Tandem Extraction/Liquid Chromatography-Mass Spectrometry Protocol for the Analysis of Acrylamide and Surfactant-Related Compounds in Complex Matrix Environmental Water Samples

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Overview

Purpose

To develop a liquid chromatography-mass spectrometry (LC-MS)-based strategy for the detection and quantitation of acrylamide and surfactant-related compounds.

Methods

A combination of solid-phase extraction (SPE) and LC-MS.

Results

Tandem SPE protocol allowed for the analysis of surfactant-related compounds and acrylamide while conserving sample volume.

Introduction

Acrylamide and surfactant-related compounds, including ethoxylated alcohols, ethoxylated alkylphenols, and alkylphenols, are emerging contaminants with many different routes into the environment.

Acrylamide, a probable carcinogen [1], is used industrially as a coagulant aid, a grouting agent, and a friction reducer. Additionally, it is formed during the heating of starch-rich foods. Acrylamide is a small, highly water-soluble polar compound (Fig. 1) that is not retained by traditional SPE media. The U.S. EPA has established a maximum contaminant level goal (MCLG) of 0 mg/L for acrylamide in drinking water and regulates acrylamide using a treatment technique requirement in lieu of an MCL because of the absence of a standardized analytical method for its measurement in water [2].

Ethoxylated alcohols and ethoxylated alkylphenols are used ubiquitously as surfactants in industrial and household products. The use of ethoxylated alcohols and ethoxylated alkylphenols as surfactants raises the possibility of toxicity to aquatic life through their degradation byproducts, including nonylphenol, an endocrine disruptor (Fig. 2) [3].

Currently, appropriate standard methods in complex matrices are not established for these classes of compounds. For example, EPA Method 8316 for the determination of acrylamide in water is based on reversed-phase HPLC with UV detection, and the limit of detection (10 µg/L) of this method is insufficient for trace-level acrylamide determinations. Described here is the application of a tandem solid-phase extraction (SPE) protocol combined with liquid chromatography-mass spectrometry (LC-MS) to analyze environmental samples for multiple classes of compounds, including ethoxylated compounds, alkylphenols, and acrylamide.

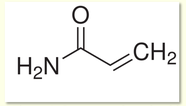

Figure 1. Structure of acrylamide.

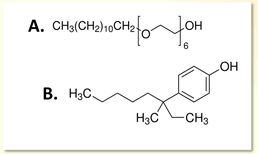

Figure 2. Examples of A) linear ethoxylated alcohol ($C_{12}EO_6$) and B) alkylphenol (nonylphenol isomer).

Methods

Tandem SPE

All samples were spiked with acrylamide-*d3*, nonylphenol-*d4*, octylphenol-*d2*, and 7 linear alcohol ethoxylate standards whose alkyl carbon chain lengths varied from C_6-C_{12} prior to extraction. A schematic representation of the tandem SPE protocol is shown in Fig. 3. Analytes of interest were extracted from water samples using an Autotrace SPE Workstation (Fig 4). The ethoxylated alcohols, ethoxylated alkylphenols, and alkylphenols were extracted using Oasis HLB 6 cc SPE cartridges (Waters). A total of 500 mL of water sample was loaded onto the cartridges. Because the highly polar acrylamide is not retained at all by the HLB cartridges, the flowthrough solution from the HLB cartridge sample loading was collected for the extraction of acrylamide (red arrow in Fig. 4), allowing the HLB cartridges to serve as a chemical filter for acrylamide [4]. The collected flowthrough was then extracted for acrylamide with activated carbon cartridges (Biotage) [5]. The SPE eluates were then concentrated to 0.5 mL under a stream of nitrogen.

LC-MS

A Dionex IonPac ICE-AS1 ion exclusion column (4 x 250 mm, 7.5 µm particle size) was used for the LC separation of acrylamide on a Dionex UltiMate 3000 HPLC system, which was coupled to a Thermo Finnigan TSQ Quantum Ultra for MS analysis. An isocratic gradient at 50/50 water/acetonitrile with 0.1% formic acid (0.16 mL/min) was used to elute the acrylamide. The 72 > 55 transition was used for quantitation.

Ethoxylated alcohol, ethoxylated alkylphenol, and alkylphenol samples were analyzed on a Waters LCT Premier TOF. Full scan mass spectra were collected for quantitation using QuanLynx, and additional MS/MS confirmation of specific ions was performed on a Varian 500-MS ion trap. An ACQUITY UPLC BEH Shield RP 18 (1.7 µm, 2.1 x 50 mm) column was used to separate the compounds. Negative-mode MS was used for the alkylphenols, and positive-mode was used for the ethoxylated alcohols and ethoxylated alkylphenols.

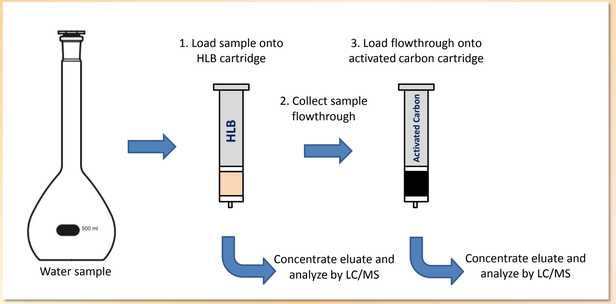

Figure 3. Schematic representation of tandem SPE protocol for analysis of surfactant-related compounds followed by acrylamide. Because the acrylamide is not retained by traditional SPE media, the first extraction acts as a chemical filter and "cleans" the sample of many of the compounds that could potentially interfere with acrylamide analysis.

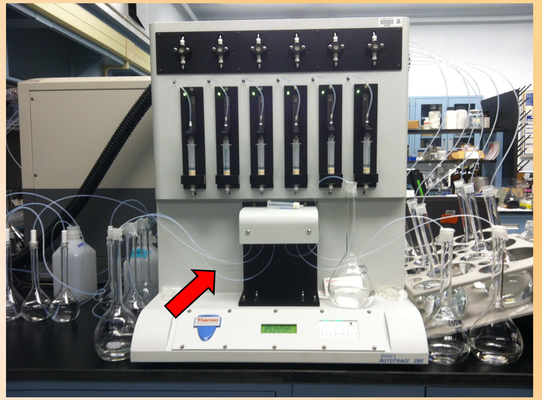

Figure 4. Autotrace SPE Workstation that was used for SPE extractions. Red arrow denotes teflon tubing that was attached to nozzles to collect flowthrough.

Results

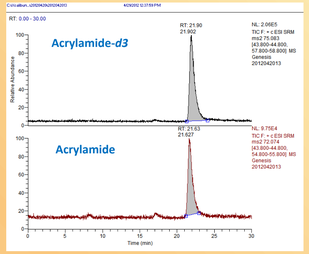

Figure 5. Example chromatogram of acrylamide and acrylamide-d3 (IS) using Dionex IonPac ICE-AS1 ion exclusion column, the only HPLC column among those tested that effectively retained acrylamide.

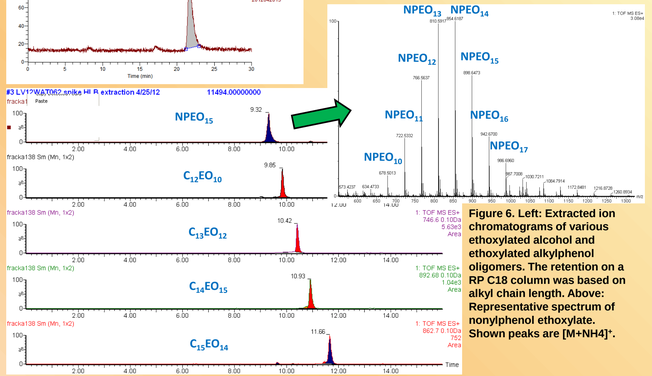

Figure 6. Left: Extracted ion chromatograms of various ethoxylated alcohol and ethoxylated alkylphenol oligomers. The retention on a RP C18 column was based on alkyl chain length. Above: Representative spectrum of nonylphenol ethoxylate. Shown peaks are $[M+NH4]^+$.

Various SPE cartridges were analyzed for their extraction efficiencies for the various compounds, including polystyrene-divinylbenzene (Waters Oasis HLB), RP C18, Isolute ENV+, graphitized carbon, and activated carbon. Acceptable recoveries of ethoxylated compounds and alkylphenols were obtained using the polystyrene-divinylbenzene SPE cartridges. Acrylamide is a highly polar compound that remains in the aqueous phase and so was not retained on any SPE cartridge except for the activated carbon cartridges. As a result of this finding, and to minimize the sample volume that was necessary to analyze both the surfactant-based compounds following polystyrene-divinylbenzene extraction and the acrylamide following activated carbon extraction, the tandem SPE protocol was performed.

Different HPLC columns were investigated for use with acrylamide, including reversed-phase C18, ion exclusion, and porous graphitic carbon. The only column that retained the acrylamide was the ion exclusion chromatography column using an isocratic elution profile. In all other columns tested, acrylamide eluted with the dead volume and was not retained.

Table 1. Recoveries of ethoxylated alcohol standards in various groundwater samples

	C_6E_5	C_8E_4	C_8E_5	$C_{10}E_4$	$C_{10}E_6$	$C_{12}E_3$	$C_{12}E_4$
Groundwater LV12WAT060	65%	68%	61%	62%	53%	33%	32%
Groundwater LV12WAT061	61%	58%	60%	60%	56%	60%	53%
Groundwater LV12WAT062	58%	61%	59%	58%	56%	48%	44%
Groundwater LV12WAT069	55%	52%	52%	48%	48%	46%	44%
Groundwater LV12WAT071	54%	53%	52%	50%	46%	45%	43%

Recoveries of acrylamide spiked into water ranged from 19-42%. Accuracy of acrylamide spike quantitation ranged from 100-108% (measured using deuterated IS).

Conclusions

- The tandem SPE protocol, involving two SPE phases, polystyrene-divinylbenzene (HLB) and activated carbon, enabled the determination of various classes of compounds, including surfactant-related compounds and acrylamide.
- The HLB cartridges first extracted out surfactant-related compounds and served as a chemical filter to clean up the samples prior to the acrylamide extraction with activated carbon.
- Longer alkyl chain ethoxylated alcohols were not recovered as well as shorter alkyl chains (Table 1), presumably due to their increased hydrophobicity [6]. More nonpolar SPE eluent is needed for better recoveries.
- Future work will aim to improve recoveries of surfactant-related compounds and acrylamide.

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References

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4. Rosen et al. *J. Chrom. A*, **2007**, *1172*, 19-24.
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