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Retention behaviour of some ring-substituted phenol derivatives on a porous graphitized carbon column

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ABSTRACT

The retentions of 24 ring-substituted phenol 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 phenol derivatives. Each phenol 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; however, according to the second principal component they showed slightly different selectivities. Calculations demonstrated that the number and not the type of substituents exerts the highest impact on the retention. Both calculation methods (based on correlation and covariance matrices) gave similar results.

INTRODUCTION

The application of silica or silica-based supports in high-performance liquid chromatography (HPLC) is limited by the low stability of silica at alkaline pH values [1] and by the undesirable electrostatic interactions between the polar substructures of solutes and the free silanol groups not covered by the hydrophobic ligand [2,3]. To decrease or eliminate the effect of residual acidic silanol group, the eluent has to be buffered or various additives have to be added to the eluent to mask the effect of silanol groups [4]. The drawbacks mentioned above necessitated the search for suitable supports other than silica, such as alumina [5], octadecyl-coated alumina [6], zirconia [7,8] and various polymer-based supports [9].

The porous graphitic carbon support (PGC) has been developed in the last decade [10,11]. PGC is characterized by the following: sufficient hardness to withstand high pressures; a well defined, reproducible and stable surface that shows no change during chromatographic work or storage; a specific surface area in the range 50–500 m^2/g to give adequate retention of solutes and maintain a reasonable linear sample capacity; a mean pore size >10 nm and the absence of micropores to ensure rapid mass transfer of solutes into and out of the particles; and uniform surface energy to give linear adsorption isotherms [12].

Until now PGC, has been mainly used to separate basic compounds [13,14]. The effect of various physico-chemical parameters of solutes on their retention behaviour has been studied in detail and the importance of electronic interactions between solutes and stationary phase on PGC has been emphasized [15]. A much greater influence of structural planarity of solutes on selectivity was observed on carbon than on C_{18} columns, presumably because the solute planarity emphasizes the interaction with the graphitic surface based on charge transfer and dispersion forces [16].

The evaluation of the performance of a new type of support generally involves the application of numerous eluents and solutes. The evaluation of such complicated data matrices is hardly possible without the application of computer-assisted multivariate mathematical–statistical methods. These methods make possible the simultaneous evaluation of an almost unlimited number of variables (chromatographic parameters), which greatly facilitates the solution of theoretical and practical problems.

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Multivariate methods have been applied in chromatography to identify basic factors that influence solute–solvent interactions and to classify solutes and solvents into groups having similar characteristics.

Principal component analysis (PCA) has frequently been applied to the evaluation of chromatographic data matrices [17]. The advantages of PCA are that it allows a reduction in the number of variables, that is, instead of the measured variables, artificial variables can be calculated that the explain the highest possible ratio of the change of the phenomenon observed (principal component variables); and it is suitable not only for the calculation of two–two variables relationship (PC variables), but also for the study of all variables of a linear correlation system. Therefore, it was assumed that PCA can be used when more solutes and more eluent systems are involved and we are interested in the similarities or dissimilarities between solutes and eluent systems.

The objectives of this investigation were to determine the retentions of 24 ring-substituted phenol derivatives on a PGC column in various eluent systems, to assess the separation power of the column without buffering the eluent and to evaluate the results with multivariate mathematical–statistical methods.

EXPERIMENTAL

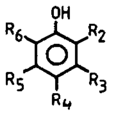
A porous graphitic carbon column (Shandon Hypercarb, 100 × 4.7 mm I.D., particle diameter 7 μm) was purchased from Shandon Scientific (Runcorn, UK). The HPLC system consisted of a Liquopump Model 312 pump (LaborMIM, Budapest, Hungary), a Cecil (Cambridge, UK) CE-212 variable-wavelength UV detector, a Valco (Houston, TX, USA) injector with a 20-μl 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 254 nm. Mixtures of methanol–water and acetonitrile–water were used as eluents. The methanol and acetonitrile concentrations ranged from 90 to 97.5 (in steps of 2.5%, v/v) and from 70 to 85% (v/v) (in steps of 5%, v/v) respectively. The use of these narrow concentration ranges was motivated by the high dependence of the retention of solutes on the organic mobile phase concentration. Buffers were not used. The structures of the ring-substituted phenol derivatives are shown in Table I. The phenol derivatives were dissolved in methanol or in 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 the traditional reversed-phase column, the retentions of compounds **14**, **17** and **18** were determined on a 150 × 4 mm I.D. Hypersil ODS (5 μm) column in water–methanol (4:6, v/v) and 0.025 *M* KH_2PO_4–methanol (4:6, v/v) (pH adjusted to 1.75 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 the phenol derivative to the plate number for toluene. We assume that this ratio reflects the influence of solute polarity on the column performance.

Principal component analysis was used to find the similarities and dissimilarities between the retention characteristics of the various eleuents and solutes. The mean retention times ± two standard deviations for phenol derivatives determined with the eight eluents formed the original data matrix for PCA. The various acetonitrile–water (four) and methanol–water (four) eluents were considered as variables, and the mean retention times ± two standard deviations for phenol derivatives were the observations. The calculation was carried out on both the correlation and covariance matrices. The calculation of the correlation matrix requires the normalization of the original data whereas the covariance matrix uses them without normalization. The explained variance was set to 99.9% in both instances. As the significance test of PCA loadings and variables is not yet known, we assumed that inserting the mean ± two standard deviations in PCA may help the elucidation of this problem. Linear correlations were calculated between PCA variables and loading 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:

TABLE I

STRUCTURES OF RING-SUBSTITUTED PHENOL DERIVATIVES



No.	R_2	R_3	R_4	R_5	R_6
1	CH_3	H	H	H	H
2	H	CH_3	H	H	H
3	H	H	CH_3	H	H
4	CH_3	H	H	H	CH_3
5	H	$N(CH_3)_2$	H	H	H
6	H	OH	CH_2CH_3	H	H
7	H	OCH_3	OCH_3	H	H
8	$C(CH_3)_3$	H	H	H	$C(CH_3)_3$
9	OCH_3	OCH_3	H	H	H
10	OCH_3	H	$CH_2CH=CH_2$	H	H
11	H	H	CH_2CN	H	H
12	CH_2OCH_3	H	H	H	H
13	CL	H	H	H	H
14	H	H	Cl	H	H
15	Cl	H	H	H	CL
16	H	Cl	H	Cl	H
17	H	Br	H	H	H
18	H	H	Br	H	H
19	Br	H	Br	H	H
20	H	F	H	H	H
21	H	H	F	H	H
22	H	H	CN	H	H
23	H	NO_2	H	H	H
24	H	H	NO_2	H	H

$$A_{cov} = a + b\,A_{corr} \qquad (1)$$

where A = PCA loadings and

$$Z_{cov} = a + b\,Z_{corr} \qquad (2)$$

where Z = PCA variable, corr = calculated from correlation matrix and cov = calculated from covariance matrix.

The two-dimensional non-linear (nl) map of PCA variables was also calculated [19,20]. Linear correlations were also calculated between the coordinates of the nl maps calculated from the covariance and correlation matrices:

$$X_{cov} = a + b\,X_{corr} \qquad (3)$$

$$Y_{cov} = a + b\,Y_{corr} \qquad (4)$$

where X and Y are the coordinates of nl maps.

The aim of the determination of correlations listed above was the comparison of the information content of PCA methods. It was motivated by the fact that the normalization needed for the calculation of the correlation matrix may cause information loss and may lead to distorted conclusions in the evaluation of retention data [21].

RESULTS AND DISCUSSION

Each phenol derivative showed narrow and symmetric peaks in each eluent system (Figs. 1 and 2), that is, the carbon column can be successfully used for the separation of ring-substituted phenol derivatives without buffering the eluent. The *ortho*, *meta*

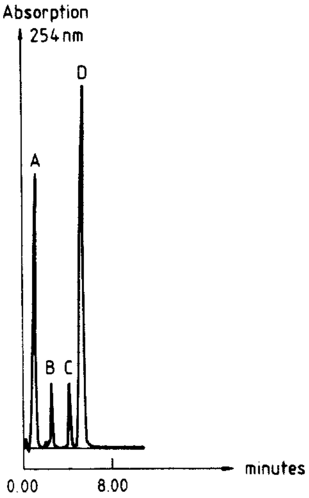


Fig. 1. Separation of phenol derivatives on the porous graphitic carbon column. Eluent, acetonitrile–water (7:3, v/v); flow-rate, 1 ml/min; detection, 254 nm. (A) 3-fluorophenol; (B) 3-bromophenol; (C) 4-nitrophenol; (D) 3-nitrophenol.

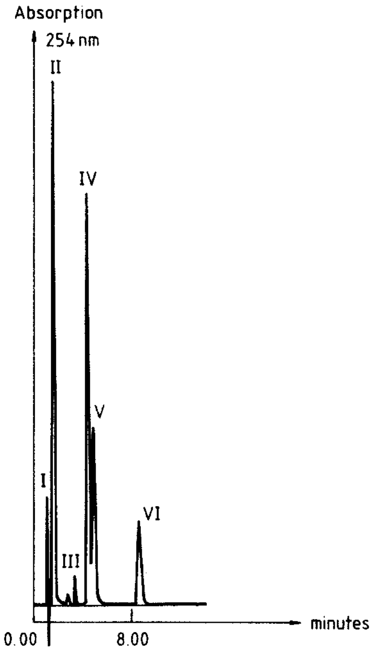


Fig. 2. Separation of phenol derivatives on the porous graphitic carbon column. Eluent, methanol–water (95:5, v/v); flow-rate, 1 ml/min; detection, 254 nm. (I) Dead time; (II) 2,3-dimethoxyphenol; (III) 3,5-dichlorophenol; (IV) 2,6-di-*tert.*-butylphenol; (V) 2,6-dichlorophenol; (VI) 2,4-dibromophenol.

and *para* isomers could be well separated on the column, which indicates the good separation capacity of PGC. The retention order of solutes does not follow the retention order expected according to their lipophilicity [22]. The more hydrophilic nitro derivatives elute after the more hydrophobic bromo and fluoro derivatives (Fig. 1) and di-*tert.*-butylphenol shows a lower retention than the corresponding dichloro derivative (Fig. 2), however, its lipophilicity is higher. These findings indicate that the retention behaviour of the PGC column differs from that of the common reversed-phase columns.

The retention times of phenol derivatives are compiled in Table II. The retention of each compound decreases with increasing concentration of organic modifier, and no anomalous retention behaviour was observed. The data support our previous qualitative conclusions that the lipophilicity of solutes does not determine their retention on a PGC column, but the eluents are typical reversed-phase eluents. The retention increases with increase in the number of substituents, which indicates the involvement of steric parameters in the retention mechanism. We are well aware that in general the molecular size is related to the lipophilicity, and therefore the influence of lipophilicity of solutes on the retention cannot be excluded. However, the relationship between the lipophilicity and molecular size is a loose one and our data rather support the hypothesis that molecular parameters other than lipophilicity govern the retention.

The data on peak symmetry and column performance are complied in Table III. The data indicate that the peak symmetry is similar on PGC in an unbuffered eluent and on an ODS column in a buffered eluent, but the relative performance of PGC column is markedly better than that of the ODS column even with a buffered eluent.

The results of PCA carried out on the covariance and correlation matrices are summarized in Tables IV and V, respectively. In both instances the fist principal component explained most of the variance, hence the main retention characteristics of the eight eluent systems applied can be expressed by only one hypothetical eluent system. PCA does not prove the existence of such an eluent system, but only indicates its mathematical possibility. Comparing the ratio of variance explained by the individual principal components, both calculation

TABLE II

RETENTION TIMES OF PHENOL DERIVATIVES (min)

Compound	Methanol (%, v/v)				Acetonitrile (%, v/v)			
	90	92.5	95	97.5	70	75	80	85
1	$3.23 \pm <10^{-3}$	$2.73 \pm <10^{-3}$	$2.29 \pm <10^{-3}$	$1.99 \pm 7.1 \cdot 10^{-3}$	$2.10 \pm <10^{-3}$	$1.92 \pm <10^{-3}$	$1.80 \pm <10^{-3}$	$1.71 \pm <10^{-3}$
2	$3.21 \pm <10^{-3}$	$2.70 \pm <10^{-3}$	$2.25 \pm <10^{-3}$	$1.97 \pm 7.1 \cdot 10^{-3}$	$2.11 \pm <10^{-3}$	$1.99 \pm <10^{-3}$	$1.86 \pm <10^{-3}$	$1.74 \pm 7.1 \cdot 10^{-3}$
3	$2.80 \pm <10^{-3}$	$2.50 \pm <10^{-3}$	$2.28 \pm <10^{-3}$	$2.05 \pm 7.1 \cdot 10^{-3}$	$2.05 \pm <10^{-3}$	$1.82 \pm <10^{-3}$	$1.77 \pm <10^{-3}$	$1.71 \pm <10^{-3}$
4	$9.11 \pm 1.4 \cdot 10^{-2}$	$7.44 \pm 3.5 \cdot 10^{-2}$	$5.88 \pm <10^{-3}$	$4.55 \pm <10^{-3}$	$3.03 \pm <10^{-3}$	$2.87 \pm 7.1 \cdot 10^{-3}$	$2.74 \pm <10^{-3}$	$2.65 \pm 2.4 \cdot 10^{-2}$
5	$8.20 \pm 9.1 \cdot 10^{-2}$	$7.14 \pm 2.8 \cdot 10^{-2}$	$5.70 \pm 7.1 \cdot 10^{-3}$	$4.82 \pm 7.1 \cdot 10^{-3}$	$3.69 \pm 2.8 \cdot 10^{-2}$	$3.26 \pm <10^{-3}$	$3.00 \pm 3.5 \cdot 10^{-2}$	$2.64 \pm 7.1 \cdot 10^{-2}$
6	$5.70 \pm <10^{-3}$	$3.58 \pm 2.8 \cdot 10^{-2}$	$2.85 \pm <10^{-3}$	$2.05 \pm 1.4 \cdot 10^{-2}$	$2.16 \pm <10^{-3}$	$2.03 \pm 7.0 \cdot 10^{-2}$	$1.85 \pm <10^{-3}$	$1.30 \pm 3.5 \cdot 10^{-2}$
7	$6.60 \pm <10^{-3}$	$4.56 \pm 7.0 \cdot 10^{-2}$	$3.44 \pm 1.4 \cdot 10^{-2}$	$2.80 \pm 3.5 \cdot 10^{-2}$	$2.62 \pm 1.4 \cdot 10^{-2}$	$2.46 \pm <10^{-3}$	$2.33 \pm <10^{-3}$	$2.27 \pm 3.5 \cdot 10^{-2}$
8	$7.46 \pm <10^{-3}$	$4.85 \pm <10^{-3}$	$3.90 \pm <10^{-3}$	$2.62 \pm <10^{-3}$	$5.65 \pm <10^{-3}$	$4.38 \pm <10^{-3}$	$3.38 \pm <10^{-3}$	$2.77 \pm 1.4 \cdot 10^{-2}$
9	$4.12 \pm <10^{-3}$	$3.24 \pm 7.1 \cdot 10^{-3}$	$2.70 \pm 8.4 \cdot 10^{-2}$	$2.12 \pm 2.1 \cdot 10^{-2}$	$1.99 \pm <10^{-3}$	$1.88 \pm <10^{-3}$	$1.82 \pm 7.0 \cdot 10^{-2}$	$1.77 \pm 4.0 \cdot 10^{-3}$
10	$7.03 \pm 1.4 \cdot 10^{-2}$	$5.90 \pm 7.1 \cdot 10^{-3}$	$5.17 \pm 1.4 \cdot 10^{-2}$	$4.08 \pm 7.1 \cdot 10^{-3}$	$4.21 \pm <10^{-3}$	$3.67 \pm <10^{-3}$	$3.29 \pm <10^{-3}$	$3.01 \pm <10^{-3}$
11	$4.17 \pm <10^{-3}$	$2.96 \pm <10^{-3}$	$2.17 \pm <10^{-3}$	$2.10 \pm 1.4 \cdot 10^{-2}$	$1.87 \pm 7.1 \cdot 10^{-2}$	$2.06 \pm <10^{-3}$	$1.65 \pm <10^{-3}$	$1.46 \pm 7.1 \cdot 10^{-3}$
12	$4.60 \pm <10^{-3}$	$3.73 \pm 1.4 \cdot 10^{-2}$	$2.71 \pm 2.1 \cdot 10^{-2}$	$2.35 \pm 1.4 \cdot 10^{-2}$	$2.19 \pm <10^{-3}$	$2.03 \pm 5.6 \cdot 10^{-2}$	$1.92 \pm <10^{-3}$	$1.82 \pm 2.4 \cdot 10^{-2}$
13	$3.54 \pm 7.1 \cdot 10^{-3}$	$3.02 \pm 3.5 \cdot 10^{-2}$	$2.55 \pm 1.4 \cdot 10^{-2}$	$2.23 \pm 7.1 \cdot 10^{-3}$	$2.45 \pm 3.54 \cdot 10^{-2}$	$2.25 \pm <10^{-3}$	$2.05 \pm 2.1 \cdot 10^{-2}$	$1.96 \pm <10^{-3}$
14	$3.90 \pm <10^{-3}$	$3.34 \pm 1.4 \cdot 10^{-2}$	$2.71 \pm 7.1 \cdot 10^{-3}$	$2.36 \pm 7.1 \cdot 10^{-3}$	$2.50 \pm <10^{-3}$	$2.29 \pm <10^{-3}$	$2.15 \pm <10^{-3}$	$2.03 \pm 1.4 \cdot 10^{-3}$
15	$8.50 \pm <10^{-3}$	$7.20 \pm 7.1 \cdot 10^{-3}$	$5.71 \pm 6.2 \cdot 10^{-2}$	$4.70 \pm 7.7 \cdot 10^{-2}$	$7.07 \pm 1.4 \cdot 10^{-2}$	$5.83 \pm <10^{-3}$	$5.31 \pm <10^{-3}$	$4.58 \pm 2.8 \cdot 10^{-3}$
16	$10.35 \pm <10^{-3}$	$8.65 \pm <10^{-3}$	$6.51 \pm 1.4 \cdot 10^{-2}$	$5.40 \pm 2.8 \cdot 10^{-2}$	$7.33 \pm <10^{-3}$	$6.01 \pm <10^{-3}$	$5.40 \pm <10^{-3}$	$4.56 \pm <10^{-3}$
17	$4.47 \pm <10^{-3}$	$3.86 \pm 7.1 \cdot 10^{-3}$	$3.37 \pm 7.1 \cdot 10^{-3}$	$2.78 \pm <10^{-3}$	$3.01 \pm <10^{-3}$	$2.74 \pm 7.1 \cdot 10^{-3}$	$2.49 \pm <10^{-3}$	$2.26 \pm 7.1 \cdot 10^{-3}$
18	$4.42 \pm <10^{-3}$	$3.96 \pm <10^{-3}$	$3.22 \pm 7.1 \cdot 10^{-3}$	$2.88 \pm <10^{-3}$	$3.15 \pm <10^{-3}$	$2.84 \pm <10^{-3}$	$2.59 \pm <10^{-3}$	$2.46 \pm <10^{-3}$
19	$16.14 \pm 7.1 \cdot 10^{-3}$	$12.88 \pm 7.1 \cdot 10^{-3}$	$10.52 \pm 1.4 \cdot 10^{-2}$	$7.93 \pm 1.4 \cdot 10^{-2}$	$3.15 \pm <10^{-3}$	$9.79 \pm <10^{-3}$	$8.75 \pm <10^{-3}$	$7.13 \pm <10^{-3}$
20	$3.53 \pm <10^{-3}$	$2.10 \pm <10^{-3}$	$1.75 \pm 7.1 \cdot 10^{-3}$	$1.65 \pm 1.4 \cdot 10^{-2}$	$1.81 \pm <10^{-3}$	$1.65 \pm <10^{-3}$	$1.56 \pm <10^{-3}$	$1.48 \pm <10^{-3}$
21	$3.26 \pm <10^{-3}$	$2.42 \pm 7.1 \cdot 10^{-3}$	$1.78 \pm 7.1 \cdot 10^{-3}$	$1.59 \pm 7.1 \cdot 10^{-3}$	$1.79 \pm <7.1 \cdot 10^{-3}$	$1.70 \pm <10^{-3}$	$1.59 \pm 2.8 \cdot 10^{-2}$	$1.53 \pm <10^{-3}$
22	$4.23 \pm 1.4 \cdot 10^{-3}$	$3.75 \pm <10^{-3}$	$3.28 \pm 2.2 \cdot 10^{-2}$	$2.77 \pm 1.4 \cdot 10^{-2}$	$2.72 \pm 1.4 \cdot 10^{-2}$	$2.43 \pm <10^{-3}$	$2.25 \pm 1.4 \cdot 10^{-2}$	$2.12 \pm 7.1 \cdot 10^{-3}$
23	$9.92 \pm 3.5 \cdot 10^{-2}$	$7.97 \pm 5.6 \cdot 10^{-2}$	$6.30 \pm 1.4 \cdot 10^{-2}$	$4.45 \pm 6.36 \cdot 10^{-2}$	$5.65 \pm 3.5 \cdot 10^{-2}$	$4.97 \pm 1.4 \cdot 10^{-2}$	$4.29 \pm 7.1 \cdot 10^{-2}$	$3.80 \pm <10^{-3}$
24	$11.88 \pm 2.8 \cdot 10^{-2}$	$8.62 \pm 7.1 \cdot 10^{-2}$	$5.37 \pm 9.9 \cdot 10^{-2}$	$4.63 \pm 2.12 \cdot 10^{-2}$	$4.56 \pm 7.1 \cdot 10^{-3}$	$4.17 \pm 1.1 \cdot 10^{-2}$	$3.74 \pm <10^{-3}$	$3.48 \pm 2.8 \cdot 10^{-2}$

TABLE III

COMPARISON OF THE RETENTION CHARACTERISTICS OF POROUS GRAPHITIZED CARBON AND HYPERSIL ODS COLUMNS

I = Porous graphitized column; II = ODS column, non-buffered eluent; III = ODS column, buffered eluent. P (%) = 100· (plate number for compound/plate number for toluene).

Compound	Asymmetry factor			P (%)		
	I	II	III	I	II	III
14	1.12	1.49	1.00	65.8	54.4	47.8
17	1.25	1.50	1.33	61.0	46.2	59.5
18	1.20	1.31	1.08	64.9	23.0	22.1

methods give similar results. The high ratio of variance explained by the first PC may make questionable the application of PCA for the evaluation of our data matrix. As the eigenvalue of the second PC (PCA on the correlation matrix) was >1, it was assumed that it also contains important information and it is not only the product of the standard deviation of the original data.

TABLE IV

RESULT OF PRINCIPAL COMPONENT ANALYSIS CARRIED OUT ON THE COVARIANCE MATRIX

No.	Eigenvalue	Variance explained (%)
1	34.657	94.49
2	1.531	98.67
3	0.378	99.70

	PCA loadings		
	1	2	3
1	1.461	−0.146	0.342
2	1.949	−0.171	0.294
3	2.535	−0.379	0.136
4	3.163	−0.605	−0.368
5	1.263	0.286	0.066
6	1.565	0.386	0.033
7	1.778	0.478	−0.067
8	2.253	0.714	−0.098

TABLE V

RESULT OF PRINCIPAL COMPONENT ANALYSIS CARRIED OUT ON THE CORRELATION MATRIX

No.	Eigenvalue	Variance explained (%)
1	7.57	94.71
2	0.31	98.66
3	0.07	99.58

	PCA loadings		
	1	2	3
1	0.968	0.192	−0.150
2	0.978	0.173	−0.076
3	0.975	0.209	0.032
4	0.956	0.215	0.197
5	0.980	−0.156	−0.051
6	0.983	−0.177	−0.012
7	0.977	−0.205	0.034
8	0.966	−0.246	0.043

The parameters of linear correlations between PC loadings and variables calculated from the covariance and correlation matrices and between the coordinates of the non-linear maps are compiled in Table VI. According to eqns. 1 and 2, highly significant correlations were found between the PCA variables and loadings. This result indicates that in our case the information content of the PCA variables and loadings computed from different matrices are similar. The distribution of PCA loadings (eluent systems) calculated according to the covariance and correlation matrices are illustrated in Figs. 3 and 4, respectively. In both figures the eluents containing different organic components (methanol or acetonitrile) form separate clusters. This result shows that methanol and acetonitrile show different selectivities, but this difference is fairly small (compare the information content of the first and second principal components in Tables IV and V).

The two-dimensional non-linear maps of PCA variables calculated from the covariance and correlation matrices are shown in Figs. 5 and 6. The centre of the circle represents the mean retention time and the radius of the circle characterizes the ±2 standard deviation value. It was assumed that the

TABLE VI

LINEAR CORRELATION OF PARAMETERS BETWEEN THE PRINCIPAL COMPONENT VARIABLES AND LOADINGS AND BETWEEN THE NON-LINEAR MAP COORDINATES

$y = a + bx$. y = Calculated from covariance matrix; x = calculated from correlation matrix; 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.

Dependent and independent variables	Parameters					
	n	a	b	S_b	r	$r_{\text{tabulated}}$
(1) PC variables	75	11.10	2.13	0.02	0.9962	0.3799 (99.9%)
(2) PC variables	75	−0.59	−1.50	0.18	0.6789	0.3799 (99.9%)
(3) PC variables	75	0.59	1.81	0.15	0.8077	0.3799 (99.9%)
(1) PCA loadings	8	51.29	−50.66	19.61	0.7257	0.7067 (95%)
(2) PCA loading	8	0.07	−2.07	0.26	0.9547	0.9249 (99.9%)
(3) PCA loading	8	0.02	−1.73	0.43	0.8516	0.8343 (99%)
(1) Coordinates	75	2.84	1.01	0.008	0.9975	0.3799 (99.9%)
(2) Coordinates	75	181.92	−1.05	0.002	0.9747	0.3799 (99.9%)

phenol derivatives can be separated in all eluents with 95% probability when the circles in Figs. 5 and 6 do not overlap each other. The experimental data (see Table II) supported this assumption. It can be concluded that this procedure may represent a new graphical approximation method including the standard deviation in PCA. The phenol derivatives

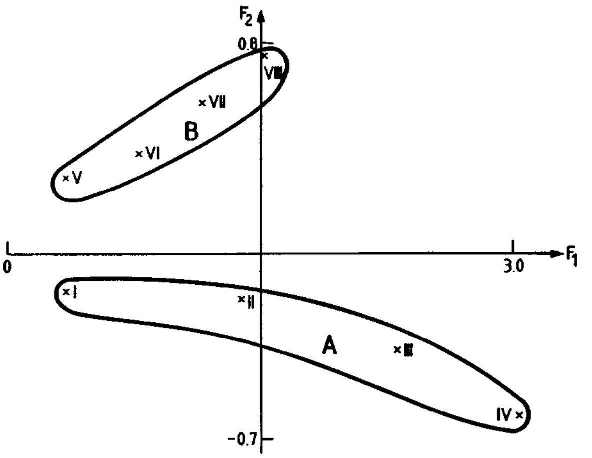


Fig. 3. Distribution of eluent systems according to the covariance matrix. (A) Methanol–water eluents; (B) acetonitrile–water eluents.

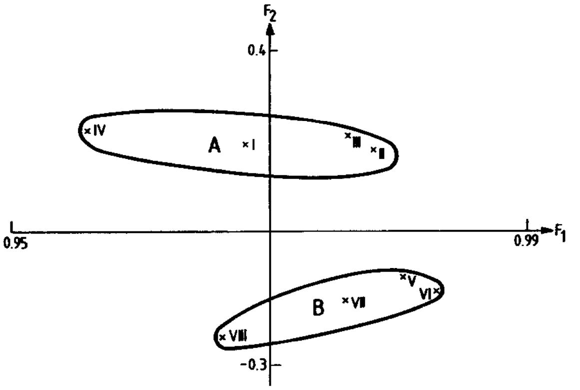


Fig. 4. Distribution of eluent systems according to the correlation matrix. (A) Methanol–water eluents; (B) acetonitrile–water eluents.

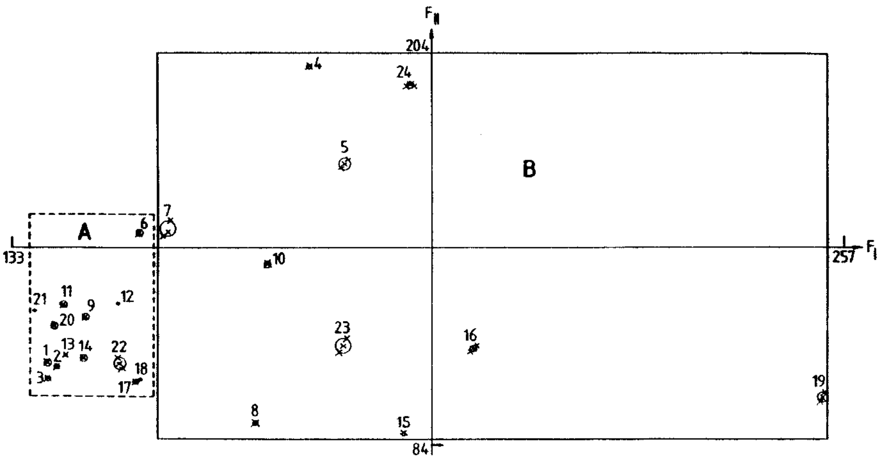


Fig. 5. Two-dimensional non-linear map of PC variables. Covariance matrix; number of iterations, 64; maximum error, $1.00 \cdot 10^{-3}$. Numbers refer to phenol derivatives in Table I.

form two distinct clusters on the basis of the number of substituents; clusters A and B contain the mono- and disubstituted phenol derivatives, respectively.

This finding supports the assumption that the retention behaviour of ring-substituted phenol derivatives on the carbon column is mainly governed by the number of substituents, which is related to the bulkiness of the whole molecule; however, the influence of lipophilicity on the retention cannot be entirely ruled out. According to our calculations, the mononitro derivatives (compounds **23** and **24** in Table I) show a similar retention behaviour to the other disubstituted compounds. This effect may be due to the bulkiness of the NO_2 substituents.

The parameters of linear correlations between the coordinates of non-linear maps are compiled in Table VI. The data demonstrate again the similar in-

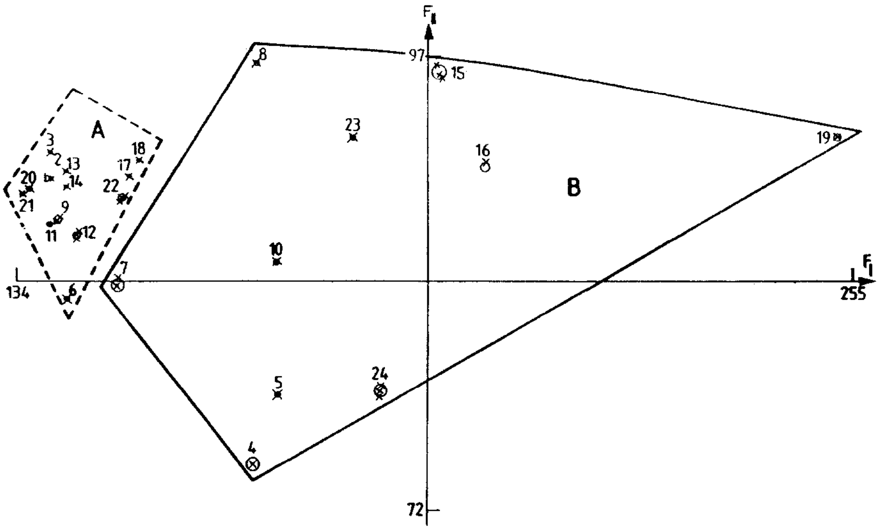


Fig. 6. Two-dimensional non-linear map of PC variables. Correlation matrix; number of iterations, 82; maximum error, $1.03 \cdot 10^{-3}$. Numbers refer to phenol derivatives in Table I.

formation content of the two calculation methods.

It can be concluded from our data that ring-substituted phenol derivatives can be well separated on a graphitized carbon column without buffering the eluent, and monosubstituted phenol derivatives generally show lower retentions than disubstituted derivatives. Calculations indicated that the retention of the ring-substituted phenol derivatives is mainly governed by the bulkiness of the solute molecule.

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