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Characterisation of reversed-phase liquid chromatographic columns by chromatographic tests
Rational column classification by a minimal number of column test parameters

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Abstract

The European Pharmacopoeia (Ph. Eur.) and other official compendia give only a general description of the stationary phase in the description of a liquid chromatographic method. Therefore the selection of a column giving suitable selectivity presents difficulties. Earlier, a test procedure was proposed that allows to measure 36 chromatographic parameters which have been described for characterising stationary phases. This procedure was carried out on 69 reversed-phase liquid chromatography (RP-LC) columns. This paper focuses on the classification of RP-LC stationary phases based on chromatographic parameters. A chemometric study was conducted using 24 parameters that could be measured in a repeatable and reproducible way. Principal component analysis was used to classify the columns and to estimate the minimal number of parameters necessary for a rational classification. It is shown that after reducing the number of parameters from 24 to four or three, similar classifications were obtained. The column classifications were compared to the European Pharmacopoeia stationary phase description and to the column properties obtained from the manufacturers.

Keywords: Column classification; Column testing; Principal component analysis; Chemometrics; Stationary phases, LC; Chromatographic tests

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1. Introduction

Today, an overwhelming number of C_{18} reversed-phase liquid chromatography columns is available on the market. Although they belong to the same type of columns, their selectivity can be considerably different. Manufacturers provide only limited information about the stationary phases, e.g. the results of their own test methods and some applications. Therefore comparison of columns from different manufacturers is difficult. Due to these facts the choice of a proper RP-LC column for a particular separation presents difficulties as was already discussed by Steffeck [1].

The situation is even more difficult when a given official method must be carried out, e.g. a monograph from the European Pharmacopoeia (Ph. Eur.), since the brand name of the column with required selectivity is not communicated. The Ph. Eur. gives the eluent composition and other experimental conditions precisely, prescribes a system suitability test, the sub-class of the stationary phase (purity, end-capping, base-deactivation) and further refers to a description of the stationary phase in the reagents part with information on e.g. pore size, specific surface area and chain length [2]. This information is insufficient to choose a suitable column and there is no method in the Ph. Eur. to characterise or check the stationary phases.

The need to classify RP-LC columns has been reported from the seventies [3–6]. Several chromatographic tests can be found in the literature to characterise RP columns [7–33]. These tests are not destructive and stationary phase properties, such as column efficiency, hydrophobicity, silanol activity, ion-exchange capacity, steric selectivity and the amount of metal impurities can be featured. Until now none of these tests has been widely accepted. Also, it has never been verified sufficiently whether columns having closely related characteristics as determined by these chromatographic tests, indeed are suitable for the same separation. Such study is the subject of a project, which the work described here belongs to.

Within the confines of this project, chromatographic tests described in the literature were reviewed. Eight different chromatographic methods were chosen and a protocol was developed, suitable for characterising chromatographic properties of RP-LC columns. This protocol allows the determination of 36 parameters summarised in Section 2 and is discussed in detail in [34]. The test procedure was carried out on 69 RP-LC columns in different laboratories. Repeatability, reproducibility and correlation of the test parameters was discussed [35]. It was also shown that column classification was not possible with only one parameter.

Principal component analysis (PCA) is used when large, complex data sets have to be investigated [36]. It is a statistical technique used for exploratory data analysis. PCA and cluster analysis were already involved in LC column classification studies [13,18,22,23,27,37,38]. A chemometric study was conducted in the frame of this project on a previously published data set consisting of 85 RP-LC columns and on a data set consisting of 47 self-tested columns. It had been shown that upon carefully reducing the original number of chromatographic parameters, the classification pattern can be retained, three parameters produce a classification similar to that obtained with five parameters [39].

In this paper PCA classifications of RP-LC columns based on 24 of the 36 parameters were performed. These 24 parameters were retained because they are reproducible [35]. The aim was to reduce the number of parameters for classification to the minimum with keeping rational clustering of RP-LC stationary phases. Characterisation with four and three parameters was examined and parameter sets which allow rational classifications were selected. The obtained clusters were compared with the classification, which is used in the Ph. Eur.

2. Experimental

2.1. Experimental conditions

After a careful study of the literature eight different methods were selected and these were described in detail [34]. General information on the test methods, the chromatographic conditions applied, the measured parameters and the estimated column properties can be found in Table 1. The test procedure with these eight methods, which allow the determination of 36 test parameters, was carried out on 69 RP-LC columns (see Table 2). For the silica gel, distinction was made between old type (Type A) and new type (Type B, ultrapure). The materials

Table 1
Examined parameters, classified according to the chromatographic method used

Parameter number	Name of the parameter	Column property tested	Chromatographic method	Mobile phase
(1)	Dihydroxynaphthalene efficiency ratio test (DERT)	Metal impurities	M1	Acetonitrile/0.025 *M* ammonium acetate (26.2:100 w/w)
2	*n* MPPH	Efficiency	M2	Acetonitrile/water/0.2 *M* potassium phosphate pH 2.3 (312:340:340 w/w)
3	$rk'_{\text{diazepam/MPPH}}$	Hydrophobicity		
4	$rk'_{\text{toluene/MPPH}}$	Hydrophobicity		
5	$rk'_{\text{diphenhydramine/MPPH}}$	Silanol activity		
(6)	$SF_{\text{diphenhydramine}}$	Silanol activity		
7	$rk'_{\text{acetylsalicylic acid/MPPH}}$	Non-specified		
8	$rk'_{o\text{-hydroxyhippuric acid/MPPH}}$	Non-specified		
(9)	peak area/peak height of acetylacetone	Metal impurities	M3	Methanol/0.5% w/v CH_3COONa (118.5:100 w/w)
(10)	$n_{\text{acetylacetone}}$	Metal impurities		
(11)	$SF_{\text{acetylacetone}}$	Metal impurities		
12	$rk'_{\text{benzylamine/phenol}}$ pH 2.7	Ion-exchange capacity at low pH	M4	Methanol/water/0.2 *M* potassium phosphate pH 2.7 (34:90:10 w/w)
(13)	$rk'_{\text{benzylamine/phenol}}$ pH 7.3	Ion-exchange capacity at high pH	M5	Methanol/water/0.2 *M* potassium phosphate pH 7.3 (34:90:10 w/w)
14	$rk'_{\text{caffeine/phenol}}$	Silanol activity	M6	Methanol/water (34:100 w/w)
15	$rk'_{\text{pyridine/caffeine}}$	Silanol activity		
16	$rk'_{\text{pyridine/phenol}}$	Silanol activity		
(17)	SF_{pyridine}	Silanol activity		
18	$k'_{\text{2,2′-dipyridyl}}$	Metal impurities		
19	$k'_{\text{2,3-dihydroxynaphthalene}}$	Metal impurities		
20	$rk'_{\text{2,3-dihydroxynaphthalene/2,2′-dipyridyl}}$	Metal impurities		
(21)	$SF_{\text{2,2′-dipyridyl}}$	Metal impurities		
(22)	$SF_{\text{2,3-dihydroxynaphthalene}}$	Metal impurities		
(23)	$rk'_{\text{theophylline/theobromine}}$	Non-specified		
(24)	$rk'_{\text{caffeine/theophylline}}$	Non-specified		
25	n_{toluene}	Efficiency	M7	Methanol/water (50:50 w/w)
26	k'_{toluene}	Hydrophobicity		
27	$k'_{\text{ethylbenzene}}$	Hydrophobicity		
28	$rk'_{\text{ethylbenzene/toluene}}$ 7	Hydrophobicity		
(29)	$SF_{p\text{-ethylaniline}}$	Silanol activity		
30	$rk'_{\text{toluene/phenol}}$ 7	Other parameter		
31	$n_{\text{amylbenzene}}$	Efficiency	M8	Methanol/water (317:100 w/w)
32	$k'_{\text{amylbenzene}}$	Hydrophobicity		
33	$rk'_{\text{ethylbenzene/toluene}}$ 8	Hydrophobicity		
34	$rk'_{\text{amylbenzene/butylbenzene}}$	Hydrophobicity		
35	$rk'_{\text{triphenylene/}o\text{-terphenyl}}$	Steric selectivity		
36	$rk'_{\text{toluene/phenol}}$ 8	Other parameter		

() excluded parameters.
k': retention factor; n: theoretical plate number; rk': relative retention factor; SF: symmetry factor; MPPH: 5-*p*-methylphenyl-5-phenylhydantoin.

Table 2
Stationary phases examined and their properties provided by the manufacturers

Column number	Name of the column	Length (mm)[a]	Particle size (μm)	Manufacturer/Supplier	Type of silica	End-capped	Base deacti-vated	Polar embedded	Pore size (Å)
1	ACE 3 C_{18}	150	3	Advanced Chrom. Tech./Achrom	B	+	+	−	100
2	ACE 5 C_{18}	250	5	Advanced Chrom. Tech./Achrom	B	+	+	−	100
3	Alltima C_{18} 3	150	3	Alltech	B	+	+	−	120
4	Alltima C_{18} 5	250	5	Alltech	B	+	+	−	120
5	Apex Basic	250	5	Jones Chromatography/Sopachem	A	+	+	−	100
6	Apex ODS II	250	5	Jones Chromatography/Sopachem	A	+	−	−	100
7	Aqua	150	5	Phenomenex/Bester	B	+	−	−	125
8	μBondapak	250	10	Waters	A	+	−	−	125
9	Brava BDS 3	150	3	Alltech	B	+	+	−	145
10	Brava BDS 5	250	5	Alltech	B	+	+	−	145
11	Chromolith	100	–	Merck	B	+	−	−	–
12	Discovery C_{18}	250	5	Supelco	B	+	−	−	180
13	Genesis C_{18} 3	100	3	Jones Chromatography/Sopachem	B	+	+	−	100
14	Genesis C_{18} 4	250	4	Jones Chromatography/Sopachem	B	+	+	−	100
15	Hypersil BDS 3	100	3	Alltech	A	+	+	−	130
16	Hypersil BDS 5	250	5	ThermoQuest	A	+	+	−	130
17	Hypersil ODS 3	100	3	Alltech	A	+	−	−	120
18	Hypersil ODS 5	250	5	ThermoQuest	A	+	−	−	120
19	HyPURITY Elite 3	150	3	ThermoQuest, SerCoLab	B	+	+	−	200
20	HyPURITY Elite 5	150	5	ThermoQuest, SerCoLab	B	+	+	−	200
21	Kromasil 100-3	100	3	Alltech	B	+	−	−	100
22	Kromasil (MN)	250	5	Macherey–Nagel/Filter Service	B	+	−	−	100
23	Kromasil (EKA)	250	5	Akzo Nobel/SerCoLab	B	+	−	−	100
24	LiChrosorb	250	5	Merck	A	−	−	−	100
25	LiChrospher	250	5	Merck	A	−	+	−	100
26	Luna	150	5	Phenomenex/Bester	B	+	−	−	100
27	Nucleosil 3	100	3	Alltech	A	+	−	−	100
28	Nucleosil 5	250	5	Macherey–Nagel/Filter Service	A	+	−	−	100
29	Nucleosil HD	250	5	Macherey–Nagel/Filter Service	B	+	−	−	100
30	Nucleosil Nautilus	250	5	Macherey–Nagel/Filter Service	B	−	−	+	100
31	OmniSpher	250	5	Varian	B	−	−	−	110
32	Pecospher C_{18}	83	3	PerkinElmer	B	+	−	−	80
33	Platinum C_{18} 3	150	3	Alltech	B	+	+	−	100
34	Platinum C_{18} 5	250	5	Alltech	B	+	+	−	100
35	Platinum EPS C_{18} 3	150	3	Alltech	B	−	+	+	100
36	Platinum EPS C_{18} 5	250	5	Alltech	B	−	+	+	100
37	Prodigy	100	3	Phenomenex/Bester	B	+	−	−	100
38	Purospher	250	5	Merck	B	+	−	−	80
39	Purospher endcapped	250	5	Merck	B	+	+	−	80
40	Purospher STAR e	250	5	Merck	B	+	+	−	80
41	SPHERI-5	250	5	PerkinElmer	B	+	−	−	80
42	Spherisorb ODS2 3	100	3	Waters	A	+ −	−	−	80
43	Spherisorb ODS2 5	250	5	Waters	A	+	−	−	80
44	Supelcosil LC-18	250	5	Supelco	A	−	−	−	120
45	Supelcosil LC-18 DB 3	150	3	Supelco	B	−	+	−	120
46	Supelcosil LC-18 DB 5	250	5	Supelco	B	−	+	−	120
47	Superspher	250	4	Merck	B	+	−	−	100
48	Symmetry 3.5	100	3.5	Waters	B	+	−	−	100
49	Symmetry 5	250	5	Waters	B	+	−	−	100

Table 2. Continued

Column number	Name of the column	Length (mm)[a]	Particle size (μm)	Manufacturer/Supplier	Type of silica	End-capped	Base deacti-vated	Polar embedded	Pore size (Å)
50	TracerExcel ODS A-3	150	3	Teknokroma/SerCoLab	NA	NA	NA	NA	120
51	TracerExcel ODS A-5	250	5	Teknokroma/SerCoLab	NA	NA	NA	NA	120
52	TSKgel ODS-80TS	150	5	TosoHaas/SerCoLab	B	+	−	−	80
53	TSKgel Super ODS	100	2	TosoHaas/SerCoLab	B	+	−	−	110
54	Uptisphere 3 $HDOC_{18}$	100	3	Interchrom/Achrom	B	+	−	−	120
55	Uptisphere 5 $HDOC_{18}$	250	5	Interchrom/Achrom	B	+	−	−	120
56	Uptisphere 3 ODB	100	3	Interchrom/Achrom	B	+	−	−	120
57	Uptisphere 5 ODB	250	5	Interchrom/Achrom	B	+	−	−	120
58	Validated C_{18}	250	5	PerkinElmer	B	+	−	−	100
59	Wakosil C_{18} HG 5–10	100	5	SGE/Achrom	B	+	−	−	120
60	Wakosil C_{18}HG 5–25	250	5	SGE/Achrom	B	+	−	−	120
61	Wakosil C_{18} RS 3–10	100	3	SGE/Achrom	B	+	−	−	125
62	Wakosil II C_{18} RS 3–25	250	3	SGE/Achrom	B	+	−	−	125
63	X-Terra 3	100	3	Waters	B	+	−	+	125
64	YMC-Hydrosphere C_{18}	150	5	YMC Sep. Techn./ThermoQuest	B	+	−	−	120
65	YMC-Pack Pro C_{18}-3	150	3	YMC Sep. Techn./ThermoQuest	B	+	+	-	120
66	YMC-Pack Pro C_{18}-5	150	5	YMC Sep. Techn./ThermoQuest	B	+	+	−	120
67	Zorbax Eclipse XDB-C_{18}	250	5	Agilent Technologies	B	+	−	−	80
68	Zorbax Extend C_{18}	250	5	Agilent Technologies	B	+	−	−	80
69	Zorbax SB-C_{18}	250	5	Agilent Technologies	B	+	−	−	80

A: old type of silica gel. B: new type of silica gel. NA: data not available.
[a] The internal diameter is always 4.6 mm.

were also characterised by indicating the presence of base deactivation, end-capping and embedded polar groups.

2.2. Principal component analysis

The PCA calculations were executed with Statistica 6.0 software (StatSoft, Tulsa, OK, USA).

3. Results and discussion

3.1. Discussion of the parameters and principal component analysis on the complete data set

The original data set consisted of 36 chromatographic parameters (variables) and 69 stationary phases (objects). It was the intention to employ as many data from this set as possible. But only repeatable and reproducible chromatographic parameters should be used in a general test method and it is also important to have trustable data during the chemometry study. Therefore parameters, which, on a limited set of columns, could not be determined with sufficient precision, were eliminated from the original data set. Reproducibility data of these parameters were published recently [35]. All parameters having a reproducibility RSD of more than 10% were excluded. Also, parameters that needed extremely long equilibrium time for several columns were omitted. Parameters excluded are indicated between parentheses in Table 1. Finally, 24 parameters were retained.

However, for the complete set of columns it was still not possible to measure all 24 parameters since sometimes no peaks were detected for some compounds because of too long retention times and/or too broad peaks. This creates a calculation problem since PCA cannot be performed if data are missing. The problem of missing data in sets to be subjected to PCA is well known and several solutions are described in the literature [40–43]. There are two prime causes of missing data. A first one is that a parameter is not measured and can take on any value. A variety of approaches are described to solve this problem, one being to replace these missing data by calculation of the mean or standard deviation of the known data. A second cause of missing data,

which was encountered in this study, involves measurement results that are either very large or very small. As some peaks were not detected because of too long retention times, these missing data represent values which are very large. Measuring these data may be impossible or may take a long chromatography time at very little benefit since for least square methods, large values can strongly bias the results, i.e. large values severely distort the results if they are included in the PCA analysis. These large data can be ''Winsorized'', which means that extreme elements are given a value closer to the mean [44]. A second way to treat the missing data is to ''guess'' the values and to fill in plausible values. This requires some knowledge of the population of objects examined [40,41], which is the case for our data set. In this column classification study, the missing data were replaced by plausible values, differing by 10% from the most extreme value measured on the other columns, which is a solution equivalent to those indicated above. It should be noticed that this treatment is allowed only when the amount of missing values is reasonably small (10% or less), which is the case in this study. A similar problem was observed in the column classification study of Olsen and Sullivan, where some arbitrary values, which were not expected to influence the results of the PCA analysis, were used [23].

The influence of our approach to the missing data on the PCA classification was examined. In a first analysis the problematic columns (5, 6, 17, 18, 24, 30, 32, 35, 36, 38, 41, 42, 43, 44, 45 and 46), on which not all 24 parameters could be measured, were eliminated from the data set. In a second analysis all columns were used but the plausible values were introduced for the missing parameters: $rk'_{\text{phenol/benzylamine}}$ pH 2.7, $k'_{\text{2,2′-dipyridyl}}$, $k'_{\text{2,3-dihydroxynaphthalene}}$, $rk'_{\text{pyridine/caffeine}}$, $rk'_{\text{pyridine/phenol}}$ and $rk'_{\text{2,2′-dipyridyl/2,3-dihydroxynaphthalene}}$.

The PCA calculations were performed on the data sets obtained after autoscaling. The PC1–PC2 score plot for all columns (using plausible values) can be seen in Fig. 1a, the score plot for a reduced number of stationary phases in Fig. 2. The score plots are quite similar, i.e. the same stationary phases appear in each other vicinity in both plots. The columns also are clustered similarly, when ignoring the problematic columns in Fig. 1. The above shows that the introduction of plausible values did not dramatically change the data structure. Therefore, the data set with all columns and 24 parameters was used for further evaluation.

A main group (Group I) can be observed in the PC1–PC2 score plot in Fig. 1a. The detailed composition of groups of columns obtained is reported in Table 3. Group I can be subdivided in subgroups I/a and I/b. Group I/a contains the stationary phases made from type B silica gel, they are all base-deactivated and/or end-capped, except Column 31, and their pore size is average (80–120 Å). Columns in Group I/a have the highest efficiency and hydrophobicity, they have low silanol activity, little metal impurity and low steric selectivity. This is deduced by comparison of the score plot and the loading plot (Fig. 1b). The loading plot shows the position of the parameters, which are grouped according to the chromatographic property they measure. In the following lines, the group number is reported in parentheses. Efficiency (1) has a small negative loading and hydrophobicity (2) has a large negative loading on PC1, therefore columns having high negative value on PC1, show high efficiency and hydrophobicity. Further, parameters related mainly to silanol activity (3) and metal impurity (5 and 6), steric selectivity (4) and non-specified (''other'' 7) properties are distinguished on the loading plot. The parameters representing metal impurity (5 and 6) are rather negatively correlated.

The Group I/b stationary phases have lower efficiency and are less hydrophobic than those of Group I/a. Group I/b consists of different types of columns. Columns 9, 10, 12, 19 and 20 are made of silica gel type B but their pore size is larger (>125 Å). Others are made of silica gel type A (15 and 16), are monolithic (11) or have a special ''hydrophilic'' surface (38). Columns 53 and 64 are in this group in spite of the fact that they are made of type B, end-capped silica gel.

A number of columns are situated below the main group (Group II). They have greater silanol activity and metal impurity level than Group I although their efficiency and hydrophobicity is similar. The non-base-deactivated, non-endcapped silica gels are in this group (24, 32, 41 and 44). Type A, non-base-deactivated, end-capped silica gels (6, 17, 18, 27, 28, 42 and 43) and type A, base-deactivated but non-

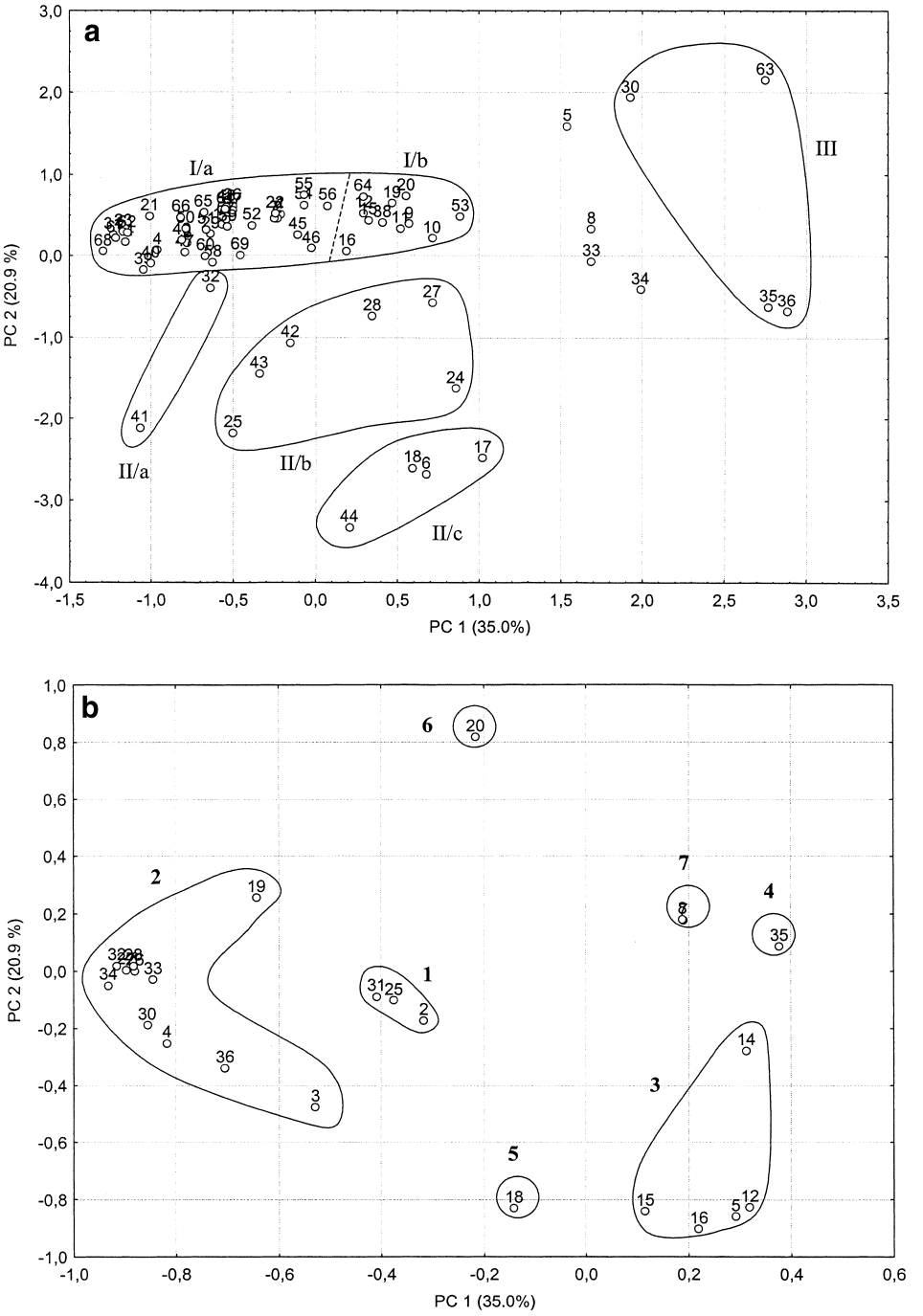

Fig. 1. (a) PC1–PC2 score plot and (b) PC1–PC2 loading plot for 69 RP-LC columns employing 24 chromatographic parameters. The bold numbers represent group numbers of parameters related to a given property (see details in the text).

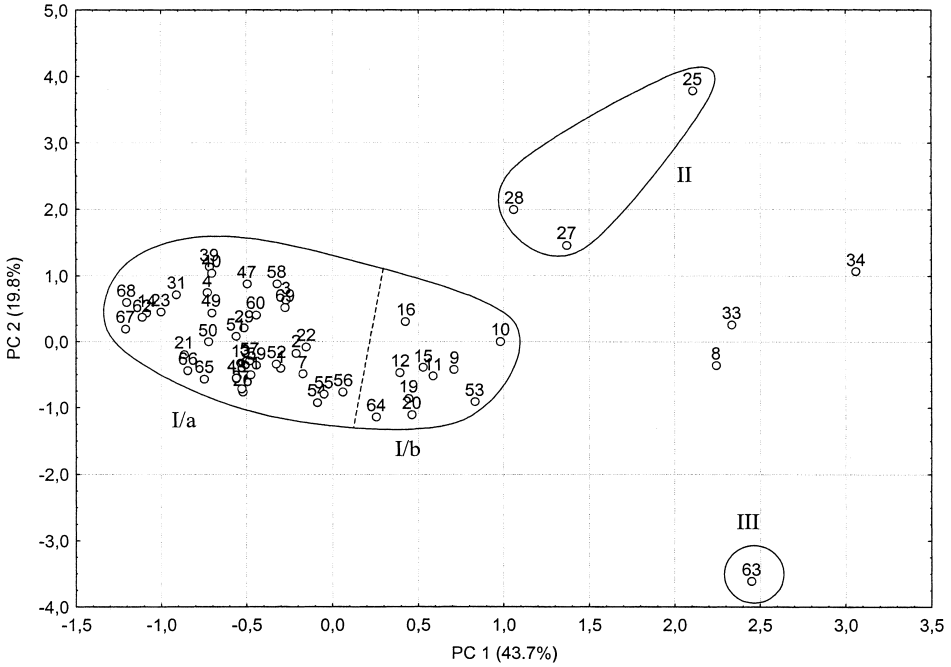

Fig. 2. PC1–PC2 score plot for 53 RP-LC columns employing 24 chromatographic parameters (see details in the text).

end-capped silica gel (25) can also be found here except Column 8, which is an outlier. Group II can be separated into 3 subgroups. Group II/a contains the non-end-capped columns made from silica gel type B (32 and 41). Group II/b contains columns with silica gel type A (24, 25, 27, 28, 41 and 43). Columns 6, 17, 18 and 44 form Group II/c. The latter two subgroups contain both end-capped and non-end-capped columns.

The remaining columns have higher scores on PC1. These columns have more steric selectivity but much less hydrophobicity and efficiency than the main group and their silanol activity is low as well. Polar-embedded columns (30, 35, 36 and 63) are in the Group III. Some columns are situated between Groups I and III, for example Columns 33 and 34, which contain silica gel with special, hydrophilic surface; Column 5 is made of type A, base-deactivated, end-capped silica gel and Column 8 is made of type A, end-capped silica gel. For these latter stationary phases it is not evident to categorise them as a group.

Since the PC1–PC2 score plot explains only 55.9% of the total variation in the data set, the PC1–PC3 plot was also considered (not shown) but no additional useful information was obtained.

In the Reagent part of the Ph. Eur. octadecylsilyl silica gels are divided into 7 groups. ‘‘Silica gel for chromatography, octadecylsilyl. 1077500’’ covers all the C_{18} silica gels. ‘‘Silica gel for chromatography, octadecylsilyl R1. 1110100’’ is an ultrapure material, containing less than 20 ppm of metals. ‘‘Silica gel for chromatography, octadecylsilyl R2’’ is also ultrapure, the carbon load is at least 20% and it is optimised for the analysis of polycyclic hydrocarbons, but there is no requirement here for the metal impurity. In the author’s opinion these classes are not well-defined. The other four classes are better defined. ‘‘Silica gel for chromatography, octadecylsilyl, base-deactivated. 1077600’’ covers silica gels pretreated by careful washing and hydrolysing most of the superficial siloxane bridges (base deactivation) before bonding of C_{18} groups. Materials in class ‘‘Silica gel for chromatography, octadecylsilyl, end-capped. 115400’’ are end-capped to cover most of the remaining free silanol groups. ‘‘Silica gel for chromatography, octadecylsilyl, end-capped, base-deactivated. 1108600’’ contains columns which are

base-deactivated and end-capped. ‘‘Silica gel for chromatography, dimethyloctadecylsilyl. 1115100.’’ covers C_{18} silica gels sterically protected with methyl groups. There are no criteria for the purity, the carbon load and the content of metals for the latter four classes. The Ph. Eur. does not distinguish between silica gels type A and B and does not mention the polar embedded columns in spite of the fact that they have different chromatographic properties. When the Ph. Eur. classification is compared to the clustering in Fig. 1a, no real correspondence can be found.

It can be concluded that based on these 24 parameters the columns can be classified in a central cluster and a number of outlying groups of columns. The stationary phases with missing values for some of the parameters or with somewhat different physico-chemical properties (e.g. polar embedded) belong to the latter group.

3.2. Reducing the number of parameters

The final purpose is to develop a simple test procedure which allows to characterise RP-LC stationary phases. Therefore it is required to reduce the number of the parameters without losing information. Table 4 classifies the 24 parameters in 7 groups according to six chromatographic properties and a seventh group contains two non-specified (‘‘other’’) parameters. The classification in 7 groups was based on the correlation between the parameters. Correlation between variables is also visualised with the PCA loading plots: those points which are close to each other represent highly correlated parameters (see Fig. 1b).

Most of the parameters were found to be classified in the class they were expected and thus they measure the stationary phase property that was claimed in the original papers. Exceptions are: $k'_{2,3\text{-dihydroxynaphthalene}}$ (19) (belongs to hydrophobicity group instead of metal impurity) [32], $rk'_{\text{toluene/phenol}}$ 7 and $rk'_{\text{toluene/phenol}}$ 8 (30 and 36) (belong to hydrophobicity group, were not specified in the literature) [25] and $rk'_{\text{benzylamine/phenol}}$ pH 2.7 (12) (belongs to silanol activity group instead of ion-exchange capacity) [15]. The $k'_{2,2'\text{-dipyridyl}}$, (18) reported to measure metal impurity and also to depend on silanol activity [45], is classified here into a separate group.

To reduce the number of parameters without losing information, the best parameter (criteria specified below) was selected from each group. Since Groups 4 (steric selectivity), 5 (silanol activity and metal impurity) and 6 (metal impurity) only have one element, these are selected automatically. These parameters can be measured using Methods 6 and 8. In Groups 1 to 3, the best parameter was selected based on the following criteria: the reproducibility of the determination, the sensitivity for differences in the concerned chromatographic property and finally, the chromatographic method required to measure the parameter. To reduce the number of methods and to simplify the final procedure, Methods 6 and 8, already selected above, are preferably used whenever possible. Therefore the theoretical plate number of amylbenzene (31) (Method 8) was chosen from Group 1 (efficiency). The retention factor of amylbenzene (32) (Method 8) was selected from Group 2 (hydrophobicity) because this parameter is sensitive enough (relative retention factors, e.g. $rk'_{\text{ethylbenzene/toluene}}$ are differing only by 10–15% within the data set). The most reproducible parameter in Group 3 is the relative retention of caffeine/phenol (14) but it is not sensitive enough (too small column-to-column difference). It also can be seen on the loading plot (see Fig. 1b) that this parameter is relatively far from the other members. Pyridine sometimes does not show a peak and therefore the parameters containing this compound are excluded. Finally, the relative retention factor of benzylamine/phenol pH 2.7 (12) was selected, which met best the requirements. From Group 7, the $rk'_{\text{acetylsalicylic acid/MPPH}}$ (7) was chosen because acetylsalicylic acid is more commonly available than *o*-hydroxyhippuric acid. The selected parameters are presented in bold in Table 4.

3.3. Principal component analysis on 4 parameters

It has been already demonstrated that PCA classification of RP-LC columns based on 4 parameters is possible [39]. Therefore PCA calculations were performed on 35 different combinations of 4 parameters selected from the seven above discussed parame-

Table 3
The composition of the groups of columns obtained by PCA with different parameter sets (see details in the text)

Parameter set	24 parameters	$k'_{\text{amylbenzene}}$, $rk'_{\text{o-terphenyl/triphenylene}}$, $rk'_{\text{benzylamine/phenol}}$ pH 2.7, $k'_{2,2'\text{-dipyridyl}}$	$rk'_{\text{o-terphenyl/triphenylene}}$, $rk'_{\text{benzylamine/phenol}}$ pH 2.7, $k'_{2,2'\text{-dipyridyl}}$, $rk'_{2,3\text{-dihydroxynaphthalene/2,2'-dipyridyl}}$	$k'_{\text{amylbenzene}}$, $rk'_{\text{benzylamine/phenol}}$ pH 2.7, $k'_{2,2'\text{-dipyridyl}}$, $rk'_{\text{acetylsalicylic acid/MPPH}}$	$k'_{\text{amylbenzene}}$, $rk'_{\text{o-terphenyl/triphenylene}}$, $k'_{2,2'\text{-dipyridyl acetylsalicylic acid/MPPH}}$	$k'_{\text{amylbenzene}}$, $rk'_{\text{o-terphenyl/triphenylene}}$, $rk'_{\text{benzylamine/phenol}}$ pH 2.7, $rk'_{2,3\text{-dihydroxynaphthalene/2,2'-dipyridyl}}$
Group I/a	1, 2, 3, 4, 7, 13, 14, 21, 22, 23, 26, 29, 31, 37, 39, 40, 45, 46, 47, 48, 49, 50, 51, 52, 54, 55, 56, 57, 58, 59, 60, 61, 62, 65, 66, 67, 68, 69	3, 4, 7, 13, 14, 21, 22, 23, 26, 29, 31, 37, 39, 40, 47, 48, 49, 50, 51, 52, 54, 55, 56, 57, 58, 59, 60, 61, 62, 64, 65, 66, 67, 68, 69	1, 2, 3, 4, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 19, 20, 21, 22, 23, 26, 29, 31, 37, 40, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 64, 65, 66, 67, 68, 69	1, 2, 3, 4, 7, 13, 14, 21, 22, 23, 26, 29, 31, 37, 39, 40, 47, 48, 49, 50, 51, 52, 54, 55, 56, 57, 58, 59, 60, 61, 62, 64, 65, 66, 67, 68, 69	1, 2, 3, 4, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 31, 32, 33, 34, 37, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 64, 65, 66, 67, 68, 69	1, 2, 3, 4, 7, 13, 14, 21, 22, 23, 26, 29, 31, 37, 39, 40, 47, 48, 49, 50, 51, 52, 54, 55, 56, 57, 58, 59, 60, 61, 62, 64, 65, 66, 67, 68, 69
Group I/b	9, 10, 11, 12, 15, 16, 19, 20, 38, 53, 64	1, 2, 8, 9, 10, 11, 12, 15, 16, 19, 20, 33, 34, 38, 45, 46, 53	8, 9, 10, 11, 12, 15, 16, 19, 20, 30, 45, 46, 53, 63	9, 10, 11, 12, 15, 19, 20, 38, 45, 46, 53		
Group II/a	32, 41	32, 41	32, 41	24, 25, 27, 28, 32, 41, 42, 43	–	32, 41
Group II/b	24, 25, 27, 28, 42, 43	24, 25, 27, 28, 42, 43	24, 25, 27, 28, 42, 43	–	8, 16, 24, 25, 27, 28, 42, 43	
Group II/c	6, 17, 18, 44	6, 17, 18, 44	6, 17, 18, 44	6, 17, 18, 44	–	6, 17, 18, 44
Group III	30, 35, 36, 63	30, 35, 36, 63	–	–	30, 35, 36, 63	30, 35, 36, 63
Outliers	5, 8, 33, 34	5	5, 30, 33, 34, 35, 36, 38, 39, 63	5, 33, 34, 35, 36, 38	5, 38	5, 33, 34

Table 3. Continued

Parameter set	$rk'_{o\text{-terphenyl/triphenylene}}$, $rk'_{\text{benzylamine/phenol}}$ pH 2.7 $k'_{2,2'\text{-dipyridyl}}$	$k'_{\text{amylbenzene}}$, $rk'_{\text{benzylamine/phenol}}$ pH 2.7 $k'_{2,2'\text{-dipyridyl}}$	$k'_{\text{amylbenzene}}$, $rk'_{o\text{-terphenyl/triphenylene}}$ $k'_{2,2'\text{-dipyridyl}}$	$k'_{\text{amylbenzene}}$, $rk'_{o\text{-terphenyl/triphenylene}}$ $rk'_{\text{benzylamine/phenol}}$ pH 2.7
Group I/a	1, 2, 3, 4, 7, 8, 9, 10, 13, 14, 15, 16, 21, 22, 23, 26, 29, 31, 33, 34, 37, 40, 45, 46, 47, 48, 49, 50, 51, 52, 54, 55, 56, 57, 58, 59, 60, 61, 62, 64, 65, 66, 67, 68, 69	1, 2, 3, 4, 7, 13, 14, 21, 22, 23, 26, 29, 31, 37, 38, 39, 40, 47, 48, 49, 50, 51, 52, 54, 55, 56, 57, 58, 59, 60, 61, 62, 64, 65, 66, 67, 68, 69	3, 4, 7, 13, 14, 21, 22, 23, 26, 29, 31, 32, 37, 40, 47, 48, 49, 50, 51, 52, 54, 55, 56, 57, 58, 59, 60, 61, 62, 64, 65, 66, 67, 68, 69	3, 4, 7, 13, 14, 21, 22, 23, 26, 29, 31, 32, 37, 39, 40, 41, 47, 48, 49, 50, 51, 52, 54, 55, 56, 57, 58, 59, 60, 61, 62, 64, 65, 66, 67, 68, 69
Group I/b	11, 12, 19, 20, 53	5, 8, 9, 10, 11, 12, 15, 16, 19, 20, 30, 45, 46, 53, 63	1, 2, 8, 9, 10, 11, 12, 15, 16, 19, 20, 33, 34, 45, 46, 53	1, 2, 8, 9, 10, 11, 12, 15, 16, 19, 20, 42, 43, 45, 46, 53
Group II/a	32, 41	24, 25, 27, 28, 32, 41, 42, 43	41	–
Group II/b	24, 25, 27, 28, 42, 43	24, 25, 27, 28, 42, 43	24, 25, 27, 28	
Group II/c	6, 17, 18, 44	6, 17, 18, 44	6, 17, 18, 44	6, 17, 18, 44
Group III	30, 35, 36, 63	–	30, 35, 36, 63	30, 35, 36, 63
Outliers	5, 38, 39	33, 34, 35, 36	5, 38, 39	5, 33, 34, 38

Table 4
Reproducible parameters classified according to the correlation matrix and the PC1–PC2 loading plot

Chromatographic property	Parameter number	Parameter name
1. Efficiency	2	n MPPH
	25	n_{toluene}
	31	$\boldsymbol{n_{\text{amylbenzene}}}$
2. Hydrophobicity	3	$rk'_{\text{diazepam/MPPH}}$
	4	$rk'_{\text{toluene/MPPH}}$
	19	$k'_{\text{2,3-dihydroxynaphthalene}}$
	26	k'_{toluene}
	27	$k'_{\text{ethylbenzene}}$
	28	$rk'_{\text{ethylbenzene/toluene}}$ 7
	30	$rk'_{\text{toluene/phenol}}$ 7
	32	$\boldsymbol{k'_{\text{amylbenzene}}}$
	33	$rk'_{\text{ethylbenzene/toluene}}$ 8
	34	$rk'_{\text{amylbenzene/butylbenzene}}$
	36	$rk'_{\text{toluene/phenol}}$ 8
3. Silanol activity	5	$rk'_{\text{diphenhydramine/MPPH}}$
	12	$\boldsymbol{rk'_{\text{benzylamine/phenol}}}$ **pH 2.7**
	14	$rk'_{\text{caffeine/phenol}}$
	15	$rk'_{\text{pyridine/caffeine}}$
	16	$rk'_{\text{pyridine/phenol}}$
4. Steric selectivity	35	$\boldsymbol{rk'_{\text{triphenylene/o-terphenyl}}}$
5. Silanol activity and metal impurity	18	$\boldsymbol{k'_{\text{2,2′-dipyridyl}}}$
6. Metal impurity	20	$\boldsymbol{rk'_{\text{2,3-dihydroxynaphthalene/2,2′-dipyridyl}}}$
7. Not specified	7	$\boldsymbol{rk'_{\text{acetylsalicylic acid/MPPH}}}$
	8	$rk'_{\text{o-hydroxyhippuric acid/MPPH}}$

k': retention factor; n: theoretical plate number; rk': relative retention factor; SF: symmetry factor; MPPH: 5-(p-methylphenyl)-5-phenylhydantoin.
The parameters selected from each class are presented in bold (see details in the text).

ters. All PCA plots were compared to that obtained with 24 parameters.

The following combination of 4 parameters provides a classification most similar to that obtained with 24 parameters: $k'_{\text{amylbenzene}}$ (32), $rk'_{\text{benzylamine/phenol}}$ pH 2.7 (12), $k'_{\text{2,2′-dipyridyl}}$ (18) and $rk'_{\text{triphenylene/}o\text{-terphenyl}}$ (35). Two of the parameters where plausible values had to be introduced ($rk'_{\text{benzylamine/phenol}}$ pH 2.7, $k'_{\text{2,2′-dipyridyl}}$) can be found in this set of 4 parameters. This is logical as the parameters which give the most diverse values will be most suited to differentiate between columns. The PC1–PC2 score plot is shown in Fig. 3, the composition of the Groups obtained is reported in Table 3. The same clusters are obtained as in Fig. 1a. There is a main group I, which is now separated even better into two sub-groups. The type B, non-end-capped columns (Group II/a) are also separated from group I. The type A end-capped and non-end-capped columns are also divided into two subgroups, Groups II/b and II/c, like in Fig. 1a. Polar-embedded columns (Group III) are also clearly distinguished. Column 5 is an outlier column. There are some differences between the two classifications obtained with 24 and 4 parameters, respectively. Columns 8, 33 and 34 were outliers in Fig. 1/a, now they are in Group I/b. Columns 1, 2, 45 and 46, which are close to the border between Groups I/a and I/b, shifted from Group I/a to Group I/b. Column 64, which was in Group I/b (Fig. 1/a) close to Group I/a, now is in Group I/a. This is a more suitable location for the latter column because it is a type B, end-capped column like the others in Group I/a. It can be concluded that classification obtained with these 4 parameters is similar to that obtained with 24 parameters. The set of parameters selected here is called ‘‘best parameter set’’ further on.

These parameters can be determined with 3 sim-

ple, fast, repeatable and reproducible methods (see Table 1). For further details the reader is referred to [34,35]. The parameters represent hydrophobicity (32), silanol activity (12, 18), metal impurity (18) and steric selectivity (35) properties of the columns.

Other PCA plots drawn from the different parameter combinations were also examined to evaluate the obtained classifications. They led to less good classifications of the columns. Examples are shown in Fig. 4, in each plot one parameter was replaced in the best parameter set. The composition of the Groups obtained is reported in Table 3.

It can be observed that these classifications differentiate less clear or not at all between the different groups defined in Fig. 3. Only Fig. 4d gives similar classification. This is not surprising if the properties of the parameters are regarded. In Fig. 4d the $k'_{2,2'\text{-dipyridyl}}$ (18) related to silanol activity and metal impurity content was replaced by $rk'_{2,3\text{-dihydroxynaphthalene}/2,2'\text{-dipyridyl}}$ (20) representing the metal impurity. Compared to Fig. 3 the same properties remain represented.

Fig. 4 also indicates that removing the parameter related to either the hydrophobicity, the silanol activity or the steric selectivity leads to a clearly less good distinction between the different columns. The $k'_{\text{amylbenzene}}$, which characterises hydrophobicity, is important in distinguishing between type A and B columns as well as between normal and large pore size stationary phases and in identification of polar embedded columns, which generally have less hydrophobicity than the non-polar-embedded ones. The latter columns are separated from Group I only if $rk'_{o\text{-terphenyl}/\text{triphenylene}}$, the steric selectivity parameter was in the parameter set. It was also noticed that the silanol activity parameters ($rk'_{\text{benzylamine}/\text{phenol}}$ pH 2.7 and $k'_{2,2'\text{-dipyridyl}}$) are necessary to recognise the old-type, end-capped columns and the old and new-type, non-end-capped columns.

3.4. Principal component analysis on 3 parameters

The number of parameters was reduced further by removing one parameter from the best parameter set to obtain 4 different combinations of 3 parameters. The score plots are shown in Fig. 5 and the detailed

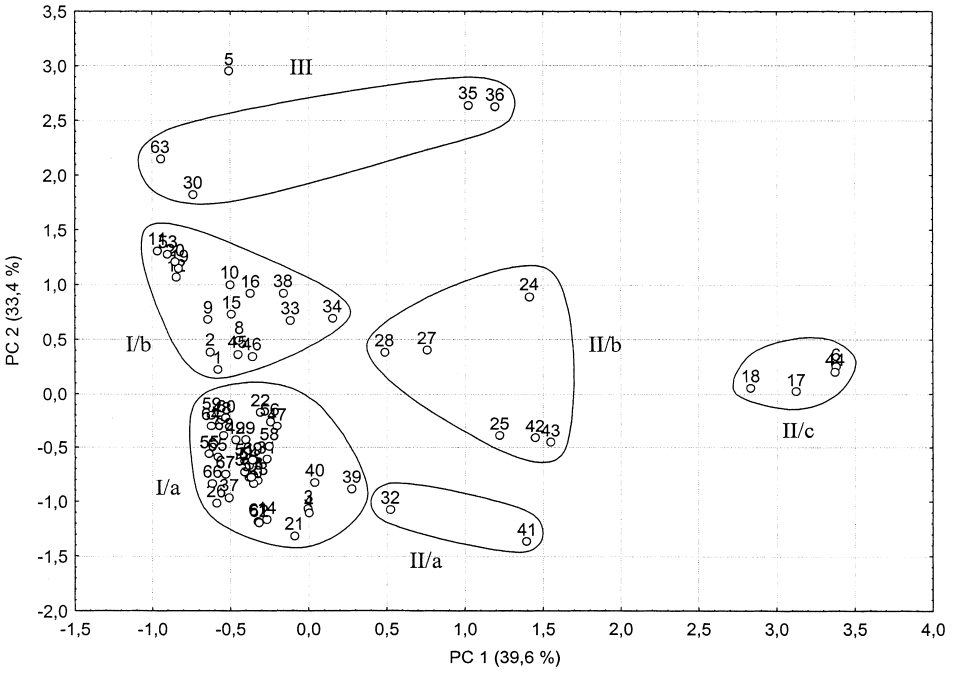

Fig. 3. PC1–PC2 score plot for 69 RP-LC columns employing 4 chromatographic parameters: $k'_{\text{amylbenzene}}$, $rk'_{o\text{-terphenyl}/\text{triphenylene}}$, $rk'_{\text{benzylamine}/\text{phenol}}$ pH 2.7 and $k'_{2,2'\text{-dipyridyl}}$.

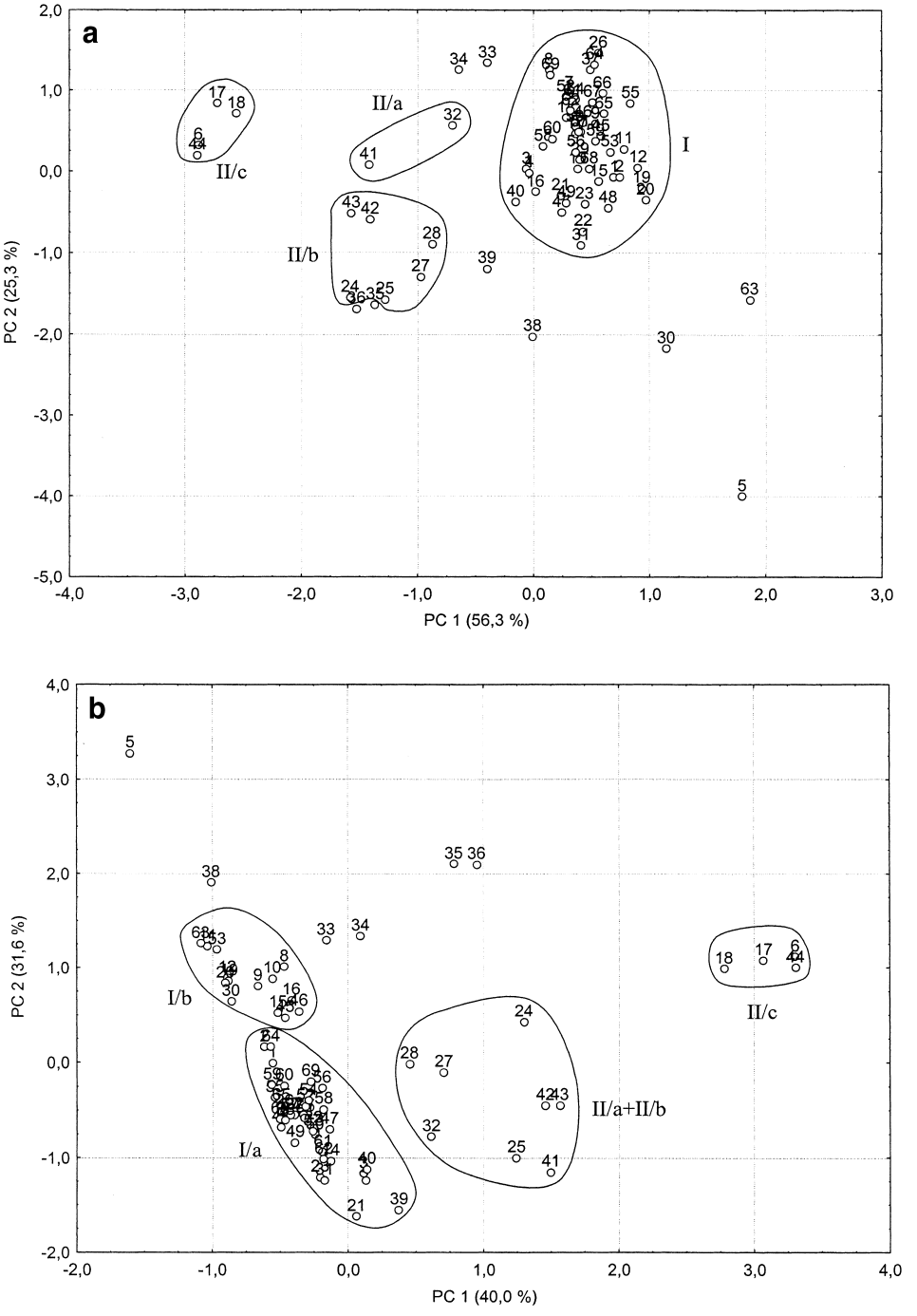

Fig. 4. PC1–PC2 score plot for 69 RP-LC columns employing 4 chromatographic parameters: (a) $rk'_{\text{2,3-dihydroxynaphthalene/2,2′-dipyridyl}}$, $rk'_{\text{o-terphenyl/triphenylene,}}$ $rk'_{\text{benzylamine/phenol}}$ pH 2.7 and $k'_{\text{2,2′-dipyridyl}}$; (b) $k'_{\text{amylbenzene}}$, $rk'_{\text{acetylsalicylic acid/MPPH,}}$ $rk'_{\text{benzylamine/phenol}}$ pH 2.7 and $k'_{\text{2,2′-dipyridyl}}$; (c) $k'_{\text{amylbenzene}}$, $rk'_{\text{o-terphenyl/triphenylene,}}$ $rk'_{\text{acetylsalicylic acid/MPPH}}$ and $k'_{\text{2,2′-dipyridyl}}$; (d) $k'_{\text{amylbenzene}}$, $rk'_{\text{o-terphenyl/triphenylene,}}$ $rk'_{\text{benzylamine/phenol}}$ pH 2.7 and $rk'_{\text{2,3-dihydroxynaphthalene/2,2′-dipyridyl}}$.

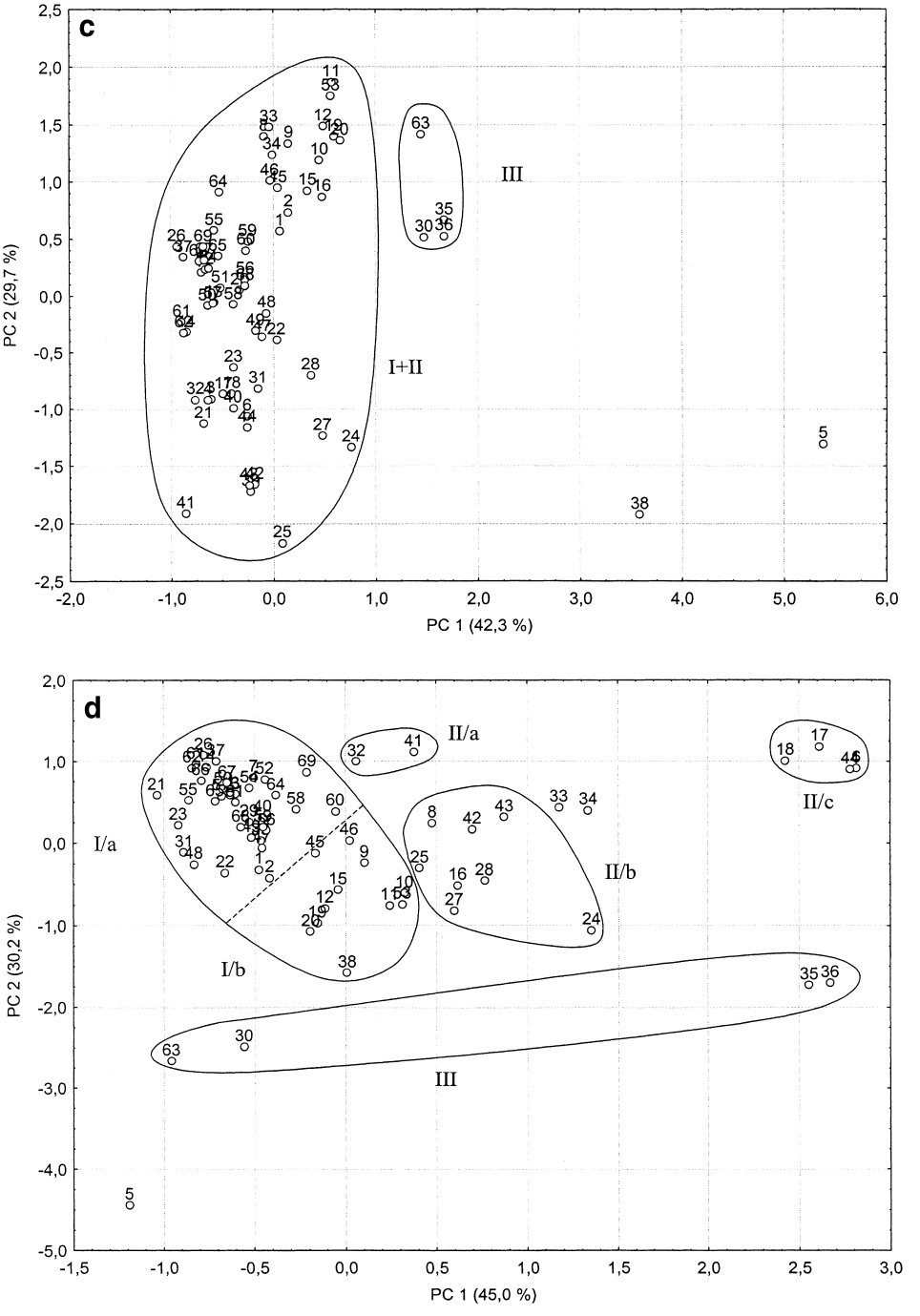

Fig. 4. (*continued*)

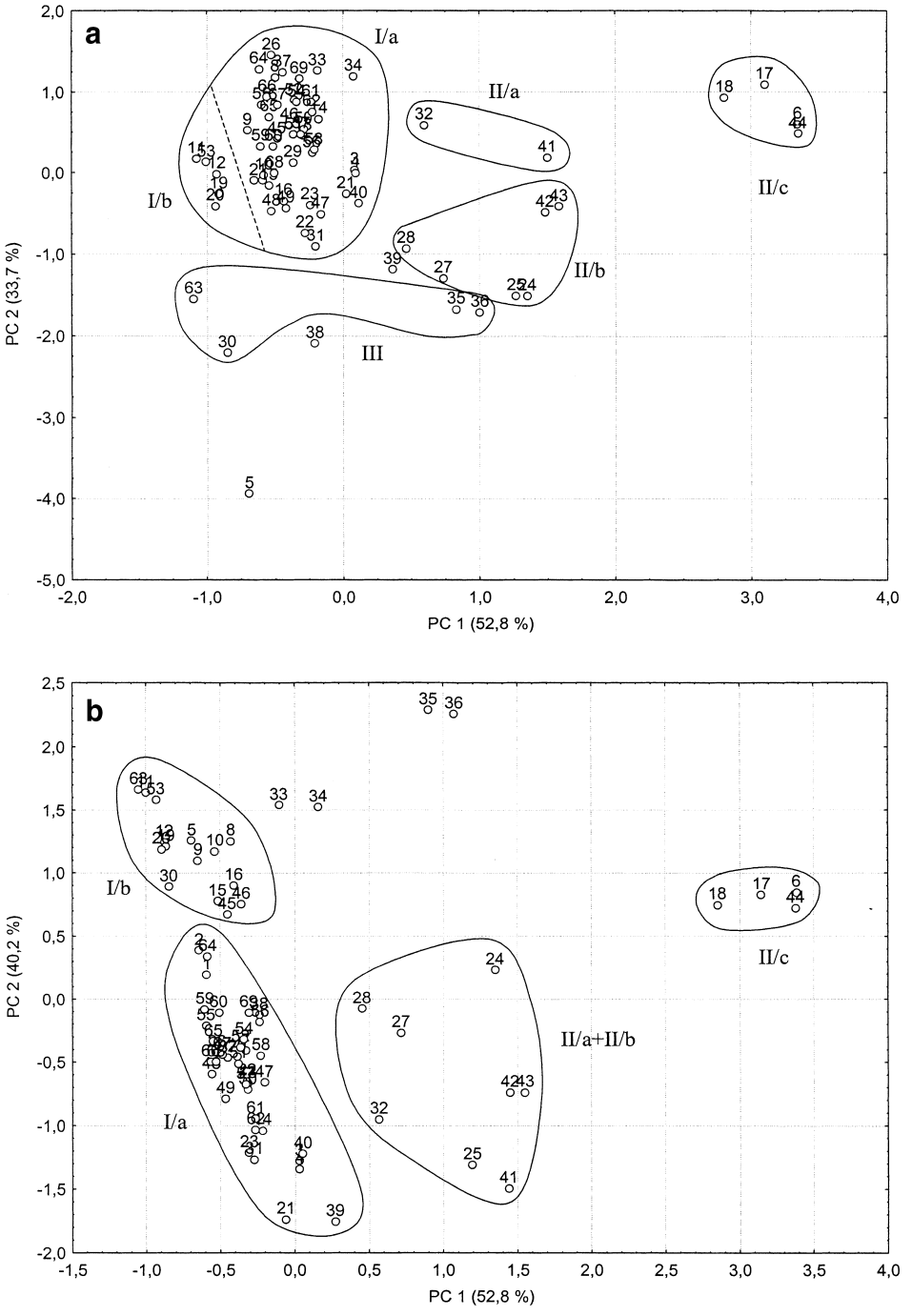

Fig. 5. PC1–PC2 score plot for 69 RP-LC columns employing 3 chromatographic parameters: (a) $rk'_{o\text{-terphenyl/triphenylene}}$, $rk'_{benzylamine/phenol}$ pH 2.7 and $k'_{2,2'\text{-dipyridyl}}$; (b) $k'_{amylbenzene}$, $rk'_{benzylamine/phenol}$ pH 2.7 and $k'_{2,2'\text{-dipyridyl}}$; (c) $k'_{amylbenzene}$, $rk'_{o\text{-terphenyl/triphenylene}}$, and $k'_{2,2'\text{-dipyridyl}}$; (d) $k'_{amylbenzene}$, $rk'_{o\text{-terphenyl/triphenylene}}$, and $rk'_{benzylamine/phenol}$ pH 2.7.

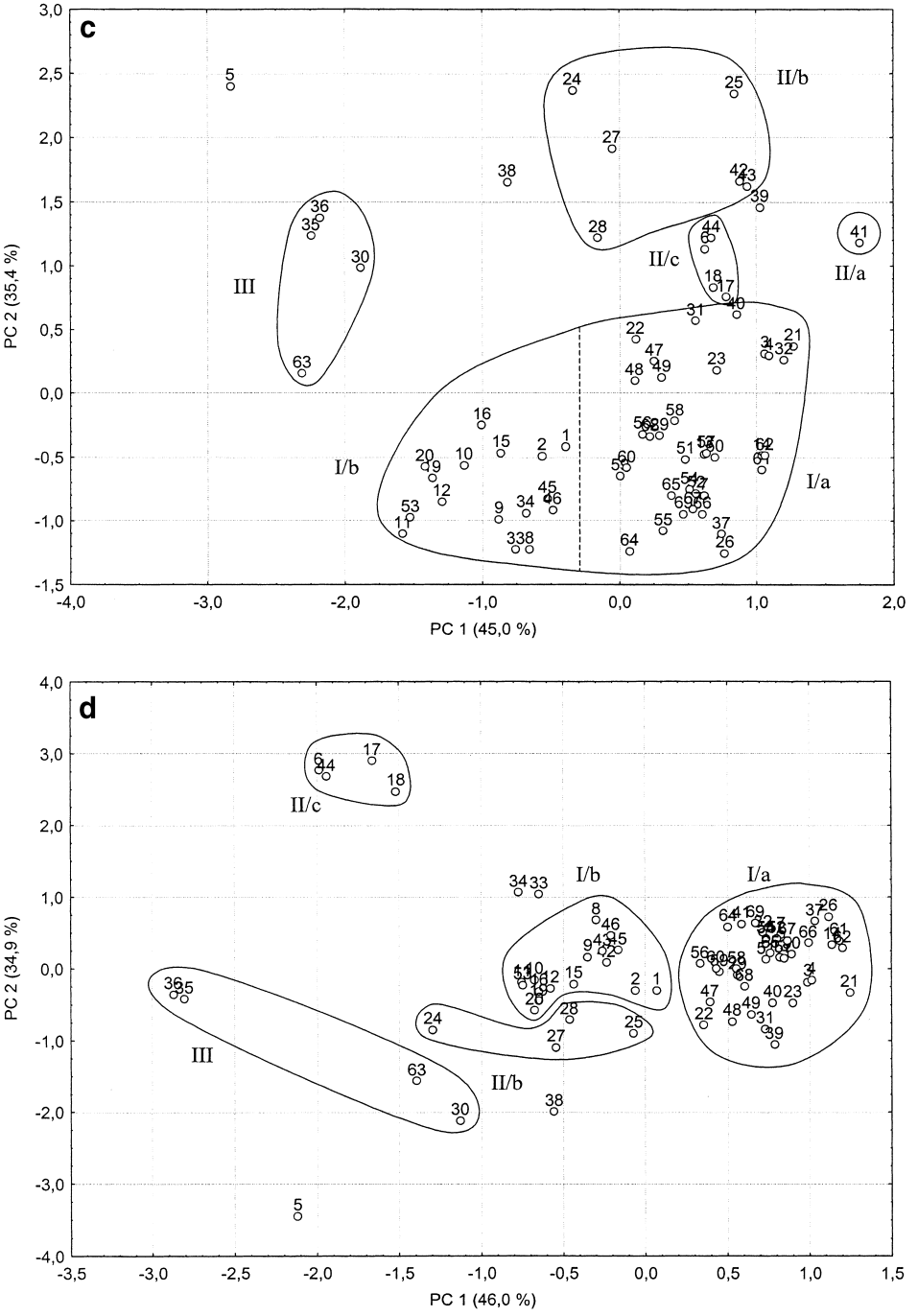

Fig. 5. (*continued*)

information about composition of Groups can be found in Table 3.

In the first combination ($rk'_{o\text{-terphenyl/triphenylene}}$, $rk'_{\text{benzylamine/phenol}}$ pH 2.7 and $k'_{2,2'\text{-dipyridyl}}$) there is no parameter measuring the hydrophobicity. The score plot is shown in Fig. 5a. The main group (I) is separated into two subgroups, Group I/a and Group I/b. Groups II/a, II/b and II/c, the type A, end-capped and non-end-capped and type B, non-end-capped columns are differentiated. Although the polar embedded columns (Column 30, 35, 36 and 63) are outliers they do not form a separate group. The classification can be considered somewhat less good than with 4 parameters (Fig. 3) but in general it is fairly similar.

In combination 2 ($k'_{\text{amylbenzene}}$, $rk'_{\text{benzylamine/phenol}}$ pH 2.7 and $k'_{2,2'\text{-dipyridyl}}$) there is no parameter measuring steric selectivity. The score plot of this combination is shown in Fig. 5b. Again, a main group with two subgroups (I/a and I/b) can be distinguished. The columns with high silanol activity (Group II) are differentiated. Type A and type B columns (Group II/a and II/b) are not separated well, Group II/c is clearly differentiated. Polar embedded columns are not separated well from the main group as might be expected, because the steric selectivity parameter is removed. In general the obtained classification is less good.

In the third combination ($k'_{\text{amylbenzene}}$, $rk'_{o\text{-terphenyl/triphenylene}}$ and $k'_{2,2'\text{-dipyridyl}}$) one of the parameters related to silanol activity is missing. The score plot is represented in Fig. 5c. Due to insufficient characterisation of silanol activity ($k'_{2,2'\text{-dipyridyl}}$ appears to be less good in representing silanol activity), the stationary phases with high silanol activity, e.g. Groups II/a, II/b and II/c are less well separated from Group I/a. Polar embedded columns (Group III) are clearly distinguished from the main group. Globally this classification can be considered somewhat less good than the one obtained with 4 parameters.

The score plot of combination 4 ($k'_{\text{amylbenzene}}$, $rk'_{o\text{-terphenyl/triphenylene}}$ and $rk'_{\text{benzylamine/phenol}}$ pH 2.7) is shown in Fig. 5d. This combination lacks the parameter measuring a combined effect of silanol activity and metal impurity. The main group is separated into two subgroups but Group II/a faded into Group I/a. Group II/c is clearly separated. Polar embedded columns are from Group III. Compared to Fig. 3 this classification is clearly less discriminating between stationary phases.

It can be concluded that classification of the columns based on three parameters can be similar to a great extent to the clustering obtained with 24 or 4 parameters. From a practical point of view the same number of different chromatographic methods (Methods 4, 6 and 8) have to be used to determine these three or four parameters. If only three parameters are measured two compounds less have to be injected in Method 8.

4. Conclusion

On 69 C_{18} columns 24 different reproducible chromatographic parameters were determined. Principal component analysis was used to deal with the enormous amount of data. Different types of columns (stationary phases made of silica gel type A and B with different pore size, end-capped/non-end-capped, base deactivated/not base deactivated, polar embedded) were distinguished with these tests. The PCA classification obtained was compared to the classes of RP columns in the Ph. Eur. and no good correspondence was found.

The number of parameters was reduced in order to develop a general but simple test procedure to characterise RP columns. Based on the correlation matrix of parameters 7 classes of parameters were distinguished. The best parameter from each class was selected. From this set 35 combinations of 4 parameters were examined and the PCA score plots were drawn and compared to that obtained with 24 parameters. The effect of the parameters on the classification was also examined.

The best parameter set, which allows to maintain the classification of columns using only 4 parameters is: $k'_{\text{amylbenzene}}$, $rk'_{o\text{-terphenyl/triphenylene}}$, $rk'_{\text{benzylamine/phenol}}$ pH 2.7 and $k'_{2,2'\text{-dipyridyl}}$. They can be determined with 3 reproducible chromatographic methods. When further reduction was applied and only 3 parameters were retained, the classification could be maintained to a great extent although some groups of columns could not be differentiated.

In a latter part of the project these classifications will be correlated to real separation parameters.

Analyses of real samples will be carried out on the examined columns according to Ph. Eur. monographs. It can be expected that stationary phases having similar test results will give similar real separation selectivity. The final aim of this project is to verify whether it is possible to formulate a simple chromatographic test procedure for the characterisation of stationary phases in order to facilitate selection of appropriate columns for a particular separation. Such a chromatographic test procedure might also be used to verify the column properties at any time of its life cycle.

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