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Chromatographic behaviour of phenylurea pesticides in high-performance liquid chromatography with nitrile- and amino-bonded stationary phases

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Abstract

The retention of phenylurea pesticides on nitrile- and amino-bonded stationary phases was measured over a broad composition range of 2-propanol-*n*-hexane and 2-propanol-water mobile phases. Minima of retention were observed on the nitrile phase in mobile phases containing 80–90% 2-propanol in water. In organic mobile phases, the plots of $\log k'$ versus the concentration of 2-propanol in *n*-hexane are significantly curved. This behaviour can be attributed to combined effects of polar and hydrophobic interactions as the driving force of the retention.

Simple two-parameter equations can be used to first-approximation description of retention on the nitrile bonded phase in mobile phases containing up to 50–60% 2-propanol in water and on the two bonded phases in mobile phases containing up to 60–80% 2-propanol in *n*-hexane. Four-parameter equations taking into account mixed mechanism of retention involving polar and hydrophobic interactions can be used to describe the retention of phenylurea compounds over the whole composition range of organic and aqueous-organic mobile phases and to allow prediction of retention and optimization of separation.

The retention of phenylurea pesticides on the amino-bonded phase in mobile phase containing 2-propanol in water is too low to allow chromatographic separation of the compounds tested. The retention in aqueous-organic mobile phases is higher on the nitrile- than on the amino-bonded phase, but the selectivity of separation is unsatisfactory for most phenylurea pesticides. Most compounds tested can be separated in 2-propanol-*n*-hexane mobile phases, but significant differences in selectivities and even in the order of elution were observed between the two bonded phases.

1. Introduction

The retention in normal-phase systems is controlled primarily by polar interactions of sample solutes with the adsorbent. Amino and nitrile stationary phases bonded on a silica gel support are less polar than non-modified (“naked”) silica gel or alumina adsorbents. The functional groups

are usually bonded on to the surface of the silica gel support by means of a short hydrocarbonaceous spacer, most often comprising three carbon atoms (aminopropyl or cyanopropyl phases). The spacer is not only responsible for the mobility of the bonded groups with respect to the surface of the silica gel [1,2], but because of the non-polar character it gives rise to possible retention of the solute by a hydrophobic (lipophilic) mechanism, especially in strongly polar mobile phases. In

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addition, the bonded chains are not long enough to provide efficient shielding of the residual silanol groups, which could not be modified in the silanization procedure because of steric reasons. In addition to the aminopropyl or cyanopropyl groups, the residual silanols may participate in polar interactions with sample solutes and contribute to the retention in non-aqueous mobile phases [3,4]. Weisser et al. [5] even assume a predominant role of the residual silanol groups in the retention on cyanopropyl bonded phases in the mobile phases with low polarities.

In organic mobile phases, the energy of interaction of the solutes and of the mobile phase with the chemically bonded amino and nitrile stationary phases is weaker and the solutes are usually less strongly retained than on the "naked" silica gel or alumina. Consequently, the interactions between the solutes and the mobile phase, which are often neglected in the description of retention on the columns packed with silica gel or alumina, become more important when polar chemically bonded stationary phases are used.

Because of different acidobasic properties of the amino group and of the silanols, the selectivity of separation on the bonded amino stationary phase differs from that on the unmodified silica gel [6,7]. This holds true also for cyanopropyl bonded phases, which can demonstrate either weakly basic (e.g. in chloroform) or weakly acidic (e.g. in methyl-*tert*-butyl ether) properties in dependence on the nature of the components of the mobile phase [8].

To describe the retention on cyanopropyl and aminopropyl bonded phases, the competitive model of adsorption can be used. This model yields, with some simplification, Eq. 1 describing the dependence of the retention (capacity factor, k') on the mole fraction of the polar solvent, N_b , in a binary mobile phase comprised of a polar solvent and a non-polar one [1,9]:

$$\log k' = \text{constant} - n \log N_b \quad (1)$$

In this equation, n is the constant giving the ratio of the molecular area on the adsorbent surface occupied by one molecule of the sample solute to that occupied by one molecule of the

polar solvent. The concentration can often be expressed as volume fraction, φ , instead of the mole fraction, for convenience sake [9]:

$$\log k' = \text{constant} - n \log \varphi \quad (2)$$

or

$$k' = k'_0 \varphi^{-n} \quad (3)$$

where $k'_0 = 10^{\text{constant}}$. Eq. 3 fails for φ equal or very close to 0.

Hennion et al. [10] suggested the retention model on amino-bonded phases, where the association of one to three molecules of a solvent on to the amino group is considered: low-polarity solvents associate one molecule and strongly polar alcohols three molecules to one polar adsorption site. Based on this model, they derived the following relationship for the retention in binary mobile phases:

$$1/k' = A' - B'\varphi + C'\varphi^2 \quad (4)$$

and in the low concentration range of strongly polar solvents [alcohols, up to 0.2% (v/v)], the quadratic term can be neglected:

$$1/k' = A' - B'\varphi \quad (5)$$

This relationship takes into account the adsorption of only one molecule of alcohol on an amino group and is formally identical with the equation proposed by Scott and Kucera [11]:

$$k' = \frac{1}{c + d\varphi} \quad (6)$$

A' , B' , C' , c and d are constants depending on the nature of the solute and of the polar solvent.

Further discussion of the retention on columns with bonded amino phases can be found in Ref. 12.

In reversed-phase systems, Eq. 7 is widely used to describe the dependence of the capacity factor on the composition of a binary aqueous-organic mobile phase [9,13]:

$$k' = a \cdot 10^{-b\varphi} \quad (7)$$

where φ is the concentration (volume fraction) of the organic solvent in the mobile phase and a and b are constants which depend on the nature of the solute and of the organic solvent.

The description of the retention as a function

of the mobile phase composition allows to predict the retention and the selectivity under changing operation conditions, which is useful for the optimization of separation.

The objective of this work was to investigate the retention behaviour of selected compounds (phenylurea herbicides) on columns packed with nitrile- and amino-bonded stationary phases in both non-aqueous and aqueous–organic two-component mobile phases.

Generally, stationary phases with polar groups bonded via a non-polar spacer onto the surface of silica gel exhibit reversed-phase behaviour in aqueous–organic mobile phases, where the retention is controlled mainly by lipophilic (hydrophobic) interactions and normal-phase behaviour in organic mobile phases, where the polar interactions between the solutes and the stationary phase are most important. However, if we gradually change the composition of the mobile phase from pure water via aqueous–organic mixtures to pure polar organic solvent and then via binary organic mixtures to the pure non-polar solvent, it would be unrealistic to expect a sharp reversal in retention mechanism exactly in the pure polar solvent—especially if we realize that different polar solvents with various polarities can be used as mobile phase components. Rather, different interactions of sample components with the stationary phase contribute simultaneously to the retention and one type of interactions is enhanced while the other is suppressed when the composition of the mobile phase is changed. Smooth transition between the predominating role of the two mechanisms results and the composition of the mobile phase where the two types of interactions are of equal magnitude is close to, but not necessarily exactly at 100% polar solvent. The retention of sample components is very low in the vicinity of this transition point and its location has practical impact on the selection of the range of mobile phase compositions for the optimization of separation.

We intended to determine the composition range where simple two-parameter equations such as Eqs. 3, 5 and 7 can be used to describe the retention and to find out whether deviations from these equations can be explained by a

mixed retention mechanism so that the curvatures of the k' versus φ plots can be characterized to allow accurate prediction of the retention data over a wide composition range of the mobile phases, which is necessary for successful optimization of separation.

2. Theoretical

Horváth and co-workers [14,15] proposed the following equation to describe the retention (k') in reversed-phase systems where the retention results from the combination of the non-polar and the polar mechanisms:

$$k' = \psi_1 K_1 + \psi_2 K_2 = k'_1 + k'_2 \quad (8)$$

where ψ_1 and ψ_2 are the phase ratios of the hydrocarbonaceous ligates and of the polar groups (silanols) in the column, respectively, K_1 is the equilibrium constant for the purely solvophobic retention and K_2 that for the polar (silanophilic) retention on the column. The capacity factor is here expressed as the sum of the solvophobic, k'_1 , and of the polar (silanophilic), k'_2 , contributions.

Using this approach to columns with polar bonded phases in aqueous–organic mobile phases and introducing Eqs. 3 and 7 for the contributions k'_1 and k'_2 , respectively, we obtain:

$$\begin{aligned} k' &= a \cdot 10^{-b\varphi_1} + k'_0 \varphi_2^{-n} \\ &= a \cdot 10^{-b\varphi_1} + k'_0 (1 - \varphi_1)^{-n} \end{aligned} \quad (9)$$

where φ_1 is the concentration of the less polar (organic) solvent and φ_2 the concentration of water in aqueous–organic mobile phases. Eq. 9 cannot be used for pure organic solvent as the mobile phase.

The first term in Eq. 9 decreases with increasing concentration of the organic solvent, φ_1 , in the mobile phase, while the second term increases. If the polar interactions contribute significantly to the retention, non-linear dependence of the logarithms of k' on φ_1 is to be expected and even minima on the log k' versus φ_1 plot may occur at higher concentrations of the organic solvent in the mobile phase [14,15].

Chemically bonded polar stationary phases are

more often used with organic than with aqueous–organic mobile phases. Mobile phases comprised of two organic solvents of different polarities are usually employed, three or more components are less frequent. In these mobile phases, polar interactions are of primary importance and the retention behaviour typical for normal-phase systems is observed, but the non-polar mechanism may contribute to the retention at higher concentrations of a more polar solvent in a less polar one. Eq. 8 can be applied to describe the combined effects of the two mechanisms on retention, like in aqueous–organic mobile phases. If φ_3 is the concentration of the more polar and φ_4 that of the less polar organic solvent in a binary mobile phase, combination of Eqs. 3, 7 and 8 results in the following retention equation, similar to Eq. 9:

$$k' = a \cdot 10^{-b\varphi_4} + k'_0 \varphi_3^{-n} \\ = a \cdot 10^{-b(1-\varphi_3)} + k'_0 \varphi_3^{-n} \quad (10)$$

Eq. 10 cannot be used for pure less polar organic solvent as the mobile phase.

The Eq. 10 is similar to Eq. 9 derived for aqueous–organic mobile phases. According to Eq. 10, the plots of the logarithms of k' versus the concentration of the polar solvent, φ_1 , are expected to deviate from straight lines at high φ_1 and possibly even retention minima may occur there.

3. Experimental

The equipment used included a Model 6000A pump, a U6K injector and a Model 440 UV detector, operated at 254 nm, all from Waters-Millipore (Milford, MA, USA). The detector signal was registered using a TZ 4200 line recorder (Laboratory Instrument Works, Prague, Czech Republic). The chromatographic columns were stainless steel, 300 × 4.2 mm I.D., packed in the laboratory with Silasorb SPH Nitrile, 7.5 μm and Silasorb SPH Amine, 7.5 μm (both from Lachema, Brno, Czech Republic), using a high-pressure slurry-packing technique. The chromatograms were measured on a HP 1090M liquid chromatograph (Hewlett-Packard, Avondale, PA, USA).

The column dead volume, V_M , was determined as the elution volumes of the pure components of the mobile phase (water in aqueous–organic mobile phases and *n*-hexane or 2-propanol in organic mobile phases), using a Model R401 refractometric detector (Waters) and the experimental values are given in Tables 2, 5 and 8.

The sample solutes (phenylurea herbicides, see Table 1), were obtained from East-Bohemian Chemical Works (Pardubice, Czech Republic) and were dissolved in 2-propanol prior to injection. The mobile phases were prepared by mixing water (double distilled in glass with addition of potassium permanganate) with 2-pro-

Table 1
Chemical structures of phenylurea herbicides and related compounds studied

Compound		Chemical structure			
No.	Name	R_1	R_2	R_3	R_4
1	Phenuron	-CH ₃	-CH ₃	-	-
2	Desphenuron	-CH ₃	-H	-	-
3	Hydroxymethoxuron	-CH ₃	-CH ₃	-Cl	-OH
4	Neburon	-CH ₃	-C ₄ H ₉	-Cl	-Cl
5	Deschloromethoxuron	-CH ₃	-CH ₃	-	-OCH ₃
6	Isoproturon	-CH ₃	-CH ₃	-	-CH(CH ₃) ₂
7	Bis-N,N'-(3-chloro-4-methylphenyl)urea	3-Chloro-4-methylphenyl	-H	-Cl	-CH ₃
8	N-Butyl-N'-phenylurea	-C ₄ H ₉	-H	-	-
9	N-Phenylurea	-H	-H	-	-

panol (spectroscopic grade, Lachema) or *n*-hexane (analytical-reagent grade; Loba, Fischamend, Austria) with 2-propanol. All the solvents were filtrated using a Millipore 0.45- μm filter and the premixed mobile phases were degassed by ultrasonication before use.

The capacity factors $k' = (V_R - V_M)/V_M$ were calculated from the arithmetic means of two or three experimental retention volumes V_R , in repeated experiments. From the plots of the experimental k' in dependence on the composition of the mobile phase, the parameters of the retention Eqs. 3, 7, 9 and 10 were determined by non-linear regression analysis using the Chemstat software (Trilobyte, Prague, Czech Republic). The values of the parameters are given in Tables 3, 6 and 9.

4. Results and discussion

4.1. Silasorb SPH Nitrile

The capacity factors of the phenylurea herbicides tested were calculated using the dead volume V_M determined as the elution volume of water in aqueous–propanolic mobile phases, which was almost identical to the elution volumes of *n*-hexane and 2-propanol in organic mobile phases. The values of V_M and of the experimental k' are given in Tables 2 and 5. Figs. 1–3 show the plots of k' in dependence on the concentration of 2-propanol, which is the stronger eluent in both aqueous–organic and organic mobile phases.

In 2-propanol–*n*-hexane mobile phases, the solutes containing polar functional groups such as hydroxyl (hydroxymethoxuron), primary amino (N-phenylurea) or methoxy (deschlor-methoxuron) on the benzene ring are most strongly retained. On the other hand, compounds with bulky non-polar substituents such as bis-N,N'-(3-chloro-4-methylphenyl)urea, neburon and N-butyl-N'-phenylurea are the least retained.

Fig. 1 shows a linear decrease (on a log–log scale) of the capacity factors with increasing volume fraction φ of 2-propanol in *n*-hexane in

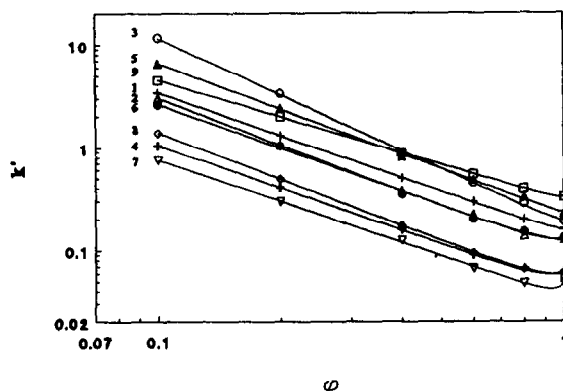


Fig. 1. Retention of phenylurea herbicides on a Silasorb SPH Nitrile column in mobile phases containing 2-propanol in *n*-hexane. Column: Silasorb SPH Nitrile, 7.5 μm , 300 \times 4.2 mm I.D. For data, see Table 2. Numbers of compounds as in Table 1. k' = capacity factor; φ = volume fraction of 2-propanol, in % (v/v) $\times 10^2$.

mobile phases containing 80% or less of 2-propanol. In mobile phases with less than 20% *n*-hexane, the plots of $\log k'$ versus $\log \varphi$ are curved. Higher values of the experimental capacity factors than those predicted from Eq. 3 were observed for all compounds tested, which suggests that the experimental behaviour in propanol-rich mobile phases is more likely to be explained by simultaneous contributions of polar and non-polar interactions to the retention than only by possible errors in measuring low ex-

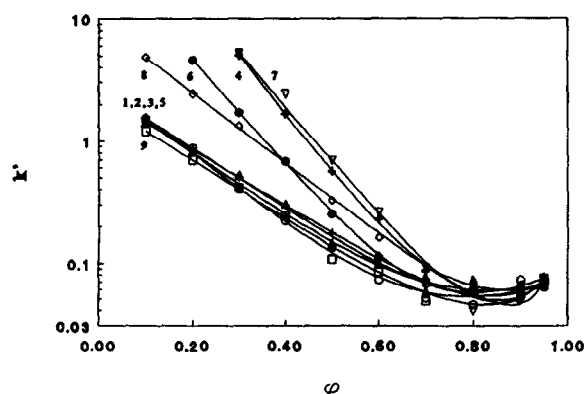


Fig. 2. Retention of phenylurea herbicides on a Silasorb SPH Nitrile column in mobile phases containing 2-propanol in water. Column: Silasorb SPH Nitrile, 7.5 μm , 300 \times 4.2 mm I.D. For data, see Table 5. Numbers of compounds as in Table 1, symbols as in Fig. 1.

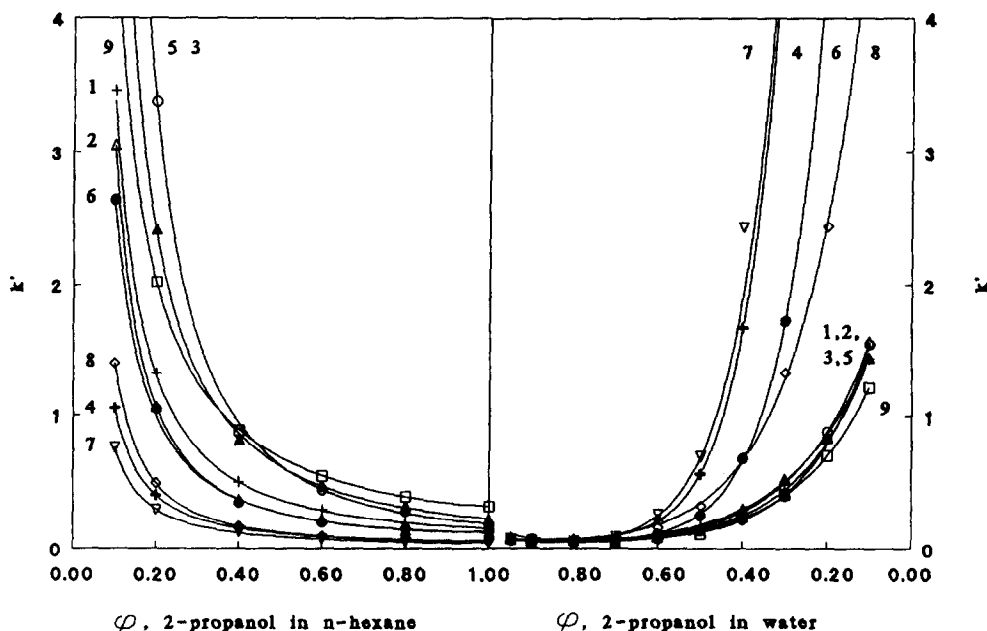


Fig. 3. Retention of phenylurea herbicides on a Silasorb SPH Nitrile column over a wide range of the mobile phase polarity from 10% 2-propanol in *n*-hexane to 10% 2-propanol in water. Column: Silasorb SPH Nitrile, 7.5 μm , 300 \times 4.2 mm I.D. For data, see Tables 2 and 5. Numbers of compounds as in Table 1, symbols as in Fig. 1.

perimental values of k' (see more detailed discussion below). This means that Eq. 3 can be used to describe the retention behaviour of phenylurea herbicides on the nitrile bonded phase only in mobile phases containing less than 80% (v/v) 2-propanol in *n*-hexane.

Fitting Eq. 3 to the experimental data in the mobile phases containing from 10 to 80% 2-propanol in *n*-hexane yielded significantly better results than fitting Eq. 6. This applied also to other chromatographic systems tested and consequently only the data calculated using Eq. 3 and Eqs. 9 and 10 are presented in this work.

Eq. 10 was fitted to the retention data in the whole composition range of mobile phases (10–100% 2-propanol in *n*-hexane) and the results in Table 2 show that this equation is well suited to describe the retention on the Silasorb SPH Nitrile column.

Table 3 lists the values of the parameters of Eqs. 3 and 10 obtained by the regression of the experimental dependencies of the capacity factors of phenylurea herbicides on the volume fraction of 2-propanol in *n*-hexane.

The parameters k'_0 and n calculated from Eq. 3 for mobile phases containing 10–80% 2-propanol agree very well with the values of the corresponding parameters of Eq. 10 measured over the whole range of mobile phase compositions.

Because the first term on the right-hand side of Eq. 10 represents the non-polar contribution to the retention whereas the second term the contribution of the polar interactions, it is possible to use the coefficients of these equations determined by regression to calculate the contributions of the two mechanisms to the retention from the ratio of the first and of the second terms to the predicted capacity factor. The values of the solvophobic contribution to retention in 2-propanol–*n*-hexane mobile phases calculated in this way from Eq. 10 indicate more significant contribution of the solvophobic mechanism to retention only in the mobile phases containing 80% or more of 2-propanol (Table 4). It is interesting to note that neburon, *N*-butyl-*N'*-phenylurea, isoproturon, phenuron and desphenuron without –OH, –OCH₃ or aromatic sub-

Table 2
Capacity factors of phenylurea herbicides on Silasorb SPH Nitrile column in mobile phases containing 2-propanol in *n*-hexane

Compound		k'					
		Concentration of 2-propanol in <i>n</i> -hexane (% v/v)					
		100	80	60	40	20	10
		V_M (ml)					
		–	3.55	3.55	–	3.56	–
1	a	0.158	0.199	0.296	0.501	1.324	3.456
	b	0.144	0.196	0.291	0.510	1.327	3.455
	c	0.158	0.200	0.291	0.507	1.322	3.456
2	a	0.126	0.139	0.221	0.374	1.064	3.057
	b	0.096	0.134	0.207	0.381	1.077	3.054
	c	0.123	0.148	0.211	0.376	1.065	3.057
3	a	0.190	0.286	0.448	0.862	3.374	11.841
	b	0.176	0.265	0.448	0.942	3.337	11.846
	c	0.193	0.270	0.448	0.931	3.334	11.846
4	a	0.062	0.067	0.091	0.161	0.408	1.055
	b	0.049	0.062	0.092	0.159	0.411	1.054
	c	0.062	0.064	0.090	0.157	0.407	1.055
5	a	0.227	0.326	0.476	0.812	2.416	6.644
	b	0.222	0.309	0.473	0.860	2.391	6.647
	c	0.231	0.312	0.473	0.859	2.389	6.649
6	a	0.132	0.152	0.201	0.311	1.046	2.643
	b	0.104	0.142	0.214	0.378	1.001	2.651
	c	0.133	0.145	0.211	0.374	0.996	2.653
7	a	0.047	0.048	0.067	0.125	0.299	0.760
	b	0.036	0.049	0.071	0.121	0.304	0.759
	c	0.047	0.048	0.069	0.119	0.302	0.760
8	a	0.055	0.066	0.094	0.174	0.494	1.402
	b	0.045	0.062	0.096	0.176	0.496	1.402
	c	0.055	0.065	0.096	0.174	0.493	1.402
9	a	0.326	0.395	0.547	0.886	2.020	4.563
	b	0.304	0.395	0.555	0.893	2.019	4.563
	c	0.326	0.394	0.551	0.889	2.014	4.564

V_M = Column dead volume; k' = capacity factor; a = experimental values; b = calculated from Eq. 3; c = calculated from Eq. 10. The parameters k'_0 , n , a and b of these equations are given in Table 3. Numbers of compounds as in Table 1.

Table 3

Experimental parameters of Eqs. 3 and 10 (10–100% 2-propanol) for phenylurea herbicides on Silasorb SPH Nitrile column in mobile phases containing 2-propanol in *n*-hexane

Compound	Parameters of equations							
	Eq. 3			Eq. 10				
	k'_0	n	R^2	k'_0	n	a	b	R^2
1	0.1438	1.381	0.9999	0.1416	1.387	0.0162	1.654	1.0000
2	0.0959	1.503	0.9998	0.0907	1.527	0.0326	0.965	0.9999
3	0.1762	1.828	0.9999	0.1756	1.829	0.0177	2.264	0.9999
4	0.0460	1.361	0.9995	0.0445	1.374	0.0178	3.616	0.9999
5	0.2225	1.475	0.9999	0.2217	1.477	0.0092	1.944	0.9999
6	0.1044	1.405	0.9983	0.1024	1.414	0.0303	4.054	0.9986
7	0.0361	1.322	0.9985	0.0353	1.333	0.0117	7.871	0.9999
8	0.0446	1.497	0.9999	0.0437	1.507	0.0152	2.353	1.0000
9	0.3041	1.176	0.9999	0.3016	1.180	0.0244	5.585	1.0000

Numbers of compounds as in Table 1.

stituents and with alkyl substituents (see Table 1) show higher non-polar contributions than the other phenylurea compounds in mobile phases containing 60–80% 2-propanol, which is in agreement with general structural effects usually observed in reversed-phase systems and supports the idea of the mixed retention mechanism.

Fig. 2 shows the dependencies of the capacity

factors of phenylurea herbicides on the volume fraction φ of 2-propanol in binary aqueous–organic mobile phases. In mobile phases containing 10–70% 2-propanol in water, bis-*N,N'*-(3-chloro-4-methylphenyl)urea, neburon, isoproturon and *N*-butyl-*N'*-phenylurea with largest alkyl or aryl substituents on the phenylurea skeleton are most strongly retained, while the compounds without (or with small) alkyl substituents (phenylurea,

Table 4

Hydrophobic (non-polar) contribution (in per cent of the first term in Eq. 10 to the capacity factor) to the retention of phenylurea herbicides on a Silasorb SPH Nitrile column in mobile phases containing 2-propanol in *n*-hexane

Compound	Relative contribution (%)				
	Concentration of 2-propanol (% v/v)				
	10	40	60	80	100
1	0.0	0.2	1.2	3.5	9.8
2	0.1	0.7	6.7	13.8	26.3
3	0.1	0.0	0.2	1.8	20.5
4	0.0	0.1	0.2	5.5	28.2
5	0.0	0.0	0.3	0.9	4.8
6	0.0	0.0	0.1	3.2	23.0
7	0.1	0.3	0.5	1.0	24.9
8	0.0	0.1	1.7	5.9	20.5
9	0.0	0.0	0.0	0.4	7.5

Numbers of compounds as in Table 1.

phenuron, desphenuron) and with a strongly polar substituent (hydroxymethoxuron) are eluted much earlier.

The dependencies of $\log k'$ on the volume fraction of 2-propanol in water in Fig. 2 show minima in 80–90% 2-propanol. This behaviour can be explained by the balance between non-polar and polar interactions in these mobile phases. Non-polar interactions control the retention in mobile phases with lower concentrations of 2-propanol, but with increasing concentration of 2-propanol the contribution of polar interactions gradually becomes more important and it predominates in mobile phases with less than 10–20% water, giving rise to the retention behaviour characteristic for a normal-phase system. As in reversed-phase systems the retention decreases with increasing concentration of the less polar solvent (2-propanol), while the opposite applies in normal-phase systems, the two opposite effects result in a minimum of retention at a certain composition of the mobile phase.

Possible experimental errors are not significant enough to explain the retention minima observed. The values of V_M in 10–100% 2-propanol in water are constant in the range 3.46–3.54 ml and an experimental error in this range may lead to deviations at the third decimal place in the values of k' . To avoid systematic errors caused by extra-column contributions, we used the shortest possible connections between the injector, the column and the detector, so that the extra-column volumes were approximately 20–30 μl , constant during the measurement of all experimental k' and V_M values, so that all the capacity factors would be affected in the same way (if this error is significant) and the absolute values of k' would be slightly modified, but the occurrence of the minima on the k' versus φ curves would not be affected at all.

The plots of $\log k'$ vs. φ are fairly linear in mobile phases containing less than 50–70% of 2-propanol, like in many reversed-phase systems with alkyl-bonded stationary phases. This is demonstrated by the agreement of the experimental capacity factors with the values calculated from the regression equation obtained by

fitting the Eq. 7 to the experimental data (Table 5).

To describe the retention in the whole composition range of the mobile phase, i.e., from 10 to 95% 2-propanol in water, the model taking into account dual mechanism of retention can be used. The values of the capacity factors calculated from the regression equations obtained by fitting Eq. 9 to the experimental data are listed in Table 5.

Table 6 lists the values of the parameters of Eqs. 7 and 9 determined by the regression of the experimental capacity factors of phenylurea herbicides in 2-propanol–water mobile phases. The correlation coefficients for Eq. 9 describing the retention in the whole range of mobile phase composition are slightly better than the correlation coefficients for Eq. 7 applied to the capacity factors in mobile phases containing 10–60% of 2-propanol in water.

The values of the contributions in this composition range of the mobile phases may be subject to some errors because of low values of the capacity factors. The values of the polar contributions to the retention of all the phenylurea herbicides tested (calculated as the per cent ratio of the second term in Eq. 9 to the value of the capacity factor) in mobile phases with different concentrations of 2-propanol in water are given in Table 7. The polar contribution to the retention can possibly be attributed mainly to the interactions of the solutes with residual silanol groups in the chemically bonded phase. For some polar compounds such as hydroxymethoxuron or chlortoluron, this contribution may be significant even in mobile phases containing 50% 2-propanol. In mobile phases with more than 70% of 2-propanol, the contribution of polar interactions to the retention increases rapidly and it amounts to 80–93% of the value of the capacity factors in 95% 2-propanol.

The data in Table 7 indicate that the equilibration of non-polar and polar contributions to the retention occurs in mobile phases with 70–90% 2-propanol in water, where the retention minima are observed (Fig. 2), supporting the idea of the reversal of the mechanism controlling the retention. Consequently, the contribution of the

Table 5

Capacity factors of phenylurea herbicides on Silasorb SPH Nitrile column in mobile phases containing 2-propanol in water

Compound		k'									
		Concentration of 2-propanol in water (% v/v)									
		95	90	80	70	60	50	40	30	20	10
		V_M (ml)									
		3.48	3.50	3.49	3.50	3.51	3.54	3.50	–	3.51	3.49
1	a	0.078	0.048	0.059	0.068	0.116	0.178	0.299	0.509	0.842	1.437
	b	0.018	0.023	0.039	0.065	0.109	0.183	0.306	0.512	0.855	1.423
	c	0.077	0.053	0.053	0.072	0.111	0.181	0.300	0.503	0.848	1.435
2	a	0.071	0.058	0.062	0.058	0.103	0.146	0.305	0.527	0.844	1.570
	b	0.014	0.018	0.031	0.055	0.096	0.167	0.291	0.509	0.889	1.552
	c	0.072	0.056	0.054	0.068	0.103	0.167	0.285	0.498	0.878	1.561
3	a	0.063	0.073	0.045	0.051	0.073	0.136	0.223	0.404	–	1.547
	b	0.007	0.009	0.018	0.034	0.063	0.120	0.227	0.430	–	1.542
	c	0.069	0.057	0.052	0.058	0.079	0.125	0.219	0.409	–	1.546
4	a	0.063	0.059	0.068	0.087	0.227	0.565	1.673	5.035	–	–
	b	0.004	0.007	0.022	0.064	0.191	0.569	1.692	5.031	–	–
	c	0.062	0.060	0.064	0.098	0.214	0.572	1.671	5.035	–	–
5	a	0.068	0.051	0.074	0.078	0.101	0.141	0.242	0.432	0.832	1.445
	b	0.014	0.015	0.027	0.047	0.084	0.148	0.261	0.462	0.817	1.443
	c	0.068	0.059	0.058	0.070	0.098	0.152	0.255	0.447	0.801	1.454
6	a	0.075	0.065	0.061	0.072	0.113	0.253	0.679	1.724	4.590	–
	b	0.003	0.005	0.014	0.037	0.097	0.253	0.665	1.747	4.584	–
	c	0.078	0.061	0.055	0.069	0.121	0.267	0.663	1.729	4.589	–
7	a	0.076	0.066	0.040	0.093	0.262	0.694	–	5.270	–	–
	b	0.008	0.012	0.034	0.094	0.257	0.703	–	5.269	–	–
	c	0.080	0.049	0.052	0.102	0.257	0.693	–	5.270	–	–
8	a	0.072	0.058	0.057	0.066	0.163	0.329	0.680	1.330	2.440	4.858
	b	0.017	0.024	0.047	0.090	0.175	0.340	0.661	1.284	2.492	4.884
	c	0.067	0.050	0.059	0.098	0.180	0.343	0.661	1.282	2.490	4.845
9	a	0.071	0.054	0.058	0.048	0.085	0.109	0.246	0.412	0.700	1.215
	b	0.012	0.016	0.028	0.048	0.082	0.140	0.241	0.412	0.706	1.211
	c	0.073	0.049	0.044	0.057	0.085	0.139	0.234	0.403	0.699	1.217

V_M = Column dead volume; k' = capacity factor; a = experimental values; b = calculated from Eq. 7; c = calculated from Eq. 9. The parameters k'_0 , n , a and b of these equations are given in Table 6. Numbers of compounds as in Table 1.

polar interactions to the retention predominates not only in the whole range of concentrations of 2-propanol in *n*-hexane, but also in mobile phases containing 90–100% 2-propanol in water.

The parameter k'_0 of Eq. 9 represents the polar contribution to the capacity factor in pure water as the mobile phase and is approximately 1–2 orders of magnitude lower than the corre-

Table 6

Experimental parameters of Eqs. 7 and 9 for phenylurea herbicides on Silasorb SPH Nitrile column in mobile phases containing 2-propanol in water

Compound	Parameters of equations							
	Eq. 7			Eq. 9				
	<i>a</i>	<i>b</i>	<i>R</i> ²	<i>a</i>	<i>b</i>	<i>k</i> ' ₀	<i>n</i>	<i>R</i> ²
1	2.387	2.230	0.9973	2.462	2.294	0.0041	0.899	0.9992
2	2.711	2.421	0.9958	2.772	2.525	0.0109	0.578	0.9992
3	2.919	2.771	0.9950	3.036	2.994	0.0223	0.357	0.9997
4	132.3	4.733	0.9993	143.0	4.854	0.0325	0.205	1.0000
5	2.551	2.473	0.9942	2.636	2.651	0.0223	0.333	0.9990
6	31.59	4.191	0.9992	32.78	4.280	0.0222	0.408	1.0000
7	108.2	4.375	0.9997	112.1	4.427	0.0042	0.965	1.0000
8	9.408	2.883	0.9989	9.427	2.893	0.0063	0.947	0.9997
9	2.075	2.340	0.9948	2.118	2.426	0.0051	0.839	0.9990

Numbers of compounds as in Table 1.

sponding parameter *k*'₀ of Eq. 10, which has the meaning of the polar contribution to *k*' in pure 2-propanol (Tables 3 and 6). The reason is increasing contribution of polar interactions to the retention when the polarity of the mobile phase is decreased.

Fig. 3 shows the dependence of the retention (capacity factors) of phenylurea herbicides on the composition of the mobile phase over a wide range of polarities, from 10% 2-propanol in *n*-

hexane to 10% 2-propanol in water. From these plots it is obvious that much better selectivities of separation can be achieved in non-aqueous mobile phases than in aqueous–propanolic media. In mobile phases containing more than 60% 2-propanol in water, all the solutes investigated are almost unretained, which contrasts to the behaviour in mobile phases with high concentrations of 2-propanol in *n*-hexane. A discontinuous change in retention of most her-

Table 7

Polar contribution (in per cent of the second term in Eq. 9 to the capacity factor) to the retention of phenylurea herbicides on a Silasorb SPH Nitrile column in mobile phases containing 2-propanol in water

Compound	Relative contribution (%)					
	Concentration of 2-propanol (% v/v)					
	10	50	70	80	90	95
1	0.4	4.4	16.7	32.7	60.4	79.2
2	0.8	9.6	30.9	50.9	73.2	85.0
3	1.5	23.0	58.6	76.9	89.5	93.9
4	–	6.1	41.6	70.3	86.7	92.4
5	1.5	18.3	47.1	65.5	81.3	89.0
6	–	10.9	52.2	77.7	87.5	93.9
7	–	1.2	12.7	38.5	76.0	91.6
8	0.0	0.5	4.3	14.8	47.8	78.2
9	0.5	6.5	25.0	45.5	71.4	85.1

Numbers of compounds as in Table 1.

bicides is apparent between 100% 2-propanol and 95% 2-propanol in water. This behaviour possibly can be explained by deactivation of the residual silanol groups as the polar adsorption centers by adsorption of water, which has leveling effect on retention and results in much lower separation selectivity for the separation of phenylurea herbicides on the bonded nitrile phases in aqueous–propanolic than in non-aqueous mobile phases.

Fig. 4 shows an example of separation of phenylurea herbicides on a Silasorb SPH Nitrile column in 2-propanol–*n*-hexane mobile phases. Strong tailing is apparent for the most strongly retained peak of unsubstituted *N*-phenylurea, which probably can be attributed to the hydrogen-bonding interactions with residual silanol groups in the stationary phase. This compound—the most simple in the phenylurea series—does not show herbicide properties and its peak is not of primary interest in the samples of herbicides. The peaks of herbicides of the *N'*-substituted *N*-phenylurea type in Fig. 4 are symmetrical and the efficiency of the column used (5000 theoretical plates) is sufficient for their separation. The shape of the peak of unsubstituted phenylurea can be possibly improved by the addition of small amounts of an alkylamine to the mobile phase.

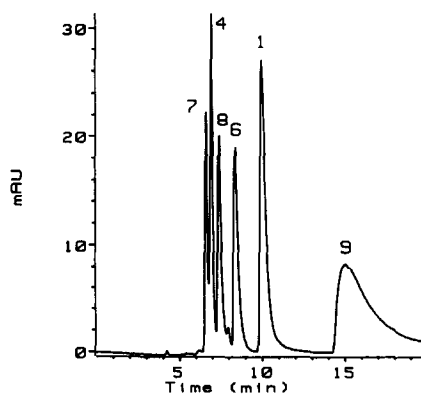


Fig. 4. Separation of phenylurea herbicides on a Silasorb SPH Nitrile column in a normal-phase system. Column: Silasorb SPH Nitrile, 7.5 μm , 300 \times 4.2 mm I.D.; mobile phase: 10% 2-propanol in *n*-hexane, flow-rate 1.0 ml/min, UV detection at 254 nm. Numbers of compounds as in Table 1.

4.2. Silasorb SPH Amine

The capacity factors of most phenylurea herbicides on a Silasorb SPH Amine column are low and close to each other in mobile phases containing 10–90% 2-propanol in water. Some solutes show retention minima in 60% 2-propanol, but with a few exceptions, the capacity factors of sample solutes are lower than 0.5 even in mobile phases with 10% 2-propanol.

In non-aqueous mobile phases, much higher retention and selectivity of separation of substituted phenylureas are observed. The elution volume of *n*-hexane was almost independent of the composition of 2-propanol–*n*-hexane mobile phases and was used as the column dead volume for calculations of capacity factors of the analytes in mobile phases containing 5–100% 2-propanol in *n*-hexane (Table 8). Fig. 5 shows the logarithmic plots of k' in dependence on the volume fraction ϕ of 2-propanol in the mobile phase. The plots are approximately linear in mobile phases containing less than 60% 2-propanol and can be described by Eq. 3.

To characterize the retention over the whole range of concentrations of 2-propanol in *n*-hexane, Eq. 10 based on the model of retention taking into account simultaneous contribution of polar and non-polar interactions yields predicted capacity factors in better agreement with the experimental k' than Eq. 3.

The parameters a , b , k'_0 and n obtained by fitting the retention Eqs. 3 and 10 to the experimental data are listed in Table 9. The parameters k'_0 and n of Eq. 3 are almost identical with corresponding parameters obtained by fitting the non-linear Eq. 10 to the retention data.

Table 10 lists the values of the non-polar contributions to the retention of the individual phenylurea herbicides, calculated as the ratios of the second term in Eq. 10 to the capacity factors calculated from this equation. The non-polar contribution to the retention increases with increasing concentration of 2-propanol in the organic mobile phase. It does not exceed 10% of k' in mobile phases containing less than 60% 2-propanol and its value is higher for compounds with bulky hydrocarbon substituents such as

Table 8
Capacity factors of phenylurea herbicides on Silasorb SPH Amine column in mobile phase containing 2-propanol in *n*-hexane

Compound		k'							
		Concentration of 2-propanol in <i>n</i> -hexane (% v/v)							
		100	80	60	40	30	20	10	5
		V_M (ml)							
		2.98	2.95	2.96	–	3.01	3.01	–	3.02
1	a	0.357	0.508	0.700	1.185	1.824	3.544	10.020	–
	b	0.308	0.432	0.667	1.231	1.903	3.513	10.020	–
	c	0.376	0.469	0.681	1.227	1.888	3.849	10.028	–
2	a	0.549	0.598	0.842	1.625	2.629	5.355	17.935	–
	b	0.335	0.491	0.807	1.628	2.678	5.402	17.922	–
	c	0.502	0.594	0.844	1.619	2.646	5.346	17.936	–
3	a	0.606	0.921	1.653	3.945	7.203	–	–	–
	b	0.564	0.904	1.662	3.992	7.211	–	–	–
	c	0.606	0.916	1.617	3.929	7.207	–	–	–
4	a	0.224	0.229	0.258	0.379	0.536	0.845	2.297	5.404
	b	0.122	0.162	0.233	0.389	0.561	0.937	2.251	5.410
	c	0.231	0.209	0.249	0.387	0.551	0.919	2.223	5.418
5	a	0.515	0.629	0.970	1.741	2.733	5.569	17.921	–
	b	0.374	0.543	0.881	1.742	2.826	5.586	17.911	–
	c	0.518	0.636	0.929	1.744	2.799	5.527	17.925	–
6	a	0.357	0.393	0.534	0.871	1.372	2.222	6.174	15.244
	b	0.274	0.371	0.545	0.939	1.382	2.380	6.029	15.273
	c	0.358	0.382	0.548	0.936	1.377	2.373	6.021	15.247
7	a	0.426	0.418	0.471	0.676	1.007	1.776	5.382	13.726
	b	0.208	0.284	0.425	0.749	1.121	1.977	5.214	13.756
	c	0.441	0.365	0.469	0.745	1.107	1.952	5.181	13.767
8	a	0.301	0.308	0.371	0.591	0.895	1.682	5.303	15.005
	b	0.154	0.217	0.337	0.626	0.972	1.806	5.209	15.020
	c	0.310	0.274	0.355	0.627	0.966	1.793	5.188	15.026
9	a	0.774	0.972	1.544	2.665	4.207	7.672	–	–
	b	0.705	0.981	1.503	2.742	4.200	7.662	–	–
	c	0.769	0.998	1.493	2.718	4.179	7.675	–	–

V_M = Column dead volume; k' = capacity factor; a = experimental values; b = calculated from Eq. 3; c = calculated from Eq. 10. The parameters k'_0 , n , a and b of these equations are given in Table 9. Numbers of compounds as in Table 1.

neburon, bis-*N,N'*-(3-chloro-4-methylphenyl)-urea and *N*-butyl-*N'*-phenylurea. These compounds are the most early eluted ones in mobile

phases containing 5–40% 2-propanol, where the non-polar contribution to retention can be neglected. On the other hand, in these mobile

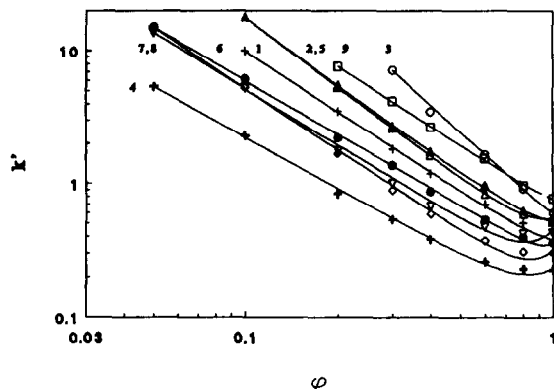


Fig. 5. Retention of phenylurea herbicides on a Silasorb SPH Amine column in mobile phases containing 2-propanol in *n*-hexane. Column: Silasorb SPH Amine, 7.5 μ m, 300 \times 4.2 mm I.D. For data, see Table 8. Numbers of compounds as in Table 1, symbols as in Fig. 1.

phases hydroxymethoxuron with polar OH group is retained most strongly, but it shows the lowest non-polar contribution to retention of all the compounds tested. This behaviour suggests that the model based on Eq. 10 is suitable to describe the experimental behaviour of phenylurea herbicides on the amino-bonded phase in organic mobile phases. Changing proportions of the polar and of the non-polar contributions to the retention can probably explain the strong dependence of the selectivity of separation on the

concentration of 2-propanol in the mobile phase (Fig. 5) accompanied with changes in the order of elution of some compounds, such as hydroxymethoxuron and phenylurea, which are the most strongly retained ones.

Because the amino group is more polar than the nitrile group, the phenylurea herbicides are retained more strongly on the Silasorb SPH Amine column than on the Silasorb SPH Nitrile column. Deschlormethoxuron is eluted before N-phenylurea, and phenuron before desphenuron on the Silasorb SPH Amine column, while the order of elution of these pairs of compounds is reversed on the Silasorb SPH Nitrile column. Fig. 6 shows an example of the separation of phenylurea herbicides on the Silasorb SPH Amine column to illustrate this behaviour. Like on the nitrile column, unsubstituted N-phenylurea is most strongly retained and its peak is unsymmetrical—probably because of the hydrogen-bonding interactions with the residual silanol groups in the stationary phase. The efficiency of the column used was rather low (approximately 1500–2000 theoretical plates) and also the peaks of less retained compounds showed some tailing, caused by the previous history of the column used for several hundreds of experiments. An example of a more efficient separation of a mixture of phenylurea herbicides

Table 9

Experimental parameters of Eqs. 3 and 10 for phenylurea herbicides on Silasorb SPH Amine column in mobile phases containing 2-propanol in *n*-hexane

Compound	Parameters of equations							
	Eq. 3			Eq. 10				
	k'_0	n	R^2	k'_0	n	a	b	R^2
1	0.3063	1.515	0.9997	0.2976	1.527	0.0779	0.962	0.9998
2	0.3292	1.736	0.9997	0.3185	1.750	0.2317	1.368	1.0000
3	0.5572	2.127	0.9998	0.5689	2.109	0.0374	1.841	1.0000
4	0.1289	1.247	0.9988	0.1158	1.284	0.1157	1.610	0.9995
5	0.3703	1.685	0.9998	0.3517	1.706	0.1670	0.686	1.0000
6	0.2715	1.345	0.9996	0.2716	1.343	0.0849	3.953	0.9997
7	0.2049	1.404	0.9990	0.2012	1.411	0.2398	2.142	0.9994
8	0.1532	1.531	0.9997	0.1514	1.535	0.1596	2.098	0.9998
9	0.6957	1.491	0.9996	0.6839	1.502	0.0853	1.552	0.9998

Numbers of compounds as in Table 1.

Table 10

Solvophobic contribution (in per cent of the first term in Eq. 10 to the capacity factor) to the retention of phenylurea herbicides on a Silasorb SPH Amine column in mobile phases containing 2-propanol in *n*-hexane

Compound	Relative contribution (%)				
	Concentration of 2-propanol (% v/v)				
	10	40	60	80	100
1	0.1	1.7	4.7	10.8	20.9
2	0.1	2.2	7.7	20.8	36.6
3	–	0.0	0.0	0.6	6.1
4	0.0	3.0	10.4	26.2	49.9
5	0.2	3.7	9.5	19.1	32.1
6	0.6	0.7	1.6	4.1	24.1
7	0.1	1.7	11.8	24.5	54.4
8	0.0	1.5	6.6	22.2	51.2
9	–	0.4	1.3	4.2	11.1

Numbers of compounds as in Table 1.

(most of which have not been studied in detail in all the mobile phases reported in this work) on a more fresh and efficient column is shown in Fig. 7.

5. Conclusions

(1) For the polar bonded stationary phases Silasorb SPH Nitrile and Silasorb SPH Amine,

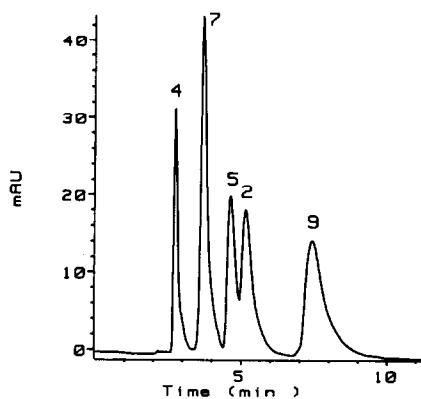


Fig. 6. Separation of phenylurea herbicides on a Silasorb SPH Amine column in a normal-phase system. Column: Silasorb SPH Amine, 7.5 μ m, 300 \times 4.2 mm I.D.; mobile phase: 40% 2-propanol in *n*-hexane, flow-rate 1.0 ml/min, UV detection at 254 nm. Numbers of compounds as in Table 1.

the dependence of the retention of phenylurea herbicides on the concentration of a polar organic solvent in a non-polar one or in water can be adequately described by two-parameter equations only over a limited composition range of the mobile phases. In aqueous–organic and in non-aqueous binary mixtures with concentrations of 2-propanol higher than 60–70%, significant curvature of the plots of $\log k'$ versus the volume fraction of propanol, or its logarithm, respectively, is observed. If the composition of the mobile phase is changed gradually from 0 to 100% 2-

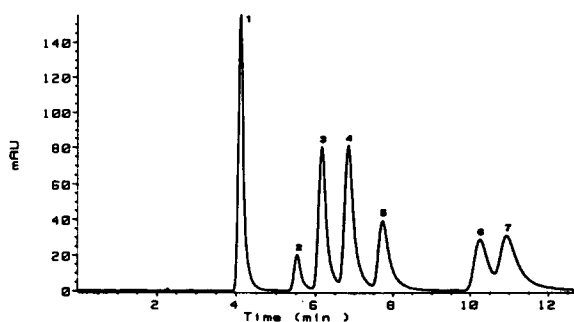


Fig. 7. Separation of phenylurea herbicides on a Silasorb SPH Amine column in a normal-phase system. Column: Silasorb SPH Amine, 7 μ m, 250 \times 4 mm I.D.; mobile phase: 20% 2-propanol in *n*-hexane, flow-rate 1.0 ml/min, UV detection at 254 nm, column temperature 40°C. Peaks: 1 = neburon; 2 = fluormethuron; 3 = diuron; 4 = monuron; 5 = phenuron; 6 = methoxuron; 7 = deschlormethoxuron.

propanol in *n*-hexane and then to 0% 2-propanol in water, minima of retention are observed in mobile phases containing 80–90% 2-propanol in water. The retention behaviour can be described over the whole range of compositions of binary mobile phases using a four-parameter equation derived on the basis of the mechanism of retention taking into account simultaneous effects of non-polar and polar interactions in the chromatographic systems studied.

(2) For the two stationary phases, the separation selectivity of phenylurea herbicides is higher in non-aqueous than in aqueous–organic mobile phases, so that binary organic solvents are to be preferred for practical separations of these compounds on chemically bonded nitrile and amino stationary phases. Because of a higher polarity of the amino group with respect to the nitrile substituent, the retention is higher on Silasorb SPH Amine than on Silasorb SPH Nitrile stationary phases and the separation selectivity differs significantly for the two stationary phases, which makes separations of phenylurea herbicides on the two columns complementary each to the other.

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References

- [1] L.R. Snyder, *Anal. Chem.*, 46 (1981) 1384.
- [2] L.R. Snyder and T.C. Schunk, *Anal. Chem.*, 54 (1982) 1764.
- [3] R.J. Hurtubise, A. Hussain and H.F. Silver, *Anal. Chem.*, 53 (1981) 1993.
- [4] C.A. Chang and C.-S. Chuang, *Anal. Chem.*, 57 (1985) 997.
- [5] E.L. Weisser, A.W. Salotto, S.M. Flach and L.R. Snyder, *J. Chromatogr.*, 303 (1984) 1.
- [6] J. Chmielovec and A.E. George, *Anal. Chem.*, 52 (1980) 1154.
- [7] K.-G. Liphard, *Chromatographia*, 13 (1980) 603.
- [8] W.T. Cooper and P.L. Smith, *J. Chromatogr.*, 335 (1986) 57.
- [9] P. Jandera and J. Churáček, *J. Chromatogr.*, 91 (1974) 207.
- [10] C.M. Hennion, C. Picard, C. Combellas, M. Caude and R. Rosset, *J. Chromatogr.*, 210 (1981) 211.
- [11] R.P.W. Scott and P. Kucera, *Anal. Chem.*, 45 (1973) 749.
- [12] L.R. Snyder, in Cs. Horváth (Editor), *High-Performance Liquid Chromatography—Advances and Perspectives*, Vol. 3, Academic Press, Orlando, FL, 1983, p. 186.
- [13] L.R. Snyder, J.W. Dolan and J.R. Gant, *J. Chromatogr.*, 165 (1979) 3.
- [14] A. Nahum and Cs. Horváth, *J. Chromatogr.*, 203 (1981) 53.
- [15] K.E. Bij, Cs. Horváth, W.R. Melander and A. Nahum, *J. Chromatogr.*, 203 (1981) 65.