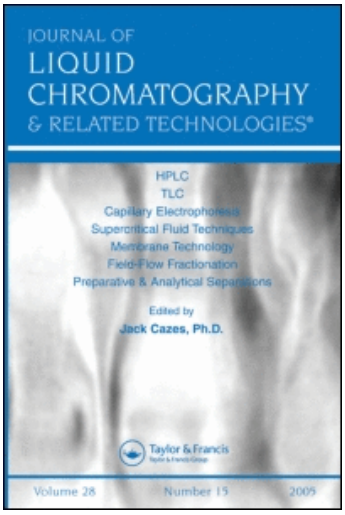

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Selecting a Suitable LC Column for Pharmaceutical Separations using a Column Characterisation System

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Abstract: Selecting a reversed phase liquid chromatographic (RP-LC) column with suitable selectivity for a particular separation is difficult if the brand name of the column is not known. Monographs of the European Pharmacopoeia and other official compendia for drug analysis only give a general description of the stationary phase to be used for a liquid chromatographic method. A project to develop a chromatographic test procedure to characterise RP-LC C_{18} columns was started earlier and resulted in a fast, simple, repeatable, and reproducible test procedure. Four column parameters allowed the characterisation and ranking of these columns. In this paper, the separations of three drug substances (amlodipine, tetracaine, and bisacodyl) from their respective impurities were examined applied on 77 RP-LC columns. It was observed, that the column ranking system was helpful in the selection of a suitable column for the separation of amlodipine and tetracaine, but also showed its limitations towards the separation of bisacodyl.

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Keywords: Amlodipine, Bisacodyl, Chromatographic tests, Column ranking, Tetracaine

INTRODUCTION

Official compendia like the European Pharmacopoeia (Ph. Eur.)[1] and the United States Pharmacopeia (USP),[2] prescribe in most of their monographs high performance liquid chromatography (HPLC) as an analysis technique. In most cases, the use of reversed phase liquid chromatography (RP-LC) is defined with a C_{18} stationary phase. However, nowadays an extended number of different C_{18} columns are commercially available on the market. Since monographs of the Ph. Eur. and other official compendia for drug analysis only give a general description of the stationary phase to be used in the operating procedure of an LC method (e.g., in terms of particle size, pore size, specific area, chain length, ...), the selection of a suitable column can be a problematic issue. For recently developed monographs, usually more information about the suitable stationary phases can be found on the Ph. Eur. website, under the box "knowledge database".[3] The information on the development column for the USP is provided in their Chromatographic reagents book and in the electronic version of USP.[4] The selection problem also arises when a column, prescribed in literature or by compendia, is not available in the laboratory. Adequate column selection is also needed during method development when an analyst wants to try columns with orthogonal selectivity. Therefore, a reliable method was needed for the characterisation of columns, based on a minimal number of test parameters.

Many different chromatographic tests for column characterisation, based on various test parameters, have been published in literature, but only the more recent ones are cited here.[3–18] Features like column efficiency, silanol activity, steric selectivity, and metal impurities were tested. Until now, none of these tests has been widely accepted. Also, it was never verified sufficiently whether columns having closely related characteristics, as determined by these chromatographic tests, are indeed suitable for the same chromatographic separation. A simple and reliable characterisation method, based on a limited number of parameters, easily determined in any laboratory, would be helpful for column comparison.

COLUMN CHARACTERISATION SYSTEM

The column characterisation project, developed in the Laboratory for Pharmaceutical Analysis, consisted of three consecutive parts. First, a procedure to measure a number of parameters, reflecting chromatographic characteristics, was developed. This number of test parameters was kept

minimal to make the procedure as user friendly as possible. After the characterization part, the second part involved ranking the RP-LC columns according to increasing deviation from a freely chosen reference column. In the final part, the relationship between the ranking of the RP-LC columns and performance in real pharmaceutical separations was examined.

The project started with collecting test methods for the characterisation of RP-LC columns from the literature. After a critical evaluation, 36 test parameters were selected, testing different properties like hydrophobicity, silanol activity, metal impurities, and steric selectivity.[19] A general procedure requires repeatable and reproducible test parameters. Therefore, all methods were examined in three different laboratories, and 24 out of the initial 36 parameters complied.[20] Similar to the approach of Iványi et al.,[21] principal component analysis (PCA) was used to reduce the number of parameters to four: the retention factor of amylbenzene (k'_{amb}), the relative retention factor benzylamine/phenol at pH 2.7 ($rk'_{ba/ph\ pH\ 2.7}$), the retention factor of 2,2′-dipyridyl ($k'_{2,2'-dip}$), and the relative retention factor triphenylene/o-terphenyl ($rk'_{tri/o-ter}$).[22]

Next, a practical approach to rank RP-LC versus a chosen reference column was introduced by using F-values. The column ranking approach starts with the choice of a reference column or of four reference parameters. For each column, the F-value versus this reference column was calculated and the columns were ranked according to increasing F-values, calculated as follows:

$$\begin{aligned} F =& (k'_{amb,ref} - k'_{amb,i})^2 + (rk'_{ba/ph\ pH\ 2.7,ref} - rk'_{ba/ph\ pH\ 2.7,i})^2 \\ &+ (k'_{2,2'-dip,ref} - k'_{2,2'-dip,i})^2 + (rk'_{tri/o-ter,ref} - rk'_{tri/o-ter,i})^2 \end{aligned} \quad (1)$$

The F-value of a column i equals the sum of squares of the differences between each parameter value of the reference column and column i. The smaller the F-value, the more similar is column i to the reference column. In order to have the same weighing of each parameter in this equation, the parameters are autoscaled using formula (2) before being introduced in Equation (1):

$$\frac{x_{ij} - \bar{x}_j}{s_j} \quad (2)$$

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_j is the standard deviation on the mean value of parameter j. With this F-value, a ranking of all columns can be obtained, indicating how close columns are to the selected reference column. Low F-values correspond to high ranking.[23]

The last part of the project consisted in performing pharmaceutical analyses on all characterized columns to check the performance of the column characterisation system in real separations. Dehouck et al.

carried out the separation of acetylsalicylic acid (aspirin) according to the Ph. Eur. monograph. An evaluation of the system suitability test (SST), as prescribed by the Ph. Eur. was made. It was concluded that this SST could not always predict the suitability of the column for the aspirin separation. As alternative criterion, the chromatographic response function (CRF) was proposed. The CRF is calculated as:

$$CRF = \prod_{i=1}^{n-1} \frac{f_i}{g_i} \tag{3}$$

where n is the total number of solutes, g the interpolated peak height, i.e., the distance between the baseline and the line connecting the two peak tops, at the location of the valley and f the depth of the valley, measured from the line connecting the two peak tops.[24,25]

Baseline separation of all peaks results in a CRF value of 1, while a value of 0 corresponds to coelution of 2 or more peaks. Partial separations lead to intermediate values. So, after ranking all columns and checking the CRF values, it was observed that the chance of selecting a suitable column clearly increased with a smaller F-value. All columns with an $F < 2$ gave baseline separation for all peaks ($CRF = 1$), while this number decreased to 43% for columns with $2 < F < 6$ and to 18% for columns with $F > 6$.[26] The performance of the system was also tested on 7 other separations, leading to promising results.[27–30] Here, a virtual, ideal column was taken as reference, calculated as the mean of all columns providing an overall baseline separation ($CRF = 1$) after applying the Grubbs' test onto the column parameters to remove possible outliers.[31]

Compared to the originally proposed chromatographic methods, some of the conditions were slightly adapted since the determination of $k'_{2,2'-dip}$ was sometimes problematic due to a poor peak shape or a very high retention time, which could not be determined properly. To overcome this problem, the mobile phases were slightly adapted. The determination of the dead volume was also simplified.[32] In order to further investigate the possibilities of this column classification system, its performance towards the separation of amlodipine, tetracaine, and bisacodyl, and their respective impurities, is examined here. For these three different separations, 76 columns were selected.

EXPERIMENTAL

Chromatographic Tests and Tested Columns

General information concerning the column test method resulting in the four final parameters was published earlier.[32] For the present analyses, 76 new C_{18} columns were used (Table 1). All were C_{18} columns, except

Table 1. List of C_{18} RP-LC columns examined and their properties as provided by the manufacturer

No.	Column name	Length (mm)	Internal diameter (mm)	Particle size (μm)	Manufacturer/ Supplier
1	Acclaim 3 μm	150	4.6	3	Dionex
2	Acclaim 5 μm	250	4.6	5	Dionex
3	ACE 5 C18	250	4.6	5	Achrom
4	Alltima AQ	250	4.6	5	Grace
5	Alltima C18	250	4.6	5	Grace
6	Alltima HP C18	250	4.6	5	Grace
7	Alltima HP C18 Amide	250	4.6	5	Grace
8	Alltima HP C18 HL	250	4.6	5	Grace
9	Brava BDS C18	250	4.6	5	Grace
10	Capcell Pak C18 ACR	250	4.6	5	Shiseido Fine Chemicals
11	Capcell Pak C18 AQ	250	4.6	5	Shiseido Fine Chemicals
12	Capcell Pak C18 MG	250	4.6	5	Shiseido Fine Chemicals
13	Capcell Pak C18 UG120	250	4.6	5	Shiseido Fine Chemicals
14	Chromolith Performance	100	4.6	/	Merck
15	Denali	250	4.6	5	Grace
16	Discovery C18	250	4.6	5	Supelco
17	Discovery HS C18	250	4.6	5	Supelco
18	Everest	250	4.6	5	Grace
19	Exsil ODS	250	4.6	5	SGE
20	Hamilton Hx Sil C18	250	4.6	5	Hamilton
21	Hydrospher C18	250	4.0	5	YMC
22	HyPURITY Advance	250	4.6	5	Thermo Electron Corporation
23	HyPURITY Aquastar	250	4.6	5	Thermo Electron Corporation
24	HyPURITY C18	250	4.6	5	Thermo Electron Corporation
25	Inertsil ODS-2	250	4.6	5	GL Sciences Inc.
26	Inertsil ODS-3	250	4.6	5	GL Sciences Inc.
27	Inertsil ODS-80A	250	4.6	5	GL Sciences Inc.
28	Inertsil ODS-P	250	4.6	5	GL Sciences Inc.
29	Kromasil KR100-5C18	250	4.6	5	EKA Chemicals
30	LiChrosorb RP-18	250	4.6	5	Merck

(*Continued*)

Table 1. Continued

No.	Column name	Length (mm)	Internal diameter (mm)	Particle size (μm)	Manufacturer/ Supplier
31	LiChrospher 100 RP-18	250	4.6	5	Merck
32	MP-Gel ODS-5	250	4.0	5	YMC/ OmniChrom
33	Nucleodur 100-5 C18 ec	250	4.6	5	Macherey-Nagel/ Filterservice
34	Nucleodur C18 Gravity	250	4.6	5	Macherey-Nagel/ Filterservice
35	Nucleodur C18 Isis	250	4.6	5	Macherey-Nagel/ Filterservice
36	Nucleodur C18 Pyramid	250	4.6	5	Macherey-Nagel/ Filterservice
37	Nucleodur Sphinx RP	250	4.6	5	Macherey-Nagel/ Filterservice
38	Omnispher 5 C18	250	4.6	5	Varian
39	Platinum C18	250	4.6	5	Grace
40	Platinum EPS C18	250	4.6	5	Grace
41	Polaris C18-A	250	4.6	5	Varian
42	Prevail Amide	250	4.6	5	Grace
43	Prevail C18	250	4.6	5	Grace
44	Prevail Select C18	250	4.6	5	Grace
45	Prontosil 120-5-C18 AQ	250	4.6	5	BISCHOFF
46	Prontosil 120-5-C18 AQ PLUS	250	4.6	5	BISCHOFF
47	Prontosil 120-5-C18-ace-EPS	250	4.6	5	BISCHOFF
48	Prontosil 120-5-C18-H	250	4.6	5	BISCHOFF
49	Prontosil 120-5-C18-SH	250	4.6	5	BISCHOFF
50	Prontosil 60-5-C18 H	250	4.6	5	BISCHOFF
51	Purospher RP-18e	250	4.6	5	Merck
52	Purospher Star RP-18	250	4.6	5	Merck
53	Pursuit 5 u C18	250	4.6	5	Varian
54	Pursuit PFP	250	4.6	5	Varian
55	Pursuit XRs C18	250	4.6	5	Varian
56	Restek Allure C18	250	4.6	5	Restek
57	Restek Pinnacle DB C18	250	4.6	5	Restek

(*Continued*)

Table 1. Continued

No.	Column name	Length (mm)	Internal diameter (mm)	Particle size (μm)	Manufacturer/ Supplier
58	Restek Pinnacle II C18	250	4.6	5	Restek
59	Restek Ultra C18	250	4.6	5	Restek
60	Supelcosil LC-18	250	4.6	5	Supelco
61	Supelcosil LC-18 DB	250	4.6	5	Supelco
62	Superspher 100 RP-18	250	4.6	5	Merck
63	Uptisphere 5 ODB-25QS	250	4.6	5	Interchrom/ Achrom
64	Wakosil II 5C18RS	250	4.6	5	SGE
65	X-Bridge C18 5 μm	250	4.6	5	Waters
66	X-Bridge Shield RP18	250	4.6	5	Waters
67	Xterra MS C18	250	4.6	5	Waters
68	Xterra RP C18	250	4.6	5	Waters
69	YMC-Pack Pro C18 3 μm	250	4.0	3	YMC
70	YMC-Pack Pro C18 5 μm	250	4.0	5	YMC
71	YMC-Pack Pro C18 RS	250	4.0	5	YMC
72	ZirChrom-PS 3 μm	150	4.6	3	ZirChrom
73	Zorbax Eclipse XDB-C18	250	4.6	5	Agilent
74	Zorbax Extend-C18	250	4.6	5	Agilent
75	Zorbax SB-Aq	250	4.6	5	Agilent
76	Zorbax SB-C18	250	4.6	5	Agilent

column no. 14 (Chromolith Performance) and column no. 72 (Zirchrom). Chromolith is a monolithic column, consisting of one porous bar of silica gel. Zirchrom is a column, which is not based on silica but on particles of polymerised zirconiumdioxide.

Samples and Reagents

All solvents were of HPLC grade and all other chemicals were of AR grade. Methanol and phosphoric acid were purchased from Acros Organics (Geel, Belgium), potassium dihydrogen phosphate from Sigma-Aldrich (Seelze, Germany), and acetonitrile from Biosolve (Valkenswaard, The Netherlands). Distilled water was purified by a Milli-Q50 system (Millipore, Billerica, MA, USA).

Amlodipine besilate and its potential impurities: diethylamlodipine besilate, impurity A, impurity B, impurity D, and phthalamic acid, as well as tetracaine and its potential impurities: 4-aminobenzoic acid (paracid), methyl(4-butylamino)benzoate (tetrabutyl) and 4-(butylamino)-benzoic acid (tetracid), and bisacodyl, its potential impurity E and bisacodyl chemical reference substance (CRS) for system suitability test (SST) containing impurity A, B, C, D, and E were donated by the European Directorate for the Quality of Medicines (Strasbourg, France).

Chromatographic Conditions

Analyses were carried out using a Varian (Walnut Creek, CA, USA) 9010 LC pump, a 9100 autosampler, and a 9050 UV-VIS detector with Chrom-Perfect 4.4.0 software (Justice Laboratory Software, Fife, UK) for data acquisition. The columns were immersed in a water bath heated by a Julabo EC thermostat (Julabo, Seelbach, Germany).

The analysis of amlodipine and bisacodyl was carried out according to the corresponding Ph. Eur. Monographs.[1] The chromatographic procedure for tetracaine was performed according to the method prescribed in a draft monograph.[33] The nomenclature of the Ph. Eur. was used. Since the elution order of the peaks can change on different stationary phases, for each substance a test mixture was prepared so that for each component different areas were obtained to facilitate peak identification in the chromatogram. No adaptation was made towards the chromatographic circumstances in order to obtain similar retention times on all columns. The chromatographic conditions used are summarised below:

Analysis of Amlodipine

The mobile phase consisted of acetonitrile-methanol-7.0 mL of triethylamine in 1 liter of water, adjusted to pH 3.0 with phosphoric acid (15:35:50 v/v/v). The mobile phase was degassed by helium and the flow rate was 1.5 mL/min. The column was kept at 25°C and the detection wavelength was fixed at 237 nm. A sample mixture, containing 3 mg of diethylamlodipine besilate, impurity D and phthalamic acid, and 6 mg of amlodipine besilate and impurity B was dissolved in 100 mL of mobile phase. The bulk sample solution was stored in a deep freezer below −15°C, protected from light, 20 µL were injected.

Analysis of Tetracaine

Mobile phase A consisted of 1.36 g KH_2PO_4 and 0.5 mL H_3PO_4 85 % in 1 liter of water. Mobile phase B was acetonitrile. Gradient program used:

0–15 min, 20 to 60% of B (linear gradient) and a further 60% of B (isocratic). The mobile phases were degassed by helium and the flow rate was 1 mL/min. The temperature of the column was kept at 30°C and the detection wavelength was fixed at 300 nm. The sample mixture consisted of 1% paracid, 8% tetracaine HCl, 16% tetracid, and 75% tetrabutyl. About 2 mg of the mixture was dissolved in 10 mL of water-acetonitrile (8:2, v/v). The bulk sample solution was stored below −15°C in a freezer, 20 μL were injected. The mobile phase and the gradient were the same for all columns.

Analysis of Bisacodyl

The mobile phase consisted of acetonitrile and a 1.58 g/L ammonium formate solution, adjusted with anhydrous formic acid to pH 5.0 (45:55, v/v). The mobile phase was degassed by helium and the flow rate was 1.5 mL/min. The column was kept at room temperature and the detection wavelength was fixed at 220 nm, which was lower than the prescribed 265 nm to increase the sensitivity. A solvent mixture was prepared consisting of acetic acid – acetonitrile – water (4:30:66, v/v/v). The sample mixture consisted of 5 mg bisacodyl CRS for SST, dissolved in 2.5 mL acetonitrile and diluted to 5 mL with solvent mixture. To this solution, 15.4 μg of impurity E was added. The sample solution was stored in a deep freezer below −15°C, protected from light, 20 μL were injected.

RESULTS AND DISCUSSION

Amlodipine

The analytical method can be found in the 'related substances' part of the Ph. Eur. Monograph.[1] However, when the method was first applied using an ACE 5 C_{18}, some adjustments needed to be made. As can be seen from Figure 1, the total analysis time is quite long. The Ph. Eur. prescribes a column with a length of 150 mm, but for this study, only 250 mm columns were available. This was considered not a problem as the Ph. Eur. allows a deviation of the prescribed column length by ±70%.; 250 mm columns may be selected for this analysis too. To reduce the total analysis time, the flow rate was increased to 1.5 mL/min and impurity A was omitted from the final sample mixture. To justify the latter adjustment, it was verified on several columns that impurity A was eluted much later than the other components of the sample mixture, and that its separation, therefore, never could be problematic.

The SST of the Ph. Eur. monograph for amlodipine besilate prescribes a minimum resolution of 4.5 between the peaks corresponding

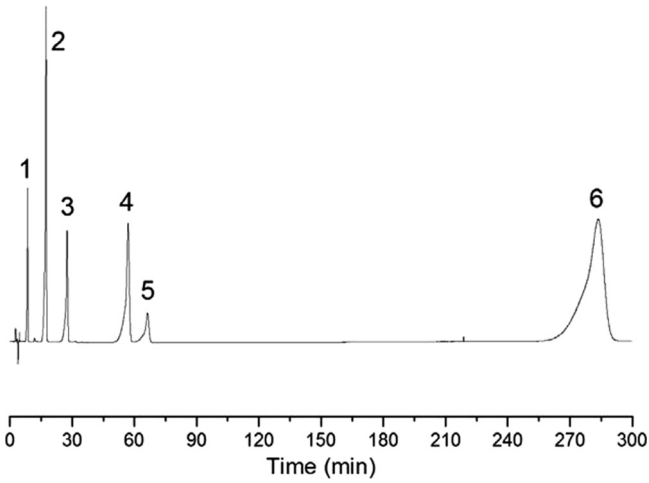

Figure 1. Separation of amlodipine besilate and its impurities: (1) impurity D, (2) amlodipine, (3) diethylamlodipine, (4) impurity B, (5) phthalamic acid and (6) impurity A. Column: ACE 5 C_{18} (No. 3).

to the first pair, i.e., amlodipine and impurity D. However, Figure 2 shows that a good resolution of this critical peak pair is, in some cases, insufficient to draw conclusions about the overall selectivity of a column.

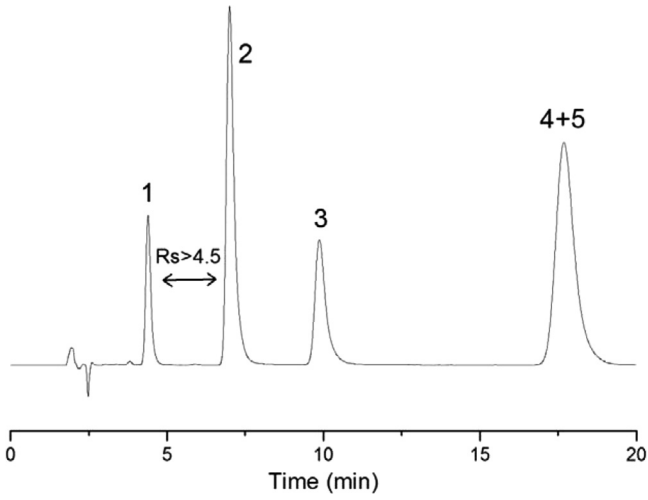

Figure 2. Separation of amlodipine besilate and its impurities. Despite the fact that the Rs between amlodipine and impurity D is above 5 and thus the SST is satisfied, the overall selectivity of the column is very poor. Column: Platinum C_{18} (No. 39). The peak numbering of Figure 1 was maintained.

Table 2. Column ranking based on the F-values, relative to virtual, ideal column parameters (k'_{amb}: 0.212, $rk'_{ba/ph\ pH\ 2.7}$: −0.130, $k'_{2,2'-dip}$: −0.095, $rk'_{tri/o-ter}$: −0.123) for the separation of amlodipine

No.	Column name	k'_{amb}	$rk'_{ba/ph\ pH\ 2.7}$	$k'_{2,2'-dip}$	$rk'_{tri/o-ter}$	F-value	CRF
36	Nucleodur C18 Pyramid	0.114	−0.150	−0.024	−0.159	0.016	1.00
64	Wakosil II 5C18RS	0.312	−0.138	−0.004	−0.162	0.020	1.00
10	Capcell Pak C18 ACR	0.381	−0.133	−0.220	−0.123	0.044	1.00
70	YMC-Pack Pro C18 5 μm	0.277	−0.149	−0.300	−0.154	0.048	1.00
25	Inertsil ODS-2	0.448	−0.148	−0.049	−0.111	0.058	1.00
58	Restek Pinnacle II C18	0.153	−0.101	−0.365	−0.130	0.078	1.00
73	Zorbax Eclipse XDB-C18	0.332	−0.125	−0.389	−0.154	0.102	1.00
48	Prontosil 120-5-C18-H	−0.078	−0.116	−0.230	−0.139	0.103	1.00
45	Prontosil 120-5-C18 AQ	−0.119	−0.113	−0.158	−0.152	0.114	1.00
63	Uptisphere 5 ODB-25QS	0.569	−0.122	−0.067	−0.144	0.129	1.00
32	MP-Gel ODS-5	0.228	−0.133	0.282	−0.098	0.143	1.00
47	Prontosil 120-5-C18-ace-EPS	0.053	−0.155	−0.451	−0.079	0.155	1.00
76	Zorbax SB-C18	−0.171	−0.100	−0.204	−0.155	0.161	0.96
15	Denali	0.597	−0.123	−0.219	−0.129	0.164	1.00
38	Omnispher 5 C18	0.653	−0.123	−0.172	−0.110	0.201	1.00
21	Hydrospher C18	−0.208	−0.145	−0.265	−0.165	0.207	1.00
74	Zorbax Extend-C18	0.622	−0.134	−0.311	−0.131	0.215	1.00
62	Superspher 100 RP-18	0.665	−0.109	0.054	−0.116	0.228	1.00
12	Capcell Pak C18 MG	0.685	−0.124	0.071	−0.154	0.252	1.00
49	Prontosil 120-5-C18-SH	0.388	−0.118	0.377	−0.131	0.254	1.00
13	Capcell Pak C18 UG120	−0.080	−0.139	−0.516	−0.151	0.264	1.00
20	Hamilton Hx Sil C18	0.327	−0.116	0.432	−0.130	0.291	1.00
17	Discovery HS C18	0.784	−0.126	−0.129	−0.128	0.329	1.00
52	Purospher Star RP-18	0.628	−0.130	0.369	−0.118	0.388	1.00

(*Continued*)

Table 2. Continued

No.	Column name	k'_{amb}	$rk'_{ba/ph\ pH\ 2.7}$	$k'_{2,2'-dip}$	$rk'_{tri/o-ter}$	F-value	CRF
35	Nucleodur C18 Isis	0.853	−0.162	0.063	−0.070	0.440	1.00
67	Xterra MS C18	−0.411	−0.122	−0.399	−0.166	0.482	1.00
3	ACE 5 C18	−0.258	−0.117	−0.670	−0.127	0.552	1.00
2	Acclaim 5 µm	0.951	−0.121	0.053	−0.145	0.568	1.00
57	Restek Pinnacle DB C18	−0.362	−0.098	−0.649	−0.128	0.637	1.00
60	Supelcosil LC-18	−0.330	0.285	−0.519	−0.140	0.646	1.00
53	Pursuit 5u C18	−0.382	−0.122	−0.636	−0.145	0.646	1.00
11	Capcell Pak C18 AQ	−0.540	−0.142	0.192	−0.145	0.649	1.00
34	Nucleodur C18 Gravity	1.021	−0.146	0.016	−0.149	0.668	1.00
33	Nucleodur 100-5 C18 ec	0.943	−0.134	0.290	−0.111	0.683	1.00
65	X-Bridge C18 5 µm	−0.417	−0.125	−0.673	−0.142	0.730	1.00
61	Supelcosil LC-18 DB	−0.582	−0.085	−0.558	−0.149	0.847	1.00
29	Kromasil KR100-5C18	1.126	−0.117	0.076	−0.125	0.865	1.00
37	Nucleodur Sphinx RP	−0.754	−0.132	0.057	−0.193	0.960	1.00
26	Inertsil ODS-3	1.132	−0.144	0.486	−0.150	1.186	1.00
5	Alltima C18	0.730	−0.123	0.888	−0.121	1.235	1.00
59	Restek Ultra C18	1.308	−0.124	0.130	−0.124	1.251	1.00
16	Discovery C18	−0.670	−0.123	−0.791	−0.132	1.262	1.00
6	Alltima HP C18	−0.769	−0.116	−0.791	−0.147	1.447	1.00
41	Polaris C18-A	−0.812	−0.122	−0.791	−0.104	1.533	1.00
9	Brava BDS C18	−0.979	−0.096	−0.469	−0.120	1.561	1.00
24	HyPURITY C18	−0.806	−0.120	−0.863	−0.123	1.626	1.00
44	Prevail Select C18	−0.837	−0.199	−0.816	−0.027	1.633	0.98
8	Alltima HP C18 HL	1.481	−0.132	0.107	−0.126	1.650	1.00
66	X-Bridge Shield RP18	−0.915	−0.149	−0.839	−0.047	1.828	1.00
51	Purospher RP-18e	1.009	−0.136	1.011	−0.092	1.861	1.00
55	Pursuit XRs C18	1.545	−0.132	0.246	−0.135	1.894	1.00
46	Prontosil 120-5-C18 AQ PLUS	0.269	−0.143	1.314	−0.096	1.989	1.00
14	Chromolith Performance	−1.110	−0.134	−0.814	−0.131	2.265	0.96
68	Xterra RP C18	−1.100	−0.136	−0.911	−0.084	2.388	1.00

(*Continued*)

Table 2. Continued

No.	Column name	k'_{amb}	$rk'_{ba/ph\ pH\ 2.7}$	$k'_{2,2'-dip}$	$rk'_{tri/o-ter}$	F-value	CRF
27	Inertsil ODS-80A	1.436	−0.134	1.094	−0.146	2.913	1.00
39	Platinum C18	−1.438	0.018	−0.624	−0.142	3.023	0.00
23	HyPURITY Aquastar	−1.522	−0.077	−0.327	0.042	3.089	0.00
19	Exsil ODS	−0.132	−0.070	1.630	−0.093	3.099	0.82
40	Platinum EPS C18	−1.552	0.120	0.125	−0.055	3.226	0.00
42	Prevail Amide	−1.439	−0.210	−0.812	−0.070	3.247	1.00
7	Alltima HP C18 Amide	−1.310	−0.170	−1.151	−0.008	3.447	1.00
4	Alltima AQ	−0.348	−0.114	1.714	−0.021	3.596	1.00
75	Zorbax SB-Aq	−1.724	−0.089	−0.337	−0.179	3.812	0.00
54	Pursuit PFP	−1.623	−0.114	−0.816	−0.014	3.899	1.00
30	LiChrosorb RP-18	−0.431	0.146	1.799	−0.084	4.078	0.77
43	Prevail C18	−0.278	−0.118	1.899	−0.027	4.226	0.00
18	Everest	−1.461	−0.147	−1.298	−0.124	4.248	1.00
50	Prontosil 60-5-C18 H	2.117	−0.141	0.771	−0.143	4.379	1.00
71	YMC-Pack Pro C18 RS	2.307	−0.150	0.409	−0.145	4.646	1.00
31	LiChrospher 100 RP-18	0.504	−0.048	2.287	−0.093	5.769	0.95
56	Restek Allure C18	2.479	−0.128	0.880	−0.130	6.092	1.00
22	HyPURITY Advance	−1.883	−0.286	−1.394	−0.062	6.105	0.36
28	Inertsil ODS-P	1.130	−0.125	5.420	0.000	31.278	0.35
72	ZirChrom -PS 3μm	−1.944	8.523	−1.524	8.536	156.548	0.00

The performance of the SST does not give any information concerning the other peaks. Therefore, to evaluate the overall separation on the different stationary phases, the CRF was used here.

The calculations started with the autoscaling of the column parameters. Then, all columns resulting in baseline separation of all peaks (CRF = 1) were selected and a Grubb's test was performed on the parameters to trace possible outliers, as applied before in ref, [27]. After omitting these outliers, all remaining columns with CRF = 1 were used to define the 4 parameters of a virtual, ideal column. Using this column as reference, the ranking of all columns was made based on ascending F-values. For amlodipine, instead of all 76 columns, only 74 were used for the calculations. Two columns, the Acclaim 3 μm (No. 1) and the YMC-Pack Pro C18 3 μm (No. 69) generated too high

back pressures using a flow of 1.5 mL/min due to their smaller particle size and therefore were omitted. The results are shown in Table 2. As before in ref. [27], columns are classified in three groups: high ranked columns with $F < 2$, intermediate columns with $2 < F < 6$, and low ranked columns with $F > 6$. In the $F < 2$ range, 50 of 52 columns (96%) show a $CRF = 1$, i.e., they give baseline separation of amlodipine from its related substances. Two columns (No. 44 and 76) did not have a value of $CRF = 1$, but 0.98 and 0.96, respectively, which corresponds to almost baseline separation. For columns with $2 < F < 6$, the chance to find a suitable one is 50% (9/18) and only 1 out of the 4 (25%) columns with $F > 6$ complied. Therefore, an analyst should select preferably a column with $F < 2$ to have a high change of selecting a column providing a baseline separation for amlodipine and its impurities. Among the columns giving $CRF = 0$, a column with enhanced polar selectivity (Platinum EPS) and 2 columns with polar endcapping functions (Zorbax SB-Aq and HyPURITY Aquastar) were observed. The Platinum EPS, however, showed an inversion in elution order between impurity B and phthalamic acid, see Figure 2). It was observed that the monolithic (Chromolith Performance, no. 14) and zirconia (Zirchrom, no. 72) columns were less suitable for this analysis because of partial and full co-elution (impurity B and phthalamic acid), respectively. Monolithic stationary phases are described to give faster elution, and this was also the case for the separation of amlodipine on the Chromolith Performance (with about 16 minutes versus an average of 80 minutes for other

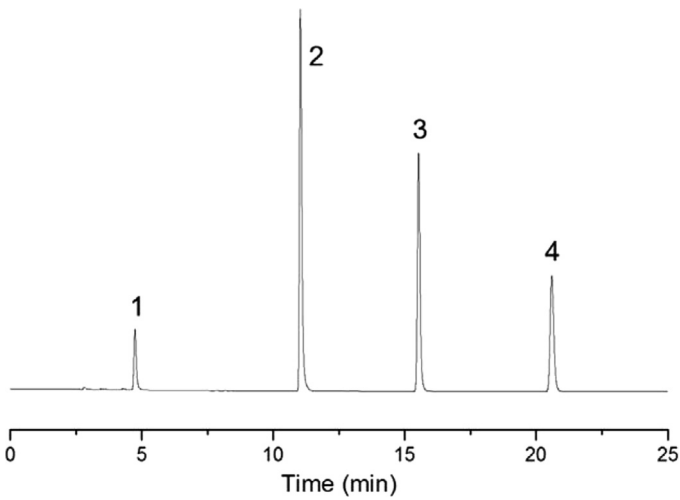

Figure 3. Separation of tetracaine and impurities: (1) paracid, (2) tetracaine, (3) tetracid and (4) tetrabutyl. Column: Zorbax Extend C_{18} (No. 74).

Table 3. Column ranking based on the F-values, relative to virtual, ideal column parameters (k'_{amb}: 0.105, $rk'_{ba/ph\ pH\ 2.7}$: −0.0004, $k'_{2,2'-dip}$: 0.107, $rk'_{tri/o-ter}$: −0.013) for the separation of tetracaine

No.	Column name	k'_{amb}	$rk'_{ba/ph\ pH\ 2.7}$	$k'_{2,2'-dip}$	$rk'_{tri/o-ter}$	F-value	CRF
36	Nucleodur C18 Pyramid	−0.123	0.149	0.009	0.157	0.062	1.00
48	Prontosil 120-5-C18-H	0.069	0.114	0.216	0.136	0.078	1.00
45	Prontosil 120-5-C18 AQ	0.110	0.111	0.144	0.150	0.087	1.00
58	Restek Pinnacle II C18	−0.162	0.099	0.353	0.128	0.094	1.00
64	Wakosil II 5C18RS	−0.321	0.136	−0.011	0.161	0.110	1.00
70	YMC-Pack Pro C18 5 μm	−0.287	0.147	0.287	0.152	0.116	1.00
76	Zorbax SB-C18	0.163	0.098	0.191	0.153	0.116	1.00
10	Capcell Pak C18 ACR	−0.391	0.131	0.207	0.121	0.128	1.00
47	Prontosil 120-5-C18-ace-EPS	−0.062	0.153	0.439	0.076	0.145	1.00
21	Hydrospher C18	0.199	0.143	0.252	0.163	0.166	1.00
25	Inertsil ODS-2	−0.457	0.146	0.035	0.109	0.167	1.00
73	Zorbax Eclipse XDB-C18	−0.341	0.123	0.377	0.152	0.172	1.00
32	MP-Gel ODS-5	−0.237	0.132	−0.299	0.095	0.212	1.00
13	Capcell Pak C18 UG120	0.071	0.137	0.504	0.149	0.235	1.00
69	YMC-Pack Pro C18 3 μm	−0.554	0.139	0.034	0.152	0.255	1.00
63	Uptisphere 5 ODB-25QS	−0.579	0.120	0.052	0.142	0.267	1.00
15	Denali	−0.606	0.121	0.206	0.127	0.296	1.00
38	Omnispher 5 C18	−0.663	0.121	0.158	0.107	0.344	1.00
74	Zorbax Extend-C18	−0.632	0.132	0.298	0.129	0.353	1.00
49	Prontosil 120-5-C18-SH	−0.398	0.116	−0.394	0.129	0.371	1.00
67	Xterra MS C18	0.402	0.120	0.386	0.164	0.382	1.00
62	Superspher 100 RP-18	−0.674	0.107	−0.069	0.113	0.383	1.00
20	Hamilton Hx Sil C18	−0.337	0.114	−0.449	0.128	0.396	1.00
12	Capcell Pak C18 MG	−0.695	0.122	−0.086	0.152	0.428	1.00

(*Continued*)

Table 3. Continued

No.	Column name	k'_{amb}	$rk'_{ba/ph\ pH\ 2.7}$	$k'_{2,2'-dip}$	$rk'_{tri/o-ter}$	F-value	CRF
60	Supelcosil LC-18	0.321	−0.292	0.507	0.138	0.448	1.00
3	ACE 5 C18	0.249	0.115	0.659	0.125	0.464	1.00
17	Discovery HS C18	−0.794	0.124	0.115	0.125	0.511	1.00
57	Restek Pinnacle DB C18	0.353	0.096	0.638	0.125	0.520	1.00
53	Pursuit 5 u C18	0.373	0.120	0.625	0.143	0.537	1.00
11	Capcell Pak C18 AQ	0.532	0.141	−0.208	0.143	0.550	1.00
52	Purospher Star RP-18	−0.638	0.129	−0.386	0.116	0.560	1.00
65	X-Bridge C18 5 µm	0.408	0.123	0.662	0.140	0.611	1.00
35	Nucleodur C18 Isis	−0.863	0.160	−0.078	0.067	0.643	1.00
61	Supelcosil LC-18 DB	0.573	0.083	0.546	0.147	0.686	0.98
2	Acclaim 5 µm	−0.961	0.120	−0.068	0.143	0.803	1.00
37	Nucleodur Sphinx RP	0.745	0.130	−0.072	0.192	0.815	1.00
33	Nucleodur 100-5 C18 ec	−0.954	0.132	−0.306	0.109	0.924	1.00
34	Nucleodur C18 Gravity	−1.031	0.145	−0.031	0.147	0.925	1.00
16	Discovery C18	0.662	0.122	0.781	0.130	1.078	1.00
29	Kromasil KR100-5C18	−1.136	0.124	−0.092	0.122	1.137	1.00
6	Alltima HP C18	0.761	0.114	0.780	0.145	1.242	1.00
9	Brava BDS C18	0.971	0.093	0.457	0.117	1.308	1.00
41	Polaris C18-A	0.804	0.121	0.781	0.102	1.309	1.00
44	Prevail Select C18	0.828	0.198	0.806	0.023	1.402	1.00
24	HyPURITY C18	0.798	0.118	0.853	0.121	1.405	1.00
5	Alltima C18	−0.740	0.121	−0.908	0.118	1.465	1.00
26	Inertsil ODS-3	−1.142	0.143	−0.504	0.148	1.497	1.00
59	Restek Ultra C18	−1.318	0.122	−0.145	0.122	1.570	1.00
66	X-Bridge Shield RP18	0.906	0.148	0.829	0.043	1.570	1.00
14	Chromolith Performance	1.102	0.132	0.804	0.129	1.982	1.00
8	Alltima HP C18 HL	−1.491	0.131	−0.122	0.124	2.011	1.00
68	Xterra RP C18	1.092	0.134	0.902	0.081	2.092	1.00

(*Continued*)

Table 3. Continued

No.	Column name	k'_{amb}	$rk'_{ba/ph\ pH\ 2.7}$	$k'_{2,2'-dip}$	$rk'_{tri/o-ter}$	F-value	CRF
46	Prontosil 120-5-C18 AQ PLUS	−0.275	0.142	−1.332	0.094	2.132	1.00
51	Purospher RP-18e	−1.020	0.134	−1.032	0.089	2.163	1.00
55	Pursuit XRs C18	−1.556	0.131	−0.262	0.133	2.281	1.00
39	Platinum C18	1.430	−0.022	0.612	0.140	2.636	1.00
23	HyPURITY Aquastar	1.514	0.075	0.314	−0.047	2.672	1.00
1	Acclaim 3 μm	1.235	0.110	1.070	0.148	2.761	1.00
40	Platinum EPS C18	1.544	−0.125	−0.141	0.052	2.801	0.42
42	Prevail Amide	1.431	0.209	0.801	0.067	2.894	1.00
7	Alltima HP C18 Amide	1.303	0.169	1.143	0.004	3.085	1.00
19	Exsil ODS	0.124	0.067	−1.654	0.091	3.169	0.98
27	Inertsil ODS-80A	−1.447	0.133	−1.115	0.144	3.337	1.00
75	Zorbax SB-Aq	1.717	0.087	0.326	0.178	3.412	1.00
54	Pursuit PFP	1.616	0.112	0.805	0.010	3.463	1.00
4	Alltima AQ	0.339	0.112	−1.738	0.017	3.616	1.00
18	Everest	1.454	0.145	1.291	0.122	3.872	1.00
30	LiChrosorb RP-18	0.423	−0.151	−1.824	0.081	4.037	1.00
43	Prevail C18	0.270	0.117	−1.925	0.024	4.282	1.00
50	Prontosil 60-5-C18 H	−2.128	0.140	−0.790	0.141	4.942	1.00
71	YMC-Pack Pro C18 RS	−2.319	0.149	−0.426	0.143	5.234	1.00
22	HyPURITY Advance	1.876	0.286	1.387	0.059	5.654	0.96
31	LiChrospher 100 RP-18	−0.514	0.045	−2.315	0.090	6.046	0.97
56	Restek Allure C18	−2.491	0.127	−0.900	0.128	6.744	1.00
28	Inertsil ODS-P	−1.140	0.123	−5.466	−0.004	32.145	1.00
72	ZirChrom -PS 3μm	1.937	−8.639	1.518	−8.652	155.353	1.00

columns). It must be noticed that no changes in chromatographic conditions were performed to optimise the separation.

Tetracaine

A typical chromatogram for the separation of tetracaine and its impurities is shown in Figure 3. As for amlodipine, the virtual, ideal reference column was defined after removing the outliers. All remaining columns

with CRF = 1 were used to define the reference parameters and F-values were calculated. The result is shown in Table 3. As before, columns are classified in three groups: high ranked columns with $F < 2$, intermediate columns with $2 < F < 6$, and low ranked columns with $F > 6$. In the $F < 2$ range, 49 of 50 columns (98%) show a CRF = 1, i.e., they give baseline separation of tetracaine from its related substances. For columns with $2 < F < 6$, the chance to find a suitable one is 86% (19/22) and 3 of the 4 (75%) columns with $F > 6$ complied. Five columns did not give baseline separation because of partial co-elution between 4-aminobenzoic acid (paracid)-tetracaine and 4-(butylamino)benzoic acid (tetracid)-methyl(4-butylamino)benzoate (tetrabutyl), respectively. The Platinum EPS and HyPURITY Advance gave either partial co-elution or inversion of peaks, as can be expected from columns with a different selectivity, due to an extended polar selectivity and an amide polar embedded function, respectively. The Zirchrom and Chromolith Performance were both suitable, while the Chromolith reduced the analysis time from an average of 25 minutes to 15 minutes.

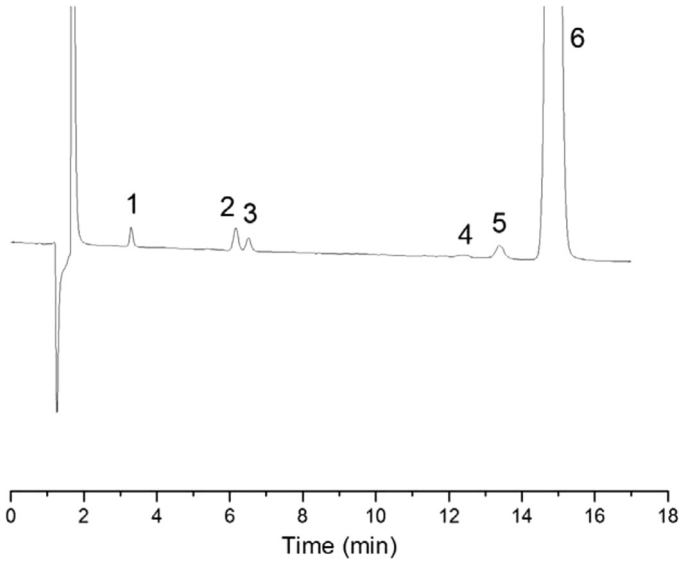

Figure 4. Separation of bisacodyl and impurities: (1) impurity A, (2) impurity B, (3) impurity C, (4) impurity D, (5) impurity E and (6) bisacodyl. Column: Capcell Pak C_{18} MG (No. 12).

Table 4. Column ranking based on the F-values, relative to virtual, ideal column parameters (k'_{amb} : 0.154, $rk'_{ba/ph\ pH\ 2.7}$: 1.740, $k'_{2,2'-dip}$: 0.286, $rk'_{tri/o-ter}$: − 0.149) for the separation of bisacodyl

No.	Column name	k'_{amb}	$rk'_{ba/ph\ pH\ 2.7}$	$k'_{2,2'-dip}$	$rk'_{tri/o-ter}$	F-value	CRF
48	Prontosil 120-5-C18-H	0.066	1.629	0.157	−0.567	0.212	0.56
20	Hamilton Hx Sil C18	0.453	1.662	0.657	−0.392	0.293	0.89
3	ACE 5 C18	−0.105	1.571	−0.177	−0.336	0.345	0.70
49	Prontosil 120-5-C18-SH	0.511	1.486	0.616	−0.412	0.371	0.77
45	Prontosil 120-5-C18 AQ	0.028	1.911	0.211	−0.831	0.516	1.00
62	Superspher 100 RP-18	0.775	2.223	0.371	−0.102	0.627	0.83
38	Omnispher 5 C18	0.764	1.110	0.200	0.015	0.805	0.65
15	Denali	0.710	1.070	0.164	−0.370	0.823	0.73
63	Uptisphere 5 ODB-25QS	0.684	1.203	0.280	−0.672	0.844	0.00
53	Pursuit 5 u C18	−0.224	1.213	−0.151	−0.698	0.914	0.72
24	HyPURITY C18	−0.629	1.343	−0.322	−0.262	1.153	0.48
29	Kromasil KR100-5C18	1.215	1.555	0.388	−0.286	1.190	0.87
6	Alltima HP C18	−0.593	1.633	−0.268	−0.730	1.214	0.79
65	X-Bridge C18 5 μm	−0.257	0.970	−0.179	−0.642	1.223	0.54
16	Discovery C18	−0.499	1.057	−0.268	−0.439	1.285	0.14
73	Zorbax Eclipse XDB - C18	0.457	0.958	0.036	−0.877	1.298	0.25
41	Polaris C18-A	−0.634	1.137	−0.268	0.127	1.369	0.35
2	Acclaim 5ìm	1.048	1.225	0.370	−0.695	1.371	0.86
17	Discovery HS C18	0.889	0.853	0.233	−0.348	1.372	0.50
5	Alltima C18	0.838	1.115	1.002	−0.206	1.376	1.00
12	Capcell Pak C18 MG	0.795	1.022	0.384	−0.871	1.459	1.00
58	Restek Pinnacle II C18	0.286	2.909	0.054	−0.396	1.496	0.56
67	Xterra MS C18	−0.251	1.150	0.029	−1.124	1.531	0.55
52	Purospher Star RP-18	0.740	0.495	0.609	−0.154	2.001	1.00
76	Zorbax SB - C18	−0.023	2.951	0.176	−0.892	2.059	1.00
59	Restek Ultra C18	1.389	1.022	0.428	−0.282	2.080	0.73
10	Capcell Pak C18 ACR	0.504	0.287	0.164	−0.257	2.265	0.73

(*Continued*)

Table 4. Continued

No.	Column name	k'_{amb}	$rk'_{ba/ph\ pH\ 2.7}$	$k'_{2,2'-dip}$	$rk'_{tri/o-ter}$	F-value	CRF
57	Restek Pinnacle DB C18	−0.204	3.137	−0.160	−0.346	2.313	0.45
32	MP-Gel ODS-5	0.358	0.268	0.544	0.258	2.446	0.00
1	Acclaim 3ìm	−1.045	2.028	−0.485	−0.800	2.538	1.00
74	Zorbax Extend - C18	0.734	0.225	0.095	−0.417	2.746	0.39
33	Nucleodur 100-5 C18 ec	1.041	0.211	0.549	−0.013	3.216	1.00
9	Brava BDS C18	−0.794	3.322	−0.025	−0.188	3.494	0.00
14	Chromolith Performance	−0.919	0.241	−0.286	−0.416	3.800	0.00
8	Alltima HP C18 HL	1.554	0.340	0.411	−0.313	3.965	0.86
64	Wakosil II 5C18RS	0.438	−0.083	0.327	−1.046	4.215	0.82
55	Pursuit XRs C18	1.615	0.335	0.516	−0.498	4.289	0.86
13	Capcell Pak C18 UG120	0.064	−0.197	−0.060	−0.811	4.322	0.65
51	Purospher RP-18e	1.104	0.059	1.095	0.379	4.667	0.68
37	Nucleodur Sphinx RP	−0.579	0.362	0.373	−1.670	4.762	0.82
68	Xterra RP C18	−0.909	0.068	−0.359	0.535	4.811	0.00
69	YMC-Pack Pro C18 3ìm	0.660	−0.367	0.293	−0.876	5.229	0.21
11	Capcell Pak C18 AQ	−0.375	−0.482	0.475	−0.703	5.568	1.00
43	Prevail C18	−0.125	1.467	1.766	1.670	5.654	1.00
4	Alltima AQ	−0.191	1.838	1.626	1.799	5.718	1.00
46	Prontosil 120-5-C18 AQ PLUS	0.397	−0.522	1.324	0.284	6.447	1.00
61	Supelcosil LC-18 DB	−0.414	4.136	−0.092	−0.772	6.590	1.00
21	Hydrospher C18	−0.058	−0.677	0.130	−1.092	6.805	0.44
54	Pursuit PFP	−1.408	1.859	−0.286	1.947	7.175	1.00
56	Restek Allure C18	2.507	0.662	0.996	−0.397	7.265	0.88
26	I nertsil ODS-3	1.221	−0.620	0.698	−0.802	7.311	1.00
27	I nertsil ODS-80A	1.652	−0.320	0.993	−0.773	7.381	1.00
34	Nucleodur C18 Gravity	1.115	−0.773	0.342	−0.782	7.652	0.72
70	YMC-Pack Pro C18 5ìm	0.405	−1.002	0.103	−0.870	8.141	0.76
75	Zorbax SB - Aq	−1.505	3.840	0.076	−1.383	8.724	0.00

(*Continued*)

Table 4. Continued

No.	Column name	k'_{amb}	$rk'_{ba/ph\ pH\ 2.7}$	$k'_{2,2'-dip}$	$rk'_{tri/o-ter}$	F-value	CRF
36	Nucleodur C18 Pyramid	0.249	−1.122	0.312	−0.978	8.898	1.00
25	I nertsil ODS-2	0.550	−1.235	0.191	−0.050	9.035	0.38
50	Prontosil 60-5-C18 H	2.161	−0.404	0.913	−0.663	9.288	0.80
18	Everest	−1.254	−0.829	−0.652	−0.272	9.484	1.00
66	X-Bridge Shield RP18	−0.732	−1.026	−0.304	1.286	10.848	0.00
47	Prontosil 120-5-C18-ace-EPS	0.191	−1.466	−0.011	0.624	10.973	1.00
71	YMC-Pack Pro C18 RS	2.343	−1.098	0.640	−0.699	13.280	0.75
19	Exsil ODS	0.014	5.411	1.563	0.345	15.365	1.00
35	Nucleodur C18 Isis	0.955	−2.031	0.378	0.808	15.795	0.38
28	I nertsil ODS-P	1.093	0.316	3.903	1.980	20.532	1.00
23	HyPURITY Aquastar	−1.311	4.819	0.083	3.078	22.072	1.00
7	Alltima HP C18 Amide	−1.110	−2.704	−0.540	2.060	26.917	0.00
30	LiChrosorb RP-18	0.622	7.165	2.060	0.355	33.036	1.00
44	Prevail Select C18	−0.658	−5.056	−0.287	1.686	50.550	1.00
42	Prevail Amide	−1.232	−5.941	−0.283	0.817	62.197	0.00
39	Platinum C18	−1.231	12.523	−0.141	−0.641	118.586	0.00
22	HyPURITY Advance	−1.657	−12.097	−0.724	0.981	197.087	0.00
40	Platinum EPS C18	−1.340	20.796	0.425	1.118	366.952	0.30
31	LiChrospher 100 RP-18	−0.271	22.884	1.691	0.534	449.623	0.89
60	Supelcosil LC-18	−0.174	34.152	−0.062	−0.590	1050.850	0.72
72	ZirChrom -PS 3um	−1.714	702.116	−0.822	174.051	520875.386	0.00

Bisacodyl

A typical chromatogram for the separation of bisacodyl and its impurities is shown in Figure 4. Also here, a virtual, ideal column was calculated using columns with CRF = 1, after omitting outliers. Based on this virtual column, a ranking was built and the above mentioned ranges were applied. It was observed that the F-values increased much faster, compared to the previous applications, pointing towards the fact that the virtual, ideal column is quite different in comparison with the other columns.

As can be seen from Table 4, only 3 columns with CRF = 1 can be found in the range with F < 2. There is also 1 column with CRF = 0 in this range. In the range 2 < F < 6 and F > 6, 7 and 13 columns with CRF = 1 were found, respectively. Here, the column classification system gives somewhat less good results. However, when checking the columns resulting in a CRF = 0, it was observed that 7 columns were situated in the F > 6 range, compared to 4 and 1 for the 2 < F < 6 and F < 2 ratio, respectively. This is consistent with earlier findings, whereas columns giving poor separations have high F-values and are, hence, found below the table.

CONCLUSION

This paper focuses on the performance of a column selection system when applied to three pharmaceutical separations. For each of them, a virtual, ideal column was calculated as the mean of the parameters of the columns giving sufficient separation after removal of outliers. A CRF value of 1 (baseline separation of all peaks) was considered as a criterion for a perfect separation. All columns were then ranked according to their F-values, based on the difference between their own test parameters and these of the virtual, ideal column. Columns were classified in three arbitrary groups: F < 2, 2 < F < 6 and F > 6.

For amlodipine and tetracaine, it was observed that the highest possibility to find a suitable column was almost 100% for F < 2. These possibilities lowered when selecting columns with 2 < F < 6 (50% and 86%) and F > 6 (25% and 75%). For bisacodyl, the generated ranking was less discriminating. It was observed that the column classification system was helpful in the selection of a suitable column for the separation of amlodipine and tetracaine, but also showed its limitations towards the separation of bisacodyl.

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REFERENCES

1. *European Pharmacopoeia,* 5th edition; Council of Europe: Strasbourg, France, 2005.
2. *United States Pharmacopeia 28*; The United States Pharmacopeial Convention: Rockville, Maryland, USA, 2005.
3. http://extranet.edqm.eu/publications/recherches_sw.shtml (2007).
4. http://www.uspnf.com/uspnf (2007).
5. Marchand, D.H.; Croes, K.; Dolan, J.W.; Snyder, L.R.; Henry, R.A.; Kallury, K.M.R.; Waite, S.; Carr, P.W. Column selectivity in reversed-phase liquid chromatography-VIII. Phenylalkyl and fluoro-substituted columns. J. Chromatogr. A. **2005**, *1062* (1), 65–78.
6. Pellett, J.; Lukulay, P.; Mao, Y.; Bowen, W.; Reed, R.; Ma, M.; Munger, R.C.; Dolan, J.W.; Wrisley, L.; Medwid, K. "Orthogonal" separations for reversed-phase liquid chromatography. J. Chromatogr. A. **2006**, *1101* (1–2), 122–135.
7. Euerby, M.R.; Petersson, P. Chromatographic classification and comparison of commercially available reversed-phase liquid chromatographic columns using principal component analysis. J. Chromatogr. A. **2003**, *994* (1–2), 13–36.
8. Euerby, M.R.; Petersson, P. Chromatographic classification and comparison of commercially available reversed-phase liquid chromatographic columns containing polar embedded groups/amino endcappings using principal component analysis. J. Chromatogr. A. **2005**, *1088* (1–2), 1–15.
9. Euerby, M.R.; Petersson, P.; Campbell, W.; Roe, W. Chromatographic classification and comparison of commercially available reversed-phase liquid chromatographic columns containing phenyl moieties using principal component analysis. J. Chromatogr. A. **2007**, *1154* (1–2), 138–151.
10. Van Gyseghem, E.; Jimidar, M.; Sneyers, R.; Redlich, D.; Verhoeven, E.; Massart, D.L.; Vander Heyden, Y. Selection of reversed-phase liquid chromatographic columns with diverse selectivity towards the potential separation of impurities in drugs. J. Chromatogr. A. **2004**, *1042* (1–2), 69–80.
11. Van Gyseghem, E.; Jimidar, M.; Sneyers, R.; De Smet, M.; Verhoeven, E.; Vander Heyden, Y. Stationary phases in the screening of drug/impurity profiles and in their separation method development: Identification of columns with different and similar selectivities. J. Pharm. Biomed. Anal. **2006**, *41* (3), 751–760.
12. Neue, U.D.; Van Tran, K.; Iraneta, P.C.; Alden, B.A. Characterization of HPLC packings. J. Sep. Sci. **2003**, *26* (3–4), 174–186.
13. Neue, U.D.; O'Gara, J.E.; Méndez, A. Selectivity in reversed-phase separations-Influence of the stationary phase. J. Chromatogr. A. **2006**, *1127* (1–2), 161–174.
14. Baczek, T.; Kaliszan, R.; Novotna, K.; Jandera, P. Comparative characteristics of HPLC columns based on quantitative structure-retention relationships (QSRR) and hydrophobic-subtraction model. J. Chromatogr. A. **2005**, *1075* (1–2), 109–115.
15. Le Mapihan, K.; Vial, J.; Jardy, A. Reversed-phase liquid chromatography column testing: robustness study of the test. J. Chromatogr. A. **2004**, *1061* (2), 149–158.

16. Le Mapihan, K.; Vial, J.; Jardy, A. Reversed-phase liquid chromatography column testing and classification: Physicochemical interpretation based on a wide set of stationary phases. J. Chromatogr. A. **2007**, *1144* (2), 183–186.
17. Forlay-Frick, P.; Fekete, J.; Héberger, K. Classification and replacement test of HPLC systems using principal component analysis. Anal. Chim. Acta. **2005**, *536* (1–2), 71–81.
18. Lesellier, E.; West, C. Combined supercritical fluid chromatographic methods for the characterization of octadecylsiloxane-bonded stationary phases. J. Chromatogr. A. **2007**, *1149* (2), 345–357.
19. Visky, D.; Vander Heyden, Y.; Iványi, T.; Baten, P.; De Beer, J.; Noszál, B.; Roets, E.; Massart, D.L.; Hoogmartens, J. Characterisation of Reversed Phase Liquid Chromatographic Columns by Chromatographic Tests-Preliminary experiments and development of the protocol. Pharmeuropa. **2002**, *14* (2), 288–297.
20. Visky, D.; Vander Heyden, Y.; Iványi, T.; Baten, P.; De Beer, J.; Kovács, Zs.; Noszál, B.; Roets, E.; Massart, D.L.; Hoogmartens, J. Characterisation of reversed-phase liquid chromatographic columns by chromatographic tests. Evaluation of 36 test parameters: repeatability, reproducibility and correlation. J. Chromatogr. A. **2002**, *977* (1), 39–58.
21. Iványi, T.; Vander Heyden, Y.; Visky, D.; Baten, P.; De Beer, J.; Lázár, I.; Massart, D.L.; Roets, E.; Hoogmartens, J. Minimal number of chromatographic test parameters for the characterisation of reversed-phase liquid chromatographic stationary phases. J. Chromatogr. A. **2002**, *954* (1–2), 99–114.
22. Visky, D.; Vander Heyden, Y.; Iványi, T.; Baten, P.; De Beer, J.; Kovács, Z.; Noszál, B.; Dehouck, P.; Roets, E.; Massart, D.L.; Hoogmartens, J. Characterisation of reversed-phase liquid chromatographic columns by chromatographic tests-Rational column classification by a minimal number of column test parameters. J. Chromatogr. A. **2003**, *1012* (1), 11–29.
23. Dehouck, P.; Visky, D.; Van den Bergh, G.; Haghedooren, E.; Adams, E.; Kerner, Á.; Vander Heyden, Y.; Massart, D.L.; Kovács, Zs.; Noszál, B.; Hoogmartens, J. Facilitated column ranking and selection in reversed-phase liquid chromatographic analysis. LC-GC Europe. **2004**, *17* (11), 592–601.
24. Naidong, W.; Hua, S.; Roets, E.; Hoogmartens, J. Assay and purity control of tetracycline by thin layer chromatography. I Qualitative aspects. J. Planar Chromatogr. **1992**, *5* (march/april), 92–98.
25. Morgan, S.L.; Deming, S.N. Optimization strategies for development of gas-liquid-chromatographic methods. J. Chromatogr. **1975**, *112* (OCT29), 267–285.
26. Dehouck, P.; Visky, D.; Vander Heyden, Y.; Adams, E.; Kovács, Z.; Noszál, B.; Massart, D.L.; Hoogmartens, J. Characterisation of reversed-phase liquid-chromatographic columns by chromatographic tests-Comparing column classification based on chromatographic parameters and column performance for the separation of acetylsalicylic acid and related compounds. J. Chromatogr. A. **2004**, *1025* (2), 189–200.
27. Visky, D.; Haghedooren, E.; Dehouck, P.; Kovács, Zs.; Kóczián, K.; Noszál, B.; Hoogmartens, J.; Adams, E. Facilitated column selection in

pharmaceutical analyses using a simple column classification system. J. Chromatogr. A. **2006**, *1101* (1–2), 103–114.
28. Haghedooren, E.; Diana, J.; Noszál, B.; Hoogmartens, J.; Adams, E. Classification of reversed-phase columns based on their selectivity towards vancomycin compounds. Talanta. **2007**, *71* (1), 31–37.
29. Haghedooren, E.; Visky, D.; Dehouck, P.; Kóczián, K.; Diana, J.; Kovács, Zs.; Noszál, B.; Hoogmartens, J.; Adams, E. Facilitated Column Selection in Reversed-Phase Liquid Chromatography for Pharmaceutical Separations. LC-GC Europe. **2007**, *20* (2), 82–96.
30. www.pharm.kuleuven.be/pharmchem/columnclassification (**2007**).
31. Massart, D.L.; Vandeginste, B.G.M.; Buydens, L.M.C.; De Jong, S.; Lewi, P. J.; Smeyers-Verbeke, J. Some Important Hypothesis Tests. In: *Handbook of Chemometrics and Qualimetrics: Part A,* 3rd Ed.; Vandeginste, B.G.M.; Rutan, S.C., Eds.; Elsevier: The Netherlands, 2003, 93.
32. Haghedooren, E.; Kerner, A.; Noszal, B.; Hoogmartens, J.; Adams, E. Application of an improved column characterisation system to evaluate the within and between batch variability. J. Pharm. Biomed. Anal. **2006**, *44* (3), 634–639.
33. *Pharmeuropa*; Council of Europe: Strasbourg, France, 2006; 18, 494.

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