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Journal of Chromatography A, 791 (1997) 1–19

JOURNAL OF
CHROMATOGRAPHY A

Characterisation and prediction of retention in isocratic and gradient-elution normal-phase high-performance liquid chromatography on polar bonded stationary phases with binary and ternary solvent systems

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Received 6 May 1997; received in revised form 18 July 1997; accepted 18 July 1997

Abstract

The retention of phenylurea herbicides on bonded nitrile- and amino-silica gel columns was studied in mixed mobile phases containing 2-propanol or dioxane in *n*-hexane. Under isocratic conditions, a three-parameter retention equation describes adequately the dependence of the retention factors on the concentration of a polar solvent in binary mobile phases. Parameters of this equation can be used for the prediction of elution volumes in gradient elution with binary solvent gradients. If dry solvents and controlled temperature are used, if the migration of sample compounds corresponding to the gradient dwell volume is accounted for and if the initial concentration of the polar solvent in gradient elution is 3% or more, the differences between the predicted gradient elution volumes and the experimental data were, in most cases, in between 0.1 and 0.25 ml, i.e., less than 5%. With gradients starting from pure non-polar solvents, the differences are more significant because of preferential adsorption of the polar solvents during the gradient. The adsorption isotherms of 2-propanol and of dioxane in *n*-hexane on the nitrile-bonded phase are less steep and column saturation occurs at a higher concentration of the polar solvent than with the unmodified silica gel column. Based on three experimental retention factors, retention can be predicted in isocratic and gradient elution with ternary mobile phases. © 1997 Elsevier Science B.V.

Keywords: Gradient elution; Retention prediction; Mobile phase composition; Pesticides

1. Introduction

Chromatography in reversed-phase systems is the most popular mode used in the contemporary practice of liquid chromatography for the separation of different classes of compounds ranging from aromatic hydrocarbons and fatty acid esters to ionizable or ionic compounds such as carboxylic acids, nitrogen bases, amino acids, peptides, proteins and sul-

phonic acids. However, columns packed with polar adsorbents usually show better separation selectivities than alkylsilica columns for various positional isomers of moderately polar compounds [1,2] or for some oligomers containing repeat polar groups [3].

In chromatography on polar adsorbents, preferential adsorption of more polar solvents may occur, especially of water. This phenomenon may be very important in gradient elution, where the concentration of a polar solvent in a non-polar one increases

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during the gradient run and preferential adsorption may lead to important deviations of the actual gradient profile from the pre-set mobile-phase composition program. To obtain reproducible results, it is necessary to keep a constant adsorbent activity [4].

This objective can be most easily achieved by using dehydrated solvents [5,6]. With solvent mixtures containing strongly polar organic solvents, such as alcohols, as mobile-phase components, possible effects of small residual trace concentrations of water present in the solvents, even after careful drying, are likely to be less significant than with mobile phases containing only non-polar or slightly polar solvents [5].

More than twenty years ago, we started our systematic investigation of gradient elution in normal-phase systems with silica gel columns and binary gradients formed using solvents with large differences in polarities [7]. We have found recently that with dry solvents, controlled temperature and using an instrument with a high accuracy of solvent mixing and mobile phase delivery, good reproducibility of the retention data in repeated gradient-elution experiments on a silica gel column can be maintained even after long-term column use [5,6]. Furthermore, the elution volumes in gradient elution chromatography with binary and ternary gradients on a silica gel column can be predicted by calculation using the parameters of simple two- and three-parameter equations describing the isocratic retention.

The objective of this work was to investigate the retention behaviour in normal-phase systems with chemically bonded polar stationary nitrile- and amino-silica gel columns and to estimate the effects of the preferential adsorption of the more polar component from the mobile phase on the accuracy of the retention data in gradient elution. For this purpose, experimental adsorption isotherms and breakthrough curves of the polar solvents were measured. Finally, the experimental elution volumes of phenylurea pesticides, which were selected as model compounds, in binary and ternary mobile phases under isocratic- and gradient-elution conditions were compared with the data calculated using equations derived on the basis of the retention models to test the suitability of the prediction methods in normal-phase systems with polar-bonded stationary phases.

2. Theoretical

The first model of retention in adsorption chromatography was developed by Snyder in the early 1960s [4,8,9]. Soczewiński and Golkiewicz [10,11] suggested a model of retention assuming adsorption in a monomolecular layer and cancellation of the solute–solvent interactions in the mobile and stationary phases. With some simplification, both models lead to an identical equation describing the retention (retention factor, k) as a function of the concentration of the stronger (more polar) solvent, φ , in binary mobile phases comprising two solvents of different polarities [7,12]:

$$k = k_0 \cdot \varphi^{-m} \quad (1)$$

where k_0 and m are experimental constants, k_0 being the retention factor in pure strong solvent.

Based on the original Snyder concept of adsorption as a competitive phenomenon, but with less simplification than in the derivation of Eq. (1), another retention equation was derived [13,14]:

$$k = (a + b \cdot \varphi)^{-m} \quad (2)$$

where a , b and m are experimental constants that depend on the solute and on the chromatographic system [$a = 1/(k_a)^m$, where k_a is the retention factor in pure non-polar solvent]. If the retention in pure non-polar solvent is very high, the term a in Eq. (2) can be neglected and this equation becomes Eq. (1) [13].

A theoretical description of linear binary gradient elution in normal-phase systems was presented by Jandera and Churáček [12,14–16]. In these gradients, the concentration of a polar solvent, φ , increases as the volume of eluate, V , increases:

$$\varphi = A + B \cdot V \quad (3)$$

Here, A is the initial concentration of the strongly polar organic solvent in the mobile phase and B is the steepness of the gradient in concentration units per ml of the eluate. If the retention in a normal-phase system can be described by the two-parameter retention equation (Eq. (1)), the retention volume, V_R , of a sample compound in gradient-elution chromatography can be calculated as [15]:

$$V_R = \frac{1}{B}[(m+1)Bk_0V_0 + A^{(m+1)}]^{1/m+1} - \frac{A}{B} + V_0 \quad (4)$$

where V_0 is the column hold-up (dead) volume.

On the other hand, if the three-parameter dependence (Eq. (2)) describes adequately the retention in a given normal-phase system, a slightly more complex equation should be used to calculate the retention volumes in gradient elution [5,14,16]:

$$V_R = \frac{1}{b \cdot B} [b \cdot B(m+1)V_0 + (a + A \cdot b)^{(m+1)}]^{1/m+1} - \frac{a + A \cdot b}{b \cdot B} + V_0 \quad (5)$$

Ternary mobile phases in normal-phase chromatography contain two different polar solvents (*i* and *j*) in a third, non-polar one [17]. Two different effects of polar solvents on retention can be distinguished, as in reversed-phase systems. If the concentration ratio 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 similar to the effect of changing the concentration of a single, more polar, solvent in binary mobile phases and can be attributed mainly to changing elution 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) mobile phases in liquid chromatography. Isoeluotropic solvent mixtures with equal solvent strengths should be used [18] to have pure selectivity effects, but it is more convenient to work with concentrations than with solvent strengths (which depend on the adsorbent and are not exactly the same with different solutes). For practical method development, it is not very important if some change in solvent strength occurs when optimizing the selectivity.

In ternary mobile phases with a constant concentration ratio of two polar solvents, $r = \varphi_i/\varphi_j$, the effect of the sum of the two concentrations, $\varphi_T = \varphi_i + \varphi_j$, on the retention can be assumed to be principally

the same as the effect of the concentration of one polar solvent in binary mobile phases, if the differences between the polarities of the solvents *i* and *j* are significantly lower than the differences between the polarities of each of the two solvents and the polarity of the third solvent. Then, a retention equation similar to Eq. (1) can be expected to describe the retention behaviour in such ternary solvent systems [19]:

$$k = k_{0T} \cdot \varphi_T^{-mT} \quad (6)$$

It should be noted that the coefficients k_{0T} and m in Eq. (6) are constant only at a constant ratio of φ_i/φ_j . In the same way, Eq. (2) with coefficients a , b and m , depending on the concentration ratio of the two polar solvents, r , can be used to describe the retention in ternary mobile phases with changing φ_T .

When the sum of the concentrations of the two polar solvents *i* and *j*, φ_T , is constant, but their ratio is changing, the elution strength in ternary mobile phases changes much less than in mobile phases where φ_T is changed. We have found that the simultaneous effects of the concentrations φ_i and φ_j can be described by Eq. (7) [9].

$$\frac{1}{k} = \alpha + \beta \cdot X + \gamma \cdot X^2 \quad (7)$$

$X = \varphi_i/\varphi_T$, is the ratio of one polar solvent to the total concentration of the two polar solvents (in the present work, the stronger of the two solvents is solvent *i*) and α , β and γ are constants that depend on the solute, the chromatographic system and on φ_T . Eq. (7) with a zero quadratic term can be derived from the additivity of reciprocal capacity factors [20,21], but we found that this equation gave a poor fit to the experimental data.

From the practical point of view, it is useful that the parameters α , β and γ of Eq. (7) can be determined from three experimental values of k , two of them in binary mobile phases with concentrations, φ , equal to the concentration, φ_T , in the ternary mobile phase, k_1 at $X=0$ and k_2 at $X=1$. Only one experimental value, k_3 , should be measured in a ternary solvent system at a known concentration ratio, X_3 . Introducing k_1 , k_2 and k_3 into Eq. (7), we obtain a set of three equations, from which we can calculate the parameters α , β and γ .

$$\alpha = \frac{1}{k_1} \quad (8)$$

$$\beta = \frac{1}{k_3 \cdot X_3 \cdot (1 - X_3)} - \frac{1 + X_3}{k_1 \cdot X_3} - \frac{X_3}{k_2 \cdot (1 - X_3)} \quad (9)$$

$$\gamma = \frac{1}{k_1 \cdot X_3} + \frac{1}{k_2 \cdot (1 - X_3)} - \frac{1}{k_3 \cdot X_3 \cdot (1 - X_3)} \quad (10)$$

If the selectivity in binary gradients is too low, ternary gradients can be used where the concentrations of two polar solvents i and j , φ_i and φ_j in a non-polar one are changing simultaneously:

$$\varphi_i = A_i + B_i \cdot V; \varphi_j = A_j + B_j \cdot V \quad (11)$$

where A_i , B_i , A_j and B_j are the initial concentration and the steepness of the gradient with respect to the individual polar solvents i and j , respectively.

With two specific types of ternary gradients, the calculation of the elution volumes is more simple than in the general case of ternary gradient elution:

(1) Especially useful are “elution strength” gradients, where the ratio of concentrations of the two polar solvents, $r = \varphi_i/\varphi_j$, is constant and their sum changes with the volume of the eluate,

$$\varphi_T = \varphi_i + \varphi_j = A_T + B \cdot V \quad (12)$$

If retention Eq. (1) applies for each binary mobile phase with solvents i and j ,

$$k_i = k_{0i} \cdot \varphi_i^{-m_i}; k_j = k_{0j} \cdot \varphi_j^{-m_j} \quad (13)$$

it was found that Eq. (1) can be used to describe the retention in mobile phases with a constant ratio, r , using k_{0T} instead of k_0 , and m_T instead of m . Then, the retention in gradient elution can be calculated as with binary gradients, using Eq. (4). (Indices i and j relate to the parameters of Eq. (1) for solutes in binary mobile phases with polar solvents i and j , respectively, and index T to ternary mobile phases with a given constant ratio, r .)

(2) The second type of ternary gradient that can be described in a relatively simple way is “selectivity” gradients, where the sum φ_T is constant and the ratio of concentrations of the two polar solvents is changed in such a way that the slope of the gradient

for solvent i , B_i is positive and the same gradient with a negative slope $B_j = -B_i$, is used for solvent j :

$$\frac{\varphi_i}{\varphi_T} = X = \frac{\varphi_{0i}}{\varphi_T} + B \cdot V = X_0 + B \cdot V \quad (14)$$

Such gradients may be useful for the separation of samples containing a pair or a group of less strongly retained compounds showing a good separation selectivity in binary mobile phases with solvent i , but a poor selectivity in binary mobile phases containing solvent j and, at the same time, another pair or group of more strongly retained compounds, which exhibit opposite separation selectivities with respect to the binary mobile phases. Here, a continuous change of selectivity during the gradient elution may improve the overall separation with respect to binary and to ternary “elution strength” gradients [22]. If the retention is controlled by Eq. (7), the net retention volume $V'_R = V_R - V_0$ under these gradient conditions can be calculated by solving Eq. (15) in the implicit form:

$$\frac{(V'_R)^3}{3} \gamma B_2 + \frac{(V'_R)^2}{2} (\beta + 2\gamma X_0) B + V'_R (\alpha + \beta X_0) + \gamma X_0^2 = V_0 \quad (15)$$

If the gradient dwell volume V_D cannot be neglected and a sample solute moves some distance along the column before the front of the gradient reaches the top of the column, the elution occurs in two steps; first, isocratic and second, gradient. This situation is equivalent to the elution with two columns in series, where the first is eluted in the isocratic mode in a mobile phase containing the strong solvent at concentration A (the starting concentration in gradient elution) and the second in the gradient mode. The contribution of the first part (column) to the total retention volume of the solute is equal to V_D . The part of the column through which the solute has migrated at the end of the first step, i.e., at the time when it is taken by the front of the gradient, has a dead (hold-up) volume, V_{01} , corresponding to the proportional part of the total column dead volume, $V_0 \cdot V_{01}/V_0 = V_D/[V_0(1+k_1)]$, where k_1 is the retention factor in the mobile phase of initial composition. Then, $V_{01} = V_D/(1+k_1)$ and the second part (column) which remains available for the gradient elution step has the dead volume $V_{02} = V_0 - V_D/(1+k_1)$. The final

retention volume is composed of (1) the contribution of the gradient step to the net retention volume, V'_{R2} , which can be calculated from Eqs. (4) and (5) after subtracting V_0 (or from Eq. (15)) and using V_{02} instead of V_0 and (2) the contribution of the gradient dwell volume V'_{R1}

$$V'_{R1} = V_D - V_{01} = V_D / [1 + (k_1)^{-1}];$$

$$\begin{aligned} V_R &= V'_{R1} + V'_{R2} + V_0 = V_D - V_{01} + V'_{R2} + V_0 \\ &= \frac{V_D}{1 + \frac{1}{k_1}} + V'_{R2} + V_0 \end{aligned} \quad (16)$$

3. Experimental

An HP 1090M liquid chromatograph equipped with a UV diode-array detector, operated at 230 nm, an automatic sample injector, a 3DR solvent delivery system, a thermostated column compartment and a Series 7994A workstation (Hewlett-Packard, Palo Alto, CA, USA) was used to acquire the elution data. Glass cartridge columns (150 × 3.3 mm I.D.), packed with chemically modified silica gel, Separon SGX Nitrile and Separon SGX Amine, both 7.5 μm, 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*-hexane and dioxane, all of HPLC grade, were purchased from Baker (Deventer, Netherlands). The solvents were dried and kept in tightly closed dark bottles over molecular sieve beads, Dusimo 5Å (Lachema, Brno, Czech Republic), previously activated at 300°C (ca. 30–40 g/l), filtered using a Millipore 0.45 μm filter and degassed by ultrasonication immediately before use. Mobile phases were prepared directly in the HP 1090M instrument from the components, which were continuously stripped by a stream of helium, according to a gradient pre-set program. Phenylurea herbicide sample compounds were obtained from Lachema and are listed in Table 1. The solutes were dissolved in the mobile phase to provide adequate an response of the UV detector. Sample volumes (5 μl) were injected in each experiment.

Under isocratic conditions, elution volumes were measured at various mobile-phase compositions and

Table 1
Compounds studied — phenylurea herbicides

1	Phenuron
2	Diuron
3	Chlorotoluron
4	Fluometuron
5	Desphenuron
6	Neburon
7	Linuron
8	Monuron
9	Isoproturon
10	Metobromuron
11	Chlorobromuron
12	bis-N,N'-(3-Chloro-4-methyl)phenylurea

mean values from three repeated experiments were used to calculate the retention factors, $k = (V_R/V_0 - 1)$ of sample solutes. The sets of experimental dependencies of k on the mobile phase composition were used to find the best-fit parameters of Eqs. (1), (2), (6), (7) by linear or non-linear regression, as appropriate, using Adstat statistical software (Trilobyte, Prague, Czech Republic).

In gradient-elution experiments, the columns were first equilibrated with the mobile phase and then the retention volumes, V_R , of the sample compounds were measured at different profiles of binary or ternary gradients. A 5-min reversed gradient and a 5-min equilibration time were used after the end of each experiment to re-equilibrate the column at a flow-rate of 1 ml/min. In agreement with previous results [6], this equilibration approach provided reproducible retention data in repeated experiments, when dry solvents were used. The mean values of V_R from three repeated experiments are compared with the data calculated using Eqs. (4), (5), (15), respecting the gradient dwell volume according to Eq. (16), and the parameters of the retention equations (Eqs. (1), (2), (6), (7)), as described previously [6]. All calculations were carried out using Quattro Pro 4.0 spread-sheet software.

Column dead (hold-up) volumes, V_0 , were determined using trichloroethylene as the marker in mobile phases containing 5% or more 2-propanol or dioxane, where V_0 did not depend on the composition of the mobile phase, which was considered as proof that the marker solute is not retained ($V_0 = 0.966$ ml with the Separon SGX Nitrile- and 0.99 ml with the

Separon SGX Amine column). This corresponds to a column porosity $\epsilon_T = 0.75$ – 0.77 , which is somewhat higher than the porosity usually found with C_{18} columns, however, the amount of bonded moieties is lower with aminopropyl- or cyanopropyl-bonded phases. The gradient dwell volume, V_D , was determined from the blank gradient run in the system without a column and was 1.00 ml, as a high-pressure filter was inserted in between the pump and the sample injector. The column breakthrough volumes, V_B , were determined in a similar way with a column in the instrument from the start of the ramp on the UV detector response trace at a low wavelength (210 nm); 2-propanol has a higher absorptivity than *n*-hexane at this wavelength.

The isotherms of 2-propanol and of dioxane in *n*-hexane on the Nitrile column were determined using the frontal-analysis method as described previously [23], except that a Waters–Millipore R 401 refractometric detector was used instead of the UV-absorbance detector.

4. Results and discussion

4.1. Binary gradient elution

The two-parameter equation, Eq. (1), can describe reasonably well the retention on the Separon SGX Nitrile and on the Separon SGX Amine columns with binary mobile phases of 2-propanol–*n*-hexane and dioxane–*n*-hexane under isocratic conditions, but the three-parameter equation, Eq. (2), provides a better fit to the experimental retention data sets on the two columns (Figs. 1–3). Four to six data points were used to fit the retention equations and the retention factors calculated using the best-fit parameters of Eq. (1) showed significantly larger deviations from the experimental data, mainly at lower concentrations of polar solvents (in the higher range of retention factors) than the results obtained using the best-fit parameters of Eq. (2). The mean error of the retention factors fitted using Eq. (2) was ten times lower with the Separon SGX Nitrile column and

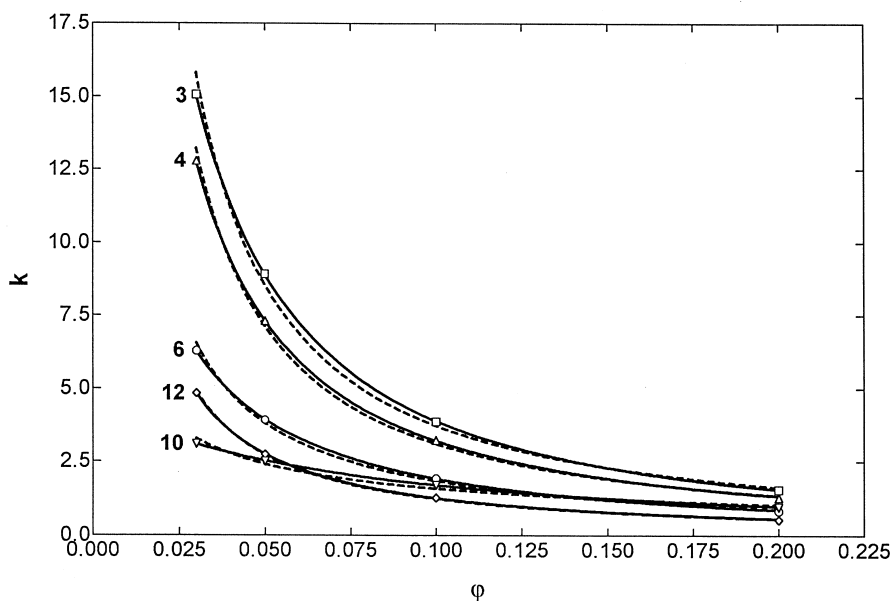


Fig. 1. Dependence of the retention factors, k , of phenylurea herbicides on the concentration of 2-propanol, ϕ (% vol 10^{-2}), in *n*-hexane. Numbers of plots agree with the numbers of compounds given in Table 1. Column: Separon SGX Nitrile. Points = experimental data; dotted lines = best-fit dependencies according to the two-parameter equation, Eq. (1); unbroken lines = best-fit dependencies according to the three-parameter equation, Eq. (2).

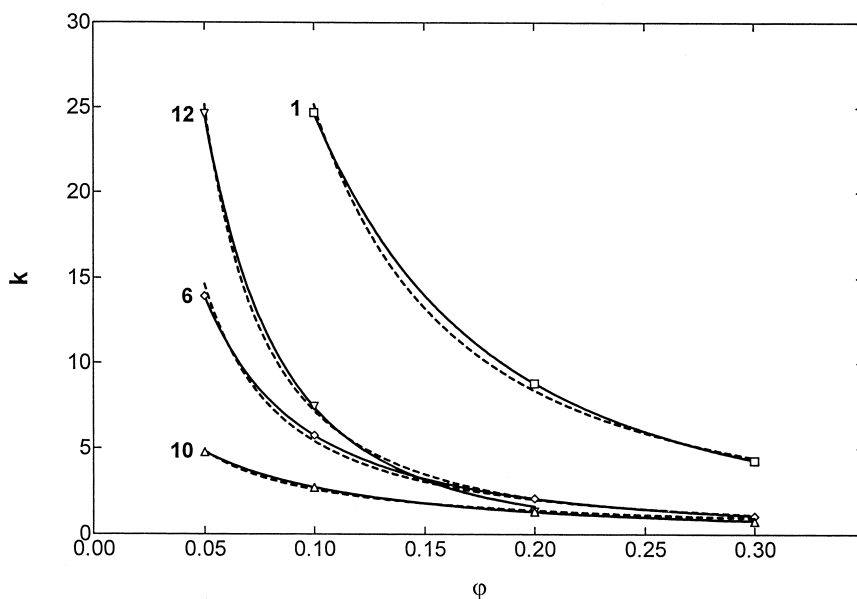


Fig. 2. Dependence of retention factors, k , of phenylurea herbicides on the concentration of dioxane, ϕ (%vol 10^{-2}), in n -hexane. Numbers of plots agree with the numbers of compounds given in Table 1. Column: Separon SGX Nitrile. Points=experimental data; dotted lines=best-fit dependencies according to the two-parameter equation, Eq. (1); unbroken lines=best-fit dependencies according to the three-parameter equation, Eq. (2).

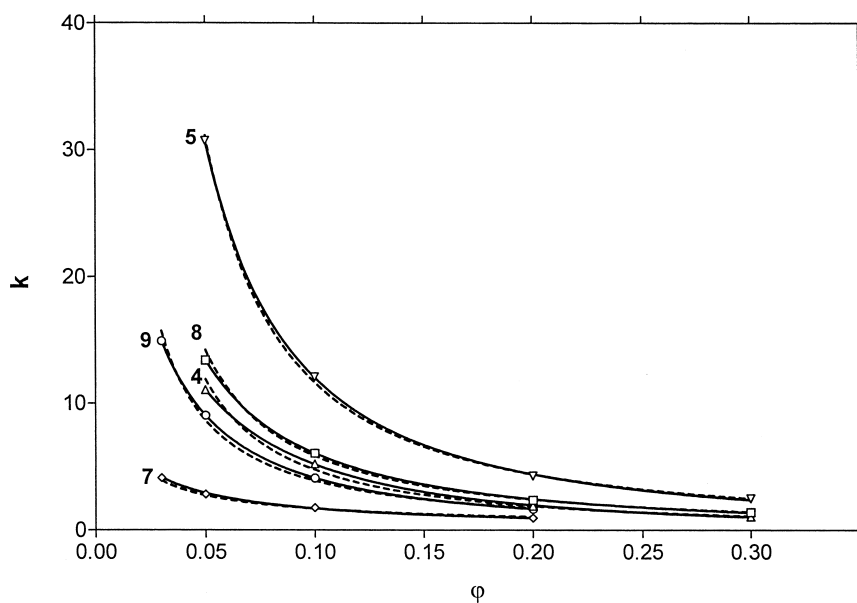


Fig. 3. Dependence of retention factors, k , of phenylurea herbicides on the concentration of 2-propanol, ϕ (%vol 10^{-2}), in n -hexane. Numbers of plots agree with the numbers of compounds given in Table 1. Column: Separon SGX Amine. Points=experimental data; dotted lines=best-fit dependencies according to the two-parameter equation, Eq. (1); unbroken lines=best-fit dependencies according to the three-parameter equation, Eq. (2).

three times lower with the Separon SGX Amine column. This is in agreement with earlier results for an unmodified Separon SGX silica gel column [5]. Therefore, Eq. (2) was used as the basis for all predictive calculations of retention volumes under gradient-elution conditions.

It is well known that, in gradient-elution normal-phase chromatography, the more polar solvent may be preferentially adsorbed from a mixed mobile phase, which may cause errors in the retention data predicted by calculation from the parameters of the isocratic retention equations (Eqs. (1) and (2)). Table 2 shows the experimental breakthrough volumes measured on the Separon SGX Nitrile and on the Separon SGX Amine columns with binary gradients of 2-propanol or dioxane in *n*-hexane, differing in the gradient time (steepness) and started at various concentrations of the polar solvent. The breakthrough volumes, V_B , are corrected for the column's hold-up volume and for the gradient dwell volume to give a measure of the preferential retention of the polar solvent under various gradient conditions.

With gradients starting in pure *n*-hexane, the breakthrough volumes of 2-propanol and dioxane on

the nitrile column are slightly lower than the V_B determined earlier on the silica gel column [5], in contrast to gradients starting at 3–9% of the polar solvent. However, the breakthrough volumes are lower than 0.1 ml with gradients starting at 6% or more 2-propanol and lower than 0.15 ml with gradients starting at 6% or more of dioxane.

To explain this behaviour, adsorption isotherms of the polar solvents were measured on the nitrile column and are compared with previously determined isotherms on a silica gel column in Fig. 4. The sorption isotherm of 2-propanol can be well described by the Langmuir isotherm (Table 2), but is less steep on the nitrile than on the silica gel column and the saturation capacity (ca. 55% of the saturation capacity of the silica gel column) is approached in the mobile phase containing 5% 2-propanol, which is a higher concentration than with the silica gel column (2% 2-propanol).

The isotherm of dioxane on the nitrile column is almost linear (Fig. 4, Table 2). Less dioxane is adsorbed on this than on the silica gel column in mobile phases with less than 4% dioxane, but in mobile phases containing more dioxane, the ad-

Table 2

Net breakthrough volumes, V_B , and parameters of the Langmuir isotherm for polar solvents in gradient elution on columns packed with a nitrile (I)- and an amino-silica (II)-bonded phase

	Column I, gradient	V_B , ml		Column I, gradient	V_B , ml		Column II, gradient	V_B , ml
1	0–50% P in 30 min	0.51	7	0–100% D in 30 min	0.51	13	0–50% P in 30 min	0.63
2	0–50% P in 60 min	0.56	8	0–100% D in 60 min	0.60	14	0–50% P in 60 min	0.68
3	0–50% P in 90 min	0.60	9	0–100% D in 90 min	0.62	15	0–50% P in 90 min	0.83
4	3–50% P in 30 min	0.31	10	3–100% D in 30 min	0.21	16	3–50% P in 30 min	0.18
5	6–50% P in 30 min	0.08	11	6–100% D in 30 min	0.15	17	6–50% P in 30 min	0.02
6	9–50% P in 30 min	0.06	12	9–100% D in 30 min	0.10	18	9–50% P in 30 min	0.00

Linear binary gradients of 2-propanol (P) or dioxane (D) in *n*-hexane. Temperature, 40°C; flow-rate, 1 ml/min.

Langmuir isotherm: $q = a \cdot c / (1 + b \cdot c)$, where q and c are the concentrations of the polar solvent in the stationary and mobile phases, respectively; $q_s = a/b$ is the theoretical column saturation capacity.

Note: The breakthrough volumes are corrected for the column dead volume ($V_0 = 0.966$ ml, column I, $V_0 = 0.99$ ml, column II) and for the gradient dwell volume ($V_D = 1.0$ ml). Langmuir isotherms on a Separon SGX Nitrile column for 2-propanol are $a = 16.52$; $b = 0.895$ % vol⁻¹; $q_s = 18$ % vol and for dioxane: $a = 5.19$; $b = 0.0124$ % vol⁻¹; $q_s = 419$ % vol (hypothetical)

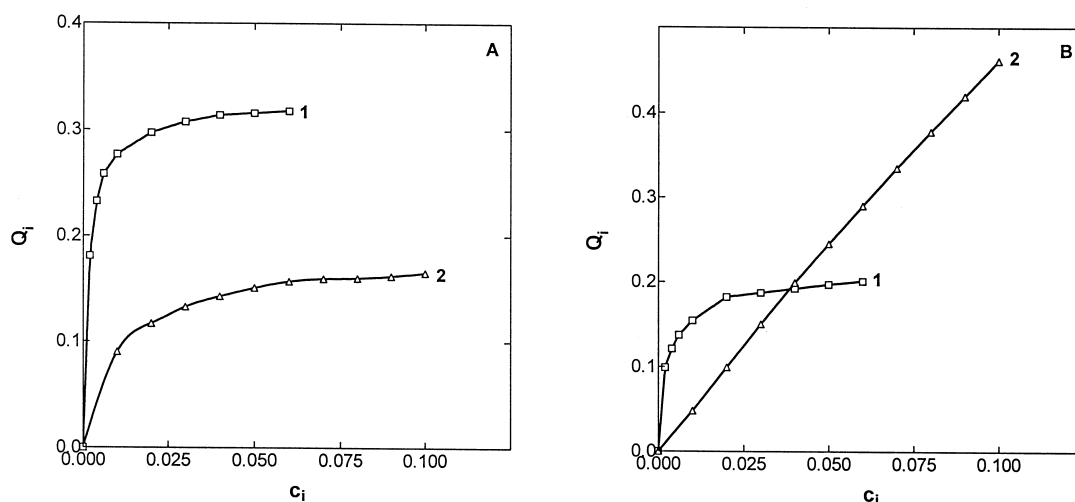


Fig. 4. Sorption isotherms of 2-propanol (1) and of dioxane (2) in *n*-hexane (A) on a silica gel Separon SGX, 7.5 μm , column (150 \times 3.3 mm I.D.); (B) on a Separon SGX Amine, 7.5 μm , column (150 \times 3.3 mm I.D.), all at 40°C. c_i =concentration of the polar solvent in the mobile phase in %vol 10^{-2} , Q_i =concentration of polar solvent in the stationary phase in %vol 10^{-2} , i.e., in ml of solvent adsorbed per ml of solid phase in the column, V_s (determined as the volume of the empty column minus the volume of the mobile phase in the column, V_0).

sorbed amount of dioxane is higher on the nitrile than on the silica gel column. The saturation capacity on the nitrile column is not approached even in the mobile phase containing 10% dioxane. This behaviour suggests, surprisingly enough, that the preferential adsorption of polar solvents on the nitrile column may affect the elution data in gradient-elution chromatography more significantly than with the silica gel column. The profile of the isotherm indicates that the interactions controlling the sorption of dioxane may be different on the nitrile than on the unmodified silica gel column. A similar isotherm profile was found for cholesterol and related compounds in non-aqueous solvents on a C_{18} column [24], where this behaviour was attributed to possible non-localised multilayer adsorption.

The elution volumes, $V_R(C)$, in gradient-elution chromatography on the nitrile and on the amine columns were calculated using Eq. (5) with the experimental regression parameters a , b and m of the isocratic retention equation, Eq. (2), taking into account the contribution to the elution corresponding to the gradient dwell volume (Eq. (16)), and are compared with the experimental data, $V_R(E)$, for various profiles of the gradients of 2-propanol and dioxane in *n*-hexane (Tables 3–5). The experimental elution volumes are, in most cases, higher than the

calculated values, which can be explained by the effect of the preferential adsorption of the polar solvent.

With gradients starting at 0% polar solvent, the mean differences between the experimental and the calculated elution volumes are larger than with gradients starting at a higher concentration and increase as the gradient time increases from 30 to 90 min, i.e., as the slope of the gradient decreases (from 4.9 to 5.6% on the nitrile column and from 1.2 to 5.3% on the amine column). The order of increasing prediction error agrees with the order of increasing breakthrough volume (Table 2). The error of prediction is generally more significant with earlier eluted than with the more strongly retained sample compounds. This behaviour is in agreement with the assumed role of the preferential adsorption of the stronger solvent.

We tried to account for this effect by adding the breakthrough volumes determined in blank gradient runs to the gradient dwell volume in the calculations, but the elution volumes calculated in this way were significantly higher than the experimental data. We are now investigating a more rigorous way of correcting for preferential adsorption by numerical calculations of the band profiles, taking into account competition between the sample solutes and the polar

Table 3

Experimental retention volumes, V_R (E), and the values calculated from Eqs. (5) and (16) using the two-step isocratic-gradient approach with the three-parameter equation (Eq. (2)), V_R (C)

Gradient		0–50% in 30 min	0–50% in 60 min	0–50% in 90 min	3–50% in 30 min	6–50% in 30 min	9–50% in 30 min
A		0	0	0	0.03	0.06	0.09
B		0.016667	0.0083333	0.0055556	0.0156667	0.0146667	0.0136667
Solute	V_R (ml)						
1	V_R (C)	9.79	13.14	15.60	8.23	6.79	5.58
	V_R (E)	10.16	14.02	16.71	8.46	7.04	5.71
2	V_R (C)	8.58	11.58	13.88	6.89	5.41	4.27
	V_R (E)	8.93	12.23	14.50	7.01	5.50	4.32
3	V_R (C)	8.77	11.81	14.09	7.13	5.66	4.50
	V_R (E)	9.10	12.13	14.70	7.21	5.58	4.56
4	V_R (C)	8.22	11.03	13.16	6.52	5.07	3.99
	V_R (E)	8.55	11.49	13.74	6.67	5.21	4.08
5	V_R (C)	9.67	13.57	16.70	7.99	6.37	5.03
	V_R (E)	10.40	14.07	17.31	8.19	6.59	5.19
6	V_R (C)	6.50	8.25	9.49	4.85	3.66	2.90
	V_R (E)	7.08	9.22	10.55	5.01	3.76	2.94
8	V_R (C)	8.59	11.62	13.93	6.91	5.42	4.28
	V_R (E)	8.94	12.04	14.57	7.02	5.62	4.38
12	V_R (C)	5.97	7.72	9.06	4.08	2.91	2.29
	V_R (E)	6.34	8.26	9.84	4.29	3.03	2.34

Parameters of Eq. (2)	a	b	m
Solute 1	0.069	2.577	1.514
2	0.043	3.688	1.431
3	0.060	3.445	1.497
4	0.035	3.884	1.352
5	0.003	3.435	1.461
6	0.081	3.624	1.280
8	0.040	3.688	1.426
12	0.015	8.086	1.162

A, B = initial concentration and slope of the gradient (Eq. (3)).

Column: Separon SGX CN, $V_0 = 0.966$ ml.

Gradients of 2-propanol in *n*-hexane, 40°C, 1 ml/min, dwell volume, $V_D = 1.0$ ml.

Numbers of solutes are the same as in Table 1.

solvent for the adsorption sites on the surface of the column packing material.

With gradients starting at 3–9% polar solvent, mean deviations of the calculated elution volumes from the experimental values are significantly lower than with gradients starting at 0% polar solvent and, with few exceptions, are in between 0.1 and 0.25 ml. The errors of predicted retention volumes generally decrease as the initial concentration of the polar solvent increases. This effect is more apparent for the gradients of dioxane than for the gradients of 2-propanol with the nitrile column. This behaviour can possibly be explained by a weaker role of the

effect of the preferential adsorption on a column partially saturated with the polar solvent at the start of the gradient run. The differences between the calculated and the experimental elution volumes are slightly higher with gradients of dioxane (Table 4) than with gradients of 2-propanol (Table 5), which could possibly be explained by a less steep profile of the sorption isotherm of dioxane (Fig. 4). Similar differences between the experimental and the calculated elution volumes as with the nitrile column were found in the experiments with the amine column (Table 5), and were similar to the results obtained earlier in gradient elution on an unmodified silica gel

Table 4

Experimental retention volumes, V_R (E), and the values calculated from Eqs. (5) and (16) using the two-step isocratic-gradient approach with the three-parameter equation (Eq. (2)), V_R (C)

Gradient		0–100% in 30 min	0–100% in 60 min	0–100% in 90 min	3–100% in 30 min	6–100% in 30 min	9–100% in 30 min
A		0	0	0	0.03	0.06	0.09
B		0.0333333	0.0166667	0.0111111	0.0333333	0.0313333	0.0303333
Solute	V_R ml						
1	V_R (C)	11.64	16.83	20.92	10.90	10.13	9.35
	V_R (E)	11.98	17.23	20.34	11.09	10.37	9.49
2	V_R (C)	9.59	13.62	16.82	8.79	7.96	7.13
	V_R (E)	10.06	14.11	17.42	9.00	8.16	7.16
3	V_R (C)	10.12	14.38	17.74	9.33	8.52	7.71
	V_R (E)	10.25	14.98	18.26	9.53	8.64	7.78
4	V_R (C)	9.12	12.82	15.72	8.31	7.49	6.66
	V_R (E)	9.56	13.42	16.26	8.52	7.66	6.77
5	V_R (C)	13.91	21.14	27.14	13.24	12.52	11.78
	V_R (E)	14.26	21.34	25.95	13.39	12.72	11.73
6	V_R (C)	7.43	10.14	12.24	6.56	5.69	4.87
	V_R (E)	8.01	11.28	12.98	6.93	6.00	5.09
7	V_R (C)	5.38	6.57	7.35	4.54	3.82	3.25
	V_R (E)	5.91	7.36	8.29	4.82	4.04	3.36
8	V_R (C)	9.59	13.56	16.68	8.80	7.98	7.16
	V_R (E)	10.07	15.57	17.50	9.06	8.20	7.34

Parameters of Eq. (2)	a	b	m
Solute 1	0.127	1.411	2.439
2	0.084	1.969	2.063
3	0.088	1.748	2.064
4	0.113	2.072	2.146
5	0.103	1.302	2.846
6	0.081	2.925	1.776
7	0.183	3.531	1.479
8	0.124	1.907	2.251

A, B = initial concentration and slope of the gradient (Eq. (3)).

Column: Separon SGX CN, $V_0 = 0.966$ ml.

Gradients of dioxane in *n*-hexane, 40°C, 1 ml/min, dwell volume, $V_D = 1.0$ ml.

Numbers of solutes are the same as in Table 1.

column [5]. However, no systematic effect of the starting concentration of 2-propanol on the mean error of prediction of retention on the Separon SGX Amine column could be determined with gradients starting at 3–9% 2-propanol.

Figs. 5 and 6 illustrate differences between the separation with binary gradients of 2-propanol in *n*-hexane and of dioxane in *n*-hexane. For the particular separation of eight phenylurea herbicides, gradients of dioxane provide significantly better selectivity than gradients of 2-propanol in *n*-hexane. The separation selectivity on the amino-bonded column is better than on the bonded nitrile column in

2-propanol–*n*-hexane gradients, but poorer than on the nitrile phase with dioxane–*n*-hexane gradients.

4.2. Isocratic and gradient elution with ternary mobile phases

Possibilities for the characterisation and prediction of retention using isocratic and gradient elution with ternary mobile phases were studied on a Separon SGX Nitrile column with mobile phases of 2-propanol–dioxane–*n*-hexane. Fig. 7 shows examples of dependencies of retention factors, k , of phenylurea pesticides, on the sum of concentrations of 2-pro-

Table 5

Experimental retention volumes, V_R (E), and the values calculated from Eqs. (5) and (16) using the two-step isocratic-gradient approach with the three-parameter equation (Eq. (2)), V_R (C)

Gradient		0–50% in 30 min	0–50% in 60 min	0–50% in 90 min	3–50% in 30 min	6–50% in 30 min	9–50% in 30 min
A		0	0	0	0.03	0.06	0.09
B		0.0166667	0.00833333	0.00555556	0.0156667	0.0146667	0.0136667
Solute	V_R ml						
1	V_R (C)	10.06	13.70	16.52	8.39	6.89	5.65
	V_R (E)	9.89	13.67	16.65	8.09	6.65	5.86
4	V_R (C)	9.63	12.89	15.22	8.12	6.69	5.46
	V_R (E)	9.82	13.96	16.11	8.02	6.50	5.59
6	V_R (C)	7.19	9.57	11.41	5.38	3.98	3.08
	V_R (E)	7.03	9.60	11.69	5.26	3.98	3.27
7	V_R (C)	5.67	6.82	7.59	4.15	3.26	2.74
	V_R (E)	5.68	7.01	8.21	4.15	3.37	2.97
8	V_R (C)	10.36	14.11	16.92	8.80	7.31	6.03
	V_R (E)	10.68	15.09	17.77	8.80	7.25	6.22
9	V_R (C)	8.96	12.01	14.27	7.34	5.90	4.74
	V_R (E)	8.82	12.17	14.80	7.04	5.67	4.83
10	V_R (C)	4.95	5.78	6.34	3.49	2.81	2.44
	V_R (E)	5.16	6.31	7.12	3.97	3.00	2.62
11	V_R (C)	5.68	6.76	7.47	4.22	3.37	2.86
	V_R (E)	5.81	7.34	8.33	4.23	3.44	3.01

Parameters of Eq. (2)	a	b	m
Solute 1	0.013	2.497	1.257
4	0.147	2.804	1.924
6	0.017	5.581	1.274
7	0.050	5.022	0.882
8	0.060	2.525	1.546
9	0.064	3.235	1.481
10	0.029	5.811	0.682
11	0.046	4.353	0.821

A, B = initial concentration and slope of the gradient (Eq. (3)). Column: Separon SGX Amine; $V_0 = 0.99$ ml.

Gradients of 2-propanol in *n*-hexane, 40°C, 1 ml/min; dwell volume $V_D = 1$ ml.

Numbers of solutes are the same as in Table 1.

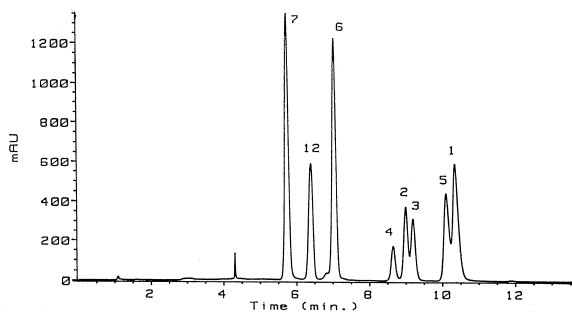


Fig. 5. Separation of phenylurea herbicides on a Separon SGX Nitrile column with a binary gradient of 2-propanol in *n*-hexane, from 0 to 50% vol. in 30 min, at 40°C and 1 ml/min. Detection, UV at 230 nm. Numbers of compounds are the same as in Table 1.

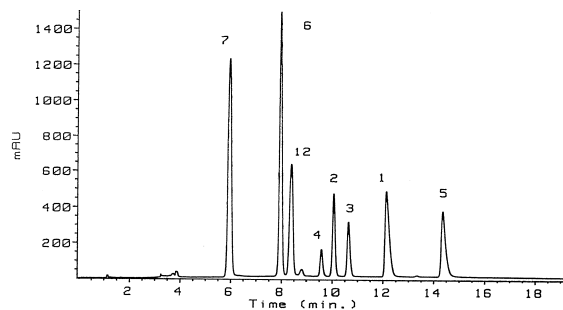


Fig. 6. Separation of phenylurea herbicides on a Separon SGX Nitrile column with a binary gradient of dioxane in *n*-hexane, from 0 to 100% vol. in 30 min, at 40°C and 1 ml/min. Detection, UV at 230 nm. Numbers of compounds are the same as in Table 1.

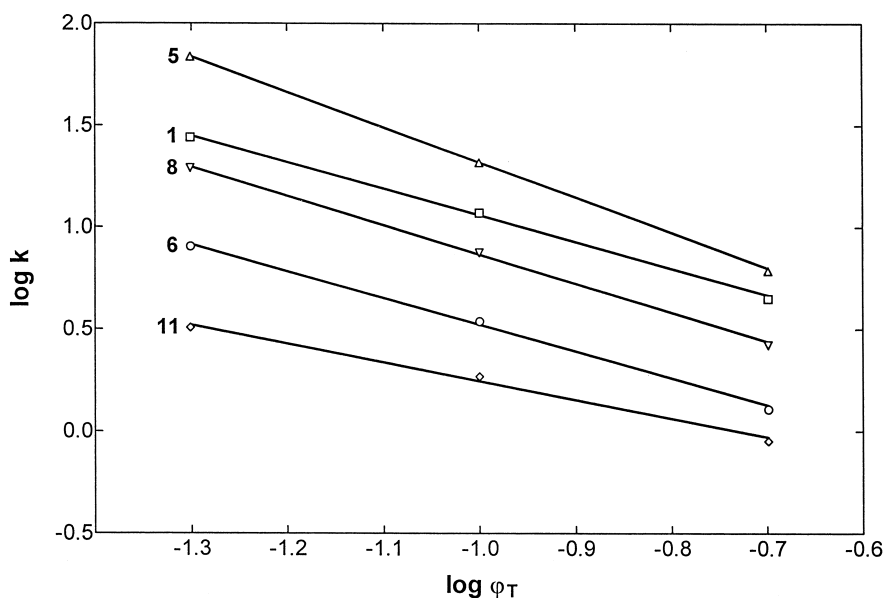


Fig. 7. Dependence of retention factors, k , of phenylurea herbicides on the sum of concentrations of 2-propanol (P) and dioxane (D), φ_T (%vol 10^{-2}), in n -hexane, at a constant concentration ratio of D/P=4:1. Column: Separon SGX Nitrile. Numbers of plots agree with the numbers of compounds given in Table 1. Points=experimental data; lines=best-fit dependencies according to Eq. (6).

panol and dioxane in n -hexane, φ_T , measured in ternary mobile phases at a concentration ratio of 2-propanol to dioxane of 1:4. The plots are linear, in agreement with the expected validity of Eq. (6).

The dependencies of the reciprocal values of the retention factors on the concentration ratio of the polar solvents, X , in the ternary mobile phases, with a constant sum of the concentrations of 2-propanol and dioxane, 20% vol., (points=experimental values) in Fig. 8 are well described by the quadratic equation (Eq. (7)) (lines=regression values). To further test the validity of the quadratic equation (Eq. (7)) in isocratic systems at three different constant sums, φ_T , of the concentrations of polar solvents (5, 10 and 20% vol.), three retention factors were used to calculate the parameters α , β and γ of this equation from Eqs. (8)–(10). For this purpose, two retention factors (k_1 and k_2) were determined in binary mobile phases containing the individual polar solvents at concentrations equal to φ_T and the third (k_3) was measured in the ternary mobile phase with a ratio of the concentrations of 2-propanol and dioxane of 1:1. The retention factors calculated in this way for ternary mobile phases with other concentration ratios of polar organic solvents are

compared with the experimental values in Table 6. In all cases, the differences between the experimental and the predicted values were less than 5%, or 0.1 ml.

Table 7 shows the experimental and calculated elution volumes of phenylurea pesticides on a bonded nitrile column with ternary “elution strength” gradients at the constant concentration ratio of 2-propanol to dioxane of 1:2. The same two-step calculation approach, taking into account the migration of sample bands in the column corresponding to the gradient dwell volume (Eqs. (4) and (16)) was used as with binary gradients. The two-parameter retention equation (Eq. (6)) was assumed to describe the retention at a constant concentration ratio of the two solvents used in these experiments. To provide a more severe test of the calculation approach, the parameters k_{OT} , m_T of this equation were not determined in ternary mobile phases with this concentration ratio of polar solvents, but from the experimental retention factors in binary mobile phases containing 5, 10 and 20% 2-propanol and 5, 10 and 20% dioxane in n -heptane and in ternary mobile phases with ratios of 2.5:2.5:95, 5:5:90 and 10:10:80 dioxane–2-propanol– n -hexane (the con-

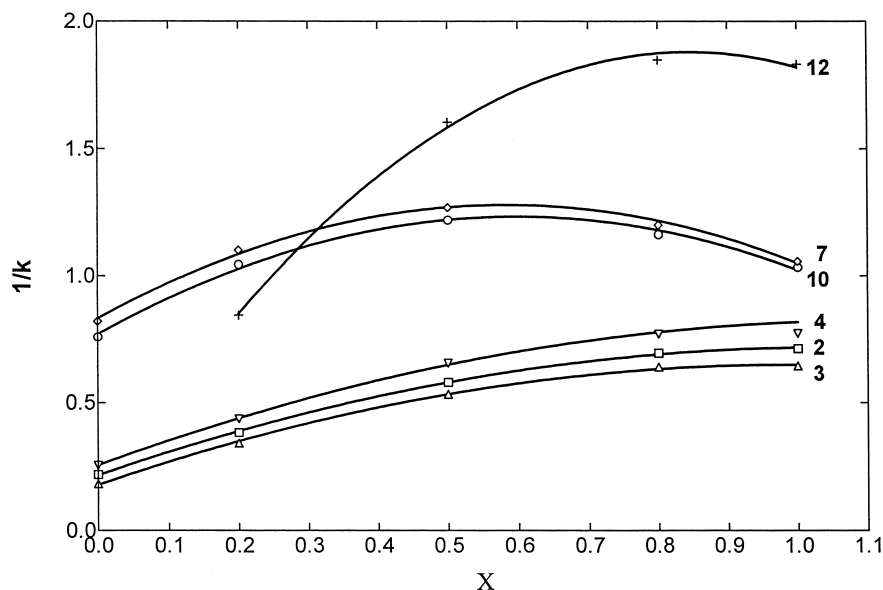


Fig. 8. Dependence of retention factors, k , of phenylurea herbicides on the concentration ratio, X , of 2-propanol, $X = \varphi_p / \varphi_T$ (φ_p = concentration of propanol in % vol 10^{-2}), in ternary mobile phases composed of 2-propanol–dioxane– n -heptane at a constant sum of the concentration of the two polar solvents of 20% vol. Column: Separon SGX Nitrile. Numbers of plots agree with the numbers of compounds given in Table 1. Points = experimental data; lines = best-fit dependencies according to Eq. (7).

centration ratio of 2-propanol–dioxane = 1:1). From three retention factors at each φ_T (concentration sum of the polar solvents at 5, 10 or 20%), the retention factors were calculated for ternary mobile phases with the following ratios: 1.67:3.33:95, 3.33:6.67:90 and 6.67:13.33:80 of 2-propanol–dioxane– n -heptane using Eqs. (7)–(10), as indicated in Table 6. By regression of the dependence of the retention factors determined in this way on the sum of concentrations of the polar solvents, φ_T , the parameters k_{0T} and m_T of Eq. (6) were found and used in Eqs. (4) and (16) to predict the gradient elution volumes. For all gradients, the correct elution order was predicted and mean deviations of the calculated elution volumes from the experimental values were -0.3 ml for gradients starting at 0% n -hexane and -0.18 to -0.24 ml for gradients starting at 3 to 9% of the polar solvents (Table 7). The absolute values of the differences decreased as the initial concentration of the polar solvents at the start of the gradient increased, suggesting the effect of preferential adsorption. The differences between the calculated and the experimental elution volumes in ternary gradient elution (shown in Table 6) were comparable to the

errors of prediction found with binary gradients on the nitrile column (Tables 3 and 4). This agreement suggests that the calculation approach used for ternary gradients is not associated with significant errors.

Table 8 shows the results of the tests of prediction of the elution volumes of phenylurea herbicides on the Separon SGX Nitrile column in gradient elution with ternary “selectivity” gradients, with the constant sum of concentrations of 2-propanol and dioxane in n -hexane equal to 10%, but their concentration ratio being changed during the elution.

Again, the two-step calculation approach, taking into account the migration of sample compounds during the gradient dwell time, was used (Eqs. (15) and (16)). The parameters α , β and γ of Eq. (7) for the individual solutes were determined from the binary and ternary retention factors using Eqs. (8)–(10), as indicated in Table 6. The effect of preferential adsorption on the elution volumes is least with gradients that had decreasing concentrations of 2-propanol (stronger solvent) and simultaneously increasing concentrations of dioxane, where the mean absolute value of the difference between the ex-

Table 6

Experimental retention factors, k (E), and the values k (C) calculated from Eq. (7) with parameters α , β and γ determined from three values, k_1 , k_2 and k_3 (Eqs. (8)–(10)), in ternary mobile phases of 2-propanol (P)–dioxane (D)– n -hexane

Solute	2	3	4	6	7	10	11
$\varphi_T = 0.05, k$							
$X=0$ (k_1)	35.15	40.20	29.36	13.93	4.54	4.78	3.70
$X=1$ (k_2)	8.33	8.91	7.30	3.94	2.55	2.50	2.67
$X=0.5$ (k_3)	11.78	12.06	10.09	5.20	2.64	2.56	2.68
$X=0.2$ (E)	19.56	20.04	16.41	8.05	3.23	3.22	3.21
$X=0.2$ (C)	18.67	19.40	15.79	7.88	3.32	3.31	3.09
$X=0.8$ (E)	9.07	9.36	7.88	4.22	2.54	2.44	2.57
$X=0.8$ (C)	9.22	9.62	8.00	4.22	2.47	2.40	2.59
$\varphi_T = 0.1, k$							
$X=0$ (k_1)	13.70	15.83	11.49	5.75	2.50	2.70	2.66
$X=1$ (k_2)	3.59	3.86	3.23	1.93	1.71	1.70	1.73
$X=0.5$ (k_3)	4.72	4.98	4.10	2.33	1.58	1.57	1.61
$X=0.2$ (E)	7.62	8.17	6.39	3.46	1.80	1.88	1.86
$X=0.2$ (C)	7.28	7.82	6.23	3.38	1.89	1.94	1.96
$X=0.8$ (E)	3.80	4.02	3.36	2.00	1.58	1.56	1.63
$X=0.8$ (C)	3.84	4.08	3.40	1.98	1.57	1.55	1.59
$\varphi_T = 0.2 k$							
$X=0$ (k_1)	4.58	5.52	3.94	2.08	1.22	1.32	1.27
$X=1$ (k_2)	1.40	1.55	1.30	0.84	0.94	0.97	0.96
$X=0.5$ (k_3)	1.72	1.87	1.53	0.93	0.79	0.82	0.81
$X=0.2$ (E)	2.61	2.93	2.29	1.29	0.91	0.96	0.89
$X=0.2$ (C)	2.56	2.85	2.23	1.29	0.93	0.98	0.96
$X=0.8$ (E)	1.44	1.56	1.30	0.83	0.83	0.86	0.85
$X=0.8$ (C)	1.45	1.59	1.32	0.83	0.82	0.84	0.84

Column: Separon SGX CN, $V_0 = 0.966$ ml.

φ_p = concentration of 2-propanol, %vol./vol. 10^{-2} .

φ_D = concentration of dioxane, %vol./vol. 10^{-2} .

$\varphi_T = \varphi_p + \varphi_D$ = total concentration of polar solvents in n -hexane.

$X = \varphi_p / (\varphi_p + \varphi_D)$ = concentration ratio.

Temperature, 40°C.

Numbers of solutes are the same as in Table 1.

perimental and the calculated elution volume was 0.06 ml. The mean differences were larger with “selectivity” gradients where the concentration of 2-propanol increased from 5 to 10% (0.11 ml) and from 0 to 10% (0.22 ml), but are comparable with the errors of prediction of the retention volumes in gradient elution with binary gradients starting at a non-zero concentration of the polar solvent.

5. Conclusions

Retention on the Separon SGX Nitrile- and Separon SGX Amine-bonded phase columns in binary mobile phases composed of 2-propanol and

n -hexane or of dioxane and n -hexane under isocratic conditions can be adequately described by a three-parameter retention equation. The parameters of this equation, determined for the sample components, can be used to predict (by calculation) the elution volumes in gradient-elution chromatography with increasing concentration of a polar organic solvent in a non-polar one. To obtain accurate results, pre-elution of the sample under isocratic conditions in the step corresponding to the gradient dwell time should be taken into account in the calculations.

The adsorption isotherms of polar organic solvents on a chemically bonded nitrile column are less steep and the saturation capacities are approached at a higher concentration of the polar solvent in the

Table 7

Experimental retention volumes, V_R (E), and the values calculated from Eqs. (4) and (16), V_R (C), using the two-step isocratic-gradient approach with the two-parameter equation (Eq. (6))

Gradient		0–33.3% P 0–66.7% D in 30 min	0–33.3% P 0–66.7% D in 60 min	1–33.3% P 2–66.7% D in 30 min	2–33.3% P 4–66.7% D in 30 min	3–33.3% P 6–66.7% D in 30 min
A_T		0	0	0.03	0.06	0.09
B		0.0333333	0.0166667	0.0323333	0.0313333	0.0303333
Solute	V_R ml					
1	V_R (C)	8.77	12.02	7.89	7.02	6.20
	V_R (E)	8.97	12.29	8.02	7.15	6.32
2	V_R (C)	7.55	10.34	6.64	5.73	4.90
	V_R (E)	7.84	10.66	6.90	6.01	5.12
3	V_R (C)	7.72	10.55	6.82	5.92	5.10
	V_R (E)	8.09	10.86	7.05	6.15	5.27
4	V_R (C)	7.22	9.83	6.30	5.39	4.57
	V_R (E)	7.73	10.16	6.64	5.66	4.77
5	V_R (C)	9.40	13.44	8.59	7.72	6.86
	V_R (E)	9.60	13.65	8.76	7.91	7.02
6	V_R (C)	6.02	7.95	5.01	4.09	3.36
	V_R (E)	6.33	8.28	5.31	4.37	3.56
7	V_R (C)	4.93	6.07	3.75	3.01	2.54
	V_R (E)	5.21	6.07	3.95	3.21	2.66
8	V_R (C)	7.55	10.34	6.65	5.74	4.91
	V_R (E)	7.95	10.84	6.94	6.01	5.12

Parameters of Eq. (6) found from three retention factors, k' , in two binary and one ternary isocratic mobile phase

Solute		k_{0T}	m_T
1		0.448	1.296
2		0.221	1.416
3		0.266	1.362
4		0.203	1.389
5		0.282	1.668
6		0.142	1.279
7		0.206	0.890
8		0.226	1.407

A_T , B = initial concentration and slope of the gradient (Eq. (12)).

Column: Separon SGX CN, $V_0 = 0.966$ ml.

Ternary “elution strength” gradients of 2-propanol–dioxane (1:2, v/v) in *n*-hexane, 40°C, 1 ml/min, dwell volume, $V_D = 1.0$ ml.

Numbers of solutes are the same as in Table 1.

mobile phase than on an unmodified silica gel column. This is possibly the reason why the elution volumes in binary gradient elution starting in pure non-polar solvent, calculated from the isocratic retention data, differ more from the experimental elution volumes than with the unmodified silica gel column. However, the experimental elution volumes in binary gradients of 2-propanol or dioxane in *n*-hexane on the Separon SGX Nitrile- and Separon SGX Amine-bonded phase columns are only 0.1–0.25 ml higher than the data predicted by calculation.

The retention in ternary mobile phases with a

constant concentration ratio of two polar solvents in a non-polar one can be described in a similar way as in binary mobile phases and a similar calculation approach can be used to predict the gradient-elution data with ternary “elution strength” gradients. In ternary mobile phases with a constant sum of concentrations of the two polar solvents, a three-parameter equation characterises the dependence of isocratic retention factors on the changing concentration ratio of the polar solvents. The parameters of this equation can be determined from the retention data measured in two binary and one ternary mobile

Table 8

Experimental retention volumes, V_R (E), and the values calculated from Eqs. (15) and (16), V_R (C), using the two-step isocratic-gradient approach with the quadratic equation (Eq. (7))

Gradient	10% P+0% D to 0% P+10% D in 30 min		0% P+10% D to 10% P+0% D in 30 min		5% P+5% D to 10% P+0% D in 30 min	
Initial ratio, X_0	1		0		0.5	
Slope, B	-0.0333333		0.0333333		0.0166667	
Solute	V_R (E)	V_R (C)	V_R (E)	V_R (C)	V_R (E)	V_R (C)
1	6.32	6.32	13.46	13.32	7.45	7.76
3	4.66	4.72	10.64	10.26	5.55	5.66
4	4.05	4.10	9.11	8.71	4.78	4.86
5	5.57	5.71	17.49	17.57	8.51	8.71
6	2.79	2.82	6.09	5.63	3.15	3.20
7	2.59	2.61	3.32	3.30	2.44	2.49
8	4.39	4.47	10.05	9.66	5.31	5.42
10	2.53	2.60	3.54	3.46	2.44	2.48
11	2.59	2.69	3.39	3.43	2.46	2.51

Parameters α , β , γ of Eq. (7) determined using Eqs. (8)–(10) from three retention factors k_1 , k_2 in two binary and k_3 in one ternary isocratic mobile phase (Table 6)

Solute	1	α	β	γ
	1	0.0404	0.2400	-0.0965
	3	0.0632	0.3554	-0.1599
	4	0.0870	0.4034	-0.1806
	5	0.0851	0.4063	-0.1797
	6	0.1738	0.6735	-0.3245
	7	0.4000	0.7529	-0.5678
	8	0.0738	0.3472	-0.1429
	10	0.3706	0.8539	-0.6376
	11	0.3766	0.7852	-0.5848

X_0 , B = the initial concentration ratio of 2-propanol to dioxane and the slope of the gradient (Eq. (14)).

Column: Separon SGX CN, V_0 = 0.966 ml.

Ternary selectivity gradients with the sum of concentrations of 2-propanol and dioxane = 10% (φ_T = 0.1, % vol./vol. 10^{-2}) in *n*-hexane, 40°C, 1 ml/min, dwell volume, V_D = 1.0 ml.

Numbers of solutes are the same as in Table 1.

phases and can be used for prediction of the retention volumes in gradient elution with ternary “selectivity” gradients. The differences between the experimental and the predicted elution volumes in ternary gradient elution are similar to the errors of prediction in binary mobile phases and are higher with the gradients starting in pure non-polar solvent.

To obtain reproducible and predictable results in normal-phase gradient elution, it is necessary to use a sophisticated, precise gradient-elution instrument, to work with dried solvents and at a controlled constant temperature. With these precautions, the main source of the errors of predicted elution volumes is the preferential adsorption of the more

polar solvent during the gradient run. This can be largely eliminated by working with gradients starting at a low concentration of the polar solvent(s) (e.g., 3% or more) instead of in pure non-polar solvent, where possible.

6. List of the symbols used

A	concentration of the strong (polar) solvent at the start of the gradient, in % vol. 10^{-2}
A_i, A_j	A of the individual polar solvents i and j
$A_T = A_i + A_j$	and their sum

B	slope (steepness) of the gradient, in vol (%) 10^{-2} /ml of the eluate	k_1	retention factor of a sample solute in the mobile phase in the dwell volume step, i.e., at the start of the gradient
B_i, B_j	B of the individual polar solvents i and j		
V	volume of the eluate from the sample injection, in ml	k_1	retention factor in a binary mobile phase where $\varphi_i = \varphi_T$ ($X=0$) — Eqs. (8)–(10)
V_B	column breakthrough volume of the polar solvent in gradient elution	k_2	retention factor in a binary mobile phase where $\varphi_j = \varphi_T$ ($X=1$) — Eqs. (8)–(10)
V_D	gradient dwell volume of the instrument, in ml		
V_R	retention volume, in ml	k_3	retention factor in a ternary mobile phase where $\varphi_i + \varphi_j = \varphi_T$ — Eqs. (8)–(10)
$V'_R = V_R - V_0$	net retention volume, in ml	m	parameter of the Eqs. (1) and (2)
V'_{R1}	contribution of the isocratic (dwell volume) step to the net retention volume	m_i, m_j	parameters of Eq. (13) for binary mobile phases containing polar solvents i and j , respectively
V'_{R2}	contribution of the gradient step to the net retention volume		
V_0	column dead (hold-up) volume	m_T	parameter of Eq. (6) at a given ratio X
V_{01}	contribution of the isocratic (dwell volume) step to V_0	r	concentration ratio of the two polar solvents i and j , φ_i/φ_j , in a ternary mobile phase
V_{02}	contribution of the gradient step to V_0		
X	ratio of the concentration of the polar solvent i , φ_i , to the sum of the concentrations of two polar solvents in a ternary mobile phase, φ_T	α, β, γ	parameters of Eq. (7)
X_0	X at the start of a gradient	φ	concentration of the strong (polar) solvent in the mobile phase (isocratic) or the instantaneous concentration of this solvent at the top of the column corresponding to the volume V of the eluate
a	parameter of the retention equation (Eq. (2))	φ_i, φ_j	φ of the polar solvents i and j in a ternary mobile phase
b	parameter of the retention equation (Eq. (2))		
k	retention factor of a sample solute	$\varphi_T = \varphi_i + \varphi_j$	sum of φ of the polar solvents i and j in ternary mobile phases
k_a	retention factor of a sample solute in pure non-polar solvent as the mobile phase	φ_{0i}	φ_i at the start of the gradient
k_i, k_j	retention factors of a sample solute in binary mobile phases containing polar solvents i and j		
k_0	retention factor of a sample solute in pure polar solvent; parameter of the retention equation (Eq. (1))		
k_{0T}	retention factor of a sample solute in a binary mobile phase containing two polar solvents at a given ratio X ; parameter of the retention equation (Eq. (6))		
k_{0i}, k_{0j}	k_0 in pure polar solvents, i and j parameters of the retention equations (Eq. (13))		

Acknowledgements

This publication is based on work under Project No. 203/94/1397 sponsored by the Grant Agency of Czech Republic.

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