Talanta 99 (2012) 492–501

Contents lists available at SciVerse ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Application of a column classification method in a selectivity study involving caffeine and its related impurities

Jarosław Szulfer [a], Alina Plenis [b], Tomasz Bączek [b,*]

[a] Polpharma SA Pharmaceutical Works, Pelplińska 19, 83-200 Starogard Gdański, Poland
[b] Department of Pharmaceutical Chemistry, Medical University of Gdańsk, Hallera 107, 80-416 Gdańsk, Poland

ARTICLE INFO

Article history:
Received 22 February 2012
Received in revised form
4 June 2012
Accepted 6 June 2012
Available online 18 June 2012

Keywords:
Column classification system
Factor analysis
LC separations
Caffeine
Impurities

ABSTRACT

In this paper a comparative study of RP-LC column selectivity as obtained by the classification method of the Katholieke Universiteit Leuven (KUL method) and the selectivity obtained in real pharmaceutical analysis is reported. The separation of caffeine and its respective impurities was performed on 35 brands of stationary phases in accordance with the method prescribed in the European Pharmacopoeia (Ph. Eur.). Evaluation concerned the probability of appropriate column selection related to the selection of two different stationary phases for reference. The comparison was based on the traditional correlation of the *F*-values with the results of a system suitability test (SST) for the columns, as well as an application of a factor analysis (FA) for graphical visualisation of the differences and similarities between the stationary phases established against four test chromatographic parameters provided by the KUL method and the retention parameters of the compounds of interest describing the column performance test. The obtained results confirmed that the class of the stationary phases selected according to the chromatographic test parameters gave comparable separation for caffeine and its impurities.

1. Introduction

Reversed-phase liquid chromatography (RP-LC) today probably belongs to the separation techniques most frequently used in pharmaceutical and biomedical analyses [1,2]. However, the hundreds of commercially available columns offered in the market in combination with the great variety of possible chromatographic systems have turned the selection of the stationary phase suitable for real application into a challenging task. The choice is additionally complicated by the fact that the RP-LC phases often belong to the same chemical class, which may suggest their similar chromatographic properties. In practice, the polar and ionic properties of the RP-LC phases responsible for the secondary intermolecular interaction mechanisms cause the stationary phases to give RP-LC columns a unique character. Therefore, the differences between the physical and/or chemical properties of stationary phases cause the analysts to confront the problem of column selection for a given separation. The same difficulties can be encountered by analysts who perform separations in accordance with the official monographs of the European Pharmacopoeia (Ph. Eur.) [3] and the United States Pharmacopeia (USP) [4]. The monographs report numerous chromatographic methods, mainly under RP conditions, but provide merely general descriptions of the stationary phases to characterise the suitable column, including e.g. the chain length, end-capping, base-deactivation, particle size, pore size, and specific surface. More detailed information about the stationary phase can only be found for the recently developed Ph. Eur. and USP monographs on their websites [5,6]. It may just as well happen that the name of the required column will be known but its application for a given analysis might be impossible because the prescribed stationary phase is not available in the laboratory or simply not attainable from the market any longer. Sometimes, the chromatographic behaviour of the RP stationary phases can alter because of the storage time or usage causing their potentially different selectivity [7]. In such a case it would be helpful to have a replacement of a suitable alternative offering separation "equivalent" to the original column. Therefore, many analysts expect reliable test methods should characterise the RP-LC columns so as to solve the problem of non-suitable column selection. For this reason, extensive investigations involving several chromatographic column tests were conducted over the last two decades [8–14]. The interesting approaches reported in the published papers were as follows: the mathematical models including the hydrophobic-subtraction (HS) model proposed by the Snyder and Dolan group [15–17], the linear solvation–energy relationship (LSER) delivered by Abraham and Sándi and Szepesy [18,19], the quantitative structure–retention relationships (QSRRs) provided

* Corresponding author. Tel.: +48 58 3493133.
E-mail address: tbaczek@gumed.edu.pl (T. Bączek).

0039-9140/$ - see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.talanta.2012.06.015

by Kaliszan et al. [20–23], and the sum of ranking differences (SRD) reported by Héberger [24]. Recently, a few general overviews of the QSRR and other test methods in determining LC column selectivity can be found in the literature [25–29]. The group of Hoogmartens [30–40] published an alternative column classification system, namely the Katholieke Universiteit Leuven method (KUL method). In this approach, each stationary phase is characterised using four chromatographic parameters: the amylbenzene (k'_{amb}) retention factor reflecting hydrophobicity, the benzylamine/phenol at pH 2.7 ($rk'_{ba/ph\ pH2.7}$) relative retention factor describing possible silanol activity, the triphenylene/o-terphenyl ($rk'_{tri/o\text{-}ter}$) relative retention factor evaluating steric selectivity, and the 2,2′-dipyridyl ($k'_{2,2'\text{-}d}$) retention factor evaluating silanol activity and metal impurities [33,36]. In its step one, the approach requires choosing a specific reference column or selecting four reference parameters corresponding to the chosen reference. Next, the *F*-value for column *i*, being the sum of the squares of the differences between each parameter value of the reference stationary phase and that of column *i*, is calculated

$$F = (k'_{amb,ref} - k'_{amb,i})^2 + (rk'_{ba/ph\ pH2.7,ref} - rk'_{ba/ph\ pH2.7,i})^2 + (rk'_{tri/ter,ref} - rk'_{tri/ter,i})^2 + (k'_{2,2'-d,ref} - k'_{2,2'-d,i})^2 \quad (1)$$

Finally, a comparison is conducted between brands of stationary phases of column *i* and the reference phases. Lower *F*-values indicate that the specific column *i* is more similar to the reference one, whereas higher *F*-values reflect more significant dissimilarities between them. In effect, a ranking list of the tested columns is established for stationary phases, arranged from high to low in accordance with the increasing *F*-value. In order to guarantee that each parameter would be given the same weight, each was autoscaled before being introduced in Eq. (1) according to the formula:

$$(x_{ij} - x_j)/s_j \quad (2)$$

where x_{ij} is the value of parameter *j* in column *i*, x_j is the mean value of parameter *j* on all tested stationary phases, and s_j is the standard deviation for parameter *j* [32,34,38].

Of course, this convenient and useful simplification combining four different contributors into a single parameter has caused all calculated *F*-values to relate to a single reference stationary phase. It is customary for columns with $F<2$ to be deemed high-ranking and offering the highest probability of finding an appropriate alternative; columns with $2<F<6$ are deemed medium, whereas columns with $F>6$ are considered low-ranking. In case of the latter, the chance of selecting a suitable stationary phase visibly decreases. It is also necessary to verify the theoretical results of the KUL method and column performance by checking whether the columns sharing similar parameters produce similar separations in the pharmaceutical practice. In the literature, one finds reports on the usefulness of the KUL test procedure for real pharmaceutical applications [31–37,39], as well as comparative studies of the KUL ranking system and other column classification methods [23,29,38]. The published papers confirm that the KUL approach can facilitate the selection of a suitable RP-LC C18 column for a specific analysis. However, those comparative studies only provided a general description of the pharmaceutical analysis, including the Ph. Eur. system suitability test (SST) and/or the chromatographic response function (CRF), in correlation to the *F*-values established under the KUL method [31–37]. Neither of the cases indicated that the separations were identical or that the tested stationary phases represented exactly the same chromatographic properties. In other words, the correlation between the test results of column characterisation or classification and their performance in real separations was evaluated without any detailed description of the similarities and differences between the tested columns during their practical test in pharmaceutical applications. Moreover, it is commonly known that the calculated *F*-values are relative to a single reference stationary phase. Yet, we do not know if the probability of appropriate column selection under the KUL method in real pharmaceutical separation would be identical, if different columns suitable for the analysis were taken into account as the reference stationary phases. It is also not known whether the probability of appropriate column selection established under the KUL method would be similar, if the general criteria or detailed description of selectivity in the real analysis were taken into account in the investigation.

In this paper, the comparative study between the KUL test results and the column performance when applied in pharmaceutical analysis was based on raw experimental data including the retention parameters of the analysed substances such as the retention time (t_R), the symmetry factor (A_s) and the resolution of the peaks of interest (R_s), which clearly distinguish each real separation. As a case study, an analysis of caffeine and its impurities (Fig. 1) in accordance with the Ph. Eur. monograph was performed on 35 stationary phases previously tested under the chromatographic test procedure. Since the Ph. Eur. knowledge database recommends Supelcosil LC-18-DB (Sup-DB) and Kromasil (Krom) for this separation, these columns were selected as the references and the ranking list of all tested stationary phases based on the four test parameters was established. Next, a verification test was conducted to check the column performance when applied for the separation of caffeine from its related compounds in order to check if the KUL method could properly predict suitability of the RP-LC columns for a specific pharmaceutical analysis. The Ph. Eur. system suitability test (SST) requires that the R_s is minimum 2.5 between the peaks due to impurities C and D, and minimum 2.5 between the peaks due to impurities F and A. The parameter was established for the above mentioned critical pairs using all RP-LC stationary phases, and the results were confronted with the *F*-values for all columns. Next, the data of the theoretical column classification and their practical application in pharmaceutical analysis were compared using the factor analysis (FA). For a clearer interpretation of the results, the same numbers from 1 to 35 arranged by the increasing *F*-values depending on the selection of Sup-DB and Krom as the

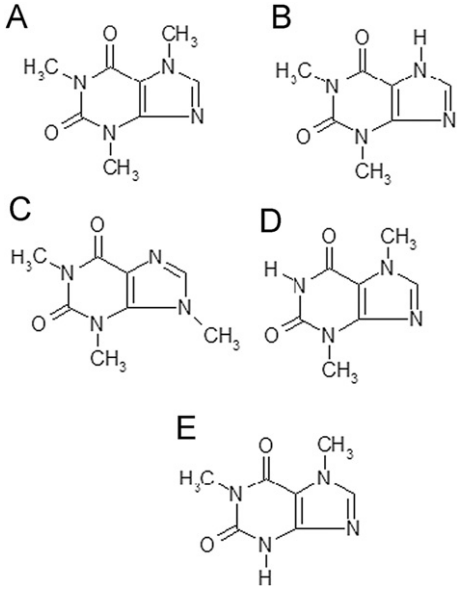

Fig. 1. Chemical structure of caffeine (A); impurity A (B); impurity C (C); impurity D (D); and impurity F (E).

reference stationary phases were assigned to both data sets. Next, the FA column classes identified based on the four test chromatographic parameters were correlated with the caffeine separation described by the t_R and R_s values for the compounds of interest, and the potential similarities and dissimilarities between them were evaluated. Finally, the SST-values were confronted with the location of the brands of individual stationary phases in the FA column classes.

2. Experimental

2.1. Chemicals

The test analytes, including uracil, benzylamine, *o*-terphenyl, triphenylene, amylbenzene (n-pentylbenzene), and 2,2′-dipyridyl, were supplied by Sigma-Aldrich (St. Louis, MO, USA) while phenol was purchased from POCH (Gliwice, Poland). *Caffeine chemical reference substance for system suitability testing* (*CRS-SST*) (containing 1,3,7-trimethyl-3,7-dihydro-1H-purine-2,6-dione (caffeine); 1,3-dimethyl-3,7-dihydro-1H-purine-2,6-dione (theophylline—impurity A); 1,3,9-trimethyl-3,9-dihydro-1H-purine-2,6-dione (isocaffeine—impurity C); 3,7-dimethyl-3,7-dihydro-1H-purine-2,6-dione (theobromine—impurity D); and 1,7-dimethyl-3,7-dihydro-1H-purine-2,6-dione (impurity F)) was delivered by EDQM (Strasbourg, France). The standard solution containing caffeine and impurities A, C, D, and F was prepared in accordance with the Ph. Eur. description to contain the active substance at the level of 200 μg mL^{-1} and four related compounds at the concentration of 2 μg mL^{-1}. All reagents and solvents were of Ph. Eur. quality. Methanol, acetonitrile, dipotassium hydrogen phosphate, and potassium dihydrogen phosphate of HPLC grade were purchased from J.T. Baker (Deventer, Netherlands), while phosphoric acid, anhydrous sodium acetate, glacial acetic acid, and tetrahydrofuran were supplied by Merck (Darmstadt, Germany). All chemicals were of the AR grade and used as received without further purification. Water was purified in the Milli-Q water purification system (Millipore, Bedford, MA, USA). All investigated stationary phases were donated by the manufacturers or distributors. Their characteristics are presented in Table 1.

2.2. Experimental conditions

All LC separations were performed in the Waters system (Milford, MA, USA) consisting of the 2695 Separation Module, Column Heater/Cooler with a three-column selector valve (Rheodyne RV500-100), and the 2996 Photodiode Array Detector. The Empower 2 software was used for data acquisition.

2.2.1. Chromatographic conditions for the KUL test procedure

In the study, three isocratic chromatographic methods were used in the order (A–B–C) defined for the selected analytes in accordance with the KUL test procedure as described in Ref. [34]. In each method, the column was thermostatted at 40 °C, and UV detection was performed at 254 nm. The flow rate was 1 mL min^{-1}, and the sample volume of 20 μL was injected into the HPLC system.

Table 1
List of RP-LC columns examined in this study and their properties as provided by the manufacturer.

Name of the column	Length (mm)	Internal diameter (mm)	Particle size (μm)	Carbon load (%)	Pore size (Å)	Surface area (m^2/g)	Silica[a]	Endcap.	Manufacturer/ supplier	Abbreviation
ACE 5 AQ	150	4.6	5	14	100	300	EP	+	ACT	AC-AQ
ACE 5 C18	150	4.6	5	15.5	100	300	B	+	ACT	AC-C18
ACE 5 C18-AR	150	4.6	5	15.5	100	300	B	+	ACT	AC-AR
ACE 5 C18-HL	150	4.6	5	20	90	400	B	+	ACT	AC-HL
Aquasil C18	150	4.6	5	12	100	310	EP	+	Thermo	Aq-sil
Aqua C18	150	4.6	5	15	125	320	B	+	Phenomenex	Aqua
Discovery C18	150	4.6	5	12	180	200	B	+	Supelco	Disc
Hypersil BDS C18	150	4.6	5	11	130	170	A	+	Thermo	Hyp-BDS
Hypersil Elite C18	150	4.6	5	15	114	250	A	+	Thermo	Hyp-Elite
Hypersil Gold aQ	150	4.6	5	12	175	220	EP	+	Thermo	Hyp-aQ
Hypersil Gold	150	4.6	5	10	175	220	B	+	Thermo	Hyp-Gold
Inertsil ODS2	150	4.6	5	18.5	150	320	B	+	GL Science	Iner-GL
Inertsil ODS2	150	4.6	5	18.5	150	320	B	+	Hichrom	Iner-HI
Kromasil 100-5 C18	150	4.6	5	19	100	340	B	+	Akzo Nobel	Krom
Luna C18	150	4.6	5	17.5	100	400	B	+	Phenomenex	Luna
Nucleodur C18 Isis	150	4.6	5	20	110	340	B	+	Macherey-Nagel	Nuc-Isis
Nucleodur C18 Pyramid	150	4.6	5	14	110	340	B	+	Macherey-Nagel	Nuc-Pyr
Nucleodur Sphinx RP	150	4.6	5	15	110	340	B	+	Macherey-Nagel	Nuc-Sph
Nucleosil 100-5 C18	150	4.6	5	15	100	350	A	+	Macherey-Nagel	Nuc-C18
Nucleosil 100-5 C18 AB	150	4.6	5	24	100	350	A	+	Macherey-Nagel	Nuc-AB
Nucleosil 100-5 C18 HD	150	4.6	5	20	100	350	A	+	Macherey-Nagel	Nuc-HD
Nucleosil 100-5 C18 Nautilus	150	4.6	5	16	100	350	EP	+	Macherey-Nagel	Nuc-Nau
Prodigy ODS3	150	4.6	5	15.5	100	450	B	+	Phenomenex	Pro-gy
Spherisorb ODS1	150	4.6	5	6.2	80	220	A	-	Waters	Sph-ODS1
Spherisorb ODS2	150	4.6	5	11.5	80	220	A	+	Waters	Sph-ODS2
SunFire C18	150	4.6	5	16	100	340	B	+	Waters	SunFire
Supelcosil LC-18-DB	150	4.6	5	11	120	170	A	+	Supelco	Sup-DB
Symmetry C18	150	4.6	5	19	100	335	B	+	Waters	Sym-ry
Symmetry Shield RP18	150	4.6	5	17	100	335	EP	+	Waters	Sym-Shield
Wakosil II 5 C18 HG	150	4.6	5	15	120	300	B	+	SGE	Wak-HG
Xbridge Shield RP18	150	4.6	5	17	130	185	EP	+	Waters	Xbr-Shield
YMC Pack ODS-AQ	150	4.6	5	14.1	120	300	B	+	YMC	Pack-AQ
Zorbax Eclipse XDB C18	150	4.6	5	10	80	180	B	+	Agilent	Zor-XDB
Zorbax SB-Aq	150	4.6	5	Proprietary	80	180	EP	-	Agilent	Zor-Aq
Zorbax SB-C18	150	4.6	5	10	80	180	B	-	Agilent	Zor-C18

[a] A—"traditional", acidic silica gel, B—"high purity", more neutral silica gel, EP—embedded or end-capped polar group.

2.2.2. Chromatographic conditions for the LC separation of caffeine

The separation of caffeine and the related compounds was performed in accordance with the Ph. Eur. monograph. The mobile phase consisted of tetrahydrofuran, acetonitrile, and aqueous solution of sodium acetate (pH 4.5) prepared by diluting 1.64 g of anhydrous sodium acetate in 2000 mL of water and adjusting the solution to the pH of 4.5 with glacial acetic acid (40:50:1910, *v*/*v*/*v*). The flow rate was 1.0 mL min^{-1}. The analysed compounds were monitored with UV detection at 275 nm. The injected sample volume was 10 μL.

2.3. Column examination

2.3.1. Column classification

In order to establish the amylbenzene (k'_{amb}) retention factor the relative retention factor of benzylamine/phenol at pH 2.7 ($rk'_{ba/ph\ pH2.7}$), the relative retention factor of triphenylene/o-terphenyl ($rk'_{tri/o\text{-}ter}$), and the retention factor of 2,2′-dipyridyl ($k'_{2,2'\text{-}d}$), as well as the retention times for the selected analytes under the three test methods A–B–C as described in Section 2.2.1 were determined for all stationary phases. The retention time of uracil in method C was used to determine the dead volume used in the calculations. The separation was performed three times on each stationary phase and the RSD values were lower than 1%. Next, the values of the four column parameters were calculated for all stationary phases. Finally, upon the choice of the Sup-DB column as the reference the *F*-values for the other stationary phases were established in accordance with Eqs. (1) and (2). Those calculations were performed using the available on-line software [39]. These results are presented in Table 2. The same calculations were repeated with the Krom column as the reference. In both cases, all columns were assigned numbers from 1 to 35 according to the increasing *F*-value.

2.3.2. Column selectivity in separation of caffeine

Column performance for 35 brands of the stationary phases was tested based on the LC separation of caffeine and the related compounds in the chromatographic conditions described in Section 2.2.2. The samples were prepared in accordance with the Ph. Eur. monograph. For this LC analysis, 5 mg of *caffeine CRS-SST* was dissolved in 5 mL of the mobile phase. Next, 2 mL of the solution was diluted to 10 mL of the mobile phase. Finally, this reference solution containing caffeine at a concentration of 200 μg mL^{-1} in the presence of 1% of its related compounds was injected into the HPLC system to check compliance with the SST requirements. The separation for each column was performed in triplicate. Typical chromatograms obtained from the sample containing the compounds of interest during the LC analysis using Sup-DB (A), Krom (B), Hyp-Elite (C) and Sph-ODS1(D) are illustrated in Fig. 2A–D, respectively. The t_R values of caffeine and its impurities, as well as the R_s and the A_s of the peaks of interest for all stationary phases were calculated with the Empower 2 software. The data are reported in Table 3. Moreover, the R_s between impurities C and D, and F and A were checked for compliance with the SST requirements.

Table 2
The column parameters and *F*-values for thirty five tested stationary phases according to the KUL test procedure.

Analytical column	Column parameters				Reference stationary phases			
	k'_{amb}	$rk'_{ba/ph\ pH2.7}$	$rk'_{tri/o\text{-}ter}$	$k'_{2,2'\text{-}d}$	Sup-DB *F*	Krom	Sup-DB Column no.	Krom
Sup-DB	3.053	0.215	1.320	5.709	0	4.177	1	23
Disc	2.780	0.087	1.447	4.408	1.214	3.741	2	22
Hyp-BDS	3.480	0.136	1.569	5.123	1.233	2.599	3	20
AC-AQ	2.232	0.077	1.322	7.271	1.329	4.582	4	24
Hyp-aQ	**1.882**	**0.089**	**1.268**	**3.877**	**1.393**	**6.342**	**5**	**29**
Hyp-Elite	**4.450**	**0.121**	**1.502**	**6.807**	**1.484**	**1.003**	**6**	**10**
Zor-C18	4.281	0.094	1.212	8.810	1.674	1.980	7	18
AC-C18	4.489	0.097	1.505	6.833	1.817	0.918	8	8
Pack-AQ	4.164	0.073	1.258	9.137	1.888	1.809	9	17
Aqua	4.932	0.096	1.277	9.243	2.134	1.021	10	11
Wak-HG	**4.913**	**0.070**	**1.353**	**7.422**	**2.208**	**0.790**	**11**	**7**
Nuc-HD	**5.099**	**0.093**	**1.482**	**8.049**	**2.456**	**0.347**	**12**	**4**
Nuc-Pyr	4.682	0.060	1.259	9.879	**2.649**	1.382	13	14
Zor-Aq	0.863	0.109	1.192	9.989	**2.679**	8.635	14	32
AC-AR	**3.483**	**0.099**	**1.698**	**8.180**	**2.884**	**2.514**	**15**	**19**
Pro-gy	**5.476**	**0.078**	**1.246**	**8.416**	**2.888**	**0.941**	**16**	**9**
Nuc-Sph	**2.738**	**0.073**	**1.000**	**9.688**	**3.014**	**6.314**	**17**	**28**
Zor-XDB	**5.762**	**0.082**	**1.284**	**8.179**	**3.123**	**0.639**	**18**	**6**
Iner-GL	**5.170**	**0.051**	**1.445**	**8.667**	**3.178**	**0.401**	**19**	**5**
Iner-HI	**4.257**	**0.062**	**1.639**	**8.431**	**3.275**	**1.330**	**20**	**13**
Luna	5.509	0.050	1.172	9.012	**3.744**	1.555	21	15
SunFire	5.479	0.038	1.231	9.124	**3.770**	1.177	22	12
Krom	6.199	0.091	1.491	9.038	**4.177**	0	23	1
Nuc-C18	**3.360**	**0.115**	**1.634**	**14.430**	**4.269**	**3.310**	**24**	**21**
AC-HL	6.369	0.087	1.535	8.950	**4.737**	0.035	25	2
Sym-ry	6.140	0.042	1.566	8.513	**5.222**	0.222	26	3
Nuc-Nau	2.734	0.023	1.827	5.828	**5.481**	5.193	27	26
Aq-sil	**2.971**	**0.163**	**1.825**	**15.163**	**6.306**	**5.651**	**28**	**27**
Nuc-AB	**3.658**	**0.097**	**1.964**	**6.021**	**6.325**	**4.904**	**29**	**25**
Nuc-Isis	**5.420**	**0.061**	**1.827**	**8.650**	**6.454**	**1.700**	**30**	**16**
Sph-ODS2	**4.044**	**0.366**	**1.657**	**17.275**	**7.227**	**7.972**	**31**	**30**
Hyp-Gold	**2.299**	**0.118**	**2.040**	**7.128**	**7.549**	**8.074**	**32**	**31**
Xbr-Shield	**2.296**	**0.046**	**2.111**	**4.456**	**10.036**	**9.788**	**33**	**34**
Sym-Shield	3.771	0.022	2.212	6.575	12.735	8.832	34	33
Sph-ODS1	**2.041**	**0.334**	**1.865**	**23.641**	**15.026**	**16.348**	**35**	**35**

Column no.—the position in the ranking list; meaning of other symbols is explained in the text. The columns non-suitable for the separation of caffeine are indicated in bold.

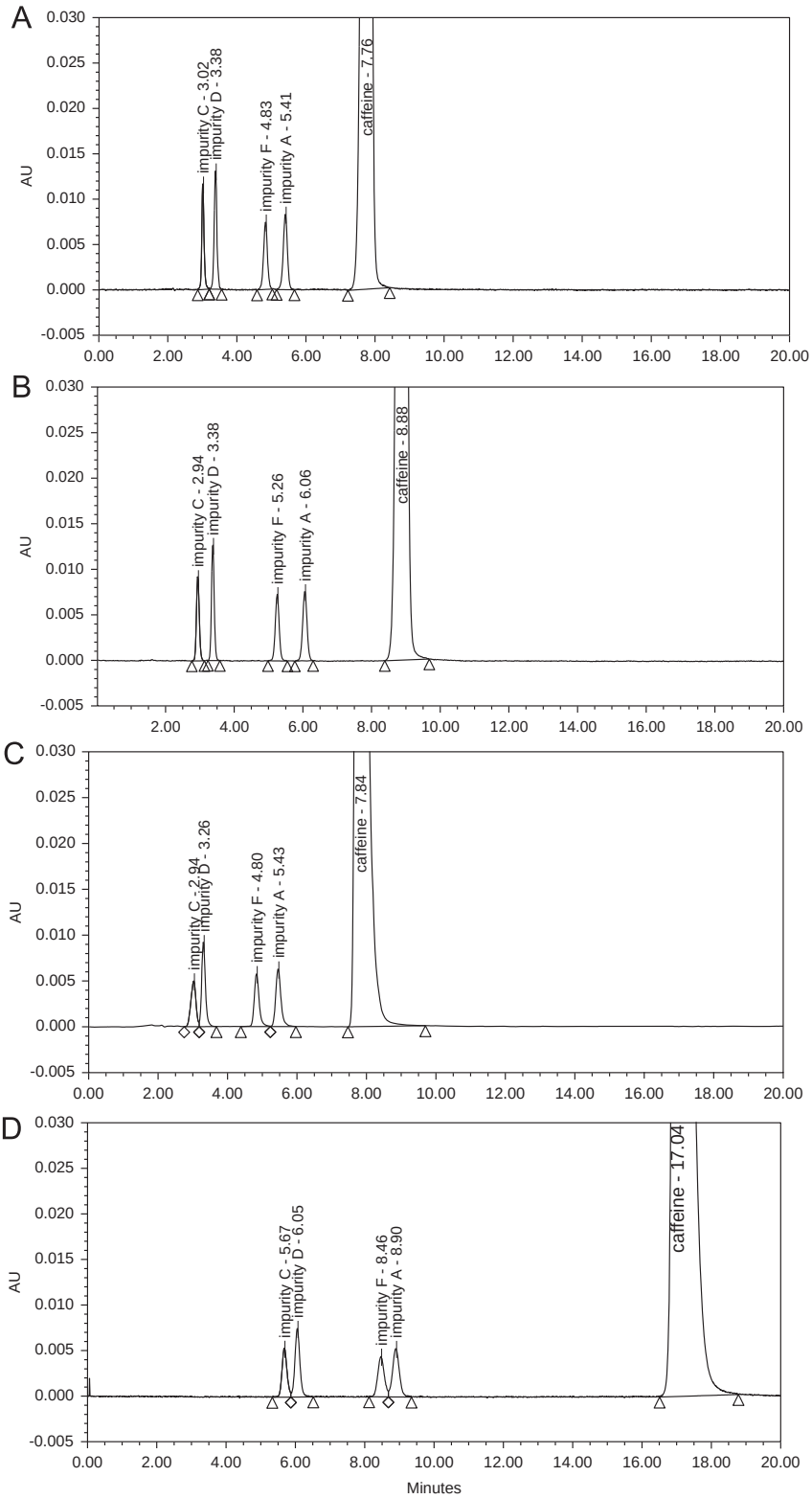

Fig. 2. LC analysis of caffeine and its four impurities: A, C, D, and F, performed on Sup-DB (A), Krom (B), Hyp-Elite (C) and Sph-ODS1(D) columns, respectively.

2.4. Data analysis

A comparative analysis of both data sets, including the four chromatographic test parameters for 35 stationary phases and their column performances for the separation of caffeine and its four impurities was performed to verify the potential of the KUL test procedure as a useful tool facilitating the RP-LC column selection. For graphic visualisation of the data sets containing many variables and objects, a multivariate data processing technique such as factor analysis (FA) was applied using the Statistica 9.0 package (StatSoft, Tulsa, USA). To begin with, an FA based on four chromatographic parameters: k'_{amb}, $rk'_{ba/ph\ pH2.7}$, $rk'_{tri/o\text{-}ter}$ and $k'_{2,2'\text{-}d}$ calculated for all stationary phases (Table 2) was performed. The FA plot picturing the variables in two-dimensional space is shown in Fig. 3. The FA plot for

Table 3
Summary of retention parameters of t_R, A_s and R_s for caffeine and related compounds in column performance test for 35 tested stationary phases.

Analysed substances	The position in the ranking list (column no.)		Impurity C		Impurity D			Impurity F			Impurity A			Caffeine		
Analytical column	Sup-DB	Krom	t_R	A_s	t_R	A_s	R_s	t_R	A_s	R_s	t_R	A_s	R_s	t_R	A_s	R_s
Sup-DB	1	23	3.02	1.14	3.38	1.02	2.83	4.83	0.97	8.37	5.41	0.95	2.76	7.76	0.94	9.12
Disc	2	22	2.92	0.90	3.19	1.07	2.54	4.46	1.00	8.39	4.98	0.99	2.88	6.95	0.98	8.84
Hyp-BDS	**3**	20	2.86	1.14	3.15	1.09	2.66	4.47	1.02	8.15	5.00	1.00	2.69	7.16	1.02	8.79
AC-AQ	4	24	3.59	1.11	4.00	1.07	2.60	5.84	1.03	9.40	6.47	1.01	2.63	*9.65*	1.06	10.55
Hyp-aQ	**5**	**29**	3.13	1.25	3.41	1.18	**1.93**	4.72	1.12	7.75	5.20	1.08	**2.45**	7.74	1.19	10.22
Hyp-Elite	**6**	**10**	2.94	1.12	3.26	1.41	**1.58**	4.80	1.32	8.49	5.43	1.26	2.79	7.84	1.36	8.47
Zor-C18	7	18	2.91	1.41	3.35	1.24	3.39	4.99	1.15	8.61	5.78	1.20	3.36	8.70	1.34	9.39
AC-C18	8	8	3.02	0.96	3.38	1.17	2.51	4.94	1.14	8.99	5.58	1.14	3.04	8.07	1.17	9.52
Pack-AQ	9	17	4.00	1.17	4.72	1.10	4.39	7.07	1.04	11.42	8.07	1.00	4.01	*12.53*	1.06	13.40
Aqua	10	11	3.84	1.09	4.55	1.03	4.28	6.93	0.97	11.00	7.95	0.97	3.76	*12.27*	0.99	12.00
Wak-HG	**11**	**7**	2.95	1.06	3.31	1.09	**2.11**	4.85	1.02	9.03	5.52	1.01	3.19	8.02	1.05	9.42
Nuc-HD	**12**	**4**	2.95	1.07	3.35	1.18	**2.22**	5.05	1.13	8.66	5.77	1.12	2.96	8.49	1.19	8.72
Nuc-Pyr	13	14	3.49	1.21	4.17	1.15	3.87	6.33	1.13	9.33	7.23	1.15	3.10	*10.93*	1.17	10.00
Zor-Aq	14	32	5.88	1.14	8.86	1.04	9.86	11.43	1.08	6.42	14.40	1.04	6.05	*23.60*	1.45	12.62
AC-AR	**15**	**19**	4.62	1.10	4.28	1.06	**1.97**	6.37	1.03	8.35	6.87	1.03	**2.04**	*11.47*	1.03	14.11
Pro-gy	**16**	**9**	3.09	0.97	3.50	1.22	**2.43**	5.34	1.18	10.03	6.13	1.15	3.51	8.95	1.16	9.91
Nuc-Sph	**17**	**28**	4.03	0.91	4.23	1.24	**1.12**	6.49	1.09	9.44	7.26	1.08	2.53	*11.48*	1.13	10.81
Zor-XDB	**18**	**6**	2.66	1.01	3.02	1.16	**2.33**	4.51	1.08	9.59	5.18	1.07	3.57	7.60	1.10	10.17
Iner-GL	**19**	**5**	3.19	1.17	3.63	1.20	**2.42**	5.48	1.12	9.05	6.27	1.10	3.14	*9.13*	1.13	9.11
Iner-HI	**20**	**13**	3.16	0.98	3.54	1.17	**1.84**	5.23	1.12	7.50	5.93	1.16	2.51	8.60	1.21	7.57
Luna	21	15	3.27	0.84	3.75	1.19	3.50	5.79	1.14	10.81	6.69	1.15	3.79	*9.85*	1.18	10.41
SunFire	22	12	3.36	0.98	3.85	1.14	3.68	5.96	1.09	10.32	6.87	1.08	3.54	*10.11*	1.08	9.89
Krom	23	1	2.94	1.10	3.38	1.07	2.93	5.26	1.02	10.64	6.06	1.00	3.66	8.88	1.00	10.12
Nuc-C18	**24**	**21**	4.44	0.81	5.10	**0.79**	2.87	7.36	**0.73**	8.24	8.15	**0.79**	**2.37**	*13.44*	**0.77**	11.66
AC-HL	25	2	2.94	1.17	3.37	1.12	2.90	5.19	1.06	10.60	5.97	1.06	3.62	8.73	1.06	10.03
Sym-ry	26	3	2.65	1.19	3.02	1.31	2.73	4.58	1.10	10.43	5.25	1.06	3.41	7.63	1.09	9.62
Nuc-Nau	27	26	3.95	0.98	4.60	0.93	2.98	5.54	0.88	7.18	7.51	0.90	2.95	*10.07*	0.91	6.33
Aq-sil	**28**	**27**	6.74	0.93	7.14	1.16	**1.27**	9.98	0.93	7.41	10.65	1.10	**1.46**	*19.11*	1.15	12.56
Nuc-AB	**29**	**25**	2.25	1.25	2.50	1.34	**1.72**	3.61	1.30	6.43	4.07	1.28	**2.34**	5.65	**1.54**	6.41
Nuc-Isis	**30**	**16**	2.53	1.14	2.90	0.94	2.54	4.34	1.08	7.78	4.97	1.08	**2.44**	7.09	1.08	6.75
Sph-ODS2	**31**	**30**	3.65	1.28	4.12	1.21	**2.49**	5.91	1.15	7.96	6.45	1.16	**2.07**	*10.59*	1.47	12.15
Hyp-Gold	**32**	**31**	3.03	1.18	3.29	1.08	**2.23**	4.46	1.00	7.51	4.91	0.98	**2.49**	6.85	1.02	8.62
Xbr-Shield	**33**	**34**	2.97	0.94	3.25	1.19	**1.62**	4.74	1.13	8.92	5.33	1.12	2.89	7.42	1.13	8.35
Sym-Shield	34	33	2.94	1.18	3.50	1.16	3.28	5.17	1.12	8.57	6.02	1.08	3.51	8.11	1.12	7.12
Sph-ODS1	**35**	**35**	5.67	1.06	6.05	1.15	**1.50**	8.46	0.96	8.33	8.90	1.18	**1.31**	*17.04*	**1.82**	16.11

Meaning of symbols is explained in the text. The columns non-suitable for the separation of caffeine in accordance with the SST requirements are indicated in bold. The columns with t_R of caffeine above 9 min are indicated in italic.

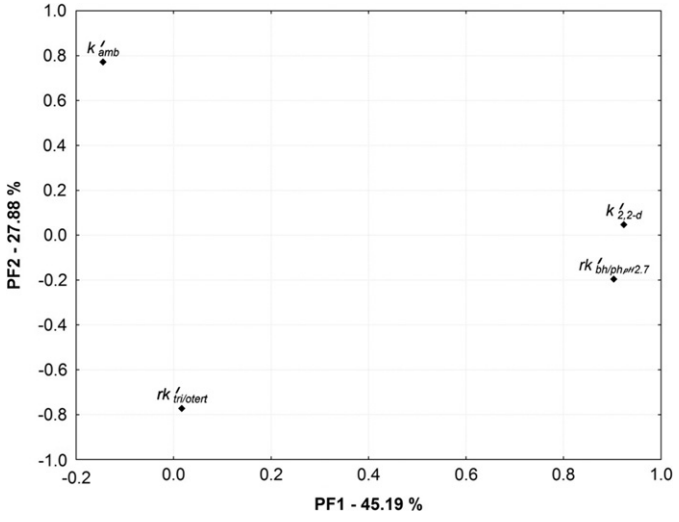

Fig. 3. A two-dimensional FA plot for four chromatographic parameters obtained by the KUL method for thirty five tested RP-LC stationary phases.

the stationary phases, where the column numbers were arranged in the order of the increasing *F*-values after the selection of the Sup-DB and Krom columns as the reference stationary phases (Table 2), is illustrated in Fig. 4A and B, respectively. Finally, the retention parameters of t_R and R_s for caffeine and the related compounds established for all stationary phases (Table 3) were evaluated in an FA. For both reference columns the same numbers 1–35 were continued for the stationary phases as reported in Table 2. The two-dimensional FA plot for the variables is presented in Fig. 5, whereas the FA plots for the columns described in accordance with the increasing *F*-value calculated after the choice of the Sup-DB and Krom column as the reference stationary phases are shown in Fig. 6A and B, respectively.

3. Results and discussion

3.1. Column classification

Column classification in the KUL ranking system is based on four chromatographic parameters, namely k'_{amb}, $rk'_{bh/ph\ pH2.7}$, $rk'_{tri-/o-tert}$ and $rk'_{2,2-d}$, which enables the establishment of the *F*-values. The parameters calculated for 35 columns examined during this study are reported in Table 2. Upon the selection of the Sup-DB column to serve as reference it was found that nine stationary phases, from Disco to Pack-AQ, were characterised by $F<2$. For them, lower $rk'_{2,2-d}$-values were observed. Eighteen other columns were found ranking in the middle ($2<F<6$), and for them higher k'_{amb} and medium $rk'_{2,2-d}$ parameters were noted. Columns Aq-sil to Sph-ODS1 shared the *F*-values >6, which indicates that their chromatographic behaviour is significantly different. In their case higher $rk'_{tri-/o-tert}$ values and

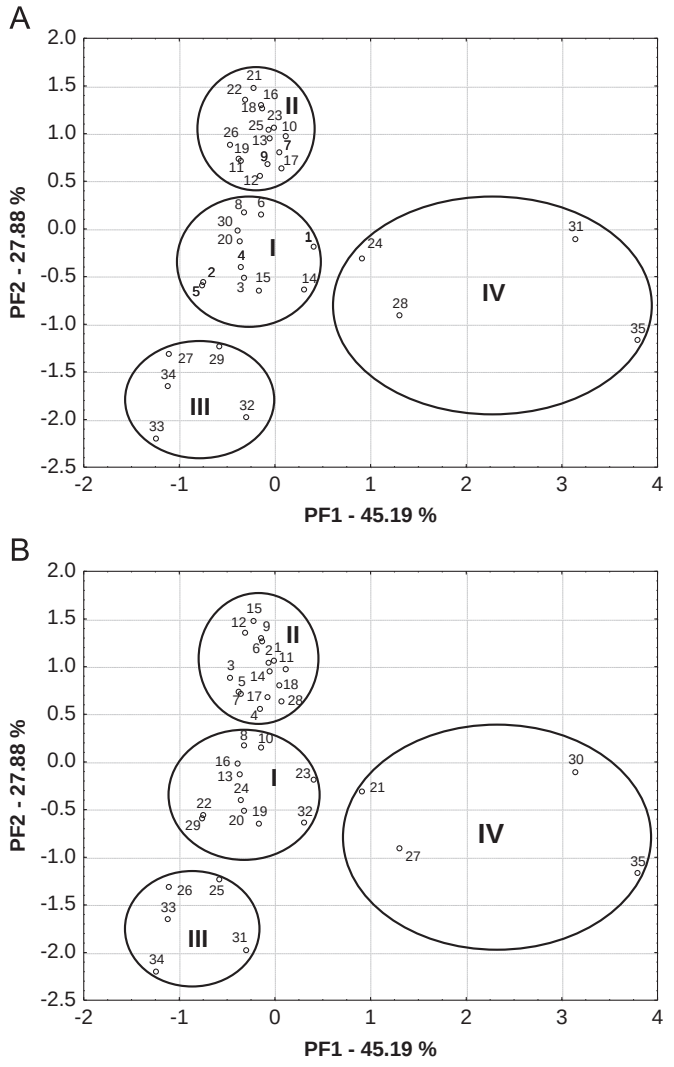

Fig. 4. Projection of 35 RP-LC stationary phases onto the space of the first two PFs from the FA of the four chromatographic test parameters obtained by the KUL method and assigned in line with the increasing *F*-values to Sup-DB (A) and Krom (B).

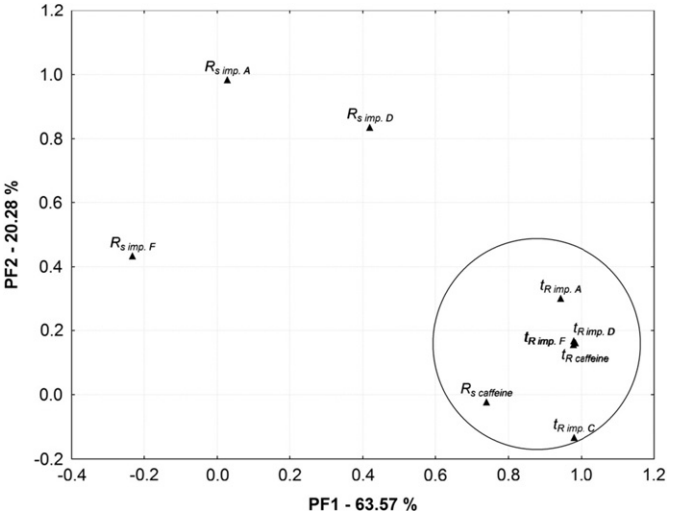

Fig. 5. A two-dimensional FA plot based on the retention parameters t_R and R_s of the analytes in the column performance test.

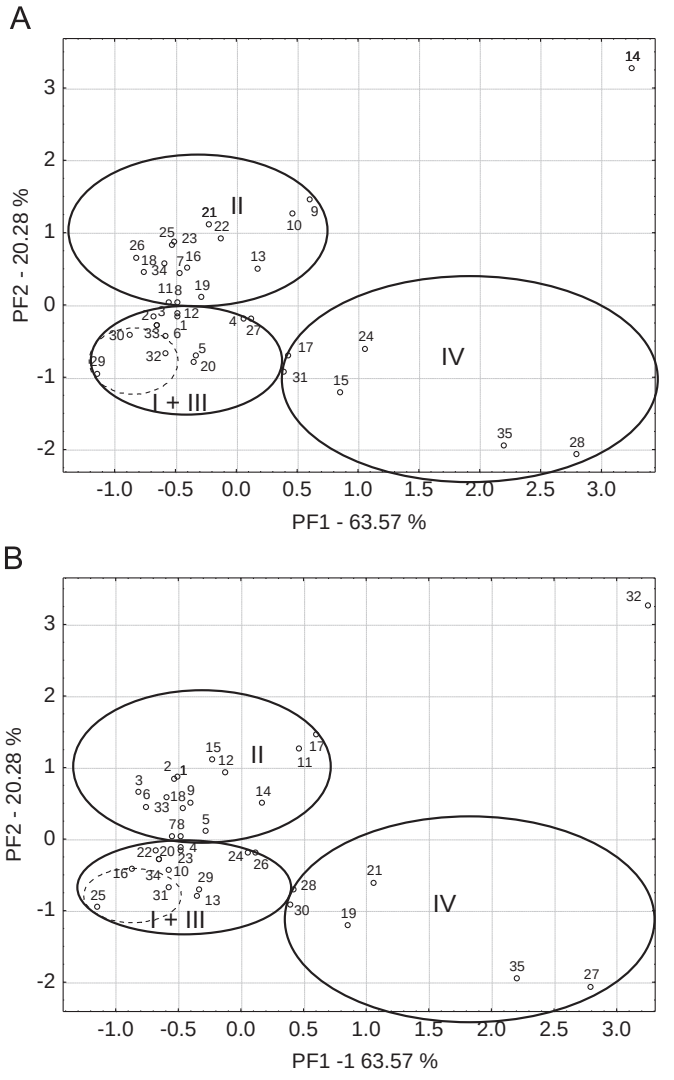

Fig. 6. Projection of 35 RP-LC stationary phases onto the space of the first two PFs from the FA of the retention parameters t_R and R_s of the analytes in the column performance test, assigned in line with the increasing *F*-values to Sup-DB (A) and Krom (B).

significant differences in the k'_{amb}, $rk'_{bh/ph\ pH2.7}$ and $rk'_{2,2\text{-}d}$ parameters were observed.

When the Krom column was selected as reference, it was noticed that the localisation of the stationary phases in the KUL ranking list was different than that for the Sup-DB. Thus, seventeen other columns from AC-HL to Zor-C18 ranked high. Nine others, including Sup-DB, were identified as ranking in the middle, whereas another eight were found in the lowest ranking positions. Among them, five stationary phases (Sph-ODS2, Hyp-aQ, Sym-Shield, Xbr-Shield and Sph-ODS1) had earlier been also found most different for Sup-DB in the KUL list ranking.

Next, for a more detailed interpretation of the theoretical KUL results for 35 brands of stationary phases an FA based on the four column parameters was performed. This statistical technique can be used to reduce the number of variables and detect structure in the relationships between the variables or objects without losing any significant information. The FA only used the variability of an item shared with the other items to identify the criteria, which affect retention and the possible correlations between the criteria themselves, and to group the columns within two- or three-dimensional space based on the data initially spread over a multi-dimensional space. The application of the chemometric methods

for column selectivity in the RP-LC, especially the principal component analysis (PCA), has already been discussed in publications [8,12,17,20–23,30,31,38,39]. Nevertheless, the FA is preferable as the chemometric method whenever the goal of the analysis is to detect the structure, whereas PCA is frequently applied as a data reduction method. In this paper, we used the FA based on the varimax criterion to seek the rotated loadings that maximise the variance of the squared loadings for each factor. Some of the loadings are as large as possible in the rotation model, whereas the rest are calculated as small as possible in terms of the absolute value.

The two-dimensional FA plot for variables derived from the four chromatographic test parameters is shown in Fig. 3. On the other hand, the FA plot for the columns where the stationary phases were described in accordance with the increasing *F*-values after the selection of the Sup-DB and Krom column as the references (Table 2) is illustrated in Fig. 4A and B, respectively. Notably, the data variability of 45.19% originated mainly from the variability of $rk'_{bh/ph\ pH2.7}$ and $rk'_{2,2\text{-}d}$, which is explained by the first principal factor (PFs). Those two variables were positioned centrally, close to each other, on the right side of the plot (Fig. 3), which is easy to explain since both parameters characterise the same feature of the stationary phase—silanol activity. The k'_{amb} variables describing hydrophobicity and $rk'_{tri\text{-}/o\text{-}tert}$ reflecting the possibility of steric selectivity were found to position themselves as outliers in the top left corner of the plot (k'_{amb}) and the bottom left corner of the graph ($rk'_{tri\text{-}/o\text{-}tert}$), respectively. These parameters were related mainly to the second PFs, which explained the 27.88% variance of the analysed variables. Thus, the observed differences in the positions of k'_{amb} and $rk'_{tri\text{-}/o\text{-}tert}$ were also correlated to different chromatographic properties of the stationary phases reflected in the parameters. As the two-dimensional FA plots in Fig. 4A and B illustrate, the columns were placed in four clusters I, II, III, and IV. The Sup-DB was observed to position itself in cluster I together with the stationary phase nos. 2–6, 8, 14, 15, 20, and 30 placed centrally on the left side of the plot (Fig. 4A). Those stationary phases all shared lower and middle values of the k'_{amb} parameters (except for Nuc-Isis—5.420) and $rk'_{2,2\text{-}d}$ (Table 2). They were also observed to share middle values of $rk'_{bh/ph\ pH2.7}$ (except for Sup-DB—0.215) and $rk'_{tri\text{-}/o\text{-}tert}$. Most columns included in cluster I had the carbon load of $\leq 15.5\%$ and silica varying in type between A, B, and EP, the latter having embedded or end-capped polar groups (EP) (Table 1).

In the case of Krom as the reference column cluster I it included four stationary phases with the *F*-values <2, five others with the *F* between 2 and 6, and two phases with the *F* parameters above 6 (Fig. 4B). Krom was observed in cluster II located in the upper left part of the plot together with the columns of the highest and high-ranked positions, as well as stationary phase no. 28. For them, higher k'_{amb} parameters above 4.164 (except for no. 28—2.738) and $rk'_{2,2\text{-}d}$ between 7.422 and 9.879 were recorded (Table 2). The $rk'_{bh/ph\ pH2.7}$ values were lower and medium, while $rk'_{tri\text{-}/o\text{-}tert}$ ranged from 1.00 to 1.566. Those columns, except for Nuc-HD, have a more neutral silica gel type B (Table 1). When Sup-DB was selected as the reference column (Fig. 4A), two columns with the $F<2$, and thirteen stationary phases with the middle-ranking positions fell in cluster II. Hence, the Sup-DB and Krom columns belong to the stationary phases of different chromatographic properties. The same is confirmed in the KUL and FA results. The finding is consistent with their chemical structure, since Sup-DB has the carbon load of 11% and silica type A while Krom belongs to the stationary phases of higher carbon loads (19%) with silica type B (Table 1). In the case of both columns nine significantly different stationary phases ranking high or highest were clearly identified (Fig. 4A and B). These columns fell in the two clusters occupying the lower left part of the plot (cluster III—nos. 27, 29, 32–34 for Sup-DB, and 25, 26, 31, 33, 34 for Krom) and the right middle side of the graph (cluster IV—nos. 24, 28, 31, 35 for Sup-DB, and 21, 27, 30 and 35 for Krom). Concerning the stationary phases included in cluster III, the lower k'_{amb}, lower and intermediate $rk'_{2,2\text{-}d}$, and significantly higher values of $rk'_{tri\text{-}/o\text{-}tert}$ were obtained in calculations conducted under the KUL method (Table 2). The same columns were characterised by a broad range of $rk'_{bh/ph\ pH2.7}$: 0.022–0.118. These differences were due to the use of the EP silica in the three columns, which resulted in lower $rk'_{bh/ph\ pH2.7}$, while the other two belonged to the stationary phases with silica types A and B (Table 1). In the case of the stationary phases falling in cluster IV, significantly higher values of the $rk'_{2,2\text{-}d}$, $rk'_{tri\text{-}/o\text{-}tert}$ and $rk'_{bh/ph\ pH2.7}$ parameters and lower value of k'_{amb} were found (Table 2). All those columns share a lower carbon load ($\leq 15\%$) and silica type A, except for Aq-sil (Table 1). Of course, the *F* parameter depends on the selection of the reference column [30–37]. Nevertheless, in both cases the KUL test procedure was used to characterise the columns whose positions in the ranking list were correlated with the *F*-values.

3.2. Column selectivity in separation of caffeine

The KUL classification system was tested by using the 35 classified stationary phases in the chromatographic separation of caffeine from its four related compounds A, C, D, and F performed in accordance with the Ph. Eur. monograph [3]. The composition of the mobile phase, the flow rate, the sample injection volume, and the wavelength for UV detection required the LC analysis to be exactly as prescribed by the Ph. Eur., even though the monograph gave only a general description of the stationary phase as *base-deactivated end-capped octadodecylsilyl silica gel for chromatography R* (*5* μm) *having 0.15* m *of length and diameter of 4.6* mm. On the other hand, the Ph. Eur. knowledge database [5] recommends the Sup-DB and Krom columns as suitable for separation of caffeine and its impurities. Still, other stationary phases enabling chromatographic separation and complying with the SST are also allowed for the analysis. The SST test for separation involves the minimum R_s of 2.5 for two "critical pairs" of impurities: C and D, and F and A. In accordance with the Ph. Eur. monograph, the desirable retention time of caffeine is about 8 min, although the parameter is not included among the SST requirements. Similarly, no specific Ph. Eur. prescription related to the symmetry factor (A_s) was introduced, which indicates that the A_s of 0.8–1.5 for a peak in the chromatograph obtained with the reference solution is required in LC separation of caffeine and its related compounds. Thus, a Sup-DB, Krom, and 33 other stationary phases having the required length and particle size (Table 1) tested earlier under the chromatographic test procedure were applied to separate caffeine and its four impurities. The experimental data set of t_R, A_s and R_s for the compounds of interest obtained for all stationary phases is reported in Table 3. The results revealed that the caffeine t_R between 7.0 and 9.0 min was observed for Sup-DB, Krom, and fifteen other columns examined. Shorter t_R of the active substance was recorded for three stationary phases, whereas a longer t_R of caffeine, ranging from 9.13 to 23.60 min, was obtained in the calculations for the remaining fifteen. However, this parameter was not considered critical in evaluation of the quality of the LC separation because the desirable t_R value of caffeine is recommended but not required for the separation. In the case of the A_s parameter it can also be noticed that the range limit of A_s for the analysed compounds required by the Ph. Eur. was extended by three columns (Nuc-C18, Nuc-AB and Sph-ODS1). One should also note that only nineteen columns gave chromatographic separation with $R_s>2.5$ for impurities C and D. Concerning the F and A pair

of impurities, the requirement was met by twenty-six stationary phases. In summary, proper LC assays of caffeine and the related compounds in compliance with the SST requirements for the two critical pairs were only attained for seventeen columns. Upon correlation of those results with the KUL results for the same 35 stationary phases it was noticed that the probability of incorrect separation of caffeine from its impurities increased in line with the increase in the *F*-values for both reference columns.

The probability for Sup-DB was found at 22.2% for the high-ranked columns ($F<2$), 50.0% for the stationary phases with the *F*-values between 2 and 6, and 87.5% for the columns of the lowest-ranked positions. Turning to Krom, eight stationary phases with $F<2$ were found non-suitable for LC separation of the compounds of interest (44.4%), just like four columns of the intermediate group in the KUL list ranking (44.4%) and six having the *F*-values >6 (75.0%). These results are similar to the findings previously reported in the literature [31–33,36–39]. However, we observed higher correlation between the KUL column classification based on the *F*-values and the column performance for the Sup-DB than for the Krom. It should also be emphasised that when the t_R values of the active substance were taken into account only twenty columns gave the separation with the desirable retention time of the last eluting compound, i.e. caffeine (about 8 min). Moreover, only nine columns were evaluated as suitable for caffeine separation in accordance with the SST requirements and the desirable t_R value of the active substance.

An FA served further detailed analysis of the results of the column performance test in the pharmaceutical practice. The positions of the variables in two-dimensional space formed in the FA are illustrated in Fig. 5, while the location of the columns in line with the increasing *F*-values upon the choice of Sup-DB and Krom as the reference columns is shown in Fig. 6A and B, respectively. The first two PFs in the plots account for more than 83.85% of the data variability where the PF1 was mainly related to the variance of t_R of the analytes and R_s of caffeine, whereas the PF2 was linked primarily to the variability of R_s for impurities A and D, and then of the R_s of impurity F. Six variables were found in the same cluster located in the bottom right corner of the graph (Fig. 5), while the R_s of impurities A, D, and F found themselves as outliers in the upper left part of the plot. In Fig. 6A and B, where the columns are presented in two-dimensional space, one can notice that they were spread between three clusters with one stationary phase Zor-Aq classifying as an outlier in the upper right corner of the plot. In the case of that particular column with silica type EP, its k'_{amb} parameter previously identified as attaining the lowest value, and significantly high t_R and R_s of the analytes were observed (Tables 1–3). Unfortunately, the information on the carbon load of that column was proprietary (Table 1). The cluster described as I+III was located centrally in the left part of the plot and included most of the columns earlier classified under the KUL method as falling in cluster I and cluster III. The Nuc-HD was also placed in the group. In their case lower and medium t_R and R_s of the analytes were noticed. Interestingly, five stationary phases (Hyp-BDS, Disc, Sup-DB, AC-AQ, and Nuc-Nau) deemed suitable for the LC analysis of the compounds of interest in accordance with the Ph. Eur. requirements were placed in the upper part of the cluster. Those columns have the silica gel of different types (A, B, and EP) and the carbon load in the range of 11–16% (Table 1). However, only three stationary phases (nos. 1, 2 and 3—Fig. 6A; nos. 23, 22, 20—Fig. 6B) gave the separation with the desirable caffeine retention time. Seven other columns yielded chromatographic separation of impurities C and D at $R_s<2.5$ (Table 3), and insufficient R_s between impurities A and F, ranging from 2.34 to 2.49, were observed for four stationary phases assigned nos. 29, 30, 5, and 32 (Fig. 6A), and nos. 25, 16, 29, and 31 (Fig. 6B). As mentioned earlier, the ranges of the k'_{amb} and $rk'_{2,2\text{-}d}$ parameters calculated under the KUL method were similar for the stationary phases included in clusters I and III (Table 2); yet the same phases had highly diverse values of the $rk'_{tri\text{-}/o\text{-}tert}$ parameter, which describes the potential steric selectivity of the specific stationary phase.

Cluster II including Krom (no. 23 or 1) and fourteen other stationary phases were positioned in the top left corner of the plot (Fig. 6A and B), respectively. They served the determination of the medium values of the analyte t_R and R_s retention parameters, except for t_R of impurity D, which were lower than for the columns of other clusters (Table 3). Among them, only six columns (nos. 7, 8, 23, 25, 26, 34—Fig. 6A; nos. 1, 2, 3, 8, 18, 33—Fig. 6B) gave the separation of caffeine with the desirable t_R of 8 min. In addition, the experimental data retention ranges were extended to include higher R_s of impurities A, D, and F. Among them, eleven stationary phases were suitable for LC separation of caffeine and the related compounds complied with the SST requirements. All columns except for Sym-Shield have silica type B (Table 1). These stationary phases positioned themselves in the central and upper parts of the cluster while non-suitable columns were positioned in the bottom part of the group. Almost all columns in cluster II (13) were earlier observed to qualify in the same cluster based on the test of four chromatographic parameters (Fig. 4A and B). Notably, AC-C18 and Sym-Shield were in the group, even though under the KUL method the columns qualified for clusters I and III (Fig. 4A and B), respectively. Six other columns having different types of silica gel (A—3, B—2 and EP—1) and the carbon load ranging from 6.2% to 15.5% (Table 1) were located in cluster IV on the right side of the plot. Out of them, four columns had also been classified in cluster IV in accordance with the four test chromatographic parameters (Fig. 4A and B), while 5 AC-AR and Nuc-Sph had earlier been located in clusters I and II, respectively. All stationary phases from cluster IV were found to share the $R_s<2.5$ for two critical pairs of impurities C and D and/or A and F (Table 3). This made them unable to offer an appropriate LC analysis of the compounds of interest so as to comply with the SST requirements.

To recapitulate, the FA results for the theoretical data set obtained under the KUL approach and the respective column performance in separation of caffeine from its four impurities A, C, D, and F, carried out in accordance with the Ph. Eur. monograph confirmed that the classes of the stationary phases in both data sets were correlated. The correlations were observed regardless of whether Sup-BD or Krom stationary phase was selected as the reference. However, the probability of selecting the suitable column, evaluated using the general description of the column performance test (in compliance with the SST requirements) and the decrease in the *F*-values were correlated better when Sup-DB was adopted as the reference column than when Krom was the reference. On the other hand, the FA results based on four chromatographic test parameters (Fig. 4A and B) and column performance for 35 tested stationary phases (Fig. 6A and B) confirmed that nine columns in cluster II (including Krom) suitable for the LC analysis of caffeine and the related compounds were more significantly distinct from non-suitable ones (clusters III and IV) than was the case of Sup-DB and five other columns found in cluster I. Only two stationary phases, Nuc-Nau and Sym-Shield, were characterised incorrectly under the KUL method and classified in cluster III grouping most columns unsuitable for caffeine analysis. The correlation between the KUL ranking list and the results of the column performance test in separation of caffeine was observed worse when the t_Rs of the active substance were taken into account for the desirable value of 8 min. It should also be emphasised that in both cases the columns with the highest ranking positions (nos. 2 and 3) gave caffeine separation satisfying the SST requirements and the desirable retention time

of the active substance. Moreover, the obtained results confirmed that most of the appropriate LC separations of caffeine and related compounds compliant with the SST were performed using stationary phases having silica type B (11/17—64.7%). The probability was lower for the columns having embedded or end-capped polar group (4/17—23.53%) and silica type A (2/17—11.76%).

4. Conclusions

The paper discusses in detail the KUL approach for characterisation or classification of the RP-LC stationary phases when applied in real separation of caffeine and its four impurities. The columns, previously tested under the KUL test procedure, were applied to perform an LC analysis of the compounds of interest in accordance with the Ph. Eur. monograph. The obtained results confirmed that higher probability of selecting a suitable column existed with the Sup-DB as the reference where the *F*-values for 35 tested stationary phases obtained under the KUL approach were directly correlated with the results of the SST test characterising column performance of the other tests. On the other hand, in the FA analysis conducted based on the experimental data (analyte parameters t_R and R_s) obtained in the practical performance test, separation was found better for Krom and other stationary phases suitable for correct LC analysis of the compounds of interest than for Sup-DB and similar columns. In other words, in the practical test Krom was qualified in the cluster grouping most phases suitable for caffeine separation, whereas Sup-DB fell in the cluster which grouped a mixture of phases, both suitable and unsuitable for the analysis. Moreover, in both cases, the stationary phases of significantly different chromatographic properties were clearly identified by the KUL method. On the other hand, when both the SST requirements and desirable retention time of last eluting compound, caffeine, were taken into account, the worse correlation between the KUL ranking list and the results of column performance test was observed. Thus, the column ranking system can be considered a helpful although not excellent tool in the selection of the suitable column required for real separation of caffeine from its related compounds. Moreover, the paper provides a list of the stationary phases suitable for the LC analysis of caffeine and its impurities, all giving separation equivalent to Supelcosil LC-18-DB and/or Kromasil, which may be attractive for the pharmaceutical industry.

Novelty statement

The obtained results confirmed that higher probability of a suitable column selection was established when the *F*-values for 35 tested stationary phases provided by the Katholieke Universiteit Leuven (KUL) approach were directly correlated with the results of the system suitability test (SST) characterising the column performance test.

Factor analysis (FA) results based on four chromatographic test parameters and the column performance test described by the retention parameters showed what column offers better separation of the compounds of interest (caffeine and its respective impurities). For this analysis, the direct descriptions of the tested columns by the *F*-values offering the possibility of finding unequivocal relationships within both data sets was used.

Moreover, the stationary phases having significantly different chromatographic properties were clearly indicated by the KUL method. Thus, the column ranking system can be considered as a helpful tool in the selection of a suitable column required for real separation of caffeine from its related compounds.

Acknowledgements

The work was supported by the Polish State Committee for Scientific Research Projects no. N405 024340.

References

[1] B.A. Olsen, B.C. Castle, D.P. Myers, Trends Anal. Chem. 25 (2006) 796–805.
[2] E. Van Gyseghem, M. Jimidar, R. Sneyers, M. De Smet, E. Verhoeven, Y. Vander Heyden, J. Pharm. Biomed. Anal. 41 (2006) 751–760.
[3] European Pharmacopoeia, 6th ed. Council of Europe, Strasbourg, France, 2008.
[4] United States Pharmacopeia 31. The United States Pharmacopeial Convention, Rockville, Maryland, USA, 2008.
[5] Ph. Eur. Knowledge Database. 〈http://extranet.edqm.eu/publications/recherches_sw.shtml〉.
[6] USP-NF. Online 〈http://www.uspnf.com/uspnf〉.
[7] H.A. Claessens, M.A. van Straten, J. Chromatogr. A 1060 (2004) 23–41.
[8] E. Cruz, M.R. Euerby, C.M. Johnson, C.A. Hackett, Chromatographia 44 (1997) 151–161.
[9] H. Engelhardt, R. Grünner, Int. Lab. 29 (1999) 34–42.
[10] H.A. Claessens, M.A. van Straten, C.A. Cramers, M. Jezierska, B. Buszewski, J. Chromatogr. A 826 (1998) 135–156.
[11] M.R. Euerby, P. Petterson, LC-GC Europe 9 (2000) 665–677.
[12] P. Forlay-Frick, J. Fekete, K. Héberger, Anal. Chim. Acta 536 (2005) 71–81.
[13] R.J.M. Vervoort, M.W.J. Derksen, A.J.J. Debets, J. Chromatogr. A 765 (1997) 157–168.
[14] P. Jandera, K. Novotná, Anal. Lett. 39 (2006) 2095–2152.
[15] N.S. Wilson, M.D. Nelson, J.W. Dolan, L.R. Snyder, R.G. Wolcott, P.W. Carr, J. Chromatogr. A 961 (2002) 171–193.
[16] D.H. Marchand, L.R. Snyder, J.W. Dolan, J. Chromatogr. A 1191 (2008) 2–20.
[17] W. Fan, Y. Zhang, P.W. Carr, S.C. Rutan, M. Dumarey, A.P. Schellinger, W. Pritts, J. Chromatogr. A 1216 (2009) 6587–6599.
[18] M.M.H. Abraham, Pure Appl. Chem. 65 (1993) 2503–2515.
[19] A. Sándi, L. Szepesy, J. Chromatogr. A 818 (1998) 1–17.
[20] T. Bączek, R. Kaliszan, K. Novotńa, P. Jandera, J. Chromatogr. A 1075 (2005) 109–115.
[21] M. Michel, T. Bączek, S. Studzińska, K. Bodzioch, T. Jonsson, R. Kaliszan, B. Buszewski, J. Chromatogr. A 1175 (2007) 49–54.
[22] J. Jiskra, H.A. Claessens, C.A. Cramers, R. Kaliszan, J. Chromatogr. A 977 (2002) 193–206.
[23] A. Plenis, E. Balakowska, T. Bączek, J. Sep. Sci. 34 (2011) 3310–3321.
[24] K. Héberger, Trends Anal. Chem. 29 (2010) 101–109.
[25] H.A. Claessens, Trends Anal. Chem. 20 (2001) 563–583.
[26] K. Héberger, J. Chromatogr. A 1158 (2007) 273–305.
[27] R. Kaliszan, Chem. Rev. 107 (2007) 3212–3246.
[28] R. Put, Y. Vander Heyden, Anal. Chim. Acta 602 (2007) 164–172.
[29] T. Németh, E. Haghedooren, B. Noszál, J. Hoogmartens, E. Adams, J. Chemometrics 22 (2008) 178–185.
[30] D. Visky, Y. Vander Heyden, T. Iványi, P. Baten, J. De Beer, Z. Kovács, B. Noszál, J. Hoogmartens, J. Chromatogr. A 1012 (2003) 11–29.
[31] 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.
[32] P. Dehouck, D. Visky, G. Van den Bergh, E. Haghedooren, E. Adams, A. Kerner, Y. Vander Heyden, D.L. Massart, Z. Kovács, B. Noszál, J. Hoogmartens, LC-GC Europe 17 (2004) 592–601.
[33] D. Visky, E. Haghedooren, P. Dehouck, Z. Kovács, K. Kóczián, B. Noszál, J. Hoogmartens, E. Adams, J. Chromatogr. A 1101 (2006) 103–114.
[34] E. Haghedooren, J. Diana, B. Noszál, J. Hoogmartens, E. Adams, Talanta 71 (2007) 31–37.
[35] E. Haghedooren, A. Kerner, B. Noszál, J. Hoogmartens, E. Adams, J. Pharm. Biomed. Anal. 44 (2007) 634–639.
[36] K. Kóczián, E. Haghedooren, S. Dragovic, B. Noszál, J. Hoogmartens, E. Adams, J. Pharm. Biomed. Anal. 44 (2007) 894–905.
[37] E. Haghedooren, T. Németh, S. Dragovic, B. Noszál, J. Hoogmartens, E. Adams, J. Chromatogr. A 1189 (2008) 59–71.
[38] S. Dragovic, E. Haghedooren, T. Nemeth, I.M. Palabiyik, J. Hoogmartens, E. Adams, J. Chromatogr. A 1216 (2009) 3210–3216.
[39] J. Szulfer, A. Plenis, T. Bączek, J. Chromatogr. A 1229 (2012) 198–207.
[40] 〈http://pharm.kuleuven.be/pharmchem/Pages/ccs.html〉.