value of the third independent variable that, in the case of Fig. 3, was set at 175 μl min^{-1}.

Statistical data analysis evidence the significant effect ($P < 0.05$) of acetonitrile concentration on acrylamide retention factor k and height both linear and second-degree terms: increasing the acetonitrile level, the retention factor decreases while peak height rises to the maximum in the region reported in Table 3. Significant interaction effects between the experimental factors were not observed. The results of the experimental design approach showed that the prevailing retention mechanism of acrylamide is the partition on lipophilic polystyrene polymer, and the concentration of formic acid has a minor influence on the acrylamide retention. Because flow rate barely affected both

Table 2
Regression coefficient and analysis of variance of the regression model for retention factor (k) and peak height

Term	Retention factor k		Height	
	Coefficient	P	Coefficient	P
Intercept parameter (b_0)	3.8034	0.01	7.908	0.01
A (ACN)	−0.0540	0.01	0.180	0.01
B (HCOOH)	−0.0195	0.33	−0.186	0.39
C (flow rate)	0.0049	0.81	−0.009	0.21
A^2	0.0003	0.29	−0.002	0.01
B^2	−0.0059	0.52	0.017	0.10
C^2	−0.00002	0.86	0.00002	0.64
AB	0.0010	0.27	−0.003	0.37
AC	0.000019	0.67	−0.00008	0.67
BC	0.000185	0.39	0.0007	0.37
Regression model	$R^2 = 0.828$		$R^2 = 0.800$	

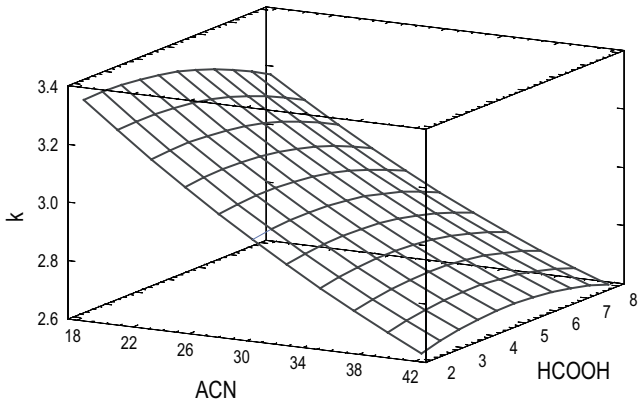

Fig. 3. Estimated response surfaces showing the influence of formic acid and acetonitrile concentration on the retention factor (k) at a flow rate of 175 μl min^{-1}.

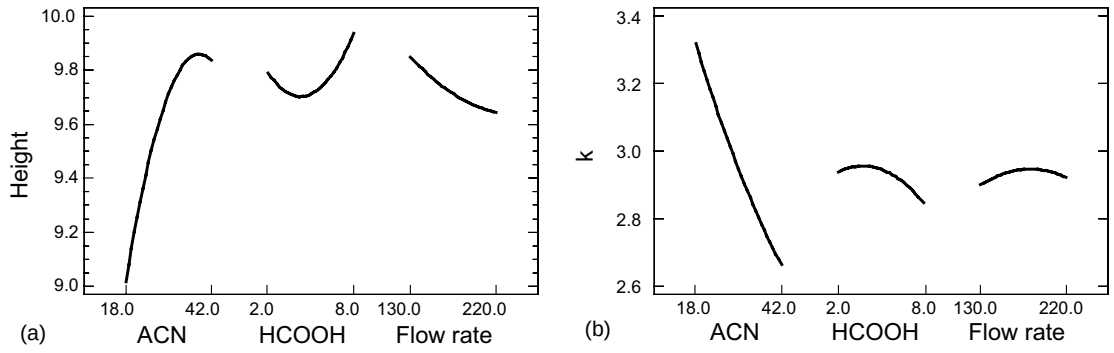

Fig. 2. Main effects plots for (a) peak height and (b) retention factor k.

Table 3
Optimized factors for acrylamide determination ($P < 0.05$)

Factor	Minimize k	Maximize h
Acetonitrile (%)	47	43
Formic acid (mM)	10	1
Flow rate ($\mu l\,min^{-1}$)	99	100

k and height, a higher flow rate was chosen ($180\,\mu l\,min^{-1}$) as a compromise between column backpressure limit and a reasonable total analysis run time. On the basis of these considerations, the eluent composition used in the following validation experiments was 40% acetonitrile and 3 mM formic acid operating at $180\,\mu l\,min^{-1}$.

Mass spectrometric conditions were optimized in a previous work [10]. Mass spectrum of acrylamide in total ion current mode is shown in Fig. 4. For the quantitative measurements, we operated in the selected-ion monitoring (SIM) mode with an exact mass of m/z 72, a span of m/z 0.2, and a dwell time of 1 s. Increasing dwell time led to a loss in linearity without a significant increase in sensitivity. Fragment ion at 55 m/z was also acquired for peak identity confirmation. A side effect of the acrylamide determination is the contamination of the source. In fact, we found carbon residues inside the inlet cone that had to be cleaned after some analytical sessions.

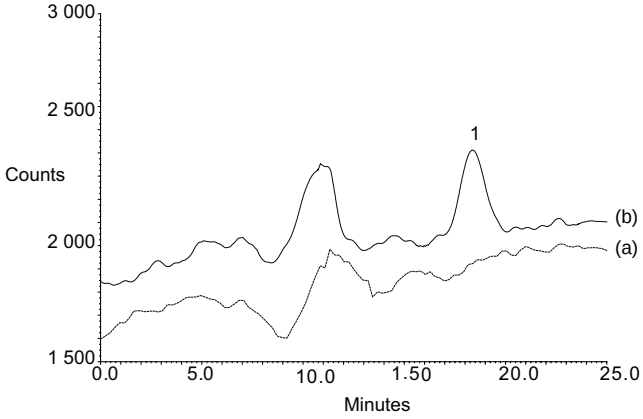

Fig. 5. (a) Tap water sample and (b) the same sample spiked with $0.5\,\mu g\,l^{-1}$ acrylamide (1). Chromatographic conditions: column: IonPac ICE-AS1 250 mm × 4 mm, 7.5 μm; eluent: 3.0 mM formic acid in CAN–water (40:60, v/v); flow rate: $0.18\,ml\,min^{-1}$; injection volume: 500 μl; MS detection: ESI+ 3.0 kV; cone: 50 V; probe temperature: 350 °C; SIM: 72 m/z; dwell time: 1 s.

We added acrylamide to drinking water from different sources and never detected interferences with MS detection in the total ion current (TIC) mode. Furthermore, the use of the SIM detection mode assures better sensitivity and selectivity to this analysis (Fig. 5).

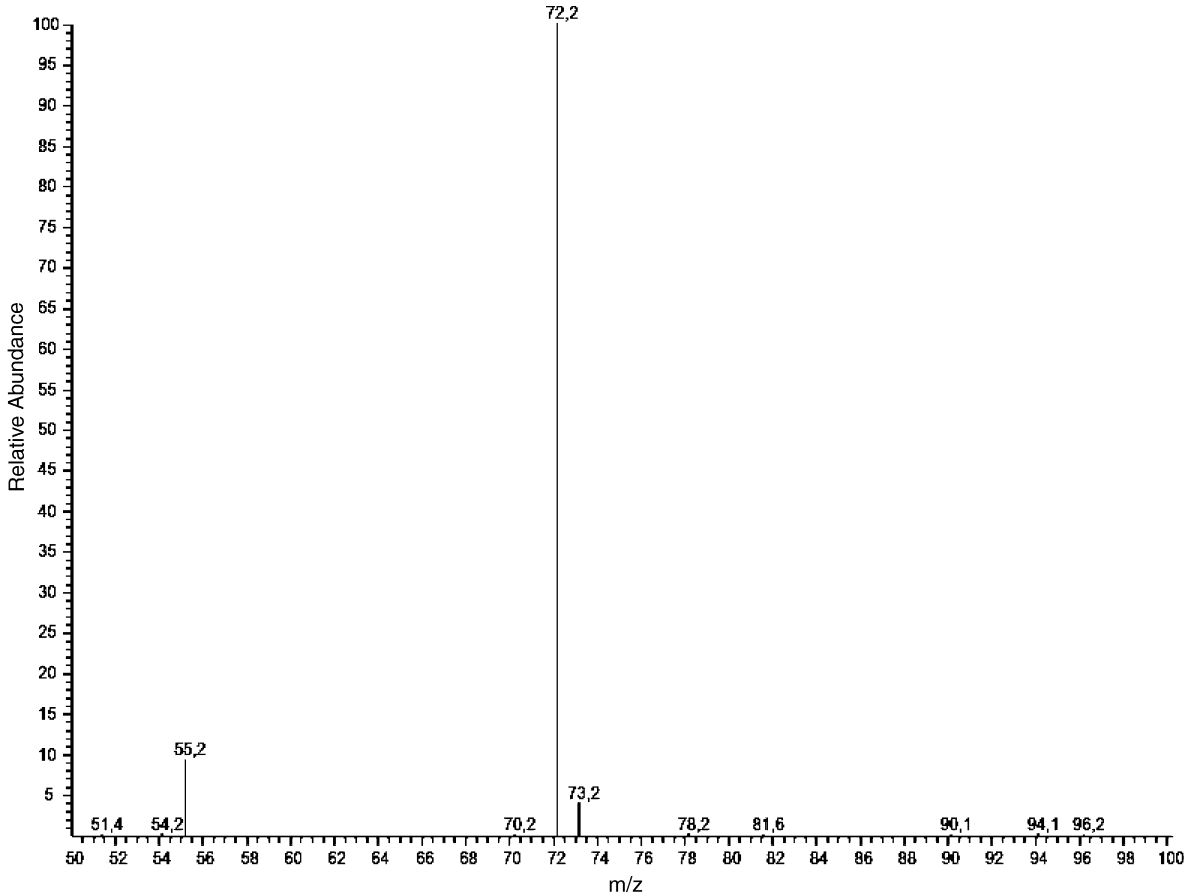

Fig. 4. MS Spectra of acrylamide. MS detection: ESI+ 3.0 kV; cone: 50 V; probe temperature: 350 °C; scan: 1 s.

To achieve sub-ppb limits of detection, as required by regulations, we explored the feasibility of using large-volume injection up to 1 ml. We verified that at injection volumes larger than 500 μl, the decrease in the efficiency largely overcomes the increase in response due to the increase of injected amount.

The preliminary validation procedure has been carried out using 500 μl injection loop and the optimized chromatographic conditions. The acrylamide calibration curve was linear up to only 5 ppb. A five-point calibration curve was created between 0.5 and 5 ppb with a very significant correlation coefficient ($R^2 > 0.9999$).

Recovery and repeatability were estimated at two levels (0.5 and 1 $\mu g\,l^{-1}$) by consecutive 10-fold injections of the samples. Recoveries were 95.5 and 97% and R.S.D.s were 16 and 12%, respectively for 0.5 and 1 ppb spiked solutions. The LOD was estimated as three-fold the standard deviation of the sample at the lowest concentration (0.5 $\mu g\,l^{-1}$) [11] giving a LOD of 0.20 $\mu g\,l^{-1}$.

4. Conclusion

Direct injection of large-volume sample in an ion-exclusion chromatography–MS system allows the determination of sub-ppb levels of acrylamide in drinking water without any sample pretreatment. Acceptable repeatability (16%) has been obtained at 0.5 $\mu g\,l^{-1}$, but the estimated detection limit is not yet sufficient for compliance to the strict regulations of the EU Directive on drinking water. Nevertheless, method sensitivity is sufficient to verify the compliance with WHO guideline and EPA requirements. A possibility to further lower the absolute sensitivity of the method could be the use of tandem MS detection, a more expensive system, as demonstrated by a recent paper on food analysis [12].

Whereas the EU Directive limit could not be reached by direct injection and simple MS detection, our method proved to be affordable and straightforward for rapid screening of sub-ppb concentration of acrylamide in drinking water for the protection of the consumer health, according to WHO guideline.

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