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Chromatographic classification and comparison of commercially available reversed-phase liquid chromatographic columns using principal component analysis

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Abstract

A total of 135 commercially available alkyl, cyano, phenyl, perfluorinated, polar embedded, enhanced polar selectivity (i.e., polar/hydrophilic endcapped), ''Aqua type'' and a variety of novel phases including some non-silica based stationary phases have been characterised in terms of their surface coverage, hydrophobic selectivity, shape selectivity, hydrogen bonding capacity and ion-exchange capacity at pH 2.7 and 7.6. Principal component analysis has been used to provide a simple graphical comparison of the differences/similarities between columns in the entire database and differing subsets such as ''Aqua type''/enhanced polar selectivity phases. The PCA has been correlated to the phase's ability to analyse a range of hydrophilic bases.

Keywords: Stationary phases, LC; Principal component analysis; Column characterization

1. Introduction

Novice and experienced analysts alike are faced with an ever-increasing array of LC stationary phase materials. The dilemma is; ''which of these columns is the optimum for a certain application?'' Unfortunately, the situation is made even more complicated by the many and varied claims that are made by the stationary phase manufacturers regarding their new and old materials. The problem is compounded by the fact that the manufacturers do not use a standardised testing procedure. Comparing manufacturer's data proves to be of little value since experimental conditions are often chosen to show one stationary phase material in a better light than another. Despite the many advances in separation science, the weak link in method development still involves stationary phase selection. Unfortunately, in too many cases, column selection is based on which column worked best previously, or worse still, which one is in the LC oven!

There have been numerous attempts by academic groups, stationary phase manufacturers and end users to characterise LC phases by chromatographic, spectroscopic and physical approaches [1–4].

Many manufacturers provide physical parameters

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relating to their packing materials such as percent carbon load, particle size distribution (μm), surface area (m^2/g), pore size (Å), pore volume (ml/g), calculated bonded phase coverage ($\mu mol/m^2$) and if the phase has been end-capped or not. These parameters are extremely useful for quality control purposes for the manufacturers [5]; however, there is often little correlation between these parameters and the chromatographic performance of the phase [5–7].

Techniques such as ^{29}Si, ^{13}C, ^{1}H NMR and FT-IR spectroscopy have been employed to investigate the surface characteristics of LC phases. The technique of NMR spectroscopy is very expensive and not widely available. Additionally, the data obtained are very complex and their relationship to chromatographic observations is uncertain and therefore is of very little relevance to the practicing chromatographer [8–11].

In contrast, the chromatographic approach is the most meaningful in that it seeks to measure and specify, discrete physico-chemical interactions between certain simple and well-characterised probes/analytes and a stationary phase. Various analytes have been used and experimental conditions are described in the literature [1–5,12–26].

The chromatographic approach has been combined with differing chemometric tools such as principal component analysis (PCA), cluster analysis and radar plots to further visual groupings, to characterise RP packing materials and to try to gain a better understanding of the underpinning molecular interactions between the analyte and the stationary phase material [6,13,23,27–31].

In order to enable the analyst to make a rational selection, we have performed an independent assessment/characterisation on 135 new and old stationary phases possessing differing chemistries, ''using a well known and recognised standard testing procedure [13,14,24,30–36]'', in order to generate an unbiased database. This work expands on our original paper, which characterised 79 differing phases [13] and on a recent paper, which re-analysed our original database plus an additional eight new phases [31]. The expanded database of 135 commercially available phases now contains alkyl, cyano, phenyl, perfluorinated, polar embedded, polar/hydrophilic endcapped (enhanced polar selectivity), ''Aqua type'' and a variety of novel phases including some non-silica based stationary phases.

The database can be interpreted in many ways: we have highlighted the usefulness of using PCA in order to:

- Identify columns with equivalent properties to eliminate the need to be tied to one manufacturer and to classify columns into pharmacopoeial types.
- Select columns of widely differing chromatographic characteristics in order to fully exploit their selectivity differences in method development. Combining the knowledge of the analyte's physicochemical properties with that of the phase's characteristics it should be feasible to weight the column selection accordingly.
- To obtain a greater understanding of the molecular interactions between the analyte and the stationary phase.

PCA [37,38] is a general tool for interpretation of large data tables. In PCA the number of variables (in this case the six column characterisation parameters) is reduced by a projection of the objects (135 stationary phases) onto a smaller number of new variables termed principal components (PC). The PCs are orientated so that the first PC describes as much as possible of the original variation between the objects. The second PC is orientated in an orthogonal manner to the first PC and is directed to describe as much as possible of the remaining variation and so on.

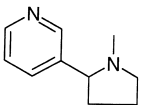
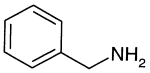
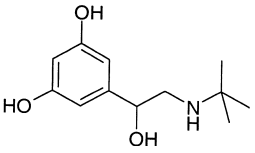
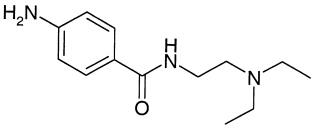
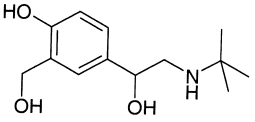
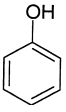
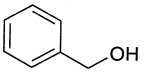
The projection of objects onto a PC is called scores—by plotting the scores for two PCs it is possible to graphically find similarities and differences between objects (stationary phases). The distance between objects in a score plot shows if they are similar or different.

How much of each of the original variables that are included in a PC is described by so-called loadings, one for each variable. By plotting the loadings for two PCs, it is possible to see which of the original variables are most important (longest distance from the origin) and if any variables are correlated (the same or opposite directions on a straight line through the origin).

The reason why two objects are different can easily be determined with a so-called contribution plot. This type of plot shows which variables (chromatographic parameters) cause a difference between two objects (stationary phases) or, alternatively, one object and the average object.

The results of the PCA have, additionally, been

Table 1
Structure, Log D and pK_a values of the hydrophilic bases

Compound number	Name	Structure	pK_a of amino groups	Log D at pH 2.7
1	Nicotine		9.13±0.40 4.23±0.12	−3.01
2	Benzylamine		9.40±0.10	−2.01
3	Terbutaline		9.45±0.50	−2.62
4	Procainamide		9.86±0.25 2.63±0.10	−2.13
5	Salbutamol		9.22±0.50	−3.08
6	Phenol		–	1.48
BA	Benzylalcohol		–	1.03

correlated with the analysis of five basic analytes of differing physico-chemical properties (see Table 1) in order to assess the scope and applicability of the characterisation procedure in the rational selection of stationary phases.

2. Experimental

2.1. Chemicals and reagents

All solvents used were of at least HPLC grade supplied by Romil (Cambridgeshire, UK) except for the water, which was provided by a Milli-Q-plus 185 ultra pure water system (Molsheim, France). Benzylamine hydrochloride, *n*-pentylbenzene, *n*-butylbenzene, triphenylene, *o*-terphenyl, caffeine, benzylalcohol and thiourea were all supplied by Sigma–Aldrich (Dorset, UK). Phenol, KH_2PO_4, H_3PO_4 and KOH were supplied by Fisher Scientific (Leicestershire, UK).

Five basic analytes of pharmaceutical relevance, covering a range of pK_a and log D values, were selected for the LC studies. These included benzylamine hydrochloride, and salbutamol sulphate supplied by Sigma–Aldrich (Poole, UK), nicotine (free base) and procainamide hydrochloride supplied by Fluka (Dorset, UK) and AstraZeneca compound terbutaline sulphate supplied from the AstraZeneca R&D Charnwood (Loughborough, UK) compound

bank—all at 0.3 mg/ml in water. The hydrophilic test mixture consisted of 100 μl of the nicotine, benzylamine hydrochloride, procainamide hydrochloride, terbutaline sulphate, salbutamol sulphate and phenol (all at 0.3 mg/ml concentration) plus 500 μl of water. The compound number, structure, pK_a and log *D* values for the components in the hydrophilic test mixture are given in Table 1.

2.2. Instrumentation

LC separations were performed on either an HP1090M Series II or Agilent Technologies 1100 liquid chromatograph with ChemStation v. 6.04 LC software (Agilent Technologies, Cheadle, Cheshire) equipped with column switching valves (Jones Chromatography, Mid-Glamorgan, Wales or Valco International, Schenkon, Switzerland) and a Mistral column oven (Spark Holland, Emmen, The Netherlands).

2.3. Liquid chromatography

All columns were new as supplied by the manufacturer/supplier. The chromatographic conditions for the LC characterisation of the phases were as reported previously [13,30,39] and are summarised below. The first disturbance of the baseline on the injection of methanol was used as dead time marker.

Six variables reflecting different chromatographic properties were used for the characterisation. Each variable is briefly described below. Injections volumes and flow-rates have been scaled for 150×4.6-mm I.D. columns.

Retention factor for pentylbenzene, k_{PB}: Reflects the surface area and surface coverage (ligand density). Chromatographic conditions: MeOH–H_2O (8:2, v/v), 1.0 ml/min, 40 °C, 5-μl injection of pentylbenzene (0.6 μg/ml).

Hydrophobicity or hydrophobic selectivity, α_{CH2}: Retention factor ratio between pentylbenzene and butylbenzene, $\alpha_{CH2} = k_{PB}/k_{BB}$. This is a measure of the surface coverage of the phase as the selectivity between alkylbenzenes differentiated by one methylene group is dependent on the ligand density. Chromatographic conditions: MeOH–H_2O (8:2, v/v), 1.0 ml/min, 40 °C, individual 5-μl injections of pentylbenzene (0.6 μg/ml) and butylbenzene (0.3 μg/ml).

Shape selectivity, $\alpha_{T/O}$: Retention factor ratio between triphenylene and *o*-terphenyl, $\alpha_{T/O} = k_T/k_O$. This descriptor is a measure of the shape selectivity, which is influenced by the spacing of the ligands and probably also the shape/functionality of the silylating reagent. Chromatographic conditions: mobile phase as above for hydrophobicity, individual 5-μl injections of *o*-terphenyl and triphenylene both at 0.05 mg/ml.

Hydrogen bonding capacity, $\alpha_{C/P}$: Retention factor ratio between caffeine and phenol, $\alpha_{C/P} = k_C/k_P$. This descriptor is a measure of the number of available silanol groups and the degree of endcapping. Chromatographic conditions: MeOH–H_2O (3:7, v/v), 1.0 ml/min, 40 °C, individual 5-μl injections of phenol (1 mg/ml) and caffeine (0.5 mg/ml).

Total ion-exchange capacity, $\alpha_{B/P}$ pH 7.6: The retention factor ratio between benzylamine and phenol, $\alpha_{B/P}$ pH 7.6 = k_B/k_P. This is an estimate of the total silanol activity. Chromatographic conditions: 20 m*M* KH_2PO_4, pH 7.6, in MeOH–H_2O (3:7, v/v), 1.0 ml/min, 40 °C, individual 5-μl injections of phenol and benzylamine HCl both at 0.5 mg/ml.

Acidic ion-exchange capacity, $\alpha_{B/P}$ *pH 2.7*: The retention factor ratio between benzylamine and phenol, $\alpha_{B/P}$ *pH* 2.7 = k_B/k_P. This is a measure of the acidic activity of the silanol groups. Chromatographic conditions: All conditions as for total ion-exchange determinations above, but using a pH 2.7 KH_2PO_4 buffer.

Hydrophilic base analysis: Chromatographic conditions: 20 m*M* KH_2PO_4, pH 2.7, in MeOH–H_2O (3.3:96.7, v/v), 1.0 ml/min, 60 °C, 5-μl injection of the hydrophilic base test mixture, detection at 210 nm.

The analytes typically eluted within 30 min in all the tests.

2.4. Software employed

2.4.1. Principal component analysis

PCA was performed using Simca-P 8.1 software (Umetrics, Sweden). All six variables from the column characterisation were included in the analysis. In order to give all variables the same impor-

tance the variables were ''auto scaled'', i.e., the average was subtracted from each variable and each variable was divided by its standard deviation.

2.4.2. Log D and pK_a predictions

Predictions of pK_a and log *D* were calculated using Advanced Chemistry Development software programmes (Toronto, Canada).

3. Results and discussion

3.1. Column characterisation parameters

To date there have been many differing types of protocols suggested for the characterisation of RP materials [1–5,12–26,32,33] that utilise differing test analytes and differing LC conditions. The Tanaka characterisation protocol [14] is a well-established approach that has been favoured by academic groups and many stationary phase manufacturers, such as ThermoHypersil-Keystone, Merck and Phenomenex to assess their phases. The research groups of Kele and Guiochon [24] and Massart and co-workers [32,33] have incorporated many of the Tanaka tests to assess the repeatability and reproducibility of commercially available RP columns and in the development of a generic pharmacopoeial test protocol for columns. The chromatographic parameters of the phase which are measured in the Tanaka protocol are highly relevant ones to the chromatographer these include surface coverage, hydrophobic selectivity, shape selectivity, hydrogen bonding capacity and ion-exchange capacity at pH 2.7 and 7.6.

The Tanaka characterisation protocol was used to extend our existing column characterisation database from 79 to 135 differing phases.

Surface metal activity was not included as a column characterisation parameter as the value for a phase is known to be dependent on the previous history of the column. For example, shipping and storage of columns in pure organic solvents such as ACN and MeOH [30] and the number of column volumes of mobile phase passed through the column will all effect the metal activity of that column [40,41] and hence the repeatability and reproducibility of the metal content of differing columns is poor [32,34]. The metal activity parameter is only valid for the column under evaluation at that moment in time and it is, therefore, potentially misleading to report such values. In addition, we have previously reported [30] that ''doping'' metals, such as iron, onto RP silica does not alter the column characterisation parameters described in this current paper.

There have been several recent papers attempting to correlate the various column characterisation procedures of Walters [18], Tanaka and co-workers [14], Galushko [19] and Engelhardt and Jungheim [17]. While the hydrophobicity terms of the various approaches often correlate, terms such as shape selectivity, silanol activity and ion-exchange capacity show poor correlations [1,4]. Claessens et al. [1] suggested that the results from the differing protocols are not interchangeable. It is not surprising that these testing procedures do not correlate since the individual tests measure different aspects of the complex ionic/polar activities of the phases towards differing analytes using differing LC conditions and calculations of parameters [4,42]. It is highly likely that the various tests are describing differing column parameters.

3.2. PCA of the complete database

The results of the column characterisation procedure performed on the 135 differing stationary phases can be located in Table 2. It has been previously reported [31] that only three of the six variables (i.e., chromatographic properties of the phase) are needed to adequately describe the variability of the phases since certain of the variables were correlated. While this may be true for C_{18} phases, we felt that these correlations may not hold for other types of phases (i.e., those with differing stationary phase chemistries) that are now included in the enlarged database, therefore it was decided to include all six variables in the PCA.

PCA score plots of the entire database of 135 differing stationary phases showed a similar profile (results not shown) to that presented in our 2000 paper which compared only 79 differing phases [13]. As expected, the non-silica phases, such as the zirconium oxide (ZiChrom PDB, column no. 123) and aluminium oxide (Spherisorb A5Y, column no. 90) based phases, were located a long distance away from the silica-based phases in the PCA score plots.

Table 2
Stationary phases characterised

Column no.	Description	k_{PB}	α_{CH2}	$\alpha_{T/O}$	$\alpha_{C/P}$	$\alpha_{B/P}$ pH 7.6	$\alpha_{B/P}$ pH 2.7	N (m^{-1})	dp (μm)	Producer/ Supplier	Re-test/ New	Type of phase
1	Ace $5C_{18}$	4.58	1.46	1.52	0.40	0.47	0.13	79 200	5	Hichrom	Yes	1
2	ACE Aq	2.30	1.35	1.22	0.48	0.32	0.11	73 500	5	Hichrom	New	1,2
3	Ace CN	0.26	1.08	1.73	0.51	0.74	0.15	35 200	5	Hichrom	New	3
4	Ace Phenyl	1.20	1.26	1.00	0.88	0.46	0.14	15 100	5	Hichrom	New	4
5	Aquasil C_{18}	4.14	1.41	1.84	0.18	2.29	0.16	85 900	5	Hypersil	New	1,2,11
6	Astec Polymer C_{18}	4.92	1.35	4.09	0.15	0.04	0.01	31 300	5	Astec	No	1,5
7	Betabasic C_{18}	4.49	1.47	1.56	0.39	0.80	0.12	83 200	5	Hypersil	New	1
8	Betabasic CN	0.18	1.06	1.82	0.43	0.77	0.17	15 100	5	Hypersil	New	3
9	BetaMax Acidic	2.84	1.33	2.04	0.29	0.55	−0.03	58 800	3	Hypersil	New	1,6
10	BetaMax Basic (CN)	0.37	1.04	2.04	0.42	1.70	0.19	23 700	3	Hypersil	New	3
11	BetaMax Neutral C_{18}	10.62	1.49	1.50	0.40	1.00	0.10	85 600	3	Hypersil	New	1
12	C_{18} multiring	1.86	1.46	2.35	0.56	1.23	0.09	35 800	5	Vydac	No	1
13	Chromolith C_{18}	4.22	1.24	1.31	0.48	0.63	0.12	108 000	Monolith	Merck	New	1
14	Curosil PFP	1.77	1.27	2.49	0.71	0.85	0.10	21 700	3	Phenomenex	New	7
15	Develosil ODS-MG-5	6.70	1.49	1.24	0.51	0.10	0.07	63 300	5	Phenomenex	No	1,2
16	Discovery C_{18}	3.32	1.48	1.51	0.39	0.28	0.10	80 300	5	Supelco	No	1
17	Discovery C_{18} HS	6.68	1.40	1.55	0.40	0.38	0.10	99 300	5	Supelco	New	1
18	Discovery C_8	1.10	1.34	1.00	0.43	0.41	0.09	33 100	5	Supelco	New	8
19	Discovery CN	0.29	1.00	1.00	1.00	1.60	0.55	12 800	5	Supelco	New	3
20	Discovery F5 HS	1.70	1.26	2.55	0.68	0.85	0.34	68 700	5	Supelco	New	7
21	Discovery PEG HS	0.23	1.06	2.57	0.02	0.07	−0.04	31 400	5	Supelco	New	9
22	Discovery RP-amide	1.65	1.35	1.81	0.49	0.44	0.19	82 600	5	Supelco	No	6
23	EU Column	6.19	1.46	1.50	0.56	1.00	0.12	64 700	5	Bischoff	New	1
24	Fluofix (ec)	0.57	1.24	0.58	0.81	2.06	0.38	60 500	5	Neos	Yes	7
25	Fluofix (nec)	0.48	1.20	0.67	1.37	3.90	0.26	68 900	5	Neos	Yes	7
26	Fluophase PFP	1.60	1.23	2.50	0.63	0.70	0.30	56 000	5	Hypersil	New	7
27	Flouphase RP	0.98	1.21	0.62	0.73	2.12	0.60	35 200	5	Hypersil	New	7
28	FluoroSep RP Octyl	1.44	1.23	0.63	0.75	4.12	0.52	62 600	5	ES Industries	New	7
29	Genesis AQ	6.07	1.49	1.26	0.53	0.42	0.11	79 900	4	Jones	New	1,2
30	Genesis C_{18}	6.25	1.50	1.41	0.44	0.29	0.10	72 600	4	Jones	No	1
31	Genesis C_8	2.09	1.33	1.01	0.55	0.60	0.12	74 500	4	Jones	No	8
32	Genesis CN	0.22	1.16	1.90	0.60	1.00	0.18	13 600	4	Jones	New	3
33	Grom0Sil 100DS-2FE	4.68	1.46	1.72	0.59	0.72	0.17	92 300	3	Grom	No	1
34	Grom-Sil ODS-0 AB	3.46	1.45	1.40	0.72	0.67	0.19	119 000	3	Grom	No	1
35	Grom-Sil ODS-4 HE	6.28	1.50	1.27	0.54	0.31	0.10	49 300	5	Grom	No	1,2,11
36	Grom-Sil ODS-7 pH	12.68	1.54	1.53	0.39	0.32	0.06	98 900	4	Grom	No	1
37	Hichrom RPB	4.56	1.40	1.21	0.36	0.18	0.11	71 900	5	Hichrom	No	10
38	Hypersil 100 HS C_{18}	7.66	1.53	1.40	0.42	1.01	0.25	78 964	5	Hypersil	No	1
39	Hypersil C_{18} BDS	4.50	1.47	1.49	0.39	0.19	0.17	74 600	5	Hypersil	No	1
40	Hypersil Elite C_{18}	4.76	1.49	1.52	0.37	0.30	0.14	75 100	5	Hypersil	No	1
41	Hypersil ODS	4.44	1.45	1.28	0.38	1.04	0.64	76 100	5	Hypersil	No	1
42	HyPURITY C_{18}	3.20	1.47	1.60	0.37	0.29	0.10	78 800	5	Hypersil	No	1
43	HyPURITY C_4	0.55	1.30	0.72	0.44	0.30	0.10	20 600	5	Hypersil	No	12
44	HyPURITY C_8	1.59	1.35	1.00	0.34	0.30	0.11	83 200	5	Hypersil	No	8
45	HyPURITY CN	0.08	1.12	1.87	0.81	2.21	0.08	30 700	5	Hypersil	Yes	3
46	HyPURITY Advance (C_8)	1.13	1.00	1.59	0.39	0.80	0.13	38 400	5	Hypersil	No	6,8
47	Inertsil CN3	0.57	1.04	1.97	0.26	3.27	0.03	22 700	5	Hichrom	New	3
48	Inertsil ODS3	7.74	1.45	1.29	0.48	0.29	0.01	130 400	3	Hichrom	New	1
49	Inertsil ODS	6.31	1.47	1.57	0.36	0.53	0.01	44 000	5	Hichrom	No	1
50	J Sphere ODS	10.60	1.51	1.59	0.39	0.43	0.06	124 800	4	YMC	New	1
51	Jupiter C_{18} 300 A	2.26	1.48	1.65	0.37	0.47	0.27	24 700	5	Phenomenex	No	1
52	Kromasil C_{18}	7.01	1.48	1.53	0.40	0.31	0.11	84 900	5	Hicrom	No	1

Table 2. Continued

Column no.	Description	k_{PB}	α_{CH2}	$\alpha_{T/O}$	$\alpha_{C/P}$	$\alpha_{B/P}$ pH 7.6	$\alpha_{B/P}$ pH 2.7	N (m^{-1})	dp (μm)	Producer/ Supplier	Re-test/ New	Type of phase
53	RP Select B (C_8)	2.76	1.32	1.21	0.66	1.40	0.14	43 300	5	Merck	No	8
54	Lichrosphere RP18	7.92	1.48	1.73	0.54	1.39	0.19	46 100	5	Merck	No	1
55	Luna C_{18}	5.97	1.47	1.17	0.40	0.24	0.08	89 700	5	Phenomenex	No	1
56	Luna 18(2)	6.34	1.47	1.23	0.41	0.26	0.06	80 700	5	Phenomenex	No	1
57	Luna CN	0.17	1.14	1.47	0.75	3.51	0.24	25 300	5	Phenomenex	New	3
58	Luna NH2	−0.17	1.01	4.05	0.47	0.43	3.41	13 300	5	Phenomenex	New	13
59	Luna Phenyl-Hexyl	2.82	1.33	1.10	0.91	0.33	0.11	85 100	3	Phenomenex	New	14
60	MetaSil Basic	2.03	1.32	1.25	0.32	0.29	0.09	96 000	3	Ansys	New	10
61	Monochrom MS	1.69	1.27	2.53	0.83	0.66	0.15	53 000	5	Ansys	New	7
62	Novapak C_{18}	4.49	1.49	1.44	0.48	0.27	0.14	70 200	4	Waters	No	1
63	Nucleodur C_{18} Gravity	7.71	1.48	1.80	0.45	0.36	0.07	67 300	5	Macherey-Nagel	New	1
64	Nucleosil C_{18}	4.80	1.44	1.68	0.70	2.18	0.13	48 800	5	Macherey-Nagel	No	1
65	Nucleosil C_{18} HD	6.04	1.48	1.54	0.40	0.47	0.10	86 700	5	Macherey-Nagel	New	1
66	Nucleosil C_{18} Nautilus	3.37	1.40	1.98	0.33	0.48	0.01	73 300	5	Macherey-Nagel	New	1,6
67	Nucleosil C_8 HD	3.05	1.38	0.91	0.49	0.51	0.13	80 000	5	Macherey-Nagel	New	8
68	Omnisphere C_{18}	8.54	1.48	1.69	0.41	0.56	0.11	119 600	3	Varian	New	1
69	Optimal ODS-L	5.87	1.48	1.26	0.51	0.30	0.09	65 200	5	Capital HPLC	No	1
70	Optimal ODS H	6.15	1.48	1.38	0.44	0.24	0.09	82 700	5	Capital HPLC	No	1
71	Perfluorphenyl HS	3.31	1.29	2.72	0.65	0.74	0.19	66 400	5	ES Industries	New	7
72	Perfluorpropyl ESI	0.16	1.15	1.00	1.15	1.47	0.39	9 200	5	ES Industries	New	7
73	Phenomenex Aqua	6.21	1.48	1.27	0.60	0.52	0.11	113 600	3	Phenomenex	New	1,11
74	Platinum C_{18}	2.12	1.39	1.23	0.81	2.82	0.21	56 800	5	Alltec	New	1
75	Platinum C_{18} EPS	0.97	1.31	1.98	2.62	10.11	0.26	57 700	5	Alltec	New	1,2
76	Polaris Amide C_{18}	2.87	1.43	2.43	0.20	0.15	−0.02	76 700	3	Ansys	New	1,6
77	Polaris C_{18} A	3.20	1.44	1.85	0.34	0.33	0.11	58 800	5	Ansys	New	1,6
78	Polaris C_{18} Ether	2.98	1.45	1.63	0.46	0.38	0.10	81 300	3	Ansys	New	1,6
79	Polaris C_8 Ether	0.82	1.29	1.49	0.50	0.56	0.31	51 300	3	Ansys	New	8,6
80	Prism NRP (C_{18} nec)	1.68	1.35	2.24	0.42	1.23	0.01	49 700	3	Hypersil	New	1,6
81	Prism RP (C_{18} ec)	2.54	1.33	1.66	0.38	0.59	0.01	70 400	3	Hypersil	New	1,6
82	Prodigy ODS2	4.94	1.49	1.43	0.37	0.50	0.01	60 500	5	Phenomenex	No	1
83	Prodigy ODS3	7.27	1.49	1.26	0.42	0.27	0.09	73 000	5	Phenomenex	No	1
84	Prontosil C_{18}-AQ	4.80	1.46	1.28	0.58	0.56	0.11	120 200	3	Bischoff	New	1,11
85	Purospher RP18	4.78	1.44	1.93	0.72	1.29	−0.07	27 600	5	Merck	No	1
86	Purospher RP18e	6.51	1.48	1.75	0.46	0.34	0.08	66 000	5	Merck	No	1
87	Resolve C_{18}	2.40	1.46	1.59	1.29	4.06	1.23	47 700	4	Waters	No	1
88	Selectosil C_{18}	4.94	1.45	1.69	0.68	1.98	0.14	61 300	5	Phenomenex	No	1
89	SMT Total coverage C_{18}	7.26	1.48	1.59	0.56	0.93	0.07	41 200	5	SMT	No	1
90	Spherisorb A5Y	0.73	1.41	1.71	0.84	1.44	13.39	25 800	5	Waters	No	15
91	Spherisorb ODS1	1.78	1.47	1.64	1.57	2.84	2.55	85 800	5	Waters	No	1
92	Spherisorb ODS2	3.00	1.51	1.56	0.59	0.76	0.23	82 600	5	Waters	No	1
93	Spherisorb ODSB	5.09	1.46	1.78	0.80	3.56	0.06	51 400	5	Waters	No	1
94	Summit ODS (W)	5.45	1.47	1.29	0.56	0.40	0.10	88 300	3	Crawford	No	1
95	Supelcogel TR-100	11.99	1.44	2.81	0.34	0.20	0.06	12 900	5	Supelco	No	1
96	Supelcosil LC_{18}	4.82	1.47	1.42	0.46	1.93	0.89	60 800	5	Supelco	No	1,5
97	Supelcosil LC_{18}DB	5.16	1.51	1.40	0.42	0.47	0.14	52 300	5	Supelco	No	1
98	Supelcosil LC-ABZ	3.14	1.37	2.23	0.24	0.20	0.03	67 500	5	Supelco	No	6
99	Superspher RP 18e	5.47	1.47	1.64	0.44	0.42	0.11	49 900	5	Merck	No	1
100	Suplex pkb 100	1.24	1.35	2.84	0.34	0.29	0.00	41 200	5	Supelco	No	16
101	Symmetry C_{18}	6.51	1.46	1.49	0.41	0.68	0.01	56 100	5	Waters	No	1
102	Symmetry Shield RP18	4.66	1.41	2.22	0.27	0.20	0.04	82 700	5	Waters	No	1,6
103	Symmetry Shield RP8	2.30	1.32	1.87	0.27	0.19	0.04	80 400	5	Waters	No	8,6
104	Synergi Max RP	4.91	1.44	1.15	0.33	0.32	0.08	87 100	4	Phenomenex	New	17
105	Synergi Polar RP	1.18	1.22	1.35	2.53	1.00	0.14	46 600	4	Phenomenex	New	6,11
106	Syngeri Hydro-RP	7.63	1.47	1.47	0.58	0.83	0.25	113 300	4	Phenomenex	New	1,11

Table 2. Continued

Column no.	Description	k_{PB}	α_{CH2}	$\alpha_{T/O}$	$\alpha_{C/P}$	$\alpha_{B/P}$ pH 7.6	$\alpha_{B/P}$ pH 2.7	N (m^{-1})	dp (μm)	Producer/ Supplier	Re-test/ New	Type of phase
107	Targa C_{18}	6.06	1.63	1.27	0.52	0.10	0.14	97 200	5	Higgins	No	1
108	TSKGel super ODS	2.22	1.47	1.65	0.33	0.33	0.10	112 600	2	TosoHaas	No	1
109	TSKGelODS-80TM	5.07	1.46	1.34	0.58	0.65	0.09	91 000	5	TosoHaas	No	1
110	TSKGelODS-80TS	4.57	1.45	1.24	0.52	0.30	0.08	95 800	5	TosoHaas	No	1
111	μBondpak	1.97	1.39	1.28	0.78	1.12	0.15	19 200	5	Waters	No	1
112	Ultracarb ODS(30)	13.27	1.52	1.39	0.48	0.73	0.06	65 400	5	Phenomenex	No	1
113	Ultrasphere ODS	6.41	1.52	1.42	0.48	0.31	0.16	66 300	5	Hichrom	No	1
114	Uptisphere 3 HDO	4.88	1.46	1.29	0.57	0.55	0.11	108 700	3	Interchim	New	1
115	Uptisphere 3 ODS	6.19	1.48	1.36	0.49	0.52	0.10	108 200	3	Interchim	New	1
116	XTerra MS C_{18}	3.52	1.42	1.26	0.42	0.35	0.10	41 500	3.5	Waters	No	1
117	XTerra Phenyl	1.26	1.28	0.88	0.62	0.35	0.11	39 300	5	Waters	New	4
118	XTerra RP18	2.38	1.29	1.83	0.33	0.20	0.07	40 700	3.5	Waters	No	1,6
119	YMC Basic (C_8)	1.40	1.26	0.98	0.57	0.51	0.27	52 100	5	YMC	No	8
120	YMC HYdrosphere C_{18}	3.90	1.34	1.20	0.56	0.39	0.08	90 700	5	YMC	New	1,16
121	YMC ODS-AQ	4.44	1.46	1.25	0.57	0.41	0.11	19 300	5	YMC	No	1,11
122	YMC ProC_{18}	7.42	1.53	1.29	0.46	0.26	0.08	79 800	5	YMC	No	1
123	ZirChrom PBD	1.28	1.41	2.08	0.32	18.77	9.20	73 300	5	ZiChrom	No	18
124	Zorbax Bonus-RP	1.74	1.43	1.60	0.31	0.30	0.04	42 300	5	Agilent	No	19.6
125	Zorbax Eclipse XDB-C_{18}	5.79	1.50	1.30	0.47	0.35	0.09	38 500	5	Agilent	No	1
126	Zorbax Extend C_{18}	6.66	1.50	1.49	0.38	0.20	0.08	86 600	5	Agilent	No	1
127	Zorbax Rx C_{18}	5.68	1.57	1.61	0.54	0.55	0.11	88 100	5	Agilent	No	1
128	Zorbax SB-C_{18}	6.00	1.49	1.20	0.65	1.46	0.13	76 900	5	Agilent	No	1
129	Zorbax SB-C_{18} 300	1.23	1.44	1.22	0.76	1.00	0.12	41 500	5	Agilent	New	1
130	Zorbax SB-C_3	0.91	1.32	1.06	2.71	0.88	0.11	63 700	5	Agilent	No	12
131	Zorbax SB-C_8	1.97	1.37	1.08	1.27	0.81	0.12	63 000	5	Agilent	No	8
132	Zorbax SB-CN	0.36	1.18	2.12	1.14	1.62	0.10	55 900	5	Agilent	No	3
133	Zorbax SB-Phenyl	1.09	1.30	1.18	3.69	1.08	0.13	69 100	5	Agilent	No	4
134	Zorbax-SB Aq	0.93	1.31	1.18	2.54	1.27	0.13	35 300	5	Agilent	New	2,16
135	Hypersil CEC Basic C_{18}	1.55	0.71	2.52	1.28	2.71	0.18	98 300	3	Hypersil	New	16

Key	Phase
1	C_{18}
2	Aqua
3	Cyano
4	Phenyl
5	Polymer
6	Polar embedded
7	Perfluorinated
8	C_8
9	Polyethylene glycol
10	Mixed alkyl
11	Polar endcapped
12	Short alkyl ligand
13	Amino
14	Phenyl-hexyl
15	Aluminium oxide
16	Unspecified
17	C_{12}
18	Zirconium oxide
nec	non-endcapped
ec	endcapped

The complete database has been included in the paper as it allows workers firstly to interpret the data as a whole and also permits sub-classes to be characterised, as carried out by Vander Heyden and Massart's group [31] on our 2000 database [13]. We have subsequently performed a PCA on a sub-group of this data, which contains only perfluorinated phases [39]. The full database contains polar embedded phases, enhanced polar selectivity phases, ''Aqua type'' phases (including polar endcapped and mixed alkyl ligands), cyano phases, phenyl phases, perfluorinated phases, short chain alkyl phases and non-silica based, in addition to standard C_{18} phases (see Table 2).

''Polymeric'' C_{18} phases, i.e., silica-based C_{18} columns prepared by polymeric silanization with di- or trifunctional silanes, possess a unique shape selectivity for compounds with rigid conformation such as polyaromatic hydrocarbons [43,44]. For example, extremely high $\alpha_{T/O}$ values are obtained with the polymeric Vydac 218TP C_{18} phase $\alpha_{T/O}$ = 3.13 [34] compared to monomeric C_{18} phases (typical $\alpha_{T/O}$ range 1.3–1.8). Engelhardt et al. [45] reported that the Tanaka [14] and Sander and Wise [46] tests correlated well in their abilities to distinguish between monomeric, intermediate and polymeric phases but did not necessarily give the same shape/steric classification, this was further verified by Wilson et al. [25]. Polymeric C_{18} type phases have not been included in the present study since it is generally believed that they are less reproducible than phases prepared from monofunctional silanes [47,48]. This has been recently verified by the work of Kele and Guiochon who highlighted the lack of reproducibility of a polymeric phase compared to monomeric ones using a similar testing protocol as described here [34–36]. For the pharmaceutical industry batch-to-batch reproducibility is of critical importance since the methods that are developed often are in use for a long period of time, 15 years or more. In previous surveys in LC–GC, column-to-column reproducibility was selected as being the most important consideration when selecting a column type [49,50].

3.3. PCA of the database of RP silica materials

Exclusion of the non-silica (columns nos. 6, 90, 95 and 123) and amino phases (column no. 58) from the PC1–PC2 score plot gave a greater differentiation between various types of phases (see Fig. 1a). Group A phases were characterised by high silanol activity and low retentivity (see the corresponding PC1–PC2 loading plot in Fig. 1b) and are mostly non-C_{18} phases and traditional C_{18} phases such as the Resolve C_{18} phase (column 87). In contrast, the group B phases are newer generation C_{18} materials, which exhibited low silanol activity and high retentivity (see Fig. 1b). Group C phases contained the polar

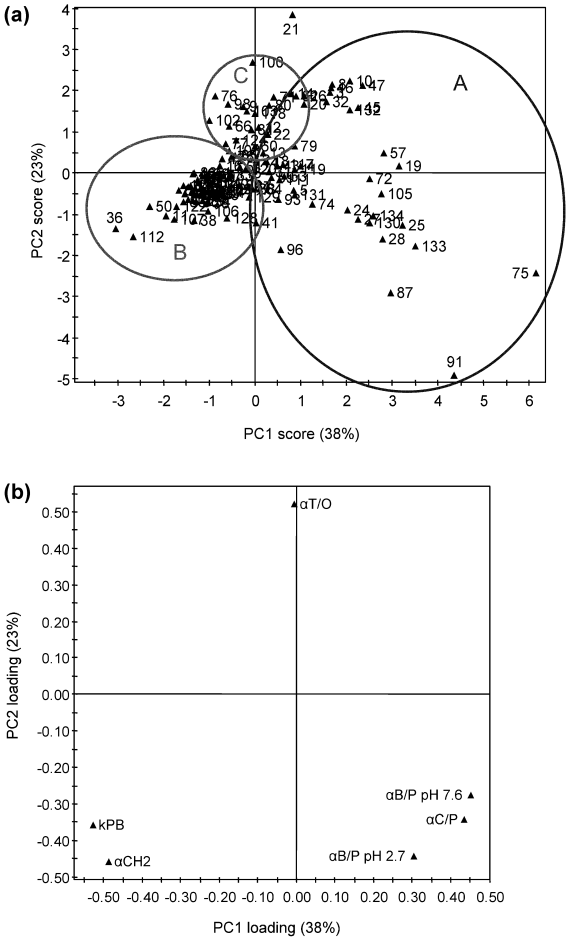


Fig. 1. (a) PC1 and 2 score plot for all columns excluding non-silica and amino phases: A, mostly non-C_{18} and traditional acidic (type A) C_{18} silica phases; B, mostly non-acidic (type B) C_{18} silica phases; C, polar embedded phases. (b) PC1 and 2 loading plot for all columns excluding non-silica and amino phases.

embedded materials and these were differentiated from the other phases due to their high shape selectivity character (see Fig. 1b). The polyethylene glycol phase (column 21) appears to be very different from the other phases in this sub-set.

Since the PC1–PC2 score plot only explained 61% of the variation, the PC1–PC3 plots were constructed and highlighted further differentiation of the phases (see Fig. 2a,b). The third PC contributed to 15% of the variability of the data. The acidic phases such as Resolve C_{18}, Spherisorb ODS1 and the Platinum EPS C_{18} (column nos. 87, 91 and 75) could be grouped together (Group E) as these possessed high acidic and total silanol activity. It is of interest to note that, in contrast to findings of Vander Heyden and Massart's group [31], the hydrogen bonding capacity did not correlate with the total ion-exchange capacity; this is partially to be expected due to the diversity of stationary phases included in the extended database.

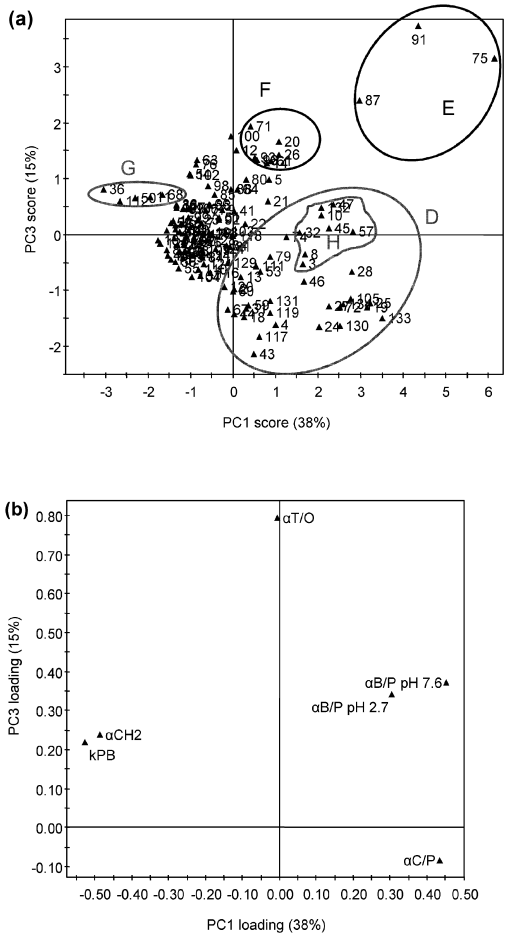


Fig. 2. (a) PC1 and 3 score plot for all columns excluding non-silica and amino phases: D, non-C_{18} phases; E, acidic phases; F, perfluorophenyl phases; G, highly hydrophobic phases; H, cyano phases. (b) PC1 and 3 loading plot for all columns excluding non-silica and amino phases.

Within the D grouping (non-C_{18} phases) it can be seen that most of the cyano phases (column nos. 3, 8, 10, 32, 45, 47, 57, 132) are grouped as a sub-set H. The PC1–PC3 score plot also differentiates the perfluorophenyl phases (grouping F—column nos. 14, 20, 26, 61, 71) on the basis of their shape selectivity parameter.

The high retentive phases such as Ultracarb ODS(30), BetaMax Neutral C_{18}, Gromsil-Sil ODS pH7, J'sphere ODS JH and the Omnisphere C_{18} (column nos. 11, 36, 50, 68, 112) which possess high carbon loads (i.e., >22% carbon) are grouped together and depicted as group G, these are characterised by their high retention factor for pentylbenzene and their high hydrophobic selectivity term.

3.4. PCA of silica-based C_8 and C_{18} phases

By further re-defining the database by excluding phenyl, perfluorinated, cyano, short chain alkyl phases and the polyethylene glycol phase, the PC1–PC2 score plot shown in Fig. 3 is obtained.

The difference in the underlying base silica can be seen by comparing the position of the three phases Hypersil C_{18}, Hypersil BDS C_{18} and HyPURITY C_{18} (see Fig. 3), which are produced by the same manufacturer. The C_{18} bonded ligand is the same in all cases, the only difference between the three phases being the purity of the base silica. Hypersil is a traditional type A silica, which contains significant amounts of metal ion impurities, Hypersil BDS uses an acid washed silica, which has significantly reduced metal impurities, compared to the Hypersil C_{18} material. The HyPURITY phase is prepared on new generation high purity silica, which is extremely low in metal contamination [51]. The acidity of the three phases is Hypersil C_{18}>Hypersil BDS C_{18}> HyPURITY C_{18}: this is depicted in the contribution plots of Hypersil BDS C_{18}-Hypersil C_{18} (Fig. 4a) and HyPURITY C_{18}-Hypersil BDS C_{18} (Fig. 4b)

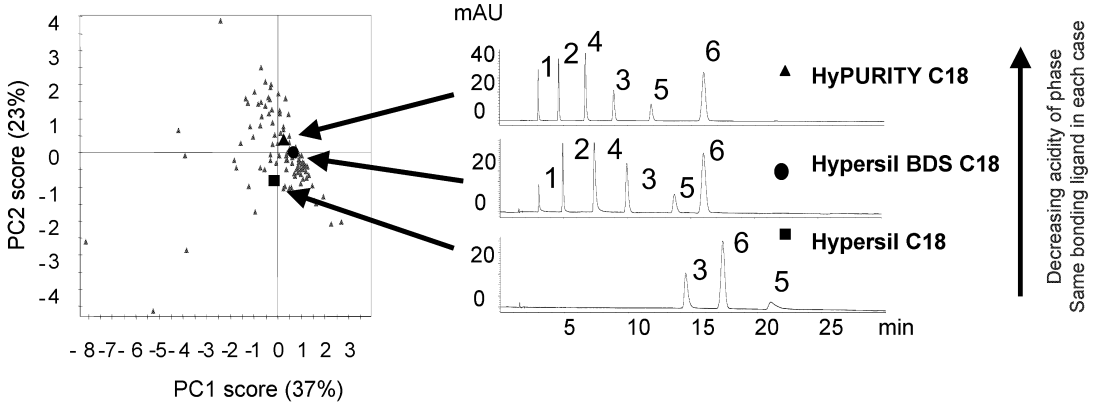


Fig. 3. PC1 and 2 score plot for columns excluding non-silica, amino, phenyl, perfluoro, cyano and PEG phases. A comparison of HyPURITY C_{18}, Hypersil BDS C_{18} and Hypersil C_{18} phases of differing silica purity in the analysis of hydrophilic bases (see Table 1 for analyte structures and experimental section for LC conditions). Bases 1, 2 and 4 are irreversibly retained on the Hypersil C_{18} phase.

which show marked lower acidic ion-exchange capacity $\alpha_{B/P}$ pH 2.7 for the BDS and HyPURITY materials compared to the traditional Hypersil material. This is reflected in their chromatographic performance for the analysis of five hydrophilic bases (see Fig. 3). Phenol (compound 6) was included in the test mixture as an assessment of the packing efficiency of the column. The peak symmetry of the five bases mirrored the acidic ion-exchange capacity and the metal content of the three phases. The best peak shape was obtained with the high purity material (HyPURITY), even the acid-washed BDS material was a vast improvement over the traditional Hypersil; however, slight peak tailing was still observed with the BDS phase. It is interesting to note that the bases (nicotine, benzylamine and procainamide; compounds 1, 2, and 4) were not eluted due to strong adsorption on the Hypersil C_{18} phase as a consequence of excessive interaction with the ionised silanol groups even at pH 2.7.

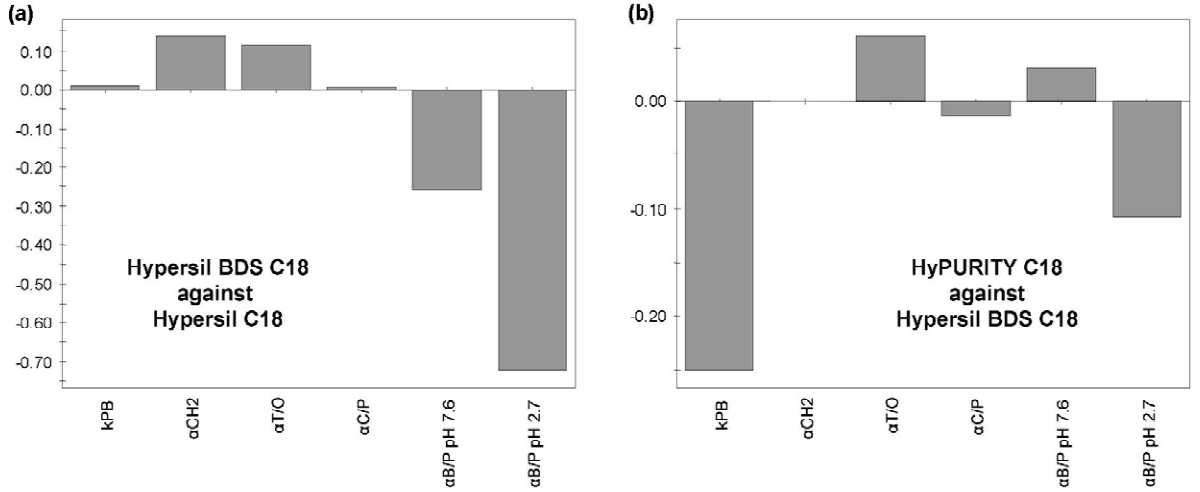


Fig. 4. (a) PC contribution plot of Hypersil BDS C_{18} against Hypersil C_{18} phases. (b) PC contribution plot of HyPURITY C_{18} against Hypersil BDS C_{18} phases.

Table 3
Robustness testing of the column characterisation procedure at two sites (Charnwood, UK and Lund, Sweden)

Column no.	Description	k_{PB}	α_{CH2}	$\alpha_{T/O}$	$\alpha_{C/P}$	$\alpha_{B/P}$ pH 7.6	$\alpha_{B/P}$ pH 2.7	N (m^{-1})	dp (μm)	Producer/ Supplier
83	Prodigy ODS3	7.27	1.49	1.26	0.42	0.27	0.09	73 000	5	Phenomenex
Ca	Prodigy ODS3V *S/N* 318940	7.23	1.48	1.21	0.44	0.33	0.10	131 800	3	Phenomenex
La	Prodigy ODS3V *S/N* 318940	6.18	1.47	1.24	0.42	0.38	0.11	121 200	3	Phenomenex
Cb	Prodigy ODS3V *S/N* 320269	7.57	1.49	1.21	0.43	0.35	0.10	97 300	3	Phenomenex
Lb	Prodigy ODS3V *S/N* 320269	6.17	1.47	1.21	0.42	0.37	0.11	122 900	3	Phenomenex
Cc	Prodigy ODS3V *S/N* 320270	8.14	1.49	1.22	0.44	0.34	0.09	132 300	3	Phenomenex
Lc	Prodigy ODS3V *S/N* 320270	6.76	1.47	1.23	0.43	0.37	0.10	150 900	3	Phenomenex

3.5. Robustness of the characterisation procedure

Three different batches of Prodigy ODS3 were analysed at two differing sites by two differing analysts and two differing types of LC system (see Table 3). From the PC1–PC2 score plot of C_{18} and C_8 phases (Fig. 5a) it can been observed that the results are similar indicating the overall robustness of the approach; however, expansion of Fig. 5a (see Fig. 5b), highlights the fact that the results from the two sites are grouped separately. On examination of the contribution plots (figures not shown), it is clear that the main difference is attributed to the retention factor of pentylbenzene and the hydrophobicity selectivity term (α_{CH2}). The apparent site differences may have been due to the difference in the accuracy of the mixing of the mobile phase at both sites as site L used a quaternary whereas site C employed a binary LC pump.

A robustness test performed by experimental design has highlighted that the column characterisation procedure is robust within the following experimental constraints MeOH±1% (v/v), temperature ±2 °C, pH±0.1 unit and buffer concentration±2 m*M* [52].

3.6. Comparison of C_8 and C_{18} phases

Further analysis of Fig. 5 reveals that the C_8 phases (column nos. 131, 53, 119, 31, 18, 67, 44 and the mixed alkyl phase 60) are grouped together and are characterised in the PC1–PC2 loading plot (see

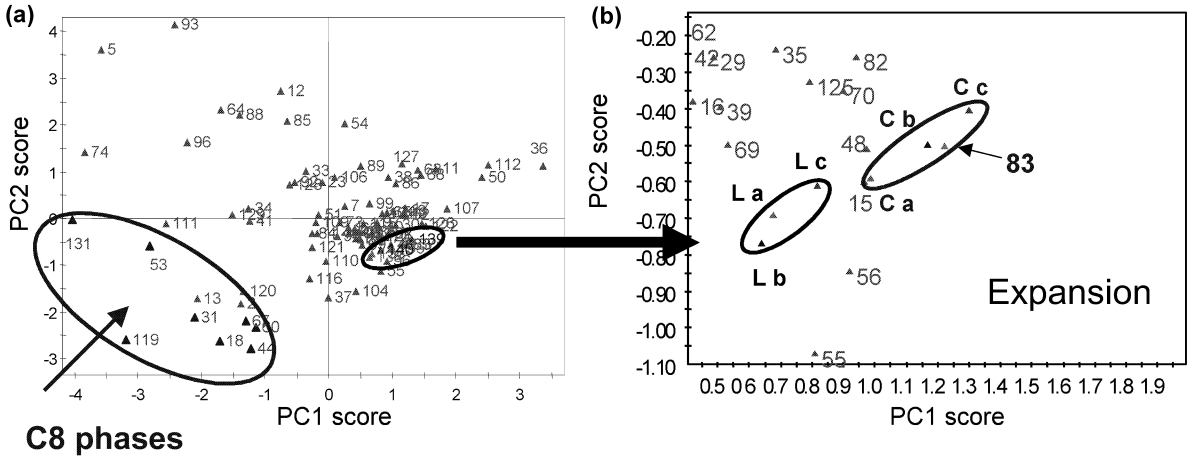


Fig. 5. (a) PC1 and 2 score plot of the C_8 and C_{18} phases. (b) PC1 and 2 score plot expansion to highlight batch to batch variability of four Prodigy ODS3 columns and interlaboratory variability.

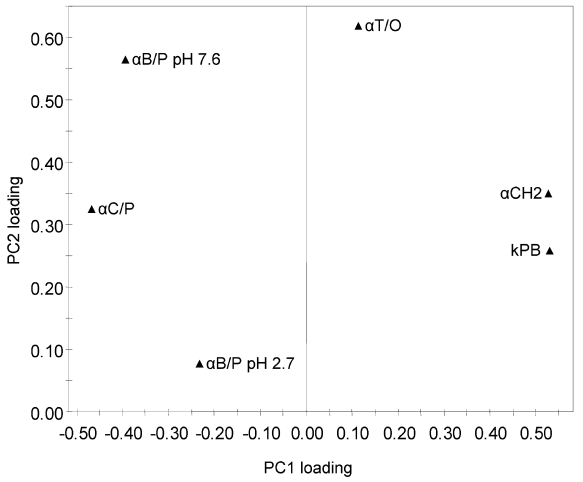


Fig. 6. PC1 and 2 loading plot of the C_8 and C_{18} phases.

Fig. 6) as exhibiting low retention and decreased shape selectivity. The difference between C_8 and C_{18} phases bonded onto the same base silica such as HyPURITY (column nos. 44 and 42), Nucleosil HD (column nos. 67 and 65), Genesis (column nos. 31 and 30) and Discovery (column nos. 18 and 16) can be clearly seen in the PCA contribution plots which show that the C_{18} phases are more retentive in addition to exhibiting a markedly greater shape selectivity (see Fig. 7 for a typical example).

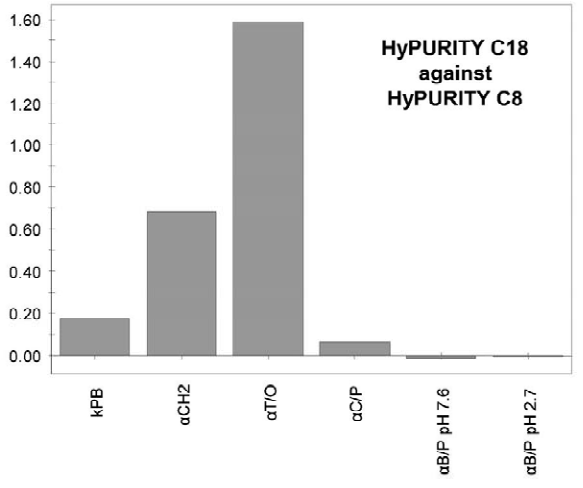


Fig. 7. PC contribution plot of HyPURITY C_{18} against HyPURITY C_8 phases.

3.7. PCA of C_{18} phases (based on non-acidic silica)

The sub-set of the database that contains only C_{18} stationary phases, based on non-acidic silica, highlighted large differences in what, from manufacturer's literature, would seem to be similar phases (see Fig. 8a,b). By using the PC1–PC2 score plot in conjunction with the PC1–PC2 loading plots, a number of various C_{18} phases could be selected for evaluation based on their differences in the six parameters. For example, if selected stationary phases were chosen from the four sectors of the PCA plots, then the differences in the columns selectivities could be exploited. The PC contribution plots for the Grom-Sil ODS-7 pH (column no. 36, positive PC1 and positive PC2), Nucleosil C_{18} (column no. 64, negative PC1 and positive PC2), μBondapak C_{18} (column no. 111, negative PC1 and PC2), Luna C_{18} (column no. 55, positive PC1 and negative PC2) versus the *average* column in the sub-set clearly highlights differences in chromatographic characteristics of the phases (see Fig. 8c). The Grom-Sil ODS-7 pH exhibits high retention due to its polymeric coating, high surface area (510 m^2/g) and carbon load of 22%, the Nucleosil C_{18} possessed a relatively high ion-exchange capacity at pH 7.6, the μBondapak C_{18} possessed low retention and high ion-exchange capacity presumably due to its low carbon load of 10% and moderate surface coverage of 330 m^2/g and the Luna C_{18} showed a low shape selectivity and low ion-exchange capacity.

3.8. PCA of polar embedded, enhanced polar selectivity and "Aqua" phases

In recent years there has been an increasing number of commercially available phases, which have been classed as polar embedded, enhanced polar selectivity, and ''Aqua'' phases; i.e., those suitable for chromatography using a high aqueous environment [53]. The latter two are sometimes classed as polar/hydrophilic endcapped phases depending on the bonding technology employed. Unfortunately, there are few details in the open literature regarding the exact nature of the ligand bonding/chemistry that has been employed. Retention of polar analytes under highly aqueous conditions can

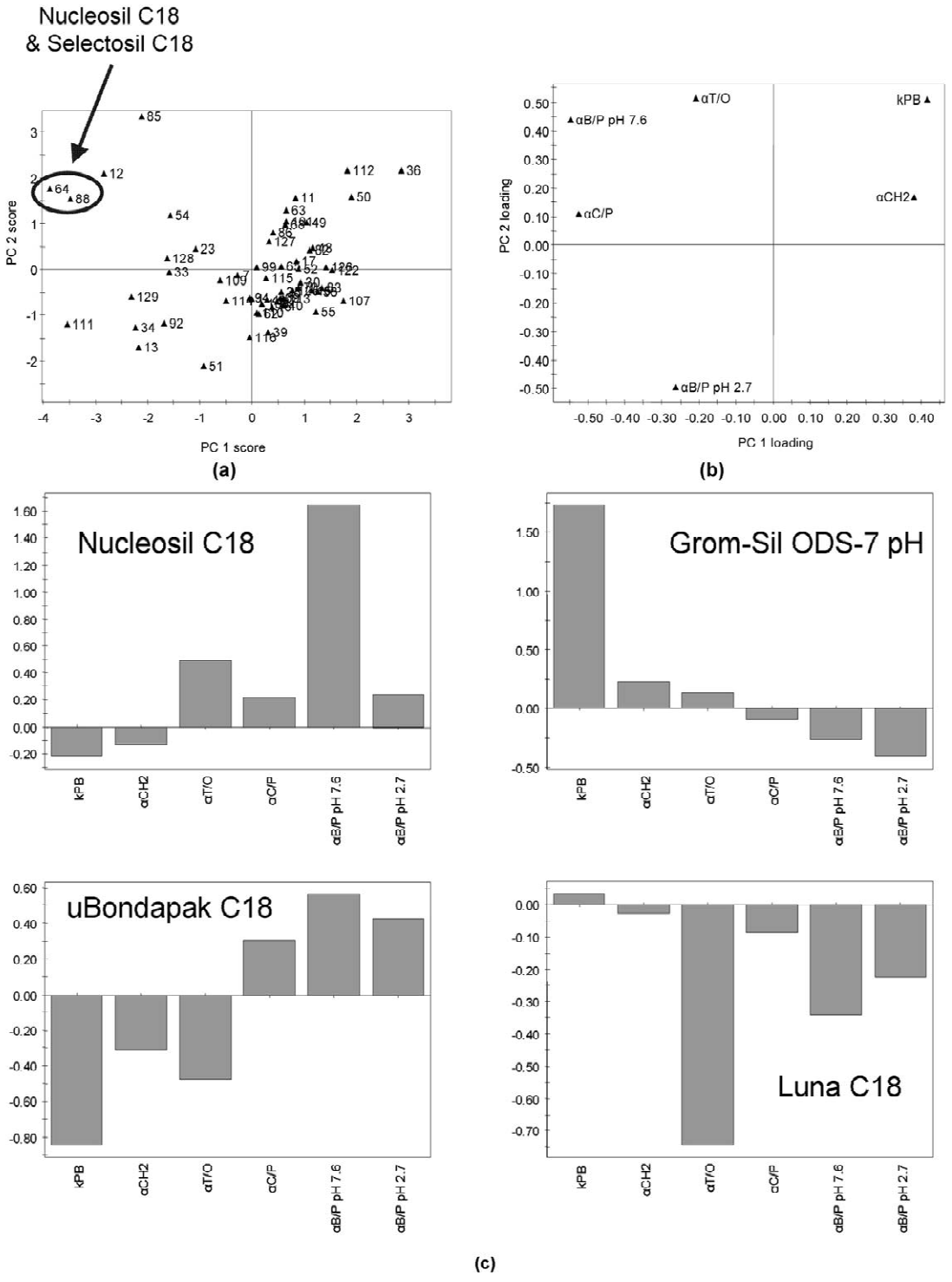


Fig. 8. (a) PC1 and 2 score plot of non-acidic (Type B) C_{18} phases. (b) PC1 and 2 loading plot of non-acidic (Type B) C_{18} phases. (c) PC contribution plot of Nucleosil, Grom-Sil ODS pH, μBondapak C_{18} and Luna C_{18} phases against the average C_{18} non-acidic silica phase.

be achieved by a number of approaches including the use of non-endcapped phases, short chain alkyl phases, hydrophilic/polar-endcapped and polar enhanced stationary phases, polar embedded phases, long chain alkyl phases and wide pore diameter phases [54,55]. In order to characterise a selection of these commercially available phases and to evaluate their potential use within the pharmaceutical environment, we have performed PCA on a sub-set of the database containing 31 phases classified by the stationary phase manufacturers as suitable for use in high aqueous containing mobile phases (see Table 4). Table 4 shows that there is a dearth of data from the manufacturers as to the exact nature of the polar embedded moieties or the hydrophilic bonding employed as this information is classified as proprietary in nature.

The PC1–PC2 score plot of this sub-set of 31 phases separates them into three distinct groups: (1) those phases which possess enhanced shape selectivity and reduced retention (see Fig. 9a,b) as typified by the polar embedded phases (which have a polar functionality, such as an amide [56], urea [57], carbamate [58] and ether moieties, inserted into the alkyl ligand close to the silica surface); (2 and 3) ''Aqua'' and enhanced polar selectivity phases, which possess greater retention, lower shape selectivity and, in some instances, enhanced hydrogen bonding capacity.

The PCA contribution plot (see Fig. 10) for an

Table 4
Phases suitable for use in highly aqueous mobile phase

Column no.	Description	Comments
2	ACE Aq	C_{18} + integral polar functionality
5	Aquasil C_{18}	C_{18} + hydrophilic endcapping
9	BetaMax Acidic	C_{18} + amide polar embedded group
15	Develosil ODS-MG-5	C_{18} unspecified
22	Discovery RP-amide	C_{16} + amide polar embedded group
29	Genesis AQ	C_{18} + short alkyl chains
35	Grom-Sil ODS-4 HE	C_{18} + hydrophilic endcapping
37	Hichrom RPB	C_{18} + C_8 alkyl chains
46	HyPURITY Advance (C_8)	C_8 + amide polar embedded group
60	MetaSil Basic	Mixture of short chain alkyl groups
66	Nucleosil C_{18} Nautilus	C_{18} unspecified polar embedded group
73	Phenonemex Aqua	C_{18} + polar endcapping
75	Platinum C_{18} EPS	C_{18}, low carbon load phase
76	Polaris Amide C_{18}	C_{18} + amide polar embedded group
77	Polaris C_{18} A	C_{18} + unspecified polar embedded group
78	Polaris C_{18} Ether	C_{18} + ether embedded polar group
79	Polaris C_8 Ether	C_8 + ether embedded polar group
80	Prism NRP (C_{18} nec)	C_{18} + unspecified polar embedded group (no endcapping)
81	Prism RP (C_{18} ec)	C_{18} + unspecified polar embedded group
84	Prontosil C_{18}-AQ	C_{18} + hydrophilic endcapping
98	Supelcosil LC-ABZ	RP + amide polar embedded group
100	Suplex pkb 100	Unspecified
102	Symmetry Shield RP18	C_{18} + carbamate polar embedded group
103	Symmetry Shield RP8	C_8 + carbamate polar embedded group
105	Synergi Polar RP	Phenyl ether linkage + polar endcapping
106	Syngeri Hydro-RP	C_{18} + proprietary polar endcapping
118	XTerra RP18	C_{18} + carbamate polar embedded group
120	YMC Hydrosphere C_{18}	C_{18} unspecified
121	YMC ODS-AQ	C_{18} + hydrophilic endcapping
124	Zorbax Bonus-RP	C_{14} + amide embedded polar group, triple endcapped, steric protected bonded phase
134	Zorbax-SB Aq	Proprietary, steric protected chemistry

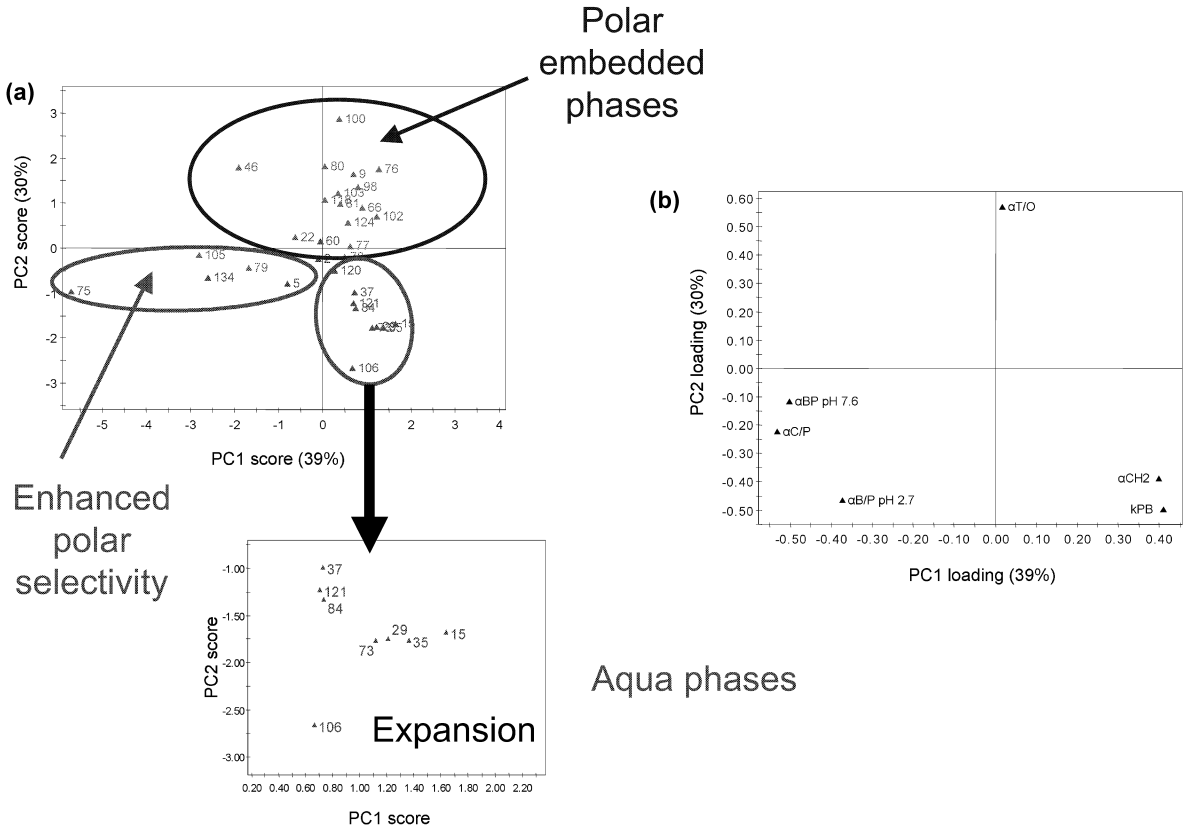


Fig. 9. (a) PC1 and 2 score plot for polar embedded, enhanced polar selectivity and ''Aqua'' phases plus a scale expansion of the ''Aqua'' phases. (b) PC1 and 2 loading plot for polar embedded, enhanced polar selectivity and ''Aqua'' phases.

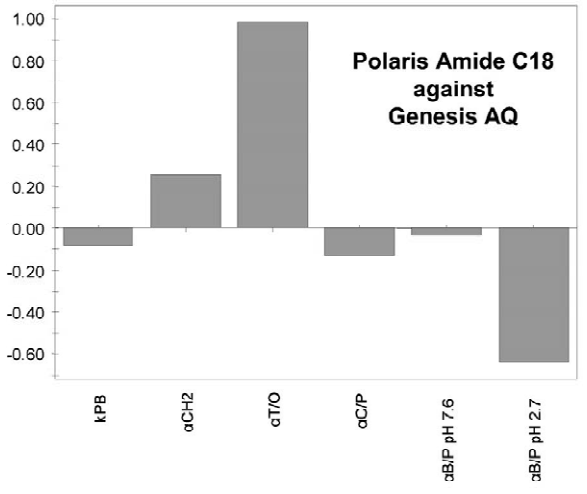


Fig. 10. PC contribution plot of Polaris Amide C_{18} against Genesis AQ phase.

amide-based polar embedded phase against a mixed alkyl stationary phase (typified by Polaris Amide C_{18}, column no. 76 and Genesis AQ, column no. 29, respectively) highlighted the fact that the amide phases possessed low acidic silanol activity and greatly enhanced shape selectivity.

The polar embedded phases produce excellent chromatography of basic analytes at pH 2.7 (see Fig. 11a,b) since they contain polar functionalities, such as amide (i.e., Advance, Discovery RP amide and Zorbax RP Bonus column nos. 46, 22 and 124), unspecified polar embedded group (i.e., Prism phases column nos. 80 and 81), carbamate (Symmetry Shield RP C_{18} and C_8 phases and XTerra RP18 column nos. 102, 103 and 116) and ether bonds (i.e., Polaris ether range column nos. 78 and 79), within the alkyl ligand. The chromatograms are typified by Gaussian peak shapes and reduced retention (i.e.,

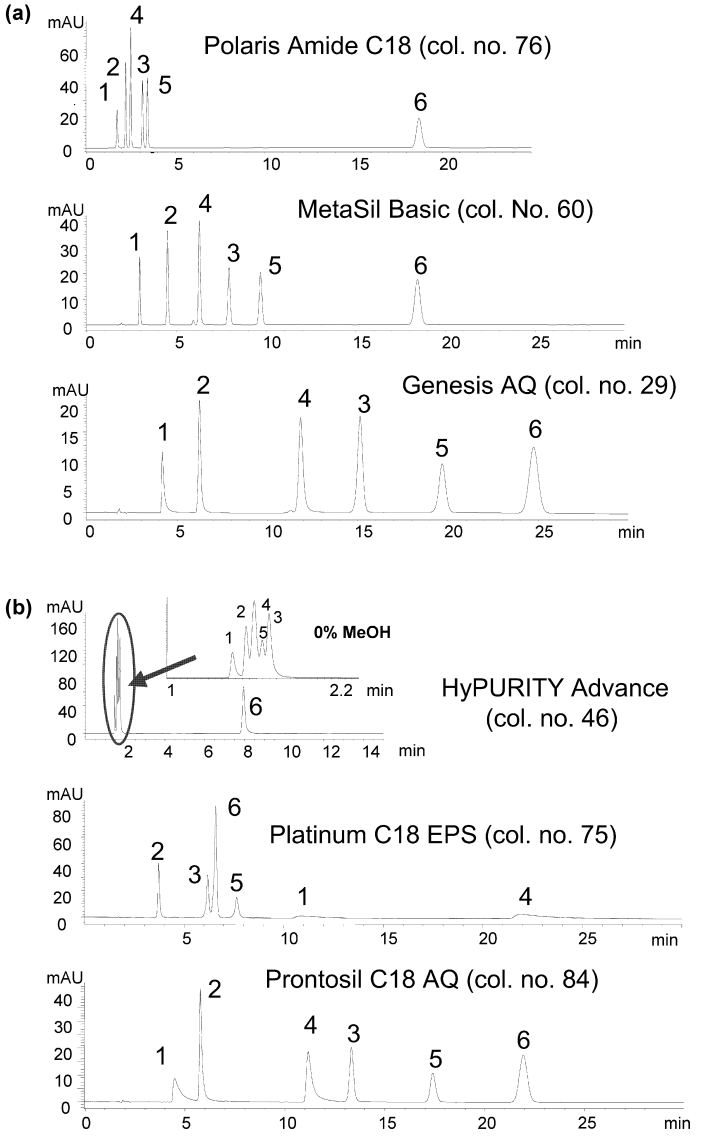


Fig. 11. Comparison of polar embedded, enhanced polar selectivity and Aqua phases in the analysis of hydrophilic bases. For LC conditions and structures of analytes see Section 2 and Table 1.

Advance and Polaris Amide C_{18}—both amide linkages).

In comparison, the other phases appear to be of two types: those with enhanced polar selectivity and the so-called ‘‘Aqua’’ phases. The former type are typified by the Platinum C_{18} EPS, Zorbax SB Aq and Aquasil phases (column nos. 75, 134 and 5), which exhibited longer retention of caffeine compared to phenol in the hydrogen bonding capacity test. This suggests that these phases possess a high density of hydrogen bonding moieties, possibly as a result of a polar endcapping or low bonding density as in the case of the Platinum C_{18} EPS. The phases appear not to be suitable for the analysis of hydrophilic bases as excessive analyte retention and peak broadening is observed, especially for nicotine and procainamide.

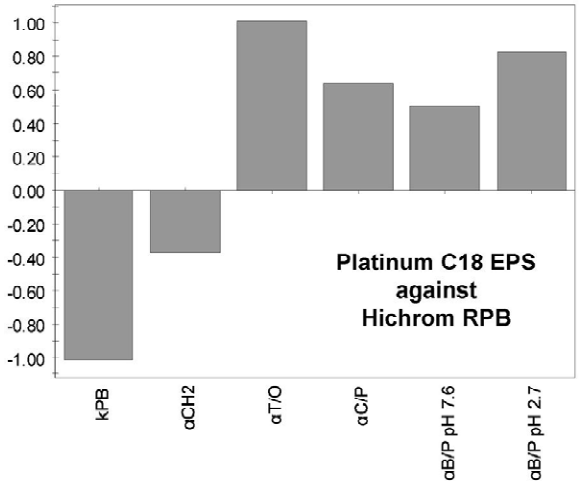


Fig. 12. PC contribution plot of Platinum C_{18} EPS against Hichrom RPB phases.

This is in sharp contrast to many of the ''Aqua'' phases, which exhibit good peak shape for the hydrophilic bases (see Fig. 11a,b).

The PCA contribution plot (see Fig. 12a) of the enhanced polar selectivity phase against the mixed alkyl phase (typified by Platinum C_{18} EPS, column no. 75 and Hichrom RPB, column no. 37, respectively) highlights the fact that the enhanced polar selectivity phases possesses low retention, high hydrogen bonding and ion-exchange capacity as well as an enhanced shape selectivity, compared to the mixed alkyl phases.

The manufacturers of the Genesis Aq and MetaSil Basic phases claim that both phases can be employed in a totally aqueous environment, as they possess alkyl ligands of mixed chain length, which prevent phase collapse in high aqueous conditions. It is possible that the other so-called ''Aqua'' phases in this sub-group may possess a similar approach to the prevention of the phase collapse.

It is of interest to note that the Prontosil C_{18} Aq phase (column no. 84) shows properties of both the enhanced polar selectivity (polar endcapping) and mixed alkyl phases. This is most strikingly shown in the analysis of the hydrophilic base test in which the increased retention and peak tailing of nicotine and procainamide is observed but is less marked than that for the Platinum C_{18} EPS and Zorbax SB Aq phases (column nos. 75 and 134, see Figs. 11b and 13, respectively).

3.9. PCA of the enhanced polar selectivity and ''Aqua'' type phases

Removing the polar embedded phases from the database allowed differences and similarities between the different types of ''Aqua'' and enhanced

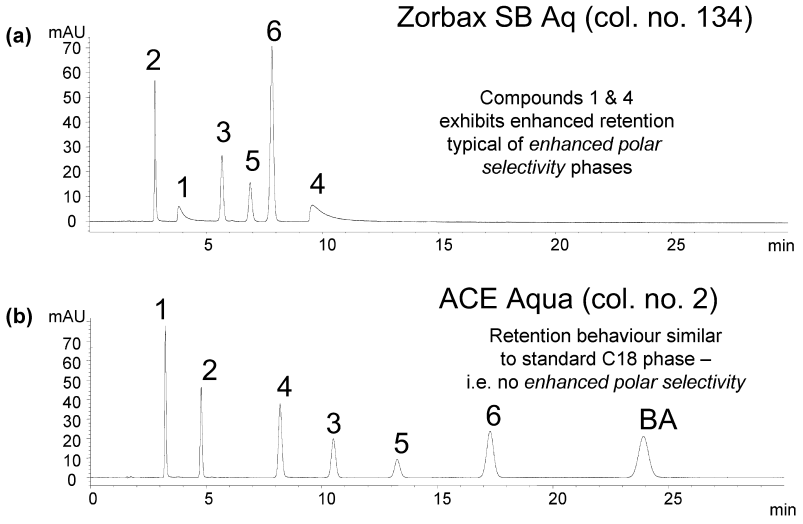


Fig. 13. Comparison of Aqua phases for the analysis of hydrophilic bases. For LC conditions and structures of analytes see Section 2 and Table 1.

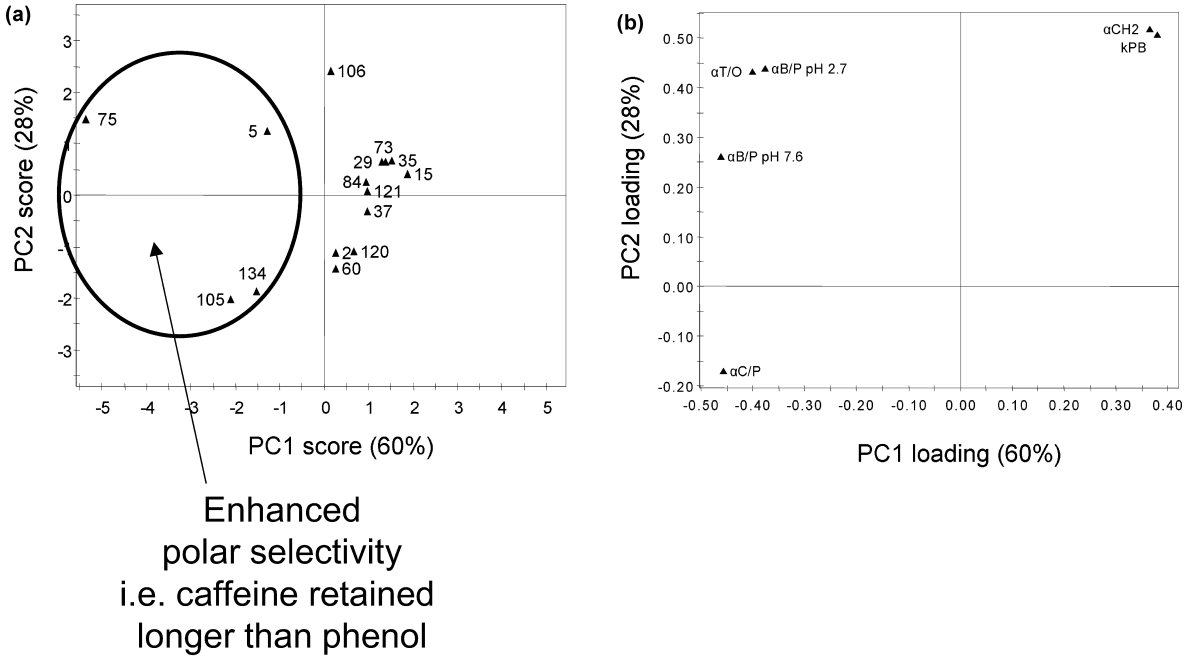


Fig. 14. PC1 and 2 score and loading plots for the Aqua type phases.

polar selectivity phases to be realised. The PC1–PC2 score plot (Fig. 14a,b) clearly differentiated between those phases which retained caffeine longer than phenol (i.e., enhanced polar selectivity phases such as Platinum C_{18} EPS, Synergi RP-Polar, Zorbax SB Aq, Aquasil phases (column nos. 75, 105, 134, 5) and those which retained the compounds on the basis of the analyte's lipophilicity. The enhanced polar selectivity of the former phases is attributed to either the introduction of polar endcapping functionality (i.e., Aquasil, and Synergi RP-Polar, column nos. 105 and 5, respectively), low surface coverage (Platinum C_{18} EPS, column no. 75) or possibly the type of silylating reagent that was used in the bonding technology (Zorbax SB-Aq, column no. 134).

The enhanced selectivity of the Platinum C_{18} EPS, Synergi RP-Polar, Zorbax SB Aq and the Aquasil phases (column nos. 75, 105, 134, 5) was further highlighted on comparison of their chromatographic selectivity towards the analysis of hydrophilic bases. These phases exhibited enhanced retention and poor peak shape for nicotine and procainamide (i.e., see Fig. 13a). In comparison, the other phases such as ACE Aqua, Genesis Aq and MetaSil Basic (see Figs. 13b and 11a) exhibited a similar selectivity to that of new generation C_{18} phases, for example see Fig. 3.

The chromatographic characteristics of the enhanced polar selectivity type phases were observed to be very similar to the Hypersil CEC Basic phase [59] (column no. 135). This phase has been specifically introduced for the analysis of basic analytes in capillary electrochromatography, as it possesses an enhanced electrosomotic flow generated by a high density of non-acidic silanol groups present on the phase; presumably via the bonding technology employed. When this phase is added to the sub-set it is located in the region of the enhanced polar selectivity phases giving strong evidence that these are very similar indeed (see Fig. 15a,b). In addition, the Hypersil CEC Basic phase shows strong retention and poor peak shape for nicotine and procainamide (see Fig. 16).

It is of interest to note that during the evaluation of these phases under a highly aqueous environment (results not presented here) no evidence of phase collapse was observed after subjecting the columns to 60 °C with a mobile phase containing only 3.3%

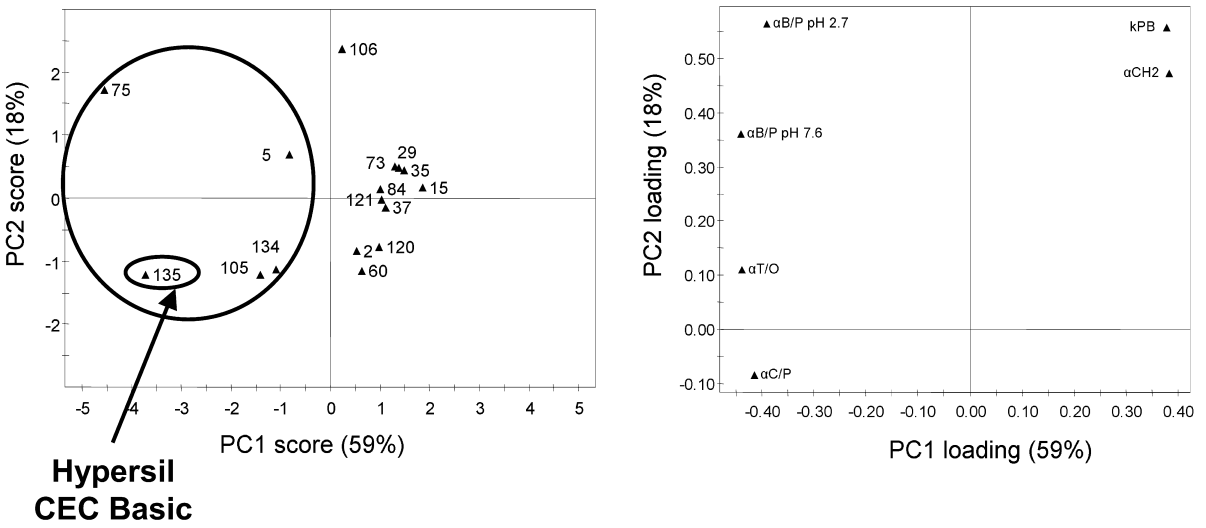


Fig. 15. PC1 and 2 score and loading plots for the Aqua type phases plus the Hypersil CEC Basic C_{18} phase.

(v/v) MeOH with an aqueous pH of 2.7 for up to 4 h.

3.10. Benefits from an independent stationary phase database

3.10.1. Identification of stationary phases of equivalent chromatographic properties

One of the advantages of the column characterisation database is its ability to unambiguously identify columns with equivalent properties (i.e., the same selectivity and retention, such as the Nucleosil and Selectosil columns {col. Nos. 64 and 88, respectively}, see Figs. 8a and 17); this can be achieved by locating columns that are positioned near to one another in the PCA score plots. An additional approach is to calculate the distance in the six-dimensional variable space between the column of interest and the other columns in the database.

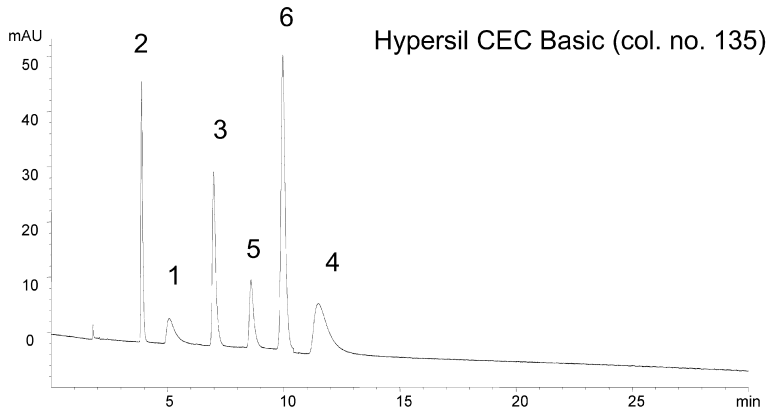
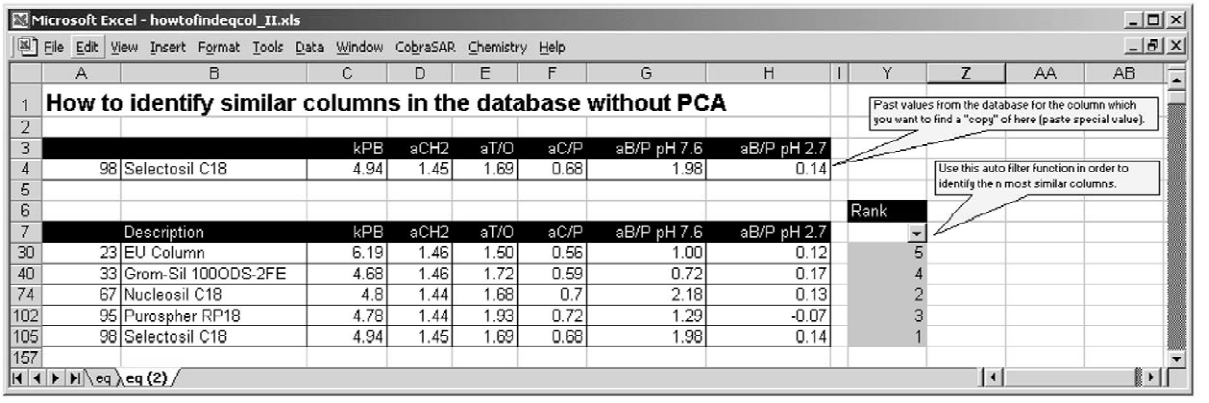


Fig. 16. Chromatographic performance of Hypersil CEC Basic C_{18} towards hydrophilic bases. For LC conditions and structures of analytes see Section 2 and Table 1.


Microsoft Excel - howtofindeqcol_II.xls

File Edit View Insert Format Tools Data Window CobraSAR Chemistry Help

How to identify similar columns in the database without PCA

Past values from the database for the column which you want to find a "copy" of here (paste special value).

Use this auto filter function in order to identify the n most similar columns.

	A	B	C	D	E	F	G	H	Y	Z	AA	AB
3			kPB	aCH2	aT/O	aC/P	aB/P pH 7.6	aB/P pH 2.7				
4	98	Selectosil C18	4.94	1.45	1.69	0.68	1.98	0.14				
6									Rank			
7		Description	kPB	aCH2	aT/O	aC/P	aB/P pH 7.6	aB/P pH 2.7				
30	23	EU Column	6.19	1.46	1.50	0.56	1.00	0.12	5			
40	33	Grom-Sil 100ODS-2FE	4.68	1.46	1.72	0.59	0.72	0.17	4			
74	67	Nucleosil C18	4.8	1.44	1.68	0.7	2.18	0.13	2			
102	95	Purospher RP18	4.78	1.44	1.93	0.72	1.29	-0.07	3			
105	98	Selectosil C18	4.94	1.45	1.69	0.68	1.98	0.14	1			

eq eq (2)

Fig. 17. Microsoft Excel spreadsheet for identifying similar phases. Nucleosil C_{18} is shown to be a useful replacement for the Selectosil C_{18} phases.

This can be achieved in a spreadsheet programme (e.g., Microsoft Excel) as follows:

(1) All variables are ''auto scaled'' by subtracting the mean value of the variable and dividing it by its standard deviation.

(2) The differences between the ''auto scaled'' variables for the column of interest and all other columns in the database are calculated.

(3) The distances between the column of interest and the other columns are calculated by Pythagoras' theorem using these differences.

(4) By sorting or ranking these distances, it is the possible to identify the most similar or dissimilar columns in the database. See Fig. 17 for an example of the Excel spreadsheet used to find an equivalent phase to the Selectosil C_{18} material. It can be observed that the most similar phase, to the Selectosil C_{18}, was the Nucleosil C_{18} (this was the same result as obtained by PCA, see Fig. 8a).

It has been suggested that RPLC columns could be classified into different categories in order to allow identification of columns with equivalent properties suitable for certain pharmacopoeial methods. Unfortunately many of the groups that can be identified in a score plot are not well resolved and the distance within a group is often larger than between groups. Consequently, there are larger differences between many standard C_{18} columns than between certain C_{18} and C_8 columns or between certain C_{18} and polar embedded/polar end capped columns! It will therefore be difficult to define groups with equivalent phases. The most effective approach would be to define one suitable phase for a particular method and then use data for this phase to identify others with equivalent properties as outlined above.

3.10.2. Rational selection of stationary phases for method development

A second reason for characterising stationary phases is the identification of those with differing selectivities that can be exploited in method development. It is evident that a phenyl phase will have a differing selectivity to a C_{18} phase. To locate C_{18} phases with different selectivity is, however, more or less impossible without the aid of column characterisation. The combination of column characterisation with PCA is an excellent tool for the identification of phases with different selectivity. By combining the information in score and loading plots it is also possible to determine phases with undesirable properties, for example, a phase with a high density of acidic silanol groups, would be detrimental for the analysis of bases.

One strategy for method development that we have found to be both powerful and rational is to automatically screen phases with different selectivity by running two slow gradients on selected columns. Optimal isocratic or gradient conditions are subsequently predicted by computer optimisation software for retention time and peak width modelling (e.g., DryLab, LCResources, Walnut Creek, CA,

USA). In order to perform peak tracking, the use of both DAD and MS detection is recommended.

3.10.3. Increased understanding of retention mechanisms

Due to the lack of detailed (and sometimes basic) information regarding stationary phase materials from certain manufacturer regarding their phases it is very difficult to make deductions regarding retention mechanisms. Therefore, a third reason for performing column characterisation is to rectify the above problem and to learn more about stationary phase chemistries [47,60,61]. It is often possible to make qualified deductions regarding the functionality of packing materials based on score and loading plots (cf. previous discussion about polar endcapped and polar embedded columns) and hence analyte–phase interactions.

4. Conclusions

The paper describes the establishment of an independent and unbiased column characterisation database containing 135 differing stationary phase materials (i.e., C_{18}, C_8, short alkyl ligands, cyano, phenyl, polar embedded, enhanced polar selectivity phases, ''Aqua'' phases—polar endcapped and mixed alkyl phases, perfluorinated phases plus a number of novel stationary phase chemistries including non-silica-based materials) using an established testing protocol. The phases have been characterised chromatographically in terms of their surface coverage, hydrophobic selectivity, shape selectivity, hydrogen bonding capacity and ion-exchange capacity at pH 2.7 and 7.6. The columns and their chromatographic parameters have been analysed by PCA in order to: (1) identify equivalent phases; (2) select columns of widely differing characteristics to fully exploit selectivity differences in method development; (3) assist in the rational selection of suitable stationary phases; (4) provide a greater understanding of the retention mechanisms and to allow qualified deduction to be made regarding the functionality and bonding chemistries that the manufacturers have employed. The latter is most desirable as there is very little reliable data available on the nature of most of the stationary phases that we use on a daily basis.

Previous PCA on smaller databases containing predominately C_{18} and C_8 phases [13,31] have shown a correlation between surface coverage and hydrophobic selectivity as well as between hydrogen bonding capacity and ion-exchange capacity at pH 7.6 (hence some of these parameters become redundant); however, with this new and enlarged database that contains many diverse stationary phase chemistries we have included all the column chromatographic parameters in the PCA as these deductions have subsequently been shown not to be valid with such diverse stationary phase chemistries.

The robustness of the column characterisation testing procedure was assessed using a reduced factorial design and was deemed to be acceptable within the following experimental conditions: MeOH content in the mobile phases $\pm 1\%$ (v/v), thermostated column temperature $\pm 2\,^{\circ}C$, aqueous component of the mobile phases of pH ± 0.1 unit and buffer concentration of the aqueous component of the mobile phase ± 2 m*M* [52].

PCA has been shown to be extremely useful in simplifying the ''data mining'' process with such a large amount of data (i.e., $135 \times 6 = 810$ values). PCA provides a simple graphical comparison of the phases within the database, for example the PCA of silica-based RP materials using first, second and third components allows the phases to be grouped into non-C_{18} phases, acidic phases, new generation phases, polar embedded phases, cyano phases, perfluorophenyl phases and highly retentive phases. These three components describe nearly 80% of the total chromatographic differences between these phases.

There appears to be a good correlation between the PCA of C_8 and C_{18} phases with that of the peak shape obtained for the analysis of hydrophilic bases.

The highly discriminating power of PCA was highlighted with the analysis of a sub-set of the database, which contained only C_{18} phases based on non-acidic silica material. The results highlighted large differences in what would appear, from the manufacturers literature, to be similar C_{18} phases. In addition, PCA ''contribution plots'' proved a rapid visualisation of differences between any two phases within the database.

PCA of phases which are suitable for use in highly aqueous mobile phases provided discrimination between phases containing polar embedded moieties, those containing mixed alkyl ligands and those containing polar/hydrophilic endcapping technologies.

The authors intend to maintain and expand this database to include other and new phases when they are released and to expand the range of test probes that are employed in the characterisation protocol.

Acknowledgements

The authors gratefully acknowledge the stationary phase manufacturers and suppliers who provided columns for this study, Dr. McKeown, Miss Merrin and Mr Hassey who assisted with the practical aspects of the column characterisation testing and to Drs. Dolan and Snyder (LCResources, Walnut Creek, CA, USA) for encouragement and advise during this work. The authors would also like to thank Dr. Danielsson (Uppsala University, Sweden) for his assistance in the spreadsheet to identify similar columns.

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