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# High temperature liquid chromatography and liquid chromatography–mass spectroscopy analysis of octylphenol ethoxylates on different stationary phases

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**Abstract**

Temperature was investigated as active parameter in the liquid chromatography (LC) analysis of octylphenol ethoxylates. Significant differences in selectivity were observed when the oligomers were analyzed by reversed phase LC (RPLC) on silica-, zirconia- and polystyrene/divinylbenzene based stationary phases at low (ambient), medium and elevated temperature with acetonitrile/water as mobile phase. As ascertained by LC–mass spectroscopy (MS), in most cases the elution order of the oligomers was completely reversed comparing ambient and high temperature separations. On a graphitized carbon type column, the selectivity remained unchanged, regardless the analysis temperature. Also in normal phase LC, the elution order remained unaffected by temperature variations both for acetonitrile/water and methanol/water mixtures as mobile phase. Surprisingly, when reversed phase LC on a octadecylsilicagel column at different temperatures was repeated with methanol instead of acetonitrile as mobile phase ingredient, the reversal of elution order did not take place. Results are evaluated in terms of thermodynamic parameters.


*Keywords:* LC; LC–MS; Temperature; Octylphenol ethoxylates; Selectivity

## 1. Introduction

The use of elevated temperatures and temperature programming in liquid chromatography (LC) is gaining momentum in recent years [1–9]. At the present time, the number of applications of high (or elevated) temperature LC is still limited mainly because of lack of suitable stationary phases and equipment. Of utmost importance is adequate control of mobile phase and column temperature. In this respect, problems could be circumvented by using packed capillary columns exhibiting low heat capacity and negligible radial temperature gradients. Instrumentation for elevated temperature conventional LC became recently available to efficiently heat the entering mobile phase to the same temperature as the column (oven) temperature. In this way, loss of separation and efficiency due to thermal mismatch between mobile and stationary phase is eliminated. The effluent temperature is actively controlled by a Peltier element to stabilize and protect the detector.

Presently, the limiting factor in using high temperature in conventional LC is the stability of bonded silica-based stationary phases. Silica-based stationary phases usually are stable at temperatures up to 90 °C; although some novel reversed phase material can be used up to 120 °C [8,9]. When using water in the mobile phase as in reversed phase type separations, loss of bonded phase from the silica support due to hydrolysis is more pronounced at high temperatures [10]. Stationary phases with higher temperature stability are based on materials other than silica e.g. graphitized carbon types, zirconium oxide based phases and polystyrene/divinylbenzene phases.

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Alkylphenol ethoxylates are important technical nonionic surfactants widely used in industrial and household applications. The production process generally does not generate one discrete molecule, but a disperse distribution of compounds. Quality and application area of the product is greatly determined by the chain length distribution and the purity of the synthesized product. To determine these characteristics, several separation techniques can be applied. Reversed phase LC (RPLC) and normal phase LC (NPLC) with UV detection are by far the most popular techniques for these analyses.

Analyses are normally carried out at ambient temperatures or temperatures slightly higher than ambient. Using acetonitrile–water as mobile phase in RPLC, a chain length distribution separation is obtained which is controlled by hydrophobic interaction [11]. The large, less hydrophobic oligomers elute before oligomers with a smaller degree of polymerization. For normal phase separations, this elution order is opposite and the larger oligomers are more retained on the column. When the reversed phase analysis is carried out at moderately elevated temperatures (e.g. 50 °C) with the same mobile phase composition, the selectivity between oligomers with different chain lengths is significantly reduced and consequently, little or no oligomeric separation takes place. At higher temperatures than this 'critical' temperature a chain length distribution separation reappears, however, with inversed elution order compared to analyses at ambient temperature.

At present, the reason for this phenomenon is not completely obvious, but size-exclusion effects seem not to cause this reversal. The effect is most interesting since reversed selectivity by temperature can be achieved on the same instrument, with the same column, and with the same mobile phase composition. Several publications describe this selectivity change for polymers with ethylene oxide (EO) units. Melander et al. [12] observed more than 20 years ago increased retention of PEG 400 at elevated temperature in RPLC. Escott and Mortimer operated an ODS column at 80 °C for a separation of a blend of PEGs and they reported an improved resolution compared to ambient temperature [13]. Lochmüller et al. [14] also observed an increased retention of PEO samples with increased temperature in RPLC on a silica-based C18 stationary phase with a mobile phase composed of water and acetonitrile. The group of Greibrokk applied inverse temperature programming in RP-packed capillary LC for improved separation of PEG oligomers [15]. Cho et al. [16] recently reported on the temperature dependence of retention of poly(ethylene oxide) (PEO) and fatty alcohol ethoxylates (FAE) [17] in RPLC. Significant selectivity changes caused by changes in analysis temperature were observed. Kamiusuki et al. [18] have reported on the separation of octylphenol ethoxylates on branched fluorinated silica gel columns. The oligomers were separated according to increasing number of ethylene oxide units. The mobile phase was a mixture of water and methanol. Increasing the temperature from 40 to 70 °C resulted in a shorter retention time, however, without affecting selectivity.

The aim of this study was to investigate the potential of using temperature as active variable in the LC analysis of octylphenol ethoxylates using the present state-of-the-art in column technology and instrumentation.

## 2. Experimental

### 2.1. Chemicals and samples

All solvents used were HPLC grade from Biosolve Ltd. (Valkenswaard, The Netherlands). Triton X-100 from Sigma-Aldrich (Bornem, Belgium) was dissolved in water/acetonitrile, 1/1 (v/v) for RPLC analyses and in water/acetonitrile, 1/9 (v/v) for NPLC analyses.

### 2.2. Instrumental

Analyses were performed using an Agilent 1100 Series LC equipped with a diode array detector (Agilent Technologies, Waldbronn, Germany) set at 225 nm. All analyses were carried out in the isocratic mode. The column temperature was controlled with a Polaratherm Series 9000 oven equipped with a mobile phase preheater and cryo-option (Selerity Technologies, Salt Lake City, UT, USA). The preheater temperature was set equal to the oven temperature and the effluent temperature at 40 °C.

The mass spectrometer was an Agilent 1100 Series Quadrupole MSD version SL equipped with an atmospheric pressure chemical ionization (APCI) source (Agilent Technologies, Waldbronn, Germany). Positive ionization was

Table 1
LC columns with details on column dimensions and packing material

| Column (stationary phase) | Dimensions $L \times$ I.D. (particle size) | Base material | Pore size (Å) |
|---|---|---|---|
| Agilent Zorbax StableBond (C18) | 150 mm × 3.0 mm (3.5 μm) | Silica, sterically protected | 80 |
| Agilent Zorbax StableBond (C18) | 150 mm × 4.6 mm (3.5 μm) | Silica, sterically protected | 300 |
| Agilent Zorbax StableBond (phenyl) | 150 mm × 3.0 mm (3.5 μm) | Silica, sterically protected | 80 |
| Selerity Blaze (C8) | 150 mm × 4.6 mm (3 μm) | Silica, poly dentate | 100 |
| Zirchrom PBD (polybutadiene) | 150 mm × 2.1 mm (3 μm) | Zirconium oxide | 300 |
| Polymer Laboratories PLRP-S (polystyrene/divinylbenzene) | 150 mm × 2.1 mm (3 μm) | Polystyrene/divinylbenzene | 100 |
| Thermo Electron Hypercarb (graphitized carbon) | 100 mm × 3.0 mm (5 μm) | Graphitized carbon | 250 |
| Phenomenex Ultremex 3 Silica (silica) | 75 mm × 4.6 mm (3 μm) | Silica | 80 |

performed in the scan mode (300–1400, *m*/*z*). Interface settings were: $N_2$ drying gas temperature 325 °C, $N_2$ drying gas flow 8 l/min, APCI vaporizer temperature 350 °C, nebulizer 50 psi, capillary voltage 3000 V.

The following LC columns were used in this work: various Zorbax StableBond columns (Agilent Technologies, Waldbronn, Germany), a Selerity Blaze C8 column (Selerity Technologies, Salt Lake City, UT, USA), a Zirchrom PBD

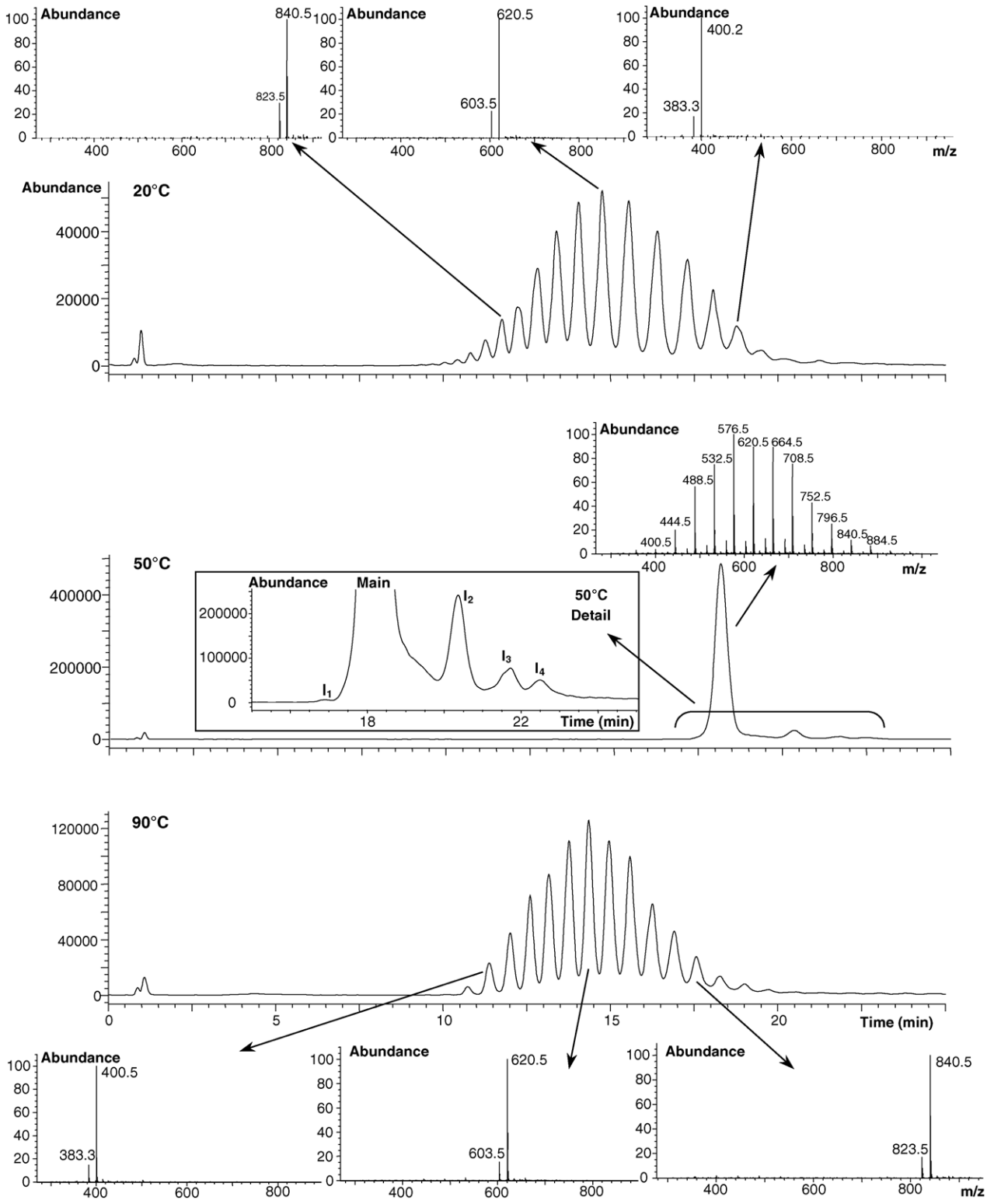


Fig. 1. LC–MS analysis of Triton X-100 (1000 ppm) on a Zorbax StableBond C18 column (150 mm *L* × 3.0 mm I.D., 3.5 μm particles, 80 Å pore size). Mobile phase: water/ACN, 50/50 (v/v), flow rate: 0.6 ml/min, injection volume: 3 μl, detection: APCI, positive ionization.

column (Zirchrom, Anoka, MN, USA), a PLRP-S column (Polymer Laboratories, Shropshire, UK), a Hypercarb column (Thermo Electron Corporation, Cheshire, UK) and an Ultremex 3 Silica (Phenomenex, Cheshire, UK). Details on column dimensions and packing material are given in Table 1.

The software used to calculate the $\log K_{O/W}$ values was KOWWIN v1.66, SRC-LOGKOW for Microsoft Windows (Syracuse Research Corporation, Syracuse, NY, USA).

## 3. Results and discussion

### 3.1. RPLC on silica-based columns

The selectivity of reversed phase separations of alkylphenol ethoxylates is influenced by the nature of the stationary phase, the temperature and the mobile phase composition. At ambient temperatures with acetonitrile–water a chain length distribution separation controlled by hydrophobic interaction is obtained [11]. Under these conditions, polymers with longer EO chains elute before polymers with shorter EO chains. The $\log K_{O/W}$ values for Triton X-100 oligomers with 1 up to 35 EO units were calculated using KOWWIN and plotted against the number of EO units. A linear plot was obtained ($R^2 = 0.9995$) with a negative slope ($y = -0.272x + 5.129$). The $\log K_{O/W}$ value switches from positive to negative between 18 and 19 EO units. This means that the longer the EO chain, the more polar the alkylphenol ethoxylate becomes. The results obtained in this contribution on various silica-based reversed phase columns with water–acetonitrile as mobile phase are in agreement with this concept. When increasing the temperature, the EO chain length selectivity deteriorates and long EO chains are more retained compared to ambient temperature separations. At a certain temperature, all oligomers elute together as a single peak and the EO selectivity completely disappears. However, at even higher temperatures a new chain length distribution separation shows up, but with reversed elution order compared to the separation at ambient temperature. This is illustrated in Fig. 1, showing the LC–mass spectroscopy (MS) analysis using an APCI source of Triton X-100 on a Zorbax StableBond C18 column at 20, 50, and 90 °C. Inserted are mass spectra of some representative oligomers. These mass spectra are made up of the molecular ion ($[M + H]^+$) and an intense ion $[M + 18]^+$ that can be attributed to ammonium adduct formation. Adduct formation is rather unusual in APCI, but this has also been reported in thermospray LC–MS analyses of surfactants by Evans et al. [19]. Jandera et al. [20,21] obtained mass spectra of ethoxylate polymers with molecular ions and sodium and potassium adducts in APCI positive ionization. At 50 °C, the transition temperature at which no oligomeric separation takes place, several side products ($I_1$–$I_4$ in Fig. 1) are detected in the vicinity of the main product. A detail of the chromatogram is inserted in Fig. 1. These impurities are isomeric oligomers of the main product, *tert*-octylphenol ethoxylate, and show a similar EO distribution pattern as for the main product. The isomers thus originate from the *tert*-octylphenol used as starting material in the polymerization procedure. The octylphenol contained impurities with more and less branched octyl chains.

Cho et al. [16,17] also observed that retention of PEO in RPLC increased as temperature increased. The thermodynamics of the chromatographic process were investigated within a temperature range of 35–49 °C. The thermodynamic parameters of a sorption process of analytes to a stationary phase can be described by:

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ = -RT \ln K \quad (1)$$

$$k' = K\phi \quad (2)$$

$$\ln k' = -\left(\frac{\Delta H^\circ}{RT}\right) + \left(\frac{\Delta S^\circ}{R}\right) + \ln \phi \quad (3)$$

The equilibrium constant ($K$) of a molecule between the stationary and mobile phase is related to changes in the Gibbs free energy ($\Delta G^\circ$) and consequently, the enthalpy ($\Delta H^\circ$) and the entropy ($\Delta S^\circ$) of the system; $T$ is the absolute temperature (in Kelvin); $R$ is the gas constant (8.31441 J/K mol); $k'$ is the retention factor and $\phi$ is the

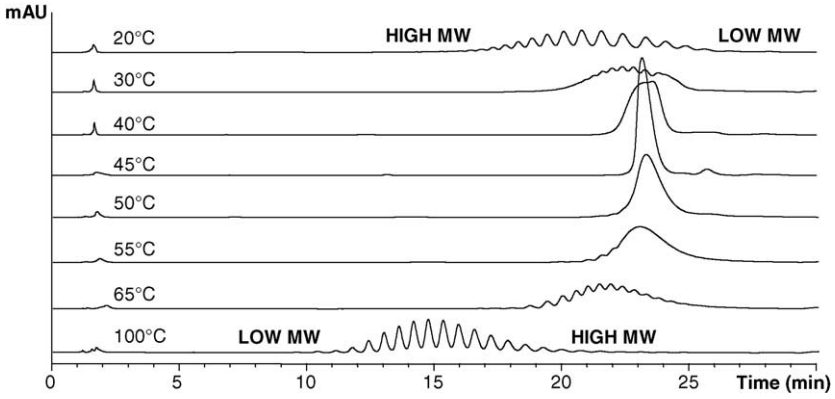


Fig. 2. LC analysis of Triton X-100 (10000 ppm) on a Selerity Blaze C8 column (150 mm $L$ × 4.6 mm I.D., 3 μm particles, 100 Å pore size). Mobile phase: water/ACN, 50/50 (v/v), flow rate: 1 ml/min, injection volume: 5 μl, detection: DAD 225 nm.

volume ratio of stationary to mobile phase. $\Delta H^\circ$ can be described as the change in enthalpy upon the transfer of one mole of analyte from the mobile to the stationary phase. Generally, in hydrophobic interaction chromatography (HIC) the sorption of a polymer to a stationary phase is an energetically unfavorable process ($\Delta H^\circ > 0$) and is entropy-driven ($\Delta S^\circ > 0$). Under these conditions the interaction strength will increase with increasing oligomer mass and the retention of polymers will increase with increasing degree of polymerization.

Cho et al. [16] noted that PEO retention increased as temperature increased. The thermodynamic parameters were calculated using van't Hoff plots and it was concluded that the sorption process of EO chains to the stationary phase is an endothermic entropy-driven process ($\Delta H^\circ > 0$ and $\Delta S^\circ > 0$). Water molecules adjacent to the solute EO chains and the stationary phase are in a low entropy state. When adsorbed to the stationary phase, the EO chains release the ordered water molecules to the more unstructured water in the mobile phase, increasing the entropy of the system. This entropy gain increases retention and its contribution increases relative to the enthalpic effect with increasing temperature as can be deduced from Eq. (3).

If an alkyl group is added to the EO chain, the thermodynamics of the sorption process is more complex. Cho et al. analyzed fatty alcohol ethoxylates with RPLC and observed opposite thermodynamic characteristics for the PEO block ($\Delta H^\circ > 0$ and $\Delta S^\circ > 0$) and the alkyl chain ($\Delta H^\circ < 0$ and $\Delta S^\circ < 0$) [17]. This means that sorption of the alkyl chain is an enthalpy-driven process, while sorption of the PEO chain is an entropy-driven process. Implications for FAE are that at low temperature (e.g. 15 °C) the resolution according to EO chain length is better, while at higher temperature (e.g. 40 °C) the resolution according to the number of EO units decreases, but the selectivity according to the alkyl chain length improves.

These results are in agreement with our data obtained for the alkylphenol ethoxylates at 20 and 50 °C in Fig. 1. However, at higher temperatures, the EO selectivity increases once more, but with inversed elution order. In the work of the group of Cho, no analyses were performed at such high temperatures.

To have an overview of the separation at various temperatures, similar analyses were performed with smaller temperature intervals on a Selerity Blaze C8 column, a silica-based polydentate column, stable at 100 °C (Fig. 2). A similar effect, ascertained with LC–MS, takes place as in Fig. 1. The separations at elevated temperature (100 °C) are better with respect to analysis time, efficiency, and resolution compared to the analysis at 20 °C.

A van't Hoff plot was constructed from the LC–MS analyses of Triton X-100 at various temperatures using the retention factor of the oligomers with an even number of EO units (2–20 U) as data points (Fig. 3A). As expected, the plot is non-linear and different effects play a role in the separation at different temperatures. Therefore, the results

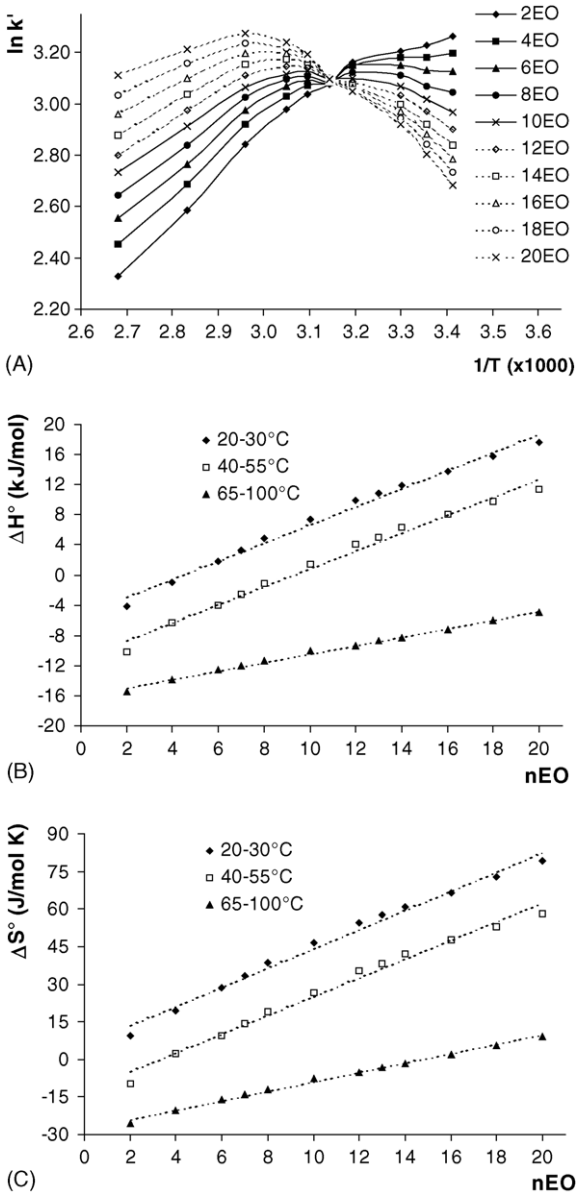


Fig. 3. van't Hoff plots of Triton X-100 (A) and influence of degree of polymerization (*n*EO = number of EO units) on enthalpy (B) and entropy (C) for the different temperature regions. Analytical conditions: see Fig. 2.

were divided in three temperature regions: a low temperature region (20–30 °C), a medium 'transition' temperature region (40–55 °C), and a high temperature region (65–100 °C). The van't Hoff plots show satisfactory linearity in both the low and high temperature region, but linearity is completely lost in the medium temperature region. This is due to the extreme influence on the retention factor of small temperature variations in this 'transition' region. The thermodynamic parameters $\Delta H^\circ$ and $\Delta S^\circ$ for each temperature region were obtained from these three separate van't Hoff plots (Fig. 3B and C).

In the low temperature region $\Delta H^\circ > 0$ and $\Delta S^\circ > 0$, except for the oligomers with short EO chains (*n*EO < 6) which is in accordance with the results of Cho et al. [17]. This means

that the sorption process on the stationary phase in this region is an entropy-driven process that results in an increase of PEO retention as temperature increases. When analyses are carried out in the medium temperature region, $\Delta H^\circ > 0$ for oligomers with 10 or more EO units, but $\Delta H^\circ < 0$ for shorter oligomers. $\Delta S^\circ$ is > 0 in this region. In this situation, enthalpic and entropic effects per EO unit compensate each other and retention is less dependent on EO chain length. Therefore, coelution of the oligomers occurs at a 'transition' temperature, which was 45 °C in this case. In the high temperature region, $\Delta H^\circ < 0$ and $\Delta S^\circ < 0$, except for the oligomers with long EO chains ($n$EO > 15). This implies that the retention process in this region is of a different nature compared to retention at (normal) low temperature. The sorption process is mainly directed by enthalpic effects and this results in the reversal of the elution order.

A significant advantage of the use of high temperature in LC is the reduced viscosity of the mobile phase. Consequently, the pressure drop over the column is decreased and higher flow rates can be applied to reduce analysis time. Efficiency is not sacrificed by the high linear velocity of the mobile phase because the solute transfer between mobile and stationary phase ($C$ term in Van Deemter equation) is greatly enhanced at elevated temperature. In this way, the analysis time could be reduced by a factor of nearly 3 for the analysis of Triton X-100 (Fig. 4) on a Zorbax StableBond C18 column at 90 °C and a flow rate of 1.5 ml/min. Although the injection volume was equal for all analyses, no loss in sensitivity was observed at higher flow rates since the signal-to-noise ratio remained unaffected. Also, efficiency and resolution were not affected by the high flow rate.

Similar results were obtained on a Zorbax StableBond Phenyl column (results not shown). The only difference was that this column provided less retention, and consequently less selectivity compared with the Zorbax StableBond C18 column. To investigate if size-exclusion effects play a role in these analyses, the experiments were repeated on Zorbax StableBond C18 packed with particles with a larger pore size (300 Å versus 80 Å). Since the 300 Å column had a larger internal diameter than the 80 Å column, the flow rate was increased in order to obtain a similar linear velocity of the mobile phase. Although the analysis time was lower on the 300 Å column compared to the 80 Å column, the overall result was similar indicating that size exclusion effects did not play a prominent role in the separation and that oligomeric separation is guided through partitioning between mobile and stationary phase.

Kamiusuki et al. [18] separated octylphenol ethoxylates on branched fluorinated silica gel columns with a methanol–water mobile phase. The oligomers were separated according to increasing number of ethylene oxide units and the selectivity was not affected by temperature changes between 40 and 70 °C. This retention behavior is contradictory to what we observed with acetonitrile–water as mobile phase and experiments were therefore repeated with methanol–water as mobile phase. The content of methanol had to be increased to 70% compared to the 50% acetonitrile to elute the oligomers in a reasonable analysis time. The interaction strength and thus retention indeed increased with increasing oligomer mass and under these conditions the sorption process is an exothermic process ($\Delta H^\circ < 0$). Disorder ($\Delta S^\circ$) is reduced in a protic solvent (methanol) compared to an aprotic solvent (acetonitrile). Rissler et al. [22–24] have investigated the influence of the organic modifier in RPLC on various polyether compounds. Methanol was better to elute samples with a higher hydrophobicity compared to acetoni-

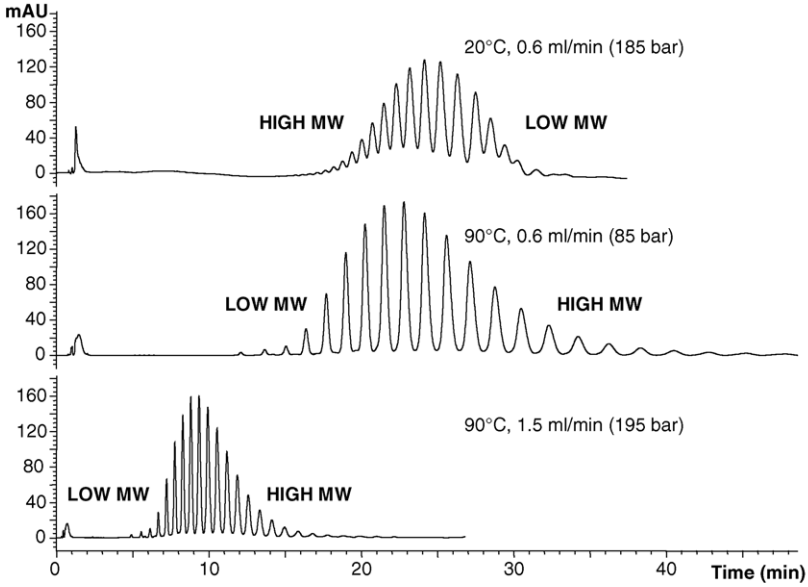


Fig. 4. LC analysis of Triton X-100 (10000 ppm) on a Zorbax StableBond C18 column (150 mm $L$ × 3.0 mm I.D., 3.5 μm particles, 80 Å pore size). Mobile phase: water/ACN, 55/45 (v/v), injection volume: 3 μl, detection: DAD 225 nm.

trile. This was attributed to the improved solvation of the polyether backbone by hydrogen bonding between the ether oxygens and the hydroxyl group of the protic solvent. The difference between methanol and acetonitrile in RPLC of ethoxylated alcohol surfactants has also been described by Jandera et al. [20]. Their findings were that the effect of the acetonitrile concentration in the mobile phase is more complex than the effect of methanol. These results are in agreement with our findings. In fact, RPLC and NPLC (see further) provides the same separation pattern with methanol as mobile phase additive.

### 3.2. RPLC on alternative columns

In an attempt to further explain the temperature dependence on the elution order of the oligomers, several less conventional columns, packed with non silica-based particles, were tested at various temperatures in LC–MS. The results are graphically summarized in Fig. 5. The retention time of three oligomers, namely 4EO (MW 382.4) 10 EO (MW 646.4), and 16 EO (MW 910.4), is plotted versus column temperature. The chromatograms at the highest analysis temperature for each column are represented in Fig. 6.

The first column was a polymeric column packed with polystyrene/divinylbenzene copolymer particles with a pore size of 100 Å (PLRP-S). The analysis was carried out at 20, 50, 90, and 120 °C, respectively with a mobile phase composed of water/ACN, 50/50 (v/v). The results obtained with this column are very similar to the results obtained on silica-based reversed phase columns. At ambient temperature large more hydrophilic oligomers elute before smaller ones and at medium temperature the oligomeric separation disappears. When the analysis temperature is further raised however, the chain length separation reappears but with a reversed elution order. Since this type of column is not silica-based, polar interactions with residual silanol groups can be excluded and the separation thus is guided only by partitioning between mobile and stationary phase.

Secondly, a column packed with zirconium oxide based particles with 300 Å pore size on which a polybutadiene stationary phase is applied (Zirchrom PBD) was used. Analyses were performed at column temperatures of 20, 50, 90, and 120 °C, respectively, with a mobile phase composed of water/ACN, 65/35 (v/v). At 20 °C, all oligomers coelute at a retention time of ca. 11.5 min. When the analytical temperature is increased to 50 °C, an oligomeric separation is starting to take place with low molecular weight oligomers eluting before high molecular weight oligomers. The complete chain length distribution elutes between 7 and 11 min. When increasing the temperature to 120 °C, the separation is further improved and all oligomers elute between 2.2 and 5 min. The chain length distribution separation directed by hydrophobic interaction at ambient temperature does not take place on this column, but a similar separation as on silica-based reversed phase columns is achieved at elevated temperatures. There is also a striking decrease in retention time when the temperature is raised.

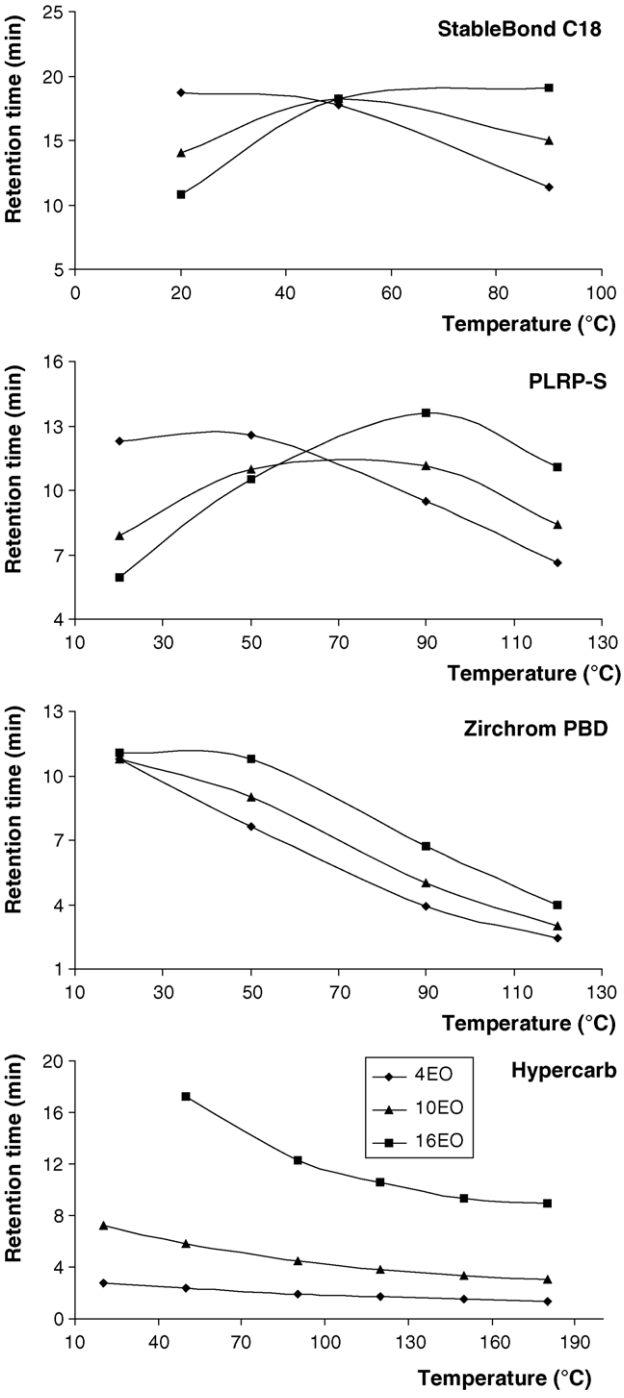


Fig. 5. Comparison of temperature influence on retention time in LC analyses of Triton X-100 (1000 ppm) on various columns at high temperature.

The last column tested was a graphitized carbon column (Hypercarb) packed with particles with a pore size of 250 Å. The pure carbon stationary phase in this column shows a significantly stronger interaction with the alkyl chains of the polymers and a mobile phase composed only of ACN and water was too weak to elute the oligomers. Therefore,

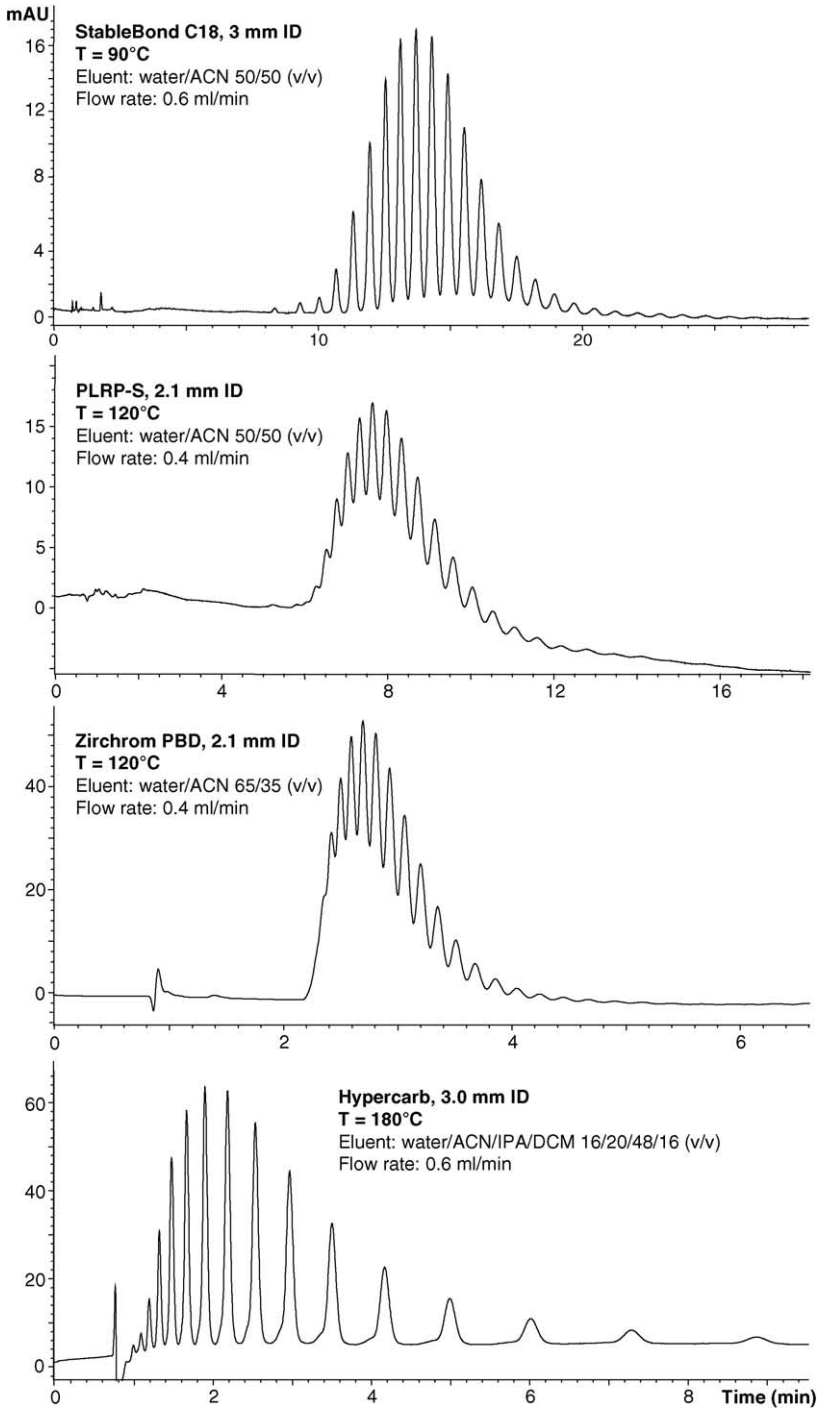


Fig. 6. Comparison of LC analyses of Triton X-100 (1000 ppm) on various columns at high temperature. Detection: DAD 225 nm.

dichloromethane (DCM) and isopropanol (IPA) were added to the mobile phase in amounts that provide satisfactory separation within a reasonable analysis time. The final mobile phase composition was water/ACN/IPA/DCM, 16/20/48/16 (v/v). The main advantage of a Hypercarb column for this type of experiments is its stability at high temperature. Analyses were carried out at 20, 50, 90, 120, 150, and 180 °C. The elution order on this column remains unaffected by temperature. Small oligomers elute before larger ones and it is clear that the chain length separation here is not entropy-driven by hydrophobic interaction. The retention of alkylphenol ethoxylates increases by increased ethylene oxide chain length at any temperature between 20 and 180 °C. This is in contrast with results obtained with any other column in this work. Similar results were described by Chaimbault et al. [25]. The only obvious effect on the separation is the decreased analysis time with increased temperature. Calculations of thermodynamic parameters showed that $\Delta H^\circ < 0$

and $\Delta S^\circ < 0$, meaning that the separation is an enthalpic-driven process. Probably, the significantly different surface of the packing in this column (pure carbon) compared to all other columns tested plays an important role in this separation mechanism. Additionally, the altered composition and water content of the mobile phase for these analyses can also influence selectivity.

### 3.3. NPLC separations

Jandera et al. [20,21,26] have investigated the retention behaviour of oligomers in normal phase. Results showed that the retention behaviour of ethoxylated alkylphenols on a diol and a nitrile column was very much affected by the propanol content of the propanol–*n*-alkane mobile phase [26]. Mixed retention mechanisms were observed that caused a non-linear increase in $\log k'$ with increasing number of EO units.

The Triton X-100 sample was also analyzed in the normal phase mode using a silica column. The mobile phase was composed of water/acetonitrile, 15/85 (v/v). The analysis temperature was varied between 15 and 150 °C. The elution order was not affected by the analytical temperature. The only clear trend is the reduced retention of all oligomers when temperature is increased. Fig. 7 shows the van't Hoff plot for the normal phase analyses. From this plot the enthalpic and entropic parameters were calculated. Both $\Delta H^\circ$ and $\Delta S^\circ$ are negative, thus the retention process is mainly directed by enthalpic effects. Although the linearity of the van't Hoff plot is questionable, it is clear that the reduction of retention time with an increased analysis temperature is monotonous in these analyses. This is in contrast to the reversed phase separations performed on silica-based stationary phases. Moreover, using methanol as co-solvent instead of acetonitrile no changes in selectivity and elution order occurred.

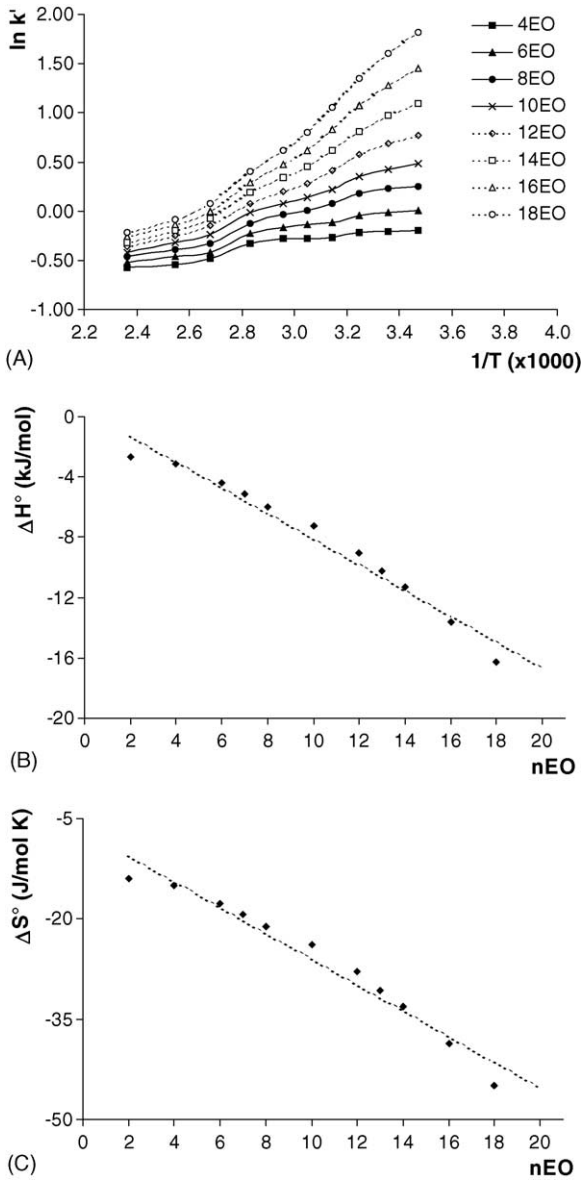


Fig. 7. van't Hoff plots of Triton X-100 (A) and influence of degree of polymerization (*n*EO = number of EO units) on enthalpy (B) and entropy (C). Data were obtained from normal phase LC–MS analyses of Triton X-100 (2000 ppm). Analytical conditions: Ultremex 3 silica column, mobile phase: water/ACN, 15/85 (v/v), flow rate: 0.8 ml/min, injection volume: 3 μl, detection: APCI, positive ionization.

## 4. Conclusions

Significant differences in selectivity were observed when analyzing octylphenol ethoxylate oligomers on silica-based reversed phase LC columns at low (ambient), medium and elevated temperature, respectively, with acetonitrile/water as mobile phase. This observation was evaluated in terms of thermodynamic parameters. The elution order of the oligomers was reversed when comparing ambient and high temperature separations. When a protic solvent such as methanol is used the elution order is reversed compared to acetonitrile at ambient temperature and no reversal in function of temperature is taking place.

With acetonitrile, at low (ambient) temperature the sorption process on the stationary phase is an endothermic entropy-driven process and larger oligomers elute before smaller oligomers. In this temperature region an increase of PEO retention occurs as temperature increases. When analyses are carried out in a intermediate temperature region (40–55 °C), enthalpic and entropic effects per EO unit compensate each other and retention is less dependent on EO chain length. Therefore, coelution of the oligomers occurs at this 'transition' temperature. At high temperatures the sorption process is mainly directed by enthalpic effect. This results in the reversal of the elution order and small oligomers elute before larger oligomers.

This effect was observed on silica-based, a zirconia based, and a polystyrene/divinylbenzene based stationary phase. The sorption process on a graphitized carbon type column appeared to be an enthalpy-driven process, regardless of the analysis temperature. The reason for this discrepancy is not

clear at the present time. In this case, small oligomers elute before larger oligomers. Possibly the nature of the pure carbon surface on the stationary phase plays a key role in this difference. Contrary to most reversed phase separations, the elution order was not affected by the analytical temperature in normal phase LC. The only obvious trend was the reduced retention time of all oligomers at elevated temperature.

## References

[1] O. Chienthavorn, R. Smith, Chromatographia 50 (1999) 485.
[2] T. Pawlowski, C. Poole, Anal. Commun. 36 (1999) 71.
[3] R. Majors, LC–GC 18 (2000) 1214.
[4] S. Fields, C. Ye, D. Zhang, B. Branch, X. Zhang, N. Okafo, J. Chromatogr. A 913 (2001) 197.
[5] A. Premstaller, P. Oefner, LC–GC Eur. 15 (2002) 410.
[6] T. Greibrokk, T. Andersen, J. Chromatogr. A 1000 (2003) 743.
[7] J. Coym, J. Dorsey, J. Chromatogr. A 1035 (2004) 23.
[8] C. Zhu, D. Goodall, S. Wren, LC–GC Eur. 17 (2004) 530.
[9] S. Marin, B. Jones, W. Felix, J. Clark, J. Chromatogr. A 1030 (2004) 255.
[10] J. Thompson, P. Carr, Anal. Chem. 74 (2002) 1017.
[11] H. Pasch, B. Trathnigg, HPLC of Polymers, Springer-Verlag, Berlin, 1998, p. 95.
[12] W.R. Melander, A. Nahum, Cs. Horvath, J. Chromatogr. 185 (1979) 129.
[13] R.E.A. Escott, N. Mortimer, J. Chromatogr. 553 (1991) 423.
[14] C. Lochmüller, M. Moebus, Q. Liu, C. Jiang, M. Elomaa, J. Chromatogr. Sci. 34 (1996) 69.
[15] T. Andersen, P. Molander, R. Trones, D.R. Hegna, T. Greibrokk, J. Chromatogr. A 918 (2001) 221.
[16] D. Cho, S. Park, J. Hong, T. Chang, J. Chromatogr. A 986 (2003) 191.
[17] D. Cho, J. Hong, S. Park, T. Chang, J. Chromatogr. A 986 (2003) 199.
[18] T. Kamiusuki, T. Monde, F. Nemoto, T. Konakahara, Y. Takahashi, J. Chromatogr. A 852 (1999) 475.
[19] K. Evans, S. Dubey, L. Kravetz, T. Dzidic, R. Mueller, J. Stork, Anal. Chem. 68 (1994) 699.
[20] P. Jandera, M. Holcapek, G. Theodoridis, J. Chromatogr. A 813 (1998) 299.
[21] P. Jandera, M. Holcapek, L. Kolarova, J. Chromatogr. A 869 (2000) 65.
[22] K. Rissler, U. Fuchslueger, H. Grether, J. Chromatogr. A 654 (1993) 309.
[23] K. Rissler, J. Chromatogr. A 667 (1994) 167.
[24] K. Rissler, U. Fuchslueger, J. Liq. Chromatogr. 17 (1994) 2791.
[25] P. Chaimbault, C. Elfakir, M. Lafosse, J. Chromatogr. A 797 (1998) 83.
[26] P. Jandera, J. Urbanek, B. Prokes, J. Churacek, J. Chromatogr. 504 (1990) 297.