Quantitation of Alcohol Ethoxylate Surfactants in Environmental Samples by Electrospray Mass Spectrometry

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ABSTRACT: This report describes a method for obtaining the concentrations of the total and individual alcohol ethoxylate (AE) species in sewage treatment plant (STP) effluents by using electrospray liquid chromatography/mass spectrometry (LC/MS). This is a more advantageous method for quantitative analysis of AE in environmental matrices as compared with a previous thermospray LC/MS method. This new method is more sensitive, uses less solvents, utilizes a deuterated internal standard blend [$C_{13}D_{27}O(CH_2CH_2O)_nH$, where n varies from 0 to 21 with an average of $n = 9$], which corresponds more closely to the AE, and it is a more robust instrumental technique. In this report, we document the results for validation of the electrospray LC/MS method by spike recovery of AE from STP effluent and influent samples.

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Surfactants are used in large volumes in a broad variety of household and commercial detergents and cleaning products (1–3). After use, the surfactants are usually disposed of into a wastewater treatment system, and the effluents ultimately are released into surface waters. Biodegradation and other removal mechanisms greatly reduce the mass and concentration of surfactants that reach the environment.

Alcohol ethoxylates (AE) of the formula $RO(CH_2CH_2O)_nH$ are an increasingly important class of nonionic surfactants. They are not single components but complex mixtures with a distribution of alkyl and ethoxylate groups. The R group (in the above formula) is typically an alkyl carbon chain with 12, 13, 14, or 15 carbon atoms (Table 1). The average number of ethoxylate groups can vary from 0 to 30. AE generally fall into two broad subclasses. One subclass represents the monobranched (primarily 2-alkyl-branched) and unbranched (linear) AE homologues, typically referred to as "essentially linear AE." Henceforth in this report, the term "essentially linear AE" shall be taken to mean both 2-alkyl-branched and linear AE. Essentially linear AE are commonly used in household applications. Highly branched AE, the other subclass, are typically derived from propene or butene oligomers. They are used more commonly in nonhousehold applications and can be present in sewage treatment plant (STP) samples.

TABLE 1
Analytical Reference Materials Used

Surfactant	Alkyl carbon no. distribution Acronym	Range	Avg.	Average EO
Linear $C_{12–15}$ AE-9 EO[a]	N25-9	12–15	13.5	9
Deuterated C_{13} AE-9[b]	D27 AE	13	13	9

[a]NEODOL® 25-9 (Shell Chemical Co., Houston, TX). The NEODOL 25-9 AE was made from $C_{12–15}$ alcohols and ethoxylated to an average of nine ethylene oxide (EO) units per mole of alcohol. The $C_{12–15}$ alcohols were derived from $C_{11–14}$ olefins by hydroformylation with a proprietary catalyst. The resulting alcohols contain approximately 80% linear alkyl groups and 20% 2-alkyl groups.
[b]Made by laboratory-scale ethoxylation of a $C_{13}D_{27}OH$ alcohol purchased from Cambridge Isotope Laboratories (Woburn, MA).

Although prior research has shown that essentially linear AE biodegrade rapidly and extensively in typical sewage treatment schemes (4), it is still not certain how much is actually discharged at trace levels into receiving waters. It is important to know these actual concentrations, especially in safety risk assessment studies of these molecules and in general monitoring studies. For such studies, sensitive and specific detection methods, such as liquid chromatography/mass spectrometry (LC/MS), are needed to quantitatively determine total AE concentrations at the parts per billion (ppb) level.

STP influents are complex mixtures of suspended solids and liquids, and they contain analytes of interest. Accurate methods for determining AE levels in STP influents are important to enable calculations of the efficiency of AE removal in STP. Although others have reported good recovery of AE from influents (3), our past efforts to validate a method for determination of AE in STP influents by spike recovery of AE have resulted in less than satisfactory recoveries (Evans, K.A., and S.T. Dubey, unpublished research). Partitioning of analytes between the aqueous phase and suspended solids can lead to difficulties in sampling and lower recoveries for spiked samples. Even if great care is taken to mix the influent samples, taking a small

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yet representative sample is challenging. Separation and extraction of AE sorbed on solids and subsequent analyses of extracts in addition to liquid-phase samples would provide the most comprehensive approach to determining the concentration of AE in influents. However, this approach would be time-consuming.

Methods for obtaining the total AE concentrations in STP effluents have been previously reported (5–9). Some of these methods are based on colorimetric detection (5) and are nonspecific methods designed to encompass all nonionics with an ethoxylate moiety. More specific methods, such as gas chromatography (GC) or high-performance liquid chromatography (HPLC) with ultraviolet (UV) or fluorescence detection (6), have also been utilized. GC methods are limited by the low volatility of the higher-molecular-weight (>EO7) ethoxylate species. Utilization of HPLC methods with UV or fluorescence detection requires derivatization of the AE because AE does not contain a chromophore (7). Dubey *et al.* (7) reported using evaporative light-scattering detection (ELSD) for determination of AE, but this method requires that all sample matrix interferences be eliminated prior to analysis of samples that contain ppb levels of AE.

Wee (8) used a hydrogen bromide (HBr) cleavage technique to obtain alkyl bromides from the AE, which were then analyzed by GC–flame-ionization detection (FID). Fendinger *et al.* (9) have used a similar method with GC/MS detection for the analysis of AE in environmental matrices. However, these latter two methods are not unequivocal; if the AE in the matrix is a complete unknown, some assumptions have to be made regarding the amount or distribution of ethoxylate groups in the AE.

More specific methods for obtaining the concentrations of the total and individual AE species in STP effluents by thermospray (TSP) LC/MS have been reported (1,2). They are more specific than the HBr cleavage GC or GC/MS methods in that the LC/MS methods detect individual AE homologues without derivatization of the samples. Recently, Crescenzi *et al.* (3) have reported an electrospray (ESP) LC/MS method for quantitative determination of total AE in environmental and drinking water samples. They used a single ethoxylate, $C_{10}H_{21}(CH_2CH_2O)_6H$, as an internal standard. The authors reported only separation of monobranched and unbranched AE (essentially linear AE), with no mention of highly branched AE species. These highly branched AE homologues can occur in STP locations where nonhousehold AE applications contribute to influent waste streams. To provide accurate measurements of essentially linear AE in receiving waters where nonhousehold applications might contribute to influents, it is important to have a method that is capable of distinguishing between the essentially linear and highly branched isomers.

In an effort to provide an improved LC/MS method for the quantitative analysis of AE in environmental matrices to support safety risk assessment studies, we have developed a new ESP LC/MS method. When compared with our previous LC/MS method (1), this new method has better sensitivity for AE, uses less solvent, and is more instrumentally robust. It also allows for the separation of essentially linear and highly branched AE isomeric homologous compounds, and it incorporates a deuterated internal standard blend, which is a more suitable internal standard for AE analysis. In addition, we have improved our approach to sample preparation for STP influents. In this report, we describe the validation of this new ESP LC/MS method by spike recovery of AE from STP effluent and influent samples.

TABLE 2
Sewage Treatment Plant Sample Sets

Effluents Set number/ sample	Initial volume	Spike	Final volume to SPE					
E1,E2,E3,E4	2.5 L	—	2.5 L					
E1,E2,E3,E4	2.5 L	10 ppb	2.5 L					
Influents								
Set number/ sample	**Bottle number**	**Initial volume**	**Spike**	**Dilution**	**Split volume**	**Dilution**	**Spike**	**Final volume to SPE**
1a,1b,1c,1d	1	900 mL/ea.	—	1:4	900 mL/ea.	1:4	—	950 mL
1e,1f,1g,1h	1	900 mL/ea.	1 ppm	1:4	900 mL/ea.	1:4	—	950 mL
2a,2b	1	900 mL/ea.	—	1:4	900 mL/ea.	1:4	—	950 mL
2c	1	900 mL	—	1:4	900 mL/ea.	1:4	1 ppm	950 mL
3a,3b	2	900 mL/ea.	—	1:4	900 mL/ea.	1:4	—	950 mL
3c,3d	2	900 mL/ea.	—	1:4	900 mL/ea.	1:4	1 ppm	950 mL
3e,3f	2	900 mL/ea.	1 ppm	1:4	900 mL/ea.	1:4	—	950 mL
4a,4b,4c[a]	2	25 mL/ea.	—	1:10	—	—	—	250 mL
4d,4e,4f[a]	2	25 mL/ea.	1 ppm	1:10	—	—	—-	250 mL
G1,G2,G3[a]	—	50 mL/ea.	—	—	—	—	—-	50 mL
G4,G5,G6[a]	—	50 mL/ea.	2 ppm	—	—	—	—-	50 mL

[a]These sample sets were prepared by the solid-phase extraction (SPE) method previously used for thermospray liquid chromatography/mass spectrometry (LC/MS) analyses. G1–G6 were samples from the Glendale STP (Glendale, OH), which were analyzed by thermospray LC/MS two years prior to the work reported here.

EXPERIMENTAL PROCEDURES

Analytical reference materials used are shown in Table 1. Influents and effluents were provided from the Jackrabbit STP (Katy, TX). These samples were preserved with 8% formalin (vol/vol). These validation samples are listed, along with volumes, dilutions, and final spike concentrations, in Table 2. The Glendale STP samples were provided by Procter & Gamble Co. (Cincinnati, OH).

Validation of the ESP LC/MS method was done by spike recovery of 1 ppm and 10 ppb NEODOL 25-9 (N25-9, see Table 1) in Jackrabbit influents and effluents, respectively. The aqueous samples were mixed thoroughly and passed through 1-g C_8 reverse-phase solid-phase extraction (SPE) cartridges (Varian Sample Preparation Products, Harbor City, CA). The cartridge was preconditioned with methanol, followed by isopropanol (10 mL each), and finally deionized (DI) water. The sample was loaded on the cartridges by using either a vacuum (manual system) or pressure (automated workstation; Zymark Autotrace, Hopkinton, MA). After loading, the cartridge was air-dried, and the surfactant was eluted with 8 mL methanol, followed by 4 mL isopropanol. The solvent was evaporated in a Zymark Turbovap system at 60°C under a gentle stream of nitrogen. The residue was reconstituted in methanol (usually 250 µL for TSP and 100 µL for ESP) with 26 ppm of the D_{27}AE internal standard [$C_{13}D_{27}O(CH_2CH_2O)_nH$, where n varies from 0 to 21 with an average of $n = 9$, Table 1] and injected into the LC/MS instrument. When volumes greater than 1 L were loaded on a cartridge, and especially when the matrix looked dirty (suspended solids were visible), a 3-g cartridge was used, and the solvent elution volumes were adjusted proportionally.

For influents, sample preparation had to be modified. Early in the validation experiments, we recognized that the suspended solids might be a source of problems (especially in influents) if they were not sampled representatively. We did not want to develop a lengthy and labor-intensive sample preparation scheme (such as separation and extraction of solids along with subsequent analyses of extracts and aqueous phases). Also, because influents have a higher surfactant concentration (typically 1 ppm or more), smaller volumes (25–50 mL) are usually sampled as compared with effluents (2.5 L). This could also lead to sampling errors associated with obtaining a representative sample.

We have developed a scheme in which 900 mL of influent was sampled and diluted with DI water 1:4 (vol/vol). If the sample was to be spiked, sufficient spike was added at this stage, resulting in a 1-ppm N25-9 concentration in the final diluted influent. Out of the 3600 mL of diluted spiked sample, 900 mL was further diluted 1:4 with DI water. From this second dilution, 950 mL was taken and passed through the SPE cartridge. After each dilution, the samples were shaken thoroughly. The samples are listed as 1e, 1f, 1g, and 1h in Table 2. The corresponding unspiked samples are listed as 1a, 1b, 1c, and 1d. Samples 2a and 2b were prepared as 1a and 1b. For sample 2c, the N25-9 spike was added in the last dilution stage. Samples 3a and 3b are similar to samples 1a and 1b; however, these samples were taken from a second (1-gallon) grab bottle, collected at the same time and location as that used for samples 1a–2c. Samples 3c and 3d are similar to sample 2c. Samples 3e and 3f are similar to 1e–1h. Samples 4a–4f were prepared similarly to samples 1a–1h except that 25-mL aliquots of influent were diluted 1:10 (vol/vol). For these smaller samples, 250 mL was loaded on the cartridge. For the sake of comparison, we have included results generated from previous (1993) TSP analyses of influent samples (Glendale STP, Glendale, OH).

All samples were taken through the SPE sample preparation as described for effluents, and the residue was spiked with 26 (µg/mL of D_{27}AE in a suitable volume of methanol and analyzed by LC/MS. Standard solutions that contained varied amounts (206, 103, 51, and 26 ppm) of N25-9 and constant amounts of D_{27}AE (26 ppm) in methanol were analyzed under the same LC/MS conditions as the environmental samples. The results from the standard analyses were used to generate relative response curves for each ethoxylate compound. The samples were then quantitated against the relative response curves by linear regression.

The injection volumes were 25 and 100 µL for ESP and TSP analyses, respectively. The HPLC columns used were Supelco LC-18 (Bellefonte, PA) 3.2 mm × 25 cm × 5 micron (ESP) and 4.6 mm × 25 cm × 5 micron (TSP). The column temperature was 40°C. The flow rates were 0.25 mL/min for ESP and 0.7 mL/min for TSP. The isocratic mobile phase used for both TSP and ESP contained 50% water in tetrahydrofuran. For TSP analyses, 0.6 mL/min 43 mM aqueous ammonium acetate was added post-column. No buffer solution was added post-column for the ESP analyses. Our method differs from that used by Crescenzi *et al*. (3) in both mobile-phase composition (THF/H_2O vs. $MeOH/H_2O$), and separation capabilities (essentially linear vs. highly branched AE). The HPLC systems were Hewlett-Packard model HP 1050 (ESP) (Palo Alto, CA) and Waters 600MS (TSP) (Milford, MA).

The mass spectrometer and conditions used for the TSP analyses are the same as previously reported (1). The mass spectrometer used for the ESP analyses was a Finnigan TSQ 7000 (San Jose, CA) with an atmospheric pressure ionization source and operated in the ESP mode. The spray voltage was set to 8 kV, and the capillary heater was operated at 200°C. The mass range was scanned from 250 to 1100 amu in 3-s intervals by using a profile averaging program to average every four scans. In addition, profile background subtraction was employed.

Data calculations were performed as previously reported (1) except that for ESP analyses, sodium adduct ions were used for quantitation of individual species instead of ammonium adduct ions (TSP analyses). ESP LC/MS analyses of AE under the conditions described yield primarily pseudomolecular ions of the $(M + Na)^+$ type, as shown in Figure 1 for a specific AE compound, $C_{14}H_{29}O(CH_2CH_2O)_8H$. Although no sodium ions were intentionally added to the mo-

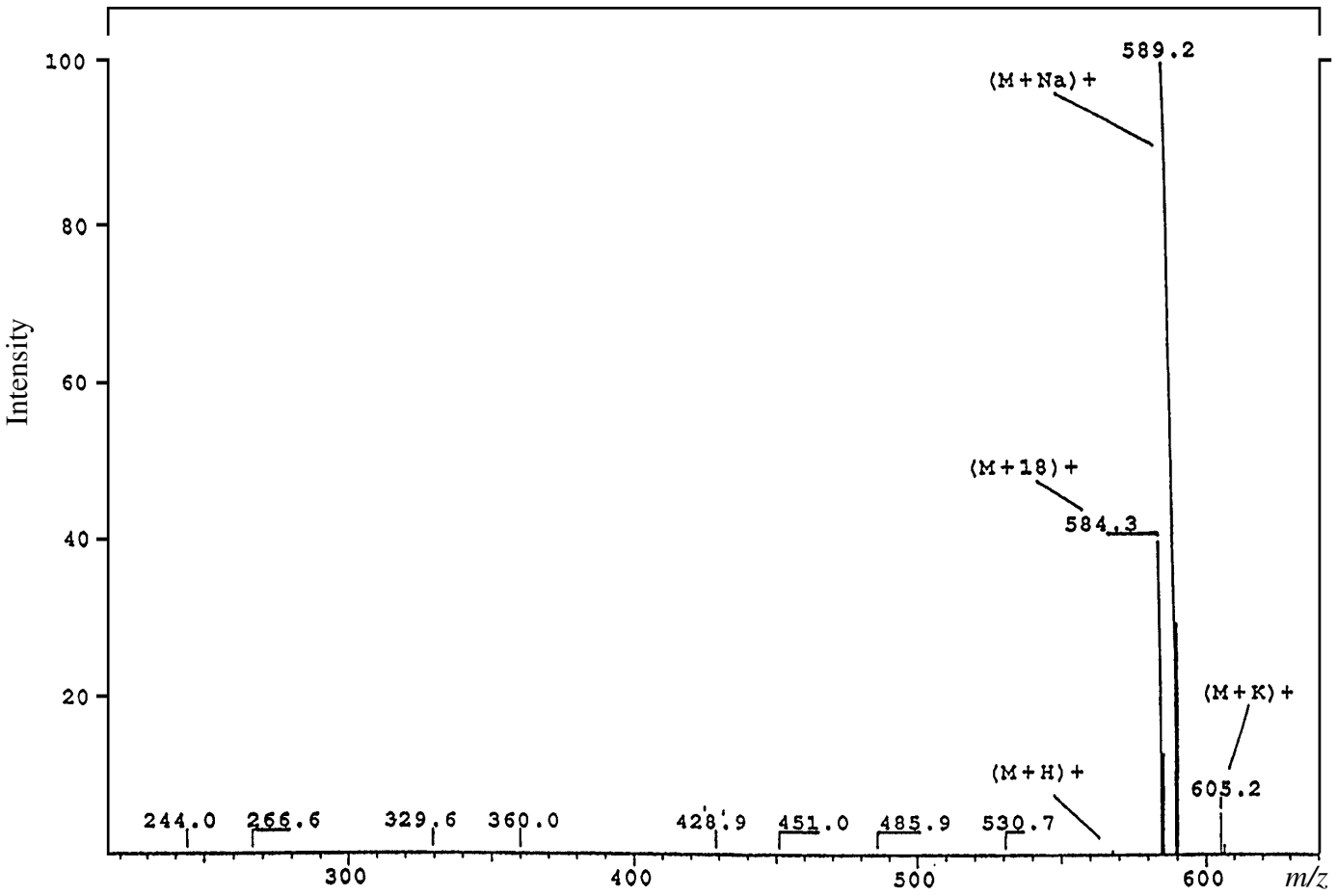

FIG. 1. Electrospray liquid chromatography/mass spectrometry spectrum of $C_{14}H_{29}O(CH_2CH_2O)_8H$.

bile phase, enough free sodium ions exist in the LC/MS system to generate intense sodium adduct ions. The original quantitation tables, used in the TSP method, were modified to contain the appropriate $(M + Na)^+$ adduct ions. Summing all adduct ions generated for each species would be expected to yield increased response, but unfortunately our experience indicates that the signal-to-noise ratio for each species is decreased by that technique. Thus, better specificity and improved signal-to-noise response is obtained by using only the most intense adduct ion, $(M + Na)^+$, for quantitation.

RESULTS AND DISCUSSION

When compared with our previous TSP LC/MS method (1), this ESP LC/MS method has better sensitivity for AE, uses less solvent, and is more instrumentally robust. When compared with the method of Crescenzi *et al.* (3), our method utilizes chromatographic separation, which allows for the separation of essentially linear and highly branched AE isomeric homologous compounds. In addition, this new method incorporates a more suitable internal standard, D_{27}AE, which corresponds more closely to the complex AE sample matrix than does a single nondeuterated ethoxylate standard.

Under the optimized instrumental conditions used here for analysis, AE species that contain less than two ethoxylate groups are not detected, regardless of concentration. This observed result is not fully understood at this time. It may be due to lower ionization efficiencies for EO0 and EO1 relative to the higher ethoxylates. Analysis of the samples by GC/MS to determine the concentrations of EO0 and EO1 would be difficult, owing to low absolute concentrations of these oligomers (typical N25-9 product contains only 3% EO0 + EO1) and the relatively small (1 μL) injection volumes used for GC/MS analysis. One would expect to see increased concentrations of EO3, EO4, and EO5 relative to the total distribution if there were corresponding relative increases in concentrations for EO0 and EO1. One would not expect a bimodal EO distribution in the effluents because there is not a bimodal EO distribution in the influents. To date, we have seen no evidence of significant changes in relative EO distribution between influents and effluents.

AE species that contain more than 18 ethoxylate groups are arbitrarily excluded from our calculations. Although it is possible to detect these species with the LC/MS techniques, typical N25-9 product contains only small amounts (<5%) of these species (>EO18). Detection of the concentrations of

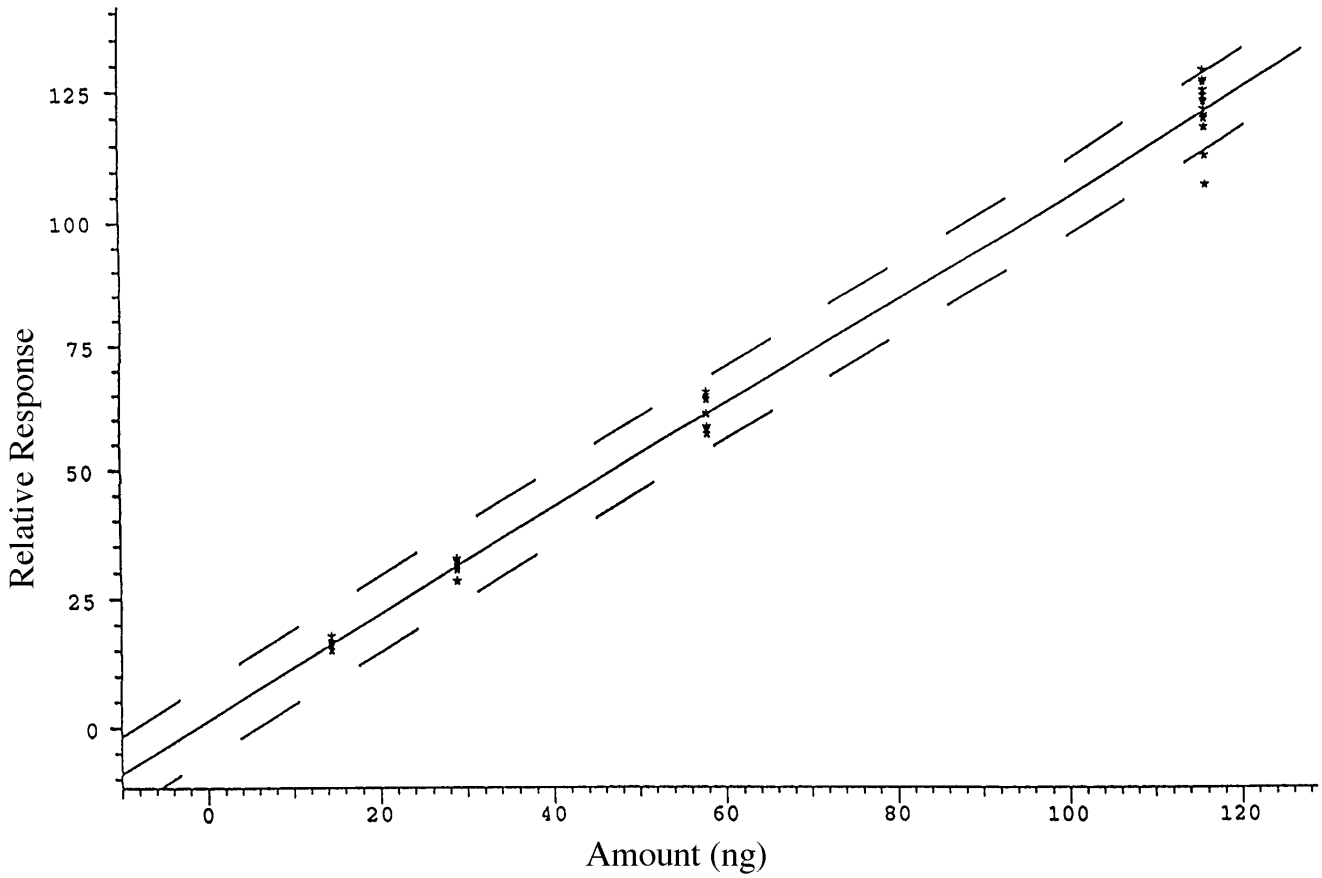

FIG. 2. Electrospray liquid chromatography/mass spectrometry relative response curve for varying concentrations (in ng) of $C_{13}H_{27}O(CH_2CH_2O)_9H$ vs. relative response (amount of reference material × area of analyte)/(area of reference material). Data were generated by multiple analyses of N25-9 reference material.

these species in the environmental samples would require additional sample injections at higher concentrations. These would add significant cost to the analyses, without a corresponding gain in value. The remaining AE species (C_{12}EO2–C_{15}EO18) constitute 92% of the N25-9 reference material and are easily quantitated from a single analysis as described in this report.

Quantitative relative response curves were generated for each AE species(C_{12}EO2–C_{15}EO18) detected in the N25-9 reference material. An example of the response curve for $C_{13}H_{27}O(CH_2CH_2O)_9H$ is shown in Figure 2. In general, the response curves from the ESP analyses had higher linear correlation coefficients compared with those from the TSP analyses. For example, the response curve for analysis of $C_{13}H_{27}O(CH_2CH_2O)_9H$ had linear correlation coefficients of 0.997 (ESP) and 0.960 (TSP). Because N25-9 is a mixture, the amounts of individual AE species are not equivalent, and thus the ranges of individual AE amounts injected varied. The range of amounts injected for one AE species, $C_{13}H_{27}O(CH_2CH_2O)_9H$, was 15–117 ng. A lower limit of detection of 0.36 ng injected, with a 10:1 signal-to-noise ratio, was determined for ESP analysis of a $C_{14}H_{29}O(EO)_8H$ single ethoxylate species (spectrum shown in Fig. 1). The lower limit of detection for TSP analysis of this $C_{14}H_{29}O(CH_2CH_2O)_8H$ standard under similar LC conditions was 36 ng injected, with a 14:1 signal-to-noise ratio. Exact limits of detection were not determined for other individual AE species because absolute standards were not available. The amounts of total AE (from reference materials) injected into the ESP LC/MS system ranged from 0.6 to 5 (µg. This compares to a range of 2 to 20 (µg injected for TSP analyses. For analyses of standard AE solutions that contained less than 2 µg (TSP) or 0.6 µg (ESP), ions representative of individual species of lowest relative concentration (i.e., EO2, EO3, EO17, and EO18) were not detected above noise level. Although these results were obtained on different instruments under different instrumental conditions, they indicate a significant increase in sensitivity (fourfold) for ESP as compared with TSP methods.

The TSP LC/MS method, previously developed in our laboratory, was validated by spike recovery of N25-9 in receiving waters at concentration levels of 25 to 100 ppb total AE and 0.06 to 2.1 ppb for individual AE species (1). The ESP LC/MS method reported here has been validated by spike recovery of total AE at levels of 10 ppb and 1 ppm in STP effluents and influents, respectively (Tables 3 and 4). The range of detected concentrations for individual AE species in the spiked (10 ppb) effluent samples, as quantitated by the ESP

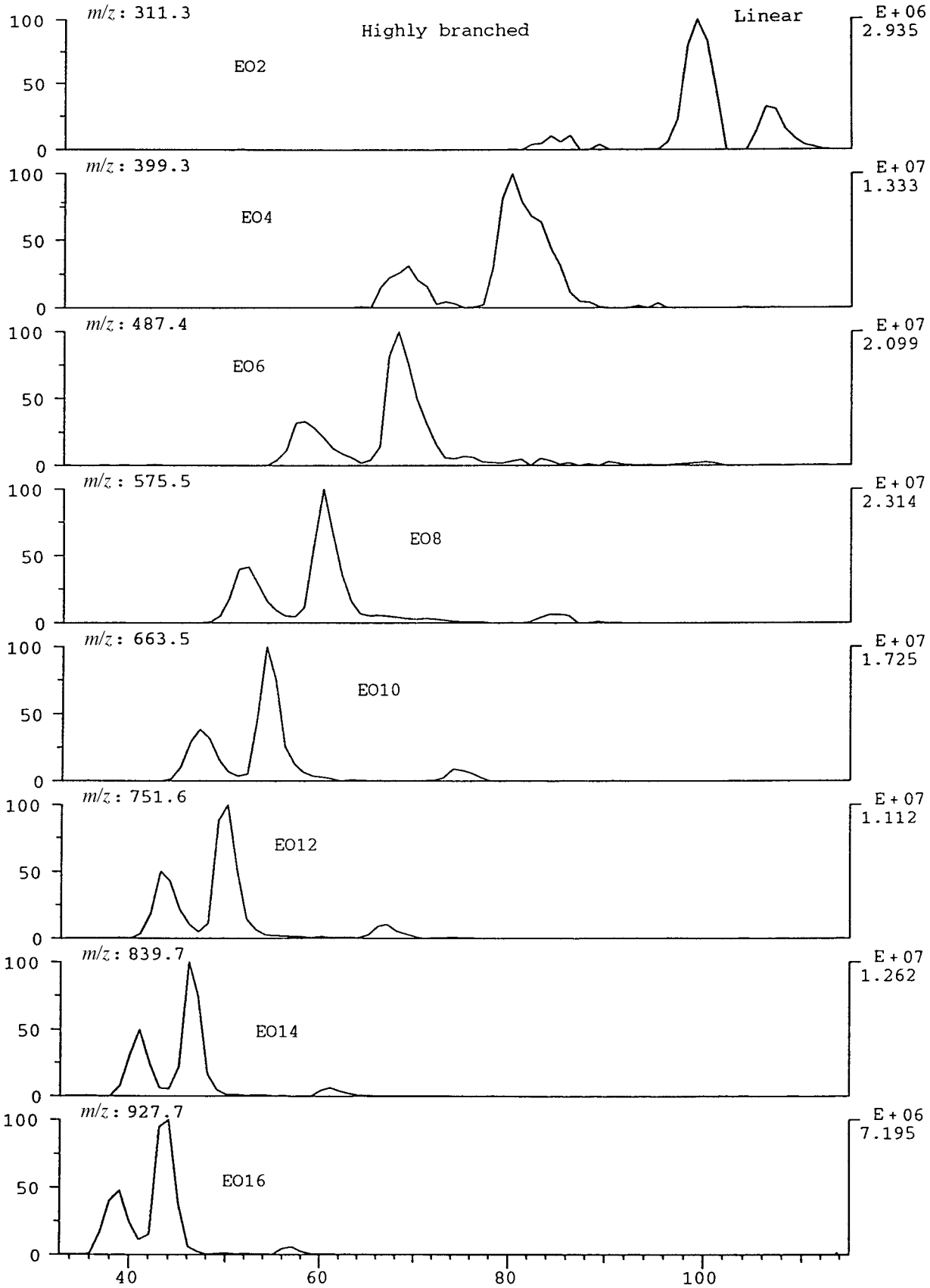

FIG. 3. Electrospray liquid chromatography/mass spectrometry selected ion chromatograms for various $C_{13}H_{27}O(CH_2CH_2O)_nHNa^+$ oligomers, detailng the separation of highly branched and essentially linear isomers.

method, was 0.01 to 0.21 ppb. These results again indicate that the ESP method is more sensitive as compared with our previous TSP method (1).

This increase in sensitivity allows for one-fourth as much sample to be injected for each ESP analysis as compared with the TSP analysis. This results in a decrease of one-fourth as much sample volume prepared for each effluent analysis.

Early investigation of the ESP system in our laboratory revealed that optimal sensitivity for AE species by this mobile phase was obtained at a flow rate of 0.25 mL/min. However, use of a standard 4.6-mm LC-18 column at this flow rate did not provide optimal chromatography. We were able to use a smaller-diameter column (3.2 mm) at the lower flow rate and achieve optimal sensitivity along with the desired chromatographic separation. This resulted in decreased solvent waste for the LC/MS separation as compared with the TSP method, which required higher flow rates (0.7 mL/min) and post-column addition of aqueous ammonium acetate (additional 0.6 mL/min). The ESP method generates approximately 1 mL/min or 80% less solvent waste as compared with the TSP method.

The chromatographic separations of individual AE homologues, obtained with this ESP method, do not differ significantly from those previously reported for the TSP method (1). As shown in Figure 3, the highly branched and essentially linear AE isomers are completely separated on the column. This separation allows for specific quantitation of the essentially linear AE compounds, even if highly branched AE are present in the sample.

Over the course of these experiments and during routine (for the past 2 yr) analyses, the ESP LC/MS instrument performed without any downtime. During the same time period, the TSP instrument suffered from more than 12 significant instrumental malfunctions. Most of these failures were due to the TSP vaporizer tip plugging and consequently being replaced. In addition, three vacuum pumps had to be replaced on the TSP instrument. The exact causes for these problems are unknown at this time. However, owing to these instrumental malfunctions, the TSP calibration curves for the AE standard reference materials had to be regenerated each time there was a malfunction. The ESP instrument did not have similar problems, even though the same types of samples were analyzed on both instruments. Our experience indicates that the ESP method is more instrumentally robust under the conditions used in our experiments.

In the previous TSP method (1), a linear primary C_{11} AE, NEODOL 1-9 (N1-9), with an average of nine ethoxylate groups, was used as the internal standard. More recently, we purchased a deuterated alcohol and ethoxylated it to an average of nine ethoxylate groups (Table 1). The TSP method was subsequently modified to use this new internal standard, D_{27}AE. This modification was not reported in external literature; however, this same D_{27}AE is used in the ESP LC/MS method as reported here. It is advantageous to use this deuterated standard because it closely resembles the analytes but does not occur naturally in the environment. And just like the N1-9 standard, use of this D_{27}AE standard blend, instead of a single ethoxylate standard, allows for more accurate calculations of individual AE species because each specific AE, $C_yH_{(2y+2)}OEO_xH$, is referenced to the corresponding deuterated AE species in the internal standard, $C_{13}D_{27}OEO_xH$. Both the D_{27}AE and the analyte of interest (N25-9) have averages of nine ethoxylate groups per molecule. Response factors within a homologous alkyl series ($C_{12}H_{25}OEO_xH$, $C_{13}H_{27}OEO_xH$, $C_{14}H_{29}OEO_xH$, $C_{15}H_{31}OEO_xH$, and $C_{13}D_{27}OEO_xH$) are arbitrarily assumed to be equal.

Prior attempts in our laboratory to validate the TSP and ESP methods by spike recovery of AE from influents resulted in spike recoveries of 40–65%, with poor repeatability [14–42% relative standard deviations (RSD) for analyses (5)]. The combined SPE sample preparation and ESP LC/MS method reported here gave improved recoveries (average of 3 data sets = 77%) of AE from influent samples. These improvements result from using more representative sampling and a modified sample preparation technique as described in the Experimental Procedures section. The key to these improvements is taking a larger initial sample of influent and diluting prior to SPE.

The average total percentage spike recoveries for sample set #1 were 98% for effluents (Table 3) and 69% for influents (Table 4). Also shown are the values of average percentage spike recoveries for individual AE species. The lower total recovery (69%) for spiked influent samples might result from sorption to particulates present in the influent sample. There are obviously some questionable results in the lower ethoxylate range. The AE species with two ethoxylate groups are the most difficult to detect. They are low in concentration, and there is a great deal of background chemical interference at the lower mass range. The automatic background subtraction program does not completely solve this latter problem. Spiking the samples with a lower distribution standard (such as N25-3 or N25-6) might help to resolve questions about the

TABLE 3
Electrospray Effluent Validation Results[a]

	Average percentage recovery			
EO number/C number	C_{12}	C_{13}	C_{14}	C_{15}
EO3	141	45	72	51
EO4	78	93	62	53
EO5	123	93	68	52
EO6	115	99	68	63
EO7	118	94	75	64
EO8	112	96	80	64
EO9	93	98	82	67
EO10	79	93	84	73
EO11	86	106	94	84
EO12	92	109	112	97
EO13	95	96	117	109
EO14	98	100	137	132
EO15	88	97	125	143
EO16	96	79	118	159
EO17	83	107	141	170
EO18	92	113	134	194
Total $C_{12-15}EO_3$–EO_{18}				98

[a]See Table 1 for abbreviations.

TABLE 4
Electrospray Influent Validation Results[a]

	Average percentage recovery			
EO number/C number	C_{12}	C_{13}	C_{14}	C_{15}
EO2	19	22	22	34
EO3	65	64	51	31
EO4	107	46	56	40
EO5	45	73	48	44
EO6	73	72	76	60
EO7	83	77	68	66
EO8	78	76	35	58
EO9	77	78	61	56
EO10	82	81	73	41
EO11	77	81	73	44
EO12	79	80	70	66
EO13	83	80	65	69
EO14	81	77	73	72
EO15	92	76	48	76
EO16	96	82	52	69
EO17	87	70	44	69
EO18	89	68	2	71
Total $C_{12-15}EO_3$–EO_{18}				98

[a]See Table 1 for abbreviations.

lower ethoxylate results. However, those experiments were not conducted at this time. The average standard deviations and average percentage RSD for the sample data sets are given in Table 5. Overall, these results are good, considering the complexity of the matrices and the low concentration level of the individual AE species. The 3 to 5% average RSD obtained is excellent when compared with the 14 to 42% values obtained from the previous TSP method (Table 5).

These initial results for influents were substantially better than those previously obtained for other STP influents analyzed in our laboratory. To further define the sample preparation necessary for influents, we undertook additional series of experiments on influents from the same STP. For sample set #2, we repeated the basic sample preparation procedure that was used for sample set #1, namely, larger samples, dilution, and splitting. However, in this set, the spike was added after dilution and immediately prior to SPE separation to minimize time for surfactant sorption. If sorption of the spike on solids was the cause of low recovery, this scheme should provide an indication. Indeed, higher recovery was obtained. As shown in Table 5, an average of 95% total AE spike recovery was obtained.

In an attempt to determine if we had a representative sample in the first two experiments, we took the samples for set #3 from a different bottle of influent, which was collected at the same date and location as the previous sample sets. Four spiked samples were prepared, two spiked prior to dilution and two spiked after dilution. The average percentage recovery for both sets was 85%, with 2–3% RSD. This would indicate little if any effect from sorption on solids when using this sample preparation scheme. Perhaps, by diluting the sample with DI water, sorption effects are minimized.

Overall, the first three sample sets indicate that taking a larger initial sample (900 mL) results in higher recovery of AE (77%), as compared with previous sample preparation schemes (40–65% recovery), in which 25 or 50 mL of influent were used with no dilution. In the previous influent validation experiments, we used samples from a different STP, so matrix effects could contribute to the results. Because no additional influent remained from the previous studies, we decided to duplicate prior sample schemes with the same influent used in the more recent experiments. This would allow a direct comparison between the two sample preparation schemes.

As shown in Table 2, sample set 4 consisted of small-aliquot (25-mL) samples from Jackrabbit STP. The sample preparation scheme used was the earlier (unsuccessful) scheme. The average percentage recovery for sample set #4 is shown to be 45% in Table 5. In addition, the percentage RSD for this spiked data set is 12%. Also, values for unspiked samples from the first three sample sets (8 samples) were consistent, with an average of 3–5% RSD. However,

TABLE 5
Effluent and Influent Validation Results

Effluent sample	Initial volume (L)		Spike (ppb)	Average percentage recovered	Average percentage RSD[a]
E1,E2,E3,E4	2.5		—	—	17
E5,E6,E7,E8	2.5		10	97	3
Influent set number/sample	**Bottle number**	**Initial volume**	**Spike (ppm)**	**Average percentage recovered**	**Average percentage RSD**
1a,1b,1c,1d	1	900 mL/ea.	—	—	5
1e,1f,1g,1h	1	900 mL/ea.	1 ppm	69	3
2a,2b	1	900 mL/ea.	—	—	5
2c	1	900 mL/ea.	1 ppm	95	—
3a,3b	2	900 mL/ea.	—	—	5
3c,3d	2	900 mL/ea.	1 ppm	85	2
3e,3f	2	900 mL/ea.	1 ppm	85	3
4a,4b,4c	2	25 mL/ea.	—	—	27
4d,4e,4f	2	25 mL/ea.	1 ppm	45	12
G1,G2,G3	—	50mL/ea.	—	—	42
G1,G2,G3	—	50mL/ea.	2 ppm	65	14

[a]RSD, relative standard deviation.

the average percentage RSD for set #4 unspiked samples was 27%. These results demonstrate that taking a larger, therefore more representative, sample is the key to improving precision as well as recovery for influent analyses. The sample preparation method for influents described here takes more manpower (time) and larger initial samples but obviously produces more reliable results as compared to the prior method.

This new SPE-ESP LC/MS method for quantitation of essentially linear AE in STP effluents and influents has been validated by using spike recovery of N25-9 in STP samples. This method will not be subject to interferences from highly branched AE (if present) in the sample matrix. The method has proven capable of quantitation of AE in effluents at levels of 10 ppb with 97% spike recovery and in influents at levels of 1 ppm with an average of 77% spike recovery. This new method can be used for calculation of removal rates of essentially linear AE from STP influents before discharge as effluents.

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