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Journal of Chromatography A, 927 (2001) 103–110

JOURNAL OF CHROMATOGRAPHY A

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Routine analysis of alcohol and nonylphenol polyethoxylates in wastewater and sludge using liquid chromatography–electrospray mass spectrometry

A. Cohen[a,*], K. Klint[b], S. Bøwadt[c,1], P. Persson[a], J.Å. Jönsson[a]

[a]*Department of Analytical Chemistry, Lund University, P.O. Box 124, S-221 00, Lund, Sweden*
[b]*Department of Chemistry, Copenhagen University, Universitetsparken DK-2200, Copenhagen, Denmark*
[c]*DHI Water & Environment, Agern Allé 11, DK-2970 Hørsholm, Denmark*

Received 1 September 2000; received in revised form 29 May 2001; accepted 22 June 2001

Abstract

A wide range of alcohol and nonylphenol polyethoxylates was determined by separation on a reversed-phase liquid chromatographic column, followed by electrospray ionisation-mass spectral analysis. The compounds were separated chromatographically according to their aliphatic chain length. The mass spectral analysis functioned as a second separation step during which homologues of the ethoxylates were separated according to their polyethoxylate chain length. In this manner a truly orthogonal separation was obtained. The compounds were detected as ammonium complexes. The analysis presented is capable of qualitative and quantitative determination of a large number of ethoxylates as well as their metabolites in a single run. The method was applied to many different sample types, ranging from primary and treated wastewater to sludge. Batches of 50 real samples were routinely analysed without the need for cleaning the mass spectrometer or regeneration of the column. By utilising the extracted mass chromatograms, detection limits of 1 to 10 μg/l could be obtained for individual compounds in water samples, while the detection limits were around 100 μg/kg in sludge, depending on the degree of pollution.

Keywords: Water analysis; Alcohol polyethoxylates; Nonylphenol polyethoxylates

1. Introduction

Alcohol and nonylphenol polyethoxylates are the most commonly used non-ionic surfactants. They are active ingredients in a long row of detergents and they are also used in pesticide formulations as well as in liquid fuels. More than 600 000 tons are discharged yearly into the environment [1]. Alcohol polyethoxylates have generally replaced nonylphenol polyethoxylates in most European countries due to the persistency and estrogenic effects of nonylphenol polyethoxylate degradation products. However, nonylphenol polyethoxylates are still used in a large number of countries worldwide. It is clearly of interest to be able to monitor the concentration and the degradation pathways of nonylphenol polyethoxylates as well as alcohol polyethoxylates once they are

*Corresponding author. Tel.: +46-46-2228-166; fax: +46-46-222-4544.

E-mail address: arieh.cohen@analykem.lu.se (A. Cohen).

[1]Presently at European Commission, Research Directorate-General, Measurement &Testing, Infrastructure, Rue de la Loi 200, B-1049 Brussels, Belgium.

0021-9673/01/$ – see front matter
PII: S0021-9673(01)01064-0

discharged to the environment, as little is known about the ultimate fate of these compounds. Alcohol and nonylphenol polyethoxylates are similar in structure as they both have a hydrophilic domain consisting of a polyethoxylate chain and a hydrophobic domain consisting of an aliphatic chain or an aryl moiety, respectively.

Unlike nonylphenols, alcohol polyethoxylates lack a chromophore making them difficult to detect with conventional liquid chromatography (LC) detectors [2]. Although these compounds have been detected by LC–UV, LC–fluorescence, gas chromatography–flame ionisation detection (GC–FID) as well as GC–mass spectrometry (MS), these techniques require a derivatisation step [3–7]. LC–MS allows direct analysis of alcohol polyethoxylates, with respect to both their aliphatic chain and their polyethoxylate chain, in a single run without derivatisation.

Alcohol and nonylphenol polyethoxylates are non-ionic and as such uncharged. It is thus necessary to charge them for MS analysis. The oxygen atoms in the polyethoxylate chain can donate their free electrons to a complexing agent and the flexible structure of the chain allows the molecule to ''wrap'' itself around the complexing ion which was confirmed by empirical and semi-empirical molecular modelling [8] which also showed a significant destabilization of crown ether type ethoxylate-ion complexes for compounds with less than four ethoxylate units. In this manner polyethoxylates form strong complexes with small cations in a way that may be similar to that of the crown ether. A number of complexing ions (H^+, Na^+, K^+, NH_4^+, Rb^+, Cs^+), have been used for the MS analysis of polyethoxylates [8,9]. In this work only ammonium was used.

In the analysis presented the compounds are separated on a C_{18} reversed-phase high-performance liquid chromatography (HPLC) column. In this step the compounds are separated according to the aliphatic chain length as the retention is primarily based on the interaction between the hydrophobic domain and the C_{18} phase and to a much lesser extent based on the solubility of the ethoxylate chains in the mobile phase [10]. Thus when the compounds enter the subsequent MS analysis they are grouped as co-eluting homologues that differ in mass by 44 mass units (corresponding to one ethoxylate unit, $-CH_2-CH_2-O-$). As the MS separates according to mass the homologues are individually detected. In this way the analytes are first separated according to alkane chain length and then according to ethoxylate chain length. Polyethoxylates were determined in samples containing solid matter (up to 25% solid matter), which has not previously been published.

2. Materials and methods

2.1. LC–MS

The chromatographic system consisted of a HP 1100 HPLC system (Hewlett-Packard, Palo Alto, CA, USA) with a Phenomex Luna 5 μm, 100 Å, 250×2.00 mm C_{18} analytical column (flow-rate 0.2 ml/min, injection volume 20 μl, column temperature 40°C). The chromatographic system was connected to an Esquire-LC mass spectrometer (Bruker Daltonics, Bremen, Germany) with an electrospray interface, operated in the positive ionisation mode. The mass spectrometer was set to scan between m/z 300 and 1400. Nitrogen was used as a drying gas and was pumped into the interface at the rate 7 l/min with a temperature of 300°C. Nitrogen was also used as a nebulizer gas and was kept at 30 p.s.i. (1 p.s.i.=6894.76 Pa). The following voltages were used: nebulizer capillary tip 4000 V, endplate at the sampling orifice 3500 V, sampling capillary exit 80 V, skimmer 1 24 V and skimmer 2 6 V.

2.2. Separation and elution solvents

Separations were performed with gradient elution. The eluents were water (A) (obtained from a Millipore purification system, Bedford, MA, USA) and methanol (B) (Merck, Darmstadt, Germany) both containing 5 m*M* ammonium acetate (Merck) and 0.5 m*M* trichloroacetic acid (Merck). The elution program consisted of a linear gradient from 80 to 90% eluent B during 30 min. The chromatographic conditions were developed to avoid the need for column regeneration.

2.3. Standards

The standards, Nonidet P40 (commercial nonyl-

phenolethoxylate), $C_{10}EO_6$ (hexaethylene glycol monodecylether), $C_{12}EO_6$ (hexaethylene glycol monododecylether), $C_{14}EO_6$ (hexaethylene glycol monotetradecylether), $C_{16}EO_6$ (hexaethylene glycol monohexadecylether), $C_{18}EO_6$ (hexaethylene glycol monooctadecylether), were purchased from Fluka (Buchs, Switzerland). Field samples were taken from wastewater treatment plants in Herning and Hillerød, Denmark. All samples contained two internal standards, hexylphenol pentaethylene glycol and ethylphenol pentaethylene glycol [synthesized with a purity better than 98% as determined by nuclear magnetic resonance (NMR), by Niels Svenstrup at the Department of Chemistry, Odense University, Denmark].

2.4. Sample pre-treatment

The extraction is part of an accredited method in accordance with Danish law [11]. Liquid–liquid extraction was chosen rather than solid-phase extraction or centrifugation for sample pre-treatment as extraction from the solid part of the samples as well the liquid also was of interest. All samples were homogenised prior to extraction. Influent and effluent samples were collected as weekly flow proportionate samples whereas sludge samples were collected twice daily. Raw wastewater was collected as 300 g samples whereas sludge samples were collected so that they contained about 10 g of dry matter (approximately 100–300 g of wet matter). At this point all samples were stored at −18°C in glass containers until further preparation. Sludge samples were slurried with 40 ml of water. The samples were adjusted to pH 10–12 with sodium hydroxide (Merck) and 100 μl of internal standard solution containing 100 ppm of hexylphenol pentaethylene glycol and ethylphenol pentaethylene glycol was added. 150 ml of dichloromethane (Rathburn, Waterburn, UK) was added, the samples were ultrasonicated for 5 min followed by 2 h on a shaking table. The samples were centrifuged with a tabletop centrifuge at 1300 rpm for 5 min. The dichloromethane phase was collected and filtered through dry sodium sulfate (Merck) to remove residual water. Finally, the solvent was evaporated and the samples were redissolved in 5 ml of buffer B. In this paper recoveries were determined for all samples by measuring the absolute recovery of the internal standards and were typically between 30 and 60%. The sample pre-treatment offered an up-concentration of typically 10 to 30 times the original sample.

3. Results

3.1. Mass spectra

As mentioned earlier, the analytes were separated by LC according to the length of their aliphatic chain. Thus, the co-eluting compounds differ in mass according to their polyethoxylate chain length. The various masses of the ethoxylates are shown in Table 1. The resulting mass spectra display a series of masses with 44 ($-CH_2CH_2O-$) mass units between each signal (see Fig. 1).

Table 1
The masses of the various ethoxylates that were investigated in this work[a]

EO units	$OPEO_x$	$NPEO_x$	C_6EO	C_7EO	C_8EO	C_9EO	$C_{10}EO$	$C_{11}EO$	$C_{12}EO$	$C_{13}EO$	$C_{14}EO$	$C_{15}EO$	$C_{16}EO$	$C_{17}EO$	$C_{18}EO$
6	488	502	384	398	412	426	440	454	468	482	496	510	524	538	552
7	532	546	428	442	456	470	484	498	512	526	540	554	568	582	596
8	576	590	472	486	500	514	528	542	556	570	584	598	612	626	640
9	620	634	516	530	544	558	572	586	600	614	628	642	656	670	684
10	664	678	560	574	588	602	616	630	644	658	672	686	700	714	728
11	708	722	604	618	632	646	660	674	688	702	716	730	744	758	772
12	752	766	648	662	676	690	704	718	732	746	760	774	788	802	816
13	796	810	692	706	720	734	748	762	776	790	804	818	832	846	860
14	840	854	736	750	764	778	792	806	820	834	848	862	876	890	904
15	884	898	780	794	808	822	836	850	864	878	892	906	920	934	948

All masses include the ammonium ion.

[a] For the internal standards ethylphenol and hexylphenol pentaethylene glycol, the masses are m/z 360 and m/z 416, respectively.

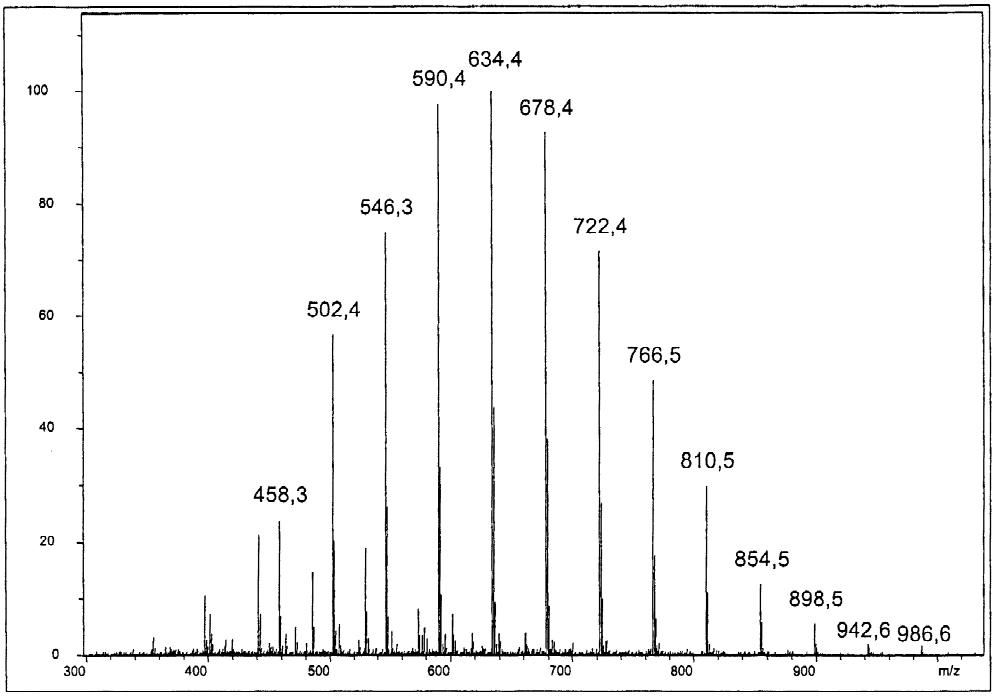

Fig. 1. Mass spectrum for nonylphenol ethoxylates. Each mass represents one ethoxylate chain length, e.g., m/z 590, which is C_9PhEO_8. The m/z difference of 44 corresponds to the mass of one ethoxylate unit.

3.2. Calibration

Four-point calibrations were performed using standard solutions containing nonylphenol EO_x, $C_{10}EO_6$, $C_{12}EO_6$, $C_{14}EO_6$, $C_{16}EO_6$, and $C_{18}EO_6$ as well as two internal standards hexylphenol pentaethylene glycol and ethylphenol pentaethylene glycol (Fig. 2). The calibration solutions contained 1, 2.5, 5 and 10 mg/l of the standards except for nonylphenol EO_x, which were at double concentration. The internal standards were present at 1 ppm in all calibration solutions. Corrected peak areas were calculated for the calibration standards and were used to plot the calibration curves. Calibration curves typically passed through the origin with a regression coefficient better than 0.999 and were similar to that for $C_{12}EO_6$. $y=0.47\pm(0.02)x+0.039\pm(0.10)$, $n=4$, the values in parentheses are 95% confidence intervals. Ethoxylates of varying chain lengths ($EO_x>5$) were found to have the same response in the MS detection (data not shown). Calibration samples were injected into the LC–MS at 10 sample intervals and no changes in performance were observed.

3.3. Field samples

Wastewater and sludge samples from Herning and Hillerød wastewater treatment plants were analysed on a routine basis. The Herning plant treats industrial wastewater (textile production, manual and manufacturing industries) while the Hillerød plant primarily treats domestic sewage. The variation of the sample sources does not pose a problem although the detection limits are affected. Table 2 shows the results of samples taken from various steps in a wastewater treatment plant. The detection limits for individual compounds in wastewater were typically 1 μg/l whereas the detection limits in sludge samples were typically 100 μg/kg, due to the more complicated matrix. Detection limits calculations were based on the standard deviations of the MS peak. The versatility of the analysis is demonstrated in Fig.

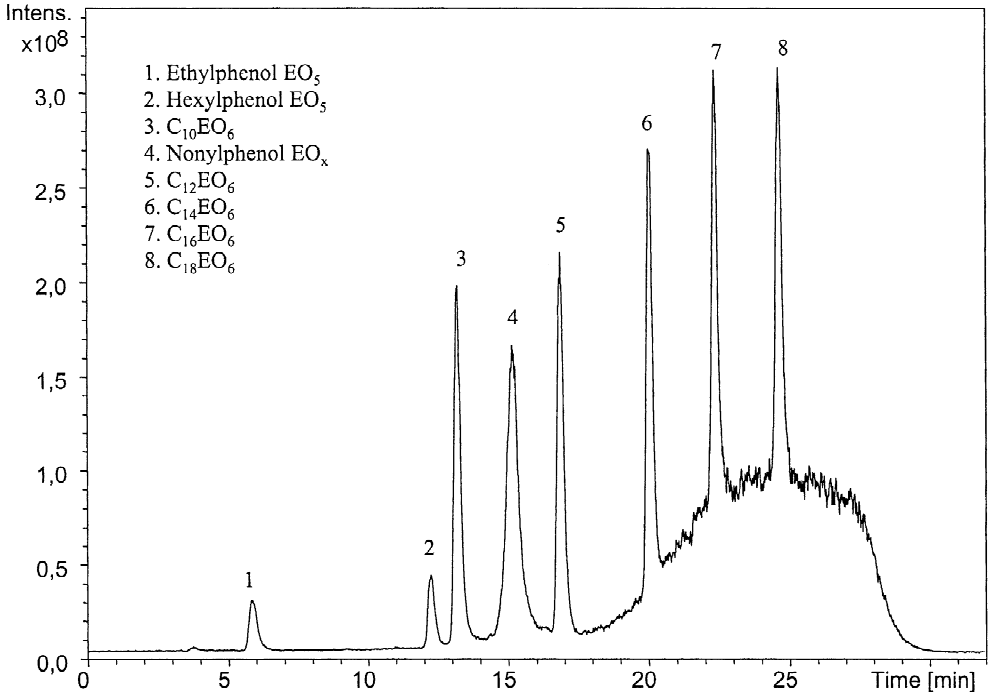

Fig. 2. Standard chromatogram used for calibration (10 ppm).

3. The figures show total ion chromatograms (TICs) and extracted chromatograms as well as mass spectra for peaks that relate to the extracted chromatograms. The multiple peaks seen in Fig. 3D are due to different branching in the aliphatic chain. Although the TICs appear to be inadequate for analytical purposes, the extracted chromatograms offer satisfactory information.

4. Discussion

With the LC–MS method presented, it was possible to measure approximately 130 compounds quantitatively as well as qualitatively in a single 30 min run. It is apparent that the large number of compounds analysed in such a short period of time gives rise to large amounts of complicated data. To simplify the data it is necessary to use extracted ion chromatograms instead of TICs, which also adds to the sensitivity of the method. It is also of utmost importance that the ionisation of the analytes is carefully controlled. Other ions present (i.e., Na^+) can also form complexes with ethoxylates causing multiple signals for a given compound. In order to avoid this problem an excess of ammonium is added to the sample as well as to the mobile phase [8]. Multiple charge states can also give rise to several signals for a single analyte. The ion trap quadrupole instrument avoids these multiply charged ions as the less stable multiply charged ions disassociate during the dwell time in the trap. Only singly charged ammonium adducts were measured for the analytes as seen in Figs. 1 and 3E, F. While developing the method it was found that the ion trap instrument was more suitable for the analysis than a quadrupole instrument due to the higher sensitivity of the ion trap [12]. A further complication is that the aliphatic chain of a homologue may have variations (i.e., linear, branched), which affects retention times. This is demonstrated in Fig. 3D in which multiple peaks are observed. The major peak in Fig. 3D is the linear form whereas the smaller peaks represent various branched forms. As the method presented is designed

Table 2
Results from the analysis of long chain nonylphenol ethoxylates and alcohol ethoxylates using LC–MS on samples from Herning waste treatment plant

Sample	Primary sludge (mg/kg)	Bio-sludge (mg/kg)	Pre-digested sludge (mg/kg)	Digested sludge (mg/kg)	Dry sludge (mg/kg)	Influent wastewater (μg/l)	Primary effluent (μg/l)	Reject water (μg/l)
	[c]	[c]	[c]	[c]	[c]	[d]	[d]	[d]
C_8EO_x	–	–	–	–	–	–	–	–
C_9EO_x	–	–	–	–	–	–	–	–
$C_{10}EO_x$	1.8	15.8	–	18.6	3.6	16.8	22.8	–
$C_{11}EO_x$	–	–	1.6	–	–	11.8	17.3	–
C_2PhEO_x	–	–	–	–	–	–	–	–
C_6PhEO_x	–	–	–	–	–	–	–	–
$OPEO_x$[a]	–	–	–	–	–	–	–	–
$NPEO_x$[b]	43.6	9.3	21.1	–	–	25.1	32.1	2.4
$C_{12}EO_x$	44.0	48.0	34.6	42.0	12.8	13.4	16.5	2.75
$C_{13}EO_x$	291	4.30	130	–	–	344	444	–
$C_{14}EO_x$	98.4	7.33	52.8	–	–	6.50	4.67	–
$C_{15}EO_x$	194	–	89.4	–	–	17.8	14.7	–
$C_{16}EO_x$	25.3	10.7	10.9	–	–	–	–	–
$C_{17}EO_x$	–	–	–	–	–	–	–	–
$C_{18}EO_x$	–	–	–	–	–	–	–	–
Recovery (%)	55	61	45	37	30	52	58	49
Total nonylphenol ethoxylate	43.6	9.3	21.1			25.1	32.1	2.4
Total alcohol ethoxylate	654	86.2	319	51.3	16.3	410	520	2.8
Dry matter	3.57	4.46	3.59	1.71	26.4	–	–	–

Each value is the average of two measurements.
[a] Octylphenol-ethoxylate C_8PhEO_x.
[b] Nonylphenol-ethoxylate C_9PhEO_x.
[c] Concentration less than 0.1 mg/kg if not detected.
[d] Concentration less than 0.1 μg/l if not detected.

to handle a wide range of samples, matrix effects is another complicating factor. By adding two internal standards, it is possible to use the one that is least affected by matrix and sample interference.

The method offers linear detection for concentrations in the resulting extracts ranging from 1 to 10 ppm. Variations in response for different compounds appear to primarily depend on their ability to complex. Clearly, signal intensities drop off drastically for compounds with a chain length of less than four ethoxylate units [8,9]. This has been verified by calculation of the ion stability of the ammonium complex through empirical molecular modelling [8].

One of the strengths of LC–MS is the high level of selectivity the technique offers. By knowing the mass as well as the retention time for a compound it is possible to unequivocally determine an analyte in terms of alkyl chain length and ethoxylate chain length. Chromatograms representing signals for specific masses (extracted chromatograms) may be reconstructed after a run is complete. Basing the quantitative analyses on the extracted chromatograms instead of the TIC allows the reduction of noise and the effects of co-eluting matrix compounds. By adding selected extracted chromatograms to each other whole groups of compounds may be analysed very precisely.

The method's ability to handle complex samples was demonstrated by analysing samples from sewage treatment plants. The data shown in Table 2 is a quantification of homologues. The most predominant homologues (EO_x, $x=6,\ldots,15$) are quantified by integrating the peaks areas of the combined extracted chromatograms. The fact that many concentrations are well above the calibrated linear range of the method is a result of data handling, e.g., the concentrations presented are a sum of 10 individual compounds. The suitability of applying this method

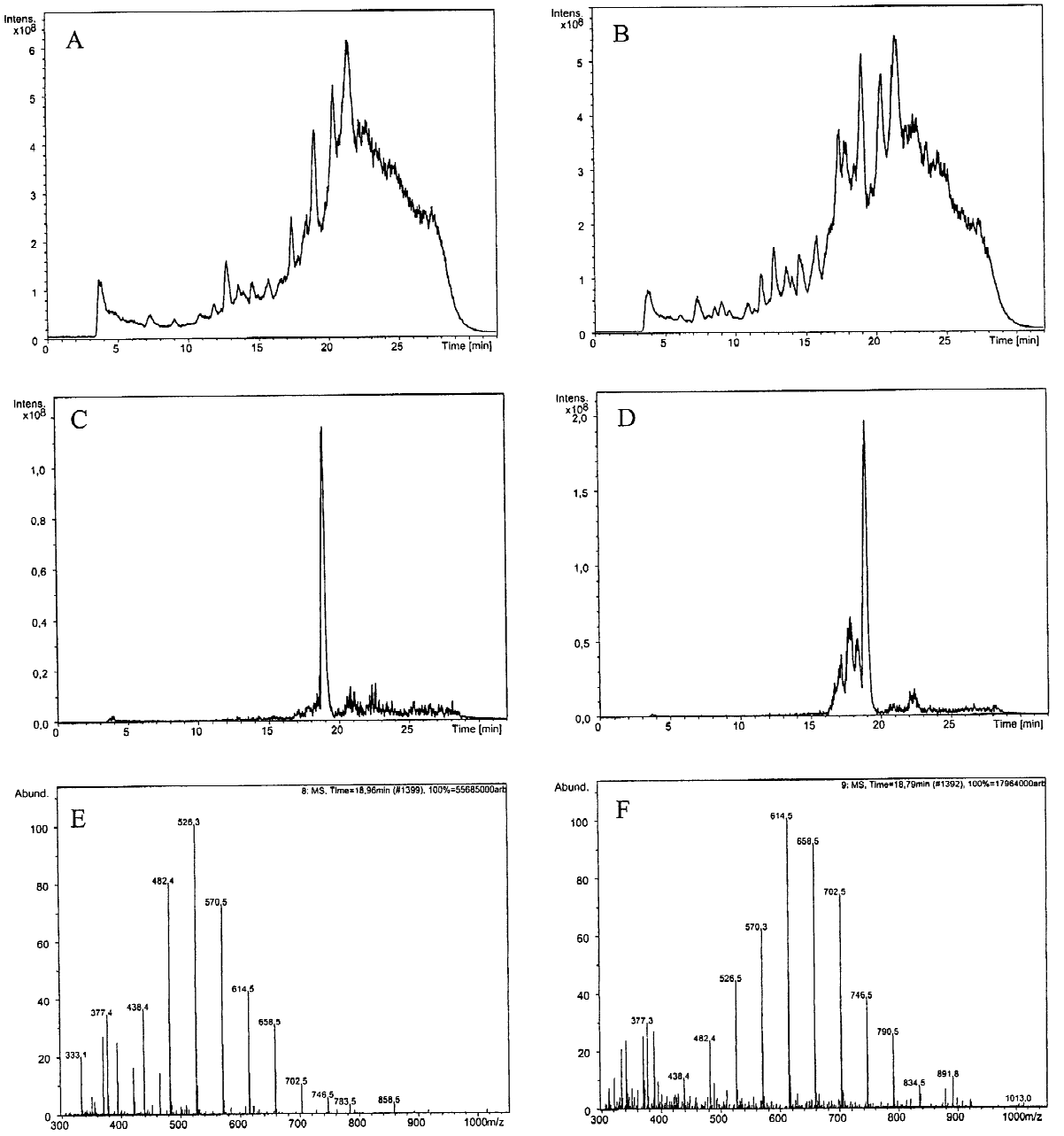

Fig. 3. Four mass chromatograms for field samples. Chromatograms A and B are TICs for primary sludge samples from Hillerød and Herning wastewater treatment plants, respectively. Chromatograms C and D are extracted chromatograms for $C_{13}EO_x$ (x=6, . . . , 15) for primary sludge samples from Hillerød and Herning, respectively. In chromatogram D several peaks appear due to variation in the branching of the alkyl chain. E and F represent the mass spectrum from the extracted chromatograms.

to routine analysis of dirty samples is demonstrated by the fact that over 200 sludge and wastewater samples have been analysed by this method without the need for column regeneration or cleaning of the instrument.

As the method offers orthogonal separation and highly selective detection, samples from a wide range of sources may readily be analysed. For the same reason, it is also possible to follow the composition profile of the polyethoxylates in the environment. This is amply demonstrated in Table 2, where the transformation of the incoming compounds can be followed through the different processes in a wastewater treatment plant. In conjunction with GC–MS analysis of carboxylate metabolites [13], it would be possible to follow the degradation pattern of the polyethoxylates.

5. Conclusion

The method presented in this work is capable of analysing a wide range of nonylphenol and alcohol polyethoxylates in complex field samples. The orthogonal separation achieved by LC–MS offers unequivocal determination of the analytes with respect to their aliphatic chain length as well as their polyethoxylate chain length.

Acknowledgements

We would like to thank DHI Water & Environment (Hørsholm, Denmark) for their generous financial support.

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