

High-performance liquid chromatographic retention behaviour of ring-substituted aniline derivatives on a porous graphitized carbon column

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ABSTRACT

The retentions of sixteen ring-substituted aniline derivatives were determined on a graphitized carbon column using unbuffered acetonitrile–water and methanol–water eluent mixtures at various organic phase concentrations. Principal component analysis calculated from both the correlation and covariance matrices was used to detect the similarities and dissimilarities between the retention behaviours of the aniline derivatives. Each aniline derivative showed narrow and symmetrical peaks in each eluent. The high percentage of variance explained in the first principal component suggests that the eluents have common elution characteristics, but according to the second principal component they showed slightly different selectivities. The retention of aniline derivatives is not governed by their lipophilicity and bulkiness. The data indicate that a mixed type of retention mechanism accounts for the retention.

INTRODUCTION

The poor stability of silica at alkaline pH values [1] and the presence of free silanol groups on the surface of silica-based reversed-phase supports [2,3] limits the application of silica materials in chromatography. In order to suppress the effect of electrostatic interactions between the polar solutes and the acidic silanol groups, the eluent has to be buffered or various eluent additives such as salts and organic amines have to be added to the eluent [4]. To increase the pH range of application, many other supports have been developed, such as alumina [5], octadecyl-coated alumina [6], zirconia [7,8] and various polymer-based supports [9]. A promising new support, porous graphitic carbon (PGC), has been developed in the last decade [10,11]. The characteristics of PGC have recently been discussed [12]. Successful separations of basic solutes have been achieved on PGC columns [13,14]. It was found that the electronic interactions between the solutes and the surface of PGC have a considerable impact on the retention [15], and the structural planarity of

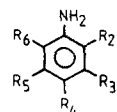
solutes exerted a much greater influence on the selectivity on PGC than on C_{18} columns [16].

Principal component analysis (PCA) has frequently been used for the evaluation of retention data matrices [17]. In situations with many variables (chromatographic systems) and observations (solutes), the comparison of the retention behaviours of chromatographic systems taking into consideration simultaneously the retention of each solute is difficult owing to the multi-dimensionality of the data matrix. PCA allows the easier evaluation of the similarities or dissimilarities between solutes and eluent systems.

EXPERIMENTAL

A porous graphitic carbon column (Hypercarb, 100×4.7 mm I.D. particle diameter $7 \mu\text{m}$) was purchased from Shandon Scientific (Runcorn, UK). The high-performance liquid chromatographic system consisted of a Liquopump Model 312 pump (LaborMIM, Budapest, Hungary), a Model CE-212 variable-wavelength UV detector (Cecil In-

TABLE I
STRUCTURES OF RING-SUBSTITUTED ANILINE DERIVATIVES



No.	R2	R3	R4	R5	R6
1	CH ₃	CH ₃	H	H	H
2	CH ₃	H	H	CH ₃	H
3	CH ₂ CH ₃	H	H	H	CH ₃
4	H	H	Cl	H	H
5	Cl	H	Cl	H	H
6	H	Cl	H	Cl	H
7	H	Br	H	H	H
8	H	H	Br	H	H
9	Br	H	Br	H	H
10	I	H	H	H	H
11	H	H	I	H	H
12	NO ₂	H	H	H	H
13	H	NO ₂	H	H	H
14	H	H	NO ₂	H	H
15	NO ₂	H	H	H	NO ₂
16	Cl	H	NO ₂	H	H

struments, Cambridge, UK), an injector (Valco, Houston, TX, USA) with a 20- μ l sample loop and a Waters Model 740 integrator (Waters-Millipore, Milford, MA, USA). The flow-rate was 1 ml/min and the detection wavelength was set at 254 nm. Mixtures of methanol-water and acetonitrile-water were used as eluents, with methanol and acetonitrile concentrations ranging from 90 to 97.5% (v/v) (in steps of 2.5%, v/v) and 85–92.5% (v/v) (in steps of 2.5%, v/v), respectively. The structures of the ring-substituted aniline derivatives are shown in Table I. The aniline derivatives were dissolved in methanol or acetonitrile at a concentration of 0.05 mg/ml. The retention time of each compound in each eluent was determined with three consecutive determinations.

To compare the performance of PGC with that of a traditional reversed-phase column, the retentions of compounds **4**, **9** and **10** were determined on a 150 \times 4 mm I.D. Hypersil ODS (5 μ m) column with water-methanol (4:6, v/v) and 0.005 M K₂HPO₄-methanol (4:6, v/v) (pH adjusted to 7.80 with orthophosphoric acid) eluents. The asymmetry factor

and the plate number for each derivative were calculated as described [18]. The plate number for toluene as a non-polar compound was also determined on both columns. The performance of the column was characterized by the ratio of the plate number for aniline derivative to the plate number for toluene. We assume that this ratio reflects the influence of solute polarity on the column performance.

In the PCA the mean retention time \pm 2 standard deviations (S.D.) of aniline derivatives determined with in eight eluents formed the data matrix. The various acetonitrile-water and methanol-water eluents (four of each) were considered as variables, and the mean retention times \pm 2S.D. of aniline derivatives were the observations. The calculation was carried out on both the correlation and covariance matrices. The application of both PCA methods for the same data matrix was motivated by the finding that the normalization (needed for the calculation of the correlation matrix) causes distortion of the data which may lead to inadequate results [19]. The explained variance was set to 99.9% in both instances. To compare the information content of PCA methods, linear correlations were calculated between PCA variables and loadings calculated from the two different matrices. Dependent variables were always those calculated from the covariance matrix. Only the PCA variables and loadings with the same serial number were correlated.

$$A_{\text{cov}} = a + b A_{\text{corr}} \quad (1)$$

$$Z_{\text{cov}} = a + b Z_{\text{corr}} \quad (2)$$

where A = PCA loadings, Z = PCA variables, corr = calculated from correlation matrix and cov = calculated from covariance matrix.

RESULTS AND DISCUSSION

Each aniline derivative showed symmetrical peaks in each eluent system (Figs. 1 and 2), that is, the carbon column can be successfully used for the separations of ring-substituted aniline derivatives without buffering the eluent. The retention order of solutes does not follow the retention order expected according to their lipophilicity. The more hydrophilic nitro derivative elutes after the more hydrophobic methoxy, chloro and iodo derivatives (Fig. 1). Substitution isomers could be well separated on

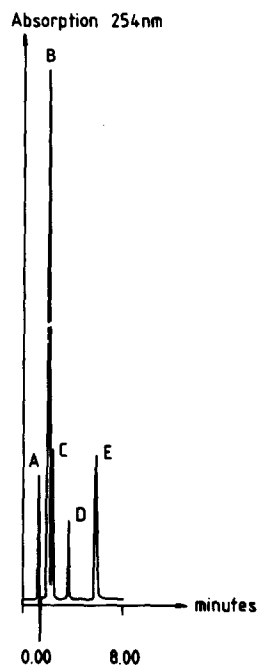


Fig. 1. Separation of aniline derivatives on a porous graphitic carbon column. Eluent, methanol-water (95:5, v/v); flow-rate, 1 ml/min; detection, 254 nm. A = Aniline; B = 2-methoxyaniline; C = 2-chloroaniline; D = 2-iodoaniline; E = 2-nitroaniline.

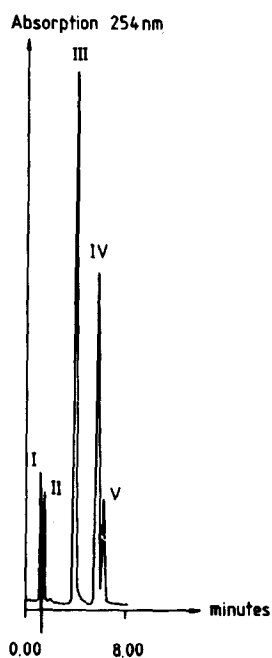


Fig. 2. Separation of aniline derivatives on a porous graphitic carbon column. Eluent, methanol-water (97.5:2.5, v/v); flow-rate, 1 ml/min; detection, 254 nm. I = Dead time; II = aniline; III = 3-nitroaniline; IV = 2-nitroaniline; V = 4-nitroaniline.

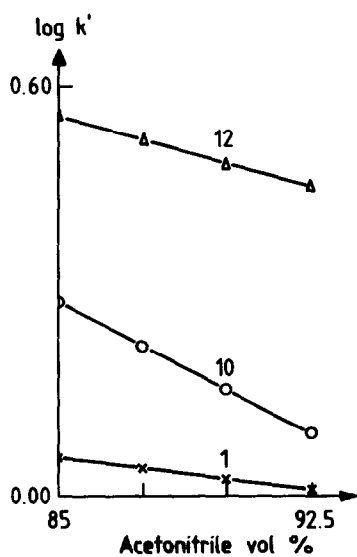
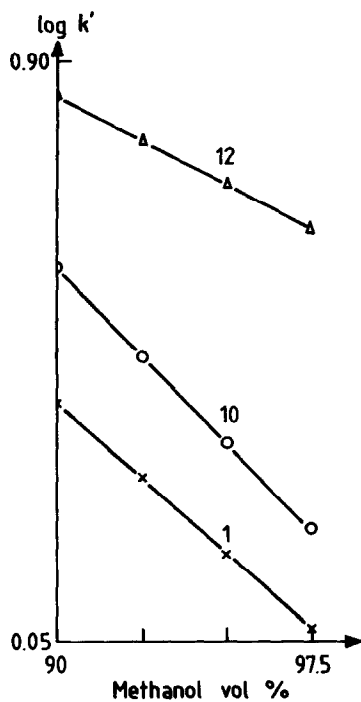


Fig. 3. Dependence of $\log k'$ values of ring-substituted aniline derivatives on the concentration of organic modifier in the eluent. Numbers refer to aniline derivatives in Table I.

TABLE II
RETENTION TIMES OF ANILINE DERIVATIVES

Compound	Retention time (min)			
	Methanol (% v/v)			
	90	92.5	95	97.5
1	$2.92 \pm 1.4 \cdot 10^{-2}$	$2.73 \pm 5.6 \cdot 10^{-2}$	$2.42 \pm 7.0 \cdot 10^{-3}$	$2.25 \pm 7.0 \cdot 10^{-3}$
2	$4.45 \pm 3.5 \cdot 10^{-2}$	$4.90 \pm 7.0 \cdot 10^{-3}$	$2.70 \pm 2.8 \cdot 10^{-2}$	$2.69 \pm 4.3 \cdot 10^{-2}$
3	$5.66 \pm <10^{-3}$	$4.95 \pm <10^{-3}$	$3.78 \pm 1.4 \cdot 10^{-2}$	$3.48 \pm <10^{-3}$
4	$3.46 \pm 2.1 \cdot 10^{-2}$	$3.00 \pm 7.0 \cdot 10^{-3}$	$2.77 \pm 3.5 \cdot 10^{-2}$	$2.02 \pm 1.4 \cdot 10^{-2}$
5	11.30 ± 0.19	$8.92 \pm 7.0 \cdot 10^{-3}$	$6.30 \pm 7.0 \cdot 10^{-3}$	$5.18 \pm <10^{-3}$
6	$10.80 \pm <10^{-3}$	8.70 ± 7.10^{-3}	$5.91 \pm 7.0 \cdot 10^{-3}$	$4.66 \pm 2.8 \cdot 10^{-2}$
7	$2.69 \pm <10^{-3}$	$2.32 \pm <10^{-3}$	$1.93 \pm 7.0 \cdot 10^{-3}$	$1.76 \pm <10^{-3}$
8	$4.13 \pm 4.9 \cdot 10^{-2}$	$3.50 \pm 1.4 \cdot 10^{-2}$	$2.81 \pm 1.4 \cdot 10^{-2}$	$2.41 \pm 1.4 \cdot 10^{-2}$
9	$16.60 \pm <10^{-3}$	$14.04 \pm 3.5 \cdot 10^{-2}$	$10.19 \pm <10^{-3}$	8.47 ± 0.12
10	$5.75 \pm <10^{-3}$	$5.25 \pm <10^{-3}$	$4.05 \pm <10^{-3}$	$3.27 \pm 7.0 \cdot 10^{-3}$
11	$5.82 \pm 6.3 \cdot 10^{-2}$	$4.83 \pm 3.5 \cdot 10^{-2}$	$3.65 \pm <10^{-3}$	$3.13 \pm 1.4 \cdot 10^{-2}$
12	$10.56 \pm 7.0 \cdot 10^{-3}$	$9.13 \pm <10^{-3}$	$6.79 \pm 2.1 \cdot 10^{-2}$	$5.53 \pm 3.5 \cdot 10^{-2}$
13	$8.10 \pm <10^{-3}$	6.53 ± 0.21	$4.84 \pm <10^{-3}$	$3.92 \pm 7.0 \cdot 10^{-3}$
14	$12.55 \pm <10^{-3}$	10.31 ± 0.11	$7.89 \pm 2.1 \cdot 10^{-3}$	$6.42 \pm 3.5 \cdot 10^{-3}$
15	$63.40 \pm <10^{-3}$	$52.30 \pm <10^{-3}$	$41.50 \pm <10^{-3}$	36.0 ± 0.21
16	$48.49 \pm <10^{-3}$	$34.04 \pm <10^{-3}$	$27.50 \pm <10^{-3}$	$21.11 \pm 7.0 \cdot 10^{-3}$

the column, indicating the importance of the steric parameters in the retention (Fig. 2).

The retention of each compound decreases with increasing concentration of organic modifier (Fig. 3) and it depends on the number and position of substituents (Table II), which indicates the involvement of steric parameters in the mechanism of retention. The peak symmetry is similar on PGC with unbuffered eluent and on an ODS column with buffered eluent, but the relative performance of the PGC column is better than that of the ODS column even with a buffered eluent (Table III).

In both PCA methods the first principal component explained most of the variance (Tables IV and V), hence the main retention characteristics of the eight eluent systems applied can be expressed by only one hypothetical eluent system. Comparing the ratio of variance explained by the individual principal components, both calculation methods give similar results. According to eqn. 1, highly significant correlations were found between the PCA variables (Table VI). This result indicates that in our case the information contents of the PCA variables computed from different matrices are similar. However, the r values show that the predictive pow-

ers of the two calculation methods are different, which means that they are not entirely interchangeable. The distributions of PCA loadings (eluent systems) calculated according to the covariance and correlation matrices are shown in Fig. 4. The coordinates (F_1 and F_2) have no concrete physical meaning; they only indicate the relative distances between the eluent systems in two dimensions.

TABLE III
COMPARISON OF RETENTION CHARACTERISTICS OF POROUS GRAPHITIZED CARBON AND HYPERSIL ODS COLUMNS

(A) Porous graphitized carbon column; (B) ODS column, non-buffered eluent; (C) ODS column, buffered eluent. $P(\%) = (\text{plate number for compound}/\text{plate number for toluene}) \cdot 100$.

Compound	Asymmetry factor			$P(\%)$		
	A	B	C	A	B	C
4	1.23	1.60	1.25	64.02	40.4	47.7
9	1.17	1.67	1.33	65.3	65.6	67.3
10	1.12	1.50	1.05	66.12	39.0	40.1

Acetonitrile (% v/v)

85	87.5	90	92.5
$2.96 \pm <10^{-3}$	$2.50 \pm 7.0 \cdot 10^{-3}$	$2.22 \pm <10^{-3}$	$1.97 \pm 7.0 \cdot 10^{-3}$
$2.46 \pm <10^{-3}$	2.33 ± 0.21	$2.14 \pm <10^{-3}$	$1.75 \pm 7.0 \cdot 10^{-3}$
$3.22 \pm <10^{-3}$	$3.01 \pm <10^{-3}$	$2.75 \pm <10^{-3}$	$2.71 \pm <10^{-3}$
$2.33 \pm <10^{-3}$	$2.20 \pm <10^{-3}$	$2.14 \pm 7.0 \cdot 10^{-3}$	$2.07 \pm <10^{-3}$
$6.53 \pm <10^{-3}$	$5.84 \pm 1.4 \cdot 10^{-2}$	$5.20 \pm <10^{-3}$	$5.08 \pm <10^{-3}$
$5.92 \pm <10^{-3}$	$5.61 \pm <10^{-3}$	$5.19 \pm <10^{-3}$	$4.65 \pm <10^{-3}$
$2.60 \pm 7.7 \cdot 10^{-3}$	$2.53 \pm 4.2 \cdot 10^{-3}$	$2.37 \pm <10^{-3}$	$2.25 \pm <10^{-3}$
$2.82 \pm 7.0 \cdot 10^{-3}$	$2.52 \pm <10^{-3}$	$2.34 \pm 7.0 \cdot 10^{-3}$	$2.05 \pm <10^{-3}$
$10.94 \pm <10^{-3}$	$9.87 \pm <10^{-3}$	$9.24 \pm <10^{-3}$	$8.32 \pm <10^{-3}$
$3.67 \pm <10^{-3}$	$3.52 \pm <10^{-3}$	$3.36 \pm <10^{-3}$	$3.17 \pm <10^{-3}$
$2.35 \pm <10^{-3}$	$2.17 \pm <10^{-3}$	$1.96 \pm 7.0 \cdot 10^{-3}$	$1.80 \pm 7.0 \cdot 10^{-3}$
$5.76 \pm <10^{-3}$	$5.24 \pm <10^{-3}$	$4.95 \pm 7.0 \cdot 10^{-3}$	$4.60 \pm <10^{-3}$
$4.26 \pm 5.6 \cdot 10^{-3}$	$4.07 \pm 2.8 \cdot 10^{-3}$	$3.88 \pm 7.0 \cdot 10^{-3}$	$3.64 \pm 7.0 \cdot 10^{-3}$
$6.22 \pm 7.0 \cdot 10^{-3}$	$5.89 \pm 1.4 \cdot 10^{-3}$	$5.55 \pm <10^{-3}$	$5.16 \pm <10^{-3}$
$39.72 \pm <10^{-3}$	$26.95 \pm <10^{-3}$	$11.71 \pm 7.0 \cdot 10^{-3}$	$6.35 \pm <10^{-3}$
$13.28 \pm <10^{-3}$	$19.65 \pm <10^{-3}$	$18.54 \pm <10^{-3}$	$17.08 \pm <10^{-3}$

Eluents containing different organic mobile phases (methanol or acetonitrile) form separate clusters. This result indicates that methanol and acetonitrile show different selectivities, but this difference is small (compare the information contents of the first and second principal components in Table IV).

TABLE IV
RESULTS OF PRINCIPAL COMPONENT ANALYSIS
CARRIED OUT ON THE COVARIANCE MATRIX

Eluent		PCA loadings ^a	
No.	Composition (v/v)	I ^b	II ^c
1	Methanol-water (90:10)	4.52	-0.24
2	Methanol-water (92.5:7.5)	5.94	-0.19
3	Methanol-water (95:5)	7.37	-0.06
4	Methanol-water (97.5:2.5)	0.62	-0.68
5	Acetonitrile-water (85:15)	2.84	1.22
6	Acetonitrile-water (87.5:12.5)	4.27	0.37
7	Acetonitrile-water (90:10)	4.03	0.31
8	Acetonitrile-water (92.5:7.5)	3.70	0.33

^a PCA loadings are related to the individual contributions of eluents to the first and second hypothetical eluent systems.

^b Eigenvalue 278.96, variance explained 98.94%.

^c Eigenvalue 2.38, variance explained 0.82%.

The relationships between the PCA variables are shown in Fig. 5. Also here the coordinates (F_I and F_{II}) have no concrete physical meaning; they only indicate the relative distances between the aniline derivatives in two dimensions. The centre of the circle represents the mean retention time and the radi-

TABLE V
RESULTS OF PRINCIPAL COMPONENT ANALYSIS
CARRIED OUT ON THE CORRELATION MATRIX

Eluent		PCA loadings ^a	
No.	Composition (v/v)	I ^b	II ^c
1	Methanol-water (90:10)	0.99	-0.09
2	Methanol-water (92.5:7.5)	0.99	-0.11
3	Methanol-water (95:5)	0.99	-0.06
4	Methanol-water (97.5:2.5)	0.99	-0.12
5	Acetonitrile-water (85:15)	0.94	0.33
6	Acetonitrile-water (87.5:12.5)	0.99	0.03
7	Acetonitrile-water (90:10)	0.99	0.03
8	Acetonitrile-water (92.5:7.5)	0.99	0.01

^a PCA loadings are related to the individual contributions of eluents to the first and second hypothetical eluent systems.

^b Eigenvalue 7.82, variance explained 97.77%.

^c Eigenvalue 0.15, variance explained 2.12%.

TABLE VI

PARAMETERS OF LINEAR CORRELATION BETWEEN THE PRINCIPAL COMPONENT VARIABLES AND LOADINGS

$$y = a + bx$$

(y = calculated from covariance matrix; x = calculated from correlation matrix).

Dependent and independent variables ^a	Parameters ^b					
	n	a	b	S_b	r	$r_{\text{tabulated}}$
(1) PCA variables	48	18.10	5.95	0.05	0.9975	0.4648 (99.9%)
(2) PCA variables	48	0.55	4.12	0.55	0.7391	0.4648 (99.9%)
(1) PCA loadings	8	37.18	0.43	0.50	0.3262	0.6215 (90.0%)
(2) PCA loadings	8	0.12	3.64	0.48	0.9518	0.9249 (99.9%)

^a PCA variables = related to the individual contributions of solutes to the first and second hypothetical solutes; PCA loadings = related to the individual contributions of eluents to the first and second hypothetical eluent systems.

^b n = Sample number; a = regression constant; b = regression coefficient; S_b = standard deviation of regression coefficient; r = correlation coefficient; $r_{\text{tabulated}}$ = critical value of correlation coefficient.

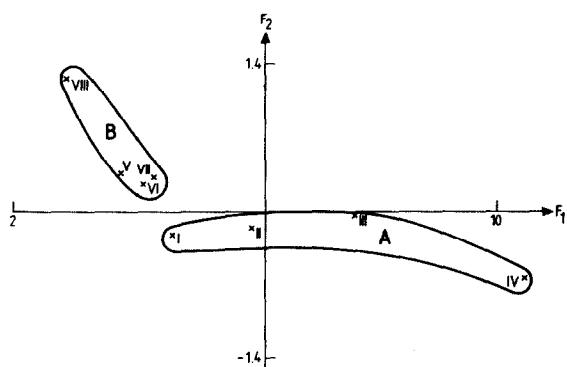


Fig. 4. Distribution of eluent systems according to the covariance matrix. A = Methanol-water eluents; B = acetonitrile-water eluents. The coordinates (F_1 and F_2) have no concrete physical meaning; they only indicate the relative distances between the eluent systems.

us of the circle characterizes the ± 2 S.D. value.

We conclude that this procedure may represent a new graphical method including the standard deviation in PCA. The aniline derivatives do not form separate clusters either according to the number of substituents or according to the lipophilicity of the substituents. This result indicates that the number of substituents, the lipophilicity of the derivatives and possibly other molecular parameters have a similar impact on the retention, that is, with the PGC column the aniline derivatives show a mixed retention mechanism.

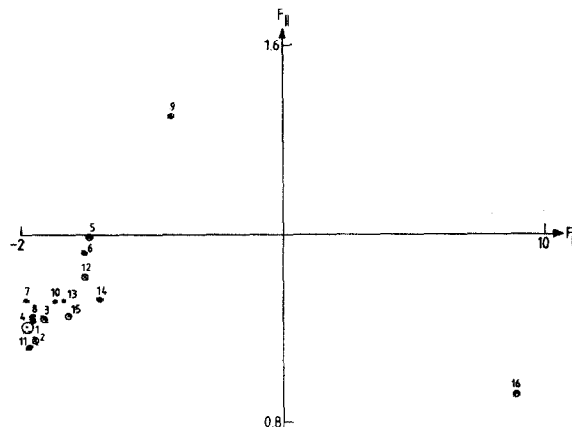


Fig. 5. Two-dimensional map of principal component variables based on the covariance matrix. Numbers refer to aniline derivatives in Table I. The coordinates (F_1 and F_{II}) have no concrete physical meaning; they only indicate the relative distances between the aniline derivatives.

ACKNOWLEDGEMENT

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