Analysis of Nonionic Surfactants in Surface Water using High Resolution Mass Spectrometry

Cindy S. Lai[1], Mark Tracy[2]
[1]Thermo Fisher Scientific, San Jose, CA
[2]Thermo Fisher Scientific, Sunnyvale, CA

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ABSTRACT

A quantitative and screening method was developed for nonylphenol ethoxylates and octophenol ethoxylates in tap water. Baseline separation for each of the ethoxylated units were achieved and the LC method robustness was showcased through highly reproducible retention time. Coupled with accurate mass and MS/MS spectral matching, quantitation and identification of these long chained environmental contaminants are possible, even without commercially available standards.

INTRODUCTION

Nonylphenol Ethoxylates (NPEOs) and Octophenol Ethoxylates (OPEOs) are a class of nonionic surfactants commonly used in industrial detergents. Their toxicity to the environment has been known and studied. Currently the EPA recommends the amount of Nonylphenol be maintained below 6.6 ug/L level in fresh water, and mass spectrometer (MS) is the ideal analytical instrumentation because of its high sensitivity and specificity. However quantitizing NPEOs via LCMS pose a few challenges. 1) Many of these surfactants, especially the longer chained ethoxylates, do not have commercially available standards, making it difficult for targeted analysis using triple-quadrupole MS approach. 2) multiple ethoxylated species need to be resolved on the chromatographic level. Here we develop a method by optimizing separation of NPEs in a fast gradient. High Resolution Accurate Mass (HRAM) mass spectrometry allows examining each one of the individual oligomers, while achieving very high sensitivity in matrix.

MATERIALS AND METHODS

Sample Preparation
Nonylphenol Monoethoxylate (NPEO1), Nonylphenol Diethoxylate (NPEO2), Octophenol Ethoxylate (OPEO1), and Octophenol Diethoxylate (OPEO2) were purchased from Sigma Aldrich for method optimization and calibration standards. Tergitol-10, a commercial emulsifier that contains a mixture of NPEOs of various degrees of ethoxylation, and Triton-X, a commercial surfactant containing OPEOs, were purchased from Sigma Aldrich for spiking surface water obtained in San Jose, CA.

Calibration curve was prepared by diluting NPEO1, NPEO2, OPEO1, OPEO2 standard mixture in tap water to build 0.5 ppb to 500 ppb calibration levels.

Sample was prepared by spiking 100 ppb of Tergitol-10 and 100 ppb of Triton-X. The resulting 100 ppb spiked sample contains a mix of highly ethoxylated surfactants. Only ethoxylates n=1 to n=15 for the NPEOs and OPEOs are studied in this experiment.

Chromatography
Chromatographic conditions were developed on a Thermo Scientific™ UltiMate™ 3000 RSLC binary system. Spiked surface waters samples are loaded onto a silica-based HPLC column (Thermo Scientific™ Acclaim™ Surfactant Plus, 3um, 2.1mm x 100mm) and separated by a 10-minute linear gradient with Acetonitrile and Water with 10mM Ammonium Formate. Initial gradient condition starts at 40%B to 100%B at 7 minute, with 2 minute hold, back to 40%B at 9.1 minute, and ends at 10 minute.

Mass Spectrometry
MS data were acquired on a Thermo Scientific™ Q Exactive™ Focus Orbitrap™ mass spectrometer, operating in Full Scan mode with data-dependent MS/MS. Resolution was set at 70,000 (FWHM) at m/z 200, and 35,000 (FWHM) at m/z 200 for MS/MS. Positive ESI source condition was optimized with targeted analyte teed in with LC flow of 400uL/min:

Sheath Gas: 60
Aux Gas: 20
Sweep Gas: 1
Spray voltage: 3kV
Ion Transfer temperature: 300C
RF Lens: 50
Aux Gas temperature: 350C

Data Analysis
Chromatographic data was processed and presented using Thermo Scientific™ Xcalibur™ Qual Browser. MS/MS Spectral database,.screening and quantiative results were processed using Thermo Scientific™ TraceFinder™ 3.3 software

RESULTS

Prior experiments have shown that NPEOs and OPEOs readily form sodium adducts with the highest intensity, as well as ammonium adducts and protonated species in positive electrospray ionization. It was also found that ammonium adducts fragment more easily when collision energy was applied, therefore [M+NH4]+ is monitored, and a mobile phase that contains ammonium formate (10mM) was prepared to aid the formation of ammonium adducts. Accurate mass for each of the NPEO adducts with n=1 to n=14 was calculated (Figure 1 and Figure 2) for positively identify each of the ethoxylated units during method development.

Figure 1. Accurate Mass m/z for Nonylphenol Ethoxylate adducts

Name	Molecular Formula	[M+NH4]+	[M+Na]+	{M+H]+
NPEO1	$C_{17}H_{28}O_2$	282.2427	287.1981	265.2162
NPEO2	$C_{19}H_{32}O_3$	326.2690	331.2244	309.2424
NPEO3	$C_{21}H_{36}O_4$	370.2952	375.2506	353.2686
NPEO4	$C_{23}H_{40}O_5$	414.3214	419.2768	397.2948
NPEO5	$C_{25}H_{44}O_6$	458.3476	463.3030	441.3211
NPEO6	$C_{27}H_{48}O7$	502.3738	507.3292	485.3473
NPEO7	$C_{29}H_{52}O_8$	546.4000	551.3554	529.3735
NPEO8	$C_{31}H_{56}O_9$	590.4263	595.3817	573.3997
NPEO9	$C_{33}H_{60}O_{10}$	634.4524	639.4078	617.4259
NPEO10	$C_{35}H_{64}O_{11}$	678.4787	683.4341	661.4521
NPEO11	$C_{37}H_{68}O_{12}$	722.5049	727.4603	705.4784
NPEO12	$C_{39}H_{72}O_{13}$	766.5311	771.4865	749.5046
NPEO13	$C_{41}H_{76}O_{14}$	810.5573	815.5127	793.5308
NPEO14	$C_{43}H_{80}O_{15}$	854.5835	859.5389	837.5570
NPEO15	$C_{45}H_{84}O_{16}$	898.6098	903.5652	881.5832

Nonylphenol Ethoxylates form [M+NH4]+, [M+Na]+, [M+H]+ adducts in positive ESI mode, with [M+Na]+ the highest intensity, and [M+NH4]+ gives the most unique MS/MS fragmentation for identification.

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Figure 2. Accurate Mass m/z for Octophenol Ethoxylate adducts

Name	Molecular Formula	M+NH4	M+Na	M+H+
OPEO1	$C_{16}H_{26}O_2$	268.2271	273.1825	251.2005
OPEO2	$C_{18}H_{30}O_3$	312.2533	317.2087	295.2268
OPEO3	$C_{20}H_{34}O_4$	356.2795	361.2349	339.2530
OPEO4	$C_{22}H_{38}O_5$	400.3057	405.2611	383.2792
OPEO5	$C_{24}H_{42}O_6$	444.3320	449.2874	427.3054
OPEO6	$C_{26}H_{46}O_7$	488.3582	493.3136	471.3316
OPEO7	$C_{28}H_{50}O_8$	532.3844	537.3398	515.3578
OPEO8	$C_{30}H_{54}O_9$	576.4106	581.3660	559.3841
OPEO9	$C_{32}H_{58}O_{10}$	620.4368	625.3922	603.4103
OPEO10	$C_{34}H_{62}O_{11}$	664.4630	669.4184	647.4365
OPEO11	$C_{36}H_{66}O_{12}$	708.4893	713.4446	691.4627
OPEO12	$C_{38}H_{70}O_{13}$	752.5155	757.4709	735.4889
OPEO13	$C_{40}H_{74}O_{14}$	796.5417	801.4971	779.5151
OPEO14	$C_{42}H_{78}O_{15}$	840.5679	845.5233	823.5413
OPEO15	$C_{44}H_{82}O_{16}$	884.5941	889.5495	867.5676

Octophenol Ethoxylates form [M+NH4]+, [M+Na]+, [M+H]+ adducts in positive ESI mode, with [M+Na]+ the highest intensity, and [M+NH4]+ gives the most unique MS/MS fragmentation for identification.

In addition to comparing mobile phase additives and adduct formation, several mobile phase conditions were also examined in optimizing the chromatographic resolution. It was found that using acetonitrile instead of methanol as the organic phase, helped with separating NPEOs and OPEOs based on their ethoxylated units. A mixture of 100 ppb Tergitol-10 and 100 ppb Triton-X are then spiked in tap water for method development. Extracted ion chromatograms (figure 3 and 4) are shown based on 5ppm m/z window. Peaks for each of the individual oligomers were baseline resolved based on their degree of ethoxylation, with the longer chain units eluting first and shorter length species eluting later in the chromatographic run.

Figure 3. Extracted Ion Chromatogram for Nonylphenol Ethoxylates (n=1 to n=14) in 100 ppb of Tergol-10 mixture spiked in Tap water

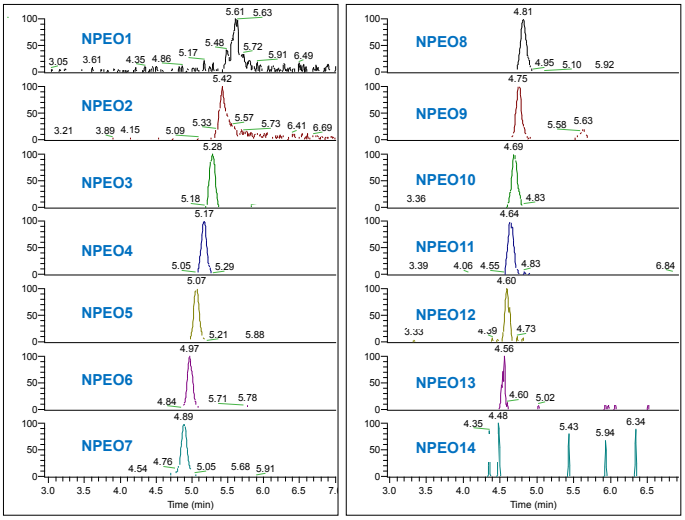
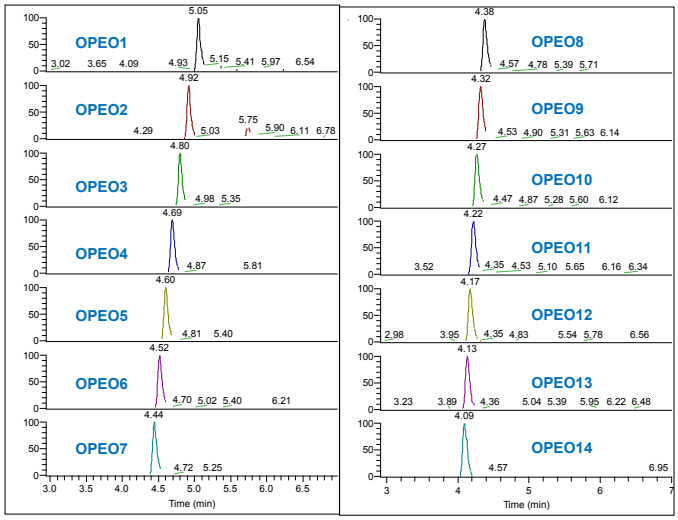
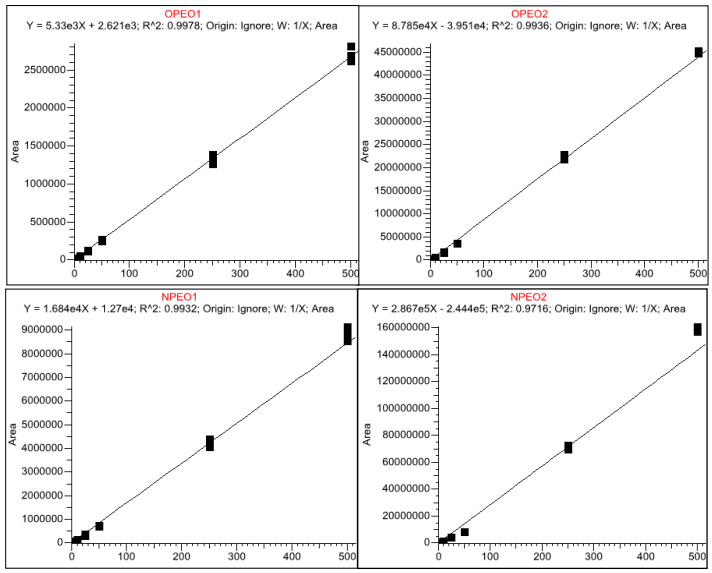


Figure 2. Extracted Ion Chromatogram for Octophenol Ethoxylates (OPEO n=1 to n=14) in 100 ppb of Triton-X mixture spiked in tap water



With the chromatography conditions optimized, calibration standards for NPEO1, NPEO2, OPEO1, and OPEO2 are prepared for assessing method detection limit using the UHPLC-MS platform. Calibration standards are prepared from 0.5ppb to 500 ppb in tap water collected in San Jose, CA., and 10uL of each calibration level was injected directly for LC/MS analysis with no additional sample preparation or pre-concentration steps. MS data was collected in full scan with data-dependent MS/MS for confirmation. Quantitation was based on extracted m/z within 5ppm window of calculated accurate mass. Method detection limit was defined as <20% in CV and RSD, based on replicates of n=3 for each calibration level. NPEO1 and OPEO1 have lower ionization efficiency and the observed MDL is reported at 2.5 ppb. Detection limit for NPEO2 and OPEO2 is reported at 1ppb.

Figure 3. Extracted Ion Chromatogram for Nonylphenol Ethoxylates (n=1 to n=14) in 100 ppb of Tergol-10 mixture spiked in Tap water



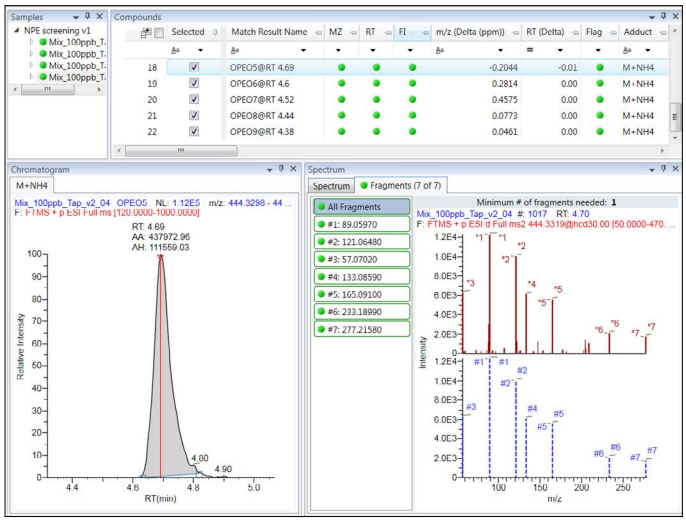
Since commercially available standards for the larger surfactants can be difficult to obtain, and in addition, the different oligomers may not have same ionization efficiency to be able to quantitate based on calibratioi curve of the monoethoxylates or diethoxylates – as evident in the calibration data presented above - a screening approach may be employed for monitoring the larger oligomers. Utilizing the optimized retention time, accurate mass, as well as MS/MS fragmentation, a workflow that allows for positively identification of NPEOs and OPEOs is investigated.

To collect MS/MS fragment data, neat standards of Tergitol-10 and Triton-X were prepared in LC/MS grade water separately and analyzed with the same chromatographic condition. An absolute collision energy (CE=30) was applied to collect MS/MS spectra for each of the individual surfactants. A list of most abundant fragment masses are then entered in theTraceFinder compound database for building the screening method.

For proof of concept, sample containing a mixture of 100ppb Tergitol-10 and 100ppb Triton-X are then prepared in tap water and injected directly for LC/MS analysis. Data collected are processed with screening criteria set at minimum of S/N of 5 for precursor m/z, and minimum of 1,000 counts for MS/MS fragment ion intensity. Retention time match window was set at 30 seconds (0.5 minutes) window, compared to previously acquired retention time information.

Figure 4 provides an example for OPEO5, showing a positive identification based on retention time, accurate mass for precursor m/z, and all seven fragment match for MS/MS spectra.

Figure 4. Screening result example for OPEO5



CONCLUSIONS

High resolution mass spectrometry has shown to be an effective methodology for quantitative and screening analysis for environmental contaminants when 1) analytical standards are difficult to obtain for targeted MS/MS analysis, and 2) retroactive data inspection is desirable. The UHPLC separation coupled to the Orbitrap MS platform provides a highly reliable solution with easy method setup for environmental pollutants studies.

In the example of Tergitol-10 and Triton-X, two commonly used industrial surfactants, having the flexibility for targeted quantitation and screening is desirable. In order to achieve method robustness for routine analysis, it is essential to start with an optimized UHPLC condition including column selection and evaluation of mobile phase conditions. Utilizing accurate mass and MS/MS fragment information, a fast and robust workflow allows nonylphenol ethoxylates and octophenyl ethoxylates to be quantified and screened in a single experiment. The simplicity and convenience of this platform will help with continuous monitoring of these environmental toxins and further investigation of health affects to human and marine life.

REFERENCES

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)

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