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Column selection for pharmaceutical analyses based on a column classification using four test parameters

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Abstract

This paper focuses on the usability of a previously developed column classification system, applied to pharmaceutical analyses. The separation of two drugs from their respective related substances was investigated on 65 new reversed-phase liquid chromatographic columns. The chromatographic procedure for fluoxetine hydrochloride was performed according to the method prescribed in the European Pharmacopoeia monograph while the separation of gemcitabine hydrochloride was carried out according to the United States Pharmacopeia monograph. It was shown that the column ranking system is a helpful tool in the selection of a suitable column.

Keywords: Chromatographic tests; Column ranking; Fluoxetine; Gemcitabine

1. Introduction

When performing analyses according to official monographs from the European Pharmacopoeia (Ph. Eur.) or the United States Pharmacopeia (USP), analysts are often confronted with the problem of column selection. In general, monographs in the Ph. Eur. only give very general information about the stationary phase to be used in terms of chain length, end-capping, base-deactivation, particle size and sometimes pore size and specific surface. Only for recently developed monographs, more information about the stationary phase can be found on the Ph. Eur. website, under "knowledge database". Even when the brand name of the column is known, this specific column is often not present in the laboratory that wants to perform the analysis. In this case, replacement by a suitable alternative would be helpful.

The selection of columns with similar selectivity could not easily be done based on the prescriptions provided by the official monographs. This can be illustrated by the separation of acetylsalicylic acid (ASA) from its known impurities according to the Ph. Eur. monograph [1]. The monograph prescribes the composition of the mobile phase as acetonitrile–water–phosphoric acid (400:600:2, v/v/v). The stationary phase is described as "a stainless steel column 0.25 m long and 4.6 mm in internal diameter packed with *octadecylsilyl silica gel for chromatography R* (5 μm)". The results shown in Fig. 1 illustrate that stationary phases, belonging to the C_{18} group, do not always result in similar separations. Column A shows baseline separation for all peaks, but columns B and C show co-elution and even change in elution order. Moreover, the situation is not facilitated by the information given by the manufacturers. Based on the information received, comparison between columns of different manufacturers is not always easy. Therefore, a method has been developed to characterise and classify reversed-phase liquid chromatography (RP-LC) columns. The aim was to improve the easiness of finding a column similar to a particular column. The same system would also allow to select a dissimilar column, as needed in orthogonal chromatography.

Many papers describing methods to characterise columns were published, but only the more recent ones are cited here [2–17]. A brief overview of the RP-LC column test parameters

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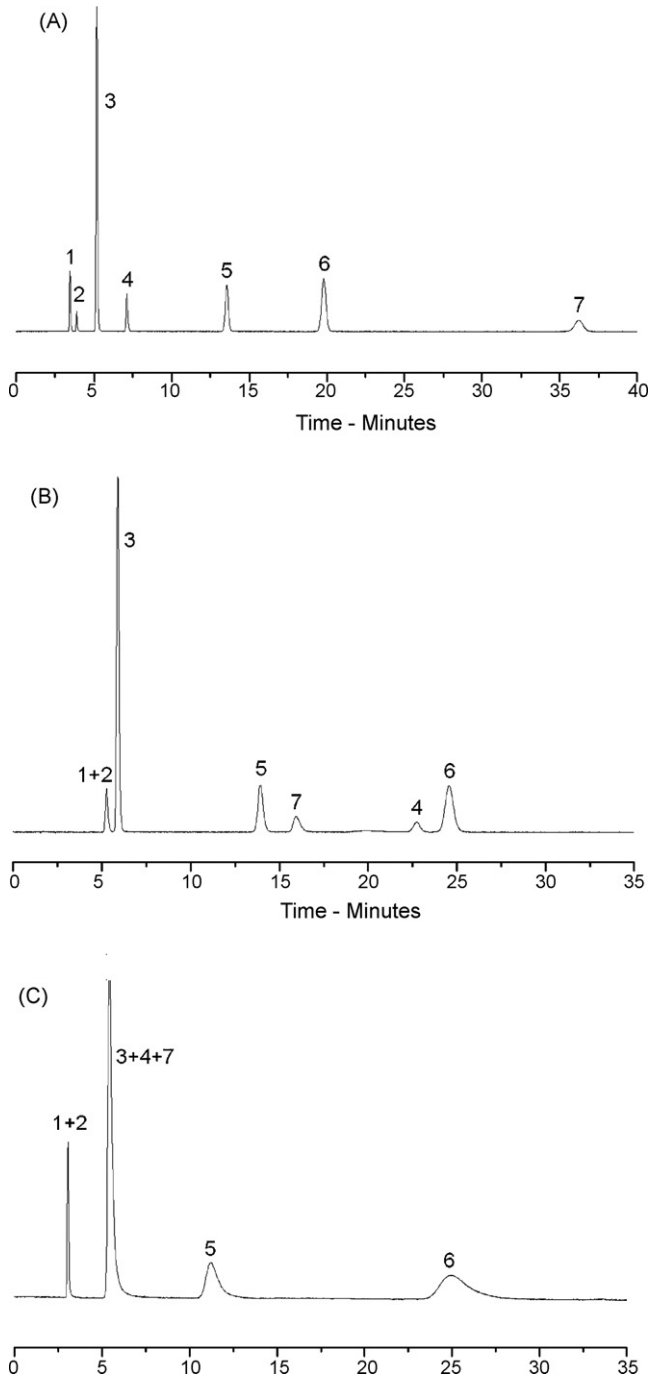

Fig. 1. Separation of ASA (3) and its impurities (1 = 4-OH benzoic acid, 2 = 4-OH isophthalic acid, 4 = salicylic acid, 5 = acetylsalicylsalicylic acid, 6 = salicylsalicylic acid, 7 = acetylsalicylic anhydride). Column (A) Tracerexcel, column (B) Nucleosil Nautilus and column (C) Apex Basic.

used can be found in literature [18]. For the characterisation and classification of the different brands of stationary phases, Principle Component Analysis (PCA) was often used to facilitate data evaluation [8,19–21].

In recent years, a simple chromatographic test procedure has been developed in our laboratory to characterise and classify RP-LC C_{18} columns. The system allows the ranking of C_{18} columns, each characterised by four parameters: the retention factor of amylbenzene, $k'_{\text{amylbenzene}}$ (k'_{amb}), the relative retention factor benzylamine/phenol at pH 2.7, $rk'_{\text{benzylamine/phenol}}$ ($rk'_{\text{ba/pH 2.7}}$), the retention factor of 2,2′-dipyridyl, $k'_{2,2'\text{-dipyridyl}}$ ($k'_{2,2'\text{-dip}}$) and the relative retention factor triphenylene/*o*-terphenyl, $rk'_{\text{triphenylene/}o\text{-terphenyl}}$ ($rk'_{\text{tri/ter}}$). PCA, as a useful chemometric tool, was used to visualise and interpret the data [18,22–24]. Next, a ranking system based on *F*-values was introduced as a considerable simplification of a four-dimensional space into a single one [25]. The initial step of this approach is the selection of four reference parameters, corresponding to a freely chosen reference column. The *F*-value for a column *i* is calculated as

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

The *F*-value of a column *i* equals the sum of squares of the differences between each parameter value of the reference column and of a column *i*. The smaller the *F*-value, the more similar is column *i* to the reference column and the higher is column *i* found in the ranking (high ranked columns). Before being introduced in Eq. (1), the parameters are autoscaled:

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

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

Of course, an important test of the characterisation method is to verify whether columns having similar parameters give similar separations in practice. In previous studies, the separation of ASA, clindamycin hydrochloride, buflomedil hydrochloride, chloramphenicol sodium succinate, nimesulide, phenoxymethylpenicillin, dihydrostreptomycin sulphate and vancomycin from their respective impurities was investigated [26–29]. After testing 69 columns, a nice relationship was demonstrated between the ranking of the columns and the selectivity in the separations of the pharmaceuticals and it was concluded that the column classification system can help analysts in the selection of a suitable RP-LC C_{18} column. The parameters of the columns used are freely accessible on a website [30], where anyone can freely define a reference column or reference parameters and a ranking of the columns is easily obtained using the *F*-values.

In order to evaluate the separation on the stationary phases, the chromatographic response function (CRF), which is a measure for the overall selectivity, was applied [31]. The CRF is calculated as

$$\text{CRF} = \prod_{i=1}^{n-1} \frac{f_i}{g_i} \quad (3)$$

where *n* is the total number of solutes, *g* the interpolated peak height for each peak pair, i.e. the distance between the baseline and the line connecting the two peak tops, at the location of the

valley and *f* is the depth of the valley, measured from the line connecting the two peak tops [26,32]. It follows that a baseline separated peak pair has an *f*/*g* ratio of 1.00, a non-separated pair has a value of 0.00 and in case of partial co-elution, an intermediate value is obtained. Columns with CRF = 1.00 show baseline separation for all peaks, but this does not mean that the separation is identical or column properties are exactly the same. It only indicates that these columns are suitable for that separation. In this paper, it was investigated whether the column ranking with the *F*-values could be successfully applied to a new set of columns. Two separations were examined on 65 RP-LC C_{18} stationary phases. The separation of fluoxetine from its related substances uses isocratic elution, while gemcitabine requires a gradient mode. Earlier, a virtual, ideal column was calculated as an average value of all columns with a good separation (CRF = 1) after omitting outliers, that were detected with the Grubbs test [27–29].

However, in practice, an analyst does not have the possibility to test a set of 65 columns and to deduce a virtual, ideal column. Even during method development, usually only a few columns are tested. From that point of view, the developed column classification system will be investigated by using a single column showing a good separation (CRF = 1) as reference column, instead of a virtual, ideal column.

Moreover, it was checked whether the selectivity and equivalency between stationary phases for a given separation could be evaluated based on the correlation of the retention times obtained on two columns.

2. Experimental

2.1. Chromatographic tests and tested columns

General information concerning the column test methods resulting in the four final parameters was published earlier [22–24]. Compared to the originally proposed chromatographic methods, some of the conditions were slightly adapted to obtain a faster elution of 2,2′-dipyridyl and a more consistent determination of the dead volume [33]. For the present analysis, 65 new C_{18} columns were used (Table 1).

2.2. Samples and reagents

Fluoxetine hydrochloride, (1*RS*)-3-(methylamino)-1-phenylpropan-1-ol (fluoxetine impurity A), *N*-methyl-3-phenylpropan-1-amine (fluoxetine impurity B), (3*RS*)-*N*-methyl-3-phenyl-3-[3-(trifluoromethyl)phenoxy]propan-1-amine (fluoxetine impurity C) and 4-trifluoromethylphenol were generous gifts from E. Lilly (Lilly Corporate Center, Indianapolis, IN, USA). Gemcitabine hydrochloride and cytosine were also obtained from E. Lilly. The gemcitabine α-anomer was prepared according to the system suitability solution in the corresponding USP monograph using 10 h conversion time [34].

All solvents and reagents were of Ph. Eur. quality. Methanol (Prolabo, Paris, France) was of LC grade, other chemicals of AR grade. Triethylamine and tetrahydrofuran were purchased from Acros Organics (Geel, Belgium). Sodium dihydrogen phosphate and potassium hydroxide were from Fluka (Buchs, Switzerland) and phosphoric acid from Sigma–Aldrich (Seelze, Germany). Water was distilled and purified (Milli-Q50, Millipore, Billerica, MA, USA) before use.

2.3. 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 ChromPerfect 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 chromatographic procedure for fluoxetine hydrochloride was performed according to the method prescribed in the Ph. Eur. monograph [35]. The separation of gemcitabine hydrochloride was carried out according to the USP monograph [34]. The chromatographic conditions given in the monographs may be adjusted when necessary to reach the SST limits. As the aim of this study was to compare the behaviour of different types of RP-LC C_{18} columns in the same chromatographic conditions, neither the mobile phase composition nor other chromatographic parameters were adapted.

The nomenclature of the Ph. Eur. was used. Since the elution order of the peaks could be changed on different stationary phases, it was desirable to have peaks with different areas for each component to facilitate peak identification. Therefore, a spiked sample had been prepared wherein the concentrations of the related substances available as reference compounds were varied.

The used chromatographic conditions are summarised below.

2.3.1. Analysis of fluoxetine hydrochloride

The Ph. Eur. method prescribes a C_8 column as stationary phase [35]. The separations on Zorbax SB C_8 column and on Zorbax SB C_{18} were found to be intrinsically the same, thus the method was considered to be applicable on C_{18} columns. The mobile phase was a mixture of 8 volumes of methanol, 30 volumes of tetrahydrofuran and 62 volumes of triethylammonium phosphate buffer. The buffer was prepared by adding 980 ml of water to 10 ml of triethylamine, adjusting this mixture to pH 6.0 with phosphoric acid, and finally diluting it to 1000 ml with water.

The flow rate was 1.0 ml/min and the columns were equilibrated for 30–60 min, dependent on their length. The sample consisted of 0.55 mg/ml of fluoxetine hydrochloride, 0.05 mg/ml of fluoxetine impurity B and 0.02 mg/ml each of fluoxetine impurity A, fluoxetine impurity C and 4-trifluoromethylphenol. The injection volume was 10 μl, the detector was set at 215 nm. The column was kept at 30.0 °C. Helium was used to degas the mobile phase.

On each column, the separation was performed in triplicate.

2.3.2. Analysis of gemcitabine hydrochloride

The USP method was applied with some slight modifications since the mixing of a pure organic solvent with an aqueous solution could cause problems. So, premixed eluents were prepared

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

Column number	Name of the column	Length (mm)	Internal diameter (mm)	Particle size (μm)	Pore size (Å)	Manufacturer/supplier
1	Acclaim 3 μm	150	4.6	3	300	Dionex
2	Acclaim 5 μm	250	4.6	5	120	Dionex
3	ACE 5 C18	250	4.6	5	100	Achrom
4	Alltima AQ	250	4.6	5	100	Alltech
5	Alltima C18	250	4.6	5	117	Alltech
6	Alltima HP C18	250	4.6	5	100	Alltech
7	Alltima HP C18 Amide	250	4.6	5	100	Alltech
8	Brava BDS C18	250	4.6	5	145	Alltech
9	Capcell Pak C18 ACR	250	4.6	5	80	Shiseido Fine Chemicals
10	Capcell Pak C18 AQ	250	4.6	5	80	Shiseido Fine Chemicals
11	Capcell Pak C18 MG	250	4.6	5	90	Shiseido Fine Chemicals
12	Capcell Pak C18 UG120	250	4.6	5	120	Shiseido Fine Chemicals
13	Chromolith Performance	100	4.6	–	20000/130[a]	Merck
14	Discovery C18	250	4.6	5	180	Supelco
15	Discovery HS C18	250	4.6	5	120	Supelco
16	Exsil ODS 5 μm	250	4.6	5	80	SGE
17	Hamilton Hx Sil 18	250	4.6	5	312	Hamilton
18	Hydrospher C18	250	4.0	5	120	YMC
19	HyPURITY Advance	250	4.6	5	190	Thermo Electron Corp.
20	HyPURITY Aquastar	250	4.6	5	190	Thermo Electron Corp.
21	HyPURITY C18	250	4.6	5	190	Thermo Electron Corp.
22	Inertsil ODS-2	250	4.6	5	150	GL Sciences Inc.
23	Inertsil ODS-3	250	4.6	5	100	GL Sciences Inc.
24	Inertsil ODS-80A	250	4.6	5	80	GL Sciences Inc.
25	Inertsil ODS-P	250	4.6	5	100	GL Sciences Inc.
26	Kromasil KR100-5C18	250	4.6	5	100	EKA Chemicals
27	LiChrosorb RP-18	250	4.6	5	100	Merck
28	LiChrospher 100 RP-18	250	4.6	5	100	Merck
29	MP-Gel ODS-5	250	4.0	5	120	YMC/OmniChrom
30	Omnispher 5 C18	250	4.6	5	110	Varian
31	Platinum C18	250	4.6	5	100	Alltech
32	Platinum EPS C18	250	4.6	5	100	Alltech
33	Polaris 5 μm C18-A	250	4.6	5	180	Varian
34	Prevail Amide	250	4.6	5	190	Alltech
35	Prevail C18	250	4.6	5	110	Alltech
36	Prevail Select C18	250	4.6	5	120	Alltech
37	Prontosil 120 5 C18 AQ	250	4.6	5	120	Bischoff
38	Prontosil 120 5 C18 AQ PLUS	250	4.6	5	120	Bischoff
39	Prontosil 120 5 C18 ace EPS	250	4.6	5	120	Bischoff
40	Prontosil 120 5 C18 H	250	4.6	5	120	Bischoff
41	Prontosil 120 5 C18 SH	250	4.6	5	120	Bischoff
42	Prontosil 60 5 C18H	250	4.6	5	60	Bischoff
43	PurospherRP-18e	250	4.6	5	90	Merck
44	Purospher Star RP-18	250	4.6	5	120	Merck
45	Pursuit 5 C18	250	4.6	5	180	Varian
46	Restek Allure C18	250	4.6	5	60	Restek
47	Restek Pinnacle DB C18	250	4.6	5	140	Restek
48	Restek Pinnacle II C18	250	4.6	5	110	Restek
49	Restek Ultra C18	250	4.6	5	100	Restek
50	Supelcosil LC-18	250	4.6	5	100	Supelco
51	Supelcosil LC-18 DB	250	4.6	5	100	Supelco
52	Superspher 100 RP-18	250	4.6	5	100	Merck
53	Uptisphere 5 HDO-25QS	250	4.6	5	120	Interchrom/Achrom
54	Uptisphere 5 ODB-25QS	250	4.6	5	120	Interchrom/Achrom
55	Wakosil II 5 C18 RS	250	4.6	5	120	SGE
56	Xterra MS C18	250	4.6	5	125	Waters
57	Xterra RP C18	250	4.6	5	125	Waters
58	YMC-Pack Pro 3 C18	250	4.6	3	120	YMC
59	YMC-Pack Pro 5 C18	250	4.6	5	120	YMC
60	YMC-Pack Pro C18 RS	250	4.6	5	80	YMC
61	ZirChrom PS 3 μm	150	4.6	3	300	ZirChrom
62	Zorbax Eclipse XDB C18	250	4.6	5	80	Agilent
63	Zorbax Extend C18	250	4.6	5	80	Agilent
64	Zorbax SB Aq	250	4.6	5	80	Agilent
65	Zorbax SB C18	250	4.6	5	80	Agilent

[a] Macropores/mesopores.

as follows: eluent A consisted of 97% of solution A (filtered and degassed solution containing 13.8 g of sodium dihydrogen phosphate and 2.5 ml of phosphoric acid in 1000 ml of water) and 3% of solution B (filtered and degassed methanol). Eluent B was pure methanol. The pH of solution A was checked to be 2.45–2.55 (2.4–2.6 was prescribed). The gradient program was adapted as follows: 0–8 min: 100% eluent A; 8–13 min: from 100% eluent A to 50% eluent A; 13–20 min: 50% eluent A; 20–25 min: from 50% eluent A to 100% eluent A. Before each injection, 15 min re-equilibration time was maintained.

The flow rate was kept at 1.2 ml/min. The sample was prepared as prescribed for the system suitability solution. About 10 mg of gemcitabine hydrochloride is transferred to a small vial, a solution containing 168 mg of potassium hydroxide per milliliter of methanol is added and the vial is capped tightly and sonicated. Then, the mixture was heated at 55 °C for 10 h, cooled down and transferred to a 100 ml volumetric flask with successive washes of 1% (v/v) phosphoric acid. After this, the solution is diluted with 1% (v/v) phosphoric acid and mixed to obtain about 0.02 mg/ml of gemcitabine α-anomer. Finally 1.0 mg/ml cytosine was added to the sample. When stored between 2 and 8 °C, this solution showed no degradation. The injection volume was 20 μl. The column was kept in a water bath at 25.0 °C and the detector was set at 275 nm. For each column, three runs were carried out.

3. Results and discussion

3.1. Column selection in pharmaceutical separations

Fluoxetine hydrochloride and gemcitabine hydrochloride analyses were performed on 65 new RP-LC C_{18} stationary phases according to methods prescribed in official compendia. Both monographs exactly prescribe the chromatographic procedure (mobile phase, flow rate, column temperature, detector wavelength), but only vague information is given about the type of the stationary phase has to be used. The monographs prescribe a stainless steel column of 0.25 m long and 4.6 mm in internal diameter packed with octylsilyl and octadecylsilyl silica gel for chromatography R (5 μm) for fluoxetine and gemcitabine, respectively. Although Chromolith Performance (no. 13), a monolithic column and Zirchrom (no. 61) with zirconium backbone do not meet these Ph. Eur. requirements, results for both columns are presented. Analysts have the freedom to select a suitable column, but their choice is often limited by the availability of the columns in their laboratories. Once a chromatographer has selected a column, it has to be checked for compliance with regard to the System Suitability Test (SST) requirements. For fluoxetine, the SST requires a maximum h/v ratio of 1.1 for the "critical pair" of impurity C and fluoxetine (where h is the distance between the top of the peak due to impurity C and baseline and v is the distance between the top of the peak due to impurity C and the lowest point of the valley between the peak due to impurity C and the peak due to fluoxetine), a retention time between 10 and 18 min for fluoxetine and a maximum retention time of 35 min for 4-trifluoromethylphenol.

In the case of gemcitabine, the SST demands a minimum resolution of 8.0 between the gemcitabine α-anomer and gemcitabine and a maximum tailing factor of 1.5 for the peak corresponding to gemcitabine. The SST also mentions relative retention times for the two impurities compared to the main compound. Problems with the use of relative retention times have been discussed elsewhere [37] and therefore this parameter was not considered as adequate to decide on the quality of a separation.

According to the monographs, only those columns, complying with the SST, are allowed to be used for the separation. It will be checked whether all these columns indeed give sufficient separation and whether other columns, not compliant with the SST, may also give good results.

The CRF was introduced as a criterion to evaluate the quality of the separations [26]. Although the CRF was a helpful tool to evaluate the separations in this study, it is difficult to prescribe it in practice as a SST, as the CRF requires the exact location of potential impurities in the chromatogram, which is often not possible in daily practice.

3.2. Column examination

After applying a separation onto all 65 columns, for each column the CRF value was calculated. All columns, giving a baseline separation of all peaks for a given separation (CRF = 1) were grouped and a double-sided single Grubb's test was performed onto the data to trace and remove outliers [36]. This test was applied on each of the four parameters (k'_{amb}, $rk'_{ba/pH\,2.7}$, $k'_{2,2'\text{-dip}}$ and $rk'_{tri/ter}$) and columns with an outlying value for one of the parameters were considered as outlier. Then, ideal column parameters were calculated by taking the average of the parameters of the remaining columns with a CRF = 1. With this virtual column as a reference, F-values were calculated and a ranking of all columns was made. The columns with $F < 2$ were considered as high ranked, columns with $F > 6$ as low ranked and columns with $2 < F < 6$ as intermediate. The probability to find a suitable column should be the highest in the range of $F < 2$. This probability should decrease for $2 < F < 6$ and be the lowest for $F > 6$. Similar columns could be preferably selected from $F < 2$ and orthogonal columns would be most likely found in the range of columns with $F > 6$. This approach has given already nice results, proving that the column classification system is a helpful tool in the selection of a suitable column [26–28].

When an analyst performs a certain routine analysis, it is not possible for him/her to test 65 different liquid chromatographic columns, then determine all CRF values and finally deduce a virtual, reference column. Even during method development, only a limited number of columns is tested. Therefore, the development column is not always the best choice for a reference column, as was published earlier [27]. Moreover, this column could have special properties and could differ substantially from other columns suitable for that separation.

Another possibility is to use a column that gives CRF = 1 for the separation studied as reference column. This approach will now be investigated and evaluated. When only one single column was used as reference, an alternative for the CRF value

can be presented by plotting the retention times of the separated compounds determined on the reference column plotted versus the retention times observed on another column. The correlation between the retention times (expressed as retention factors k) obtained on both columns could be investigated by calculating the coefficient of determination, resulting in a value between 0 and 1. The closer the value is to 1, the more similar the two columns are for that particular separation. A similar approach to compare the selectivity and equivalency of columns was used by Dolan and co-workers by means of log–log plots of the retention factor (k) for one column versus the other for a given separation. If there is a linear correlation of $\log k$ values with no deviation of data points (standard deviation, S.D. = 0), the two columns are said to correlate perfectly, i.e., the two columns could be regarded as equivalent in terms of selectivity [3].

3.3. Separation of fluoxetine from its related substances

A typical chromatogram of a fluoxetine separation is presented in Fig. 2. The values in Table 2 that did not comply to a SST, are indicated in bold. As a conclusion for the SST, two symbols were used: +, when the column complied to all requested SST, and −, when the column failed for at least one SST.

As the analysis involves the separation of two critical peak pairs (fluoxetine impurity A–fluoxetine impurity B and fluoxetine impurity C–fluoxetine), only six columns gave a CRF of 1.00. The Grubbs' test was applied onto these six columns and two columns, Alltima C18 (no. 5) and Capcell Pack AQ (no. 10) were found to be outliers. Then, the averages of the parameters of the remaining four columns with a CRF value of 1 were calculated and used as reference values, representing a virtual, ideal column for fluoxetine, in the ranking system. As before, columns were classified in three groups: high ranked columns ($F < 2$), intermediate columns ($2 < F < 6$) and low ranked columns ($F > 6$) [25]. All columns with CRF = 1 are situated in the group of columns with $F < 2$. It should be noted that for this separation, all aqua columns from our dataset proved to be suitable. The

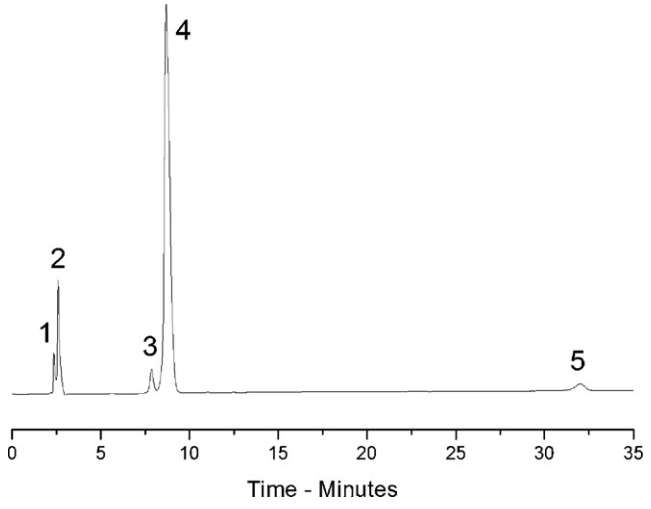

Fig. 2. Separation of fluoxetine hydrochloride and its impurities. 1 = impurity A, 2 = impurity B, 3 = impurity C, 4 = fluoxetine and 5 = 4-trifluoromethylphenol. Column: Discovery HS (CRF = 0.82).

Alltima AQ (no. 4), Capcell Pak AQ (no. 10) and Prontosil 120 5 C_{18} AQ (no. 37) column are all synthesised with polar endcapping. It was observed that the zirconia (Zirchrom, no. 61) and monolithic column (Chromolith Performance, no. 13) are not suitable for this analysis because of partial and full co-elution (impurities A and B), respectively. Since monolithic stationary phases are described to give faster elution, the separation of fluoxetine on the Chromolith Performance was apparently too fast, resulting in co-elution. It must be noticed that no changes in chromatographic conditions were performed to optimise the separation.

It can be observed from Fig. 2, that a CRF of 0.82 still gives a very acceptable separation. Therefore, the criterion of an acceptable separation could also be set at CRF > 0.80. In the range of $F < 2$ range, 31 of 36 columns (86%) show a CRF > 0.80, i.e. they give a sufficient separation between fluoxetine and all its related substances. For columns with $2 < F < 6$, the chance to find a suitable one is 16/24 (67%). Only two of the five (40%) columns with $F > 6$ complied (Table 2).

Although many columns give a high CRF value, eight columns only comply with all SST requirements and the $F < 2$ range includes six of the eight SST compliant columns. When checking the h/v ratio, 26 out of 36 columns of the high ranked columns (with $F < 2$) comply, whereas only 13 out of 24 columns with $2 < F < 6$ have a ratio h/v under 1.1. The lowest compliance (one out of five columns) with h/v could be seen in the group with $F > 6$. For the retention time of fluoxetine, 14 out of 36 columns with $F < 2$ comply. For columns with $2 < F < 6$ and $F > 6$, only 5 out of 24 and 1 out of 5 columns comply, respectively. Most columns have a retention time for 4-trifluoromethylphenol lower than 35. Except for Platinum EPS C_{18} (column no. 32, CRF = 0), all SST compliant columns provide a CRF value higher than 0.92.

The correlation between CRF > 0.8 and a complying SST was examined. After checking all columns, two possible situations were considered: either the chance to find a complying SST in case of a suitable separation (CRF > 0.8), or the probability to find a non-complying SST when a poor separation is encountered. When selecting all good separations, only 7 out of 49 columns complied with the requested SST. For poor separations, 15 of the 16 columns had a non-complying SST. This implies that 42 different RP-LC columns that gave a good separation, cannot be used in analyses due to a non-complying SST. This is an indication that the SST prescribed by the Ph. Eur. does not always provide relevant information.

Selection of a single reference column instead of a virtual, ideal column, obviously should be made from the group of columns with CRF = 1. To choose between these six columns, an additional criterion should be found. This could be the column efficiency. Column efficiency provides a measure of how peaks broaden while they pass through a chromatographic column. It is function of particle size and shape, viscosity of the stationary phase, diffusion coefficients of the analyte in the mobile and stationary phases, solvent viscosity, flow rate, and uniformity of the packing material. Since the advent of HPLC, column efficiency has been an important column parameter [38]. Euerby and Petersson also implemented efficiency in their column

Table 2
Column ranking obtained with the *F*-values, relative to the mean parameter values (k'_{amb} : 4.37, $rk'_{ba/pH\,2.7}$: 0.11, $k'_{2,2'\text{-d}}$: 15.29, $rk'_{tri/ter}$: 1.87) for the separation of fluoxetine

No.	Column name	k'_{ab}	$rk'_{ba/pH}$	k'_{dip}	$rk_{tri/o\text{-}ter}$	*F*-value	CRF	h/v	Rt Fluox	Rt 4-trifluoro	SST
17	Hamilton Hx Sil C18	0.348	−0.128	0.406	−0.136	0.440	0.95	1.01	**8.87**	33.17	–
29	MP Gel ODS-5	0.247	−0.144	0.255	−0.106	0.475	0.88	1.09	**9.09**	23.96	–
38	Prontosil 120 C18 AQ PLUS	0.289	−0.153	1.295	−0.105	0.497	0.96	**1.46**	11.41	30.59	–
10	Capcell Pak AQ	−0.536	−0.153	0.164	−0.150	0.504	1.00	1.02	11.43	31.61	+
41	Prontosil 120 5C18 SH	0.410	−0.130	0.351	−0.137	0.556	0.93	1.07	10.12	29.63	+
16	Exsil ODS 5 μm	−0.120	−0.085	1.614	−0.102	0.698	0.83	**7.08**	14.13	**18.83**	–
4	Alltima AQ	−0.340	−0.126	1.698	−0.035	0.862	1.00	1.06	16.56	27.36	+
44	Purospher Star RP-18	0.655	−0.142	0.342	−0.125	0.919	0.86	1.02	**8.90**	34.45	–
5	Alltima C18	0.760	−0.134	0.865	−0.127	0.919	1.00	1.00	12.31	32.20	+
55	Wakosil II 5 C18 RS	0.333	−0.148	−0.034	−0.166	0.952	0.93	1.00	10.37	**35.33**	–
37	Prontosil 120 5 C18 AQ	−0.106	−0.125	−0.189	−0.156	0.957	1.00	1.07	**18.32**	33.05	–
53	Uptisphere 5 HDO-25QS	0.387	−0.133	−0.016	−0.160	0.981	0.90	1.00	**8.71**	**35.97**	–
65	Zorbax SB C18	−0.160	−0.113	−0.236	−0.159	1.044	0.97	**1.14**	10.88	19.82	–
40	Prontosil 120 5 C18 H	−0.065	−0.129	−0.261	−0.144	1.111	1.00	1.05	**21.15**	28.46	–
27	LiChrosorb RP-18	−0.425	0.116	1.784	−0.093	1.114	0.87	**4.05**	14.29	20.54	–
18	Hydrosphere C18	−0.197	−0.155	−0.297	−0.168	1.173	0.90	1.07	**9.17**	26.60	–
35	Prevail C18	−0.269	−0.130	1.885	−0.041	1.222	1.00	1.05	15.42	28.55	+
58	YMC-Pack Pro 3 C18	0.570	−0.151	−0.079	−0.158	1.334	0.98	1.00	10.88	**36.39**	–
52	Superspher 100 RP-18	0.692	−0.122	0.025	−0.123	1.364	0.90	1.05	11.23	**35.98**	–
11	Capcell Pak MG	0.713	−0.135	0.041	−0.158	1.380	0.81	1.00	**7.50**	33.98	–
54	Uptisphere 5 ODB-25QS	0.595	−0.133	−0.097	−0.149	1.402	0.87	1.03	**8.01**	32.43	–
22	Inertsil ODS-2	0.452	−0.161	−0.215	−0.120	1.417	0.92	1.00	10.87	27.61	+
9	Capcell Pak ACR	0.403	−0.144	−0.252	−0.130	1.432	0.84	1.07	**7.11**	25.88	–
59	YMC-Pack Pro 5 C18	0.298	−0.159	−0.333	−0.158	1.493	0.83	1.10	**8.22**	25.71	–
56	Xterra MS C18	−0.404	−0.134	−0.432	−0.170	1.526	0.00	**1.15**	**6.53**	22.97	–
48	Restek Pinnacle II C18	0.170	−0.114	−0.398	−0.136	1.531	0.89	1.04	**8.86**	25.09	–
43	Purospher RP-18e	1.044	−0.147	0.990	−0.100	1.579	0.62	**1.14**	**8.64**	30.40	–
39	Prontosil 120 ace EPS	0.069	−0.164	−0.485	−0.089	1.679	0.92	1.03	**8.77**	**42.71**	–
30	Omnispher 5 C18	0.680	−0.134	−0.204	−0.117	1.741	0.86	1.03	**8.29**	28.10	–
62	Zorbax Eclipse XDB C18	0.353	−0.136	−0.422	−0.158	1.758	0.88	**1.13**	**8.02**	21.87	–
12	Capcell Pak UG120	−0.067	−0.150	−0.551	−0.155	1.799	0.78	1.03	**7.19**	23.17	–
15	Discovery HS C18	0.815	−0.137	−0.160	−0.134	1.910	0.82	**1.15**	**8.82**	31.99	–
50	Supelcosil LC-18	−0.321	0.245	−0.553	−0.145	1.944	0.00	∞	16.08	21.66	–
23	Inertsil ODS-3	1.169	−0.154	0.461	−0.155	1.970	0.84	**1.03**	**7.49**	**37.74**	–
2	Acclaim 5 μm	0.984	−0.133	0.024	−0.150	1.971	0.85	**1.01**	**8.08**	34.82	–
63	Zorbax Extend C18	0.649	−0.145	−0.343	−0.137	1.984	0.67	**1.15**	**5.41**	21.14	–
51	Supelcosil LC-8DB	−0.579	−0.100	−0.593	−0.154	2.044	0.96	**1.66**	**9.10**	22.64	–
45	Pursuit 5 C18	−0.375	−0.133	−0.672	−0.150	2.152	0.83	1.03	**8.46**	26.13	–
47	Restek Pinnacle DB C18	−0.354	−0.111	−0.684	−0.134	2.180	0.83	1.02	**7.60**	21.31	–
3	ACE 5 C18	−0.248	−0.129	−0.706	−0.133	2.221	0.90	1.03	**8.59**	23.27	–
8	Brava BDS C18	−0.984	−0.109	−0.503	−0.127	2.278	0.96	**1.11**	**9.63**	18.90	–
26	Kromasil KR100-5C18	1.163	−0.129	0.047	−0.131	2.387	0.85	1.03	**7.65**	33.01	–
32	Platinum EPS C18	−1.568	0.092	0.096	−0.066	2.405	0.00	1.03	12.28	15.15	+
28	LiChrospher 100 RP-18	0.529	−0.065	2.277	−0.101	2.758	0.98	**1.13**	13.31	27.57	–
14	Discovery C18	−0.669	−0.135	−0.827	−0.138	2.820	0.82	1.02	**8.22**	22.10	–
49	Restek Ultra C18	1.348	−0.136	0.101	−0.131	2.849	0.88	1.02	**7.64**	31.90	–
6	Alltima HP C18	−0.770	−0.128	−0.827	−0.152	2.926	0.83	1.01	**8.07**	21.71	–
33	Polaris 5 μm C18-A	−0.813	−0.134	−0.828	−0.112	2.978	0.85	1.03	**8.20**	23.06	–
36	Prevail Select C18	−0.838	−0.205	−0.852	−0.040	3.098	0.91	1.03	**7.56**	26.98	–
20	HyPURITY Aquastar	−1.537	−0.092	−0.360	0.025	3.122	0.99	1.01	10.29	16.19	+
21	HyPURITY C18	−0.807	−0.132	−0.900	−0.130	3.209	0.86	1.00	**8.42**	21.11	–
24	Inertsil ODS-80A	1.630	−0.151	0.853	−0.154	3.339	0.69	**1.18**	**6.33**	31.60	–
13	Chromolith Performance	−1.117	−0.144	−0.851	−0.137	3.523	0.29	**7.55**	**3.03**	6.67	–
31	Platinum C18	−1.451	−0.003	−0.659	−0.147	3.676	0.82	**1.12**	14.41	16.03	–
64	Zorbax SB Aq	−1.744	−0.103	−0.369	−0.182	3.734	0.00	∞	12.40	19.74	–
57	Xterra RP C18	−1.107	−0.146	−0.949	−0.093	3.831	0.56	**1.16**	**6.59**	24.68	–
34	Prevail Amide	−1.452	−0.215	−0.848	−0.080	4.250	0.95	1.00	**9.60**	31.89	–
1	Acclaim 3 μm	−1.252	−0.124	−1.117	−0.155	4.734	0.00	**1.61**	**4.15**	10.62	–
7	Alltima HPAmide	−1.321	−0.178	−1.191	−0.023	5.172	0.71	**1.22**	**6.98**	19.62	–
42	Prontosil 60 5 C18 H	2.173	−0.152	0.748	−0.149	5.614	0.74	**5.00**	11.51	24.43	–
60	YMC-Pack Pro RS	2.368	−0.160	0.382	−0.150	6.734	0.74	**1.25**	**6.31**	24.36	–
46	Restek Allure C18	2.543	−0.140	0.858	−0.136	7.505	0.88	**1.13**	**8.45**	35.14	–
19	HyPURITY Advance	−1.906	−0.286	−1.435	−0.072	7.875	0.74	**1.21**	**6.66**	18.28	–
25	Inertsil ODS-P	1.032	−0.144	4.734	−0.026	17.121	0.97	**1.39**	11.98	28.80	–
61	Zirchrom PS 3 μm	−1.968	7.918	−1.566	7.932	137.803	0.00	1.00	**54.39**	**352.00**	–

Table 3
Fluoxetine separation

		Reference column						
		Alltima AQ ($N=3770$)	Prevail C18 ($N=3506$)	Alltima C18 ($N=3474$)	Capcell Pak AQ ($N=2824$)	Prontosil 120 5 C18 H ($N=2486$)	Prontosil 120 5 C18 AQ ($N=2127$)	Xterra RP ($N=2874$)
CRF > 0.8	$F<2$	7/7 (100.0%)	6/6 (100.0%)	27/30 (90.0%)	37/45 (82.2%)	36/42 (85.7%)	36/42 (85.7%)	20/30 (66.7%)
	$2<F<6$	33/40 (82.5%)	28/34(82.4%)	18/23(78.3%)	10/15(66.7%)	10/17 (58.8%)	8/17 (47.1%)	17/18 (94.4%)
	$F>6$	9/18 (50.0%)	15/25 (60%)	4/12 (33.3%)	2/5 (40.0%)	3/6 (50.0%)	3/6 (50.0%)	12/17 (70.6%)
av R^2 log k	$F<2$	0.994	0.995	0.989	0.988	*0.940*	0.970	*0.971*
	$2<F<6$	0.964	0.972	0.988	0.982	*0.948*	0.968	*0.995*
	$F>6$	0.938	0.958	0.953	0.950	*0.893*	0.928	*0.965*
av R^2k	$F<2$	*0.966*	*0.972*	*0.965*	*0.958*	*0.730*	*0.897*	*0.908*
	$2<F<6$	*0.866*	*0.901*	*0.971*	*0.952*	*0.810*	*0.904*	*0.994*
	$F>6$	*0.871*	*0.923*	*0.912*	*0.977*	*0.739*	*0.902*	*0.927*

Overview of the number of suitable columns (CRF > 0.8) within three different ranges ($F<2$, $2<F<6$ and $F>6$). For comparison coefficient of determination (k, log k) results were added.

classification system by measuring the amount of theoretical plates of *n*-pentylbenzene [39,40].

The results of column classification using each of the six columns with CRF = 1 as the reference column can be seen in Table 3.

In Table 3, a distinction can be made based on the efficiency. Columns with higher efficiency, used as reference column, result in classifications where the probability is higher to find, within the $F<2$ range, a column providing an acceptable separation. For five out of the six columns, this probability lowers for $2<F<6$ and is the lowest for $F>6$, which confirms the earlier seen tendency.

As already explained, not only CRF values, but also coefficients of determination (CoD) were considered to evaluate a given separation on different columns.

The coefficient of determination (CoD) was determined using the k values and the log k values calculated for each peak. Van Gyseghem and co-workers preferred the use of k values over log k values since the investigated data focused on low retention times and no extreme differences were noticed between the HPLC systems [41]. On the other hand, Dolan and co-authors used the log k values [3]. So, both CoDs were calculated here and the average value of the R^2 values for $F<2$, $2<F<6$ and $F>6$ were checked for a decreased tendency. Only when using log k values (see Table 3), a tendency could be seen according to that observed above with the CRF values.

It was checked whether for a column with a CRF value $\neq 1$ as the reference column, the ranking shows a less clear tendency. XTerra RP (CRF = 0.56) was chosen (see Table 3) as an example and nor the CRF, neither the CoD gave good information. This is an indication that the parameter efficiency can be helpful in selecting a good reference column.

Table 4 shows the detailed classification with an Alltima AQ as reference column, having the highest efficiency ($N=3770$).

3.4. Separation of gemcitabine from its related compounds

The separation of gemcitabine from its related compounds requires gradient elution. A typical chromatogram is shown in Fig. 3. The following columns did not fulfil the SST criteria: columns 4, 7, 13, 19, 25, 27, 28, 31, 32, 34, 36, 50, 57, 61 and 64. Although none of these columns were suitable according to the SST, only columns 19, 32 and 61 did not give baseline separation of all peaks (CRF < 1.00). As was done for fluoxetine, a CRF = 1 and a complying SST were considered to be criteria for a suitable separation. After checking all columns with CRF = 1, it was observed that 50 out of the 62 columns complied with the SST. When selecting columns with CRF $\neq$ 1, 3 of the 3 columns did not comply with the SST. It must be noted here that almost all columns gave good results for the separation. However, this indicates that the SST prescribed by the USP does not always provide relevant information. Therefore, it was checked whether the column classification could help in the selection of a suitable column. Sixty-two of the 65 columns gave overall baseline separation (CRF = 1.00) for the peaks investigated (Table 5). For the calculation of the reference parameter values from the columns with CRF = 1.0, the columns Platinum C18 (no. 31), Lichrosorb RP 18 (27), Supelcosil LC 18 (no. 50), Hypurity Aquastar (no. 20) and Inertsil ODS-P (no. 25) were found to be outliers and

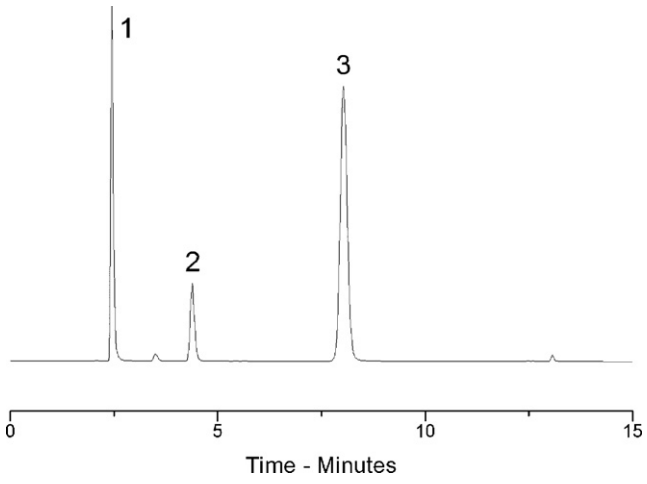

Fig. 3. Separation of gemcitabine and its related compounds. 1 = cytosine B, 2 = gemcitabine α-anomer and 3 = gemcitabine. Column: Restek Pinnacle DB C_{18}.

Table 4
Column ranking obtained with the *F*-values, relative to Alltima AQ column (k'_{amb} : 4.04, $rk'_{ba/pH\,2.7}$: 0.12, $k'_{2,2'\text{-d}}$: 21.04, $rk'_{tri/ter}$: 2.40) for the separation of fluoxetine

No.	Column name	k'_{ab}	$rk'_{ba/pH}$	k'_{dip}	$rk_{tri/o\text{-}ter}$	*F*	CRF	R^2k	Average	$R^2 \log k$	Average
4	Alltima AQ	−0.340	−0.126	1.698	−0.035	0.000	1.00	1.000	0.966	1.000	0.994
35	Prevail C18	−0.269	−0.130	1.885	−0.041	0.040	1.00	0.993		0.999	
16	Exsil ODS 5	−0.120	−0.085	1.614	−0.102	0.062	0.83	0.964		0.994	
27	LiChrosorb RP 18	−0.425	0.116	1.784	−0.093	0.076	0.87	0.988		0.997	
38	Prontosil 120 C18 AQ PLUS	0.289	−0.153	1.295	−0.105	0.564	0.96	0.914		0.985	
28	LiChrospher 100 RP-18	0.529	−0.065	2.277	−0.101	1.098	0.98	0.974		0.996	
5	Alltima C18	0.760	−0.134	0.865	−0.127	1.912	1.00	0.925		0.988	
17	Hamilton Hx Sil C18	0.348	−0.128	0.406	−0.136	2.155	0.95	0.832	0.866	0.975	0.964
41	Prontosil 120 5 C18 SH	0.410	−0.130	0.351	−0.137	2.390	0.93	0.887		0.976	
10	Capcell Pak C18 AQ	−0.536	−0.153	0.164	−0.150	2.408	1.00	0.908		0.984	
43	Purospher RP 18e	1.044	−0.147	0.990	−0.100	2.423	0.62	0.841		0.965	
29	MP Gel ODS-5	0.247	−0.144	0.255	−0.106	2.434	0.88	0.908		0.949	
44	Purospher Star RP-18	0.655	−0.142	0.342	−0.125	2.838	0.86	0.822		0.971	
55	Wakosil II 5 C18 RS	0.333	−0.148	−0.034	−0.166	3.471	0.93	0.855		0.978	
53	Uptisphere 5 HDO-25QS	0.387	−0.133	−0.016	−0.160	3.482	0.90	0.809		0.969	
37	Prontosil 120 5 C18 AQ	−0.106	−0.125	−0.189	−0.156	3.631	1.00	0.997		0.995	
65	Zorbax SB C18	−0.160	−0.113	−0.236	−0.159	3.789	0.97	0.983		0.988	
23	Inertsil ODS-3	1.169	−0.154	0.461	−0.155	3.825	0.84	0.772		0.978	
11	Capcell Pak C18 MG	0.713	−0.135	0.041	−0.158	3.870	0.81	0.790		0.959	
52	Superspher 100 RP-18	0.692	−0.122	0.025	−0.123	3.875	0.90	0.868		0.957	
40	Prontosil 120 5 C18 H	−0.065	−0.129	−0.261	−0.144	3.928	1.00	0.962		0.990	
58	YMC-Pack Pro 3 C18	0.570	−0.151	−0.079	−0.158	4.003	0.98	0.855		0.923	
18	Hydrosphere C18	−0.197	−0.155	−0.297	−0.168	4.020	0.90	0.890		0.959	
54	Uptisphere 5 ODB-25QS	0.595	−0.133	−0.097	−0.149	4.111	0.87	0.809		0.961	
32	Platinum EPS C18	−1.568	0.092	0.096	−0.066	4.121	0.00	0.843		0.978	
22	Inertsil ODS-2	0.452	−0.161	−0.215	−0.120	4.296	0.92	0.926		0.978	
9	Capcell Pak C18 ACR	0.403	−0.144	−0.252	−0.130	4.366	0.84	0.827		0.954	
59	YMC-Pack Pro 5 C18	0.298	−0.159	−0.333	−0.158	4.548	0.83	0.866		0.939	
56	Xterra MS C18	−0.404	−0.134	−0.432	−0.170	4.559	0.00	0.817		0.902	
1	Acclaim 5 μm	0.984	−0.133	0.024	−0.150	4.572	0.85	0.797		0.959	
24	Inertsil ODS-80A	1.630	−0.151	0.853	−0.154	4.609	0.69	0.768		0.938	
30	Omnispher 5 C18	0.680	−0.134	−0.204	−0.117	4.665	0.86	0.851		0.969	
48	Restek Pinnacle II C18	0.170	−0.114	−0.398	−0.136	4.666	0.89	0.894		0.972	
15	Discovery HS C18	0.815	−0.137	−0.160	−0.134	4.795	0.82	0.838		0.964	
39	Prontosil 120 5 C18 ace EPS	0.069	−0.164	−0.485	−0.089	4.937	0.92	0.781		0.948	
62	Zorbax Eclipse XDB C18	0.353	−0.136	−0.422	−0.158	4.992	0.88	0.899		0.970	
26	Kromasil KR100-5C18	1.163	−0.129	0.047	−0.131	4.994	0.85	0.801		0.956	
12	Capcell Pak C18 UG120	−0.067	−0.150	−0.551	−0.155	5.146	0.78	0.848		0.956	
63	Zorbax Extend C18	0.649	−0.145	−0.343	−0.137	5.157	0.67	0.797		0.919	
50	Supelcosil LC-18	−0.321	0.245	−0.553	−0.145	5.220	0.00	0.956		0.976	
8	Brava BDS C18	−0.984	−0.109	−0.503	−0.127	5.269	0.96	0.972		0.991	
51	Supelcosil LC-18 DB	−0.579	−0.100	−0.593	−0.154	5.319	0.96	0.922		0.982	
49	Restek Ultra C18	1.348	−0.136	0.101	−0.131	5.412	0.88	0.808		0.951	
45	Pursuit 5 C18	−0.375	−0.133	−0.672	−0.150	5.630	0.83	0.858		0.964	
20	HyPURITY Aquastar	−1.537	−0.092	−0.360	0.025	5.671	0.99	0.999		0.993	
47	Restek Pinnacle DB C18	−0.354	−0.111	−0.684	−0.134	5.685	0.83	0.882		0.960	
3	ACE 5 C18	−0.248	−0.129	−0.706	−0.133	5.797	0.90	0.890		0.972	
64	Zorbax SB Aq	−1.744	−0.103	−0.369	−0.182	6.266	0.00	0.994	0.871	0.993	0.938
14	Discovery C18	−0.669	−0.135	−0.827	−0.138	6.496	0.82	0.887		0.966	
6	Alltima HP C18	−0.770	−0.128	−0.827	−0.152	6.575	0.83	0.883		0.963	
33	Polaris 5 μm C18-A	−0.813	−0.134	−0.828	−0.112	6.610	0.85	0.876		0.965	
36	Prevail Select C18	−0.838	−0.205	−0.852	−0.040	6.760	0.91	0.828		0.965	
31	Platinum C18	−1.451	−0.003	−0.659	−0.147	6.817	0.82	0.883		0.984	
21	HyPURITY C18	−0.807	−0.132	−0.900	−0.130	6.977	0.86	0.900		0.968	
13	Chromolith Performance	−1.117	−0.144	−0.851	−0.137	7.113	0.29	0.909		0.920	
42	Prontosil 60 5 C18 H	2.173	−0.152	0.748	−0.149	7.235	0.74	0.974		0.992	
57	Xterra RP C18	−1.107	−0.146	−0.949	−0.093	7.597	0.56	0.809		0.937	
34	Prevail Amide	−1.452	−0.215	−0.848	−0.080	7.731	0.95	0.850		0.967	
2	Acclaim 3 μm	−1.252	−0.124	−1.117	−0.155	8.774	0.00	0.857		0.878	
46	Restek Allure C18	2.543	−0.140	0.858	−0.136	9.030	0.88	0.819		0.959	
60	YMC-Pack Pro RS	2.368	−0.160	0.382	−0.150	9.080	0.74	0.822		0.929	
7	Alltima HP Amide C18	−1.321	−0.178	−1.191	−0.023	9.311	0.71	0.861		0.873	
25	Inertsil ODS-P	1.032	−0.144	4.734	−0.026	11.101	0.97	0.946		0.990	
19	HyPURITY Advance	−1.906	−0.286	−1.435	−0.072	12.298	0.74	0.853		0.960	
61	Zirchrom PS 3 μm	−1.968	7.918	−1.566	7.932	141.476	0.00	0.730		0.675	

Table 5
Column ranking obtained with the *F*-values, relative to the mean parameter values (k'_{amb} : 5.16, $rk'_{ba/pH\,2.7}$: 0.093, $k'_{2,2'\text{-d}}$: 10.14, $rk'_{tri/ter}$: 1.55) for the separation of gemcitabine

No.	Name	k'_{ab}	$rk'_{ba/pH}$	k'_{dip}	$rk_{tri/o\text{-}ter}$	*F*	CRF	Rs	Tf	SST
55	Wakosil II 5 C18 RS	0.333	−0.148	−0.034	−0.166	0.037	1.00	12.69	0.75	+
53	Uptisphere 5 HDO-25QS	0.387	−0.133	−0.016	−0.160	0.061	1.00	28.07	0.72	+
37	Prontosil 120 5 C18 AQ	−0.106	−0.125	−0.189	−0.156	0.086	1.00	11.73	1.06	+
40	Prontosil 120 5 C18 H	−0.065	−0.129	−0.261	−0.144	0.094	1.00	10.75	1.14	+
29	MP Gel ODS-5	0.247	−0.144	0.255	−0.106	0.096	1.00	13.45	0.85	+
59	YMC-Pack Pro 5 C18	0.298	−0.159	−0.333	−0.158	0.113	1.00	16.64	0.74	+
9	Capcell Pak ACR	0.403	−0.144	−0.252	−0.130	0.115	1.00	14.35	1.16	+
22	Inertsil ODS-2	0.452	−0.161	−0.215	−0.120	0.128	1.00	14.02	1.06	+
48	Restek Pinnacle II C18	0.170	−0.114	−0.398	−0.136	0.133	1.00	15.19	0.73	+
65	Zorbax SB C18	−0.160	−0.113	−0.236	−0.159	0.133	1.00	11.86	0.95	+
58	YMC-Pack Pro 3 C18	0.570	−0.151	−0.079	−0.158	0.185	1.00	10.63	1.21	+
18	Hydrosphere C18	−0.197	−0.155	−0.297	−0.168	0.186	1.00	13.57	0.72	+
62	Zorbax Eclipse XDB C18	0.353	−0.136	−0.422	−0.158	0.194	1.00	14.25	0.88	+
54	Uptisphere 5 ODB-25QS	0.595	−0.133	−0.097	−0.149	0.208	1.00	16.31	0.74	+
39	Prontosil 120 ace EPS	0.069	−0.164	−0.485	−0.089	0.209	1.00	11.20	1.00	+
41	Prontosil 120 5 C18 SH	0.410	−0.130	0.351	−0.137	0.221	1.00	12.85	0.82	+
17	Hamilton Hx Sil C18	0.348	−0.128	0.406	−0.136	0.237	1.00	13.50	0.90	+
52	Superspher 100 RP-18	0.692	−0.122	0.025	−0.123	0.306	1.00	17.75	0.66	+
12	Capcell Pak UG120	−0.067	−0.150	−0.551	−0.155	0.310	1.00	12.47	1.05	+
30	Omnispher 5 C18	0.680	−0.134	−0.204	−0.117	0.317	1.00	15.88	0.98	+
11	Capcell Pak MG	0.713	−0.135	0.041	−0.158	0.332	1.00	16.57	1.09	+
63	Zorbax Extend C18	0.649	−0.145	−0.343	−0.137	0.351	1.00	12.99	0.89	+
44	Purospher Star RP-18	0.655	−0.142	0.342	−0.125	0.405	1.00	16.19	1.23	+
56	Xterra MS C18	−0.404	−0.134	−0.432	−0.170	0.457	1.00	10.53	1.09	+
15	Discovery HS C18	0.815	−0.137	−0.160	−0.134	0.466	1.00	17.80	0.68	+
10	Capcell Pak AQ	−0.536	−0.153	0.164	−0.150	0.502	1.00	13.32	0.92	+
3	ACE 5 C18	−0.248	−0.129	−0.706	−0.133	0.602	1.00	12.84	0.90	+
50	Supelcosil LC-18	−0.321	0.245	−0.553	−0.145	0.630	1.00	9.78	**2.50**	−
47	Restek Pinnacle DB C18	−0.354	−0.111	−0.684	−0.134	0.668	1.00	13.47	1.10	+
45	Pursuit 5 C18	−0.375	−0.133	−0.672	−0.150	0.672	1.00	16.38	1.20	+
2	Acclaim 5 μm	0.984	−0.133	0.024	−0.150	0.712	1.00	21.99	0.74	+
51	Supelcosil LC-18 DB	−0.579	−0.100	−0.593	−0.154	0.832	1.00	15.02	1.38	+
26	Kromasil KR100-5C18	1.163	−0.129	0.047	−0.131	1.047	1.00	16.74	0.99	+
5	Alltima C18	0.760	−0.134	0.865	−0.127	1.192	1.00	15.71	0.85	+
14	Discovery C18	−0.669	−0.135	−0.827	−0.138	1.285	1.00	14.32	0.97	+
23	Inertsil ODS-3	1.169	−0.154	0.461	−0.155	1.301	1.00	24.93	1.15	+
7	Alltima HP Amide	−0.770	−0.128	−0.827	−0.152	1.459	1.00	14.02	1.07	+
49	Restek Ultra C18	1.348	−0.136	0.101	−0.131	1.472	1.00	17.73	1.16	+
8	Brava BDS C18	−0.984	−0.109	−0.503	−0.127	1.489	1.00	11.49	0.98	+
33	Polaris 5 μm C18-A	−0.813	−0.134	−0.828	−0.112	1.542	1.00	11.89	1.21	+
36	Prevail Select C18	−0.838	−0.205	−0.852	−0.040	1.643	1.00	**6.18**	1.11	−
21	HyPURITY C18	−0.807	−0.132	−0.900	−0.130	1.650	1.00	12.52	1.05	+
38	Prontosil 120 C18 AQ PLUS	0.289	−0.153	1.295	−0.105	1.793	1.00	11.37	**1.64**	+
43	Purospher RP-18e	1.044	−0.147	0.990	−0.100	1.865	1.00	8.08	0.85	+
13	Chromolith Performance	−1.117	−0.144	−0.851	−0.137	2.252	1.00	**6.13**	**1.62**	−
57	Xterra RP C18	−1.107	−0.146	−0.949	−0.093	2.396	1.00	**4.65**	1.29	−
16	Exsil ODS 5 μm	−0.120	−0.085	1.614	−0.102	2.794	1.00	9.62	1.26	+
31	Platinum C18	−1.451	−0.003	−0.659	−0.147	2.948	1.00	**7.35**	1.21	−
20	HyPURITY Aquastar	−1.537	−0.092	−0.360	0.025	2.953	1.00	9.07	1.06	+
32	Platinum EPS C18	−1.568	0.092	0.096	−0.066	3.001	0.86	**2.36**	**2.34**	−
24	Inertsil ODS-80A	1.630	−0.151	0.853	−0.154	3.001	1.00	14.59	1.02	+
1	Acclaim 3 μm	−1.252	−0.124	−1.117	−0.155	3.117	1.00	9.57	1.15	+
34	Prevail Amide	−1.452	−0.215	−0.848	−0.080	3.214	1.00	**4.13**	1.15	−
4	Alltima AQ	−0.340	−0.126	1.698	−0.035	3.249	1.00	**7.14**	**1.73**	−
6	Alltima HP C18	−1.321	−0.178	−1.191	−0.023	3.491	1.00	**4.94**	1.17	−
64	Zorbax SB Aq	−1.744	−0.103	−0.369	−0.182	3.674	1.00	**6.41**	1.05	−
27	LiChrosorb RP-18	−0.425	0.116	1.784	−0.093	3.700	1.00	8.28	**2.27**	−
35	Prevail C18	−0.269	−0.130	1.885	−0.041	3.867	1.00	8.17	1.41	+
42	Prontosil 60 5 C18 H	2.173	−0.152	0.748	−0.149	4.737	1.00	8.32	1.08	+
60	YMC-Pack Pro RS	2.368	−0.160	0.382	−0.150	5.127	1.00	14.57	0.83	+
28	LiChrospher 100 RP-18	0.529	−0.065	2.277	−0.101	5.502	1.00	**7.96**	1.23	−
19	HyPURITY Advance	−1.906	−0.286	−1.435	−0.072	6.182	0.74	**0.99**	1.13	−
46	Restek Allure C18	2.543	−0.140	0.858	−0.136	6.559	1.00	15.81	1.00	+
25	Inertsil ODS-P	1.032	−0.144	4.734	−0.026	23.554	1.00	**4.44**	**2.74**	−
61	Zirchrom PS 3 μm	−1.968	7.918	−1.566	7.932	136.679	0.92	**1.65**	**2.61**	−

Table 6
Gemcitabine separation

		Reference column				
		Uptiphere 5 HDO (CRF value = 1.00, N = 103,984)	Inertsil ODS-3 (CRF value = 1.00, N = 66,503)	Acclaim 5u (CRF value = 1.00, N = 24,921)	Inertsil ODS-P (CRF value = 1.00, N = 850)	Platinum EPS C18 (CRF value = 0.86, N = 890)
CRF < 1	$F < 2$	0/40	0/28	0/32	0/1	1/18
	$2 < F < 6$	1/22	0/25	0/23	0/0	1/34
	$F > 6$	2/3	3/12	3/10	3/64	1/13
av R^2 log k	$F < 2$	0.99800	*0.99870*	0.99960	*1*	*0.99300*
	$2 < F < 6$	0.99500	*0.99898*	0.99850	*0*	*0.99600*
	$F > 6$	0.98800	*0.99100*	0.99100	*0.998*	*0.99800*
av $R^2 k$	$F < 2$	0.99880	0.99920	0.99900	*1*	*0.99350*
	$2 < F < 6$	0.99300	0.99890	0.99600	*0*	*0.99352*
	$F > 6$	0.97000	0.97980	0.96700	*0.995*	*0.99230*

Overview of the number of suitable columns (CRF > 0.8) within three different ranges ($F < 2$, $2 < F < 6$ and $F > 6$). For comparison coefficient of determination (k, log k) results were added.

were excluded. Results of the column ranking are presented in Table 5. Out of the three columns with CRF < 1, two columns are situated in the $F > 6$ range, and one in the intermediate range ($2 < F < 6$). Correlation with the SST shows that all columns with $F < 2$, except for the Prevail select (no. 36) comply with the minimum resolution of 8.0 between the gemcitabine α-anomer and the main peak (β-anomer). From the columns with $F > 2$, 12 of the 21 columns do not comply. The tailing factor of gemcitabine is higher than 1.5 (not compliant) for 6 out of the 21 columns with $F > 2$, whereas only two columns do not comply in the group of columns with $F < 2$ (Table 5). The conclusion is that the column ranking system could help analysts with their column selection for this analysis too.

When checking the Zirchrom and Chromolith Performance column, it was seen that the Zirchrom gives partial co-elution between cytosine, gemcitabine α-anomer and the main peak (β-anomer). Chromolith Performance gave a good separation, and moreover, instead of about 10 min for an average C_{18} column, the retention time dropped now to 3 min. Here, the faster elution did not lead to co-elution.

Like for the fluoxetine separation, one single column with a suitable separation (CRF = 1) was also selected as reference column. The efficiency of the main peak, i.e. gemcitabine, was also used here as a criterion. Four columns with CRF = 1 were chosen: the three columns with the highest N value and the one with the lowest value. The Platinum EPS column (no. 32) was also added as a column with CRF < 1 for this separation (Table 6).

For the four columns with CRF = 1 taken as reference, the three non-suitable columns can be found in the $2 < F < 6$ or $F > 6$ range. When using Platinum EPS (CRF = 0.86) as reference column, the non-suitable columns can be found on positions 1, 27 and 65. When comparing the ranking based on the Inertsil ODS-P (CRF = 1, but lowest efficiency), it can be seen that all F-values are higher than 6, which means that this approach is not the best.

When checking correlation between retention times obtained on different columns, it was seen that the CoD value follows the expected tendency for k values in all situations but for log k in two of the three cases. When using a column with CRF = 0.86 (Platinum EPS), the CoD values do not match with the ranking.

4. Conclusion

This paper focuses on the performance of a column selection system when applied to two pharmaceutical separations. The columns were ranked according to their F-values, which were calculated based on four chromatographic parameters. In the examples described, first, a virtual ideal column was used as reference column. The chromatographic parameters for this virtual column were calculated as the means of the parameters of the columns giving sufficient separation after correcting for outliers. The relationship between the ranking of the columns and their separation performance was discussed. Secondly, single columns with CRF = 1 (high and low efficiency) and CRF ≠ 1 were selected as reference. In this study, the CRF value and SST value were used to evaluate the separations. Also, CoD values were calculated and the correlation with quality of separation was discussed. For fluoxetine, the SST refers to just one of the critical pairs while the CRF gives a better overview of the separation because it takes into account all peak pairs. Moreover, many columns yielding a poor SST showed adequate overall separation. Columns were classified in three arbitrary groups: $F < 2$, $2 < F < 6$ and $F > 6$. The CoD values correlated best when using the log k values.

For gemcitabine, a useful classification was obtained whether using a virtual, ideal column as reference column or a column with CRF = 1 and a high efficiency. Here, the CoD values correlated better when the k value was calculated. The usage of CoD as additional criterion may be useful, but should be investigated on more separations.

The efficiency appears to be a useful parameter in the selection of a reference column. The CoD could be an additional parameter, supplementary to the CRF value, e.g. where the CRF makes no distinction between columns since most of the columns give an overall baseline separation (CRF = 1). Depending on the separation, k or log k values appear to provide better results.

The developed column classification system is considered as a helpful tool to find a suitable column for a given separation. As expected, column selection is less a problem for rather simple

separations (gemcitabine) than for more complex separations (fluoxetine).

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References

[1] European Pharmacopoeia, 5th ed., Council of Europe, Strasbourg, France, 2005, pp. 917–918.
[2] L.R. Snyder, A. Maule, A. Heebsh, R. Cuellar, S. Paulson, J. Carrano, L. Wrisley, C.C. Chan, N. Pearson, J.W. Dolan, J.J. Gilroy, J. Chromatogr. A 1057 (2004) 49–57.
[3] J.W. Dolan, A. Maule, D. Bingley, L. Wrisley, C.C. Chan, M. Angod, C. Lunte, R. Krisko, J.M. Winston, B.A. Homeier, D.V. McCalley, L.R. Snyder, J. Chromatogr. A 1057 (2004) 59–74.
[4] L.R. Snyder, J.W. Dolan, P.W. Carr, J. Chromatogr. A 1060 (2004) 77–116.
[5] D.H. Marchand, K. Croes, J.W. Dolan, L.R. Snyder, J. Chromatogr. A 1062 (2005) 57–64.
[6] D.H. Marchand, K. Croes, J.W. Dolan, L.R. Snyder, R.A. Henry, K.M.R. Kallury, S. Waite, P.W. Carr, J. Chromatogr. A 1062 (2005) 65–78.
[7] J. Pellett, P. Lukulay, Y. Mao, W. Bowen, R. Reed, M. Ma, R.C. Munger, J.W. Dolan, L. Wrisley, K. Medwid, J. Chromatogr. A 1101 (2006) 122–135.
[8] M.R. Euerby, P. Petersson, J. Chromatogr. A 994 (2003) 13–36.
[9] M.R. Euerby, P. Petersson, J. Chromatogr. A 1088 (2005) 1–15.
[10] E. Van Gyseghem, M. Jimidar, R. Sneyers, D. Redlich, E. Verhoeven, D.L. Massart, Y. Vander Heyden, J. Chromatogr. A 1042 (2004) 69–80.
[11] M. Kele, G. Guiochon, J. Chromatogr. A 830 (1999) 41–54.
[12] W. Verstraeten, J. de Zeeuw, J. Crombeen, N. Vonk, Int. Lab. (2000) 20–29.
[13] U.D. Neue, K. Van Tran, P.C. Iraneta, B.A. Alden, J. Sep. Sci. 26 (2003) 174–186.
[14] K. Le Mapihan, J. Vial, A. Jardy, J. Chromatogr. A 1061 (2004) 149–158.
[15] R. Kaliszan, M.A. van Straten, M. Markuszewski, C.A. Cramers, H.A. Claessens, J. Chromatogr. A 855 (1999) 455–486.
[16] T. Baczek, R. Kaliszan, K. Novotna, P. Jandera, J. Chromatogr. A 1075 (2005) 109–115.
[17] P. Forlay-Frick, J. Fekete, K. Héberger, Anal. Chim. Acta 536 (2005) 71–81.
[18] D. Visky, Y. Vander Heyden, T. Iványi, P. Baten, J. De Beer, B. Noszál, E. Roets, D.L. Massart, J. Hoogmartens, Pharmeuropa 14 (2002) 288–297.
[19] D.V. McCalley, R.G. Brereton, J. Chromatogr. A 828 (1998) 407–420.
[20] U.D. Neue, B.A. Alden, T.H. Walter, J. Chromatogr. A 849 (1999) 101–116.
[21] K. Le Mapihan, J. Vial, A. Jardy, J. Chromatogr. A 1030 (2004) 135–147.
[22] D. Visky, Y. Vander Heyden, T. Iványi, P. Baten, J. De Beer, Zs. Kovács, B. Noszál, E. Roets, D.L. Massart, J. Hoogmartens, J. Chromatogr. A 977 (2002) 39–58.
[23] T. Iványi, Y. Vander Heyden, D. Visky, P. Baten, J. De Beer, I. Lázár, D.L. Massart, E. Roets, J. Hoogmartens, J. Chromatogr. A 954 (2002) 99–114.
[24] D. Visky, Y. Vander Heyden, T. Iványi, P. Baten, J. De Beer, Z. Kovács, B. Noszál, P. Dehouck, E. Roets, D.L. Massart, J. Hoogmartens, J. Chromatogr. A 1012 (2003) 11–29.
[25] P. Dehouck, D. Visky, G. Van den Bergh, E. Haghedooren, E. Adams, A. Kerner, Y. Vander Heyden, D.L. Massart, Zs. Kovács, B. Noszál, J. Hoogmartens, LC-GC Europe 17 (2004) 592–598.
[26] P. Dehouck, D. Visky, Y. Vander Heyden, E. Adams, Z. Kovács, B. Noszál, D.L. Massart, J. Hoogmartens, J. Chromatogr. A 1025 (2004) 189–200.
[27] D. Visky, E. Haghedooren, P. Dehouck, Zs. Kovács, K. Kóczián, B. Noszál, J. Hoogmartens, E. Adams, J. Chromatogr. A 1101 (2006) 103–114.
[28] E. Haghedooren, J. Diana, B. Noszál, J. Hoogmartens, E. Adams, Talanta 71 (2007) 31–37.
[29] E. Haghedooren, D. Visky, P. Dehouck, K. Kóczián, J. Diana, Zs. Kovács, B. Noszál, J. Hoogmartens, E. Adams, LC-GC Europe 20 (2007) 82–103.
[30] www.pharm.kuleuven.ac.be/pharmchem/columnclassification, 2007.
[31] W. Naidong, S. Hua, E. Roets, J. Hoogmartens, J. Planar Chromatogr. 5 (1992) 92–98.
[32] S.L. Morgan, S.N. Deming, J. Chromatogr. 112 (1975) 267–285.
[33] E. Haghedooren, A. Kerner, B. Noszál, J. Hoogmartens, E. Adams, J. Pharm. Biomed. Anal. 44 (2007) 634–639.
[34] The United States Pharmacopeia, 28th ed., United States Pharmacopeial Convention, Rockville, USA, 2005, pp. 892–894.
[35] European Pharmacopoeia, 5th ed., Council of Europe, Strasbourg, France, 2005, pp. 1615–1617.
[36] D.L. Massart, B.G.M. Vandeginste, L.M.C. Buydens, S. De Jong, P.J. Lewi, J. Smeyers-Verbeke, Handbook of Chemometrics and Qualimetrics: Part A, 3rd ed, Elsevier, Amsterdam, The Netherlands, 2003.
[37] P. Dehouck, D. Visky, Z. Kovács, B. Noszál, E. Adams, D.L. Massart, J. Hoogmartens, LC-GC Europe 16 (2003) 764–771.
[38] K.L. Rowlen, K.A. Duell, J.P. Avery, J.W. Birks, Anal. Chem. 63 (1991) 575–579.
[39] M.R. Euerby, P. Petersson, LC-GC Europe 13 (2000) 665–677.
[40] M.R. Euerby, P. Petersson, J. Sep. Sci. 28 (2005) 2120–2129.
[41] E. Van Gyseghem, S. Van Hemelryck, M. Daszykowski, F. Questier, D.L. Massart, Y. Vander Heyden, J. Chromatogr. A 988 (2003) 77–93.