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Separation of some chlorophenoxyacetic acid congeners on a porous graphitized carbon column

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ABSTRACT

The retentions of twelve phenoxyacetic acid derivatives were measured on a porous graphitized carbon (PGC) column using dioxane–water mobile phases without additives and with added sodium acetate, acetic acid and lithium chloride. Good linear correlations were found between the capacity factors and the dioxane concentration in the mobile phase. The number of substituents on the benzene ring and their hydrophilicity most strongly affected the retention behaviour of phenoxyacetic acid derivatives with dioxane–water eluents. Sodium acetate had the greatest effect on both retention and selectivity, emphasizing the considerable role of the degree of dissociation of solutes on their retention on a PGC column.

INTRODUCTION

Chlorophenoxyacetic acid herbicides are applied extensively in chemical plant protection of rice [1], winter wheat [2,3] and soybean [4]. To increase their effect, phenoxyacetic acids are frequently used in combination with other herbicides [5,6]. Many HPLC methods have been developed for the determination of phenoxyacetic acid herbicides [7]. Separation of phenoxyacetic acids has been carried out on octadecylsilica using methanol–water as the eluent, acidified with trifluoroacetic acid [8] or acetic acid [9]. An octylsilica column has also been used for the separation of these herbicides with acetic acid and methanol–water as eluents [10]. The addition of the ion-pairing agent iron(II) 1,10-phenanthroline to the mobile phase considerably improved the separation of the herbicides [11]. The detection sensitivity was enhanced by the formation of 9-anthryldiazomethane derivatives and by using fluorescence detection [12] and particle beam mass spectrometry [13]. Porous polymer sorbents such as PLRP-S [14] and PRP-1 have also been used for the separation of chlorophenoxyacetic acid congeners [15,16].

Porous graphitized carbon (PGC) supports have gained increasing acceptance and application in HPLC. A carbon adsorbent with organic solvents as the eluent was suitable for the separation of isomers [17]. PGC showed lower equilibration times than a DIOL column and the water content of the organic mobile phase had no significant effect on the equilibration time [18]. Polar phenol derivatives have been successfully separated on a PGC column without buffering the eluent [19]; steric and electronic parameters of the solutes had the greatest effect on retention [20]. The application of PGC in biomedical research has recently been reviewed (21).

The retention mechanism on PGC columns has not been elucidated in detail. It has been established that the retention of anionic compounds is dominated by electronic interactions between the solute and the delocalized electron bonds on the graphitized carbon, whereas cationic compounds are retained mainly by reversed-phase interactions with the hydrophobic carbon surface [22].

The objectives of this investigation were to determine the retentions of twelve phenoxyacetic acids

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on a PGC column with various eluent systems, to assess the effect of several eluent additives on the separation power of the column and to evaluate the results with multivariate methods.

EXPERIMENTAL

A PGC column (Shandon Hypercarb, 100 × 4.7 mm I.D., particle diameter 7 μm) was purchased from Shandon Scientific (Runcorn, UK). The HPLC system consisted of a Liquopump Model 312 pump (Labor MIM, Budapest, Hungary), a Cecil (Cambridge, UK) CE-212 variable-wavelength UV detector, a Valco (Houston, TX, USA) injector with a 20-μl loop and a Waters Model 740 integrator (Waters–Millipore, Milford, MA, USA). The flow-rate was 0.6 ml/min and the detection wavelength was 230 nm. Dioxane–water mixtures were used as eluents with dioxane concentrations ranging from 30 to 85% (v/v) (in steps of 5%). To study the effect of eluent pH and salt concentration, the retention of the solutes was also determined in dioxane–water (7:3, v/v) containing 50, 25, 10 and 5 m*M* LiCl, 50 m*M* sodium acetate or 50 m*M* acetic acid (end concentration). As sodium acetate caused a large decrease in the retention, its effect was also determined in dioxane-water (4:6, v/v).

The structures of the phenoxyacetic acid congeners are shown in Table I. They were dissolved in the eluents at a concentration of 0.1 mg/ml. The retention time of each compound in each eluent was determined with three consecutive injections. As the correlation between log k' and the organic phase concentration is generally linear in HPLC, we applied linear equations to describe the relationship between he capacity factor and the dioxane concentration in the mobile phase:

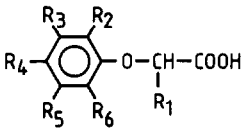
$$\log k' = \log k'_0 + bC \quad (1)$$

where k' = capacity factor, k'_0 = capacity factor extrapolated to zero concentration of organic component in the mobile phase (intercept, related to the retention strength of the solute), b = change in log k' caused by unit change (1%, v/v) in dioxane concentration (slope, related to the specific surface area of the solute contacting the stationary phase) and C = concentration of organic component (%, v/v).

To prove the validity of the hypothesis outlined in ref. 23 that for a homologous series of solutes the slope and intercept values are stongly intercorrelated, the linear correlation was calculated between the slope and intercept values of eqn. 1. A modified Free–Wilson analysis [24] was applied to select the substituents of the solutes having the greatest effect on the retention behaviour of these compounds. Free–Wilson analysis was developed for the calculation of the contributions of substituents to the biological activity of a homologous series of compounds, the activity of the unsubstituted molecule being equal to zero. In the traditional Free–Wilson analysis, the presence of independent variables (substituents) that exert no significant influence on the dependent variable (biological activity) lessens the significance level of the independent variables that significantly influence the dependent variable. To overcome this difficulty, the modified Free–Wilson analysis automatically eliminates from the selected equation the insignificant independent variables (substituents), increasing in this manner the information power of the calculation. The same calculation can be used for the selection of substituents of solutes having the greatest effect on their retention under given chromatographic conditions. The slope and intercept values of eqn. 1 were the depen-

TABLE I

STRUCTURES OF PHENOXYACETIC ACID CONGENERS



R_{1-6} = H except where stated otherwise.

No.	R_1	R_2	R_3	R_4	R_5	R_6
1						
2				Cl		
3		Cl		Cl		
4		Cl	Cl			
5		Cl				Cl
6		Cl			Cl	
7			Cl	Cl		
8			Cl		Cl	
9		Cl		Cl	Cl	
10	CH_3	CH_3		Cl		
11	CH_3	Cl		Cl	Cl	
12	β-Naphthoxyacetic acid					

dent variables and the individual substituents and the sum of substituents on the benzene ring were the independent variables. The acceptance level for the independent variables was set to the 95% significance level. Owing to the small number of compounds, the number of accepted variables was limited to one.

To correlate the retention behaviour of phenoxyacetic acids with their physico-chemical parameters, stepwise regression analysis [25] was applied. The physico-chemical parameters included in the stepwise regression analysis as independent variables were as follows: π = Hans–Fujita substituent constant characterizing hydrophobicity; M-RE = molar refractivity; F and R = Swain–Lupton electronic parameters characterizing inductive and resonance effects, respectively; σ = Hammett's constant, characterizing the electron-withdrawing power of a substituent; E_s = Taft's constant, characterizing the steric effects of a substituent; and B_1, B_4 = Sterimol width parameters determined by the distance of substituents at their maximum point perpendicular to the attachment bond axis. The other conditions were the same as in the Free–Wilson analysis.

The effects of various eluent additives on the solvent strength and selectivity were calculated by the spectral mapping technique [26–28]. The capacity factors of the phenoxyacetic acids were the observations and the following eluents were the variables: dioxane–water (7:3, v/v) without additive, with 50 mM LiCl and acetic acid and dioxane–water (4:6, v/v) with 50 mM sodium acetate.

To study the effect of eluent additives on the performance of the PGC column, the theoretical plate number and the asymmetry factor were calculated for each solute in each eluent.

RESULTS AND DISCUSSION

Eluent additives do not change the retention order of chlorophenoxyacetic acids (Fig. 1). Sodium acetate considerably decreases the retention whereas the effects of acetic acid and LiCl are similar; however, the separation is better with LiCl as additive. This observation can be explained by the assumption that sodium acetate increases the dissociation of the polar solutes, and the dissociated form shows a lower retention on the carbon surface. Both acetic acid and LiCl probably suppress the dissociation, resulting in enhanced retention.

Each phenoxyacetic acid shows a regular retention behaviour (Fig. 2), the retention decreasing linearly with increasing concentration of dioxane in the eluent. The parameters of eqn. 1 are compiled in Table II. The relationship between log k' and the dioxane concentration in the eluent was significantly linear in each instance, that is, the retention of

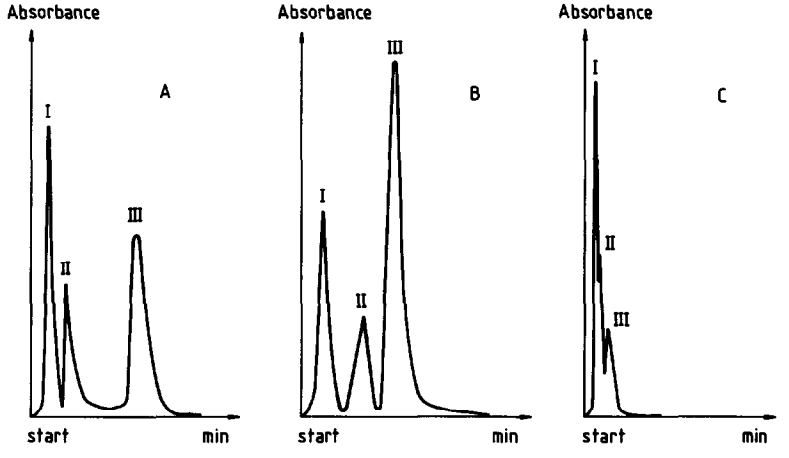


Fig. 1. Separation of chlorophenoxyacetic acids on the PGC column. I = 4-Chlorophenoxyacetic acid; II = 2,4-dichlorophenoxyacetic acid; III = 2, 4, 5-trichlorophenoxyacetic acid. A = dioxane–water (7:3, v/v), 50 mM acetic acid end concentration; B = dioxane–water (7:3, v/v), 50 mM LiCl end concentration; C = dioxane–water (7:3, v/v), 50 mM sodium acetate end concentration.

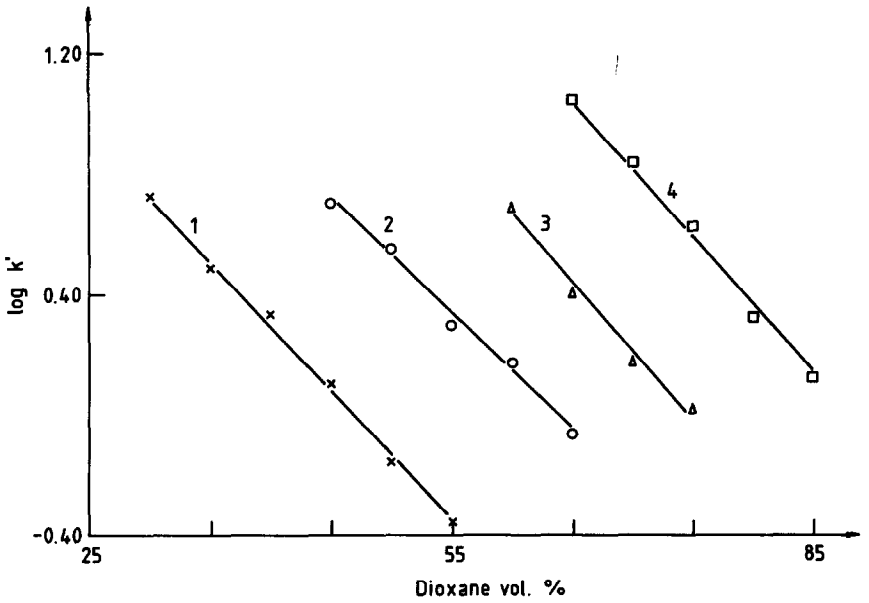


Fig. 2. Dependence of the capacity factor of phenoxyacetic acid derivatives on the dioxane concentration in the eluent. 1 = Phenoxyacetic acid; 2 = 4-chlorophenoxyacetic acid; 3 = 2,4-dichlorophenoxyacetic acid; 4 = 2,4,5-trichlorophenoxyacetic acid.

phenoxyacetic acid derivatives decreases linearly with increasing concentration of the organic component. The significance level in most instances was higher than 99.9%, confirming the applicability of eqn. 1. This indicates that the phenoxyacetic acid derivatives follow the general rule on PGC columns, and no anomalous retention behaviour was observed. The intercept values differ considerably from each other, which means that the these derivatives can be separated easily on the PGC column.

TABLE II

PARAMETERS OF THE LINEAR CORRELATION BETWEEN THE CAPACITY FACTOR (LOG k') OF PHENOXYACETIC ACID DERIVATIVES AND THE DIOXANE CONCENTRATION [C, (v/v)] IN THE MOBILE PHASE

Compounds numbers refer to phenoxyacetic acid derivatives in Table I.

$\log k' = \log k'_0 + bC$

Compound	Log k'_0	$-b \times 10^2$	Significance level (%)
1	1.96 ± 0.46	4.18 ± <0.01	99.9
2	2.47 ± 0.33	3.85 ± 0.02	99.9
3	3.43 ± 0.35	4.59 ± 0.3	95.0
4	3.18 ± 0.35	4.39 ± <0.01	99.9
5	2.35 ± 0.16	3.78 ± 0.03	99.9
6	3.28 ± 0.38	4.39 ± 0.02	99.0
7	3.48 ± 0.36	4.53 ± 0.01	95.0
8	3.45 ± 0.39	4.52 ± 0.02	99.0
9	3.94 ± 0.35	4.46 ± <0.01	99.9
10	3.52 ± 0.29	4.45 ± 0.02	99.9
11	3.88 ± 0.36	4.56 ± <0.01	99.9
12	3.08 ± 0.24	3.69 ± 0.01	99.9

The parameters in Table II make possible the calculation of retention time differences for each pair of derivatives at each eluent composition:

$$t_1 - t_2 = t_0(10^{a_1+b_1C} - 10^{a_2+b_2C}) \quad (2)$$

where a and b are the intercept and slope values for compounds 1 and 2 at a dioxane concentration C.

The relationship between the intercept (hydrophobicity) and the slope (specific hydrophobic surface area) values of eqn. 1 is significant, but the correlation is not strong enough to substitute the parameters with each other:

$$\log k'_0 = -(2.42 \pm 0.61) + (1.31 \pm 0.42) \cdot 10^2 b$$
$$r = 0.6966; n = 12 \quad (3)$$

According to ref. 23, a series of solutes can be considered homologous from the chromatographic point of view when there is a strong linear correlation between the corresponding slope and intercept

values. The relatively low correlation coefficient indicates that the phenoxyacetic acids cannot be considered as a homologous series of solutes according to their retention behaviour on the PGC column.

The results of modified Free–Wilson and stepwise regression analysis are compiled in Table III. The number of substituents on the benzene ring exerts the greatest effect on both the intercept (a values) and slope (b values) of eqn. 1 (see eqns. 4 and 5 in Table III). This result emphasizes the importance of steric parameters in the retention mechanism of PGC. The lipophilicity of phenoxyacetic acids significantly influences their retention (see eqn. 6 in Table III). As the substituents are apolar, and the differences between their lipophilicity is not very high, the conclusions drawn from eqns 4–6 are in good agreement: in this special case the higher the number of substituents, the higher is the lipophilicity of the compound.

The retention of the polar phenoxyacetic acids increases with increasing LiCl concentration (Fig. 3). This finding is in good agreement with the findings in refs. 29 and 30 that the dissociated ions markedly modify the retention (lipophilicity) of bioactive compounds containing one or more polar

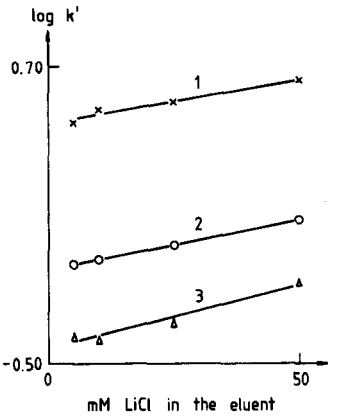


Fig. 3. Dependence of the capacity factors of phenoxyacetic acid derivatives on the LiCl concentration in the dioxan–water (7:3, v/v) eluent. 1 = 2,4,5-Trichlorophenoxyacetic acid; 2 = 3,5-dichlorophenoxyacetic acid; 3 = 2,3-dichlorophenoxyacetic acid.

substructures. The data emphasize again the role of lipophilicity in the retention. The salt suppresses the dissociation of polar substituents, increasing the apparent lipophilicity of the solute (salting-out effect).

The retention of solutes in the eluents dioxane–water (7:3, v/v) decreased in the sequence 50 mM sodium acetate > 50 mM acetic acid > water > 50 mM LiCl. This finding emphasizes again the importance of the degree of dissociation of the solute in the retention of polar molecules on a PGC column. The eluent additives form two separate clusters on the two-dimensional non-linear selectivity map (Fig. 4). The distribution of eluent additives indi-

TABLE III

RELATIONSHIP BETWEEN THE RETENTION PARAMETERS OF PHENOXYACETIC ACID DERIVATIVES AND THE PHYSICO-CHEMICAL PARAMETERS OF THE SUBSTITUENTS

Results of Free–Wilson and stepwise regression analysis (n = 12).

$$\log k'_0 = a + b_1 x_1 \quad (4)$$

$$b = a + b_1 x_1 \quad (5)$$

$$\log k'_0 = a + b_1 x_2 \quad (6)$$

x_1 = number of substituents on the benzene ring; x_2 = lipophilicity of substituents; $r_{95\%}$ = 0.5760; $r_{99\%}$ = 0.7079; $r_{99.9\%}$ = 0.8233.

Parameter	Number of equation		
	4	5	6
a	2.35	3.89	1.98
s_a	0.61	0.33	0.61
b_1	0.47	0.22	0.82
s_{b_1}	0.14	0.08	0.16
r	0.7356	0.6561	0.8561

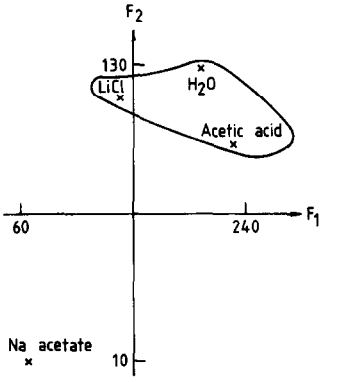


Fig. 4. Two-dimensional non-linear selectivity map of eluent additives. Number of iterations: 41. Maximum error: $4.02 \cdot 10^{-3}$.

TABLE IV

EFFECT OF ELUENT ADDITIVES ON THE THEORETICAL PLATE NUMBER (TPN) AND ASYMMETRY FACTOR (ASF) OF CHLOROPHENOXYACETIC ACID CONGENERS

Compound numbers refer to solutes in Table I.

No. of solute	Eluent additive							
	None		Sodium acetate		Acetic acid		LiCl	
	TPN	ASF	TPN	ASF	TPN	ASF	TPN	ASF
4	269	0.71	224	0.72	243	0.90	505	0.86
5	318	0.83	413	0.79	457	0.93	1022	0.93
10	209	0.66	201	0.95	299	0.83	918	0.95
11	255	0.45	192	0.91	316	0.84	920	0.91

cates that sodium acetate has the greatest effect on the selectivity whereas the effects of acetic acid and LiCl are of secondary importance.

The highest theoretical plate number and the best asymmetry factor were found with eluents containing LiCl (Table IV). The theoretical plate numbers were similar in eluents without an additive and with sodium acetate and acetic acid, whereas the peak asymmetry was the highest in the eluent without an additive. As far as we are aware, the influence of various eluent additives on the performance of PGC columns has not been studied in detail. Unfortunately, our data are not sufficient to be able to draw valid theoretical conclusions about the effect of eluent additives on theoretical plate number and peak asymmetry. These effects are not clearly understood, but our data lend support to the hypothesis that the addition of salts to the eluent may improve the separation efficiency of PGC columns for polar solutes.

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