

Facilitated Column Ranking and Selection in Reversed-Phase Liquid Chromatographic Analysis

Pieter Dehouck,^a Dóra Visky,^{a,c} Guy Van den Bergh,^a Erik Haghedooren,^a Erwin Adams,^a Ágnes Kerner,^{a,d} Yvan Vander Heyden,^b Désiré L. Massart,^b Zsuzsanna Kovács,^{a,c} Béla Noszál^c and Jos Hoogmartens.^a

^aKatholieke Universiteit Leuven, Belgium

^bVrije Universiteit Brussel (VUB), Belgium

^cSemmelweis University, Budapest, Hungary

^dNational Institute of Forensic Toxicology, Budapest, Hungary

This article presents a column ranking system to facilitate column selection in reversed-phase liquid chromatographic analysis. The system ranks columns based on their similarity to a reference column of choice and the authors demonstrate its applicability to a real pharmaceutical analysis. The column ranking system is freely available on-line.

Introduction

Selection of reversed-phase liquid chromatography (RPLC) C18 columns with suitable selectivity for a given analysis is difficult. *The European Pharmacopoeia (Ph. Eur.)* and other official compendia provide only a general description of the required stationary phase in the operating procedure of an LC method.^{1,2} This information is often insufficient when selecting a suitable column from a market offering more than 600 brands. To compound the problem, manufacturers provide only limited information about their columns. The need for a general test method to characterize RPLC columns has existed since the 1970s and several chromatographic column tests have been published.^{3–35}

A project to define a suitable procedure characterizing RPLC C18 columns by chromatographic tests was started in our laboratory. Different tests were selected from the literature. A procedure to measure 36 column parameters was applied, and the repeatability and reproducibility of the parameters were examined in three laboratories.^{36,37} Of these thirty six parameters, twenty four were found to be reproducible. Subsequently, a classification of 69 RPLC C18 columns, based on these twenty four parameters, was obtained by principal component analysis (PCA, Figure 1).³⁸ However, the measurement of these twenty four parameters was labour-intensive and time-consuming. Through additional studies it was deemed possible to reduce the number of column

parameters from twenty four to just four, without losing essential information; that is, without losing column classification (Figure 2).³⁸ The four selected parameters were

- the retention factor of amylbenzene, $k'_{\text{amylbenzene}}$ (i.e., hydrophobicity)
- the relative retention factor of triphenylene/*o*-terphenyl, $rk'_{\text{triphenylene/o-terphenyl}}$ (i.e., steric selectivity)
- the relative retention factor of benzylamine/phenol determined at pH 2.7, $rk'_{\text{benzylamine/phenol pH 2.7}}$ (i.e., silanol activity)
- the retention factor of 2,2'-dipyridyl, $k'_{2,2'\text{-dipyridyl}}$ (i.e., silanol activity and metal impurity).

These parameters can be determined using three simple, fast, repeatable and reproducible methods (Table 1).

The correlation between column classification, obtained in the PCA plot, and column selectivity, as determined by the *Ph. Eur.* separation of acetylsalicylic acid (ASA) and its impurities, has been demonstrated.³⁹ It was concluded that columns belonging to the same group showed similar selectivities for the separation of ASA and its impurities. Although a correlation was found, definition of column groups based on the PCA plot was difficult. Therefore, a more practical approach has been developed. This approach begins with the selection of a reference column; for example, the column on which a method has been developed or a column mentioned in the literature. Other columns are then ranked against this reference column,

based on the aforementioned four column parameters. This approach has one obvious advantage; that is, the relationship between columns can be expressed using a single value (F-value). This, in turn, allows the system to be made available on-line.

Experimental

The chromatographic conditions for column characterization are summarized in Table 1. Analyses were performed using a Varian (Walnut Creek, California, USA) 9010 LC pump, 9100 autosampler and 9050 UV/vis detector, with ChromPerfect 4.4.0 software (Justice Laboratory Software, Fife, UK) for data acquisition. Full details on the practical application of this characterization procedure are given elsewhere.^{36–39}

The mobile phase for the separation of ASA and related compounds was acetonitrile/water/phosphoric acid, 400:600:2 (v/v/v). Helium was used to degas the mobile phase. Columns were equilibrated for 30–90 min, depending on their length. The flow-rate was 1 mL/min, the injection volume was 20 μ L and the detection wavelength 237 nm. Column temperature was maintained at 30 ± 0.1 °C by immersion in a water bath, and the laboratory was air-conditioned to 25 °C. The separation was performed in triplicate on each column. A typical chromatogram is shown in Figure 3.

Stationary-phase suitability for the separation was examined by calculating the chromatographic response function (CRF), a measure of overall selectivity. The CRF was calculated as:

$$CRF = \prod_{i=1}^{n-1} \frac{f_i}{g_i} \quad [1]$$

where n is the total number of peaks, g is the interpolated peak height between two peaks, (i.e., the distance between the baseline and a line connecting the two peak apexes, at the location of the valley), and f is the depth of the valley, measured from the line connecting two peak apexes.^{40,41} This

means that a baseline-separated peak pair has an f/g ratio of 1, a co-eluting pair has a value of 0, and a partly separated peak pair has an intermediate value.

The full identification of the columns used in this study has been described previously,^{36–39} but is included here in Tables 2 and 3.

Results

Easy column classification system: Earlier, it was mentioned that defining column groups, based on the PCA plot, is difficult. Therefore, a more practical approach to column classification has been developed. This approach begins with the selection of a reference column. A ranking of RPLC C18 columns, based on the similarity of their four test parameters with those of the reference column, can be obtained, by introducing an F-value.³³

$$F = (k'_{\text{amylbenzene, ref}} - k'_{\text{amylbenzene, } i})^2 + (rk'_{\text{benzylamine/phenol pH 2.7, ref}} - rk'_{\text{benzylamine/phenol pH 2.7, } i})^2 + (k'_{\text{2,2'-dipyridyl, ref}} - k'_{\text{2,2'-dipyridyl, } i})^2 + (rk'_{\text{triphenylene/o-terphenyl, ref}} - rk'_{\text{triphenylene/o-terphenyl, } i})^2 \quad [2]$$

The F-value of a column i equals the sum of squares of the differences between each parameter value of the reference column and those of a column i . The smaller the F-value, the more similar column i is to the reference column. To ensure the same weighting of each parameter in this equation, the parameters are autoscaled using Equation 3 before being introduced into Equation 2:

$$\frac{x_{ij} - \bar{x}_j}{s_{x_{ij}}} \quad [3]$$

where (x_{ij}) is the value of parameter j on column i ; (\bar{x}_j) is the mean of parameter j on all tested columns, and $(s_{x_{ij}})$ is the standard deviation on the mean parameter value.

It could be expected that a ranking based on F-values

Figure 1: PC1-PC2 scores plot for 69 RPLC C18 columns based on 24 column parameters.

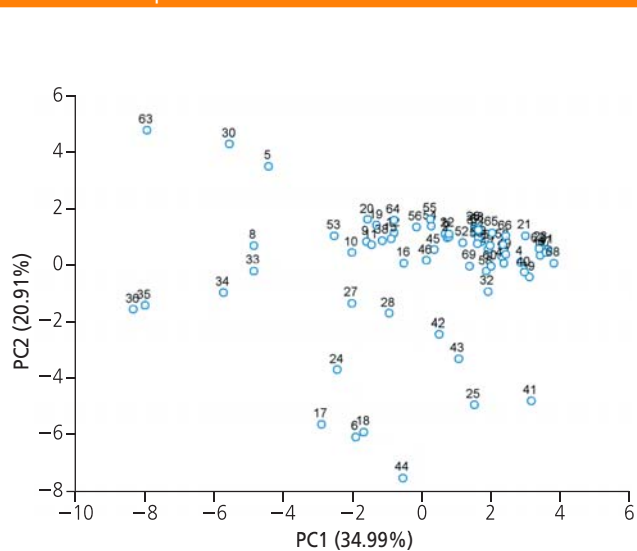
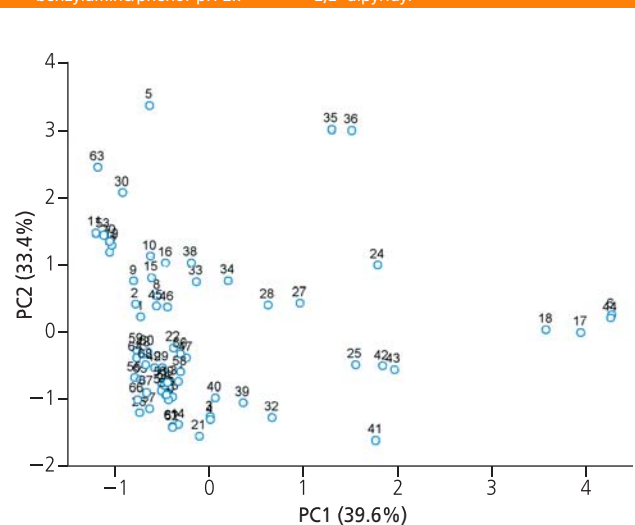


Figure 2: PC1-PC2 scores plot for 69 RPLC C18 columns based on 4 column parameters, $k'_{\text{amylbenzene}}$, $rk'_{\text{triphenylene/o-terphenyl}}$, $rk'_{\text{benzylamine/phenol pH 2.7}}$ and $k'_{\text{2,2'-dipyridyl}}$.



correlates to column positions on the PC1-PC2 scores plot (Figure 2). To examine this, column 4 (Alltima 5), situated in the central cluster of the PC1-PC2 plot, was selected as the reference column and the F-values were calculated (Table 2). In order to discriminate very similar columns, F-values are presented to three decimal places. Table 2 shows that columns 6, 5, 44, 36 and 35, all situated at the border of the PCA plot (Figure 2), have F-values above 20 and are therefore ranked in the lowest positions (low-ranked columns). The most similar column with an F-value = 0.037, was column 3 (Alltima 3). This seems logical as the reference column and column 3 differ only in dimensions and not in type of stationary phase. A good correlation is found between the column rankings and their position on the PCA plot relative to that of the reference column. Columns close to column 4 on the PCA plot have small F-values and are found high in column ranking (high-ranked columns). Small differences between the PCA plot and column ranking can be explained because the PC1-PC2 scores plot only explains about 73% of the variation. The remaining variation can be found in PC3 and PC4. However, it was not possible to retrieve interesting information from PC3 and PC4. Equation 2, on the other hand, includes all information available in the parameters.

Real life application of the column ranking system: It was concluded that a rational ranking of RPLC C18 columns, based on a single F-value, can be obtained. However, do high-ranked columns show the same selectivity as the reference column in real separations? The aim of this project is to facilitate both column selection in analyses prescribed by the *Ph. Eur.*, *United States Pharmacopoeia (USP)* or other official compendia, and the application of methods described in the literature when the column referred to in the paper is not immediately available. Therefore, the separation of ASA and its impurities, as prescribed by the *Ph. Eur.*,¹ was selected as a case study. In a previous paper, the correlation between PCA plot position and selectivity for this separation was shown.³⁹ In this study, the Tracerexcel 5 column (column 51, Teknokroma, Barcelona, Spain), producing the chromatogram of Figure 3, was chosen as the reference column and an examination of whether high-ranked columns showed a similar selectivity for the separation of ASA and its impurities was performed.

A ranking of the columns, based on their F-values, was made

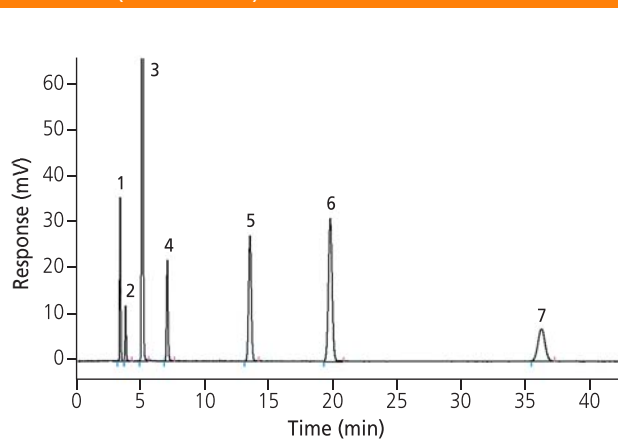
Table 1. The four test parameters, the chromatographic property they represent and the mobile phase composition, in order of execution.

	Chromatographic property	Mobile phase composition
rk' benzylamine/phenol	Silanol activity	Methanol–water–0.2 M KH_2PO_4 pH 2.7 (34:90:10, w/w)
k' 2,2'-dipyridyl	Silanol activity and metal impurity	Methanol–water (34:100, w/w)
k' amylbenzene	Hydrophobicity	Methanol–water
rk' triphenylene/o-terphenyl	Steric selectivity	(317:100, w/w)

(Table 3). The CRF value, which is also included in Table 3, was used as a measure of overall selectivity. A CRF value of 1 indicates complete baseline separation of all substances, while a CRF value of 0 indicates that two or more peaks co-elute. Intermediate values indicate that two or more peaks are not baseline separated. All 0.25 m columns (i.e., length prescribed by the *Ph. Eur.*) are in bold, shorter columns are not. Table 3 shows that all 0.25 m columns with F-values < 2.0 are suitable for the analysis of ASA and its impurities (CRF = 1). For columns with $F > 2.0$ the probability of complete baseline separation clearly decreases. When $F = 2-6$, four out of eight (50%) 0.25 m columns gave a baseline separation of all compounds, while for F-values above 6, only two out of eleven (18%) showed an appropriate selectivity. Although suitable columns can be found among the low-ranked columns, the probability has clearly decreased. In addition, the differences between the parameter values of the low-ranked columns are larger and if such a column is selected as the reference column, only a few columns with similar characteristics ($F < 2$) are found. Table 3 demonstrates that the classification system can help analysts to find a suitable column for their analysis more easily. Although not all suitable columns are high-ranked ($F < 2$), all high-ranked 0.25 m columns are found suitable. Therefore, the chance of selecting a suitable column has clearly increased, up to 100% if a 0.25 m column with $F < 2$ is selected.

Columns of shorter length than the prescribed one can have chromatographic test parameters closely related to those of the reference column, but this does not necessarily lead to the desired separation. Of the 16 shorter columns with an F-value < 2, only seven gave a baseline separation of all compounds. This discrepancy between the closeness of test parameters with those of the reference column (i.e., low F-value, thus similar stationary phase properties) and the observed separation of the ASA mixture, may be the result of a lower column efficiency (number of theoretical plates), which is not expressed by the F-value. Moreover, in these experiments the chromatographic conditions were never optimized to improve the separation.

Figure 3: Separation of ASA and its related substances on column 51 (Tracerexcel 5).



Peaks: 1 = 4-OH benzoic acid, 2 = 4-OH isophthalic acid, 3 = acetylsalicylic acid, 4 = salicylic acid, 5 = acetylsalicylsalicylic acid, 6 = salicylsalicylic acid, 7 = acetylsalicylic anhydride.

Table 2. Column classification based on F-values. Column 4 (Alltima 5) is taken as reference column ($F = 0$). 0.25 m columns are indicated in bold.

No.	Column name i.d. (mm), particle size, manufacturer	F-value	CRF
4	Alltima 5, 250 × 4.6, 5 µm, Alltech	0.000	1.00
3	Alltima 3, 150 × 4.6, 3 µm, Alltech	0.037	0.00
40	Purospher Star, 250 × 4.6, 5 µm, Merck	0.197	1.00
22	Kromasil EKA, 250 × 4.6, 5 µm, Akzo Nobel	0.690	1.00
57	Uptispher ODB5, 250 × 4.6, 5 µm, Interchrom	1.036	1.00
50	Tracerexcel 3, 150 × 4.6, 3 µm, Teknokroma	1.039	1.00
13	Genesis C18, 100 × 4.6, 3 µm, Jones Chrom.	1.076	0.74
49	Symmetry, 250 × 4.6, 5 µm, Waters	1.105	1.00
61	Wakosil RS, 100 × 4.6, 3 µm, SGE	1.142	0.89
47	Superspher, 250 × 4.6, 4 µm, Merck	1.200	1.00
58	Validated C18, 250 × 4.6, 5 µm, PerkinElmer	1.203	1.00
32	Pecosphere C18, 83 × 4.6, 3 µm, PerkinElmer	1.270	0.50
31	OmniSpher, 250 × 4.6, 5 µm, Varian	1.385	1.00
51	Tracerexcel 5, 250 × 4.6, 5 µm, Teknokroma	1.419	1.00
29	Nucleosil HD, 250 × 4.6, 5 µm, Macherey-Nagel	1.445	1.00
23	Kromasil NM, 250 × 4.6, 5 µm, Macherey-Nagel	1.486	1.00
68	Zorbax Extend C18, 250 × 4.6, 5 µm, Agilent	1.675	1.00
39	Purospher endcapped, 250 × 4.6, 5 µm, Merck	1.782	1.00
56	Uptispher ODB3, 100 × 4.6, 3 µm, Interchrom	1.899	0.94
52	TSKgel ODS-80TS, 150 × 4.6, 5 µm, TosoHaas	2.172	0.84
7	Aqua 5, 150 × 4.6, 5 µm, Phenomenex	2.214	1.00
67	Zorbax Eclipse XDB, 250 × 4.6, 5 µm, Agilent	2.230	1.00
54	Uptispher HDO3, 100 × 4.6, 3 µm, Interchrom	2.282	1.00
65	YMC-Pack-Pro C18-3, 150 × 4.6, 3 µm, YMC	2.373	1.00
66	YMC-Pack-Pro C18-3, 150 × 4.6, 5 µm, YMC	2.378	1.00
60	Wakosil HG, 250 × 4.6, 5 µm, SGE	2.614	1.00
37	Prodigy 3, 100 × 4.6, 3 µm, Phenomenex	2.885	0.81
59	Wakosil HG, 100 × 4.6, 5 µm, SGE	2.901	0.66
55	Uptispher HDO, 250 × 4.6, 5 µm, Interchrom	3.199	1.00
69	Zorbax SB-C18, 250 × 4.6, 5 µm, Agilent	3.409	1.00
26	Luna 5, 150 × 4.6, 5 µm, Phenomenex	3.723	1.00
41	Spheri-5, 250 × 4.6, 5 µm, PerkinElmer	3.853	0.96
28	Nucleosil NM, 250 × 4.6, 5 µm, Macherey-Nagel	3.863	1.00
1	ACE C18-3, 150 × 4.6, 3 µm, Achrom	3.881	0.93
2	ACE C18-3, 250 × 4.6, 3 µm, Achrom	4.828	1.00
43	Spherisorb ODS2, 250 × 4.6, 5 µm, Waters	5.308	1.00
25	LiChrospher, 250 × 4.6, 5 µm, Merck	5.367	1.00
64	YMC Hydrospher C18, 150 × 4.6, 5 µm, YMC	5.938	1.00
45	Supelcosil LC-18 DB, 150 × 4.6, 3 µm, Supelco	6.006	0.86
46	Supelcosil LC-18 DB, 250 × 4.6, 5 µm, Supelco	6.305	0.71
38	Purospher, 250 × 4.6, 5 µm, Merck	6.954	0.00
16	Hypersil BDS, 250 × 4.6, 5 µm, Alltech	7.053	0.91
9	Brava BDS 3, 150 × 4.6, 3 µm, Alltech	8.632	0.63
10	Brava BDS 5, 250 × 4.6, 5 µm Alltech	8.716	0.67
19	HyPurity Elite 3, 150 × 4.6, 3 µm, ThermoQuest	10.098	0.80
20	HyPurity Elite 3, 150 × 4.6, 5 µm, ThermoQuest	10.182	0.66
12	Discovery C18, 250 × 4.6, 5 µm, Supelco	10.232	0.96
8	Bondapak, 250 × 4.6, 10 µm, Waters	10.333	1.00
34	Platinum 5, 250 × 4.6, 5 µm, Alltech	11.613	0.24
33	Platinum 3, 150 × 4.6, 3 µm, Alltech	11.771	0.40
53	TSKgel Super ODS, 100 × 4.6, 2 µm, TosoHaas	12.660	0.83
30	Nucleosil Nautilus, 250 × 4.6, 5 µm, Macherey-Nagel	13.028	0.00
11	Chromolit, 100 × 4.6, ---, Merck	13.430	0.83
18	Hypersil ODS, 250 × 4.6, 5 µm, Alltech	19.713	0.88
35	Platinum EPS 3, 150 × 4.6, 3 µm, Alltech	21.327	0.09
36	Platinum EPS 5, 250 × 4.6, 5 µm, Alltech	21.779	0.42
44	Supelcosil LC18, 250 × 4.6, 5 µm, Supelco	25.630	0.00
5	Apex Basic, 250 × 4.6, 5 µm, Jones Chrom.	26.163	0.00
6	Apex ODS, 250 × 4.6, 5 µm, Jones Chrom.	26.272	0.67

Table 3. Column classification based on F-values. Column 51 (Tracerexcel 5) is taken as reference column ($F = 0$) for the separation of ASA and its impurities. CRF = chromatographic response function. 0.25 m columns are indicated in bold.

No.	Column name i.d. (mm), particle size, manufacturer	F-value	CRF
51	Tracerexcel 5, 250 × 4.6, 5 µm, teknochroma	0.000	1.00
57	Uptispher ODB5, 250 × 4.6, 5 µm, interchrom	0.041	1.00
13	Genesis C18, 100 × 4.6, 3 Mm, Jones Chromatogr.	0.055	0.74
50	Tracerexcel 3, 150 × 4.6, 3 µm, teknochroma	0.076	1.00
65	YMC-Pack-Pro C18-3, 150 × 4.6, 3 µm, YMC	0.143	1.00
58	Validated C18, 250 × 4.6, 5 µm, PerkinElmer	0.145	1.00
67	Zorbax Eclipse XDB, 250 × 4.6, 5µm, Agilent	0.162	1.00
29	Nucleosil HD, 250 × 4.6, 5 µm, Macherey-Nagel	0.162	1.00
52	TSKgel ODS-80TS 150 × 4.6, 5 µm, TosoHaas	0.218	0.84
56	Uptispher ODB3, 100 × 4.6, 3 µm, Interchrom	0.223	0.94
54	Uptispher HDO3, 100 × 4.6, 3 µm, Interchrom	0.255	1.0
60	Wakosil HG, 250 × 4.6, 5 µm, SGE	0.274	1.00
66	YMC-Pack-Pro C18, 150 × 4.6, 5 µm, YMC	0.279	1.00
7	Aqua 5, 150 × 4.6, 5 µm, YMC	0.299	1.00
68	Zorbax Extend C18, 250 × 4.6, 5 µm, Agilent	0.347	1.00
59	Wakosil HG, 100 × 4.6, 5µm, SGE	0.363	0.66
55	Uptispher HDO, 250 × 4.6, 5 µm, Interchrom	0.418	1.00
61	Wakosil HG, 100 × 4.6, 3µm, SGE	0.506	0.89
37	Prodigy 3, 100 × 4.6, 3µm, Phenomenex	0.632	0.81
69	Zorbax SB-C18, 250 × 4.6, 5 µm, Agilent	0.788	1.00
49	Symmetry, 250 × 4.6, 5µm, Waters	0.885	1.00
1	ACE C18-3, 150 × 4.6, 3 µm, Achrom	1.093	0.93
26	Luna 5, 150 × 4.6, 5 µm, Phenomenex	1.119	1.00
47	Superspher, 250 × 4.6, 4 µm, Merck	1.160	1.00
3	Alltima 3, 150 × 4.6, 3 µm, Alltech	1.344	0.00
4	Alltima 5, 250 × 4.6, 5 µm, Alltech	1.419	1.00
23	Kromasil NM, 250 × 4.6, 5 µm, Macherey-Nagel	1.487	1.00
22	Kromasil EKA, 250 × 4.6, 5 µm, Akzo Nobel	1.510	1.00
2	ACE C18, 250 × 4.6, 5µm, Achrom	1.588	1.00
40	Purospher Star, 250 × 4.6 5 µm, Merck	1.797	1.00
64	YMC Hydrospher C18, 150 × 4.6, 5 µm, YMC	1.877	1.00
45	Supelcosil LC-18 DB, 150 × 4.6, 3 µm, Supelco	1.998	0.86
46	Supelcosil LC-18 DB, 250 × 4.6, 5 µm, Supelco	2.152	0.71
32	Pecosphere C18, 83 × 4.6, 3µm, PerkinElmer	2.366	0.50
31	OmniSpher, 250 × 4.6, 5 µm, Varian	2.786	1.00
16	Hypersil BDS, 250 × 4.6, 5 µm, Alltech	3.300	0.91
9	Brava BDS 3, 150 × 4.6, 3 µm, Alltech	3.434	0.63
10	Brava BDS 5, 250 × 4.6, 5 µm, Alltech	4.005	0.67
28	Nucleosil NM, 250 × 4.6, 5 µm, Macherey-Nagel	4.339	1.00
8	Bondapak, 250 × 4.6, 10 µm, Waters	4.719	1.00
12	Discovery C18, 250 × 4.6, 5 µm, Supelco	4.768	0.96
19	HyPurity Elite 3, 150 × 4.6, 3µm, ThermoQuest	4.919	0.80
20	HyPurity Elite 5, 150 × 4.6, 5µm, ThermoQuest	5.123	0.66
39	Purospher endcapped, 250 × 4.6, 5 µm, Merck	5.565	1.00
33	Platinum 3, 150 × 4.6, 3 µm, Alltech	5.949	0.40
53	TSKgel Super ODS, 100 × 4.6, 2 µm TosoHaas	6.334	0.83
34	Platinum 5, 250 × 4.6, 5 µm, Alltech	6.372	0.24
11	Chromolit, 100 × 4.6, ---, Merck	6.810	0.83
38	Purosphere, 250 × 4.6, 5 µm, Merck	7.408	0.00
41	Spheri-5, 250 × 4.6, 5 µm, PerkinElmer	7.811	0.96
43	Spherisorb ODS2, 250 × 4.6, 5 µm, Waters	8.443	1.00
25	LiChrospher, 250 × 4.6, 5 µm, Merck	10.087	1.00
30	Nucleosil Nautilus, 250 × 4.6, 5 µm, Macherey-Nagel	10.350	0.00
35	Platinum EPS 3, 150 × 4.6, 3 µm, Alltech	17.906	0.09
36	Platinum EPS 5, 150 × 4.6, 5 µm, Alltech	18.728	0.42
18	Hypersil ODS, 250 × 4.6, 5 µm, Alltech	20.392	0.88
5	Apex Basic, 250 × 4.6, 5 µm, Jones Chrom.	24.584	0.00
44	Sypelcosil LC18, 250 × 4.6, 5 µm, Supelco	27.346	0.00
6	Apex ODS, 250 × 4.6, 5 µm, Jones Chrom.	27.642	0.67

For several columns, both short- and long-type are included in the study. It should be noted that for short columns, having an F-value < 2, but failing to give baseline separation, increasing column length enables baseline separation of all compounds. Examples are column pairs 3/4 and 56/57. However, the difference between short and long columns is of minor importance in this context, as the literature, the *Ph. Eur.* and other compendia always specify the column length to be used.

The above shows that column ranking can facilitate column selection for this separation. Even though many RPLC C18 columns are commercially available, not all laboratories will have column 51 in stock. The ranking presented here allows the determination of columns with similar selectivity. Analysts preferably should select a 0.25 m column with a low F-value (e.g., < 2), in order to achieve a good baseline separation for ASA and its impurities. Figure 4 shows chromatograms obtained using columns 57 (F = 0.041) and 40 (F = 1.797). The selectivity of these two high-ranked columns is similar to that of reference column 51 (Figure 3). Figure 5 shows chromatograms obtained using columns 36 (F = 18.728) and 5 (F = 24.584). These low-ranked columns do not give baseline separations. On column 5, salicylic acid and salicysalicylic acid are not even eluted.

It should be noted that this column ranking does not distinguish between “good” and “bad” columns. The fact that a column is ranked with a high F-value only indicates that it has properties different from that of the reference column. For other applications developed on another type of column, such a low-ranked column may be ranked much higher. Even a

column that ranks low for many applications may be of great value for some specific applications.

This system can also be used to improve column descriptions in official compendia. Compendia such as the *Ph. Eur.* and *USP* are not allowed to communicate stationary phase brands. However, once well-performing columns are characterized by their four parameters, compendia may publish their values as reference with no further need to publish brand names. Based on these four parameter values, analysts can easily identify suitable columns with the required selectivity.

Easy Accessibility

The column ranking system described in this article is freely accessible on our website: <http://www.pharm.kuleuven.ac.be/pharmchem/columnclassification>. The database already contains more than 50 types of RPLC C18 columns and new types are being characterized in our laboratory at present. Analysts can either classify all columns from the database with respect to a freely chosen reference column, or they can input the four parameter values determined on their own column. The latter method allows the ranking of columns available in any laboratory, but not included in the database.

This column classification system allows the selection of columns with a selectivity similar to or different from a reference column. A similar selectivity may be needed during routine analysis, when a column prescribed in the literature is not available in the laboratory. By using the classification system, analysts can find available columns with similar

Figure 4: Typical chromatograms for ASA and its related substances on (a) column 57 (F = 0.041) and (b) column 40 (F = 1.797). See Figure 3 for peak identification.

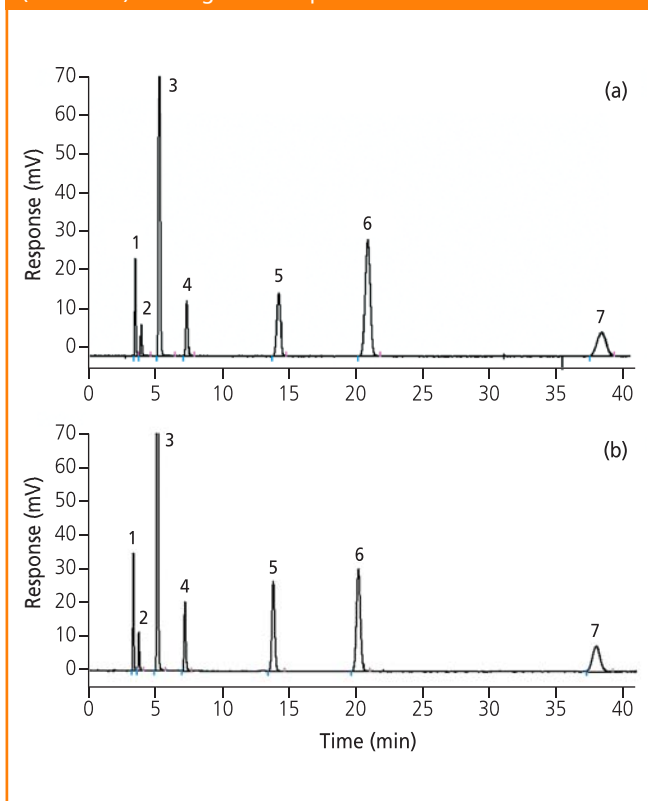
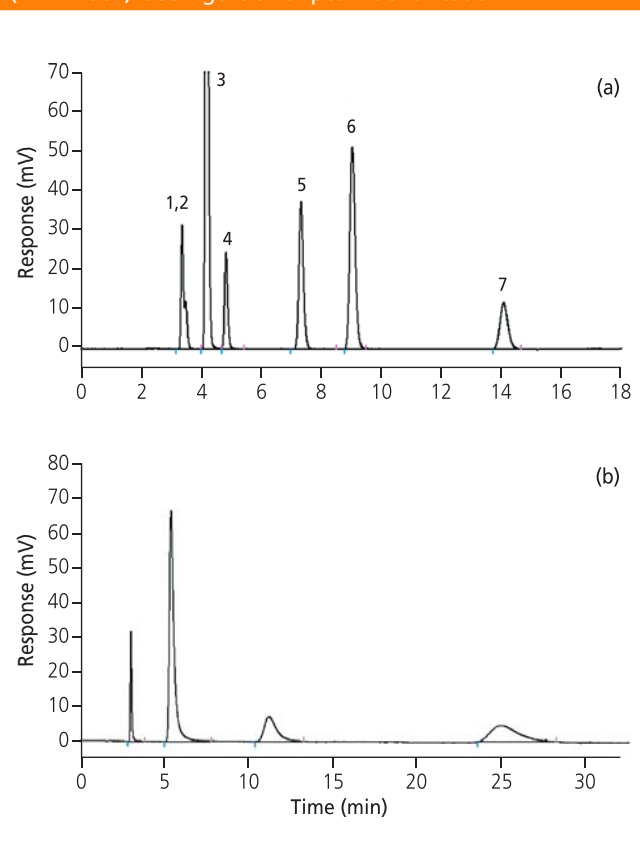


Figure 5: Typical chromatograms for ASA and its related substances on (a) column 36 (F = 18.728) and (b) column 5 (F = 24.584). See Figure 3 for peak identification.



selectivities; that is, columns with small F-values (e.g., $F < 2$). The column ranking system can also be used during method development, in which analysts may require columns with different selectivities. These columns will have large F-values and might be found among the low-ranked columns.

A further advantage of this ranking system is that it allows column ageing to be monitored; that is, analysts can check whether the characteristics of a column have changed over time. They can compare the four parameter values of a new column with those of the same column after a period of use. The system allows the two parameter sets to be input and F-values calculated. The first parameter set is automatically considered as the reference while the second is classified among the other columns in the database. When a new column is chosen as the reference, an F-value close to zero and a high position in the ranking is expected for the used column if little change in column parameters has occurred with time. Different batches of one column type can also be compared in the same way.

Figure 6 shows what the website looks like. Analysts can select a column from the database and press the “classify” button to obtain a column ranking. A ranking of all columns in the database, including F-values and column parameter values

...this ranking facilitates column selection in a real pharmaceutical analysis.

is obtained. Analysts can also enter their RPLC C18 column's four parameter values and then press “classify” to update the database. A second parameter set can also be entered in order to compare two columns, two column batches or to monitor column ageing. The first parameter set is automatically considered as the reference.

Conclusions

A column ranking system is presented. This simple, fast, repeatable and reproducible test procedure characterizes RPLC C18 columns using four column parameters. These parameters enable columns to be ranked using a single F-value, which is a measure of column similarity when compared with a freely chosen reference column. It has been shown that this ranking facilitates column selection in a real pharmaceutical analysis. Although not all suitable 0.25 m columns were found high-ranked, all high-ranked 0.25 m columns were found suitable for this analysis. Therefore, analysts can select a suitable column, which is available in their laboratory, more easily. As the ranking is relative to a reference column or four reference parameters, the selection of a good reference column is important. This can be the column on which the method has been developed or a column mentioned in the literature. The column ranking system has been made freely accessible on a website. The database on this website contains more than 50 types of RPLC C18 columns and additional types will be added in the near future. The correlation with other real separations is under investigation.

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Figure 6: The column classification system website, with (top) selection of a column from the database and (bottom) the column ranking obtained.

Column	k'omb	r'ba/pH2.7	r'ba/rtz	k'2.2
AD1 C18(5), 250 x 4.6 mm ID, 5 µm, Adrenum	0.8	4.26	111.67	1.51
AD2 C18(5), 250 x 4.6 mm ID, 5 µm, Adrenum	0.83	4.01	111.69	1.5
Adrenum 111, 250 x 4.6 mm ID, 5 µm, ZGI	0.839	5.23	111.67	1.43
Hypocel HRS, 250 x 4.6 mm ID, 5 µm, Alltech	0.875	2.96	113.31	1.58
Pharmacia 101A-C18(4), 250 x 4.6 mm ID, Nova-ff	0.87	4.06	111.67	1.47
Waters 110, 250 x 4.6 mm ID, 5 µm, ZGI	0.883	5.14	111.69	1.41
Supelco L1-C18(10), 250 x 4.6 mm, 10 µm, Supelco	0.887	4.3	111.76	1.4
Supelco L1-C18(10), 250 x 4.6 mm ID, 10 µm, Supelco	0.888	3.88	113.94	1.41
Hypocel C18, 250 x 4.6 mm ID, 5 µm, Alltech	0.892	5.79	111.69	1.49
Boehr HRS, 250 x 4.6 mm ID, 5 µm, Alltech	0.893	3.03	113.14	1.5
Hypocel 110, 250 x 4.6 mm ID, 5 µm, Alltech	0.894	3.17	111.61	1.55
Hypocel 110, 250 x 4.6 mm ID, 5 µm, Alltech	0.894	3.58	111.67	1.58
Dynacore 110, 250 x 4.6 mm ID, 5 µm, Nova	0.895	3.04	111.61	1.5
Pharmacia 101A-C18(4), 250 x 4.6 mm ID, Nova-ff	0.896	4.76	111.65	1.48
Adrenum 111, 250 x 4.6 mm ID, 5 µm, Pharmacia	0.898	6.11	111.61	1.48
Boehr HRS, 250 x 4.6 mm ID, 5 µm, Alltech	0.898	3.17	111.66	1.47
Dynacore 110, 250 x 4.6 mm ID, 5 µm, Nova	0.898	4.67	111.69	1.44
Waters 110, 250 x 4.6 mm ID, 5 µm, Nova	0.897	4.41	111.67	1.41
Waters 110, 250 x 4.6 mm ID, 5 µm, Nova	0.894	5.73	111.61	1.37
Hypocel 110, 250 x 4.6 mm ID, 5 µm, Alltech	0.897	5.08	111.61	1.37

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Pieter Dehouck acquired his PhD at the Laboratory of Pharmaceutical Chemistry and Drug Analysis, Leuven, and did research in the field of uncertainty determination in pharmaceutical analysis and column classification.

Dóra Visky was a post-doctoral researcher of the Laboratory of Pharmaceutical Chemistry and Drug Analysis, Leuven, working in the field of column classification and LC-MS.

Guy Van den Bergh is a technical member of the Catholic University of Leuven, responsible for informatics at the Department of Pharmaceutical Sciences.

Erik Haghevooren is a PhD student of the Laboratory of Pharmaceutical Chemistry and Drug Analysis, Leuven, working in the field of column classification.

Erwin Adams is a post-doctoral fellow of the Fund for Scientific Research — Flanders (Belgium) at the Laboratory of Pharmaceutical Chemistry and Drug Analysis, Leuven. He is mainly working in the field of LC, LC-MS and column classification.

Ágnes Kerner was a post-doctoral researcher of the Laboratory of Pharmaceutical Chemistry and Drug Analysis, Leuven, working in the field of column classification.

Yvan Vander Heyden is Professor of Analytical Chemistry at the Free University of Brussels and heads a research group on chemometrics and separation sciences.

Désiré L. Massart is part-time Professor at the Free University of Brussels, and performs research on chemometrics in process analysis and its use in the detection of counterfeiting products or illegal manufacturing processes.

Zsuzsanna Kovács is a PhD student at Semmelweis University, Department of Pharmaceutical Chemistry, Budapest, Hungary, using separation techniques (HPCE, HPLC), electroanalytical and spectroscopic (NMR, UV) methods to characterize and analyse drugs and biomolecules.

Béla Noszál is Professor and Head of Pharmaceutical Chemistry at Semmelweis University, Budapest, working in the fields of pharmaceutical and biomolecular analysis and microspeciation.

Jos Hoogmartens is Professor and Head of the Laboratory of Pharmaceutical Chemistry and Drug Analysis, Catholic University of Leuven, Belgium.

