Rapid liquid chromatography-tandem mass spectrometry-based method for the analysis of alcohol ethoxylates and alkylphenol ethoxylates in environmental samples

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Accepted for publication in the *Journal of Chromatography A.* in July 2013.

Final version published as:
DeArmond P.D., DiGoregorio A.L. Rapid liquid chromatography-tandem mass spectrometry-based method for the analysis of alcohol ethoxylates and alkylphenol ethoxylates in environmental samples. *Journal of Chromatography A,* Aug 30; 1305: 154-63 (2013).

Abstract

A sensitive and selective method for the determination of alcohol ethoxylates (AEOs) and alkylphenol ethoxylates (APEOs) using solid-phase extraction (SPE) and LC-MS/MS was developed and applied to the analysis of water samples. All AEO and APEO homologues, a total of 152 analytes, were analyzed within a run time of 11 min, and the MS allowed for the detection of ethoxymers containing 2-20 ethoxy units (n_{EO} = 2-20). The limits of detection (LOD) were as low as 0.1 pg injected, which generally increased as n_{EO} increased. Additionally, the responses of the various ethoxymers varied by orders of magnitude, with ethoxymers with n_{EO} = 3-5 being the most sensitive and those with n_{EO} > 15 producing the least response in the MS. Absolute extraction recoveries of the analytes ranged from 37% to 69%, with the recovery depending on the length of the alkyl chain. Abiotic stability studies were performed, and C_{14-18} ethoxylates showed significant degrees of degradation. Water samples from the Colorado River were analyzed for AEOs and APEOs, which contained concentrations greater than 100 ng/L of total APEOs.

Key Words

liquid chromatography-tandem mass spectrometry, alcohol ethoxylates, alkylphenol ethoxylates, solid-phase extraction, nonionic surfactants

1. Introduction

The predominance of surfactant compounds in industrial and household cleaning products over the past four decades has led to environmentally relevant concentrations of alcohol ethoxylates (AEOs) and alkylphenol ethoxylates (APEOs) in ground and surface waters [1-6]. AEOs and APEOs are a class of nonionic surfactants that are common components of detergent formulations and household and industrial

cleaning products. AEOs and APEOs are also used as surfactants during oil and gas extraction [7]. They are high-production volume (HPV) chemicals, with estimates of 275,000 tonnes of AEOs being used in the year 2002 in European household detergents [8]. In the U.S., the consumption of nonylphenol ethoxylates (NPEOs) has been estimated at between 300-400 million lbs per year [9]. AEOs possess a chemical formula of CH_3-$(CH_2)_y$-$(OCH_2CH_2)_x$-OH, with values of *y* typically ranging from 11 to 17 and values of *x* ranging from 0 to 20. The alkyl portion of the molecule can be either linear or branched. In this work, APEOs refer to either octylphenol ethoxylates (OPEOs) or NPEOs.

NPEOs are considered toxic to many aquatic species and are a major contributor to nonylphenol (NP) in the environment, a persistent endocrine-disrupting compound [10,11]. AEOs biodegrade more rapidly and are considered less ecotoxic than APEOs; hence, NPEOs are gradually being phased out and replaced with AEOs. The European Union has banned NPEOs for household use due to their toxicity, but the U.S. has not prohibited their use. The U.S. Environmental Protection Agency (EPA) has, however, added $NPEO_1$, $NPEO_2$, $NPEO_3$, and $NPEO_4$ (i.e., the mono-, di-, tri-, and tetraethoxylates of NP, respectively) to the Toxic Substances Control Act (TSCA) Section 4(e) Priority Testing List [12]. Additionally, many detergent manufacturers in the U.S. are voluntarily replacing NPEOs with AEOs. While considered safe to humans [8], AEOs are not completely environmentally benign themselves, and many studies have investigated the ecotoxicity of alcohol ethoxymer species in various organisms, including estimating the quantitative structure-activity relationships (QSARs) and no-observed-effect concentrations (NOECs) in algae, Daphnia, and various fish species [8,13-15]. Cardellini et al. [16] studied the teratogenic and toxic effects of AEOs in frog embryos and tadpoles and determined median lethal concentrations (LC_{50}) of 4.59 mg/L. The biodegradation rates of AEOS vary among the different isomers of the same chemical formula, e.g., AEOs with branched 2-alkyl chains were previously shown to degrade slower than linear AEOs [17]. Surveys of wastewater treatment plant effluents have shown that wastewater treatment plants typically remove > 99% of AEOs from the influent [1,18]; however, often the more toxic species, i.e., the high-carbon alkyl chain and low-ethoxylate ethoxymers, are less efficiently

removed [18]. Due to the ubiquity of AEOs and APEOs in both household and industrial detergents and surfactants, it is highly unlikely that the sources of ethoxylated compounds in environmental waters and sediments can be pinpointed, e.g., whether they are due to residential down-the-drain disposal or from nearby industrial processes. However, the determination of AEOs and APEOs in environmental waters is necessary to assess potential risks to aquatic life.

Methods for the detection and quantitation of AEOs and APEOs from water samples typically utilize an extraction step followed by LC-MS [2,3] or LC-MS/MS [19-21]. The quantitation of AEOs and APEOs has been challenging due to a lack of certified standards, and assumptions are often made about instrument response for the various ethoxymers or about the concentrations of ethoxymers in the technical mixtures used as standards. The LC conditions often require long (i.e., 30 min – 1 h) run times to separate the homologues [3,22]. Deuterated $C_{13}EO_x$ [23] and ^{13}C-labeled $NPEO_x$ [4] have been synthesized for more accurate quantitation, but these compounds are not commercially available. Additionally, derivatization with 2-fluoro-*N*-methylpyridinium *p*-toluensulfonate (Pyr+) has been used to increase MS sensitivity [24], especially for mono- and diethoxylate species of the alcohols, but the derivatization process is subject to the purity and moisture content of the Pyr+ reagent and is time-consuming [24].

In this work, we developed an analytical method for the rapid determination and quantitation of individual alcohol and alkylphenol ethoxymers that does not require the use of derivatization reagents for quantitation. The method utilized solid-phase extraction (SPE) followed by a short LC-MS/MS run. The use of scheduled multiple-reaction monitoring (sMRM) mode was crucial for monitoring more than 100 MRM transitions in 11 min. We also show that the responses of the AEOs and APEOs vary considerably as a function of ethoxymer and that it is necessary to know the concentrations of each ethoxymer for accurate quantitation. We demonstrate the applicability of this approach by measuring AEOs and APEOs in river water samples. While only C_{12}-C_{16} and C_{18} AEOs and APEOs were investigated in this work, this method is applicable to the analysis of C_8-C_{11} AEOs, providing appropriate standards can be obtained.

2. Materials and Methods

2.1 Standards and reagents

Neodol 25-9, a commercial formulation of AEOs composed of C_{12}-C_{15} homologues, with an average ethoxylation of 9 units, was obtained as a white, waxy solid from Shell Chemical Company (Houston, TX) for use as AEO standards. The composition of the Neodol 25-9 was approximately C_{12}: 20%, C_{13}: 30%, C_{14}: 30%, and C_{15}: 20%, and the mol% of each ethoxymer was provided and is shown in Table S1. The Neodol 25-9 was "essentially linear", but approximately 20% of the ethoxymers were 2-alkyl branched [24]. Hexaethylene glycol monodecyl ether ($C_{10}EO_6$) and Triton X-100, a common laboratory detergent used as an $OPEO_x$ standard, were purchased from Sigma (St. Louis, MO). Polyoxyethylene (POE) (20) nonylphenol, POE (10) cetyl alcohol ether ($C_{16}EO_x$), and POE (10) stearyl alcohol ether (C_{18}EOx) were obtained from Chem Service (West Chester, PA). Tergitol NP-10 was purchased from Fisher Scientific (Pittsburgh, PA). HPLC-grade methanol (MeOH) was purchased from Fox Scientific (Alvarado, TX), and HPLC-grade isopropanol (IPA) was obtained from J.T. Baker (Center Valley, PA). HPLC-grade methyl tert-butyl ether (MTBE), dichloromethane (DCM), and acetonitrile (ACN) were received from Burdick and Jackson (Honeywell International, Muskegon, MI). Ultrapure water was generated in-house from a Barnstead NANOpure water purification system. Stock solutions (0.5-1 mg/mL) of individual standards and standard mixtures were prepared by dissolving accurate amounts of the standard compounds in MeOH. Working standard solutions were obtained by further dilution of stock solutions with MeOH.

The choice of laboratory detergent is critical when cleaning glassware, as many detergents contain AEOs or APEOs. All glassware was cleaned with Alconox powdered detergent, which does not contain ethoxylates.

2.2 SPE extraction of target analytes

Samples were extracted using an Autotrace SPE Workstation (Dionex, Sunnyvale, CA). Various types of SPE extraction cartridges were evaluated, including Oasis HLB cartridges (200 mg, 6 cc) (Waters, Milford, MA), Enviro-Clean divinylbenzene (endcapped, 500 mg, 6 cc) (United Chem Service, Bristol PA), Enviro-Clean C18 (endcapped, 500 mg, 6 cc) (United Chem Service), Enviro-Clean C18 (unendcapped, 500 mg, 6cc) (United Chem Service), and Enviro-Clean C8 (endcapped, 500 mg, 6 cc) (United Chem Service). The SPE cartridges from United Chem Service were constructed with glass. The cartridges were first conditioned with 5 mL MeOH and 5 mL water at a flow rate of 5 mL/min. After conditioning, 500 mL of sample was passed through the cartridges at 5 mL/min. To ensure quantitative recovery, the sample flasks were then rinsed with 50 mL water, and the rinsate was loaded onto the cartridges. The SPE cartridges were rinsed with 2 mL water before drying with N_2 gas for 30 min. The analytes were eluted off the cartridges with 10 mL of various solvents, including 90:10 MTBE/MeOH, MeOH, DCM, and 60:40 ACN/IPA, at 3 mL/min. The eluate was then concentrated and solvent exchanged with a TurboVap Concentrator (Biotage, Charlotte, NC) to 0.5 mL in MeOH and transferred to HPLC sample vials for analysis. Prior to extraction, $C_{10}EO_6$ was added to the samples as a surrogate standard.

2.3 HPLC-MS/MS

Analyses were performed on an AB Sciex 4000 Q Trap MS interfaced with a Shimadzu HPLC system. Detection was performed using the AB Sciex 4000 Q Trap MS in the triple quadrupole mode. The MS was equipped with a Turbo V Ion Source, which utilized the TIS source probe for positive-mode electrospray ionization (ESI+). The HPLC system consisted of LC-20AD pumps, an SIL-20AC HT autosampler, and a CTO-20A column oven. The injection volume was set at 25 µL. The AEOs and APEOs were separated on a Waters Acquity UPLC BEH C18 column (1.7 µm, 2.1 x 50 mm). Mobile phase A was 2 mM ammonium acetate in water, and mobile phase B was 2 mM ammonium acetate in acetonitrile. The initial mobile phase composition was 50% B, which was ramped up to 100% B over 8

min and held for 3 min. The mobile phase composition was then brought back to 50% B over 1 min and held there to equilibrate for 3 min prior to the next injection. The flow rate was 0.3 mL/min, and the column temperature was maintained at 30°C. During the first 2 min of the gradient, the mobile phase was redirected to waste and not to the mass spectrometer.

The AB Sciex software Analyst version 1.5.2 was used for data acquisition and analysis. Because of the number of analytes (i.e., approximately 152) that were investigated within a relatively short amount of time (i.e., 11 min), scheduled multiple-reaction monitoring (sMRM) mode was performed on each of the individual ethoxymers for identification and quantitation purposes. The sMRM mode requires the user to program the data acquisition with the retention time of each analyte and an appropriate MRM detection window. The MS parameters for each individual ethoxymer were optimized to ensure the most favorable ionization and ion transfer conditions and attain optimum signal of both the precursor and product ions by infusing the analytes into 50% B at 0.3 mL/min and manually turning the parameters. The source parameters were identical for all of the analytes: curtain gas, 35 psi; IonSpray voltage, 5500 V; source temperature, 250°C; ion source gas 1 (nebulizer gas), 55 psi; ion source gas 2 (auxiliary gas), 25 psi; and the interface heater was on. The ESI probe *y*-axis was set to 9.5 mm, and the *x*-axis was positioned at 6.5 mm. The unique MS sMRM parameters for each ethoxymer are shown in Tables S2-S9, and the collision gas was set to a value of 7 for all of the analytes. General *m/z* values for the AEOs and APEOs investigated in this study are shown in Table 1.

The instrumental limit of detection (LOD) was defined as the minimum amount of compound analyzed in the LC-MS/MS that produced a signal-to-noise (S/N) ratio of 3. The instrumental limit of quantitation (LOQ) was defined as the minimum amount of compound that produced a S/N of 10.

2.4 Samples

Initially, ultrapure water was spiked with the analytes of interest, and the extraction recoveries (percent of standard added to sample that was recovered following extraction) of the analytes were evaluated. Once completed, four river water samples collected from the Colorado River in January 2013 and a drinking water sample from Colorado were evaluated for AEOs and APEOs. Each river water sample was also spiked with the analytes of interest to determine the extraction recoveries. Laboratory blanks and laboratory-fortified blanks were also evaluated to ensure that the analytical method and laboratory equipment were free from outside contamination and to compare recoveries.

2.5 Determination of the compositions of the $NPEO_x$, $OPEO_x$, $C_{16}EO_x$, and $C_{18}EO_x$ standards

The composition of the Neodol 25-9 AEO standard was supplied by Shell Chemical Co. (Table S1), but the compositions of the $NPEO_x$, $OPEO_x$, $C_{16}EO_x$, and $C_{18}EO_x$ standards were uncharacterized. For quantitative purposes, the ethoxymer distribution of every standard was needed. Therefore, the MS responses of the Neodol 25-9 AEO homologues were first determined as a function of the number of ethoxy units (n_{EO}) by dividing the raw response R of the signal of each ethoxymer by the mass m injected into the MS (the mol% values in Table S1 were first converted to %wt using the mass of each ethoxymer). Triplicate injections were used for the calculations, and the average R/m values were utilized. For each homologue (i.e., C_{12}, C_{13}, C_{14}, or C_{15}), the R/m values (R/m_{norm}) were normalized using Eq. 1:

$$R/m_{i,norm} = \frac{R_i/m_i}{\sum_{i=1}^{n}(R_i/m_i)} \times 100\% \qquad \text{Eq. 1}$$

where R_i is the response obtained for ethoxymer i, and m_i is the mass of i injected in pg.

For each n_{EO} value, the four R/m_{norm} values from the four homologues were averaged to arrive at a mean R/m_{norm} ($\overline{R/m_{i,norm}}$). Assuming that the predominant factor that contributed to the responses of ethoxylated compounds were the n_{EO} values, the $\overline{R/m_{i,norm}}$ values were used to calculate the ethoxymer

distributions of the $NPEO_x$, $OPEO_x$, $C_{16}EO_x$, and $C_{18}EO_x$ standards. This was accomplished using Eq. 2 for each value of n_{EO}:

$$\%EO_j = \frac{\frac{R_j}{R_j m_{Lnorm}}}{\sum_{i,j=1}^{n}\left(\frac{R_j}{R_j m_{Lnorm}}\right)} \times 100\% \qquad \text{Eq. 2}$$

where $\%EO_j$ is the %wt of the NP, OP, C_{16}, or C_{18} ethoxymer j, and R_j is the response of j. This calculation was performed using triplicate injections of the uncharacterized AEO and APEO standards to arrive at mean $\%EO$ values for each ethoxymer.

2.6 Stability studies

The stabilities of the ethoxylates were investigated over a period of four weeks to determine appropriate holding times for the analytes. A stock solution of ethoxylates was prepared in ultrapure water and stored at 4°C, and aliquots of the water sample were periodically sampled and analyzed using LC-MS/MS.

2.7 Quantitation

The compounds were identified by their retention times and their specific MRM transitions. Quantitation was performed using external quantitation with standard solution mixtures. Calibration standards were prepared at nine calibration levels that ranged from low to high over a factor of 40. The calibration standards contained 100-4000 µg/L Neodol 25-9 and 25-1000 µg/L of Triton X-100, $NPEO_x$, $C_{16}EO_x$ and $C_{18}EO_x$, from which the concentrations of the individual ethoxymers were known based on their %wt values. Standards and MeOH blanks were injected periodically to ensure that the instrument

response was not drifting and that the blanks were free of analytes. The concentrations were not corrected for the SPE recovery rates.

2.8 Quality Assurance and Quality Control

Rigorous quality assurance was applied throughout this research as required by the EPA Hydraulic Fracturing Quality Management Plan (QMP) [25]. During the course of this research, technical systems audits, audits of data quality, and audits of data usability were performed as described in the QMP and the quality assurance project plan associated with this research [26].

3. Results and Discussion

3.1 Selection of extraction conditions

Different extraction conditions have previously been suggested for optimum recoveries of AEOs, such as liquid-liquid extraction [2] and C18 [1,20], C8 [23], C2+SAX+SCX [3,18], graphitized carbon black [5-6], and HLB SPE [19-20], but SPE conditions vary from study to study. Therefore, we initially compared the extraction recoveries between various SPE cartridges and elution conditions to determine the optimal SPE conditions for ethoxylated alcohols and ethoxylated alkylphenols (Fig. 1). The different types of SPE cartridges investigated in this study included Oasis HLB, glass Enviro-Clean divinylbenzene, glass Enviro-Clean C18 (both endcapped and unendcapped), and glass Enviro-Clean C8 (endcapped) cartridges. The different solvents used to desorb the analytes from the SPE cartridges included MeOH, 90:10 MTBE/MeOH, DCM, and 60:40 ACN/IPA. The volume of solvent used for elution remained constant at 10 mL. As shown in Fig. 1, the extraction recoveries from the HLB cartridges using 90:10 MTBE/MeOH as the elution solvent were greater than those from any other cartridge for nearly all the ethoxylate homologues (with exception to unendcapped C18 SPE cartridges, which produced highest recovery of C_{18} ethoxylates). While it is preferable to select SPE conditions that

selectively extract only the analytes of interest, in this case the general-purpose HLB cartridges produced the highest recoveries. The HLB cartridges consist of a copolymer of *N*-vinylpyrrolidone-divinylbenzene, and they are also applicable for the extraction of a broad range of compounds, especially polar compounds, making their utilization advantageous for nontargeted compounds as well. For example, the alkylphenols NP and OP can also be extracted using the HLB media; however, glass cartridges must be utilized because NP and OP have been observed in our lab as contaminants in the standard polypropylene HLB cartridges. No increases in extraction recoveries were gained from adjusting the sample pH to acidic (pH = 3) or basic (pH = 11) conditions prior to SPE; therefore, a pH of 7 was maintained in all future extractions. Additionally, Sep Pak tC18 SPE cartridges (Waters) were briefly investigated, but were shown to result in comparable recoveries to the C18 cartridges shown in Fig. 1 (data not shown).

3.2 HPLC-MS/MS conditions

Ammonium acetate mobile phases were chosen because of the preferential formation of the ammonium adducts ($[M+NH_4]^+$) of the ethoxylate species. Mobile phases containing formic acid as the modifier were also investigated for the analysis of ethoxylated alcohols, but the predominant ionized species in these cases were the sodium adduct ($[M+Na]^+$) of the analytes of interest, not the desired $[M+H]^+$, due to the ubiquitousness of sodium. The $[M+Na]^+$ is much more stable of an ionized species and is not as susceptible to MS/MS fragmentation [5,22]. Therefore, the $[M + NH_4]^+$ adducts were generated through the addition of ammonium acetate to the mobile phases. This was preferred due to the ability to perform MS/MS, which allowed greater selectivity of the analytes of interest. All ethoxymers were ionized except for those with 0 or 1 ethoxy units (n_{EO} = 0-1), which was the only disadvantage to the lack of derivatization involved with this method. The derivatization technique developed by Dunphy et al. [24] imparts a cationic charge on the molecules, enabling the ionization of ethoxymers with n_{EO} = 0-1.

A representative chromatogram is shown in Fig. 2. The AEOs were predominantly separated by the length of their alkyl chain. The reversed-phase C18 HPLC column retained the longer alkyl chains more effectively, and so the order of elution with regard to retention time was $OPEO_x$ < $NPEO_x$ < $C_{12}EO_x$ < $C_{13}EO_x$ < $C_{14}EO_x$ < $C_{15}EO_x$< $C_{16}EO_x$ < $C_{18}EO_x$. If lower-carbon alkyl chain ethoxylates (e.g., C_{10} or C_{11} ethoxylates) were also investigated, they would most likely overlap with the $OPEO_x$ and $NPEO_x$ chromatographic peaks. Longer, more gradual elution gradients would more efficiently separate the various ethoxylate homologues, but a short gradient was desired for higher analytical throughput. The chromatographic peaks were not completely Gaussian in shape due to the presence of various isomers. To the best of our knowledge, the 11-min gradient is the shortest gradient used for the analyses of a range of AEOs and APEOs simultaneously. For example, Eadsforth et al. [3] utilized a gradient that was longer than 60 min to monitor AEOs, while Cohen et al. [2], Morrall et al. [18], and Loos et al. [19] used gradients of 30 min. Recently González et al. [27] and Lara-Martín et al. [20] utilized 13-min and 11-min gradients, respectively, for the analysis of ethoxylates, essentially the same gradients as the 11-min gradient used here.

Because the structures of the AEOs and APEOs are related and are quite similar, many of the product ions produced during the collision-induced dissociation (CID) process among the various AEOs and APEOs were identical. For example, the *m/z* values of the product ions corresponding to EO_1, EO_2, EO_3…EO_x were predominant and were commonly found in all MS/MS spectra. For the purposes of this study, the MRM transitions were chosen to be as selective as possible. Specifically, the loss of ammonia during the $[M+NH_4]^+ > [M+H]^+$ transition was monitored for each analyte. It should be noted that the $[M+NH_4]^+ > [M+H]^+$ transition was not the most sensitive reaction to monitor during the MRM experiment; however, it produced product ions that were unique to the precursor ions from which they originated so that there could be no significant quantitative influences from ion carryover.

When dealing with large numbers of analytes, conventional MRM experiments, in which the MS continually cycles through the entire list of analytes while monitoring each individual MRM transition,

either are not fast enough to capture a sufficient number of data points across each analyte's chromatographic peak, or they suffer from losses in sensitivity due to the speed of the duty cycle. In this study, sMRM was performed to ensure that an adequate number of data points across the peaks (i.e., > 10) were collected at a sufficient sensitivity. The sMRM mode requires the user to program the data acquisition with the specific retention time of each analyte and an appropriate detection window. Using the sMRM feature, more than 20 data points were typically collected across each peak, allowing the peaks to be accurately defined. Newer MS instrumentations generally possess the ability to perform sensitive MRM experiments at fast enough speeds for processing large numbers of analytes, potentially making sMRM unnecessary. However, with the 152 compounds that were investigated here, the sMRM feature was necessary.

3.3 Determination of response as a function of ethoxymer

The normalized response as a function of n_{EO} for the AEOs was investigated using Eq. 1, and the plot is shown in Fig. 3A. These values were calculated by dividing the response of the instrument by the amount of each analyte injected (based on the supplied composition of the Neodol 25-9) and then normalizing to 100% for each AEO homologue. The RSD values of the normalized data points in Fig. 3A were less than 10% for n_{EO} = 3-19. The RSD values of the normalized data points for $C_{12}E_{20}$, $C_{14}E_2$, $C_{14}E_{20}$, and $C_{15}E_{20}$, however, were 31%, 12%, 10%, and 18%, respectively. The highest sensitivity per amount of AEO was observed for AEOs with n_{EO} = 3-5 (Fig. 3A). The normalized plots of the four AEO homologues were virtually identical; therefore, the length of the alkyl chain from C_{12} to C_{15} did not appear to contribute to any differences in sensitivity. Contributing factors most likely included the ionization efficiency and fragmentation efficiency, which appeared to depend considerably on the number of ethoxy units present in the molecule. Whether this observation was based solely on the size of the molecule, the additional ethoxy units themselves contributing to chemical and/or folding effects [28], or a combination

of both was not investigated. Additionally, the sensitivity as a function of n_{EO} is likely instrument-specific, as Crescenzi et al. [29] investigated the sensitivity for the C_{12} AEO homologue and found that the sensitivity peaked at n_{EO} = 8. Regardless of the variations from instrument to instrument, the observation that the sensitivity changes dramatically as n_{EO} increases or decreases highlights the fact that the accuracy of quantitation of AEOS and APEOS will suffer if calibration curves are not based on each individual ethoxymer, including the cases in which only one ethoxymer standard is used to calibrate for every ethoxymer within a given homologue class [2] or, more commonly, those in which the quantitation is based on the "total" amounts of AEOs and APEOs in the calibrations standards [20,27,29 ,30]. The use of calibration standards to determine the "total" amounts of AEOs and APEOs in samples would only be accurate if the distributions of the AEO and APEO ethoxymers are similar between the standards and the samples; however, the distributions of AEO and APEO ethoxymers in standards likely differ from those in real-world samples.

The compositions of the Triton X-100 ($OPEO_x$), $NPEO_x$, $C_{16}EO_x$, and $C_{18}EO_x$ standards were determined by applying the normalized values of the $C_{12-15}EO_x$ standards to the responses of the uncharacterized ethoxylate standards, which was accomplished by assuming that the same factors responsible for influencing the sensitivity differences in the C_{12}-C_{15} AEOs were similar to those in the uncharacterized ethoxylates. The distributions of the four C_{12}-C_{15} homologues and the calculated distributions of the uncharacterized AEO and APEO homologues are shown in Fig. 3B. While the actual amounts of each of the four Neodol 25-9 homologues (i.e., C_{12}-C_{15}) varied, the normalized distributions of the AEOs were nearly identical in shape. Each $C_{12-15}EO_x$ distribution was centered at approximately n_{EO} = 11 (Fig. 3B), which had no relationship to the MS sensitivity of each ethoxymer (Fig. 3A). The distribution of the $OPEO_x$ in the Triton X-100 was more narrow than those of the AEOs from the Neodol 25-9; however, the distribution of $OPEO_x$ was centered at approximately n_{EO} = 10, which was similar to the AEOs. The amounts of OP ethoxymers in the Triton X-100 decreased sharply from the apex of the distribution (beyond n_{EO} = 8-12), and ethoxymers with $n_{EO} < 4$ and $n_{EO} > 18$ were especially low in

abundance. The distribution of NP ethoxymers from the POE (20) nonylphenol standard was shifted far to the right towards higher n_{EO} values and was centered at approximately n_{EO} = 16. Additionally, the lack of NP ethoxymers with n_{EO} < 7 meant that it would be especially difficult to quantitate any NP ethoxymers with n_{EO} < 7. Therefore, to ensure accurate quantitation at these lower n_{EO} values, a more appropriate $NPEO_x$ standard, Tergitol NP-10, was chosen for a calibration standard (Fig. 3B). The ethoxymer distributions of the Tergitol NP-10, $C_{16}EO_x$, and $C_{18}EO_x$ were centered at approximately n_{EO} = 10 (Fig. 3B).

3.4 Calibration, LOD values, and LOQ values

Calibration curves for most analytes were linear over a factor of 40 (i.e., from 1x to 40x) with r^2 values greater than 0.99. Because the individual ethoxymer analytes within the ethoxylate mixtures varied, the concentrations of each ethoxymer in the calibrations standard mixtures were unique. For example, the calibration standard concentrations of $C_{12}E_{11}$ and $C_{12}E_{14}$ ranged from 1.7-66.8 ppb and 1.2-48.4 ppb, respectively. At $n_{EO} \geq 18$, the curves were more likely to not include some of the lower calibration levels due to sensitivity issues. However, nine calibration standards were used to construct the calibration curves, so if a few of the lower calibration levels were not used due to poor data quality, the curves could still be generated with at least 5-6 data points. Also, for NPE_{2-3} and OPE_{2-3}, calibration curves were unable to be generated due to the low abundances of these ethoxymers in the standards that were used (see Fig. 3B).

LOD and LOQ values were next determined for the C_{12-15} AEOs and the APEOs, as shown in Tables 2 and 3. LOD values increased with increasing n_{EO}, which was also observed by Loyo-Rosales et al. [21] for $NPEO_x$. This is due to the larger molecules being more resistant to the CID process, as their larger size and higher number of vibrational degrees of freedom enable the molecules to better resist fragmentation. The LOD values ranged from 3-49 pg for C_{12}, 5-75 pg for C_{13}, 2-76 pg for C_{14}, 1-51 pg for

C_{15}, 1-80 pg for OP, and < 0.1-300 pg for NP ethoxylates, while the LOQ values ranged from 8-98 pg, 13-110 pg, 13-110 pg, 2-110 pg, 4- > 80 pg, and 0.2-800 pg, respectively. Theoretically, LOQ values should be approximately 3.3 times that of LOD values. For the most part, the observed LOQ values were greater than 3 times the LOD values.

3.5 Extractions in clean matrices

Extractions were performed from ultrapure water to investigate the extraction reproducibility in clean matrices. The extraction recoveries for C_{12}, C_{13}, C_{14},C_{15}, C_{16}, C_{18}, OP, and NP ethoxylates averaged 65%, 58%, 55%, 49%, 41%, 37%, 68%, and 69%, respectively, in DI water (n = 5). As the alkyl chains increased in length, the extraction recovery decreased. This effect of decreasing recovery with increasing alkyl chain length has been observed before [30] and has been attributed to the increasing hydrophobicity of the longer alkyl chains. The precisions of the extraction efficiencies, measured as the RSD, for C_{12}, C_{13}, C_{14},C_{15}, C_{16}, C_{18}, OP, and NP ethoxylates ranged from 14-17%, 18-23%, 16-19%, 15-18%, 13-14%, 13-23%, 8-10%, and 13-14%, respectively, and averaged 16%, 20%, 18%, 16%, 14%, 17%, 9%, and 13%, respectively. These estimates of the precision of the extraction recovery are comparable to other studies in the literature [24,30].

3.6 Stability studies

To determine appropriate holding times for the ethoxylates, a water sample spiked with a known concentration of ethoxylates and stored at 4°C was periodically sampled and analyzed using LC-MS/MS to test for degradation of the analytes (Fig. 4). For simplicity, only n_{EO}=7-11 were investigated for each homologue. It became apparent from the data that degradation was a considerable issue, especially with the C_{14}, C_{15}, C_{16}, and C_{18} ethoxylates. The rate of degradation increased as the length of the alkyl chain increased and was not as significant for the OP, NP, C_{12}, and C_{13} ethoxylates. Specifically, after 28 days, an average of 91%, 80%, 78%, and 75% of the original amounts of OP, NP, C_{12}, and C_{13} ethoxylates, respectively, remained (Fig. 4A-D). In contrast, only 43%, 15%, 8%, and 14% of the original amounts of

C_{14}, C_{15}, C_{16}, and C_{18} ethoxylates, respectively, remained after 28 days (Fig. 4E-H). The decrease in concentration as a function of time appeared to be linear for the OP, NP, and C_{12-14} ethoxylates, in contrast to the C_{15}, C_{16}, and C_{18} ethoxylates. No source of microorganisms was used to intentionally inoculate the sample, and AEOs are not expected to undergo abiotic degradation processes [8]. Because the flask used to store the sample had not been sterilized prior to use, aerobic biodegradation was the probable cause of the loss of analyte. Previous studies have also highlighted the rapid biodegradation of ethoxylated alcohols in environmental samples [17,31-34], with half-lives ranging from 1.3-1.5 days for C_{12} and C_{16} ethoxylates at 25°C in river water [31]. While most of the AEOs and APEOs did not degrade as quickly as the results from previous biodegradation studies, it was clear that the stabilities of the different ethoxylates in water matrices were poor and that samples must be extracted immediately after sampling.

3.7 AEOs and APEOs in water samples

Four water samples collected along the Colorado River labeled A-D and a drinking water sample (sample E) were obtained and were analyzed for the presence of AEOs and APEOs. The pH in each of the samples was approximately 7. The samples were extracted, and the extraction recoveries were estimated by spiking additional aliquots of sample with the analytes of interest and subtracting the measured amount in the unspiked samples from the measured amount in the spiked samples. Certain C_{12} and C_{14} ethoxymers were identified in the laboratory blanks at considerable concentrations (i.e., > 10 ng/L $C_{12}EO_{7-12}$ and > 30 ng/L $C_{14}EO_{6-12}$); therefore, the concentrations of C_{12} and C_{14} ethoxylates were not determined in the river water samples or the drinking water sample. The extraction recoveries of the AEOs and APEOs from samples A, B, C, D, and E averaged 45%, 43%, 30%, 37%, and 46%, respectively, which were slightly lower than the extraction recoveries from ultrapure water. The average recoveries of OP, NP, C_{13}, C_{15}, C_{16}, and C_{18} ethoxylates among the five samples were 39 ± 12%, 42 ± 9%, 45 ± 10%, 41 ± 12%, 33 ± 12%, and 39 ± 8%, respectively (mean ± SD). Again, these average values were slightly lower than what was observed in ultrapure water. The recovery of the surrogate standard

$C_{10}EO_6$, which was also added to each sample, ranged from 91-122%. The higher extraction recoveries of the surrogate were due to the lower carbon-containing alkyl chain. The measured values were not corrected for extraction recoveries, based on guidelines from IUPAC for correcting for recoveries [35]. While the extraction recoveries were determined by spiking the analytes into the samples, the absolute extraction recoveries might have been biased low due to degradation, as the extractions of the spiked samples were not conducted until 5 days after the extractions of the unspiked samples.

Plots of the measured ethoxylate concentrations in the river water and drinking water samples are shown in Fig. 5A-D and Fig. 5E, respectively. Most of the concentrations of the individual ethoxymers were in the low ng/L range, typically 1-15 ng/L. However, the drinking water sample contained significant levels of $NPEO_{4-11}$ between 20-60 ng/L, despite having negligible concentrations of all other ethoxylates (Fig. 5E). The total amount of all NP ethoxymers measured approximately 350 ng/L in the drinking water. Sample A and sample B contained as high as 20 ng/L $NPEO_4$ and $OPEO_{9-12}$, respectively (100 and 170 ng/L total NP ethoxylates and total OP ethoxylates, respectively), while also measuring low concentrations of the other ethoxylates (Fig. 5A-B). Sample C contained low levels of all the ethoxylates (Fig. 5C), while sample D did not measure levels of ethoxylates that were significantly different from the laboratory blank (Fig. 5D). The predominant species observed in most samples were the OP and NP ethoxylates, which were the ethoxylate species that degraded slowest during the stability studies (see above). The APEOs are generally considered more toxic than the AEOs, as NP ethoxylates have been shown to degrade to nonylphenol, an endocrine-disrupting compound [10,11]. The C_{13} and C_{15} ethoxylates were for the most part measured at very low concentrations, i.e., < 5 ng/L, as were the C_{16} and C_{18} ethoxylates, except for samples B and C in which between 10-15 ng/L C_{18} ethoxylates were determined.

4. Conclusions

The development of a method for the rapid, sensitive detection and quantitation of AEOs and APEOs was described. The range of n_{EO} that could be detected with the LC-MS/MS system in this work ranged from 2 to 20, with LOD values for most ethoxymers in the low pg range without requiring derivatization. The LC-MS/MS method allowed for the simultaneous analysis of 152 analytes within 11 min. The extraction recoveries of the AEOs and APEOs in clean matrices and river water samples ranged from 37-69% and 39-45%, respectively. During the development of this method, a few key criteria necessary for the accurate quantitation of AEOs and APEOs became obvious. First, the selection of appropriate standards is crucial. For example, POE (20) nonylphenol was initially chosen in this work as the standard for $NPEO_x$; however, its ethoxymer distribution was shifted towards higher n_{EO} values than was desired, and quantitation at lower n_{EO} values proved challenging. Therefore,Tergitol NP-10 was substituted for the $NPEO_x$ standard. The known concentration of each ethoxymer is also necessary, as the various ethoxymers produced different responses that appeared to depend on the length of the molecule, or n_{EO}. Therefore, in this work, the compositions of C_{16}, C_{18}, NP, and OP ethoxylates were calculated for accurate quantitation, but the commercial availability of characterized reference standards would also be useful. Second, commercially available isotopically labeled standards are also desirable, as this would enable the use of isotope dilution approaches, making corrections for recovery more feasible. Third, contamination from other sources is problematic for ethoxylates, as they are quite ubiquitous in many cleaning products. For example, during the analysis of the Colorado River samples, we observed contamination from C_{12} and C_{14} ethoxylates, which prevented their analysis. It is unclear whether the use of a cleaning product containing these specific ethoxylates was used in the vicinity of our laboratory space or the glassware had become contaminated. Fourth, the degradation of the ethoxylates was shown to be a significant issue for certain ethoxylates, notably the $C_{14\text{-}18}$ ethoxylates. The use of preservation agents has been suggested in the literature when analyzing ethoxylates, however, Petrović and Barceló previously demonstrated that the stabilities of ethoxylates in aqueous matrices were poor even after using acid or formaldehyde as preservation agents [34]. Therefore, the best approach would be the analysis of samples immediately after sampling to prevent the loss of analyte. The method described here enabled the

analysis of AEOs and APEOs at ng/L levels. While only C_{12}-C_{16}, C_{18}, OP, and NP homologues were investigated here, this method is applicable for the C_8-C_{11} homologues as well.

Acknowledgements

The United States Environmental Protection Agency, through its Office of Research and Development, funded and managed the research described here. It has been subjected to the Agency's peer and administrative review and has been approved for publication. Mention of trade names or commercial products in this paper does not constitute endorsement or recommendation by the EPA.

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The authors thank Phil Dorn at Shell Chemical Company for providing the composition of the Neodol 25-9 and Tammy Jones-Lepp (EPA) for providing the water samples.

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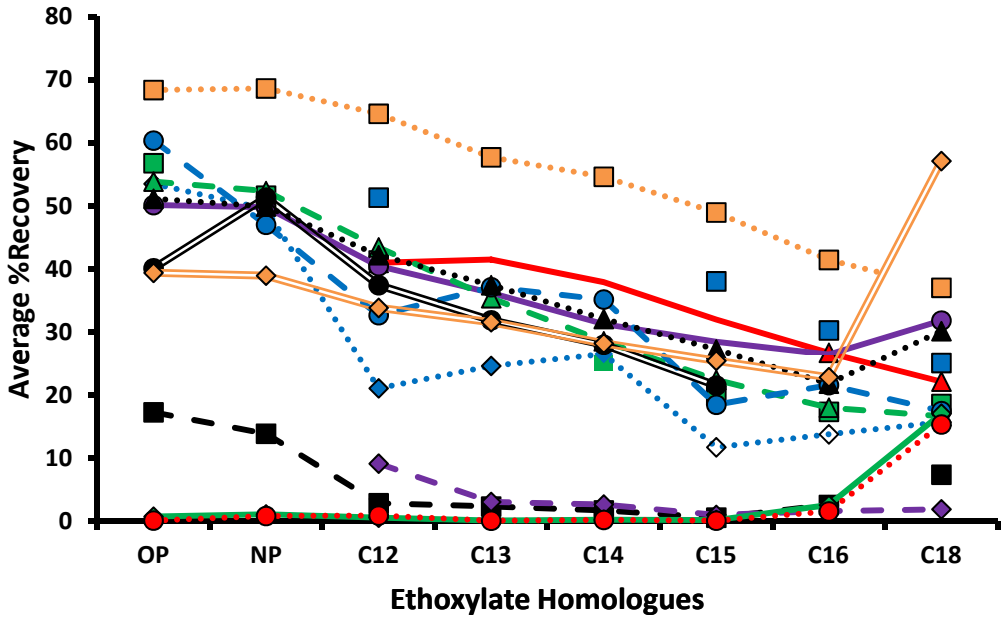

Figure 1. Various SPE conditions used to optimize the ethoxylate extraction recovery. Endcapped C18 (eC18), 9:1 MTBE/MeOH; eC18, MeOH; eC18, 6:4 ACN/IPA; endcapped C8 (eC8), 9:1 MTBE/MeOH; eC8, MeOH; eC8 6:4 ACN/IPA; endcapped divinylbenzene (eDVB), 9:1 MTBE/MeOH; eDVB, MeOH; eDVB, 6:4 ACN/IPA; HLB, DCM; HLB, 9:1 MTBE/MeOH; unendcapped C18 (uC18), MeOH; uC18, 6:4 ACN/IPA; uC18, DCM; uC18, 9:1 MTBE/MeOH.

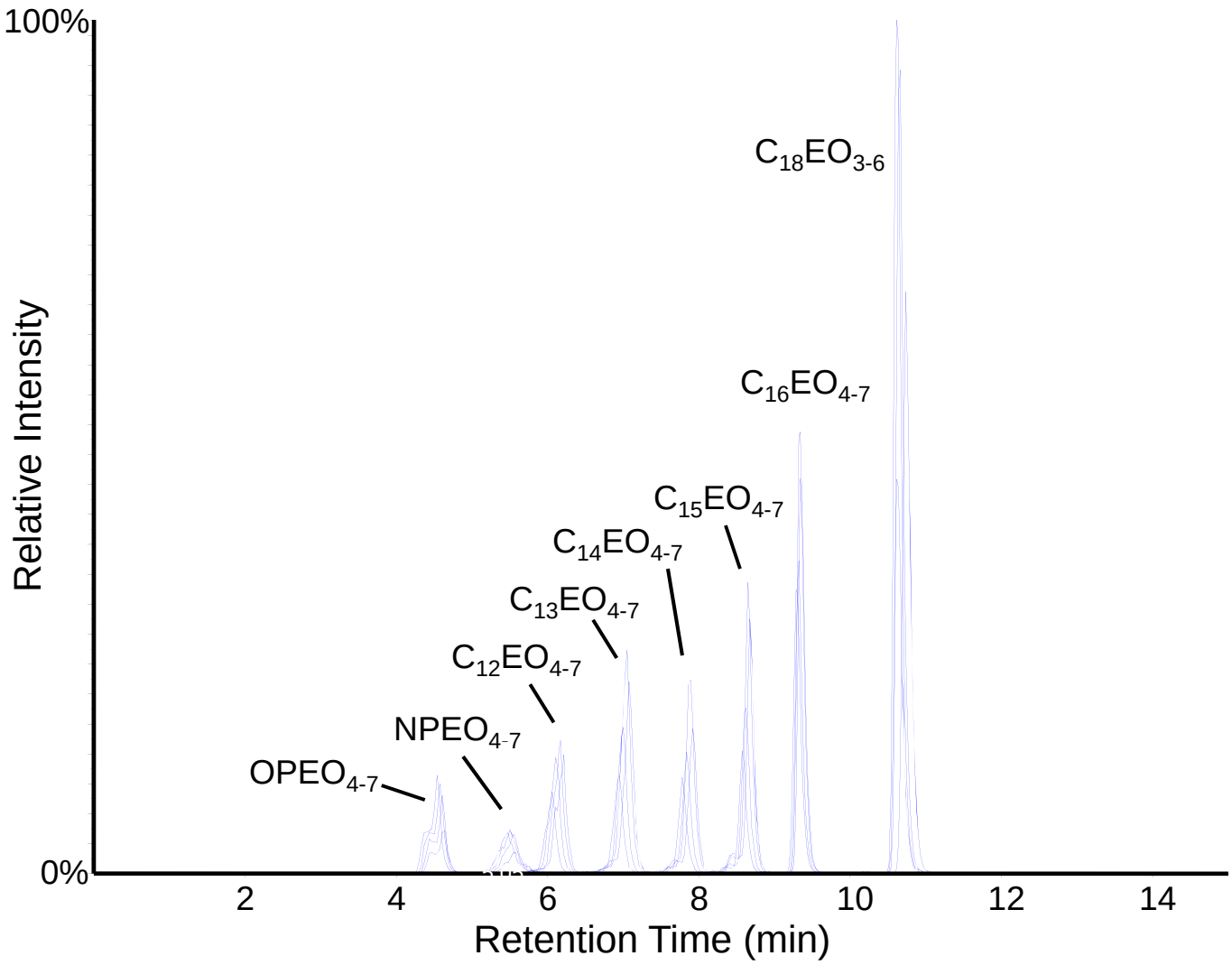

Figure 2. Representative chromatogram of a subset of the AEOs and APEOs investigated in this study. Only 4 ethoxymers from each homologue are shown in the chromatogram for better clarity. The ethoxylated compounds were predominantly separated as a function of the length of the alkyl or alkylphenol chain preceding the ethoxy groups, but minor differences in the RTs were observed as a function of ethoxymer.

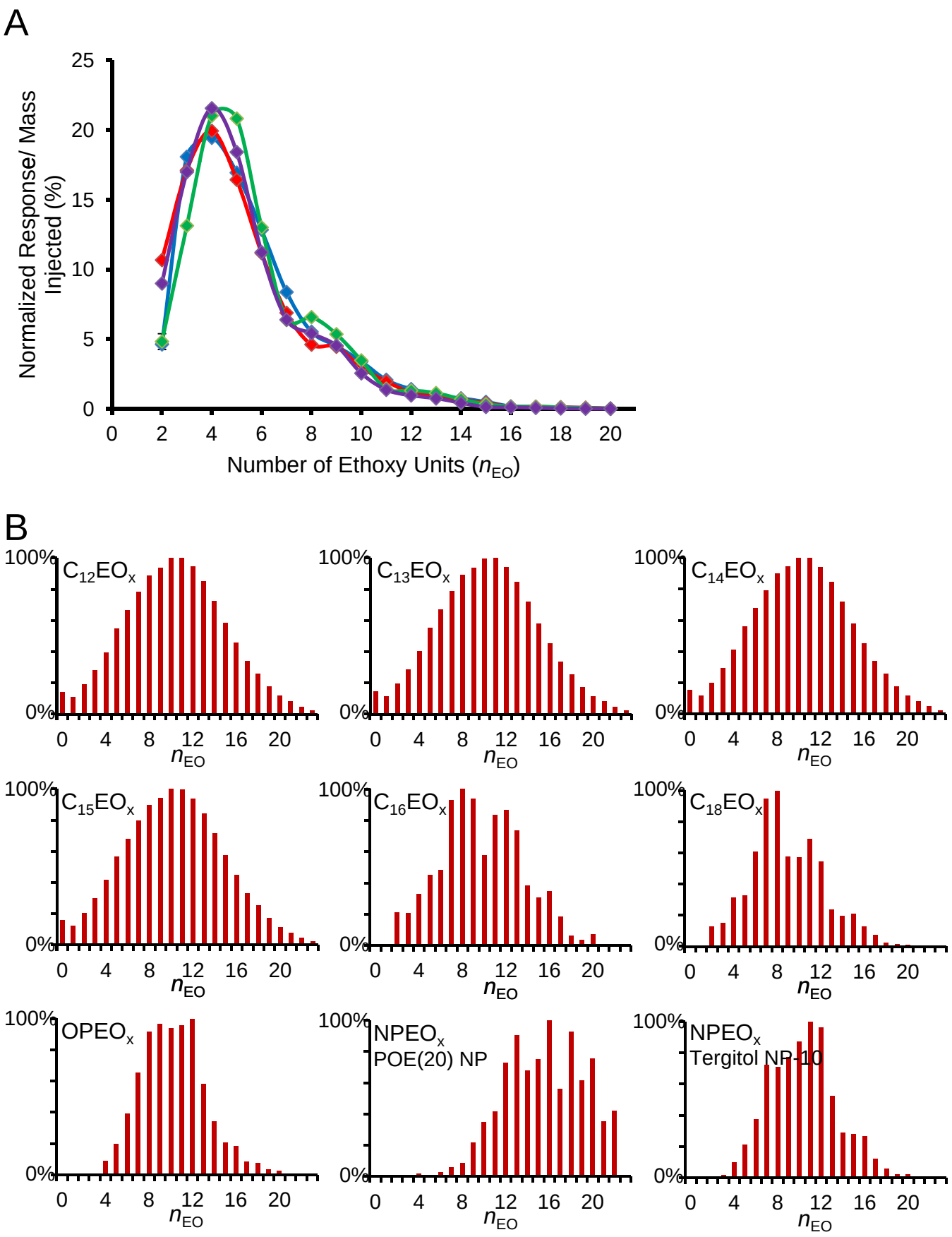

Figure 3. (A) Response as a function of ethoxymer. Error bars represent 1 standard deviation (n=3). Blue = C12, Red= C13, Green = C14, Purple = C15. (B) Distributions of AEO and APEO homologues. The C16, C18, OP, and NP distributions were calculated based on the C12-15 responses as a function of ethoxymer. The OPEOx was from Triton X-100 solution, and the NPEOx, C16EOx, and C18EOx were from technical mixtures. The POE(20) NP standard composition was shifted towards higher nEO, making quantitation of NP ethoxymers with lower nEO values difficult.

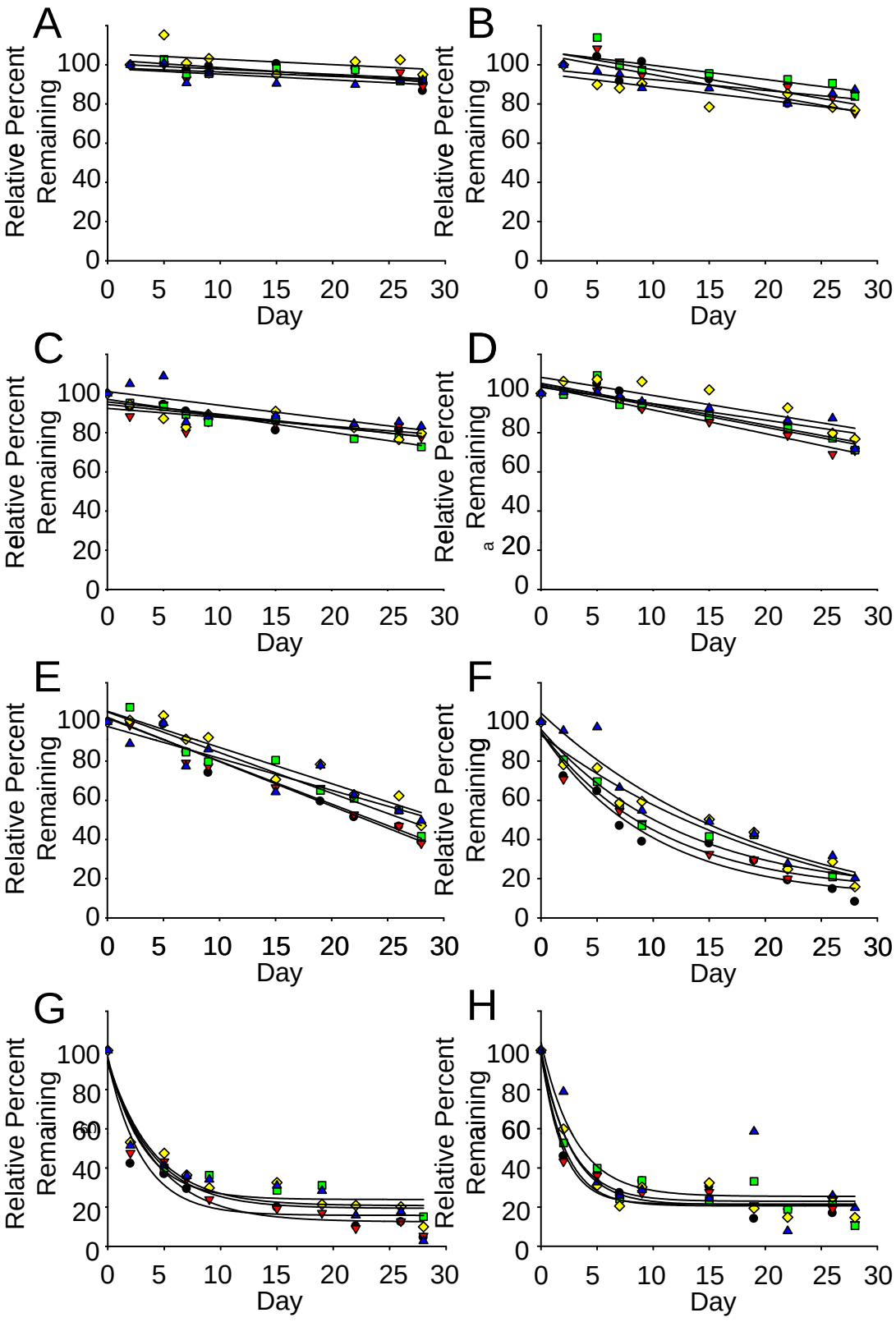

Figure 4. Stability studies of (A) $OPEO_x$, (B) $NPEO_x$, (C) $C_{12}EO_x$, (D) $C_{13}EO_x$, (E) $C_{14}EO_x$, (F), $C_{15}EO_x$, (G) $C_{16}EO_x$, (H) and $C_{18}EO_x$ over 28 days in water at 4°C.

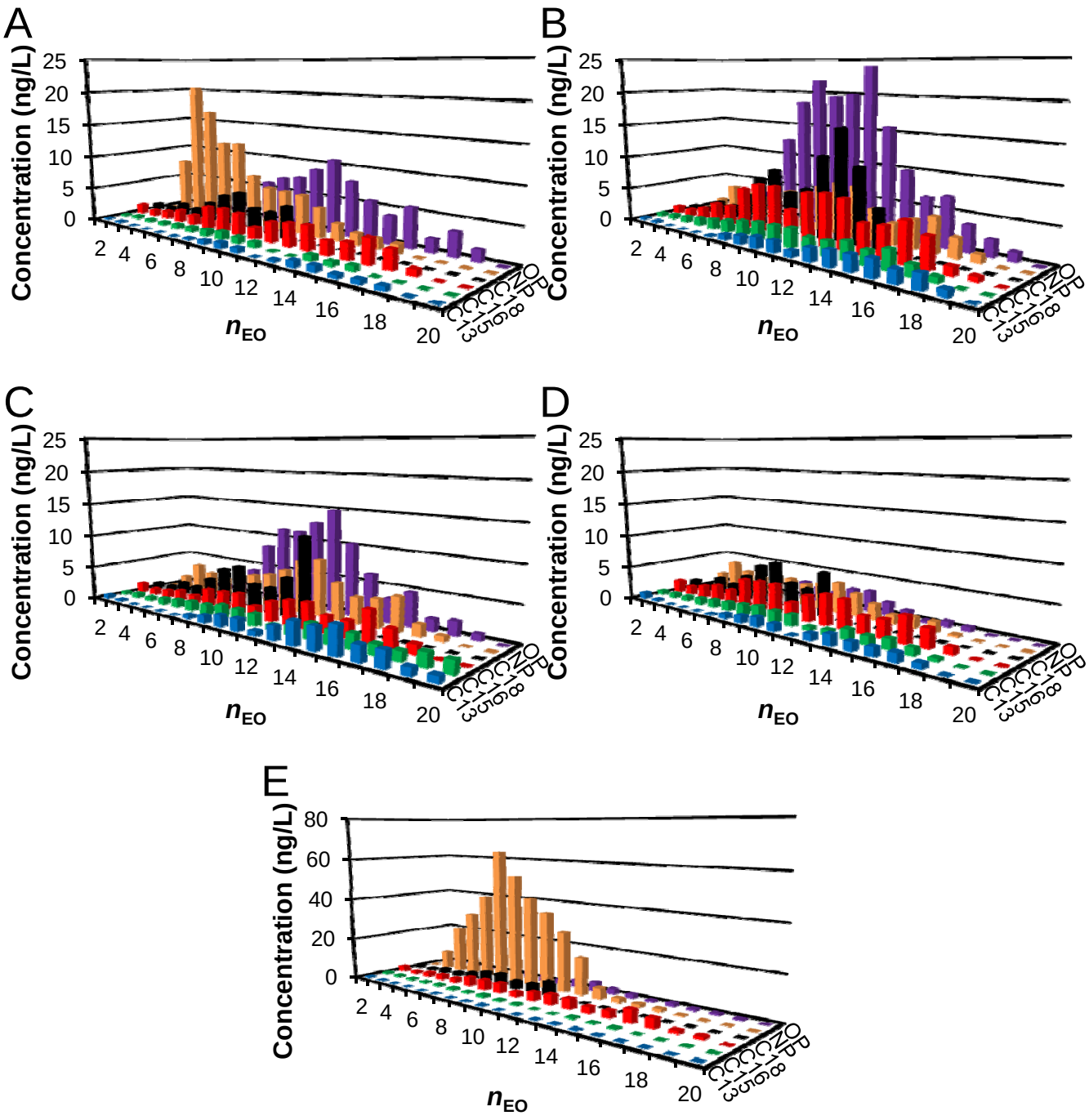

Figure 5. Measured values of AEOs and APEOs in Colorado River water samples A-D and drinking water sample E. The scale of the y-axis for sample E is different from the scale of samples A-D The most prevalent species that were observed were the APEOs. The total $NPEO_x$ in sample A measured approximately 100 ng/L, while sample B contained approximately 170 ng/L total $OPEO_x$, both in addition to low concentrations of other ethoxylates (i.e., C_{16} and C_{18}). Sample C contained low concentrations of multiple ethoxylates (i.e., C_{16}, C_{18}, NP, and OP), and D contained negligible concentrations of most ethoxylates. The drinking water sample E contained high concentrations of $NPEO_x$, in total approximately 350 ng/L.

Table 1. *m/z* values of AEO and APEO analytes.

Compounds	Number of ethoxy units	Precursor $[M+NH_4]^+$ ion *m/z*
$C_{12}EO_x$	n_{EO} =2-20	292, 336, $n \times 44$…1084
$C_{13}EO_x$	n_{EO} =2-20	306, 350, $n \times 44$…1098
$C_{14}EO_x$	n_{EO} =2-20	320, 364, $n \times 44$…1112
$C_{15}EO_x$	n_{EO} =2-20	334, 378, $n \times 44$…1126
$C_{16}EO_x$	n_{EO} =2-20	348, 392, $n \times 44$…1140
$C_{18}EO_x$	n_{EO} =2-20	376, 420, $n \times 44$…1168
$NPEO_x$	n_{EO} =2-20	326, 370, $n \times 44$…1118
$OPEO_x$	n_{EO} =2-20	312, 356, $n \times 44$…1104

Table 2. LOD and LOQ values for AEOs.

Cmpd	LOD (pg)	LOQ (pg)	Cmpd	LOD (pg)	LOQ (pg)	Cmpd	LOD (pg)	LOQ (pg)	Cmpd	LOD (pg)	LOQ (pg)
$C_{12}EO_2$	3	8	$C_{13}EO_2$	10	13	$C_{14}EO_2$	7	13	$C_{15}EO_2$	1	2
$C_{12}EO_3$	2	12	$C_{13}EO_3$	9	18	$C_{14}EO_3$	2	15	$C_{15}EO_3$	1	5
$C_{12}EO_4$	3	17	$C_{13}EO_4$	5	21	$C_{14}EO_4$	3	13	$C_{15}EO_4$	1	4
$C_{12}EO_5$	3	11	$C_{13}EO_5$	7	28	$C_{14}EO_5$	3	15	$C_{15}EO_5$	1	5
$C_{12}EO_6$	2	14	$C_{13}EO_6$	9	43	$C_{14}EO_6$	4	17	$C_{15}EO_6$	1	6
$C_{12}EO_7$	2	16	$C_{13}EO_7$	6	40	$C_{14}EO_7$	2	21	$C_{15}EO_7$	1	14
$C_{12}EO_8$	4	19	$C_{13}EO_8$	5	46	$C_{14}EO_8$	2	23	$C_{15}EO_8$	2	8
$C_{12}EO_9$	5	31	$C_{13}EO_9$	7	48	$C_{14}EO_9$	2	49	$C_{15}EO_9$	2	8
$C_{12}EO_{10}$	7	33	$C_{13}EO_{10}$	8	51	$C_{14}EO_{10}$	5	65	$C_{15}EO_{10}$	5	22
$C_{12}EO_{11}$	8	33	$C_{13}EO_{11}$	13	51	$C_{14}EO_{11}$	8	65	$C_{15}EO_{11}$	5	22
$C_{12}EO_{12}$	8	32	$C_{13}EO_{12}$	30	48	$C_{14}EO_{12}$	25	61	$C_{15}EO_{12}$	17	33
$C_{12}EO_{13}$	14	36	$C_{13}EO_{13}$	27	54	$C_{14}EO_{13}$	44	55	$C_{15}EO_{13}$	19	38
$C_{12}EO_{14}$	15	30	$C_{13}EO_{14}$	23	46	$C_{14}EO_{14}$	47	94	$C_{15}EO_{14}$	25	64
$C_{12}EO_{15}$	24	48	$C_{13}EO_{15}$	19	37	$C_{14}EO_{15}$	38	76	$C_{15}EO_{15}$	26	51
$C_{12}EO_{16}$	19	38	$C_{13}EO_{16}$	29	58	$C_{14}EO_{16}$	30	59	$C_{15}EO_{16}$	20	60
$C_{12}EO_{17}$	28	43	$C_{13}EO_{17}$	22	43	$C_{14}EO_{17}$	44	66	$C_{15}EO_{17}$	15	45
$C_{12}EO_{18}$	22	33	$C_{13}EO_{18}$	33	49	$C_{14}EO_{18}$	33	50	$C_{15}EO_{18}$	23	110
$C_{12}EO_{19}$	15	37	$C_{13}EO_{19}$	34	56	$C_{14}EO_{19}$	34	110	$C_{15}EO_{19}$	38	77
$C_{12}EO_{20}$	49	98	$C_{13}EO_{20}$	75	110	$C_{14}EO_{20}$	76	110	$C_{15}EO_{20}$	51	77

Table 3. LOD and LOQ values for APEOs.

Cmpd	LOD (pg)	LOQ (pg)	Cmpd	LOD (pg)	LOQ (pg)
$NPEO_2$	[a]	[a]	$OPEO_2$	[a]	[a]
$NPEO_3$	[a]	[a]	$OPEO_3$	[a]	[a]
$NPEO_4$	< 0.2	0.2	$OPEO_4$	1	4
$NPEO_5$	< 0.1	0.1	$OPEO_5$	2	6
$NPEO_6$	< 0.1	0.1	$OPEO_6$	3	6
$NPEO_7$	< 0.1	0.1	$OPEO_7$	2	6
$NPEO_8$	0.1	0.2	$OPEO_8$	3	9
$NPEO_9$	0.1	1	$OPEO_9$	3	9
$NPEO_{10}$	0.6	2	$OPEO_{10}$	3	6
$NPEO_{11}$	2	6	$OPEO_{11}$	3	6
$NPEO_{12}$	10	20	$OPEO_{12}$	7	10
$NPEO_{13}$	10	30	$OPEO_{13}$	6	20
$NPEO_{14}$	20	40	$OPEO_{14}$	10	20
$NPEO_{15}$	40	50	$OPEO_{15}$	9	20
$NPEO_{16}$	60	70	$OPEO_{16}$	20	30
$NPEO_{17}$	40	80	$OPEO_{17}$	7	10
$NPEO_{18}$	70	100	$OPEO_{18}$	10	60
$NPEO_{19}$	100	400	$OPEO_{19}$	10	30
$NPEO_{20}$	300	800	$OPEO_{20}$	80	[b]

[a]: Ethoxymer was not present at detectable levels in standards
[b]: LOQ was greater than the levels that were tested.

Table S1. Mol% of AEO homologues in Neodol 25-9 (provided by Shell Chemical Company).

Number of ethoxy units (n_{EO})	C_{12} Homologue	C_{13} Homologue	C_{14} Homologue	C_{15} Homologue
0	0.741	1.111	1.111	0.741
1	0.470	0.705	0.705	0.470
2	0.680	1.020	1.020	0.680
3	0.870	1.305	1.305	0.870
4	1.078	1.617	1.617	1.078
5	1.326	1.989	1.989	1.326
6	1.453	2.179	2.179	1.453
7	1.558	2.336	2.336	1.558
8	1.625	2.437	2.437	1.625
9	1.585	2.377	2.377	1.585
10	1.568	2.352	2.352	1.568
11	1.468	2.202	2.202	1.468
12	1.300	1.950	1.950	1.300
13	1.105	1.658	1.658	1.105
14	0.889	1.333	1.333	0.889
15	0.680	1.020	1.020	0.680
16	0.505	0.757	0.757	0.505
17	0.358	0.537	0.537	0.358
18	0.260	0.390	0.390	0.260
19	0.170	0.254	0.254	0.170
20	0.109	0.163	0.163	0.109
21	0.073	0.110	0.110	0.073
22	0.040	0.060	0.060	0.040
23	0.019	0.029	0.029	0.019

Table S2. Table of sMRM parameters for C12 AEOs.

Compound	Precursor m/z	Fragment m/z	RT[a]	DP[b] (V)	EP[c] (V)	CE[d] (V)	CXP[e] (V)
C12EO2	292.3	275.3	5.5	46	10	11	10
C12EO3	336.3	319.3	5.5	51	10	13	10
C12EO4	380.3	363.3	5.4	56	10	15	10
C12EO5	424.4	407.4	5.4	66	10	17	10
C12EO6	468.4	451.4	5.3	81	10	19	12
C12EO7	512.4	495.4	5.3	71	10	21	14
C12EO8	556.4	539.4	5.2	96	10	23	16
C12EO9	600.5	583.5	5.2	91	10	25	10
C12EO10	644.5	627.5	5.1	101	10	26	10
C12EO11	688.5	671.5	5.0	111	10	27	10
C12EO12	732.5	715.5	5.0	86	10	29	10
C12EO13	776.6	759.6	4.9	90	10	30	11
C12EO14	820.6	803.6	4.9	90	10	30	12
C12EO15	864.6	847.6	4.8	90	10	32	13
C12EO16	908.7	891.7	4.8	95	10	33	14
C12EO17	952.7	935.7	4.8	95	10	34	14
C12EO18	996.7	979.7	4.7	80	15	35	16
C12EO19	1040.7	1023.7	4.7	65	15	36	17
C12EO20	1084.7	1067.7	4.7	64	15	35	18

[a]RT = retention time
[b]DP = declustering potential
[c]EP = entrance potential
[d]CE = collision energy
[e]CXP = collision cell exit potential

Table S3. Table of sMRM parameters for C13 AEOs.

Compound	Precursor m/z	Fragment m/z	RT[a]	DP[b] (V)	EP[c] (V)	CE[d] (V)	CXP[e] (V)
C13EO2	306.3	289.3	6.5	41	6	11	8
C13EO3	350.3	333.3	6.5	48	10	14	10
C13EO4	394.3	377.3	6.5	50	14	16	11
C13EO5	438.4	421.4	6.4	57	8	18	10
C13EO6	482.4	465.4	6.4	64	9	20	10
C13EO7	526.4	509.4	6.4	72	13	22	10
C13EO8	570.5	553.5	6.4	85	10	23	10
C13EO9	614.5	597.5	6.3	87	10	25	10
C13EO10	658.5	641.5	6.3	90	10	26	10
C13EO11	702.5	685.5	6.2	95	10	28	10
C13EO12	746.6	729.6	6.1	100	10	29	11
C13EO13	790.6	773.6	6.1	95	10	30	12
C13EO14	834.6	817.6	6.0	105	10	31	13
C13EO15	878.6	861.6	6.0	95	10	33	14
C13EO16	922.7	905.7	5.9	95	10	34	15
C13EO17	966.7	949.7	5.9	75	10	35	16
C13EO18	1010.7	993.7	5.9	70	10	36	17
C13EO19	1054.7	1037.7	5.8	60	10	38	18
C13EO20	1098.8	1081.8	5.8	50	10	39	19

[a]RT = retention time
[b]DP = declustering potential
[c]EP = entrance potential
[d]CE = collision energy
[e]CXP = collision cell exit potential

Table S4. Table of sMRM parameters for C14 AEOs.

Compound	Precursor m/z	Fragment m/z	RT[a]	DP[b] (V)	EP[c] (V)	CE[d] (V)	CXP[e] (V)
C14EO2	320.3	303.3	7.5	68	14	12	8
C14EO3	364.3	347.3	7.5	73	11	14	10
C14EO4	408.4	391.4	7.5	68	12	16	10
C14EO5	452.4	435.4	7.4	72	10	18	10
C14EO6	496.4	479.4	7.4	76	10	20	10
C14EO7	540.5	523.5	7.3	78	10	22	10
C14EO8	584.5	567.5	7.3	90	10	24	10
C14EO9	628.5	611.5	7.3	95	10	25.5	10
C14EO10	672.5	655.5	7.2	101	10	27	10
C14EO11	716.6	699.5	7.1	104	10	29	10
C14EO12	760.6	743.6	7.1	105	10	29	11
C14EO13	804.6	787.6	7.0	90	9	30	12
C14EO14	848.6	831.6	7.0	96	8	31	13
C14EO15	892.7	875.7	7.0	100	10	33	14
C14EO16	936.7	919.7	6.9	98	13	34	15
C14EO17	980.7	963.7	6.9	70	10	35	16
C14EO18	1024.7	1007.7	6.8	70	10	35	17
C14EO19	1068.7	1051.8	6.8	50	10	37	18
C14EO20	1112.8	1095.8	6.7	50	10	38	19

[a]RT = retention time
[b]DP = declustering potential
[c]EP = entrance potential
[d]CE = collision energy
[e]CXP = collision cell exit potential

Table S5. Table of sMRM parameters for C15 AEOs.

Compound	Precursor m/z	Fragment m/z	RT[a]	DP[b] (V)	EP[c] (V)	CE[d] (V)	CXP[e] (V)
C15EO2	334.3	317.3	8.5	60	10	12	10
C15EO3	378.4	361.4	8.4	55	10	14	11
C15EO4	422.4	405.4	8.4	65	10	16	12
C15EO5	466.4	449.4	8.4	70	10	18	14
C15EO6	510.4	493.4	8.3	80	10	21	15
C15EO7	554.5	537.5	8.3	83	10	22	9
C15EO8	598.5	581.5	8.3	90	10	24	10
C15EO9	642.5	625.5	8.2	95	10	26	10
C15EO10	686.5	669.5	8.2	102	10	27	10
C15EO11	730.6	713.6	8.2	100	10	29	11
C15EO12	774.6	757.6	8.1	105	10	30	12
C15EO13	818.6	801.6	8.0	103	10	30	13
C15EO14	862.6	845.6	7.9	100	10	31	14
C15EO15	906.7	889.7	7.9	92	10	32	15
C15EO16	950.7	933.7	7.8	90	10	33	16
C15EO17	994.7	977.7	7.7	80	10	35	16
C15EO18	1038.8	1021.8	7.7	58	10	36	17
C15EO19	1082.8	1065.8	7.7	55	10	37	18
C15EO20	1126.8	1109.8	7.6	50	10	38	19

[a]RT = retention time
[b]DP = declustering potential
[c]EP = entrance potential
[d]CE = collision energy
[e]CXP = collision cell exit potential

Table S6. Table of sMRM parameters for $NPEO_x$.

Compound	Precursor m/z	Fragment m/z	RT[a]	DP[b] (V)	EP[c] (V)	CE[d] (V)	CXP[e] (V)
NPEO2	326.4	309.4	4.8	45	10	13	9
NPEO3	370.4	353.4	4.8	50	10	15	9
NPEO4	414.4	397.4	4.8	50	10	15	9
NPEO5	458.4	441.4	4.7	55	7	19	9
NPEO6	502.5	485.5	4.7	60	8.9	20	9
NPEO7	546.5	529.5	4.7	65	10	23	11
NPEO8	590.5	573.5	4.6	70	10	22	13
NPEO9	634.5	617.5	4.6	78	10	25	9
NPEO10	678.5	661.5	4.6	95	10	28	10
NPEO11	722.6	705.6	4.5	92	12	28	11
NPEO12	766.6	749.6	4.4	90	12	28.5	11
NPEO13	810.6	793.6	4.4	85	9.2	31	13
NPEO14	854.6	837.6	4.4	70	7	33	13
NPEO15	898.6	881.6	4.3	60	15	34	15
NPEO16	942.6	925.6	4.3	40	15	35.5	15
NPEO17	986.6	969.6	4.3	42	11	36	15
NPEO18	1030.7	1013.7	4.2	30	9	37	10
NPEO19	1074.7	1057.7	4.2	30	9	38	9
NPEO20	1118.7	1101.7	4.2	25	9	40	9

[a]RT = retention time
[b]DP = declustering potential
[c]EP = entrance potential
[d]CE = collision energy
[e]CXP = collision cell exit potential

Table S7. Table of sMRM parameters for $OPEO_x$.

Compound	Precursor m/z	Fragment m/z	RT[a]	DP[b] (V)	EP[c] (V)	CE[d] (V)	CXP[e] (V)
OPEO2	312.3	295.3	3.9	55	10	11	10
OPEO3	356.3	339.3	3.8	55	10	12	11
OPEO4	400.3	383.3	3.8	55	10	16	12
OPEO5	444.4	427.4	3.8	60	10	18	13
OPEO6	488.4	471.4	3.7	70	9	21	14
OPEO7	532.4	515.4	3.7	80	10	23.5	8
OPEO8	576.4	559.4	3.6	90	10	25	9
OPEO9	620.4	603.4	3.6	90	10	27	10
OPEO10	664.4	647.4	3.6	95	10	28	10
OPEO11	708.5	691.5	3.6	100	10	29	10
OPEO12	752.5	735.5	3.5	105	10	30	11
OPEO13	796.5	779.5	3.5	110	10	31	12
OPEO14	840.6	823.6	3.5	107	10	31.5	13
OPEO15	884.6	867.6	3.4	102	10	33	14
OPEO16	928.6	911.6	3.4	90	10	34	15
OPEO17	972.6	955.6	3.4	95	10	35	16
OPEO18	1016.7	999.7	3.4	70	10	36	17
OPEO19	1060.7	1043.7	3.3	60	10	37	18
OPEO20	1104.8	1087.8	3.3	50	10	38	19

[a]RT = retention time
[b]DP = declustering potential
[c]EP = entrance potential
[d]CE = collision energy
[e]CXP = collision cell exit potential

Table S8. Table of sMRM parameters for C16 AEOs.

Compound	Precursor m/z	Fragment m/z	RT[a]	DP[b] (V)	EP[c] (V)	CE[d] (V)	CXP[e] (V)
C16EO2	348.4	331.4	9.3	35	10	13	8
C16EO3	392.4	375.4	9.3	50	8	15	10
C16EO4	436.4	419.4	9.3	55	9	18	10
C16EO5	480.4	463.4	9.3	65	10	19	7
C16EO6	524.4	507.4	9.2	72	14	21	7
C16EO7	568.5	551.5	9.2	75	14	24	8
C16EO8	612.5	595.5	9.1	85	15	26	9
C16EO9	656.5	639.5	9.1	90	15	27	10
C16EO10	700.6	683.6	9.0	98	15	27	10
C16EO11	744.6	727.6	9.0	100	15	29	11
C16EO12	788.6	771.6	9.0	105	15	30	12
C16EO13	832.6	815.6	8.9	100	15	31	13
C16EO14	876.7	859.7	8.9	100	15	32	14
C16EO15	920.7	903.7	8.9	100	15	34	15
C16EO16	964.7	947.7	8.8	100	15	34	16
C16EO17	1008.7	991.7	8.8	90	15	36	17
C16EO18	1052.8	1035.8	8.7	85	15	36	19
C16EO19	1096.8	1079.8	8.7	70	15	38	20
C16EO20	1140.8	1123.8	8.7	65	15	40	20

[a]RT = retention time
[b]DP = declustering potential
[c]EP = entrance potential
[d]CE = collision energy
[e]CXP = collision cell exit potential

Table S9. Table of sMRM parameters for C18 AEOs.

Compound	Precursor m/z	Fragment m/z	RT[a]	DP[b] (V)	EP[c] (V)	CE[d] (V)	CXP[e] (V)
C18EO2	376.4	359.4	10.6	45	10	13	10
C18EO3	420.4	403.4	10.7	55	10	16	10
C18EO4	464.4	447.4	10.7	60	10	18	7
C18EO5	508.5	491.5	10.8	70	15	20	7
C18EO6	552.5	535.5	10.8	75	10	22	8
C18EO7	596.5	579.5	10.9	80	9	24	9
C18EO8	640.5	623.5	10.9	90	14	26	10
C18EO9	684.6	667.6	11.0	95	15	27.5	11
C18EO10	728.6	711.6	11.0	95	15	29	11
C18EO11	772.6	755.6	11.0	100	15	30	12
C18EO12	816.6	799.6	11.0	105	15	31	13
C18EO13	860.7	843.7	11.0	105	15	32	14
C18EO14	904.7	887.7	11.0	105	15	34	15
C18EO15	948.7	931.7	11.0	105	15	34	16
C18EO16	992.8	975.8	11.0	95	10	35	16
C18EO17	1036.8	1019.8	11.0	90	10	37	17
C18EO18	1080.8	1063.8	11.0	90	10	37	18
C18EO19	1124.8	1107.8	11.0	70	15	38	21
C18EO20	1168.9	1151.9	11.0	65	15	40	21

[a]RT = retention time
[b]DP = declustering potential
[c]EP = entrance potential
[d]CE = collision energy
[e]CXP = collision cell exit potential