Journal of Chromatography, 371 (1986) 253–267
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMSYMP. 971

FACTOR ANALYSIS AND EXPERIMENT DESIGN IN HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

VI. COMPARISON OF THE RETENTION MECHANISM FOR FOURTEEN OCTADECYLSILICA PACKINGS

J. R. CHRETIEN* and B. WALCZAK*
Laboratoire de Chimie Analytique Industrielle et Chimiométrie, Université d'Orléans, B.P. 6759, 45067 Orléans Cedex 2 (France)
and
L. MORIN-ALLORY, M. DREUX and M. LAFOSSE
Laboratoire de Chimie Organique Physique et Chromatographies, Université d'Orléans, B.P. 6759, 45067 Orléans Cedex 2 (France)

SUMMARY

The selectivity of 63 compounds on 14 ODS packings for reversed-phase high-performance liquid chromatography has been studied. Plots of logarithmic retention factors, log k', measured on column pairs with the same mobile phase, are used to compare the energetics of retention and to study the similarity of the retention mechanisms on all the possible pairs of packings. To discuss the specific properties of the packings, a new criterion of the similarity of retention mechanism is proposed. The chi-squared (χ^2) distance can be used to describe the deviation from proportionality between the capacity factors measured on the column pairs. Correspondence factor analysis (CFA) gives access to the χ^2 distance in the reduced space of the main factors affecting solute selectivity. Additionally, the relative importance of the "hydrophobic" and non-hydrophobic effects can be estimated. The extracted factors allow the $a_{jj'}$ parameters to be recreated, *i.e.*, the ratio of the Gibbs free energy for the jth and j'th phase pair.

INTRODUCTION

The differences that exist among commercially available packing materials for reversed-phase high-performance liquid chromatography (RP-HPLC) are of considerable interest from both a theoretical and practical point of view. The effect of the length of the bonded alkyl chain on solute selectivity has been studied extensively[1–13], but remains a subject of discussion. Significant differences have been shown among columns which have the same bonded functional groups[14–21]. The chromatographic differences observed between similarly prepared columns are due to differ-

* On leave from Silesian University, Katowice, Poland.

0021-9673/86/$03.50

ences in the characteristics of the silica material used as a support and in the technique used to form the bonded phase. The surface coverage[7,22,23], carbon loading[24], end-capping[25,26], surface area[5,8,27,28], pore size and pore volume[27] are variables that directly affect retention and selectivity. Other parameters that can affect the chromatographic behaviour of solutes include the particle shape[29] and size[30], the size distribution[31], the presence of trace elements in the silica matrix[32,33] and the surface pH[14]. The differences among monomeric and polymeric C_{18} phases prepared on a variety of silica substrate materials have also been reported[27,34,35].

However, there is little general agreement on the relative importance of the above-mentioned factors. Our systematic study dealing with factor analysis[36] and experiment design in HPLC[37–41] prompted us to re-examine the main trends in the influence of packing properties on the solute selectivity under conditions of solvophobic chromatography. Correspondence factor analysis (CFA)[36–43] was applied to extract the main factors influencing solute selectivity and to estimate their relative importance.

To grasp the broader generalizations that can be applied to the classification of different commercially available packings, with respect to differences in selectivity, a large set of structurally diverse compounds was used as a probe. Fourteen ODS RP-HPLC packings are taken into consideration. The conclusions are based on the chromatographic behaviour of two series of compounds: one including the sixteen compounds most often proposed for testing RP-HPLC packing materials, and another including 47 chalcones ($X\text{–}C_6H_4\text{–}CH=CH\text{–}CO\text{–}C_6H_4\text{–}Y$) diversely substituted.

THEORETICAL

The aim of the study was to grasp the differences between the studied stationary phases as revealed by the use of a judicious set of compounds. Therefore it is advisable to employ the same constant mobile phase composition for all the systems and to compare the stationary phases j and j' on the basis of differences in the Gibbs free energy, ΔG°, for the binding of the chromatographed samples, i and i'. Such a difference in free energy of two samples i and i' analysed successively on two stationary phases j and j' can be expressed as:

$$\Delta(\Delta G^\circ)_{jj'} = \Delta G^\circ_j - \Delta G^\circ_{j'} = -RT \ln \alpha^j_{ii'} + RT \ln \alpha^{j'}_{ii'} = RT \ln \frac{\alpha^{j'}_{ii'}}{\alpha^j_{ii'}} \qquad (1)$$

This difference influences the dependence

$$\ln k'_{ij} = \ln k'_{ij'} + \ln \psi_j - \ln \psi_{j'} - \frac{\Delta G^\circ_j - \Delta G^\circ_{j'}}{RT} \qquad (2)$$

where k'_{ij} is the capacity factor of the ith solute in the jth chromatographic system, $\alpha^j_{ii'} = k'_{ij}/k'_{i'j}$ and $\psi_j = n_s/n_m$ is the ratio of the number of moles, n, of the mobile (m) and stationary (s) phases in the jth chromatographic system. So the difference in free energy, $\Delta(\Delta G^\circ)_{jj'}$, of samples analysed on two stationary phases j and j' can involve one of the three following cases:

$$\Delta G^\circ_j = \Delta G^\circ_{j'} \quad \text{then } \ln k'_{ij} = \ln k'_{ij'} + \ln \psi_j - \ln \psi_{j'} \tag{3}$$

$$\Delta G^\circ_j = a_{jj'} \Delta G^\circ_{j'} \text{ then } \ln k'_{ij} = a_{jj'} \ln k'_{ij'} + \ln \psi_j - a_{jj'} \ln \psi_{j'} \tag{4}$$

where $a_{jj'}$ is a factor of proportionality

ΔG°_j not proportional to $\Delta G^\circ_{j'}$ then no linear dependence exists between $\ln k'_{ij}$ and $\ln k'_{ij'}$ (5)

Thus, investigation of the relationship $\ln k'_{ij}$ *vs.* $\ln k'_{ij'}$ can furnish information concerning the difference in the retention energetics, expressed as $\Delta(\Delta G^\circ)_{jj'} = \Delta G^\circ_j - \Delta G^\circ_{j'}$, and allows the retention mechanism on the jth stationary phase to be classified as: homoenergetic (the same) if $\Delta G^\circ_j = \Delta G^\circ_{j'}$ (eqn. 3), homeoenergetic (similar) if $\Delta G^\circ_j = a_{jj'} \Delta G^\circ_{j'}$ (eqn. 4) or heteroenergetic (different) if $\Delta G^\circ_j \neq a_{jj'} \Delta G^\circ_{j'}$ (eqn. 5), according to Melander *et al.*[13].

In terms of solubility parameters[44], $\Delta(\Delta G^\circ)_{jj'}$ for the two compounds i and i' having an equal volume ($v_i = v_{i'}$) can be given in the form

$$\Delta(\Delta G^\circ)_{jj'} = 2\, v_i\, (\delta_{i'} - \delta_i)\, (\delta_j - \delta_{j'}) \tag{6}$$

where δ is the solubility parameter, defined as $\sqrt{-E/v}$ where E is the cohesive energy required to transfer 1 mol of a substance from the ideal gas to its liquid state; v is the molar volume of the liquid.

The specific properties of the individual stationary phases can be elucidated with the help of the multicomponent solubility parameter model[45,46], applied to liquid chromatography by Schoenmakers *et al.*[44], which assumes that

$$\delta^2 = \delta^2_{\mathrm{d}} + \delta^2_{\mathrm{o}} + 2\delta_{\mathrm{ind}}\, \delta_{\mathrm{d}} + 2\, \delta_{\mathrm{a}} \delta_{\mathrm{b}} \tag{7}$$

where d, o, ind, a and b denote the dispersive, orientation, induction and the acid–base interactions, respectively. Then, for the jth and j'th systems with the same mobile phase:

$$\begin{aligned} \Delta(\Delta G^\circ)_{jj'} = 2\, v_i\, [&(\delta_{\mathrm{d}i'} - \delta_{\mathrm{d}i})\, (\delta_{\mathrm{d}j} - \delta_{\mathrm{d}j'}) + (\delta_{\mathrm{o}i'} - \delta_{\mathrm{o}i})\, (\delta_{\mathrm{o}j} - \delta_{\mathrm{o}j'}) + \\ &(\delta_{\mathrm{d}i'} - \delta_{\mathrm{d}i})\, (\delta_{\mathrm{ind}\ j} - \delta_{\mathrm{ind}\ j'}) + (\delta_{\mathrm{ind}\ i'} - \delta_{\mathrm{ind}\ i})\, (\delta_{\mathrm{d}j} - \delta_{\mathrm{d}j'}) + \\ &(\delta_{\mathrm{a}i'} - \delta_{\mathrm{a}i})\, (\delta_{\mathrm{b}j} - \delta_{\mathrm{b}j'}) + (\delta_{\mathrm{b}i'} - \delta_{\mathrm{b}i})\, (\delta_{\mathrm{a}j} - \delta_{\mathrm{a}j'})] \end{aligned} \tag{8}$$

So, the difference $\Delta(\Delta G^\circ)_{jj'}$ depends simultaneously on the properties of the compounds and of the stationary phases. The differences in the individual parameters δ of the stationary phases will be more visible if the corresponding parameters δ of the chromatographed compounds differ strongly.

Factor analysis is used for a deeper insight into the "true" complexity of factors influencing $\Delta(\Delta G^\circ)_{jj'}$. Furthermore, this data processing technique helps to distinguish the parameters which depend upon the characteristics of the stationary phases and those which depend upon the nature of the solutes.

In our study, correspondence factor analysis seems particularly useful as the chi-squared (χ^2) distance is used to describe the deviation from proportionality between rows and columns of the data matrix, elements of which are the capacity factors of the compounds on the stationary phases considered.

EXPERIMENTAL

Reagents

The chalcones and test compounds considered are listed in Table I. The mobile phase consisted of HPLC-grade methanol (E. Merck, Darmstadt, F.R.G.) and of Millipore purified water.

Chromatographic procedure

Prior to the measurements, the columns were washed with the methanol–water (7:3) mobile phase until a constant value was obtained for the retention of the compounds. Sample solutions (2 mg per 25 ml) were prepared in dichloromethane (chalcones), or in methanol (test compounds). All data points were collected by averaging three reproducible separations. The mobile phase and 1 μl of 10^{-3} *M* sodium nitrate,

TABLE I

CHALCONES AND TEST COMPOUNDS MOST OFTEN USED TO CHARACTERIZE HPLC PACKINGS

Me = methyl; Et = ethyl; Pr = propyl; Bu = butyl.

E-s-cis chalcone $X\text{-}C_6H_4\text{-}CH=CH\text{-}C(=O)\text{-}C_6H_4\text{-}Y$		*Z-s-cis chalcone* $X\text{-}C_6H_4\text{-}CH=CH\text{-}C(=O)\text{-}C_6H_4\text{-}Y$		*No.*	*Test compound*
No.	*X-Y*	*No.*	*X-Y*		
1	H-CF_3	26	H-CF_3	48	Nitrobenzene
2	H-*tert.*-Bu	27	H-*tert.*-Bu	49	Naphthalene
3	H-*iso*-Pr	28	H-*iso*-Pr	50	Phenanthrene
4	H-H	29	H-H	51	Methyl benzoate
5	F-H	30	F-H	52	Biphenyl
6	H-F	31	H-F	53	Diethyl phthalate
7	H-Et	32	H-Et	54	Anthracene
8	H-Me	33	H-Me	55	*p*-Cresol
9	F-Me	34	F-Me	56	2-Phenylethanol
10	F-F	35	F-F	57	Benzophenone
11	MeO-Me	36	MeO-Me	58	Benzyl alcohol
12	Me-MeO	37	Me-MeO	59	3-Phenylpropanol
13	F-MeO	38	F-MeO	60	4-Phenylbutanol
14	H-NO_2	39	H-NO_2	61	6-Phenylhexanol
15	F-NO_2	40	F-NO_2	62	9-Phenylnonanol
16	NO_2-Me	41	NO_2-Me	63	α-Nitronaphthalene
17	NO_2-H	42	NO_2-H		
18	MeO-MeO	43	MeO-MeO		
19	MeO-NO_2	44	NO_2-F		
20	NO_2-F	45	NO_2-MeO		
21	NO_2-MeO	46	NO_2-NO_2		
22	NO_2-NO_2	47	H-OH		
23	NH_2-H				
24	H-OH				
25	NMe_2-NO_2				

detected at 210 nm, were used to determine the dead time, t_0, for each column. The capacity factor, k', was calculated from the solute retention time, t_R, according to $k' = (t_R - t_0)/t_0$.

Instruments and columns

The HPLC equipment included the following components: a Bruker LC-31 pump, a Rheodyne Model 7125 injection valve, a Schoeffel Model SF 770 spectrophotometer set at 300 nm (chalcones) or 254 nm (test compounds) and a Shimadzu C-RIB data processor.

The commercially available columns or columns prepared in our laboratory by slurry-packing at 6000 p.s.i. with carbon tetrachloride, followed by methanol, are presented in Table II.

Data processing

A set of "abstract" factors affecting the selectivity in RP-HPLC systems was extracted by CFA[42,43]. To determine the number of factors in a data matrix, *i.e.*, the primary set of eigenvectors, the imbedder error function (IE)[47] and the factor indicator function (INF)[47] were used. It was found that six main factors emerge from principal component analysis[47], and that five factors ought to be considered in the CFA results[43]. To transform the abstract CFA factors into chemically significant ones, "target testing"[47] was applied.

RESULTS AND DISCUSSION

The capacity factors, k', of the investigated compounds in fourteen RP-HPLC systems with the same methanol–water mobile phase and different ODS stationary

TABLE II

COMMERCIALLY AVAILABLE PACKINGS

No.	*Column packing**	*Dimension (mm × mm)*	*Supplier*
1	RSIL C_{18} LL*	90 × 4	Alltech
2	RSIL C_{18} HL*	90 × 4	Alltech
3	Partisil ODS*	90 × 4	Whatman
4	Partisil ODS 2*	90 × 4	Whatman
5	Partisil ODS 3*	90 × 4	Whatman
6	Spherisorb ODS-2*	90 × 4	Phase Separations
7	μBondapak C_{18}*	90 × 4	Waters
8	Hypersil C_{18}	90 × 4	Shandon
9	Spherosil XOA 600 C_{18}*	90 × 4	Prolabo
10	Nucleosil C_{18}*	90 × 4	Macherey-Nagel
11	Nova Pak C_{18}	100 × 5	Waters
12	Resolve C_{18} Radial Pak	100 × 8	Waters
13	μBondapak C_{18} Radial Pak	100 × 8	Waters
14	Zorbax ODS	150 × 4.6	Du Pont

* Packed in our laboratory.

TABLE III

THE CAPACITY FACTORS, k', OF THE 63 COMPOUNDS SEPARATED IN THE 14 CHROMATOGRAPHIC SYSTEMS WITH METHANOL–WATER (7:3, v/v) AS MOBILE PHASE

Solute	*Chromatographic system*													
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*
1	6.58	20.05	2.84	19.40	11.05	13.91	10.40	10.40	22.96	14.86	15.46	12.48	16.69	27.14
2	12.80	54.01	4.87	46.46	25.52	34.79	22.14	24.55	58.49	33.99	40.51	28.86	33.07	65.19
3	10.10	36.57	4.08	33.72	18.38	24.29	16.21	17.55	41.93	25.27	27.22	21.71	26.12	47.02
4	4.25	8.98	2.08	9.67	5.36	6.46	5.31	4.86	12.51	8.73	6.82	7.08	8.04	11.91
5	4.08	8.65	2.01	9.15	5.25	6.09	5.22	4.67	11.71	8.12	6.55	6.68	8.10	11.62
6	4.25	9.61	2.08	9.98	5.75	6.89	5.68	5.20	12.79	8.79	7.28	7.29	8.69	13.04
7	7.92	23.13	3.39	23.75	12.66	16.64	11.65	12.07	30.63	18.66	17.97	16.00	17.24	31.79
8	5.97	14.77	2.73	15.81	8.36	10.57	7.92	7.77	20.55	13.09	11.16	11.01	11.64	20.55
9	5.62	14.13	2.57	14.58	8.23	9.93	7.92	7.33	18.71	12.35	10.41	10.38	12.28	21.19
10	4.02	9.61	1.95	9.30	5.63	6.53	5.68	5.01	11.83	8.21	6.72	6.77	8.73	12.43
11	7.19	16.03	3.12	18.97	8.85	11.49	8.78	7.98	25.91	15.47	11.16	12.62	14.00	24.14
12	7.40	17.15	3.20	20.29	9.36	12.05	9.19	8.48	26.93	15.95	12.61	13.25	14.65	23.33
13	4.83	9.77	2.29	11.15	5.77	6.51	5.87	4.91	14.31	9.56	6.72	7.69	8.61	13.33
14	4.28	9.13	2.07	9.86	5.30	6.53	5.46	4.95	12.96	8.47	6.59	7.11	8.73	13.25
15	3.99	9.03	1.70	8.86	5.25	6.04	5.51	4.75	11.36	8.01	6.66	6.45	8.63	12.23
16	5.31	11.53	2.41	12.26	6.80	8.25	6.96	6.03	16.69	10.69	8.53	9.11	10.91	17.66
17	3.83	7.22	1.91	7.87	4.42	4.98	4.62	3.85	10.33	7.27	5.37	5.95	7.27	10.23
18	5.80	11.22	2.73	13.59	6.16	7.12	6.33	5.15	18.13	11.47	7.18	8.97	9.39	14.67
19	5.13	11.25	2.76	11.93	5.80	7.03	6.10	5.23	15.69	9.94	7.29	8.39	9.11	14.65
20	3.81	7.72	1.84	7.93	4.79	5.33	4.98	4.19	10.21	7.18	5.68	5.96	8.00	11.45
21	4.64	8.25	2.25	9.63	4.97	5.40	5.24	4.17	13.06	8.64	5.57	7.05	8.42	11.95
22	3.64	8.46	1.75	7.44	4.42	4.79	4.87	3.95	10.19	6.76	5.54	5.48	7.25	10.52
23	2.53	3.27	1.45	3.44	2.01	1.70	2.07	1.62	4.16	3.72	1.69	2.87	3.17	3.29
24	2.62	4.65	1.45	4.04	2.75	2.62	2.89	2.40	4.86	4.33	2.62	3.03	4.44	4.52
25	8.02	17.08	3.43	20.30	8.16	11.44	9.15	8.06	27.38	16.45	10.53	15.81	14.73	23.73
26	4.43	12.95	2.09	12.21	7.51	8.89	6.96	7.04	13.83	9.69	9.84	7.93	10.83	17.08
27	8.20	33.39	3.39	27.96	16.79	21.60	14.42	16.16	33.36	20.83	25.22	17.63	21.30	40.16
28	6.51	22.62	2.84	20.38	12.10	15.00	10.55	11.54	23.80	15.49	16.97	13.15	16.84	28.51
29	2.80	5.80	1.51	5.89	3.73	4.10	3.65	3.33	7.16	5.47	4.44	4.47	5.43	7.47
30	3.04	6.49	1.61	6.58	4.07	4.58	4.01	3.69	8.06	5.98	4.89	4.94	6.10	8.51
31	2.87	6.24	1.52	6.26	3.98	4.41	3.87	3.59	7.56	5.69	4.72	4.64	5.80	8.23
32	5.03	14.16	2.34	14.38	8.35	10.09	7.62	7.88	17.39	11.47	10.95	9.46	11.05	18.90
33	3.73	8.89	1.89	8.98	5.48	6.30	5.16	5.01	10.96	7.78	6.77	6.46	7.47	12.06
34	4.02	10.05	2.00	9.94	6.07	7.10	5.80	5.53	12.19	8.67	7.28	7.24	8.85	14.77
35	3.13	7.16	1.62	7.04	4.26	4.88	4.24	3.91	8.56	6.30	4.91	5.10	6.40	9.06
36	4.56	10.62	2.19	11.32	6.21	7.57	6.07	5.65	14.53	9.67	7.63	7.81	9.43	15.60
37	4.32	9.84	2.12	10.48	5.94	6.79	5.74	5.23	12.86	9.01	7.31	7.18	8.85	12.90
38	3.35	6.71	1.75	7.19	4.19	4.51	4.24	3.62	8.76	6.53	4.62	5.23	6.14	8.90
39	2.98	5.99	1.59	6.36	3.69	4.20	3.77	3.41	7.83	5.75	4.25	4.55	5.80	8.36
40	3.34	6.87	1.51	7.27	4.01	4.58	4.15	3.72	8.89	6.67	4.83	5.03	6.39	9.05
41	3.91	7.72	1.97	8.38	4.75	5.45	4.87	4.23	10.63	7.75	5.44	6.27	7.43	11.44
42	2.92	5.05	1.59	5.59	3.21	3.47	3.42	2.83	6.99	5.44	3.58	4.38	5.19	6.93
43	3.64	7.16	1.90	7.84	4.27	4.62	4.34	3.62	9.86	7.09	4.77	5.47	6.34	9.19
44	3.07	5.36	1.65	6.03	3.38	3.66	3.53	3.03	7.41	5.70	3.63	4.46	5.45	7.66
45	3.31	5.39	1.79	6.26	3.42	3.51	3.65	2.91	7.93	6.09	3.47	4.80	5.64	7.44
46	3.25	5.81	1.75	6.21	3.07	3.23	3.37	2.75	7.89	5.87	3.44	4.25	4.89	6.98

TABLE III (*continued*)

Solute	*Chromatographic system*													
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*
47	1.73	2.89	1.04	2.36	1.86	1.65	1.96	1.62	2.73	2.70	1.66	2.04	2.95	2.79
48	1.58	2.15	1.03	2.16	1.58	1.56	1.75	1.44	2.70	2.65	1.47	2.45	2.69	2.95
49	3.08	7.98	1.72	7.46	4.41	5.56	3.69	4.39	8.93	6.78	6.61	6.22	6.10	11.68
50	6.68	22.21	3.27	22.38	10.20	15.51	7.63	10.75	27.59	17.55	19.21	15.79	13.15	33.95
51	1.76	2.70	1.12	2.67	1.99	1.86	1.96	1.68	3.27	3.12	2.03	2.80	3.01	3.76
52	4.38	13.70	2.31	12.47	7.25	9.35	5.69	7.06	14.83	10.50	11.96	9.75	9.68	20.66
53	1.86	2.85	1.18	2.73	2.20	1.86	2.12	1.76	3.21	3.21	2.16	2.67	3.37	3.79
54	7.40	26.17	3.58	26.38	11.49	18.34	8.52	12.35	32.43	20.03	22.50	18.77	14.73	40.20
55	1.19	1.51	0.81	1.41	1.22	1.01	1.33	1.07	1.67	1.87	1.10	1.55	2.00	1.79
56	1.22	1.42	0.84	1.44	1.16	0.94	1.27	1.03	1.66	1.86	1.00	1.57	1.88	1.72
57	2.58	4.89	1.57	4.75	3.32	3.27	3.07	2.73	5.78	5.08	3.82	4.34	4.95	6.92
58	1.03	1.12	0.75	1.10	0.95	0.76	1.09	0.85	1.30	1.51	0.78	1.34	1.59	1.29
59	1.49	1.93	0.98	2.01	1.52	1.28	1.55	1.31	2.30	2.37	1.47	1.91	2.36	2.45
60	1.91	2.79	1.20	2.93	2.05	1.83	1.99	1.80	3.31	3.22	2.19	2.47	3.15	3.63
61	3.56	6.96	2.02	7.26	4.47	4.40	3.93	3.94	7.99	6.85	5.99	5.09	6.61	9.73
62	11.64	34.29	5.57	35.43	18.13	20.62	13.95	16.74	37.99	27.13	33.73	21.13	25.96	52.04
63	2.93	5.32	1.73	5.61	3.29	3.61	3.13	2.94	6.66	5.87	3.91	4.67	5.07	7.48

phases are presented in Table III.

Based on the average capacity factor, $k'_j = \sum_{i=1}^{63} k'_{ij}/63$, the order of "retention power" of the chromatographic systems is: Zorbax ODS > Spherosil XOA C_{18} > Partisil ODS 2 > RSIL C_{18} HL > Nucleosil C_{18} > μBondapak C_{18} Rp > Nova Pak C_{18} > Resolve C_{18} > Spherisorb ODS-2 > Partisil ODS 3 > μBondapak C_{18} > Hypersil C_{18} > RSIL C_{18} LL > Partisil ODS.

According to the solvophobic theory, the differences in the retention behaviour of a given sample, using the same eluent and the same alkyl ligand, are essentially due to the different phase ratios. It is also known, from experimental practice, that the differences in the silica material used as a support and the differences in the technique used to form the bonded phase can affect not only solute retention but also solute selectivity.

Plots of log k'_{ij} versus log $k'_{ij'}$

A comparison of the separation mechanisms on the particular phase pair j and j' can be made based on the relationship log k'_{ij} *versus* log $k'_{ij'}$. The resulting correlation coefficients, r, and the slope, a, for all pairs of the stationary phases, j and j', are listed in Table IV. The similarity of the separation mechanism is analysed in terms of the following criteria[13]:

if $r > 0.95$ and $0.90 < a < 1.10$ the mechanism is homoenergetic (eqn. 3)
if $r > 0.95$ and $a < 0.90$ or $a > 1.10$ the mechanism is homeoenergetic (eqn. 4)
if $r < 0.95$ the mechanism is heteroenergetic (eqn. 5)

From the r and a values in Table IV, it appears that in all chromatographic systems

TABLE IV

COMPARISON OF THE RETENTION MECHANISMS FOR 91 PAIRS OF PACKINGS

Correlation coefficients, r, and slope, a, according to eqn. 2. ●, Homoenergetic mechanism ($r > 0.95$, $a = 1.0 \pm 0.1$); ▲, homeoenergetic mechanism ($r > 0.95$, $a < 0.9$ or $a > 1.10$). Identification numbers of the chromatographic systems as in Table II.

	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*
1	*r*	0.98	0.99	0.98	0.98	0.98	0.98	0.97	0.99	0.99	0.97	0.99	0.99	0.98
	a	1.46	0.76	1.48	1.24	1.48	1.15	1.30	1.51	1.22	1.52	1.24	1.18	1.53
2	▲		0.97	0.99	1.00	1.00	0.99	1.00	0.99	0.99	0.99	0.99	0.99	0.99
			0.50	1.00	0.85	1.01	0.78	0.89	1.01	0.82	1.05	0.83	0.80	1.04
3	▲	▲		0.98	0.96	0.96	0.96	0.96	0.98	0.99	0.96	0.98	0.97	0.97
				1.90	1.60	1.90	1.46	1.66	1.94	1.57	1.96	1.60	1.50	1.97
4	▲	●	▲		0.99	0.99	0.99	0.99	1.00	1.00	0.99	0.99	0.99	0.99
					0.84	1.01	0.77	0.88	1.02	0.82	1.04	0.84	0.79	1.04
5	▲	▲	▲	▲		1.00	0.99	1.00	0.98	0.99	0.99	0.99	1.00	0.99
						1.19	0.91	1.04	1.18	0.96	1.23	0.98	0.93	1.22
6	▲	▲	▲	●	▲		0.99	1.00	0.99	0.99	0.99	0.99	0.99	1.00
							0.76	0.88	0.99	0.80	1.03	0.82	0.78	1.03
7	▲	▲	▲	▲	●	▲		0.99	0.99	0.99	0.98	0.98	1.00	0.98
								1.13	1.29	1.04	1.32	1.06	1.02	1.32
8	▲	▲	▲	▲	●	▲	▲		0.98	0.99	1.00	0.99	0.99	0.99
									1.12	0.91	1.17	0.93	0.89	1.17
9	▲	●	▲	●	▲	●	▲	▲		1.00	0.98	0.99	0.99	0.99
										0.80	1.01	0.82	0.77	1.02
10	▲	▲	▲	▲	●	▲	●	●	▲		0.98	1.00	0.99	0.99
											1.26	1.02	0.96	1.27
11	▲	●	▲	●	▲	●	▲	▲	●	▲		0.99	0.99	0.99
												0.79	0.75	0.99
12	▲	▲	▲	▲	●	▲	●	●	▲	●	▲		0.99	0.99
													0.94	1.24
13	▲	▲	▲	▲	●	▲	●	▲	▲	●	▲	●		0.99
														1.30
14	▲	●	▲	●	▲	●	▲	▲	●	▲	●	▲	▲	

the separation mechanism is homo- or homeoenergetic. For 27 of the 91 possible pairs of stationary phases, the selectivity is the same ($a = 1.00 \pm 0.10$). Even for the systems differing in retention power (see, *e.g.*, the capacity factors of solutes separated on Nova Pak C_{18} and Zorbax ODS), the selectivity is the same. The greatest differences in selectivity are observed for Partisil ODS relative to the remaining phases, *e.g.*, the slope a for Partisil ODS 2 *vs.* Partisil ODS equals 1.90 and for Nova Pak C_{18} *vs.* Partisil ODS is 1.96.

Nevertheless, despite its interest, such a comparison of the retention mechanism does not give details about the specific properties of particular phases or compounds. So, even if the correlation coefficients of the plots of log k'_{ij} *versus* log $k'_{ij'}$

are very high, the selectivity of, for example, the naphthalene (n) and α-nitronaphthalene (nn) test compounds (see Table V) cannot be predicted with the $a_{jj'}$ parameters.

The specificity of the packings could be discussed in terms of the deviations from proportionality between log k'_{ij} and log $k'_{ij'}$ for the particular compounds and for all the possible phase pairs. However, to do so, one must consider the hyperspace of phases and compounds. To reduce this space, without loss of information, factor analysis is required.

Correspondence factor analysis

From the set of "abstract" factors, extracted by CFA, and from IE function, we can determine and delete the eigenvectors, which are composed of pure error, and choose the five remaining ones, which belong to the true primary set and reflect the real complexity of the data space. The first five main axes contribute 67.96, 16.71, 8.54, 2.42 and 2.27%, respectively to the total cluster inertia. The projections of the stationary phases and compounds onto the plane defined by the two main axes of inertia are presented in Fig. 1 and the coordinates of the phases on the five main axes are listed in Table VI. The factors which emerge from CFA are only "abstract" ones because they have no real physical or chemical meaning. To convert them into meaningful factors, the target procedure[47] is necessary.

Using a stepwise procedure it was found that only the axis 1 correlates well with the "hydrophobicity" of compounds and that introduction of the remaining axes does not improve this correlation. The "hydrophobicity" parameters were taken as a sum of Rekker's hydrophobic fragmental constants[48], f, which were obtained from octanol–water partition data for a large series of benzene derivatives

TABLE V

THE SLOPES OF LOG k'_{ij} *VERSUS* LOG $k'_{ij'}$ PLOTS FOR 63 COMPOUNDS, $a_{jj'}$(63), AND FOR NAPHTHALENE (n) AND NITRONAPHTHALENE (nn), $a_{jj'}$(n, nn)

j' = Zorbax ODS stationary phase.

*Chromatographic system**, j	$a_{jj'}$ *(63)*	$a_{jj'}$ *(n, nn)*
1	0.65	0.10
2	0.96	0.92
3	0.51	−0.02
4	0.96	0.64
5	0.82	0.66
6	0.97	0.97
7	0.76	0.37
8	0.85	0.90
9	0.98	0.66
10	0.79	0.33
11	1.01	1.18
12	0.81	0.64
13	0.77	0.41

* The identification numbers are as in Table II.

$$\log P = \sum_{1}^{n} c_n f_n$$

where P is the partition coefficient and c is a numerical factor indicating the influence of a given fragment in the structure.

As the axis 1 reflects the differences in the "hydrophobicity" of the solutes, the remaining axes could be considered as the factors responsible for the non-hydrophobic effects. The relative contributions of these effects can be estimated in the following manner: the χ^2 distance between two stationary phases, j and j', in the multidimensional space is defined[43] as

$$\chi^2(j,j') = k \sum_{i=1}^{n} (k'_{ij}/k_{.j} - k'_{ij'}/k_{.j'})/k_{i.}$$

where p and n are respectively the number of stationary phases and of compounds, and

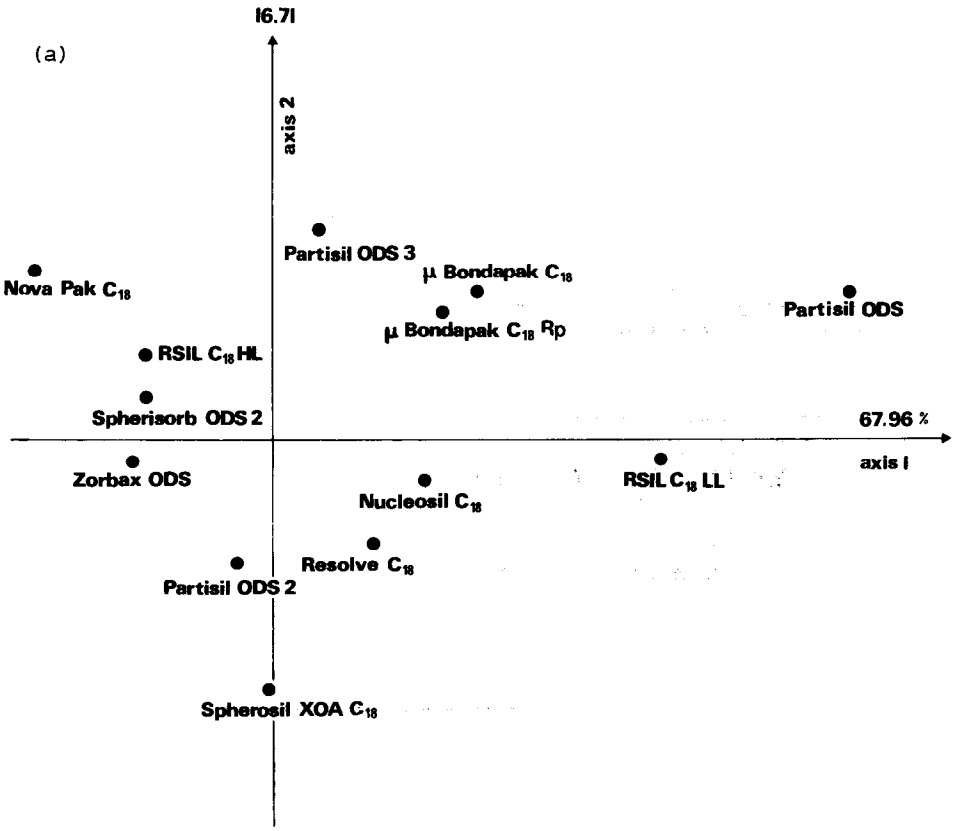

Fig. 1.

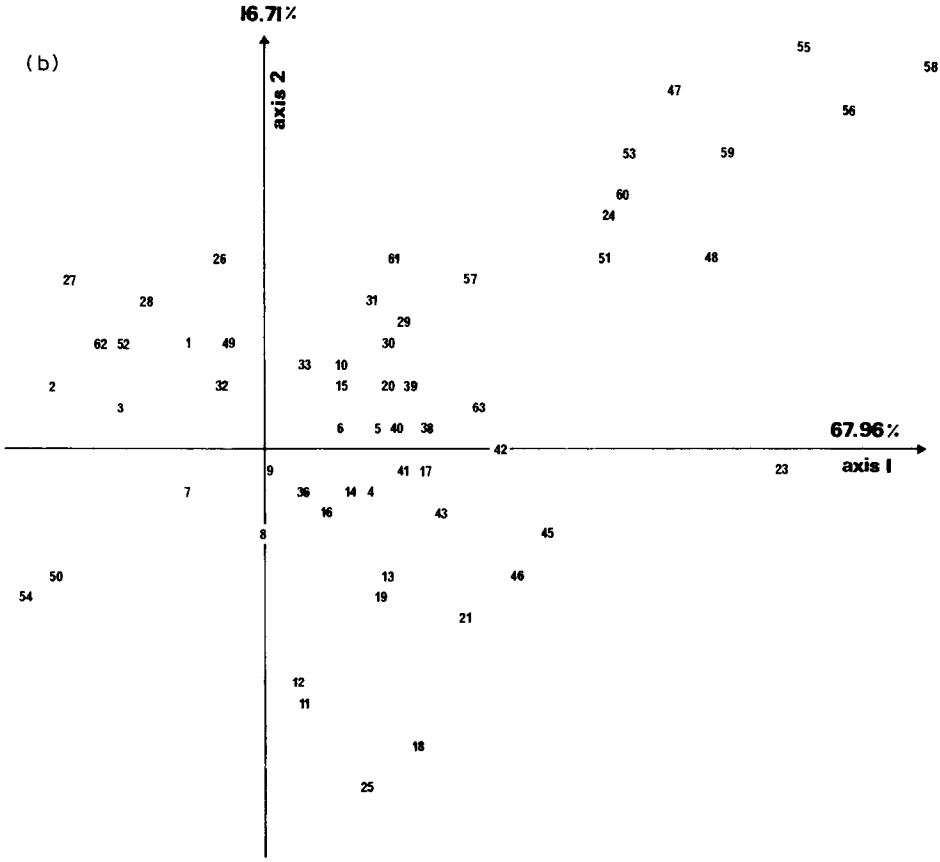

Fig. 1. CFA of the behaviour of 63 compounds on 14 ODS reversed-phase chromatographic systems. Projection on the plane defined by the main axes of inertia 1 and 2: (a) of the 14 chromatographic systems; (b) of the 63 compounds.

$$k_{i.} = \sum_{j=1}^{p} k'_{ij}$$

$$k_{.j} = \sum_{i=1}^{n} k'_{ij}$$

$$k = \sum_{i=1}^{n} \sum_{j=1}^{p} k'_{ij}$$

This distance between two stationary phases is a very useful measurement of the differences in the solute selectivity on the jth and j'th phases. It reflects the deviation from the proportionality between the capacity factors k'_{ij} and $k'_{ij'}$ of all 63 compounds. If k'_{ij} is proportional to $k'_{ij'}$, then the selectivity on the jth and j'th phases is the same

TABLE VI

THE COORDINATES OF THE 14 RP-HPLC SYSTEMS ON THE FIRST FIVE MAIN AXES OF INERTIA AS DEDUCED FROM CFA

*Chromatographic system**	*Coordinates*				
	Axis 1	*Axis 2*	*Axis 3*	*Axis 4*	*Axis 5*
1	0.208	−0.006	0.027	−0.002	0.019
2	−0.073	0.025	−0.021	0.024	0.022
3	0.307	0.044	0.099	0.029	0.014
4	−0.023	−0.044	−0.003	−0.008	0.018
5	0.022	0.064	−0.021	−0.001	0.001
6	−0.075	0.012	−0.027	0.024	−0.023
7	0.106	0.043	−0.063	−0.007	−0.007
8	−0.021	0.059	−0.011	0.017	−0.011
9	−0.009	−0.085	−0.027	−0.001	0.005
10	0.077	−0.019	0.022	0.002	0.006
11	−0.137	0.052	0.046	−0.009	0.020
12	0.052	−0.034	0.032	0.024	−0.029
13	0.089	0.041	−0.028	−0.032	−0.007
14	−0.083	−0.007	0.031	0.019	−0.019

* The identification numbers are as in Table II.

and $\chi^2 = 0$. In addition, the χ^2 distance does not depend on the absolute values of the capacity factors. It does not reflect the differences in the "retention power" of the jth and j'th phases, only the differences in the solute selectivity. In the constructed space, the χ^2 distance can be expressed as

$$\chi^2(j,j') = (x_{1j} - x_{1j'})^2 + (x_{2j} - x_{2j'})^2 + (x_{3j} - x_{3j'})^2 + (x_{4j} - x_{4j'})^2 + (x_{5j} - x_{5j'})^2$$

where x_{ij} is the coordinate of the jth phase on the ith axis. Then, the relative contribution of the "hydrophobic" effect to χ^2, denoted as a^h, is:

$$a^h = (x_{1j} - x_{1j'})^2 / \chi^2(j,j')$$

From the phase coordinates, listed in Table VI, one can easily estimate the a^h parameters for each phase pair. These parameters for the pairs of packings which include Zorbax ODS are presented in Table VII.

The greatest value of the χ^2 distance is observed for the pair Zorbax ODS–Partisil ODS and for the Zorbax ODS–RSIL C_{18} LL ones, being 0.163 and 0.086 respectively. This means that the solute selectivity on these pairs differs to a high degree. The relative contributions of the hydrophobic effect, a^h, to the $\chi^2(j,j')$ distance are 0.93 and 0.98, respectively, so the "hydrophobicity" of these packings is the main factor influencing solute selectivity. The lowest χ^2 values are observed for the pairs Zorbax ODS–Spherisorb ODS-2, Zorbax ODS–RSIL C_{18} HL, Zorbax ODS–Partisil ODS 2 and Zorbax ODS–Nova Pak C_{18}. This means that the solute selectivity on these phases is very similar to that on Zorbax ODS.

The CFA results, based on the χ^2 distance, indicate the similarity of the phase

TABLE VII

$\chi^2(j, j')$ MEASUREMENT OF THE DIFFERENCES IN THE SOLUTE SELECTIVITY ON THE jth AND j'th STATIONARY PHASES AND CORRESPONDING RELATIVE CONTRIBUTIONS, a^h, OF THE HYDROPHOBIC EFFECT

j' is the Zorbax ODS phase.

*Chromatographic system**, j	$\chi^2(j, j')$	a^h
1	0.086	0.98
2	0.007	0.01
3	0.163	0.93
4	0.008	0.47
5	0.019	0.56
6	0.006	0.01
7	0.047	0.75
8	0.011	0.34
9	0.016	0.35
10	0.027	0.95
11	0.008	0.35
12	0.021	0.87
13	0.036	0.83

* The identification numbers are as in Table II.

selectivity, reflect the true complexity of the data space, estimate the relative importance of the extracted factors and depict the compound and phase specificities. Additionally, it is possible to recreate the $a_{jj'}$ parameters. Let us define the $\overline{k'_i}$ parameters as:

$$\overline{k'_i} = \sum_{j=1}^{14} k'_{ij}/14$$

Then the slope of the log k'_{ij} *versus* log $\overline{k'_i}$ relationship, denoted as $\overline{a_j}$, can be expressed as a linear combination of the jth phase coordinates

$$\overline{a_j} = 1.01 - 1.35\, x_{1j} - 0.83\, x_{2j} - 0.21\, x_{3j} - 0.42\, x_{4j} + 0.28\, x_{5j}$$

where x_{1j}, x_{2j}, x_{3j}, x_{4j} and x_{5j} are the jth phase coordinates on the ith axis (i = 1, 2, 3, 4, 5), respectively. The $a_{jj'}$ parameters for the phase pair j and j' are then equal to

$$a_{jj'} = \overline{a_j}/\overline{a_{j'}} = \frac{1.01 - 1.35\, x_{1j} - 0.83\, x_{2j} - 0.21\, x_{3j} - 0.42\, x_{4j} + 0.28\, x_{5j}}{1.01 - 1.35\, x_{1j'} - 0.83\, x_{2j'} - 0.21\, x_{3j'} - 0.42\, x_{4j'} + 0.28\, x_{5j'}}$$

Such a possibility is important from a physico-chemical point of view and is currently being investigated in our laboratory. It underlines once more the interest of CFA.

CONCLUSIONS

Plots of $\log k'_{ij}$ values *versus* $\log k'_{ij'}$ values, obtained on two stationary phases, j and j', with the same mobile phase, can serve as a useful tool for comparing the energetics of solute retention on different packings. Nevertheless, even if the statistical criteria, proposed by Melander *et al.*[13] are fulfilled, and the separation mechanism on the two phases is classified as homo- or homeoenergetic, deviations from proportionality are observed for particular compounds, and these deviations ought to be considered as the main source of information about the specific properties of the packings.

The interest in this correspondence factor analysis (CFA) is to reduce the dimensions of the multidimensional space of packings and of compounds. CFA helps to estimate the true complexity of the chromatographic data and the relative importance of the main factors affecting solute selectivity. By use of the χ^2 (j,j') distance as the measure of the differences in the solute selectivity, no information about the phase specificity is lost.

From the CFA carried out for the 63 compounds separated on the 14 RP-HPLC packings, it appears that five factors influence the solute selectivity on the ODS packings of different origins. The first factor representing 68% of the information content is the "hydrophobicity" of the phases, and the remaining ones are the chemical and/or steric factors influencing solute selectivity. It is possible to estimate the relative importance of the hydrophobic and the non-hydrophobic effects.

Using factor analysis of the retention behaviour of large series of compounds, as a probe of the retention mechanism in RP-HPLC, work is underway in two complementary directions: extension of the comparison of packings to polar, chemically bonded phases; study of the selectivity of the compounds with the emphasis on specific interactions.

REFERENCES

1 K. K. Unger, *Porous Silica (Journal of Chromatography Library*, Vol. 16), Elsevier, Amsterdam, 1979.
2 R. E. Major and M. J. Hopper, *J. Chromatogr. Sci.*, 12 (1974) 767.
3 H. Hemetsberger, W. Maasfeld and H. Ricken, *Chromatographia*, 9 (1976) 303.
4 K. K. Unger, N. Becker and P. Roumeliotis, *J. Chromatogr.*, 125 (1976) 115.
5 K. Karch, J. Sebestian and J. Halász, *J. Chromatogr.*, 122 (1976) 3.
6 H. Hemetsberger, M. Kellermann and M. Ricken, *Chromatographia*, 10 (1977) 726.
7 M. C. Hennion, C. Picard and M. Caude, *J. Chromatogr.*, 166 (1978) 21.
8 P. Roumeliotis and K. K. Unger, *J. Chromatogr.*, 149 (1978) 211.
9 H. Hemetsberger, P. Behrensmeyer, J. Henning and R. Ricken, *Chromatographia*, 12 (1979) 71.
10 C. H. Lochmüller and D. R. Wilder, *J. Chromatogr. Sci.*, 17 (1979) 574.
11 G. E. Berendsen and L. de Galan, *J. Chromatogr.*, 196 (1980) 21.
12 N. Tanaka, K. Sakagami and M. Araki, *J. Chromatogr.*, 199 (1980) 327.
13 W. Melander, J. Stoveken and Cs. Horváth, *J. Chromatogr.*, 199 (1980) 35.
14 H. Engelhardt and H. Müller, *J. Chromatogr.*, 218 (1981) 395.
15 R. P. W. Scott and P. Kucera, *J. Chromatogr.*, 142 (1977) 213.
16 J. L. DiCesare and M. W. Dong, *Chromatogr. Newsl.*, 10 (1982) 12.
17 C. Gonnet, C. Bory and G. Lachatre, *Chromatographia*, 16 (1982) 242.
18 B. Shaikh and J. E. Tomaszewski, *Chromatographia*, 17 (1983) 675.
19 M. Zakaria and P. R. Brown, *J. Chromatogr.*, 255 (1983) 151.
20 G. Schomburg, A. Deege, J. Köhler and U. Bien-Vogelsang, *J. Chromatogr.*, 282 (1983) 27.
21 A. P. Goldberg, *Anal. Chem.*, 54 (1982) 342.

22 S. A. Wise and W. E. May, *Anal. Chem.*, 55 (1983) 479.
23 S. A. Wise, L. C. Sander and W. E. May, *J. Liq. Chromatogr.*, 6 (1983) 2709.
24 P. Spacek, M. Kubin, S. Vozka and B. Porsch, *J. Liq. Chromatogr.*, 3 (1980) 1465.
25 C. Dewaele, P. Mussche and M. Verzele, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 5 (1982) 616.
26 C. H. Lochmüller and D. B. Marshal, *Anal. Chim. Acta*, 142 (1982) 63.
27 L. C. Sander and S. A. Wise, *J. Chromatogr.*, 316 (1984) 163.
28 J. F. K. Huber and F. Eisenbeiss, *J. Chromatogr.*, 149 (1978) 127.
29 M. Verzele, J. Van Dijck, P. Mussche and C. Dewaele, *J. Liq. Chromatogr.*, 5 (1982) 1431.
30 D. E. Domagalska and C. R. Loscombe, *Chromatographia*, 15 (1982) 657.
31 C. Dewaele and M. Verzele, *J. Chromatogr.*, 260 (1983) 13.
32 M. Verzele, M. de Potter and J. Bhysels, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 2 (1979) 151.
33 M. Verzele and C. Dewaele, *J. Chromatogr.*, 217 (1981) 399.
34 L. C. Sander and S. A. Wise, *Anal. Chem.*, 56 (1984) 504.
35 M. Verzele and P. Mussche, *J. Chromatogr.*, 254 (1983) 117.
36 R. F. Hirsch, R. Gaydosh and J. R. Chrétien, *Anal. Chem.*, 52 (1980) 723.
37 B. Walczak, M. Dreux, J. R. Chrétien, K. Szymoniak, M. Lafosse, L. Morin-Allory and J. P. Doucet, *J. Chromatogr.*, 353 (1986) 109.
38 B. Walczak, J. R. Chrétien, M. Dreux, L. Morin-Allory, M. Lafosse, K. Szymoniak and F. Membrey, *J. Chromatogr.*, 353 (1986) 123.
39 B. Walczak, L. Morin-Allory, J. R. Chrétien, M. Lafosse and M. Dreux, *Chemometrics Intell. Lab. Systems*, 1 (1986) 79–90.
40 B. Walczak, J. R. Chrétien, M. Dreux, L. Morin-Allory and M. Lafosse, *Chemometrics, Intell. Lab. Systems*, in press.
41 B. Walczak, M. Lafosse, J. R. Chrétien, M. Dreux and L. Morin-Allory, *J. Chromatogr.*, 369 (1986) 27.
42 T. Foucart, *Analyse Factorielle*, Masson, Paris, 2nd ed., 1985.
43 J. P. Benzécri, *l'Analyse des Données*, Vol. 2, Dunod, Paris, 1973.
44 P. J. Schoenmakers, H. A. H. Billiet and L. de Galan, *Chromatographia*, 15 (1982) 205.
45 R. A. Keller, B. L. Karger and L. R. Snyder, in R. Stok (Editor), *Gas Chromatography 1970*, Institute of Petroleum, London, 1971, p. 125.
46 B. L. Karger, L. R. Snyder and C. Eon, *J. Chromatogr.*, 125 (1976) 71.
47 E. R. Malinowski and D. C. Howery, *Factor Analysis in Chemistry*, Wiley, New York, 1980.
48 G. G. Nys and R. F. Rekker, *Eur. J. Med. Chem. Chim. Ther.*, 9 (1974) 361.