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# Description and prediction of retention in normal-phase high-performance liquid chromatography with binary and ternary mobile phases

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## Abstract

The suitabilities of several earlier reported models for description and prediction of retention in normal-phase systems with mobile phases comprised of two organic solvents — a polar and a non-polar one — were tested on the chromatographic behaviour of phenylurea herbicides and alkyl-, aryl- and nitrophenols as sample solutes with a silica-gel column and 2-propanol, *n*-heptane and dioxane as mobile phase components. With few exceptions, the data obtained from the best-fit three-parameter retention equation differ from the experimental capacity factors at less than 0.1, or 2%, and for most compounds the fit is better than 1%. Of the two two-parameter equations derived from theoretical models, one failed to describe the retention behaviour. The fit of the other two-parameter equation to the retention data of phenols is slightly inferior than with the three-parameter equation. The differences between the fitted data and the experimental capacity factors of substituted phenylureas were  $\leq 0.2$ , or 5%. Three-parameter equations were suggested to describe the retention behaviour in three-component mobile phases either at a constant sum or at a constant ratio of the two stronger solvents in ternary mobile phases. To fit the three-component data at any combination of concentrations of the three solvents with the error comparable to that in binary mobile phases, a nine-parameter equation is necessary. Suitability of these equations to describe the experimental behaviour of substituted phenylureas and phenols was verified in ternary mobile phases with various concentrations of 2-propanol, dioxane and *n*-heptane. Finally, a method was suggested for the prediction of retention in ternary mobile phases with varying concentration ratios of the two polar solvents from the parameters of best-fit equations in binary mobile phases and a single experimental capacity factor in a ternary mobile phase. For most capacity factors, the error of prediction was lower than 0.2, or 5%. Dried solvents were used to improve the reproducibility of the results and the temperature was controlled to  $\pm 0.1^\circ\text{C}$  in all experiments. With these precautions, differences between the original retention volumes and the data from repeated experiments measured after ten months of use of the column in the system tested were less than 0.2 ml for 85% of the values compared.

**Keywords:** Mobile phase composition; Retention prediction; Retention models; Pesticides; Phenols

## 1. Introduction

Chromatography in reversed-phase systems is the most popular mode used in the contemporary practice of liquid chromatography. This is justified by the general applicability of reversed-phase chromatog-

raphy for separation of different classes of compounds ranging from non-polar aromatic hydrocarbons and fatty acid esters to ionizable or ionic compounds such as carboxylic acids, nitrogen bases, amino acids, peptides, proteins and sulphonic acids. The main advantage is unrivalled 'hydrophobic selectivity' of this method for compounds with even minor differences in the structure of the hydrophobic

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part of their molecules or for compounds with functional groups differing in polarities. On the other hand, columns packed with polar adsorbents usually show better selectivity than reversed-phase columns for various positional isomers of moderately polar compounds [1,2], or for some oligomers containing repeat polar groups [3]. Furthermore, columns packed with unmodified adsorbents are not subject to 'bleeding', i.e. gradual loss of the bonded stationary phase which usually occurs during the lifetime of the column, decreasing slowly the retention.

Chromatography on polar adsorbents suffers from another inconvenience — preferential adsorption of more polar solvents, especially water, which is often connected with long equilibration times if separation conditions are changed. To get reproducible results it is necessary to keep a constant adsorbent activity [4]. To maintain a constant content of water in the mobile phase, 'isohydric' organic solvents with equilibrium water concentrations should be used [5]. A closed 'constant moisture system' with a certain volume of solvent containing the required concentration (a few ppm) of water was suggested, in which the eluent from the column and detector is recycled through a large column packed with alumina adsorbent back to the solvent reservoir, so that constant adsorbent activity is guaranteed [6]. However, equilibrating such a system takes a long time and after a certain number of cycles the mobile phase becomes excessively contaminated with the sample solutes, so that it is necessary to exchange both the solvent and the adsorbent in the large column and re-equilibrate the system. This procedure is tedious and not very practical, especially when switching to another composition of mobile phase is required, which is obviously the reason why this approach has not become widely used. That is why we preferred to use dehydrated solvents kept dry over activated molecular sieves and filtered just before use to improve the reproducibility and to work under controlled temperature conditions. The objective of the present work was to investigate the possibilities of description of retention in binary and ternary mobile phases in chromatography on polar adsorbents and to evaluate errors connected with characterization and prediction of retention in such systems, if these precautions and a modern instrument with high accuracy and reproducibility of solvent mixing and mobile phase delivery are used.

## 2. Theoretical

The retention in normal-phase systems as a function of the composition of two-component (binary) mobile phases can be described using theoretical models of adsorption. The first model of retention in adsorption chromatography was developed by Snyder in early 1960s [4,7,8]. Flat adsorption in a monomolecular layer on a homogeneous adsorption surface was assumed. The adsorption was understood to be a competition phenomenon between the molecules of the solute and of the solvent on the adsorbent surface. The interactions in the mobile phase were assumed to be less significant and were neglected. Later, corrections were introduced for preferential adsorption on localized adsorption centers [9,10]. Soczewinski [11,12] suggested a model of retention assuming adsorption in a monomolecular layer on a heterogeneous surface of adsorbent and cancellation of the solute — solvent interactions in the mobile and in the stationary phases. With some simplification, both models lead to identical equations describing the retention (capacity factor,  $k'$ ) as a function of the concentration of the stronger (more polar) solvent,  $\varphi$ , in binary mobile phases comprised of two solvents of different polarities [7,12,13]:

$$k' = k'_0 \varphi^{-m} \quad (1)$$

$k'_0$  and  $m$  are experimental constants,  $k'_0$  being the capacity factor in a pure strong solvent. This equation has become known as the Snyder–Soczewinski model equation [8].

Scott and Kucera [14–17] in their model of adsorption defined the distribution coefficient of a solute between the stationary and the mobile phases as the ratio of the forces acting on the solute in the two phases, set to the product of the probability of interactions and the interacting forces in each phase. In mobile phase, both polar and dispersive forces were considered, but dispersive forces on the adsorbent surface were neglected. The number of the adsorption sites was considered independent of the composition of the mobile phase when the concentration of the polar solvent in the mobile phase was above 3% and decreasing retention with increasing concentration of the more polar solvent in these mobile phases was attributed mainly to increasing interactions in the mobile phase. With these assumptions, they derived a retention equation different from Eq. (1):

$$\frac{1}{k'} = a + b\varphi \quad (2)$$

where  $a$  and  $b$  are experimental constants.

It is interesting to note that both Eq. (1) and Eq. (2) can be derived on the basis of molecular statistical–mechanical theory of adsorption chromatography [18].

Departing from the original theoretical basis of the Snyder original model, another retention equation was derived [19,20]:

$$k' = (a + b\varphi)^{-m} \quad (3)$$

Here again,  $a$ ,  $b$  and  $m$  are experimental constants depending on the solute and on the chromatographic system ( $a = 1/(k'_a)^m$ , where  $k'_a$  is the capacity factor in pure non-polar solvent). If the retention in pure non-polar solvent is very high, the term  $a$  in Eq. (3) can be neglected and this equation becomes Eq. (1) [13]. On the other hand, if the molecule of the polar solvent occupies an area on the adsorbent surface approximately equal to the area occupied by a molecule of solute,  $m=1$  and Eq. (3) is then identical to Eq. (2) [19,20].

Ternary and more complex mobile phases in normal-phase chromatography contain two or more different polar solvents in a non-polar one [21]. Two different effects of polar solvents on retention can be distinguished here, like in reversed-phase systems. If the ratio of concentrations of the two polar solvents in the non-polar one is constant, but the sum of the two concentrations is being changed, the effect of this change on retention is much the same as when the concentration of more polar solvent is changed in binary mobile phases and can be attributed mainly to changing solvent strength. On the other hand, if the sum of concentrations of the two polar solvents is constant but their ratio is changing, specific types of polar interactions of the two solvents with sample components (dipole–dipole and proton-donor–acceptor interactions) are changing too, which gives rise to larger effects on the selectivity of separation than in the systems where this ratio is constant. Such selectivity tuning is the main purpose of using ternary (or even more complex) solvent systems as mobile phases in liquid chromatography. More exactly, to have pure selectivity effects, isoelutropic solvent mixtures with equal solvent strengths and non-equal concentrations should be used [22], but it

is more convenient to work with concentration than with solvent strength (which depends on the adsorbent) and for practical method development it is not very important if some change in solvent strength occurs when optimizing selectivity. Some changes in selectivity occur very often even when changing only the concentration of polar solvent in a binary mobile phase, i.e., the solvent strength of the mobile phase [19], so that it is virtually impossible in practice to use the method development approach where the selectivity and the retention are changed independently of each other.

To describe the retention in ternary and more complex mixed solvents, it is possible to use Snyder's description of adsorption chromatography, with elution strength contributed to by all solvents in the mobile phase [23,24]. It is necessary to consider competition effects between various solvents in the mobile phase for localized adsorption centers on the adsorbent surface and to correct correspondingly the solvent strength [25], which is difficult to do in a straightforward manner.

In ternary mobile phases with a constant concentration ratio of two polar solvents,  $\varphi_1/\varphi_2$ , the effect of the sum of the two concentrations,  $\varphi_T = \varphi_1 + \varphi_2$ , on the retention can be assumed principally to be the same as the effect of the concentration of one polar solvent in binary mobile phases and the retention equation similar to Eq. (1) can be expected to describe the retention behaviour in such ternary solvent systems [26]:

$$k' = k'_0 \varphi_T^{-m} \quad (4)$$

When the sum of the concentrations of the two polar solvents,  $\varphi_T$ , is constant, but their ratio,  $\varphi_1/\varphi_2$ , is changing, the elution strength in ternary mobile phases is subject to minor changes only, so that the assumptions adopted in the derivation of Eq. (2) are more likely to be justified than with binary mobile phases with variable concentration  $\varphi$ . From Eq. (2) it follows that the capacity factor in pure non-polar solvent  $k'_0 = 1/a$  and this equation can be rewritten as follows:

$$\frac{1}{k'} = \frac{1}{k'_0} \left(1 + \frac{b}{a}\varphi\right) \quad (5)$$

The right-hand term in the parentheses describes the diminishing effect of the concentration of the polar

solvent on the capacity factor in a binary solvent system. In ternary mobile phases, the simultaneous effects of the concentrations  $\varphi_1$  and  $\varphi_2$  of two polar solvents 1, 2 can be expressed in analogy with Eq. (5) as:

$$\frac{1}{k'} = \frac{1}{k'_0} \left(1 + \frac{b_1}{a_1} \varphi_1\right) \left(1 + \frac{b_2}{a_2} \varphi_2\right) = A + BX + CX^2 \quad (6)$$

where indices 1 and 2 relate to the parameters of Eq. (2) for solutes in binary mobile phases with polar solvents 1 and 2, respectively;  $A$ ,  $B$  and  $C$  are constants depending on the solute, the chromatographic system and  $\varphi_T$ . Here,  $X = \varphi_1/\varphi_T$  is the ratio of one polar solvent (the stronger of the two solvents can be selected as solvent 1) to the total concentration of the two polar solvents. Eq. (6) with a zero quadratic term can be derived from the addition of reciprocal capacity factors suggested by Martire [18] and Jaroniec [27,28], but we found a poor fit of such an equation to the experimental data.

The dependencies of the parameters  $A$ ,  $B$  and  $C$  of Eq. (6) on  $\varphi_T$  should be known to make possible a prediction of retention in ternary mobile phases with any combination of concentrations  $\varphi_1$  and  $\varphi_2$  of two polar solvents 1, 2. We tried various forms of such dependencies, from which a second-degree polynomial function of  $\log \varphi_T$  yielded the best fit to the set of experimental data with varying  $X = \varphi_1/\varphi_T$  and  $\varphi_T = \varphi_1 + \varphi_2$ . Introducing these empirical relationships with constants  $a_1, a_2, a_3, b_1, b_2, b_3, c_1, c_2$  and  $c_3$  depending on the solute and on the chromatographic system into Eq. (6), we obtain:

$$\begin{aligned} \frac{1}{k'} = & a_0 + a_1 \log \varphi_T + a_2 (\log \varphi_T)^2 \\ & + [b_0 + b_1 \log \varphi_T + b_2 (\log \varphi_T)^2] X \\ & + [c_0 + c_1 \log \varphi_T + c_2 (\log \varphi_T)^2] X^2 \end{aligned} \quad (7)$$

Eq. (7) requires determination of the individual coefficients by regression analysis of a large number of data, which is tedious and inconvenient. From the practical point of view it would be more useful if we could use the parameters of the retention equations in binary systems and as few experimental data in ternary systems as possible. Eq. (6) can be adapted to allow this approach, as we can determine its parameters  $A$ ,  $B$  and  $C$  from three experimental

values of  $k'$ . Two of these values can be selected to represent the data in binary mobile phases with the concentrations  $\varphi$  equal to the concentration  $\varphi_T$  in the ternary mobile phase,  $k'_1$  at  $X=0$  and  $k'_2$  at  $X=1$ , and can be calculated from the appropriate retention equations for binary mobile phases, e.g. Eq. (1) or Eq. (3). Only one experimental value,  $k'_3$ , remains to be measured in a ternary solvent system at a known concentration ratio  $X$ . Introducing  $k'_1$ ,  $k'_2$  and  $k'_3$  into Eq. (6), we obtain a set of three equations, from which we can calculate the parameters  $A$ ,  $B$  and  $C$  that should be known to make possible using Eq. (6) for the prediction of retention in various ternary solvent systems:

$$A = \frac{1}{k'_1} \quad (8)$$

$$B = \frac{1}{k'_3 X(1-X)} - \frac{1+X}{k'_1 X} - \frac{X}{k'_2(1-X)} \quad (9)$$

$$C = \frac{1}{k'_1 X} + \frac{1}{k'_2(1-X)} - \frac{1}{k'_3 X(1-X)} \quad (10)$$

### 3. Experimental

A HP 1090M liquid chromatograph equipped with a UV diode-array detector operated at 230 nm, an automatic sample injector, a DR5 solvent delivery system, a thermostatted column compartment and a Series 7994A workstation (Hewlett-Packard, Palo Alto, CA, USA) was used to acquire the elution data. Glass cartridge columns, 150 mm  $\times$  3.3 mm I.D., packed with silica-gel Separon SGX, 7.5  $\mu$ m were obtained from Tessek, Prague, Czech Republic. The flow-rate of the mobile phases was kept at 1 ml/min and the temperature at 40°C in all experiments.

2-Propanol, *n*-heptane and dioxane, all of HPLC grade, were purchased from Baker (Deventer, The Netherlands). The solvents were dried and kept in tightly closed dark bottles over molecular sieve beads Dusimo 5 Å (Lachema, Brno, Czech Republic), previously activated at 300°C (ca. 30–40 g/l). The solvents were filtered using a Millipore 0.45  $\mu$ m filter and degassed by ultrasonication before use. Mobile phases were either pre-mixed in the required volume ratios or were prepared directly in the HP 1090M instrument from the components

Table 1  
Compounds studied

I. Phenylurea herbicides	II. Phenols
1: Diuron	12: <i>o</i> -Cresol
2: Chlortoluron	13: <i>m</i> -Cresol
3: Fluometuron	14: <i>p</i> -Cresol
4: Desphenuron	15: 2,3-Dimethylphenol
5: Metoxuron	16: 2,5-Dimethylphenol
6: Linuron	17: 2,4-Dimethylphenol
7: Deschlormetoxuron	18: 3-Phenylphenol
8: Isoproturon	19: 2-Phenylphenol
9: Monuron	20: 2-Nitrophenol
10: Chlorbromuron	21: 3-Nitrophenol
11: Neburon	22: 4-Nitrophenol
	23: 3-Methyl-2-nitrophenol
	24: 4-Methyl-3-nitrophenol
	25: 2-Methyl-5-nitrophenol
	26: 2,5-Dinitrophenol
	27: 2,6-Dinitrophenol

continuously stripped by a stream of helium. Phenylurea herbicides, alkyl-, aryl- and nitrophenols sample compounds were obtained from Lachema and are listed in Table 1. The solutes were dissolved in the mobile phase to provide an adequate response of the UV detector. 5  $\mu$ l sample volumes were injected in each experiment.

The columns were first equilibrated with the mobile phase and then the retention volumes,  $V_R$ , of the sample compounds were measured at different mobile phase compositions. The mean values of  $V_R$  from three repeated experiments were used for calculations of the capacity factors,  $k' = (V_R/V_0 - 1)$ . Column dead volumes,  $V_0$ , were determined using trichloroethylene as the dead volume marker ( $V_0 = 0.905$  ml for the column used in experiments with phenylurea herbicides and  $V_0 = 0.94$  ml for the columns used for chromatography of phenols). An Adstat software package (Trilobyte, Prague, Czech Republic) and a 486 AT personal computer were used for linear and non-linear single- and multiple-parameter regression of the experimental sets of retention data in dependence on the concentrations of the mobile phase components.

#### 4. Results and discussion

To test the reproducibility of chromatography with

mixed binary or ternary mobile phases prepared from dehydrated solvents kept dry over molecular sieves before use we compared the retention volumes of several phenylurea herbicides in binary and ternary mobile phases prepared from dried solvents measured on a fresh silica-gel column with the data from the experiments repeated on the same column after 10 months of use. The results are shown in Table 2. The differences between the retention volumes measured in the experiments repeated in this way are lower than 0.25 ml, or 3%, and 3/4 of the repeated data show differences of less than 0.1 ml. This is a reproducibility of retention at least comparable to that usually achieved with most bonded phases.

Table 3 compares the experimental capacity factors of phenylurea herbicides in binary mobile phases 2-propanol-*n*-heptane and dioxane-*n*-heptane with the values calculated from the best-fit regression equations, Eqs. (1) and (3). Linear regression was used to fit the logarithmic form of Eq. (1) to the experimental data set, whereas non-linear regression was used to fit Eq. (3). With few exceptions, the data obtained from the three-parameter retention Eq. (3) differed from the experimental capacity factors by less than 0.1 units, or 2%, and for most compounds the fit is better than 1%.

Of the two two-parameter equations derived from theoretical models, Eq. (2) completely failed to describe the retention behaviour. The values of  $k'$  calculated using this equation fitted to the experimental data deviated from the experiment by 20–100% in mobile phases containing 5% or less polar solvent, and, therefore, are not given in Table 3. These differences demonstrate failure of the underlying model in the systems tested.

The fit of Eq. (1) to the retention data of phenols was comparable to the three-parameter Eq. (3) and the differences of the fitted data from the experimental capacity factors of substituted phenylureas were  $\leq 0.2$ , or 5%, in mobile phases with 20% or more 2-propanol and with 40% or more dioxane. At lower concentrations of polar solvents the three-parameter Eq. (3) described the experimental data significantly better than the two-parameter Eq. (1). The dependencies of the capacity factors of several phenylurea herbicides on the concentration (% v/v) of 2-propanol in *n*-heptane,  $\phi$ , are demonstrated on a logarithmic scale in Fig. 1, and Fig. 2 shows similar plots

Table 2

Comparison of the retention volumes,  $V_R$ , of phenylurea herbicides measured on a Separon SGX, 7  $\mu\text{m}$ , 150 $\times$ 3.3 mm I.D., silica-gel column in binary and ternary mobile phases

Mobile phase	$V_R$ (ml)	Solute						
		1	2	5	6	8	10	11
I	1	2.96	2.65	4.66	1.48	2.82	1.77	–
	2	2.97	2.63	4.49	1.52	2.83	1.52	–
II	1	4.70	4.03	8.19	1.85	4.38	1.95	1.77
	2	4.77	4.08	8.23	1.94	4.38	1.96	1.83
III	1	9.62	7.91	19.08	2.80	8.45	2.80	2.84
	2	9.52	7.94	18.52	2.79	8.61	2.87	2.92
IV	1	2.72	2.52	3.63	–	2.45	–	–
	2	2.59	2.36	3.68	–	2.44	–	–
V	1	4.69	3.88	6.70	2.12	3.76	2.10	1.91
	2	4.68	3.90	6.67	2.12	3.80	2.13	1.91
VI	1	4.05	3.49	5.78	1.95	3.34	2.03	1.72
	2	4.02	3.51	5.84	2.08	3.43	2.07	1.73
VII	1	6.43	5.03	9.75	2.23	4.92	2.28	2.29
	2	6.43	5.17	9.83	2.26	5.15	2.40	2.32

The phases contained 40% dioxane (I), 30% dioxane (II), 20% dioxane (III), 30% 2-propanol (IV), 10% 2-propanol+10% dioxane (V), 16% 2-propanol+4% dioxane (VI) and 4% 2-propanol+16% dioxane (VII) in *n*-heptane.

$V_R$ : 1=original retention volumes, 2=retention volumes measured on the same column after 10 months of use.

Conditions: temperature 40°C, flow-rate 1 ml/min, dried solvents.

Numbers of compounds as in Table 1.

obtained with binary mobile phases dioxane–*n*-heptane. The plots are close to straight lines, in agreement with Eq. (2), but slight curvature is apparent for some dependencies at low concentrations of polar solvents in binary mobile phases. These deviations from linearity are accounted for by Eq. (3). Furthermore, significant changes in selectivity of separation are apparent as  $\varphi$  is changed, even connected with the opposite order of elution of metoxuron (compound 4) and isoproturon (compound 8) in mobile phases with low and high concentrations of propanol.

In Table 4, a similar comparison of the experimental and calculated capacity factors is given for alkyl-, aryl- and nitrophenols in mobile phases with various concentrations of 2-propanol in *n*-heptane. Except for seven values calculated from Eq. (1) and two values calculated from Eq. (3), the differences between the experimental capacity factors and the data calculated from best-fit equations are lower than 0.1 ml. For these compounds, the two-parameter Eq. (1) describes well the retention behaviour, and the three-parameter Eq. (3) offers a slight advantage only in mobile phase containing 1% 2-propanol. The retention behaviour in binary mobile phases diox-

ane–*n*-heptane (not shown here) is similar to that in propanolic systems.

To investigate the retention behaviour in ternary mobile phases, retention volumes and capacity factors of phenylurea herbicides and phenols were measured in mixtures containing various concentrations of 2-propanol and dioxane in *n*-heptane (Tables 5 and 6). First, the validity of Eq. (4) was tested in mobile phases at three different constant ratios of the concentrations of 2-propanol to dioxane in the ternary mobile phase,  $\varphi_1/\varphi_2$ : 1:1, 1:4 and 4:1. For each constant concentration ratio, the sum of the concentrations of the two polar solvents in the ternary phase,  $\varphi_T = \varphi_1 + \varphi_2$ , was changed from 3 to 20% and Eq. (4) was fitted to the experimental dependencies of  $\log k'$  versus  $\log \varphi_T$  using linear regression. Fig. 3 shows several examples of the plots for phenols in ternary mobile phases with a 1:1 concentration ratio of 2-propanol to dioxane. Most plots are very close to straight lines, suggesting good validity of Eq. (4) in the systems studied, similar to the validity of the analogous Eq. (1) in the binary mobile phases studied. Furthermore, important selectivity changes are apparent as the sum of the

Table 3

Comparison of the experimental and the best-fit values of the capacity factors,  $k'$ , of phenylurea herbicides measured on a Separon SGX, 7  $\mu\text{m}$ ,  $150 \times 3.3$  mm I.D., silica-gel column in binary mobile phases

Mobile phase	$k'$	Solute								
		2	3	4	5	6	7	8	9	10
I	$k'_e$	1.60	1.64	1.37	3.06	–	3.44	1.70	1.86	–
	$k'_1$	1.70	1.72	1.40	3.26	–	3.61	1.80	1.94	–
	$k'_2$	1.63	1.66	1.38	3.10	–	3.47	1.81	1.88	–
II	$k'_e$	3.09	3.25	2.85	6.53	1.35	7.01	3.22	3.67	1.39
	$k'_1$	3.02	3.13	2.79	6.19	1.60	6.76	3.15	3.57	1.47
	$k'_2$	3.05	3.17	2.80	6.37	1.47	6.89	3.23	3.64	1.51
III	$k'_e$	8.55	9.10	9.14	19.57	2.63	20.82	8.70	10.63	2.61
	$k'_1$	8.03	8.75	9.00	18.56	2.66	19.76	8.14	10.17	2.49
	$k'_2$	8.56	9.11	9.15	19.92	2.51	20.85	8.51	10.64	2.54
IV	$k'_e$	21.93	23.64	28.62	53.28	4.54	55.53	21.37	27.97	4.40
	$k'_1$	21.38	24.46	29.07	55.67	4.40	57.71	21.09	28.92	4.21
	$k'_2$	21.93	23.64	28.62	53.00	4.27	55.53	21.47	27.97	4.25
V	$k'_e$	–	–	–	–	6.36	–	40.57	–	6.33
	$k'_1$	–	–	–	–	6.39	–	42.50	–	6.20
	$k'_2$	–	–	–	–	6.30	–	40.55	–	6.21
VI	$k'_e$	1.19	1.04	3.30	2.27	0.41	2.95	1.30	1.40	0.41
	$k'_1$	1.25	1.09	3.37	2.33	0.50	3.03	1.36	1.47	0.56
	$k'_2$	1.19	1.05	3.28	2.26	0.44	2.98	1.32	1.40	0.44
VII	$k'_e$	1.91	1.68	5.69	3.96	0.68	5.03	2.12	2.30	0.68
	$k'_1$	1.92	1.70	5.68	3.93	0.68	4.88	2.11	2.29	0.76
	$k'_2$	1.92	1.69	5.77	4.00	0.65	4.93	2.10	2.28	0.66
VIII	$k'_e$	3.51	3.19	11.66	8.09	1.14	9.09	3.84	4.22	1.17
	$k'_1$	3.36	3.00	11.12	7.72	1.02	9.03	3.72	4.06	1.13
	$k'_2$	3.48	3.09	11.52	8.03	1.07	9.22	3.81	4.21	1.09
IX	$k'_e$	7.77	6.84	27.91	19.47	2.08	21.26	8.51	9.59	2.17
	$k'_1$	7.38	6.69	28.66	20.02	1.82	21.49	8.26	9.11	1.97
	$k'_2$	7.80	7.01	27.98	19.50	2.07	21.19	8.60	9.64	2.12
X	$k'_e$	27.13	25.54	–	–	5.38	–	31.46	34.75	5.44
	$k'_1$	28.30	26.37	–	–	4.88	–	32.36	36.23	5.06
	$k'_2$	27.11	25.43	–	–	5.54	–	31.39	34.71	5.74
XI	$k'_e$	–	–	–	–	11.31	–	–	–	11.99
	$k'_1$	–	–	–	–	13.07	–	–	–	13.04
	$k'_2$	–	–	–	–	11.91	–	–	–	12.44

The mobile phases contained 30% (I), 20% (II), 10% (III), 5% (IV), 3% (V) 2-propanol, 50% (VI), 40% (VII), 30% (VIII), 20% (IX), 10% (X) and 5% (XI) dioxane in *n*-heptane.

$k'_e$  = experimental values;  $k'_1$  = calculated using Eq. (1);  $k'_2$  = calculated using Eq. (3).

Conditions: temperature 40°C, flow-rate 1 ml/min, dried solvents.

Numbers of compounds as in Table 1.

concentrations of the two polar solvents,  $\varphi_T$ , is changed in a ternary mobile phase at a constant concentration ratio  $\varphi_1/\varphi_2$ . Only six values of 216 differences between the experimental capacity factors and  $k'$  calculated from the best-fit Eq. (4) exceed 0.2, which proves the suitability of this equation in describing the retention behaviour in ternary mobile phases with constant concentration ratios of polar solvents.

The same set of experimental data was used to test the validity of Eq. (6) at four different constant sums of concentrations,  $\varphi_T$ , of 2-propanol and dioxane (20, 10, 5 and 3%). For each sum, Eq. (6) was fitted using non-linear regression to experimental dependencies of  $1/k'$  on concentration ratio  $X = \varphi_1/\varphi_T$  varying from 0 to 0.2, 0.5, 0.8 and 1.0. Fig. 4 shows several examples of the plots of phenylurea herbicides in ternary mobile phases at total concen-

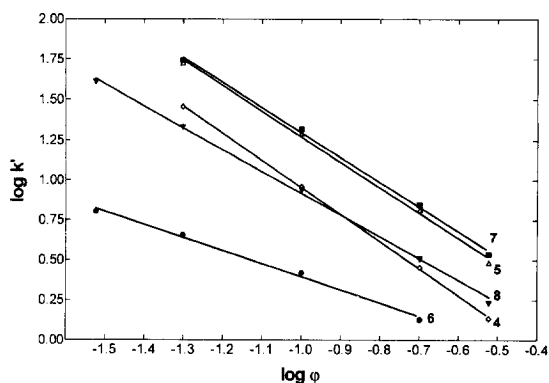


Fig. 1. Dependence of capacity factors,  $k'$ , of phenylurea herbicides on the concentration of 2-propanol,  $\varphi$  (%  $v/v \times 10^{-2}$ ), in  $n$ -heptane. Numbers of plots agree with the numbers of compounds in Table 1. Points = experimental data; lines = best-fit dependencies according to Eq. (1).

trations of 20% 2-propanol + dioxane, while Fig. 5 illustrates the retention behaviour of several phenols in mobile phases with the sum of concentrations equal to 10%. For some compounds, minima or maxima of retention were observed at certain concentration ratios of the two polar solvents and for many sample compounds important changes in the separation selectivity and even in the order of elution occur as the ratio  $X$  is changed. Eq. (6) fits well the experimental data as only a few values calculated from the best-fit equations differ from the experimental capacity factors by more than 0.15 (ex-

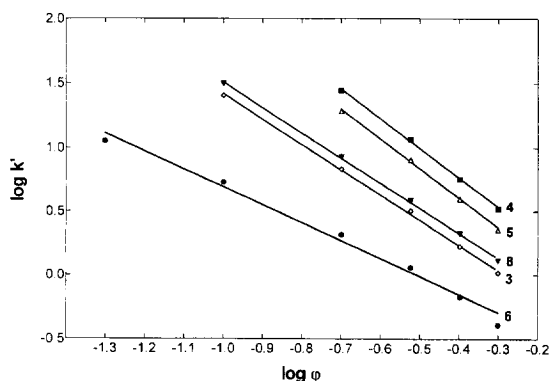


Fig. 2. Dependence of capacity factors,  $k'$ , of phenylurea herbicides on the concentration of dioxane,  $\varphi$  (%  $v/v \times 10^{-2}$ ), in  $n$ -heptane. Numbers of plots agree with the numbers of compounds in Table 1. Points = experimental data; lines = best-fit dependencies according to Eq. (1).

cept for slightly worse agreement in mobile phase containing 1% 2-propanol and 4% dioxane).

After verifying the validity of Eqs. (4,6) in ternary mobile phases with either constant concentration ratios or a concentration sum of polar solvents, we investigated the possibilities of describing the retention in ternary mobile phases where the concentrations of the two polar solvents,  $\varphi_1$  and  $\varphi_2$ , are changed independently of each other. This is equivalent to the situation where both the sum  $\varphi_T$  and the ratio  $X$  of the concentrations of the two polar solvents are variable. It should be noted that  $\varphi_T$  and  $X$  are independent of each other, like the concentrations of the individual polar solvents in the ternary mobile phase,  $\varphi_1$  and  $\varphi_2$ , and are used because of the convenience of describing the solvent strength and the selectivity in ternary mobile phases. To find a suitable equation describing the retention in a ternary solvent system over a broad concentration range of the concentrations of its individual components, a second-degree polynomial equation was fitted using non-linear regression to the dependencies of the coefficients  $A$ ,  $B$  and  $C$  in Eq. (6) on the logarithm of the concentration sum,  $\varphi_T$ . Eq. (7) with best-fit parameters determined in this way was used to calculate the capacity factors of sample compounds. Calculated values are compared in Table 5 with experimental capacity factors. Of 108 calculated values, only 8 differ by more than 0.2 from the experimental  $k'$ , with a maximum difference corresponding to 0.37, which is 5% of the retention time.

The nine-parameter Eq. (7) may seem too complex to describe the retention behaviour in ternary solvent systems. We investigated the possibilities of its simplification and found that in some specific cases one or more second-degree terms in this equation can be neglected, but, generally, this neglect leads to poorer agreement with the experiment. Eq. (7) is an empirical equation and for such an equation it usually applies that the more parameters, the better the fit to the experimental data. On the other hand, a high number of parameters necessitates a large amount of experimental data to be collected and is more liable to be affected by experimental errors. This means that this equation gives a good description of the retention data in normal-phase ternary systems tested, but would not be very practical for prediction of retention. For this reason,



Table 4

Comparison of the experimental and the best-fit values of capacity factors,  $k'$ , of phenols measured on a Separon SGX, 7  $\mu\text{m}$ , 150 $\times$ 3.3 mm I.D., silica-gel column in binary mobile phases

Solute	Mobile phase											
	I			II			III			IV		
	$k'_e$	$k'_1$	$k'_2$	$k'_e$	$k'_1$	$k'_2$	$k'_e$	$k'_1$	$k'_2$	$k'_e$	$k'_1$	$k'_2$
12	0.58	0.60	0.58	1.03	1.07	1.01	1.50	1.51	1.50	3.20	3.14	3.18
13	0.57	0.59	0.57	1.14	1.20	1.13	1.86	1.83	1.82	4.54	4.51	4.50
14	0.65	0.65	0.66	1.24	1.26	1.17	1.72	1.89	1.80	4.87	4.54	4.86
15	0.57	0.60	0.57	1.00	1.03	0.98	1.45	1.40	1.41	2.71	2.68	2.67
16	0.53	0.55	0.53	0.89	0.96	0.90	1.36	1.33	1.32	2.68	2.64	2.67
17	0.60	0.63	0.61	1.18	1.26	1.15	1.81	1.82	1.79	4.15	4.02	4.10
18	0.57	0.61	0.58	1.23	1.14	1.16	1.79	1.83	1.88	4.84	5.03	4.81
19	0.47	0.45	0.47	0.69	0.67	0.67	0.84	0.90	0.87	1.73	1.67	1.73
20	0.22	0.21	0.21	0.23	0.24	0.24	0.24	0.26	0.25	0.31	0.31	0.31
21	0.84	0.82	0.83	1.58	1.64	1.64	2.80	2.72	2.72	8.23	8.16	8.26
22	1.16	1.15	1.15	2.47	2.54	2.55	4.73	4.56	4.58	–	–	–
23	0.43	0.47	0.43	0.65	0.60	0.62	0.69	0.73	0.76	0.98	1.08	1.00
24	0.74	0.78	0.77	1.87	1.69	1.70	3.02	3.00	3.03	10.32	10.25	10.11
25	0.51	0.50	0.52	1.08	1.04	1.02	1.67	1.77	1.71	5.99	5.64	5.97
26	0.41	0.42	0.41	0.59	0.56	0.56	0.66	0.70	0.70	1.10	1.11	1.10
27	1.37	1.33	1.36	2.05	2.03	2.02	2.56	2.77	2.73	5.49	5.42	5.57

The phases contained 10% 2-propanol (I), 5% 2-propanol (II), 3% 2-propanol (III) and 1% 2-propanol (IV) in *n*-heptane.

$k'_e$  = experimental values;  $k'_1$  = calculated using Eq. (1);  $k'_2$  = calculated using Eq. (3).

Conditions: temperature 40°C, flow-rate 1 ml/min, dried solvents.

Numbers of compounds as in Table 1.

we were investigating the possibility of using retention data measured (or predicted from best-fit equations) in binary mobile phases to predict the retention behaviour in ternary mobile phases. All attempts to get reasonably accurate prediction of retention in ternary mobile phases from the data measured only in binary solvent systems failed, and, therefore, we tried to combine the binary retention data with as few as possible experimental values measured in ternary mobile phases for this purpose. As shown in the theoretical part, Eq. (6) combined with Eqs. (8–10) suggests the possibility of predicting the retention in ternary mobile phases from three experimental capacity factors only, one determined in a ternary mobile phase and the others in two binary mobile phases, provided the elution strength, i.e., the sum of the concentrations of the polar solvents in the ternary mobile phase, is constant and equal to the concentration of polar solvents in the individual binary mobile phases.

The approach using two experimental capacity factors in binary mobile phases together with a single experimental value of  $k'$  determined in a ternary

mobile phase to predict the retention in other ternary phases with Eq. (6) and parameters  $A$ ,  $B$  and  $C$  calculated from Eqs. (8–10) is illustrated by several examples in Table 6 for phenylurea herbicides and in Table 7 for phenols. Here, the experimental and the calculated  $k'$  in binary mobile phases 2-propanol–*n*-heptane and dioxane–*n*-heptane are listed in columns I, II, VI and VII, together with the experimental values of the capacity factors in ternary mobile phases with concentration ratio 1:1 2-propanol–dioxane (columns III and VIII). Predicted capacity factors in ternary mobile phases with other concentration ratios, 4:1 and 1:4 are compared with experimental values in columns IV and V (phenylurea herbicides) and IX and X (phenols). It should be noted that the data in these four columns have not been used in prediction and represent an independent test set of  $k'$  values.

The binary capacity factors used to predict ternary data were calculated using three-parameter Eq. (3) for phenylurea herbicides and two-parameter Eq. (1) for phenols. As could be expected, the agreement between the predicted and the experimental capacity

Table 5

Comparison of the capacity factors,  $k'$ , of phenols measured on a Separon SGX, 7  $\mu\text{m}$ , 150 $\times$ 3.3 mm I.D., silica-gel column in ternary mobile phases

Mobile phase	$k'$	Solute							
		12	15	16	17	23	25	26	27
I	$k'_e$	0.39	0.35	0.34	0.41	0.51	0.44	0.47	1.28
	$k'_c$	0.39	0.35	0.34	0.41	0.52	0.44	0.47	1.28
II	$k'_e$	0.29	0.26	0.24	0.30	0.38	0.31	0.34	1.12
	$k'_c$	0.29	0.26	0.25	0.30	0.37	0.31	0.34	1.13
III	$k'_e$	0.61	0.56	0.53	0.67	0.83	0.77	0.77	1.73
	$k'_c$	0.62	0.57	0.54	0.67	0.78	0.79	0.77	1.76
IV	$k'_e$	0.76	0.69	0.67	0.83	0.76	0.85	0.71	2.14
	$k'_c$	0.74	0.67	0.64	0.81	0.79	0.82	0.72	2.08
V	$k'_e$	0.55	0.50	0.47	0.60	0.54	0.58	0.51	1.69
	$k'_c$	0.58	0.53	0.50	0.62	0.56	0.59	0.52	1.61
VI	$k'_e$	1.22	1.14	1.08	1.42	1.11	1.61	1.28	3.22
	$k'_c$	1.17	1.06	1.02	1.33	1.20	1.50	1.26	3.22
VII	$k'_e$	1.45	1.30	1.25	1.71	0.95	1.72	1.03	3.11
	$k'_c$	1.56	1.39	1.34	1.86	1.11	1.75	1.07	3.20
VIII	$k'_e$	1.04	0.94	0.90	1.20	0.69	1.13	0.70	2.17
	$k'_c$	1.27	1.18	1.17	1.48	0.79	1.24	0.73	2.27
IX	$k'_e$	2.64	2.42	2.17	3.16	1.46	3.58	1.82	5.93
	$k'_c$	2.50	2.29	2.16	3.12	1.66	3.46	1.98	5.80
X	$k'_e$	2.40	2.22	2.10	3.10	1.19	2.99	1.28	3.94
	$k'_c$	2.31	2.04	1.98	2.95	1.21	2.83	1.24	3.67
XI	$k'_e$	1.82	1.59	1.56	2.25	0.82	1.94	0.82	2.73
	$k'_c$	1.63	1.48	1.42	2.01	0.87	1.92	0.81	2.80
XII	$k'_e$	4.03	3.97	3.57	5.47	1.67	6.68	2.44	7.20
	$k'_c$	3.86	3.79	3.46	5.13	1.74	6.38	2.37	6.83

The phases contained 10% 2-propanol+10% dioxane (I), 16% 2-propanol+4% dioxane (II), 4% 2-propanol+16% dioxane (III), 5% 2-propanol+5% dioxane (IV), 8% 2-propanol+2% dioxane (V), 2% 2-propanol+8% dioxane (VI), 2.5% 2-propanol+2.5% dioxane (VII), 4% 2-propanol+1% dioxane (VIII), 1% 2-propanol+4% dioxane (IX), 1.5% 2-propanol+1.5% dioxane (X), 2.4% 2-propanol+0.6% dioxane (XI) and 0.6% 2-propanol+2.4% dioxane (XII) in *n*-heptane.

$k'_e$  = experimental values;  $k'_c$  = calculated using Eq. (7).

Conditions: temperature 40°C, flow-rate 1 ml/min, dried solvents.

Numbers of compounds as in Table 1.

factors is slightly less good than with the values predicted from the best-fit retention Eq. (7), but still it demonstrates the practical feasibility of this approach in predicting the retention in ternary normal-phase systems. Most differences between the predicted and the experimental  $k'$  are lower than 0.2; one fourth of the predicted values in Tables 6 and 7 exceeds this value, with a prediction error of 4–10%. It should be noted that the retention maximum of 3-methyl-2-nitrophenol (compound 23) in the mobile phase X was accurately predicted from lower values in mobile phases VI–VIII. In our opinion, the prediction error of this approach would be in most cases acceptable for practical method development. If necessary, the accuracy of prediction can be

further improved by measuring an additional experimental  $k'$  value in another ternary mobile phase to correct originally predicted values.

## 5. Conclusions

Using dry organic solvents in normal-phase chromatography, reproducible retention data may be obtained over a long period of column use. The retention in binary mobile phases comprised of solvents with different polarities can be accurately described by a three-parameter retention equation. In many instances, a two-parameter retention equation based on the Snyder–Soczewinski model of retention

Table 6

Comparison of experimental capacity factors,  $k'_e$ , of phenylurea herbicides measured on a Separon SGX, 7  $\mu\text{m}$ , 150  $\times$  3.3 mm I.D., silica-gel column in ternary mobile phases IV and V with  $k'_e$  calculated using Eqs. (6,8–10) from  $k'_c$  in binary mobile phases I and II and  $k'_c$  in ternary mobile phase III

Solute	$k'$	Mobile phase				
		I	II	III	IV	V
1	$k'_e$	3.62	9.52	4.17	3.45	6.11
	$k'_c$	3.42	9.65	–	3.55	5.95
2	$k'_e$	3.09	7.77	3.31	2.88	4.71
	$k'_c$	3.05	7.80	–	2.98	4.64
5	$k'_e$	6.53	19.47	6.40	5.45	9.86
	$k'_c$	6.37	19.50	–	5.91	9.45
6	$k'_e$	1.35	2.08	1.35	1.31	1.60
	$k'_c$	1.48	2.07	–	1.35	1.60
7	$k'_e$	7.01	21.26	6.19	5.34	9.77
	$k'_c$	6.76	21.19	–	5.97	9.21
8	$k'_e$	3.22	8.61	3.19	2.79	4.69
	$k'_c$	3.23	8.60	–	2.98	4.57
10	$k'_e$	1.39	2.17	1.35	1.29	1.65
	$k'_c$	1.51	2.12	–	1.36	1.61

The phases contained 16% 2-propanol+4% dioxane (IV), 4% 2-propanol+16% dioxane (V), in *n*-heptane, 20% 2-propanol (I) and 20% dioxane (II) in *n*-heptane, and 10% 2-propanol+10% dioxane in *n*-heptane (III).

$k'_c$  in binary mobile phases I and II were predicted from Eq. (3). Numbers of compounds as in Table 1.

can be used for characterization of retention without significant loss of accuracy.

Simple two- or three-parameter equations can be

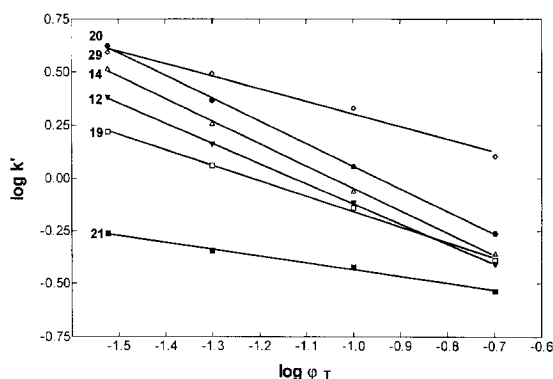


Fig. 3. Dependence of capacity factors,  $k'$ , of phenols on the sum of concentrations of 2-propanol and dioxane,  $\varphi_T$  (%), in *n*-heptane at the constant concentration ratio of the two polar solvents 1:1. Numbers of plots agree with the numbers of compounds in Table 1. Points = experimental data; lines = best-fit dependencies according to Eq. (4).

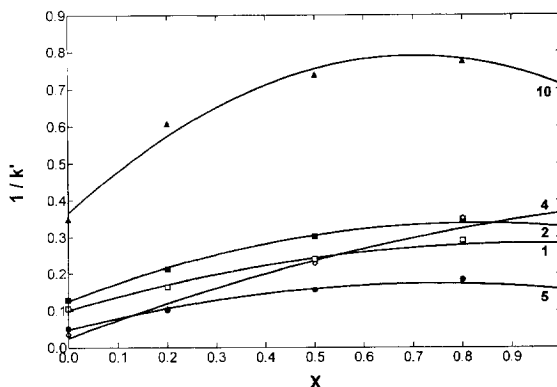


Fig. 4. Dependence of capacity factors,  $k'$ , of phenylurea herbicides on the concentration ratio  $X$  of 2-propanol,  $X = \varphi/\varphi_T$  ( $\varphi$  in %,  $v/v \times 10^{-2}$ ), in ternary mobile phases 2-propanol–dioxane–*n*-heptane at the constant sum of concentrations of the two polar solvents, 20% vol. Numbers of plots agree with the numbers of compounds in Table 1. Points = experimental data; lines = best-fit dependencies according to Eq. (6).

used to characterize the retention in ternary mobile phases comprised of two polar solvents and a non-polar one, provided that either the concentration ratio of the two polar solvents is kept constant and the sum of these concentrations is changed, or that this ratio is varied at a constant sum of the two concentrations. A nine-parameter equation can describe the retention in ternary mobile phases where both the

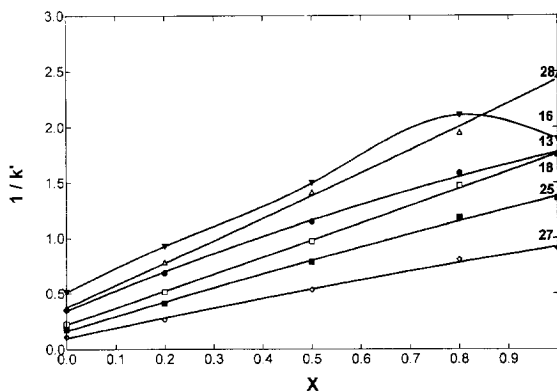


Fig. 5. Dependence of capacity factors,  $k'$ , of phenols on the concentration ratio  $X$  of 2-propanol,  $X = \varphi/\varphi_T$  ( $\varphi$  in %,  $v/v \times 10^{-2}$ ), in ternary mobile phases 2-propanol–dioxane–*n*-heptane at the constant sum of concentrations of the two polar solvents, 10% ( $v/v$ ). Numbers of plots agree with the numbers of compounds in Table 1. Points = experimental data; lines = best-fit dependencies according to Eq. (6).

Table 7

Comparison of experimental capacity factors,  $k'_e$ , of phenols measured on a Separon SGX, 7  $\mu\text{m}$ , 150 $\times$ 3.3 mm I.D., silica-gel column in ternary mobile phases IX and XV with  $k'_c$  calculated using Eqs. (6,8–10) from  $k'_c$  in binary mobile phases VI and VII and  $k'_c$  in ternary mobile phase VIII

Solute	$k'$	Mobile phase				
		VI	VII	VIII	IX	X
18	$k'_e$	1.23	9.86	2.07	1.31	4.08
	$k'_c$	1.15	10.34	–	1.40	3.99
23	$k'_e$	0.23	1.26	0.44	0.69	1.46
	$k'_c$	0.24	1.34	–	0.70	1.51
25	$k'_e$	1.08	9.22	1.72	1.13	3.58
	$k'_c$	1.04	9.04	–	1.21	3.2
26	$k'_e$	0.59	4.51	1.03	0.70	1.82
	$k'_c$	0.56	4.81	–	0.69	1.98

The phases contained 4% 2-propanol+1% dioxane (IX) and 1% 2-propanol+4% dioxane (X) in *n*-heptane, 5% 2-propanol (VI) and 5% dioxane (VII) in *n*-heptane, 2.5% 2-propanol+2.5% dioxane in *n*-heptane (VIII).

$k'_c$  in binary mobile phases VI and VII were predicted from Eq. (1).

Numbers of compounds as in Table 1.

ratio and the sum of concentrations of the two polar solvents are variable. This equation is not very suitable for prediction of retention in various ternary solvent mixtures, but this is possible using two capacity factors determined in binary mobile phases with each of the polar solvents and the retention measured at least at one composition of the ternary systems, if the sum of the concentrations of polar solvents is kept constant during this activity.

Application of the approaches described here in normal-phase systems with polar bonded-phase columns is currently being investigated. Furthermore, the description of retention in isocratic solvent systems can be used for predicting retention in gradient elution. The accuracy of prediction can be affected by the preferential sorption of the more polar solvent on the column packed with a polar adsorbent. The extent of errors caused by this effect and possible ways of eliminating or suppressing them are discussed elsewhere [29]. Finally, appropriate model equations for the description of retention as a function of the composition of binary and ternary mobile phases can be used for predictive optimization of isocratic or gradient-elution separation in these systems.

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