

Simultaneous effect of organic modifier and physicochemical parameters of barbiturates on their retention on a narrow-bore PGC column

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Abstract

The retention time of 22 barbituric acid derivatives was measured on a narrow-bore porous graphitized carbon (PGC) column using water–dioxane mixtures as mobile phases. The capacity factor (k), theoretical plate number (N), and asymmetry factor (AF) were calculated for each solute in each mobile phase. The relationships between chromatographic characteristics and physicochemical parameters of solutes were elucidated by stepwise regression analysis (SRA). SRA indicated that the binding of barbiturates to the PGC surface is of mixed character electrostatic and apolar interactive forces are equally involved. Sterical correspondence between the surface of the stationary phase and the solutes also exert a significant influence on the retention behavior.

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1. Introduction

Because of their considerable importance in health care much effort has been devoted to the development and application of various chromatographic methods for the separation and quantitative determination of barbituric acid derivatives such as gas [1] and thin-layer chromatography [2], etc. Numerous high-performance liquid chromatographic (HPLC) techniques with high separation capacity and elevated sensitivity have also found employment in the analysis of barbiturates [3]. Thus, the successful use of ion-pair chromatography [4], micellar HPLC [5], 3-(1,8-naphthalimido)propyl-modified silica [6] has been reported. The advantageous retention characteristics of porous graphitized carbon (PGC) stationary phase [7,8] have also been exploited in the analysis of barbituric acid derivatives [9,10]. Microcolumn HPLC combined with post-column photochemical derivatization has been recently applied for the determination of barbiturates [11].

The quantitative relationship between the structure and retention (QSRS) of barbiturates has been vigorously dis-

cussed the results depending considerably on the HPLC system employed. The marked impact of molar volume [12], hydrophobicity [13], and electrostatic interactive forces [14] has been demonstrated. In order to increase the reliability of QSRS, linear correlations are frequently calculated between the retention parameter (retention time, capacity factor (k) or logarithm of capacity factor) selected and the parameters of the equation (slope and/or intercept values) are used for the elucidation of the quantitative relationship between retention and solute structure. This procedure necessitates the application of two separate calculation steps using more computer time and complicating the evaluation of results.

Linear and multiple linear regression analyses have been extensively used to find the relationship between one dependent and one or more independent variables. Because of their simplicity and good predictive power they have been successfully applied in various chromatographic techniques too [15]. These methods have been recently employed in chromatography for the investigation of the molecular mechanism of separation [16], for the classification of modern stationary phases [17], for structure–retention relationship study in HPLC [18] and in GC [19,20], and for the elucidation of the correlation between retention and biological activity [21].

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The objectives of the work were the determination of the retention characteristics (capacity factor, theoretical plate number (N), and asymmetry factor (AF)) of some barbituric acid derivatives on a narrow-bore PGC column using water–dioxane mixtures as mobile phases, the elucidation of the simultaneous effect of organic modifier and the physicochemical parameters of solutes on the retention and the assessment of the suitability of stepwise regression analysis (SRA) for this qualitative structure–retention relationship study [22].

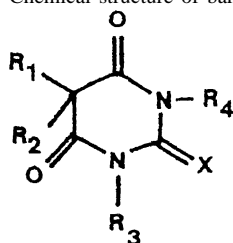
2. Experimental

Dioxane of HPLC quality was purchased from Romil Ltd. (Cambridge, UK). The chemical structure of barbiturates are compiled in Table 1. They were separately dissolved in the mobile phases at the concentration of 1.0 mg/ml and aliquots of 0.5 μ l were injected. HPLC measurements were performed with a Waters LC Module I HPLC instrument with an injection device of variable volume and a Waters 746 Data Module integrator (Waters–Millipore Inc., Milford, Massachusetts, USA). Analyses were carried out on a Hypersil Hypercarb PGC column (100 mm \times 2.1 mm

i.d., particle size, 7 μ m) (HYPERASIL, Runcorn, Cheshire). The column was not thermostated, measurements were performed at ambient temperature (21 ± 1 °C). Mobile phases consisted of water–dioxane mixtures the dioxane concentrations being 52, 55, 57, 60, 62, and 65% (v/v). The flow rate was 0.10 ml/min, barbiturates were detected at 240 nm. Three parallel measurements were carried out for each solute and the retention time, theoretical plate number and asymmetry factor were calculated. The dead volume of the column was determined by three parallel injections of 0.1% aqueous NaNO₂.

SRA has been employed for the elucidation of the simultaneous effect of dioxane concentration and molecular parameters on the retention characteristics. In the traditional multivariate regression analysis, the presence of the independent variables that exert no significant influence on the dependent variable lessens the significance level of the independent variables that significantly influence the dependent variable. To overcome this difficulty SRA automatically eliminates from the selected equation the insignificant independent variables increasing in this manner the information power of the calculation. SRA has been carried out three times. The dependent variables were separately the capacity factor, theoretical plate number, and asymmetry factor of 22 barbituric acid derivatives measured at six different concentrations of dioxane (altogether 132 observations). The independent variables were in each instance the actual concentration of dioxane and the following physicochemical parameters of solute molecules: π , Hansch–Fujita's substituent constants 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 Luton's electronic parameters characterizing the inductive and resonance effects; σ , Hammett's constants characterizing the electron-withdrawing power of the substituents; E_s , Taft's constant characterizing the steric effects of substituents; and B_1 and B_4 , Sterimol width parameters determined by distance of substituents at their maximum point perpendicular to attachment. Except dioxane concentration independent variables were calculated according to the additivity rule from the fragmental constants. The number of accepted independent variables was not limited and the acceptance limit was set to the 95% significance level. The inclusion of the dioxane concentration into the set of physicochemical parameters of barbituric acid derivatives was motivated by the following considerations: it has been previously demonstrated that barbiturates show regular retention behavior on PGC column, their retention decreases monotonously with increasing concentration of the organic modifier in the mobile phase. Furthermore, it can be assumed that the retention of these analytes depends not only on the dioxane concentration but also on their physicochemical parameters. Therefore, their common inclusion in the calculation as independent variables is justified. This procedure makes possible the performance of QSRS computation in one step taking into consideration simultaneously the effect

Table 1
Chemical structure of barbituric acid derivatives



Compound no.	R ₁	R ₂	X
1	H	H	O
2	Methyl	Methyl	O
3	3-Pentyl	Methyl	O
4	Methyl	1-Methylpentyl	O
5	Ethyl	Ethyl	O
6	Ethyl	1-Methylbutyl	O
7	Ethyl	3-Methylbutyl	O
8	Ethyl	1-Methylpropyl	O
9	Ethyl	<i>n</i> -Pentyl	O
10	Butyl	1-Methylpropyl	O
11	Butyl	1-Methylbutyl	O
12	Butyl	3-Methylbutyl	O
13	Ethyl	<i>n</i> -Octyl	O
14	Ethyl	3-Dimethyloctyl	O
15	Allyl	<i>i</i> -Propyl	O
16	Allyl	<i>i</i> -Butyl	O
17	Allyl	1-Methylbutyl	O
18	Allyl	1-Methylcyclohexenyl	O
19	Allyl	2-Cyclopentyl	O
20	Ethyl	1-Cyclohexenyl	O
21	Ethyl	Ethyl	S
22	Ethyl	1-Methylbutyl	S

General structure R₃ = R₄ = H.

of organic modifier and physicochemical parameters. Moreover, this method allows the evaluation and comparison of the relative impact of organic modifier and solute parameters on the retention.

As QSRS generally concentrates on the retention time and derivated parameters, the relationships between other retention characteristics such as theoretical plate number and asymmetry factor, and solute parameters are computed in the majority of cases. It is somewhat surprising because it is well known that these characteristics also depends on the type of solute molecule and may influence the efficacy of the separation.

Software for SRA was purchased from Compudrug Ltd. (Budapest, Hungary).

3. Results and discussion

Retention time of each solute decreased monotonously with increasing concentration of dioxane in the mobile phase proving again their regular retention. The high differences among the capacity factors suggested that barbituric acid derivatives can be well separated on narrow-bore PGC column. Interestingly, both the theoretical plate numbers and asymmetry factors showed marked variations among the solutes (data not shown). This finding indicates that even these retention parameters may depend on the type of solute, therefore, their inclusion in QSRS calculations is justified.

SRA found significant relationships between the retention characteristics and physicochemical parameters of barbiturates in each instance. The parameters of the equations are compiled in Table 2. The significance level was over 99% in all cases illustrating that these physicochemical parameters have a marked impact on the chromatographic characteristics (compare $F_{\text{calc.}}$ values with tabulated ones). However, the ratios of variance explained (see r^2 (%) values) were relatively low. This result draws the attention to the fact that other physicochemical parameters not included in the calculation may also exert a considerable influence on the retention behavior of this set of solutes. SRA selected more than one independent variables in each instance. As it has been expected the capacity factor decreased linearly with increasing concentration of dioxane (Eq. (1) b_1 value) proving again the regular retention behavior of solutes. The molecular hydrophobicity (π), polarity (H-Ac, H-Do), and sterical parameters (M_{RE} , B_4) influenced equally the retention. This result demonstrates that mainly hydrophobic, electrostatic, and sterical interactions account for the strength of retention of solutes.

The involvement of more than one type of binding forces in the determination of retention strength implies the mixed character of retention mechanism. It can be assumed that solutes bind to the apolar surface of PGC by hydrophobic forces.

However, the sterical correspondence between the hexagonal structures on the PGC surface and the heterocyclic

Table 2

Parameters of significant relationships between the retention characteristics and physicochemical parameters of barbiturates

Parameter	Equation (1)	Equation (2)	Equation (3)
a	5.89	1.69×10^4	1.28
b_1	-0.08	1.16×10^5	2.40
s_{b_1}	0.02	3.31×10^4	0.64
b_2	5.49	-5.15×10^3	-1.03
s_{b_2}	0.79	2.01×10^3	0.32
b_3	-2.72	-	-2.51
s_{b_3}	0.62	-	0.46
b_4	-2.08	-	-
s_{b_4}	0.61	-	-
b_5	-0.48	-	-
s_{b_5}	0.07	-	-
b_6	-0.33	-	-
s_{b_6}	0.12	-	-
b'_1 (%)	3.02	57.81	29.10
b'_2 (%)	46.43	42.19	26.08
b'_3 (%)	3.60	-	44.82
b'_4 (%)	2.75	-	-
b'_5 (%)	37.82	-	-
b'_6 (%)	6.38	-	-
r^2 (%)	42.92	8.92	34.51
$F_{\text{calc.}}$	15.66	6.32	22.48
$F_{99\%}$	2.95	4.78	3.94

Results of stepwise regression analysis. For symbols see Section 2. ($n = 132$; C is the concentration of dioxane in the mobile phase, vol.%).

$$k = a + b_1C + b_2\pi + b_3\text{H-Ac} + b_4\text{H-Do} + b_5M_{\text{RE}} + b_6B_4, \quad (1)$$

$$N = a + b_1\sigma + b_2E_s, \quad (2)$$

$$\text{AF} = a + b_1F + b_2R + b_3\sigma. \quad (3)$$

ring of barbiturates (stacking interactions) influences the retention. The negative effect of polarity parameters on the retention can be explained by the supposition that the components of the mobile phase can interact with the solutes via electrostatic forces modifying this manner their distribution between the stationary and mobile phase. The normalized slope values (path coefficients b'_i %) indicate that the hydrophobicity and molecular refraction have the highest impact on the capacity factor while the effect of other independent variables is negligible.

Dioxane concentration did not influence significantly the theoretical plate number and the asymmetry factor indicating that under these chromatographic conditions the impact of the concentration of organic modifier is negligible. Similarly to the capacity factor, the theoretical plate number also depended significantly on the electrostatic and sterical parameters of barbiturates the ratio of variance explained being very low.

Asymmetry factor depended exclusively on the electrostatic parameters. This finding demonstrates that barbiturates containing more polar substituents can be eluted with irregular peak shape.

4. Conclusions

It can be concluded from the results that SRA can be successfully used for the elucidation of the relationships

between chromatographic characteristics of barbituric acid derivatives on a narrow-bore PGC column and their physico-chemical parameters. The results indicated mixed retention mechanism with the involvement of hydrophobic, hydrophilic interactive forces and sterical correspondence between the solutes and the surface of the stationary phase.

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