



**IMPROVED SENSITIVITY OF HPLC-ESI/MS ANALYSIS  
OF THE OF BIODEGRADATION PRODUCTS OF ALKYL  
ETHOXYLATES AND ALKYLPHENOL ETHOXYLATES  
BY POST-COLUMN LITHIUM ADDITION**

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**ABSTRACT**

Biodegradation of nonylphenol ethoxylates and dodecyl ethoxylates ( $C_9H_{19}-C_6H_4-(OCH_2CH_2)_n-OH$  and  $C_{12}H_{25}-(OCH_2CH_2)_n-OH$  respectively) in river water in a static die-away test was performed. By using high performance liquid chromatography with electrospray ionization mass spectrometric detection (HPLC-ESI/MS), surfactants containing 2-4 ethoxylene units were detected as biodegradation products. Post-column addition of a number of metal cation was tested in order to improve the sensitivity of HPLC-ESI/MS analysis. Among the metal cations taken into account, the use of a lithium cation gave the best results provided that the cone voltage was increased to 40-50 V.

Keywords: non-ionic surfactants, biodegradation, HPLC-ESI/MS, post-column addition

**INTRODUCTION**

Nowadays, post-column addition/reactions is an important way of improving the quality/utility of high performance liquid chromatography with electrospray ionization mass spectrometric detection (HPLC-ESI/MS). The most interesting examples are as follows: Lévêque et al. have used silver nitrate in order to improve the sensitivity of HPLC-ESI/MS of triacylglycerols as well as to identify the regioisomers in this class of compounds [1]. Raina and Etter, upon HPLC-ESI/MS analysis in the negative ion mode, have applied the post-column reagent addition of ammonia for determination of a number of acid

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herbicides and their degradation products in surface water [2]. By post-column addition of formic acid, Verardo et al. have generated abundant  $[M+HCOO]^-$  ions (M stands for underivatized oligosaccharide molecule) which were further successfully fragmented [3]. Lien et al. have applied tropylium post-column derivatization for the analysis of polycyclic aromatic hydrocarbons [4]. Mawhinney et al. have demonstrated that post-column addition of an organic solvent enhances the ESI response of alkyl methylphosphonic acids [5]. Rijke et al. have used post-column chloride attachment in order to identify *N*-glucosyl ethanolamine in wine [6]. Takino et al., by using silver nitrate as a post-column reagent, have determined ten polycyclic aromatic hydrocarbons in river water [7]. Kohler and Leary have demonstrated that post-column addition of metal chlorides may be useful in the structural analysis of carbohydrates by HPLC-ESI/MS [8]. Yamaguchi et al., have shown that post-column addition of 2-(2-methoxyethoxy)ethanol enables identification of rat urinary metabolites of esonarimod [9]. Shou et al. have improved the sensitivity of HPLC-ESI/MS analysis of organoselenium compounds by post-column crown ether complexation [10]. Shen and Brodbelt have analysed a number of quinoline antibiotics by post-column metal complexation [11]. Post-column addition may also be useful in peptide analysis [12-15]. A post-column ligand-exchange reaction may also be applied for the selective detection of metal ligands [16]. A number of compounds have been analysed by Karlsson, who used post-column addition of alkali metal cation in microcolumn liquid chromatography/mass spectrometry [17]. Kempen et al. have used post-column reaction for screening metal binding selectivities of various macrocyclic compounds [18].

Alkyl- and alkylphenol ethoxylates (AEs and APEs) are the most widely used non-ionic surfactants. They have been used in domestic detergents, pesticide formulations and industrial products. There are a number of interesting papers dedicated to the analysis of these compounds with the use of HPLC-ESI/MS [19-27]. In this work, the effect of post-column metal cation addition for the HPLC-ESI/MS analysis of the products of biodegradation of nonylphenol ethoxylate, namely Rokafenol N-10, and dodecyl ethoxylate, namely Tergitol TMN-10 ( $C_9H_{19}-C_6H_4-(OCH_2CH_2)_n-OH$  and  $C_{12}H_{25}-(OCH_2CH_2)_n-OH$ , respectively) has been studied.

## EXPERIMENTAL

The water used in the biodegradation study was sourced from the River Warta (September 2010 Poznań, Poland; the physical and chemical parameters of the water are available from <http://www.poznan.pios.gov.pl/glowna/index.php>). Dodecyl ethoxylates (Tergitol TMN-10) were obtained from Sigma-Aldrich (Poznań, Poland). Nonylphenol ethoxylates (Rokafenol N-10) was obtained from PCC Rokita S.A., (Brzeg Dolny, Poland). A standard solution of nonylphenol monoethoxylate and nonylphenol diethoxylate (acetone solution, 5 µg/ml) was obtained from Sigma-Aldrich.

The tests were performed in 200 ml bottles; a bottle was filled with river water and then 5 mg of a surfactant was added (two bottles were used: one for dodecyl ethoxylates and one for nonylphenol ethoxylates). The bottles were left opened (aerobic conditions), stored at a room temperature and protected against dust. Each day, a 0.5 ml sample was collected from each bottle and 2 ml of acetonitrile was added to the collected portion in order to stop the biodegradation process. The samples collected were subjected to HPLC/ESI-MS analysis.

In order to prove that the observed changes in the surfactant concentration are, indeed, a result of biodegradation, the next two bottles were filled with mixtures of 9/1 river water/acetonitrile (microorganisms are unlikely to survive such conditions) and the next two bottles were filled with tap water (tap water contains very low amounts of microorganisms, compared with river water). To each bottle, a portion of the surfactant was added. No changes in the homolog concentrations (described further for the bottles filled with river water) were observed either in the bottles filled with river water/acetonitrile or with tap water.

The HPLC/ESI-MS analyses were performed using a Waters model 2690 HPLC pump (Milford, MA, USA), a Waters/Micromass ZQ2000 mass spectrometer (single quadrupole type instrument equipped with electrospray ion source, Z-spray, Manchester, UK). The software used was MassLynx V3.5 (Manchester, UK). Using an autosampler, the sample solutions were injected onto the XBridge C18 column (3.5  $\mu\text{m}$ , 100 mm x 2.1 mm i.d., Waters). The injection volume was 10  $\mu\text{l}$ . A gradient methanol and 9:1 v/v water : methanol mixture, with a flow rate of 0.2 ml/min, was applied. The linear gradient started from 5% of methanol reaching 100% of methanol after 5 minutes and the latter concentration was maintained for 10 min. Respective homologous ethoxylate molecules were not separated under such conditions, which allowed recording a signal of each molecule in one mass spectrum. For HPLC-ESI/MS post-column addition analysis, a metal chloride-containing solution (concentration  $10^{-4}$  mol/dm<sup>3</sup>) was introduced post-column by using a Harvard pump, into the LC flow at a flow-rate of 0.04 ml/min via a mixing-T before entering the mass spectrometer. The electrospray source potentials were: capillary 3 kV, lens 0.5 kV, extractor 4 V and cone voltage 30 V, or three different values of cone voltage (during the HPLC/ESI-MS analysis the mass spectrometer was switched in the fast mode between the three cone voltages, e.g. 20, 30 and 40 V). The source temperature was 120°C and the desolvation temperature 300°C. Nitrogen was used as the nebulising and desolvation gas at the flow rates of 100 and 300 dm<sup>3</sup>/h, respectively.

## RESULTS AND DISCUSSION

As described in the previous paper, under the conditions applied, biodegradation of the surfactants used consists in shortening of the ethoxylene chain [28]. Figure 1 shows the ESI mass spectra of Rokafenol N-10 obtained for a 0 day sample (initial day of biodegradation test, Fig. 1a) and for a thirteen-day old sample (Fig. 1b).

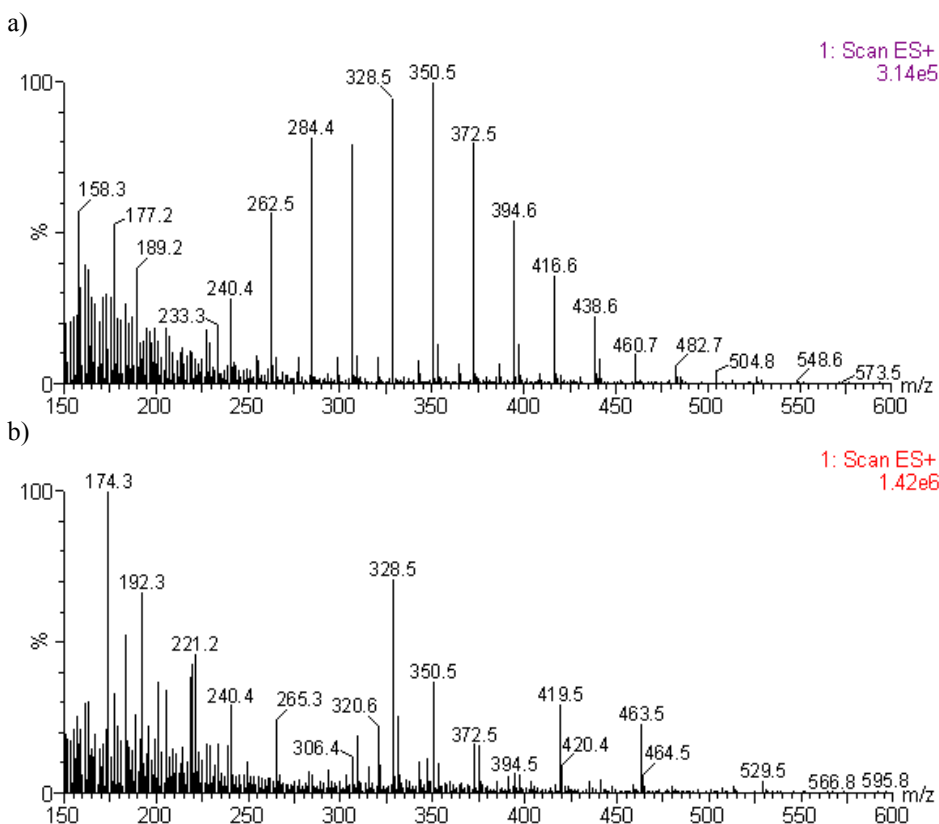


Fig. 1. ESI mass spectra of Rokafenol N-10 obtained for a 0 day sample (initial day of biodegradation test, Fig. 1a) and for a thirteen day sample (Fig. 1b). Ions  $[\text{C}_9\text{H}_{19}\text{-C}_6\text{H}_4\text{-(OCH}_2\text{CH}_2)_n\text{-OH+Ca}]^{2+}$  are at  $m/z$   $174+(22)_m$ , ions  $[\text{C}_9\text{H}_{19}\text{-C}_6\text{H}_4\text{-(OCH}_2\text{CH}_2)_n\text{-OH+Na}]^+$  are at  $m/z$   $287+(44)_m$ .

ESI mass spectrum obtained before the biodegradation process shows an abundant presence of doubly charged ions  $[\text{M+Ca}]^{2+}$ . The most abundant ions originated from molecules containing 9 and 10 ethoxylene units ( $[\text{C}_9\text{H}_{19}\text{-C}_6\text{H}_4\text{-(OCH}_2\text{CH}_2)_9\text{-OH+Ca}]^{2+}$   $m/z$  328,  $[\text{C}_9\text{H}_{19}\text{-C}_6\text{H}_4\text{-(OCH}_2\text{CH}_2)_{10}\text{-OH+Ca}]^{2+}$   $m/z$  350). Usually, ESI mass spectra of ethoxylates show  $[\text{M+Na}]^+$  ions. The abundant ions  $[\text{M+Ca}]^{2+}$  observed indicate that the water used contains a notable amount of calcium (it does not disturb the detection of Rokafenol N-10). In the ESI mass spectrum of the 13-day old sample the most abundant ions originated from the molecule containing two ethoxylene units, namely ( $[\text{C}_9\text{H}_{19}\text{-C}_6\text{H}_4\text{-(OCH}_2\text{CH}_2)_2\text{-OH+Ca}]^{2+}$  at  $m/z$  174). The ions  $[\text{M+Na}]^+$  were also observed. Because the molecule  $[\text{C}_9\text{H}_{19}\text{-C}_6\text{H}_4\text{-(OCH}_2\text{CH}_2)_2\text{-OH}]$  contains only two ethoxylene units, its complexes with sodium and calcium cations are less stable than those formed by the molecules containing more ethoxylene units. As a consequence, the molecule  $[\text{C}_9\text{H}_{19}\text{-C}_6\text{H}_4\text{-(OCH}_2\text{CH}_2)_2\text{-OH}]$  has a lower ESI response than other molecules. The cations  $\text{Ca}^{2+}$  and  $\text{Na}^+$  have similar radii (106 and 98 pm, respectively [29]).

Because of the stronger electrostatic attractions in the calcium-containing ions, compared with the sodium-containing ions, the differences between the stabilities of the  $[\text{C}_9\text{H}_{19}\text{-C}_6\text{H}_4\text{-(OCH}_2\text{CH}_2)_2\text{-OH+Ca}]^{2+}$  ion and other  $[\text{M+Ca}]^{2+}$  ions are lower than the differences between the stabilities of  $[\text{C}_9\text{H}_{19}\text{-C}_6\text{H}_4\text{-(OCH}_2\text{CH}_2)_2\text{-OH+Na}]^+$  and other  $[\text{M+Na}]^+$  ions. This explains why the abundance of the  $[\text{C}_9\text{H}_{19}\text{-C}_6\text{H}_4\text{-(OCH}_2\text{CH}_2)_2\text{-OH+Ca}]^{2+}$  ion is higher than the abundances of all other  $[\text{M+Ca}]^{2+}$  ions, whereas the abundance of  $[\text{C}_9\text{H}_{19}\text{-C}_6\text{H}_4\text{-(OCH}_2\text{CH}_2)_2\text{-OH+Na}]^+$  is higher only compared with that of  $[\text{C}_9\text{H}_{19}\text{-C}_6\text{H}_4\text{-(OCH}_2\text{CH}_2)_3\text{-OH+Na}]^+$  but not higher than that of the other  $[\text{M+Na}]^+$  ions.

In order to increase the signal intensities of cationized molecule of  $\text{C}_9\text{H}_{19}\text{-C}_6\text{H}_4\text{-(OCH}_2\text{CH}_2)_2\text{-OH}$ , post-column addition of the metal cation was applied. Sodium and lithium were chosen first. Figure 2 shows the ESI mass spectra obtained and Figure 3 shows single ion chromatograms of respective cationized molecules of  $\text{C}_9\text{H}_{19}\text{-C}_6\text{H}_4\text{-(OCH}_2\text{CH}_2)_2\text{-OH}$ , obtained by the post-column addition of lithium (Fig. 3a) and sodium (Fig. 3b) upon HPLC-ESI/MS analysis of the 13-day-old sample.

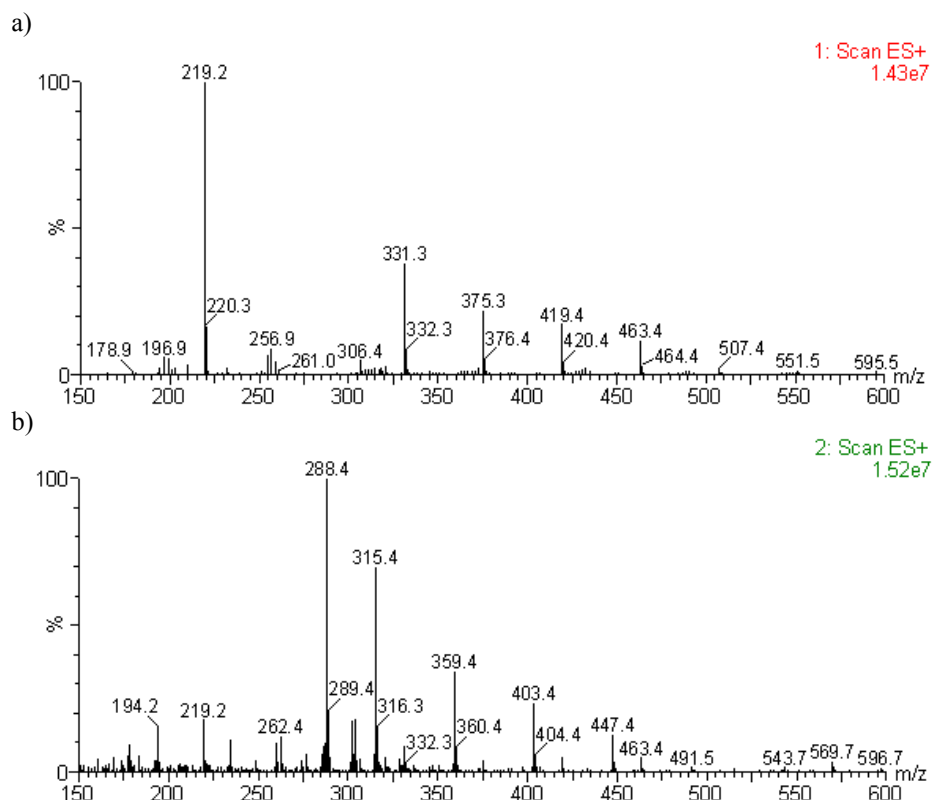


Fig. 2. ESI mass spectra obtained upon HPLC-ESI/MS analysis of a thirteen day sample of Rokafenol N-10 with post-column addition of sodium (a) and lithium (b) cations. Ions  $[\text{C}_9\text{H}_{19}\text{-C}_6\text{H}_4\text{-(OCH}_2\text{CH}_2)_n\text{-OH+Na}]^+$  are at  $m/z\ 287+(44)_m$ , ions  $[\text{C}_9\text{H}_{19}\text{-C}_6\text{H}_4\text{-(OCH}_2\text{CH}_2)_n\text{-OH+Li}]^+$  are at  $m/z\ 271+(44)_m$ .

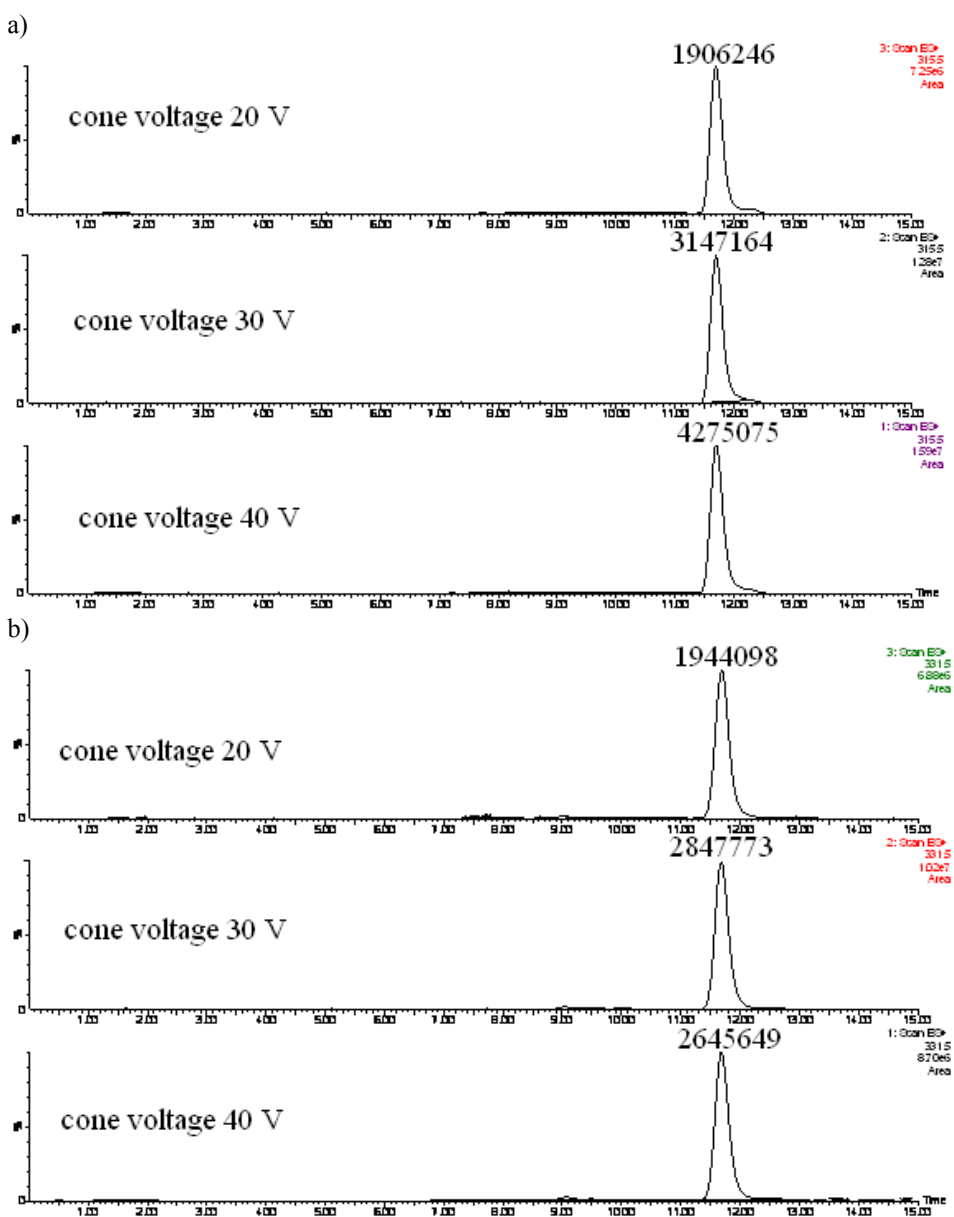


Fig. 3. Single ion chromatograms obtained upon HPLC-ESI/MS analysis of a 13-day old sample of Rokafenol N-10, with different cone voltages, by lithium (a)  $[(C_9H_{19}-C_6H_4-(OCH_2CH_2)_2-OH+Li]^+$  and sodium (b)  $(C_9H_{19}-C_6H_4-(OCH_2CH_2)_2-OH+Na]^+$  post-column addition. The peaks are assigned by peak areas.

It is expected that, because of low ionic radii of the lithium cation (78 pm, [29]), the molecule containing only two ethoxy units will form stable complexes (especially in the gas phase) with the lithium cation. As indicated in Fig-

ure 3, the analyses were performed with different cone voltages (during the HPLC/ESI-MS analyses the mass spectrometer was switched in the fast mode between 20, 30 and 40 V). Cone voltage has the most profound effect on the mass spectra obtained. An increase in this voltage leads to the so-called “in-source” fragmentation/dissociation, but a too low cone voltage may cause a drop in sensitivity. The generated chromatographic peaks (Fig. 3) are assigned by peak areas. For the cone voltages of 20 and 30 V the peak areas of the ions  $[\text{C}_9\text{H}_{19}\text{-C}_6\text{H}_4\text{-(OCH}_2\text{CH}_2)_2\text{-OH+Na}]^+$  and  $[\text{C}_9\text{H}_{19}\text{-C}_6\text{H}_4\text{-(OCH}_2\text{CH}_2)_2\text{-OH+Li}]^+$  are similar, whereas for the cone voltage 40 V the peak area of the ion  $[\text{C}_9\text{H}_{19}\text{-C}_6\text{H}_4\text{-(OCH}_2\text{CH}_2)_2\text{-OH+Li}]^+$  is the highest (Fig. 3). In other words, an increase in the cone voltage to 40 V caused lowering of the signal intensity of the ion  $[\text{C}_9\text{H}_{19}\text{-C}_6\text{H}_4\text{-(OCH}_2\text{CH}_2)_2\text{-OH+Na}]^+$  (because of its decomposition). However, an increase in the cone voltage to 40 V caused an increase in the signal intensity of the ion  $[\text{C}_9\text{H}_{19}\text{-C}_6\text{H}_4\text{-(OCH}_2\text{CH}_2)_2\text{-OH+Li}]^+$ , the ion is more stable in the gas phase, compared with  $[\text{C}_9\text{H}_{19}\text{-C}_6\text{H}_4\text{-(OCH}_2\text{CH}_2)_2\text{-OH+Na}]^+$ .

The HPLC-ESI/MS analysis with the post-column addition of the lithium cation was also performed at other cone voltages (in addition to the 20, 30 and 40 V discussed above), namely at 30, 40, 50 V and 40, 50, 60 V. Figure 4 shows the peak areas obtained, which are directly proportional to the abundances of the ions  $[\text{C}_9\text{H}_{19}\text{-C}_6\text{H}_4\text{-(OCH}_2\text{CH}_2)_n\text{-OH+Li}]^+$  for  $n = 2, 3$  and 4.

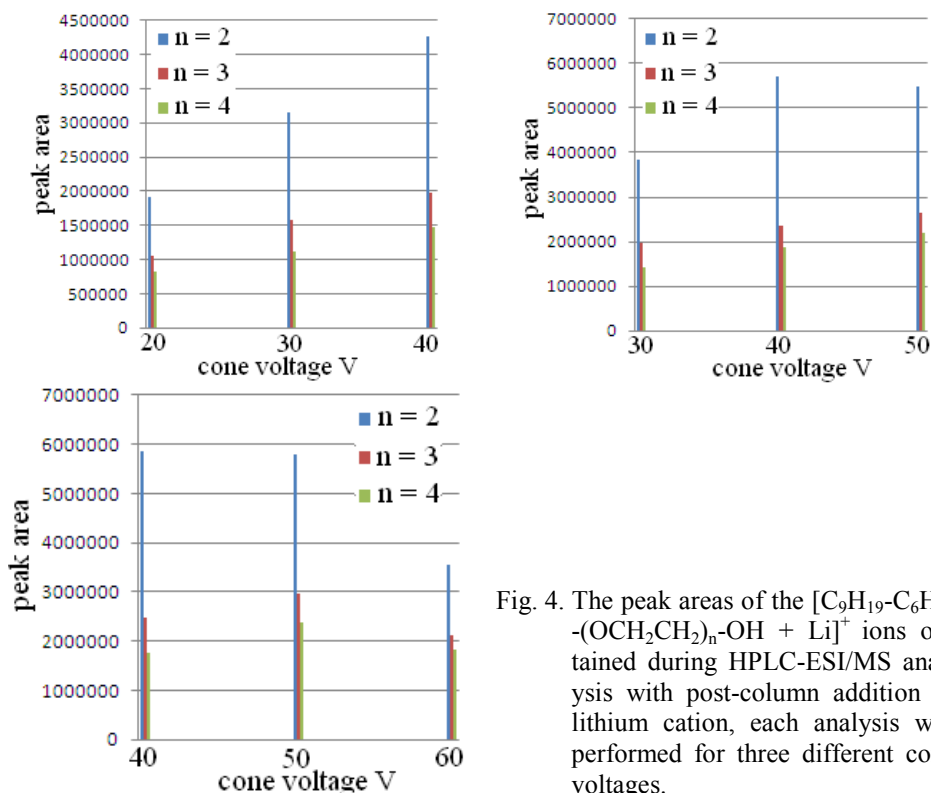


Fig. 4. The peak areas of the  $[\text{C}_9\text{H}_{19}\text{-C}_6\text{H}_4\text{-(OCH}_2\text{CH}_2)_n\text{-OH + Li}]^+$  ions obtained during HPLC-ESI/MS analysis with post-column addition of lithium cation, each analysis was performed for three different cone voltages.

In all cases the highest, and comparable, signal intensities of the ion  $[C_9H_{19}-C_6H_4-(OCH_2CH_2)_2-OH+Li]^+$  were obtained for 40 and 50 V. For the other  $[M+Li]^+$  ions the cone voltage higher than 30 V also allowed a better sensitivity. Moreover, the signal intensities observed for 50 V were higher than those observed for 40 V. The use of a cone voltage of 60 V resulted in lowering the signal intensities of the ions  $[M+Li]^+$ . In other words, for the HPLC-ESI/MS analysis of Rokafenol N-10 biodegradation products using post-column addition of the lithium cation, the cone voltage 50 V seems to be optimum.

Tergitol TMN-10 ( $C_{12}H_{25}-(OCH_2CH_2)_n-OH$ ) was the second compound studied in this work and, as described previously, its biodegradation also resulted in shortening of the ethoxylene chain [28]. Figure 5 shows the ESI mass spectra obtained for a 0 day sample (initial day of biodegradation test, Fig. 5a) and for the thirteen day old sample (Fig. 5b).

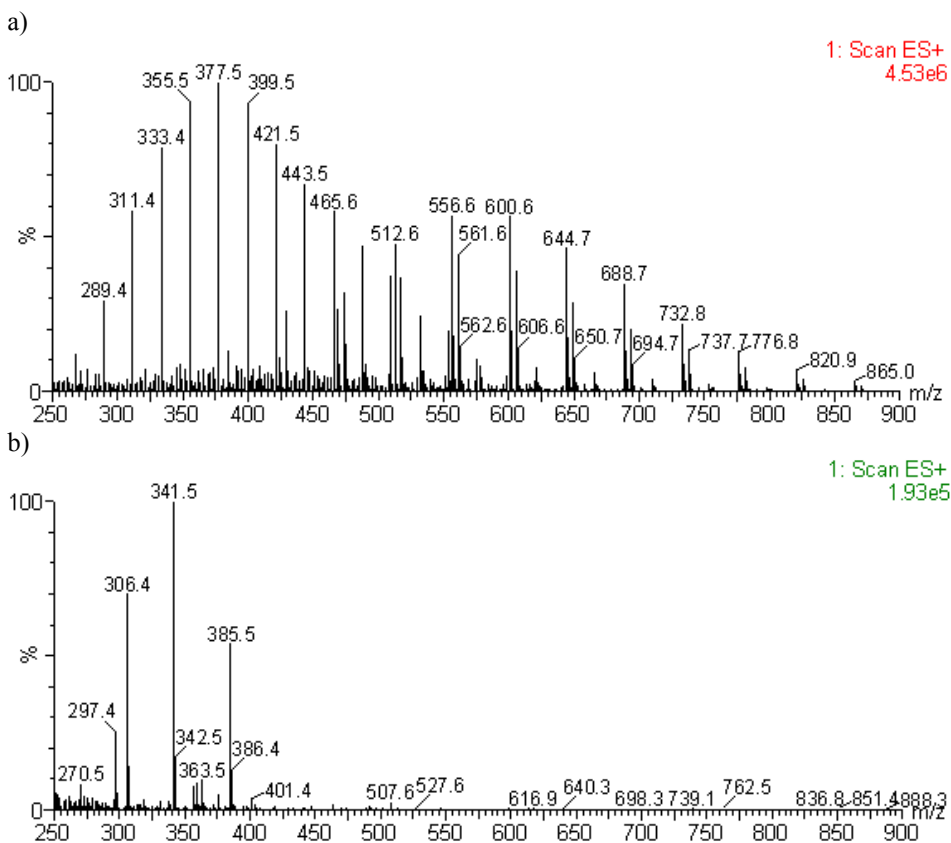


Fig. 5. ESI mass spectra obtained for a 0 day sample (initial day of biodegradation test, Fig. 5a) and for a thirteen day sample, Fig. 5b) of Tergitol TMN-10. Ions  $[C_{12}H_{25}-(OCH_2CH_2)_n-OH+Ca]^{2+}$  are at  $m/z 289+(22)_m$ , ions  $[C_{12}H_{25}-(OCH_2CH_2)_n-OH+Na]^+$  are at  $m/z 297+(44)_m$ , ions  $[C_{12}H_{25}-(OCH_2CH_2)_n-OH+NH_4]^+$  are at  $m/z 468 + (44)_m$ .



Same as for Rokafenol N-10, post-column addition of the lithium cation resulted in increasing the signal intensities of Tergitol TMN-10 biodegradation products. Figure 6 shows ESI mass spectrum.

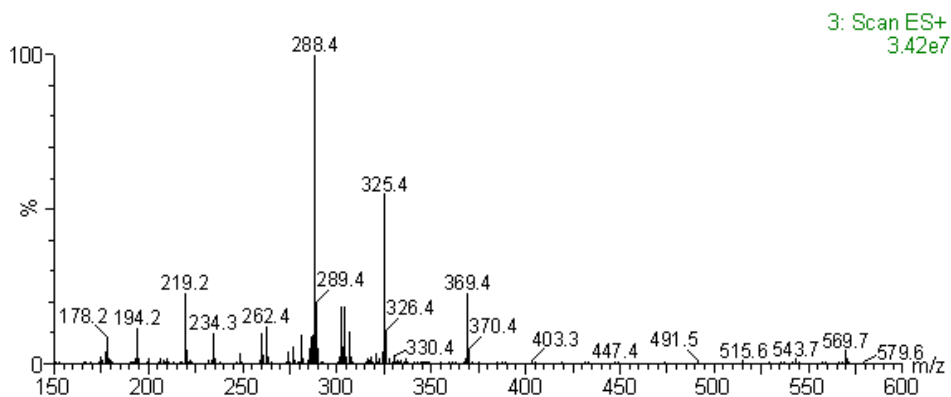


Fig. 6. ESI mass spectrum obtained for a thirteen day sample of Tergitol TMN-10 with post-column addition of lithium cation. Ions  $[C_{12}H_{25}-(OCH_2CH_2)_n-OH+Li]^+$  are at  $m/z 281+(44)_m$ .

However, the cone voltage effect was not the same as that observed for Rokafenol N-10. When the cone voltage was increased from 30 to 50 V, a notable increase in the signal intensities was observed for the ions  $[C_{12}H_{25}-(OCH_2CH_2)_3-OH+Li]^+$  and  $[C_{12}H_{25}-(OCH_2CH_2)_4-OH+Li]^+$ , but only minor increase in the signal intensity was observed for the ion  $[C_{12}H_{25}-(OCH_2CH_2)_2-OH+Li]^+$ , as presented in Figure 7.

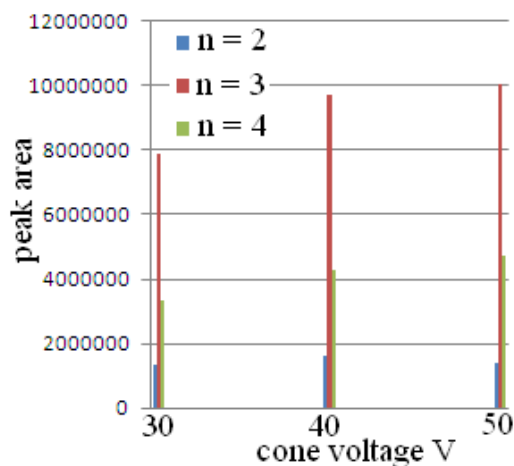


Fig. 7. The peak areas of the  $[C_{12}H_{25}-(OCH_2CH_2)_n-OH + Li]^+$  ions obtained during HPLC-ESI/MS analysis with post-column addition of lithium cation, the analysis was performed for three different cone voltages.

The homologues containing one ethoxylene unit  $C_{12}H_{25}-(OCH_2CH_2)-OH$  and  $C_9H_{19}-C_6H_4-(OCH_2CH_2)-OH$  were not formed under the biodegradation conditions referred to in this work. Obviously, these compounds may be formed under other biodegradation conditions [30]. Thus, it is reasonable to study the effect of post-column metal cation addition on the sensitivity of the HPLC-ESI/MS analysis of the homologues containing one ethoxylene unit. It is also interesting to check the effect of other metals, in addition to lithium and sodium, on the analysis. Therefore, a commercially available standard containing  $C_9H_{19}-C_6H_4-(OCH_2CH_2)-OH$ , and also  $C_9H_{19}-C_6H_4-(OCH_2CH_2)_2-OH$ , was subjected to the HPLC-ESI/MS analysis with post-column metal cation addition. Figure 8 shows the peak areas of the respective ions obtained.

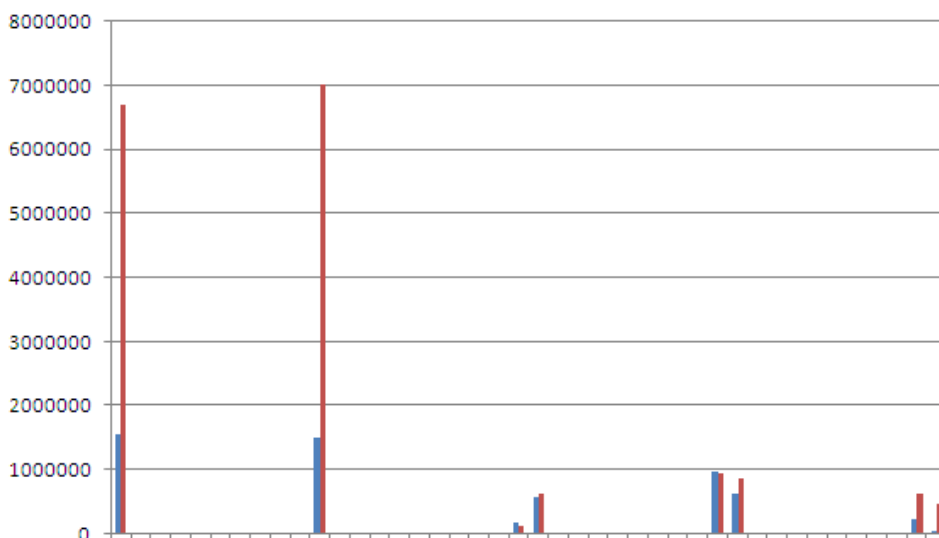


Fig. 8. The peak areas of ions detected upon HPLC-ESI/MS analysis of a standard containing  $C_9H_{19}-C_6H_4-(OCH_2CH_2)-OH$  and  $C_9H_{19}-C_6H_4-(OCH_2CH_2)_2-OH$ , with post-column metal cation addition. The blue series from the left:  $[C_9H_{19}-C_6H_4-(OCH_2CH_2)-OH+Li]^+$ ,  $[C_9H_{19}-C_6H_4-(OCH_2CH_2)-OH+Na]^+$ ,  $[C_9H_{19}-C_6H_4-(OCH_2CH_2)-OH+Mg]^{+2}$ ,  $[C_9H_{19}-C_6H_4-(OCH_2CH_2)-OH+MgCl]^+$ ,  $[C_9H_{19}-C_6H_4-(OCH_2CH_2)-OH+Ca]^{+2}$ ,  $[C_9H_{19}-C_6H_4-(OCH_2CH_2)-OH+CaCl]^+$ ,  $[C_9H_{19}-C_6H_4-(OCH_2CH_2)-OH+Cu]^+$ ,  $[C_9H_{19}-C_6H_4-(OCH_2CH_2)-OH+CuCl]^+$ , the red series from the left:  $[C_9H_{19}-C_6H_4-(OCH_2CH_2)_2-OH+Li]^+$ ,  $[C_9H_{19}-C_6H_4-(OCH_2CH_2)_2-OH+Na]^+$ ,  $[C_9H_{19}-C_6H_4-(OCH_2CH_2)_2-OH+Mg]^{+2}$ ,  $[C_9H_{19}-C_6H_4-(OCH_2CH_2)_2-OH+MgCl]^+$ ,  $[C_9H_{19}-C_6H_4-(OCH_2CH_2)_2-OH+Ca]^{+2}$ ,  $[C_9H_{19}-C_6H_4-(OCH_2CH_2)_2-OH+CaCl]^+$ ,  $[C_9H_{19}-C_6H_4-(OCH_2CH_2)_2-OH+Cu]^+$ ,  $[C_9H_{19}-C_6H_4-(OCH_2CH_2)_2-OH+CuCl]^+$ .

As results from Figure 8, the post-column addition of the lithium and sodium cation yielded much more abundant ions, compared with the addition of other metals. HPLC-ESI/MS with lithium cation analysis of a standard were also performed at different cone voltages. The peak areas of the  $[C_9H_{19}-C_6H_4-$

$-(\text{OCH}_2\text{CH}_2)_2\text{-OH+Li}^+$  ions obtained confirmed the conclusion drawn from the analysis, discussed earlier, of the 13-day old sample from the Rokafenol N-10 biodegradation test. However, the effect of cone voltage increase on the abundances of the  $[\text{C}_9\text{H}_{19}\text{-C}_6\text{H}_4\text{-(OCH}_2\text{CH}_2\text{)-OH+Li}^+]$  ions was less pronounced, as shown in Figure 9.

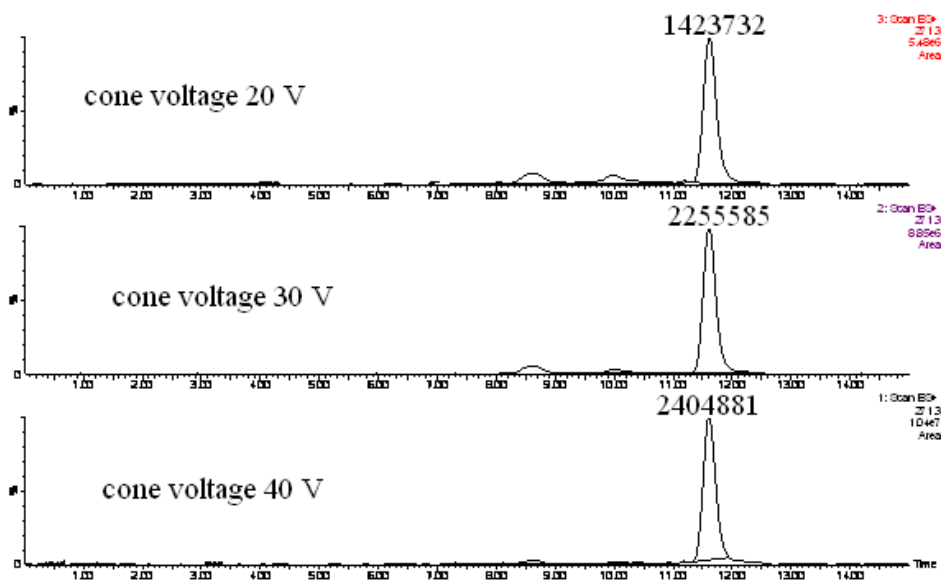


Fig. 9. Single ion chromatograms of ion  $[\text{C}_9\text{H}_{19}\text{-C}_6\text{H}_4\text{-(OCH}_2\text{CH}_2\text{)-OH+Li}^+]$  ( $m/z$  271) obtained upon HPLC-ESI/MS with post-column lithium addition analysis of standard solution, with different cone voltages. The peaks are assigned by peak areas.

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