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Journal of Chromatography A, 954 (2002) 99–114

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Minimal number of chromatographic test parameters for the characterisation of reversed-phase liquid chromatographic stationary phases

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Received 26 September 2001; received in revised form 11 February 2002; accepted 13 February 2002

Abstract

This paper focuses on the classification or differentiation of RP-HPLC columns based on measured chromatographic properties. A chemometric study has been conducted on a published data set consisting of 85 RP-HPLC columns and on a data set consisting of 47 self-tested columns. Principal component analysis enables determination of the number of parameters necessary for a rational differentiation. The results show that reducing the number of parameters for such differentiation still allows classification of the columns just as a higher number did. It is shown that three test parameters produce a classification similar to that obtained with five parameters.

Keywords: Stationary phases, LC; Principal component analysis; Chemometrics; Test parameters; Column testing

1. Introduction

Chromatographic separations described in official methods (European Pharmacopoeia, US Pharmacopeia) may fail because of the lack of information given in a monograph to characterise a suitable column. The large number of commercially available RP-HPLC columns and their potentially very different selectivity [1,2] require a classification of the stationary phases in order to facilitate the selection of appropriate columns for a given application. The classification should be based on chemical properties that chromatographically can be measured, as e.g. column efficiency, hydrophobicity, steric selectivity, silanol activity, ion-exchange capacity, metal impurity and polar interactions, and which are directly related to the actual chromatographic performance of the column [1,3–7]. The chemical properties are more relevant than the physical ones (e.g. specific surface, particle size, pore size, chain length) which are now defined in compendial methods, since these latter are only indirectly related to the chromatographic behaviour of the RP stationary

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0021-9673/02/$ – see front matter
PII: S0021-9673(02)00166-8

phases [2,8], and are often insufficient to choose a suitably performing column. Therefore a project was started to characterise and classify RP-stationary phases based on test parameters derived from chromatographic tests [9] and to correlate the results of these parameters to the selectivity of the columns for a given mixture. Correlation of the obtained classifications with the physical properties of the stationary phases on the other hand is not the issue here; lack of such correlation was reported earlier [2,8].

The project consists of three parts. First, one needs to characterise the stationary phases. A suitable protocol should allow measuring a number of parameters that reflect chromatographic characteristics. An overview of the chromatographic properties and of the many parameters measured to estimate them is given in Ref. [9]. However, the question is which and how many of those parameters are necessary for a suitable test procedure. Those maintained should be reproducible, fast and simple.

The second step is to classify columns with closely related characteristics. Preferably this classification should be based on a minimal number of test parameters. However, stationary phase characterisation can result in many measured parameters [1,3,5,7–10]. Traditional plotting in a Cartesian representation of data is limited to a maximum of three parameters at the time. When three or more parameters are considered multivariate techniques can be used to obtain (two-dimensional) graphical representations of the classifications. Principal component analysis (PCA), for instance, could be a helpful tool for such visualisation [1,5,7,8,11–13].

The third step consists in converting the obtained information to a procedure suitable for use in pharmaceutical monographs. It demands correlating the test parameter results to the selectivity required by the monograph, e.g. for the separation of a pharmaceutical substance from impurities. This correlation should lead to the definition of system suitability test limits for the test parameters, which would allow distinguishing between more and less suitable columns.

The work described in this paper is related to the second part, namely the search for a minimal number of measured parameters which are still able to achieve a meaningful classification. Chemometric techniques have been used previously in the literature to classify columns with similar characteristics [11,12]. Hamoir et al. [13] used a PCA variant, namely spectral mapping analysis, for this purpose. Olsen et al. [8] determined five chromatographic properties (hydrophobic and free silanol interactions, trace metal activity, silanol interaction and shape selectivity) of seventeen octadecylsilyl phases in order to examine column similarities and differences for column selection in method development. They used principal components and cluster analysis to analyse their data. Cruz et al. [5] characterised 30 different commercially available columns. The data set was according to Ref. [3], measuring five chromatographic properties (amount of alkyl chains, hydrophobicity, steric selectivity, hydrogen-bonding capacity, ion-exchange capacity at $pH>7$ and $pH<3$), while also an efficiency parameter for each column was added. The column properties were graphically presented using adapted Tanaka radar plots [3,5]. PCA and cluster analysis were used to group columns having similar chromatographic properties. Euerby et al. [1] increased the Cruz data set to 85 columns. The classification was performed using PCA. They found by an initial PCA that three of the seven parameters were highly correlated, hence only five parameters were included in the column characterisation procedure.

In the context of the project, we would like to make a relevant visual classification based on a minimal number of parameters which in earlier publications was not the goal. Therefore, the aim of this paper is to evaluate if PCA offers a possibility of reducing the number of parameters while maintaining the classification. In the first instance, the data set of Euerby et al. [1] is used. Then, whether the results found in the Euerby data set could be confirmed on a second set consisting of 47 self-tested columns is evaluated.

The other techniques, such as the Tanaka plot and the cluster analysis, were not considered. Tanaka plots are made for each column individually and for large numbers of columns it is not easy to compare them visually or to make classifications. The dendrograms resulting from the cluster analyses were not evaluated either since we considered it less evident to visually derive groups of similar or dissimilar columns than is the case from the plots resulting from a PCA analysis.

2. Theory

2.1. Principal component analysis

PCA is a chemometric tool for data reduction that allows the representation of high-dimensional data in fewer dimensions [1,14,15]. For instance, in our case, three or more parameters measured for each column which are represented in two dimensions. The original data set is an $m \times n$ table or matrix in which m represents the number of objects (chromatographic stationary phases in our case) and n the variables (here, test parameters). To visualise the content of such data table one should be able to draw n-dimensional plots, which graphically is impossible. The PCA reduces the n variables to a few latent variables or principal components without losing significant information. These principal components can be considered as new axes drawn in the original n-dimensional space. Often PCA is performed, not on the original data set, but on transformed data. Here autoscaling was performed first, i.e. from each value of a matrix column the mean (average value of a given parameter) is subtracted (=centering) and the result is divided by the standard deviation of that parameter. Autoscaling removes occasional differences in the order of magnitude between the different variables. By performing this data treatment all variables are expressed on a same scale in standard deviation units.

For centered or autoscaled data, the first principal component (PC1) is the latent variable passing through the centre of the data set and explaining the largest variation in the data. The second principal component (PC2), which is orthogonal to the first, goes through the centre of the data and explains the largest amount of the remaining variation. The third PC (PC3) is orthogonal to PC1 and PC2 and explains the largest amount of the still remaining variation, etc. The projections of the objects onto the PCs are called scores. A score plot represents the scores of the objects on two of the PCs (examples are shown in Results and discussion). The score plots give information about the objects and allow to identify those with closely related properties, i.e. the columns with similar test characteristics. Mathematically, a score on a principal component is a weighted sum of the original variables. The weights are called loadings. Each original variable has a loading on each PC. A loading plot represents the loadings of the variables on two of the PCs (examples are shown in Results and discussion). The loading plots give information about the original variables (test parameters) i.e. about their influence and importance on the PCs. Moreover, they also give information on the correlation of the variables.

3. Experimental

3.1. Chemicals

Solvents were of HPLC grade, other chemicals were AR grade. Methanol was obtained from BDH (Poole, UK), acetonitrile from BDH and Biosolve (Valkenswaard, The Netherlands). All other substances were obtained from Acros Organics (Beerse, Belgium). Water was purified by the Milli-Q water purification system (Millipore, Milford, MA, USA).

3.2. pH Measurements

Consort C831 (Consort, Turnhout, Belgium) and Ankersmit A520 (Orion, Boston, MA, USA) pH meters equipped with a glass electrode were used for pH measurements. The electrodes were calibrated daily with appropriate buffers.

3.3. Chromatography

Analyses were carried out on two instruments. The first consisted of a 9010 LC pump, a 9100 autosampler and a 9050 UV–Vis detector, all from Varian (Walnut Creek, CA, USA). CHROMPERFECT 4.4.0 software (Justice Laboratory Software, Fife, UK) was used for data acquisition and treatment. The second chromatograph was composed of a Merck–Hitachi (Tokyo, Japan) L-6200 Intelligent Pump equipped with a Rheodyne (Rheodyne, Cotati, CA, USA) injector, a T-6300 column thermostat, a L-4000 UV detector and a D-2500 Chromato-Integrator.

The details concerning the chromatographic conditions applied can be found in Refs. [5,9]. All tested columns were donated by the manufacturers or distributors.

3.4. Calculations

The autoscaling and PCA calculations were executed with a MATLAB 4.0 program (MathWorks, Natick, MA, USA).

4. Results and discussion

We first examined a published data set, taken from Euerby et al. [1], which consists of 85 HPLC columns (objects) and seven chromatographic parameters (variables). The investigated columns were mainly silica based C_8 and C_{18} columns, but there are some exceptions as e.g. C_4 or C_{16} aluminium oxide or zirconium oxide based and polymer columns. The classification performed is focused only on the silica based C_8 and C_{18} columns, since they are most commonly used in the compendial analyses. It is also shown earlier that the differences in selectivity within C_{18} and C_8 columns might be as large as those between the two types of support [2]. All other stationary phases were eliminated so that 74 columns remained (Table 1). The column numbers of Ref. [1] were maintained. The seven variables were: (i) the retention factor of pentylbenzene (k_{PB}) and (ii) the number of plates per metre (N) from the pentylbenzene peak, reflecting the column efficiency; (iii) the selectivity factor between pentylbenzene and butylbenzene (α_{CH_2}) reflecting the hydrophobicity; (iv) the selectivity factor between triphenylene and *o*-terphenyl ($\alpha_{T/O}$) reflecting the steric selectivity; (v) the selectivity factor between caffeine and phenol ($\alpha_{C/P}$) reflecting hydrogen-bonding capacity; and (vi–vii) the selectivity factors between benzylamine and phenol at pH 7.6 ($\alpha_{A/P\ pH\ 7.6}$) and at pH 2.7 ($\alpha_{A/P\ pH\ 2.7}$) reflecting the ion-exchange capacity.

The data set consisting of the 47 self-tested columns is shown in Table 2. All columns were C_{18} silica based, with the exception of columns 1 and 2, which were C_8 ones. In Table 2 the column efficiency is expressed as n, the number of plates per column length, for reasons explained later.

4.1. PCA on the Euerby data

The PCA shown in the paper by Euerby et al. [1] was carried out with five parameters on the autoscaled data set [14] and was in the first instance repeated in this study. The theoretical plate number and the retention factor of pentylbenzene were eliminated, due to the high correlation with the hydrophobicity, which means that those three parameters give the same information. Euerby et al. [1], prior to chemometrical analysis, classified the columns according to the type of supports. Three groups were distinguished: (i) non-endcapped columns (A) with poor surface coverage based on acidic silica– (columns 47, 51); (ii) polar-embedded columns (B) having a polar group (e.g. a carbamate, amide, urea or other moiety) embedded between the silica and the alkyl chain to mask silanol groups (columns 6, 21, 58, 60, 62, 63, 72, 77); and (iii) other C_8 and C_{18} columns (C) with different degrees of endcapping and differing silicas. The score and loading plots on PC1 and PC2 are shown in Fig. 1. The classes as defined in Ref. [1] are indicated.

The three types of support are somewhat differentiated, but some columns from different groups have related characteristics, i.e. are close to each other on the score plot. The non-endcapped columns are clearly separated from the others. They have the highest ion-exchange capacities at low and at high pH and the highest hydrogen-bonding capacities. This can be seen from the loadings in Fig. 1b and from the results of Table 1. These variables namely have the highest loadings on PC1 while on PC2 they are close to zero. The scores of these columns on PC1 are also high while they are close to zero on PC2, meaning they are mainly determined by the above variables. The differentiation of the polar-embedded columns is not so straightforward on the PC plot of Fig. 1a. Columns 6, 21, 58, 60, 62, 63, 72, all have high steric selectivity values and are grouped, but column 77 is obviously closely related to the third group and its steric selectivity is lower. Furthermore, columns 3 and 45, which are located near to the polar-embedded columns do not have polar-embedded groups.

The above indicates that the classification based on the type of support is different from that which would be made when only the test parameter results are used without taking into account this background information. Fig. 2 shows the classification made without taking into account prior knowledge about

Table 1
Characterization of RPLC columns, data set of Euerby et al. [1]

No.	Description	k_{PB}	α_{CH_2}	$\alpha_{T/O}$	$\alpha_{C/P}$	$\alpha_{A/P}$ pH 7.6	$\alpha_{A/P}$ pH 2.7	N (m^{-1})	dp (μm)	Supplier/ producer
1	ACE5 C_{18}	3.96	1.48	1.34	0.35	0.63	0.13	59 800	5	Hichrom
3	C_{18} multiring	1.86	1.46	2.35	0.56	1.23	0.09	35 800	5	Vydac
4	Develosil ODS-G-5	6.70	1.49	1.24	0.51	0.10	0.07	63 300	5	Phenomenex/ Nomura Chemicals
5	Discovery C_{18}	3.32	1.48	1.51	0.39	0.28	0.10	80 300	5	Supelco
6	Discovery RP-amide C_{16}	1.65	1.35	1.81	0.49	0.44	0.19	82 600	5	Supelco
9	Genesis C_{18}	6.25	1.50	1.41	0.44	0.29	0.10	72 600	4	Jones Chromatography
10	Genesis C_8	2.09	1.33	1.01	0.55	0.60	0.12	74 500	4	Jones Chromatography
11	Grom-Sil 100ODS-2FE	4.68	1.46	1.72	0.59	0.72	0.17	92 300	3	GROM
12	Grom-Sil ODS-0 AB	3.46	1.45	1.40	0.72	0.67	0.19	119 000	3	GROM
13	Grom-Sil ODS-4 HE	6.28	1.5	1.27	0.54	0.31	0.10	49 300	5	GROM
14	Grom-Sil ODS-7 pH	13.68	1.54	1.53	0.39	0.32	0.06	98 900	4	GROM
15	Hichrom RPB	4.56	1.40	1.21	0.36	0.18	0.11	71 900	5	Hichrom
16	Hypersil 100 C_{18}	7.66	1.53	1.40	0.42	1.01	0.25	79 000	5	TCS
17	Hypersil BDS	4.50	1.47	1.49	0.39	0.19	0.17	74 600	5	TCS
18	Hypersil Elite	4.76	1.49	1.52	0.37	0.30	0.14	75 100	5	TCS
19	Hypersil Hypurity Elite C_{18}	3.20	1.47	1.60	0.37	0.29	0.10	78 900	5	TCS
20	Hypersil ODS	4.44	1.45	1.28	0.38	1.04	0.64	76 100	5	TCS
21	Hypurity Advance (C_8)	1.13	1.00	1.59	0.39	0.80	0.13	38 400	5	TCS
23	Hypurity C_8	1.59	1.35	1.00	0.34	0.30	0.11	83 200	5	TCS
25	Intersil ODS	6.31	1.47	1.57	0.36	0.53	0.01	44 000	5	Hichrom
26	Jupiter C_{18} 300A	2.26	1.48	1.65	0.37	0.47	0.27	24 700	5	Phenomenex
27	Kromasil C_{18}	7.01	1.48	1.53	0.4	0.31	0.11	84 900	5	Hichrom
28	Lichrosphere RP Select B (C_8)	2.76	1.32	1.21	0.66	1.40	0.14	43 300	5	Merck
29	Lichrosphere RP18	7.92	1.48	1.73	0.54	1.39	0.19	46 100	5	Merck
30	Lunar C_{18} (16.5%C load)	5.97	1.47	1.17	0.40	0.24	0.08	89 700	5	Phenomenex
31	Lunar C_{18} (19%C load)	6.34	1.47	1.23	0.41	0.26	0.06	80 700	5	Phenomenex
32	Magellen C_{18}	6.19	1.50	1.24	0.41	0.27	0.15	111 300	3	Phenomenex
33	Novapak C_{18}	4.49	1.49	1.44	0.48	0.27	0.14	70 200	4	Waters
34	Nucleosil C_{18}	4.80	1.44	1.68	0.70	2.18	0.13	48 800	5	Hichrom
35	Optimal ODS-L	5.87	1.48	1.26	0.51	0.30	0.09	65 200	5	Capital HPLC
36	Optimal ODS-H	6.15	1.48	1.38	0.44	0.24	0.09	82 700	5	Capital HPLC
37	Prodigy ODS2	4.94	1.49	1.43	0.37	0.50	0.01	60 500	5	Phenomenex
38	Prodigy ODS3	7.27	1.49	1.26	0.42	0.27	0.09	73 000	5	Phenomenex
39	Prodigy ODS3 V S/N 318940 (C)	7.23	1.48	1.21	0.44	0.33	0.10	131 800	3	Phenomenex
40	Prodigy ODS3 V S/N 318940 (L)	6.18	1.47	1.24	0.42	0.38	0.11	121 200	3	Phenomenex
41	Prodigy ODS3V S/N 320269(C)	7.57	1.49	1.21	0.43	0.35	0.10	97 300	3	Phenomenex
42	Prodigy ODS3V S/N 320269 (L)	6.17	1.47	1.21	0.42	0.37	0.11	123 000	3	Phenomenex
43	Prodigy ODS3V S/N 320270 (C)	8.14	1.49	1.22	0.44	0.34	0.09	132 300	3	Phenomenex

Table 1. Continued

No.	Description	k_{PB}	α_{CH_2}	$\alpha_{T/O}$	$\alpha_{C/P}$	$\alpha_{A/P}$ pH 7.6	$\alpha_{A/P}$ pH 2.7	N (m^{-1})	dp (μm)	Supplier/ producer
44	Prodigy ODS3V S/N 320270 (L)	6.76	1.47	1.23	0.43	0.37	0.10	150 900	3	Phenomenex
45	Purosphere RP18	4.78	1.44	1.93	0.72	1.29	−0.07	27 600	5	Merck
46	Purosphere RP18e	6.51	1.48	1.75	0.46	0.34	0.08	66 000	5	Merck
47	Resolve C_{18}	2.40	1.46	1.59	1.29	4.06	1.23	47 700	4	Waters
48	Selectosil C_{18}	4.94	1.45	1.69	0.68	1.98	0.14	61 300	5	Phenomenex
49	SMT total coverage C_{18}	7.26	1.48	1.59	0.56	0.93	0.07	41 200	5	Separation Method Technologies
51	Spherisorb ODS1	1.78	1.47	1.64	1.57	2.84	2.55	85 800	5	Waters
52	Spherisorb ODS2	3.00	1.51	1.56	0.59	0.76	0.23	82 600	5	Waters
53	Spherisorb ODSB	5.09	1.46	1.78	0.80	3.56	0.06	51 400	5	Waters
54	Summit ODS (W)	5.45	1.47	1.29	0.56	0.40	0.10	88 300	3	Crawford
56	Supelcosil LC_{18}	4.82	1.47	1.42	0.46	1.93	0.89	60 800	5	Supelco
57	Supelcosil $LC_{18}DB$	5.16	1.51	1.40	0.42	0.47	0.14	52 300	5	Supelco
58	Supelcosil LC-ABZ	3.14	1.37	2.23	0.24	0.20	0.03	67 500	5	Supelco
59	Superspher RP18e	5.47	1.47	1.64	0.44	0.42	0.11	49 900	5	Merck
60	Suplex pkb 100	1.24	1.35	2.84	0.34	0.29	0.00	41 200	5	Supelco
61	Symmetry C_{18}	6.51	1.46	1.49	0.41	0.68	0.01	56 100	5	Waters
62	Symmetry Shield RP18	4.66	1.41	2.22	0.27	0.20	0.04	82 700	5	Waters
63	Symmetry Shield RP8 (C_8)	2.30	1.32	1.87	0.27	0.19	0.04	80 400	5	Waters
64	Targa C_{18}	6.06	1.63	1.27	0.52	0.10	0.14	97 200	5	Higgins Analytical
65	TSKGel super ODS	2.22	1.47	1.65	0.33	0.33	0.10	112 600	2	TosoHaas
66	TSKGelODS-80TM	5.07	1.46	1.34	0.58	0.65	0.09	91 000	5	TosoHaas
67	TSKGelODS-80TS	4.57	1.45	1.24	0.52	0.30	0.08	95 800	5	TosoHaas
68	μBondapak C_{18}	1.97	1.39	1.28	0.78	1.12	0.15	19 200	5	Waters
69	Ultracarb ODS(30)	13.27	1.52	1.39	0.48	0.73	0.06	65 400	5	Phenomenex
70	Ultrasphere ODS	6.41	1.52	1.42	0.48	0.31	0.16	66 300	5	Hichrom
71	Xterra MS C_{18}	3.52	1.42	1.26	0.42	0.35	0.10	41 500	3.5	Waters
72	Xterra RP18	2.38	1.29	1.83	0.33	0.20	0.07	40 700	3.5	Waters
73	YMC Basic (C_8)	1.40	1.26	0.98	0.57	0.51	0.27	52 100	5	YMC
74	YMC ODS-AQ	4.44	1.46	1.25	0.57	0.41	0.11	19 300	5	YMC
75	YMC $ProC_{18}$	7.42	1.53	1.29	0.46	0.26	0.08	79 800	5	YMC
77	Zorbax Bonus-RP	1.74	1.43	1.60	0.31	0.30	0.04	42 300	5	Agilent Tech.
78	Zorbax Eclipse XDB-C_{18}	5.79	1.50	1.30	0.47	0.35	0.09	38 500	5	Agilent Tech.
79	Zorbax Extend C_{18}	6.66	1.50	1.49	0.38	0.20	0.08	86 600	5	Agilent Tech.
80	Zorbax Rx C_{18}	5.68	1.57	1.61	0.54	0.55	0.11	88 100	5	Agilent Tech.
81	Zorbax SB-C_{18}	6.00	1.49	1.20	0.65	1.46	0.13	76 900	5	Agilent Tech.
83	Zorbax SB-C_8	1.97	1.37	1.08	1.27	0.81	0.12	63 000	5	Agilent Tech.

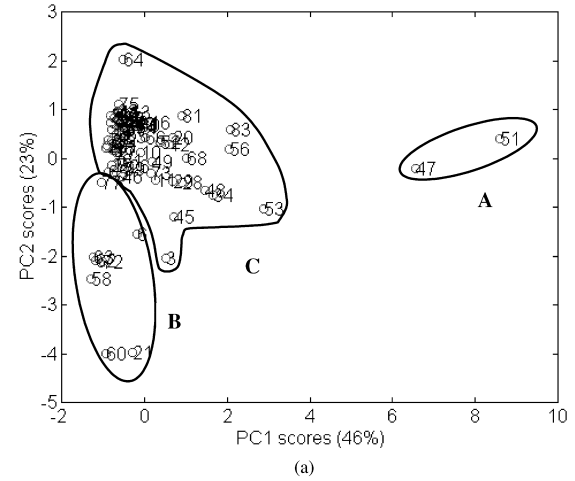
No., column number; N, efficiency, plates per metre; dp = particle diameter, μm. Other symbols are explained in the text.

the columns. It consists of a central group C, two relatively distinct groups A and B, and two somewhat outlying columns 53 and 64. Considering this latter classification is exactly our intention since there is no guarantee that columns with a given support would exhibit similar selectivity for a given separation. Furthermore, the total population of columns acceptable for a given compendial method, regardless of the subclass they belong to, is our group of interest and in compendial methods a subdivision within the C_{18} columns is not indicated either. Finally, later in the above-mentioned project the correlation with real separations has to be made. One then expects that columns with similar parameter results, rather than those belonging to the same type of support, will behave similarly, at least if the

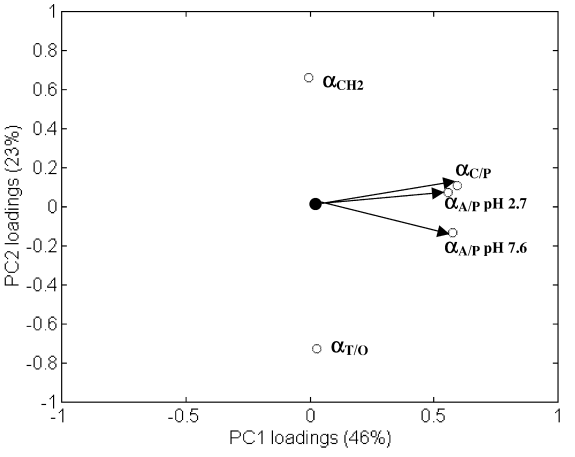
Table 2
Data set of the self-tested columns

No.	Description	k_{PB}	α_{CH_2}	$\alpha_{T/O}$	$\alpha_{C/P}$	$\alpha_{A/P}$ pH 7.3	$\alpha_{A/}$ pH 2.7_P	n	dp (μm)	Length (mm)	Manufacturer/ supplier
1	Discover C_8 [a]	1.13	1.34	1.00	0.39	0.44	0.060	1200	5	50	Supelco
2	Zorbax SB-C_8 [a]	1.77	1.37	0.93	1.27	1.13	0.066	3800	3.5	75	Agilent
3	Genesis C_{18-3}	6.43	1.48	1.38	0.44	0.25	0.078	9800	3	100	Jones Chromatography
4	Genesis C_{18-3}	6.35	1.48	1.37	0.43	0.33	0.083	8600	3	100	Jones Chromatography
5	Kromasil C_{18}	8.28	1.48	1.52	0.37	0.28	0.077	7500	5	100	Alltech
6	Nucleosil C_{18}	4.14	1.43	1.71	0.81	1.98	0.068	8600	3	100	Alltech
7	Hypersil BDS-3	3.89	1.46	1.52	0.36	0.27	0.12	7700	3	100	Alltech
8	Hypersil ODS-3	3.69	1.45	1.28	0.42	1.36	0.69	7800	3	100	Alltech
9	Spherisorb ODS2	5.43	1.45	1.56	0.63	1.47	0.22	9300	3	100	Waters
10	Symmetry C_{18}	6.08	1.47	1.56	0.37	0.28	0.024	9500	3.5	100	Waters
11	TSKGel super ODS	2.26	1.44	1.46	0.44	0.45	0.071	7800	2	100	TosoHaas/Sercolab
12	Uptispher HDO-3	5.50	1.47	1.28	0.46	0.35	0.064	8200	3	100	Interchim/Achrom
13	Uptispher ODB-3	5.36	1.45	1.42	0.47	1.00	0.095	9800	3	100	Interchim/Achrom
14	ACE-3 C_{18}	4.89	1.47	1.50	0.37	0.31	0.084	18 700	3	150	ACT/Achrom
15	YMC-Hydrosphere-3 C_{18}	4.51	1.47	1.18	0.53	0.23	0.0085	13 300	5	150	YMC/ThermoQuest
16	HyPurity Elite-3 C_{18}	3.10	1.46	1.55	0.37	0.29	0.086	14 400	3	150	ThermoQuest
17	HyPurity Elite-3 C_{18}	3.20	1.46	1.56	0.37	0.27	0.087	13 500	3	150	ThermoQuest
18	HyPurity Elite-5 C_{18}	3.12	1.46	1.58	0.36	0.29	0.080	10 700	5	150	ThermoQuest
19	HyPurity Elite-5 C_{18}	3.26	1.46	1.57	0.35	0.29	0.083	11 100	5	150	ThermoQuest
20	TSKGel ODS-80TS	5.62	1.47	1.27	0.46	0.32	0.060	13 100	5	150	TosoHaas/Sercolab
21	YMC-Hydrosphere-5 C_{18}	4.17	1.46	1.19	0.52	0.31	0.032	14 100	5	150	YMC/ThermoQuest
22	YMC-Pack Pro-3 C_{18}	5.71	1.47	1.32	0.44	0.29	0.047	19 900	3	150	YMC/ThermoQuest
23	YMC-Pack Pro-5 C_{18}	5.96	1.49	1.26	0.44	0.28	0.035	16 400	5	150	YMC/ThermoQuest
24	ACE-5 C_{18}	4.56	1.46	1.50	0.36	0.30	0.084	24 200	5	250	ACT/Achrom
25	Apex Basic C_{18}	2.14	1.40	2.32	0.79	0.95	0.0010	16 200	5	250	Jones Chromatography
26	Genesis C_{18-4}	6.99	1.49	1.32	0.43	0.30	0.078	24 000	4	250	Jones Chromatography
27	Genesis C_{18-4}	7.01	1.48	1.34	0.44	0.24	0.073	24 700	4	250	Jones Chromatography
28	Hypersil BDS-5	3.55	1.46	1.56	0.34	0.33	0.13	18 900	5	250	ThermoQuest
29	Hypersil ODS-5	3.56	1.45	1.30	0.42	1.31	0.68	17 400	5	250	ThermoQuest
30	Kromasil	6.17	1.45	1.62	0.41	1.97	0.065	18 800	5	250	Macherey–Nagel
31	Nucleosil	4.40	1.44	1.66	0.73	1.57	0.11	20 100	5	250	Macherey–Nagel
32	Nucleosil HD	6.01	1.47	1.44	0.41	0.33	0.081	20 800	5	250	Mach-N/Filter Service
33	Nucleosil HD Nautilus	3.21	1.40	1.95	0.33	0.39	0.012	18 300	5	250	Mach-N/Filter Service
34	OmniSpher	7.55	1.48	1.66	0.34	0.30	0.080	22 600	5	250	Varian
35	Supelcosil ABZ [a]	2.54	1.39	2.56	0.21	0.46	0.0022	4600	5	250	Supelco
36	Supelcosil LC-18-DB	4.12	1.47	1.40	0.41	0.32	0.13	6100	5	150	Supelco
37	Spherisorb ODS2	5.44	1.45	1.54	0.64	1.61	0.26	23 300	5	250	Waters
38	Symmetry C_{18}	6.50	1.47	1.56	0.38	0.31	0.022	22 300	5	250	Waters
39	Uptispher HDO-5	5.63	1.46	1.13	0.50	0.52	0.082	16 900	5	250	Interchim/Achrom
40	Uptispher ODB-5	6.27	1.47	1.36	0.44	0.38	0.070	17 600	5	250	Interchim/Achrom
41	Zorbax Eclipse XDB-C_{18}	6.03	1.50	1.29	0.40	0.30	0.072	22 100	5	250	Agilent
42	Zorbax Eclipse XDB-C_{18}	6.12	1.49	1.29	0.42	0.24	0.071	21 000	5	250	Agilent
43	Zorbax Extend C_{18}	6.16	1.50	1.47	0.36	0.43	0.067	19 200	5	250	Agilent
44	Zorbax Extend C_{18}	6.28	1.49	1.47	0.38	0.36	0.078	21 000	5	250	Agilent
45	Zorbax SB-C_{18}	4.97	1.47	1.22	0.56	0.61	0.067	21 200	5	250	Agilent
46	Zorbax SB-C_{18}	4.94	1.46	1.22	0.57	0.56	0.067	18 300	5	250	Agilent
47	Xterra RP18	2.07	1.33	1.82	0.32	0.15	0.045	5800	3.5	100	Waters

No., column number; *n*, efficiency; plates in terms of the length of the column, dp, particle diameter.
[a] Used, before testing.



(a)



(b)

Fig. 1. (a) PC1–PC2 score plot for 74 columns and 5 parameters (α_{CH_2}, $\alpha_{T/O}$, $\alpha_{C/P}$, $\alpha_{A/P\ pH\ 7.6}$ and $\alpha_{A/P\ pH\ 2.7}$); (b) PC1–PC2 loading plot. ● origin (0,0) of loading plot.

parameters used for classification give information about the mechanisms governing the retention and selectivity.

A chromatographic system suitability test is in practice only interesting when columns can be differentiated based on a very limited number of test parameters. Therefore in this paper it is verified that a reduced number of test parameters still allows differentiation between the columns to be made.

Although studying the PC1–PC2 score plot of Fig. 1 was sufficient for the purposes of Ref. [1], this plot

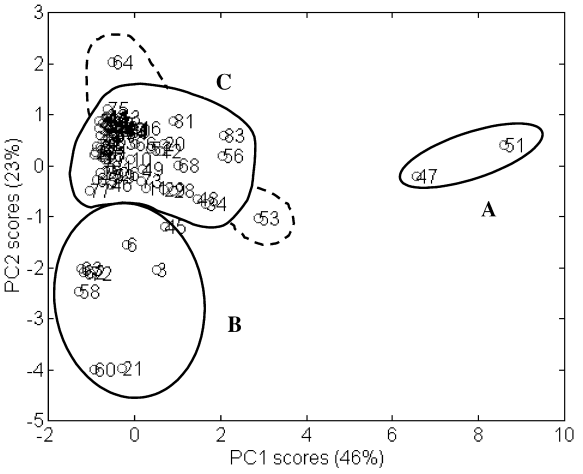


Fig. 2. PC1–PC2 score plot for 74 columns. Classification based on related scores, i.e. similar test parameter results.

describes only 69% of the total variation in the data. Therefore, the PC1–PC3 score plot was also checked (Fig. 3). It is observed that PC3 explains another 17.5% of the 31% remaining variation. A different classification can be distinguished consisting of a group A with columns 47 and 51; a central group C, a group D with columns 10, 21, 23, 28, 68, 73 and 83, and three outlying columns (3, 53 and 60). The columns of group D have low hydrophobicity and

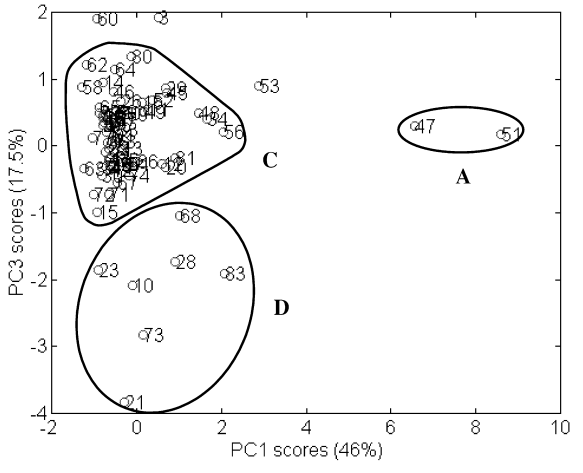


Fig. 3. PC1–PC3 score plot for 74 columns and 5 parameters (α_{CH_2}, $\alpha_{T/O}$, $\alpha_{C/P}$, $\alpha_{A/P\ pH\ 7.6}$ and $\alpha_{A/P\ pH\ 2.7}$).

steric selectivity values. It turned out they all are C_8 columns, except column 68.

4.2. Classification based on a reduced number of test parameters

To be practical, the number of test parameters to differentiate between columns preferably should be minimised. A reduction in parameters without loss of the classification would be possible if variables are correlated or if they do not have a relevant influence on the classification. In Fig. 1b the loadings for the hydrogen-bonding capacity and the ion-exchange capacities at high and at low pH are located near to each other. Parameters, for which the angle between the vectors connecting the origin of the plot with the loading of the parameter is small, are correlated [14,15]. This means they contain similar information. This is confirmed by calculating the correlation coefficients (r) between the parameters (Table 3). It can be seen that the correlation between $\alpha_{C/P}$ and $\alpha_{A/P\ pH\ 7.6}$ ($r=0.722$), between $\alpha_{C/P}$ and $\alpha_{A/P\ pH\ 2.7}$ ($r=0.666$) and between $\alpha_{A/P}$ at pH 7.6 and $\alpha_{A/P}$ at pH 2.7 ($r=0.584$) is highest. Even though the observed correlations are not extremely high it will be verified that the parameters contain enough similar information so that eliminating two of them does not lead to an important loss in information.

It could be argued that if only three parameters are considered one could make three-dimensional data plots without performing calculations. This is true, but we did not consider this possibility since it is often far from evident to visually define groups in such a cloud of data points. Further, we preferred to maintain one kind of data treatment independently of the number of parameters applied. Another risk we took by applying PCA on three parameters is that the dimensionality reduction would not be very successful since PCA is intended to work well only when correlated variables are available, which is exactly what is avoided here.

4.2.1. PCA on the Euerby data using three test-parameters: α_{CH_2}, $\alpha_{T/O}$ and $\alpha_{A/P\ pH\ 2.7}$

In Fig. 4 the PC1–PC2 score and loading plots for 74 columns and the variables α_{CH_2}, $\alpha_{T/O}$ and $\alpha_{A/P\ pH\ 2.7}$ are shown. Again three groups are clearly distinguished: group A with columns 20, 56, 47, 51 which have the highest ion-exchange capacities at pH 2.7. This is the previous group A (47, 51) obtained in the classification using five parameters, but now also including columns 20 and 56. These two columns are located closer to the origin than column 47 meaning they have lower $\alpha_{A/P}$ at pH 2.7. Group B (columns 3, 6, 21, 45, 58, 60, 62, 63, 72) is the same as that obtained using all five parameters. Group C: all other columns are included into this group which is also the main group when using five parameters (only difference: columns 20 and 56). It is also obvious that column 64, which has the highest hydrophobicity value, is again located separated from the rest.

Since the PC1–PC2 score plot explained only 71% of the variation, the PC1–PC3 plots were again checked (Fig. 5). It gave additional information about the columns. The columns 3, 6, 45, 58, 60, 62, 63 and 72 (group B) are recognised as on the PC1–PC2 score plot. Column 21 is now found completely separated, due to its low steric selectivity. Additionally the group of columns 10, 23, 28, 68, 73 and 83 (group D) which was also obtained on PC1–PC3 with five parameters is again formed (without column 21) and but now containing columns 15 and

Table 3
Correlation coefficients (r) between the test parameters; results of [1] were used

	α_{CH_2}	$\alpha_{T/O}$	$\alpha_{C/P}$	$\alpha_{A/P\ pH\ 7.6}$	$\alpha_{A/P\ pH\ 2.7}$
α_{CH_2}	1				
$\alpha_{T/O}$	−0.139	1			
$\alpha_{C/P}$	0.014	−0.084	1		
$\alpha_{A/P\ pH\ 7.6}$	−0.039	0.157	*0.722*	1	
$\alpha_{A/P\ pH\ 2.7}$	0.031	−0.003	*0.666*	*0.584*	1

The highest r values are italicised.

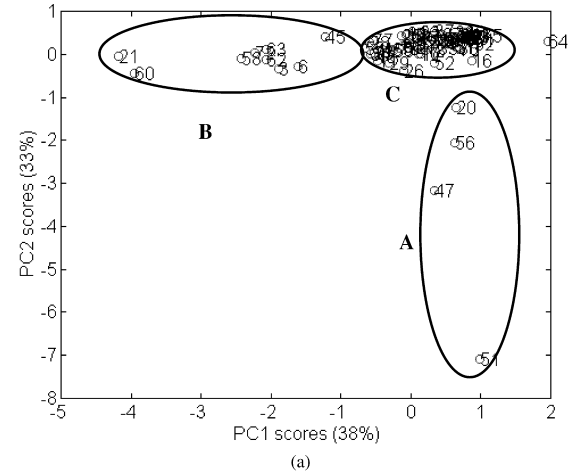


Fig. 4. (a) PC1–PC2 score plot for 74 columns and three parameters (α_{CH_2}, $\alpha_{T/O}$ and $\alpha_{A/P\ pH\ 2.7}$); (b) PC1–PC2 loading plot.

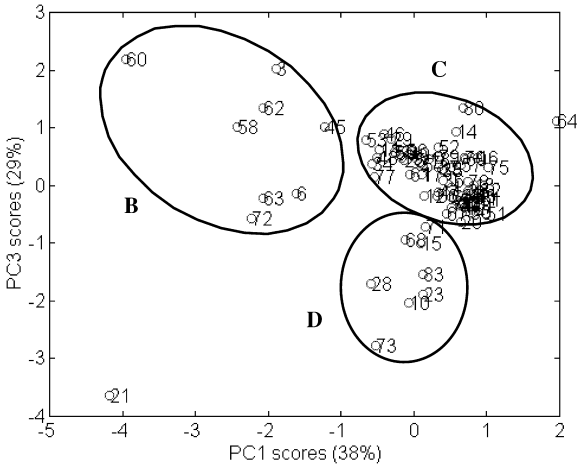


Fig. 5. (a) PC1–PC3 score plot for 74 columns and three parameters (α_{CH_2}, $\alpha_{T/O}$ and $\alpha_{A/P\ pH\ 2.7}$).

71. Columns 47 and 51 (group A) however, belong in this plot to the central cluster, group C.

4.2.2. PCA on the Euerby data using three variables: α_{CH_2}, $\alpha_{T/O}$ and $\alpha_{C/P}$

Another possibility to reduce the number of parameters is to eliminate the variables describing the ion-exchange capacities. In Fig. 6 the PC1–PC2 score and loading plots for 74 columns and the variables α_{CH_2}, $\alpha_{T/O}$ and $\alpha_{C/P}$ are shown. In this case four groups are distinguished, indicating that fewer parameters sometimes even discriminate more between columns. Group A: columns 47, 51 are grouped together with column 83 and are characterised by a high hydrogen-bonding capacity. Group B: columns 3, 6, 45, 58, 60, 62, 63 and 72 with a high steric selectivity. Column 21 is again found separated from the rest. Group D: columns 10, 23, 28, 34, 48, 68 and 73 are located near to each other, but were before only differentiated on PC1–PC3 plots (Figs. 3 and 5). The group now also includes column 12. They all have the lowest hydrophobicities. Group C: all other columns are included into one central group having similar hydrophobicity, again with the exception of column 64. For these three parameters no additional information was found on the PC1–PC3 score plot.

From the above it is observed that, on the PC1–PC2 score plot, these three parameters allow a better differentiation between the columns than the five of Fig. 2 and the three of Fig. 4. If this differentiation is also relevant for a given separation it will have to be demonstrated in the third step of the project. It was also observed that for this classification the loadings are more evenly distributed around the origin than in the previous situations. This resulted in a score plot in which the different columns were more spread and

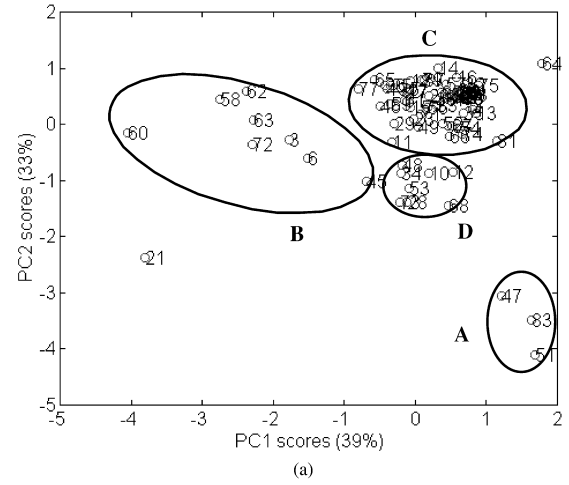


Fig. 6. (a) PC1–PC2 score plot for 74 columns and three parameters (α_{CH_2}, $\alpha_{T/O}$ and $\alpha_{C/P}$); (b) PC1–PC2 loading plot.

in which a clearer distinction between different groups of columns could be made.

4.2.3. PCA on the Euerby data using three variables: α_{CH_2}, $\alpha_{T/O}$ and $\alpha_{A/P\ pH\ 7.6}$

Three clusters are distinguished on the PC1–PC2 score plot (Fig. 7). Group A: columns 3, 29, 34, 45, 47, 48, 51, 53 and 56 have the highest ion-exchange capacity values; group B: columns 6, 58, 60, 62, 63 and 72 are grouped as before (Fig. 5), but without columns 3 and 45, which are included in group A;

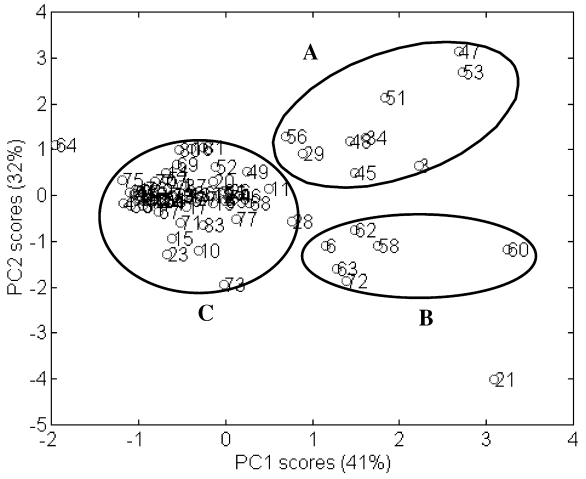
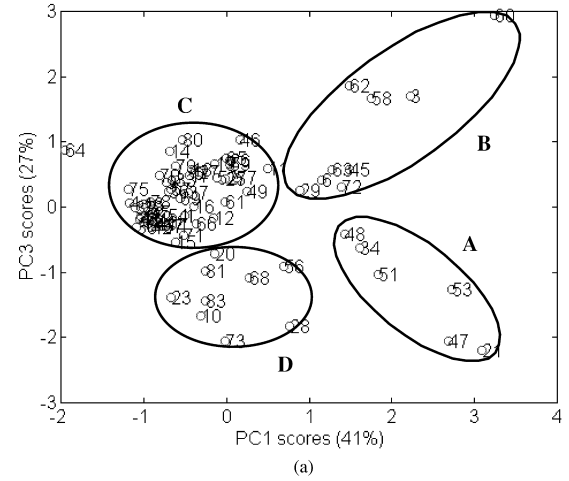


Fig. 7. (a) PC1–PC2 score plot for 74 columns and 3 parameters (α_{CH_2}, $\alpha_{T/O}$ and $\alpha_{A/P\ pH\ 7.6}$).

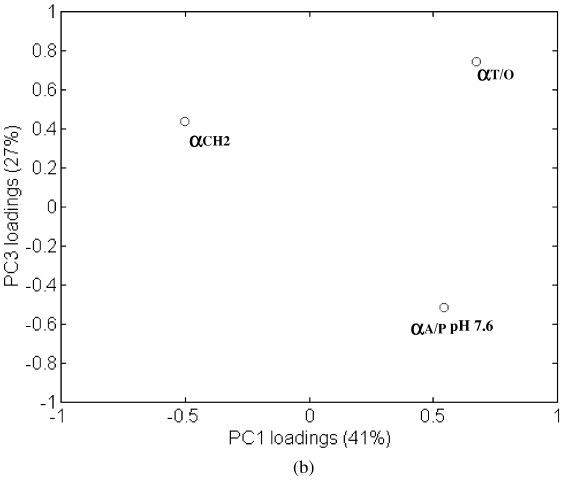
group C: all the others are grouped and have similar hydrophobicity. Exceptions are again columns 21 and 64 that do not belong to any group. Within group C a subgroup consisting of columns 10, 15, 23, 28, 71, 73, 77, 83 can be distinguished.

The PC1–PC3 score plot is shown in Fig. 8a and PC3 explains 27% of the remaining variation in the data. Four groups are clearly separated: group A: the loading plot (Fig. 8b) indicates that columns 21, 34, 47, 48, 51 and 53 have the highest ion-exchange capacities; group B: columns 3, 6, 29, 45, 58, 60, 62, 63 and 72 show the highest steric selectivities. The group now also includes column 29, compared to Figs. 5 and 6. Group D consists of columns 10, 20, 23, 28, 56, 68, 73, 81 and 83 and can be distinguished as a separate group. They have the lowest steric selectivity values. Group C contains the remaining columns; again column 64 is an outlier. The clear differentiation between the four groups seems again to be a consequence of the fact that the loadings are well distributed around the origin (Fig. 8b).

In summary, we observed that including only three parameters into the data analysis offers possibilities to approach a similar classification as with five parameters (Fig. 4). Moreover, the differentiation sometimes was even more detailed (Fig. 6). When



(a)



(b)

Fig. 8. (a) PC1–PC3 score plot for 74 columns and 3 parameters (α_{CH_2}, $\alpha_{T/O}$ and $\alpha_{A/P\ pH\ 7.6}$); (b) PC1–PC3 loading plot.

PC3 still explains a large amount of variation, considering PC1–PC3 plots can lead to additional differentiation among the columns (Figs. 3, 5 and 8).

One could wonder whether the members of the central group C, which always contains the majority of columns, would behave similarly for a chromatographic separation and if not, whether this group can be further differentiated into subgroups. After elimination of the columns belonging to the other clusters, the measured results of the remaining data set were autoscaled and a new PCA analysis was performed. It was observed that performing PCA analysis on the columns of the central cluster indeed allows differentiating several subgroups (Fig. 9).

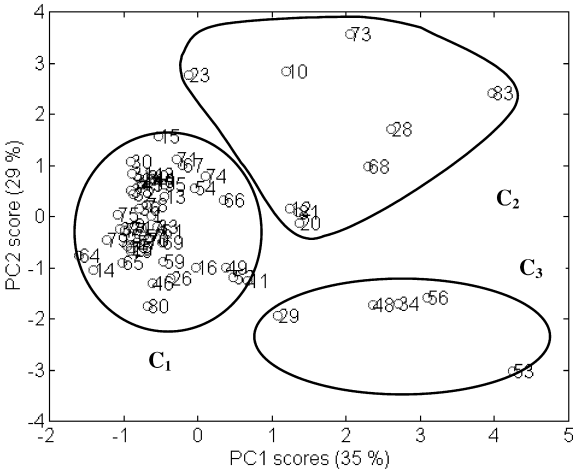


Fig. 9. PC1–PC2 score plot after autoscaling and PCA on the members of the C cluster in Fig. 2.

4.3. PCA on 47 self-tested columns

A total of 47 columns with different lengths (5, 7.5, 10, 15 and 25 cm) and packed with different C_{18} stationary phases were tested (Table 2). All columns were new, except the Supelcosil LC-ABZ (25 cm), Discover C_8 (5 cm) and Zorbax SB-C_8 (7.5 cm) columns which previously had been used and of which the latter two were C_8.

For several 10- and 15-cm columns the equivalent 25-cm column was available, so that the influence of the length on the characteristics and classification of the columns can be found. In order to compare the self-tested columns with those tested by Euerby et al. [1], the same test parameters were considered. In contrast with Ref. [1] we considered the theoretical plate number (n) found on a column with a given length and not the number of plates per metre (N), so as to be able to distinguish in the parameter results identical stationary phases packed in columns of different lengths. If N is used then the parameter is expected to be similar for equivalent short and long columns. This can be seen in the following example, N for Kromasil 10 cm (Table 2, column 5) is 75 100 and for Kromasil 25 cm (Table 2, column 30) it is 75 200, while n is 7500 and 18 800, respectively. If both columns show a difference in selectivity, e.g. for a given separation a peak pair is sufficiently separated on the longer column while not on the

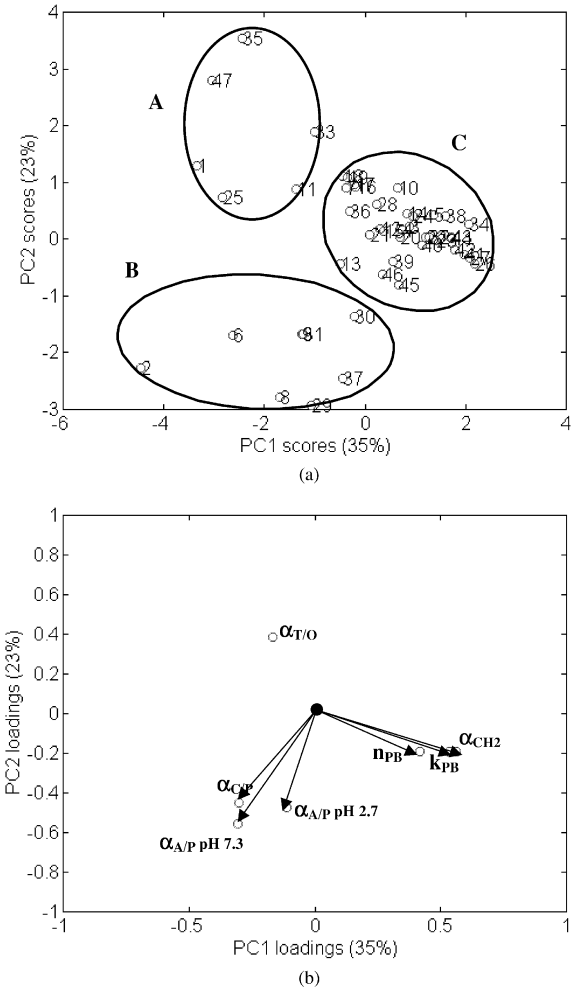


Fig. 10. (a) PC1–PC2 score plot for 47 columns and 7 parameters (k_{PB}, α_{CH_2}, $\alpha_{T/O}$, $\alpha_{C/P}$, $\alpha_{A/P\ pH\ 7.3}$, $\alpha_{A/P}$ pH 2.7 and n_{PB}); (b) PC1–PC2 loading plot. ● origin (0,0) of loading plot.

shorter, then it is more likely that such phenomenon can be related to n rather than to N (on the condition that the parameter is maintained in a classification).

Another difference was the highest buffer pH used to test the ion-exchange capacity, which was pH 7.3, as in Ref. [9] and not pH 7.6 as in Ref. [1].

4.3.1. Classification of self-tested columns based on seven parameters

Fig. 10 shows the PC1–PC2 score and loading plots for the seven parameters. In this way it was checked whether the parameters that reflect the column efficiency or the hydrophobicity (n_{PB}, k_{PB}, α_{CH_2}) are correlated as mentioned in Ref. [1]. The parameters k_{PB}, α_{CH_2} and n_{PB} are indeed located near to each other on the loading plot (Fig. 10b). The parameters $\alpha_{C/P}$, $\alpha_{A/P\ pH\ 7.3}$ and $\alpha_{A/P\ pH\ 2.7}$ also are again situated close to each other, though less than before (Fig. 1b). In order to confirm this, the correlation coefficients (r) between all parameters were calculated (Table 4). The highest correlations can be found between the three first mentioned parameters, giving the possibility to eliminate k_{PB} and n_{PB} to reduce the number of parameters as was done in Ref. [1]. Parameters $\alpha_{C/P}$ and $\alpha_{A/P\ pH\ 7.3}$ also seem to be correlated, while parameter $\alpha_{A/P\ pH\ 2.7}$ is less correlated, especially with parameter $\alpha_{C/P}$.

On the PC1–PC2 score plot three groups are distinguished: Group A: columns 1, 11, 25, 33, 35 and 47. The numbers 25, 33, 35 and 47 have the highest steric selectivities. Group B: columns 2, 6, 8, 9, 29, 30, 31 and 37, which have the highest hydrogen-bonding capacity and ion-exchange capacities at low and high pH. Group C: the other columns having similar efficiency values.

Table 4
Correlation coefficients (r) between the test parameters

	k_{PB}	α_{CH_2}	$\alpha_{T/O}$	$\alpha_{C/P}$	$\alpha_{A/P\ pH\ 7.3}$	$\alpha_{A/P\ pH\ 2.7}$	n_{PB}
k_{PB}	1						
α_{CH_2}	*0.767*	1					
$\alpha_{T/O}$	−0.218	0.296	1				
$\alpha_{C/P}$	−0.204	−0.272	−0.224	1			
$\alpha_{A/P\ pH\ 7.3}$	−0.126	−0.243	0.111	*0.545*	1		
$\alpha_{A/P\ pH\ 2.7}$	−0.103	0.029	−0.177	−0.004	*0.437*	1	
n_{PB}	*0.506*	*0.522*	−0.029	−0.114	−0.043	−0.038	1

Italicised r values represent the most correlated test parameters.

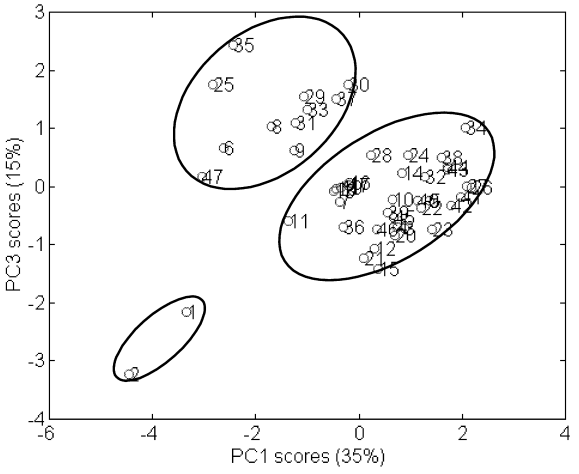


Fig. 11. PC1–PC3 score plot for 47 columns and 7 parameters (k_{PB}, α_{CH_2}, $\alpha_{T/O}$, $\alpha_{C/P}$, $\alpha_{A/P\ pH\ 7.3}$, $\alpha_{A/P\ pH\ 2.7}$ and n_{PB}).

In the PC1–PC3 plot (PC3 explains another 15% of the variation) three groups are observed (Fig. 11). It mainly shows that columns 1 and 2 are separated from the others. These are the shortest columns with the lowest efficiencies and the only C_8 columns in the data set.

4.3.2. Classification of the 47 columns based on a reduced number of parameters

Due to the correlation between the parameters k_{PB}, α_{CH_2} and n_{PB}, the classification can be performed using only five parameters as in Ref. [1]. Some changes in the groups (Fig. 12) can be seen compared to Fig. 10. Group A does not contain columns 1 and 11 anymore. Columns 1 and 11 have lower steric selectivities than the columns in group A. The composition of group B is the same as in Fig. 10. Group D is poorly distinguished from group C and contains columns 1, 11, 13, 39, 45 and 46. The PC1–PC3 plot did not give additional useful information. Comparing Fig. 12a with the classification of the Euerby data set (Fig. 2) shows that Fig. 12 does not contain such extreme columns as those of group A from Fig. 2 (non-endcapped columns on acidic silica). The clusters B, C and D are in fact subclasses of what is defined as group C in Fig. 2. It is another illustration of what was mentioned in Section 4.2.3 and shown in Fig. 9. This also could be

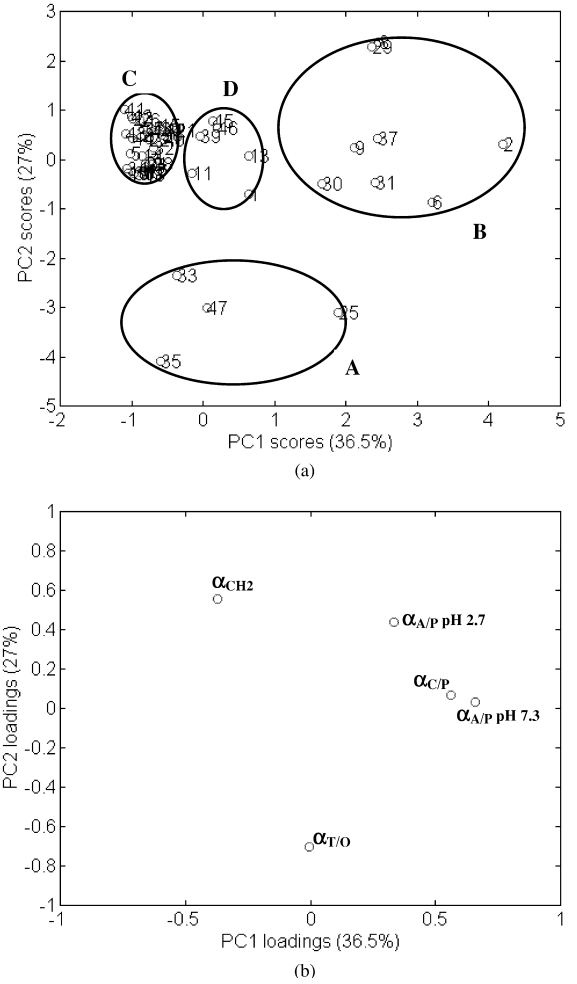
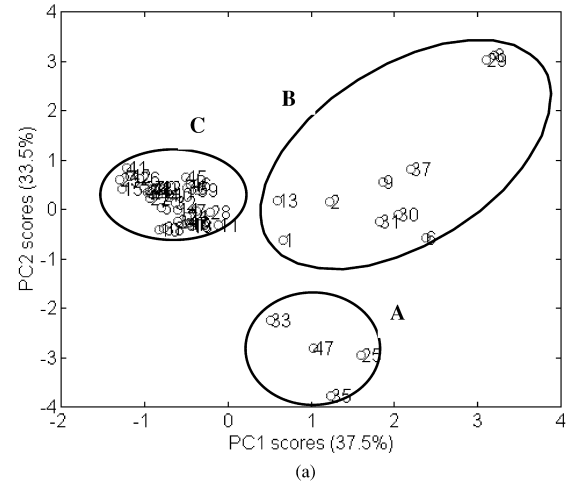


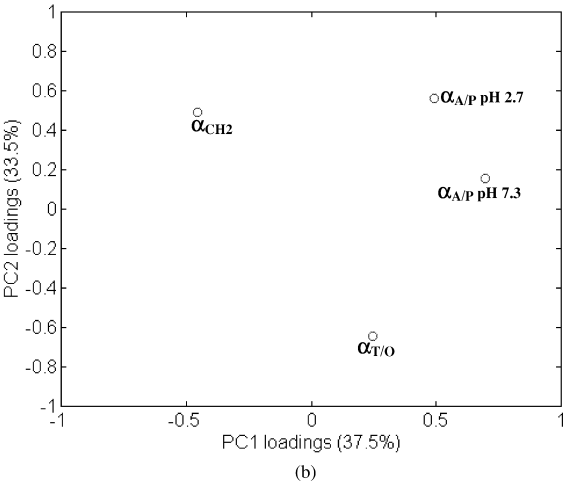
Fig. 12. (a) PC1–PC2 score plot for 47 columns and five parameters (α_{CH_2}, $\alpha_{T/O}$, $\alpha_{C/P}$, $\alpha_{A/P\ pH\ 7.3}$ and $\alpha_{A/P\ pH\ 2.7}$); (b) PC1–PC2 loading plot.

verified by performing PCA on the combined data set.

According to the loading plot (Fig. 12b) and the correlation coefficients (Table 4) either the hydrogen-bonding capacity or the ion-exchange capacity at high pH can be eliminated from the parameters. Using the variables, α_{CH_2}, $\alpha_{T/O}$, $\alpha_{C/P}$ and $\alpha_{A/P}$ at pH 2.7, three groups are distinguished (not shown). Similar results were observed when the hydrogen-bonding capacity ($\alpha_{C/P}$) was not considered, but $\alpha_{A/P\ pH\ 7.3}$ was (Fig. 13). The groups furthest away from the central group C in Fig. 12, namely group A with columns 25, 33, 35 and 47 and group B with



(a)



(b)

Fig. 13. (a) PC1–PC2 score plot for 47 columns and 4 parameters (α_{CH_2}, $\alpha_{T/O}$, $\alpha_{A/P\ pH\ 7.3}$ and $\alpha_{A/P\ pH\ 2.7}$); (b) PC1–PC2 loading plot.

columns 1, 2, 6, 8, 9, 13, 29, 30, 31 and 37 were also observed in Fig. 13.

It was known from Fig. 6 that parameters α_{CH_2}, $\alpha_{T/O}$ and $\alpha_{C/P}$ gave the best differentiation on PC1–PC2 for the Euerby data set. This also proved to be the case for the 47 columns. The same three groups were observed as when using four parameters (i.e. including parameter $\alpha_{A/P\ pH\ 2.7}$). Also parameters α_{CH_2}, $\alpha_{T/O}$ and $\alpha_{A/P\ pH\ 7.6}$ gave a very good differentiation in the Euerby data set, especially when plotting PC1–PC3 (Figs. 7 and 8). Fig. 14 shows that these three parameters (α_{CH_2}, $\alpha_{T/O}$ and

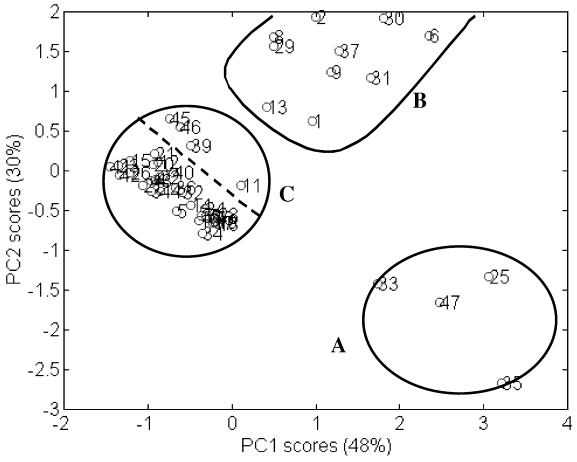


Fig. 14. PC1–PC2 score plot for 47 columns and 3 parameters (α_{CH_2}, $\alpha_{T/O}$ and $\alpha_{A/P\ pH\ 7.3}$).

$\alpha_{A/P\ pH\ 7.3}$) allow differentiating the same three groups as the four parameters in Fig. 13. This confirms the earlier conclusion that three parameters can classify columns as well as four or five.

Since equivalent short and long columns were tested, the PCA offers a possibility to compare their properties. In general, the situation of similar stationary phases on the score plots is independent of the column length. Some examples are identified in Fig. 15 (e.g. columns 9–37 Spherisorb ODS2; 8–29 Hypersil ODS; 6–31 Nucleosil). The other column

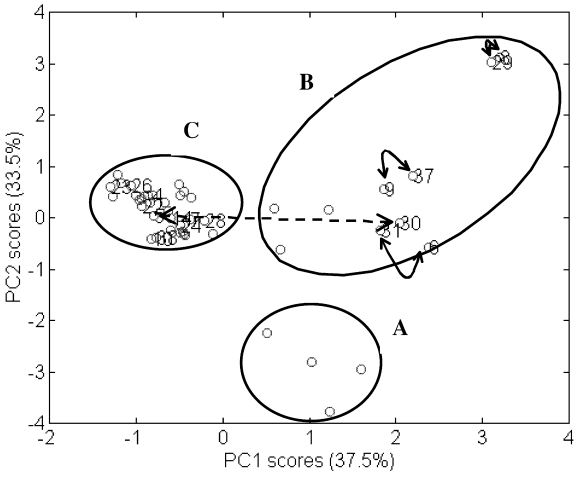


Fig. 15. PC1–PC2 score plot for 47 columns and 4 parameters (α_{CH_2}, $\alpha_{T/O}$, $\alpha_{A/P\ pH\ 7.3}$ and $\alpha_{A/P\ pH\ 2.7}$). Some equivalent short and long columns are indicated.

pairs are situated in cluster C. The equivalent columns are located near to each other. The Kromasil columns (5 and 30) were the only exceptions. The shorter one (column 5) is located into the group C, while the longer is in group B. It can be noticed that the Kromasil columns formed the only pair which had particles of equal size. In the other columns the short contained smaller diameter particles (3–3.5 μm) than the longer (4–5 μm).

The fact that column pairs are situated close to each other and that one expects these columns to have similar separation properties strengthens the idea that it will be possible to classify and differentiate columns based on a limited number of test parameters and that these classifications can be related to differences in separation behaviour of the columns. This also would offer the possibility to define system suitability test limits for the considered parameters.

5. Conclusion

The applied chemometric analysis (PCA) of the chromatographic parameters of RP-HPLC columns offers the possibility to evaluate column clustering or column differentiation. The number of parameters could be reduced due to their correlation, and the columns classified based on a minimal number of column tests. Only three or four chromatographic parameters could be considered without much loss of information. Moreover, fewer parameters sometimes gave more detailed results. It can also be helpful, not only to consider PC1–PC2, but also PC3, because it sometimes provides additional information.

In Ref. [9] about 40 parameters were selected from the literature which are to be tested on a large set of columns. In a next step it then will be examined which sets of three to five uncorrelated parameters out of the about 40 tested allow differentiation in the examined columns. Afterwards correlations will have to be made between the classifications and the separation observed in a monograph method for a pharmaceutical substance and its impurities. This should lead to the definition of suitable intervals for the test parameters, which allow identifying columns with appropriate separation properties.

Acknowledgements

Thanks to all column manufacturers and distributors for providing the columns. T. Iványi and D. Visky thank the Ministry of the Flemish Community for financial support. Y. Vander Heyden is a postdoctoral fellow of the Fund for Scientific Research (FWO)-Vlaanderen, Belgium.

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