

Retention prediction in ternary solvent reversed-phase liquid chromatography systems based on the variation of retention with binary mobile phase composition

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Abstract

An extension of the treatment adopted in a recent paper [P. Nikitas, A. Pappa-Louisi, P. Agrafiotou, *J. Chromatogr. A* 946 (2002) 33] was used to derive expressions describing the variation of solute retention k with composition in ternary reversed phase liquid chromatography, RP-LC, solvent systems. The equation of the partition model obtained in this way for a ternary mobile phase was identical to that previously derived using the solubility parameter concept. This equation as well as two new expressions of $\ln k$ versus organic modifiers content were tested in a variety of ternary solvent systems in order to examine the possibility of predicting retention behavior of solutes under ternary solvent mixture elution conditions from known retention characteristics in binary mobile phases. It was demonstrated the superiority of both new equations derived in this paper to that previously proposed and applied to date in ternary solvent mixtures.

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1. Introduction

Optimization of the mobile phase composition is an important aspect of method development in HPLC and as the complexity of separations increases the need of optimized procedures based on the existence of theoretical or empirical models of the dependence of retention on composition in mobile phases also increases. Today is generally accepted that RP-LC analyses poorly performed by using isocratic elutions in binary solvent mixtures may be enhanced by isocratically running ternary solvents as well as by using binary or ternary gradients. The main purpose of using ternary or even more complex mobile phases in liquid chromatography is their larger effects on the selectivity of separations than in binary solvent systems [1–4]. However, little attention has been paid to HPLC isocratic methods for the separation of solute complex mixtures with ternary mobile phases, even though the

optimization is easier than gradient methods [5–11]. In a previous paper, both empirical and theoretical equations proposed to describe the dependence of reversed-phase retention, k , on organic modifiers in ternary and higher-order solvent systems were reviewed and compared. It was concluded that no one of these expressions describes adequately the retention in such eluent systems [4]. Consequently, it is still desirable, according to our knowledge, to more accurately model solute retention in multi-component solvent systems.

For this reason, in this paper the treatment recently proposed in [12,13] is extended to mobile phases that contain more than one organic modifier and two simplified expressions for the retention description in ternary mobile phases based on either the adsorption or partition mechanism are derived. Additionally two simple three-parameters equations that have been shown to describe satisfactorily retention in binary mobile phases [14] are adequately extended to ternary mobile phases and their applicability is tested in a variety of ternary eluents. However, the main goal of the present paper is not to investigate if the new derived equations can describe the

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retention in ternary mobile phases but to explore the possibility of using these equations for prediction retention in ternary mobile phases from a minimum number of chromatographic data obtained in binary mobile phases, taking into account that ternary solvents provide a smooth transition between two limiting binary mixtures.

2. Theoretical part

In recent papers we have presented a new method to develop expressions for the retention factor k in terms of the concentration of a single organic modifier in the mobile phase [12,13]. This method is adopted to study the effect of ternary mobile phases on k . The extension to quaternary mobile phases and in general to m organic modifiers is straightforward and it will be presented in a future communication.

2.1. Thermodynamic treatment for $\ln k$

The fundamental equation of liquid chromatography may be expressed as [13]:

$$\ln k = \ln k^* + \lim_{\varphi_A \rightarrow 0} \ln \frac{\varphi_A^s}{\varphi_A^m} \quad (1)$$

where φ_A^m , φ_A^s are the volume fractions of the solute A in the mobile phase (m) and in/on the stationary phase (s), respectively. This equation is valid independently of the mobile phase composition and the retention mechanism. However, the ratio φ_A^s/φ_A^m depends on both these two factors.

In particular, consider that the mobile phase is a ternary mixture consisting of water S and two organic modifiers, B and C. If the retention mechanism is due to partition, an equilibrium $A^s \Leftrightarrow A^m$ of the solute molecules between the stationary and the mobile phase is established and the ratio φ_A^s/φ_A^m may be determined from the following equation

$$\ln \frac{\varphi_A^s}{\varphi_A^m} + \ln \frac{f_A^s}{f_A^m} = \ln \beta \quad (2)$$

which comes directly from the equilibrium process $A^s \Leftrightarrow A^m$. Here, β is the thermodynamic equilibrium constant of this process and f_A^s , f_A^m are the activity coefficients of the analyte in the stationary and mobile phase, respectively.

If the retention mechanism is due to adsorption, then, to a first approximation, the equilibrium processes may be represented as [12,13] $A^m + S^s \Leftrightarrow A^s + S^m$, $B^m + S^s \Leftrightarrow B^s + S^m$, $C^m + S^s \Leftrightarrow C^s + S^m$, which readily yield the following system of adsorption isotherms:

$$\ln \frac{\varphi_A^s}{1 - \varphi_B^s - \varphi_C^s} = \ln \beta_A - \ln \frac{f_A^s}{f_S^s} + \ln \frac{f_A^m}{f_S^m} + \ln \frac{\varphi_A^m}{1 - \varphi_B^m - \varphi_C^m} \quad (3)$$

$$\ln \frac{\varphi_j^s}{1 - \varphi_B^s - \varphi_C^s} = \ln \beta_j - \ln \frac{f_j^s}{f_S^s} + \ln \frac{f_j^m}{f_S^m} + \ln \frac{\varphi_j^m}{1 - \varphi_B^m - \varphi_C^m}, \quad j = B \text{ or } C \quad (4)$$

The ratio φ_A^s/φ_A^m can be obtained from Eq. (3), but this ratio may be expressed analytically in terms of the mobile phase composition only if the adsorption isotherms represented by Eq. (4) can be solved with respect to φ_B^s and φ_C^s . This is achieved if we approximately assume that $f_i^s = f_i^m = 1$, $i = B, C, S$. Then Eq. (4) yields the Langmuir type isotherms

$$\varphi_j^s = \frac{b_j \varphi_j^m}{1 + b_B \varphi_B^m + b_C \varphi_C^m}, \quad j = B \text{ or } C \quad (5)$$

where $b_j = \beta_j - 1$. Note that these isotherms are thermodynamically consistent only if the stationary phase has approximately equal adsorption capacities for the two solvents of the mobile phase. For strong polar solvents, as in our case, this is a reasonable approximation [12,13].

It is seen that the ratio φ_A^s/φ_A^m and therefore the final expressions of $\ln k$ depend on the expressions of the activity coefficients f_A and f_S in the two phases present in the column. The activity coefficients may be determined by extending properly the method proposed in [13]. Thus if G^e is the excess free energy of a quaternary mixture, which may be either the mobile phase or the adsorbed layer on the hydrocarbon chains of the stationary phase, we may write it as a sum of six terms of the form $A_i \varphi_j \varphi_k$ ($A_i^m \varphi_j^m \varphi_k^m$ or $A_i^s \varphi_j^s \varphi_k^s$ depending on whether the mixture is formed in the mobile or on the stationary phase), where $i = 1, 2, \dots, 6$, and $j, k = A, B, C, S$. Note that if an one-to-one correspondence between components A, B, C, and S and numbers 1, 2, 3, 4 is established, then j and k should fulfil the condition $j < k$. In this case we readily find that the activity coefficients of A and S either in the mobile phase or at the stationary phase may be expressed as

$$\ln f_A^p = r D_1^p + r D_2^p \varphi_B^p + r D_3^p \varphi_C^p + r D_4^p (\varphi_B^p)^2 + r D_5^p (\varphi_C^p)^2 + r D_6^p \varphi_B^p \varphi_C^p \quad (6)$$

$$\ln f_S^p = C_1^p (\varphi_B^p)^2 + C_2^p (\varphi_C^p)^2 + C_3^p \varphi_B^p \varphi_C^p \quad (7)$$

Here, r is a size ratio coefficient indicating that each A solute molecule is r times greater than that of a water molecule S, $D_1^p = A_3^p$, $D_2^p = A_1^p - A_3^p - A_5^p$, $D_3^p = A_2^p - A_3^p - A_6^p$, $D_4^p = A_5^p$, $D_5^p = A_6^p$, $D_6^p = A_5^p + A_6^p - A_4^p$, $C_1^p = D_4^p$, $C_2^p = D_5^p$, $C_3^p = D_6^p$, and $p = s$ or m .

We should point out that in the partition model the stationary phase consists of the solute molecules inside the cavities of the hydrocarbon chains. Therefore, in this model we can assume that $f_A^s = \text{constant}$.

Based on the above relationships we readily obtain the following expressions for $\ln k$:

2.1.1. Partition model

$$\ln k = a + a_1\varphi_B + a_2\varphi_C + a_3\varphi_B^2 + a_4\varphi_C^2 + a_5\varphi_B\varphi_C \quad (8)$$

where for simplicity we have dropped superscript (m) from the volume fractions of the mobile phase, $a = \ln k(\varphi = 0)$, and $a_i = rD_{i+1}^m$. This equation has been previously proposed and applied by Schoenmakers et al. [1].

2.1.2. Adsorption model

$$\ln k = a - \ln(1 + b_B\varphi_B + b_C\varphi_C) - \frac{c_B\varphi_B + c_C\varphi_C}{1 + b_B\varphi_B + b_C\varphi_C} + d_B\varphi_B + d_C\varphi_C \quad (9)$$

where $a = \ln k(\varphi = 0)$, $c_B = D_2^s\beta_B$, $c_C = D_3^s\beta_C$, $d_B = D_2^m$ and $d_C = D_3^m$.

Obviously, if φ_B or φ_C in Eqs. (8) and (9) becomes zero, then the above equations result in the following already known equations describing retention for binary solvent systems [13]

$$\ln k = a + a_{1,2}\varphi_j + a_{3,4}\varphi_j^2, \quad j = B \text{ or } C \quad (10)$$

and

$$\ln k = a - \ln(1 + b_j\varphi_j) - \frac{c_j\varphi_j}{1 + b_j\varphi_j} + d_j\varphi_j, \quad j = B \text{ or } C \quad (11)$$

From this observation it becomes clear that using Eq. (9) it is possible to predict the retention data in mixed ternary mobile phases from known retention characteristics of original binary mobile phases. In other words, the values of constants a , b_j , c_j , and d_j in Eq. (9) can be determined by regression of $\ln k$ versus φ_j data for the individual binary mobile phases using Eq. (11).

On the contrary, Eq. (8), which is commonly used to describe retention for ternary solvent systems, is inadequate to be used as the basis for predictions in ternary mixtures from retention data in binary mobile phases, because the estimation of the regression constant a_5 in Eq. (8) requires the fitting of this equation to ternary mobile phase experimental data. This problem can be overcome if a_5 is expressed in terms of the other coefficients, a_1 to a_4 . Note that according to our treatment, such a relationship does not exist, because a_i is given by $a_i = rD_{i+1}^m$ and D_{i+1} are mutually independent coefficients. Despite this, Schoenmakers et al. [1] based on the solubility theory have suggested the calculation of a_5 from the following equation:

$$a_5 = \sqrt{a_3a_4} \quad (12)$$

However, a prerequisite for the application of Eq. (12) is that both parameters a_3 and a_4 must be positive numbers, which is not always true [1]. Additionally, according to the same reference [1], the six-parameter Eq. (8) is preferred over its five-parameter version, in which a_5 is calculated from Eq. (12), because it yields a better fit in the regression analysis.

2.2. Empirical expressions of $\ln k$

The derivation of Eqs. (8) and (9) presented above is strictly thermodynamic. It is based on the thermodynamic equilibrium established when the solute molecules are either partitioned between the mobile and the stationary phase, according to Dill's model [15,16], or adsorbed on the chains of the stationary phase. The calculation of the chemical potentials from the excess free energy after its expansion to a power series is also a standard thermodynamic procedure. Moreover, the approximations adopted for the derivation of Eqs. (8) and (9) are related to the ideal behavior of certain species in the stationary or/and the mobile phase, which is a common practice in thermodynamic studies. However, in a recent paper [13] we have shown that although Eqs. (10) and (11) are based on the partition model the first and the adsorption model the latter, these equations do not, in fact, express the properties of the partition or adsorption mechanism due to the rough approximations involved in their derivation. For this reason they should be treated as simple mathematical equations applied to a system irrespective of the retention mechanism that governs the properties of this system. It is evident that the same is valid for Eqs. (8) and (9), which are extensions of Eqs. (10) and (11), respectively.

Moreover in [13,14] we have shown that Eq. (11) can be significantly simplified if we put $d_j = 0$ and additionally eliminate the logarithm term, because its effect is largely absorbed by the rational function $c_j\varphi/(1 + b_j\varphi)$. If we adopt these empirical amendments for Eq. (9), we obtain

$$\ln k = a - \frac{c_B\varphi_B + c_C\varphi_C}{1 + b_B\varphi_B + b_C\varphi_C} \quad (13)$$

which is the extension of the following equation valid in binary mobile phases

$$\ln k = a - \frac{c_j\varphi_j}{1 + b_j\varphi_j}, \quad j = B \text{ or } C \quad (14)$$

We should point out here that the three constants, a , c_j , and b_j , may be all treated as adjustable parameters or the value of b_j at a certain modifier may be taken from literature [14]. In the latter case Eq. (14) is a two-parameter equation since only a and c_j can be treated as adjustable parameters.

Finally, we have recently proposed the following three-parameter equation of $\ln k$ [14]

$$\ln k = m' + \frac{n'}{1 + b_t\varphi_j} + \frac{q'}{1 + b^*\varphi_j}, \quad j = B \text{ or } C \quad (15)$$

which is valid in binary mobile phases and combines simplicity, linearity of its adjustable parameters, m' , n' , q' , and the highest applicability. Here, parameters b_t , b^* depend only on the nature of modifier and their values in aqueous mobile phases modified by MeOH, ACN, iPrOH or THF are given in [14]. From a mathematical point of view Eq. (15) is identical

to the following equation

$$\ln k = a - \frac{c_{1j}\varphi_j}{1 + b_{1j}\varphi_j} - \frac{c_{2j}\varphi_j}{1 + b_{2j}\varphi_j}, \quad j = B\eta' C \quad (16)$$

Now if we take into account the correspondence between Eqs. (13) and (14), we readily conclude that the empirical extension of Eq. (16) valid to a ternary mobile phase should be the following

$$\ln k = a - \frac{c_{1B}\varphi_B + c_{1C}\varphi_C}{1 + b_{1B}\varphi_B + b_{1C}\varphi_C} - \frac{c_{2B}\varphi_B + c_{2C}\varphi_C}{1 + b_{2B}^*\varphi_B + b_{2C}^*\varphi_C} \quad (17)$$

We observe that Eqs. (13) and (17), like Eq. (9), can predict the retention in ternary solvent systems from retention data obtained from studies in binary mobile phases.

2.3. Limiting expressions of $\ln k$

The above expressions of $\ln k$ for ternary mobile phases are significantly simplified if the volume fractions of the organic constituents of the mobile phase fulfil one of the relationships: $\varphi_B + \varphi_C = \lambda$ or $\varphi_C/\varphi_B = \lambda$, where λ is a constant. Thus we have the following cases:

2.3.1. $\varphi_B + \varphi_C = \lambda$

Eqs. (8), (9), (13) and (17) are reduced to the following equations, respectively:

$$\ln k = A + A_1\varphi_B + A_2\varphi_B^2 \quad (18)$$

where $A = a + a_2\lambda + a_4\lambda$, $A_1 = a_1 - a_2 - 2a_4\lambda + a_5\lambda$, and $A_2 = a_3 + a_4 - a_5$

$$\ln k = A - \ln(1 + B\varphi_B) - \frac{C_1 + C_2\varphi_B}{1 + B\varphi_B} + D\varphi_B \quad (19)$$

where $A = a + \ln(1 + b_C\lambda) + d_C\lambda$, $B = (b_B - b_C)/(1 + b_C\lambda)$, $C_1 = c_C\lambda/(1 + b_C\lambda)$ and $C_2 = (c_B - c_C)/(1 + b_C\lambda)$

$$\ln k = A - \frac{C_1 + C_2\varphi_B}{1 + B\varphi_B} \quad (20)$$

where $A = a$, $C_1 = c_C\lambda/(1 + b_C\lambda)$, $C_2 = (c_B - c_C)/(1 + b_C\lambda)$, and $B = (b_B - b_C)/(1 + b_C\lambda)$

$$\ln k = A - \frac{C_{1t} + C_{2t}\varphi_B}{1 + B_t\varphi_B} - \frac{C_{1*} + C_{2*}\varphi_B}{1 + B^*\varphi_B} \quad (21)$$

where $A = a$, $C_{1t} = c_{1C}\lambda/(1 + b_{1C}\lambda)$, $C_{2t} = (c_{1B} - c_{1C})/(1 + b_{1C}\lambda)$, $B_t = (b_{1B} - b_{1C})/(1 + b_{1C}\lambda)$, $C_{1*} = c_{2C}\lambda/(1 + b_{2C}^*\lambda)$, $C_{2*} = (c_{2B} - c_{2C})/(1 + b_{2C}^*\lambda)$ and $B^* = (b_{2B}^* - b_{2C}^*)/(1 + b_{2C}^*\lambda)$.

2.3.2. $\varphi_C/\varphi_B = \lambda$

Here, we obtain again Eqs. (18)–(21) but with different coefficients: $A = a$, $A_1 = a_1 + a_2\lambda$, $A_2 = a_3 + a_4\lambda^2 + a_5\lambda$ for Eq. (18); $A = a$, $B = b_B + \lambda b_C$, $C_1 = 0$, $C_2 = c_B + \lambda c_C$ for Eq. (19); $A = a$, $B = b_B + \lambda b_C$, $C_1 = 0$, $C_2 = c_B + \lambda c_C$ for Eq. (20); $A = a$, $C_{1t} = 0$, $C_{2t} = c_{1B} + \lambda c_{1C}$, $C_{1*} = 0$, $C_{2*} = c_{2B} + \lambda c_{2C}$, $B_t = b_{1B} + \lambda b_{1C}$, $B^* = b_{2B}^* + \lambda b_{2C}^*$ for Eq. (21).

3. Experimental

In order to examine the possibility of predicting the retention of a solute at any ternary mobile phase composition from retention data in binary mobile phases, the retention factors of seven catechol-related solutes, 3,4-dihydroxyphenyl glycol (hpg), serotonin (5ht), vanillylmandelic acid (vma), 3,4-dihydroxy phenylacetic acid (dopac), 5-hydroxytryptophol (htoh), 5-hydroxyindole-3-acetic acid (hiao), and homovanillic acid (hva) were measured using binary and ternary mobile phases. In particular, we first used three different mobile phase compositions in each of four binary aqueous mobile phase systems modified with methanol (MeOH), acetonitrile (ACN), isopropanol (iPrOH) or tetrahydrofuran (THF), respectively, and second nine different mobile phase compositions in each of six aqueous ternary mobile phase systems modified with two organic solvents (MeOH–ACN, MeOH–THF, MeOH–iPrOH, ACN–iPrOH, iPrOH–THF or ACN–THF). Ternary mobile phases were prepared by automatically mixing appropriate binary mobile phases at the required volume ratio (1/3, 1/1 or 3/1). The aqueous mobile phase component was a phosphate buffer of pH 2.5. The total ionic strength of the mobile phases was held constant at $I = 0.02$ M. All chemicals were used as received from commercial sources. Catechol-related compounds were available from Sigma or Aldrich. The liquid chromatography system consisted of a Shimadzu LC-10AD pump, a model 7125 syringe loading sample injector fitted with a 20 μ L loop (Rheodyne, Cotati, CA), a 250 mm \times 4 mm MZ-Analysentechnik column (5 μ m Inertsil ODS-3) thermostated by a CTO-10AS Shimadzu column oven at 25 $^{\circ}$ C, and a Gilson EC detector (Model 141) equipped with a glassy carbon electrode. The detection of the analytes was performed at 0.8 V versus the Ag/AgCl reference electrode. The eluent flow rate was varied from 0.75 to 1.25 ml/min depending on the mobile phase composition. The hold-up time, t_0 , was measured for every mobile phase composition by injection of water, methanol or inorganic salt (KNO_3) and searching the start of the main first perturbation on the electrochemical chromatograms. It was found that different hold-up time markers give nearly identical results and that t_0 changes in the experimental ranges of mobile phase compositions studied. The obtained experimental data in terms of $\ln k$ versus φ are shown in Tables 1–7.

Calculations reported in this paper have been performed using Excel 2000 spreadsheets and the determination of the fitting parameters have been done by Solver of Excel.

4. Results and discussion

The experimental retention data of all solutes, measured in the four binary mobile phases adopted in the present study, have been fitted to Eqs. (10), (14) and (16) yielding a set of coefficients for each particular solute and regression equation in each mobile phase system. In this regression procedure the

Table 1

Experimental retention values ($\ln k$) of catechol-related compounds in binary aqueous mobile phases modified with MeOH, ACN, iPrOH and THF

Modifier	φ	hpg	5ht	vma	dopac	htoh	hiao	hva	t_0 (min)
MeOH	0.04	0.787	1.943	1.729	3.176	3.652	4.023	4.574	1.831
MeOH	0.10	0.207	1.057	1.087	2.429	2.765	3.096	3.628	1.800
MeOH	0.20	-0.576	-0.144	0.234	1.455	1.652	1.919	2.467	1.800
ACN	0.04	0.404	1.352	1.356	2.632	3.069	3.418	3.838	1.785
ACN	0.10	-0.337	0.084	0.525	1.496	1.867	2.145	2.489	1.691
ACN	0.20	-0.655	-0.390	-0.263	0.304	0.686	0.854	1.077	1.628
iPrOH	0.04	-0.208	0.150	0.474	1.719	1.995	2.266	2.666	1.750
iPrOH	0.10	-1.174	-1.174	-0.539	0.635	0.748	0.954	1.366	1.695
iPrOH	0.20	-1.279	-1.757	-0.959	-0.166	-0.162	-0.040	0.301	1.710
THF	0.04	0.259	0.273	1.150	2.300	2.340	2.759	2.871	1.712
THF	0.10	-0.073	-0.217	0.716	1.757	1.704	2.105	2.105	1.605
THF	0.20	-0.231	-1.167	0.286	1.228	1.086	1.480	1.395	1.540

Table 2

Experimental retention values ($\ln k$) of catechol-related compounds in ternary aqueous mobile phases modified with MeOH and ACN

φ_{total}	φ_{MeOH}	φ_{ACN}	hpg	5ht	vma	dopac	htoh	hiao	hva	t_0 (min)
0.04	0.030	0.010	0.707	1.789	1.633	3.041	3.507	3.869	4.365	1.823
0.04	0.020	0.020	0.597	1.653	1.539	2.901	3.356	3.715	4.174	1.826
0.04	0.010	0.030	0.490	1.492	1.439	2.759	3.205	3.557	3.995	1.815
0.10	0.075	0.025	0.113	0.888	1.008	2.266	2.598	2.927	3.405	1.756
0.10	0.050	0.050	-0.020	0.652	0.872	2.038	2.364	2.681	3.113	1.733
0.10	0.025	0.075	-0.184	0.376	0.704	1.781	2.119	2.419	2.808	1.708
0.20	0.150	0.050	-0.550	-0.223	0.231	1.274	1.475	1.737	2.210	1.758
0.20	0.100	0.100	-0.663	-0.377	0.073	0.966	1.219	1.456	1.848	1.702
0.20	0.050	0.150	-0.732	-0.383	-0.104	0.629	0.944	1.152	1.454	1.657

Table 3

Experimental retention values ($\ln k$) of catechol-related compounds in ternary aqueous mobile phases modified with MeOH and THF

φ_{total}	φ_{MeOH}	φ_{THF}	hpg	5ht	vma	dopac	htoh	hiao	hva	t_0 (min)
0.04	0.030	0.010	0.528	1.083	1.443	2.726	2.984	3.384	3.644	1.778
0.04	0.020	0.020	0.448	0.679	1.295	2.512	2.664	3.069	3.256	1.751
0.04	0.010	0.030	0.325	0.450	1.216	2.391	2.480	2.892	3.038	1.713
0.10	0.075	0.025	0.064	0.263	0.921	2.063	2.136	2.514	2.724	1.695
0.10	0.050	0.050	0.029	0.025	0.806	1.891	1.885	2.279	2.403	1.683
0.10	0.025	0.075	-0.031	-0.125	0.766	1.815	1.777	2.155	2.234	1.629
0.20	0.150	0.050	-0.445	-0.680	0.305	1.281	1.277	1.606	1.797	1.741
0.20	0.100	0.100	-0.392	-0.911	0.279	1.238	1.163	1.509	1.577	1.679
0.20	0.050	0.150	-0.243	-1.014	0.314	1.249	1.142	1.504	1.504	1.575

a -coefficient was treated as an adjustable parameter that has the same value for a given solute regardless of the nature of the organic modifier. The uniform a -values not only indicate that the extrapolation procedure is reliable but have also prac-

tical significance that is related to the use of Eqs. (8), (13) and (17) for predicting retention in ternary systems from regression coefficients estimated by binary mobile phase data. For this reason the experimental retention data of each solute in

Table 4

Experimental retention values ($\ln k$) of catechol-related compounds in ternary aqueous mobile phases modified with MeOH and iPrOH

φ_{total}	φ_{MeOH}	φ_{iPrOH}	hpg	5ht	vma	dopac	htoh	hiao	hva	t_0 (min)
0.04	0.030	0.010	0.365	1.230	1.223	2.588	2.997	3.324	3.779	1.794
0.04	0.020	0.020	0.118	0.781	0.899	2.225	2.577	2.884	3.311	1.780
0.04	0.010	0.030	0.014	0.418	0.661	1.942	2.256	2.542	2.948	1.764
0.10	0.075	0.025	-0.321	0.155	0.455	1.677	1.921	2.198	2.644	1.756
0.10	0.050	0.050	-0.559	-0.283	0.104	1.249	1.429	1.671	2.092	1.714
0.10	0.025	0.075	-0.825	-0.825	-0.232	0.888	1.028	1.254	1.680	1.716
0.20	0.150	0.050	-0.990	-1.310	-0.314	0.710	0.794	1.003	1.460	1.750
0.20	0.100	0.100	-1.192	-1.422	-0.645	0.331	0.339	0.508	0.927	1.735
0.20	0.050	0.150	-1.254	-1.612	-0.823	0.036	0.041	0.184	0.563	1.718

Table 5

Experimental retention values ($\ln k$) of catechol-related compounds in ternary aqueous mobile phases modified with ACN and iPrOH

φ_{total}	φ_{ACN}	φ_{iPrOH}	hpg	5ht	vma	dopac	htoh	hiao	hva	t_0 (min)
0.04	0.030	0.010	0.209	0.989	1.087	2.362	2.755	3.074	3.489	1.783
0.04	0.020	0.020	0.027	0.672	0.856	2.127	2.479	2.776	3.188	1.779
0.04	0.010	0.030	-0.081	0.401	0.665	1.920	2.231	2.516	2.919	1.753
0.10	0.075	0.025	-0.470	-0.151	0.331	1.362	1.642	1.899	2.277	1.699
0.10	0.050	0.050	-0.611	-0.469	0.091	1.131	1.338	1.566	1.958	1.677
0.10	0.025	0.075	-0.773	-0.852	-0.141	0.895	1.047	1.257	1.651	1.686
0.20	0.150	0.050	-0.808	-0.807	-0.387	0.316	0.572	0.744	1.022	1.692
0.20	0.100	0.100	-1.040	-1.048	-0.621	0.135	0.293	0.442	0.753	1.719
0.20	0.050	0.150	-1.180	-1.313	-0.847	-0.061	0.016	0.152	0.477	1.715

Table 6

Experimental retention values ($\ln k$) of catechol-related compounds in ternary aqueous mobile phases modified with ACN and THF

φ_{total}	φ_{ACN}	φ_{THF}	hpg	5ht	vma	dopac	htoh	hiao	hva	t_0 (min)
0.04	0.030	0.010	0.342	0.897	1.282	2.484	2.732	3.116	3.390	1.746
0.04	0.020	0.020	0.288	0.579	1.212	2.379	2.533	2.931	3.132	1.729
0.04	0.010	0.030	0.290	0.404	1.174	2.326	2.416	2.825	2.976	1.712
0.10	0.075	0.025	-0.202	-0.168	0.629	1.590	1.761	2.093	2.295	1.646
0.10	0.050	0.050	-0.194	-0.325	0.640	1.609	1.689	2.048	2.162	1.629
0.10	0.025	0.075	-0.056	-0.310	0.672	1.674	1.675	2.057	2.105	1.611
0.20	0.150	0.050	-0.490	-0.498	-0.024	0.654	0.837	1.098	1.198	1.609
0.20	0.100	0.100	-0.532	-0.607	0.076	0.830	0.900	1.214	1.216	1.596
0.20	0.050	0.150	-0.486	-1.121	0.160	0.972	0.967	1.322	1.274	1.589

the four binary mobile phases has been simultaneously fitted to each of Eqs. (10), (14) and (16). Thus the adjustable parameters were 9 for each equation. In particular, the adjustable parameters of Eq. (10) were the common in the four modifiers a -coefficient and eight $a_{1,2}$ and $a_{3,4}$ -coefficients for four modifiers, whereas those of Eqs. (14) and (16) were a , b_j , c_j , and a , c_{1j} , c_{2j} , respectively, where j denotes one of the four modifiers.

The results of this regression analysis are presented in Table 8 for all seven solutes in the four modifier systems studied. The last column in Table 8 lists the average deviation, a.d., between calculated and experimental values. According to the results depicted in Table 8, both three-parameter Eqs. (14) and (16) give an accurate description of the variation of retention with composition in binary systems for all solutes despite the fact that a fixed a -term was adopted. The average deviation for all data points, 84, is about 0.042 corresponding to an error of 4% in retention factor values, k . Additionally, Eqs. (14) treated as a three or a two-parameter

equation as well as Eq. (16) seem to yield good estimates for the a -coefficient, $a = \ln k_{\varphi=0}$, since these estimates are in good agreement with the values of $\ln k_{\varphi=0}$ obtained by direct measurements in a series of experiments carried out previously [14].

In contrast, Eq. (10) leads to a rather poor description of the retention of solutes in binary eluent systems. The average error in k values is approximately 11%, i.e. the data points show an average deviation from the regression curves of 0.107 in $\ln k$ values, which is much higher than that obtained when the same data points are fitted to Eq. (14) treated as a two-parameter equation by assuming a constant b_j -value for each modifier. The reason for this poor fit should be the fact that the regression procedure adopted acknowledges the a -term in each regression equation as a constant, independent of the nature of the organic modifier, although previous studies have underlined that a shortcoming of Eq. (10) is that the extrapolated retention data towards $\varphi=0$ vary significantly with the nature of the binary organic modifier-aqueous buffer

Table 7

Experimental retention values ($\ln k$) of catechol-related compounds in ternary aqueous mobile phases modified with iPrOH and THF

φ_{total}	φ_{iPrOH}	φ_{THF}	hpg	5ht	vma	dopac	htoh	hiao	hva	t_0 (min)
0.04	0.030	0.010	-0.072	0.189	0.729	1.934	2.124	2.447	2.717	1.733
0.04	0.020	0.020	0.060	0.227	0.902	2.076	2.209	2.569	2.770	1.719
0.04	0.010	0.030	0.165	0.251	1.039	2.197	2.279	2.671	2.821	1.711
0.10	0.075	0.025	-0.595	-0.598	0.055	1.081	1.138	1.400	1.630	1.656
0.10	0.050	0.050	-0.389	-0.532	0.315	1.355	1.359	1.675	1.810	1.640
0.10	0.025	0.075	-0.210	-0.380	0.543	1.586	1.558	1.914	1.978	1.613
0.20	0.150	0.050	-0.902	-1.300	-0.501	0.332	0.332	0.539	0.681	1.655
0.20	0.100	0.100	-0.707	-1.126	-0.198	0.651	0.647	0.914	0.936	1.623
0.20	0.050	0.150	-0.507	-1.112	0.069	0.977	0.890	1.221	1.196	1.579

Table 8

Coefficients of Eqs. (10), (14) and (16) obtained from regression analysis of all binary mobile phases experimental data adopting an a -term independent of the nature of organic modifier

Solute	a	δa^a	MeOH		ACN		iPrOH		THF		a.d. ^b
			$a_{1,2}$	$a_{3,4}$	$a_{1,2}$	$a_{3,4}$	$a_{1,2}$	$a_{3,4}$	$a_{1,2}$	$a_{3,4}$	
Eq. (10)											
hpg	0.935	0.40	-5.710	-9.49	-16.62	43.14	-31.66	103.1	-15.92	50.83	0.047
5ht	1.804	0.88	-1.588	-41.56	-19.79	43.30	-43.40	128.3	-31.15	82.71	0.158
vma	1.812	0.49	-4.815	-15.79	-14.26	19.15	-34.73	104.7	-15.65	40.41	0.064
dopac	3.189	0.63	-4.049	-23.68	-17.74	16.20	-36.56	99.4	-20.80	55.42	0.092
htoh	3.601	0.84	-3.642	-31.26	-17.95	16.42	-40.42	108.5	-28.65	81.11	0.120
hiaa	3.990	0.84	-4.160	-31.73	-19.04	16.29	-43.06	115.1	-28.21	78.97	0.121
hva	4.397	0.97	-1.598	-41.17	-19.07	11.81	-42.79	112.1	-34.77	99.68	0.145
Solute	a	δa	MeOH		ACN		iPrOH		THF		a.d.
			c_j	b_j	c_j	b_j	c_j	b_j	c_j	b_j	
Eq. (14)											
hpg	1.454	0.12	17.90	3.879	44.48	15.78	91.4	27.28	82.0	43.68	0.050
5ht	2.757	0.07	21.40	2.397	57.20	12.76	126.3	22.73	124.2	28.05	0.097
vma	2.233	0.07	13.45	1.729	27.04	5.84	81.4	20.20	46.1	19.08	0.025
dopac	3.689	0.13	14.04	1.273	30.94	4.13	79.1	15.61	60.4	19.99	0.025
htoh	4.280	0.16	17.32	1.578	36.60	5.18	92.5	15.92	94.5	25.16	0.030
hiaa	4.663	0.16	17.66	1.423	37.09	4.73	95.7	15.44	90.5	23.97	0.032
hva	5.183	0.18	17.22	1.322	39.61	4.65	100.4	15.73	114.0	25.63	0.045
Solute	a	δa	MeOH		ACN		iPrOH		THF		a.d.
			c_j	b_j	c_j	b_j	c_j	b_j	c_j	b_j	
Eq. (14) treated as a two-parameter equation											
hpg	1.093	0.25	11.27	2	22.09	7	51.2	15	40.4	25	0.078
5ht	2.446	0.24	17.56		35.98		87.3		103.1		0.143
vma	2.237	0.07	13.95		29.57		66.6		56.0		0.056
dopac	3.843	0.02	16.84		41.36		80.8		76.3		0.056
htoh	4.352	0.09	18.94		43.21		90.8		96.6		0.048
hiaa	4.785	0.04	20.13		46.18		96.8		97.6		0.055
hva	5.304	0.06	19.91		49.54		100.2		116.2		0.066
Solute	a	δa	MeOH		ACN		iPrOH		THF		a.d.
			c_{1j}	c_{2j}	c_{1j}	c_{2j}	c_{1j}	c_{2j}	c_{1j}	c_{2j}	
Eq. (16)											
hpg	1.654	0.32	-4.171	27.59	3.28	65.51	1.35	126.5	0.625	116.9	0.064
5ht	2.969	0.29	3.001	24.66	7.71	81.70	6.36	174.1	7.535	193.4	0.108
vma	2.436	0.13	2.769	16.45	11.59	36.86	5.38	120.3	5.054	92.3	0.037
dopac	3.877	0.06	6.470	12.72	18.82	31.33	10.05	118.1	6.315	113.0	0.020
htoh	4.496	0.06	6.084	17.35	18.13	43.20	11.32	137.8	6.457	160.7	0.027
hiaa	4.889	0.06	7.268	16.66	20.01	41.65	12.43	142.7	6.784	157.6	0.024
hva	5.415	0.05	7.563	16.01	21.72	43.58	12.69	148.8	7.533	190.3	0.015

^a $\delta a = |a - (\ln k_{\varphi=0})_{\text{exp}}|$.^b a.d. is the average deviation of experimental data points from the regression curves defined from $\text{a.d.} = \frac{1}{N} \sum |\ln k_{\text{calc}} - \ln k_{\text{exp}}|$ and N is the number of data points included in the regression analysis, $N = 12$ throughout this analysis.

system [1,17–19]. As a result, the uniform a -values found in this case differ from the corresponding experimental data in pure aqueous buffer, $(\ln k_{\varphi=0})_{\text{exp}}$, by values up to about 0.97, see Eq. (10) in Table 8. A correct estimate for the a -term can be obtained in some cases by incorporating into Eq. (10) an extra term that is proportional to the square root of φ [17]. However, this extension of Eq. (10) does not necessarily yield reliable results for the retention in pure aqueous buffer. On the other hand the inclusion of additional terms in an equation

is not an attractive way to improve the description of experimental retention data from a practical point of view, since the number of experiments required for the estimation of model coefficients depends on the order of the model. For this reason, the extension of Eq. (10) proposed in Ref. [17] as well as Eq. (11) due to its term $d_j \varphi_j$ (four-parameter equation) are not applied to our data sets obtained for the binary solvent systems as the purpose of this study is to evaluate the possibility of predicting retention for any composition of ternary eluent

Table 9

Coefficients according to Eq. (10) determined by regression analysis of experimental retention data for the individual binary mobile phases modified by MeOH, ACN, iPrOH and THF without the assumption of a uniform a -value

Solute	$(\ln k_{\varphi=0})_{\text{exp}}$	MeOH			ACN			iPrOH			THF		
		a	$a_{1,2}$	$a_{3,4}$	a	$a_{1,2}$	$a_{3,4}$	a	$a_{1,2}$	$a_{3,4}$	a	$a_{1,2}$	$a_{3,4}$
hpg	1.338	1.220	−11.27	11.48	1.127	−20.37	57.29	0.812	−29.26	94.03	0.580	−9.00	24.73
5ht	2.683	2.602	−17.16	17.16	2.607	−35.47	102.4	1.439	−36.29	101.5	0.567	−7.01	−8.30
vma	2.303	2.211	−12.60	13.56	2.060	−19.09	37.37	1.465	−27.96	79.20	1.513	−9.81	18.38
dopac	3.820	3.742	−14.84	17.02	3.565	−25.07	43.84	2.695	−26.90	62.98	2.756	−12.33	23.49
htoh	4.438	4.335	−17.98	22.79	4.075	−27.20	51.31	3.118	−31.00	73.00	2.874	−14.47	27.66
hiao	4.826	4.734	−18.68	23.03	4.474	−28.48	51.91	3.440	−32.33	74.62	3.312	−14.97	29.06
hva	5.364	5.309	−19.40	25.96	4.948	−29.83	52.37	3.808	−31.29	68.77	3.523	−17.71	35.36

systems within a design space from a minimum number of binary mobile phase experimental data.

Once the variation of retention with binary solvent systems composition was obtained, the regression parameters for the individual solutes shown in Table 8 can be used in Eqs. (8), (13) and (17) to predict retention in ternary mobile phases. However, Eq. (8) cannot be used for calculating retention for MeOH-containing ternary systems, because the values of $a_{3,4}$ -parameters estimated for all seven solutes in MeOH-containing binary mobile phases are negative. Consequently, parameter a_5 in Eq. (8) can be estimated from Eq. (12) only for the ternary mobile phases modified by ACN–iPrOH, ACN–THF or iPrOH–THF. The retention calculated in this way for the above ternary eluents are compared with the experimental values and the mean value of the absolute differences between predicted and experimental $\ln k$ data was found to be 0.166, corresponding to an error of 18% in retention factors, a really very poor retention prediction.

To test further the validity of Eq. (8) the experimental retention data for the individual binary systems modified by MeOH, ACN, iPrOH and THF were fitted again to Eq. (10) but this time without the assumption of a uniform a -value. The resulting coefficients for each solute and organic modifier are given in Table 9. Obviously, Eq. (10) now gives an excellent description of retention with composition in all binary systems, because three independent data points are used to estimate three parameters in Eq. (10). However, the regression parameters for the individual solutes shown in Table 9 can be strictly used only for the retention prediction within the φ -range studied, because, as is seen in Table 9, the a -coefficients do not give an accurate estimate for the retention in pure aqueous buffer and errors in extrapolating the retention data affect significantly the quality of predictions. Additionally, different values of the a -term for the same

solute in different binary systems, as shown in Table 9, make the application of Eq. (8) to ternary solvent systems impossible. To overcome this obstacle, we may follow the empirical remedy proposed in [19]. According to this suggestion, a depends on the composition of the ternary mixture, say φ_B , φ_C if the mixture consists of modifiers B and C, and it is calculated from

$$a = \frac{a_B \varphi_B + a_C \varphi_C}{\varphi_B + \varphi_C} \quad (22)$$

where the two a -regression coefficients, a_B and a_C , are obtained from the corresponding individual binary mobile phases data. We should clarify that Eq. (22) is adopted only for the application of Eq. (8), because the other two Eqs. (13) and (17) do not need such a remedy. We found that the combination of Eqs. (8) and (22) using the regression parameters of Table 9 results in a significant improvement in the description of experimental data in ternary mobile phases. The average deviation between the experimental values for $\ln k$ and the calculated ones for all data except those of 5ht was found to be 0.123, which corresponds to a 13% error in the values of k (see Table 10). The negative value of the $a_{3,4}$ -coefficient obtained for 5ht in THF-containing binary systems does not permit the a_5 -coefficient in Eq. (8) to be computed by means of Eq. (12) for all ternary systems. For this reason, the retention data of 5ht are not included in the estimation of mean deviations of the calculated by different equations $\ln k$ values from the experimental ones.

However, even if we combine Eq. (8) with Eq. (22) the performance of Eq. (8) remains lower than that of Eqs. (13) and (17). Table 10 depicts the degree of agreement between retention prediction using Eqs. (8), (13) and (17) and the real experimental retention obtained for six solutes at nine different compositions in each of six different ternary

Table 10

Average deviation between experimental and calculated $\ln k$ values using different equations for all data points except those of 5ht

	Eq. (8) ^a	Eq. (8) ^b	Two parameter Eq. (13) ^b	Eq. (13) ^b	Eq. (17) ^b
Binary systems	0.000	0.098	0.060	0.034	0.031
Ternary systems	0.123	0.160 ^c	0.084	0.081	0.069

^a Using coefficients of Table 9.

^b Using coefficients of Table 8.

^c Determined in ternary mobile phases when $a_{3,4} > 0$.

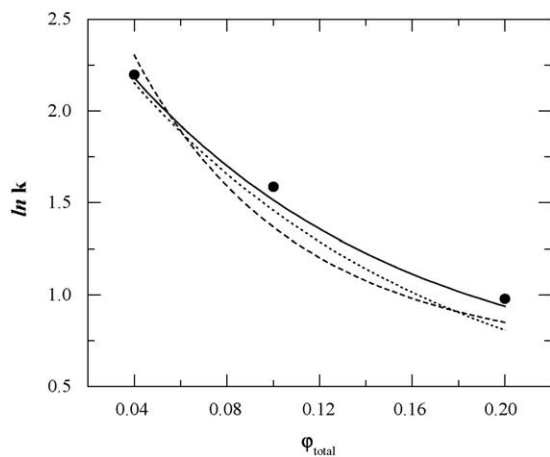


Fig. 1. Dependence of $\ln k$ of dopac on the total volume fraction, φ_{total} , of iPrOH–THF in a ternary aqueous mobile phase with a constant volume fraction ratio of the two organic modifiers, $\varphi_{\text{iPrOH}}/\varphi_{\text{THF}} = 1/3$. Points are experimental data. Solid and dashed line constructed using Eqs. (17) and (8), respectively, and the relevant coefficients in Table 8, whereas dotted line constructed using Eq. (8) and coefficients in Table 9. Parameter a_5 of Eq. (8) is calculated from Eq. (12).

solvent systems. It can be seen that Eq. (13) treated either as a three- or a two-parameter equation using regression coefficients from Table 8 can describe reasonably well the retention in ternary mobile phases but Eq. (17) provides an even better prediction of the experimental retention behavior of the same solutes in ternary solvent systems. Indeed, the average prediction error obtained by Eq. (17) is 0.069 in $\ln k$ values or about 7% in k values that can be considered satisfactory for retention prediction under ternary conditions without resorting to experimental measurements in ternary systems.

The superiority of Eqs. (13) and (17) to Eq. (8) in predicting retention behavior of solutes under ternary solvent mixture elution conditions from a few experimental data obtained in binary mobile phases is also shown in Figs. 1–3. The experimental retention behavior of two solutes with a significant retention in some binary and ternary eluents, such as dopac and htoh, was selected and compared with the corresponding theoretical description of their retention by Eqs. (8), (13) and (17). In more details, Fig. 1 shows an example of dependence of $\ln k$ of dopac on the total volume fraction, φ_{total} , of iPrOH–THF in ternary aqueous mobile phases at a constant volume fraction ratio of iPrOH to THF equal to 1/3. The plots in this figure calculated using Eq. (17) and regression coefficients from Table 8 show an excellent agreement between theoretical predictions and experiment data, whereas Eq. (8) using either parameters of Table 8 or parameters of Table 9 totally fails to describe the retention behavior of dopac in this ternary solvent system. This behavior of Eq. (8) suggests that the a_5 coefficient of this equation may be treated as an adjustable parameter estimated by regression of experimental data in ternary mobile phase and not calculated from Eq. (12). Similarly, in Fig. 2, Eq. (8) can describe rea-

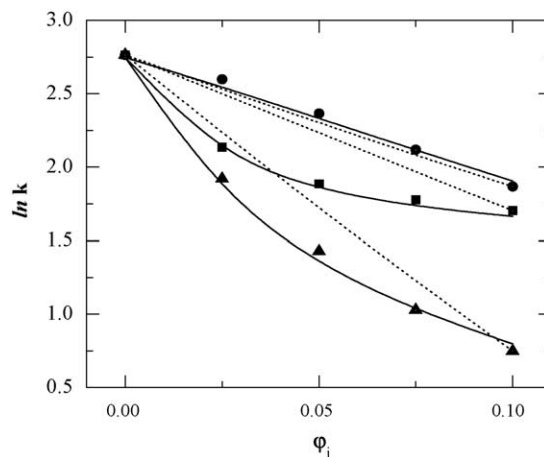


Fig. 2. Dependence of $\ln k$ of htoh on the volume fraction of ACN (●), iPrOH (▲) and THF (■) in ternary aqueous mobile phases modified by MeOH–ACN, MeOH–iPrOH and MeOH–THF, respectively, with a constant sum of the two organic modifier fractions, $\varphi_{\text{total}} = 0.1$. Solid lines constructed using Eq. (17) and the relevant coefficients in Table 8, whereas dotted lines constructed using Eq. (8) and coefficients in Table 9. Parameter a_5 of Eq. (8) is calculated from Eq. (12).

sonably well the retention of htoh in binary mobile phases containing MeOH, ACN, iPrOH, or THF as organic modifier as well as in ternary mobile phase modified by MeOH–ACN. However, it shows significantly large deviations from the experimental data obtained for this solute by MeOH–iPrOH or MeOH–THF containing ternary solvent systems. In contrast, the retention of htoh in all binary and ternary mobile phases depicted in Figs. 2 and 3 is adequately described by both Eqs. (13) and (17) using the relevant parameters of Table 8.

Thus we conclude that retention behavior under ternary solvent mixture elution conditions can be accurately predicted using either Eq. (13) or (17) with parameters obtained by regression analysis of experimental data in binary mobile phases following the procedure outlined in this study. That

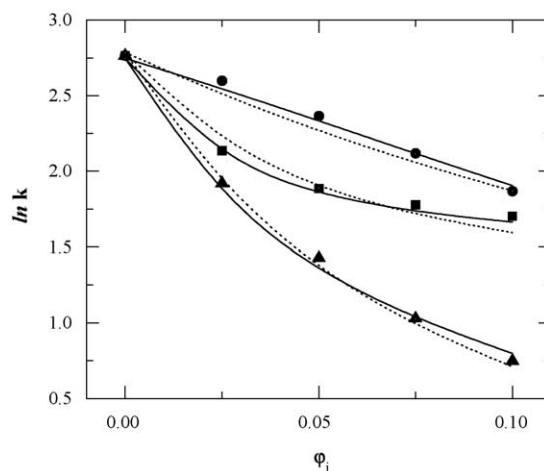


Fig. 3. As in Fig. 2 but using Eq. (13) instead of Eq. (8).

is, without using experimental data in ternary eluents except to confirm the accuracy of predictions.

Finally, it should be emphasized that based on the regression coefficients shown in Table 8, Eqs. (13) and (17) can be used to calculate the retention in the general case of ternary systems, i.e. at any ternary mobile phase composition within the ranges of individual binary mobile phases studied, and not only in two specific types of ternary systems where either $\varphi_C/\varphi_B = \lambda$ or $\varphi_{\text{total}} = \varphi_B + \varphi_C = \lambda$. However, if the volume fractions of the organic constituents of ternary mobile phases fulfil one of the above restrictions, as in the case of our experimental data, then the simplified expressions for $\ln k$ derived in the theoretical part can be applied directly to the ternary mobile phase retention data and the coefficients of Eqs. (20) and (21) can be obtained by regression using these data. Moreover, the regression coefficients estimated in this way can be used to calculate the parameters for each organic solvent in simple binary mobile phases and thus to predict the retention as function of any binary or ternary mobile phase composition without experiments. For example, Eq. (20) was used as the basis for predictive calculations of retention in binary mobile phases from ternary solvent experimental retention data. Thus, starting from twelve experimental data concerning htoh retention in four mobile phases modified by MeOH–THF, MeOH–iPrOH, iPrOH–THF and ACN–THF with a constant volume fraction ratio of the two organic modifiers, $\varphi_{\text{MeOH}}/\varphi_{\text{THF}} = \varphi_{\text{MeOH}}/\varphi_{\text{iPrOH}} = \varphi_{\text{iPrOH}}/\varphi_{\text{THF}} = \varphi_{\text{ACN}}/\varphi_{\text{THF}} = 3/1$ and a variable φ_{total} , $\varphi_{\text{total}} = 0.04, 0.1$ and 0.2 , the nine A , B_i , and C_{2i} regression coefficients of Eq. (20), $C_{1i} = 0$ in this case, can be determined for each of the above four ternary systems denoted by i . After that the nine parameters of Eq. (14), a , b_j , and c_j , for htoh in the four modifiers containing MeOH, ACN, iPrOH or THF can be calculated and consequently the retention of this solute can be predicted by using Eq. (13) in any binary or ternary mobile phase modified with one or two of these organic solvents. The predictive results were sufficiently accurate since it was found that the average prediction error in $\ln k$ values is 0.065 for all data points, 54, obtained under ternary conditions and 0.102 for 12 data obtained in binary mobile phases.

5. Conclusions

The unique feature of our approach is that once the variation of retention with binary solvent systems is obtained for some solutes, the retention of these solutes can be calculated by either Eq. (13) or (17) at every ternary mobile phase composition in a designed space without resorting to experimental data obtained in ternary eluents. Such experimental data are required only to confirm the predictions. In contrast, Eq. (8), applied to date in ternary solvent systems, fails to describe satisfactorily the retention under ternary eluent conditions from known retention characteristics in binary mobile phases. Additionally it was found that, departing from a min-

imum number of experimental retention data obtained in a specific type of ternary mobile phases in which the ratio of the volume fractions of the two organic modifiers is constant, Eq. (20) allows the prediction of retention in any binary or ternary mobile phase. Therefore, the results of this study open the practical possibility of using Eqs. (13) or (17) for optimization separations under ternary solvent mixture isocratic and/or gradient elution conditions.

6. Nomenclature

a	$= \ln k(\varphi = 0)$, the value of $\ln k$ when the mobile phase consists of water only
a_B, a_C	a -values of Eq. (22) obtained using aqueous binary mobile phases modified by the solvent B and C, respectively
a_i	($i = 1, 2, \dots, 5$) parameters of the retention Eq. (8)
$a_{1,2}, a_{3,4}$	parameters of the retention Eq. (10)
A	the constant term of the retention Eqs. (18)–(21)
A^m, A^s	a solute molecule in the mobile (m) and in/on the stationary (s) phase, respectively
A_i^m, A_i^s	coefficients of the excess free energy of a quaternary mixture formed in the mobile and on the stationary phase, respectively
A_1, A_2	parameters of the retention Eq. (18)
b_B, b_C	parameters of Eqs. (5), (9), (11), (13) and (14) related to the adsorption equilibrium constants β_j through the equation $b_j = \beta_j - 1$
b_t, b^*	parameters of the retention Eqs. (15), (16)
b_j, b_j^*	($j = B$ or C) parameters of the retention Eq. (17)
B	parameter of the retention Eqs. (19) and (20)
B_t, B^*	parameters of the retention Eq. (21)
B^m, B^s	a solvent molecule of type B in the mobile and on the stationary phase, respectively
c_B, c_C	parameters of the retention Eqs. (9), (11), (13) and (14)
c_{1j}, c_{2j}	($j = B$ or C) parameters of the retention Eqs. (16) and (17)
C^m, C^s	a solvent molecule of type C in the mobile and on the stationary phase, respectively
C_1, C_2	parameters of the retention Eqs. (19) and (20)
$C_{1t}, C_{2t}, C_{1*}, C_{2*}$	parameters of the retention Eq. (21)
C_i^m, C_i^s	parameters of the activity coefficient f_s^m of water in the mobile and in/on the stationary phase, respectively
d_B, d_C	parameters of the retention Eqs. (9) and (11)
D	parameter of the retention Eq. (19)
D_i^m, D_i^s	parameters of the activity coefficient f_A of the solute in the mobile or in/on the stationary phase, respectively
f_j^m, f_j^s	activity coefficient of species j ($j = A, B, C, S$) in the mobile and in/on the stationary phase, respectively
k	retention factor of the sample solute
m'	parameter of the retention Eq. (15)

n' parameter of the retention Eq. (15)
 q' parameter of the retention Eq. (15)
 r size ratio coefficient indicating that each solute molecule is r times greater than that of a water molecule
 RP-LC reversed phase liquid chromatography
 S^m, S^s a water molecule in the mobile and on the stationary phase, respectively

Greek letters

β partition equilibrium constant of Eq. (2)
 β_j ($j = A, B$ or C) adsorption equilibrium constants of Eqs. (3), (4) and (5)
 $\lambda = \varphi_B + \varphi_C$ or $\lambda = \varphi_C / \varphi_B$
 φ_j volume fractions of species j ($j = A, B$ or C) in the mobile phase
 φ_j^m, φ_j^s volume fractions of species j ($j = A, B$ or C) in the mobile and in/on the stationary phase, respectively

References

- [1] P.J. Schoenmakers, H.A.H. Billiet, L.D. Galan, J. Chromatogr. 218 (1981) 261.
- [2] P. Jandera, L. Petranek, M. Kucerova, J. Chromatogr. A 791 (1997) 1.
- [3] D. Bolliet, C.F. Poole, Anal. Commun. 35 (1998) 253.
- [4] K. Valko, L.R. Snyder, J.L. Glajch, J. Chromatogr. A 656 (1993) 501.
- [5] J. Li, D.S. Shah, J. Chromatogr. A 954 (2002) 159.
- [6] R. Gonzalo-Lumbreras, R. Izquierdo-Hornillos, J. Chromatogr. B 742 (2000) 1.
- [7] R. Gonzalo-Lumbreras, R. Izquierdo-Hornillos, J. Chromatogr. B 742 (2000) 47.
- [8] H.J. Metting, P.M.J. Coenegracht, J. Chromatogr. A 728 (1996) 45.
- [9] P. Chaminade, A. Baillet, D. Bayloq-Ferrier, Analisis 22 (1994) 55.
- [10] J.A. Martinez-Pontevedra, L. Pensado, M.C. Casais, R. Cela, Anal. Chim. Acta 515 (2004) 127.
- [11] A.M. Siouffi, R. Phan-Tan-Luu, J. Chromatogr. A 892 (2000) 75.
- [12] P. Nikitas, A. Pappa-Louisi, P. Agrafiotou, J. Chromatogr. A 946 (2002) 9.
- [13] P. Nikitas, A. Pappa-Louisi, P. Agrafiotou, J. Chromatogr. A 946 (2002) 33.
- [14] A. Pappa-Louisi, P. Nikitas, P. Balkatzopoulou, C. Malliakas, J. Chromatogr. A 1033 (2004) 29.
- [15] K.A. Dill, J. Phys. Chem. 91 (1987) 1980.
- [16] K.A. Dill, J. Naghizadeh, J.A. Marquise, Ann. Rev. Phys. Chem. 39 (1988) 425.
- [17] P.J. Schoenmakers, H.A.H. Billiet, L.D. Galan, J. Chromatogr. 282 (1983) 107.
- [18] P.J. Schoenmakers, Optimisation of Chromatographic Selectivity, Elsevier, Amsterdam, 1986, pp. 59–61.
- [19] P. Jandera, in: R.M. Smith (Ed.), Retention and Selectivity in Liquid Chromatography, Elsevier, Amsterdam, 1995, pp. 243–247.