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Influence of physico-chemical parameters of some barbituric acid derivatives on their retention on an amide embedded RP silica column

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Abstract

Retention parameters of 45 different barbituric acid derivatives were determined on an amide embedded RP silica column (Discovery RP-AmideC16) using non-buffered water–acetonitrile eluent systems. Linear correlation were calculated between the logarithm of the capacity factor and the acetonitrile concentration in the eluent. To determine the retention behavior of barbituric acid derivatives, stepwise regression analysis (SRA) and principal component analysis (PCA) followed by two-dimensional nonlinear and modified nonlinear mapping was used. It can be concluded, the retention of barbituric acid derivatives are governed mainly by the steric parameters of the substituents. Principal component analysis indicated that the barbituric acid derivatives have mixed retention on this amide embedded RP silica column in water–acetonitrile eluent.

Keywords: Amide embedded RP silica column; Barbituric acid derivatives; Structure–retention relationship

1. Introduction

The application of silica or silica based supports in reversed-phase high performance liquid chromatography (RP-HPLC) is limited by the low stability of silica at high pH values [1] and by the undesirable electrostatic interactions between the polar substructures of solutes and the free silanol groups not covered by the hydrophobic ligand of the support. This necessitated the search for other than silica support as alumina [2], octadecyl or polymer coated alumina [3,4], titana or polymer coated titana and zirconia [5,6], porous graphitic carbon [7] and various polymer based supports [8]. Such supports are still not generally used owing to their price and uncleared retention mechanisms. Using silica based phases have to decrease or eliminate the effect of residual acidic silanol groups. Partial solution for problem mentioned above consist of end-capping [9,10], introduction of bulkier substituents on the silica atom of the silanol reagent in the place of methyl groups [11,12], use of bidentate ligands [13] or mixed trifunctional silanes [14], and addition of buffers or various additives to the eluent to mask the effect of the silanol groups [15,16].

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A totally different approach to minimize the effect of residual silanol groups is to generate a functionality on the modified reversed phase silica surface. Internal polar groups [17] as amide [18] or carbamate [19] groups can react with the residual silica silanols through electrostatic or hydrogen bonding interaction, resulting to weaken 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 [20]. Such amide embedded columns have an excellent resolution of polar compound and different elution profiles compared to C18 phases. There are lot of articles in the literature dealing with the elucidation of relationships between the retention parameters and the molecule structure. However, most of these articles discussing the retention mechanisms of the traditional reversed-phase and the adsorption columns. Since the amide embedded columns have been introduced for few years, to the best of our knowledge the relationships between the retention parameters of analytes on this specific stationary phase and their physicochemical parameters has not been studied in details.

The aim of this paper was to determine the retention behavior of different barbituric acid derivatives on amide embedded silica column in acetonitrile-water eluent systems using different mathematical statistical methods: stepwise regression analysis (SRA) and principal component analysis (PCA) followed by two-dimensional nonlinear and modified nonlinear mapping techniques.

Table 1
The calculated AF_5 and N values and theirs standard deviations S(AF_5), S(N), respectively

No.	AF_5	S(AF_5)	N	S(N)
1	–	–	–	–
2	1.27	0.62	421	87
3	2.14	0.29	3015	2015
4	1.33	0.25	4192	6317
5	1.52	0.36	513	78
6	1.58	0.50	906	290
7	2.20	0.65	1067	407
8	1.55	0.57	869	476
9	1.95	0.82	1071	394
10	1.15	0.26	819	216
11	1.40	0.47	1190	253
12	1.86	0.35	1370	372
13	1.98	0.51	2517	968
14	1.99	0.50	1841	605
15	1.75	0.29	831	419
16	1.82	0.43	860	333
17	–	–	–	–
18	1.93	0.47	1113	557
19	1.64	0.49	795	276
20	0.96	0.39	236	84
21	1.18	0.13	599	236
22	1.37	0.44	1246	479
23	1.74	0.29	1362	391
24	2.00	0.45	1473	643
25	1.55	0.33	714	244
26	1.65	0.54	1194	387
27	0.89	0.09	371	155
28	1.99	0.91	3415	101
29	1.94	0.58	2126	375
30	1.56	0.51	956	763
31	–	–	–	–
32	–	–	–	–
33	1.85	0.44	2566	651
34	1.28	0.43	1500	460
35	1.36	0.61	1929	457
36	1.83	0.72	2871	722
37	2.05	1.38	1676	348
38	1.93	0.34	2657	485
39	1.74	0.63	2123	595
40	1.47	0.75	1508	538
41	1.80	0.58	2457	343
42	1.68	0.36	1346	480
43	1.42	0.81	561	247
44	1.20	0.22	576	150
45	1.38	0.40	482	164

2. Experimental

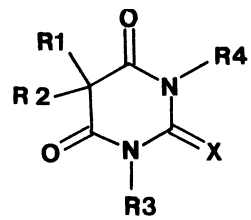
The silica column with embedded amide groups (Discovery RP-AmideC16, 125×4.6 mm I.D., particle size 5 μm) was received as a present from Supelco (Bellafonte, USA). The HPLC system consisted of a Waters LC Module I and Waters 746 integrator (Waters, Milford, MA). The flow rate was 0.8 ml min^{-1} and the detection wavelength was set to 240 nm. Mixtures of non-buffered acetonitrile-water were used as eluents, acetonitrile concentration ranged from 30 to 55 v/v (minimum at five different concentrations).

Table 2
Results of linear regression between the logarithm of capacity factor and acetonitrile concentration (C) in the eluent

No.	$\log k_0 \times 10^2$	$S(\log k_0) \times 10^2$	$b \times 10^2$	$S(b) \times 10^2$	r
1	231.27	47.66	−6.87	5.94	0.8559
2			Not significant		
3	226.77	0.56	−3.37	0.17	0.9990
4	204.17	1.36	−3.47	0.39	0.9994
5	21.10	2.79	−1.27	2.16	0.9824
6	161.28	3.07	−3.09	0.99	0.9963
7	148.71	3.45	−2.94	1.17	0.9949
8	101.14	4.03	−2.30	1.73	0.9887
9	146.19	3.48	−2.87	1.21	0.9945
10	187.75	1.40	−3.07	0.45	0.9923
11	186.29	1.18	−3.03	0.39	0.9944
12	192.62	1.50	−3.16	0.47	0.9917
13	230.74	1.00	−3.45	0.29	0.9969
14	212.54	1.30	−3.36	0.38	0.9945
15	106.95	10.53	−2.74	3.65	0.9488
16	120.36	3.15	−2.58	1.21	0.9944
17			Not significant		
18	130.96	3.09	−2.64	1.17	0.9949
19	117.09	3.52	−2.56	1.37	0.9930
20	−6.93	5.24	−0.99	4.79	0.9097
21	100.01	6.10	−2.28	2.61	0.9741
22	245.37	3.18	−4.13	0.76	0.9822
23	264.55	3.26	−4.39	0.73	0.9800
24	183.36	3.56	−3.34	1.06	0.9958
25	107.14	4.59	−2.59	1.75	0.9884
26	159.69	2.44	−3.06	0.80	0.9976
27	251.58	7.10	−4.54	1.54	0.9860
28	331.58	0.16	−4.71	0.03	1.0000
29	227.75	0.44	−3.44	0.13	0.9994
30	151.50	6.22	−3.05	2.02	0.9890
31			Not significant		
32			Not significant		
33	280.04	0.30	−4.15	0.07	0.9998
34	231.95	0.48	−3.80	0.13	0.9994
35	316.83	1.79	−4.85	0.37	0.9950
36	315.01	0.10	−4.60	0.02	1.0000
37	180.16	0.75	−2.83	0.26	0.9974
38	240.63	0.40	−3.62	0.11	0.9995
39	233.02	1.14	−3.61	0.32	0.9963
40	253.30	3.25	−4.46	0.73	0.9980
41	245.70	0.01	−3.74	0.00	1.0000
42	474.90	16.84	−8.77	1.73	0.9029
43	−15.94	1.99	−0.71	2.72	0.9718
44	29.21	2.69	−1.35	1.96	0.9854
45	−6.44	0.82	−0.75	1.09	0.9955

$\log k = \log k_0 + bC$. $S(\log k_0)$ and $S(b)$ are the standard deviations of the $\log k_0$ and b values, respectively.

Nonbuffered eluents were used because the shapes of the peaks were sufficient on this column. The experiments were carried out at room temperature (21–23 °C). The barbituric acid derivatives (Fig. 1) were dissolved in methanol at the concentration of 0.1 mg ml^{-1}. The retention time, asymmetric



No.	R1	R2	R3	R4	X
1	H	H	H	H	O
2	methyl	methyl	H	H	O
3	3-penthyl	methyl	H	H	O
4	methyl	1-methylpenthyl	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	buthyl	1-methylpropyl	H	H	O
11	buthyl	1-methylbutyl	H	H	O
12	buthyl	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	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	*p*-Cl-benzoyl	H	O
30	ethyl	ethyl	*p*-NO_2-benzoyl	H	O
31	ethyl	phenyl	*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

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

Fig. 1. Chemical structure of barbituric acid derivatives.

factor and the theoretical plate values of each compound in each eluent were determined three times.

The asymmetry factors (AF_5) were calculated according to $AF_5 = B/A$, where A and B can be measured by drawing a perpendicular line from the apex of the peak to the baseline and measure the front (A) and back (B) widths of the peak at 5% height. The number of the theoretical plates were calculated according to $N = 16(t_r/t_w)$ formula, where t_r is the retention time and t_w is the band width measured at the baseline. The N values were taken into calculation because the N values are depending on the structure of the molecules and the property of the stationary phase, because of that there are several methods and analytes used for testing the columns and calculating the N value [21].

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

$$\log k = \log\ k_0 + bc \quad (1)$$

where $\log k$ is the logarithm of the capacity factor; c is the acetonitrile 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) [22] 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 contract with support [23].

The overwhelming majority of quantitative structure–retention relationship (QSRR) studies employ linear, nonlinear, multilinear or other multivariate methods where the dependent variable is generally one selected retention parameter. This methods can be successfully used for the elucidation of the relationship between one retention parameter and any number of physico-chemical characteristics. However, these methods are unsuitable for the assessment of relationships between more than on retention parameters and physico-chemical characteristics.

To find the physico-chemical parameters of the barbituric acid derivatives significantly influence their retention parameters ($\log k_0$, b, AF_5, N, $\log k_0/b$) stepwise regression analysis (SRA) was used. In the common multivariate regression analysis the presence of independent variables which exert no significant influence on the dependent variable lessens the significancia level of those independent variables which do significantly influence the dependent variables. To overcome this difficulty the SRA automatically eliminates from the selected equation the insignificant independent variables. The physico-chemical parameters included in the calculation were: π = Hansch–Fujita's constant characterizing hydrophobicity; H-Ac and H-Do are indicator variables for proton acceptor and donor properties; M-Re is the molar

Table 3
Effect of various physico-chemical parameters of barbituric acid derivatives on their retention parameters on RP AmideC16 column

y[a]	Parameters[b]											
	a	b_1	x_1	S_{b1}	b_1 (%)	b_2	x_2	S_{b2}	b_2 (%)	r^2	F	n
$\log(k_0)$	0.51	0.06	M-Re	0.01	67.34	−0.28	B_1	0.13	32.66	0.4969	18.76	41
b	−0.035	0.006	Es	0.001	57.22	0.007	B1	0.002	42.78	0.3202	8.95	41
$\log(k_0/b)$	−14.12	10.68	B1	4.17	38.30	−8.75	B4	2.12	61.70	0.5095	19.74	41
N	−26.38	138.5	B4	30.18	–	–	–	–	–	0.3505	21.05	41
AF_5	1.10	0.13	π	0.05	–	–	–	–	–	0.1813	7.75	37

[a] Results of stepwise regression analysis: $y = a + b_1x_1 + b_2x_2$.

[b] a = intercept; b_1 and b_2 = regression coefficients; S_{b1} and S_{b2} = standard deviation of regression coefficients b_1 and b_2; b_1 (%) and b_2 (%) = path coefficients (dimensionless numbers indicating the relative impact of the individual independent variables on the dependent variable); r^2 = 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.

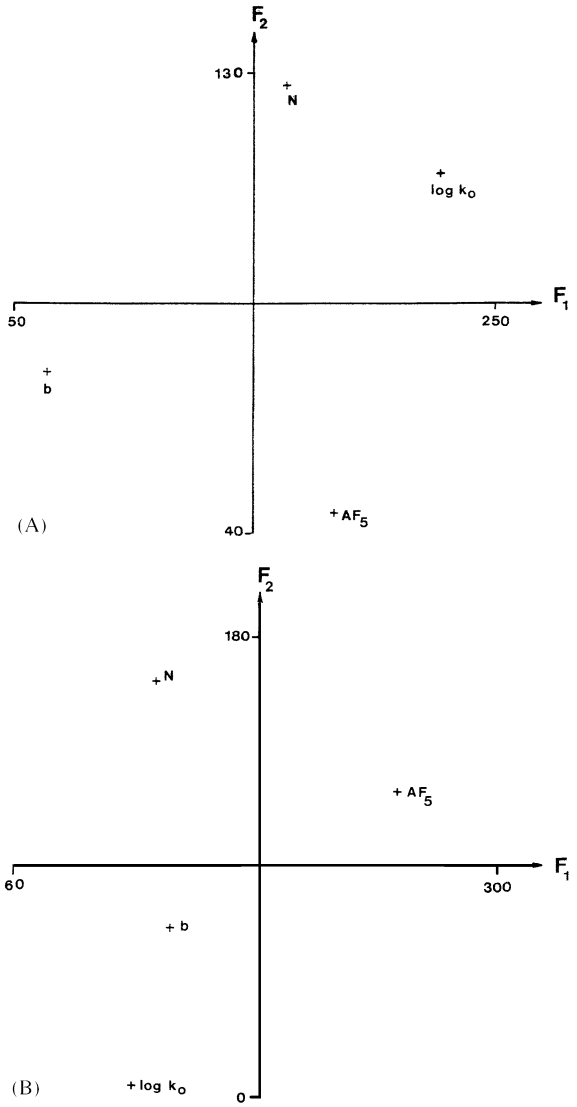


Fig. 2. Two-dimensional nonlinear map calculated from the original PC loadings (A) and from the absolute values of PC loadings (B). Number of iteration: 23 (A), 62 (B); maximum error: $4.7 \cdot 10^{-3}$ (A), $5.05 \cdot 10^{-5}$ (B). For symbols see in the text.

refractivity; F and R are the electronic parameters characterizing the inductive and resonance effects; σ = Hammett's constant, characterizing the electron withdrawing power; Es is Taft's constant, characterizing steric effects; B_1 and B_4 are the Sterimol's width parameters determined by distance of the molecule at their maximum point perpendicular to attachment. SRA was carried out five times. The dependent variables were the different retention parameters ($\log k_0$, b, AF_5, N, $\log k_0/b$) separately and the independent variables were the physico-chemical parameters of the barbituric acid derivatives. The acceptance level for the individual independent variables was set to 95% significance level.

Principal component analysis (PCA) was used to find the similarities and dissimilarities between the chromatographic parameters and peak characteristics of barbituric acid derivatives. The parameters of Eq. (1) ($\log k_0$, b), AF_5 and N values were considered as variables and the barbituric acid derivatives were the observations. The two-dimensional nonlinear maps [24] of PCA variables and loadings were also calculated. Although PCA reduces the dimensionality of the original data matrix the resulting matrixes of principal component (PC) loadings and variables are sometimes even multidimensional. As the capacity of human brain to evaluate data distributed in multidimensional space is limited, the dimensions of the matrixes of PC loadings and variables can be reduced two by nonlinear mapping technique. Both traditional nonlinear mappings take into consideration the positive and negative signs of the correlations by constructing the corresponding maps. Necessarily, the variables with strong negative correlation are far from each other on the map. Theoretically, this discrepancy can be avoided by using only the absolute values for the constructing of the map [25].

3. Results and discussion

Each barbituric acid derivative showed more or less symmetric peak in the eluent systems. The lists of the averaged AF_5 and N values for each barbituric acid derivatives are shown in Table 1. The relationship between the $\log k$ and the acetonitrile concentration was linear at each barbituric acid derivative and the correlation coefficient in most cases was above 0.99 (Table 2) proving the applicability of the Eq. (1). The slope and the intercept values of the barbituric acid derivatives differ in most cases, suggesting that the molecules

can be successfully separated on this column in water–acetonitrile eluent system. The standard deviation values are low, showing the good reproducibility.

Significant linear correlation was found between the slope (b) and the intercept ($\log k_0$) value of Eq. (1).

$$\log k_0 = -61.88b - 22.65, \quad r = 0.9143, \quad n = 41, \quad r_{99.9\%} = 0.4896 \qquad (2)$$

Eq. (2) indicates that the barbituric acid derivatives can be considered as a homogenous series of compounds.

Stepwise regression analysis found significant relationship between the chromatographic and physico-chemical parameters of the barbituric acid derivatives (Table 3). The selected structural descriptors including in the equations account for relatively low ratio of change these parameters (see r^2 values in Table 3) but the equation are significant. These descriptors can not be explained properly the change of the chromatographic parameters, this indicating that other structural descriptors not including in the SRA calculations may have not negligible impact on the $\log k_0$, $\log k_0/b$, b, N and AF_5 values. The results indicated that the steric effects have the great influence on the retention parameters and that the effect of the electronic parameters is negligible. This finding can be explained by the presence of polar interaction between the analyte molecule and the embedded amide group placed in the stationary phase. It can be established that proper size and shape of the barbituric acid molecule have the major influence for reaching to the embedded amide groups among the hydrophobic alkyl chains and for interaction with them.

Three principal components explain the majority of variance indicating that the four original variables can be substituted by three background (abstract) variables with only 0.88% loss of information. Unfortunately PCA does not prove existence of such background variables as concrete physico-chemical entities, but only indicate their mathematical possibility. The two-dimensional nonlinear maps calculated from original PC loadings are shown in Fig. 2. Maps show marked differences in the distribution of variables indicating the considerable impact of the modification of the mode of calculation. The $\log k_0$ value is far away from the b value on the map A calculated from the original PC loadings (Fig. 2A). However, the data in Table 4 clearly show the negative relationship between the $\log k_0$ and b value. This finding support our previous theoretical conclusions that the information contained in the two-dimensional nonlinear map may be misleading when both negative and positive correlation occur between the variables. The distribution of variables on the map B calculated from the absolute values (Fig. 2B) corresponds to the data in Table 4.

Table 4
Similarities and dissimilarities between chromatographic parameters and peak characteristics of barbituric acid derivatives on RP-AmidC16 column

Parameter	No. of principal component		
	1	2	3
$\log k_0$	0.95	−0.29	0.02
b	−0.87	0.38	0.28
AF_5	0.55	0.72	0.42
N	0.69	0.30	0.65
	Eigenvalue	Variance explained (%)	Total variance explained (%)
	2.43	60.81	60.81
	0.85	21.24	82.06
	0.68	17.07	99.12

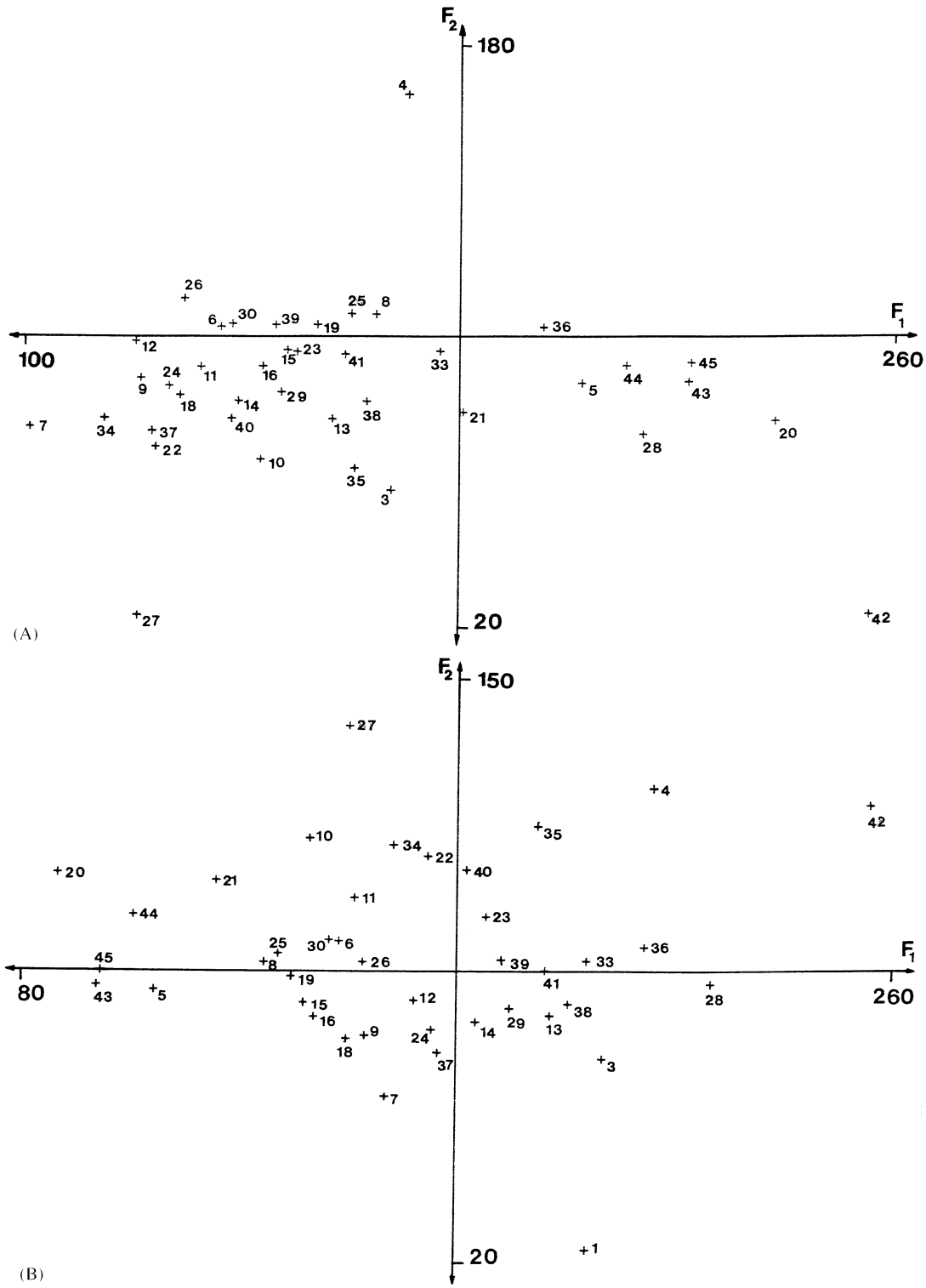


Fig. 3. Distribution of barbituric acid derivatives according to their retention behaviour on RP-AmidC16 column. Two-dimensional nonlinear map calculated from the original PC variables (A) and from the absolute values of PC variables (B). Number of iteration: 209 (A), 149 (B); maximum error: $7.55 \cdot 10^{-3}$ (A), $1.00 \cdot 10^{-3}$ (B). Numbers refer to barbituric acid derivatives are shown in Fig. 1.

Barbituric acid derivatives do not form separate clusters either to the bulkiness of the substituents or according to the hydrophobicity of the substituents. This results confirmed by the results obtained by SRA, indicating a mixed retention mechanisms of barbituric acid derivatives on Discovery RP-AmideC16 column (Fig. 3A,B). That means not only the hydrophobicity but the steric parameters of the substituents influencing on the retention behavior.

It can be concluded from our data that barbituric acid derivatives can be well separated on Discovery RP-AmideC16 column using non-buffered acetonitrile–water as eluent. Use of different mathematical statistical methods the absolute values of principal component loadings for the calculations of two-dimensional nonlinear maps prevents the occurrence of error originated from the different signs of the variables. Stepwise regression analysis indicated that the retention parameters of barbituric acid derivatives are mainly governed by the steric parameters of the substituents. Principal component analysis followed by two-dimensional nonlinear mapping elucidated that the barbituric acid derivatives have mixed retention on Discovery RP-AmideC16 column.

References

[1] G.B. Cox, J. Chromatogr. 656 (1993) 353–367.
[2] J.A. Blackwell, Chromatographia 35 (1993) 133–138.
[3] J.J. Sun, J.S. Fritz, J. Chromatogr. 522 (1990) 95–105.
[4] J.R. Garbow, J. Asrar, C.J. Hardiman, Chem. Mater. 5 (1993) 869–875.
[5] A. Kurganov, U. Trüdinger, T. Isajeva, K. Unger, Chromatographia 42 (1996) 217–221.
[6] J. Zhao, P.W. Carr, Anal. Chem. 72 (2000) 302–309.
[7] E. Forgács, T. Cserháti, Anal. Lett. 32 (1999) 1867–1878.
[8] J. Ng, D. Froom, Can. Chem. News 50 (1998) 24–26.
[9] L.C. Sanders, S.A. Wise, CRC Crit. Rev. Anal. Chem. 18 (1987) 299–305.
[10] H.H. Freiser, M.P. Nowlan, D.L. Gooding, J. Liq. Chromatogr. 12 (1989) 827–843.
[11] R. Zhang, Z. Xie, R. Zhao, X. Li, G. Liu, M. Aguilar, M.T.W. Hearn, Anal. Chem. 63 (1991) 1861–1867.
[12] J.J. Kirkland, J.W. Henderson, J. Chromatogr. Sci. 32 (1994) 473–480.
[13] R.K. Gilpin, M.E. Gangoda, J. Chromatogr. Sci. 28 (1990) 277–285.
[14] M.J. Wirth, H.O. Fatunmbi, Anal. Chem. 64 (1992) 2783–2786.
[15] S.O. Akapo, C.F. Simpson, Anal. Proc. 26 (1989) 394–397.
[16] M.A. Stadalius, J.S. Berus, L.R. Snyder, LC–GC 6 (1988) 494–505.
[17] D.V. McCalley, J. Chromatogr. A 844 (1999) 23–38.
[18] T.L. Ascah, K.M.R. Kallbury, C.A. Szafranski, S.D. Corman, F. Liu, J. Liq. Chromatogr. Rel. Tech. 19 (1996) 3049–3073.
[19] J.E. O'Gara, B.A. Alden, T.H. Walter, J.S. Peterson, C.L. Niederlander, U.D. Neue, Anal. Chem. 67 (1995) 3809–3813.
[20] P. Shieh, N. Cooke, R. Gant, R. Eksteen, Am. Lab. 30 (1998) 66–70.
[21] H. Engelhardt, M. Jungheim, Chromatographia 29 (1990) 59–68.
[22] K. Valkó, S. Olajos, T. Cserháti, J. Chromatogr. 499 (1990) 361–371.
[23] C. Horváth, W. Melander, J. Molnár, J. Chromatogr. 125 (1976) 129–156.
[24] T. Cserháti, B. Bordás, E. Ekiert, J. Bojarski, J. Chromatogr. 287 (1984) 385–390.
[25] E. Forgács, A. Kósa, G. Csiktusnádi-Kiss, T. Cserháti, R. Kaliszan, P. Haber, A. Nasal, J. Liq. Chromatogr. Rel. Tech. 21 (1998) 2523–2534.