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Retention behaviour of some barbituric acid derivatives on a polyethylene-coated silica column

Esther Forgács*, Tibor Cserháti

Central Research Institute for Chemistry, Hungarian Academy of Sciences, POB 17, H-1525 Budapest, Hungary

Abstract

The retention of 45 barbituric acid derivatives was determined on a polyethylene-coated silica column (PEE) in unbuffered methanol–water eluent mixtures. Each derivative showed a symmetric peak shape in each eluent and the capacity factor decreased monotonously with increasing concentration of methanol in the eluent. Stepwise regression analysis indicated that the retention of barbituric acid derivatives is mainly governed by the molecular lipopholicity and, to a lesser extent, by steric effects of the various substituents. This finding indicates that the polyethylene-coated silica behaves as a real reversed-phase chromatographic support with slightly different retention characteristics.

1. Introduction

The reversed-phase separation mode is the most widely used technique in high-performance liquid chromatography (HPLC). The support generally is silica with covalently bonded hydrocarbons on the surface. More recently, polymer-coated silicas have become popular since they combine the advantageous mechanical properties of silica with the dynamic chemical properties of polymers. Various polymers such as poly (alkyl aspartamide) [1], alkyl polysiloxanes [2], polyvinylpyrrolidone [3], poly(2-sulfoethyl aspartamide) [4], polyethyleneimine [5], polyamine [6], poly(butadiene-maleic acid) [7], polyvinylimidazole [8], and polypyrrole chloride [9] have been coated on silica. Polymer coating improves not only the selectivity but also the chemical stability of stationary phases. Polymer-coated silica supports have been successfully used for the separation of various alkaline compounds [10], peptides [11] and proteins [12].

The objectives of the present investigation were to determine the retention of 45 barbituric acid derivatives (Table 1) on a polyethylene-coated silica column in unbuffered methanol–water eluent mixtures at various organic phase concentrations, to evaluate the retention data by multivariate mathematical statistical methods, and to find the relationship between the retention characteristics and the physicochemical parameters of barbituric acid derivatives.

2. Experimental

The HPLC system consisted of a Liquopump Model 312 pump (LaborMIM, Budapest, Hun-

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* Corresponding author.

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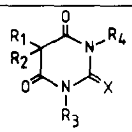
Table 1
Chemical structure of barbituric acid derivatives



General structure

No. of compounds	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	iso-propyl	H	H	O
16	allyl	iso-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	propyl	H	H	O
45	methyl	methyl	methyl	H	O

gary), a Cecil CE-212 variable wavelength UV detector (Cecil Instr., Cambridge, UK), a Valco injector (Valco, Houston, TX, USA) with a 20-μl sample loop and a Waters 740 integrator (Waters-Millipore, Milford, MA, USA). The column was a polyethylene-coated silica column (further PEE column) prepared in our laboratory (250 × 4 mm I.D.). The packing used was silica (particle size 5.0 ± 1.5 μm, pore diameter 50 Å, pore volume 0.8 mg/l; BET surface 450 m^2/g; Macherey-Nagel, Düren, Germany), the carbon and hydrogen contents of the PEE packing were 8.05 and 2.22%, respectively. The separation of some ring substituted aniline derivatives on PEE column has been previously reported [13]. The flow-rate as 0.8 ml/min and the detection wavelength was set at 240 nm. Mixtures of methanol–water were used as eluents. Methanol concentrations ranged from 30–70% in 5% (v/v) steps. Buffers were not used. The barbituric acid derivatives were dissolved in methanol at a concentration of 0.05 mg/m. The dead volume of the column was determined by injecting $NaNO_3$. Each determination was carried out in quadruplicate.

As the correlations between the log k' value and the organic phase concentration are generally linear in HPLC we also applied linear equations to describe the dependence of the log k' value on the organic mobile phase concentration:

$$\log k' = \log k'_0 + b \cdot C \qquad (1)$$

where log k' is the logarithm of the capacity factor; log k'_0 is the logarithm of the capacity factor extrapolated to zero concentration of the organic component in the mobile phase (intercept, related to molecular lipophilicity or retention capacity of solutes) [14]; b is the change of the log k' value caused by a unit change (1 vol.%) in the organic mobile phase concentration (slope, related to the specific hydrophobic surface area in contact with the support) [15], and C is the methanol concentration in the eluent (vol.%).

To test the validity of the hypothesis that in the case of a homologous series of solutes the intercepts (lipophilicity) and slope (specific hydrophobic surface area) values are intercorrelated [16], a linear correlation was calculated between the corresponding parameters of Eq. (1).

To find the molecular parameters that significantly influence the retention, the combined dependent variable log k'_0/b was correlated with the physicochemical characteristics of barbituric acid derivatives (independent variables). It was assumed that the combined variable log k'_0/b contains in one variable the information contents intercept and intercept values. A similar combined variable has been previously used to describe the retention behaviour of barbituric acid derivatives on a porous graphitized carbon column [17]. The physicochemical parameters included in the calculation were:

π = Hansch and 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 and Lupton's electronic parameters characterizing the inductive and resonance effect, respectively.

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

E_s = Taft's constant, characterizing steric effects of the substituent.

B_1 and B_4 = Sterimol width parameters determined by the distance of substituents at their maximum point perpendicular to attachment.

The inclusion of the above physicochemical parameters in the calculation was motivated by the fact that these parameters have been related to the retention behaviour of some solutes in various HPLC systems such as ring substituted anilines [18] and phenols on a porous graphitized carbon column [19], and barbituric acid derivatives on a β-cyclodextrin polymer coated column [20].

The calculation was carried out by stepwise regression analysis [21], the combined variable log k'_0/b being the dependent variable and the physicochemical parameters listed above the independent variables, respectively. The acceptance level for the individual independent variables was set at the 95% significance level. 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 have significant influence on the dependent variables. To overcome this difficulty the stepwise regression analysis automatically eliminates the insignificant independent variables from the selected equation, increasing in this manner the reliability of the calculation.

3. Results and discussion

All barbituric acid derivatives showed symmetric peaks in each eluent system (Fig. 1). The retention order of solutes followed the expected retention order on a traditional reversed-phase column, *i.e.* the less hydrophobic barbituric acid derivative (A) elutes earlier than the more hydrophobic barbituric acid derivatives (B, C and D).

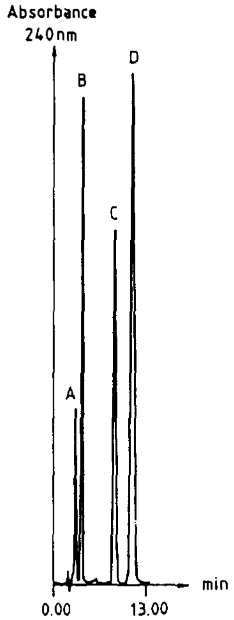


Fig. 1. Separation of some barbituric acid derivatives on PEE column. Eluent, methanol–water (3:7, v/v), flow-rate 0.8 ml/min, detection 240 nm. A, B, C and D refer to barbituric acid derivatives Nos. 1, 2, 10 and 16 in Table 1, respectively.

The parameters of eq. (1) are listed in Table 2. S_b and r values are the standard deviation of the slope "b" and the coefficient of correlation indicating the fitness of the equation to the experimental data. The relationship between the log k' and organic phase concentration was linear in each case. In most cases the coefficient of correlation was greater than 0.99 confirming the applicability of eq. (1). The slope and intercept values considerably differ from each other indicating that the barbituric acid derivatives can be easily separated on the PEE column in methanol–water eluent systems.

The calculated coefficient of correlation ($r = 0.7292$) was higher than the tabulated value corresponding to the 99.9% significance level ($r_{99.9\%} = 0.5189$). This indicates that the slope and intercept values of eq. (1) are intercorrelated (Fig. 2). This result indicates that the barbituric acid derivatives can be considered as a homogenous series of compounds not only on the bases of the similar chemical structures but also on the bases of their retention behaviour on the PEE column.

Stepwise regression analysis revealed significant linear correlation between the combined retention parameter (intercept/slope of eq. 1.) and physicochemical parameters of barbituric acid derivatives:

$$\log k'_0/b = -12.87 - (8.12 \pm 1.87)\cdot\pi + (0.95 \pm 0.42)\cdot E_s \qquad (2)$$

$$n = 38, \quad r^2 = 0.4363, \quad F_{\text{calc.}} = 13.54, \quad F_{99.9\%} = 8.75$$

Eq. (2) fits will to the experimental data, the significance level being over 99.9% (compare the $F_{\text{calc.}}$ value with the tabulated F value $F_{99.9\%}$ indicating the fitness of the equation to the experimental data in multivariate regression analysis). Only the lipophilicity (π) (path coefficient 65.62%) and Taft's constant related to the steric effect (E_s) of substituents (path coefficient 34.38%) of barbituric acid derivatives have a significant influence on the retention behaviour

Table 2
Parameters of linear correlations between the logarithm of capacity factors ($\log k'$) and methanol concentration (C, v/v%) in the eluent[a]

Compound No.	$\log k'_0$	$-b \cdot 10^{-3}$	$S_b \cdot 10^{-4}$	r
1	0.11	8.72	8.5	0.9961
2	0.31	13.23	2.7	0.9931
3	1.75	23.40	2.9	0.9774
4	1.46	25.30	2.1	0.9930
5	0.78	22.12	2.4	0.9881
6	1.46	29.49	0.7	0.9932
7	1.28	24.47	8.2	0.9905
8	0.93	19.00	4.6	0.9863
9	1.38	25.56	2.6	0.9906
10	0.94	18.77	2.6	0.9909
11	0.96	20.49	4.7	0.9591
12	0.87	14.80	1.5	0.9848
13	1.59	20.60	3.3	0.9749
14	1.46	21.01	3.4	0.9961
15	1.07	22.02	7.6	0.9988
16	1.13	25.05	2.5	0.9987
17	1.87	38.70	4.7	0.9953
18	1.28	27.12	1.9	0.9747
19	1.27	26.31	6.6	0.9946
20	1.12	27.35	1.3	0.9979
21	0.96	27.16	2.8	0.9994
22	1.20	21.20	2.6	0.9946
23	1.87	34.67	6.3	0.9923
24	1.14	21.03	1.7	0.9907
25	2.15	38.10	2.2	0.9827
26	1.03	19.00	1.3	0.9987
27	1.48	22.45	2.7	0.9909
28	1.00	24.12	4.6	0.9966
29	0.88	18.20	3.1	0.9973
30	1.86	25.23	4.2	0.9958
31	0.66	12.90	2.5	0.9947
32	0.87	20.45	1.2	0.9911
33[b]		not significant		
34	1.45	37.12	2.8	0.9956
35	1.80	21.98	2.5	0.9943
36	1.33	38.66	5.2	0.9876
37	1.45	22.12	7.9	0.9924
38[b]		not significant		
39	2.45	24.78	8.0	0.9970
40	1.65	34.33	2.1	0.9943
41	1.88	32.59	1.9	0.9931
42	1.67	32.12	5.6	0.9945
43	1.98	29.23	0.5	0.9977
44	1.55	22.12	7.2	0.9823
45	1.45	19.99	3.3	0.9902

[a] $\log k' = \log k'_0 + b \cdot C$.

[b] Compounds 33 and 38 showed low retention in the eluent systems, therefore their retention behaviour cannot be adequately determined.

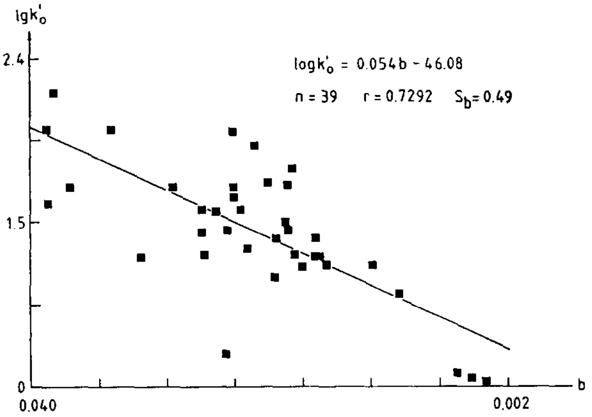


Fig. 2. Relationship between the lipophilicity (log k'_0) and specific hydrophobic surface area (b) of barbituric acid derivatives.

of barbituric acid derivatives on the PEE column. Path coefficient are dimensionless numbers indicating the relative impact of independent variables (in our case: physicochemical parameters) on the dependent variable (in our case: the retention parameter of barbiturates) without taking into consideration the original dimensions of the variables. The fact that the selected independent variables account only for a relatively (43.63%, see r^2 value) small change in the retention behaviour indicates that other physicochemical parameters not included in the calculation may exert considerable influence on the retention behaviour of the PEE column slightly differs from that of a traditional reversed-phase column [22,23] since not only lipophilicity (π) but also the Taft constants (E_s) of barbituric acid derivatives have a significant effect on the retention.

4. Conclusion

Our data show that barbituric acid derivatives can be well separated on the polyethylene-coated silica column without using buffered eluent systems. The retention behaviour of the PEE column slightly differs from that of traditional reversed-phase column and the steric parameters of the substituents also influence the retention.

5. Acknowledgment

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