Profiling Analysis of the Degradation Products of Alkylphenol Polyethoxylates by LC-MS Using an Acclaim® Surfactant Column with Mass Spectrometric Detection

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INTRODUCTION

Alkylphenol polyethoxylates (APEOs, with ethoxylate units $(EO)_n$, n = 9–40) are among the most commonly used non-ionic surfactants, with annual global production over 650,000 tons,[1] consisting of approximately 80% nonylphenol ethoxylates (NPEOs) and nearly 20% octylphenol ethoxylates (OPEOs).[2] Numerous studies have revealed the wide prevalence of APEO degradation products with known estrogenic potency and persistency (n ≤ 3, OPEO1, OPEO2 and OPEO3; NPEO1, NPEO2 and NPEO3) in water, sediments, sewage sludge, and food.[2,3,4] Meanwhile, degradation products with different EO chains (n = 0–3) show different estrogenic potencies.[5] APs and APEOs are regulated by many agencies, including the European Commission and U.S. EPA.[6,7] It is crucial to determine the profile and distribution of APEO degradation products. To achieve this purpose, an analytical method for quantitative profiling degradation products is required.

Due to the complexity of the mixtures, quantitative determination of APs and APEOs remains a challenge. Published methods involve either gas chromatography (GC) or liquid chromatography (LC) coupled with various forms of detection. Normal-phase liquid chromatography (NPLC) has been applied to separate APEOs based on EO units, and reversed-phase LC (RPLC) to separate APEOs based on hydrophobic characteristics. To the authors' best knowledge, simultaneous chromatographic separations for APEOs based on EO units and hydrophobic alkyl chain have not been reported. When mass spectrometry has been used for detection, exact mass extraction was applied to resolve different APEOs from a single chromatographic peak. Under these circumstances, without isotope labeled analytes as internal standards (IStds), quantification accuracy can be problematic due to ionization suppression from coeluting species.[8]

This study presents a sensitive and accurate quantification method for simultaneous determination of OP, OPEO1, OPEO2, OPEO3 and NP, NPEO1, NPEO2, and NPEO3. This method takes advantage of mixed retention mechanisms (hydrophobic and hydrophilic interactions) to retain and resolve APs and APEOs based on the differences in alkyl chain as well as EO units in a single chromatographic run, and provides sensitivity and selectivity through mass spectrometric detection.

EXPERIMENTAL SECTION

Instrumentation

HPLC:	P680 dual ternary pump
	ASI-100 autosampler
	TCC-100 column temperature compartment
	UVD340U detector
Detector:	MSQ™ Plus single quadruple mass spectrometer with electrospray ionization (ESI) interface
Software:	Chromeleon® Chromatography Management Software (6.8, SP3)

Chromatographic Conditions

Column:	Acclaim Surfactant (150 × 2.1 mm, 5 µm)
Column Temperature:	30 °C
Mobile Phase:	A: CH_3OH; B: NH_4OAc 100 mM pH 5.2; C: H_2O

Time/min	%A	%B	%C
–5.0	66	3	31
8.5	66	3	31
9.5	86	3	11
16.0	86	3	11
17.0	66	3	31

Flow Rate:	0.4 mL/min
Injection:	10 µL

Mass Spectrometric Conditions

Ionization Interface:	Electrospray ionization (ESI)
Detection Mode:	Selected ion monitoring (SIM)
Probe Temperature:	350 °C
Needle Voltage:	3000 V
N_2 Nebulizer Gas Pressure:	80 psi
Scan Events:	See Table 1 for details.

Standard Preparation

IGEPAL CA-210 and IGEPAL CO-210 were used as standard mixtures for OPEOs and NPEOs, respectively. Each standard mixture was diluted to 0.1% (1000 ppm total for all oligomers) in CH_3CN/H_2O (50/50, v/v) as a standard stock solution. OP, tert-OP and NP were weighed on analytical balance and dissolved in CH_3CN/H_2O to 1000 ppm as standard stock solutions.

Calibration standards were diluted from above stock solutions to eight levels: 0.1, 0.2, 0.5, 1, 2, 5, 10 and 20 ppm (w/v). Concentrations of individual APEO were calculated based on relative peak areas (Table 2).

Table 1. Scan Functions and Scan Events

Full Scan							
Name	**Start Mass *(m/z)***	**End Mass *(m/z)***	**Time Range (min)**	**Scan Time (sec)**	**Polarity**	**Cone Voltage (V)**	
Positive Full Scan	200	600	0.0 – 14.0	0.2	Pos.	40	
Negative Full Scan	200	600	12.0 – 17.0	0.2	Neg.	40	
SIM Scan							
Name	**Adduct**	**Mass *(m/z)***	**Span *(m/z)***	**Time Range (min)**	**Dwell Time (sec)**	**Polarity**	**Cone Voltage (V)**
SIM Group 1							
OPEO1	$[M+NH_4]^+$	268.2	0.5	5.0 – 10.0	0.5	Pos.	40
OPEO2	$[M+NH_4]^+$	312.3	0.5	5.0 – 10.0	0.5	Pos.	40
OPEO3	$[M+NH_4]^+$	356.3	0.5	5.0 – 10.0	0.5	Pos.	40
SIM Group 2							
NPEO1	$[M+NH_4]^+$	282.2	0.5	8.0 – 14.0	0.5	Pos.	40
NPEO2	$[M+NH_4]^+$	326.3	0.5	8.0 – 14.0	0.5	Pos.	40
NPEO3	$[M+NH_4]^+$	370.3	0.5	8.0 – 14.0	0.5	Pos.	40
SIM Group 3							
OP, tert-OP	$[M+CH_3COO]^-$	206.2	0.5	12.0 – 17.0	0.25	Neg.	40
NP	$[M+CH_3COO]^-$	220.2	0.5	12.0 – 17.0	0.25	Neg.	40

Table 2. Concentrations of Calibration Standards

Level	Conc. /ppb	OPEO1	OPEO2	OPEO3	NPEO1	NPEO2	NPEO3	OP	t-OP	NP
% Area *	—	77.2%	19.4%	2.0%	48.5%	35.2%	7.6%	—	—	—
1	100	77.20	***19.4***	***2.00***	48.5	***35.2***	***7.60***	100	100	100
2	200	***154.4***	38.8	4.00	97.0	70.4	15.2	200	200	200
3	500	386	97.0	10.0	***242***	176	38.0	***500***	***500***	***500***
4	1000	772	194	20.0	485	352	76.0	1000	1000	1000
5	2000	1544	388	40.0	970	704	152	2000	2000	2000
6	5000	3860	970	100	2425	1760	380	5000	5000	5000
7	10000	7720	1940	200	4850	3520	760	10000	10000	10000
8	20000	15440	3880	400	9700	7040	1520	20000	20000	20000

* % Area was calculated based on relative peak areas by UV detection at 225 nm.

RESULTS AND DISCUSSION

Column Selection for Quantification

Three column candidates (Acclaim Mixed-Mode HILIC-1, WAX-1, and a Surfactant column) were selected for evaluation to separate APEO oligomers. The comparison is shown in Figure 1.

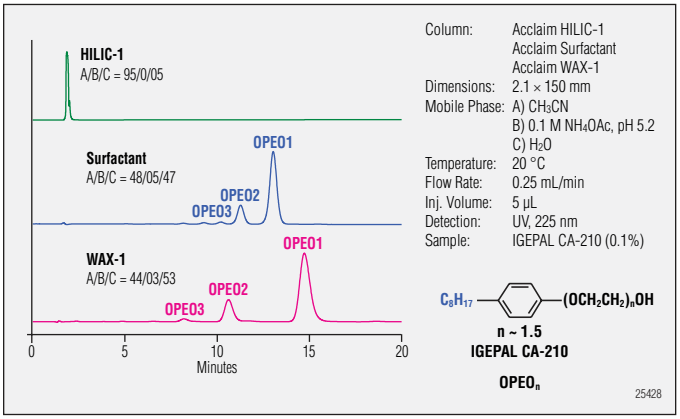

Figure 1. Resolving efficiency of three mixed-mode columns.

The retention of APEO oligomers on Acclaim WAX-1 and Surfactant columns follows the inverse order of EO number. APEOs with more EO units eluted earlier, which made these two columns good candidates for APEO degradation products analysis. The HILIC-1 column showed a reversed order of elution relative to the other two columns, i.e., APEO with less EO units eluted earlier. This column was eliminated from further evaluation.

The WAX-1 column showed the best separation efficiency for APEO oligomers. However, the resolution for oligomer groups, i.e. OPEOs and NPEOs, was not sufficient for chromatographic baseline separation. The Surfactant column showed a balanced resolution for APEO oligomers and oligomer groups, and it was selected as the analytical column for this study.

Resolution for APEO Oligomers

APEO oligomers were generally chromatographically resolved using normal phase, by amino functional group. The eluting order for APEOs agrees with the observation on the HILIC-1 column in normal-phase mode: APEOs with a longer EO chains have longer retention times. For APEO degradation products analysis, which involves mostly short chain APEOs, normal-phase chromatography could be time consuming due to the column cleanup required to elute APEOs with long EO chains. When using the Surfactant column, longer chain APEOs were easily eliminated or specifically retained by adjusting the mobile phase composition. As shown in Figure 2, the retention time of OPEOs (OPEO2) could be tuned from 6.5 minutes to 21.0 minutes or longer by lowering the mobile-phase CH_3CN concentration. If better resolution is desired for longer chain APEO degradation products analysis, such as APEO4 and APEO5, the WAX-1 column would be preferred.

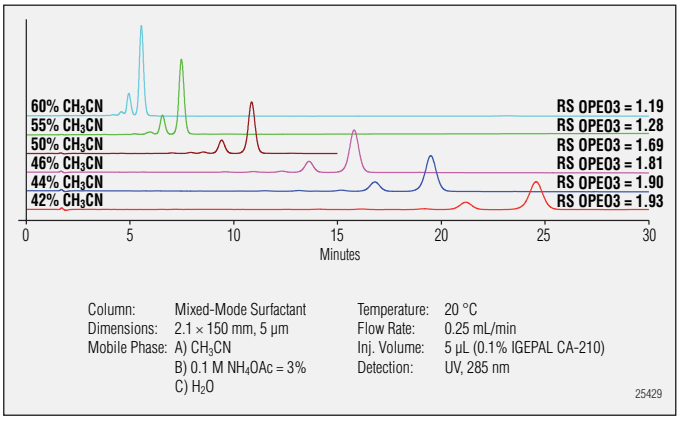

Figure 2. Resolution for OPEO oligomers on Surfactant columns.

Resolution for APEO Oligomer Groups (OPEOs & NPEOs)

To achieve complete resolution for APEOs, group separation is required between OPEOs and NPEOs. As described above, the Surfactant column was selected over WAX-1 for this reason. The effect on OPEOs and NPEOs group resolution is shown in Table 3. Retention times of OPEO1 (last-eluted peak in OPEOs) and NPEO3 (first-eluted NPEOs of interest) were compared. The group resolution for OPEOs and NPEOs increases with decreasing CH_3CN%. Chromatographic baseline separation for OPEO1 and NPEO3 was achieved from 50% CH_3CN and below. OPEO1 and NPEO4 were separated to baseline from 44% CH_3CN and below.

Table 3. Retention Times and Resolution for APEO Oligomer Groups

CH_3CN%	60%	55%[a]	50%	46%	44%[b]	42%
OPEO1	5.6	7.5	10.9	15.8	19.5	24.6
NPEO3	5.7	7.9	11.9	18.1	23.0	30.0

[a] Baseline separation of OPEO1 and NPEO3

[b] Baseline separation of OPEO1 and NPEO4 (tested from IGEPAL-NP4, $NPEO_n$, n~4)

Organic Solvent Selection for Mass Spectrometry

As the two most commonly used organic solvents in LC, CH_3CN and CH_3OH, both demonstrated satisfactory resolution for APEOs on the Surfactant column. Under mobile phase conditions offering similar chromatographic resolution, CH_3OH provided better MS response for APEOs. Figure 3 shows the comparison of OPEOs with CH_3CN and CH_3OH as the organic solvent component in the mobile phase. For OPEO3, CH_3OH showed slightly better MS response for the adduct ion $[M+NH_4]^+$ = 356.2 m/z. For OPEO2, CH_3OH showed nearly a sevenfold higher response than that of CH_3CN ($[M+NH_4]^+$ = 312.3 m/z). A more significant difference was observed for OPEO1, where CH_3OH offered a strong base peak response $[M+NH_4]^+$ = 268.2 m/z and no responding peak was found in the MS spectrum for OPEO1 when using CH_3CN. A similar result was observed for NPEOs. No significant difference was observed for MS response of phenols.

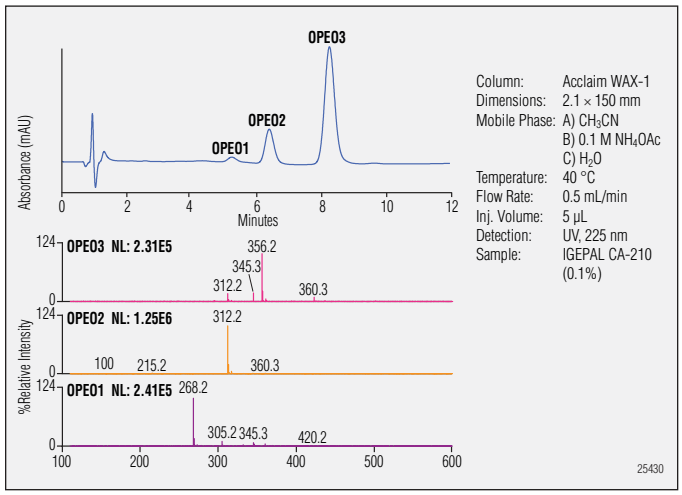

Figure 3A. Chromatograms and mass spectra for OPEOs using CH_3OH.

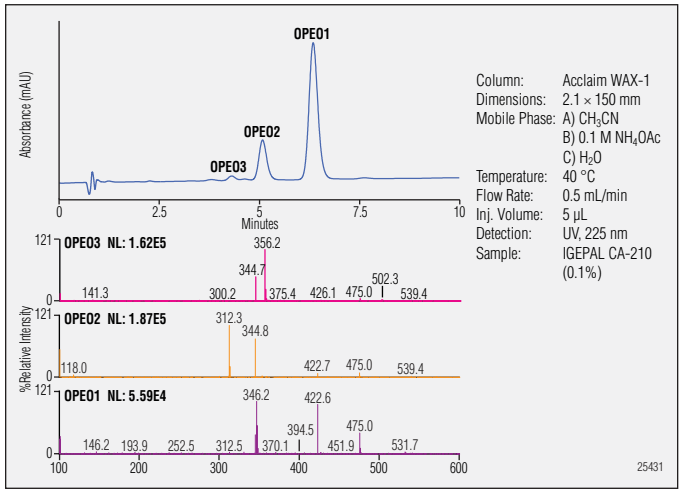

Figure 3B. Chromatograms and mass spectra for OPEOs using CH_3CN.

Buffer Selection

APEOs showed strong affinity for alkali metals, and the adduct ions were detected as base peaks in MS spectra. Shang et al. studied MS response for different adducts with respect to sensitivity and selectivity, and recommended 0.5 mM NaOAc in the mobile phase to produce the most sensitive and reproducible results without sacrificing system stability.[8] The present experiments found that, even using 0.5 mM NaOAc in mobile phase, salt condensation was observed on the MS entrance cone and there was a significant response decrease within 2 hours of continuous operation. Cleaning the entrance cone with water spray solved the sensitivity decrease, which suggests frequent interruptions would be required to maintain system integrity. An NH_4OAc buffer was used instead to prevent the formation of salt condensation on entrance cone due to its volatility.

Chromatography was then re-optimized based on CH_3OH as the organic component in the mobile phase with NH_4OAc buffer. A gradient program was applied to elute phenols that are more polar than APEOs and retain longer on the Surfactant column. Figure 4 shows overlay chromatograms of the UV trace and SIM traces.

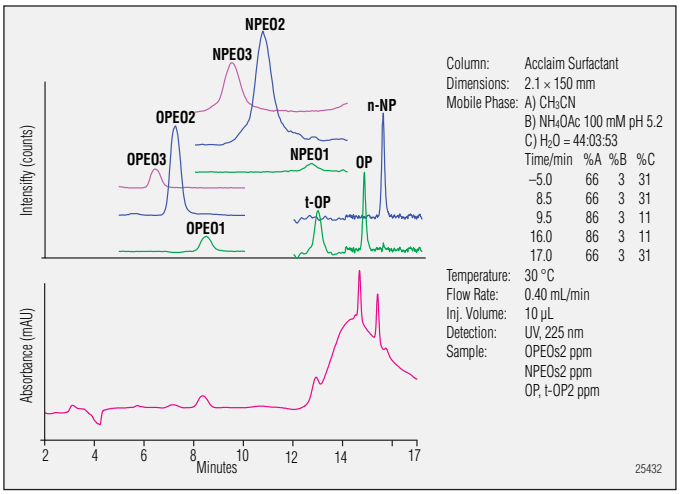

Figure 4. Chromatograms of alkylphenols and their mono- to tri-ethoxylates.

Linearity and Quantification Limits

Linearity was achieved for each analyte from individual lower quantification limit to 200 ng/injection (upper limit for individual APEO calculated based on relative peak area). Figure 5 presents calibration curves for OPEO2, NPEO2, and 4-NP. Lower limit of quantification was defined as the injected lowest concentration (10 µL injection volume) in calibration standards that showed signal-to-noise ratio greater than 10 (S/N > 10). Table 2 shows the lower quantification limits in bold italic font: OPEO1 (1.5 ng/injection), OPEO2 (0.19 ng/injection), and OPEO3 (0.02 ng/injection); NPEO1 (2.4 ng/injection), NPEO2 (0.35 ng/injection), and NPEO3 (0.08 ng/injection); OP, t-OP and NP (5 ng/injection).

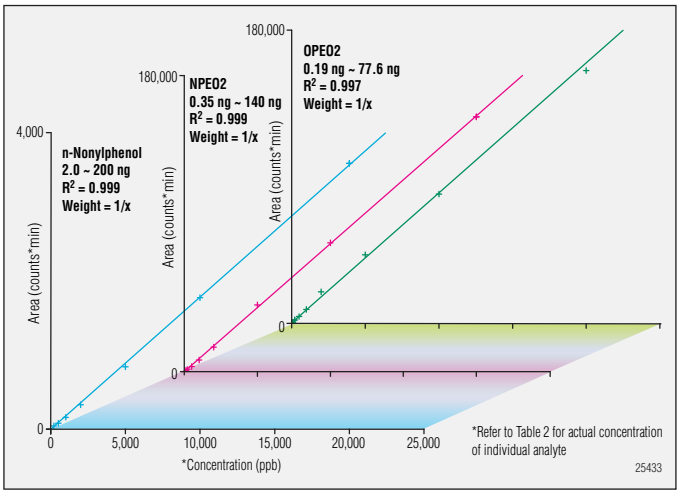

Figure 5. Calibration Curves for n-nonylphenol, NPEO2, and OPEO2.

CONCLUSION

An LC/MS method was developed for quantitative analysis of APEO degradation products. Total chromatographic separation for APEOs and APs was achieved on a Surfactant column and MS detection was applied for selective and sensitive detection. Lower quantification limits for APEOs were estimated in the range of 0.05 to 2.4 ng/injection, and 5 ng/injection for APs. Linearity was achieved for each analyte within two orders of magnitude.

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