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Dependence of the retention of some barbituric acid derivatives on a porous graphitized carbon column on their physicochemical parameters*

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Abstract: The retentions of 45 barbituric acid derivatives were determined on a porous graphitized carbon column (PGC) in unbuffered methanol–water eluent mixtures at various organic phase concentrations and the retention data were correlated with the various hydrophobic and electronic parameters of barbituric acid derivatives. Each derivative showed symmetric peaks in each eluent proving the good separation characteristics of the PGC column without buffering the eluent. Significant linear correlations were found between the logk' value and the concentration of the organic mobile phase in the eluent. Stepwise regression analysis indicated that the retention of barbituric acid derivatives is mainly governed by the electronic parameters, and the lipophilicity of various substituents did not affect significantly the retention, although the eluents were typical reversed-phase eluents.

Keywords: *Porous graphitized carbon column; barbituric acid derivatives; electronic interactions.*

Introduction

Development of highly pH stable columns such as the porous graphite carbon column (PCG) [1, 2] was motivated by the fact that 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 [3] and by the undesirable electrostatic interactions between the polar substructures of solutes and the free silanol groups not covered by the hydrophobic ligand [4]. The main characteristics of PGC column are: sufficient hardness to withstand high pressures; a well defined, reproducible and stable surface that shows no change during chromatography work or storage; a specific surface area in range 50–500 $m^2\ g^{-1}$ to give adequate retention of solutes and maintain a reasonable linear sample capacity, a mean pore size >10 nm and 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. PGC columns have been used for the separation of diastereomers [5], geometrical isomers [6], and successful separations of various alkaline compounds such as tioconazole derivatives have been carried out on PGC columns [7]. Influence of physicochemical parameters of some ring-substituted phenol derivatives on their retention on PGC column have also been studied. It has been established that the retention of ring-substituted phenol derivatives is mainly governed by the sterical parameters, electron-withdrawing power and hydrogen donor capacity of the substituents [8, 9]. Reversed-phase HPLC has been frequently used to determine the hydrophobicity of various compounds [10]. To increase the accuracy of the lipophilicity determination, linear correlations were calculated between the logk' value and the concentration of organic modifier in the eluent. The intercept of the correlation was considered as the best estimate of lipophilicity [11] and slope was considered to be related to the specific hydrophobic surface area [12]. The good correlation between the intercept and slope values indicates the structural homogeneity of solutes [13].

The objectives of the present investigation were to determine the retention of 45 barbituric acid derivatives on PGC columns in unbuffered methanol–water eluent mixtures at various organic phase concentrations, to evaluate the retention data by multivariate

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mathematical statistical methods and to find the relationship between retention characteristics and physicochemical parameters of barbituric acid derivatives.

Experimental

The porous graphite carbon column (Shandon Hypercarb 100 × 4.7 mm i.d., particle diameter 7 μm) was purchased from Shandon Scientific (UK). The HPLC system consisted of a Liquopump Model 312 (LaborMIM, Budapest, Hungary) pump, a Cecil CE-212 variable wavelength UV detector (Cecil Instr., Cambridge, UK), a Valco injector (Valco Inc., Houston, TX, USA) with a 20 μl sample loop and a Waters 740 integrator (Waters–Millipore Inc., Milford, MA, USA). The flow rate was 0.8 ml min^{-1} and the detection wavelength was set to 240 nm. Mixtures of methanol–water were used as eluents, methanol concentration ranged from 60 to 95 (in steps of 5% v/v). Buffers were not used. The chemical structure of barbituric acid derivatives are shown in Table 1. The barbituric acid derivatives were dissolved in methanol at the concentration of 0.05 mg ml^{-1}. The retention time of each compound in each eluent was determined with three consecutive determinations. As the correlations between the logk' value and the organic phase concentration is generally linear in HPLC we also applied linear equations to describe the dependence of the logk' value on the organic mobile phase concentration

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

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

To find the molecular parameters significantly influencing the retention, the combined dependent variable logk'_0/b was correlated with physicochemical characteristics of barbituric acid derivatives (independent variables). It was assumed that the combined variable logk'_0/b concentrated in one variable the information content of both the intercept and slope values. The physicochemical parameters included in the calculation 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; and

$B1$ and $B4$ = sterimol width parameters determined by distance of substituents at their maximum point perpendicular to attachment bond axis [14].

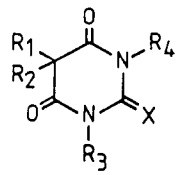
The calculation was carried out by linear stepwise regression analysis [15]. In the common multivariate regression analysis the presence of independent variables which exert no significant influence on the dependent variable lessens the significance level of those independent variables which do significantly influence the dependent variable. To overcome this difficulty the stepwise regression analysis automatically eliminates from the selected equation the insignificant independent variables. The number of accepted variables was not limited their acceptance limit was set to 95% significance level.

Results and Discussion

Each barbituric acid derivative showed symmetric peaks in each eluent system (Figs 1 and 2). The retention order of solutes deviates from their expected retention order on a traditional reversed-phase column: the more lipophilic barbituric acid derivative (no. 26 in Table 1) is eluted before the less lipophilic barbituric acid derivative (no. 27 in Table 1). The barbituric acid derivatives with identical hydrophobicity (nos 6 and 7 in Table 1) can be well separated on the column (see Fig. 2). These findings indicate that the retention behaviour of the PGC column differs from that of reversed-phase columns.

The parameters of equation (1) are compiled in Table 2 (S_b = standard deviation of the slope 'b' value). The relationship between logk' and organic phase concentration was linear in each case. The correlation coefficient in most cases was greater than 0.99 confirming the applicability of equation (1). The slope and

Table 1
Chemical structure of barbituric acid derivatives



Compound no.	R_1	R_2	R_3	R_4	X
1	H	H	H	H	O
2	methyl	methyl	H	H	O
3	3-pentyl	methyl	H	H	O
4	methyl	1-methylpentyl	H	H	O
5	ethyl	ethyl	H	H	O
6	ethyl	1-methylbutyl	H	H	O
7	ethyl	3-methylbutyl	H	H	O
8	ethyl	1-methylpropyl	H	H	O
9	ethyl	*n*-penthyl	H	H	O
10	butyl	1-methylpropyl	H	H	O
11	butyl	1-methylbutyl	H	H	O
12	butyl	3-methylbutyl	H	H	O
13	ethyl	*n*-octyl	H	H	O
14	ethyl	3-dimethyloctyl	H	H	O
15	allyl	*i*-propyl	H	H	O
16	allyl	*i*-butyl	H	H	O
17	allyl	1-methylbutyl	H	H	O
18	allyl	1-methylcyclo-hexenyl	H	H	O
19	allyl	2-cyclopentyl	H	H	O
20	ethyl	1-cyclohexenyl	H	H	O
21	ethyl	ethyl	H	H	S
22	ethyl	1-methylbutyl	H	H	S
23	allyl	1-methylbutyl	H	H	S
24	ethyl	1,3-dimethylbutyl	H	H	S
25	ethyl	phenyl	H	H	O
26	ethyl	ethyl	phenyl	H	O
27	ethyl	ethyl	benzoyl	H	O
28	ethyl	ethyl	benzoyl	benzoyl	O
29	ethyl	ethyl	*p*-Cl-benzoyl	H	O
30	ethyl	ethyl	*p*-NO_2-benzoyl	H	O
31	ethyl	ethyl	*p*-NO_2-benzoyl	*p*-NO_2-benzoyl	O
32	ethyl	phenyl	phenyl	H	O
33	ethyl	phenyl	benzoyl	methyl	O
34	ethyl	phenyl	*p*-NH_2-benzoyl	methyl	O
35	ethyl	phenyl	*o*-NO_2-benzoyl	methyl	O
36	ethyl	phenyl	*p*-NO_2-benzoyl	methyl	O
37	ethyl	phenyl	*m*-NO_2-benzoyl	methyl	O
38	ethyl	ethyl	*p*-NO_2-benzoyl	methyl	O
39	ethyl	ethyl	benzoyl	methyl	O
40	methyl	phenyl	benzoyl	H	O
41	methyl	phenyl	benzoyl	methyl	O
42	ethyl	phenyl	benzoyl	H	O
43	ethyl	methyl	H	H	O
44	ethyl	ethyl	propyl	H	O
45	methyl	methyl	methyl	H	O

intercept values considerably differ from each other. This means that the barbituric acid derivatives can be easily separated on the PGC column in methanol–water systems.

Significant linear correlation was found between the slope and intercept value of equation (1).

$$\log k'_0 = -108.22 - 0.73b, \qquad (2)$$

with $n = 35$; $r = 0.7422$; $S_b = 0.348$; and $r_{99.9\%} = 0.5189$. This result indicates that from the chromotographic point of view, the barbituric acid derivatives can be considered as a homogenous series of compounds.

Stepwise regression analysis selected only one physicochemical parameter accounting for the retention behaviour of barbituric acid derivatives:

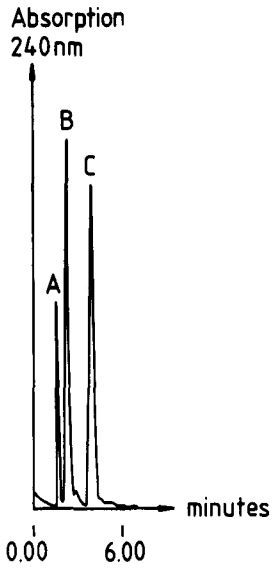


Figure 1
Separation of some barbituric acid derivatives on porous graphitized carbon column. Eluent, methanol–water (95:5, v/v); flow rate, 0.8 ml min^{-1}; detection, 240 nm. A, B and C refer to barbituric acid derivatives nos 26, 27 and 28 in Table 1, respectively.

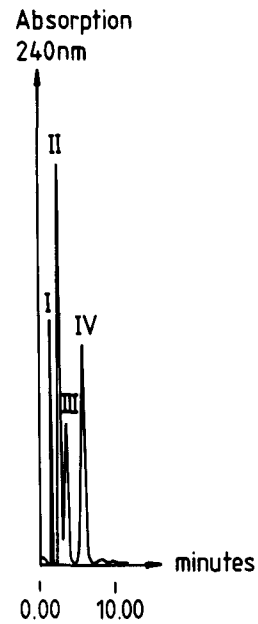


Figure 2
Separation of some barbituric acid derivatives on porous graphitized carbon column. Eluent, methanol–water (8:2, v/v); flow rate, 0.8 ml min^{-1}; detection, 240 nm. I, II, III and IV refer to barbituric acid derivatives nos 6, 7, 11 and 14 in Table 1, respectively.

$$\log k'_0/b = -31.05 - 84.39F, \qquad (3)$$

with $n = 35$; $r = 0.8157$; $S_b = 1.41$; and $r_{99.9\%} = 0.5189$.

Table 2
Parameters of linear correlations between the logarithm of capacity and methanol concentration in the eluent ($\log k' = \log k'_0 + bC$). S_b = standard deviation of the slope 'b' value

Compound no.	$\log k'_0$	$-b$	$S_b \times 10^{-2}$	r
1	99.48	1.31	1.5	0.9973
2	100.65	1.40	0.4	0.9898
3	102.85	1.49	1.4	0.9844
4	111.65	5.76	4.6	0.9776
5		not significant		
6	112.41	7.19	5.1	0.9929
7	125.95	13.58	1.3	0.9998
8	134.54	18.70	6.5	0.9954
9	122.72	12.07	0.8	0.9982
10	101.50	2.86	0.5	0.9155
11	103.23	3.05	0.3	0.9563
12	100.85	2.51	0.2	0.9909
13	100.69	2.14	1.1	0.9941
15	147.33	24.68	4.5	0.8983
16	126.38	13.24	1.8	0.9908
17	114.49	7.67	0.5	0.9947
18	136.35	18.59	3.0	0.9266
19	114.55	7.10	5.2	0.9982
20	133.60	18.01	1.7	0.9687
21	152.86	27.63	3.9	0.9967
22	112.59	6.65	0.5	0.9770
23	106.84	4.08	0.3	0.9798
24		not significant		
25	120.38	10.33	0.9	0.9710
26	113.81	8.09	0.9	0.9554
28	108.59	2.94	0.2	0.9698
29	101.46	0.66	0.1	0.9992
30	131.91	1.45	0.3	0.9958
31	116.01	0.58	0.3	0.9815
32	100.11	0.79	0.4	0.9873
33	109.17	0.89	0.1	0.9823
34	111.07	0.75	0.1	0.9753
35	113.02	0.89	0.5	0.9903
36	115.73	0.79	0.3	0.9897
37	113.57	0.90	0.5	0.9899
38	114.75	0.98	0.2	0.9799
39	107.19	2.30	0.3	0.9847
40	106.14	1.32	0.2	0.9907
41	106.23	1.35	0.1	0.9799
42	106.02	0.76	0.1	0.9959
43	100.01	1.84	0.1	0.9912
44		not significant		
45	134.60	20.11	2.3	0.9914

The equation fit well to the experimental data — the significance level was over 99.9% (see r value). This physicochemical parameter explains 66.54% of the total variance. The effect of the other electronic and structural parameters was not significant, therefore their contribution to the retention of barbituric acid derivatives is probably negligible. The lipophilicity values of substituents did not influence significantly the retention characteristics, that is the separation capacity of the PGC column considerably differs from that of

the common reversed-phase columns. It can be concluded from our data that barbituric acid derivatives can be well separated on porous graphitized carbon column without buffering the eluent. Stepwise regression analysis indicated that the retention of such derivatives is mainly governed by electronic parameters.

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