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A quantitative method is described for the analysis of the metabolites of alkylphenol ethoxylate (APEO) surfactants in estuarine water and sediment samples using reversed-phase high-performance liquid chromatography with electrospray mass spectrometry detection. Nonyl- and octylphenols, nonyl- and octylphenol mono-, di-, and triethoxylates, halogenated nonylphenols, and nonylphenol ethoxycarboxylates were concentrated from water samples using a C_{18} solid-phase extraction procedure. A novel, continuous-flow, high-temperature, sonicated extraction system was developed to isolate APEO metabolites from sediment samples. Quantitative LC–MS was performed in the negative ion mode for nonylphenols, octylphenols, and halogenated nonylphenols and in the positive ion mode for nonyl- and octylphenol ethoxylates using selected ion monitoring with isotopically labeled surrogate standards. Recoveries for sediment and water analyses ranged between 78 and 94%, and detection limits for APEO metabolites were between 1 and 20 pg injected on column. This is a significant improvement over previously reported methods. Suppression of analyte response was encountered in the presence of matrix components in sediment samples, but this effect was eliminated by careful selection of surrogate and internal standards. Individual APEO metabolite concentrations of 1–320 ng/L and 5–2000 ng/g are reported for water and sediment samples, respectively, from Jamiaca Bay, NY.

There is significant interest in the environmental fate of the metabolites of alkylphenol ethoxylate (APEO) surfactants because of the potential effects of these compounds as endocrine disruptors in aquatic organisms. APEO surfactants are manufactured by sequential ethylene oxide addition to a hydrophobic alkylphenol. The most common alkylphenol (AP) used for this application is the technical product known as 4-nonylphenol (NP). This product consists of a mixture of branched nonyl chain isomers. The end result of APEO synthesis using NP is a complex mixture of polyethoxylated, mixed alkyl isomer compounds collectively known as nonylphenol ethoxylates (NPEOs). A less common, but important AP used in APEO synthesis is 4-(1,1,3,3-tetramethylbutyl)phenol, more commonly known as 4-octylphenol (OP). APEOs manufactured from OP are termed octylphenol ethoxylates (OPEOs). Biotransformation occurs when APEO surfactants are exposed to secondary wastewater treatment,[1] and a complex mixture of metabolites is released to the aquatic environment via discharge of the treated sewage. Metabolites include the short (typically 1–3) ethoxy chain length NPEOs and OPEOs, the APs themselves (NP and OP), carboxylated APEOs (NPEC and OPEC), and ring-brominated or -chlorinated APs (such as Br-NP or Cl-NP). There is evidence that many of these substances can cause estrogenic effects in fish and other aquatic organisms,[2] and these compounds may persist in aquatic sediments[3] where they may undergo remobilization and bioaccumulate into the food chain.

Several analytical methods have been used to monitor these contaminants in various environmental matrixes. Methods include GC/MS with electron ionization (EI)[4–6] and chemical ionization (CI)[6–8] and HPLC with UV absorbance[9] and fluorescence[10–14] detection. Due to the limited volatility of highly ethoxylated and carboxylated APEOs, GC/MS is restricted to the analysis of APs and short ethoxyl chain APEOs unless derivatization is incorporated into the protocol. The high resolving power of capillary GC allows separation of many of the alkyl isomers of NP and NPEOs, resulting in compositional information, but detection at reduced signal-to-noise ratios. In addition, APs and APEOs tend to fragment extensively under EI conditions, providing valuable structural

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information, but limiting the sensitivity of the analysis in selected ion monitoring (SIM) mode. The application of CI to GC/MS of APEO metabolites provides molecular weight confirmation for each compound, but this approach has not generally been used for quantitative analysis.[6-8] HPLC with fluorescence detection is very sensitive for the analysis of APEO metabolites; however, no chromatographic separation has been reported that can simultaneously separate APEOs on the basis of alkyl and ethoxy chain length. In addition, UV absorbance and fluorescence detection lacks the specificity inherent to the mass spectrometer, and interferences from complex environmental matrixes may complicate the analysis.

HPLC with mass spectrometry detection has been used less frequently to analyze APEOs, their metabolites, and other nonionic surfactants in the environment. Early attempts included LC–MS with particle beam (PB) interface[15] and thermospray (TS) ionization.[16] The development of robust and sensitive atmospheric pressure ionization (API) sources has greatly simplified coupling of HPLC to MS, while significantly enhancing sensitivity for polar and ionic compounds. Several authors have reported the analysis of ethoxylated nonionic surfactants and their metabolites by atmospheric pressure chemical ionization (APCI)[17] and electrospray (ESI)[18-20] LC–MS. HPLC with ESI-MS detection offers excellent sensitivity for ionic and polar molecules, making it an ideal candidate for the quantitative determination of APEO metabolites. Crescenzi et al.[18] reported excellent sensitivities for alcohol ethoxylate surfactants as well as long ethoxy chain NPEOs in sewage, drinking water, and river water using HPLC–ESI-MS. Their method, however, was not readily amenable to short-chain ethoxylated analytes, and important metabolites such as APs and APECs were not detected. The HPLC–ESI-MS method of Shang et al.[20] was able to detect NPs as well as NPEOs in marine sediments, but did not detect NPECs or the halogenated NPs. This method utilized normal-phase HPLC, attained only modest sensitivity, and required long run times. Neither method included the OPEO metabolites, which may be important contaminants in some aquatic systems.

The objective of this study was to develop a method that could overcome the limitations of previous analytical approaches to measuring the important APEO metabolites in the aquatic environment. A comprehensive technique is reported that allows for quantitative determination of alkylphenols, halogenated alkylphenols, and APEOs with short (<5) ethoxyl chains in both water and sediment. Highly ethoxylated APEOs and APECs were also detected in water by this method, although quantitative analysis was not performed. Reversed-phase HPLC–MS with electrospray ionization was optimized for specific and sensitive analysis of the APEO metabolites. In addition, sample preparation methods were improved, and a novel sediment extraction technique was developed for the analysis of APEO metabolites in contaminated sediments. The entire method was validated with environmental samples from a sewage-impacted urban estuary. Important limitations of ESI-MS analysis of environmental samples were considered, including sample matrix-derived interferences, competitive ionization suppression, and interferences from multiple-charged ions. These limitations were examined in the context of quantitative determination of APEO metabolites in sediment and water samples. The method provides the highest currently available sensitivity (1–20 pg/component injected) for APEO biotransformation products and is the first technique to demonstrate applicability to a full range of important metabolites likely to be encountered in the aquatic environment.

EXPERIMENTAL SECTION

Materials. Stable isotope-labeled surrogate standards ([$^{13}C_6$]-*n*-nonylphenol, plus mono-, di-, and triethoxylates) were purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA). Mono-, di-, and triethoxylated 4-*n*-nonylphenol internal standards were synthesized from 4-*n*-nonylphenol (Lancaster Synthesis Inc., Windham, NH) and 2-chloroethanols (Aldrich, Milwaukee, WI) according to the procedure of Mansfield and Locke.[21] Synthetic products were purified by gel permeation chromatography (>95% by ESI-MS). Monochlorinated NP was prepared by chlorination of technical nonylphenol using sulfuryl chloride according to the method of Stokker et al.,[22] and monobrominated NP was synthesized using elemental bromine.[23] Halogenated nonylphenols were purified by reversed-phase HPLC (>98% by ESI-MS). Authentic standards of technical grade NP, NPEO (characterized for percentage composition of each ethoxymer), and nonylphenoxyacetic acid (sodium salt) were provided by Dr. Carter Naylor (Huntsman Corporation, Austin, TX). OP was purchased from Aldrich, and a mixture of octylphenol mono-, di-, and triethoxylates was obtained from Chem Service (West Chester, PA). The octylphenol mono-, di-, and triethoxylate material was characterized for percentage ethoxymer composition by GC-FID response. All solvents were HPLC grade or equivalent and were purchased from Burdick and Jackson (Muskegon, MI).

Due to the ubiquitous occurrence of alkylphenols and alkylphenol ethoxylate materials in plastics and detergents, glassware and sampling apparatus required special treatment prior to use. No detergents were allowed to contact any glassware. All glassware was solvent rinsed (methanol, acetone, and dichloromethane) and baked at 450 °C prior to use. No plastics, other than Teflon and PEEK, were used in any part of the procedure. All adsorbents used in chromatographic cleanup or extraction were Soxhlet extracted with methanol for 24 h.

Water Samples. Estuarine water samples (1 L) were collected from the surface of Jamaica Bay, NY, using a stainless steel and Teflon Kemerrer bottle (Wildco Instruments, Saginaw, MI). Particulate material was immediately filtered onto precombusted GF/F (0.7 μm nominal) glass fiber filters (Whatman, Maidstone, England) under vacuum. The filtered water samples were spiked with [$^{13}C_6$]-labeled surrogate standards and acidified to pH <2

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by dropwise addition of concentrated sulfuric acid. Five milliliters of methanol was added to aid extraction, and the water samples were stored in glass-stoppered Erlenmeyer flasks (1 L) until extraction (within 8 h).

APs and APEOs were isolated from water samples using solid-phase extraction. Previous workers have used C_{18}-bonded silica,[24] hydrophobic polymers,[25] and graphitized carbon black[18] as sorbents for extraction of APEOs and their metabolites from aqueous solution. We found that the use of 1.0 g of octadecylsilane (Bondesil 40 μm, Varian, Sugarland, TX) provided efficient recovery of the analytes in the current work. SPE cartridges were custom packed using 6-mL glass columns and PTFE frits (Supelco, Bellefonte, PA). Cartridges were prerinsed with 10 mL of acetone, 10 mL of methanol, and 10 mL of acidified Milli-Q water, prior to addition of sample. Water samples were applied via suction through custom-modified Teflon siphon tubes. Field blanks, consisting of Milli-Q water that had been carried through the sampling procedure described above, were extracted along with water samples. Sample vessels were rinsed three times with acidified Milli-Q water, and the rinses were passed through the SPE cartridges. Cartridges were air-dried under vacuum, and elution was performed with 2 × 3 mL acetone. Extracts were taken gently to dryness under a nitrogen stream, reconstituted in 500 μL of 60% methanol/water, and spiked with internal standards. No further treatment was necessary for APEO analysis of water extracts; however, it was necessary to add 50 mg of mixed-bed strong ion-exchange resin (AG 11 A8, Bio-Rad, Melville, NY) to the extracts prior to AP analysis to remove interferences from chromatographically unresolved APECs. It should be noted that qualitative determination of APECs in water extracts by LC–MS was performed prior to the addition of ion-exchange resin to the sample.

Sediment Samples. Sediment samples had been collected previously from the New York Harbor Complex and freeze-dried to remove water. Subsamples were thoroughly ground and homogenized using a mortar and pestle. Previous workers have reported quantitative extraction of APs and APEOs from sediments using Soxhlet extraction with nonpolar solvents[26] or exhaustive steam distillation.[9,27] We attempted to adapt both of these methods to our sediment analysis, but found that they provided insufficient recovery. In the case of Soxhlet extraction with pentane, up to 30% of NPEO that had been spiked onto wet sediment remained adsorbed to the sediment after extraction (18 h), as determined by exhaustive extraction of the sediment residue by high-power sonic probe extraction (600 W, Cole Palmer Instrument Co., Vernon Hills, IL) in methanol and dichloromethane. Even using acetone or acetone/hexane (50:50) as a Soxhlet extraction solvent, up to 20% of NPEO remained on the sediment after the extraction. However, in a recent study,[20] high recoveries of NPEOs were reported using Soxhlet extraction with 70:30 hexane/2-propanol. The success of this approach may have been due to the use of the protic solvent, 2-propanol, in the extraction.

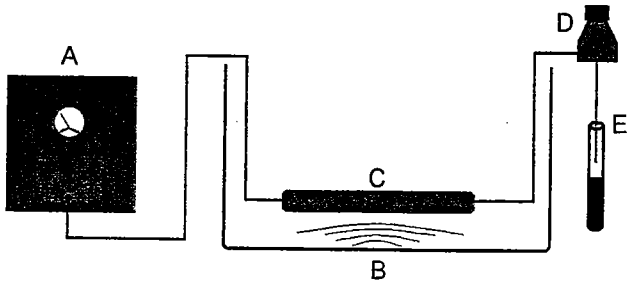

Figure 1. Diagram of the high-temperature continuous-flow sonication extraction apparatus, showing the solvent pump (A), heated ultrasonic bath (B), packed sediment extraction column (C), back-pressure regulator (D), and extract collection tube (E).

An efficient sediment extraction method was developed which provided quantitative recovery of APEOs with minimal time and solvent requirements. The extraction of dried sediments was performed under solvent flow, with elevated temperatures and ultrasonic irradiation. A diagram of the experimental apparatus is shown in Figure 1. Dried sediment (1–2 g) was packed into a 150-mm stainless steel column (4.6-mm i.d.) fitted with 0.2-μm stainless steel frits (Alltech Chromatography, Deerfield, IL) and spiked with [$^{13}C_6$]-labeled surrogate standards. The column void volume was filled with sea sand which had been baked at 450 °C overnight. The sediment column was immersed in a heated ultrasonic bath (65 °C), and methanol was pumped through the extraction cell at 0.5 mL/min. A back-pressure regulator (Alltech Chromatography) was used to maintain the solvent as a liquid under the elevated temperatures. This approach, which uses low-power ultrasonic energy to aid extraction, is different from the high-energy ultrasonic probe extraction method (i.e., USEPA method 3550) mentioned above for determining extraction efficiency. Sediment extraction using the apparatus described in Figure 1 was complete (further extraction yielded no more APEO) after 7 min with a total solvent consumption of 3.5 mL/sample. Blank sediment extractions, consisting of baked sea sand spiked with surrogate standards and packed into extraction columns, were performed alongside actual samples.

A two-step cleanup approach was used to isolate APEOs from the sediment extracts. First, a normal-phase SPE approach, using NH_2-modified silica, was utilized (modified from ref 26). Aminopropyl silica (Supelco; 750 mg) SPE cartridges were constructed from glass columns and Teflon frits. Columns were prerinsed with 2 × 5 mL of acetone and then conditioned with 2 × 5 mL of pentane. Sediment extracts (in 5 mL of hexane) were passed through the columns. Columns were rinsed with 2 mL of pentane, and APs and APEOs were eluted with 5 mL of 25% acetone/pentane. This normal-phase cleanup step served to remove APEOs > 5 EO as well as coextracted pigments and other polar material.

A second, reversed-phase cleanup technique was used to further purify the extracts. This step served to remove material that proved to be insoluble in the more aqueous HPLC–MS mobile phase. It also removed salts and some nonpolar material which would otherwise foul the analytical column. Eluents from the aminopropyl SPE cartridges were taken to dryness under nitrogen flow and resuspended in methanol. An automated, semipreparative HPLC fractionation scheme was utilized in the cleanup. Two analytical (4.6 mm i.d. × 25 cm) C_{18} columns were placed in series and connected to a UV absorbance detector and

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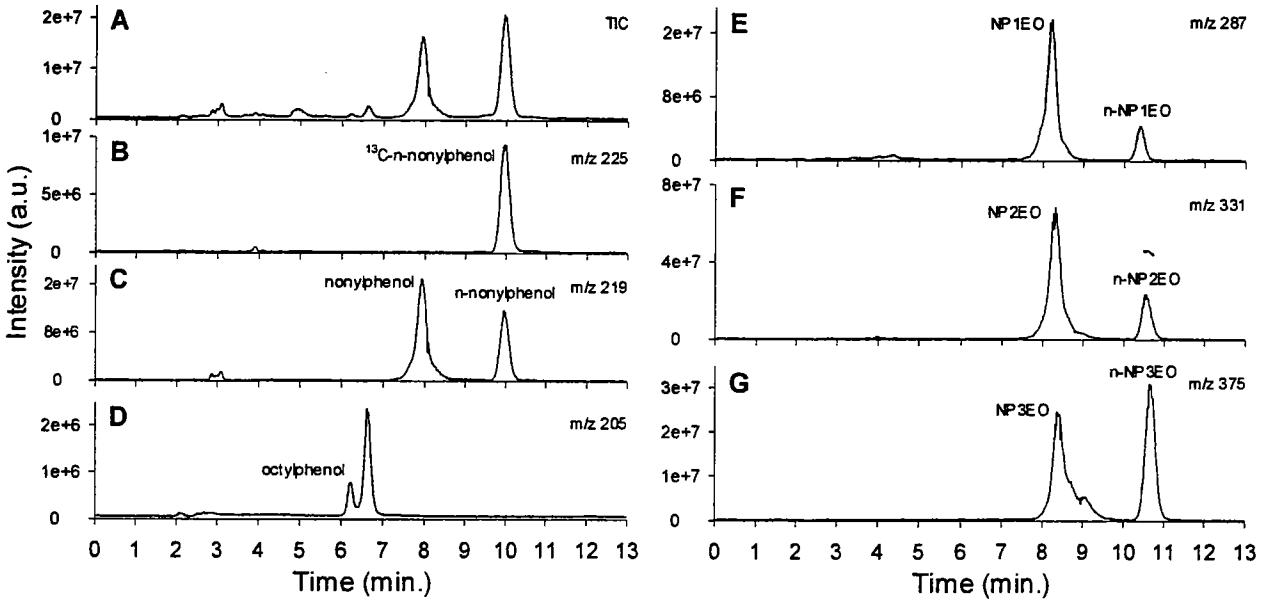

Figure 2. Total ion chromatogram (A) and reconstructed ion chromatograms (B–G) from negative (A–D) and positive (E–G) LC–ESI-MS (selected ion monitoring) runs of a sediment extract. Alkylphenols are separated chromatographically from each other and the surrogate and internal standards (A–D). Nonylphenol ethoxylates (E–G) coelute and are separated by *m/z* in the mass spectrometer.

a Gilson 203 fraction collector. Extracts (200 μL) were injected using a WISP 210B (Waters Associates, Milford, MA) autosampler. Chromatographic run conditions were isocratic 100% methanol at 2 mL/min. The fraction containing APs and APEOs was collected between 2 and 3 min. The run was continued until the UV detector response returned to baseline, typically 25 min. Collected fractions were again taken to dryness, resuspended in 60% methanol/water, and spiked with internal standards.

Chromatographic Conditions. A Hewlett-Packard 1100 series HPLC, consisting of a G1312A binary pump and a G1313A autosampler, with a 15-cm narrow-bore (2.1-mm i.d.) C_8 column (5-μm particle size, Keystone Scientific, Inc., Bellefonte, PA), was used for LC–MS analyses. The separation was performed under isocratic conditions with 80% methanol/water at ambient temperature (200 μL/min) and an injection volume of 20 μL. These conditions resulted in a run time of 13 min with no column equilibration step required between injections.

Mass Spectrometry. LC–MS analysis was performed using a Platform LCZ single-quadrupole mass spectrometer (Micromass, Manchester, U.K.) equipped with a Z-Spray ion source fitted with a pneumatically assisted electrospray probe. The use of the orthogonal Z-Spray interface allowed the entire column effluent (200 μL/min) from the HPLC to be directed into the source without flow splitting and contributed to the greatly enhanced sensitivity. As in an earlier application reported by Shang et al.,[20] it was necessary to perform two separate analyses for each sample in order to quantify both APs and APEOs. APs and halogenated APs were detected in negative ion mode as $(M - H)^-$. In the negative ion mode, the capillary voltage was −2.52 kV, and the cone was set at −45 V. APEOs were analyzed in positive ion mode as sodium adducts with capillary and cone voltages of 2.69 kV and 31 V, respectively. In both the positive and negative ion modes, source gas flow (250 °C) was 600 L/h and the source block temperature was 150 °C.

Quantitation. Quantitative analysis was performed using selected ion monitoring in order to maximize sensitivity. Analyte concentrations were calculated relative to [$^{13}C_6$]-*n*-NP(0–3)EO surrogate standards, which were added to the samples prior to extraction, at a level that would give a final extract concentration of 100 ng/mL. Recovery of the surrogate standards was calculated relative to *n*-NP(0–3)EO internal standards, which were added to the final extracts at a concentration of 100 ng/mL. Five-point quantitation calibrations were performed daily for all analytes and the surrogate standards over the linear range of the instrument, which was typically 3 orders of magnitude above the analyte-dependent instrument detection limit (approximately 0.1–5 pg injected, based on the amount giving a peak with S/N = 3). Concentrations of analytes in calibration standards ranged from <1 ng/mL to 900 ng/mL. The precision of the method was determined from extraction and analysis of triplicate samples. Method detection limits were calculated as 3 × standard deviation of analyte concentrations determined in field blank samples (n = 3). Qualitative spectra were acquired under full-scan conditions.

RESULTS AND DISCUSSION

Chromatography. With isocratic elution on a C_8 reversed-phase column, alkylphenolic compounds were separated on the basis of alkyl chain length (Figure 2A–D). Distinct peaks are evident in the TIC (Figure 2A) for OP, NP, and *n*-NP. The separation of the 4-*n*-nonylphenol internal standard from the technical 4-nonylphenol analyte (a mixture of branched nonyl isomers, containing no detectable *n*-nonylphenol) provides an advantage in SIM analysis (Figure 2C). These compounds have the same molecular weight; therefore, they can be monitored using a single *m/z* channel, increasing the relative instrument dwell time and enhancing sensitivity. The [$^{13}C_6$]-4-*n*-nonylphenol surrogate standard coelutes with the unlabeled 4-*n*-nonylphenol internal standard (Figure 2B,C); however these signals are easily resolved by the mass spectrometer. Octylphenol is the least hydrophobic AP, so it is the first to elute (Figure 2D). Like the APs, the APEOs separate on the basis of alkyl chain length under the specified chromatographic conditions; however, the ethoxymers

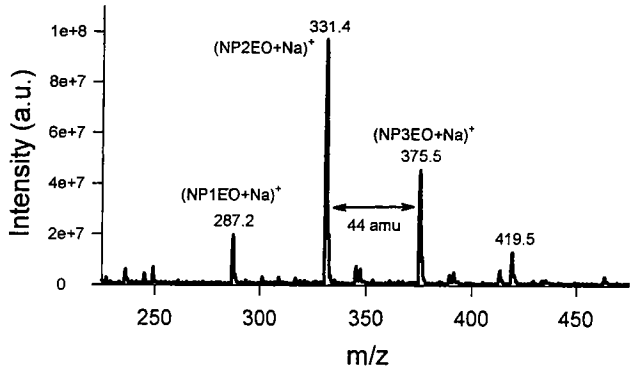

Figure 3. Positive ion electrospray spectrum of a mixture of NPEOs infused into the mass spectrometer under full-scan conditions. NPEOs give evenly spaced (44 Da) sodium adduct peaks in the positive ion mode.

of a given alkyl chain length coelute (Figure 2E–G). This presents a problem for quantitation of the individual ethoxymers when using reversed-phase HPLC with spectroscopic detection; however, as is evident in Figure 2E–G, the coeluting APEOs are resolved by the mass spectrometer in reversed-phase LC–ESI-MS due to their characteristic mass shift of 44 Da (mass of one ethoxylate moiety).

Mass Spectrometry. Under negative ion conditions, APs gave exclusively $(M - H)^-$ ions, consistent with previous reports in the literature for this class of compounds.[20] These ions are formed by loss of a proton from the phenolic moiety.

Polyethoxylated molecules show a remarkable affinity for alkali metal ions,[28] which is reflected in the positive ion electrospray spectra of these analytes (Figure 3). Even in the absence of added electrolyte, APEOs were detected as Na^+ adducts, presumably due to the ubiquity of this metal in the solvents and surfaces employed. As reported previously by Crescenzi et al.[18] for alcohol ethoxylates, an increase in absolute response in the ESI-MS signal with increasing degree of alkylphenol ethoxylation was observed. In Figure 3, NP1EO was present at higher concentration than NP2EO, yet the intensity of the $(NP2EO + Na)^+$ ion is much greater than that of the $(NP1EO + Na)^+$ ion. The ratio of the NP2EO response factor to that of NP1EO was 5.4. Crescenzi et al.[18] reported that this increase in response continued to ~6 EO, after which the response appeared to level off.

Because of a possible reduction in APEO ionization due to insufficient metal ion availability, it was necessary to fortify the samples extracts with 10 μM sodium acetate prior to injection. This provided sufficient sodium for analyte–sodium adduct formation. In the absence of this spike, response decreased for minor APEO components due to competition between the ethoxymers for the limited sodium pool. A related effect was noticed by Crescenzi et al.[18] in their analysis of alcohol ethoxylate surfactants. Injection of multiple ethoxymers simultaneously resulted in overall suppression of the analyte signals relative to those obtained on injection of the ethoxymers individually. This effect was attributed to competition between ethoxymers for sodium adduction. The suppression was much more pronounced for lower-numbered ethoxylates in the presence of equal amounts of higher-numbered ethoxylates.[18] The addition of sodium to the mobile phase was reportedly not sufficient to eliminate the effect. It should be noted that these results were based on co-injection of up to 60 ng of alcohol ethoxylates and that each ethoxylate was present at the same concentration. This situation may be markedly different from that in the present technique, in which injections of total APEO usually did not exceed low-nanogram quantities, and due to the normal-phase SPE cleanup technique, highly ethoxylated alkylphenols were not present in the standards or the sediment extracts. Competition for sodium metal adduct ions by APEOs may not completely account for the reported analyte signal suppression. Other mechanisms, such as saturation of the electrospray ion current or modification of ion evaporation processes from small droplets may produce similar results. Further consequences of these processes will be discussed later with regard to matrix effects.

To examine the competitive ionization suppression of less ethoxylated APEO oligomers by highly ethoxylated APEO, experiments were conducted in which increasing concentrations of a "polydisperse" NPEO mixture (average 9 EO, range 0–17 EO) were added to a constant (50 ng/L) level of pure NP2EO. The mixtures were analyzed by LC–ESI-MS in positive ion mode. No significant suppression of the NP2EO signal was evident until the concentration of the polydisperse NPEO mixture reached 10 times that of the NP2EO. Even when the concentration of polydisperse NPEO was a factor of 40 higher than NP2EO, the signal of the latter was only suppressed by 30% relative to the response of pure NP2EO alone, and in this case, the linear response of the instrument for total NPEO had been exceeded. In this study, the concentration of highly ethoxylated APEOs never exceeded that of the mono-, di-, and triethoxylated oligomers by more than a factor of 2 in any of the water samples analyzed (as evidenced by the paucity of higher mass peaks in the spectra of analyzed APEO samples, not shown). In fact, due to the nature of the samples analyzed in this work, the mono- and diethoxylates tended to be, by far, the most concentrated neutral APEO metabolites present (due to biodegradation processes during and after sewage treatment). This is consistent with previous work which showed that NP1–2EOs were the dominant neutral NPEO metabolites in a sewage-impacted river.[29] As a result, it appears that competitive ionization suppression from highly ethoxylated APEOs did not compromise quantitative results derived from the present method.

Because the samples studied in this work contained primarily mono-, di-, and triethoxylated APEOs, and these compounds coelute from the HPLC column, LC–ESI-MS experiments were performed in order to address the possible effects of coelution of these analytes on signal response. Pure *n*-NP3EO was added at increasing concentrations to solutions containing *n*-NP1EO at a steady concentration (50 ng/mL). In this case, no positive ion ESI signal suppression was noted for *n*-NP1EO until the concentration of *n*-NP3EO had exceeded the linear range of the instrument (a factor of 20 higher than *n*-NP1EO). The scenario tested here can be considered to be the worst possible case, since, as noted by Crescenzi et al.,[18] signal suppression was most severe for less ethoxylated analytes in the presence of more highly ethoxylated compounds. Concentrations of mono-, di, and triethoxylated APEOs in the present study were always within the linear range of the instrument, and in most cases, the AP1,2EOs were more

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Table 1. Method Validation Parameters

compound	% recovery (SD)[a]		environmental concn (RSD)[b]		MDL (ng/L) in water
	water	sediment	water (ng/L)	sediment (ng/g)	
nonylphenol	78.2 (4.0)	82.8 (5.4)	201 (3)	846 (12)	0.92
NP1EO	90.2 (5.6)	93.5 (6.7)	157 (4)	2120 (5)	0.20
NP2EO	87.4 (5.5)	84.3 (5.9)	320 (3)	1020 (2)	0.24
NP3EO	90.5 (4.6)	80.4 (4.7)	202 (4)	110 (4)	0.36
octylphenol			3.27 (3)	8.11 (5)	0.08
OP1EO			26.0 (12)	60.7 (5)	0.20
OP2EO			4.89 (8)	13.0 (3)	0.21
OP3EO			1.04 (30)	5.96 (8)	0.04
Cl-NP			nd[c]	4.38 (10)	0.21[d]
Br-NP			nd	9.38 (3)	0.27[d]

[a] Recovery of ^{13}C-labeled surrogate standards ($n = 12$). [b] Based on triplicate sample measurements. RSDs given in percent. [c] nd, not detected. [d] Extrapolated from sediment MDLs.

concentrated than the AP3EOs (see Table 1). Consequently, it is unlikely that the co-occurrence of AP1−3EO in the samples analyzed compromised analyte quantitation in the positive ion mode.

Method Validation. APEO metabolites were quantified in triplicate sediment and water samples from a sewage-impacted urban estuary (Jamaica Bay, NY) using the present method. Results from these analyses are shown in Table 1. Concentrations of NP and NPEO were consistently higher than those of OP and OPEO in both sediment and water (Table 1). This trend reflects usage patterns of these surfactants, where 80% of the APEO market is composed of NPEOs and 15−20% is made up of OPEOs and other ethoxylated alkylphenols.[30] In sediment, APs and AP-(1−2)EO made up the bulk of the APEOs measured, with minor contribution of AP3EO. NPEOs in water showed a different pattern, where NP3EO was present at approximately the same concentration as NP, and NP2EO was the most concentrated APEO metabolite measured. Overall precision of the data was excellent, with relative standard deviations of the measured AP and APEO concentrations falling generally between 3 and 5%, with some higher values, especially in the case of OPEOs in water. These higher RSDs may have been related to the generally low concentrations of these analytes.

Recoveries of spiked [$^{13}C_6$]-labeled *n*-nonylphenol and *n*-NPEO surrogates were consistently between 80 and 90% for both water and sediment (Table 1). These recoveries were calculated relative to the *n*-nonylphenol and *n*-NPEO internal standards, which were added immediately prior to injection. No trend in recovery was evident with regard to degree of ethoxylation for either water or sediment.

The high sensitivity of this analytical technique is illustrated by the method detection limits (MDLs) for water samples reported in Table 1. These MDLs represent injections of 1−4 pg for APEOs and 1.5−30 pg for APs. MDLs for sediments were dependent on the amount of dry sediment extracted, but were typically 0.2 ng/g for 1 g of sediment. Instrument detection limits were typically a factor of 5−10 lower than the MDLs for each analyte. These detection limits are an improvement over previously reported mass sensitivities for normal-phase LC−ESI-MS analyses of AP and APEO (1−4 ng injected),[20] and are nearly 1 order of magnitude lower than results reported for reversed-phase LC−ESI-MS analysis of APEOs (20 pg injected).[18] The significant increases in sensitivities reported in this work are due to (1) the use of second generation pneumatically assisted electrospray source technology, enabling high flow rates and orthogonal ion sampling; (2) the employment of aqueous mobile phases that increase electrospray efficiency; and (3) the ability of this method to analyze the entire 0.2 mL/min. HPLC eluent without the necessity for postcolumn splitting or makeup flow.

Other APEO Metabolites Identified. Short-chain APEOs and APs are not the only metabolites that have been reported to arise from exposure of APEOs to wastewater treatment processes. Many authors have reported the presence of carboxylated alkylphenols (such as the nonylphenol ethoxycarboxylates (NPECs)) in sewage effluent and receiving waters.[19,31,32] NPECs were detected in estuarine surface waters using the current technique (Figure 4). These analytes were detected in both negative (Figure 4A) and positive (Figure 4B) electrospray mode. No standards were available at the time of analysis; therefore, quantitative analysis was not possible. As with APEOs, the NPECs gave regularly spaced peaks in the mass spectra, due to the 1 EO difference in the various structures. Sodiated ions were dominant in the positive ion spectrum, and the corresponding $(M - H)^-$ ions were present in negative ion spectra, reflecting the loss of an acidic proton. The profile of pseudomolecular ions in both positive and negative ion spectra reveals that the carboxylated NPEOs are dominated by short ethoxyl chain oligomers.

Several authors have reported the formation of halogenated residues of APEO metabolites during wastewater and drinking water chlorination and their occurrence in the aquatic environment.[33−35] To examine the possible persistence of these compounds in the environment, sediment extracts were analyzed for the presence of monochlorinated and monobrominated NP. An example of a reconstructed ion chromatogram for Br-NP in a standard (Figure 5A) and a sediment sample (Figure 5B) is shown. The doublet signal in the mass spectrum of Br-NP due to the bromine isotope contribution (^{79}Br:^{81}Br = 50.5:49.5) provides ad-

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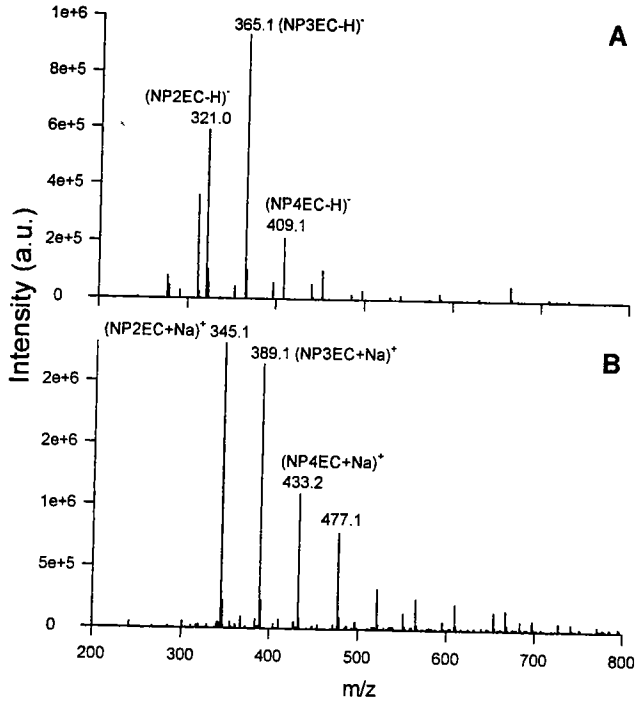

Figure 4. Negative (A) and positive (B) ion electrospray spectra of NPECs from full-scan LC–MS injection of a Jamaica Bay, NY, water extract. Shown are a range of $(M - H)^-$ ions in negative mode and the corresponding $(M + Na)^+$ ions in positive mode, each separated by an *m/z* of 44 Da, corresponding to a difference in mass of one ethoxyl moiety between adjacent oligomers. This spectrum was averaged over a partially resolved peak, which eluted before NPEOs.

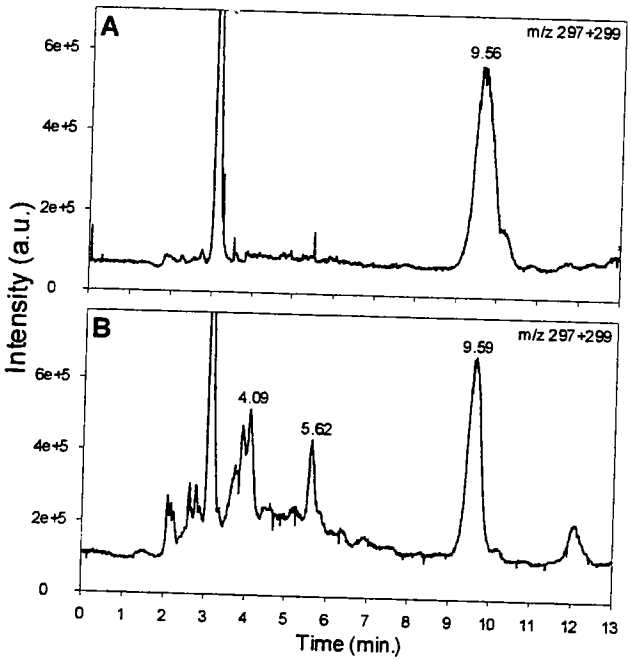

Figure 5. Reconstructed ion chromatograms of monobrominated NP in a standard (A) and a sediment sample (B). Chromatograms were reconstructed by combining the SIM channels corresponding to the bromine isotope doublet of the $(M - H)^-$ ion. The peak eluting at ~9.6 min is monobrominated NP.

ditional confirmation of the presence of this compound in sediment samples, in addition to that given by the retention time match with the standard. It is clear from the low concentrations of the halogenated NPs in sediments that these compounds constitute a relatively minor component of the persistent pool of APEO biotransformation products in the marine environment (as little as 0.2% of NPEO metabolites in sediment). However, this class of APEO metabolites may prove useful as a tracer of input of chlorinated sewage or pulp and paper mill effluent to nearshore environments.

Potential Limitations of the Present Method. The quantitative analysis of APEOs by reversed-phase HPLC–ESI-MS presents several important problems which must be addressed before reliable measurements can be made. The effect of coeluting APEO analytes on signal intensity has been discussed above. Another potential problem was highlighted by Shang et al.[20] during their work on normal-phase HPLC–MS analysis of NPEOs. In that study, it was noted that isobaric interferences from doubly charged ions of highly ethoxylated NPEOs could impact quantitation of less ethoxylated NPEOs under conditions in which all ethoxymers coelute. It was also discovered during the course of the present work that analyte signal suppression due to the presence of an environmental extract matrix must be corrected in order to prevent artifacts in the quantitative analysis of APEO metabolites by electrospray HPLC–MS.

The interference of doubly charged, highly ethoxylated APEO ions with singly charged ions of less ethoxylated APEOs is unique to the NPEO series. The doubly sodiated ion of NP15EO gives a nominal *m/z* of 463. This also happens to be the nominal mass of NP5EO + Na. In reversed-phase LC–MS, this creates a potential problem, since all APEOs of a given alkyl chain length coelute under a single peak, and hence, the NP15EO doubly charged ion can contribute to the peak area of NP5EO in the extracted ion chromatogram of *m/z* 463. Shang et al.[20] reported that the error caused by this effect can be up to 40%, and it is not limited to the NP15EO/NP5EO pair. All odd-numbered highly ethoxylated NPEOs caused interferences in the spectrum of less ethoxylated NPEO to some degree, depending on the relative concentrations of the NPEO pairs. The previous authors[20] solved the problem by separating all ethoxymers chromatographically with normal-phase HPLC. In the present technique, the problem was eliminated by the removal of highly ethoxylated APEOs by normal-phase SPE from all sediment extracts. Water extracts were not cleaned up by normal-phase SPE, but examination of full-scan ESI spectra revealed that interferences from double-charged APEO ions were not an issue. Even-numbered highly ethoxylated APEOs gave double-charged ions which were spaced 22 Da between the peaks for single-charged ions of less ethoxylated APEOs. The absence of peaks corresponding to these even-numbered double-charged ions in all water sample spectra indicated that the interfering peaks, 22 Da on either side, were also absent. This result is consistent with the typically low abundance of APEO ions with EO > 5 found in the spectra of water extracts analyzed in this study.

The intensity of a given analyte ion signal in ESI-MS is a function of both the concentration of the analyte and the total ions in solution. As the concentration of co-occurring compounds increases beyond a certain threshold, the relative contribution of the analyte to the total ion current decreases, resulting in suppression of the analyte signal.[36] This "matrix effect" has been noticed in the positive ion ESI-MS analysis of alkyl ethoxysulfates.[37]

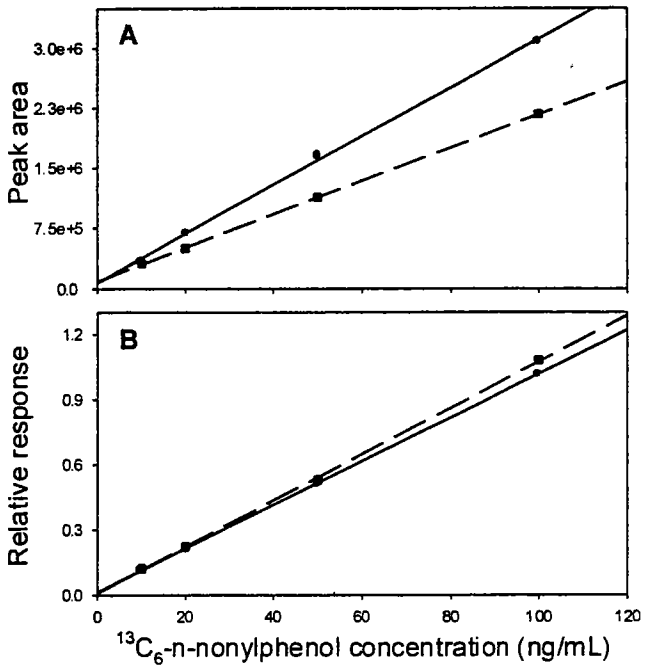

Figure 6. Absolute (A) and relative (B) response of [$^{13}C_6$]-*n*-nonylphenol spiked into clean solvent (●, solid line) and a sediment extract (■, dashed line), with increasing concentration. Response in (B) is relative to *n*-nonylphenol internal standard, spiked at constant concentration into the calibration solutions. (A) clearly shows an effect of the sediment matrix on the analyte absolute response. The suppression of analyte signal is well accounted for by normalization to internal standards (B).

Matrix-induced analyte signal suppression is likely to be of major concern in the analysis of complex environmental samples by HPLC–ESI-MS. Removal of inorganic ions and other extraneous material by sample cleanup can help to reduce, but not eliminate, the potential for coeluting species to cause matrix effects; however, it is usually impractical or impossible to remove all possible interferences. To examine the impact of matrix effects on the present technique, we prepared two spike series containing the surrogate standard [$^{13}C_6$]-*n*-NP and the internal standard *n*-NP: one in clean solvent, and one in sediment extract. The solutions were spiked with increasing concentrations of [$^{13}C_6$]-*n*-NP (treated as the analyte) and constant concentrations of *n*-NP. When these standards were run under negative ion HPLC–ESI-MS conditions, the response for [$^{13}C_6$]-*n*-NP was considerably lower in the presence of sediment extract (Figure 6A). It should be noted that the results shown are for alkylphenolic compounds in the negative ion ESI mode; therefore, the signal intensity does not reflect the contribution of adduct ion availability, as might the APEOs in positive ion mode. Because of this, the decreased response in the sediment extract can be attributed to a genuine matrix effect and not to competition for adduct ions. It is clear from the results shown that calculation of analyte concentrations in actual environmental samples based on external standard response relative to "clean" calibration standards would result in significant errors. Separate experiments indicated that without the reversed-phase HPLC cleanup step, the signal intensity of [$^{13}C_6$]-*n*-NP was reduced by 70% in a particular sediment extract by the matrix effect. This signal reduction was limited to 50% in that same sediment extract after the reversed-phase HPLC cleanup.

When the [$^{13}C_6$]-*n*-NP response was normalized to the internal standard (*n*-NP in this case), the relative response was nearly identical for clean solvent and sediment extract (Figure 6B). This result indicates that the use of an appropriate internal standard reduces the net effect of matrix-induced signal suppression on calculated analyte concentration. It is clear, therefore, that internal and surrogate standards should be chosen which closely mimic the ionization behavior of the analytes of interest in order to increase the quantitative reliability of ESI-MS in environmental analysis.

Since in the present study the surrogate and internal standards did not coelute with the analytes of interest, it was necessary to confirm that the matrix-induced ionization suppression observed at the retention time of the standards was predictive of that observed for the analytes of interest. Sequential dilution of a sediment extract containing NP, and spiked with surrogate and internal standards, showed that the decrease in response with increasing sediment extract matrix concentration did not differ significantly among the internal and surrogate standards (*n*-NP and [$^{13}C_6$]-*n*-NP) and analytes (NP and OP) tested (data not shown). This suggested that, over a limited retention time window, there was little change in matrix-induced suppression of analyte ionization and that the use of the chosen internal and surrogate standards was appropriate.

CONCLUSIONS

Reversed-phase HPLC–ESI-MS has been shown to be a highly sensitive, rapid, and robust method for the analysis of the full suite of APEO metabolites in environmental samples. The high sensitivity of the technique allows the use of very small sample sizes, making it possible to determine the concentrations of APEO biotransformation products in suspended particulates trapped on filters and in water samples far removed from contaminant sources. The technique could also be adapted to the analysis of small biological tissue samples and low-concentration matrixes, such as air. This flexibility will enable a more complete study of the aquatic fate of the APEO metabolites. The ability to simultaneously monitor APEOs and APs of varying alkyl chain length may prove useful for contaminant source tracing, as variations in the ratios of OP to NP in the environment may be reflective of specialized use patterns in industrial or municipal applications. It appears that the present method will be most useful for analyzing APEO metabolites in waters and sediments where there has been much degradation of highly ethoxylated APEOs. The complementary normal-phase HPLC–MS method recently developed by Shang et al.[20] may be more appropriate for analyzing less degraded APEO mixtures in the environment. Finally, this and other papers have shown the effect of matrix-associated effects on analyte signal response in environmental samples. It is clear that the application of electrospray LC–MS techniques to quantitative problems in environmental analytical chemistry must take these effects into account in order to reduce artifacts introduced by the sample matrix.

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