ORIGINAL PAPER

Environmental analysis of alcohol ethoxylates and nonylphenol ethoxylate metabolites by ultra-performance liquid chromatography–tandem mass spectrometry

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Abstract Surfactants and their metabolites can be found in aquatic environments at relatively high concentrations compared with other micropollutants due in part to the exceptionally large volumes produced every year. We have focused our attention here on the most widely used nonionic surfactants, alcohol ethoxylates (AEOs), and on nonylphenol ethoxylate (NPEO) degradation products (short-chain nonylphenol ethoxylates, NP1-3EO, nonylphenol, NP, and nonylphenol ethoxycarboxylates, NP1-2EC), which are endocrine-disrupting compounds. Our main objective in this work was to develop a methodology aimed at the extraction, isolation, and improved analysis of these analytes in environmental samples at trace levels. Extraction recoveries of target compounds were determined for sediment samples after ultrasonic extraction and purification using HLB or C18 solid-phase extraction minicolumns. Recovery percentages were usually between 61 and 102% but were lower for longer AEO ethoxymers. Identification and quantification of target compounds was carried out using a novel ultra-performance liquid chromatography coupled to tandem mass spectrometry (UPLC–MS-MS) approach, a combination that provides higher sensitivity and faster analysis than prior methods using conventional high-performance liquid chromatography–mass spectrometry. Limits of detection were usually below 0.5 ng/g, being higher for monoethoxylate species (>5 ng/g) because of poor ionization. The method was used for analyzing surface sediment samples collected at Jamaica Bay (NY) in 2008. The highest values (28,500 ng/g for NP, 4,200 ng/g for NP1-3EO, 22,400 ng/g for NP1-2EC, and 1,500 ng/g for AEOs) were found in a sampling station from a restricted water circulation area that is heavily impacted by wastewater discharges.

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Introduction

Endocrine disruptor compounds (EDCs) comprise a wide variety of organic compounds that can alter the normal functioning of the endocrine system in organisms. Among them, metabolites formed during the degradation of the surfactant nonylphenol ethoxylates (NPEOs) usually show the highest concentrations in aquatic environments, where they may persist for decades because of their relatively low biodegradability in sediments [1]. Nonylphenol (NP), a persistent degradation product, shows the highest estrogenicity of all metabolites, although other weakly estrogenic intermediates such as short-chain NPEOs (typically NP1-3EO) and nonylphenol mono- and diethoxycarboxylates (NP1-2EC) are frequently monitored in the environment [2]. Concentrations up to several milligrams per kilogram have been reported to occur in sewage-impacted sediments [3], those values being significantly lower for more the hydrophilic NP1-2EC [4]. The main sources associated with the

presence of these chemicals in aquatic systems are both treated and untreated wastewater discharges. Taking into account their potential adverse effects, NPEOs were recently banned in the European Union for household applications. Although their demand in the USA increased from 360 to 380 million pounds during the last 4 years, some major detergent suppliers to the consumer sector have agreed to not use NPEOs in their products, favoring more biodegradable alcohol ethoxylates (AEOs) instead [5]. AEOs are nonionic-type surfactants with the highest worldwide production (e.g., 300 ktons/year in Europe just for household cleaning products [6]). Concentrations of AEOs in sediments can be comparable to those found for NPEOs [7], and they are expected to rise as this surfactant replaces NPEOs in many household applications. Additionally, AEOs have proven to be toxic for certain aquatic species [8]. However, they do not act as EDCs, so they have received less attention than NPEOs and their metabolites from the scientific community. Their analysis is also more tedious as AEOs are commercially sold as complex mixtures of both different homologues that can be resolved by high-performance liquid chromatography (HPLC) and ethoxymers (e.g., C14EO6 is equivalent to the specific ethoxylated alcohol having a 14-carbon alkyl chain and 6 ethoxylate units).

Analysis of both classes of compounds, AEOs and NPEO metabolites, can be carried out using several techniques. The first quantitative methods were usually based on the use of HPLC coupled to UV fluorescence detectors [9, 10], and of gas chromatography coupled to mass spectrometry (GC–MS) [11, 12]. Determination of NPEO metabolites and AEOs was carried out by separate methods in most cases, and derivatization was used as some of the analytes do not absorb UV radiation or are nonvolatile [10, 11]. Considerable progress has occurred during the last decade because of advances in HPLC–MS. This combination has led to the development of methods that are superior in terms of detection limits, specificity, number of compounds that can be determined in the same run, and speed of analysis. Two early examples of application of HPLC–MS for determination of trace levels of NPEOs and some of their metabolites (NP and NP1-3EO) in marine sediments employed normal-phase and reversed-phase HPLC, respectively, coupled to single quadrupole mass spectrometers via electrospray interface (ESI) [1, 13]. Other authors [4, 14] have applied different methods using the same kind of detectors for monitoring the occurrence of these analytes and also NP1-2EC in sludge, wastewater, suspended particulate matter, and/or surface water. In contrast, the number of papers related to the determination of AEOs in environmental samples is significantly lower than those for NPEO analysis. Many of them focused on measuring AEO concentrations in surface water, sludge, and wastewater [12, 15], sometimes with simultaneous measurement of NPEOs [16–19] by

means of HPLC–single quadrupole/ion trap-MS. In recent years, the main tool for trace analysis of organic contaminants has been tandem mass spectrometry (MS-MS) because higher confidence and lower detection limits can be achieved compared with other MS analyzers. Hence, most recent papers describe the optimization and use of HPLC–MS-MS for the determination of NP, NPEOs, and NP1-2EC in surface water [20] and effluents from wastewater treatment plants (WWTPs) [21], as well as in sediments [22], showing detection limits usually lower than 0.01 μg/L and 10 ng/g, respectively. So far, AEOs have been analyzed in seawater by HPLC–MS-MS after solid-phase microextraction (SPME) by Droge and co-workers [23].

Today, mass spectrometry is often combined with ultra-performance liquid chromatography (UPLC), which uses sub-2-μm column particles that provide enhanced separation, faster analysis, and improved sensitivity over HPLC, boosting laboratory efficiency by saving time and decreasing solvent consumption. This combination was recently applied to the analysis of several classes of micropollutants such as pharmaceuticals [24]. In this work we go a step further than previously HPLC–MS methods by combining for the first time the speed of UPLC columns with the selectivity and sensitivity of MS-MS detectors to achieve faster and simultaneous analysis of NPEO metabolites and AEOs at trace levels in aquatic environments. Additionally, the new method developed here was applied to samples collected during a sampling campaign in a sewage-impacted bay (Jamaica Bay, NY), allowing for a direct comparison between the concentration levels of NPEO metabolites and AEOs in recent sediments (collected in 2008).

Material and methods

Chemicals and standards

All solvents and reagents were of chromatography quality, purchased from Merck. The individual AEO ethoxymers (C12, C14, C16, and C18 homologues having 1, 2, 3, 6, and 8 EO units; >98% pure) were purchased from Sigma-Aldrich, whereas nonylphenol (NP), NPEO ethoxymers (1, 2, and 3 EO units), and nonylphenol mono- and diethoxycarboxylates (NP1EC and NP2EC) standards were purchased from Dr. Ehrenstorfer GmbH. Deuterated internal standards used in positive (NP2EO-D2) and negative (NP-D8 and NP1EC-D2) ionization modes were also purchased from Dr. Ehrenstorfer GmbH.

Extraction and isolation of target compounds

Freeze-dried sediments (0.5 g per sample) were extracted using ultrasonic irradiation. Extraction conditions were 50 °C,

3 cycles (30 min each), and methanol as solvent (30 mL). Blank sediment extractions, consisting of nonpolluted muddy sediments, were performed alongside actual samples. After extraction, solvent was separated from sediment samples by centrifugation and evaporated to 2 mL under a nitrogen stream. Later, target compounds were isolated from these extracts by solid-phase extraction (SPE) using two types of cartridges: Oasis HLB 6 mL 500 mg (Waters) and Bond Elut C18 6 mL 500 mg (Varian). SPE cartridges were conditioned with 8 mL of methanol and 5 mL of HPLC water. Extracts were reconstituted in 100 mL of HPLC water before being passed through the SPE cartridges. Sample vessels were rinsed twice with HPLC-grade water and once with methanol (5% of total volume), and the rinses were then passed through the SPE. Cartridges were rinsed with 5 mL of HPLC water before being air-dried under vacuum. Elution was performed with 8 mL of methanol and 4 mL of dichloromethane. Extracts were then evaporated to dryness under a gentle nitrogen stream and reconstituted in 1 mL of methanol. Further dilutions were made in methanol/water (50:50) and spiked with internal standards (NP-D8, NP1EC-D2, and NP2EO-D2) prior to UPLC–MS-MS analysis.

Identification and quantification of target compounds

Target compounds were determined by UPLC–MS-MS using an Acquity UPLC chromatograph (Waters). Sample injection volume was 10 µL, and analytes were separated with a Purospher STAR RP-18 (50×2 mm, 1.8 µm particle size) UHPLC column (Merck). LC conditions for NP and NP1-2EC were as follows: mobile phase A was acetonitrile and mobile phase B was 10 mM formic acid/10 mM ammonium formate buffer in HPLC water. Flow rate was constant (0.4 mL/min) and initial solvent composition was 20% A. A linear gradient was employed with a final solvent composition of 100% A in 10 min, which was held for 1 min. The initial solvent conditions were then restored and the column was allowed to re-equilibrate for 4 min. LC conditions for NPEO and AEO compounds were similar, but initial solvent composition was 50% A.

A TQD mass spectrometer (Waters) was operated in multiple reaction monitoring (MRM) mode. Negative electrospray ionization (ESI−) was used for the determination of NP and NP1-2EC, whereas ESI+was selected for NPEOs and AEOs. Dwell time, cone gas flow, cone voltage, and collision energy were optimized to obtain the maximum signal for every transition. Other MS parameters were capillary voltage set to 3,000 V (ESI−) or 3,500 V (ESI+), desolvation and source temperature set to 350 and 150 °C, respectively, and 600 L/h and 0.19 mL/min as desolvation and collision gas flow rates, respectively. Identification of target compounds was based on monitoring transitions from their quasimolecular ions $[M−H]^-$ (ESI−) or $[M+NH_4]^+$ adducts (ESI+), and confirmed by comparison of the retention time of the compounds with that of a standard. Quantification was carried out using a six-point calibration curve (from 1 to 500 µg/L) that was constructed for each analyte. The analyte response was normalized to that of the internal standards.

Sampling and validation of the analytical procedure

The sampling area (Fig. 1), Jamaica Bay, located on the southwestern shore of Long Island, NY (USA), has been the subject of a number of studies investigating wastewater-derived contaminants [3, 25, 26]. Six WWTPs discharge roughly 1.1×10^9 L/day of biologically treated sewage, constituting by far the largest input of freshwater to Jamaica Bay. Tidal exchange of water in this estuarine embayment is restricted by the single opening to the greater New York Bight. Surface sediment (0–2 cm) samples were collected from station JB03 to station JB23 by means of Van Veen grabs during the summer of 2008. The sediments were freeze-dried prior to analysis.

Extraction recoveries of target compounds were determined for triplicate nonpolluted sediment samples spiked at 500 ng/g. Recoveries were determined by comparing the concentrations obtained with the initial spiking levels. Two blanks were also performed within every batch of samples. The precision of the method was expressed as the standard deviation (SD) of replicate measurement among values obtained from the triplicate spiking experiment. Sediment samples collected from Jamaica Bay were extracted and analyzed in duplicate. Limits of detection were determined from spiked sediment samples as the minimum detectable amount of analyte with a signal-to-noise ratio of 3, and taking into account the amount of sample extracted and extraction recovery percentages. Comparison of the signal intensity of spiked internal standards in methanol/water (50:50) and in sediment sample extracts was carried out to evaluate ionization suppression due to matrix effects.

Results and discussion

Method performance

Extraction recoveries of target compounds were determined from spiked sediments. Results are shown in Table 1. Three different experiments were carried out to evaluate methods for extraction and sample purification by SPE. First, samples were sonicated using methanol as solvent during 3 cycles (30 min per cycle). Extracts were evaporated, diluted with HPLC water by a factor of 2, and analyzed by UPLC–MS-MS with no further cleanup. Recoveries were satisfactory for most compounds, ranging between 61 and
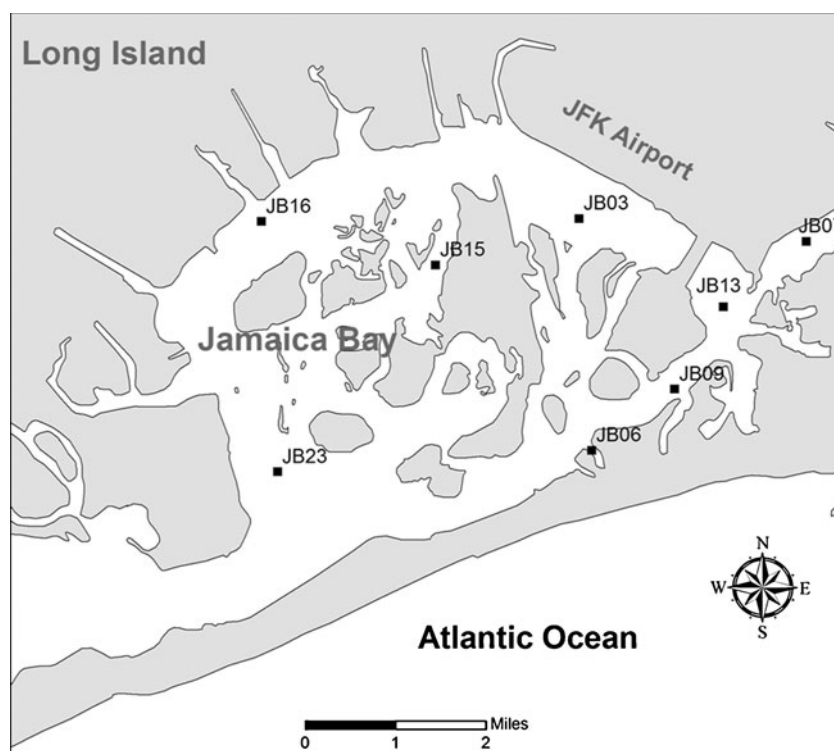
102% for NPEO metabolites and between 69 and 85% for AEO ethoxymers independent of alkyl chain length (see Table 1, first column). Significantly lower values (from 45 to 53%) were observed for those AEO components having 8 EO units. Previous works have reported the same trend during the extraction of long-chain NPEOs from solid matrixes [22]. This may be explained by enhanced hydrophilic interactions between the ethoxylated chain and mineral surfaces, which may increase the sorption of these compounds onto sediment as observed in laboratory experiments [27]. Use of more nonpolar chlorinated solvents or hexane during the last extraction cycle could increase these recovery percentages [22], which unfortunately leads to greater co-extraction of more nontarget compounds and, therefore, enhancement of matrix-induced suppression of ionization in ESI. In any case, SD is low enough (typically below 10%) to ensure reproducibility during the analysis of environmental samples. This was further confirmed by duplicate extraction and analysis of eight different sediment samples collected from Jamaica Bay (more information on this is presented in the next section). The extraction efficiency was also comparable to that of other methods based on this [14] and other extraction techniques for the same target compounds, such as NPEO metabolites [22] and AEOs [19] extracted by pressurized liquid extraction (PLE), or Soxhlet extraction of NP and other EDC [12]. Compared with other extraction techniques such as PLE or supercritical fluid extraction (SFE), ultrasonic extraction allows simultaneous processing

of a greater number of samples within the same batch in a shorter time (within less than 3 h). One drawback is that extraction is not automated as it requires centrifugation after each cycle. Solvent consumption may be also slightly higher (approximately 30 mL per sample), although it has been reduced compared with prior works that used between 60 and 150 mL of chlorinated solvents (dichloromethane) per sample [14, 17].

During the second and third experiments, sample extracts were purified by octadecylsilica (C18) or Oasis HLB SPE cartridges, two of the most commonly used sorbents for the isolation of micropollutants such a wide variety of pharmaceuticals [25] and surfactants [18]. As can be observed in Table 1 (second and third columns), extraction efficiencies of target compounds follow the same trend described above for the first experiment, although recoveries are generally reduced between 10 and 20% for most analytes as a new step is added. The biggest drop can be observed for the relatively poorly soluble NP and AEO ethoxymers having only 1 EO unit, which may be attributed to the higher sorption of these analytes in the SPE sorbent compared with more polar compounds. This problem has been already discussed by Krogh and co-workers [15] while testing several types of SPE cartridges and elution solvents for the extraction of AEOs and alkylamine ethoxylates from aqueous samples. Nevertheless, results from recovery experiments (52–84%) are comparable to those previously reported by other authors using both types of sorbents for isolating NPEO metabolites from aqueous samples [21] and

Table 1 Recovery percentages± SD for target compounds

Compound	No SPE	C18	HLB
NP	92±9	65±3	64±4
NP1EC	83±10	72±3	70±5
NP2EC	102±15	88±8	84±7
NP1EO	75±11	64±13	62±13
NP2EO	70±6	56±4	58±6
NP3EO	61±5	49±4	52±4
C12AEO1	88±14	34±7	32±8
C12AEO2	72±6	52±3	56±5
C12AEO3	76±7	55±4	58±5
C12AEO6	69±9	51±5	50±4
C12AEO8	47±10	33±5	34±4
C14AEO1	69±19	34±8	43±7
C14AEO2	75±13	36±0	51±5
C14AEO3	72±9	40±1	49±4
C14AEO6	70±8	49±4	48±4
C14AEO8	53±12	38±5	40±5
C16AEO1	85±20	44±12	65±8
C16AEO2	73±11	43±6	65±8
C16AEO3	66±9	40±3	54±7
C16AEO6	69±12	51±7	56±7
C16AEO8	45±11	34±6	40±6
C18AEO1	–	–	–
C18AEO2	70±9	45±11	76±15
C18AEO3	69±11	48±10	74±10
C18AEO6	46±7	36±6	46±6
C18AEO8	45±11	34±7	44±7

extracts from sludges [12]. In our case, we have also expanded the range of AEOs up to C18 homologues compared with some prior work that mostly focused on AEOs with short alkyl chains, typically C12 to C14 or C16 [12, 19]. Finally, Oasis HLB SPE cartridges were selected over those of C18 type as slightly better recoveries were obtained in most cases. In addition, nontargeted compounds such as pharmaceuticals and other EDCs, which were also measured successfully on the samples analyzed here under different LC–MS conditions (data not shown), can be also co-extracted using HLB cartridges as they have been proven to be better suited than C18 for the isolation of polar compounds [25].

Separation, identification, and quantification of target compounds in sediment extracts were achieved by UPLC–MS-MS. ESI was used in both modes (positive mode for NP1-3EO and AEOs, negative mode for NP and NP1-2EC) and run time was 11 min per sample for each UPLC method performed. This represents a significant advance over an average time of 30–50 min required to achieve necessary resolution of analytes for most HPLC methods [12, 14, 18, 21]. Using a C18 reversed-phase UPLC column allows complete separation of AEO homologues (Fig. 2) as retention is based on the interaction between the hydrocarbon chain and the stationary phase. On the other hand, AEO and NPEO ethoxymers are only partially separated (Figs. 2 and 3). Chromatographic separation of homologues is recommended as it prevents mass overlap of several species having the same nominal masses. This is a well-known issue when working with polyethoxylated compounds as they have high affinity for alkali metal ions with which they form several types of adducts. Even in the absence of added electrolyte to the mobile phase, AEO and NPEO sodium adducts are detected as a result of the ubiquity of this metal in solvents and samples. Many
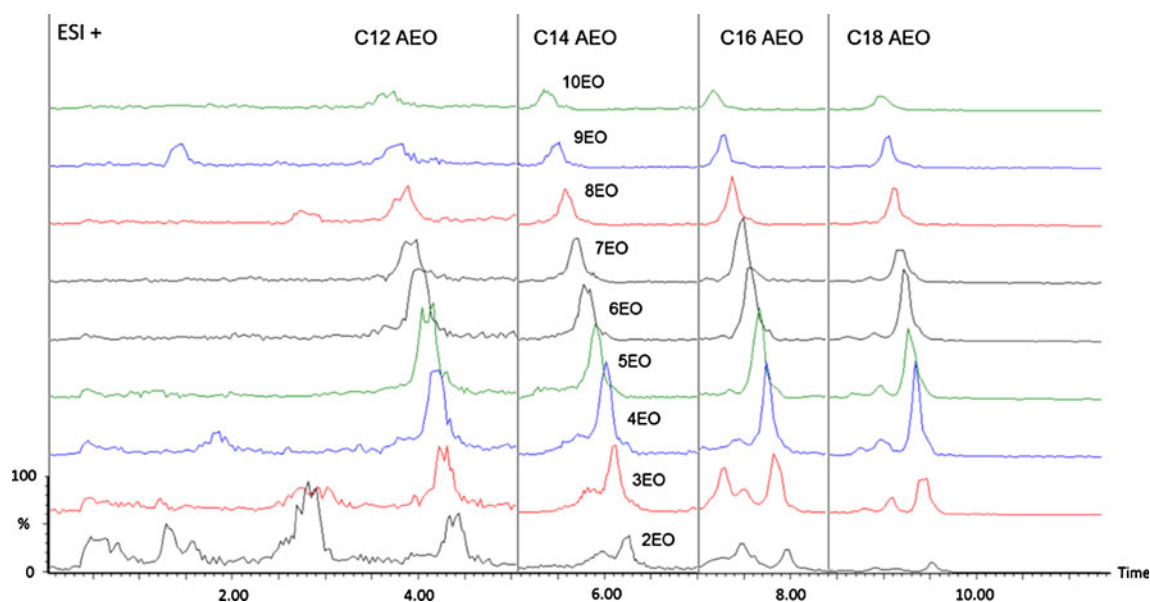

Fig. 2 UPLC–ESI-MS-MS extracted ion chromatograms showing the occurrence of AEOs in a sediment sample
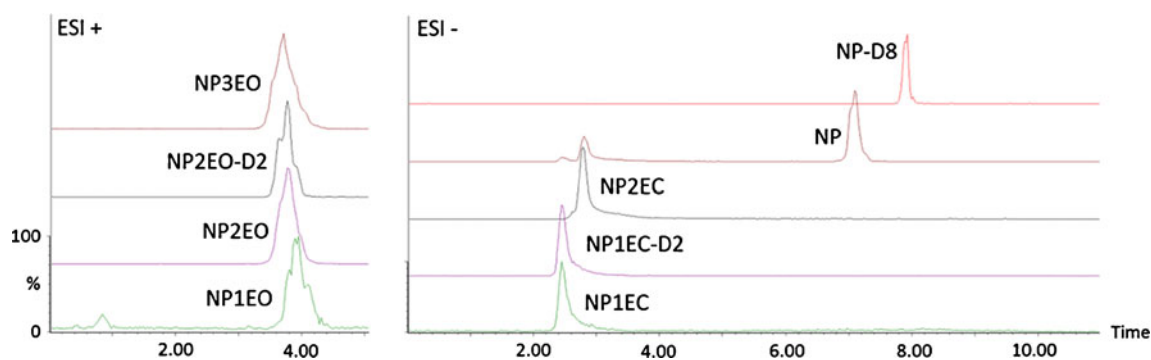
Fig. 3 UPLC–ESI-MS-MS extracted ion chromatograms showing the occurrence of NPEO metabolites in a sediment sample

authors exploit this and fortify sample extracts or the mobile phase with sodium acetate [13, 14, 18] to provide sodium for target compounds so $[M+Na]^+$ adducts can be used for their quantification using single quadrupole or ion trap MS. As a drawback, isobaric interferences have been described to occur between selected singly charged lower molecular weight and doubly charged higher molecular weight NPEO sodium (and to a lesser extent ammonium) adducts of co-eluting ethoxymers [21]. Additionally, Evans and co-workers [28] have described interferences that may happen between proton adducts from ^{13}C isotopes and ammonium adducts when analyzing low molecular weight AEOs. MS-MS helps avoid isobaric interferences that may cause errors in identification and quantification. To this end, ammonium formate was used in the mobile phase to form $[M+NH_4]^+$ adducts for NPEO and AEO ethoxymers, which are more labile and, therefore, susceptible to fragmentation than the very stable $[M+Na]^+$ adducts [20, 22]. Precursor-to-product transitions are described in Table 2 for each analyte. A detailed study on the fragmentation of NPEO metabolites by tandem mass spectrometry in both ESI negative and positive modes was carried out by Loyo-Rosales and co-workers [29]. With respect to AEO components, it can be observed that product ions correspond to the loss of ammonium.

Cone voltages and collision energies were optimized for each analyte (Table 2). Ionization conditions were set for other analytes when standards were not commercially available (e.g., C12 to C18AEO with 4 and 5 EO units). It can be observed that cone voltages and collision energies increase for longer AEO and NPEO ethoxymers, whereas the effects of changing these parameters were much less important if we only consider the length of the alkyl chain (Table 2). This is due to the stability of the ammonium adducts, which is higher as the number of EO units increases so the ethoxylated chain can accommodate the ammonium or any alkali metal better [17]. On the other hand, the lack of stability of short ethoxylated chain AEO and NPEO adducts results in reduced ionization of these ethoxymers, as has been previously reported. This decrease

appears to mostly affect those ethoxymers having 4–5 or less EO units in the case of AEOs [14, 16] and NPEOs [13], whereas longer ethoxymers show similar responses in the MS detector [17]. The same trend was observed in our case: calibration curve slopes changed for each AEO component, being significantly much higher for C12-18AEO1 (0.15–0.57) than for the rest of ethoxymers (<0.004), which results in higher detection limits for monoethoxylated species (Table 2). Ionization has been noted to be particularly poor for ethoxymers containing 1 and 2 EO units in prior works [14]. As a result, compounds having only 1 or 2 EO units can be difficult to determine by many HPLC–MS methods, especially so for AEOs [14, 17, 28]. Using MS-MS partly solves this issue, although the quantification of NP1EO and C12-18AEO1 in sediment samples is still challenging because of their low responses. Ion signal intensities for these compounds were maximized by using higher dwell times (250 ms, compared to 100 ms used for the rest of analytes in ESI+) and optimizing cone gas flow. We found that 30 mL/min was optimal for most target compounds in both positive and negative ionization modes, but ion signal intensity for NP1EO and C12-18AEO1 was very low under these conditions. By increasing this flow to 90 mL/min the signal increased by a factor of 10 for these compounds and remained very similar for more ethoxylated analytes. It also became evident that ionization efficiency improved as the aqueous content of the mobile phase decreases, so longer AEO homologues such as C16 and C18 show appreciably better detection limits than C12 and C14 (Table 2), which elute earlier in the chromatogram (Fig. 2). More information on how the MS response factor of different AEO homologues and ethoxymers can vary depending on the composition of the mobile phase and the lengths of the alkyl and ethoxylated chains can be found in a relatively recent work by Bernabé-Zafón and co-workers [30]. In any case, even if detection limits are significantly higher for monoethoxylated NPEO and AEO oligomers (8.4–27.3 ng/g) than those of the other target compounds (<0.5 ng/g), LOD values were still low enough to ensure quantification of these analytes when 0.5 g of sewage-

Table 2 Retention time (Rt), limits of detection (LOD), and MS parameters for target compounds

Compound	Rt (min)	Transitions	Cone voltage (V)	Collision energy (%)	LOD (ng/g)
NP	7.119	219.2→133.2	40	20	2.2
NP-D8	7.912	227.2→112.2	40	20	Internal std.
NP1EC	2.423	277.2→219.2	25	20	1.3
NP1EC-D2	2.423	279.2→219.2	25	20	Internal std.
NP2EC	2.745	321.2→219.2	25	20	1.1
NP1EO	3.891	282.4→127.2	10	9	27.3
NP2EO	3.742	326.3→183.2	15	11	2.6
NP2EO-D2	3.742	328.3→185.2	15	11	Internal std.
NP3EO	3.682	370.3→227.2	15	12	0.5
C12AEO1	4.55	248.2→231.2	9	6	9.5
C12AEO2	4.371	292.2→275.2	9	8	2.1
C12AEO3	4.281	336.3→319.3	10	10	0.3
C12AEO6	3.981	468.3→451.3	20	14	0.2
C12AEO8	3.772	556.4→539.4	25	16	0.2
C14AEO1	6.522	276.3→259.3	9	7	8.4
C14AEO2	6.279	320.3→303.3	10	8	1.7
C14AEO3	6.157	364.3→347.3	12	10	0.6
C14AEO6	5.852	496.4→479.4	20	14	0.5
C14AEO8	5.629	584.5→567.4	25	16	0.6
C16AEO1	8.318	304.3→287.3	10	7	11.8
C16AEO2	8.115	348.3→331.3	10	8	0.5
C16AEO3	7.993	392.4→375.3	12	10	0.1
C16AEO6	7.709	524.4→507.4	20	15	0.2
C16AEO8	7.506	612.5→595.5	25	18	0.2
C18AEO1	9.698	332.3→315.3	10	8	>50
C18AEO2	9.536	376.3→359.3	12	9	0.1
C18AEO3	9.414	420.4→403.4	15	10	0.0
C18AEO6	9.252	552.5→535.4	20	15	0.1
C18AEO8	9.13	640.5→623.5	25	18	0.0

impacted sediments were extracted in this work. The LOD values determined here are considerably lower than [1, 12, 14, 17], or at least comparable to [13, 19, 29], those determined in prior methods that do not rely on derivatization. The only exception found was C18AEO1, which could not be determined at environmentally relevant concentrations using this method because of a combination of extremely poor ionization and poor recovery related to its low solubility.

Case study

This section shows results from a survey in Jamaica Bay (NY) in order to demonstrate the applicability of the methodology proposed above. Concentrations of target compounds in surface sediments are shown in Fig. 4 for every sampling station (from JB03 to JB23), ranging between 250 and 28,500 ng/g for NP, 470 and 4,200 ng/g for NP1-3EO, 540 and 22,400 ng/g for NP1-2EC, and 60

and 1,500 ng/g for AEOs. These values are an average from two replicates per sample, with relative SD between 10 and 24%, depending on the analyte. Estimated concentrations were also corrected for recovery from spiked sediments. The effect of ion suppression was accounted for with deuterated internal standards in both ionization modes and turned out to be between 41 and 65% for NP, 7 and 31% for NP1-2EC (ESI−), and less than 5% for ethoxylated compounds (ESI+). Individual response curves were used to calculate concentration values for individual target compounds in these samples, but, since standards for specific AEO ethoxymers were not available (e.g., C12 to C18AEO having 4 and 5 EO units), calibration curves had to be interpolated in these cases.

The highest concentrations of all analytes were detected at station JB03, located within an area known as Grassy Bay (Fig. 1). This deep basin was formed in 1939 when a large area in the northeastern portion of the bay was
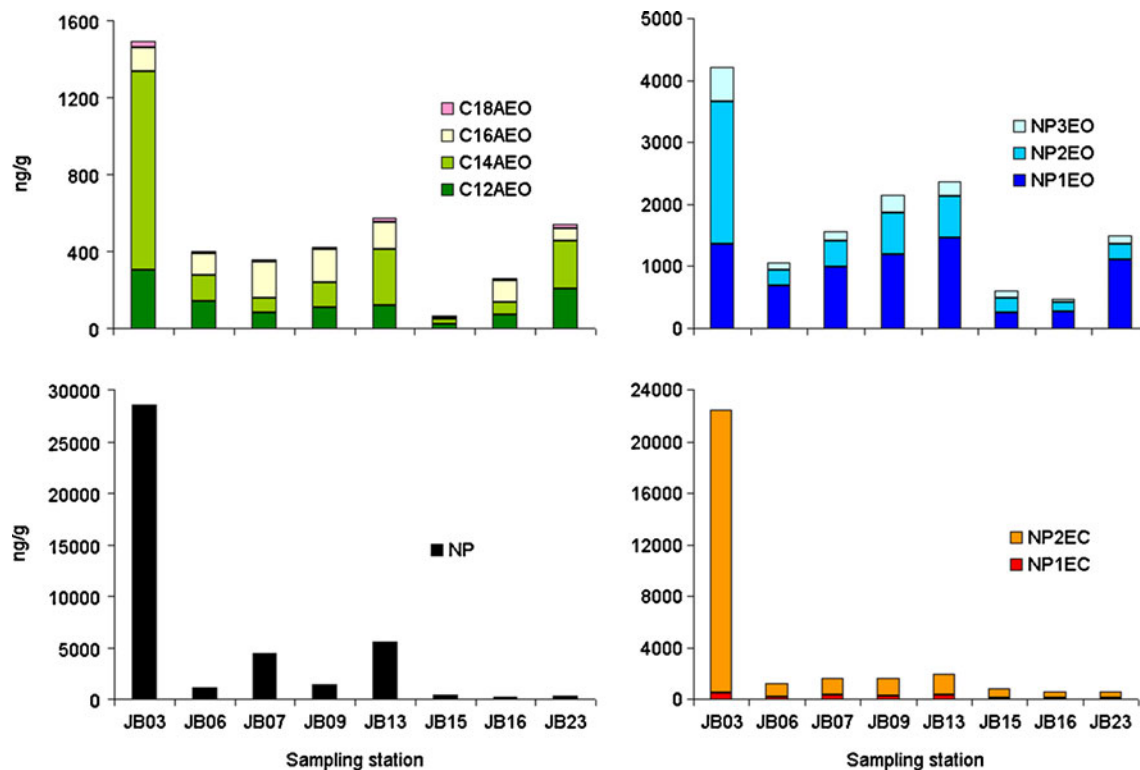
Fig. 4 Concentrations of target compounds (AEOs and NPEO metabolites) in surface sediments from Jamaica Bay (NY)

dredged to provide fill for the construction of what is now John F. Kennedy (JFK) International Airport. It is the deepest area in Jamaica Bay (up to 15 m) and characterized by high rates of sediment deposition (1–2 cm/year), as well as the highest levels of organic wastewater contaminants in both sediments and overlying waters [3, 25, 26]. This sampling station is also heavily impacted by wastewater from the Jamaica WWTP, a plant located within 1 km from this point that discharges roughly 3×10^8 L/day of biologically treated sewage. A previous study focused on the distribution and fate of NPEOs in Jamaica Bay was carried out a decade ago [3]. In that study only NP1-3EO and NP concentrations were determined, ranging between 186 and 16,300 ng/g, and 30 and 13,700 ng/g, respectively, for those stations that were sampled again in 2008 (JB03, JB15, JB16, and JB23). The highest concentrations on both occasions were found at sampling point JB03. In agreement with the earlier study, NP and NP1EO were the most abundant neutral NPEO metabolites consistent with wastewater inputs after secondary biological treatment. In the present study, these two metabolites account for 80±10% of total NP plus NP1-3EO. The concentrations of more soluble NP1-2EC (up to 500 and 22,000 ng/g for NP1EC and NP2EC, respectively) were also determined here and found to be comparable to those reported for NP and NP1EO in the same samples (Fig. 4). These carboxylated intermediates are the main products coming from NPEO degradation during secondary treatment, which

can explain their abundance in sediments in spite of their relatively low sorption capacity. For example, Loyo-Rosales and co-workers [22] measured the removal of NPEO parent compounds during wastewater treatment to be approximately 99%, whereas NP1-2EC was formed and accounted for up to 90% of the mass in the effluent (24 μg/L). Reports on concentrations of NP1-2EC in sediments, however, are scarce, being sporadically found on the Dutch coast at concentrations up to 118 ng/g [4]. Although weakly estrogenic compared with NP, these metabolites may represent a significant fraction of the potential exposure to environmental estrogens if we consider their relatively high concentrations in sewage-impacted surface waters and sediments, as well as their solubility that may lead to greater bioavailability from sediments. Additional monitoring of NP1-2EC in both aqueous and particulate phases is recommended.

AEO concentrations are below those measured for NPEO metabolites in the same samples (Fig. 4), although their spatial distribution in Jamaica Bay sediments is similar (the maximum is also located at JB03, 1,490 ng/g), suggesting commonalities in their sources and behavior. However, AEOs are significantly more particle reactive than most NPEO metabolites, showing higher affinity for suspended solids and sediments and having a greater dispersion all over the sampling area. Thus, NP and NP1-2EC concentrations decrease by average factors of 96 and 37, respectively, from station JB03 toward those stations

located in the eastern part of the bay (JB15, JB16, and JB23), whereas AEOs and NP1-3EO (both nonionic species) values decrease by less than 10 times. The relatively lower concentrations of AEOs in Jamaica Bay may be related to their very effective removal in WWTPs, typically above 95% [31], where they undergo degradation by cleavage of the ether bond and sorption on sludge. Sediments from other areas that are subjected to untreated wastewater discharges show the highest values, e.g., more than 10 mg/kg at some points on the Spanish coast [7]. Further studies monitoring the occurrence and fate of AEOs and their possible metabolites in Jamaica Bay and other aquatic systems are currently under way.

Conclusions

This work describes the development of a methodology aimed at the trace level determination of estrogenic NPEO degradation metabolites and AEOs in environmental samples . These nonionic surfactants are the most used worldwide and are now replacing NPEOs in household applications. This method was based on the use of UPLC–MS-MS, and we have shown how this combination of techniques provides enhanced separation, faster analysis, higher confidence, and lower detection limits than more conventional HPLC–single quadrupole-MS approaches. Thus, total run time was 11 min per sample, after ultrasonic extraction, which allows processing of a considerable number of samples simultaneously within 3 h. Extraction efficiency depends on the nature of the target compound, ranging between 61 and 102% for most analytes, although improvement is necessary for monoethoxylated species because of their low solubility. Limits of detection were usually below 0.5 ng/g per analyte, excepting those compounds having 1 EO group, which were also included in the method but having higher detection limits (>5 ng/g) because of poor ionization. The utility of the method was demonstrated by analyzing samplings from a survey in a sewage-impacted area (Jamaica Bay), where concentrations ranging between 250 and 28,500 ng/g for NP, 470 and 4,200 ng/g for NP1-3EO, 540 and 22,400 ng/g for NP1-2EC, and 60 and 1,500 ng/g for AEOs were measured in surface sediments. The abundance of NP, NP1EO, and NP1-2EC was remarkable as their high concentrations indicated that sampling area is heavily impacted by wastewater discharges. Further monitoring of ethoxycarboxylate intermediates in the particulate phase is recommended.

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