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INFLUENCE OF PHYSICOCHEMICAL PARAMETERS OF SOME RING-SUBSTITUTED PHENOL DERIVATIVES ON THEIR RETENTION ON A POROUS GRAPHITIZED CARBON COLUMN

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ABSTRACT

The retention characteristics of 29 phenol derivatives were determined on a porous graphitized carbon column in unbuffered acetonitrile - water and methanol - water eluent mixtures at various organic phase concentrations. Each phenol derivative showed symmetric peaks in each eluent without buffers. Good linear correlations were found between the $\log k'$ value and the organic mobile phase concentration in the eluent. Principal component analysis indicated that methanol and acetonitrile expose different selectivities. Stepwise regression analysis proved that the retention of ring - substituted phenol derivatives is mainly governed by the steric parameters, electron-withdrawing power and hydrogen donor capacity of substituents. According to the results of Free-Wilson analysis, the substituents with large steric parameters, strong electron-withdrawing power and hydrogen donor capacity have the highest impact on the retention. The lipophilicity of phenol derivatives did not affect significantly the retention, although the eluents were typical reversed-phase eluents.

INTRODUCTION

The application of silica or silica - based supports in high-performance liquid chromatography (HPLC) is limited by the low stability of silica at high 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. To decrease or eliminate the effect of residual acidic silanol groups, buffers or various additives have to be added to the eluent to mask the effect of silanol groups (2). The drawbacks mentioned above necessitated the search for other than silica support as alumina (3), octadecyl coated alumina (4) and various polymer based supports (5). The porous graphitic carbon support (PGC) recently appeared in chromatographic practice. According to our knowledge the first paper on carbon column was presented at the 14th International Symposium on Chromatography (6). Results concerning the application of carbon columns were reviewed in 1989 (7). PGC columns have been used for the separation of diastereoisomers, geometrical isomers (8) and various alkaline compounds (9,10). The separation on the porous graphitic carbon column of small ionizable compounds of pharmaceutical interest was also described (11). PGC column is characterized by the followings:

1. Sufficient hardness to withstand high pressures;
2. A well - defined, reproducible and stable surface that shows no change during chromatographic work or storage;
3. A specific surface area in the range of 50 to 500 m^2 /g to give adequate retention of solutes and maintain a reasonable linear sample capacity;
4. A mean pore size > 10 nm and the absence of micropores to ensure rapid mass transfer of solutes into and out of the particles;

5. Uniform surface energy to give linear adsorption isotherm.

The effect of various physicochemical parameters of solutes on their retention behavior on PGC column has been studied in detail and the importance of electronic interactions between solutes and stationary phase has been emphasized (12). Multivariate mathematical statistical methods as stepwise regression analysis (13), factor analysis (14), principal component analysis (PCA) (15) etc. have been frequently used to extract maximal information of great retention data matrices. The advantages of the application of PCA in the chromatography is that it allows a reduction in the number of variables whilst maintaining information content; PCA is suitable not only for the calculations of two - two variables relationship, but also for the study of all variables relationship.

The objectives of our investigation were to determine the retention 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, to evaluate the results with multivariate mathematical statistical methods and to find the relationship between the background variables of PCA and the physicochemical parameters of phenol derivatives. Free - Wilson analysis (16) was applied to select the substituents of phenol derivatives having the highest impact on the retention behavior of these compounds.

MATERIALS AND METHODS

The porous graphitic carbon column (Shandon Hypercarb 100*4.7 mm ID, particle diameter 7 μ m) was purchased from Shandon Scientific (England). The HPLC system consisted of a Liquepump Model 312 (Labor MIM, Budapest, Hungary) pump, a Cecil CE-212 variable wavelength UV

detector (Cecil Instr., Cambridge, England), a Valco injector (Valco Inc., Houston, Texas, USA) with a 20 μ l sample loop and a Waters 740 integrator (Waters-Millipor Inc., Milford, Massachusetts, USA). The flow rate was 1.0 ml/min and the detection wavelength was set to 254 nm. Mixtures of methanol : water and acetonitrile : water were used as eluents. Methanol and acetonitrile concentrations ranged from 90 to 97.5 and from 65 to 85 % (v/v), respectively. Buffers were not used. The chemical structures of the ring-substituted phenol derivatives are shown in Table 1. The phenol derivatives were dissolved in methanol or in acetonitrile at the concentration of 0.05 mg/ml. The retention time of each compound in each eluent was determined with three consecutive determinations. As the correlations between the log k' value and the organic phase concentration is generally linear in HPLC we also applied linear equations to describe the dependence of log k' value on the organic mobile phase concentration.

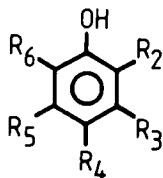
$$\log k' = \log k'_0 + b.C \quad (1)$$

where: log k' = logarithm of capacity factor; log k'_0 = logarithm of capacity factor extrapolated to zero mobile phase concentration (intercept); b = change of log k' value caused by unit change (1 vol %) of organic mobile phase concentration (slope) and C = organic mobile phase concentration (vol.%).

The data matrix for PCA consisted of the parameters of Eqn. 1 (slope and intercept values for methanol and acetonitrile) as 4 variables and of the phenol derivatives as 29 observations. The calculations were carried out on the correlation matrix, the variance explained was set to 99.9%.

TABLE 1.

Chemical structure of ring substituted phenol derivatives.



General structure

No of compounds	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.	CHCH ₂ CH ₃	H	H	CH ₃	H
6.	H	N(CH ₃) ₂	H	H	H
7.	H	OCH ₃	OCH ₃	H	H
8.	H	OH	CH ₂ CH ₃	H	H
9.	C(CH ₃) ₃	H	C(CH ₃) ₃	H	H
10.	C(CH ₃) ₃	H	H	H	C(CH ₃) ₃
11.	OCH ₃	OCH ₃	H	H	H
12.	OCH ₃	H	CHCH ₂ CH ₂	H	H
13.	H	OH	H	H	H
14.	H	H	CH ₂ CN	H	H
15.	CHOCH ₃	OH	H	H	H
16.	NH ₂	H	H	H	H
17.	Cl	H	H	H	H
18.	H	H	Cl	H	H
19.	Cl	H	H	H	Cl
20.	H	Cl	H	H	Cl
21.	H	Br	H	H	H
22.	H	H	Br	H	H
23.	Br	H	Br	H	H
24.	H	F	H	H	H
25.	H	H	F	H	H
26.	H	H	CN	H	H
27.	NO ₂	H	H	H	H
28.	H	NO ₂	H	H	H
29.	H	H	NO ₂	H	H

To find the physicochemical parameters of solutes that significantly influence their retention behavior, stepwise regression analysis was applied (13). Dependent variables were always the PC variables and the independent variables were the different physicochemical parameters of the phenol derivatives. The acceptance level for the individual independent variables was set to 95% significance level. The physicochemical parameters (17) were :

π = Hansch - Fujita's substituent constant characterizing hydrophobicity;

H - Ac and H - Do = indicator variables for proton acceptor and proton donor properties, respectively;

M - RE = molar refractivity;

F and R = Swain - Lupton's electronic parameters characterizing the inductive and resonance effect, respectively;

σ = Hammett's constant, characterizing the electron - withdrawing power of the substituent;

Es = Taft's constant, characterizing steric effects of the substituent;

B1 and B4 = Sterimol width parameters.

As the first three PC variables contained the overwhelming majority of variance only these were included into the calculations.

In the Free - Wilson analysis the dependent variables were the three PC variables and the indicator variables indicating the presence or absence of various substituents in different positions were the independent variables (altogether 35 ones). The acceptance level for the individual independent variables was set to 99.9% significance level.

RESULTS AND DISCUSSION

Each phenol derivative showed symmetrical peaks in each eluent system (Figs.1 and 2). The retention order

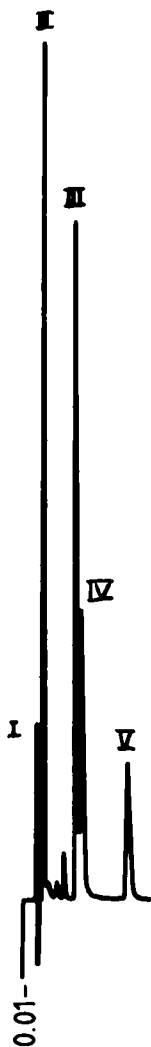


Figure 1. Separation of some phenol derivatives on the porous graphitic carbon column. Eluent : acetonitrile : water (8 : 2)v/v. Flow rate: 1ml/min, detection 254nm. I. dead time, II. 2,3-dimethoxyphenol, III. 2,6-ditertierbutylphenol, IV. 2,6-dichlorophenol, V. 2,4-dibromophenol

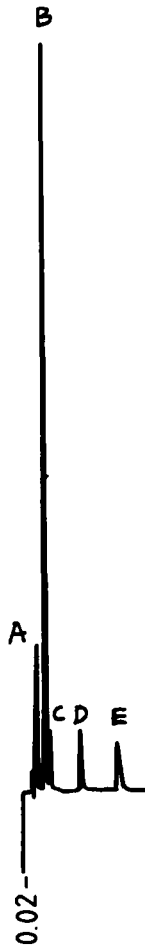


Figure 2. Separation of some phenol derivatives on the porous graphitic carbon column. Eluent : methanol:water (8:2) v/v . Flow rate : 1ml/min, detection 254nm.
A: phenol, B: 2-chlorophenol, C: 4-bromophenol, D: 2,6 dichlorophenol, E: 2,4-dibromophenol

of solutes in Fig 1. deviates from their expected retention order on a traditional reversed - phase column, the highly lipophilic di-tertiary-butyl derivatives is eluted before the less lipophilic dichloro and dibromo derivatives. This finding indicates that not only the lipophilicity of the compounds influences their retention, but also other physicochemical parameters may have a considerable impact on the strength of retention.

The parameters of eqn. 1. are compiled in Tables 2 and 3. The relationship between the logarithm of capacity factor and the concentration of organic phase in the eluent was significantly linear in each case. The value of correlation coefficient in most cases was over 0.99 proving the applicability of eqn. 1. The slope and intercept values of the correlations considerably differ from each other. This finding indicates that these compounds can be easily separated on PGC column both in acetonitrile - water and methanol - water eluents. As the slopes and intercept values of a solute determined in eluents containing methanol or acetonitrile organic modifier are different from each other, the eluent systems show different selectivity.

The results of PCA are summarized in Table 4. The calculations prove that three background components contain the majority of the information content of the four chromatographic parameters (slope and intercept values for acetonitrile and methanol organic mobile phases). As the slope and intercept values of eqn. 1. determined in acetonitrile - water eluents have high loadings in the first principal component, the first PC can be considered to be related to the effect of acetonitrile. The second and third PC mainly consist of the loadings of methanol -water eluent systems. This result proves again the different selectivity of methanol and acetonitrile.

TABLE 2.

Parameters of linear correlations between the logarithm of capacity factor and the methanol concentration in the eluent.

$$\log k' = \log k'_0 + b.C$$

No of com pounds	Parameters			
	$\log k'_0$	$-b \cdot 10^{-2}$	$s_b \cdot 10^{-3}$	r
1.	5.31	5.67	1.5	0.9993
2.	5.46	5.85	1.5	0.9994
3.	3.60	3.89	7.6	0.9988
4.	5.26	4.95	2.0	0.9966
5.	9.31	9.75	9.0	0.9958
6.	4.98	4.15	1.4	0.9966
7.	6.94	7.04	2.5	0.9970
8.	9.04	9.45	6.5	0.9953
9.	12.00	12.53	1.7	0.9982
10.	8.19	8.33	7.6	0.9918
11.	6.50	6.81	3.5	0.9933
12.	4.28	4.01	2.2	0.9909
13.	9.79	10.56	14.0	0.9910
14.	5.97	6.27	6.2	0.9904
15.	6.57	6.81	4.2	0.9907
16.	10.11	1.10	6.2	0.9922
17.	4.72	4.95	2.0	0.9982
18.	4.98	5.17	2.9	0.9968
19.	4.79	4.46	2.5	0.9969
20.	5.06	4.66	3.0	0.9958
21.	4.20	4.20	3.8	0.9920
22.	3.90	3.90	2.4	0.9981
23.	5.19	4.15	1.6	0.9964
24.	3.78	4.40	1.8	0.9992
25.	9.83	10.70	7.1	0.9935
26.	3.91	3.92	2.5	0.9920
27.	6.76	6.32	3.3	0.9934
28.	5.91	5.62	4.6	0.9935
29.	7.03	6.79	4.3	0.9966

TABLE 3.

Parameters of linear correlations between the logarithm of capacity factor and the acetonitrile concentration in the eluent.

$$\log k' = \log k'_0 + b.C$$

No of com pounds	Parameters			
	$\log k'_0$	$- b \cdot 10^{-2}$	$s_b \cdot 10^{-3}$	r
1.	1.06	1.77	4.1	0.9979
2.	0.98	1.63	0.7	0.9970
3.	0.95	1.64	1.0	0.9927
4.	0.62	0.67	0.05	0.9911
5.	1.45	1.94	0.8	0.9981
6.	1.40	1.59	0.7	0.9948
7.	0.64	0.08	0.5	0.9945
8.	1.25	1.98	1.0	0.9974
9.	2.50	10.72	1.3	0.9985
10.	2.71	3.09	0.7	0.9995
11.	0.40	0.09	0.2	0.9992
12.	1.42	1.50	0.7	0.9976
13.	0.41	1.49	1.2	0.9937
14.	2.68	4.04	3.8	0.9871
15.	0.75	1.25	1.3	0.9912
16.	0.19	1.13	0.9	0.9970
17.	1.07	1.57	0.8	0.9923
18.	0.92	1.33	0.6	0.9965
19.	1.70	1.51	1.2	0.9940
20.	1.88	1.71	0.9	0.9956
21.	1.26	1.58	0.4	0.9994
22.	1.10	1.32	1.1	0.9930
23.	2.12	1.70	1.0	0.9913
24.	1.26	2.32	1.7	0.9948
25.	1.03	1.98	0.2	0.9948
26.	1.13	1.53	0.7	0.9952
27.	1.64	1.55	0.5	0.9989
28.	1.78	1.75	0.2	0.9983
29.	1.22	1.14	0.6	0.9970

TABLE 4.

Result of principal component analysis carried out on the correlation matrix.

	Eigen value	Total variance explained%			
	1. 1.76	44.01			
	2. 1.08	71.06			
	3. 0.94	94.53			
	4. 0.22	100.00			
PCA loadings					
	1.	2.	3.	4.	
slope(ACN)	- 0.73	0.50	0.39	0.26	
intercept(ACN)	0.94	0.07	- 0.07	0.33	
slope(MET)	- 0.04	0.72	- 0.69	- 0.05	
intercept(MET)	0.59	0.55	0.55	- 0.20	

The parameters of linear correlations between PC variables and physicochemical parameters of phenol derivatives are compiled in Table 5. Eqns. I - III fit well to the retention data, the significance level being over 95% (see F values). The molar refraction and steric effects of solutes account for 61.51% of the change of the dependent variable (see r^2 value of Eqn. I). This result supports our previous conclusions that the retention behavior of the solutes does not depend on their lipophilicity, that is the retention capacity of PGC column markedly differs from that of common reversed-phase columns. The proton donor properties and the electron - withdrawing power of the substituents also influences the retention, they account for 37.11% of the change of the dependent variable (see r^2 value of Eqn.II). However, the importance on these two physicochemical parameters in the influence of the retention is lower due to the lower ratio of variance explained by the second principal component. The proton donor properties of solutes account for 17.98%

TABLE 5.

Result of stepwise regression analysis

Parameters	Number of equation.		
	I.	II.	III.
a	1.16	0.06	0.14
b ₁	- 0.05	- 1.14	- 1.32
s _{b1}	0.02	0.55	0.54
b ₂	0.42	0.99	-
s _{b2}	0.12	0.41	-
r ²	0.6151	0.3711	0.1798
b ₁ %	39.99	45.73	-
b ₂ %	60.67	54.25	-
F	20.78	7.69	5.92

No. of equation

- I. First PCV = $a + b_1 \cdot M-RE + b_2 \cdot E_S$
 II. Second PCV = $a + b_1 \cdot H-Do + b_2 \cdot \sigma$
 III. Third PCV = $a + b_1 \cdot H-Do$

PCV = principal component variable

of the change of the dependent variable (see r^2 value of Eqn. III). The correlation between the variables is the strongest for the first PC and it is the weakest (however significant) for the third PC. This finding is in good agreement with the capacity of PCA to cumulate the information content in the first principal component. The total absence of the significant impact of the lipophilicity on the retention in Eqns. II and III is in good agreement with our previous conclusions that the retention of phenol derivatives on PGC column is not governed by their lipophilicity.

The results of Free-Wilson analysis are summarized in Table 6. Each equation fits well to the experimental data, the significance level is in each case over 99.9%

TABLE 6

Effect of various substituents on the retention of some ring-substituted phenol derivatives on the PGC column (sample number = 28).

Parameters	Equations		
	A	B	C
a	0.35	- 0.14	0.30
b ₁	- 2.33	4.04	- 1.94
s _{b1}	0.53	0.75	0.56
b ₂	- 2.33	-	- 3.78
s _{b2}	0.53	-	0.56
b ₃	- 3.62	-	- 1.06
s _{b3}	0.74	-	0.31
r ²	0.8601	0.6892	0.7115
b ₁ %	25.32	-	25.88
b ₂ %	35.28	-	50.42
b ₃ %	39.39	-	23.63
F	51.24	24.43	20.55

Equation:

$$\text{A. First PCV} = a + b_1 (4\text{-CH}_2\text{CN}) + b_2 (2\text{-C(CH}_3)_3) + b_3 (4\text{-C(CH}_3)_3)$$

$$\text{B. Second PCV} = a + b_1 (2\text{-NO}_2)$$

$$\text{C. Third PCV} = a + b_1 (2\text{-NH}_2) + b_2 (2\text{-NO}_2) + b_3 (3\text{-OH})$$

PCV = Principal component variable

(see F values). Only three substituents from the 35 included in the calculations explain 86.01% of the change of the dependent variable (see r² value of Eqn. A). The fact that the molar refraction and steric parameters of the selected substituents are high, supports the con-

clusions drawn from the data in Table 5. The NO_2 substituent at position 2 accounts for 68.92% of the variance of dependent variable. The position and the capacity of NO_2 group to form hydrogen bonds probably contributes to its considerable effects. The polar character and hydrogen bond forming capacity of the selected substituents may cause their high impact on the third PC variable. However, we have to emphasize that the relatively low ratio of variance explained by the third PC variable (23%) lessens the importance of these interactions.

It can be concluded from our data that ring-substituted phenol derivatives can be well separated on the graphitized carbon column without buffering the eluent. Various multivariate mathematical-statistical calculations indicated that the retention of the ring-substituted phenol derivatives is mainly governed by the sterical parameters, electron-withdrawing power and hydrogen donor capacity of the substituent.

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