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Journal of Pharmaceutical and Biomedical Analysis 41 (2006) 751–760
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
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Stationary phases in the screening of drug/impurity profiles and in their separation method development: Identification of columns with different and similar selectivities

E. Van Gyseghem [a], M. Jimidar [b], R. Sneyers [c], M. De Smet [b], E. Verhoeven [b], Y. Vander Heyden [a,*]

[a] *Department of Analytical Chemistry and Pharmaceutical Technology, Vrije Universiteit Brussel-VUB, Laarbeeklaan 103, B-1090 Brussels, Belgium*
[b] *Global Analytical Development, Johnson & Johnson Pharmaceutical Research & Development, A division of Janssen Pharmaceutica N.V., Turnhoutseweg 30, B-2340 Beerse, Belgium*
[c] *Drug Evaluation-Analytical Chemistry, Johnson & Johnson Pharmaceutical Research & Development, A division of Janssen Pharmaceutica N.V., Turnhoutseweg 30, B-2340 Beerse, Belgium*

Received 7 November 2005; accepted 2 January 2006
Available online 8 February 2006

Abstract

The classification or characterization of stationary phases based on chromatographic parameters, in general, requires different test solutes/mixtures and several mobile phases. To simplify the classification/characterization of reversed-phase liquid chromatographic columns, to be used in separating drug/impurity profiles, a new test procedure was proposed. It consists of injecting two mixtures of relatively similar active substances applying a standard gradient. The aim was to evaluate from this approach the selectivity differences and overall separation quality of newly tested columns compared to that in an earlier selected set of eight stationary phases. The selectivity differences of the columns were evaluated by correlation coefficient-based weighted-average-linkage dendrograms and color maps. Derringer's desirability functions were used to rank similar stationary phases according to their overall separation quality. Four columns of 27 examined were, for instance, considered different from the earlier selected eight and could be added to the selection. A number of tested stationary phases might be considered as alternatives for some from the initial set. For three columns the newly tested stationary phases did not contain alternatives.

Keywords: RP-HPLC column selection; Weighted-average-linkage dendrogram; Pearson-correlation coefficients color map; Derringer's desirability function; Selectivity differences

1. Introduction

The number of commercially available stationary phases for reversed-phase HPLC separations yearly increases. The column characteristics also become more diverse because of special modifications such as bidentate structure [1], hybrid packing [2,3], fluorinated stationary phases [4], polar-embedding [2–5] and polar-endcapping [2,3,6]. These adaptations with regard to the traditional silica-based columns lead to an extension of the applicable pH-range [1–3] and/or to the use of (almost) 100% aqueous mobile phases [1–5]. Such columns can provide alternative and complementary separations with a different selectivity compared to conventional C_{18} or C_8 stationary phases [4]. As a consequence, for the chromatographer, it is not an easy task to select an appropriate column for a given separation. Therefore, chromatographic tests to evaluate stationary phase properties, such as efficiency, steric selectivity, ion-exchange and H-bonding capacity [7], are described to characterize and classify columns [8–13].

Developing chromatographic methods is laborious, expensive and difficult. It makes the definition of a strategy to find rapidly initial separation conditions very interesting. Chromatographic approaches in which several columns are screened for their separation efficiency and selectivity have been applied [14]. Chromatographic tests were performed on 28 silica-based reversed-phase stationary phases, and eight chromatographic

* Corresponding author. Tel.: +32 2 477 47 34; fax: +32 2 477 47 35.
E-mail address: yvanvdh@vub.ac.be (Y. Vander Heyden).

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doi:10.1016/j.jpba.2006.01.002

parameters, representing properties as hydrophobicity, steric selectivity, efficiency, silanol activity, H-bonding capacity and ion-exchange capacity, were determined [14]. Eight columns were selected from that data set based on chromatographic expertise (two of which were replaced later on) to develop screening systems for drug/impurity profiles by subjecting each to mobile phases with four different pH-values, creating 32 systems [15]. The initial separation conditions for an unknown mixture, which can be used for further method development, then may be selected from these screening systems. A later study on the most orthogonal systems allowed reducing the screening set to 16 [15].

Since continuously new columns become available one should have a simple and quick testing procedure that allows evaluating the potential usefulness of these phases. In order to select faster an appropriate column for a given purpose or to replace an earlier selected one, a new test was proposed. It applies two mixtures of drug substances and uses only one mobile phase.

A chromatographic test for column characterization usually applies simple test molecules, such as aromatic hydrocarbons, because they are easily accessible and detectable, stable, and generally usable [8]. The tests applied in Ref. [14] used also in-house acidic, neutral and basic pharmaceuticals to determine silanol activity, H-bonding capacity and ion-exchange capacity. This enables to select stationary phases that are better suited for particular in-house applications. However, the column testing procedure was rather time-consuming and labour-intensive [14].

Therefore, and since we were more interested in classification than in characterization, a simplified and faster column classification method was proposed. It consists of injecting two mixtures of 11 and 7 in-house developed structurally relatively similar active compounds, subjected to a linear gradient at pH 7.0. The mixtures were analyzed with LC–MS–DAD. The test was utilized to reveal the selectivity differences and separation properties of 35 stationary phases, i.e. 27 new phases and the 8 columns that were chosen earlier for screening purposes [15].

The test mixtures were applied to select new stationary phases with good overall separation quality having either a different or a similar selectivity towards the earlier chosen columns. First, the selectivity differences of the stationary phases were visually examined by weighted-average-linkage dendrograms [16–18] and correlation-coefficients color maps [15,19], as these techniques performed well to select orthogonal chromatographic systems [15,19,20]. A classification of columns with similar and different selectivities was made. Second, within a group, the stationary phases were ranked according to their overall separation quality using a multicriteria decision-making method, here Derringer's desirability functions [21–24]. This technique was already applied for the selection of columns [14] and systems [15] exhibiting good overall separation properties. The best stationary phases of each group, if exhibiting different selectivities or better overall separation quality, can then either be added to or replace a column from the set of the eight initially selected [15].

2. Experimental

2.1. Reagents

Two mixtures, one composed of 11 and the other of 7 substances (all Janssen Pharmaceutica N.V., Beerse, Belgium), were used. The first mixture consisted of azaconazole, cinnarizine, domperidone, enilconazole, flubendazole, itraconazole, ketoconazole, levamisole hydrochloride, liarozole hydrochloride, miconazole nitrate, and sabeluzole; the second of astemizole, closantel, droperidol, etomidate hydrochloride, ketanserin, prucalopride succinate, and risperidone. The solvent was prepared by mixing methanol for HPLC (Acros Organics, Geel, Belgium), tetrahydrofuran and dimethyl formamide (Merck, Darmstadt, Germany) in a ratio of 40%:40%:20% (v/v/v).

The mobile phase was prepared using acetonitrile for HPLC far UV (Acros Organics), ammonium acetate Microselect (Fluka Chemie, Buchs, Switzerland), and water of Milli-Q quality, the latter obtained with a Millipore purification system (Millipore, Molsheim, France).

2.2. Chromatographic conditions

The 35 stationary phases are described in Table 1. A linear gradient at pH 7.0 (Table 2) and a flow rate of 1.0 ml/min were applied. All experiments were carried out at 35 °C. The injection volume was 5 μl.

The LC–MS–DAD analyses were performed on an on-line coupling of four high-performance liquid chromatographs, each consisting of a Waters 2695 Separations Module (= alliance), HPLC compartment (Waters, Milford, MA), a Mistral column oven (Spark Holland, Emmen, The Netherlands), a column switcher (VICI AG, Schenkon, Switzerland) and a Waters 996 Photodiode Array Detector (Waters), linked with a single quadrupole mass spectrometer, Waters Micromass ZQ (Waters/Micromass, Manchester, UK), a multiplexed 4-channel MUX-interface (Waters/Micromass) using electrospray in the positive ionization mode. The injection was performed simultaneously on a 4-channel CTC PAL injector (CTC Analytics, Zwingen, Switzerland). The effluent from the HPLC instruments was splitted prior to diode array detection using a zero-dead-volume T-piece so that about 100 μl/min per channel enters the interface. Typical parameters of the ion source are capillary voltage: 3.6 kV; cone voltage: 20 V; source temperature: 150 °C; desolvation temperature: 100 °C; and cone gas flow: 113 l/h N_2. The specifications for the mass spectrometer are scan range: 165–750 Da; scan time: 0.23 s; interscan time: 0.1 s; photomultiplier voltage: 550 V; resolution: for low mass, 13.0 u, and for high mass, 13.5 u. The chromatographic methods were created and the data treated using both Millennium^{32} Version 4.0 software (Waters) for the spectral data, and MassLynx Version 4.0 software (Waters/Micromass) for the mass spectral data.

3. Results and discussion

The linear gradient elution (Table 2) was applied to all stationary phases. For all compounds, their normalized retention time

Table 1
The 35 columns tested with their dimensions and distributors

No	Column	Dimensions	Distributed by
1	SunFire C_{18}	100 mm × 4.6 mm i.d., 3.5 μm	Waters, Milford, MA
2	Atlantis dC_{18}	100 mm × 4.6 mm i.d., 3 μm	Waters
3	XTerra MS C_{18}	100 mm × 4.6 mm i.d., 3.5 μm	Waters
4	XTerra RP_{18}	100 mm × 4.6 mm i.d., 3.5 μm	Waters
5	XTerra Phenyl	100 mm × 4.6 mm i.d., 3.5 μm	Waters
6	SymmetryShield RP_{18}	100 mm × 4.6 mm i.d., 3.5 μm	Waters
7	Zorbax Extend-C18	100 mm × 4.6 mm i.d., 3.5 μm	Agilent, Palo Alto, CA
8	Zorbax Bonus-RP	100 mm × 4.6 mm i.d., 3.5 μm	Agilent
9	Zorbax Eclipse XDB-C_{18}	150 mm × 4.6 mm i.d., 3.5 μm	Agilent
10	Zorbax SB-C18	150 mm × 4.6 mm i.d., 3.5 μm	Agilent
11	YMC-Pack Pro C18	100 mm × 4.6 mm i.d., 3 μm	YMC c/o Waters, Milford, MA
12	YMC-Pack C4	100 mm × 4.6 mm i.d., 3 μm	YMC c/o Waters
13	Platinum EPS C18	100 mm × 4.6 mm i.d., 3 μm	Alltech, Deerfield, IL
14	FluoroSep-RP Phenyl	150 mm × 4.6 mm i.d., 3 μm	ES Industries, West Berlin, NJ
15	Pursuit C18	100 mm × 4.6 mm i.d., 3 μm	Varian, Palo Alto, CA
16	Pursuit DP	100 mm × 4.6 mm i.d., 3 μm	Varian
17	Polaris Amide-C18	100 mm × 4.6 mm i.d., 3 μm	Varian
18	Polaris C18-A	100 mm × 4.6 mm i.d., 3 μm	Varian
19	Inertsil Phenyl-3	100 mm × 4.6 mm i.d., 3 μm	Varian
20	Alltima HP C18	100 mm × 4.6 mm i.d., 3 μm	Alltech
21	Alltima HP C18 HiLoad	100 mm × 4.6 mm i.d., 3 μm	Alltech
22	Alltima HP C18 Amide	100 mm × 4.6 mm i.d., 3 μm	Alltech
23	Synergi Polar-RP	150 mm × 4.6 mm i.d., 4 μm	Phenomenex, Torrance, CA
24	Uptisphere C18-ODB	100 mm × 4.6 mm i.d., 3 μm	Interchim, Montluçon, France
25	Uptisphere 3 C18-HDO	100 mm × 4.6 mm i.d., 3 μm	Interchim
26	Capcell Pak C_{18} MG	100 mm × 4.6 mm i.d., 3 μm	Shiseido, Tokyo, Japan
27	Capcell Pak C_{18} AQ	100 mm × 4.6 mm i.d., 3 μm	Shiseido
28	Capcell Pak C_{18} UG120	100 mm × 4.6 mm i.d., 3 μm	Shiseido
29	Capcell Pak C_{18} ACR	100 mm × 4.6 mm i.d., 3 μm	Shiseido
30	Acquity (C_{18})	100 mm × 4.6 mm i.d., 3 μm	Waters
31	Synergi Max-RP	100 mm × 4.6 mm i.d., 4 μm	Phenomenex
32	Synergi Hydro-RP	100 mm × 4.6 mm i.d., 4 μm	Phenomenex
33	Synergi Fusion-RP	100 mm × 4.6 mm i.d., 4 μm	Phenomenex
34	Curosil PFP	100 mm × 4.6 mm i.d., 3 μm	Phenomenex
35	Hypersil GOLD	100 mm × 4.6 mm i.d., 3 μm	Thermo, Cheshire, UK

τ on each column was calculated, resulting in a 35 × 18 matrix. τ is defined as the difference between the retention time and the dead time, divided by the dead time, measured under gradient conditions. The first aim is to select dissimilar and similar stationary phases. Pearson's correlation coefficients, $r(\tau)$, were calculated between each pair of columns, leading to a 35 × 35 matrix. From this matrix weighted-average-linkage dendrograms and color maps were built as visualization methods to detect dissimilar (r = low) and similar (r = high) phases. The correlation coefficient values reflect the selectivity differences exhibited by the columns [15,19,20].

Table 2
Conditions for the gradient run

Time (min)	10 mM ammonium acetate in water (%)	Acetonitrile (%)
0	95	5
20	0	100
25	0	100
27	95	5
35	95	5

Weighted-average-linkage, or Weighted Pair Group Method using arithmetic Averages (WPGMA) [25], is an agglomerative hierarchical clustering technique [16–18]. It starts with clusters containing a single object (one column), and successively merges two clusters, using a dissimilarity criterion, here $1-|r|$. At each step, the two least dissimilar clusters are fused. The more dissimilar the clusters (stationary phases), the higher the branches connecting them in the resulting tree. In Fig. 1, the WPGMA-dendrogram for the 35 columns is shown. The r-matrix was also represented as a color map (Fig. 2). The phases in the map were ranked according to increasing dissimilarities in the tree [15,19].

Fig. 1 demonstrates that selectivity differences are observed between selections from the groups A, B, C and D. Columns 13 and 14, for instance, show very high $1-|r|$-values towards all others, and can be considered dissimilar. Yet both phases 13 and 14 are similar and hence grouped (group A). This is confirmed in Fig. 2, as they show correlation coefficients below 0.4 when compared to other columns, and high r-values when compared to each other. Stationary phases 19, 23 and 34 are similar (group B), with an even closer relationship between columns 23 and 34 (group B_1). All three exhibit low r-values towards the other

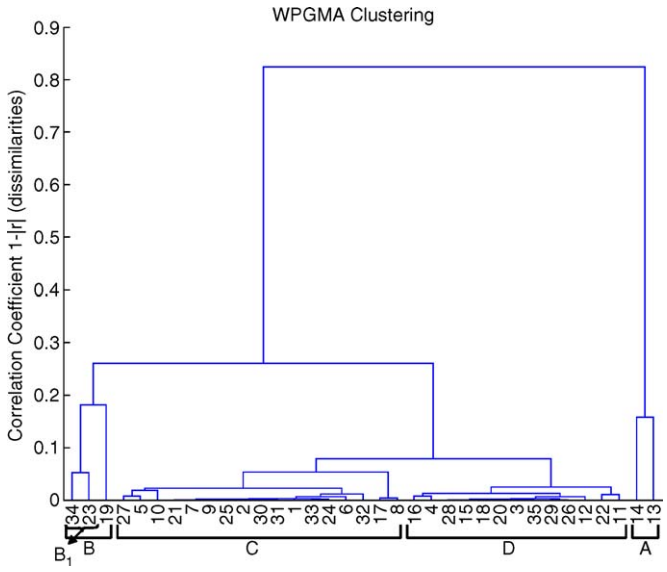

Fig. 1. WPGMA-dendrogram of 35 stationary phases based on Pearson-correlation coefficients between the normalized retention times of the 18 substances. The abscissa shows the column numbers and groups of similar stationary phases (A–D).

phases. These five columns thus can provide additional information compared to the initially selected, i.e. stationary phases 3–8, 11 and 12 [15]. The figures also show two large groups of similar phases, denoted as C and D. Those columns show very analogous selectivities. The dendrogram and color map were redrawn eliminating the five most dissimilar stationary phases (groups A and B) to better reveal occasional relationships within the groups of similar columns. The subgroups obtained are indicated in Fig. 2.

It can be concluded that a number of newly tested columns is dissimilar (group A) or rather dissimilar (group B) with respect to the formerly selected eight phases. As a consequence, for additional selectivity differences, one column from each group A and B (occasionally 19 and one from B_1) might be added to the set of eight. On the other hand, since the eight are situated in the groups of similar phases (C or D), one could consider replacing them by another from that group.

From the above data treatment, it is not clear which stationary phases within the groups have the best overall separation qualities. Therefore, the columns were characterized regarding their future application, i.e. the separation of drug/impurity profiles. This is also the reason why the test compounds were injected as two mixtures of 11 and 7 substances, because it represents the complexity in such a profile. The normalized retention times of the compounds were sorted ascending (for substances eluting after the gradient, a retention time of 25 min was used) and five parameters were determined, i.e. the minimum and maximum normalized retention time τ_{min} and τ_{max}, the minimum resolution Rs_{min}, the geometric mean of the resolutions Rs_i, and the corrected plate height h. Resolutions were calculated between successive peaks within the mixtures. τ_{min} was considered to verify whether a column retains all compounds and τ_{max} to describe the time needed to elute all components. It will reveal phases with too long analysis times. Rs_{min} and Rs_i were included to characterize the worst and overall separations, respectively. Finally, h was determined from the peak most likely to exhibit tailing, i.e. levamisole, reflecting the efficiency of the column in the worst case.

The overall separation quality of the stationary phases was quantified using Derringer's desirability functions [21–24]. The responses are transformed into a dimensionless desirability (d) scale via a desirability function. The desirability scale ranges

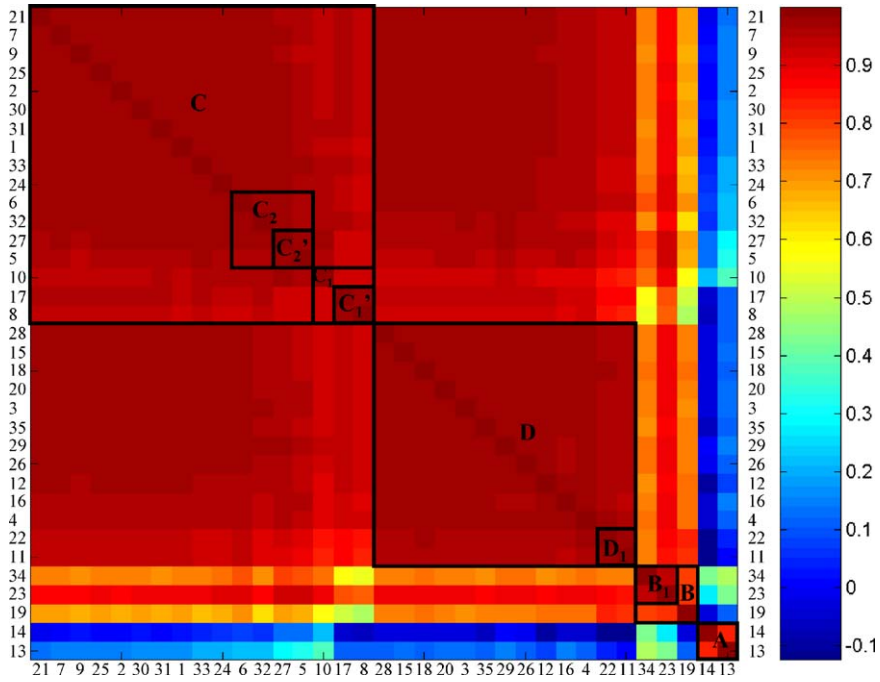

Fig. 2. Color map of Pearson-correlation coefficients for the 35 columns ranked according to increasing dissimilarities in the WPGMA-dendrogram of Fig. 1.

between $d = 1$ and $d = 0$ for a fully desirable and a completely undesired response, respectively. The transformation function can be linear or not. The transformation can be one-sided or two-sided, depending whether the desired response is either extreme (i.e. to be maximized or minimized) or has a target value. Then, the geometric mean (D) of the desirability values is calculated, which represents the overall performance of a column. The stationary phase exhibiting the best combination of responses will have the highest D-value.

For each response, a linear one-sided transformation was performed. To prevent that transformed values become either 0 or 1 (except for outliers), the interval in which the desirability function was calculated was determined following some rules [14,15]. First, obvious outliers were removed. Then, the remaining interval for a given response was extended to [lowest value −10% of interval range; highest value +10% of interval range]. The extended maximum and minimum then were assigned a d-value of 0 or 1. The Derringer's desirability functions applied are visualized in Fig. 3. The d-value increasing with τ_{min} (Fig. 3a) indicates that it is desired that substances are retained, and that τ_{min} is preferably as high as possible. A decreasing function for τ_{max} (Fig. 3b) takes into account (too) long analysis times, which are not desired. The larger Rs_{min} and Rs_i, the better the peaks will be separated (Fig. 3c and d). A stationary phase is more efficient with a smaller reduced plate height (Fig. 3e). Table 3 summarizes the individual d- and the global D-values for the columns, with the phases ranked within their groups (A–D). The columns exhibiting the best overall separation performances are located high in their class. From Figs. 1 and 2 and Table 3, phases representing both selectivity differences and good overall separation qualities can be selected.

To address the newly tested phases as appropriate alternatives for or as an addition to the initial eight [15] the following methodology was used. First, it was evaluated where the 27 new columns are situated in the classification compared to the original, i.e. it is determined which stationary phases exhibit similar or dissimilar selectivities. Secondly, it was investigated which of the new columns could be an alternative for

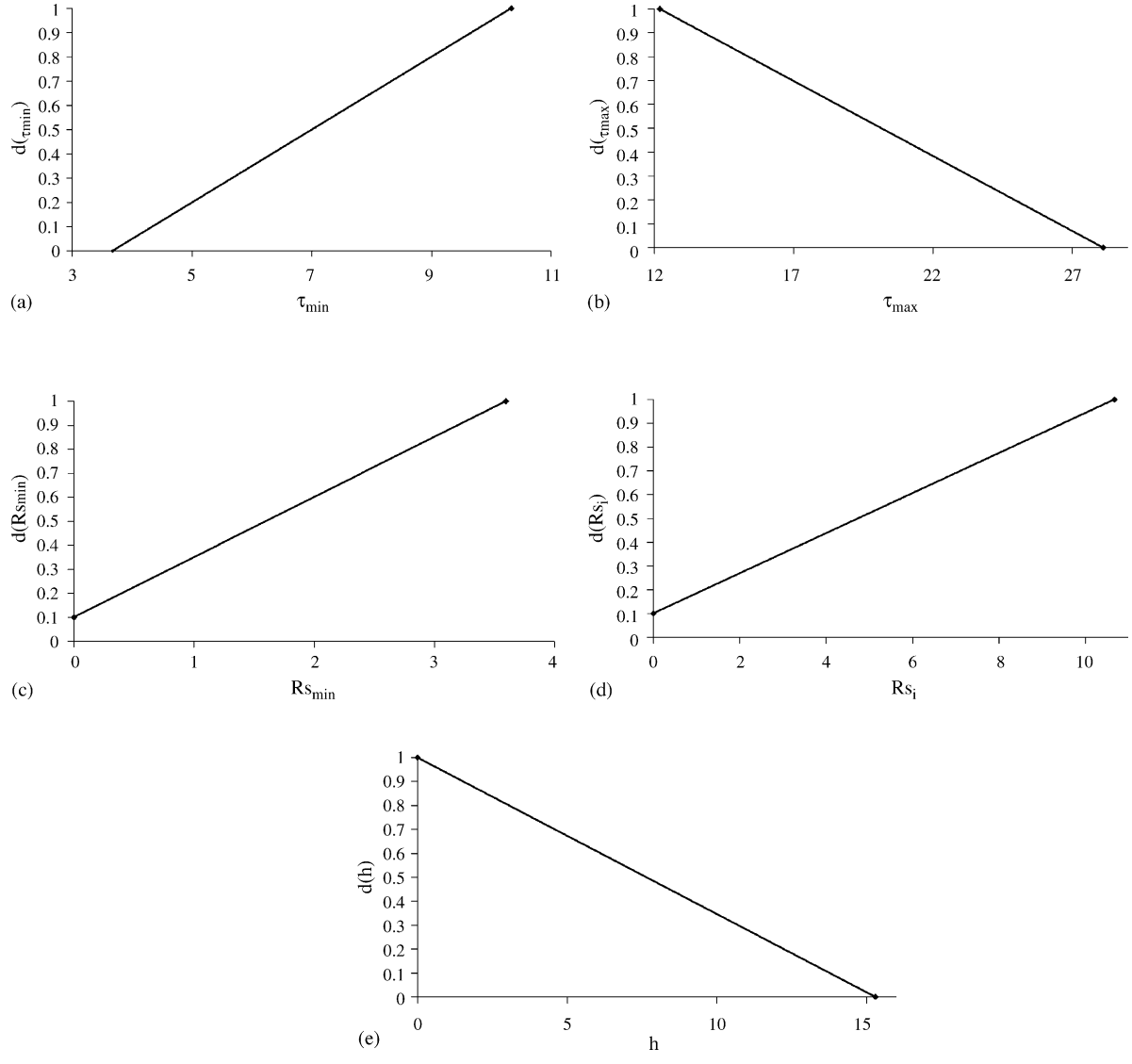

Fig. 3. Derringer's desirability functions applied for (a) τ_{min}, (b) τ_{max}, (c) Rs_{min}, (d) Rs_i, and (e) h.

Table 3
The d(i)- and D-values of the Derringer's approach

Group	Column number	Column name	$d(\tau_{min})$	$d(\tau_{max})$	$d(Rs_{min})$	$d(Rs_i)$	d(h)	D
A	14	FluoroSep-RP Phenyl	0.546	0.666	0.100	0.100	0.780	0.309
	13	Platinum EPS C18	0.917	0.083	0.100	0.100	0.951	0.236
B	19	Inertsil Phenyl-3	0.709	0.083	0.100	0.100	0.000	0.000
B_1	34	Curosil PFP	0.845	0.468	0.100	0.100	0.978	0.329
	23	Synergi Polar-RP	0.434	0.917	0.100	0.100	0.911	0.325
C	1	SunFire C_{18}	0.478	0.554	0.675	0.774	0.944	0.666
	31	Synergi Max-RP	0.442	0.576	0.650	0.658	0.867	0.624
	24	Uptisphere C18-ODB	0.564	0.484	0.438	0.686	0.876	0.590
	9	Zorbax Eclipse XDB-C_{18}	0.083	0.900	0.830	0.907	0.884	0.549
	7	Zorbax Extend-C18	0.273	0.581	0.173	0.663	0.896	0.439
	33	Synergi Fusion-RP	0.527	0.586	0.100	0.100	0.938	0.311
	2	Atlantis dC_{18}	0.506	0.514	0.100	0.100	0.887	0.297
	30	Acquity (C_{18})	0.369	0.608	0.100	0.100	0.923	0.291
	25	Uptisphere 3 C18-HDO	0.519	0.482	0.100	0.100	0.820	0.290
	21	Alltima HP C18 HiLoad	0.412	0.523	0.100	0.100	0.802	0.280
C_1	10	Zorbax SB-C18	0.258	0.884	0.249	0.605	0.855	0.494
C_1'	8	Zorbax Bonus-RP	0.109	0.728	0.100	0.100	0.593	0.216
	17	Polaris Amide-C18	0.171	0.678	0.100	0.100	0.191	0.186
C_2	32	Synergi Hydro-RP	0.456	0.515	0.758	0.628	0.899	0.632
	6	SymmetryShield RP_{18}	0.584	0.659	0.100	0.100	0.952	0.326
C_2'	5	XTerra Phenyl	0.382	0.752	0.100	0.100	0.954	0.307
	27	Capcell Pak C_{18} AQ	0.627	0.550	0.100	0.100	0.474	0.277
D	26	Capcell Pak C_{18} MG	0.446	0.507	0.918	0.918	0.909	0.704
	35	Hypersil GOLD	0.336	0.679	0.621	0.630	0.832	0.595
	16	Pursuit DP	0.291	0.820	0.516	0.574	0.898	0.576
	3	XTerra MS C_{18}	0.353	0.614	0.345	0.667	0.860	0.533
	29	Capcell Pak C_{18} ACR	0.421	0.573	0.245	0.734	0.903	0.523
	20	Alltima HP C18	0.325	0.615	0.385	0.706	0.714	0.522
	12	YMC-Pack C4	0.363	0.722	0.309	0.566	0.674	0.499
	15	Pursuit C18	0.341	0.605	0.100	0.100	0.888	0.283
	18	Polaris C18-A	0.315	0.637	0.100	0.100	0.867	0.281
	28	Capcell Pak C_{18} UG120	0.291	0.614	0.100	0.100	0.742	0.266
	4	XTerra RP_{18}	0.211	0.717	0.100	0.100	0.835	0.263
D_1	11	YMC-Pack Pro C18	0.377	0.481	0.100	0.100	0.743	0.267
	22	Alltima HP C18 Amide	0.135	0.681	0.100	0.100	0.089	0.152

Columns, within groups, are ranked according to decreasing D-values.

the initial eight, based on an evaluation of the D-values. For the dissimilar phases, the best performing according to overall separation quality can be added to the original selection. The columns with additional differences in selectivity (phases in groups A, B, and B_1) compared to the initial set do not exhibit the best global separation performances. However, these stationary phases remain interesting due to their different selectivity, and one from each group might be added to the original selection.

The original eight columns are situated in groups C (7 = Zorbax Extend-C18), C_1' (8 = Zorbax Bonus-RP), C_2 (6 = SymmetryShield RP_{18}), C_2' (5 = XTerra Phenyl), D (3 = XTerra MS C_{18}; 12 = YMC-Pack C4, and 4 = XTerra RP_{18}), and D_1 (11 = YMC-Pack Pro C18). Phases 13 and 14 (group A) are dissimilar towards those eight, and similar to each other (Figs. 1 and 2), i.e. one might be added to the initial selection. Column 14 is categorized as having better overall separation properties (Table 3) while its r-values are lower (Fig. 1). Therefore, stationary phase 14 is preferred to 13.

Three other columns, 19 (group B), 23 and 34 (group B_1), show intermediately low correlation coefficients with the original stationary phases. The D-values (Table 3) are similar for 34 and 23, but 34 has lower r-values when compared to the initial set.

Phases 1, 2, 9, 21, 24, 25, 30, 31 and 33 (group C) have high correlation coefficients with the original column 7. Table 3 shows that some stationary phases (1, 9, 24 and 31) exhibit better separation properties, and therefore can be considered alternatives for column 7. Chromatograms to demonstrate the better overall separation quality of the eventually favoured phases are displayed in Fig. 4. Fig. 4a and b (column 1) and Fig. 4c and d (column 7) exhibit a very similar elution order for both mixtures—only one peak pair is switched in the first test solution. Better resolutions, narrower peaks and improved elution spread of the substances in the interval $\tau_{min}-\tau_{max}$ for phase 1, which justifies the above preference.

Phase 17 is very similar to 8 from the original set (C_1), while they are somewhat less with 10 (C_1'). Since column 8 performs better than 17, and provides more selectivity differences, it should not be replaced. Phase 10 might be added to the set, because of slightly different selectivity and good overall separation properties.

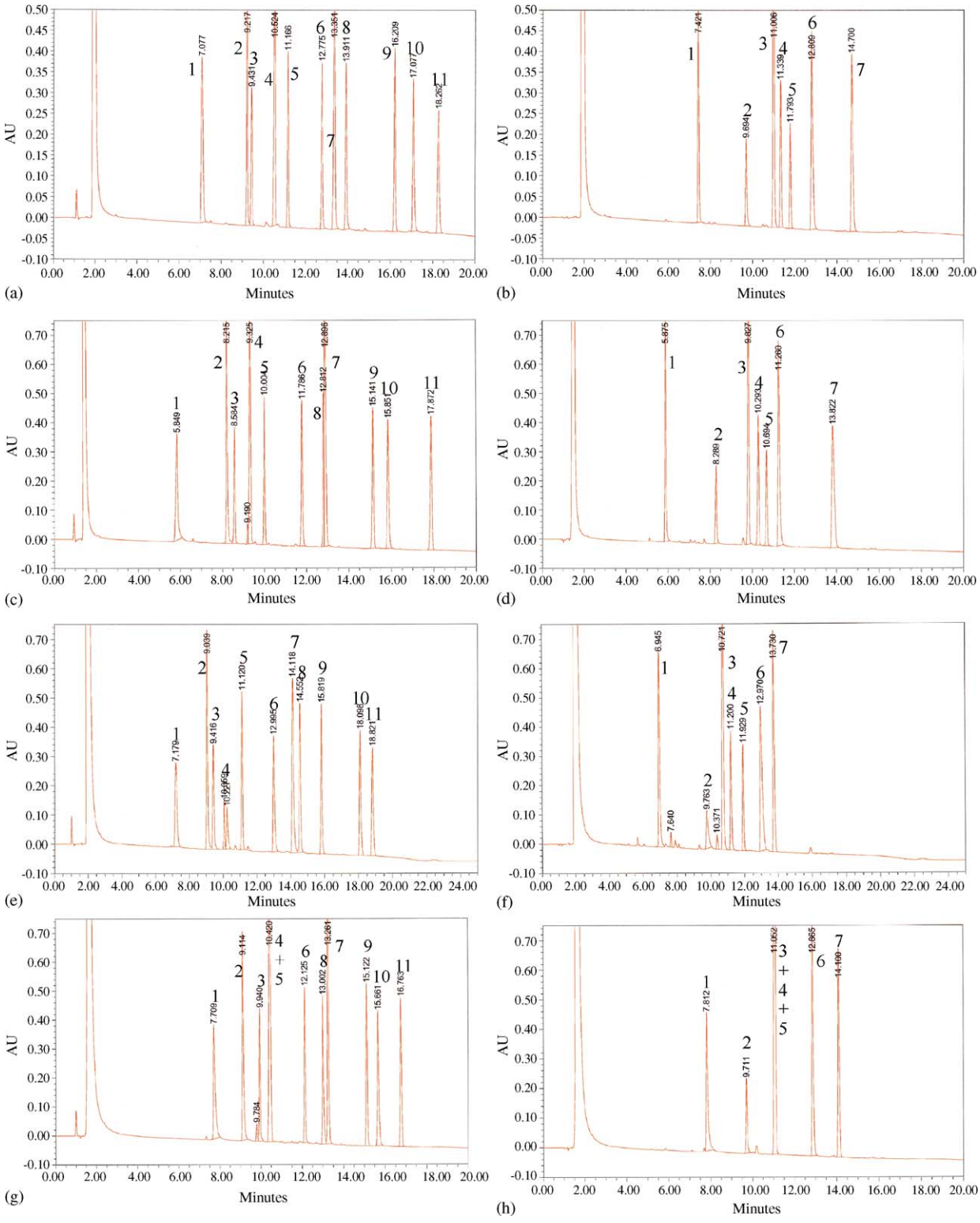

Fig. 4. Chromatograms of the mixtures of 11 and 7 substances, respectively, for the columns 1 (a and b), 7 (c and d), 32 (e and f), 6 (g and h), 26 (i and j), 3 (k and l), 12 (m and n), 4 (o and p), 11 (q and r), and 22 (s and t). The first mixture consisted of (1) levamisole hydrochloride, (2) liarozole hydrochloride, (3) domperidone, (4) flubendazole, (5) azaconazole, (6) ketoconazole, (7) sabeluzole, (8) enilconazole, (9) itraconazole, (10) miconazole nitrate, and (11) cinnarizine; the second of (1) prucalopride succinate, (2) risperidone, (3) ketanserin, (4) droperidol, (5) etomidate hydrochloride, (6) astemizole, and (7) closantel.

Analogously, column 32 (Fig. 4e and f) can replace the original phase 6 (group C_2) (Fig. 4g and h) to improve the quality of separation. For the same elution order the former provides markedly better resolutions and more regular peak spacing in the $\tau_{min}-\tau_{max}$ interval for comparable corrected peak heights. Phase 27, though exhibiting similar selectivity to the initially selected column 5 (C_2'), does not have improved separation performance and should not replace the original.

Phases 15, 16, 18, 20, 26, 28, 29 and 35 (group D) exhibit very analogous selectivities as three columns from the original group, i.e. 3, 4 and 12. Stationary phase 26, having the highest *D*-value, would be the best choice to replace one or more of

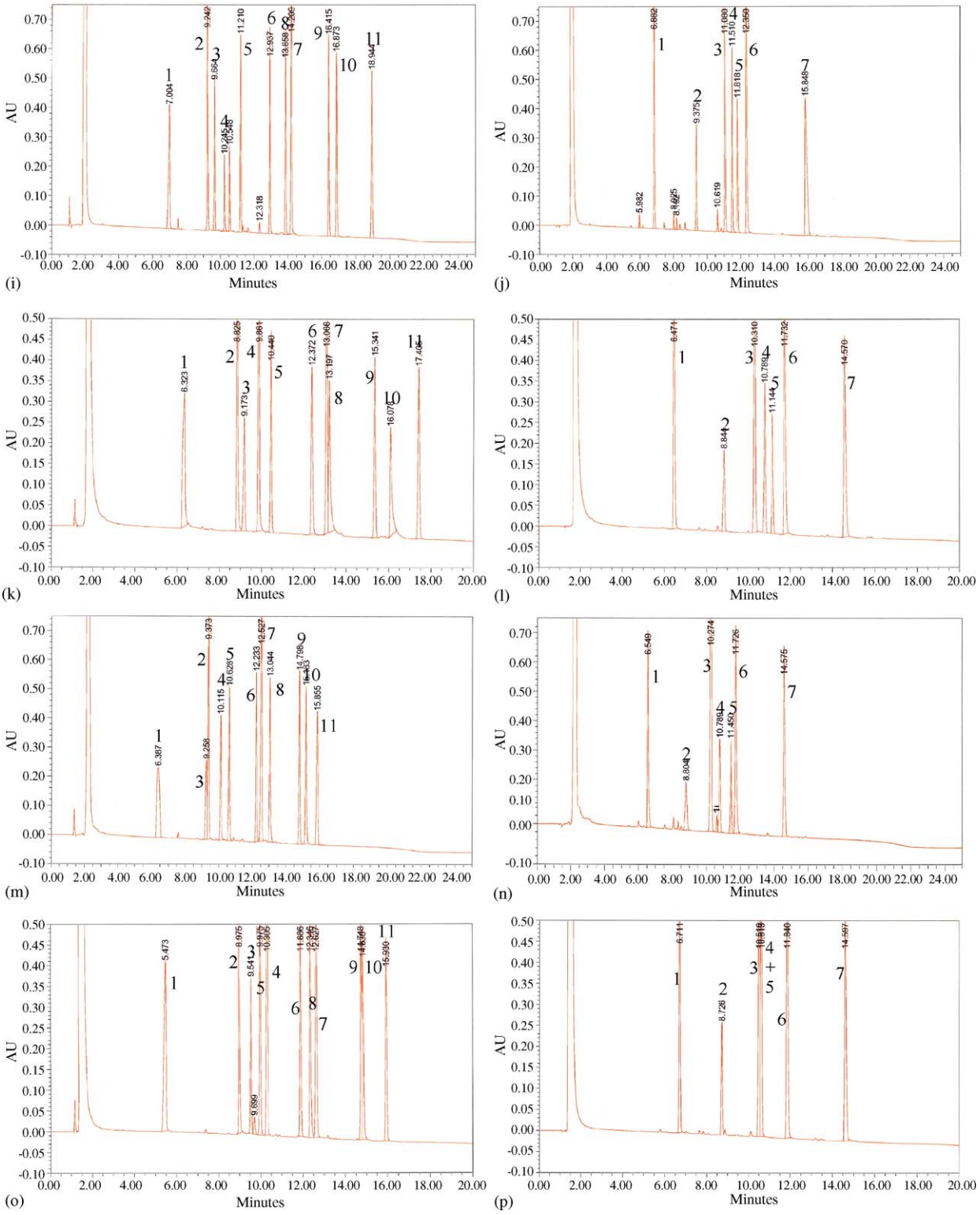

Fig. 4. (*Continued*).

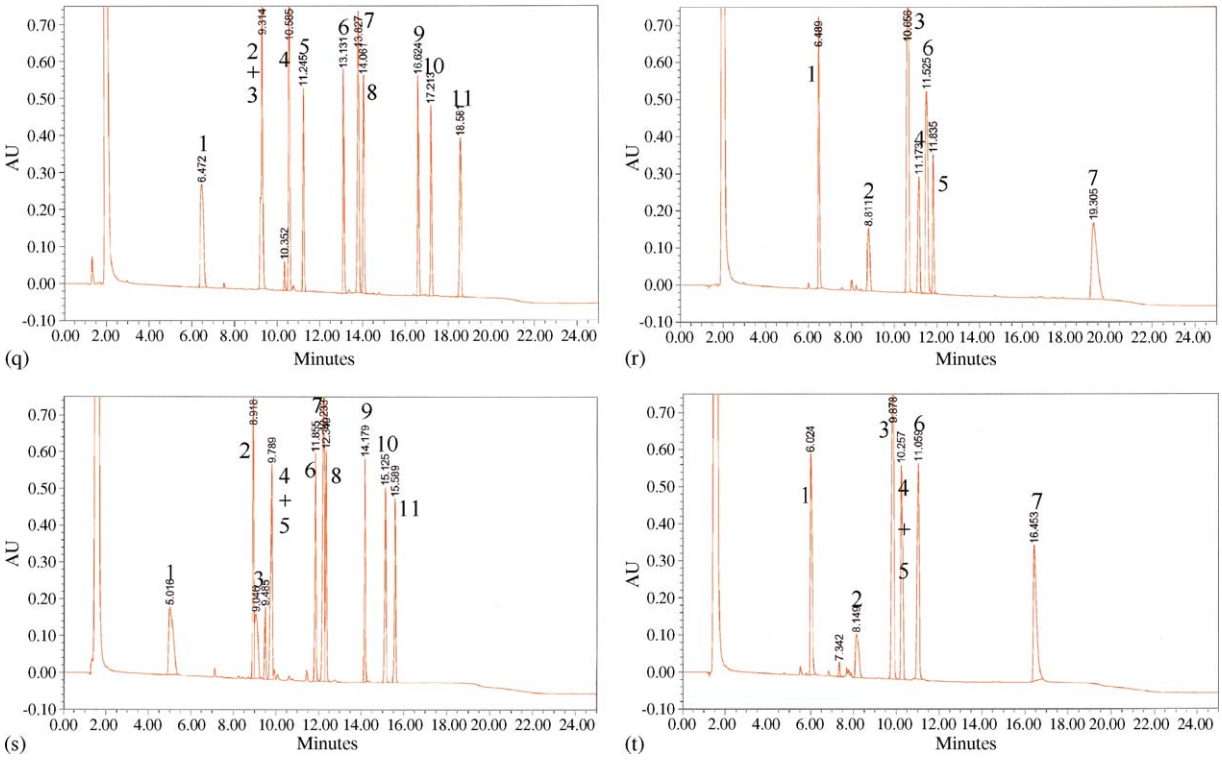

Fig. 4. (*Continued*).

them. Fig. 4i and j (phase 26) show a very similar elution order compared to Fig. 4k and l (column 3), Fig. 4m and n (column 12) and Fig. 4o and p (column 4)—only one or two peak pairs are switched in the first mixture. However, narrower peaks and better resolutions are obtained with the first. A decreasing trend in overall separation quality can be seen from phase 26 over 3, 12 and 4.

Finally, the earlier selected column 11 (D_1) (Fig. 4q and r) is very similar to phase 22 (Fig. 4s and t), but performs better according to Derringer's approach. Narrower peaks and better resolutions were obtained with 11.

In summary, it was possible to add some new columns to and to propose substitutes for the original set of phases [15]. They very likely will increase the selectivity differences and/or the overall separation quality. Columns 14 (FluoroSep-RP Phenyl), 19 (Inertsil Phenyl-3), 34 (Curosil PFP) and 10 (Zorbax SB-C18) can be added, while 1 (SunFire C_{18}) can replace 7 (Zorbax Extend-C18), 32 (Synergi Hydro-RP) can substitute 6 (SymmetryShield RP_{18}), 26 (Capcell Pak C_{18} MG) can take the place of 3 (XTerra MS C_{18}), 12 (YMC-Pack C4) and/or 4 (XTerra RP_{18}). For phases 8 (Zorbax Bonus-RP), 5 (XTerra Phenyl) and 11 (YMC-Pack Pro C18) of the original set no valuable alternatives were found within the tested columns.

If, for some additional reason, such as rapid column deterioration or less pH-stability, the preferred stationary phase is less good, it can be replaced by another from the same group. For instance in group C_1', phase 8 is preferred over 17. However, when extreme pH-values are needed, column 17 is more suited, since its applicable pH-range (1.5–10.0) is wider than that of phase 8 (2.0–8.0).

4. Conclusion

Two mixtures of structurally related active substances were injected applying a single gradient elution condition, buffered at pH 7.0, as a new test procedure for classification of RP-HPLC stationary phases based on selectivity differences and overall separation performances. The compounds/mixtures were chosen with a view to the eventual use of the columns, i.e. their implementation in methods to separate drug/impurity profiles.

The weighted-average-linkage dendrograms, $r(\tau)$-color maps and Derringer's desirability function approaches allowed to deduce dissimilar and similar columns and to rank the phases according to their overall separation performances. A methodology was proposed in which the most dissimilar stationary phases and/or those with the best overall separation qualities relative to the original eight were defined.

From the 27 new columns, four might be added because of selectivity differences; three could replace five of the original because of similar selectivities but better separation qualities. For three of the initially selected eight no alternatives were found in the test set. This approach eventually enables to increase the throughput of stationary phases testing taking into account information concerning selectivity differences and overall separation quality.

Acknowledgements

Prof. D.L. Massart for providing the possibility to start the PhD study. This research is funded by a PhD grant of the Institute

for the Promotion of Innovation through Science and Technology in Flanders (IWT-Vlaanderen).

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