Journal of Chromatography A, 1217 (2010) 5957–5964

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Evaluation of ternary mobile phases for reversed-phase liquid chromatography: Effect of composition on retention mechanism

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ARTICLE INFO

Article history:
Received 15 February 2010
Received in revised form 1 July 2010
Accepted 22 July 2010
Available online 30 July 2010

Keywords:
Reversed-phase chromatography
Ternary mobile phase
Retention mechanism
van't Hoff
LSER

ABSTRACT

The effect of varying mobile phase composition across a ternary space between two binary compositions is examined, on four different reversed-phase stationary phases. Examined stationary phases included endcapped C8 and C18, as well as a phenyl phase and a C18 phase with an embedded polar group (EPG). Mobile phases consisting of 50% water and various fractions of methanol and acetonitrile were evaluated. Retention thermodynamics are assessed via use of the van't Hoff relationship, and retention mechanism is characterized via LSER analysis, as mobile phase composition was varied from 50/50/0 water/methanol/acetonitrile to 50/0/50 water/methanol acetonitrile. As expected, as the fraction of acetonitrile increases in the mobile phase, retention decreases. In most cases, the driving force for this decrease in retention is a reduction of the enthalpic contribution to retention. The entropic contribution to retention actually increases with acetonitrile content, but not enough to overcome the reduction in the enthalpic contribution. In a similar fashion, as methanol is replaced with acetonitrile, the *v*, *e*, and *a* LSER system constants change to favor elution, while the *s* and *c* constants change to favor retention. The *b* system constant did not show a monotonic change with mobile phase composition. Overall changes in retention across the mobile phase composition range varied, based on the identity of the stationary phase and the composition of the mobile phase.

1. Introduction

1.1. Use of ternary mobile phases

Although binary mobile phases are most commonly used in reversed-phase liquid chromatography, ternary mobile phases can be employed in order to exploit unique chromatographic selectivities. In practice, ternary mobile phases generally consist of water and two mutually miscible organic modifiers. In this work, ternary mobile phase systems consisting of water, methanol, and acetonitrile will be considered, from the point of view of retention mechanism.

While the vast majority of work investigating reversed-phase chromatographic retention mechanisms has focused on binary mobile phases, there are some reports in the literature investigating the use of ternary mobile phases as well. Initial work with ternary mobile phases for RPLC examined the unique selectivities possible when these solvent systems are employed [1–4]. An early work by Schoenmakers et al. [5] systematically examined retention as a function of ternary mobile phase composition, using both water–methanol–acetonitrile and water–methanol–tetrahydrofuran mobile phases. Subsequent work focused on optimization of ternary solvent mixtures, primarily based on retention data from binary mobile phase systems [6,7]. Later work used physicochemical modeling to describe solute retention when ternary mobile phase systems were employed [8–10]. Chemometric [11] and other computer-based modeling techniques [12,13] have also been employed to optimize ternary mobile phases, or to describe their retention properties. More recently, the solvation parameter model has been used to design ternary mobile phases [14,15]. Correlations between retention in binary gradient reversed-phase chromatography and isocratic ternary mobile phase chromatography have also been presented [16,17]. Recent manuscripts of a more fundamental nature examine retention theory using ternary mobile phases [18], as well as sorption isotherms of mobile phase components on the stationary phase when ternary mobile phases are used [19].

Ternary mobile phases have found a variety of applications [20–31]. These include analysis of natural product extracts [20,21], oligomers [22], metabolites [23–25], aromatic hydrocarbons [26], triglycerides [27], and pesticides and pollutants [28–31]. In nearly all these cases, the unique selectivities presented by ternary mobile phase systems allowed for improved separations over their binary counterparts.

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doi:10.1016/j.chroma.2010.07.056

1.2. van't Hoff analysis

The focus of this work is an investigation of the effect of ternary mobile phase composition on retention mechanism. One method by which retention mechanism can be studied is via use of van't Hoff analysis [32–39]. In brief, retention of a solute of interest is measured as a function of temperature. The van't Hoff equation (Eq. (1)) can then be used to determine the thermodynamic values associated with the retention process.

$$\ln k = \frac{-\Delta H^\circ}{RT} + \frac{\Delta S^\circ}{R} + \ln \Phi \quad (1)$$

In this form of the van't Hoff equation, k is the chromatographic retention factor, ΔH° and ΔS° are the standard-state enthalpy and entropy changes associated with the retention process, R is the gas constant, T is the absolute temperature, and Φ is the phase ratio of the chromatographic system (that is, the volume of the stationary phase divided by the volume of the mobile phase). Retention data as a function of temperature can then be plotted as $\ln k$ vs. $1/T$; the slope of such a plot can be used to determine ΔH°; the intercept can be used to find ΔS° if the phase ratio is known.

The use of the van't Hoff relationship in this manner requires one of two assumptions: first, that the standard-state enthalpy change is constant over the temperature range investigated, or that the phase ratio does not vary with temperature. It can be difficult to verify either of these assumptions. If either assumption fails, a non-linear van't Hoff plot may result. Alternatively, a linear plot due to mutually compensating changes in ΔH° and Φ may be produced [36]. However, if the temperature range investigated is modest, the value of ΔH° should be more or less constant and the assumption of constancy valid. The same holds true for the phase ratio: over a modest temperature range, changes in Φ with temperature, which could be due to effects such as stationary phase solvation or conformational change, should be minimal.

An alternate approach, which eliminates the need to make estimations or assumptions on the phase ratio, is to examine the terms of the van't Hoff equation individually. At some given temperature, the first term, $-\Delta H^\circ/RT$, gives the enthalpic contribution to $\ln k$ at that temperature. The second two terms, $\Delta S^\circ/R + \ln \Phi$, provides the entropic contribution. In this context, the phase ratio is considered an entropic term, in that it represents the entropy of dilution associated with transfer of solute from the mobile phase to the stationary phase. Because phase ratios (V_s/V_m) are usually less than 1.0, the $\ln \Phi$ term is negative. However, the overall entropic contribution may still be positive, if the $\Delta S^\circ/R$ term is large. There is no need to make any assumption as to the behavior of this term with temperature. The sum of these two components is $\ln k$. Under isothermal conditions, examining each of these contributions to retention as a chromatographic variable is changed (i.e., mobile phase composition) provides insight as to how the thermodynamic driving force for retention is changing.

1.3. LSER analysis

While thermodynamic analysis of retention via a van't Hoff approach provides information on the driving force for retention, it does not provide information on specific intermolecular interactions that cause retention. This type of data can be obtained by use of a linear solvation energy relationship, or LSER, for retention [38–52]. This model assumes that the overall retention is due to the sum of contributions from various intermolecular interactions (such as dispersive forces, cavity formation, hydrogen bonding, etc.). Solutes participate in these types of interactions in both the stationary and mobile phases; if the interaction is stronger in the mobile phase, the interaction favors elution, if it is stronger in the stationary phase, it favors retention.

Eq. (2) is a typical LSER equation for reversed-phase chromatography.

$$\log k = c + vV + eE + sS + aA + bB \quad (2)$$

In this equation, k is the chromatographic retention factor, V, E, S, A, and B are solute descriptors related to molecular size, excess molar refraction relative to the molar refraction of a similar-sized alkane (this provides a measure of excess polarizability), dipolarity/polarizability, hydrogen bond acidity, and hydrogen bond basicity, respectively [40]. The coefficients c, v, e, s, a, and b, collectively called system constants, describe the chromatographic system's response to solutes participating in that type of interaction [40,41,44]. These values are relative between the stationary and mobile phase, so the sign of a system constant indicates in which phase a given interaction is stronger. A negative system constant indicates that a specific intermolecular interaction is stronger in the mobile phase (and thus favors elution); a positive system constant indicates that the interaction is stronger in the stationary phase, favoring retention. For the a and b system constants, the values refer to the relative basicity and acidity of the system—that is, its ability to interact with acids and bases. The c system constant is related to the phase ratio of the system, as well as any other interactions not accounted for in the other terms. System constants are determined by chromatographing a series of solutes with known solutes descriptors using a fixed set of chromatographic conditions, then regressing the measured retention factors against the solute descriptors to obtain a set of system constants. In examining the effect of a chromatographic variable on retention mechanism, a set of system constants are determined, some variable (such as mobile phase composition or stationary phase type) is changed, and the process repeated. In this manner, the effect of changing a chromatographic variable on a specific type of retention interaction can be observed.

In this work, the effect of ternary mobile phase composition on retention will be assessed on four different stationary phases, using mobile phases consisting of 50% water and 50% organic modifier (methanol + acetonitrile, in various ratios). The effect of changing mobile phase composition on the enthalpic and entropic contributions to overall retention will be examined via a van't Hoff approach. Changes in retention mechanism will be examined by quantifying the difference in sets of LSER system constants between different mobile phase compositions, as well as between different stationary phases across the ternary mobile phase space.

2. Experimental

2.1. Equipment and supplies

All chromatography experiments were performed using a Shimadzu (Columbia, MD, USA) Prominence HPLC system. The system consisted of a model DGU-20A5 mobile phase degasser, a model LC-20AD pump with low-pressure quaternary gradient module, a model SIL-20A autosampler, a model CTO-20AC column oven, and model SPD-20A UV–vis detector. Instrument control and data collection utilized Shimadzu EZStart software, version 7.3.

Four different reversed-phase stationary phases were examined in this work. All were Agilent (Wilmington, DE, USA) Zorbax phases, and included Eclipse XDB-C8, Eclipse XDB-C18, Eclipse XDB-Phenyl, and Bonus-RP, which is an alkyl phase with an embedded polar group (EPG). All columns were 150 mm × 4.6 mm is dimension, with 5 μm silica base particles having a surface area of 180 m^2/g and pore diameter of 80 Å.

Mobile phases consisted of various combinations of water, methanol, and acetonitrile. Water was purified using a Continental Water Systems water purifications systems. Methanol and acetoni-

Table 1
Solute descriptors for use in LSER analysis. Values are from Refs. [47–49].

Solute	*V*	*S*	*A*	*B*	*E*
Benzene	0.7164	0.52	0.00	0.14	0.610
Toluene	0.8573	0.52	0.00	0.14	0.601
Ethylbenzene	0.9982	0.51	0.00	0.15	0.613
Propylbenzene	1.1391	0.50	0.00	0.15	0.604
n-Butylbenzene	1.2800	0.51	0.00	0.15	0.600
Ethyl paraben	1.2722	1.35	0.69	0.45	0.860
Butyl paraben	1.5540	1.35	0.69	0.45	0.860
1,4-Dichlorobenzene	0.9612	0.75	0.00	0.02	0.825
3,4-Dichlorophenol	1.0199	1.14	0.85	0.03	1.020
Acetone	0.5407	0.70	0.04	0.49	0.179
Benzyl alcohol	0.9160	0.87	0.33	0.56	0.803
p-Chlorophenol	0.8975	1.08	0.67	0.20	0.915
Phenol	0.7751	0.89	0.60	0.30	0.805
m-Cresol	0.916	0.88	0.57	0.34	0.822
Theophylline	1.2223	1.60	0.54	1.34	1.500
n-Benzyl formamide	1.1137	1.80	0.40	0.63	0.990
3-Phenyl-1-propanol	1.1978	0.90	0.30	0.67	0.821
Phenyl ethyl alcohol	1.0569	0.91	0.30	0.64	0.811
Acetopheneone	1.0139	1.01	0.00	0.48	0.818
Benzonitrile	0.8711	1.11	0.00	0.33	0.742
Methyl benzoate	1.0726	0.85	0.00	0.46	0.773
Anisole	0.9160	0.75	0.00	0.29	0.708
p-Nitrotoluene	1.0315	1.11	0.00	0.28	0.870
Benzophenone	1.4808	1.50	0.00	0.50	1.447
Bromobenzene	0.8914	0.73	0.00	0.09	0.882
Naphthalene	1.0854	0.92	0.00	0.20	1.340
p-Xylene	0.9982	0.52	0.00	0.16	0.613
Nitrobenzene	0.8906	1.11	0.00	0.28	0.871
Caffeine	1.3632	1.60	0.00	1.33	1.500

trile were ACS grade, obtained from Fisher Scientific (Pittsburgh, PA, USA). For the purposes of this manuscript, mobile phase component "A" is always water, "B" is methanol, and "C" is acetonitrile. Thus, a 50/20/30 mobile phase is 50% water, 20% methanol, and 30% acetonitrile by volume. Test solutes for van't Hoff analysis were acetophenone, 3,4-dichlorophenol, and 1,4-dichlorobenzene. These solutes were selected due to their significantly different behavior in terms of hydrogen bonding, based on their LSER solute descriptors. Acetophenone is a relatively strong hydrogen bond base; 3,4-dichlorophenol is a relatively strong hydrogen bond acid, and 1,4-dichlorobenzne does not have any significant hydrogen boding ability. For the LSER analysis, the test solutes and their solvatochromic descriptors are listed in Table 1.

All test solutes were obtained from Sigma–Aldrich (St. Louis, MO, USA). For both sets of experiments, uracil was used for the void time marker. All test solutions were made up in acetonitrile. An injection volume of 5 μL was used for all chromatographic runs. The flow rate was 1.50 mL/min. Detection was by UV absorbance at 220 and 254 nm. The extracolumn volume of the system was measured and eliminated from all retention calculations.

2.2. Experimental procedure

For use in van't Hoff analysis, temperature dependent retention data were collected at 25, 35, 45, and 55 °C for each of the three van't Hoff test solutes. The mobile phase composition was systematically varied from 50/50/0 water/methanol/acetonitrile to 50/0/50 water/methanol/acetonitrile, in increments of 10% strong solvent. The water content was always kept at 50%. In effect, one mobile phase modifier was replaced with another in a stepwise fashion, while traversing the ternary mobile phase space. Experiments were repeated for each of the four stationary phases. All chromatographic runs were performed in duplicate.

For the LSER analysis, the same mobile phase conditions were used, but the temperature was held constant at 35 °C for all runs. LSER system constants were determined by multivariable linear least squares regression of the retention data against the solute descriptors, using Eq. (2) as the model.

The LINEST function in Microsoft Excel 2003 was used for regression of $\ln k$ vs. $1/T$ (for van't Hoff analysis) as well as for the LSER linear regression. This function returns standard deviations of the regression parameters, which were used to construct the error bars in the figures.

3. Results and discussion

3.1. van't Hoff analysis

Retention thermodynamics were assessed for three solutes: acetophenone, 3,4-dichlorophenol, and 1,4-dichlorobenzene, with each of the four stationary phases over the entire mobile phase range. These solutes were selected due to their significantly different values in LSER solute descriptors, especially with regards to hydrogen bonding. Overall retention at 35 °C, as $\ln k$, was deconvoluted into contributions from enthalpy and entropy, as described in Section 1.2.

Fig. 1 illustrates the effect of mobile phase composition on retention, using the C8 stationary phase. As would be expected, as the acetonitrile content of the mobile phase increases, retention (plotted here as $\ln k$) decreases. In general, the enthalpic contribution to retention (the slope of the van't Hoff plot, divided by the absolute temperature, at a temperature of 35 °C or 308 K) is greater than the overall retention. The entropic contribution, which has contributions both from relative cavity formation in the phases and from the phase volumes, is negative. The sum of these two terms provides the measured retention.

As acetonitrile replaces methanol in the mobile phase, the enthalpic term generally becomes more favorable for *elution*, while then entropic term generally becomes more favorable for *retention*. Because retention decreases with acetonitrile content in the mobile phase, it is clear that the change in the enthalpic contribution to retention is more significant than the change in the entropic term. Stated another way, the decrease in retention as acetonitrile content increases is due to a decrease in the enthalpic contribution, even with a change in the entropic contribution which, taken by itself, should lead to an increase in retention. This can be compared to the results reported by Ranatunga and Carr [35], in which the enthalpic and entropic contributions to retention were reported for binary water/acetonitrile mobile phases. In their work, the enthalpic contribution to retention decreased as *water* was replaced by acetonitrile; in this work, we observe a decrease in the enthalpic contribution to retention as *methanol* is replaced by acetonitrile. Different behavior is observed, however, with regards to the entropic contribution to retention. In Ranatunga and Carr's work, as acetonitrile replaces water, the entropic contribution becomes more negative, indicating an entropic shift favoring elution, while in this work, the change in the entropic contribution to retention as acetonitrile replaces methanol is positive, favoring retention. In summary, for binary water/acetonitrile mobile phases, as acetonitrile replaces water, both the enthalpic and entropoic contributions to retention change to favor elution [35], but in ternary mobile phases with a constant water content, as acetonitrile replaces methanol, the enthalpic contribution to retention shifts to favor elution while the entropic contribution shifts to favor retention.

The same general trends are observed for the other three stationary phases, with only a few exceptions. Figs. 2–4 show retention data on the C18, embedded polar group, and phenyl phases, respectively. One exception to this behavior occurs for acetophenone on the C18 phase, for which the changes in thermodynamic value did not appear to follow a trend (although the overall retention did

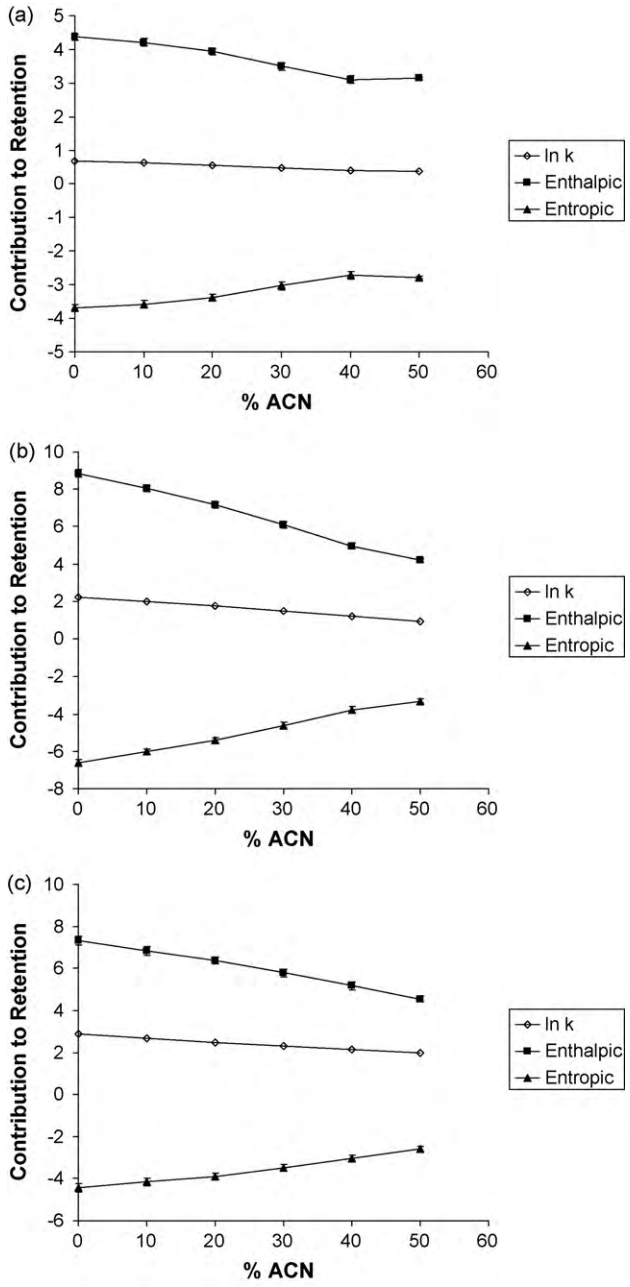

Fig. 1. Overall retention (as ln k), and enthalpic and entropic contributions to retention, for (a) acetophenone; (b) 3,4-dichlorophenol; and (c) 1,4-dichlorobenzene on the Zorbax Eclipse XDB C8 stationary phase. All mobile phases were 50% water, with the remainder being some combination of methanol and acetonitrile.

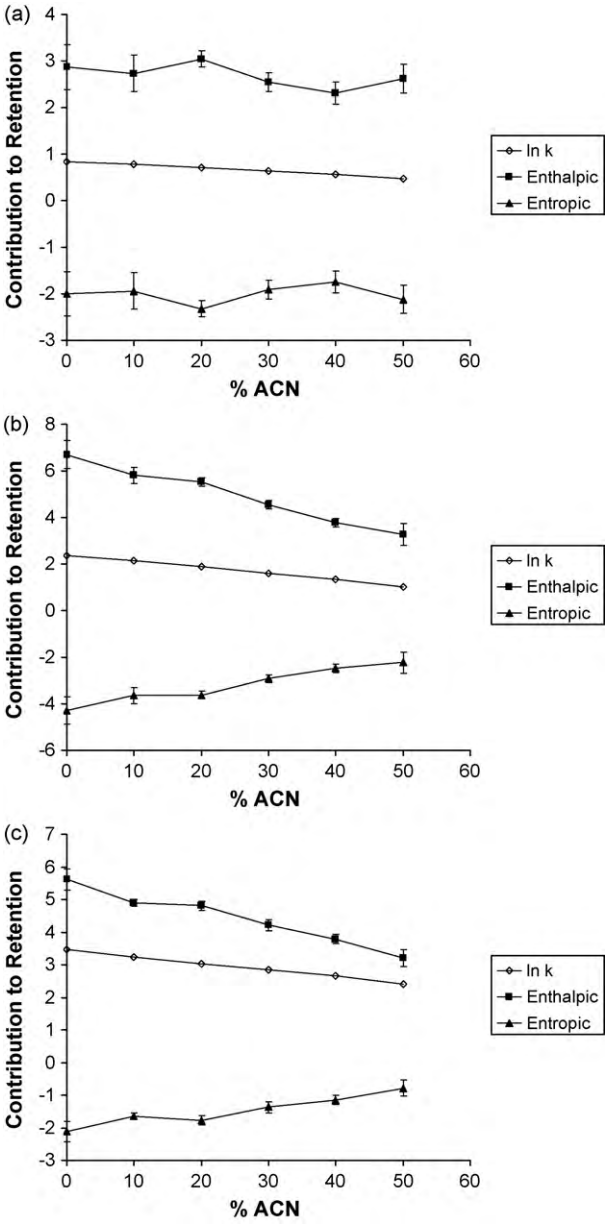

Fig. 2. Overall retention (as ln k), and enthalpic and entropic contributions to retention, for (a) acetophenone; (b) 3,4-dichlorophenol; and (c) 1,4-dichlorobenzene on the Zorbax Eclipse XDB C18 stationary phase. All mobile phases were 50% water, with the remainder being some combination of methanol and acetonitrile.

decrease with the addition of acetonitrile to the mobile phase). One other interesting point can be noted when acetophenone is used as a test solute. When the mobile phase composition changes form 50/40/10 to 50/0/50 (that is, the last step, when methanol is removed from the mobile phase), the enthalpic contribution to retention increases, while the entropic contribution to retention decreases. This is in contrast to the general trend of the enthalpic contribution to retention decreasing while the entropic contribution increases as the acetonitrile content increases.

Of primary interest is how the overall retention changes over the ternary mobile phase space. That is, are the stepwise changes in retention similar in size over the ternary phase space, or do the size of the steps change as a function of mobile phase composition? This can be investigated by examining the incremental changes in these values at each mobile phase composition. Table 2 lists the incremental changes in retention the three solutes, on each of the four stationary phases. In many cases, the size of the retention "steps" across the ternary mobile phase space changes in a monotonic fashion. The embedded polar group and phenyl phases are most predictable in this regard. For all three solutes on these phases, the retention steps decrease in size as methanol is removed and acetonitrile is added. As a result, the largest changes in retention occur with higher amounts of methanol in the ternary mobile phase. However, on the alkyl (C8 and C18) stationary phases, this trend of decreasing step size with increasing acetonitrile content is only observed for 1,4-dichlorobenzene. The other two solutes exhibit different behavior. For acetophenone on the alkyl phases, no trends are seen in the step size as mobile phase composition is varied. When 3,4-dichlorophenol is examined, the steps in retention generally increase in size with increasing acetonitrile content.

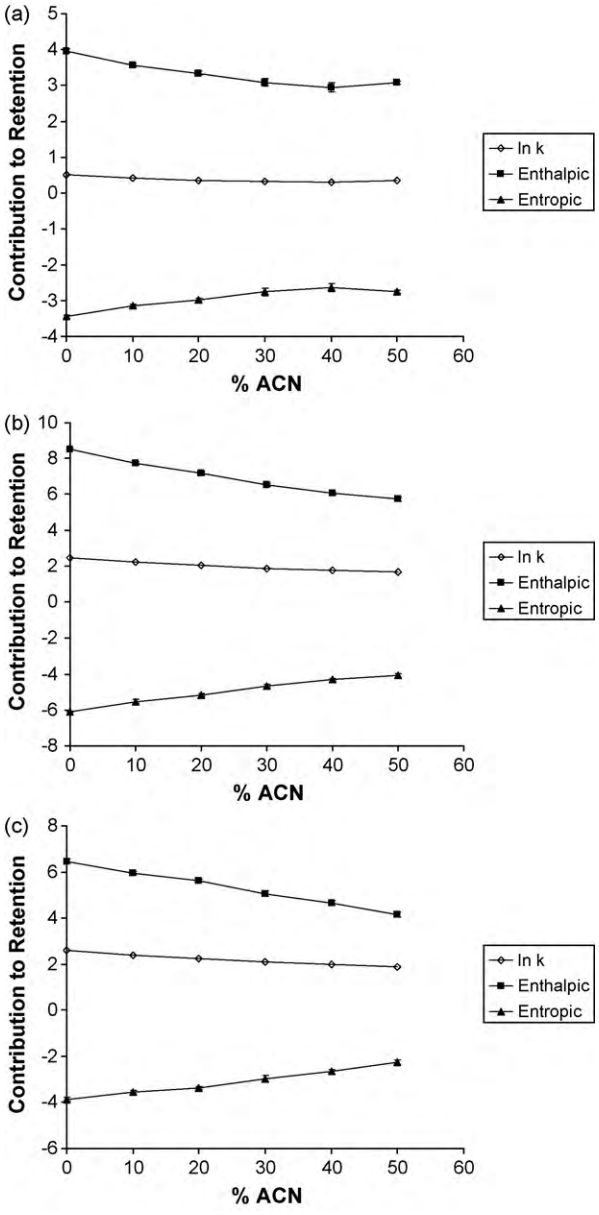

Fig. 3. Overall retention (as ln k), and enthalpic and entropic contributions to retention, for (a) acetophenone; (b) 3,4-dichlorophenol; and (c) 1,4-dichlorobenzene on the Zorbax Bonus-RP (embedded polar group) stationary phase. All mobile phases were 50% water, with the remainder being some combination of methanol and acetonitrile.

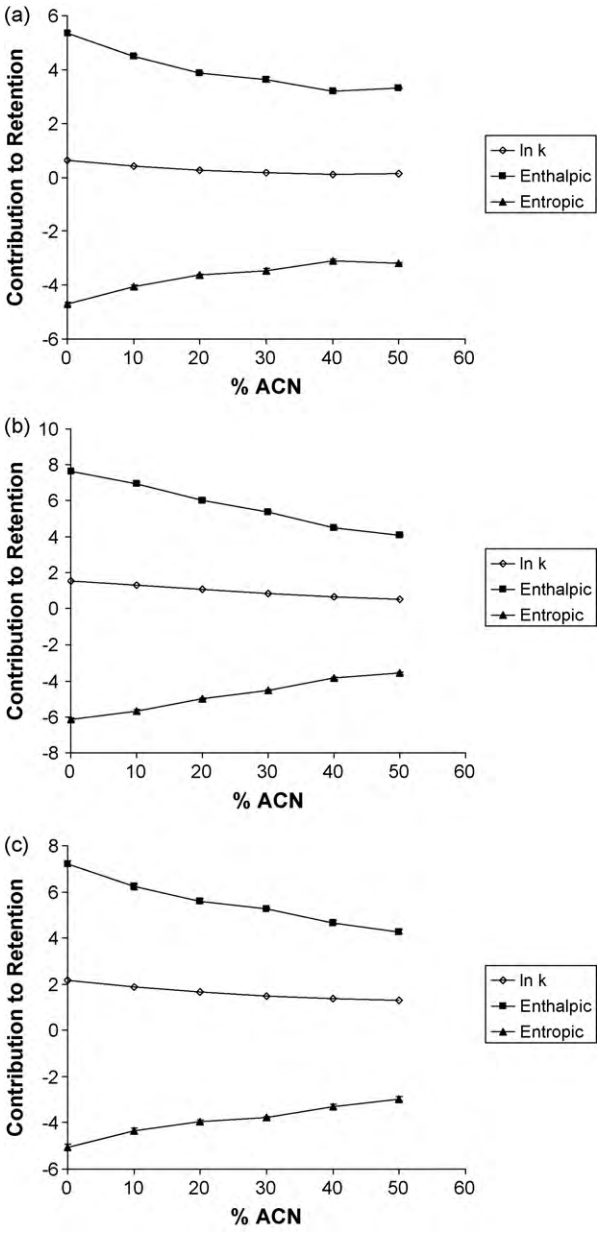

Fig. 4. Overall retention (as ln k), and enthalpic and entropic contributions to retention, for (a) acetophenone; (b) 3,4-dichlorophenol; and (c) 1,4-dichlorobenzene on the Zorbax Eclipse XDB phenyl stationary phase. All mobile phases were 50% water, with the remainder being some combination of methanol and acetonitrile.

The one exception is on the C8 stationary phase, when methanol is removed from the mobile phase.

3.2. LSER analysis

While van't Hoff analysis allows elucidation of the thermodynamic driving force for retention, it does not provide specific indication of the types of intermolecular interactions occurring between the analyte, mobile phase, and stationary phase. This can be determined by the use of LSER analysis, as detailed in Section 1.3. LSER analysis was performed using the same stationary and mobile phase combinations as used for van't Hoff analysis. The changes in LSER system constants, which describe a chromatographic system's response to a specific solute interaction, are shown in Figs. 5–7.

For all four stationary phases examined, there were some general trends seen in the changes of the LSER system constants as methanol is replaced by acetonitrile. In all cases, the v, e, and a system constants, which represent cavity formation, excess polarizability, and relative basicity of the chromatographic phases, trend negative as methanol is replaced by acetonitrile. This is illustrated in Fig. 5. Since, in general, acetonitrile is a "stronger" mobile phase modifier than methanol, it is these interactions which lead to decreased retention as the fraction of acetonitrile increases. These trends easily explained based on the types of intermolecular interaction possible with methanol and acetonitrile. The cavity formation term, v, consists of contributions from dispersive interactions between solute and the mobile or stationary phase, and from the ease with which a cavity is formed in a given phase. It is much easier to form a cavity in an acetonitrile-containing mobile phase than one containing methanol, because of the reduction in hydrogen bonding between the molecules of the solvent. The decrease in the e term, which reflects excess polarizability, is due to the

Table 2
Incremental change in the $\ln k$ for acetophenone, 3,4-dichlorophenol, and 1,4-dichlorobenzene as the ternary mobile phase space is traversed. Column 1 indicates the fraction of acetonitrile in the two mobile phase compositions compared, for example, "10 → 20" indicates a comparison of 50/40/10 and 50/30/20 H_2O/MeOH/ACN.

	C8	C18	EPG	Phenyl
Acetophenone				
0 → 10	−0.055	−0.057	−0.097	−0.221
10 → 20	−0.074	−0.074	−0.064	−0.159
20 → 30	−0.085	−0.083	−0.034	−0.105
30 → 40	−0.078	−0.060	−0.018	−0.050
40 → 50	−0.030	−0.095	+0.035	+0.016
3,4-Dichlorophenol				
0 → 10	−0.226	−0.232	−0.230	−0.229
10 → 20	−0.250	−0.242	−0.187	−0.239
20 → 30	−0.277	−0.282	−0.152	−0.219
30 → 40	−0.294	−0.282	−0.128	−0.186
40 → 50	−0.258	−0.315	−0.091	−0.133
1,4-Dichlorobenzene				
0 → 10	−0.200	−0.235	−0.207	−0.304
10 → 20	−0.186	−0.207	−0.166	−0.212
20 → 30	−0.172	−0.197	−0.132	−0.157
30 → 40	−0.170	−0.179	−0.118	−0.115
40 → 50	−0.169	−0.239	−0.113	−0.081

presence of the carbon–nitrogen triple bond in acetonitrile. The final term which changes to favor elution is the *a* system constant, or the relative basicity of the stationary and mobile phases. Both methanol and acetonitrile have lone pairs of electrons that may interact with hydrogen bond acids. When methanol is present, however, its hydrogen bond acidity may "block" this interaction from occurring with a solute. As it is removed from the mobile phase, more hydrogen-bond base sites become available, increasing the relative basicity of the mobile phase, leading to a reduction in the *a* system constant.

As seen in Fig. 6, two of the system constants actually seem to favor an increase in retention as the mobile phase organic modifier changes from methanol to acetonitrile. These are the *s* term, which represents polar interactions, and the *c* term, which contains contributions form interaction not assessed in the other system constants, as well as from the phase ratio of the system. Methanol is a more polar solvent overall than acetonitrile, so this trend in the *s* system constant is expected. Changes in the *c* term are more difficult to explain, but one factor may be mobile phase adsorption into or onto the stationary phase. In general, acetonitrile adsorbs onto the stationary phase to a much greater extent than methanol [19]; this would have the effect of increasing the stationary phase volume if this adsorbed solvent were taken to be part of the stationary phase. This would in turn increase the effective phase ratio, and thus the *c* system constant.

The one system constant that does not change monotonically as methanol is replaced with acetonitrile is the *b* term, which represents the relative acidity of the stationary and mobile phases. This is illustrated in Fig. 7. In fact, when compared to the other system constants, this term does not change much at all. However, there does seem to be a slight minimum in *b*, generally around 30% acetonitrile. This suggests that the replacement of methanol with acetonitrile affects the relative acidity of both the mobile and stationary phases; it does not just affect one or the other.

The effect of ternary mobile phase composition on overall retention mechanism can be determined from examination of the LSER system constants. Of particular interest is how these values change as the ternary mobile phase space is traversed. An overall difference in the retention mechanism can be quantified by treating the system constants as elements of a vector, and determining the angle between two vectors representing two different conditions. This type of analysis was introduced by Ishihama and Asakawa [53] and

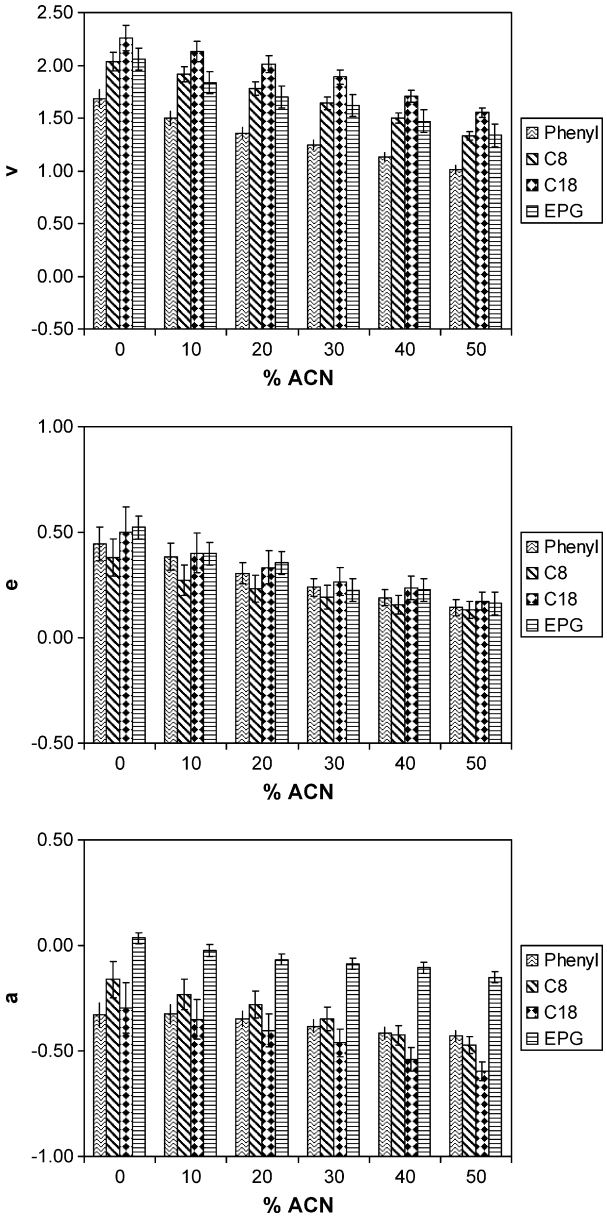

Fig. 5. Values of the *v*, *e*, and *a* system constants as a function of mobile phase composition, on the four stationary phases examined. In general, these constants changed to favor elution as methanol was replaced with acetonitrile as organic modifier in the mobile phase.

has subsequently been used by several other authors [50–52] to quantify differences between two different sets of chromatographic conditions.

In brief, the cosine of an angle between two vectors is determined by dividing the dot product of the vectors by the products of their lengths:

$$\cos \Theta = \frac{\mathbf{ab}}{\|\mathbf{a}\| \|\mathbf{b}\|} \quad (3)$$

In this equation, **a** and **b** are vectors, and Θ is the angle between them. This quantity can be directly used, or it can be converted to an angle between the two vectors. In this work the quantity will be reported as an angle, so smaller values are indicative of more similar systems, and larger value indicate systems which are more different.

When LSER system constants are compared, the *e*, *s*, *v*, *a*, and *b* terms are used. The *c* term is not used, to force the vectors through

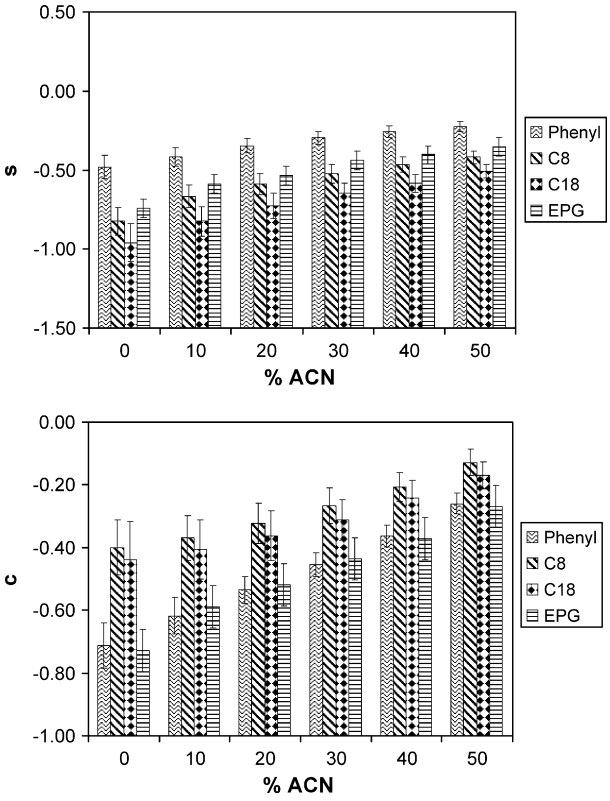

Fig. 6. Values of the *s* and *c* system constants as a function of mobile phase composition, on the four stationary phases examined. In general, these constants changed to favor retention as methanol was replaced with acetonitrile as organic modifier in the mobile phase.

the origin of a five-dimensional space [51]. The vectors **a** and **b** in Eq. (3) are the LSER system constants under two different sets of chromatographic conditions. Using this method, the amount of dissimilarity between two different sets of conditions can be quantified.

Differences in retention mechanism across the ternary phase space can be compared by finding the angle between the LSER vectors for two adjacent sets of conditions. In this way, it can be determined which steps produce more significant changes in the chromatographic system, and which steps are produce less significant changes. The angles between the steps for each of the four phases are shown in Table 3. With all four stationary phases, the step differences were generally small, with angles between the LSER vectors of between 1° and 5°. On the phenyl phase, the

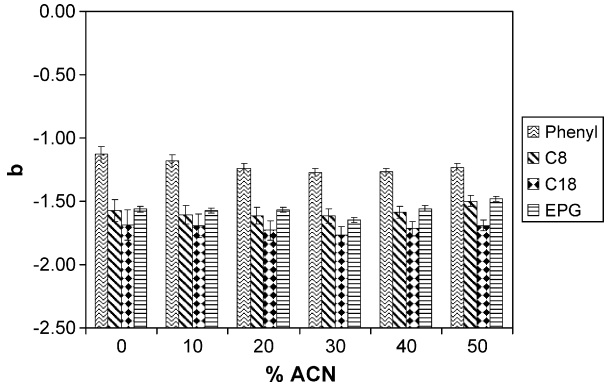

Fig. 7. Values of the *b* system constant as a function of mobile phase composition, on the four stationary phases examined. In general, the *s* constant did not vary appreciably with mobile phase composition.

Table 3
Angles between the LSER vectors across the ternary mobile phase space, on the four different stationary phases.

	C8	C18	EPG	Phenyl
Mobile phase change				
0 → 10	4.42°	3.19°	4.59°	4.46°
10 → 20	2.81°	3.09°	2.43°	4.68°
20 → 30	3.22°	3.19°	4.68°	4.01°
30 → 40	3.27°	3.12°	1.36°	3.47°
40 → 50	2.78°	3.39°	2.44°	2.85°

Table 4
Angles between the LSER vectors between different stationary phases, across the ternary mobile phase space. Mobile phase compositions are reported as water/methanol/acetonitrile.

Column pair	50/50/0	50/40/10	50/30/20	50/20/30	50/10/40	50/0/50
C8–C18	3.0°	3.7°	3.2°	2.6°	2.9°	5.6°
C8–phenyl	8.7°	7.3°	6.4°	5.7°	5.1°	10.6°
C8–EPG	5.4°	5.7°	5.8°	6.7°	8.3°	12.1°
C18–phenyl	7.2°	5.9°	5.7°	5.8°	5.9°	11.1°
C18–EPG	7.1°	7.5°	7.5°	8.7°	9.9°	13.3°
EPG–phenyl	10.4°	9.3°	9.1°	10.1°	11.1°	16.0°

step size – that is, the angle between two adjacent LSER vectors – decreased as the amount of acetonitrile increased. Stated another way, on the phenyl phase, retention mechanism changed more rapidly when acetonitrile was first introduced as compared to when it was removed.

The other three phases did not seem show any distinct trends in step size across the ternary mobile phase space. For the C8 phase, the largest step was when acetonitrile was first introduced, but subsequent steps were similar in size. The C18 phase had consistent step sizes across the entire mobile phase range. The step sized on the EPG phase were more variable, perhaps due to interaction of the various mobile phases with the embedded polar group.

A second comparison can be made between the LSER vectors of two different stationary phases at a common mobile phase composition. This allows for determination of which ternary mobile phase compositions allow for greater differences in stationary phase selectivity. This data is shown in Table 4. Several trends from this data can be described. For every pair of phases compared, the largest difference is LSER vectors is seen with the 50/0/50 H_2O/MeOH/ACN mobile phase. This suggests that these chromatographic systems act "most different" with this mobile phase. Trends in phase similarity can also be observed for each of the stationary phase pairs. The C8 and C18 phases behave in the most similar fashion across the ternary mobile phase space. This is not unexpected, as both are traditional alkyl phases. When the C8 and phenyl phases are compared, the angles between the LSER vectors gets smaller as acetonitrile is added, until the last step, when methanol is removed from the system. This contrasts with a comparison of the C18 and phenyl phases, for which the step size remains more or less constant (except for the last step). When the EPG phase is compared with either of the alkyl phases, the steps get larger across the mobile phase space. However, this trend does not hold when the EPG and phenyl phases are compared. In this case, the steps are more-or-less the same size.

4. Conclusions

Retention mechanism, as determined thermodynamically via van't Hoff analysis or specifically via LSER analysis, seems to change in a monotonic and predictable manner as mobile phase composition is changed from 50/50/0 water/methanol/acetonitrile to 50/0/50 water/methanol/acetonitrile. Thermodynamic analysis shows that the decrease in retention as acetonitrile becomes the

dominant organic solvent is due to enthalpic effects, which overrule entropic effects that would, on their own, suggest an increase in retention as the amount of acetonitrile is increased. In terms of an LSER model, the decrease in retention as acetonitrile content increases is due to changes in the cavity formation, excess polarizability, and relative basicity (v, e, and a) terms. This overrules changes in other terms that suggest increases of retention with acetonitrile.

As the ternary mobile phase space is traversed, vectors of LSER system constants can be used to quantify change in retention mechanism. When the phenyl stationary phase is used, these changes become larger as the amount of acetonitrile in the mobile phase increases. This trend is not seen on the three alkyl phases, including the one containing an embedded polar group. Differences in LSER constants between different stationary phases at a given mobile phase composition were also examined. The inclusion of methanol in the mobile phase reduced the difference in LSER vectors between two stationary phases. In other words, greater differences in selectivity due to the identity of the stationary phase were seen with binary water/acetonitrile mobile phases, when compared to ternary mobile phases or water/methanol binary mobile phases.

Acknowledgements

This work was supported by the College of Arts and Sciences of the University of South Alabama. JWC would like to thank Dr. Thomas Chester of the University of Cincinnati for thoughtful and insightful comments during the revision of this manuscript, and Masanari Fujikawa for assistance with initial data collection.

References

[1] N. Tanaka, H. Goodell, B.L. Karger, J. Chromatogr. 158 (1978) 233.
[2] J.L. Glajch, J.J. Kirkland, K.M. Squire, J.M. Minor, J. Chromatogr. 199 (1980) 57.
[3] E. Roggendorf, R. Spatz, J. Chromatogr. 204 (1981) 263.
[4] P. Jandera, J. Chromatogr. 352 (1986) 111.
[5] P.J. Schoenmakers, H.A.H. Billiet, L. De Galen, J. Chromatogr. 218 (1981) 261.
[6] P.J. Schoenmakers, A.C.J.H. Drouen, H.A.H. Billiet, L. De Galen, Chromatographia 15 (1982) 688.
[7] P. Jandera, H. Colin, G. Guiochon, Chromatographia 16 (1982) 132.
[8] C.H. Lochmuller, M.A. Hamzavi-Abedi, C. Ou, J. Chromatogr. 387 (1987) 105.
[9] T. Kowalska, Chromatographia 28 (1989) 354.
[10] T. Kowalska, Chromatographia 29 (1990) 389.
[11] P.M.J. Coenegracht, H.J. Metting, A.K. Smilde, P.J.M. Coenegracht-Lamers, Chromatographia 27 (1989) 135.
[12] P. Chaminade, A. Baillet, D. Bayloq-Ferrier, Analusis 22 (1994) 55.
[13] S. Heinisch, P. Riviere, J.L. Rocca, Chromatographia 39 (1994) 216.
[14] D. Bolliet, C.F. Poole, Anal. Commun. 35 (1998) 253.
[15] W. Kiridena, C.F. Poole, Chromatographia 48 (1998) 607.
[16] J.W. Dolan, L.R. Snyder, N.M. Djordjevic, D.W. Hill, T.J. Waeghe, J. Chromatogr. A 857 (1999) 1.
[17] A. Pappa-Louisi, P. Nikitas, M. Karageorgaki, J. Chromatogr. A 1091 (2005) 21.
[18] M. Borowko, B. Oscik-Mendyk, P. Borowko, J. Phys. Chem. B 109 (2005) 21056.
[19] M. Wang, J. Mallette, J.F. Parcher, Anal. Chem. 61 (2009) 984.
[20] F. Delaroza, I.S. Scarminio, J. Sep. Sci. 31 (2008) 1034.
[21] G. Pages, M. Mazarin, M. Sergent, R. Phan-Tan-Luu, Anal. Bioanal. Chem. 385 (2006) 716.
[22] K. Rissler, Chromatographia 51 (2000) 656.
[23] M. Rozbeh, R.J. Hurtubise, J. Liq. Chromatogr. 17 (1994) 33351.
[24] J.L. Edwards, R.L. Edwards, K.R. Reid, R.T. Kennedy, J. Chromatogr. A 1172 (2007) 127.
[25] C. Danel, C. Barthelemy, D. Azarzar, H. Robert, J.-P. Bonte, P. Odou, C. Vaccher, J. Chromatogr. A 1163 (2007) 228.
[26] T.H. Dzido, H. Engelhardt, Chromatographia 39 (1994) 67.
[27] S. Heron, A. Tchapla, Analusis 22 (1994) 114.
[28] P. Jandera, B. Prokes, J. Liq. Chromatogr. 14 (1991) 3125.
[29] C.W. Henry, M.E. McCarroll, I.M. Warner, J. Chromatogr. A 905 (2001) 319.
[30] O. Jauregui, E. Moyano, M.T. Galceran, J. Chromatogr. A 823 (1998) 241.
[31] J.L. Martinezvidal, P. Parrilla, A.R. Fernandezalba, R. Carreno, F. Herrera, J. Liq. Chromatogr. 18 (1995) 2969.
[32] L.A. Cole, J.G. Dorsey, Anal. Chem. 64 (1992) 1317.
[33] L.A. Cole, J.G. Dorsey, Anal. Chem. 64 (1992) 1324.
[34] C.S. Lee, W.J. Cheong, J. Chromatogr. A 848 (1999) 9.
[35] R.P.J. Ranatunga, P.W. Carr, Anal. Chem. 72 (2000) 5679.
[36] T.L. Chester, J.W. Coym, J. Chromatogr. A 1003 (2003) 101.
[37] J.W. Coym, J.G. Dorsey, J. Chromatogr. A 1035 (2004) 23.
[38] J.W. Coym, J. Sep. Sci. 31 (2008) 1712.
[39] S.D. Allmon, J.G. Dorsey, J. Chromatogr. A 1216 (2009) 5106.
[40] M.H. Abraham, A. Ibrahim, A.M. Zissimos, J. Chromatogr. A 1037 (2004) 29.
[41] C.F. Poole, The Essence of Chromatography, Elsevier, Amsterdam, 2003.
[42] J.A. Blackwell, P.W. Carr, HRC J. High Resolut. Chromatogr. 21 (1998) 427.
[43] A.S. Wang, P.W. Carr, J. Chromatogr. A 965 (2002) 3.
[44] M. Vitha, P.W. Carr, J. Chromatogr. A 1126 (2006) 143.
[45] D.S. Van Meter, N.J. Oliver, A.B. Carle, S. Dehm, T.H. Ridgeway, A.M. Stalcup, Anal. Bioanal. Chem. 393 (2009) 283.
[46] Y. Sun, A.M. Stalcup, J. Chromatogr. A 1126 (2006) 276.
[47] J. Zhao, P.W. Carr, Anal. Chem. 71 (1999) 2623.
[48] A. Wang, L.C. Tan, P.W. Carr, J. Chromatogr. A 848 (1999) 21.
[49] A. Berthod, C.R. Mitchell, D.W. Armstrong, J. Chromatogr. A 1166 (2007) 61.
[50] S.K. Poole, C.F. Poole, J. Sep. Sci. 31 (2008) 1118.
[51] C. West, E. Lesellier, J. Chromatogr. A 1191 (2008) 21.
[52] C. West, E. Lesellier, J. Chromatogr. A 1203 (2008) 105.
[53] Y. Ishihama, N. Asakawa, J. Pharm. Sci. 88 (1999) 1305.