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Relationship between retention characteristics and physicochemical parameters of solutes on porous graphitized carbon column

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Abstract

The retention of 44 barbituric acid derivatives was determined on porous graphitized carbon (PGC) column using dioxane–water mixtures as eluents. Linear correlations were calculated between the logarithm of the capacity factor and the dioxane concentration in the eluent. Free–Wilson analysis combined with stepwise regression analysis was used to elucidate the role of individual substituents in the retention behaviour. Calculations indicated that the apolar substituents lie parallel to the surface of PGC surface increasing in this manner the retention and—as opposed to the retention characteristics of traditional reversed-phase supports—the position of substituents also exerts a marked influence on the retention.

Keywords: Porous graphitized carbon column; Barbituric acid derivatives; Structure-retention relationship

1. Introduction

Many efforts have been devoted for the elucidation of the influence of the chemical character of substituents and that of various molecular substructures on the retention parameters in each field of chromatography [1] such as thin-layer [2,3] gas–liquid [4,5] and high performance liquid chromatography [6–9]. As generally large data matrices are used for such a type of study [10], many multivariate methods such as factor analysis [11,12] and principal component analysis [13] are frequently applied for the extraction of the maximal information regarding structure-retention relationships.

Free–Wilson [14] analysis has been developed for the determination of the contribution of individual substituents to the overall biological activity of a homologous series of drugs. Free–Wilson analysis considers the activity of the unsubstituted molecule being zero. In the structure-retention calculations, the biological activity of the molecules can be replaced with any of their retention parameters, the results give the contribution of the individual substituents to the retention characteristics observed. The prerequisite for the successful application of Free–Wilson analysis is

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that the substituents do not interact with each other and their activity contributions are independent from each other. However, this method has the same drawbacks as the traditional multilinear regression analysis, the presence of independent variables (substituents) that exert no significant influence on the dependent variable (retention parameter) lessens the significance level of the independent variables that significantly influence the dependent variable. To overcome this difficulty, stepwise regression analysis [15] can be combined with the Free–Wilson analysis. This procedure automatically eliminates from the selected equation the insignificant independent variables (substituents having no significant impact on the retention) increasing in this manner the information power of the calculation. This combined method has been successfully used for the elucidation of the influence of various substituents on the retention behaviour of some ring-substituted phenol derivatives on porous graphitized carbon (PGC) column [16] and for the determination of molecular substructures accounting for the complex forming capacity of barbituric acid derivatives with hydroxypropyl-β-cyclodextrin [17].

PGC support was developed more than ten years ago [18–20]. It has been used frequently for the separation of a wide variety of solutes such as polar compounds [21], pesticides [22], etc. Its application and retention mechanism has been reviewed recently [23].

The objectives of the work presented in this paper was to study the retention characteristics of a PGC column using barbituric acid derivatives as solutes and to apply multivariate mathematical-statistical methods for the elucidation of the role of various substituents in the determination of the retention parameters. The results may facilitate the separation and quantitative determination of barbituric acid derivatives both in pharmaceutical formulations and tissues and may help the optimization of the separation process of any set of barbiturates.

2. Experimental

The PGC 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 (Labor MIM, Budapest, Hungary) pump, a Cecil CE-212 variable wavelength W detector (Cecil, Cambridge, UK), a Valco injector (Valco, Houston, TX) with a 20 μl sample loop and a Waters 740 integrator (Water-Millipore, Milford, MA). The flow-rate was 0.8 ml min^{-1} and the detection wavelength was set to 240 nm. Mixtures of dioxane–water were used as eluents, dioxane concentration ranged from 60 to 70 v/v (in steps of 2.5% v/v). The column was not thermostated each determination was run at room temperature (21–23°C). The chemical structure of barbituric acid derivatives are shown in Table 1. The barbituric acid derivatives were dissolved in the eluents at the concentration of 0.05 mg ml^{-1}. The retention time of each compound in each eluent was determined with three consecutive determinations.

2.1. Theory

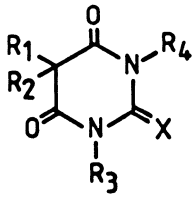
Linear correlation was used to describe the dependence of the $\log k$ value on the concentration of dioxane:

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

where $\log k'$ is the logarithm of capacity factor; $\log k'_0$ is the logarithm of capacity factor extrapolated to zero dioxane concentration in the eluent (intercept, related to the retention capacity of the column); b is the change of $\log k'$ value caused by unit change (1 vol%) of dioxane concentration (slope, related to the specific surface area of solutes in contact with the PGC surface); and C is the dioxane concentration in the eluent (vol%). To determine the influence of various substituents of the barbituric acid derivatives on their retention parameters ($\log k'_0$ and b) Free–Wilson analysis combined with stepwise regression analysis was applied:

A. The intercept ($\log k'_0$) value of Eq. (1) was the dependent variable and the substituents were the independent variables. The same substituent at different position was considered as separate independent variable (34 variables in total).

Table 1
Chemical structure of barbituric acid derivatives



General structure

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*-Pentyl	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-Methylcyclohexenyl 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	H	O
40	Methyl	Phenyl	Benzoyl	Methyl	O
41	Ethyl	Phenyl	Benzoyl	H	O
42	Ethyl	Methyl	H	H	O
43	Ethyl	Ethyl	Propyl	H	O
44	Methyl	Methyl	Methyl	H	O

B. The slope (b) value of Eq. (1) was the dependent variable and the independent variables were as in analysis A.

C. The intercept ($\log k'_0$) value of Eq. (1) was the dependent variable and the substituents were the independent variables. Substituents were included in the calculation without taking into consideration their position in the molecule (24 variables in total).

D. The slope (b) value of Eq. (1) was the dependent variable and the independent variables were as in analysis C.

The inclusion of analysis C and D was motivated by the supposition that the comparison of the results of analysis A–C and B–D contained information about the impact of substituent position on the retention behaviour of barbituric acid derivatives on PGC column. In each instance, the number of accepted independent variables was not limited, the acceptance limit was set to 95% significance level.

3. Results and discussion

The parameters of Eq. (1) are listed in Table 2. Parameters S_b and r are the standard deviation of slope b and the coefficient of correlation, respectively. The linear relationship between the logarithm of the capacity factor and the dioxane concentration in the eluent was significant in each instance proving the regular retention behaviour of barbituric acid derivatives on PGC column and the applicability of Eq. (1). The retention parameters differ markedly form each other, that means that the barbituric acid derivatives can be easily separated on the PGC column using dioxane–water mixtures as eluents. The retention parameters listed in Table 2 make possible the calculation of the retention time differences for any pairs of barbiturates and that of the optimal eluent composition corresponding to maximum retention time difference.

The results of the Free–Wilson analysis combined with stepwise regression analysis are listed in Table 3. As the information content of analysis D was considerably lower than that of analysis B these data are not included in Table 3. Each equation fits well to the experimental data the significance level being in each case over 99.9%

Table 2
Parameters of linear correlations between the logarithm of capacity factor and dioxane concentration (C) in the eluent[a,b]

Compound no.	$\log k'_0$	$-b \cdot 10^2$	$S_b \cdot 10^3$	r_{calc}
1	72.11	1.07	0.1	0.9901
2	69.79	0.99	2.9	0.9870
3	72.51	1.14	2.1	0.9340
4	84.60	2.41	0.5	0.9912
5	53.12	3.47	4.2	0.9925
6	105.21	3.25	2.1	0.9340
7	106.24	7.15	1.1	0.9210
8	99.42	10.55	0.6	0.9917
9	93.34	7.15	6.4	0.9921
10	75.22	0.84	1.2	0.9250
11	69.94	1.47	0.1	0.9999
12	79.60	2.00	0.2	0.9347
13	92.20	0.75	3.2	0.9861
14	93.20	0.89	4.5	0.9261
15	120.00	17.02	2.2	0.9921
16	106.33	7.80	6.2	0.9740
17	108.21	5.61	1.2	0.9360
18	110.11	9.52	0.2	0.9993
19	100.00	5.72	1.4	0.9921
20	106.41	7.40	1.6	0.9001
21	140.20	17.60	0.2	0.9783
22	94.70	3.40	2.5	0.9450
23	90.10	1.89	1.7	0.9564
24	21.24	7.60	6.2	0.9189
25	87.42	6.51	6.3	0.9340
26	65.20	5.12	1.3	0.9912
27	67.92	0.85	2.7	0.9234
28	174.25	1.09	0.8	0.9910
29	72.20	0.51	1.2	0.9250
30	110.15	1.00	0.2	0.9250
31	99.94	0.32	3.0	0.9664
32	74.56	0.42	1.4	0.9810
33	97.12	0.50	2.4	0.9889
34	102.20	0.50	1.8	0.9570
35	94.40	0.56	2.4	0.9150
36	90.81	0.70	7.2	0.9664
37	97.20	0.79	4.1	0.9894
38	92.51	0.34	2.8	0.9341
39	91.83	0.99	0.6	0.9992
40	90.21	0.81	0.6	0.9990
41	89.12	0.59	0.6	0.9897
42	75.54	0.78	0.3	0.9554
43	72.14	4.14	0.8	0.9214
44	64.87	8.90	0.7	0.9924

[a] S_b, standard deviation of the slope b value.
[b] $\log k' = \log k'_0 + b \cdot C$.

(see F_{calc} values). Only a few substituents (five for Equation I and three for Equations II and III) from those included in the calculation explain a considerable part of the variance (see r_2 values). The path coefficients (b_{1-5} % values) indicate that each substituent has similar impact on the retention.

The fact that Equations I and III (including the positional effect of substituents) explain a higher ratio of variance than Equations II and IV (Equation IV not shown) indicates that the position of substituents has a considerable influence on the retention behaviour. We assume that this effect may be due to planar character of the PGC surface. Graphite is composed of layers of hexagonally arranged, covalently-bonded carbon atoms, the layers themselves being held together by weak van-der Waals forces. The solute molecules probably lie parallel to the PGC surface, the solute–support interaction is mainly determined by the intermolecular dispersion forces involving both hydrophilic and hydrophobic interactions. The relative importance of the various interactive forces between the PGC surface and the solute molecules considerably depends on the structure and physicochemical character of the solute molecules. This retention mechanism is highly different from that of traditional octadecylsilica support where the hydrophobic forces between the apolar octadecyl alkyl chains and the hydrophobic substructures of solutes govern the retention. The position of substituents generally have a negligible influence on the retention. This phenomenon can be explained by the supposition that the alkyl substituents of the solutes are more or less submerged in the liquid-like apolar hydrocarbon layer independently of their position in the molecule.

Table 3
Influence of various substituents on the retention characteristics of barbituric acid derivatives on porous graphitized carbon column[a,b]

Parameter	Equation I[c]	Equation II[d]	Equation III[e]
a	75.43	85.76	3.53
b_1	17.51	20.04	13.49
S_{b1}	5.56	8.51	3.44
b_2	30.36	−64.52	−2.88
S_{b2}	7.54	19.40	1.34
b_3	−71.70	20.49	4.09
S_{b3}	14.60	6.89	1.80
b_4	−23.06	–	–
S_{b4}	10.52	–	–
b_5	81.31	–	–
S_{b6}	14.60	–	–
b_1 (%)	17.92	27.32	46.78
b_2 (%)	22.49	38.21	25.85
b_3 (%)	23.06	34.47	27.37
b_4 (%)	10.37	–	–
b_5 (%)	26.16	–	–
r_2	0.6762	0.3930	0.4071
F_{calc}	15.87	8.63	9.16

[a] x_1, ethyl substituent at position R_1; x_2, allyl substituent at position R_1; x_3, 1,3-dimethylbutyl substituent at position R_2; x_4, phenyl substituent at position R_3; x_5, p-NO_2-benzoyl substituent at position R_4; x_6, number of benzoyl substituents at each position; x_7, i-propyl substituent at position R_2; x_8, methyl substituent at position R_4; x_9, substitution of oxygen by sulfur at position X.

[b] Result of Free–Wilson and stepwise regression analysis, $n = 44$.

[c] I. $\log k'_0 = a + b_1 \cdot x_1 + b_2 \cdot x_2 + b_3 \cdot x_3 + b_4 \cdot x_4 + b_5 \cdot x_5$.

[d] II. $\log k'_0 = a + b \cdot x_2 + b_2 \cdot x_3 + b_3 \cdot x_6$.

[e] III. $b = a + b_1 \cdot x_7 + b_2 \cdot x_8 + b_3 \cdot x_9$.

The significant effect of various alkyl substitutions on the retention on PGC can be explained by the supposition that the barbituric acid derivatives contact the support surface the energy of adsorption being maximal. As the PGC surface is relatively apolar, the hydrophobic alkyl chains may interact with this surface modifying the retention. The substitution of oxygen atom by sulfur also increases the solute surface in contact with the stationary phase. This result can be easily explained by the larger bulk of sulfur substituent.

The comparison of the separation capacity of PGC and the traditional octadecylsilica (ODS) column for barbituric acid derivatives is of considerable interest both for practical and theoretical point of view. The retention behaviour of a group of 28 barbiturates was recently studied and the regular and predictable retention behaviour was established [24]. As a different set of barbiturates was used in the experiments the two methods cannot be compared. To the best of our knowledge the retention behaviour of these solutes on ODS column has never been studied in detail.

It is highly probable that not only the position and character of the apolar substituents but also the pK_a value influences the retention of barbituric acid derivatives on PGC column. Unfortunately, the majority of barbituric acid derivatives was experimental product therefore their pK_a values were not determined and cannot be included in the calculations.

It can be concluded from the results that Free–Wilson analysis combined with stepwise regression analysis is a suitable method for the assessment of the relationships between the retention behaviour of barbituric acid derivatives on PGC column and their chemical structure. Calculations indicated that the apolar substituents lie parallel to the surface of PGC surface increasing in this manner the retention and-oppositely to the retention characteristics of traditional reversed-phase supports-the position of substituents also exerts a marked influence on the retention.

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