



ELSEVIER

Journal of Chromatography B, 770 (2002) 227–236

JOURNAL OF
CHROMATOGRAPHY B

www.elsevier.com/locate/chromb

Determination of the retention behavior of barbituric acid derivatives in reversed-phase high-performance liquid chromatography by using quantitative structure–retention relationships

Annamaria Jakab^{a,*}, Gábor Schubert^b, Miklos Prodan^a, Esther Forgács^a

^a*Institute of Chemistry, Chemical Research Center, Hungarian Academy of Sciences, P.O. Box 17, 1525 Budapest, Hungary*

^b*Institute of Isotope and Surface Chemistry, Chemical Research Center, Hungarian Academy of Sciences, P.O. Box 77, 1525 Budapest, Hungary*

Abstract

Retention parameters of 45 barbituric acid derivatives were determined on an amide embedded RP silica column using non-buffered water–dioxan eluent systems. Linear correlations were calculated between the logarithm of the capacity factor and the dioxan concentration in the eluent. Six different retention parameters of each barbituric acid derivative were correlated with different conventional and quantum chemical structural descriptors using quantitative structure–retention relationship (QSRR). The different parameters were: intercept ($\log k_0$) and slope (b) values of the linear, the combined retention parameter ($\log k_0/b$), asymmetry factor (AF_s) and theoretical plate values (N_{USP} and N_{JP} , according to the United States and Japanese Pharmacopoeia calculations). Stepwise regression analysis (SRA) and principal component analysis (PCA) followed by two-dimensional nonlinear mapping were used to determine the retention behavior of barbituric acid derivatives. SRA and PCA led to similar results. The results indicated that the retention of barbituric acid derivatives are mainly governed by the polaric and steric parameters of the substituents. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Retention behaviour; Derivatization, LC; Quantitative structure–retention relationship, QSRR; Barbituric acid

1. Introduction

The application of silica or silica-based supports in reversed-phase high-performance liquid chromatography (RP-HPLC) is limited by the pH sensitivity [1] and by the undesirable electrostatic interactions between the polar substituents of solutes and the free silanol groups. Using other than silica support such

as polymer-coated alumina, titania or zirconia [2,3], porous graphitic carbon [4] and various polymer-based supports [5] could be one solution. Such supports are still not generally used owing to their price and uncleared retention mechanisms. Another solution consists of using modified silica-based supports such as end-capped [6,7], introduction of bulkier substituents on the silica atom of the silanol reagent in the place of methyl groups [8,9], use of bidentate ligands [10] or mixed trifunctional silanes [11]. Another possibility is addition of buffers or various additives to the eluent to mask the effect of the silanol groups [12,13].

*Corresponding author.

E-mail address: janna@chemres.hu (A. Jakab).

A totally different approach to minimize the effect of residual silanol groups is to generate a functionality on the modified reversed-phase silica surface [14]. Internal polar groups [15] such as amide [16] or carbamate [17] groups can react with the residual silica silanols through electrostatic and/or hydrogen bonding interaction, resulting in a weakening of the interaction between the polar analytes and the silanol groups. Columns with embedded amide groups were first introduced by Supelco who recently produced a version of the original phase based on pure octadecyl-coated silica [18]. Such amide embedded columns have an excellent resolution of polar compounds and different elution profiles compared to C_{18} phases.

Elucidating the complex retention mechanisms in liquid chromatography, quantitative structure–retention relationship (QSRR) has been widely used since the end of the 1980s [19]. QSRR is based on the correlation between the chromatographic parameters and the different descriptors of analytes. For understanding the retention mechanisms in HPLC, three main types of methods have been established. In the beginning (i) correlating the logarithms of the capacity factor extrapolated to 100% water concentration ($\log k_0$) with the logarithms of *n*-octanol–water partition coefficients ($\log P$) has been generally applied [20–22]. Further on (ii) correlating the $\log k_0$ with different conventional structural descriptors has also been reported [23,24]. Conventional structural descriptors were used to describe the different characteristics of the molecules by Hanch and Leo [25], Abraham et al. [26], and Corr et al. [27]. Recently (iii) correlating the $\log k_0$ values with quantum chemical descriptors have been extensively applied [28,29]. In the recent studies quantum chemical descriptors have been employed alone or in combination with the above mentioned descriptors [30–34]. Computational chemistry has assisted the routine development of molecular quantum mechanical calculations. Using quantum mechanical calculations many of the electronic and geometric properties of the molecules can be expressed and new molecular descriptors have been obtained [29]. The basic weakness of quantum chemical calculations is to relate to vacuum and not include the bulk effect, that means the interactions between the solvent and analyte are not included in such calculations. In some of the new quantum chemical methods molecu-

lar descriptors have been calculated in water, but not in commonly used reversed-phase solvents.

The aim of our work was to determine the retention behavior of barbituric acid derivatives on an amide embedded RP silica column in dioxan–water eluent systems and to elucidate the relationship between the retention characteristics and different structural descriptors using stepwise regression analysis (SRA) and principal component analysis (PCA) followed by two-dimensional nonlinear mapping techniques.

2. Experimental

RP silica column with embedded amide groups (Discovery RP-AmideC16, 125×4.6 mm I.D., particle size 5 μm) was received as a present from Supelco (Bellafonte, USA). The HPLC analyses were carried out using a Shimadzu system consisting of high pressure gradient system (LC10-AD, FCV-10AL), autoinjector (SIL-10AD), on-line membrane degasser (DGU-14A), column oven (CTO-10AS) and a photodiode array detector (SPD-M10A). The flow-rate was 0.8 ml min^{-1} . Column oven was set to 25 °C and the detection wavelength was 240 nm. Mixtures of non-buffered dioxan–water were used as eluents, dioxan concentration ranged from 50 to 70 v/v% (minimum at five different concentrations). The shape of the peaks was sufficient for not buffering the eluent. The barbituric acid derivatives (Table 1) were dissolved in dioxan at the concentration of 0.1 mg ml^{-1} .

The capacity factor (or retention factor, *k*), asymmetric factor (AF_5) and the theoretical plate values (N_{USP} , N_{JP}) of each compound in each eluent were determined in triplicate. The capacity factor *k* is a thermodynamic parameter of the system, related to the distribution coefficient through the phase ratio, while *N* (theoretical plate) is a kinetic parameter as a numerical expression of the column efficiency. The capacity factor, asymmetry factors (AF_5) and theoretical plate values (N_{USP} , N_{JP}) were calculated according to Eqs. (1)–(3), respectively

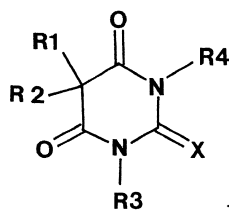
$$AF_5 = \frac{W_{0.05}}{2a_{0.05}} \quad (1)$$

$$N_{\text{USP}} = 16 \left(\frac{t_R}{W} \right)^2 \quad (2)$$

Table 1
Chemical structure of barbituric acid derivatives^a

| 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 | <i>n</i> -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 | <i>n</i> -Octyl | H | H | O |
| 14 | Ethyl | 3-Dimethyloctyl | H | H | O |
| 15 | Allyl | <i>i</i> -Propyl | H | H | O |
| 16 | Allyl | <i>i</i> -Butyl | H | H | O |
| 17 | Allyl | 1-Methylbutyl | H | H | O |
| 18 | Methyl | Cyclohexenyl | Methyl | H | O |
| 19 | Allyl | Cyclopentenyl | 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 | O |
| 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 | <i>p</i> -Cl-benzoyl | H | O |
| 30 | Ethyl | Ethyl | <i>p</i> -NO ₂ -benzoyl | H | O |
| 31 | Ethyl | Phenyl | <i>p</i> -NO ₂ -benzoyl | <i>p</i> -NO ₂ -benzoyl | O |
| 32 | Ethyl | Phenyl | Phenyl | H | O |
| 33 | Ethyl | Phenyl | Benzoyl | Methyl | O |
| 34 | Ethyl | Phenyl | <i>p</i> -NH ₂ -benzoyl | Methyl | O |
| 35 | Ethyl | Phenyl | <i>o</i> -NO ₂ -benzoyl | Methyl | O |
| 36 | Ethyl | Phenyl | <i>p</i> -NO ₂ -benzoyl | Methyl | O |
| 37 | Ethyl | Phenyl | <i>m</i> -NO ₂ -benzoyl | Methyl | O |
| 38 | Ethyl | Ethyl | <i>p</i> -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 |

^a Barbituric acid derivatives were synthesized by Professor J. Bojarski (Academy of Medicine, Krakow, Poland) and co-workers



$$N_{JP} = 5.54 \left(\frac{t_R}{W_{0.5}} \right)^2 \quad (3)$$

where $W_{0.5}$, $W_{0.05}$ and W are the peak width at 50%, 5% of peak height and at the baseline, respectively. t_R and $a_{0.05}$ are the retention time and width of first half of peak at 5% peak height, respectively.

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

$$\log k = \log k_0 + bC \quad (4)$$

where $\log k$ is the logarithm of the capacity factor; C is the dioxan concentration in the eluent (vol.%). $\log k_0$ and b are constants to be determined by the least square method. The $\log k_0$ is the logarithm of the capacity factor extrapolated to zero concentration of the organic component in the mobile phase (related to molar lipophilicity) [35] and b is the change of the $\log k$ value caused by a unit change (1% vol.) in the organic mobile phase concentration (related to the specific hydrophobic surface area in contact with support) [36].

To find if the physico-chemical parameters of the barbituric acid derivatives significantly influence their retention parameters ($\log k_0$, b , AF_5 , N_{USP} , N_{JP} , $\log k_0/b$) SRA was used. $\log k_0/b$ is a chromatographic hydrophobicity index, which reflects the organic phase concentration of the mobile phase

(v/v, %) at equal molar distribution of the compound between the mobile and the stationary phase [37]. This means that at value the retention time of the compound is exactly double the dead time. The dependent variables in the SRA calculation were separately $\log k_0$, b , $\log k_0/b$ and the average value of N_{USP} , N_{JP} and AF_5 of each barbituric acid derivative and the independent variables were the structural descriptors of the barbituric acid derivatives. The acceptance level for the individual independent variables was set to 95% significance level. The list of both the conventional and quantum chemical structural descriptors considered in this study is given in Table 2. The quantum chemical descriptors of the barbiturates were calculated using the Gaussian 94 [38] software package. The calculations were carried out on the triketo forms of the barbiturates in every case. Firstly, geometry optimization was done using the AM1 semi-empirical method [39]. The starting geometries were predicted by using the usual bond length for organic molecules. The barbiturate ring was assumed to be planar. Single point energy and molar volume calculations were carried out on the optimized geometries at the Hartree–Fock/3-21G level of theory. From the results of this second series of calculations total energies (E_T), molar volumes (Vol) and dipole moments (μ) were obtained for each of the different barbiturates. Quantum chemical descriptors (E_T , Vol , μ) were correlated with the conventional structural

Table 2
List of the molecular structural descriptors used in SRA and PCA studies

| Molecular structural descriptors | |
|----------------------------------|---|
| Conventional descriptors | |
| π | Hansch–Fujita's constant characterizing hydrophobicity |
| H-Ac | Indicator variable for proton acceptor properties |
| H-Do | Indicator variable for proton and donor properties |
| M-Re | Molar refractivity |
| F | Electronic parameter characterizing the inductive effect |
| R | Electronic parameter characterizing the resonance effects |
| σ | Hammett's constant, characterizing the electron withdrawing power |
| E_s | Taft's constant, characterizing steric effects |
| B_1 and B_4 | Sterimol's width parameters determined by distance of the molecule at their maximum point perpendicular to attachment |
| Quantum chemical descriptors | |
| E_T | Total energies |
| Vol | Molar volumes |
| μ | Dipole moments |

descriptors (π , H-Ac, H-Do, M-Re, F , R , σ , Es , B_1 , B_4) using SRA.

PCA was used to find the similarities and dissimilarities between the chromatographic parameters and the different descriptors of barbituric acid derivatives. The $\log k_0$, b , AF_5 , N_{USP} , N_{JP} , $\log k_0/b$ values together with the molecular descriptors were considered as variables (19 different) and the barbituric acid derivatives were the observations. PCA reduces the dimensionality of the original data matrices, but the resulting matrices of PC loadings and variables are sometimes even multidimensional. The dimensions of the matrices of PC loadings and variables can be reduced too by using a nonlinear mapping technique. The two-dimensional nonlinear maps of principal component (PC) loadings and variables were calculated from the PC loadings and variables, respectively.

3. Results and discussion

The slope (b) and the intercept ($\log k_0$) values of the barbituric acid derivatives were found different in most cases, indicating the appropriate separation of the barbituric acid derivatives on the Discovery RP-AmideC16 column in dioxan–water eluent system (Table 3). The standard deviations are low in each instance indicating the good reproducibility of the retention time and the stability of the amide embedded RP silica column. The correlation coefficients are above 0.9900 in most cases (Table 3) confirming the applicability of Eq. (4). The lists of the averaged AF_5 , N_{USP} and N_{JP} values for each barbituric acid derivative are shown in Table 3. Most of the barbituric acid derivatives showed a symmetric peak in dioxan–water eluent systems. The AF_5 values are around 1.0 in most cases (Table 3). Comparing the N_{USP} values with the N_{JP} values it can be considered that the N_{JP} values are somewhat higher in most cases.

Stepwise regression analysis found a significant relationship between the chromatographic parameters and the structural descriptors of the barbituric acid derivatives in five cases (Table 4). SRA did not find any significant equations explaining the asymmetry factor (AF_5) using the descriptors included in this study. This finding indicates that the retention characteristics of the solutes, except the AF_5 values,

on Discovery RP-AmideC16 column, considerably depends on the physico-chemical properties of the solutes included in this calculation. Results indicating that the hydrophobicity (π) and the total energy (E_T) have great influence on the retention, and the effect of the steric, electronic and the proton acceptor or donor properties is negligible. The $\log k_0$ and b values can be explained using the hydrophobicity (π) and the total energy (E_T) parameters of the barbituric acid derivatives. The combined retention parameter ($\log k_0/b$) can be explained using only the hydrophobicity (π) parameters of the barbituric acid derivatives. The relatively high r values for the equations of the $\log k_0$, b and $\log k_0/b$ parameters indicate that hydrophobicity (π) and the total energy (E_T) parameters can be successfully used for describing the retention behavior of barbituric acid derivatives on this column. In equations of the N_{USP} parameter only the total energy parameter (E_T) is included, and in the equation of the N_{JP} the steric parameter (M-Re) is also included. The selected structural descriptors included in the equations of theoretical plate values (N_{USP} , N_{JP}) account for relatively low ratio of change of these parameters, the r values are 0.3371 and 0.3787, respectively (Table 4). These descriptors cannot explain properly the change of the theoretical plate chromatographic parameters (see the r values). This indicates that other structural descriptors (not included in the SRA calculations) may also have significant impact on the theoretical plate values.

From the SRA results it can be concluded that E_T (total energy) and the hydrophobicity (π) have major impact on the chromatographic parameter. In order to understand the meaning of E_T , the quantum chemical descriptors were correlated with the conventional descriptors used in SRA. The results are shown in Table 5. E_T can be considered as a combined descriptor, which can be described using steric (M-Re) and polaric (σ , H-Ac, H-Do) conventional descriptors. The high r value (0.961) suggests that this set of parameters can be used for describing the E_T parameter. It is well understood, that the Vol (molar volume) parameter can be described with the steric (M-Re), and the μ (dipole moment) can be described with the inductive (F) and steric (Es) parameters.

Six principal components explain the majority of variance, indicating that the 19 original variables can

Table 3

Results of linear regression between the logarithm of capacity factor and dioxan concentration (C) in the eluent ($\log k = \log k_0 + bC$), and the calculated AF_5 , N_{USP} and N_{JP} values with their standard deviations $S(AF_5)$, $S(N_{USP})$, $S(N_{JP})$, respectively. r is the correlation coefficient of Pearson product moment

| No. | $\log k_0 \times 10^2$ | $b \times 10^2$ | r | AF_5 | $S(AF_5)$ | N_{USP} | $S(N_{USP})$ | N_{JP} | $S(N_{JP})$ |
|-----|------------------------|-----------------|--------|-----------------|-----------|-----------|--------------|----------|-------------|
| 1 | | | | Not significant | | | | | |
| 2 | -29.39 | -0.51 | 0.8708 | 1.10 | 0.08 | 1885 | 1488 | 2735 | 1620 |
| 3 | 318.34 | -4.39 | 0.9974 | 1.03 | 0.06 | 1725 | 544 | 1899 | 440 |
| 4 | 242.24 | -3.59 | 0.9980 | 1.01 | 0.17 | 1811 | 1169 | 1946 | 1087 |
| 5 | 20.68 | -0.99 | 0.9909 | 1.31 | 0.16 | 1364 | 343 | 1432 | 363 |
| 6 | 203.58 | -3.21 | 0.9973 | 1.14 | 0.02 | 1706 | 964 | 1745 | 943 |
| 7 | 154.42 | -2.51 | 0.9979 | 0.93 | 0.16 | 1046 | 545 | 1266 | 341 |
| 8 | 106.61 | -1.96 | 0.9967 | 1.17 | 0.20 | 1281 | 305 | 1285 | 356 |
| 9 | 188.02 | -3.02 | 0.9977 | 0.95 | 0.18 | 1676 | 749 | 1721 | 745 |
| 10 | 285.03 | -4.16 | 0.9969 | 1.11 | 0.12 | 2387 | 2109 | 2322 | 2113 |
| 11 | 284.06 | -4.13 | 0.9978 | 1.10 | 0.08 | 1633 | 170 | 1718 | 209 |
| 12 | 137.21 | -1.95 | 0.9863 | 1.11 | 0.12 | 1269 | 641 | 1354 | 639 |
| 13 | 317.56 | -4.38 | 0.9976 | 1.04 | 0.07 | 1683 | 528 | 1818 | 455 |
| 14 | 291.59 | -4.10 | 0.9973 | 1.12 | 0.15 | 1631 | 465 | 1746 | 404 |
| 15 | 83.80 | -1.70 | 0.9959 | 1.03 | 0.17 | 2181 | 2418 | 2076 | 2240 |
| 16 | 124.41 | -2.17 | 0.9971 | 1.08 | 0.01 | 1212 | 203 | 1200 | 256 |
| 17 | | | | Not significant | | | | | |
| 18 | 120.71 | -2.11 | 0.9977 | 1.12 | 0.08 | 1372 | 339 | 1349 | 386 |
| 19 | 115.61 | -2.10 | 0.9972 | 1.20 | 0.25 | 1256 | 290 | 1238 | 313 |
| 20 | 113.23 | -2.07 | 0.9978 | 1.09 | 0.39 | 1313 | 276 | 1307 | 315 |
| 21 | 111.38 | -1.95 | 0.9932 | 1.11 | 0.07 | 1360 | 265 | 1335 | 320 |
| 22 | 254.91 | -3.59 | 0.9991 | 1.05 | 0.14 | 1644 | 347 | 1594 | 403 |
| 23 | 287.06 | -4.01 | 0.9977 | 1.11 | 0.14 | 2297 | 1432 | 2402 | 1266 |
| 24 | 230.66 | -3.51 | 0.9975 | 1.14 | 0.13 | 1967 | 988 | 2037 | 974 |
| 25 | 78.54 | -1.73 | 0.9974 | 1.12 | 0.02 | 1226 | 231 | 1242 | 299 |
| 26 | 113.01 | -2.08 | 0.9982 | 1.23 | 0.22 | 1211 | 278 | 1212 | 327 |
| 27 | 200.86 | -3.30 | 0.9961 | 1.06 | 0.09 | 1703 | 825 | 1762 | 826 |
| 28 | 287.77 | -4.23 | 0.9968 | 1.13 | 0.12 | 1771 | 888 | 1829 | 840 |
| 29 | 305.01 | -4.38 | 0.9979 | 1.14 | 0.17 | 2966 | 2882 | 3012 | 2589 |
| 30 | | | | Not significant | | | | | |
| 31 | | | | Not significant | | | | | |
| 32 | | | | Not significant | | | | | |
| 33 | 288.29 | -4.30 | 0.9971 | 1.11 | 0.11 | 1946 | 835 | 1995 | 879 |
| 34 | 217.88 | -3.72 | 0.9933 | 1.18 | 0.07 | 1612 | 730 | 1613 | 714 |
| 35 | 316.15 | -4.73 | 0.9967 | 1.03 | 0.01 | 1919 | 966 | 1968 | 1007 |
| 36 | 285.99 | -3.74 | 0.9977 | 1.01 | 0.21 | 891 | 481 | 1071 | 311 |
| 37 | 196.60 | -3.05 | 0.9977 | 1.06 | 0.02 | 2020 | 826 | 2057 | 842 |
| 38 | 171.78 | -2.48 | 0.9971 | 1.14 | 0.13 | 1662 | 433 | 1683 | 431 |
| 39 | 227.51 | -3.52 | 0.9971 | 1.03 | 0.04 | 1884 | 900 | 1937 | 913 |
| 40 | 81.09 | -2.10 | 0.9725 | 1.22 | 0.09 | 1394 | 735 | 1491 | 909 |
| 41 | 38.64 | -1.34 | 0.9894 | 0.97 | 0.45 | 1676 | 347 | 1757 | 383 |
| 42 | 275.99 | -4.42 | 0.9889 | 1.38 | 0.26 | 1407 | 1142 | 1503 | 1123 |
| 43 | -18.46 | -0.55 | 0.9937 | 1.12 | 0.06 | 1311 | 310 | 1370 | 351 |
| 44 | 32.88 | -1.14 | 0.9975 | 1.26 | 0.05 | 1223 | 214 | 1255 | 273 |
| 45 | -24.24 | -0.43 | 0.9950 | 1.08 | 0.05 | 1353 | 327 | 1437 | 382 |

be substituted by six background variables with only 7.98% loss of information (Table 6). These six theoretical variables are sufficient to describe the relationship between the structural descriptors and

the chromatographic parameters of barbituric acid derivatives on this column. Unfortunately PCA does not prove existence of such background variables as concrete physico-chemical entities, only indicates

Table 4

Relationship between the structural descriptors of barbituric acid derivatives and their retention behavior. Results of stepwise regression analysis

| y ^a | Parameters ^b | | | | | | | | | |
|-----------------------|-------------------------|-----------------------|----------------|--------------------|----------------------|----------------|--------------------|--------|-------------------|----|
| | a | b ₁ | x ₁ | b ₁ (%) | b ₂ | x ₂ | b ₂ (%) | r | F _{calc} | n |
| log k ₀ | -1.725 | 0.579 | π | 63.8 | -0.001 | E _T | 36.2 | 0.7703 | 8.3 | 40 |
| b | 0.012 | -6 × 10 ⁻³ | π | 59.5 | 2 × 10 ⁻⁵ | E _T | 40.5 | 0.7560 | 8.3 | 40 |
| log k ₀ /b | -7.386 | -11.43 | π | 100 | - | - | - | 0.7052 | - | 40 |
| N _{USP} | 1105 | -0.556 | E _T | 100 | - | - | - | 0.3371 | - | 40 |
| N _{JP} | 1011 | -1.426 | E _T | 57.7 | -13.55 | M-Re | 42.3 | 0.3787 | 8.3 | 40 |

$$^a y = a + b_1 x_1 + b_2 x_2.$$

^b a, Intercept; b₁, b₂, b₃, regression coefficients; b₁ (%), b₂ (%), b₃ (%), path coefficients (dimensionless numbers indicating the relative impact of the individual independent variables on the dependent variable); r, coefficient of determination (indicates the ratio of variance explained by the independent variables); F, calculated value of the Fisher significance test; n, number of the barbituric acid derivatives were included in calculation.

their mathematical possibility. Almost every parameter has high loading in the first PC, indicating that these parameters have significant influence on the chromatographic parameters. Chromatographic parameters together with the hydrophobicity parameter (π) have high loadings in the second PC indicating the marked influence of these physico-chemical parameters on the retention. The fact that both the chromatographic parameters and the different structural descriptors have high loadings in more than one PC indicates that the relationship between the chromatographic behavior and descriptors is probably complex. It can be considered, that the retention mechanism can be explained with more than just the hydrophobic interaction.

Two-dimensional nonlinear map calculated from the original PC loadings is shown in Fig. 1. The log k₀ value together with N_{USP} and N_{JP} values form cluster with the hydrophobicity (π) and H-Do polaric

parameters, and this cluster is far away from the b and the log k₀/b values on the map.

Few clusters can be seen on the two-dimensional nonlinear maps calculated from the PC variables (Fig. 2). Barbituric acid derivatives substituted with nitro-benzoyl or amino-benzoyl groups (compounds 34–38), substituted with benzoyl group on the R₃ position and substituted with small groups (compounds 2, 5, 43–45) form separate clusters. The cluster formation suggests that the structure of the substituents on the barbituric acid ring of the solutes somewhat influences the retention behavior, probably having different interactions with the support.

Both results obtained from the SRA and PCA calculations indicate that the retention mechanism is complex and does not depend only on the hydrophobicity (π). This finding suggests that the retention mechanism of barbituric acid derivatives on Discovery RP-AmideC16 column is different from that of

Table 5

Relationship between the quantum chemical descriptors and the conventional structural descriptors. Results of stepwise regression analysis

| y ^a | Parameters ^b | | | | | | | | | | | | | | | |
|----------------|-------------------------|----------------|----------------|--------------------|----------------|----------------|--------------------|----------------|----------------|--------------------|----------------|----------------|--------------------|-------|-------------------|----|
| | a | b ₁ | x ₁ | b ₁ (%) | b ₂ | x ₂ | b ₂ (%) | b ₃ | x ₃ | b ₃ (%) | b ₄ | x ₄ | b ₄ (%) | r | F _{calc} | n |
| E _T | 524.5 | -9.09 | M-Re | 36.5 | -449.2 | σ | 35.4 | 119.0 | H-Ac | 19.9 | -126.9 | H-Do | 9.5 | 0.961 | 5.9 | 40 |
| Vol | 85.3 | 2.15 | M-Re | 100 | - | - | - | - | - | - | - | - | - | 0.895 | - | 40 |
| μ | 0.727 | -0.255 | Es | 53.7 | 2.032 | F | 46.3 | - | - | - | - | - | - | 0.871 | 8.3 | 40 |

$$^a y = a + b_1 x_1 + b_2 x_2 + b_3 x_3 + b_4 x_4.$$

^b a, Intercept; b₁, b₂, b₃, b₄, regression coefficients; b₁ (%), b₂ (%), b₃ (%), b₄ (%), path coefficients (dimensionless numbers indicating the relative impact of the individual independent variables on the dependent variable); r, coefficient of determination (indicates the ratio of variance explained by the independent variables); F, calculated value of the Fisher significance test; n, number of the barbituric acid derivatives were included in the calculation.

Table 6

Similarities and dissimilarities between the molecular descriptors and retention characteristics of barbituric acid derivatives on Discovery RPAmideC16 column in dioxan–water eluent. Results of principal component analysis calculated from the correlation matrix

| No. of principal component | Eigenvalue | Variance explained (%) | Total variance explained (%) |
|----------------------------|------------|------------------------|------------------------------|
| 1 | 9.679 | 50.94 | 50.94 |
| 2 | 2.788 | 14.67 | 65.61 |
| 3 | 1.936 | 10.19 | 75.81 |
| 4 | 1.275 | 6.71 | 82.52 |
| 5 | 0.980 | 5.16 | 87.68 |
| 6 | 0.826 | 4.35 | 92.02 |

Principal component loadings

| Parameter | No. of principal component | | | | | |
|--------------|----------------------------|---------------|--------------|--------------|--------------|--------------|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| $\log k_0$ | 0.662 | -0.643 | -0.010 | -0.099 | -0.081 | -0.177 |
| b | -0.680 | 0.616 | -0.016 | 0.098 | 0.020 | 0.191 |
| AF_5 | -0.037 | 0.137 | -0.321 | 0.373 | 0.699 | -0.489 |
| N_{USP} | 0.363 | -0.410 | 0.773 | -0.004 | 0.127 | -0.135 |
| N_{JP} | 0.296 | -0.288 | 0.874 | -0.053 | 0.149 | -0.065 |
| $\log k_0/b$ | -0.488 | 0.525 | 0.457 | -0.032 | 0.161 | 0.201 |
| π | 0.526 | -0.673 | -0.356 | -0.043 | -0.001 | 0.086 |
| H-Ac | 0.867 | 0.409 | 0.054 | -0.070 | 0.070 | 0.053 |
| H-Do | 0.163 | -0.399 | 0.067 | 0.618 | 0.267 | 0.579 |
| M-Re | 0.961 | 0.037 | -0.188 | -0.052 | 0.072 | 0.037 |
| F | 0.828 | 0.478 | 0.136 | 0.010 | -0.129 | -0.117 |
| R | 0.258 | -0.041 | 0.057 | 0.786 | -0.482 | -0.146 |
| σ | 0.819 | 0.454 | 0.132 | 0.102 | -0.203 | -0.149 |
| E_S | -0.935 | -0.246 | 0.049 | -0.025 | -0.094 | -0.074 |
| B_1 | 0.925 | 0.092 | -0.024 | -0.013 | 0.133 | 0.224 |
| B_4 | 0.947 | -0.047 | -0.120 | -0.132 | 0.110 | 0.108 |
| E_T | -0.947 | -0.075 | -0.033 | -0.154 | 0.054 | 0.060 |
| Vol | 0.866 | -0.049 | -0.178 | -0.220 | -0.024 | 0.122 |
| M | 0.803 | 0.391 | 0.057 | 0.052 | 0.080 | 0.012 |

the traditional ODS (octadecyl silica) support where the hydrophobic forces between the apolar octadecyl alkyl chains and the hydrophobic substructures of the solutes govern the retention. The retention behavior of barbituric acid derivatives on different columns have been recently studied in the literature [4,24,40–43], but the different results can only be compared if the same set of barbituric acid derivatives are included in the calculations. The retention mechanism of PGC (porous) column was studied with the same set of barbituric acid derivatives [4,24,41–43]. As the support of the two columns (PGC, Discovery RP-AmideC16) is totally different, one is graphitized carbon- and the other is RP silica-based, it is well-understood that the retention mechanism of the two columns obtained from the statistical calculations is different. The retention mechanism of barbituric acid

derivatives on PGC column is mainly governed by the electronic parameter of the substituents, while on the amide embedded RP silica column the effect of the electronic parameter on the retention is not significant.

4. Conclusions

It can be concluded that barbituric acid derivatives can be well separated on an amide embedded RP silica column without buffering the system using a dioxan–water eluent. Different statistical methods are suitable to elucidate the retention mechanism. Stepwise regression analysis (SRA) and principal component analysis (PCA) found the same results, that barbituric acid derivatives have a complex

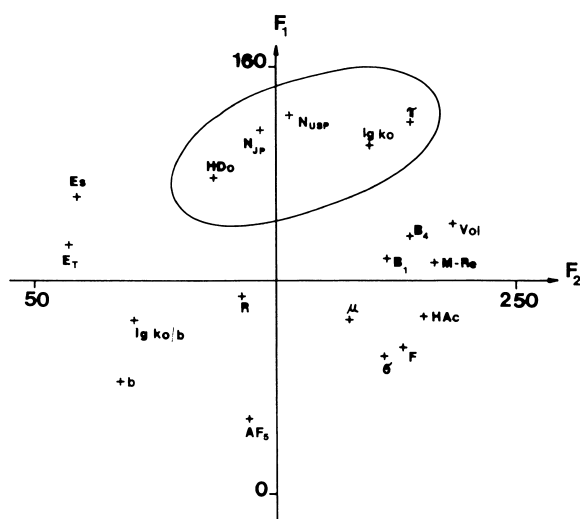


Fig. 1. Relationship between the structural descriptors of barbituric acid derivatives and their retention behavior. Two-dimensional nonlinear map calculated from the original PC loadings (number of iterations: 189, maximum error: 4.74×10^{-2}). For symbols see Table 1 and the Experimental section.

retention mechanism on this column, because more than one property of the analytes influences the retention behavior. In this case the hydrophobicity

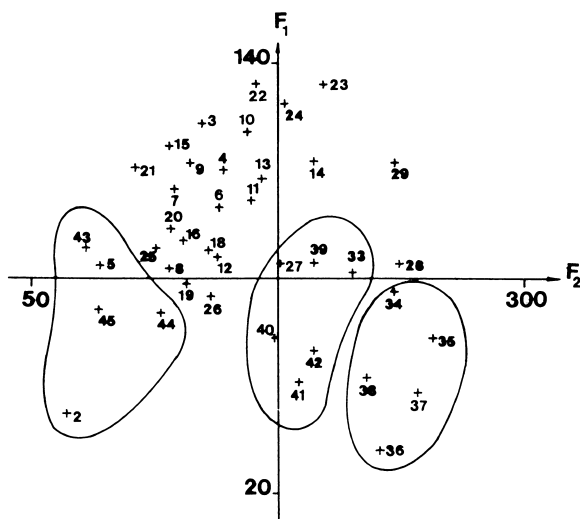


Fig. 2. Distribution of barbituric acid derivatives according to their retention behavior. Two-dimensional nonlinear map of principal component variables calculated from the original correlation matrices (number of iterations: 111, maximum error: 2.48×10^{-2}). For symbols see Table 1.

and the steric and polaric parameters of the substituents have a major influence on the retention. This finding suggests that the retention mechanism of barbituric acid derivatives on Discovery RP-AmideC16 column is different from that of the traditional ODS (octadecyl silica) support.

References

- [1] G.B. Cox, *J. Chromatogr.* 656 (1993) 353.
- [2] J.J. Sun, J.S. Fritz, *J. Chromatogr.* 522 (1990) 95.
- [3] J.R. Garbow, J. Asrar, C.J. Hardiman, *Chem. Mater.* 5 (1993) 869.
- [4] E. Forgács, T. Cserhádi, *Anal. Lett.* 32 (1999) 1867.
- [5] J. Ng, D. Froom, *Can. Chem. News* 50 (1998) 24.
- [6] L.C. Sanders, S.A. Wise, *CRC Crit. Rev. Anal. Chem.* 18 (1987) 299.
- [7] H.H. Freiser, M.P. Nowlan, D.L. Gooding, *J. Liq. Chromatogr.* 12 (1989) 827.
- [8] R. Zhang, Z. Xie, R. Zhao, X. Li, G. Liu, M. Aguilar, M.T.W. Hearn, *Anal. Chem.* 63 (1991) 1861.
- [9] J.J. Kirkland, J.W. Henderson, *J. Chromatogr. Sci* 32 (1994) 473.
- [10] R.K. Gilpin, M.E. Gangoda, *J. Chromatogr. Sci.* 28 (1990) 277.
- [11] M.J. Wirth, H.O. Fatunmbi, *Anal. Chem.* 64 (1992) 2783.
- [12] S.O. Akapo, C.F. Simpson, *Anal. Proc.* 26 (1989) 394.
- [13] M.A. Stadalius, J.S. Berus, L.R. Snyder, *LC-GC* 6 (1988) 494.
- [14] H. Engelhardt, R. Grüner, M. Scherer, *Chromatographia* 53 (2001) 154.
- [15] D.V. McCalley, *J. Chromatogr. A.* 844 (1999) 23.
- [16] T.L. Ascach, K.M.R. Kallbury, C.A. Szafranski, S.D. Corman, F. Liu, *J. Liq. Chromatogr. Relat. Technol.* 19 (1996) 3049.
- [17] J.E. O'Gara, B.A. Alden, T.H. Walter, J.S. Peterson, C.L. Niederlander, U.D. Neue, *Anal. Chem.* 67 (1995) 3809.
- [18] P. Shieh, N. Cooke, R. Gant, R. Eksteen, *Am. Lab.* 30 (1998) 66.
- [19] R. Kaliszan, *Quantitative Structure–Chromatographic Relationships*, John Wiley, Chichester, 1987.
- [20] M.H. Abraham, H.S. Chadha, R.A.E. Leitao, R.C. Mitchell, W.J. Lambert, R. Kaliszan, A. Nasal, P. Habel, *J. Chromatogr. A* 766 (1997) 35.
- [21] B.J. Herbert, J.G. Dorsey, *Anal. Chem.* 67 (1995) 744.
- [22] Z. Hanfa, Z. Yukui, H. Mingfang, L. Peizhang, *Prog. Nat. Sci.* 5 (1995) 106.
- [23] E. Forgács, T. Cserhádi, *J. Chromatogr. A* 668 (1994) 395.
- [24] E. Forgács, T. Cserhádi, *J. Pharm. Biomed. Anal.* 10 (1992) 861.
- [25] C. Hansch, A. Leo, *Substituent Constants for Correlation Analysis in Chemistry and Biology*, John Wiley, Chichester, 1979.
- [26] M.H. Abraham, A. Nasal, P. Haber, R. Kaliszan, F. Forgács, T. Cserhádi, *J. Liq. Chromatogr. Relat. Technol.* 21 (16) (1998) 2523.

- [27] J. Galvez, R. Garcia-Domenech, M.T. Salabert, R. Soler, J. Chem. Inf. Comput. Sci. 34 (1994) 520.
- [28] H. Toshihiko, H. Hiroyuki, N. Noriyuki, K. Toshio, J. Liq. Chromatogr. 16 (1993) 1453.
- [29] M. Karelson, V.S. Lobanov, A.R. Katritzky, Chem. Rev. 96 (1996) 1027.
- [30] K. Azzaoui, L. Morin-Allory, Chromatographia 40 (1995) 690.
- [31] M.L.C. Montanari, Q.B. Cass, C.A. Montanari, Chromatographia 51 (2000) 723.
- [32] M.P. Montana, N.B. Pappano, N.B. Debattista, J. Raba, J.M. Luco, Chromatographia 51 (2000) 727.
- [33] M.C. Hennion, V. Coquart, S. Guenu, C. Sella, J. Chromatogr. A 712 (1995) 287.
- [34] K. Azzaoui, L. Morin-Allory, Chromatographia 42 (2000) 389.
- [35] K. Valkó, S. Olajos, T. Cserhádi, J. Chromatogr. 499 (1990) 361.
- [36] C. Horváth, W. Melander, J. Molnár, J. Chromatogr. 125 (1976) 129.
- [37] K. Valkó, P. Slégel, J. Chromatogr. 631 (1993) 49.
- [38] M.J. Frisch, G.W. Trucks, H.B. Schlegel et al., Gaussian 94 (Revision D.1), Gaussian Inc, Pittsburgh, PA, 1995.
- [39] M.J.S. Dewar, E.G. Zoebisch, E.F. Healy, J. Am. Chem. Soc. 107 (1985) 3902.
- [40] R.K. Waters, R.D. Jee, R.A. Watt, A.C. Moffat, Pharm. Sci. 1 (1995) 593.
- [41] E. Forgács, T. Cserhádi, Anal. Chim. Acta 348 (1997) 481.
- [42] E. Forgács, T. Cserhádi, J. Pharm. Biomed. Anal. 18 (1998) 505.
- [43] E. Forgács, T. Cserhádi, Anal. Sci. 14 (1998) 991.