APPLICATION NOTE

Liquid Chromatography/
Mass Spectrometry

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LC/MS/MS: Analysis of Alkylphenol Ethoxylates (APEO) And Alkylphenols (AP) In Textiles

Introduction

Alkylphenol ethoxylates (APEOs) are common surfactants used in in industrial and consumer detergents and cleaners, as well plastics and many industrial applications. They are discharged by factories and washed out of the final products ending up in the environment where they hardly break down and can cause damage to aquatic ecosystems. Nonylphenol ethoxylates (NPEOs) belong to the group of non-ionic surfactants and are of particular interest, as they tend to degrade to nonylphenol through biochemical and photochemical processes.[1] Because of the degradative processes to which APEOs are subject, they have recently been identified as restricted substances. The degradation of APEOs in the environment is well known as the process progresses, there is a loss of ethoxy units until free alkylphenol is released. This results in increased hydrophobicity and subsequent bioaccumulation of substances of increasing toxicity compared to the original APEOs. There is significant evidence that NPEOs, and especially their degradation products, act as endocrine disruptors, which can have adverse effects on human's health. As persistent pollutants with difficult degradation in nature, the problem of wastewater treatment and discharge gains considerable importance. Several publications cover the analysis of those compounds specially in wastewater[2,3,4,5,6,] effluents[7], but there are not many publications on textiles samples.[8]

Although these compounds can be determined by HPLC[9], LC/MS/MS represents the most effective and reliable way to analyze those compounds, as it provides more sensitivity and specificity.

This application note reports a newly developed LC/MS/MS method for the determination of Alkylphenol Ethoxylates (APEO) and Alkylphenols (AP) in textiles using PerkinElmer UHPLC LX-50™ System coupled to the PerkinElmer QSight™ 210 Triple Quadrupole LC/MS/MS.

Regulations

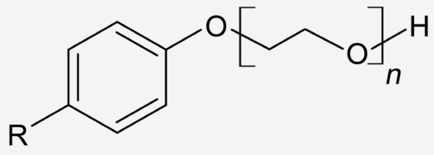
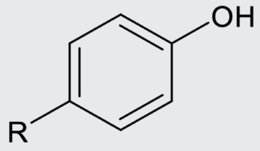
Following the identification of NPEO degradation products as toxic substances by the European Integrated Pollution Prevention and Control Bureau, the European Union has restricted the marketing and use of NPEOs since January 2005 (EU Directive 2003/53/EC included as part of EU Regulation 1907/2006 (REACH), Annex XVII restriction 46). REACH annex XVII set the conditions of restriction on the manufacture, placing on the market and use of certain dangerous substances. Entry 46a is referred to nonylphenol ethoxylates and states that after February 2021 textiles containing concentrations of those compounds greater than 0.01% by weight should not be placed on the market.

ISO 18254-1:2016 - describes analyses that are used to detect extractable alkylphenol ethoxylates (nonylphenol ethoxylates and octylphenol ethoxylates) in textile products. This document provides a method that uses Liquid Chromatograph (LC) with Mass Spectrometry (MS) system to detect and quantify alkylphenol ethoxylates of defined ethoxylate chain length.

UNI EN ISO 18254-1:2016 - provides a method that uses Liquid Chromatograph (LC) with Mass Spectrometry (MS) system to detect and quantify alkylphenol ethoxylates of defined ethoxylate chain length.[10]

UNI EN ISO 21084:2019 - specifies the method for the determination of extractable AP without derivatization step in textile and textile products.[11]

Table 1: Chemical structures and formulas of alkylphenol ethoxylates and alkylphenols.

Chemical structure	Nomenclature	Chemical formula
	Alkylphenol ethoxylate (APEO) Nonylphenol ethoxylate (NPEO) R=C_9H_{19} ; n=1-18 Octylphenol ethoxylate (OPEO) R=C_8H_{17} ; n=1-18	$C_{15}H_{24}O(C_2H_4O)_n$ $C_{14}H_{22}O(C_2H_4O)_n$
	Alkylphenol (AP) Nonylphenol (NP) R=$(CH_2)CH(CH_3)(CH_2)_3CH(CH_3)_2$ 4-n-Nonylphenol (4-n-NP) R=$(CH_2)_8CH_3$ 4-n-Octylphenol (4-n-OP) R=$(CH_2)_7CH_3$ 4-tert-Octylphenol (4-t-OP) R=$C(CH_3)_2CH_2C(CH_3)_3$	$C_{15}H_{24}O$ $C_{15}H_{24}O$ $C_{14}H_{22}O$ $C_{14}H_{22}O$

Experimental

Hardware / Software

Chromatographic separation of non-ionic surfactants was conducted on a PerkinElmer Altus A30 UHPLC System and analyte determination was achieved using a PerkinElmer QSight 210 LC/MS/MS with an ESI source. All instrument control, data acquisition and data processing were performed using PerkinElmer Simplicity 3Q™ Software.

PerkinElmer QSight Triple Quadrupole LC/MS/MS

Instrumental Parameters

The UHPLC method is shown in Table 2A for APEO and 2B for alkylphenols. MS source parameters are shown in Table 3A and B. A list of the multiple reaction monitoring (MRM) mode transitions and parameters of the studied compounds is shown 4. The MRM transitions, collision energies (CE), entrance voltages (EV) and collision cell lens 2 (CCL2) for each analyte were detected and optimized by direct infusion of the standards. MS source conditions, such as drying, nebulizer gas flow, and temperature settings were optimized by flow injection analysis (FIA).

Table 2: UHPLC Parameters for APEO and AP analysis.

Column	Bischoff ProntoPearl TPP C18 Ace EPS, 2.0 X 50 mm, 2.2 µm P/N 0502F18ATP022				
Mobile Phase APEO	**Solvent A:** 10 mM Ammonium acetate, pH 3 with formic acid **Solvent C:** Methanol				
Mobile Phase AP	**Solvent B:** 10 mM Ammonium acetate **Solvent C:** Methanol				
Gradient	**Step**	**Time (min)**	**Flow Rate (ml/min)**	**%A**	**%C**
	1	0	0.4	95	5
	2	1	0.4	95	5
	3	6	0.4	2	98
	4	7.5	0.4	2	98
	5	8	0.4	95	5
	6	13.0	0.4	95	5
Injection Volume	5 µL				
Autosampler Temperature	22 °C				
Column Temperature	40 °C				

Table 3: QSight ESI Source Parameters for APEO (A) and AP (B).

Native Analytes	(A) APEO	(B) AP
Ionization Mode	ESI positive	ESI negative
Drying gas	120	120
HSID temperature	300 °C	300 °C
Nebulizer gas	300	300
Spray voltage	+5500 V	-5000V
Source Temperature	350 °C	350 °C
Dwell time	20 ms	150 ms

Table 3: QSight ESI Source Parameters for APEO (A) and AP (B).

(A) APEO

Compound	n	Precursor [m/z]	Product [m/z]	CE	EV	CCL2
NPEO $C_{15}H_{24}O(C_2H_4O)_n$	2	326.2	183.0	-14	13	-58
	3	370.3	227.2	-16	11	-66
	4	414.3	271.2	-19	11	-78
	5	458.3	441.3	-17	4	-82
	6	502.3	485.3	-19	5	-90
	7	546.3	133.1	-32	10	-110
	8	590.4	133.1	-33	9	-118
	9	634.4	133.1	-35	10	-134
	10	678.4	133.1	-36	21	-134
	11	722.4	133.1	-38	20	-142
	12	766.5	133.1	-38	38	-162
	13	810.5	133.1	-41	35	-174
	14	854.6	133.1	-43	33	-178
	15	898.6	133.1	-46	40	-178
OPEO $C_{14}H_{22}O(C_2H_4O)_{ns}$	4	444.2	315.2	-23	19	-84
	5	400.3	271.1	-20	16	-76
	6	488.3	359.2	-23	17	-96
	7	576.4	559.3	-23	26	-112
	8	532.3	515.3	-21	17	-96
	9	620.4	133.1	-36	25	-120
	10	664.4	133.1	-38	24	-120
	11	708.4	133.1	-38	24	-128
	12	752.5	133.1	-41	28	-144
	13	796.5	133.1	-42	12	-136
	14	840.5	133.1	-44	36	-156
	15	884.6	133.1	-47	41	-156

(B) AP

Compound	Precursor [m/z]	Precursor [m/z]	RT [min]	CE	EV	CCL2
4-t-OP	205.2	133.2	7.32	31	-5	60
NP	219.2	133.2	7.54	68	-5	56
4-n-OP	205.1	106.1	7.69	27	-5	52
4-n-NP	219.1	106.1	7.86	27	-10	56

Standard preparation

TStandard for OPEOs (Triton® X-100) and NPEOs (IGEPAL® CO-630) were purchased from SigmaAldrich. Standards for APs were purchased from UltraScientific. All solvents used for both sample extraction and mobile phase were of LC-MS grade

(Scharlau UHPLC-MS Grade) and were purchased from Scharlab (Barcelona, Spain). Stock solution for all compounds were prepared in methanol at 200 mg/l and preserved in the dark at 4 °C. From this stock, appropriate dilutions were prepared to build the calibration curve (calibration range = 0.005 - 0.5 mg/L).

Sample Preparation

Sample preparation for both AP and APEO starts from 1 g of textile sample (homogenized cuts of 5 mm x 5 mm pieces) placed in a glass tube (Vial EPA). 10 ml of methanol is added to the sample for extraction by a ultrasonication in a water bath at 70 °C for 60 min. Subsequently extract is filtered through a 0.45 µm polypropylene (Uniprep) for both APEO and AP. Other filters made from different materials (for example nylon) were tested and showed some contamination for APs. Samples were directly analyzed by the LC/MS/MS system without further sample preparation.

Method Performance Parameters

Two sets of data were acquired in two different days. Time elapsed between the two data acquisitions was 13 days. Calibrators were kept in the autosampler at 22 °C all the time to check the stability of the compounds.

NPEO and OPEO are composed of different congeners (n=2 to n=15 for NPEO and n=4 to n=15 for OPEO). Chromatogram showing the different congeners are shown in Figure 1 for NPEO and Figure 2 for OPEO.

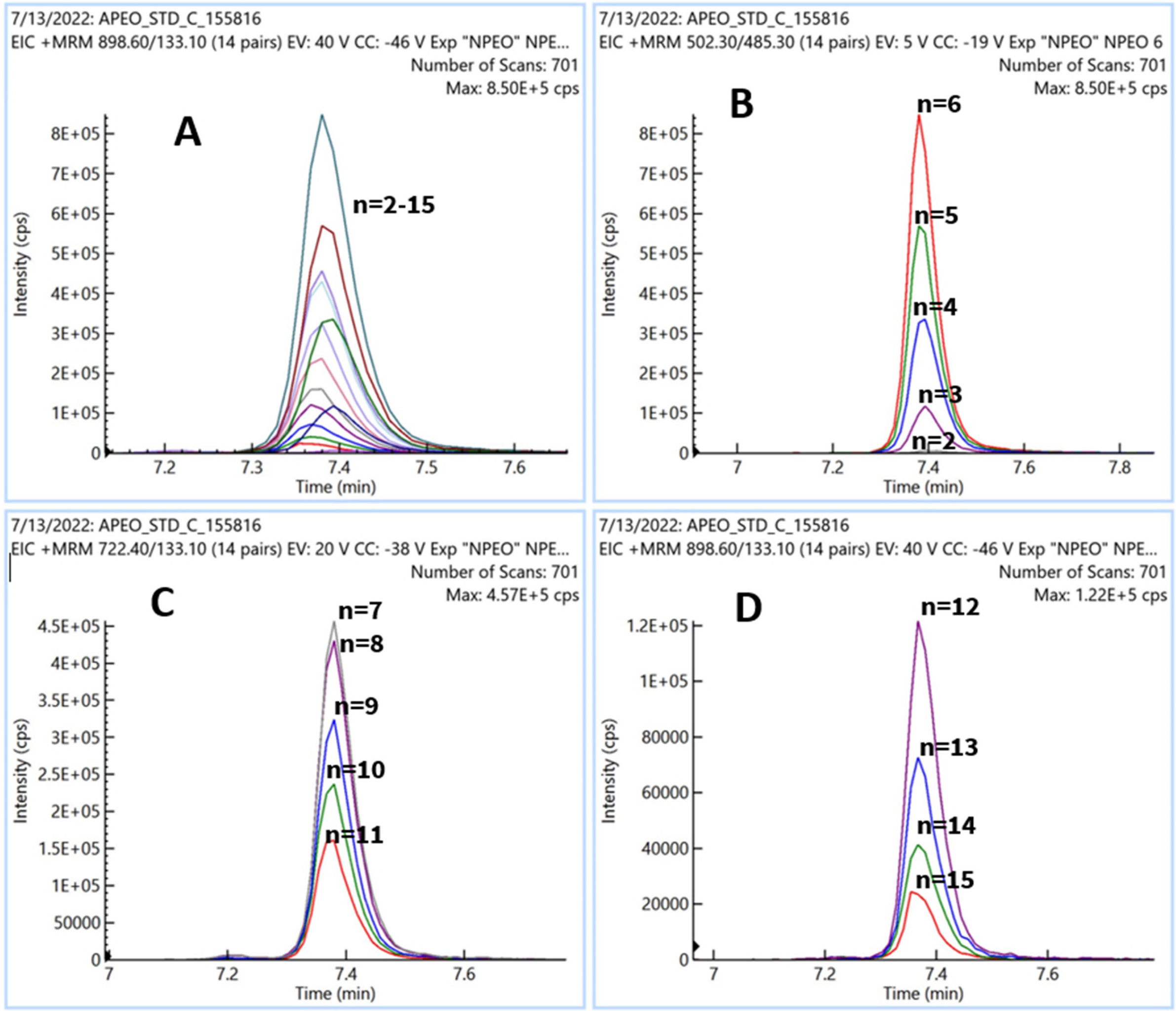


Figure 1: (A) Representative example of overlayed NPEO (n=2-15) in neat solution. (B) Overlapped NPEO (n=2 to 6). (C) Overlapped NPEO (n=7 to 11). (D) Overlapped NPEO (n=12 to 15).

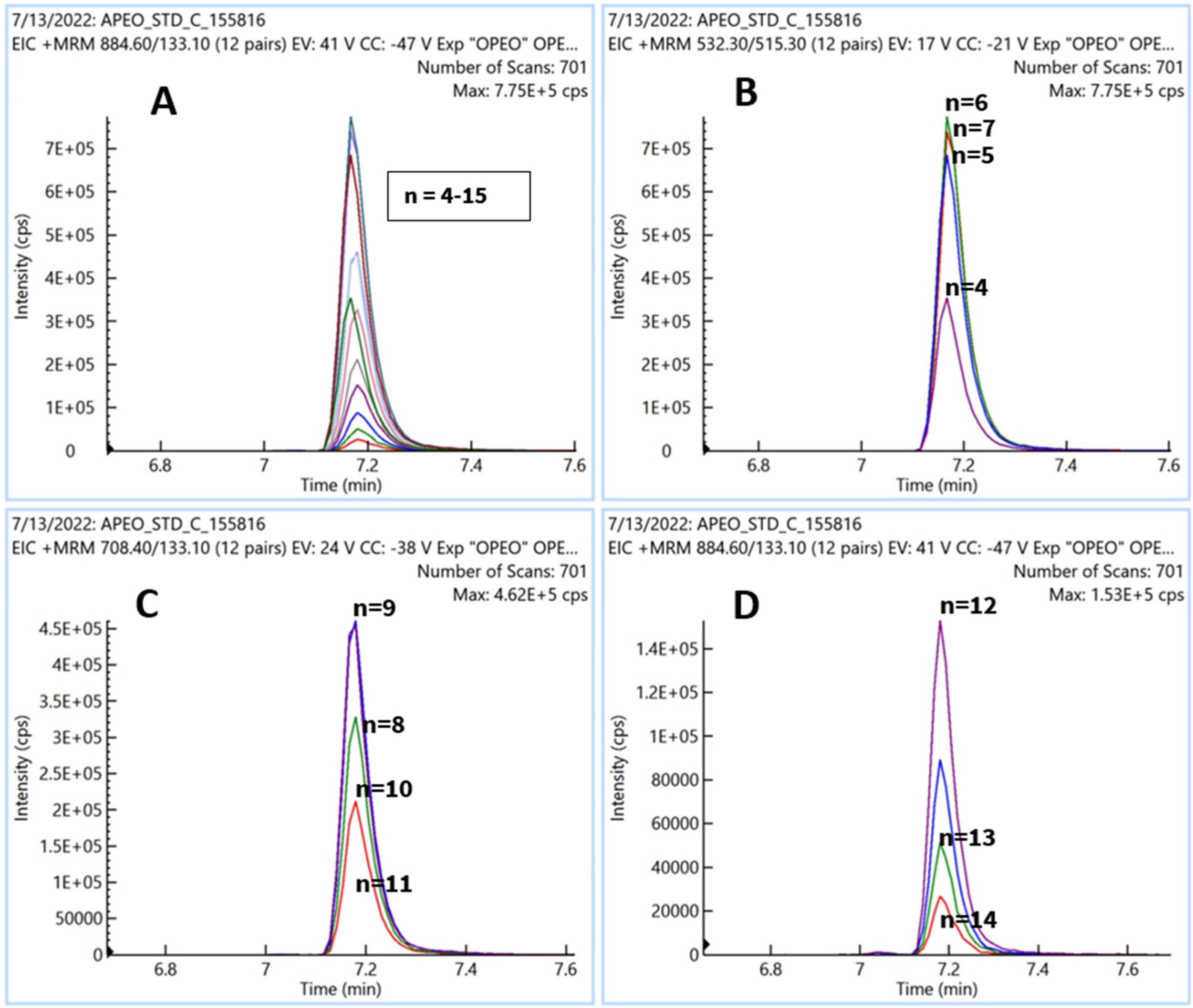


Figure 2: (A) Representative example of overlayed OPEO (n=4-15) in neat solution. (B) Overlapped OPEO (n=4 to 6). (C) Overlapped OPEO (n=9 to 11). (D) Overlapped OPEO (n=12 to 14).

Those congeners show a typical distribution like shown in the Figure 1 for NPEO and Figure 2 for OPEO. Same pattern is also observed by Jeannot et al[2] in environmental samples. There are no variations in the concentration distribution on the two batches regardless that the calibrators were stored at 22 °C in the autosampler for 13 days. The most abundant compounds for NPEO are n=4 to n=8 with the most abundant compound being n=6. For OPEO, the most abundant congeners are n=5, n=6 and n=7. The most critical compounds are n=2 and n=15 for NPEO and n=15 for OPEO. Due to the low abundance of those congeners, it's not recommended to add a calibrator lower than 0.005 mg/L. The concentration of NPEO n=2 at this level is 0.29 ng/mL (290 ppt) and OPEO N15 is 0.28 ng/mL (280 ppt).

Calculation of the concentration for each congener is performed by first calculating a concentration factor (peak area of each congener divided by the total peak area). Then the total concentration of each calibration level is multiplied by the concentration factor for each congener. Those calculations were done using an excel table. The calculated individual concentration for each congener was then copied and pasted into the Simplicity 3Q Software to build the calibration curve for each compound. Table 5 shows an example of the calculation performed for NPEO.

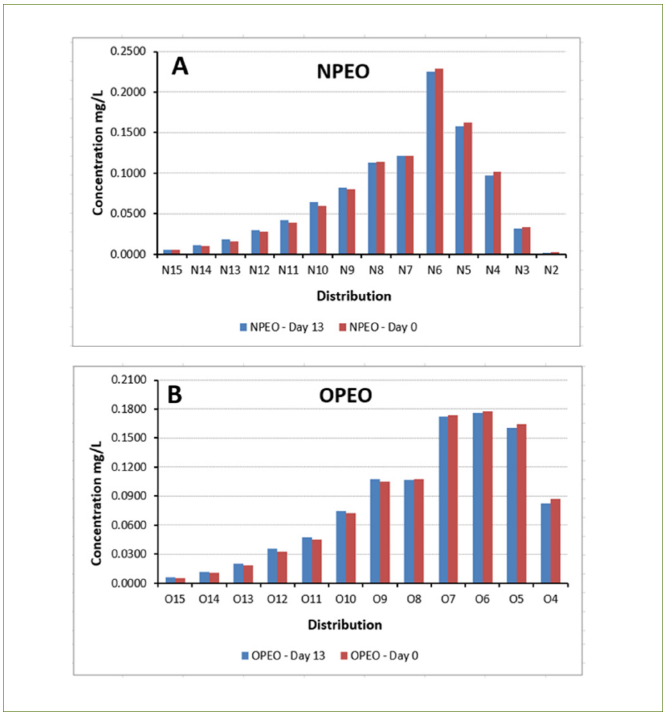


Figure 3: Concentration distribution for NPEO (A) and OPEO (B) measured on two different days. In red measures on day 0 and in blue day 13.

Table 5: Example of calculation of congeners concentration for NPEO.

NPEO	Peak Area	F.C.	STD 0.05	STD 0.1	STD 0.5
N15	93753	0.0057	0.0002907	0.0005814	0.00290701
N14	178825	0.0109	0.00055448	0.00110897	0.00554485
N13	298090	0.0181	0.00092429	0.00184858	0.00924292
N12	493381	0.0299	0.00152983	0.00305967	0.01529833
N11	693805	0.0421	0.00215129	0.00430258	0.0215129
N10	1053145	0.0639	0.0032655	0.006531	0.032655
N9	1350070	0.0819	0.00418618	0.00837236	0.04186179
N8	1858158	0.1128	0.00576161	0.01152323	0.05761615
N7	2002971	0.1215	0.0062	0.01242128	0.06210638
N6	3717681	0.2256	0.01152746	0.02305492	0.11527461
N5	2595138	0.1575	0.00804678	0.01609356	0.08046778
N4	1593436	0.0967	0.00494079	0.00988158	0.04940788
N3	520598	0.0316	0.00161422	0.00322845	0.01614255
N2	31030	0.0019		0.00019243	0.00096215
Sum	**16480081**		**0.0511**	**0.1022**	**0.5110**

Retention times for NPEO and OPEO are respectively 7.39 and 7.17 minutes (Figure 4A). Several gradients and different LC columns were tested, but it was not possible to separate chromatographically the NPEO or OPEO congeners. An impurity peak was found at 8.4 minutes on the NPEO trace, found more pronounced on n=2, n=3 and n=4 congeners. This contaminant is also present on the methanol blank (Figure 4 B). This peak could be generated by some compounds present on the samples (E.g., other surfactants with different branched alkylic portions, or lanolin residues, like sterols, fatty acids or waxes), that accumulates on column and are released at the end of the gradient, instead of an impurity in the methanol used for sample extraction. GC/MS analysis performed of those samples in the same laboratory seems to confirm this assumption.

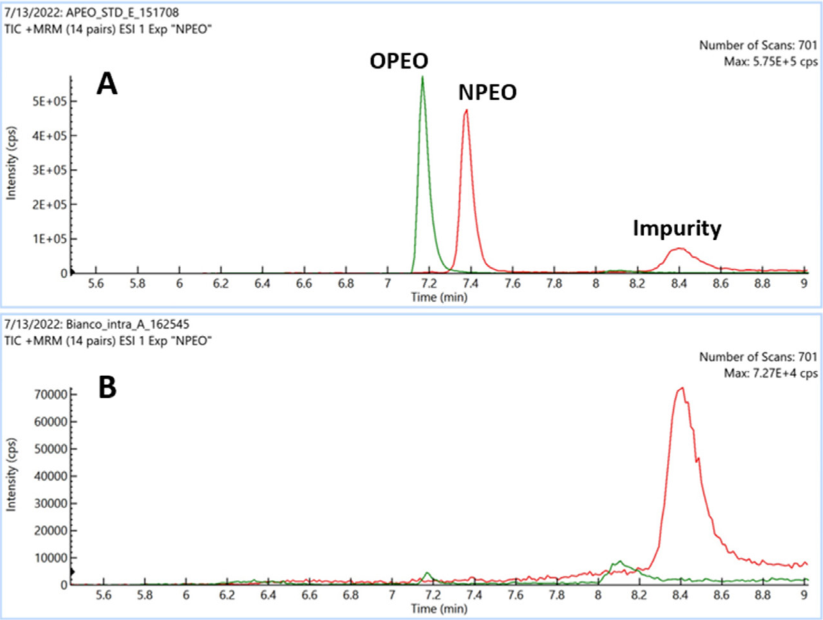


Figure 4: (A) TIC Overlay of NPEO (red) and OPEO (green) standard at 50 ng/mL). (B) Blank Methanol. In red NPEO in green OPEO.

Calibration curves were acquired at three different concentrations (0.005, 0.1 and 0.5 mg/L) with two replicates for each point. A calibration point at 2 mg/L was initially included but it was observed the bad linearity due to the high concentration of this standard. Samples showing concentration outside the calibration range are diluted enough to fit calibration curves and re-analyzed. All curves show R^2 greater than 0.99 (Figure 5 and Figure 6), as requested by the regulations for the analysis of APEO/AP in textiles (UNI EN ISO 21084 – 8.1, UNI EN ISO 18254-1 – 6.2).

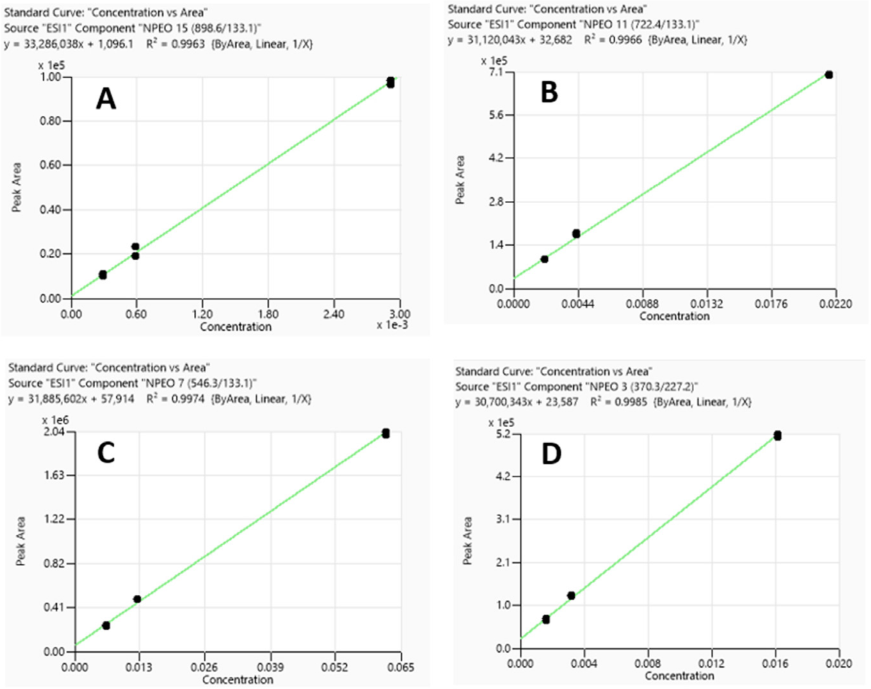


Figure 5: Representative examples of calibration curves for selected NPEO, (A) n=15, (B) n=11, (C) n=7 and (D) n=3. Two injections for each calibration level were performed.

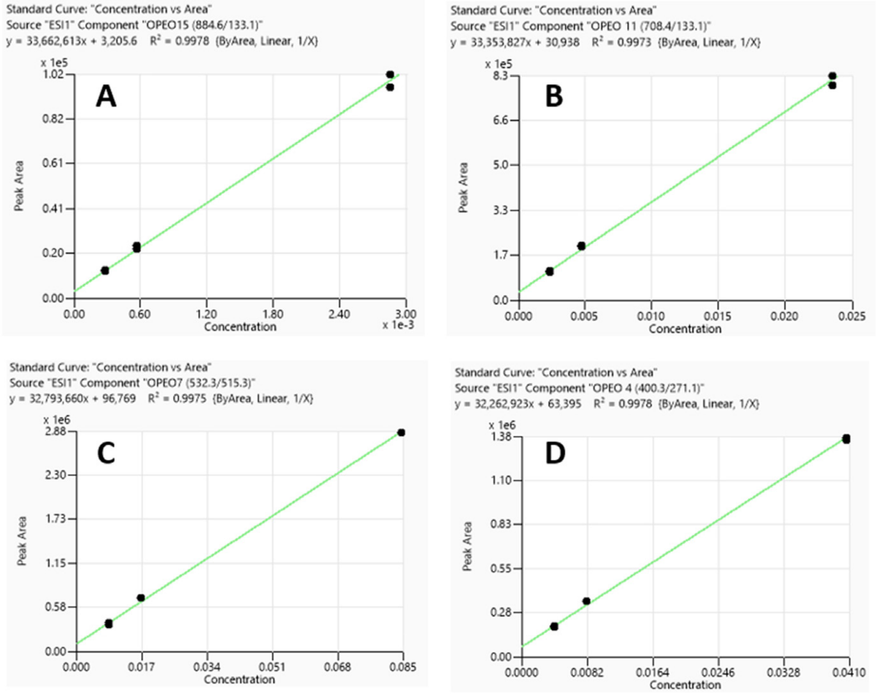


Figure 6: Representative examples of calibration curves for selected OPEO, (A) n=15, (B) n=11, (C) n=7 and (D) n=4. Two injections for each calibration level were performed.

Standard at 0.1 mg/L was repeated five times as quality control to check reproducibility inter and intra-day. Repeatability was measured by injecting five times the standard at 0.1 mg/L on both days.

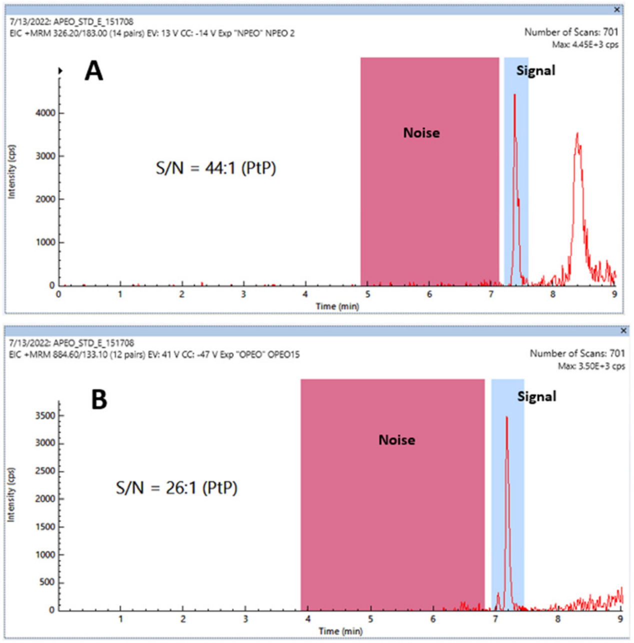
%CV are below 5% except for the two last sensitive NPEO, n=15 (%CV 6.98 on day 1 and 7.1 on day 13) and n=2 (%CV 10.43 on day 1 and 8.44 on day 13) (Table 6).

Table 6: % CV for 5 replicates for NPEO and OPEO concentration distribution (n=2 to 15 for NPEO and n=4 to15 for OPEO) for standard at 0.1 mg/L.

NPEO			OPEO		
n	%CV Batch A	%CV Batch B	n	%CV Batch A	%CV Batch B
15	6.98	7.1	15	3.98	3.74
14	4.98	2.69	14	2.13	2.2
13	3.21	2.83	13	1.6	1.2
12	1.31	3.4	12	2.49	1.99
11	2.62	3.91	11	1.84	3.44
10	2.56	2.04	10	1.4	2.81
9	2.3	2.1	9	1.7	2.58
8	1.03	2.38	8	1.9	1.62
7	2.99	2.23	7	1.13	3.11
6	1.5	0.96	6	0.97	2.39
5	1.65	1.22	5	1.53	2.9
4	1.64	1.06	4	0.79	1.76
3	2.1	1.69			
2	10.43	8.44			

Signal to noise for the less abundant congeners were measured using Peak-to-Peak method. Values are 44:1 for NPEO n=2 and 26:1 for OPEO n=15 (Figure 7).

Congener NPEO n=2 at the lowest calibration point (50 ng/mL) corresponds to a concentration of 96 ng/L (ppt). Considering the calculated signal to noise of 44:1 for this congener, a LOQ (signal to noise 10:1) can be estimated to be around 24 ng/L (ppt). The concentration of congener OPEO n=15 at the lowest calibration level of 50 ng/mL is 271.5 ng/L (ppt). LOQ estimated for this congener is around 170 ng/L (ppt).



7/13/2022: APEO_STD_E_151708
EIC +MRM 326.20/183.00 (14 pairs) EV: 13 V CC: -14 V Exp "NPEO" NPEO 2
Number of Scans: 701
Max: 4.45E+3 cps

7/13/2022: APEO_STD_E_151708
EIC +MRM 884.60/133.10 (12 pairs) EV: 41 V CC: -47 V Exp "OPEO" OPEO15
Number of Scans: 701
Max: 3.50E+3 cps

Figure 7: Signal to Noise (peak to peak) for NPEO n=2 and OPEO n=15 at lowest calibration level (50 ng/mL).

Alkylphenol compounds show a good chromatographic separation as shown in Figure 8A. Compounds are well separated from the impurity detected in the transition of 4-NP (m/z 219.2 / 133.2) at 8.4 minutes (Figure 8B). To avoid any possible contaminants coming from the mobile phases a shield column (PerkinElmer Brownlee C18 4.6 X 50mm, 5µm) was installed in the pump outlet, just after the mixer and before the autosampler.

Calibration curves at three different concentrations (0.05, 0.1 and 0.5 mg/L) were acquired in two different days, the same as for APEO, but in triplicate. R^2 are greater than 0.99 for all compounds in both days.

Figure 9 shows an example of data processing for 4-NP, showing the accuracy and CV % for the replicates. Standard at 100 ng/mL was injected five times to check repeatability. %CV are lower than 5 % for all compounds and very similar between the two batches.

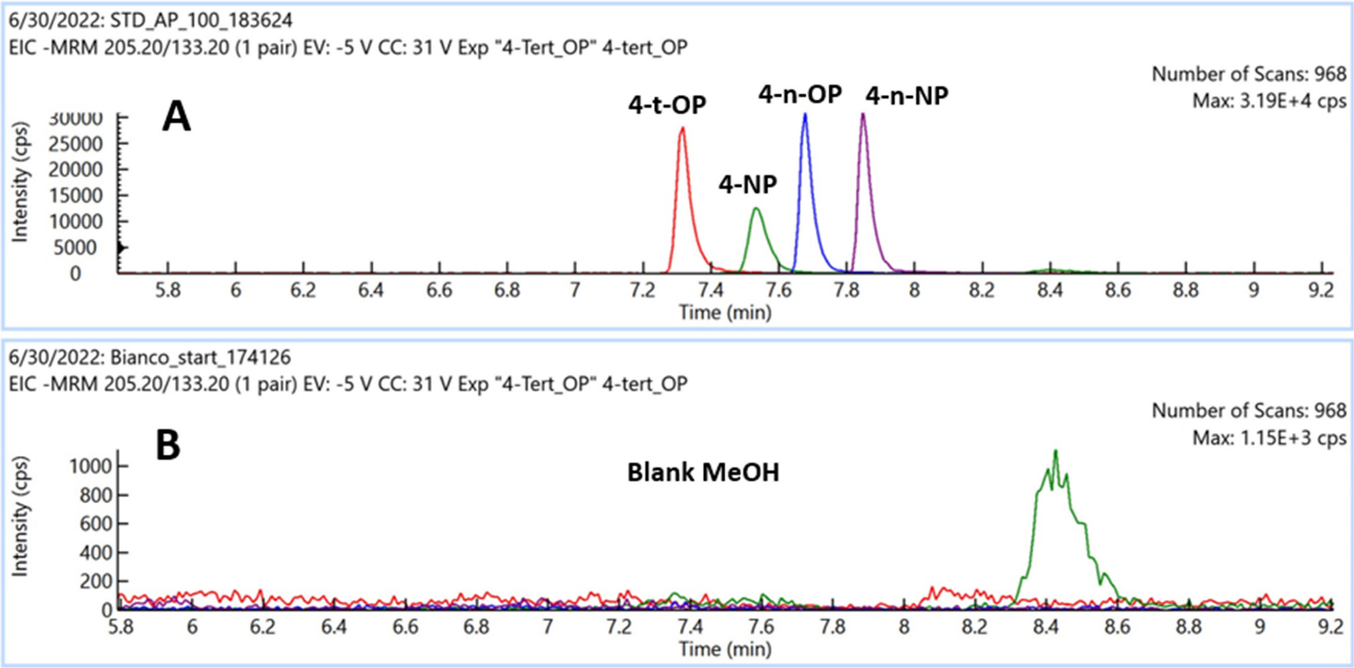
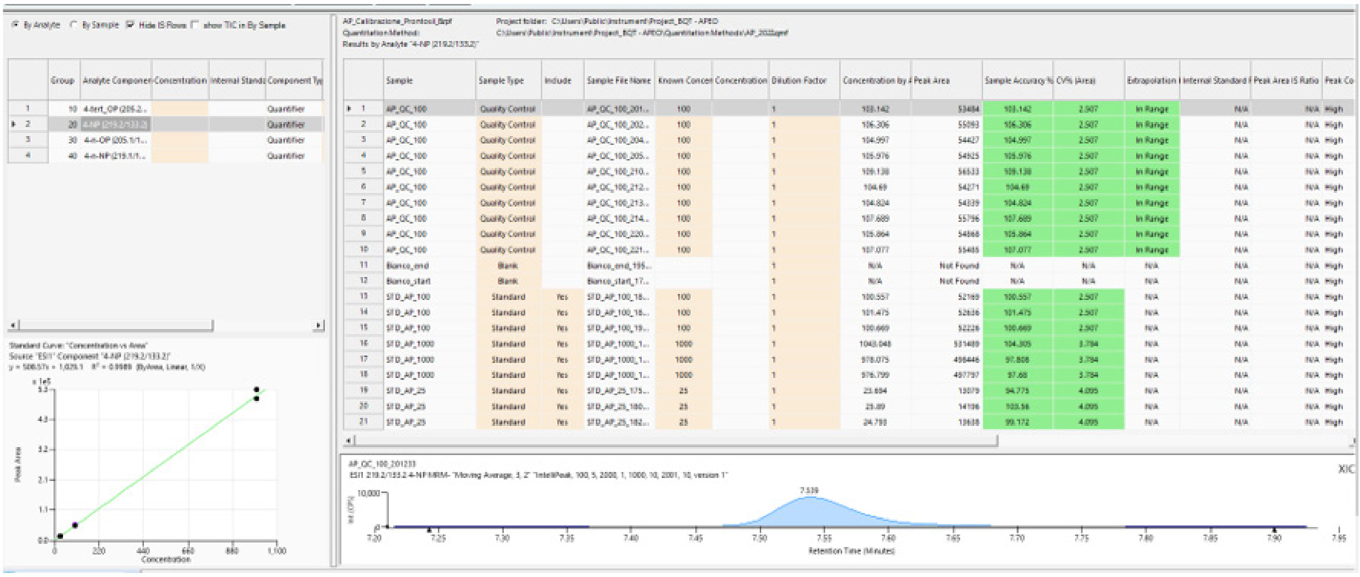


Figure 8: (A) Chromatographic separation for alkylphenol compounds. Standard at 100 ng/mL.. (B) Chromatographic trace of impurity detected in blank MeOH.



	Group	Analyte Componen	Concentration	Internal Standa	Component Ty
1	10	4-tert_OP (205.2...			Quantifier
2	20	4-NP (219.2/133.2)			Quantifier
3	30	4-n-OP (205.1/1...			Quantifier
4	40	4-n-NP (219.1/1...			Quantifier

Standard Curve: "Concentration vs Area"
Source "ESI1" Component "4-NP (219.2/133.2)"
y = 508.57x + 1,029.1   R² = 0.9989 (ByArea, Linear, 1/X)

AP_Calibrazione_Prontosil_8qf
Quantitation Method:
Results by Analyte "4-NP (219.2/133.2)"

Project folder: C:\Users\Public\Instrument\Project_BQT - APEO
C:\Users\Public\Instrument\Project_BQT - APEO\Quantitation Methods\AP_2022qmf

	Sample	Sample Type	Include	Sample File Name	Known Concen	Concentration	Dilution Factor	Concentration by A	Peak Area	Sample Accuracy %	CV% (Area)	Extrapolation	Internal Standard	Peak Area IS Ratio	Peak Co
1	AP_QC_100	Quality Control		AP_QC_100_201...	100		1	103.142	53484	103.142	2.507	In Range	N/A	N/A	High
2	AP_QC_100	Quality Control		AP_QC_100_202...	100		1	106.306	55093	106.306	2.507	In Range	N/A	N/A	High
3	AP_QC_100	Quality Control		AP_QC_100_204...	100		1	104.997	54427	104.997	2.507	In Range	N/A	N/A	High
4	AP_QC_100	Quality Control		AP_QC_100_205...	100		1	105.976	54925	105.976	2.507	In Range	N/A	N/A	High
5	AP_QC_100	Quality Control		AP_QC_100_210...	100		1	109.138	56533	109.138	2.507	In Range	N/A	N/A	High
6	AP_QC_100	Quality Control		AP_QC_100_212...	100		1	104.69	54271	104.69	2.507	In Range	N/A	N/A	High
7	AP_QC_100	Quality Control		AP_QC_100_213...	100		1	104.824	54339	104.824	2.507	In Range	N/A	N/A	High
8	AP_QC_100	Quality Control		AP_QC_100_214...	100		1	107.689	55796	107.689	2.507	In Range	N/A	N/A	High
9	AP_QC_100	Quality Control		AP_QC_100_220...	100		1	105.864	54868	105.864	2.507	In Range	N/A	N/A	High
10	AP_QC_100	Quality Control		AP_QC_100_221...	100		1	107.077	55485	107.077	2.507	In Range	N/A	N/A	High
11	Bianco_end	Blank		Bianco_end_195...			1	N/A	Not Found	N/A	N/A	N/A	N/A	N/A	High
12	Bianco_start	Blank		Bianco_start_17...			1	N/A	Not Found	N/A	N/A	N/A	N/A	N/A	High
13	STD_AP_100	Standard	Yes	STD_AP_100_18...	100		1	100.557	52169	100.557	2.507	N/A	N/A	N/A	High
14	STD_AP_100	Standard	Yes	STD_AP_100_18...	100		1	101.475	52636	101.475	2.507	N/A	N/A	N/A	High
15	STD_AP_100	Standard	Yes	STD_AP_100_19...	100		1	100.669	52228	100.669	2.507	N/A	N/A	N/A	High
16	STD_AP_1000	Standard	Yes	STD_AP_1000_1...	1000		1	1043.048	531489	104.305	3.784	N/A	N/A	N/A	High
17	STD_AP_1000	Standard	Yes	STD_AP_1000_1...	1000		1	978.075	498446	97.808	3.784	N/A	N/A	N/A	High
18	STD_AP_1000	Standard	Yes	STD_AP_1000_1...	1000		1	976.799	497797	97.68	3.784	N/A	N/A	N/A	High
19	STD_AP_25	Standard	Yes	STD_AP_25_175...	25		1	23.694	13079	94.775	4.095	N/A	N/A	N/A	High
20	STD_AP_25	Standard	Yes	STD_AP_25_180...	25		1	25.89	14196	103.56	4.095	N/A	N/A	N/A	High
21	STD_AP_25	Standard	Yes	STD_AP_25_182...	25		1	24.793	13638	99.172	4.095	N/A	N/A	N/A	High

AP_QC_100_201233
ESI1 219.2/133.2 4-NP MRM- "Moving Average, 3, 2" "IntelliPeak, 100, 5, 2000, 1, 1000, 10, 2001, 10, version 1"

Figure 9: Example of data processing for Alkylphenol 4-NP.

Sample Results

A sample showing low congeners concentration (sample number 256/10) was analyzed five times to check the repeatability. The total NPEO and OPEO concentrations in sample are calculated accordingly to the following equations:

$$\text{Sample Conc}\left(\frac{\text{mg}}{\text{Kg}}\right) = \frac{(\sum_{n=15}^{n=2} \text{NPEO measured concentration in mg/L}) \text{ X Dilution Factor X Extraction volume })}{\text{Sample weight}}$$

$$\text{Sample Conc}\left(\frac{\text{mg}}{\text{Kg}}\right) = \frac{(\sum_{n=15}^{n=4} \text{OPEO measured concentration in mg/L}) \text{ X Dilution Factor X Extraction volume })}{\text{Sample weight}}$$

Table 6 shows an example of calculation of the sample concentration calculated using the formula above. In this example the calculation is:

$$\text{Sample Conc}\left(\frac{\text{mg}}{\text{Kg}}\right) = \frac{(0.069 \ \frac{\text{mg}}{\text{L}} \text{ x } 1 \text{ x } 0.020\text{L })}{1.0007 \text{ g}} = 1.4 \text{ mg/kg}$$

Table 6: Calculation of total NPEO and OPEO concentrations (in mg/Kg) for one replicate of the sample 256/10.

Alkylphenol Ethoxylates				
Sample	**Sample Weight (g)**	**Ext Volume (mL)**	**Dilution Factor**	
			NPEO	**OPEO**
256/10-A	1.0007	20	1	1
File quant.	APEO_29_6_22.rpf			
Data	29/06/22			

NPEO		**OPEO**	
NPEO 15	0	OPEO 15	0.002
NPEO 14	0	OPEO 14	0
NPEO 13	0	OPEO 13	0
NPEO 12	0.001	OPEO 12	0
NPEO 11	0.002	OPEO 11	0
NPEO 10	0.005	OPEO 10	0
NPEO 9	0.008	OPEO 9	0.003
NPEO 8	0.011	OPEO 8	0
NPEO 7	0.008	OPEO 7	0
NPEO 6	0.018	OPEO 6	0
NPEO 5	0.009	OPEO 5	0
NPEO 4	0.005	OPEO 4	0
NPEO 3	0.002		
NPEO 2	0		
	mg/L		mg/L
Total NPEO (mg/kg)	1.4	Total NPEO (mg/kg)	NR

Table 7 shows the results for Sample 256/10 for all five replicates and Figure 10 shows the TIC for the sample 256/10 (A) and the extracted ion chromatogram for NPEO N=2, 6 and 10 (Figure 10 B, C, D). Each replicate corresponds to a separate sample extraction.

Table 7: Results for NPEO and APEO for the five replicates. Total concentration in mg/Kg in textile sample 256/10. OPEO was not detected in this sample.

Injection	NPEO (mg/Kg)	OPEO (mg/Kg)
1	1.4	NR
2	1.4	NR
3	1.3	NR
4	1.3	NR
5	1.3	NR

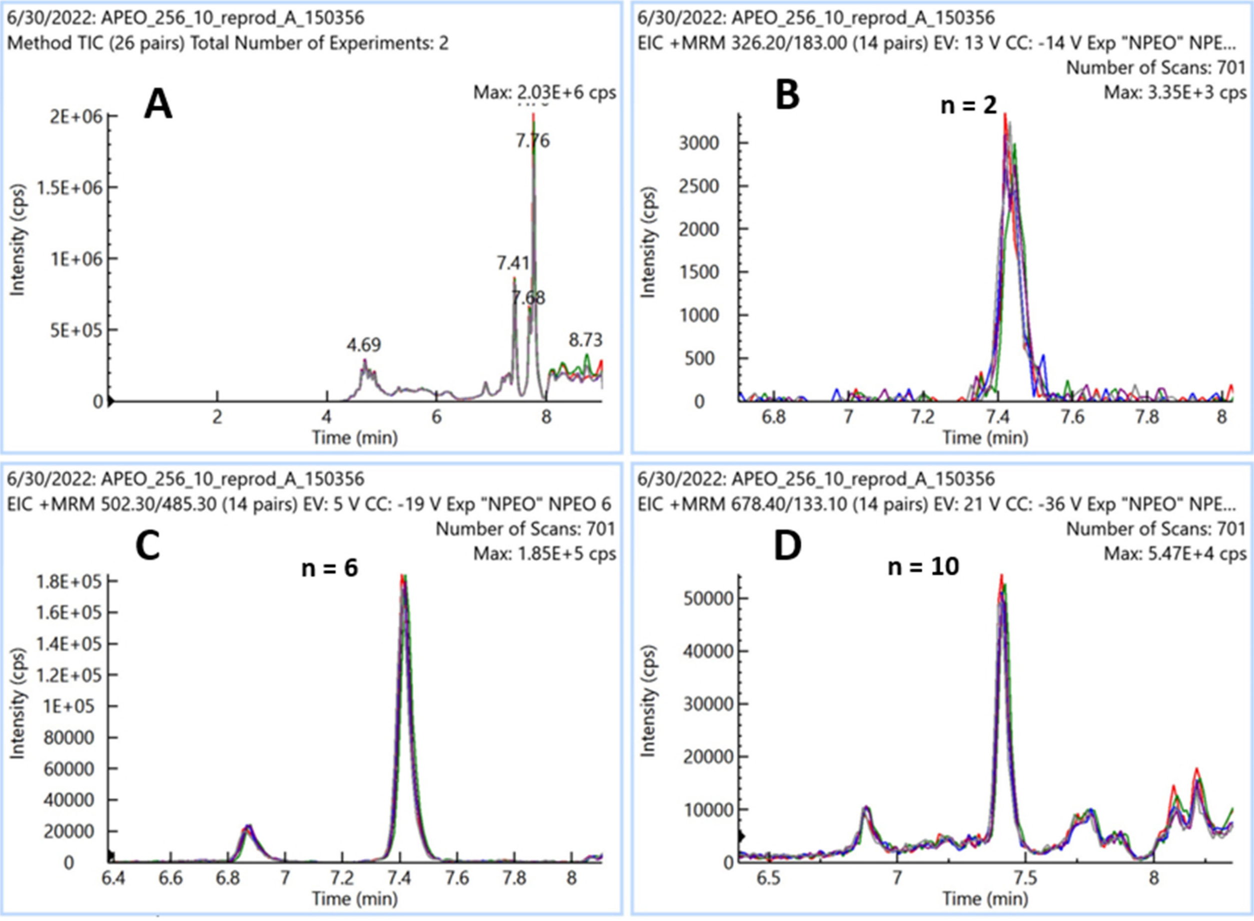


Figure 10: Overlays of five replicates of sample 256/10 (A) TIC (B,C,D) extracted ion chromatograms for NPEO N=2, 6,10.

Conclusion

For the analysis of Alkylphenol ethoxylates (APEO) and Alkylphenol (AP) compounds an LC/MS/MS method was developed following the main indications of the regulation UNI EN ISO 18254-1. The method was adapted to accommodate the analysis of APs. The main modifications were the replacement of acetonitrile by methanol in the mobile phase and the LC gradient and chromatographic column. The method removes the need for changing LC column and gradient, as APs show better chromatography when methanol is used. By replacing acetonitrile by methanol for APEO, sensitivity and peak shape are increased. Additionally, the method allows to reduce the injection volume, thanks to the system higher sensitivity, and it reduces the need for cleaning and extends the time between maintenance.

Calibrators and samples were prepared following the indications of the regulation UNI EN ISO 18254-1. Two sets of analysis were performed at two weeks of distance, showing identical results. Repeatability was also tested using a control sample, showing %CV lower than 5% for all congeners.

Results of the analysis show that the distribution of the APEO agree with the results published by by Jeannot et al[2] in water form sewage treatment plant. APEO (OPEO and NPEO) elutes at, respectively, 7.2 and 7.4 minutes and are chromatographically separated from an impurity eluting at 8.4 minutes. Repeatability at low concentration levels show good results with %CV's under 5 % for the majority of the compounds.

The sensitivity of the method, highlighted by the signal to noise calculation at the lower calibrator level, shows that the PerkinElmer QSight 210 LC/MS/MS has good sensitivity, with last abundant congeners being detected at ppt levels. The same sample was analyzed in replicates, providing consistent results, highlighting the robustness of the method. HSID interface on QSight LC/MS/MS System provides robustness when working on difficult matrices, like for example in textiles, and more reliable results.

This newly developed method shows potential for extended analysis on different types of textiles using the same extraction method and can include some anionic surfactants similar to the APEOs, as for example sulfonated compounds or congeners with different ramification.

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