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# Relationship between the retention characteristics on an alumina column and physico-chemical parameters of some environmental pollutants

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## Abstract

The retention behavior of 16 environmental pollutants was studied on alumina and porous graphitized carbon (PGC) columns using *n*-hexane as eluent. The relationship between the logarithm of the capacity factors determined on the alumina column and the physico-chemical characteristics of the solutes was elucidated by principal component analysis (PCA) followed by two-dimensional nonlinear mapping. The 12 original variables can be reduced to four with only a 10% loss of information. The logarithm of the capacity factor formed a cluster with the hydrogen donor and acceptor properties of the solutes and their Taft's constant on the two-dimensional nonlinear map of PC loadings indicating that both steric and electronic parameters play a considerable role in the retention mechanism on alumina support. Alcohols, aromatics and chlorinated alkanes formed separate clusters on the two-dimensional nonlinear map of PC variables suggesting that their retention mechanism may be different on the alumina column. Solutes were not retained on the PGC surface proving that under normal-phase conditions the retention capacity of alumina can be higher than that of PGC. © 2000 Elsevier Science B.V. All rights reserved.

**Keywords:** Structure–retention relationships; Principal component analysis; Chemometrics; Porous graphitic carbon; Alumina support; Volatile organic compounds

## 1. Introduction

The overwhelming majority of separations in high-performance liquid chromatography (HPLC) are performed on silica [1] or on silica-based stationary phases [2–4]. The widespread acceptance and application of these stationary phases are due to their good separation capacity, high mechanical stability [5,6] and the relatively easy to carry out synthetic methods to modify the silica surface. Besides the advantageous chromatographic parameters, silica and

silica-based supports show marked drawbacks too. The acidic surface silanol groups interact with the alkaline sub-structures of solutes resulting in distorted peak shape and decreased column efficiency [7,8]. Moreover, silica-based supports are not stable at extreme, especially alkaline pH making the separation of highly basic compounds difficult [9]. Due to its higher isoelectric point and higher stability in the alkaline pH range, alumina partially or totally overcomes the difficulty arising from the low pH stability of silica [10]. The alumina stationary phase has been successfully applied as a normal-phase stationary phase [11]. Separation of transition metal ions [12], alkyl- and phenyl-naphthalenes [13], various aromatic compounds [14,15], proteins [16], drugs

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[17], tributylphenol ethylene oxide oligomers [18], commercial pesticides [19] and heroin derivatives [20] were previously reported.

Porous graphitized carbon (PGC) stationary phase has also been developed as an extremely inert HPLC support [21,22]. The theoretical aspects of the retention mechanism of various solutes on PGC [23] and the practical applications (peptides, pharmaceuticals, drugs, environmental pollutants, etc.) have been recently reviewed [24]. PGC has been recently employed for the separation of industrial oligoglycerols [25].

The exact knowledge of the physico-chemical parameters of solutes exerting a significant impact on the retention is of paramount importance in any field of chromatographic separation [26]. This class of information facilitates the assessment of theoretical questions, promotes the more profound understanding of the retention mechanism resulting in the rational design of any practical separation problems. The application of various multivariate mathematical statistical methods is more and more frequent in the elucidation of the relationship between solute characteristics and retention behavior [27]. Principal component analysis (PCA) can be employed for the study of structure–retention relationships [28]. PCA has been frequently used for the evaluation of large data matrices in chromatography. Thus, the retention mechanism of the PGC column using benzene derivatives as solutes and *n*-hexane mobile phase was evaluated by PCA [29,30]. PCA was further employed for the characterization of hydrophobic interaction chromatography media [31], for the determination of the origin of cinnamon [32] and for the identification of white wines according to chromatographic retention data [33].

The objectives of the study were the determination of the retention behavior of some environmental pollutants on both alumina and PGC columns and to elucidate the relationship between the physico-chemical parameters of solutes and their retention by using PCA.

## 2. Experimental

The HPLC system consisted of a ISCO Pump Model 2360 (ISCO, Lincoln, NE, USA) a Waters

991 photodiode array detector (Millipore Corp., Waters Chromatography Division, Milford, MA, USA), a NEC PowerMate SX/16 computer with PDA program and a Valco injector (Valco, Houston, TX, USA) with a 20- $\mu$ l sample loop. A column of 250 cm $\times$ 4 mm I.D. filled with alumina (particle size 5  $\mu$ m) and a PGC column (Shandon Hypercarb, 100 $\times$ 4.7 mm I.D., particle diameter 7  $\mu$ m, Shandon Scientific, UK) were used in each experiment. Eluent was *n*-hexane, the flow-rate was set to 1 and 0.7 ml/min for alumina and PGC, respectively. The detection wavelength ranged from 190 to 230 nm. The environmental pollutants are listed in Table 1. In order to check the separation capacity of the HPLC system in some cases both the analytical and technical quality pollutant was analyzed. They were dissolved in the eluent, and each determination was run in quadruplicate.

PCA was employed for the elucidation of the quantitative relationship between retention behavior and physico-chemical parameters of solutes. The physico-chemical parameters included in the calculation were:  $\pi$ =Hansch–Fujita's substituent constant characterizing hydrophobicity [34,35], H-Ac and H-Do=indicator variables for proton acceptor and

Table 1  
Capacity factors and the relative standard deviations of some environmental pollutants on an alumina HPLC column using *n*-hexane as eluent

Solute		Capacity factor	RSD (%)
No.	Name		
1	Carbon tetrachloride	0.083	0.86
2	Trichloromethane	0.578	1.41
3	Dichloromethane	0.539	0.62
4	Dichloroethane	0.183	1.16
5	Trichloroethylene	0.191	1.50
6	Benzene	0.117	1.13
7	Toluene	0.217	0.92
8	Xylene	0.217	1.48
9	Methanol	3.513	1.09
10	1-Propanol	2.487	1.10
11	1-Butanol	3.674	1.24
12	Tetrahydrofuran	0.887	1.19
13	Dioxane	0.239	1.34
14	Diethyl ether	0.235	0.53
15	Acetone	0.204	1.42
16	Ethyl acetate	3.683	1.46
17	Dimethylformamide	Strongly retained	

proton donor properties, respectively [36], M-RE= molar refractivity,  $F$  and  $R$ =Swain and Luton's electronic parameters characterizing the inductive and resonance effects [37],  $\sigma_m$  and  $\sigma_{o+p}$ =Hammett's constants, characterizing the electron-withdrawing power of the substituent at *meta* and *ortho+para* positions [38],  $E_s$ =Taft's constant, characterizing steric effects of the substituent [39,40],  $B_1$  and  $B_4$ =Sterimol width parameters determined by distance of substituents at their maximum point perpendicular to attachment [41,42]. H-Ac and H-Do values were characterized by dummy variables (0, 1 or 2) simply indicating the number of molecular substructures with hydrogen acceptor or hydrogen donor capacities. This special parameter set was employed because they previously found application in the calculation of the relationship between retention behavior and solute structure. Thus, molar refractivity for the study of the correlation between retention between structure and retention on polybutadiene-coated alumina stationary phase [43], Taft's constant for the assessment of selectivity in gas-liquid chromatography (GLC) [44], and various steric and electronic parameters for quantitative structure retention studies both in GLC [45,46] and HPLC [47].

The parameters were calculated according to the additivity rule from the fragmental constants and they are compiled in Table 2. We are well aware that

the application of the additivity rule may lead to serious misinterpretation when intermolecular interactions between the various molecular substructures occur. In order to reduce the possibility of such intramolecular interactions we selected very simple and small solute molecules as model compounds. The variance explained by the principal components was set to 99.5%. In order to facilitate the evaluation of the multi-dimensional data matrices of PC components and loadings, cluster analysis [48] and nonlinear mapping technique [49] were employed to reduce the dimensionality of matrices to one or to two, respectively. The iteration of the two-dimensional nonlinear map was carried out to the point where the difference between the last two iterations was lower than  $10^{-8}$ .

Software for PCA, cluster analysis and nonlinear mapping was prepared by Dr. Barna Bordás, Plant Protection Institute, Hungarian Academy of Sciences (Budapest, Hungary).

### 3. Results and discussion

Solutes were not retained on the PGC surface using *n*-hexane as mobile phase. The retention of solutes in normal-phase liquid chromatography is governed mainly by the binding of solute molecules

Table 2  
Calculated physico-chemical parameters of environmental pollutants<sup>a</sup>

No.	Name	$\pi$	H-Ac	H-Do	M-RE	$F$	$R$	$\sigma_m$	$\sigma_{o+p}$	$E_s$	$B_1$	$B_4$
1	Carbon tetrachloride	3.40	0	0	29.77	1.60	-0.73	1.41	0.75	-5.12	5.12	5.64
2	Trichloromethane	2.69	0	0	23.74	1.19	-0.58	1.04	0.52	-4.15	3.32	5.64
3	Dichloromethane	1.98	0	0	17.71	0.78	-0.43	0.67	0.29	-3.18	3.32	3.84
4	Dichloroethane	2.44	0	0	22.36	0.77	-0.40	0.67	0.31	-3.25	5.12	6.57
5	Trichloroethylene	2.95	0	0	29.08	1.30	-0.53	1.16	0.67	-4.71	5.20	6.69
6	Benzene	1.96	0	0	25.36	0.08	-0.08	0.06	-0.01	-3.82	3.11	3.11
7	Toluene	2.52	0	0	31.01	0.04	-0.21	-0.01	-0.18	-5.06	3.11	5.15
8	Xylene	3.08	0	0	36.66	0	-0.34	-0.08	-0.35	-6.30	3.11	7.19
9	Methanol	-0.11	1	1	8.50	0.25	-0.77	0.05	-0.54	-1.79	2.87	3.97
10	1-Propanol	0.88	1	1	17.81	0.23	-0.72	0.05	-0.50	-2.15	2.87	5.34
11	1-Butanol	1.46	1	1	22.46	0.23	-0.72	0.04	-0.53	-2.18	2.87	6.35
12	Tetrahydrofuran	1.11	1	0	20.10	0.24	-0.49	0.06	-0.25	-0.61	1.95	4.66
13	Dioxane	0.53	2	0	22.32	0.54	-0.87	0.25	-0.35	0.08	1.95	5.49
14	Diethyl ether	1.40	1	0	22.77	0.17	-0.54	0.03	-0.39	-2.11	1.52	6.33
15	Acetone	0.01	1	0	16.83	0.28	0.07	0.31	0.33	-3.84	3.42	2.93
16	Ethyl acetate	1.07	1	0	23.12	0.29	0.02	0.30	0.28	-4.54	3.42	6.33
17	Dimethylformamide	0.14	1	1	21.61	0.17	-0.49	0.05	-0.34	-3.68	3.04	5.65

<sup>a</sup> For symbols see Experimental.

to the adsorption centers of the polar stationary phase involving hydrophilic (electrostatic) interactive forces. The lower retention capacity of environmental pollutants on PGC indicates that the surface of alumina stationary phase is more polar than that of PGC. It has to be emphasized that the conclusions

discussed above are not the results of the concrete determination of surface polarity, therefore, they are valid only for these type of solutes. The extrapolation of the conclusions to other set of solutes may lead to serious misinterpretation of the results.

Each pollutant was eluted from the alumina col-

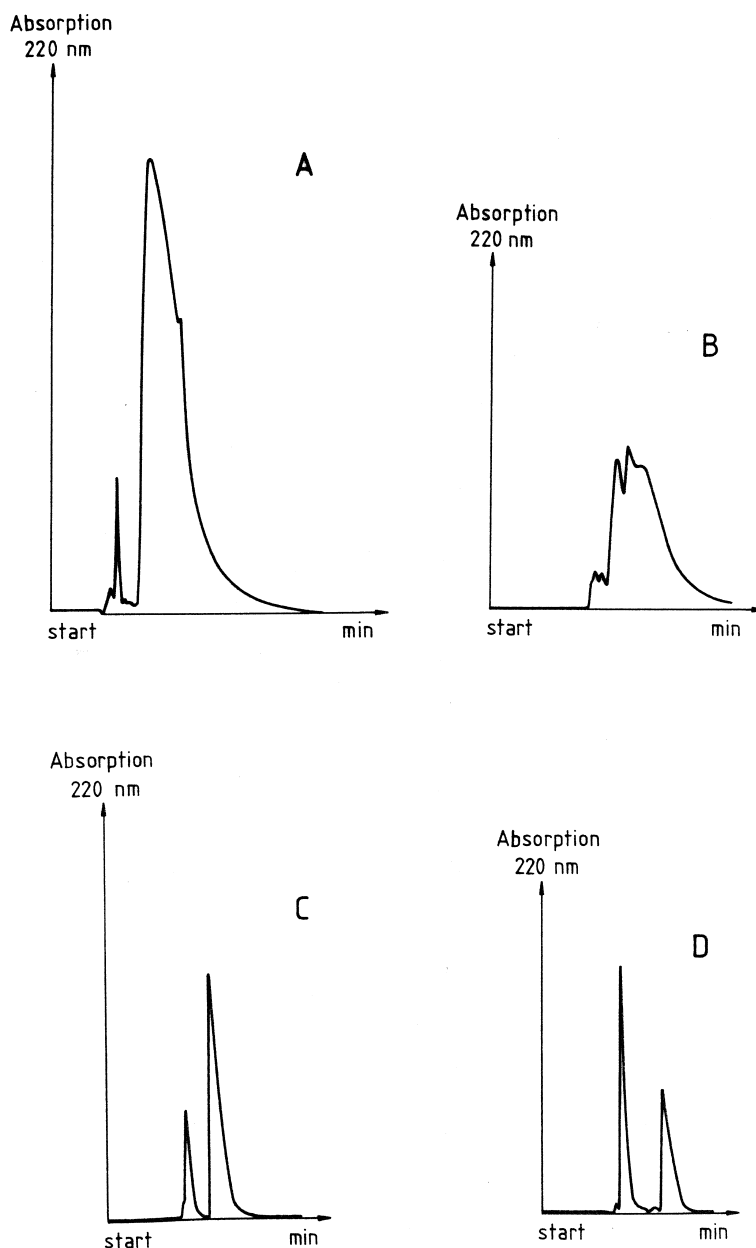


Fig. 1. Chromatogram of technical diethyl ether (A), technical tetrahydrofuran (B), technical dichloromethane (C) and technical dichloroethane (D). Alumina column, *n*-hexane mobile phase, flow-rate 1 ml/min, detection wavelength 220 nm.

umn with *n*-hexane, however, the peak shape differed considerably (Fig. 1). Methanol, 1-propanol, 1-butanol, dimethylformamide and ethyl acetate showed asymmetric peaks, however, they were easily detectable by the diode array system. The peak tailing can be explained by the supposition that the polar solutes can form hydrogen bonds with the adsorptive centers on the surface of alumina support. As the distribution of the strength of interaction between the polar substructures of solutes and the active centers probably is not of Gaussian type, the asymmetric distribution of interaction energies results in distorted peak shape. Technical diethyl ether (Fig. 1A) and technical tetrahydrofuran (Fig. 1B) showed impurities but the separation of fractions was not suitable for the exact quantitative analysis. Benzene, toluene and xylene were eluted as narrow and symmetric peaks, but their retention times were similar, therefore this method is not suitable for the purity control of benzene and benzene derivatives. Other pollutants showed good retention characteristics, the retention time was in each instance under 5 min and the peaks were symmetric. The impurities of technical dichloromethane (Fig. 1C) and technical dichloroethane (Fig. 1D) were well separated, and

they can be quantitatively determined. The capacity factors and the relative standard deviations of the capacity factors are compiled in Table 1. The good reproducibility of the log  $k'$  values indicates the stability and reliability of the HPLC system. The results of PCA are compiled in Table 3. Variables having a high loading (<0.50) in a given principal component are underlined. PCA proved that the 12 original variables can be reduced to four background theoretical variables with only a 10% loss of information. Unfortunately, PCA does not define the background variables as concrete physico-chemical entities only indicates its mathematical possibility. The majority of physico-chemical parameters together with the capacity factor have high loadings in the first principal component. This finding suggests that these set of parameters considerably influences the retention of solutes and their inclusion in the calculation of quantitative structure–retention relationship is justified. The two-dimensional nonlinear map of principal component loadings is shown in Fig. 2. As the reduction of the dimensionality of a data set distributed in a multi-dimensional space can never be perfect, the two-dimensional map is necessarily more or less nonlinear. The coordinates of the

Table 3

Similarities and dissimilarities between the retention characteristics and physico-chemical parameters of solutes on alumina column – results of principal component analysis

No. of principal components	Eigen value	Variance explained (%)	Total variance explained (%)	
1	5.56	46.36	46.36	
2	2.19	18.29	64.65	
3	1.69	14.15	78.80	
4	1.32	10.99	89.79	

Parameter	Principal component loadings			
	No. of principal components			
	1	2	3	4
Log $k'$	<u>-0.58</u>	0.32	0.22	<u>0.60</u>
$\pi$	<u>0.87</u>	-0.11	0.39	-0.03
H-Ac	<u>-0.81</u>	0.17	-0.02	-0.31
H-Do	<u>-0.62</u>	0.44	0.30	0.48
M-RE	<u>0.66</u>	-0.47	<u>0.51</u>	-0.14
$F$	<u>0.71</u>	<u>0.67</u>	-0.08	-0.19
$R$	0.21	<u>-0.71</u>	-0.48	0.35
$\sigma_m$	<u>0.78</u>	<u>0.57</u>	-0.17	-0.11
$\sigma_{o+p}$	0.02	<u>0.85</u>	0.23	-0.40
Es	<u>-0.75</u>	0.36	-0.10	-0.47
$B_1$	<u>0.69</u>	0.41	-0.09	0.46
$B_4$	0.29	0.07	<u>0.84</u>	-0.07

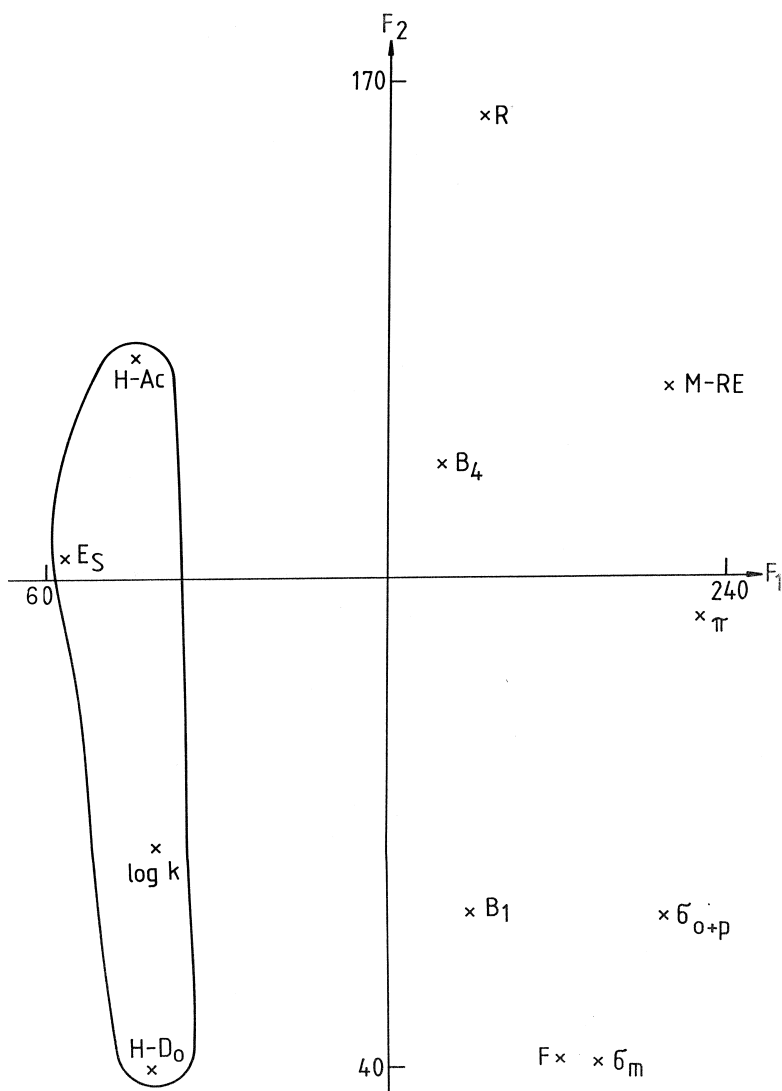


Fig. 2. Similarities and dissimilarities between the retention characteristics and physico-chemical parameters of solutes on the alumina column. Two-dimensional nonlinear map of principal component loadings. No. of iterations: 155; maximum error:  $4.30 \cdot 10^{-2}$ . For symbols see Experimental.

map are fictitious values characterizing the distribution of data points on the two-dimensional plane. They were calculated from the PC loadings but they are not identical to them. The logarithm of the capacity factor formed a cluster with the hydrogen donor and acceptor properties of the solutes and their Taft's constant. This finding indicates that both steric and electronic parameters play a considerable role in the determination of the retention mechanism of this

class of solutes on alumina support. It can be assumed that the steric parameter governs the availability of adsorption centers on the surface of alumina for the solutes whereas electrostatic interactive forces bind the solutes to the polar adsorption centers. The two-dimensional nonlinear map of principal component variables is shown in Fig. 3. Alcohols (cluster A), aromatics (cluster B) and chlorinated alkanes (cluster C) formed separate clusters indicating that

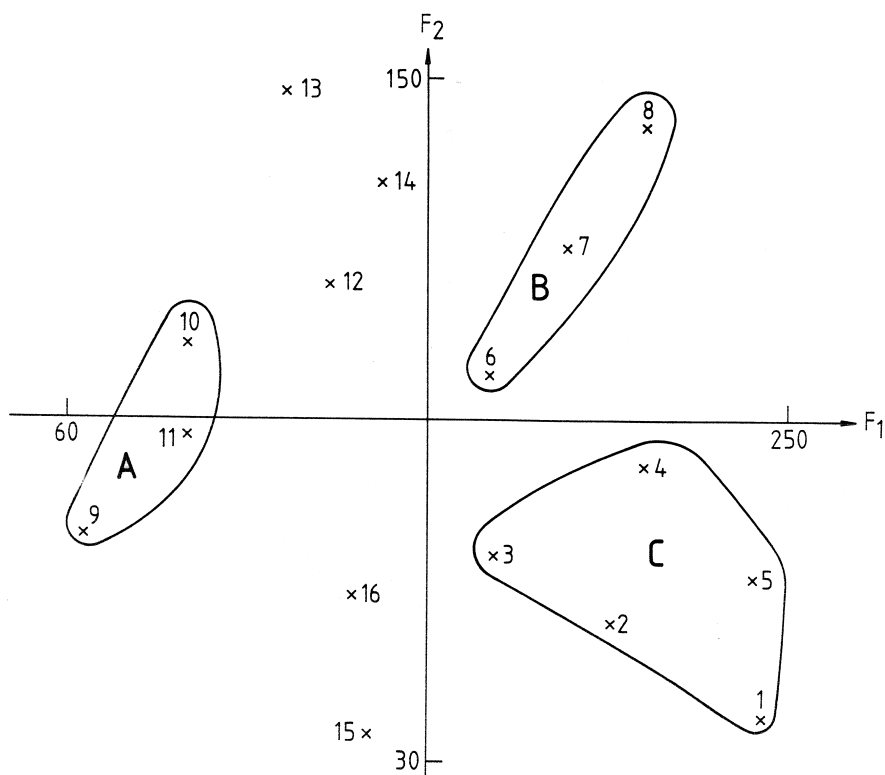


Fig. 3. Similarities and dissimilarities between the solutes taking into consideration their retention characteristics and physico-chemical parameters. Two-dimensional nonlinear map of principal component variables. No. of iterations: 150; maximum error:  $4.23 \cdot 10^{-2}$ . Numbers refer to solutes in Table 1.

their retention mechanism may be different on alumina column. Cluster analysis uses only one dimension for the projection of data points distributed in a multi-dimensional space, whereas two-dimensional nonlinear mapping technique employs a two-dimensional plane for visualization that facilitates the evaluation of multi-dimensional data matrices.

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### References

- [1] Y.S. Nikitin, S.N. Lanin, A. Al-Ahmed, *Chromatographia* 45 (1997) 408.
- [2] J.W. Dolan, L.R. Snyder, N.M. Djordevic, D.W. Hill, T.J. Waeghe, *J. Chromatogr. A* 857 (1999) 1.
- [3] J.W. Dolan, L.R. Snyder, N.M. Djordevic, D.W. Hill, T.J. Waeghe, *J. Chromatogr. A* 857 (1999) 21.
- [4] J.W. Dolan, L.R. Snyder, N.M. Djordevic, D.W. Hill, T.J. Waeghe, *J. Chromatogr. A* 857 (1999) 41.
- [5] M. Hanson, K.K. Unger, *LC-GC Int.* 9 (1996) 741.
- [6] Y. Shen, Y.J. Yang, M.L. Lee, *Anal. Chem.* 69 (1997) 628.
- [7] A. Nahum, Cs. Horváth, *J. Chromatogr.* 203 (1981) 53.
- [8] K.E. Bij, Cs. Horváth, W.R. Melander, A. Nahum, *J. Chromatogr.* 203 (1981) 65.
- [9] J.P.B. Brunell, *Pure Appl. Chem.* 50 (1978) 1211.
- [10] R. Poisson, J.P. Brunelle, P. Nortier, in: A.B. Styles (Ed.), *Catalytic Supports, Supported Catalysis*, Butterworth, Boston, MA, 1987, p. 11.
- [11] K. Cabdera, D. Lubda, G. Jung, *Kontakte (Darmstadt)*, (1992) 32.
- [12] D.K. Singh, P. Mehrotra, *Chromatographia* 23 (1987) 747.
- [13] J. Puncochárová, J. Vareka, L. Vodicka, J. Kriz, *J. Chromatogr.* 498 (1989) 248.
- [14] K.K. Unger, W. Messer, K.F. Krebs, *J. Chromatogr.* 149 (1978) 1.
- [15] T. Cserhádi, *Chromatographia* 29 (1990) 593.

- [16] C.J.C.M. Laurent, H.A.H. Billiet, L. de Galan, F.A. Buytenhuis, F.P.B. van de Maeden, *J. Chromatogr.* 287 (1984) 45.
- [17] H. Lingeman, H.A. van Muster, J.H. Beyben, W.J.M. Underber, A. Hulshoff, *J. Chromatogr.* 352 (1986) 61.
- [18] E. Forgács, T. Cserhádi, *J. Chromatogr. A* 661 (1994) 239.
- [19] T. Cserhádi, E. Forgács, *J. Chromatogr. A* 668 (1994) 495.
- [20] C.J.C.M. Laurent, H.A.H. Billiet, L. De Galan, *J. Chromatogr.* 285 (1984) 164.
- [21] J.H. Knox, K. Unger, H. Müller, *J. Liq. Chromatogr.* 6 (1983) 1.
- [22] J.H. Knox, B. Baur, G.R. Millward, *J. Chromatogr.* 352 (1986) 3.
- [23] J.H. Knox, P. Ross, *Adv. Chromatogr.* 37 (1997) 73.
- [24] P. Ross, J.H. Knox, *Adv. Chromatogr.* 37 (1997) 121.
- [25] P. Chambault, S. Cassel, S. Claude, C. Debaig, T. Benvegna, D. Plusquelle, P. Rollin, M. Lafosse, *Chromatographia* 50 (1999) 239.
- [26] R. Kaliszan, *Structure and Retention in Chromatography. A Chemometric Approach*, Harwood Academic Publishers, Australia, 1997.
- [27] T. Cserhádi, E. Forgács, *Adv. Chromatogr.* 36 (1995) 1.
- [28] K.V. Mardia, J.T. Kent, J.M. Bibby, in: *Multivariate Analysis*, Academic Press, London, 1979, p. 213.
- [29] B.J. Bassler, R. Kaliszan, R.J. Hartwick, *J. Chromatogr.* 461 (1989) 139.
- [30] R. Kaliszan, K. Osmialowski, B.J. Bassler, R.A. Hartwick, *J. Chromatogr.* 499 (1990) 333.
- [31] P. Karsnas, T. Lindblom, *J. Chromatogr.* 599 (1992) 131.
- [32] K.G. Miller, C.F. Poole, T.M.P. Pawloski, *Chromatographia* 42 (1996) 639.
- [33] C.M. Garcia-Jares, M.S. Garcia-Martin, N. Carro-Marino, R. Cela-Torrijos, *J. Sci. Food Agric.* 69 (1995) 175.
- [34] A. Leo, C. Hansch, M. Ames, *J. Pharm. Sci.* 64 (1975) 559.
- [35] T. Fujita, J. Iwasa, C. Hansch, *J. Am. Chem. Soc.* 86 (1964) 5175.
- [36] C. Hansch, A. Leo, in: *Substituent Constants for Correlation Analysis in Chemistry and Biology*, Wiley, New York, 1979, p. 1.
- [37] L. Pauling, D. Pressman, *J. Am. Chem. Soc.* 67 (1945) 1003.
- [38] R.W. Taft, I.C. Lewis, *J. Am. Chem. Soc.* 80 (1958) 2436.
- [39] L.P. Hammett, *Chem. Rev.* 17 (1935) 125.
- [40] R.W. Taft, *J. Am. Chem. Soc.* 74 (1952) 3120.
- [41] A. Verloop, J. Tipker, *Pestic. Sci.* 7 (1976) 379.
- [42] A. Verloop, W. Hoogenstraaten, J. Tipker, in: J. Ariens (Ed.), *Drug Design*, Vol. VII, Academic Press, New York, 1976, p. 165.
- [43] R. Kaliszan, K. Osmialowski, *J. Chromatogr.* 506 (1990) 3.
- [44] K. Szymoniak, J.R. Chretien, *J. Chromatogr.* 404 (1987) 11.
- [45] T.F. Woloszyn, P.C. Jurs, *Anal. Chem.* 64 (1992) 3059.
- [46] O. Mekenyan, N. Dimov, V. Enchev, *Anal. Chim. Acta* 260 (1992) 69.
- [47] P. Jandera, *J. Chromatogr. A* 656 (1993) 437.
- [48] P. Willett, *Similarity and Clustering in Chemical Information Systems*, Research Studies Press, New York, 1987.
- [49] J.W. Sammon Jr., *IEEE Trans. Computers* C18 (1969) 401.