Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1088 (2005) 16–23
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Reversed-phase liquid chromatography testing Role of organic solvent through an extended set of columns

K. Le Mapihan, J. Vial *, A. Jardy

Laboratoire Environnement et Chimie Analytique, UMR 7121, Ecole Supérieure de Physique et de Chimie Industrielles de la Ville de Paris, 10 Rue Vauquelin, 75005 Paris, France

Available online 19 February 2005

Abstract

Column testing is a primary concerns for analysts. It is of use not only for the choice of set of development columns with different behaviors, but also for a substitution column in a validated method or as a quality control of new batches of stationary phase. A validated chromatographic procedure for column testing was applied to 42 commercially available columns, including alkyl, polar embedded and Aqua type stationary phases. This procedure was based on the use of two different solvents: MeOH and MeCN; and two different solvent/aqueous buffer fractions. Principal component analysis has been combined to hierarchical cluster analysis to provide both rational and objective classifications. The solvent effects were then studied on the obtained representations, revealing the necessity for considering both the solvent nature and its fraction in RPLC column testing. Differences observed depending on the solvent nature and fractions revealed quite different chromatographic behaviors according to these parameters.

Keywords: Column testing; Stationary phases; LC; Solvent effects; Principal component analysis; Cluster analysis; Drugs

1. Introduction

To date, the choice of an adapted RPLC column for an actual separation has still been a seemingly impossible task considering the overwhelming number of commercially available stationary phases [1–3]. This already critical problem for the analyst gets more complicated when basic compounds are involved, due to the appearance of additional retention mechanisms. The literature proposed many tests intended to characterize and classify stationary phases [1,2,4–12]. Even if such chromatographic testing procedures had contributed to a better understanding of the retention properties of the column, they left often unaddressed the role of the organic modifier. As it had been demonstrated that the solvent nature had to be considered for the stationary phase characterization [13], we had developed a new testing procedure from a set of 12 stationary phases. Our approach was not based on a priori postulated functions for solutes but on a rational selection of the optimal conditions from an extended test [14]. After having validated our testing procedure it should be applied to a larger set of columns [15]. This work expands the number of columns to 42, including alkyl grafted, polar embedded groups or aqua type phases. The procedure used for generating column classification in an objective way combined two chemometric tools: principal component analysis (PCA) and hierarchical cluster analysis (HCA). PCA [16–18] is a powerful tool for the interpretation of large data tables. Actually, this projection method is able to extract the main information from the original data set by affecting it to a dimensionally reduced space. This space is defined by linear combination of variables, called principal components (PCs). PCs are computed iteratively in such a way that they convey less and less information while being orthogonal. The plots of individuals, i.e. column/solvent couples, in the new defined set of coordinate axes are called score plots whereas the representations of the initial descriptors constitute the loading plots. In the present study, data were centered and standardized in order to give all variables the same importance and a cross validation was performed to assess the reliability of the obtained results. However, it must be underlined that PCA provides more a fair column mapping than a relevant clas-

* Corresponding author. Tel.: +33 1 40794779; fax: +33 1 40794776.
E-mail address: jerome.vial@espci.fr (J. Vial).

0021-9673/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2005.02.023

sification: the use of another data treatment is then required to perform an objective classification, that is, cluster analysis. This tool, which is employed for pattern recognition, is based on the detection of similarities between objects according to the distance between them. The hierarchical method (HCA) is an agglomerative process that works as follows. At the beginning, each individual constitutes a cluster. At each step, the distance between points is calculated and the two closest ones are gathered to form a new cluster until all the original points are together into one group [18,19]. The results are represented in tree diagrams also called dendograms. In this work, the method of clustering was based on the Euclidean distance (centroid criterion) and performed on autoscaled PC-scores. Such an approach is equivalent to perform a cluster analysis based on Mahalanobis distance from the original coordinates. It confers the advantage of a definitely better fit of elongated clusters [17,20]. In the end, the hyphenation of PCA and HCA provided an objective classification. The present study focused particularly on the impact of the organic solvent on chromatographic behavior, both its nature and its fraction [21–24], through more comprehensive classifications generated according to the methodology described previously.

2. Experimental

2.1. Chemicals and reagents

Acetonitrile (MeCN, HPLC ultra gradient grade) and methanol (MeOH, HPLC gradient grade) were purchased from Mallinckrodt Baker (Deventer, The Netherlands). Water was produced by a Milli-Q Plus ultrapure water purification system (Millipore, Molsheim, France). Sodium acetate and acetic acid volumetric standard (1.031 mol L^{-1}, $d = 1.010$) were obtained from Aldrich and used as received.

The test solutes were constituted of amiodarone hydrochloride (Sigma), ampicillin sodium salt (Fluka), atropine sulfate salt (Sigma), *n*-butylbenzene (Aldrich), caffeine (Fluka), clofazimine (Sigma), cyanocobalamine (Sigma), digitoxin (Fluka), *n*-pentylbenzene (Aldrich), strychnine hemisulfate salt (Sigma), *o*-terphenyl (Fluka), triphenylene (Fluka) and D-tubocurarine chloride (Sigma). The set of selected solutes had log *P* values distributed from −0.07 to 7.66, with molecular weights comprised between 92 and 1450 g mol^{-1} and acidity constants pK_a ranging from 1.9 to 10.0 if concern.

2.2. Apparatus

Three different LC systems were used. The first was composed of an HP 1050 quaternary pump, a Waters 715 UltraWisp autosampler, a Waters 2487 UV detector (Waters, Saint-Quentin en Yvelines, France) set at 230 nm plus a Varian 2050 UV detector (Varian, Les Ulis, France) set at 254 nm. The data acquisition was performed thanks to Class-VP 4.2 (Shimadzu, Columbia, MD, USA). The second LC system consisted of a HP 1050 quaternary pump, a HP 1050 autosampler and a HP 1100 variable wavelength detector operated at 230 or 254 nm (see Table 1) with Chemstation 6.03 (Agilent Technologies, Waldbronn, Germany). The third chromatographic system consisted of a Varian Prostar 230 ternary pump (Varian, Les Ulis, France), a Waters 717 Wisp autosampler, a Jasco 875-UV detector set at 230 nm plus an LDC Spectromonitor III set at 254 nm with Azur 3.0. The rate of data acquisition was at least 25 Hz for all systems. Concerning temperature regulation, all the tested columns were placed in an Alltech water jacket connected to a water bath set at 40 °C (±0.1 °C with the water bath Bioblock 18205 for the first and the third LC system, ±0.03 °C with the water bath Neslab RTE-101 for the second LC system). All the columns were operated using a flow rate of 1 mL min^{-1}. It had been verified previously that both data acquisition systems were able to produce equivalent measurements from a common chromatogram on the two kinds of recorded parameters: retention times and asymmetries.

2.3. Running conditions

The protocol of the testing procedure was based on the previously described one [14]. This study demonstrated that acetate buffer at pH 5 was the best option concerning quality of information. Consequently, such a choice maximized the discriminating power of our testing procedure. It must also be underlined that pH was revealed a critical parameter [15], a particular care had to be taken when preparing the buffer solution as the purely aqueous value for pH had to be comprised between 4.95 and 5.05 at 25 °C. All buffers were filtered through 0.45 μm HA type filters, (Millipore, Molsheim, France), before addition of the organic modifier. Mobile phases were freshly prepared just before use imperatively by weight [15] for each experiment within the ratios indicated in Table 1, which summarizes the whole condi-

Table 1
Conditions of the test

Conditions	Solvent	Solvent fraction (%)	Level	Solute
Acetate buffer at pH 5.00 $T = 40$ °C, flow rate = 1 mL min^{-1} λ = 254 nm for solutes marked with *, λ = 230 nm otherwise	MeOH	70	A	Digitoxin, clofazimine, amiodarone, butylbenzene*, pentylbenzene*, *o*-terphenyl*, triphenylene*
	ACN	59		
	MeOH	15	D	Strychnine*, caffeine*, *d*-tubocurarine, atropine, ampicillin, cyanocobalamin
	ACN	9		

tions of the test, including the detection conditions towards the corresponding solutes.

All compounds were injected at 50 ppm except for *o*-terphenyl (12 ppm), triphenylene (3 ppm), atropine (400 ppm), ampicillin (200 ppm), strychnine (100 ppm) and D-tubocurarine (100 ppm). At least 1-h equilibration was performed for each mobile phase before the 10 μL injection of mixtures in duplicates. The column void volume was determined by the injection of thiourea (Aldrich) in the acetonitrile rich mobile phase. All samples were stored at 4 °C or less.

2.4. Tested columns

The testing procedure has been applied to 42 columns. The chosen set of columns consisted of 35 different stationary phases that are commonly used in the pharmaceutical industry. All columns were 150 mm × 4.6 mm I.D. apart from Symmetry C18 (250 mm × 4.6 mm I.D.), SymmetryShield RP8 and Chromolith Performance RP-18e (both 100 mm × 4.6 mm I.D.). The chosen columns differed from each other in the moiety length (C8, C16 or C18 graft), in the kinds of protection against residual silanol groups and the applications they are designed for. For the sake of simplicity, the same abbreviations as for [14] were employed. Thus, stationary phases were identified as following:

(1) The first letter referred to the category the stationary phase belongs to: A, C, E and P correspond respectively to Aqua type, purely Carbonaceous moiety, polymer Encapsulated and a Polar embedded group columns;
(2) The figures express the number of carbon of the entire graft (including the polar group if need be);
(3) The final number means the testing order of the stationary phase in the category.

The available characteristics of the tested columns are reported in Table 2. The 35 different stationary phases tested are representative of the special base stationary phases and give a good idea of their wide diversity.

For legibility reasons, the nature of the testing solvent was figured by a symbol: a triangle for acetonitrile and a circle for methanol.

2.5. Figures of merit

Only the principal component analyses based on retention factors will be considered as this chromatographic parameter proved to be the only robust and informative one for all the solutes amongst the classical recorded parameters, i.e. retention factors, asymmetries and efficiencies [15].

2.6. Softwares

The Unscrambler 7.5 (Camo Asa, Oslo, Norway) was used to perform principal component analyses while cluster analysis was carried out with JMP 4.0.5 (S.A.S. Institute, Carry, NC, USA).

3. Results and discussion

3.1. Overall PCA

Fig. 1 compares the score plots obtained with 24 column/solvent couples used previously for the development step and the current 84 couples resulting of the increase of the number of columns tested.

The two first principal components still convey the major part of the available information (76% versus 82% for the former classification). If the new representation remained V-shaped, it also revealed an unexpected loss of solvent nature effect: as emphasized by the solid symbols, the split into two half-planes according to the solvent nature encountered to several mismatches.

Moreover, it can be also noticed that the relative spread of the individuals is reduced by the increase in the number of columns. Such results, rather than refuting our previous ones, would indicate a perturbation responsible for the observed

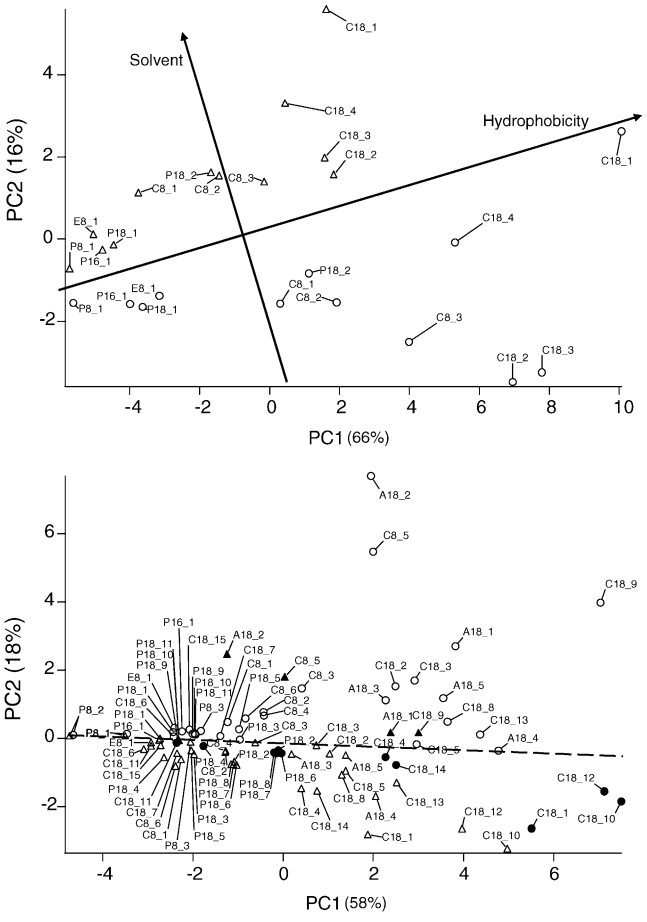

Fig. 1. Score plot based on all the retention factors of the 12 stationary phases [14] (up) and of the 42 columns (down). Solid symbols correspond to mismatches in the split into two half-planes according to the solvent nature.

fuzziness. The resulting loss of discriminating power could be a consequence of the presence of stationary phases that exhibit a particular chromatographic behavior in comparison to those we had tested before. For example, the Aqua type phases are designed for being operated with purely aqueous eluents, whereas alkyl phases are not enabled to do. Therefore, it could be conceivable that the chromatographic behavior at low solvent fractions would much differ between these two types of stationary phases, originating from different and/or additional retention mechanisms or differences in chain conformation. As the perturbation could be the result of performing the test with two solvent fractions, an evalua-

Table 2
Tested stationary phases and their characteristics

Stationary phase	$\bar{D}$	d_p	%C	S_p	Supplier	Remarks[a]	Abbreviation
Capcell Pak C8 UG 120	12	5	10	300	Shisheido	1a; 3b; 4c	E8_1
XTerra RP 8	12.5	3.5	13.37	174	Waters	1b; 2a; 5a	P8_1
Discovery RP amide C16	18	5	12.04	198	Supelco	1a; 2b; 5a	P16_1
XTerra RP 18	12.5	3.5	14.42	172	Waters	1b; 2a; 5a	P18_1
SymmetryShield RP 18	10	3.5	17.04	339	Waters	1a; 2a; 5a	P18_2
Zorbax eclipse XDB-C8	8	5	7.6	180	Agilent	1a; 5a	C8_1
Symmetry C8	10	5	12.15	344	Waters	1a; 5a	C8_2
Kromasil C8	10	5	12	340	Akzo Nobel	1a; 5a	C8_3
J'sphere ODS-H80	8	4	22	–	YMC	1a; 3a; 4c; 5c	C18_1
Zorbax stable Bond C18	8	3.5	10	180	Agilent	1a; 4b	C18_2
Nucleosil C18 HD	10	5	20	350	Macherey-Nagel	1a; 5a	C18_3
Symmetry C18	10	5	19.45	341	Waters	1a; 5a	C18_4
Aquasil C18	10	5	12	310	ThermoHypersil	1a; 5b	A18_1
Zorbax stable bond Aq	8	5	–	180	Agilent	1a; 4c; 5c	A18_2
YMC-Pack ODS-AQ	12	4	14	300	YMC	1a; 5b	A18_3
Synergi Hydro-RP	8	4	19	475	Phenomenex	1a; 5b	A18_4
Uptisphere HDO	12	5	16	324	Interchim	1a; 4c; 5b	A18_5
Luna C8 (2)	9.7	5	14.15	421	Phenomenex	1a; 5a	C8_4
Zorbax stable bond C8	8	3.5	5.5	180	Agilent	1a; 4a	C8_5
Zorbax eclipse XDB-C8	8	5	7.6	180	Agilent	1a; 5a	C8_6
Symmetry C18 (used)	10	5	–	–	Waters	1a; 5a	C18_5
Chromolith performance RP-18e	13	Eq. 5	18	300	Merck	1c; 5a	C18_6
Discovery C18	18	5	12.44	199	Supelco	1a; 5a	C18_7
Uptisphere ODB	12	5	17.4	330	Interchim	1a; 5a	C18_8
Uptisphere NEC	12	5	16	333	Interchim	1a	C18_9
Uptisphere HSC	–	5	19.2	–	Interchim	1a; 5a	C18_10
Uptisphere TF	–	5	13.3	–	Interchim	1a; 3c; 4c; 5a	C18_11
BetaMax neutral	6	5	29	–	ThermoHypersil	1a; 5a	C18_12
Nucleodur 100-5 C18 EC	11	5	17.5	–	Macherey-Nagel	1a; 5a	C18_13
Nucleodur C18 gravity	11	5	18	–	Macherey-Nagel	1a; 5a	C18_14
Hypersil gold	17.5	5	10	220	ThermoHypersil	1a; 5a	C18_15
Hypurity advance	–	5	–	–	ThermoHypersil	1a; 2b; 5a	P8_2
SymmetryShield RP8	10	3.5	14.73	343	Waters	1a; 2a; 5a	P8_3
Polaris C18-A	8	5	–	220	Ansys	1a; 2d; 5a	P18_3
Polaris amide C18	8	5	–	220	Ansys	1a; 2b; 5a	P18_4
Polaris C18-Ether	8	5	–	220	Ansys	1a; 2c; 5a	P18_5
Symmetry shield RP 18	10	3.5	17.5	340	Waters	1a; 2a; 5a	P18_6
Symmetry shield RP 18	10	3.5	17.48	340	Waters	1a; 2a; 5a	P18_7
Symmetry shield RP 18	10	3.5	17.46	340	Waters	1a; 2a; 5a	P18_8
XTerra RP 18	12.5	3.5	14.77	182	Waters	1b; 2a; 5a	P18_9
XTerra RP 18	12.5	3.5	14.62	176	Waters	1b; 2a; 5a	P18_10
XTerra RP 18	12.5	3.5	14.6	176	Waters	1b; 2a; 5a	P18_11

$\bar{D}$: Pore diameter (nm); d_p: particle diameter (μm); %C: percentage carbon and S_p: surface area.

[a]

Key	Remarks	a	b	c	d
1	Silica type	Ultrapure and spherical	Hybrid and spherical	Monolithic	
2	Polar embedded group	Carbamate	Amide	Ether	Not specified
3	Bonding type**	Polymeric	Polymer encapsulation	Trifunctional	
4	Graft base***	Diisopropylalkyle	Diisobutylalkyle	Not specified	
5	Endcapping*	Apolar	Polar	Not specified	

* If applicable, ** if not monomeric and *** if not diisomethylalkyle.

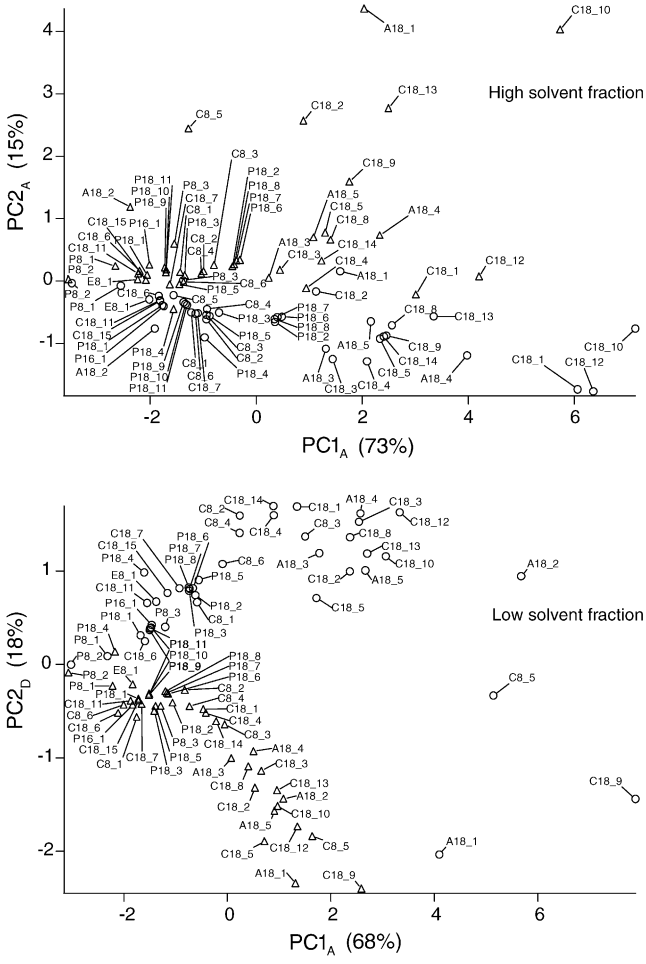

Fig. 2. Score plots based on the retention factors obtained at high solvent fraction (up) and at low solvent fraction (down).

tion of the obtained results according to the organic solvent fraction was carried out.

3.2. PCAs, according to the solvent fraction

The score plots obtained respectively at high and low solvent fractions are depicted on Fig. 2. To facilitate the recognition of the fraction, the principal components were indexed according to the letter that identifies the eluent: A for high solvent fraction and D for low solvent fraction.

The obtained PCA score plots at high and low solvent fractions accounted respectively for 88 and 86% of the available information with two principal components. Both representations were still V-shaped and above all, the split due to solvent nature was restored, apart from 2 to 3 couples (that could be considered as outliers considering the total number of individuals: 84). However, it clearly appears that the general patterns were quite different. It corresponded to different chromatographic behaviors leading to different gathering based on similarities between column/solvent couples. To go further, the study had been deepened by revealing the column mapping organization thanks to cluster analysis. HCA was performed on the two first principal components, which described equivalent information amounts. Therefore, it enabled a fair comparison of their respective dendrograms, which were "cut" at similar distance from the central trunk to give 21 groups. For the sake of legibility, the 21 D-clusters (obtained in the D eluent) were identified thanks to a color code while the 21 A-clusters (from the A eluent) were numbered, as can be seen on Fig. 3. The attributed colors of the D eluent clustering were kept during the cluster analysis performed on the A eluent. As a consequence, a double clustering was finally available and mismatches would be then revealed immediately in case of mixture of colors in a same numbered cluster.

First, almost all column/solvent couples were gathered according to their testing solvent apart from very few exceptions that all corresponded to poorly retentive phases: the need for considering the solvent nature for column characterization was reasserted. It also clearly appeared that several groups belonging to a particular colored cluster did not gathered in the numbered clusters, yielding the solvent fraction dependence of column mapping. Therefore, it was necessary to consider the solvent fraction in addition to its nature. To stress the necessity of taking into account solvent fraction impact on column characterization, chromatographic illustrations are given in Fig. 4.

Actually, the chromatographic behaviors of C8_3 (Kromasil C8) and P18_5 (Polaris C18-Ether) were close at high solvent fraction with equivalent average retention. Nevertheless, it was no longer the case at low solvent fraction where C8_3 exhibited a much stronger retention (two-times stronger). Such discrepancies made the use of two different classifications according to solvent fraction necessary. In addition, it could be noticed that the segregation of couples according to the solvent nature occurred at a smaller similarity for the D eluent, resulting of a more balanced dendrogram. This phenomenon could be related to the score plot of Fig. 2: the V-shape was more widely opened at low solvent fraction than at high one, signifying a more pronounced impact of the solvent nature for the D eluent. This result underlined the differences concerning retention mechanism in relation to the solvent fraction.

At this step, we have obtained objective classifications of the tested stationary phases. In order to go beyond purely descriptive classifications, it could be useful to give a meaning to principal components.

3.3. PCA interpretations

Fig. 5 shows the score plots of interest after performing hierarchical cluster analysis.

It first appeared that all polar embedded phase are gathered in a rather reduced portion of the PC space, for the both solvent fractions. Concerning Aqua type phases, they are all the more scattered as the solvent fraction is low. The general observed order along $PC1_A$ was the following: polar group em-

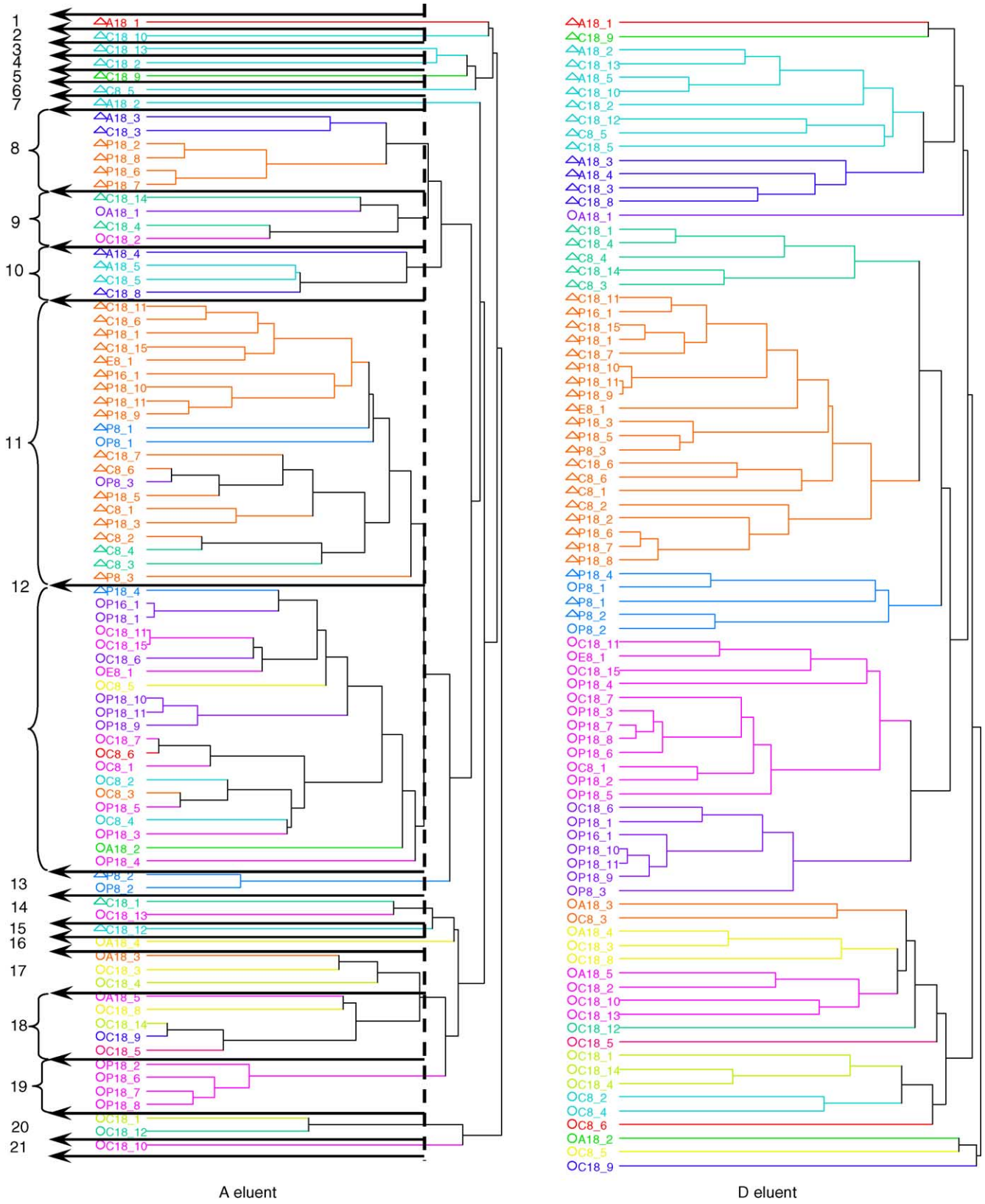

Fig. 3. Cluster analyses; the vertical doted line stands for the dendrogram truncation.

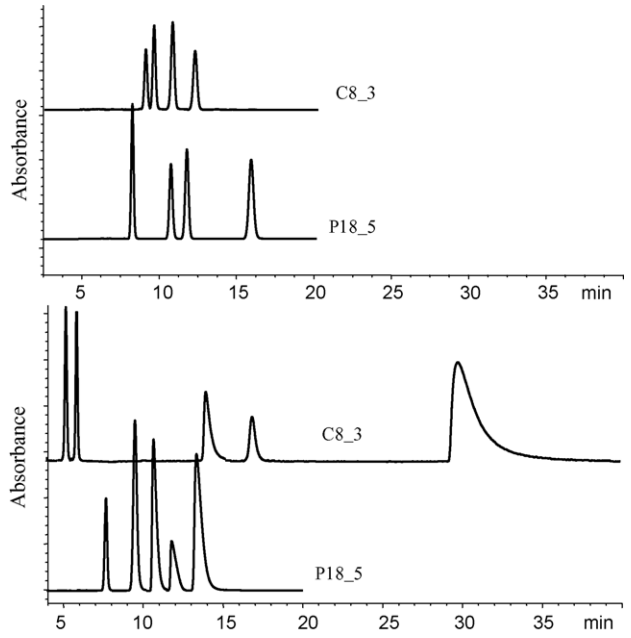

Fig. 4. Chromatograms of C8_3 (Kromasil C8) and P18_5 (Polaris C18-Ether) with methanol as the organic modifier; (up) at high solvent fraction, injection of hydrophobic neutral compounds; (down) at low solvent fraction, injection of hydrophilic solutes.

bedded in a C8 graft < polar group embedded in a C18 graft ∼ high bonding density C8 < C18 graft < high bonding density C18. The stationary phases seemed to be distributed according to their hydrophobicity along $PC1_A$. This interpretation was confirmed by the examination of the chromatographic behavior of several neutral hydrophobic compounds that initially belonged to the Tanaka's test, i.e. butylbenzene, pentylbenzene, *o*-terphenyl and triphenylene. Such solutes can be considered as good probes for reflecting the hydrophobicity of the tested stationary phases, as they interact mainly by dispersive interactions.

Fig. 6 illustrates the chromatographic behavior of such compounds, which were injected in the same chromatographic conditions on three C18 stationary phases. Elution order was systematically checked by individual injections for each compound. These phases differed only by their $PC1_A$ coordinate. The increasing order that is noticed between Discovery C18 and J'Sphere ODS H80 on the PC1-PC2 score plot just express the shift of the Tanaka's solute barycenter towards higher retentions, meaning a likely rise in the accessible hydrocarbon surface. As the mean retention for the hydrophobic Tanaka's compounds increased twofold between the first and the third columns, this example highlights, if need be, the wide diversity of C18 stationary phases and the resulting impossibility to choose an adapted column towards a new separation only from the data provided by the suppliers.

While $PC2_A$ and $PC2_D$ were related to the solvent nature, that expressed differently according to the solvent fraction and the nature of the stationary phase, $PC1_D$ seemed to be

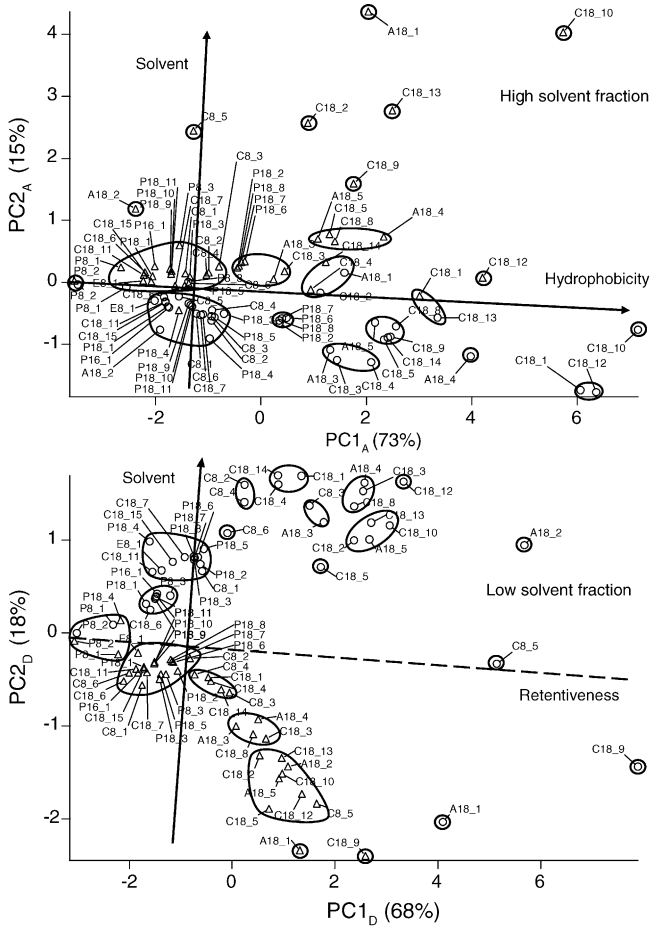

Fig. 5. Twenty-one group clustered PC1-PC2 score plots at high and low solvent fractions.

more or less organized according to the hydrophobicity of columns, but only partially. Actually, the presence of C8_5 at high values of $PC1_D$, which more correspond to C18 stationary phases, was not in accordance with such an interpretation. Complementary investigations would be required for a more comprehensive interpretation. Table 3 summarizes the result of this primary interpretation.

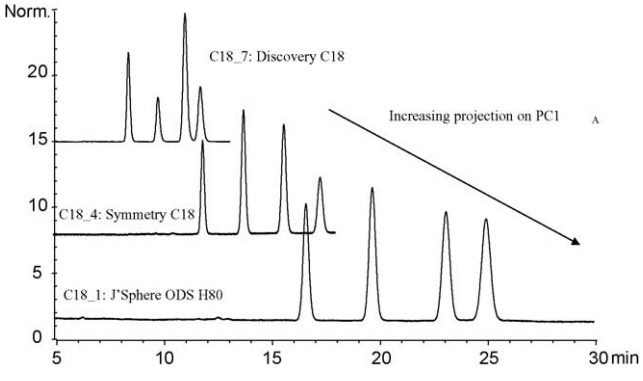

Fig. 6. Chromatograms of butylbenzene, *o*-terphenyl, triphenylene and pentylbenzene injected on three C18 columns at high fraction of acetonitrile.

Table 3
Meaning of the principal components at high and low solvent fraction

Organic solvent fraction	High (A)		Low (D)	
PC	Explained variance (%)	Interpretation	Explained variance (%)	Interpretation
PC1	73	Hydrophobicity	68	Retentive power
PC2	15	Related to solvent	18	Related to solvent
Interpreted information amount	88		86	

4. Conclusions

The introduction of many different stationary phases to the initial set has demonstrated the necessity for considering not only the solvent nature, confirming our previous results, but also the solvent fraction to perform a reliable column test. It is not only true for the design of the testing procedure itself but also for the data processing and interpretation. Therefore, it had been possible to show quite different clustering patterns according to the solvent fraction. These clusterings had been validated chromatographically, indicating the existence of different retention mechanisms depending also on the solvent fraction. Henceforth, it would be of interest to elucidate which physicochemical phenomena govern these mechanisms.

Acknowledgments

We would like to thank Aventis Pharma (Centre de Recherche de Vitry-Alfortville) for supporting this work and all suppliers for their participation.

References

[1] H. Engelhardt, M. Arangio, T. Lobert, LC–GC (1997) 856.
[2] M.R. Euerby, P. Petersson, LC–GC Eur. (2000) 665.
[3] M.R. Euerby, P. Petersson, J. Chromatogr. A 994 (2003) 13.
[4] U.D. Neue, E. Serowik, P. Iraneta, B.A. Alden, T.H. Walter, J. Chromatogr. A 849 (1999) 87.
[5] U.D. Neue, B.A. Alden, T.H. Walter, J. Chromatogr. A 849 (1999) 101.
[6] E. Cruz, M.R. Euerby, C.M. Johnson, C.A. Hackett, Chromatographia 44 (1997) 151.
[7] K. Kimata, K. Iwaguchi, S. Onishi, K. Jinno, R. Eksteen, K. Hosoya, M. Araki, N. Tanaka, J. Chromatogr. Sci. 27 (1989) 721.
[8] D.V. McCalley, J. Chromatogr. A 769 (1997) 169.
[9] R.J.M. Vervoort, E. Ruyter, A.J.J. Debets, H.A. Claessens, C.A. Cramers, G.J. de Jong, J. Chromatogr. A 931 (2001) 67.
[10] H.A. Claessens, Trends Anal. Chem. 20 (2001) 563.
[11] H.A. Claessens, M.A. van Straten, C.A. Cramers, M. Jezierska, B. Buszewski, J. Chromatogr. A 826 (1998) 135.
[12] C. Stella, P. Seuret, S. Rudaz, P.-A. Carrupt, J.-Y. Gauvrit, P. Lanteri, J.-L. Veuthey, J. Sep. Sci. 25 (2002) 1351.
[13] K. Le Mapihan, J. Vial, A. Jardy, Chromatographia 57 (2003) 163.
[14] K. Le Mapihan, J. Vial, A. Jardy, J. Chromatogr. A 1030 (2004) 135.
[15] K. Le Mapihan, J. Vial, A. Jardy, J. Chromatogr. A 1061 (2004) 149.
[16] D.L. Massart, B.G.M. Vandeginste, L.M.C. Buydens, S.D. Jong, P.J. Lewi, J. Smeyers-Verbeke, in: D.L. Massart, B.G.M. Vandeginste, L.M.C. Buydens, S.D. Jong, P.J. Lewi, J. Smeyers-Verbeke (Eds.), Handbook of Chemometrics and Qualimetrics: Part A, Elsevier, Amsterdam, 1997, p. 519.
[17] D.L. Massart, B.G.M. Vandeginste, L.M.C. Buydens, S.D. Jong, P.J. Lewi, J. Smeyers-Verbeke, in: D.L. Massart, B.G.M. Vandeginste, L.M.C. Buydens, S.D. Jong, P.J. Lewi, J. Smeyers-Verbeke (Eds.), Handbook of Chemometrics and Qualimetrics: Part B, Elsevier, Amsterdam, 1997, p. 87.
[18] J.N. Miller, J.C. Miller, in: J.N. Miller, J.C. Miller (Eds.), Statistics and Chemometrics for Analytical Chemistry, Prentice Hall, Harlow, 2000, p. 214.
[19] D.L. Massart, B.G.M. Vandeginste, L.M.C. Buydens, S.D. Jong, P.J. Lewi, J. Smeyers-Verbeke, in: D.L. Massart, B.G.M. Vandeginste, L.M.C. Buydens, S.D. Jong, P.J. Lewi, J. Smeyers-Verbeke (Eds.), Handbook of Chemometrics and Qualimetrics: Part B, Elsevier, Amsterdam, 1997, p. 57.
[20] B. Massart, Q. Guo, F. Questier, D.L. Massart, C. Boucon, S. de Jong, B.G.M. Vandeginste, Trends Anal. Chem. 20 (2001) 35.
[21] J.H. Park, Y.K. Lee, Y.C. Weon, L.C. Tan, J. Li, L. Li, J.F. Evans, P.W. Carr, J. Chromatogr. A 767 (1997) 1.
[22] L.C. Tan, P.W. Carr, J. Chromatogr. A 775 (1997) 1.
[23] A. Vailaya, C. Horváth, J. Chromatogr. A 829 (1998) 1.
[24] P. Nikitas, A. Pappa-Louisi, P. Agrafiotou, J. Chromatogr. A 1034 (2004) 41.