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Determination of aliphatic alcohols after on-line microwave-assisted derivatization by liquid chromatography-photodiode array detection

Gerson Chávez, Bélgica Bravo, Nolberto Piña, Mónica Arias, Eliseo Vivas, Fredy Ysambertt, Nelson Márquez, Ana Cáceres*

Laboratorio de Petroquímica y Surfactantes, Departamento de Química, Facultad de Ciencias, La Universidad del Zulia, Maracaibo, P.O. Box Apartado Postal #15840, C.A. 4005 Zulia, Venezuela

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Abstract

In this study, high-performance liquid chromatography (HPLC) in conjunction with continuous derivatization for the determination of aliphatic and polyethoxylated alcohol is reported. Reaction of alcohol group with phenyl isocyanate or benzyl chloride reagents assisted with microwaves (MW) irradiation is carried out in an on-line system coupled to HPLC with photodiode array detection (PDA). Reactor was placed into a microwave oven at 450 W. The flow rate, reagent amounts, irradiation time, and chromatographic conditions were optimized. The continuous analysis using the system MW-HPLC-PDA provided high sensitivity, reduce the amount of reagents and analysis time. This proposed method can be used for the analysis of commercial alcohol polyethoxylated mixture.

Keywords: Continuous analysis; On-line systems; Microwave assisted reaction; Polyethoxylated alcohol surfactants

1. Introduction

The hydroxyl groups are commonly found in compounds with wide industrial interest like surfactants, flavors, stabilizers, etc., and they are also presents in compounds that can occur naturally after hydrolysis or enzymatic reduction reactions. In the group of industrial importance, nonionic ethoxylated surfactants (i.e., alcohol ethoxylates) became popular since they are more environmental friendly than most other surfactants [1,2].

Commercial polyethoxylated alcohols surfactants (PAS) and carboxylic acids contain compounds that differ not only by the number of oxyethylene groups, but also by the lengths (and possibly branching) of the alkyl chains in their parent compounds. The analysis of these surfactants have been performed by several chromatographic techniques. The use of gas chromatography is limited because of low volatility of high oligomers and derivatization is necessary [3]. Efficient separations of ethoxylated non-ionic surfactants can be achieved by high-performance liquid chromatography (HPLC) both in normal-phase systems on columns packed with unmodified silica gel or with amino-, nitrile- or diol-chemically bonded phases and in reversed-phases systems [4]. Two different strategies can be adopted for the analysis of complex mixtures of surfactants with bimodal distribution either the separation into groups with the same number of EO units and with different alkyl lengths, or into groups with the same alkyls, but with different numbers of EO units [5].

High performance liquid chromatographic (HPLC) offers high sensitivity and selectivity but most PAS have poor absorption in the ultraviolet (UV) region the wider detection system used in LC. Therefore, wide variety of derivatization reactions have been reported in order to increase absorption in the UV–vis region [5–7]. Phenyl isocyanate and benzyl chloride reagents have been reported for PAS derivatization [5].

Derivatization techniques for HPLC analysis have received special attention because they enable highly sensitive detection of these compounds by bonding a chromophore that results in products with strong UV absorption. There

* Corresponding author. Tel.: +58 261 7598125; fax: +58 261 7598125.
E-mail addresses: caceresana7@cantv.net, A.Caceres@shu.ac.uk (A. Cáceres).

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are two alternatives for PAS derivatization, by reaction before or after chromatographic separation, using off-line or on-line system. The pre-column derivatization is used more frequently than the post-column [8–10]. PAS derivatization required some minimum reaction condition to produce the product. Most of the derivatization reaction reported the used of solvent and heating in boiling water to get the product. On the other hand, microwave (MW) heating can advantageously replace conventional heating. The two main systems in MW technique use either a domestic multimode oven equipped with a teflon coil, in which numerous organic synthesis have been conducted, some of them under high pressure (up to 1400 kPa) and temperatures up to 200 °C [11–17]. The advantages of MW heating over conventional heating is that the reaction mixture absorbs the energy directly. Thus, the temperature gradient rises steeply, leading to a acceleration of the reaction, an essential condition in a flow-through system. Another important advantage of MW heating in a such system is the "on/off condition", i.e., the possibility to turning the MW source on or off instantaneously. This allows one to carry out MW irradiation in a sequential mode, impossibility with conventional heating.

The aid of this work was the development of an on-line system for the derivatization of PAS using microwave irradiation and coupling it to HPLC-PAD system for PAS separation according to the nature of non polar aliphatic chain. The reaction conditions for the following reagents: phenyl-isocyanato (PIC) and benzoyl chloride (BC) were established. The analysis using the continuous microwave reactor (CMWR) coupled with HPLC-PDA system provided high sensitivity, reduced the amount of reagent and shorter analysis times. The developed method system was applied to real samples of PAS.

2. Experimental

2.1. Materials and methods

Acetonitrile (MeCN) and methanol (MeOH) used were HPLC grade from Baker Chemicals. Deionized water was used as the mobile phase. All solvents were ultrasonically degassed. Different high purity (98 to >99% active material) alcohols, kindly provided by the producers, were used as reference materials during the whole investigation. Linear alcohols (C_nOH with n between 4 and 16 carbon atoms number) were used (Merck). The studied PAS samples were as follows: ALEX (Ethoxyl, Arch Chemical), which are a mixture of even linear primary C_{12}, C_{14} and C_{16} fatty alcohol polyethoxylates; Ukanil36 and Ukanil50 (Ircha, France) that are a mixture of even linear primary C_9 and C_{11} fatty alcohol polyethoxylated; and Ukanil43 and Ukanil87 (Ircha, France), which are a mixture of even linear primary C_{13} and C_{15} fatty alcohol polyethoxylated. The reagents used to obtain the PAS adducts were phenyl isocyanate (PIC) and benzoyl chloride (BC) (purity over 99%), they were supplied by

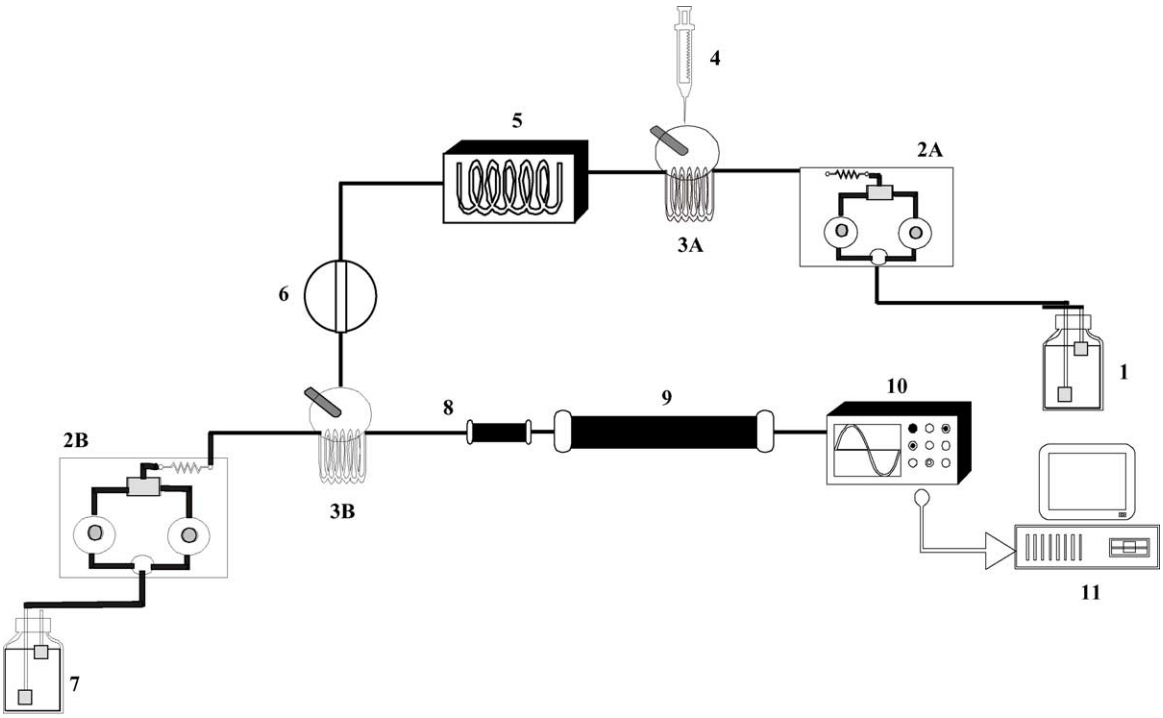

Fig. 1. Continuos MW reaction HPLC-PDA on-line systems: (1) solvent carrier (MeCN); (2) A and B pump; (3) injection valve A (100 μL) and B (10 μL); (4) fatty alcohol plus reagent; (5) coil reactor and MW oven (450 W); (6) switching valve; (7) mobile phase; (8) pre-column; (9) RP-column; (10) PDA detector; and (11) PC processador.

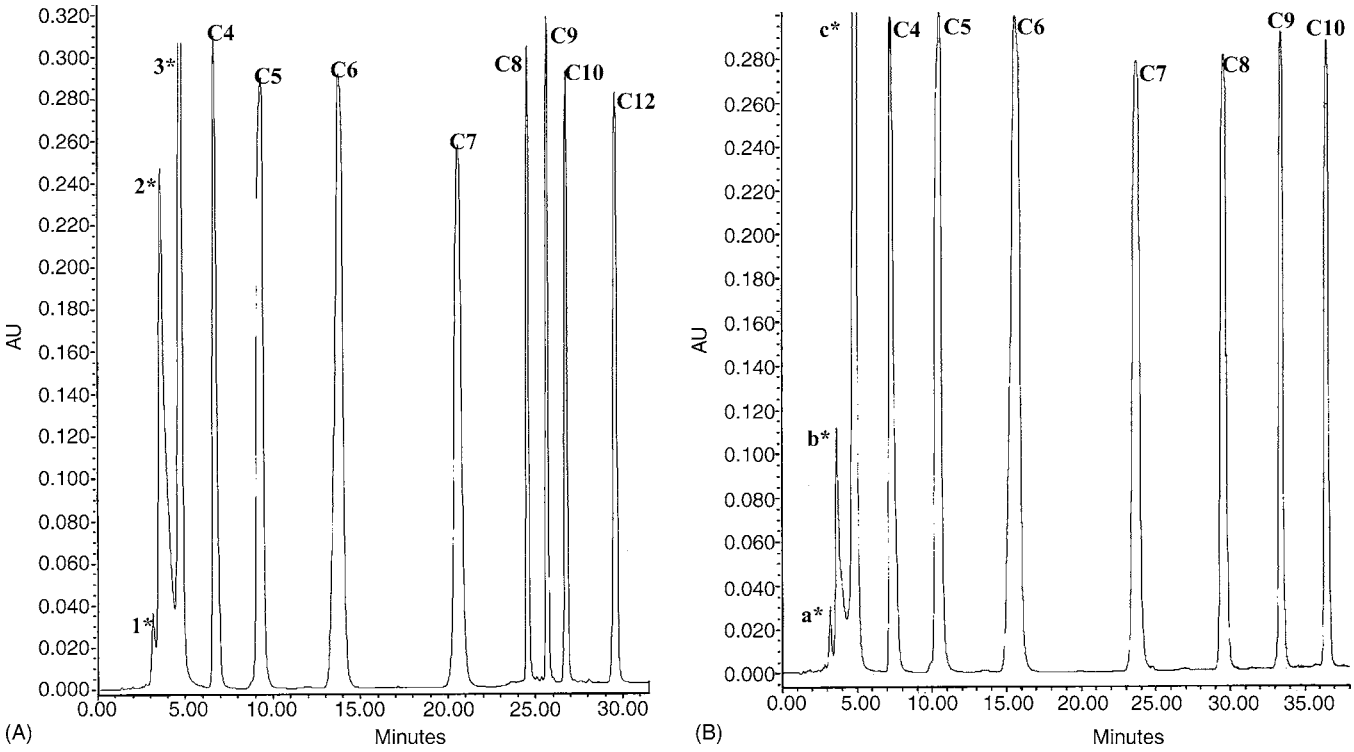

Fig. 2. Chromatogram of the alkyl alcohol-PIC adducts (A) and alkyl alcohol-BC adducts (B), with length chain between C_4OH and $C_{12}OH$. The symbol (*) is the excess of derivatizer agents or by-products of the reactions. Conditions: mobile phase MeOH/H_2O 70:30; Lichrocart C18 column (5 μm); flow 1 mL min^{-1}; PDA detection (235 nm).

Merck Company. Samples were prepared by measurement of appropriate volume and dissolving in MeCN.

HPLC separations were performed with a set-up from Waters Corporation consisting of a 510 pump and a U6K injector valve with a sample loop 10 μL, photodiode array detector (PDA), model 996 coupled to a PC loaded with Millennium software. Lichosphere C18 bonded silica column (250 mm × 4.0 mm i.d., 5 μm particle size) was supplied by Merck. Novapack C8 bonded silica column (150 mm × 3.9 mm i.d., 4 μm particle size) was supplied by Waters Corporation. On-line derivatization system consisted of an isocratic high pressure pump model 510 from Waters, a Rheodyne injection valve model 5020 and switching valve model 5011. Domestic microwave ovens operate at 450 W and spiral Teflon coil with 150 cm of length and 0.1 mm of internal diameter was used. Fig. 1 shows the CMWR-HPLC-PDA used. Optimized derivatization conditions are shown in Table 1.

Table 1
Optimized derivatization conditions for linear alcohol-PIC adducts and linear alcohol-BC adducts

Instrumental and reaction conditions	
Solvent carrier	Acetonitrile (MeCN)
Carrier flow (pump 2A)	0.5 mL min^{-1}
Loop volume (valve 3A)	100 μL
Spiral teflon coil (length × i.d.)	1500 mm × 0.25 mm
MW irradiation	450 W (60 s)
HPLC injection volume (valve 3B)	10 μL
Mobile phase flow (pump 2B)	1.0 mL min^{-1}
Sample concentration	4 mg L^{-1}
PIC concentration	46 mM (46 μmol)
BC concentration	43 mM (43 μmol)

2.2. Procedure

The continuous system developed for the on-line derivatization of samples is depicted in Fig. 1. Solution containing 4 mg L^{-1} of each fatty alcohol and PAS in MeCN medium and derivatized reagent PIC (46 μmol) or BC (43 μmol) filled the loop of injection valve (3A). By switching the injection valve, loop content was inserted into the MeCN carrier stream and driven to the reactor coil located inside the microwave oven. In this system, coil was wrapped around an assay tube. At the same time that sample was injected, the microwave oven was switched on at 400 W for 60 s. Due to the low power of the microwave used, not excessive heating was rinsed, and in this way, bubble formation was avoid. After derivatization process, the loop of injection valve (3B) was automatically filled and by switching it, the reaction products were separated by HPLC.

3. Results and discussion

3.1. Reaction conditions

Initially reaction was carried out under conventional conditions as heating in a water bath; in this case, time

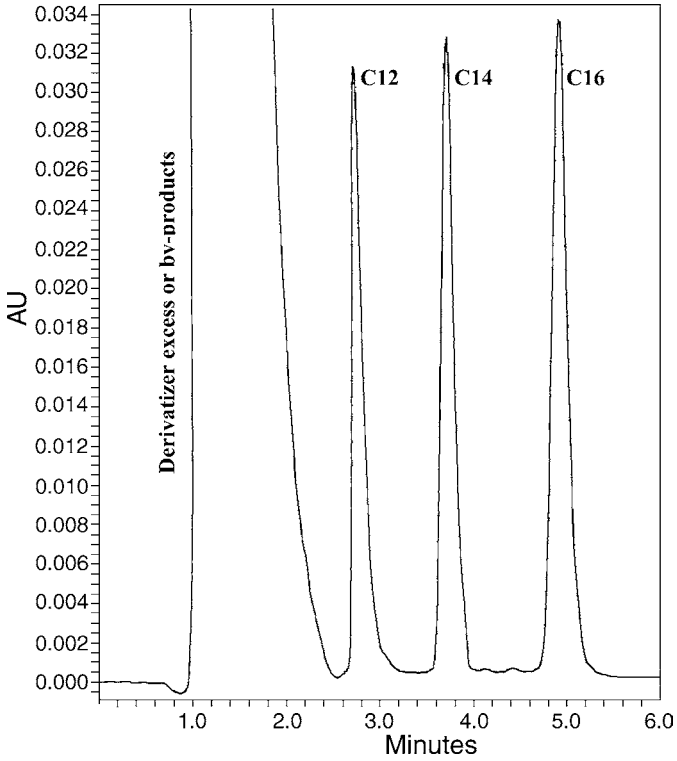

Fig. 3. Chromatogram of the fatty alcohol-PIC derivates standard (C_{12}OH, C_{14}OH and C_{16}OH). Conditions: MeOH/H_2O 90:10 mobile phase; Novapack C8 column (4 μm); flow 1 mL min^{-1}; PDA detection (235 nm).

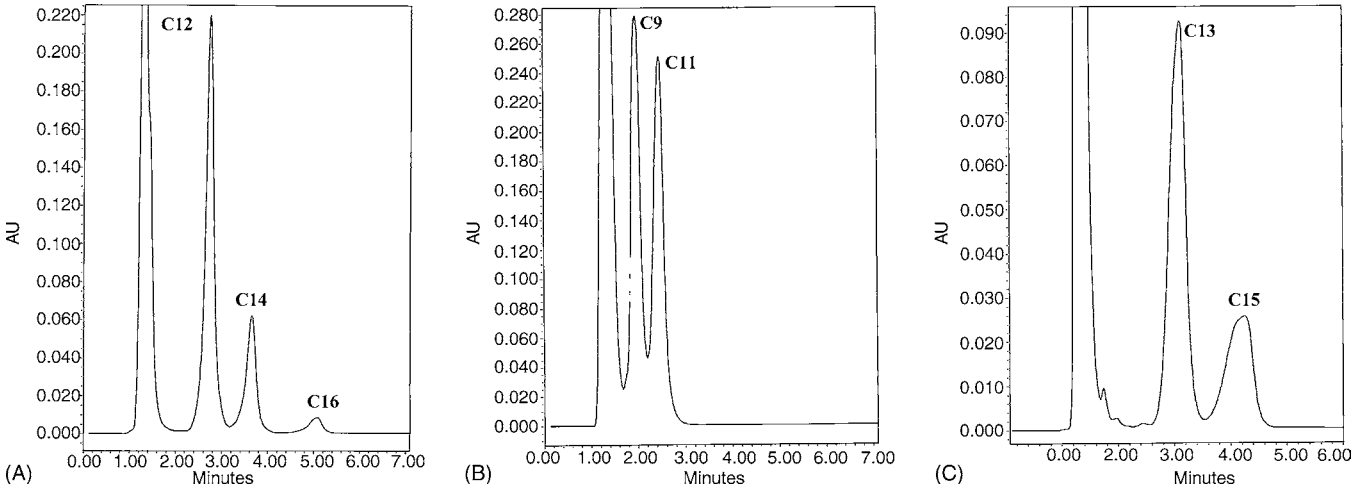

Fig. 4. Chromatogram of the PAS-PIC adducts: (A) ALEX4.0, (B) Ukanil36, and (C) Ukanil43. Conditions: MeOH/H_2O 90:10 mobile phase; Novapack C8 column (4 μm); flow 1 mL min^{-1}; PDA detection (235 nm).

Table 2
Retention time and carbon atom number content (X ± S.D.), and R.S.D.% of the fatty chain of PAS samples (ALEX) in four repeated analysis by CMWR-HPLC-PDA

Samples	C_{12}		C_{14}		C_{16}		CAN[a]
	t_r (min)	$X_{C_{12}} \times 100\%$	t_r (min)	$X_{C_{14}} \times 100\%$	t_r (min)	$X_{C_{16}} \times 100\%$	
ALEX4.0	2.727 ± 0.023	71.38	3.660 ± 0.110	24.61	5.227 ± 0.120	4.01	12.66
ALEX6.0	2.813 ± 0.020	72.43	3.643 ± 0.100	23.62	5.230 ± 0.150	3.95	12.62
ALEX12.0	2.793 ± 0.050	72.26	3.743 ± 0.090	23.90	5.333 ± 0.100	3.84	12.63
Average	2.817 ± 0.067 (2.37)	72.02 ± 0.82 (1.14)	3.20 ± 0.03 (0.93)	24.04 ± 0.70 (2.91)	5.251 ± 0.170 (3.23)	3.93 ± 0.17 (4.33)	12.64 ± 0.017 (0.13)

[a] CAN: carbon atom number determined by $CAN = 12X_{C_{12}} + 14X_{C_{14}} + 16X_{C_{16}}$.

Table 3
Retention time and carbon atom number content ($X \pm$ S.D.), and R.S.D.% of the fatty chain of PAS samples (Ukanil) in four repeated analysis by CMWR-HPLC-PDA

Samples	C_9		C_{11}		C_{13}		C_{15}		NAC[a]
	t_r (min)	X_{C_9} (%)	t_r (min)	$X_{C_{11}}$ (%)	t_r (min)	$X_{C_{13}}$ (%)	t_r (min)	$X_{C_{15}}$ (%)	
Ukanil36	1.962 ± 0.05	51.32	2.462 ± 0.04	48.68	–	–	–	–	9.97
Ukanil50	1.911 ± 0.03	51.15	2.412 ± 0.02	48.85	–	–	–	–	9.98
Ukanil43	–	–	–	–	3.108 ± 0.05	68.88	4.208 ± 0.03	31.12	13.62
Ukanil87	–	–	–	–	3.107 ± 0.05	69.28	4.257 ± 0.04	30.72	13.61
Average	1.937 ± 0.03	50.95 ± 0.30	2.437 ± 0.025	49.05 ± 0.30	3.108 ± 0.005	69.02 ± 0.18	4.233 ± 0.02	30.98 ± 0.18	

[a] CAN: carbon atom number determined by $CAN = 9X_{C_9} + 11X_{C_{11}}$ and $CAN = 13X_{C_{13}} + 15X_{C_{15}}$.

consumption was between 40 and 60 min at 60 °C. On the other hand, when reaction was carried out with microwave irradiation, reaction time decreased to 2 min at 450 W, using any of the derivatized agent. This fact, corroborates that the use of microwave produce an acceleration of the reaction due to the heating is more effective [18]. Hence, microwave irradiation procedure was selected for the derivatization step.

Linear alcohols were derivatized according to their respective phenyl urethanes and phenyl esters using different concentrations of C_nOH, PIC and BC, reaction times and flow of the carrier solvent in the continuous system (Fig. 1). Irradiation time from 10 to 60 s were tested and it was observed that after 45 s peak intensity was almost the same. In the on-line system, the reaction time is determined by the reactor volume and flow rate. Broad peak was obtained using a reactor coil of 1.5 m length and 0.8 mm internal diameter. When the internal diameter was decreased to 0.1 mm better peak shape was observed due to dispersion reduction. Different flow rates were tested to decreased analysis time. With flow rates higher that 0.5 mL min^{-1} no peaks were observed. To link the continuous reactor system with the HPLC separation, a switching valve was added. The addition of this valve in system allows to introduce in the separation system only the effective portion of product.

Using BC, the amount of unreacted reagents was very low. Nevertheless, absorption of derivatized product is ten times higher than without derivatization. The maximum absorption for the phenyl ester was observed at 230 nm. On the other hand, when PIC was used peak intensity was higher than with BC products, also the amount of by-products was smaller that with the previous one, as it is shown in Fig. 2A and B. Other advantages of using PIC is that the retention time of the products is shorter than with BC; using PIC reaction the total analysis time is 30 min while more than 35 min are necessary to separate until $C_{10}OH$ from the BC reaction. The main absorption for PIC products occurs at 235 nm.

Comparing these reactions, it was found that PAS can be readily converted into their corresponding products by the used of any of this reagent. However, the signal intensity for the product from the reaction of PIC is higher than obtained with BC.

3.2. Separation analysis

PAS derivatized were separated using a C18 column with a MeOH/H_2O 70:30 mobile phase, all the products were separated, a good commitment exists between resolution and analysis time. With this mobile phase the excess of derivatization reagent and by-products (peaks 1*, 2*, 3*, a*, b*, and c* in Fig. 2A and B) were separated from the fatty alcohol products. The spectrum observed for the by-products and excess of PIC and BC were different to the spectrum obtained for the fatty alcohol derivatized.

3.3. Sample analysis

The continuous developed system was applied to analyze three different samples of PAS. When fatty alcohols from C_9OH to $C_{16}OH$ were used as standards, to identify the alkyl chain of the commercial mixture of PAS, retention time for these alcohols was too long more than 40 min. In order to reduce the long retention time C8 column was used instead of the C18 (see Fig. 3). All other condition were the same for sample analysis. Fig. 4A shows three peaks for C_{12}, C_{14} and C_{16} of the ALEX 4.0 sample, the higher proportion was for C_{12} (approximately 72%). Fig. 4B shows the two peaks correspond to C_9 and C_{11} of the Ukanil36 sample; in this mixture the proportion of both components is almost the same (approximately 51% for C_9). Fig. 4C shows two peaks, which are C_{13} and C_{15} from the Ukanil43 sample, C_{13} has the higher proportion (approximately 69%). The results obtained for all the samples are shown in Tables 2 and 3. Standard deviation and reproducibility (R.S.D.%) of each PAS sample were satisfactory.

4. Conclusions

Adding derivatization step to PAS analysis by HPLC-PDA increased sensitivity in more than 10 times. Additionally alcohol derivatization allows to used 235 nm as wavelength for detection. The comparison of these reagents, permit to said that the better derivatized reagent was PIC. Microwave irradiation, at 450 W, was more efficient for the derivatization than the conventional conditions such as heating in a wa-

ter bath. Reaction time decrease in more than 10 times. The continuous analysis using the MW-HPLC-PDA system provided high sensitivity, reduced the amount of reagent used and shorter analysis times and it can be applied to the separation of long aliphatic chains of alcohol polyethoxylated.

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