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Homologue separation of linear alcohol ethoxylates by high-performance liquid chromatography

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Abstract

The optimization of a mobile phase and temperature for RP-HPLC separation of 3,5-dinitrobenzoyl derivatives of linear alcohol ethoxylates (LAEs) according to the alkyl chain length is reported. Partial inversion of the elution order of ethoxymers with a change of acetonitrile concentration is employed. A decrease of the temperature allows a further improvement in homologue separation. The addition of sodium perchlorate reduces the retention of higher ethoxymers. This is used for final separation improvement.

The optimal conditions found were: stationary phase: LiChrospher 100 RP-18 endcapped, 5 μm; column: LiChroCart 250×4 mm I.D.; mobile phase: acetonitrile–water (98:2, v/v) with 0.0011 mol/l sodium perchlorate; flow-rate: 1.5 ml/min; temperature: 5°C; detection: UV absorption at 233 nm. These conditions were successfully applied to the separation of a mixture of C_{12}–C_{18} LAEs and nonylphenol ethoxylates.

Keywords: Mobile phase composition; Temperature effects; Alcohol ethoxylates; Ethoxylates; Surfactants

1. Introduction

Linear alcohol ethoxylates (LAEs, see structure below) are widely used as non-ionic surfactants. In detergent formulations, they can substitute nonylphenol ethoxylates (NPEs), which are less acceptable than LAEs from the environmental point of view [1]

$$C_nH_{2n+1}\text{–}(O\text{–}CH_2\text{–}CH_2)_m\text{–}OH$$

The linear primary alkyl chain with mainly 12–18 carbon atoms (n) represents the hydrophobic part of LAE molecule. The hydrophilic part is created by a polyoxyethylene chain, typically with an average ethoxylation degree of 5–10 (m) in the case of LAEs used for detergency purposes. The separation according to the length of the alkyl or oxyethylene chain is called homologue by homologue or oligomer by oligomer separation, respectively. The goal of the first one is a recognition of homologues and a suppression of oligomer separation at the same time.

Kudoh [2] reported the RP-HPLC separation of underivatized LAEs, in accordance with the alkyl group, using a C_{18} stationary phase and a mixture of acetone–water as a mobile phase. The finding of the optimal ratio of acetone–water was essential. The presented data [2] show that inversion in the elution order of oligomers (ethoxymers) occurs. The separation of underivatized LAEs by the length of the alkyl chains was also achieved in the work of Rockwood and Higuchi [3]. They used an LC–MS hyphenated technique. Evans et al. [4] used LC–MS for the homologue as well as the ethoxymer recognition of partially separated alcohol ethoxylates (AEs).

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RP-HPLC with a linear gradient of mobile phase [methanol–water (80:20) to 100% methanol] was used for the homologue separation of AEs derivatized with phenyl isocyanate [5]. The authors direct the attention to the fact that ethoxylated alkylphenols give a large peak at a retention time corresponding to C_{11} AEs.

The LAEs derivatized with naphthyl isocyanate were successfully separated on a C_{18} column with a binary mobile phase (acetonitrile–water or acetonitrile–methanol) [6,7]. The optimization of the mobile phase composition for the homologue separation was described [6]. It was emphasised that a small change in the mobile phase composition (±1%) causes a worsening of the homologue separation.

Okada [8] described the AE analysis according to the alkyl as well as the oxyethylene chain. He derivatized AEs with 3,5-dinitrobenzoyl chloride. As stationary phases, C_8- and C_{18}-alkyl bonded silica gel and a styrene–divinylbenzene copolymer were tested. A good homologue separation was attained with the last one and with the mobile phase acetonitrile–methanol. Okada also briefly evaluated the influence of temperature. Smaller differences in retention times of the ethoxymers of one homologue were observed for higher temperatures.

Jandera [9] showed that inversion of the elution order of the oligomers can be found for different φ values [organic solvent fraction (v/v) in a mobile phase].

The influence of conformational changes of the polyoxyethylene chain on a compound's retention in RP-HPLC was studied by Melander et al. [10] and Okada [11]. The homologue separation of underivatized AEs was also explained by applying the principle of the so-called liquid chromatography under critical conditions [12,13].

In the present work, the optimization of homologue by homologue separation of LAEs derivatized with 3,5-dinitrobenzoyl chloride is described. The nature and amount of organic solvent, the temperature and the salt concentration were optimized. In comparison to Okada's work [8], a good separation was achieved on a C_{18} silica-based column and the temperature was optimized to reach a satisfying coelution of ethoxymers but also an improvement of the homologue separation. The optimal conditions were applied to the chromatographic separation of LAEs. In the end, the problem of the homologue separation for a mixture of AEs with linear as well as branched alkyl chains is mentioned.

2. Experimental

The chromatographic work was carried out using a Spectra-Physics liquid chromatograph (pump SP 8700, UV–Vis absorption detector SP 8440, all Spectra-Physics, San Jose, CA, USA). The chromatograms were recorded at 233 nm. The data acquisition and handling were executed with the use of a Chromatography Station for Windows CSW version 1.0 (DataApex, Prague, Czech Republic). The samples were injected by a 50-μl syringe (Hamilton, Reno, NV, USA) in a manual 7125 injector equipped with a 10-μl loop (Rheodyne, Cotati, CA, USA).

The separations were performed using a 250×4 or 125×4 mm I.D. LiChrospher 100 RP-18 endcapped 5 μm LiChroCart column (E. Merck, Darmstadt, Germany). The temperature of the column was controlled with a precision of ±0.1°C, using a glass water jacket and a laboratory water thermostat equipped with a freon cooler. The flow-rate was 1.5 or 1.25 ml/min, for the longer or shorter column, respectively.

The mobile phases were prepared by mixing accurately weighed components. As these components, HPLC-grade acetonitrile and tetrahydrofuran (E. Merck, Darmstadt, Germany), UV-grade methanol (Lachema, Brno, Czech Republic), redistilled water and sodium perchlorate monohydrate p.a. (Fluka, Buchs, Switzerland) were used.

Hold-up volumes were determined by two injections of methanol (0.5 μl) after each change of experimental conditions. The detection wavelength was 200 nm. Mean values of two retention times were used for calculation of retention factors.

Linear primary C_{11}–C_{16} and C_{18} alcohols ($C_{11}OH$–$C_{16}OH$, $C_{18}OH$) (all Aldrich, Milwaukee, WI, USA), diethyleneglycol ($C_{12}E_2$), tetraethyleneglycol ($C_{12}E_4$), hexaethyleneglycol ($C_{12}E_6$), heptaethyleneglycol ($C_{12}E_7$), octaethyleneglycol ($C_{12}E_8$) and nonaethyleneglycol ($C_{12}E_9$) monododecyl ethers (all Fluka, Buchs, Switzerland) were used as standards. Marlipal 28/100, Lialet 125/7 and

Triton N101 were used as samples of ethoxylates (all gifts of Dr. M. Zanette, University of Venice, Venice, Italy). Marlipal 28/100 is a mixture of even linear primary fatty alcohol (C_{12}–C_{18}) ethoxylates with an average number of 10.2 oxyethylene units. Lialet 125 /7 is a mixture of even and odd, linear and branched primary C_{12}–C_{15} alcohol ethoxylates with an average oxyethylene chain of 7 units. Triton N101 is a mixture of nonylphenol ethoxylates with an average of 10 oxyethylene units. Stock solutions of the compounds (concentration of standards≈4 mg/ml, concentration of samples≈5 mg/ml) were prepared in acetonitrile or acetone (UV grade, Lachema, Brno, Czech Republic).

The derivatization reaction was performed as follows: adequate amounts of 3,5-dinitrobenzoyl chloride p.a. (Fluka, Buchs, Switzerland), acetonitrile, a work solution of standard (≈1–2 mg/ml) or a stock solution of sample and distilled pyridine p.a. (Lachema, Brno, Czech Republic) were added and mixed in a glass flask. The closed flask was heated in an oven at 60°C for 65 min. After cooling and filling up with acetonitrile the solution was ready for injection.

3. Results and discussion

First, the effect of the nature and amount of an organic solvent in the mobile phase was evaluated. Tetrahydrofuran, methanol and acetonitrile were tested in the experiments with Marlipal 28/100. For the stationary phase used, only the last one enables to change the elution order of ethoxymers with the same alkyl chain and to consequently achieve their co-elution in a narrow zone. Acetonitrile also allows to perform the analyses in an acceptable time. The optimum lies between 98:2 and 96:4 (acetonitrile–water, v/v).

The inversion of the elution order of the standards is not complete (see Table 1) and it is less marked in comparison with C_{14} LAEs derivatized with naphthyl isocyanate [6]. Higher ethoxymers show relatively higher (in the set of C_{12} standards) retention for a mobile phase richer in acetonitrile content. $C_{12}E_9$ does not represent sufficiently the chromatographic behaviour of ethoxymers with a longer oxyethylene chain, since at $\varphi = 0.98$ even $C_{12}E_9$ is not eluted later than $C_{12}OH$. That is why the optimal acetonitrile content for the co-elution of ethoxymers was not computed as in the quoted work [6], but it was found by trial and error procedure using Marlipal 28/100. The goal of this procedure was to achieve the highest possible ratio of the space between zones of single homologues to the width of these zones. At 20°C (the role of the temperature is discussed below) the mixture of acetonitrile–water (96.6:3.4, v/v) was estimated as optimal. The conditions for the elution of ethoxymers in the narrow zone were found, but it was not possible to attain the same retention time for all of them.

The partial inversion is further demonstrated in Fig. 1. The chromatograms in the mobile phases close to the optimum are shown. The tailing of peaks in chromatogram a is due to the higher retention of the ethoxymers with a longer oxyethylene chain. The peaks of alcohols are inside the zones of single homologues. In the case of a total inversion we could expect them to elute in the start of the zones. The alcohols are eluted in the end of the zones for $\varphi = 0.96$ (see chromatograms c and d in Fig. 1). The experiment with a lower content of acetonitrile ($\varphi = 0.90$) showed that the peaks of alcohols as well as lower ethoxymers are overlapped by higher ethoxy-

Table 1
Retention factors of standards at different acetonitrile fraction φ (v/v) in the mobile phase.

φ (v/v)	Retention factor							
	$C_{12}OH$	$C_{12}E_2$	$C_{12}E_4$	$C_{12}E_6$	$C_{12}E_7$	$C_{12}E_8$	$C_{12}E_9$	$C_{11}OH$
0.90	6.92	5.98	5.32	4.91	4.73	4.55	4.40	4.96
0.96	3.05	2.80	2.66	2.60	2.61	2.63	2.68	2.26
0.98	2.25	2.12	2.07	2.07	2.09	2.14	2.21	1.69

Stationary phase: LiChrospher 100 RP-18 endcapped, 5 μm; column: LiChroCart 250×4 mm I.D.; mobile phase: acetonitrile–water; flow-rate: 1.5 ml/min; temperature: 20°C; detection: UV absorption at 233 nm.

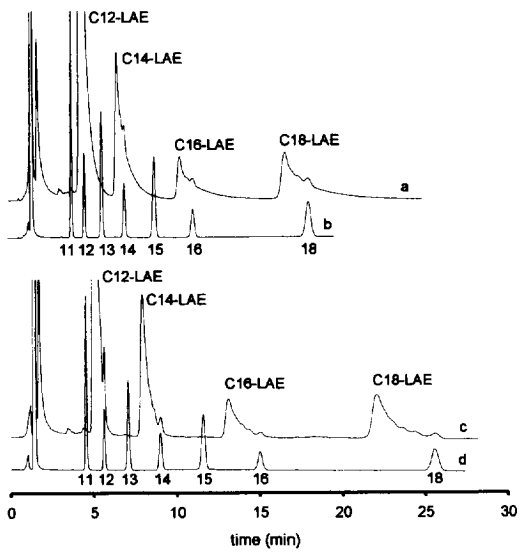

Fig. 1. RP-HPLC chromatograms of 3,5-dinitrobenzoyl derivatives of Marlipal 28/100 (C_{12}-LAE, C_{14}-LAE, C_{16}-LAE, C_{18}-LAE) (a and c) and alcohols (11=C_{11}OH, 12=C_{12}OH, 13=C_{13}OH, 14=C_{14}OH, 15=C_{15}OH, 16=C_{16}OH, 18=C_{18}OH) (b and d). Stationary phase: LiChrospher 100 RP-18 endcapped, 5 μm; column: LiChroCart 250×4 mm I.D.; mobile phase: acetonitrile–water (98:2, v/v) (a and b) and (96:4, v/v) (c and d), flow-rate: 1.5 ml/min. Temperature: 20°C. Detection: UV absorption at 233 nm.

mers with a longer alkyl chain (see also retention factors of C_{11}OH and $C_{12}E_{6-9}$ in Table 1).

The inversion of the elution order can be explained by the equilibrium of the conformers of the polyoxyethylene chain. It was shown that for a gauche conformer the retention becomes smaller with an increase of the oxyethylene unit number and for a trans conformer it is vice versa [10,11]. The equilibrium depends on the temperature as well as on the medium polarity, which is affected by a change of the mobile phase composition (content of acetonitrile).

The shorter column (125×4 mm I.D.) can also be used for a successful separation. It allows to reduce the analysis time as well as the consumption of a mobile phase. The shorter column and an adjustment of the acetonitrile–water ratio were sufficient for the separation of the C_{12}–C_{18} even homologue mixture (Marlipal 28/100). The other two parameters (the temperature and the concentration of sodium perchlorate) were optimized in endeavour to improve the separation of a more complicated mixture.

For the evaluation of the temperature influence, the separation factor S and S_{max} [6,14] were used. Separation factor S involves the influence of selectivity, as well as capacity on the separation. It can be easily computed from the experimental or predicted retention data.

$$S = \frac{(k_2 - k_1)}{(k_2 + k_1 + 2)} \quad (1)$$

where k_2 and k_1 are the retention factors ($k_2 > k_1$).

S_{max} is the maximum S found for two compounds from the evaluated set (C_{12}OH and C_{12} ethoxylates in this work).

$$S_{max} = \frac{(k_{max} - k_{min})}{(k_{max} + k_{min} + 2)} \quad (2)$$

where k_{max} and k_{min} represent the retention factors of the most and least retained compound in the evaluated set. A lower value of S_{max} means a worse separation of ethoxymers of single homologues and then narrower homologue zones.

The dependencies of S and S_{max} on the temperature are shown in Fig. 2. We can see greater differences between S and corresponding S_{max} at lower temperatures. This means a marked improve of homologue separation in comparison to the separation of ethoxymers. The region of abscissa corresponding to $S_{max} > S(C_{11}OH–C_{12}OH)$ represents temperatures for which $C_{12}E_9$ ethoxymer is eluted earlier than the alcohol of the preceding homologue (C_{11}OH).

The experiments with Marlipal 28/100 also showed that the separation of homologues can be improved owing to a diminution of temperature. Of course at the same time this is accompanied with some broadening of the single homologue zones and prolongation of analysis time.

In the dependence of the sample complexity the temperature can be chosen similarly as the column length. The adequate increase of the temperature has a practical effect similar to the use of a shorter column.

The change of temperature can also influence the elution order. For example in the mobile phase acetonitrile–water (98:2, v/v) during the change of

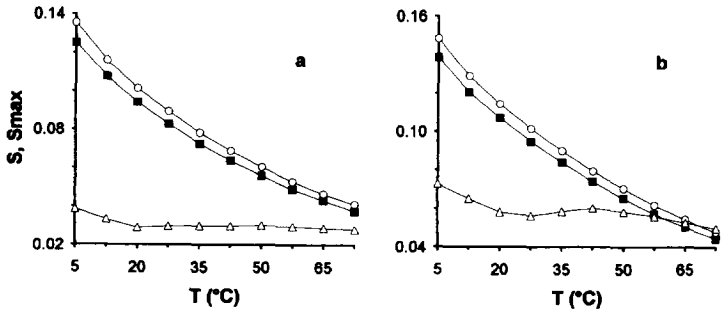

Fig. 2. Separation factors $S(C_{11}OH–C_{12}OH)$ (■), $S(C_{12}OH–C_{13}OH)$ (○) and S_{max} (△) versus the temperature. Mobile phase: acetonitrile–water (98:2, v/v) (a) and (96:4, v/v) (b). Stationary phase, column, flow-rate and detection as in Fig. 1.

temperature from 72.5 to 5°C, the retention factor is increasing 4.98× for $C_{12}OH$, 4.99× for $C_{12}E_2$, but 5.54× for $C_{12}E_9$. The elution order has changed from $C_{12}E_6$, $C_{12}E_7$, $C_{12}E_8$, $C_{12}E_4$, $C_{12}E_9$, $C_{12}E_2$, $C_{12}OH$ to $C_{12}E_4$, $C_{12}E_6$, $C_{12}E_7$, $C_{12}E_2$, $C_{12}E_8$, $C_{12}OH$, $C_{12}E_9$.

As it was stated, the organic solvent content in a mobile phase as well as the temperature have an influence on the relative retention of ethoxymers with the same alkyl chain. This means that these parameters have to be optimized at the same time. For different temperatures different optimal compositions of a mobile phase should be found.

The concentration of sodium perchlorate in the mobile phase was the last parameter to be optimized. The impact of a salt on the retention is negligible for alcohols and lower ethoxymers, but is increasing with the extension of an oxyethylene chain, with the increase of the ability to form a charged complex of oxyethylene chain with Na^+ (see Fig. 3).

The salt can be used effectively to decrease the retention of higher ethoxymers. Therefore, the mixture of acetonitrile–water (98:2, v/v), in which these compounds are eluted later than the corresponding alcohol, was chosen for the optimization of the salt concentration. The optimization was made by a trial and error procedure with the use of Marlipal 28/100. It allows to evaluate the influence of higher ethoxymers (more than nine oxyethylene units) on the separation. Using standards the dependence of S and

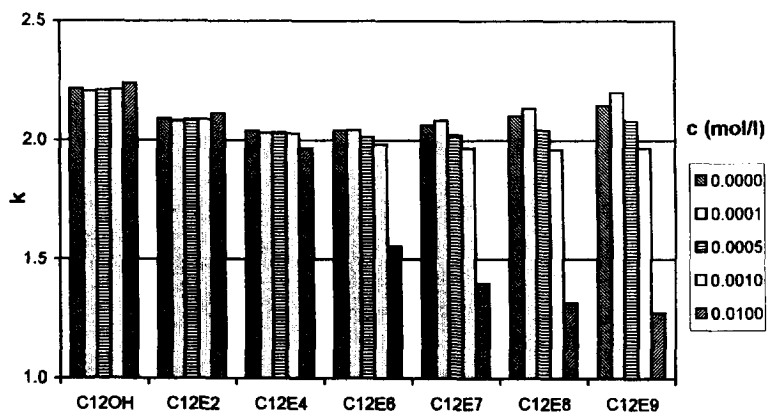

Fig. 3. Retention factors of standards at different sodium perchlorate concentrations. Mobile phase: acetonitrile–water (98:2, v/v) with the addition of sodium perchlorate at the following concentrations: 1=0.0000, 2=0.0001, 3=0.0005, 4=0.0010, 5=0.0100 mol/l. Other conditions as in Fig. 1.

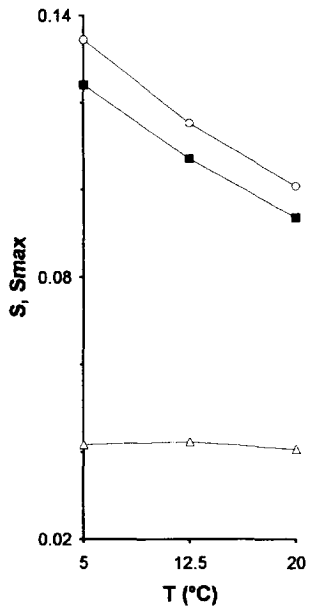

Fig. 4. Separation factors $S(C_{11}OH-C_{12}OH)$ (■), $S(C_{12}OH-C_{13}OH)$ (○) and S_{max} (△) versus the temperature. Mobile phase: acetonitrile–water (98:2, v/v) with 0.001 mol/l sodium perchlorate. Stationary phase, column, flow-rate and detection as in Fig. 1.

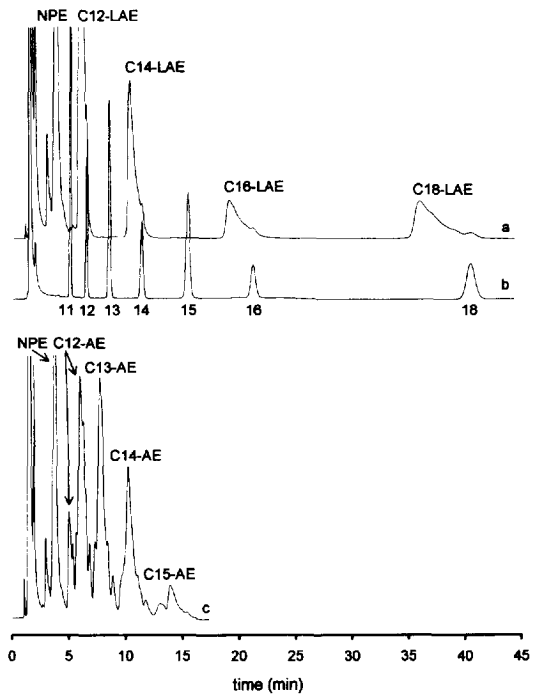

Fig. 5. RP-HPLC chromatograms of 3,5-dinitrobenzoyl derivatives of the following mixtures: Marlipal 28/100 (C_{12}-LAE, C_{14}-LAE, C_{16}-LAE, C_{18}-LAE) and Triton N101 (NPE) (a), alcohols ($11=C_{11}OH$, $12=C_{12}OH$, $13=C_{13}OH$, $14=C_{14}OH$, $15=C_{15}OH$, $16=C_{16}OH$, $18=C_{18}OH$) (b), Lialet 125/7 (C_{12}-AE, C_{13}-AE, C_{14}-AE, C_{15}-AE) and Triton N101 (NPE) (c). Mobile phase: acetonitrile–water (98:2, v/v) with 0.0011 mol/l sodium perchlorate. Temperature: 5°C. Stationary phase, column, flow-rate and detection as in Fig. 1.

S_{max} on the temperature was measured in the working range from 5 to 20°C (Fig. 4). The S and S_{max} values are similar to the ones shown in Fig. 2 (mobile phase without salt). The optimization of $NaClO_4$ concentration allowed to improve the co-elution of ethoxymers, to use a mobile phase with a higher elution strength and consequently to reduce the analysis time. This time was changed from 23.60 min (optimum at 20°C without salt, $\varphi=0.966$) to 18.50 min (optimum at 20°C with salt, $\varphi=0.980$, $[NaClO_4]=0.0009$ mol/l). For the flow-rate used (1.5 ml/min) the shorter analyses meant a significant saving of mobile phase.

The chromatograms under the optimized conditions are depicted in Fig. 5. The good separation of NPEs and C_{12}–C_{18} even homologues is demonstrated (chromatogram a). Based on the widths of LAE homologue zones and the positions of the alcohol standard peaks, we can expect the possibility to separate C_{11}–C_{18} even and odd homologues of LAEs. Their incomplete separation in the presence of branched AEs (Lialet 125/7 mixture) is seen in the chromatogram c. The good separation of NPEs from the components of Lialet 125/7 was achieved (chromatogram c).

For the solution of the homologue separation of the linear–branched AE mixture, e.g., different types of stationary phases could be tested. The other possibility is of course to use the LC–MS technique where it is enough to separate compounds with the same molecular mass [4].

4. Conclusions

The different parameters (nature and amount of an organic solvent, temperature and salt concentration) were optimized to achieve and to improve the homologue separation of LAEs. Each of these parameters contributes to the final optimal separation.

The use of optimal conditions allows to separate C_{12}–C_{18} even homologues, NPEs from C_{12}–C_{18} even homologues, as well as from C_{12}–C_{15} even and odd homologues. The separation of a C_{12}–C_{15} mixture in the presence of branched AEs was not achieved.

The effect of all optimized parameters on the separation was described and then a similar procedure could be used for the optimization of a separation of LAEs derivatized with other derivatizing agents.

Acknowledgments

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