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Selection of reversed-phase liquid chromatographic columns with diverse selectivity towards the potential separation of impurities in drugs

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Abstract

To select appropriate stationary phases from the continuously expanding supply of potentially suitable HPLC columns, the properties of 28 frequently applied stationary phases were determined by measuring several chromatographic parameters. From these results, based on chromatographic expertise, eight stationary phases with different properties and selectivities were selected. The aim of this study is to apply chemometric tools to evaluate the initially selected set of columns, i.e. a more systematic approach for making such a selection is examined. Starting from the information obtained on the 28 stationary phases, the re-evaluation was performed independently based on the chemometric techniques Pareto-optimality, principal component analysis (PCA), and Derringer's desirability functions. The aim was to select a set of efficient columns exhibiting large selectivity differences. The chemometrically selected stationary phases were divided in groups based on hydrophobicity, a critical retention-determining property in reversed-phase chromatography. This allowed to further reducing the selection to three columns. It is demonstrated that the selection by the chemometric approaches in general is fairly comparable with the initial selection.
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1. Introduction

The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), the organization that establishes the quality standards for drugs on a world-wide scale, demands that all impurities exceeding a certain threshold in a pharmaceutical product should be characterized, i.e. identified and/or quantified [1]. These drug impurities, which may cause undesired side effects, can arise during synthesis, purification or degradation during storage [1]. To characterize all components, it is necessary to develop a (chromatographic) separation method. A set of orthogonal chromatographic systems having different selectivities may provide the initial separation conditions for method development [2,3]. Applying in parallel two or more of such systems to impurity profiling of drugs maximizes the possibility that all substances can be unveiled [2,4]. The type of stationary phase, the mobile phase composition and pH have the most important influence on the orthogonality of chromatographic systems [2,3,5]. Prior to the selection/definition of orthogonal systems the columns involved should be characterized.

The characterization of the stationary phases can be performed by chromatographic, spectroscopic or physical approaches [6–11]. The physical parameters (e.g. carbon load, particle size, surface area) often show little correlation with the performance of the column [12], while the spectroscopic techniques (e.g. ^{29}Si NMR) deliver com-

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plex data of surface characteristics for which the relation to chromatographic observations is uncertain [13]. The chromatographic approaches specify physico-chemical interactions between simple well-characterized analytes and a stationary phase [14,15]. Many different tests to determine silica-based reversed-phase column properties, e.g. efficiency, hydrophobicity, steric selectivity, hydrogen-bonding capacity, ion-exchange capacity, and silanol activity, are described [6,7,10–12,14–22].

In this study, 28 frequently applied columns, selected for their pH-stability and potential differences in selectivity, were characterized through the measurement of eight parameters, representing hydrophobicity, steric selectivity, efficiency, silanol activity, H-bonding capacity and ion-exchange capacity. The *hydrophobicity* is measured by the methylene activity of the stationary phase. It reflects the possibility of the phase to separate two molecules only different in one methylene group, e.g. amylbenzene and butylbenzene, or ethylbenzene and toluene [8,9,11]. Other tests use the retention factor of, for instance, chrysene [23], toluene [21] or acenaphthene [24] as a criterion for hydrophobicity. In this study, the retention factor of amylbenzene ($k_{\text{amylbenzene}}$) was determined. Amylbenzene is an apolar hydrocarbon that strongly interacts with the hydrophobic reversed phase. The interactions are more intense when the hydrophobicity of the phase increases, leading to an increased retention factor.

The *steric selectivity* expresses the possibility of a stationary phase to separate two molecules differing in their three-dimensional structure. This property is determined from the separation of the aromatic hydrocarbons *o*-terphenyl (*o*-ter) and triphenylene (triphen) [11,16,25]. Both molecules have a similar molecular weight and hydrophobicity, but triphenylene is a rigid planar structure while o-terphenyl is twisted [9,11,16]. The better separated (expressed as selectivity factor $\alpha_{o\text{-ter/triphen}}$) the higher the steric selectivity of the stationary phase [11].

The *efficiency* gives information about the quality of the filling process and the physical properties of the particles. The efficiency depends on the particle size and on the alkyl-chain density on the silica surface [16,26]. Higher densities and smaller particles result in narrower and better-separated peaks. To measure column efficiency, apolar components, usually aromatic hydrocarbons (benzene, toluene, ethylbenzene, butylbenzene or amylbenzene) are injected [11,16]. The efficiency was calculated as the corrected plate height from the triphenylene peak, $h_{\text{triphenylene}}$. The corrected plate height is used to enable comparing stationary phases with different particle sizes. It should be as low as possible to have the best efficiency.

The *silanol activity* reflects the influence on the solutes' retention caused by remaining silanol groups at the silica surface and is pH-dependent. The differences in silanol activity between stationary phases can be explained by differences in silica, pretreatments and bonding procedures [9,16]. Several compounds and parameters to express this property are described: (i) the selectivity factor of caffeine and theophyllin [27], of either amitriptyline or propranolol to acenaphthene [12], or of nitrobenzene to benzene and indole to phenanthrene [28], (ii) the relative retention time of diphenhydramine to 5-(*p*-methylphenyl)-5-phenylhydantoin (MPPH) [29], (iii) the peak symmetry and retention factor of nitrobenzene, benzonitrile, benzylalcohol [30], (iv) the retention time or peak symmetry of *N*,*N*-diethylaniline [26], (v) the peak symmetry of diphenhydramine [21], amitriptyline or propranolol [12]. In this study, the tailing factors (t_1, t_2 and t_3) of an in-house basic, neutral and acidic compound, respectively, were determined at pH 7.0. Since low silanol activity implies symmetrical and narrow peak shapes, which are favorable for high selectivities and sensitivities [8], it is desired that t_1, t_2 and t_3 are as low as possible.

The parts of silanol activity determined by either H-bonding or ion-exchange, are calculated from the selectivity of two components, the retention of the first, caffeine, depending on both hydrophobic and H-bonding interactions, and of the second, benzylamine, on ion-exchange and hydrophobic interactions, towards the retention of phenol, which solely depends on hydrophobic interactions [8,17,25]. In our study, the selectivity factors between the in-house basic and neutral component at pH 7.0 (α_1), and between the acidic and neutral component at pH 2.5 (α_2), were determined. The ion-exchange capacity (α_1) is a measure of the ion-exchange between the basic substance and the residual silanol groups. At pH 7.0, the silanol groups appear ionized, and a strong interaction is possible with positively charged basic solutes [8]. The hydrogen-bonding capacity (α_2) is determined at pH 2.5 because the residual silanol groups, which are not dissociated, will undergo dipole interactions [9]. As both properties can be considered a part of the silanol activity, it will increase when α_1 decreases and α_2 increases. Therefore, columns with separations for which α_2 is as low and α_1 is as high as possible are the most desired. At pH 7.0, if silanol activity is encountered, it causes more retention of the base, which will move towards the neutral substance's peak, decreasing α_1. At pH 2.5, the silanol activity is lower since the hydroxyl groups are not dissociated anymore. However, now H-bonding occurs. The acidic component is more neutral at pH 2.5 and shows more retention in case of higher silanol activity, leading to an increased α_2-value.

An initial set of eight stationary phases with desired properties was selected based on chromatographic expertise. The aim of this study is to select a set of suitable columns by applying different well-established chemometric tools. A proper tool for this problem should not only allow selecting a set of suitable columns from the 28 tested, but also should easily enable evaluating newly tested phases and reconsidering the selection from the new set of columns. Once a proper chemometric tool is identified, the user should not have extensive chemometric knowledge to apply it.

Selecting a suitable column set is finding a good compromise between the various stationary phase properties. It

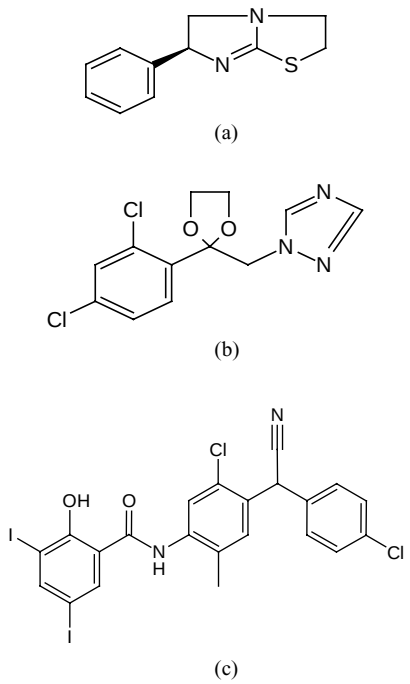


Fig. 1. Structures of: (a) levamisole, (b) azaconazole, and (c) closantel.

involves a multicriteria decision-making problem. The different chemometric approaches tested to select a proper set of columns are: (i) the Pareto-optimality concept, (ii) principal component analysis (PCA), and (iii) Derringer's desirability functions. The Pareto-optimality concept is a method to obtain a compromise between criteria in mutual conflict [31,32]. Principal component analysis [33,34] is a feature reduction technique, which enables to visualize multidimensional data sets. In the literature [8,14,15,25], it has been shown that PCA can distinguish groups of stationary phases with different properties. Derringer's desirability functions allow combining several responses into one [35–38]. This method can be applied to select columns with the best overall separation performances. Finally the results of the different approaches were compared with the initially selected columns.

2. Experimental

2.1. Reagents

The test mixture for hydrophobicity, steric selectivity and efficiency contains uracil (0.10 mg/ml) to determine the dead time, *o*-terphenyl (0.20 mg/ml), amylbenzene (1.50 mg/ml) and triphenylene (1.00 mg/ml) (Fluka, Buchs, Switzerland), dissolved in methanol/tetrahydrofuran/25% ammonium hydroxide 40:40:20% (v/v/v). The in-house test mixture for silanol activity consists of an acidic (closantel), basic (levamisole) and neutral (azaconazole) compound (all Janssen Pharmaceutica N.V., Beerse, Belgium) at a concentration of 0.10 mg/ml, and dissolved in methanol. Their structures are shown in Fig. 1. The sample solutions were prepared using methanol for HPLC (Acros Organics, Geel, Belgium), tetrahydrofuran (Fluka) and ammonium hydroxide (Merck, Darmstadt, Germany).

The mobile phases were prepared using methanol for HPLC and acetonitrile for HPLC far U.V. (Acros Organics) and ammonium acetate Microselect (Fluka). To examine silanol activity, the pH of the mobile phase was adjusted with ammonium hydroxide (Merck) and trifluoroacetic acid for protein sequence analysis (Fluka). Milli-Q water, prepared with the Millipore purification system (Millipore, Molsheim, France), was used in the test mixtures and mobile phases.

2.2. Chromatographic conditions

The 28 stationary phases tested are summarized in Table 1. The test conditions for hydrophobicity, steric selectivity and efficiency consisted of an isocratic elution with methanol/water 80:20% (v/v), while for silanol activity a linear gradient (Table 2) was applied. The detection wavelength was 220 nm, and the volume injected 10 μl. The flow rate depended on column and particle dimensions (Table 3), and was adapted to obtain the same linear velocity on each column [39]. The mixtures were injected until three stable retention times were achieved for all components.

Table 1
The tested columns with their dimensions, distributors and properties

Column number	Column	Column dimensions	Distributor	Stationary phase properties
1	ZirChrom-PBD	100 mm × 4.6 mm i.d., 3 μm	ZirChrom Separations, Anoka, MN	Zirconia-based phase coated with polybutadiene-polymer
2	SymmetryShield RP_8	150 mm × 4.6 mm i.d., 3.5 μm	Waters, Milford, MA	C_8-Silica shielded through an embedded polar group
3	Discovery RP-AmideC16	150 mm × 4.6 mm i.d., 5 μm	Supelco, Bellefonte, PA	High-purity C_{16}-silica with a polar-embedded amide function bonded to the silica surface with a propyl group
4	Zorbax Bonus-RP	150 mm × 4.6 mm i.d., 5 μm	Agilent Technologies, Palo Alto, CA	Triple-endcapped ultrapure C_{14}-silica with embedded polar amide group and sterically protecting diisopropyl group

Table 1 (*Continued*)

Column number	Column	Column dimensions	Distributor	Stationary phase properties
5	XTerra RP_{18}	100 mm × 4.6 mm i.d., 3.5 μm	Waters	Hybrid C_{18}-silica shielded through embedding a polar group
6	XTerra MS C_{18}	100 mm × 3.0 mm i.d., 3.5 μm	Waters	Hybrid C_{18}-silica with trifunctional bonding and embedded polar group
7	Hypurity Elite C18	125 mm × 4.0 mm i.d., 5 μm	Thermo Hypersil Keystone, Cheshire, UK	Metal-free, endcapped C_{18}-silica
8	Discovery C18	150 mm × 4.6 mm i.d., 5 μm	Supelco	High-purity endcapped C_{18}-silica
9	Hypersil Elite C18	125 mm × 4.0 mm i.d., 5 μm	Thermo Hypersil Keystone	Highly base-deactivated, endcapped C_{18}-silica
10	YMC ODS-AQ	100 mm × 4.6 mm i.d., 3 μm	YMC c/o Waters, Milford, MA	C_{18}-Silica with hydrophilic endcapping
11	Luna Phenyl-Hexyl	100 mm × 4.6 mm i.d., 3 μm	Phenomenex, Torrance, CA	Ultrapure silica with high-density bonded, hexyl-linked phenyl
12	SymmetryShield RP_{18}	100 mm × 4.6 mm i.d., 3.5 μm	Waters	C_{18}-Silica shielded through an embedded polar group
13	Hypersil C18-BDS	150 mm × 3.0 mm i.d., 5 μm	Thermo Hypersil Keystone	Base-deactivated C_{18}-silica with high ligand bonding
14	Eurospher 100 C18	125 mm × 4.0 mm i.d., 5 μm	Knauer, Berlin, Germany	Endcapped C_{18}-silica
15	Zorbax SB-C18	150 mm × 3.0 mm i.d., 5 μm	Agilent Technologies	Monofunctional, non-endcapped ultrapure C_{18}-silica with sterically protecting bulky diisobutyl side chain groups
16	Zorbax Eclipse XDB-C_{18}	250 mm × 4.6 mm i.d., 5 μm	Agilent Technologies	Densely bonded, double-endcapped ultrapure C_{18}-silica with dimethyl side chains
17	YMC ODS-H80 J'sphere	100 mm × 4.6 mm i.d., 4 μm	YMC c/o Waters	Polymeric bonded, fully-endcapped C_{18}-silica with high carbon loading
18	Alltima C18	100 mm × 4.6 mm i.d., 3 μm	Alltech, Deerfield, IL	Base-deactivated, polymerically bonded, double-endcapped C_{18}-silica
19	Zorbax Extend-C18	150 mm × 4.6 mm i.d., 5 μm	Agilent Technologies	Bidentate bonded and double-endcapped ultrapure C_{18}-silica
20	Prodigy ODS-3	100 mm × 4.6 mm i.d., 3 μm	Phenomenex	Ultrapure, inert, bonded, endcapped C_{18}-silica
21	Zorbax Eclipse XDB-C_{18}	75 mm × 4.6 mm i.d., 3.5 μm	Agilent Technologies	Densely bonded, double-endcapped ultrapure C_{18}-silica with dimethyl side chains
22	YMC-Pack Pro C18	100 mm × 4.6 mm i.d., 3 μm	YMC c/o Waters	C_{18}-Silica with high-coverage carbon bonding and an endcapping procedure utilizing Lewis acid-base chemistry
23	Symmetry C_{18}	75 mm × 4.6 mm i.d., 3.5 μm	Waters	High-purity C_{18}-silica with high bonding density and minimal residual silanol activity
24	Luna C18	100 mm × 4.6 mm i.d., 3 μm	Phenomenex	High-density bonded, ultrapure C_{18}-silica
25	Purospher Star RP-18	150 mm × 4.6 mm i.d., 5 μm	Merck, Darmstadt, Germany	Ultrapure, endcapped C_{18}-silica
26	Omnispher 5 C18	150 mm × 4.6 mm i.d., 5 μm	Varian, Palo Alto, CA	Ultrapure, inert, monofunctionally bonded C_{18}-silica
27	Inertsil ODS-3	100 mm × 4.6 mm i.d., 3 μm	GL-science, Tokyo, Japan	Ultrapure, fully-endcapped, inert C_{18}-silica
28	Zorbax ODS	150 mm × 4.6 mm i.d., 5 μm	Agilent Technologies	Single-endcapped C_{18}-silica with dimethyl side chains

Table 2
Gradient run conditions for silanol activity

Time (min)	0.02M aqueous ammonium acetate (% (v/v))	Acetonitrile (% (v/v))
0	90	10
20	10	90
25	10	90

Table 3
Flow rate (ml/min) as a function of the internal column diameter (i.d.) and the particle size of the stationary phase

Particle size (μm)	i.d. (mm)		
	3.0	4.0	4.6
3	0.5	1.0	1.2
4	0.7	1.2	1.5
5	1.0	1.9	2.3

The experiments were performed on an Agilent 1100 HPLC system equipped with a Photodiode Array Detector (Agilent, Walbronn, Germany) and a column switcher (VICI AG, Schenkon, Switzerland). The data were processed by the Chemstation software (Agilent).

3. Results and discussion

3.1. Column characteristics from chromatographic parameters

To obtain comparable results, on each column the tests were performed in the same order, i.e. measuring first hydrophobicity, steric selectivity and efficiency, followed by silanol activity, ion-exchange capacity and hydrogen-bonding capacity.

The results for hydrophobicity, steric selectivity, efficiency, and silanol activities (t_1, t_2 and t_3, α_1 and α_2) are displayed in Table 4. In practice, when developing a method to separate a given mixture only the most interesting (i.e. the most orthogonal, or complementary) columns will be screened. These stationary phases will provide the highest probability to find appropriate starting conditions for method optimization.

Based on a visual evaluation of the data matrix, the initial set of selected columns was defined with emphasis on efficient stationary phases covering a broad selectivity range: Zorbax Extend-C18, XTerra MS C_{18}, YMC-Pack Pro C18, SymmetryShield RP_{18}, XTerra RP_{18}, Luna Phenyl-Hexyl, Zorbax Bonus-RP and Hypersil C18-BDS. These columns were chosen because it is also believed that they are complementary in method development. Columns that are too similar to Hypersil C18-BDS (the typical column applied in method development) were rejected, because they provide too little additional information.

The initial selection was based on a visual approach and personal experience, and not from mathematically interpreted results. In order to evaluate the accuracy of the initial selection and to define a systematic approach, a re-evaluation is performed using several chemometric techniques. Criteria to select diverse columns are defined for the applied techniques: Pareto-optimality method, PCA, and Derringer's desirability functions approach.

First, the desired results for the eight parameters were considered. The eventually selected stationary phases have to comply with two conditions: they have to be efficient and selective. Since efficiencies are expressed by corrected plate heights and tailing factors, these parameters should be as low as possible. The selectivity can be attributed to differences in hydrophobicity, steric selectivity, ion-exchange and hydrogen-bonding capacities. As hydrophobicity is the most important retention mechanism on reversed-phase columns [40], differences in this property represent other contributions to the total retention mechanism, and can therefore lead to selectivity differences [41]. Thus, the hydrophobicity should be as diverse as possible among the selected stationary phases. Further on, (a) the steric selectivity and the ion-exchange capacity α_1 should be as high as possible, and (b) the hydrogen-bonding capacity α_2 should be as low as possible to obtain desirable selectivities.

It can be remarked that the column set of Table 1 does not contain different batches of a given phase. The reason for this is the fact that nowadays manufacturers produce stationary phases with stable physical, chemical and chromatographic properties [12,42,43], leading to highly reproducible results [43–49]. Therefore, it was expected that the difference between the performance of columns from different manufacturers will be much higher than the batch-to-batch variability of a given stationary phase and no columns were replicated.

3.2. Pareto-optimality

The optimal conditions for different responses measured on a given stationary phase may be in mutual conflict, and a compromise has to be found [31,32]. One can apply multicriteria decision-making methods, like Pareto-optimality [31,32], to find such compromise. An object is Pareto-optimal when there is no other object which gives a better result for one criterion without having a worse result for another criterion. The Pareto-optimality concept was applied to make either a pair-wise or a simultaneous evaluation of the responses. In the pair-wise comparison, two responses are plotted on Cartesian axes [31,32]. When all responses are considered simultaneously, Pareto-optimal objects are calculated and visualization is no longer possible.

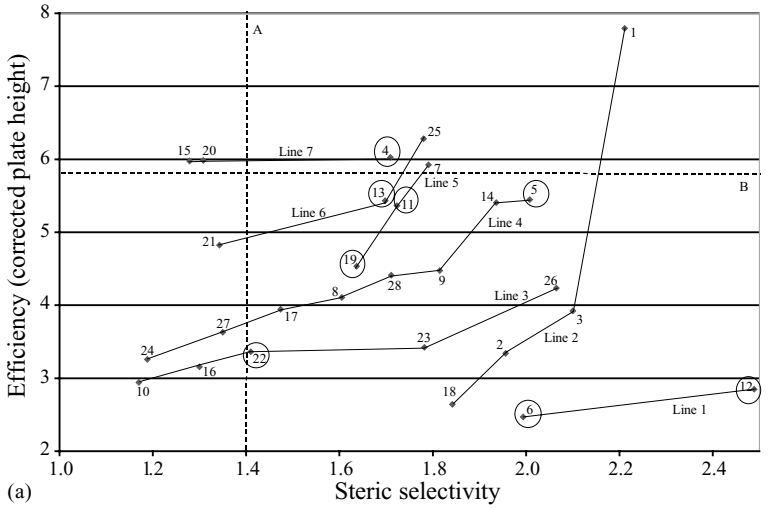
3.2.1. Pair-wise comparison

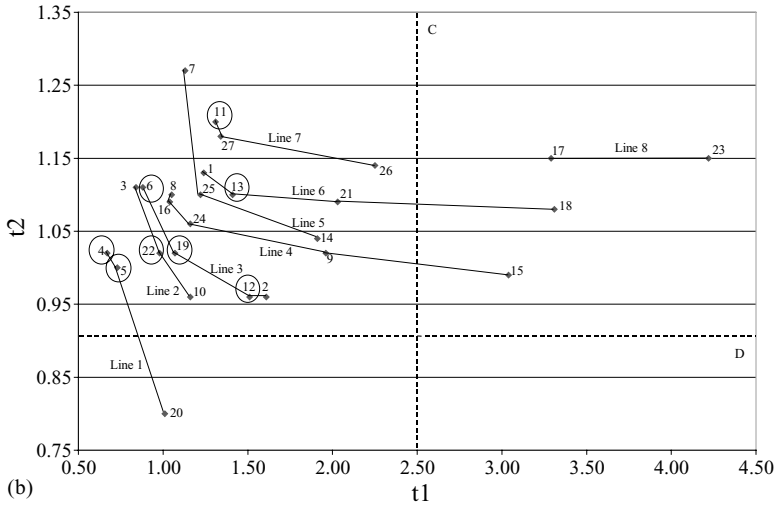
Since the stationary phases to select should be efficient and exhibit high separation capacities, corrected plate height and steric selectivity were considered major criteria and plotted (Fig. 2a). Steric selectivity is considered important, as it reflects the column's ability to separate conformational isomers while the efficiency indicates the separation power of

Table 4
Results for eight chromatographic test parameters on 28 columns

Column number	Column	Hydrophobicity ($k_{amylbenzene}$)	Steric selectivity ($\alpha_{o\text{-ter/triphen}}$)	Efficiency ($h_{triphenylene}$)	Silanol activity 1 (t_1)	Silanol activity 2 (t_2)	Silanol activity 3 (t_3)	Ion-exchange capacity (α_1)	H-bonding capacity (α_2)
1	ZirChrom-PBD	1.23	2.21	7.79	1.24	1.13	1.41	1.15	3.07
2	SymmetryShield RP_8	2.27	1.96	3.34	1.61	0.96	1.09	1.40	2.12
3	Discovery RP-AmideC16	2.50	2.10	3.92	0.84	1.11	1.45	2.89	2.66
4	Zorbax Bonus-RP	2.62	1.71	6.02	0.67	1.02	1.48	2.21	2.68
5	XTerra RP_{18}	2.86	2.01	5.45	0.73	1.00	1.54	2.79	2.42
6	XTerra MS C_{18}	2.92	1.99	2.47	0.88	1.11	1.35	2.64	2.42
7	Hypurity Elite C18	3.19	1.79	5.92	1.13	1.27	1.22	2.40	2.42
8	Discovery C18	3.97	1.61	4.11	1.05	1.10	1.27	2.71	2.37
9	Hypersil Elite C18	4.28	1.82	4.48	1.96	1.02	0.95	2.35	2.40
10	YMC ODS-AQ	4.90	1.17	2.94	1.16	0.96	1.11	1.99	2.11
11	Luna Phenyl-Hexyl	5.32	1.72	5.37	1.31	1.20	1.52	1.65	2.03
12	SymmetryShield RP_{18}	5.34	2.49	2.85	1.51	0.96	1.14	1.43	1.93
13	Hypersil C18-BDS	5.63	1.70	5.43	1.41	1.10	1.32	2.39	2.28
14	Eurospher 100 C18	5.92	1.94	5.41	1.91	1.04	1.03	1.26	1.93
15	Zorbax SB-C18	6.05	1.28	5.98	3.04	0.99	1.04	1.89	2.19
16	Zorbax Eclipse XDB-C_{18}	6.20	1.30	3.16	1.04	1.09	1.61	2.04	2.38
17	YMC ODS-H80 J'sphere	6.25	1.47	3.95	3.29	1.15	0.97	1.44	2.24
18	Alltima C18	6.37	1.84	2.64	3.31	1.08	0.89	0.96	1.95
19	Zorbax Extend-C18	6.77	1.64	4.53	1.07	1.02	1.70	2.54	2.43
20	Prodigy ODS-3	7.37	1.31	5.99	1.01	0.80	1.01	1.83	2.13
21	Zorbax Eclipse XDB-C_{18}	7.49	1.34	4.82	2.03	1.09	1.14	1.95	2.27
22	YMC-Pack Pro C18	7.50	1.41	3.36	0.98	1.02	1.16	2.24	2.12
23	Symmetry C_{18}	7.55	1.78	3.42	4.22	1.15	1.16	1.38	1.92
24	Luna C18	8.31	1.19	3.26	1.16	1.06	1.44	1.73	2.15
25	Purospher Star RP-18	8.56	1.78	6.28	1.22	1.10	1.45	2.13	2.20
26	Omnispher 5 C18	8.86	2.07	4.24	2.25	1.14	1.52	2.06	2.29
27	Inertsil ODS-3	9.26	1.35	3.63	1.34	1.18	2.43	1.72	2.12
28	Zorbax ODS	10.14	1.71	4.41	18.70	1.58	–	0.73	2.24

o-ter: *o*-terphenyl; triphen: triphenylene; –: missing value.





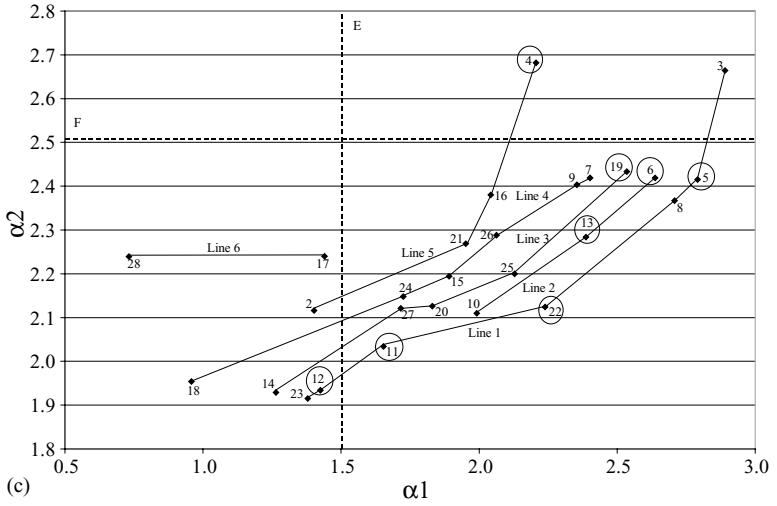


Fig. 2. (a) The efficiency (corrected plate height) vs. the steric selectivity for 28 columns; lines 1–7, sequential Pareto-optimal compromises for the two responses, (b) tailing factors t_2 vs. t_1 for 27 columns (column 28 not included, too large t_1); lines 1–8, sequential Pareto-optimal compromises for the two responses, and (c) H-bonding capacity, α_2, vs. ion-exchange capacity, α_1, for 27 columns (column 1 not included, too large α_2); lines 1–6, sequential Pareto-optimal compromises for the two responses. The circles indicate the eight columns initially selected based on experience. Dotted lines A–F, arbitrarily chosen desirability thresholds.

the stationary phase. The columns showing poor compromises for these two criteria will be excluded from further evaluation (undesired phases).

Secondly, the two expressions of silanol activity, i.e. tailing factors t_1 and t_2, are plotted (Fig. 2b). Thirdly, the selectivities α_1 and α_2, reflecting ion-exchange and H-bonding capacity, respectively, are plotted (Fig. 2c). The responses t_1, t_2, α_1, and α_2 were considered equally important. Columns 28 and 1 were not plotted in Fig. 2b and c because of too large t_1 and α_2 values, respectively. Since the hydrophobicity has to be as diverse as possible, this criterion is not enabling the selection of Pareto-optimal objects and was used to classify selected columns into groups with low, intermediate and high hydrophobicity. Tailing factor t_3 (closantel) was considered less important, because at pH 7.0 an acidic compound is negatively charged and will not interact with the residual silanol groups.

The Pareto-optimality concept was applied on all graphs. It was not used in its most narrow sense (only border points, i.e. line 1 selected), but in each plot a series of lines were selected. The different lines can be considered as sequential Pareto-optimal points after elimination of previously selected ones, i.e. after removing the first set of Pareto-optimal points (line 1), line 2 connects these that now become Pareto-optimal, etc. This approach was followed since our aim is to select a set of columns (comparable in number to the initial selection), and application of the concept in its most narrow sense would lead to a much too limited selection.

The Pareto-optimal phases from Fig. 2a were first selected. Columns 6 and 12 are Pareto-optimal stricto senso (line 1). Secondly, if these columns are ignored, columns 1, 2, 3 and 18 are selected (line 2); then columns 10, 16, 22, 23 and 26 (line 3); followed by columns 5, 8, 9, 14, 17, 24, 27 and 28 (line 4); columns 7, 11 and 19 (line 5); columns 13, 21 and 25 (line 6); and finally, columns 4, 15 and 20 (line 7). It might be (wrongly!) concluded from this ranking that columns 5, 6, 12 and 22, initially selected, show good compromises for the considered responses, whereas columns 4, 11, 13 and 19 are situated on the last lines and therefore exhibit rather poor compromises. This way of reasoning would be correct if phases from line 1 have a better compromise between responses than those from line 2, from line 3, etc. However, this is not true since Pareto-optimal points do not necessarily indicate good compromises. For instance, column 1 has the worst result for efficiency and is already selected on the second line. Also column 10 that is worst for steric selectivity was already selected on the third. Therefore, to make a meaningful selection of acceptable stationary phases based on Pareto-optimality, columns with less good properties have to be excluded. This was done by arbitrarily defining exclusion or undesirability limits for the responses considered. They were defined so that about 25% of the stationary phases with the worst responses were eliminated. Thus in Fig. 2a, only columns situated at the right of line A and below line B are considered (Table 5).

Table 5
Selection of columns based on the pair-wise Pareto-optimal lines in Fig. 2a-c, sorted per line according to column number

	Selection from $h_{triphenylene}$ vs. $\alpha_{o\text{-ter/triphen}}$	Selection from t_2 vs. t_1	Selection from α_2 vs. α_1
Line 1	6	4	5
	12	5	8
			11
			22
Line 2	2	3	6
	3	10	10
	18	22	13
Line 3	22	2	19
	23	6	20
	26	12	25
		19	27
Line 4	5	8	7
	8	9	9
	9	16	15
	14	24	24
	17		26
	28		
Line 5	11	7	16
	19	14	21
		25	
Line 6	13	1	
		13	
		21	
Line 7		11	
		26	
		27	

Analogously, a selection of stationary phases was performed from interpreting Fig. 2b and c (Table 5). In Fig. 2b, columns at the left of line C and above line D were considered; in Fig. 2c, it were those at the right of line E and below line F. Finally, only phases considered in all three Pareto-optimal selections were maintained. This led to the selection of nine columns (5, 6, 8, 9, 11, 13, 19, 22 and 26) (Table 6). Six of them correspond with those initially selected (Table 6). Columns 4 and 12, also initially selected, show poor results for efficiency (column 4) and selectivities α_1 (column 12) or α_2 (column 4). Since hydrophobicity is

Table 6
Subset selection of the columns from Table 5

Column number	Column
5[a]	XTerra RP_{18}
6[a]	XTerra MS C_{18}
8	Discovery C18
9	Hypersil Elite C18
11[a]	Luna Phenyl-Hexyl
13[a]	Hypersil C18-BDS
19[a]	Zorbax Extend-C18
22[a]	YMC-Pack Pro C18
26	Omnispher 5 C18

[a] Columns also initially selected.

Table 7
Division in groups based on hydrophobicity

Hydrophobicity class	Column	Hydrophobicity
Low	5	2.86
	6	2.92
	8	3.97
	9	4.28
Intermediate	11	5.32
	13	5.63
	19	6.77
High	22	7.50
	26	8.86

an important factor in the retention on reversed-phase stationary phases [40], it was used to group columns. Three groups were distinguished: one with relatively low, one with intermediate and one with high hydrophobicity (Table 7). It might be recommended to select a phase from each group during the search for initial separation conditions for a drug and its related compounds. These three columns differ considerably in hydrophobicity, therefore increasing the possibility of having selected more orthogonal stationary phases [40,41].

3.2.2. Simultaneous evaluation

The simultaneous evaluation was considered of no use to be performed for this data set, as to obtain comparable results with the pair-wise evaluation, the same arbitrary limits for the responses (lines A–F in Fig. 2a–c) should be respected. Using those thresholds already led to a subset of only nine columns with good compromises for the six considered responses (see above), which made an occasional further selection from a Pareto-optimality approach redundant.

In summary, the initial selection consisted of eight columns (4, 5, 6, 11, 12, 13, 19 and 22), the one by Pareto-optimality of nine columns: 5, 6, 8, 9, 11, 13, 19, 22 and 26. Thus six columns (5, 6, 11, 13, 19 and 22) were selected by both approaches. Columns 4 and 12 were not selected via the Pareto-optimality concept, because of their poor response for efficiency (column 4), selectivity α_1 (column 12) or α_2 (column 4). In conclusion, it can be said that the Pareto-optimality approach led to a relevant selection of stationary phases but the selection is not so straightforward. Construction of sequential Pareto-optimal lines and the definition of arbitrary limits were found necessary to exclude less performing columns from the selection. Further on, when additional phases need to be evaluated against the earlier evaluated ones, these columns cannot be immediately situated. The Pareto-optimality procedure(s) need to be repeated, which is another disadvantage of the approach.

3.3. Principal component analysis

Principal component analysis [33,34] on the autoscaled data of columns 1–27 was also performed, since it enables

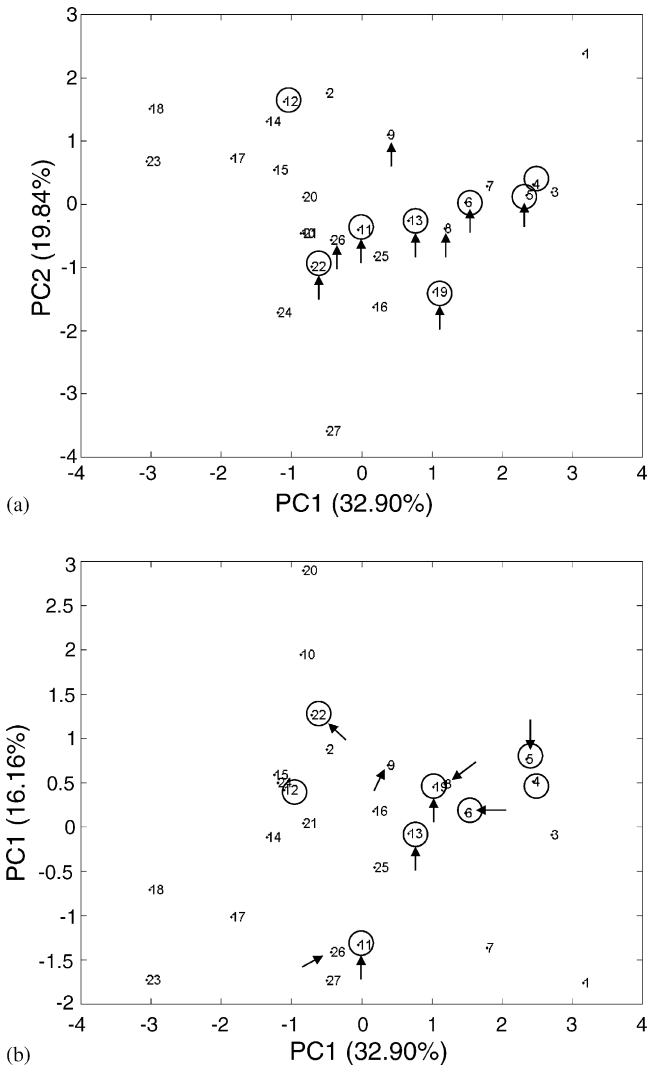


Fig. 3. (a) PC1–PC2 and (b) PC1–PC3 score plots of the autoscaled data for 27 columns and eight chromatographic test parameters (column 28 not included because of missing value). The circles refer to the columns initially selected and the arrows to those by Pareto-optimality.

classification of stationary phases [8,14,15,25]. Column 28 was eliminated because of missing data. All parameters were considered, as here the hydrophobicity may help to distinguish between groups of phases. In Fig. 3, the score plots on PC1–PC2 and on PC1–PC3 are displayed. The columns selected initially and by the Pareto-optimality approach are indicated. Because the variance explained by PC1 and PC2 was hardly 53%, also PC3 was evaluated. The three dimensions account for about 70% of the variance in the data set. The selections made by either the initial or the Pareto-optimality approaches are generally found in each other's vicinity on the score plots, but no clear classification of stationary phases was observed. To make a similar or relevant selection only based on the PCA score plots is far from being evident. It can be concluded that the principal component analysis is not a straightforward technique to select efficient and selective columns from our data set.

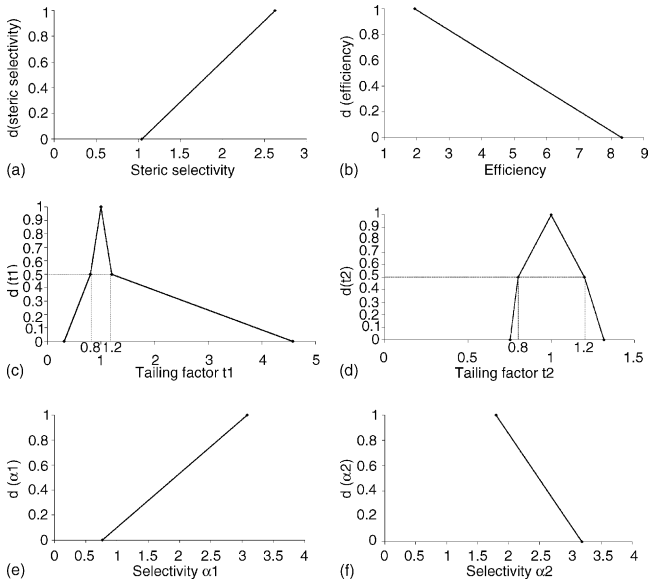


Fig. 4. The Derringer's desirability functions defined for: (a) steric selectivity, (b) efficiency, (c) silanol activity t_1, (d) silanol activity t_2, (e) ion-exchange capacity α_1, and (f) H–bonding capacity α_2.

3.4. Derringer's desirability functions

The application of Derringer's desirability functions [35–38] is another multicriteria approach to evaluate responses simultaneously. It allows determining conditions that result in the most desirable combination of properties. The measured response values are transformed into a dimensionless desirability (d) scale, ranging between 0 and 1 for a completely undesirable and a fully desired situation, respectively [38]. For each response, a desirability function is defined. Two types of transformation are possible, i.e. the one-sided and the two-sided transformation. The first is applied when a response is either to be minimized or maximized [35,36], and the second when it has an optimal value within the interval of possible values [36]. After transforming each response, the overall quality of a stationary phase is calculated as the geometric mean (D) of the individual d-values. The column for which the combination of the different properties is globally best will have the highest D [35].

Six of the eight parameters were considered. The hydrophobicity and tailing factor t_3 were not taken into account, for the reasons mentioned in 3.2. Hydrophobicity was again used to group the stationary phases into classes. In Fig. 4, the Derringer's desirability functions defined for the different responses are shown. The one-sided transformations were obtained as follows: first, the interval for the response, excluding strong outliers, is determined in the data set. Then, an extended interval is defined as [lowest value −10% of interval range; highest value +10% of interval range]. A d-value of either 0 or 1 (depending on the desirability of the response value) is assigned to the extremes of this interval and a linear desirability transformation function was calculated in it. This approach avoids that transformed values (with the exception of outliers) become either 0 or 1.

The steric selectivity (Fig. 4a) (interval [1.038, 2.621]) should be as high as possible and thus an increasing desirability function is defined, and for the corrected plate height [1.936, 8.327], which should be as low as possible, a decreasing one (Fig. 4b). Further on, substances should elute as symmetrical peaks, and the tailing factors should preferably be between 0.8 and 1.2. Values outside this range are less desirable. For t_1 [0.315, 4.575] and t_2 [0.753, 1.317], two-sided transformations were used in which desirability $d = 1$ was defined for $t = 1$, $d = 0.5$ for $t = 0.8$ and 1.2, and $d = 0$ based on the measured extremes applying the above-mentioned criteria. The functions are visualized in Fig. 4c and d. The selectivity α_1 [0.765, 3.084] should be as high, and α_2 [1.799, 3.188] as low as possible. The functions used are drawn in Fig. 4e and f, respectively.

The geometric mean of the d-values for the different columns resulted in the D-values represented in Table 8. Five of the eight stationary phases preferred by the initial selection (5, 6, 12, 19 and 22) are among the seven predicted to have the best overall performance according to Derringer's approach. Only columns 4, 11 and 13 have somewhat lower D-values. Six of the phases selected via Pareto-optimality (5, 6, 8, 9, 19 and 22) are also situated among the eight considered having the best overall performance. Columns 11, 13 and 26 have a somewhat lower, but still reasonably high, D-value.

The stationary phases selected based on Derringer's desirability functions can again be divided into groups with different hydrophobicity. The group of low hydrophobicity, for instance, is formed by columns 3, 5 and 6, the one of intermediate hydrophobicity by 8, 9 and 12, and one of high hydrophobicity by columns 19 and 22 when a selection of eight phases is considered. As mentioned earlier, one column of each group could be selected if one likes to further reduce the number of stationary phases.

It can be concluded that the application of Derringer's desirability functions is a favorable technique to select or characterize HPLC columns exhibiting good overall performances. It is shown that this method allows making a column selection largely similar to the one based on chromatographic expertise. The Derringer approach may be preferred in comparison with the Pareto-optimality or PCA ones. Moreover, it is very easy to evaluate new stationary phases, as they are characterized by one D-value, expressing their overall desirability. It quickly can be decided whether the new column is performing well or not and where it is situated relative to the other phases of the data set.

It also can be remarked that the above approach with the Derringer's desirability functions can easily be used when other chromatographic tests are applied to evaluate the stationary phase properties, i.e. when alternative test substances or chromatographic conditions are used [11,14]. To avoid confusion it might be worthwhile positioning the D-value relative to other recently applied column ranking techniques

Table 8
Columns, sorted by decreasing *D*-value, obtained from Derringer's desirability functions

Column number	$d(\alpha_{o-\text{ter/triphen}})$	$d(h_{\text{triphenylene}})$	$d(t_1)$	$d(t_2)$	$d(\alpha_1)$	$d(\alpha_2)$	D	Hydrophobicity ($k_{\text{amylbenzene}}$)
6EP	0.604	0.917	0.700	0.725	0.807	0.554	0.708	2.92
12E	0.917	0.857	0.454	0.900	0.285	0.903	0.660	5.34
22EP	0.235	0.777	0.950	0.950	0.636	0.766	0.657	7.50
8P	0.358	0.661	0.875	0.750	0.838	0.591	0.652	3.97
19EP	0.378	0.593	0.825	0.950	0.763	0.544	0.647	6.77
3	0.671	0.689	0.600	0.725	0.917	0.377	0.641	2.50
5EP	0.613	0.450	0.428	1.000	0.874	0.557	0.621	2.86
9P	0.491	0.603	0.387	0.950	0.685	0.565	0.590	4.28
2	0.580	0.780	0.439	0.900	0.275	0.772	0.580	2.27
26P	0.649	0.640	0.344	0.650	0.560	0.648	0.568	8.86
13EP	0.417	0.453	0.469	0.750	0.699	0.651	0.558	5.63
16	0.166	0.809	0.900	0.775	0.551	0.582	0.557	6.20
25	0.469	0.320	0.497	0.750	0.588	0.711	0.535	8.56
14	0.567	0.457	0.395	0.900	0.215	0.906	0.512	5.92
10	0.083	0.842	0.600	0.900	0.529	0.776	0.500	4.90
11EP	0.433	0.463	0.484	0.500	0.383	0.831	0.499	5.32
4E	0.424	0.360	0.366	0.950	0.621	0.364	0.479	2.62
27	0.197	0.735	0.479	0.550	0.410	0.768	0.479	9.26
24	0.095	0.793	0.600	0.850	0.414	0.749	0.478	8.31
20	0.171	0.366	0.975	0.500	0.459	0.765	0.469	7.37
21	0.193	0.548	0.377	0.775	0.512	0.662	0.468	7.49
7	0.476	0.376	0.675	0.201	0.706	0.554	0.460	3.19
18	0.508	0.889	0.187	0.800	0.083	0.888	0.414	6.37
17	0.275	0.685	0.190	0.625	0.291	0.683	0.406	6.25
15	0.152	0.368	0.227	0.975	0.486	0.716	0.403	6.05
23	0.470	0.767	0.053	0.625	0.265	0.916	0.377	7.55
1	0.741	0.083	0.494	0.675	0.166	0.084	0.257	1.23

E and P: the columns selected initially and by the Pareto-optimality method, respectively.

[41,50]. The *D*-values obtained from Derringer's approach lead to a unique ranking for a set of phases. The ranking obtained using the so-called column selectivity function [41,50] is not unique. The column selectivity function leads to an F_s- [41] or *F*-value [50], which is resulting from a comparison of the test parameters measured on two stationary phases. When for a set of columns one is used as reference, a ranking is obtained. Using another phase as reference another ranking will be the result and as many different rankings as reference columns considered can be created.

3.5. Conclusions

Some chemometric techniques were evaluated and compared for their potential ability to make a suitable selection of HPLC columns. Suitable stationary phases should be both efficient and selective. A set of complementary columns to be used in initial separation conditions for drug/impurity mixtures preferably should have diverse hydrophobicities as well. In this study, 28 stationary phases were characterized by eight chromatographic parameters. Column selection using Pareto-optimality, principal component analysis and Derringer's desirability functions was evaluated. The outcome was compared to the initial selection and resulted in a similar selection for the Derringer approach. The Derringer approach provides a systematic alternative that fairly corresponds to the chromatographists' experience, and is easily applicable on additional columns as well as on data sets where stationary phases are characterized by other chromatographic test parameters. The Pareto-optimality concept also led to an appropriate subset selection after choosing arbitrary thresholds for the considered responses, but seems less flexible when new additional columns are to be evaluated. The principal component analysis was not found suitable for the intended subset selection.

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