

# Determination of nonionic polyethoxylate surfactants in wastewater and sludge samples of sewage treatment plants by liquid chromatography-mass spectrometry

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**Abstract.** An analytical method is proposed in order to determine the major nonionic surfactants, octylphenolpolyethoxylates (OPEOs), nonylphenolpolyethoxylates (NPEOs) and aliphatic alcoholpolyethoxylates (AEOs), and their metabolites, nonylphenol and octylphenol in wastewater and sludge samples of sewage treatment plants. This method involved a Soxtec extraction step and a solid phase extraction step for the nonionic surfactant extraction from sludge and wastewater samples, respectively. With both methodologies, all recovery values were higher than 70 %. Quantitation and identification of individual compound were carried out by liquid chromatography-mass spectrometry (LC/MS) using electrospray (ESP) as ionization mode. Reverse phase LC chromatography allowed the elution of all oligomer constituents of each homolog component into a single peak. Gathering all the oligomers into a single peak increased the peak intensity and provided a way to determine low concentration of APEOs and AEOs in different environmental matrices. Information on the oligomers distribution of APEOs and AEOs could be obtained by the extraction of selected  $m/z$  ions from the TIC chromatograms. Positive ESP LC/MS analyses of AEOs and APEOs yielded primary pseudo-molecular ions of the  $[M + Na]^+$  type for each oligomer. The negative ionization mode allowed the determination of the two metabolites, nonylphenol and octylphenol, characterized by the pseudo molecular  $[M-H]^-$  ion. Limits of detection achieved were 0.1  $\mu\text{g/L}$  in wastewater sample and 10  $\text{ng/g}$  in sludge samples. The developed LC-ESP/MS method was applied to the analysis of wastewater and sludge samples of two sewage treatment plants located in Orléans (France).

**Keywords.** Nonionic surfactants – alcohol polyethoxylates – nonylphenol polyethoxylates – octylphenol polyethoxylates – sludge – wastewater – LC/MS.

## Introduction

Nonionic surfactants are used in a broad spectrum of household products and industrial applications [1]. The major part of nonionic surfactants consists of aliphatic alcohol ethoxylates (AEOs) and alkylphenol ethoxylate (APEOs), octylphenolpolyethoxylates (OPEOs) and nonylphenolpolyethoxylates (NPEOs) being the major compounds of this later group. AEOs and APEOs are acronyms used to indicate very complex surfactant mixtures. Each nonionic surfactant molecule consists of an hydrophobic and an hydrophilic moiety. The hydrophobic moieties (alkyl chains) can vary in length leading to various homolog compounds. In addition, AEOs and APEOs can be unbranched, monobranched and highly branched species leading to various isomeric homolog compounds. The hydrophilic moieties (ethoxy chain) may range from 1 to 20 ethoxy units in detergent formulations. The number of ethoxy unit defines an oligomer. After use, nonionic surfactants are usually disposed to sewage treatment plants (STPs). During sewage treatment, AEOs and APEOs are degraded by the hydrolytic removal of ethoxylate groups into short chain ethoxylates, carboxylic acid derivatives and finally to alkylalcohol and alkylphenol themselves [2,3].

Short chain ethoxylate compounds are hydrophobic compounds and tend to accumulate into sludges. In addition, APEOs breakdown products, mainly nonylphenol and octylphenol are regarded as more toxic than their parent compounds to aquatic organisms and elicit weak estrogenic activities [4]. As a consequence, throughout northern Europe, a voluntary ban on APEOs use in household cleaning products began in 1995 and restrictions on industrial cleaning products are set to follow in coming years [5]. Moreover, the ECC directive 86/278 set the maximum limits for nonylphenol, NPEO<sub>1</sub> and NPEO<sub>2</sub> at 50  $\text{mg/kg}$  in sludge used on a land. Up to date various monitoring programs have shown the presence of AEOs and APEOs and their metabolites in sewage effluent and surface water at concentrations ranging from nanograms to milligrams per liter meanwhile in sediment and sludge concentrations were found to be higher than milligrams per kilograms [6, 7].

Nonionic surfactants are commonly Soxhlet extracted from sludge using methanol [8]. In an attempt to shorten the sample extraction time, CO<sub>2</sub> supercritical fluid extraction with on line acetylation [9] or with methanol as modifier [10] has been proposed. However, when using aged

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sediment samples, the extraction yields are drastically reduced as compared to spiked samples, being 51 % and 49 % for NPEOs and OPEOs, respectively. Pressurized liquid extraction (PLE) has shown to fail for the extraction of NPEOs from sediments [10] although a PLE method was recently proposed for the extraction of nonylphenol [11]. Finally, extraction of polar nonylphenol polyethoxycarboxylate metabolites from sludge is performed using subcritical (hot) water extraction with ethanol as a modifier [12]. The extraction of APEOs or AEOs from water are readily achieved by solid phase extraction (SPE) involving either C-18 [13] or graphitized carbon black material [14].

Application of gas chromatography mass spectrometry has been limited to the determination of the compounds with less than five ethoxy units due to the high polarity, low volatility and thermal instability associated with the higher oligomers [15, 16]. As a consequence, liquid chromatography-mass spectrometry (LC/MS) is gaining acceptance for the characterization of APEOs and AEOs in different environmental matrices [17, 18, 19]. Normal phase chromatography has allowed the determination of individual NPEOs oligomers [19] while NPEOs and AEOs were separated according to the alcoholic or phenolic alkyl chain length as a reverse phase system was employed [13, 21].

In view of the different approaches to determine nonionic surfactants in wastewater and solid matrices, the main aims of this work were the followings:

- To develop a routine analytical method based on LC/MS able to quantify and identify APEOs and AEOs in sludge and wastewater samples.
- To apply the developed analytical method to real samples from two STPs.

## Experimental

### Reagents

LC solvents were obtained from Merck (Darmstadt, Germany). Triethylamine and acetic acid were from Fluka (Buchs, Switzerland). 4-nonylphenol and 4-octylphenol were purchased from Aldrich (L'Isle-d'Abeau, France) while hexaethylene glycol hexadecyl ether ( $C_{16}EO_6$ ), and hexaethylene glycol octadecyl ether ( $C_{18}EO_6$ ) were obtained from Fluka. NEODOL 25-9 was a gift from Shell Chemical Co (Houston, Tx). The NEODOL 25-9 AEOs was made from  $C_{12-15}$  alcohols and ethoxylated to an average of nine ethylene oxide units per mole of alcohol and was used for AEOs quantitation purposes. The relative proportion of polyethylene glycol dodecyl ether ( $C_{12}EO_x$ ), polyethylene glycol tridecyl ether ( $C_{13}EO_x$ ), poly-ethylene glycol tetradecyl ether ( $C_{14}EO_x$ ) and poly-ethylene glycol pentadecyl ether ( $C_{15}EO_x$ ) was found to be 21 %, 20 %, 28 % and 31 %, respectively. The Triton X-100, made from 4-tert-octylphenol ethoxylated (OPEOx) to an average of nine ethylene oxide units was obtained from Aldrich and was used for octylphenol oligomer quantitation purposes. Nonidet P40

Substitute, made of nonylphenol ethoxylated (NPEOx) to an average of nine ethylene oxide units was purchased from Fluka and was used for nonylphenol oligomer quantitation purposes. Standards were prepared by dissolving compounds in gradient grade methanol and stored in dark bottle at 4 °C.

### Samples collection

Influent and effluent water samples of two sewage treatment plants of Orléans were collected in Pyrex borosilicate glass containers. Samples were preserved by adding 3 % formaldehyde (v/v) and stored in the dark at 4 °C before analysis. Formaldehyde treatment is necessary to prevent changes in the oligomer composition due to biodegradation. Sludge samples available for use in agricultural fields were also collected, oven dried at 40 °C, sieved and stored at - 20 °C until analysis. Only fractions below 150  $\mu$ m were analysed.

### Analytical procedure

#### Sludge sample spiking

200  $\mu$ l of a methanolic solution of APEOs and AEOs was added to a slurry of 10 g of a sludge sample dissolved in 10 ml of methanol. After an equilibration time of 24 h, the solvent was eliminated with a gentle stream of nitrogen and the sludge was left stand for at least one week. Prior to extraction, the water content was adjusted to 10 % (w/w) and the material was allowed to swell for one day. Blank samples were prepared in the same way using pure methanol as spiking agent.

#### Sample preparation and preconcentration

Wastewater samples: 250 mL of influent and effluent water samples were filtered with a 0.45  $\mu$ m cellulose acetate membrane filter. A loading volume of 250 mL was applied to C-18 cartridges (1 g, 6 mL) from Supelco (Bellefonte, USA) as recommended [13] at a flow rate of 5 mL/min. The sorbent was conditioned with 5 mL of methanol and 5 mL of water at 1 mL/min. The elution step was performed using successively 2  $\times$  5 mL of hexane/dichloromethane (9/1) and 2  $\times$  5 mL of methanol/dichloromethane (9/1), the last fraction containing the compounds of interest. Total evaporation of the methanolic extracts was carried out with a stream of nitrogen. The extract was reconstituted to a final volume of 1 mL in appropriate HPLC mobile phase prior to analysis.

Sludge samples: Sludge samples were Soxtec extracted prior to clean up. 10 g aliquot of the homogenized sample were Soxtec extracted with 50 mL of methanol. Sample was immersed in methanol during 45 min and then rinsed for at least 4 h. The methanolic extract was diluted with water to obtain a matrix of water/methanol (70/30) and then was passed through C-18 SPE cartridges (1 g, 6 mL). Cartridge conditioning and elution were carried out in a similar way as for water analysis. The extract was reconstituted to a final volume of 1 mL in appropriate HPLC mobile phase prior to analysis.

## Analysis

HPLC mobile phase was delivered by a 9012 Varian elution gradient pump. Mobile phase consisted of a mixture of A (50 % methanol/50 % acetonitrile) and B (water). Mobile phase was acidified with 0.5 % (v/v) acetic acid for running in positive ionization mode (PI). 0.1 % (v/v) triethylamine was added to the mobile phase for running in negative ionization mode. The mobile phase composition was 70 % A at the beginning of the gradient and then linearly increased to 100 % in 28 min. Isocratic during 2 min. Nonionic surfactants were separated by means of an Hypersyl Green Env column (150 × 4.6 mm i.d., 5 μm particle size) equipped with a guard column both from Interchim (Montluçon, France). The flow rate was 0.8 mL/min and the eluent was splitted so that 0.3 mL/min entered the mass spectrometer source. 20 μl of the SPE extracts were injected into the LC system.

For electrospray MS experiments, a SSQ 7000 mass spectrometer (Finnigan, San Jose, CA) equipped with a standard atmospheric pressure ionization source was used. The electrospray voltage and the collision voltage on quadrupole were set to 4.5 kV and 10 V, respectively. The temperature of the heated capillary was 250 °C. Nitrogen was used as nebulizing gas at a pressure of 5 bar. In positive ionization mode (PI), the m/z range was from 200 to 1200. In negative ionization mode (NI), two ions at m/z 205 and m/z 219 were monitored.

## Results and discussion

### Optimization of the extraction conditions

The efficiency of the SPE and Soxtec extraction procedures for quantitatively recover AEOs and APEOs from water and sludge samples was assessed by spiking water and sludge samples with known appropriate volumes of the working composite standard solution (see experimental part). Table I

**Table I.** Mean recovery values (R) and relative standard deviations (RSD,  $n = 5$ ) of AEOs, OPEOs, NPEOs, nonylphenol and octylphenol determined from spiked wastewater and sludge samples. Spiking level: 20 μg/L in wastewater samples and 200 ng/g in sludge samples.

	wastewater			Sludge		
	R (%)	RSD (%)	LODs <sup>a</sup>	R (%)	RSD (%)	LODs <sup>b</sup>
C12EOx	75	8	0.1	90	15	10
C13EOx	82	11	0.1	88	14	10
C14EOx	84	9	0.1	92	11	10
C15EOx	88	8	0.1	85	15	10
Nonylphenol	102	12	0.1*	72	16	1*
Octylphenol	98	11	0.1*	74	16	1*
Octylphenol-EOx	92	7	0.5	69	12	20
Nonylphenol-EOx	94	8	0.5	71	12	20

a: LODs in μg/L; b: LODs in ng/g; \* LODs calculated using SIM mode of detection.

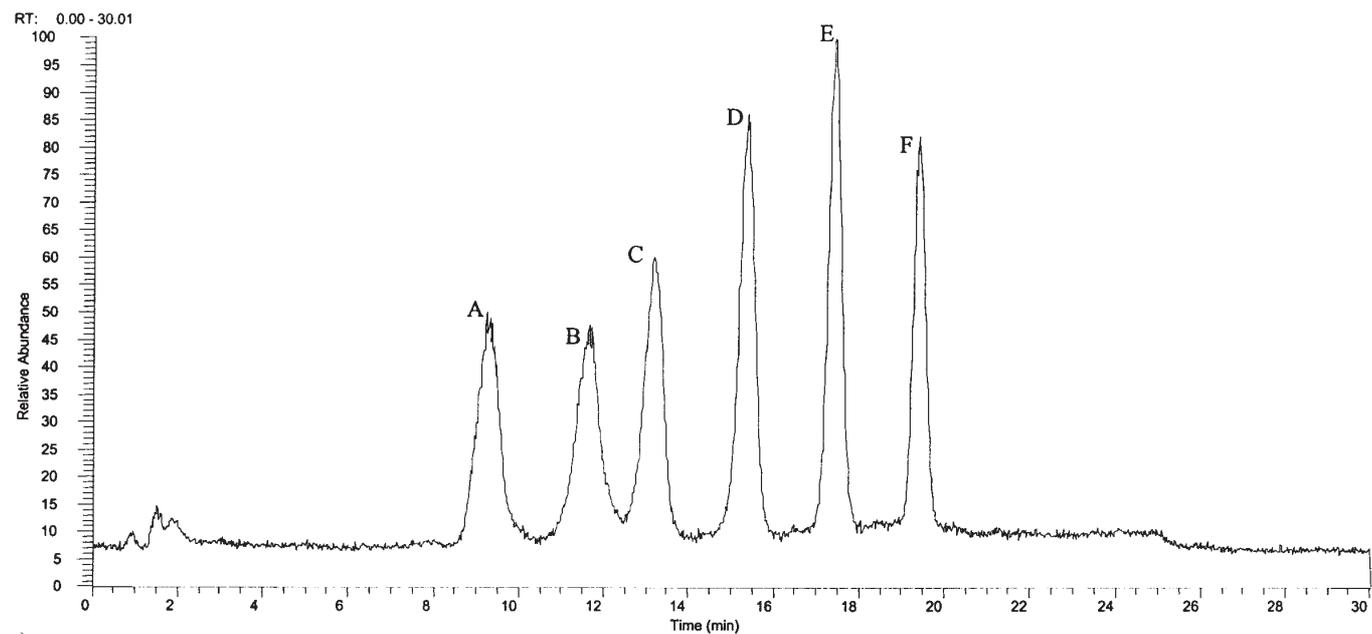
shows the recovery values obtained with the two matrices. All recovery values were higher than 70 % showing the good performances of the SPE and Soxtec methods used for preconcentration of NPEOs and OPEOs from wastewater and sludge samples, respectively. When dealing with LC analysis, additional clean-up than SPE clean-up has not turned out to be necessary for sludge analysis.

### Performances of the analytical method

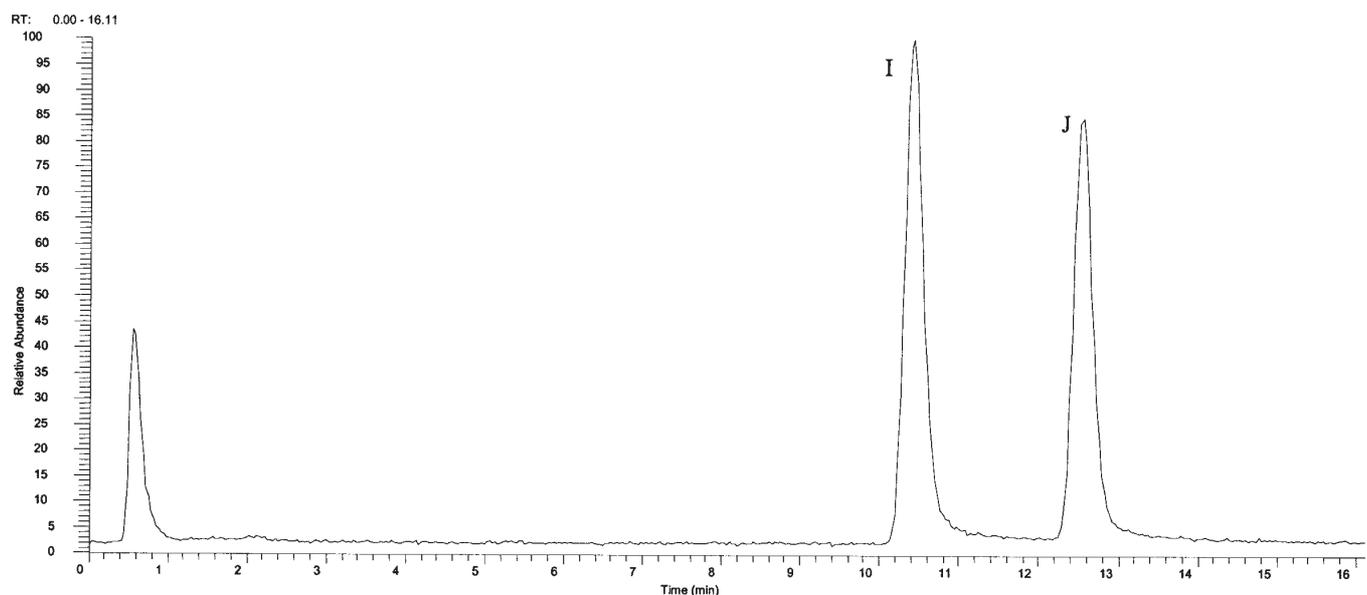
As mentioned in the literature [17], reverse phase LC chromatography allowed the elution of all oligomer constituents of each homolog component into a single peak. Gathering all the oligomers into a single peak increased the peak intensity and provided a way to determine low concentration of APEOs and AEOs. Information on the oligomers distribution of APEOs and AEOs could be obtained by the extraction of selected m/z ions from the TIC chromatograms. Figure 1a presents the elution profile of the six commercial standards (OPEOx, NPEOx, C<sub>12</sub>EOx, C<sub>13</sub>EOx, C<sub>14</sub>EOx, C<sub>15</sub>EOx) in positive ESP ionization mode while figure 1b shows the detection of 4-nonylphenol and 4-octylphenol in negative ESP ionization mode. The use of acetic acid in PI mode led to thinner peaks while the addition of triethylamine to the mobile phase greatly enhanced the detection of the 4-nonylphenol and 4-octylphenol in NI mode. Optimal sensitivity for AEOs and APEOs was obtained by splitting the LC column flow rate so that only 0.3 mL/min entered into the mass spectrometer source.

As far as compound identification is concerned, PI-ESP LC/MS analyses of AEOs and APEOs yielded primary pseudo-molecular ions of the [M + Na]<sup>+</sup> and [M + K]<sup>+</sup> type for each oligomer, [M + Na]<sup>+</sup> being the base peak. Response intensity of the [M + Na]<sup>+</sup> ion steadily increased with the number of ethoxy units. EO<sub>1</sub> and EO<sub>2</sub> oligomers of APEOs and AEOs were not detected owing to low absolute concentration of these oligomers in the standards. In a similar way, oligomers with a number of ethoxy units higher than 18 appeared not to be present in the standards. Nonionic polyethoxylated surfactants were identified by checking correspondence with the following expressions: AEO<sub>*n,x*</sub> [C<sub>*n*</sub>H<sub>2*n*+1</sub>(OCH<sub>2</sub>CH<sub>2</sub>)<sub>*x*</sub>OH] should correspond to Mw = 14*n* + 44*x* + 41; OPEOx [C<sub>8</sub>H<sub>17</sub>-C<sub>6</sub>H<sub>4</sub>(OCH<sub>2</sub>CH<sub>2</sub>)<sub>*x*</sub>OH] to Mw = 229 + 44*x*; NPEOx [C<sub>9</sub>H<sub>19</sub>-C<sub>6</sub>H<sub>4</sub>(OCH<sub>2</sub>CH<sub>2</sub>)<sub>*x*</sub>OH] to Mw = 243 + 44*x*. As an illustration, figure 2a and 2b depict the mass spectrum of NPEOx and C<sub>12</sub>EOx, respectively. The negative ionization mode allowed the determination of the two metabolites, 4-nonylphenol and 4-octylphenol, characterized by the pseudo molecular ions [M-H]<sup>-</sup> at m/z 219 and m/z 205, respectively.

External calibration was used for quantitation purposes. No internal standard was used as the broad range of pollutants present in the samples made difficult its selection. Calibration curves were obtained by injecting the available standards in a range of concentration encompassing the sample's concentration that is between 1 mg/L to 100 mg/L (injection volume: 20 μl). The detector was linear over 3 order of magnitude with coefficients of correlation



a)

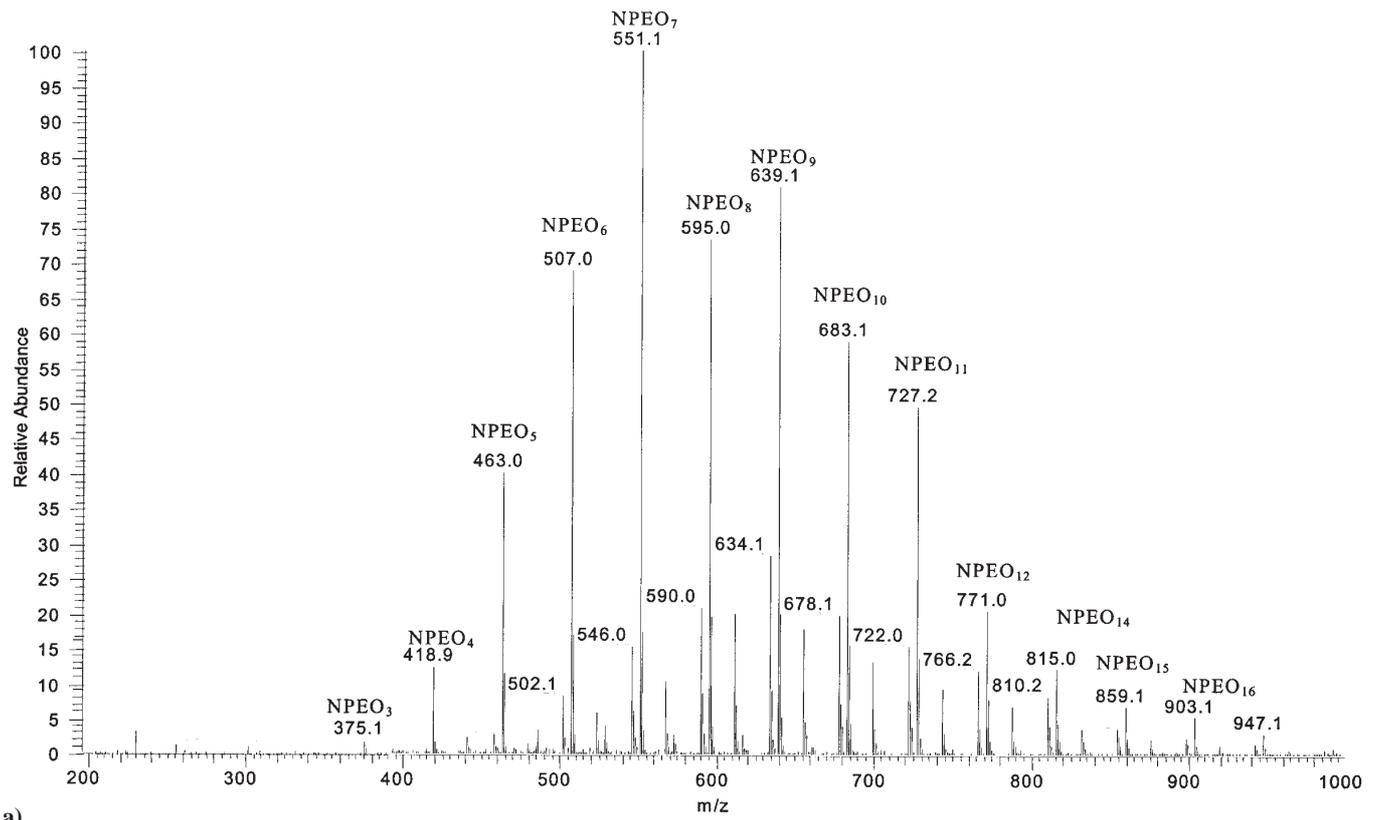


b)

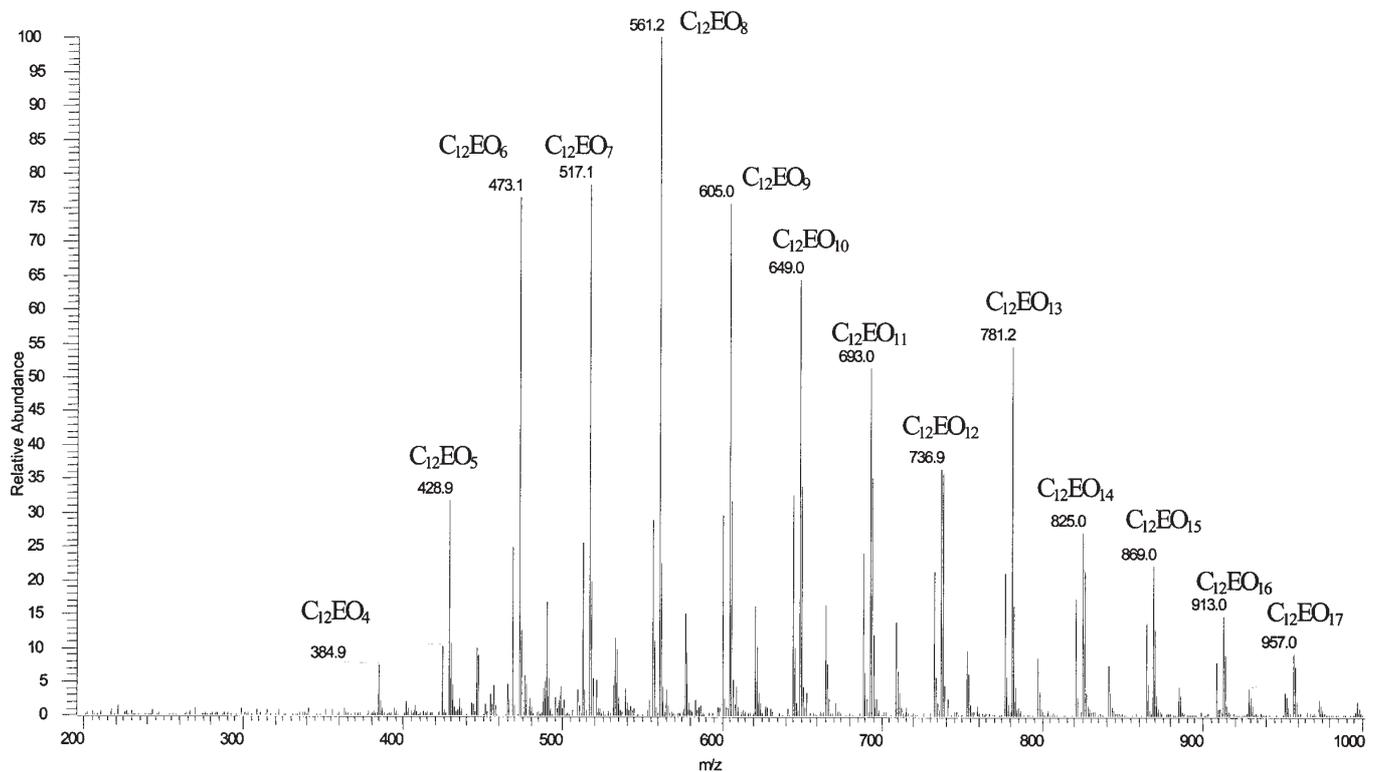
**Figure 1.** a) TIC chromatogram in PI ESP mode of a mixture of standards, 50 ng each. A (OPEOx); B (NPEOx); C ( $C_{12}$ EOx); D ( $C_{13}$ EOx); E ( $C_{14}$ EOx); F ( $C_{15}$ EOx). b) SIM chromatogram in NI ESP mode of a mixture of standards, 5 ng each. I (4-octylphenol); J (4-nonylphenol).

$r^2 > 0.0994$ . AEOs, NPEOs and OPEOs quantitation was based on the assumption that the average ethoxylate chain length in samples was close to the ethoxylate chain length of the reference standards [22]. Limits of detection (LODs) were calculated from a signal-to-noise ratio of 3:1. Table I reports the LODs achieved with the developed analytical

method using full scan conditions or SIM conditions in the case of 4-nonylphenol and 4-octylphenol. After preconcentrating 250 mL of wastewater, LODs ranged from 0.1 to 0.5  $\mu\text{g/L}$  according to the compound. After extracting 10 g of sludge sample, LODs were between 1 and 20 ng/g according to the compound.



a)



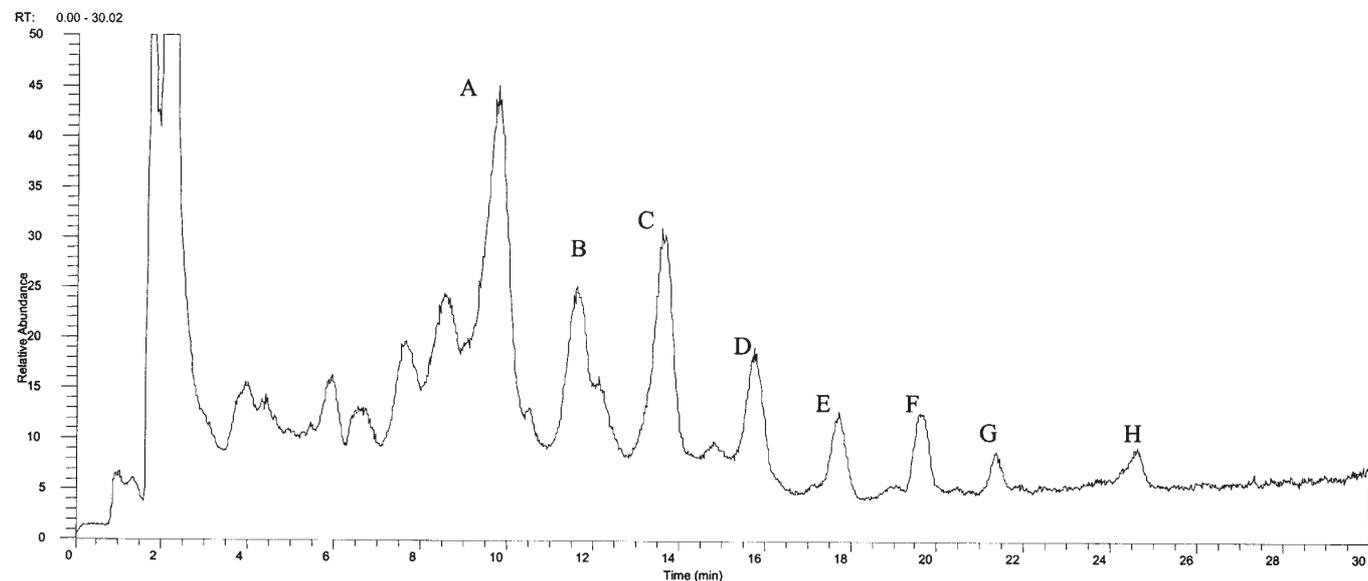
b)

Figure 2. a) mass spectrum of NPEOx. b) mass spectrum of C<sub>12</sub>EOx.

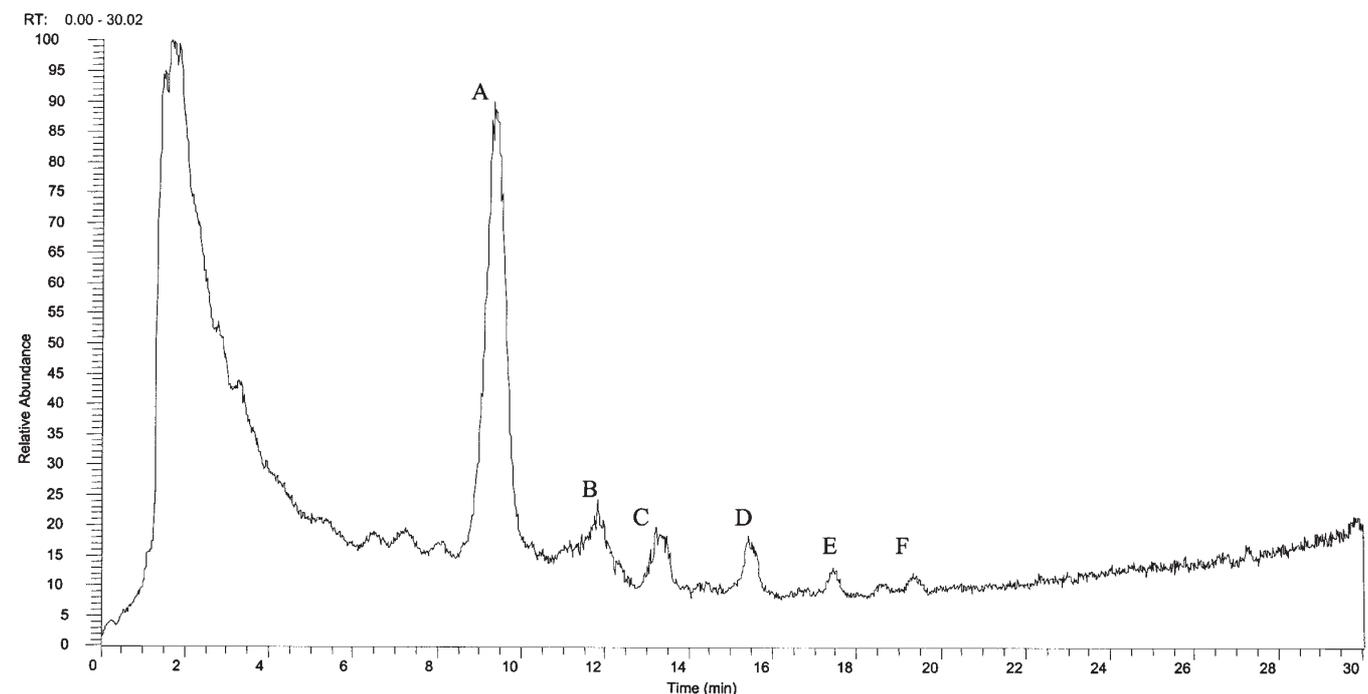
## Environmental analysis

The developed LC-ESP/MS method was applied to the analysis of the influent, effluent and sludge samples of two STPs located in Orléans. Figure 3a shows the analysis of an influent sample of a STP meanwhile figure 3b shows the analy-

sis of an effluent sample of the same STP. Both chromatograms were recorded under positive ESP ionization mode. The influent chromatogram allowed the quantitation of OPEOx, NPEOx, C<sub>12</sub>EOx, C<sub>13</sub>EOx, C<sub>14</sub>EOx, C<sub>15</sub>EOx compounds at concentration levels of 150, 38, 136, 52, 32, 40 µg/L, respectively. Other compounds were present in the



a)

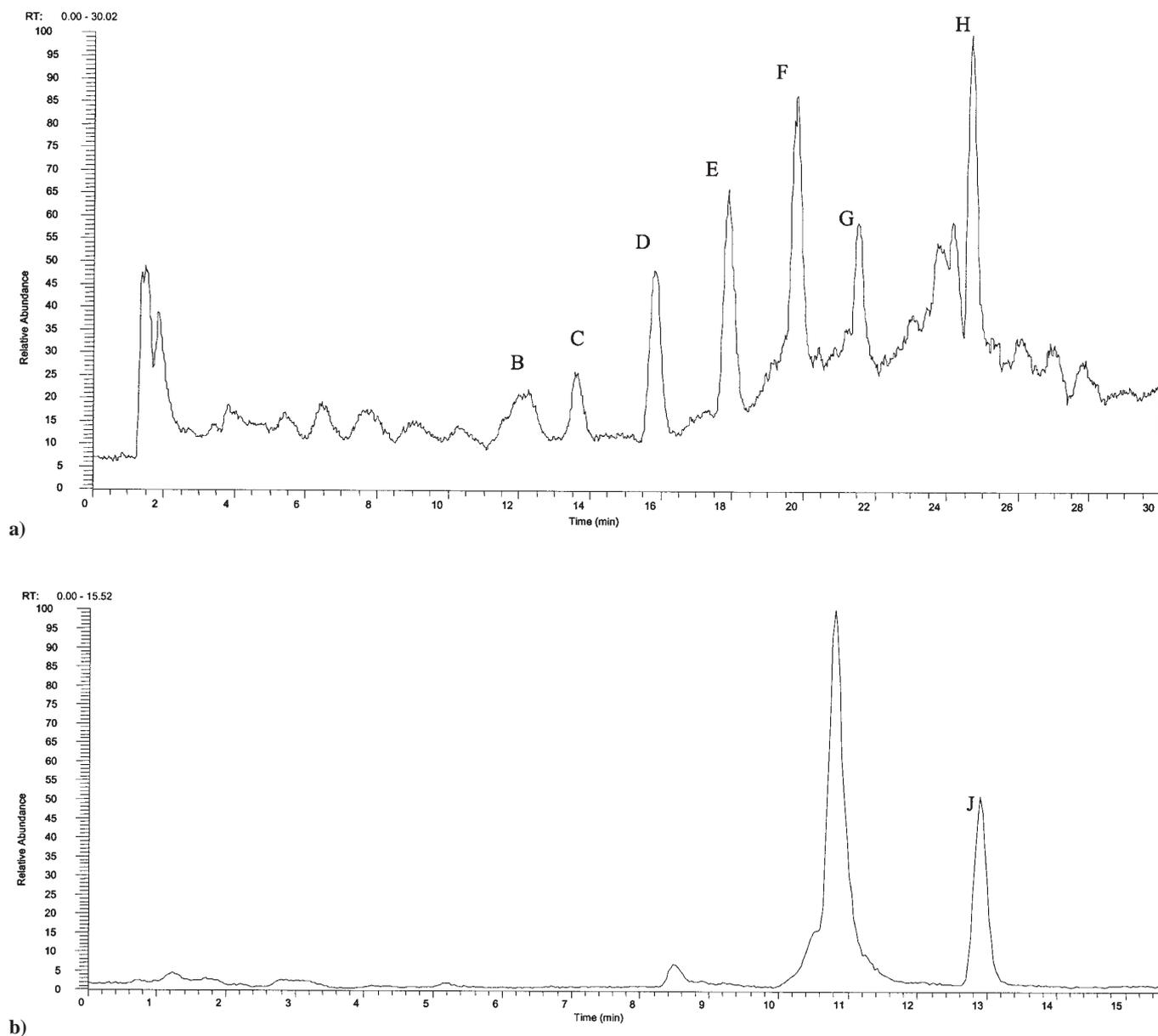


b)

**Figure 3.** a) TIC chromatogram in PI ESP mode of a STP influent sample obtained after preconcentration of 250 mL of water. A (OPEOx, 150 µg/l); B (NPEOx, 38 µg/l); C (C<sub>12</sub>EOx, 136 µg/l); D (C<sub>13</sub>EOx, 52 µg/l); E (C<sub>14</sub>EOx, 32 µg/l); F (C<sub>15</sub>EOx, 40 µg/l); G (C<sub>16</sub>EOx, not quantified); H (C<sub>18</sub>EOx, not quantified). b) TIC chromatogram in PI ESP mode of a STP effluent sample obtained after preconcentration of 250 mL of water. A (OPEOx, 138 µg/l); B (NPEOx, 31 µg/l); C (C<sub>12</sub>EOx, 18 µg/l); D (C<sub>13</sub>EOx, 12 µg/l); E (C<sub>14</sub>EOx, 5 µg/l); F (C<sub>15</sub>EOx, 3 µg/l); G (C<sub>16</sub>EOx, not quantified); H (C<sub>18</sub>EOx, not quantified).

sample such as  $C_{16}EO_x$  and  $C_{18}EO_x$ . These compounds could be identified by comparing their LC retention time with those of  $C_{16}EO_6$  and  $C_{18}EO_6$  standards and by locating in their spectra the ions at  $m/z$  529 and  $m/z$  557 which are the base peak of the  $C_{16}EO_6$  and  $C_{18}EO_6$  spectra, respectively. The effluent chromatogram still shows the presence of OPEOx, NPEOx,  $C_{12}EO_x$ ,  $C_{13}EO_x$ ,  $C_{14}EO_x$ ,  $C_{15}EO_x$ , at concentration levels of 138, 31, 18, 12, 5, 3  $\mu\text{g/L}$ , respectively. Actually, AEOs was effectively removed during the sewage treatment while NPEOs and OPEOs have appeared to be more stable against the biodegradation activity. Figure 4a and figure 4b

correspond to a sludge sample analysis in PI mode and NI mode, respectively. The PI mode allowed the determination of NPEOx,  $C_{12}EO_x$ ,  $C_{13}EO_x$ ,  $C_{14}EO_x$ ,  $C_{15}EO_x$  at concentration levels of 1.3, 2.8, 4.1, 5.4, 8.5  $\mu\text{g/g}$  meanwhile the NI mode confirmed the presence of nonylphenol at concentration level of 1.5  $\mu\text{g/g}$ . Other compounds were also identified such as  $C_{16}EO_6$  and  $C_{18}EO_6$ . These results demonstrate, once more, that NPEOs and APEOs tend to accumulate in sludge during sewage treatments. High amount of nonionic surfactants may be a source of concern when use of sludge for agricultural practices is considered.



**Figure 4.** a) TIC chromatogram in PI ESP mode of a Soxtec extracted STP sludge sample. B (NPEOx, 1.3  $\mu\text{g/g}$ ); C ( $C_{12}EO_x$ , 2.8  $\mu\text{g/g}$ ); D ( $C_{13}EO_x$ , 4.1  $\mu\text{g/g}$ ); E ( $C_{14}EO_x$ , 5.4  $\mu\text{g/g}$ ); F ( $C_{15}EO_x$ , 8.5  $\mu\text{g/g}$ ); G ( $C_{16}EO_x$ , not quantified); H ( $C_{18}EO_x$ , not quantified). b) SIM chromatogram in NI ESP mode of the same extract as in a) J (nonylphenol, 1.5  $\mu\text{g/g}$ ).

## Conclusion

Liquid chromatography-mass spectrometry using electrospray as ionization mode enabled the determination of the major class of nonionic surfactants in different environmental matrices such as sludge and wastewater samples. Reverse phase LC chromatography allowed the elution of all oligomer constituents of each homolog component into a single peak. Gathering all the oligomers into a single peak increased the peak intensity and provided a way to determine low concentration of APEOs and AEOs in different environmental matrices. Limits of detection achieved with this methodology were 0.1 µg/L for wastewater analysis and 10 ng/g for sludge sample analysis. Information on the oligomers distribution of APEOs and AEOs could be obtained by the extraction of selected m/z ions from the TIC chromatograms. As far as extraction procedures is concerned, classical approaches such as Soxtec extraction with methanol for solid matrices and solid phase extraction for liquid matrices allowed to obtain recovery values higher than 70 % in all cases. First results concerning real sample analysis have shown that NPEOs and OPEOs are less degraded than AEOs during sewage treatment. Furthermore, NPEOs and AEOs tend to accumulate in sludge where their concentration are far above 1 µg/g level. Further work on nonionic surfactants will include the analysis of the polar carboxylic acid APEOs metabolites in wastewater and sludge samples in order to better understand the fate of nonionic surfactants during sewage treatments.

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