Evaluation of Orthogonal/Dissimilar RP-HPLC Systems Sets for Their Suitability as Method-Development Starting Points for Drug Impurity Profiles

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Abstract

Orthogonal or dissimilar separation systems provide different selectivities and their application can facilitate the development of methods to identify and quantify impurities in a drug substance. Two sets of chromatographic systems potentially applicable for method development were evaluated using four drug/impurity profiles. The sets consist of orthogonal or dissimilar systems and systems with good overall separation properties, selected in earlier studies. The aim of this study is to evaluate these systems for selectivity differences in the impurity profiles. These differences should allow determining the number of compounds occurring in an impurity profile. Then, one or a very limited number of systems is to be proposed for further method development. To examine the selectivity changes and separation quality for each impurity profile, both the normalized retention times τ and the resolutions between pairs of consecutively eluting peaks were plotted on parallel axes, representing the systems. For each profile, several systems of the studied sets can serve as potential starting points for further method development. All impurities could be separated from the active substance and from each other on at least one system. However, for the different profiles, different systems were selected as best, which makes that each system in a given set has its importance, depending on the properties of the profile.

Introduction

In method development for pharmaceutical analysis, one must separate impurities from each other and from the active substance in order to enable identification/quantitation of the impurities (1). It is often challenging to develop a method that fulfills these demands. Impurities can arise from many different sources (e.g., from changes in the synthetic process, reaction conditions, purification processes, or during process scale up) (2). As a result, different impurities can appear, and the safety of the drug needs to be questioned, making method development frequently required (3). The use of a set of orthogonal or dissimilar chromatographic systems could be helpful to screen the unknown mixtures and to reach the goal of identification and quantitation. When comparing various one-dimensional systems (i.e., systems containing one stationary phase) for instance, as potential starting points for method development, orthogonal systems are defined as systems "that differ significantly in chromatographic selectivity" (3). This means that systems with a low correlation coefficient (r) (but different from zero) between the retention data are also considered or called orthogonal. It means too that (e.g., when comparing pairs of systems) terms as more orthogonal (or more dissimilar, or with more selectivity differences) and rather orthogonal can be applied. For reasons of analogy with previous publications (4–8) usually the term orthogonal, rather than dissimilar is used. The results on the different systems allow determining the number of compounds in a mixture and the most appropriate system then can be used as starting point for further method development (4–8).

In the literature, besides our own work (4–8) not so many publications can be found concerning the application and, in particular, the selection of orthogonal systems or techniques. Tagliaro et al. (9) compared two capillary zone electrophoresis (CZE) and one micellar electrokinetic capillary chromatography (MECC) methods for a number of forensic substances, and revealed the non-correlatedness, thus complementary applicability of the MECC and one CZE method. Another study (10) reported the investigation of the migration behavior of nicotine and related tobacco alkaloids using microemulsion electrokinetic chromatography (MEEKC) and non-aqueous capillary electrophoresis (NACE), next to free-solution CE (FSCE), as three orthogonal separation techniques. Steuer et al. (11) compared high-performance liquid chromatography (HPLC), supercritical fluid chromatography (SFC) and CZE to evaluate the orthogonality of the information they provided and examined their properties towards several substance groups. Xue et al. (3) described the parallel application of seven orthogonal HPLC methods in a

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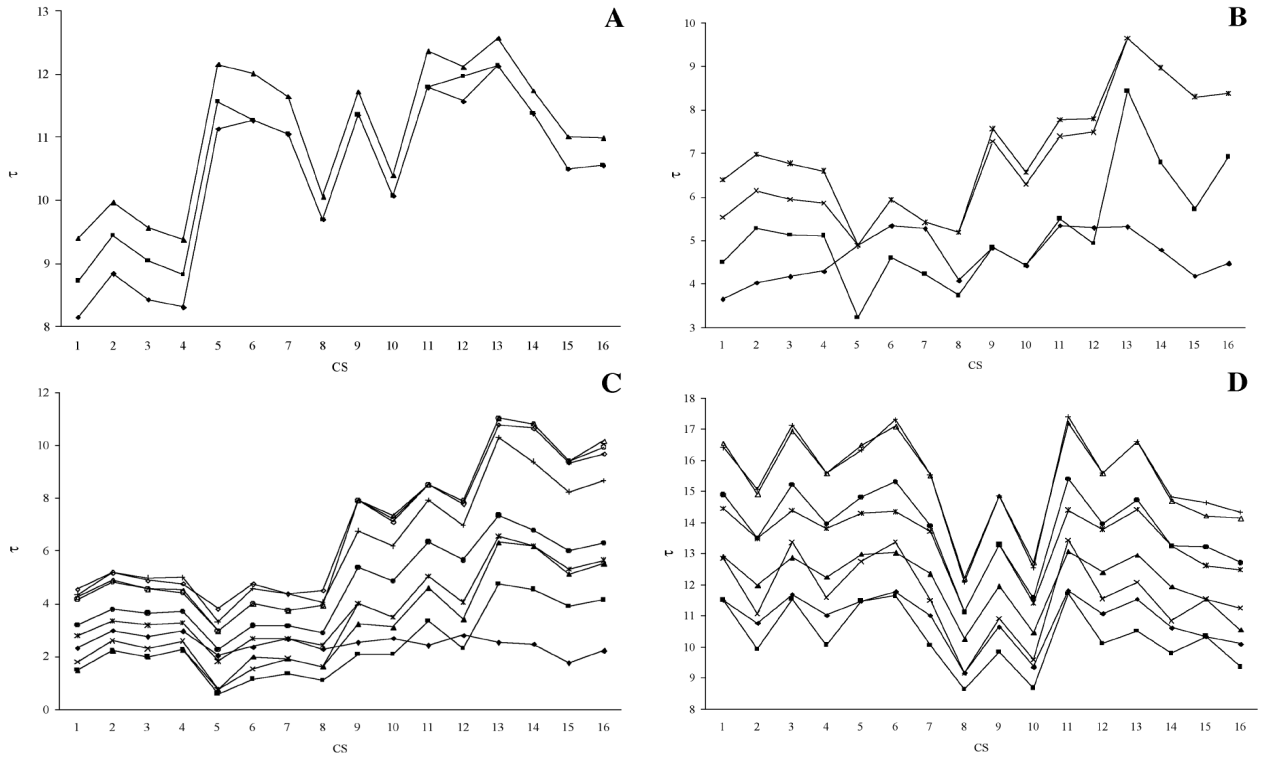

Figure 1. Normalized retention times on the 16 chromatographic systems for impurity profiles: 1 (A); 2 (B); 3 (C); and 4 (D). For part A: ♦, AS1; ▫, 1A; ▪, 1B. For part B: ♦, 2A; ▫, 2B; × 2C and AS2; *, 2D. For Part C: ♦, 3A; ▫, 3B; ▪, 3C; × AS3; *, 3D; ▲, 3E; +, 3F; Δ, 3G; ▲3H; ◊ 3I. For part D: ♦, 4A; ▫, 4B; ▪, 4C; ×, 4D; *, 4E; ●, AS4; +, 4F; –, 4G.

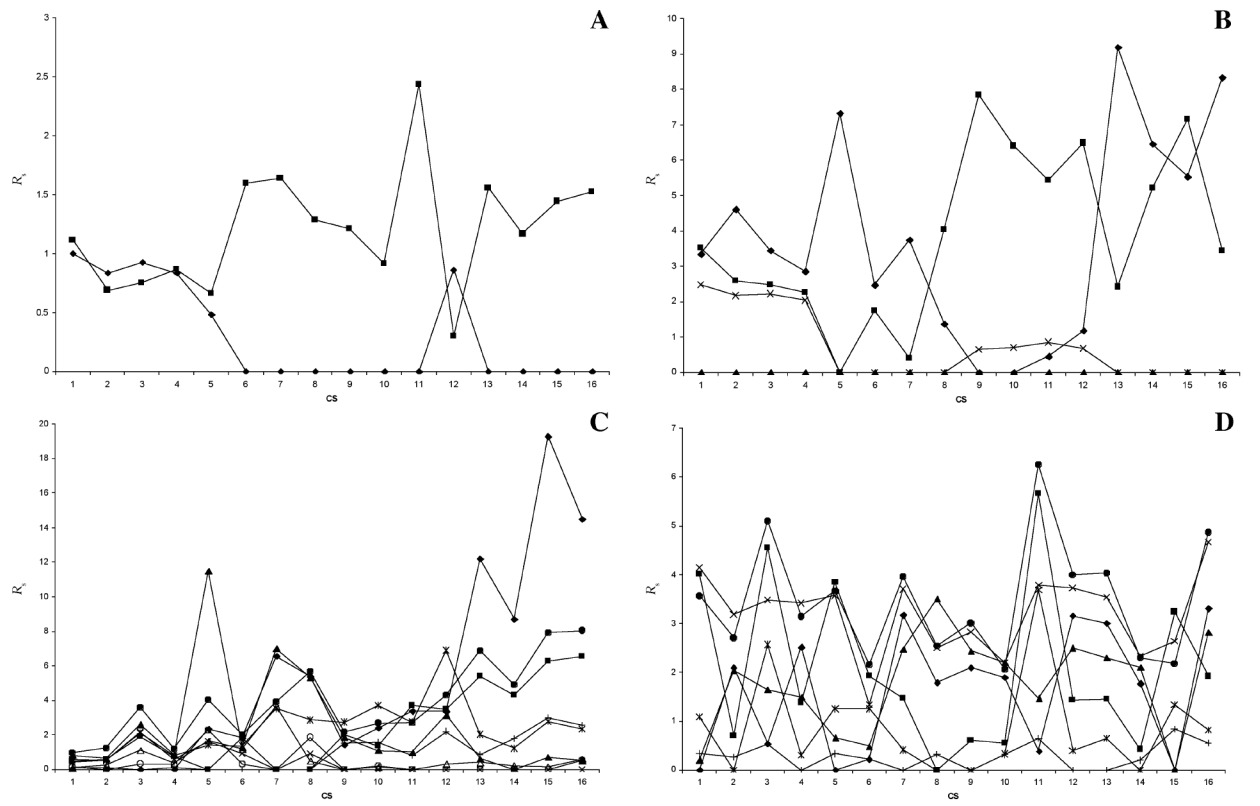

Figure 2. Resolution plots for the 16 systems for impurity profiles 1 (A); 2 (B); 3 (C); and 4 (D). The resolutions are symbolized as: ♦, peak pair 1; ▫, 2; ▪, 3; ×, 4; *, 5; ▲, 6; +, 7; Δ, 8; and ▲, 9.

fully automated generic impurities screening approach using hyphenated ultraviolet–mass spectrometric (UV–MS) detection.

Table IA. Composition of the Mobile Phases

pH	Phase A	Phase B
2.5	Ammonium acetate in water–acetonitrile 95:5% (v/v) + trifluoroacetic acid	Ammonium acetate in water–acetonitrile 30:70% (v/v) + trifluoroacetic acid
4.8	Ammonium acetate in water–acetonitrile 95:5% (v/v) + acetic acid	Ammonium acetate in water–acetonitrile 30:70% (v/v) + acetic acid
7.0	Ammonium acetate in water–acetonitrile 95:5% (v/v)	Ammonium acetate in water – acetonitrile 30:70% (v/v)
9.0	Ammonium acetate in water–acetonitrile 95:5% (v/v) + diethylamine	Ammonium acetate in water–acetonitrile 30:70% (v/v) + diethylamine

Neue et al. (12) measured the selectivity differences between RPLC systems.

In our previous work the selection of orthogonal/dissimilar systems is based on the injection of test substances. In the literature (5), 27 chromatographic systems, potentially applicable for method development, were examined for selectivity differences using two sets of test substances, in total 83 compounds. The systems consisted of eight silica-based stationary phases selected for their good efficiency and selectivity (6), with mobile phases at pH-values 2.5, 4.8, and 7.0, and for three of them, also at pH 9.0. The set was reduced to 15 systems (four at pH 2.5, 4.8 and 7.0, and three at pH 9.0) consisting of orthogonal ones

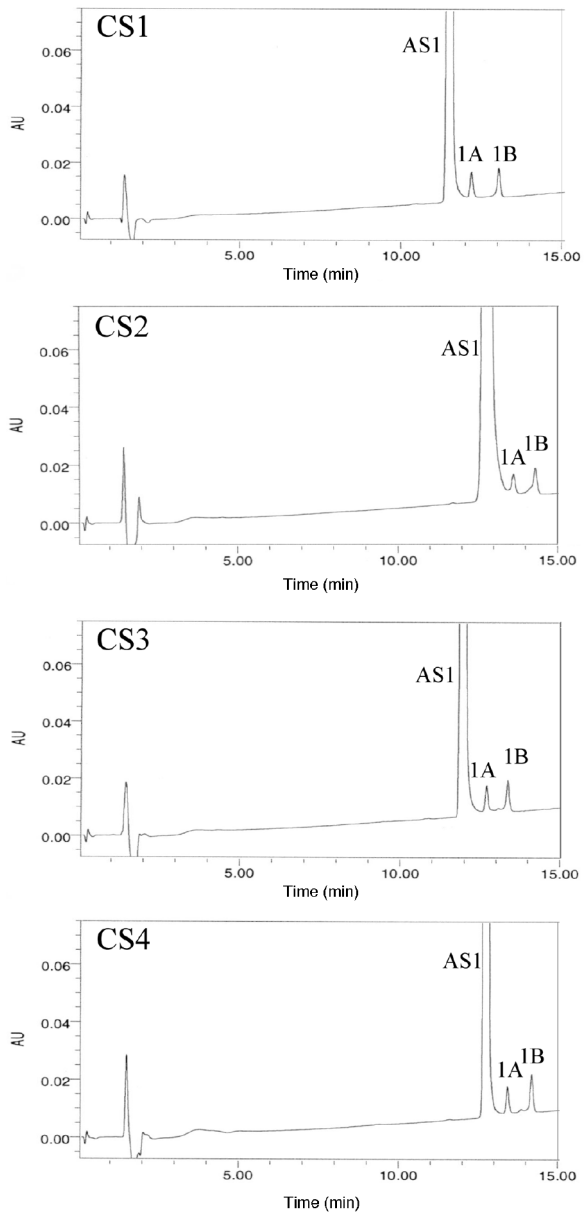

Figure 3A. Best chromatograms from the 16 systems for impurity profile 1 (CS1, CS2, CS3, and CS4).

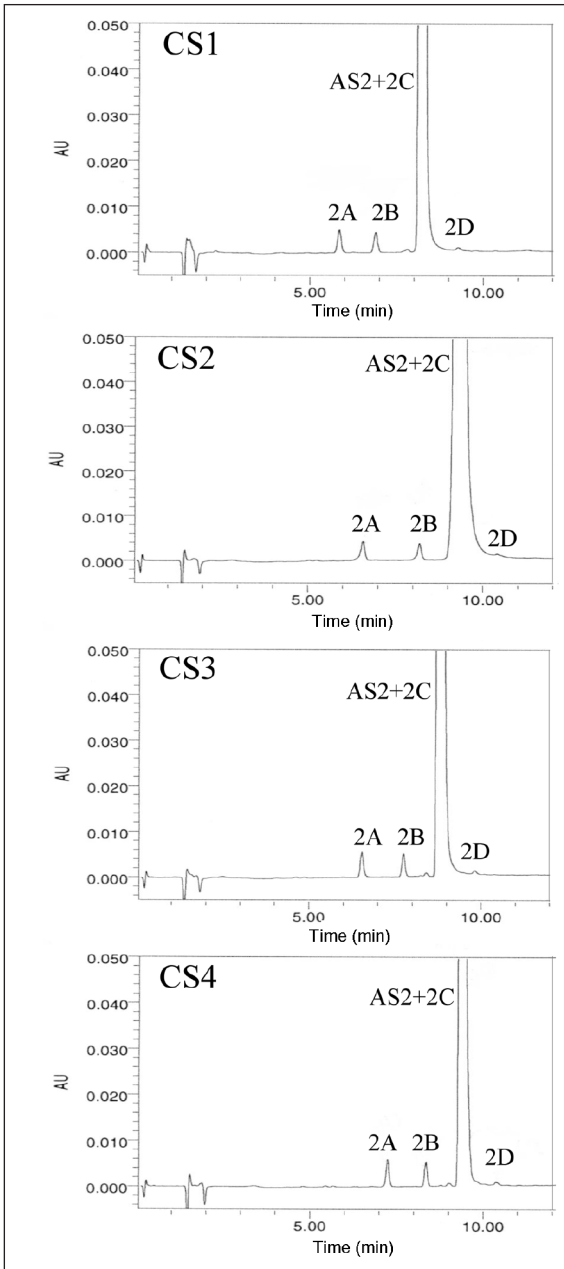

Figure 3B. Impurity profile 2 (CS1, CS2, CS3, and CS4).

and systems with good overall separation quality, the latter based on five parameters, being 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. For more information we refer to the literature (5). A second set, consisting of 17 systems, was selected in another study (7). Contrary to the above set, very diverse stationary phases were included (i.e., silica-, zirconia-, carbon-, and polymer-based ones) while also different organic modifiers, buffer pH-values, and column temperatures were considered. In this study, 68 substances were injected. This was also the case in the references (4,5,8).

However, the selection of the dissimilar systems, based on the retention differences of the test substances, does not guarantee that these systems also would show selectivity differences for drug/impurity profiles, in which the compounds are much more similar. The occasional occurrence of these differences is examined in this study. The aim of this study is to evaluate, by means of four impurity profiles differing in complexity, whether the above sets indeed could serve as screening sets allowing the selection of one or a small number of systems as potential starting points for further method development. If the previous selections of dissimilar systems (5,7) were appropriate this indeed should be the case.

Table IB. Gradient Elution Scheme

Time (min)	Phase A	Phase B
0	100%	0%
20	0%	100%
25	0%	100%

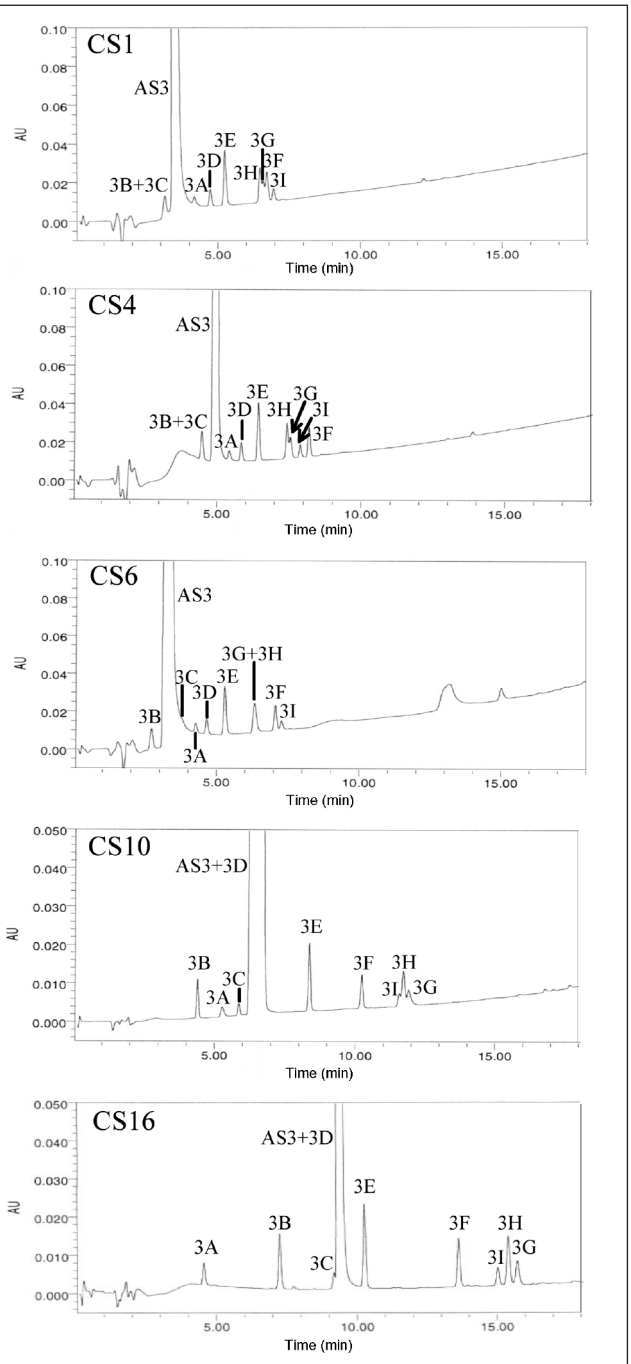

Figure 3C. impurity profile 3 (CS1, CS4, CS6, CS10, and CS16).

Experimental

Drugs and reagents

Four impurity profiles (all Johnson & Johnson Pharmaceutical Research and Development, a division of Janssen Pharmaceutica N.V., Beerse, Belgium) are used to evaluate the first set of systems. They consisted of the active substance and a number of impurities, the latter present in an amount of about 0.1–0.5% (m/m) relative to the active compound. Impurity profile 1 contains the active substance (AS1) and two impurities (1A and 1B). The second profile consists of the active substance (AS2)

Table II. The Columns Used at the Four pH-Values

pH 2.5	
	Zorbax Bonus-RP (CS1)
	Waters XTerra MS C18 (CS2)
	Waters SymmetryShield RP18 (CS3)
	YMC-Pack Pro C18 (CS4)
pH 4.8	
	Zorbax Bonus-RP (CS5)
	Waters SymmetryShield RP18 (CS6)
	YMC-Pack Pro C18 (CS7)
	Waters XTerra Phenyl (CS8)
pH 7.0	
	Waters XTerra MS C18 (CS9)
	YMC-Pack C4 (CS10)
	Waters SymmetryShield RP18 (CS11)
	YMC-Pack Pro C18 (CS12)
pH 9.0	
	Zorbax Extend-C18 (CS13)
	Waters XTerra MS C18 (CS14)
	Waters XTerra RP18 (CS15)
	Polaris C18-A (CS16)

and four impurities (2A, 2B, 2C, and 2D). The third and fourth profiles contain the active substances (AS3 and AS4, respectively), and nine and seven impurities, denoted as 3A to 3I, and 4A to 4G, respectively. The concentration of impurity profile 1 was 0.12 mg/mL in methanol; of profile 2, 0.50 mg/mL in methanol; of profile 3, 0.48 mg/mL in water–methanol 95:5% (v/v), and of profile 4, 0.10 mg/mL in dimethylsulfoxide. Methanol for HPLC (Acros Organics, Geel, Belgium) and dried dimethylsulfoxide (Merck, Darmstadt, Germany) were used.

For the evaluation of the second set of systems, impurity profile 3 consisted of AS3 and 3A–3F; while in impurity profile 4, 4E was not included.

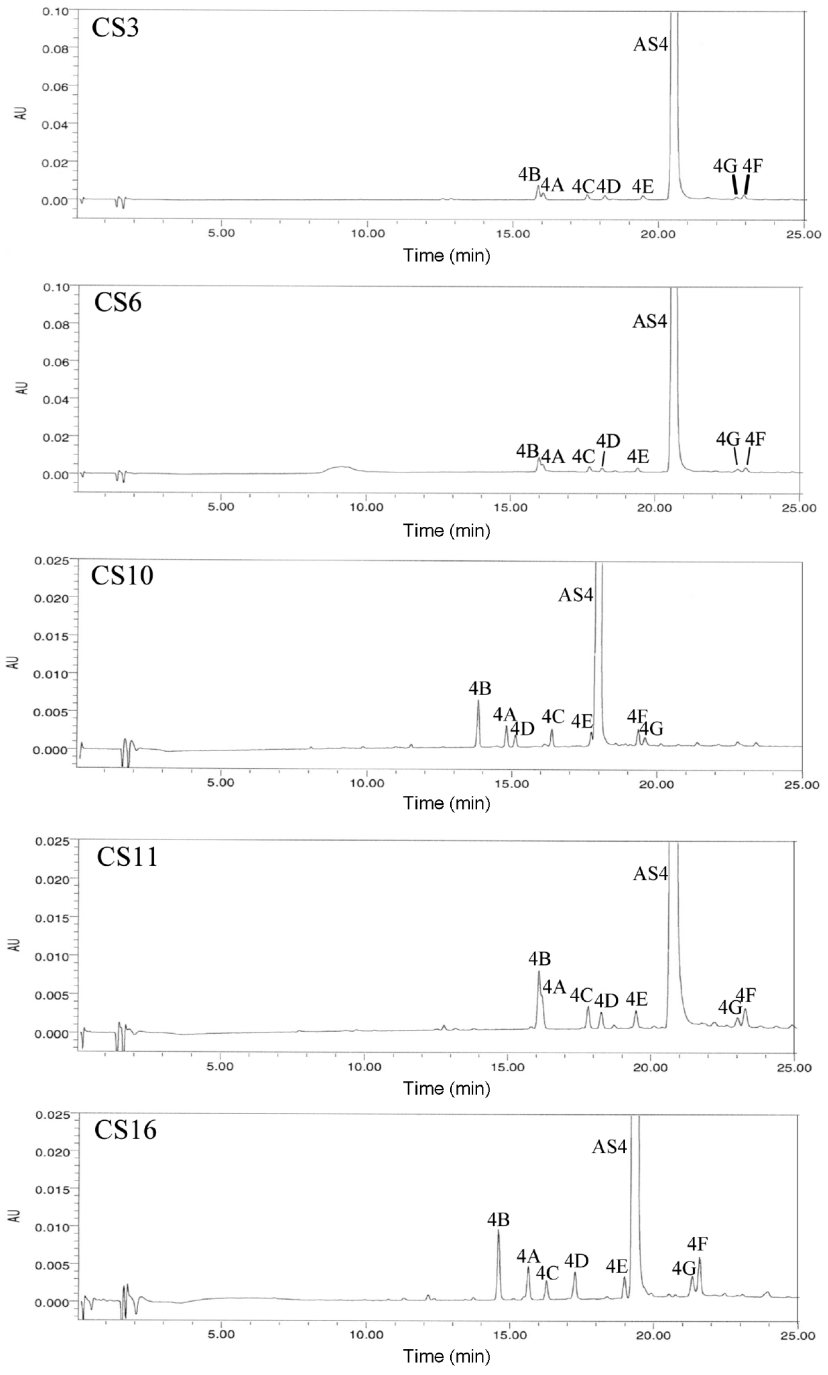

Figure 3D. Impurity profile 4 (CS3, CS6, CS10, CS11, and CS16).

In the mobile phases, methanol for HPLC, acetonitrile for HPLC far UV (Acros Organics), acetic acid TraceSelect for trace analysis, ammonium acetate Ultra, ammonium formate Ultra, ammonium hydrogen carbonate MicroSelect, diethylamine puriss. plus (GC), formic acid puriss. p.a., and trifluoroacetic acid for protein sequence analysis, all from Fluka Chemie (Buchs, Switzerland), are used. Milli-Q water, prepared with the Millipore purification system (Molsheim, France), is used in mobile phases and solvents.

Chromatographic conditions

The experiments were performed on an LC–MS-DAD instrument consisting of a Waters 2695 Separations Module (= alliance) HPLC compartment (Milford, MA), a Mistral column oven (Spark Holland, Emmen, The Netherlands), a column switcher (VICI, Schenkon, Switzerland) and a Waters 996 Photodiode Array Detector, linked with a single quadrupole mass spectrometer, Waters Micromass ZQ (Waters/Micromass, Manchester, UK), using multi-plexed electrospray ionization, applied in the positive ionization mode. The effluent from the HPLC was splitted prior to diode array detection with an Acurate Post-Column Splitter (LC Packings, Amsterdam, The Netherlands) (split = 1/20). Typical parameters of the ion source are: capillary voltage, 3.28 kV; cone voltage, 31 V; source temperature, 100°C; desolvation temperature, 350°C; and cone gas flow, 100 l/h N_2. The specifications for the mass spectrometer are: scan range, 20 to 1225 (M_r); scan time, 1.00 s; interscan time, 0.1 s; photomultiplier voltage, 650 V; resolution, 15.0 u. One ionization energy was used for all compounds. The chromatographic methods were created and the data treated using Millennium[32] Version 4.0 software (Waters) for both the diode array and the mass spectrometry data.

The first set (16 systems) [i.e., the 15 systems from (5) were increased to 16 with a Polaris C18-A column (see below) at pH 9.0 for practical reasons] comprised nine stationary phases: (A) Zorbax Bonus-RP, (150 mm × 3.0 mm i.d., 5 µm) (Agilent, Palo Alto, CA), a triple-endcapped ultrapure C_{14}-silica with embedded polar amide group and sterically protecting diisopropyl group; (B) XTerra MS C_{18} (150 mm × 3.0 mm i.d., 5 µm) (Waters), a hybrid C_{18}-silica with trifunctional bonding and embedded polar group; (C) SymmetryShield RP_{18} (150 mm × 3.0 mm i.d., 5 µm) (Waters), a C_{18}-silica shielded through an embedded polar group; (D) YMC-Pack Pro C18 (150 mm × 3.0 mm

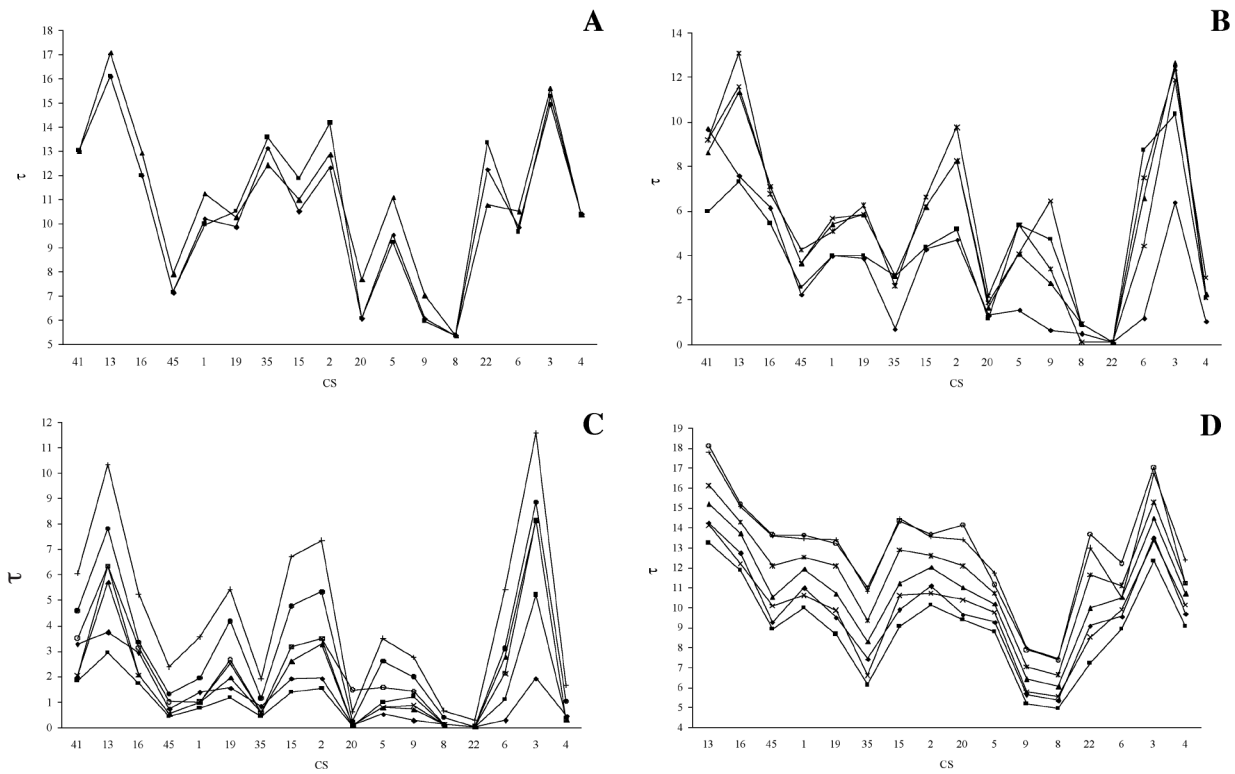

Figure 4. Normalized retention times on the 17 chromatographic systems for impurity profiles 1 (A), 2 (B), 3 (C), and 4 (D). In Part 4(A): ♦ AS1; ■ 1A; ▲ 1B. In Part 4(B): ♦ 2A; ■ 2B; ▲ AS2; × 2C; * 2D. In Part 4(C): ♦ 3A; ■ 3B; ▲ 3C; × AS3; ○ 3D; ● 3E; + 3F. In Part 4(D): ♦ 4A; ■ 4B; ▲ 4C; × 4D; * AS4; ○ 4F; + 4G. No measurements were made with profile 4 on CS41.

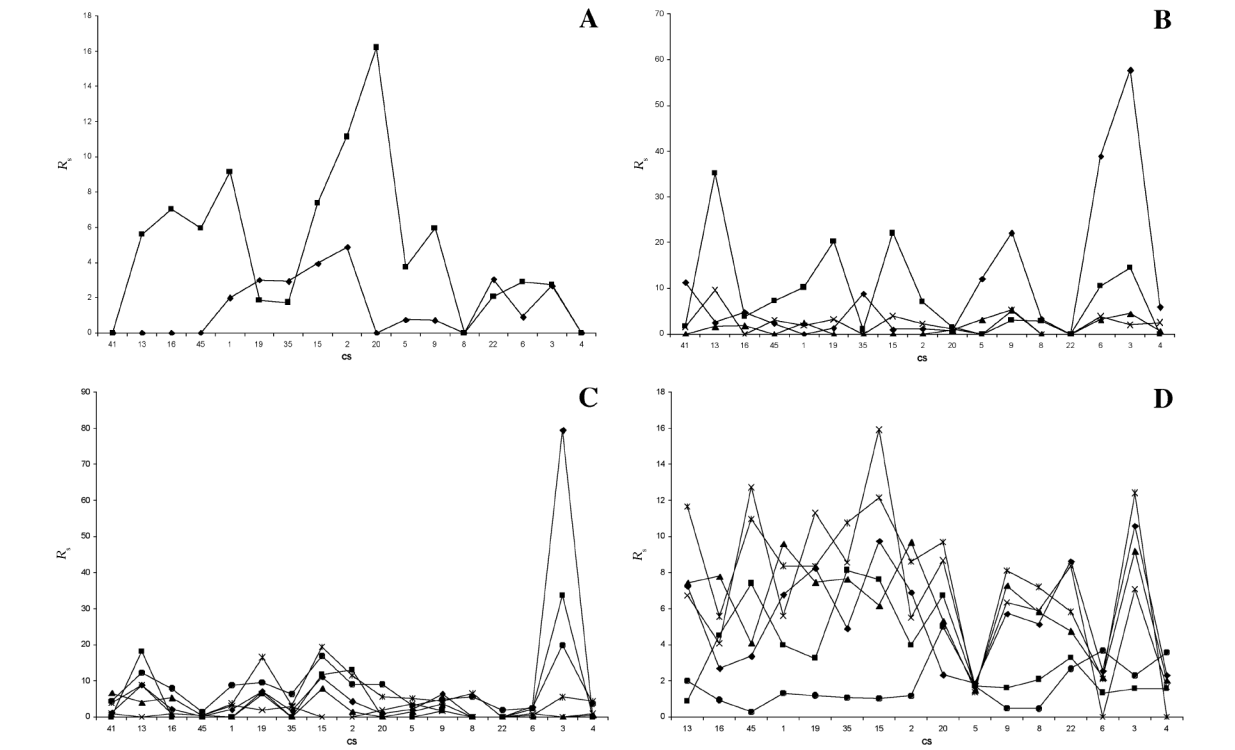

Figure 5. Resolution plots for the 17 systems for impurity profiles 1 (A), 2 (B), 3 (C), and 4 (D). The resolutions are symbolized as: ♦ (peak pair 1), ■ (2), ▲ (3), × (4), * (5), and ● (6).

i.d., 5 µm) (YMC c/o Waters), a C_{18}-silica with high-coverage carbon bonding and an endcapping procedure utilizing Lewis acid-Lewis base chemistry; (E) XTerra Phenyl (150 mm × 3.0 mm i.d., 5 µm) (Waters), a hybrid phenyl-silica with difunctional bonding; (F) YMC-Pack C4 (100 mm × 4.6 mm i.d., 3 µm) (YMC c/o Waters), a fully-endcapped C_4-silica with high-coverage monomeric bonding; (G) Zorbax Extend-C18 (150 mm × 3.0 mm i.d., 5 µm) (Agilent), a bidentate bonded and double-endcapped ultrapure C_{18}- silica stationary phase; (H) XTerra RP_{18} (150 mm × 3.0 mm i.d., 5 µm) (Waters), a hybrid C_{18}-silica shielded through embedding a polar group; and (I) Polaris C18-A (150 mm × 3.0 mm i.d., 5 µm) (MetaChem Technologies, Torrance, CA), a column with a polar group between the primary C_{18}-chain and the silica surface.

Four mobile phases, each with a different pH (2.5, 4.8, 7.0, and 9.0), were used. In Table IA, their composition is described. Four stationary phases were used at each pH, creating 16 chromatographic systems, denoted as CS1–CS16 (Table II). For all systems, the same gradient elution scheme was applied (Table IB). Afterwards, the method returned to starting conditions using a fast gradient of 2 min, and the stationary phase was re-equilibrated at starting conditions for 8 min. All experiments were thermostated at 45°C. The flow used in the literature (5), 1.0 mL/min, was adapted to 0.6 mL/min when columns with internal diameters of 3.0 mm were used. The injection volume was 10 µL.

The second set, consisting of 17 systems, is also composed of nine stationary phases: (A) Chromolith Performance, RP-18e (100 mm × 4.6 mm i.d.) (Merck), a monolithic silica phase; (B) Zorbax Extend-C18 (150 mm × 4.6 mm i.d., 3.5 µm) (Agilent); (C) ZirChrom-PS (100 mm × 4.6 mm i.d., 3 µm) (ZirChrom Separations, Anoka, MN), a polystyrene-coated zirconia-based phase; (D) Zorbax Eclipse XDB-C_8 (150 mm × 4.6 mm i.d., 5 µm) (Agilent), a densely bonded, double-endcapped ultrapure C_8-silica with dimethyl side chains; (E) Betasil Phenyl Hexyl (100 mm × 4.6 mm i.d., 5 µm) (Thermo Hypersil Keystone, Cheshire, UK), a phenyl-hexyl-silica column; (F) Suplex pKb-100 (150 mm × 4.6 mm i.d., 5 µm) (Supelco, Bellefonte, PA), a polar-embedded hexadecylsilica; (G) ZirChrom-PBD (100 mm × 4.6 mm i.d., 3 µm) (ZirChrom Separations), a polybutadiene-coated zirconia-based phase; (H) Shodex RSpak DE-413 (150 mm × 4.6 mm i.d., 4 µm) (Showa Denko, Tokyo, Japan), a polymethacrylate-packed column; and (I) Discovery RP-AmideC16 (100 mm × 4.6 mm i.d., 5 µm) (Supelco), a high-purity hexadecylsilica with a polar-embedded amide function bonded to the silica surface by a propyl group. The original systems are described in the literature (7). Since impurity profiles, implying structurally similar substances, are studied, mass spectrometry becomes necessary as a detection dimension besides the previously applied photodiode array detection (3). Therefore, the inorganic buffer of the initial systems was replaced with an organic. The gradient time applied in the lit (7) is adapted to 20 min, and the flow rate to 1.0 mL/min. In Table III, the systems are described. Once the final gradient conditions were reached, the elution conditions remained constant for 5 min. The mobile phase composition was then returned to starting conditions in 2 min, and the column re-equilibrated during 5 min.

Table III. The 17 Chromatographic Systems of the Second Set. System Numbers are as in the Literature (7)

CS	Stationary phase	Mobile phase conditions and column temperature
1	Chromolith Performance	Methanol–0.08M ammonium formate buffer pH 3.0 from 10:90 to 75:25% (v/v); 40°C
2	Chromolith Performance	Methanol–0.08M ammonium acetate buffer pH 6.8 from 10:90 to 75:25% (v/v); 40°C
3	Zorbax Extend-C18	Methanol–0.08M ammonium hydrogen carbonate-diethylamine buffer pH 10.0 from 10:90 to 75:25% (v/v); 40°C
4	ZirChrom-PS	Methanol–0.08M ammonium formate buffer pH 3.0 from 10:90 to 70:30% (v/v); 40°C
5	ZirChrom-PS	Methanol–0.08M ammonium acetate buffer pH 6.8 from 10:90 to 70:30% (v/v); 40°C
6	ZirChrom-PS	Methanol–0.08M ammonium hydrogen carbonate-diethylamine buffer pH 10.0 from 10:90 to 70:30% (v/v); 40°C
8	ZirChrom-PS	Acetonitrile–0.04M ammonium formate buffer pH 3.0 from 10:90 to 70:30% (v/v); 40°C
9	ZirChrom-PS	Acetonitrile–0.04M ammonium acetate buffer pH 6.8 from 10:90 to 70:30% (v/v); 40°C
13	Zorbax Eclipse XDB-C8	Methanol–0.04M ammonium acetate buffer pH 6.8 from 10:90 to 70:30% (v/v); 40°C
15	Zorbax Eclipse XDB-C8	Acetonitrile–0.04M ammonium acetate buffer pH 6.8 from 10:90 to 70:30% (v/v); 40°C
16	Betasil Phenyl Hexyl	Methanol–0.04M ammonium formate buffer pH 3.0 from 10:90 to 70:30% (v/v); 40°C
19	Betasil Phenyl Hexyl	Acetonitrile–0.04M ammonium acetate buffer pH 6.8 from 10:90 to 70:30% (v/v); 40°C
20	Suplex pKb-100	Methanol–10mM ammonium acetate buffer + 0.1% trifluoroacetic acid pH 2.5 from 30:70 to 75:25% (v/v); 40°C
22	ZirChrom-PBD	Methanol–10mM ammonium acetate buffer + 0.1% trifluoroacetic acid pH 2.5 from 30:70 to 75:25% (v/v); 40°C
35	ZirChrom-PBD	Acetonitrile–0.04M ammonium formate buffer pH 3.0 from 10:90 to 70:30% (v/v); 75°C
41	Shodex RSpak DE-413	Methanol–0.04M ammonium formate buffer pH 3.0 from 10:90 to 70:30% (v/v); 40°C
45	Discovery RP-AmideC16	Acetonitrile–0.04M ammonium formate buffer pH 3.0 from 10:90 to 70:30% (v/v); 40°C

Results and Discussion

Chromatograms from DAD detection were generated at 240, 275, 230, and 310 nm for the first, second, third, and fourth impurity profile, respectively. Since dissimilar systems are applied, the retention order of the impurities might change significantly, which complicates identification. Possible co-elutions make the interpretation even more difficult and UV-peak areas do not allow identification either.

Therefore, the mass spectrometry data is a valuable source of information: via the mass-to-charge ratios (and relative peak area in case of equal *m/z*-values or isomer pairs) the compounds were identified and using a peak tracking approach they were matched for the different chromatograms. The MS and DAD data enabled the determination of the normalized retention times τ of the substances on all systems, as well as the resolutions between consecutive peaks. With normalized retention times the equivalent of the retention factors, measured under gradient conditions, is meant.

Evaluation of the silica-based set of 16 systems

The aim is to evaluate whether the set allows selecting one or a couple of systems for which all or most substances in an impurity profile are (nearly) separated, preferably exhibiting a good peak distribution over the chromatogram. These systems can then occasionally serve as a starting point for further method optimization.

The separation and the elution order changes were visualized by plotting the normalized retention times on parallel axes (Figure 1) (13,14). Parallel axes resolution plots, representing the resolution of the consecutive peak pairs in the impurity profiles, also were drawn (Figure 2). Finally a visual inspection of the best chromatograms (Figure 3) is performed to evaluate peak shape, and to verify whether the systems selected indeed are suitable to separate the impurity profile.

For impurity profile 1 (Figures 1A, 2A, and 3A), both impurities (1A, 1B) and the active substance (AS1) are best separated on CS1–CS4. They seem suitable for further method optimization. Possible approaches to further optimize are discussed below.

Although no changes in elution order were observed, important selectivity shifts between the CS were obtained: from a clear separation between the three peaks to a complete co-elution of AS1 and 1A, or an additional overlap between 1A and 1B (Figures 1A and 2A). Figure 3A allows ranking the systems CS1–CS4 according to decreasing separation quality. Thus CS1, CS3, CS4 can be considered as better, and CS2 as less performing systems.

Such ranking (also for the other profiles) was obtained using some rules: (*i*) the minimal resolution should be as high as possible; (*ii*) the threshold for baseline separation is generally defined at a resolution of 1.5. The more peak pairs with a resolution above 1.5, the more preferred a system; (*iii*) for two systems exhibiting comparable resolutions between peak pairs, visual evaluation of the chromatograms might help deciding.

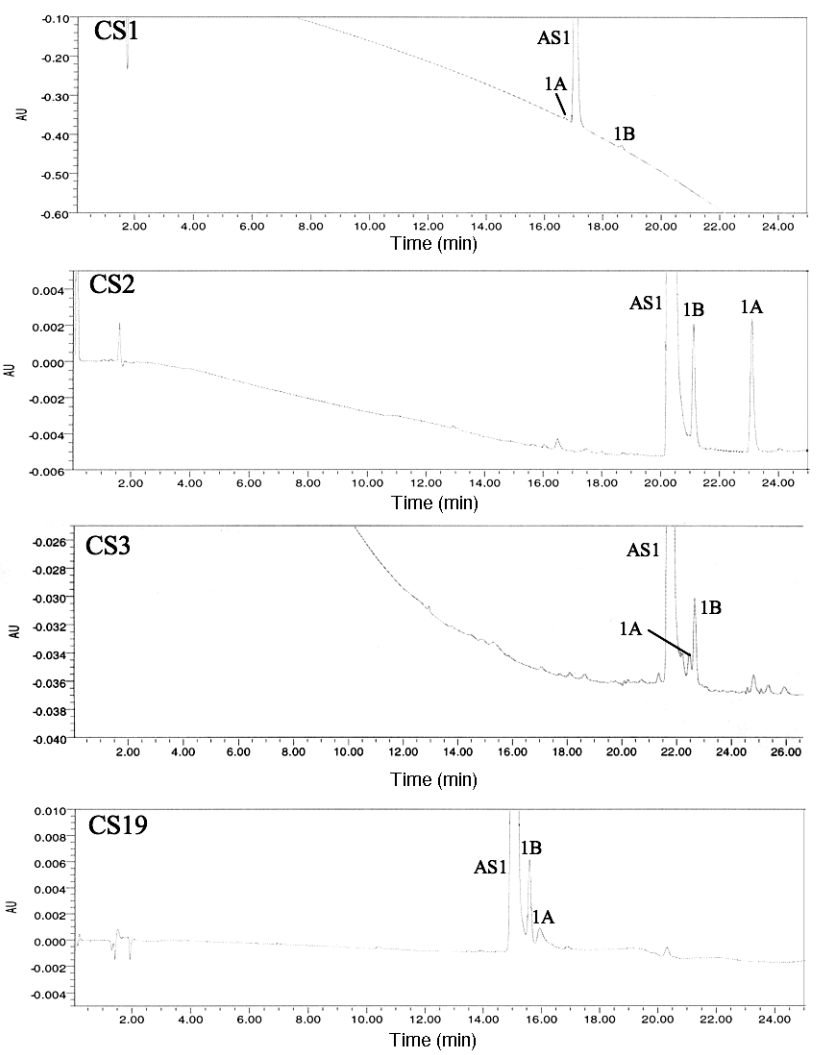

Figure 6A. Best chromatograms from the 17 systems for impurity profile 1 (on CS1, CS2, CS3, and CS19).

Even though no resolutions above 1.5 were obtained, systems CS1, CS3, and CS4 seem to reflect a good probability to achieve complete separation of the impurity profile during optimization.

To optimize, the method could, for instance, be changed to an isocratic (16–19) and the separation between AS1 and 1A, which is eluting in the tail of AS1, could be improved. Final selection of CS1, CS3, or CS4 might depend here on the preference of the analyst. It can also be noticed (Figure 2A) that beside CS1–CS4 only CS12 had a chromatogram without co-elution. In case it is required, this system also could be given a closer look.

For impurity profile 2, changes in elution order were observed for 2A and 2B (Figure 1B). On some CS, 2A and/or 2D co-elute or overlap with the active substance AS2 (Figures 1B and 2B). Impurity 2C never was separated from the active substance. The other impurities could be separated well from AS2 with baseline separation (resolution above 1.5) on CS1–CS4. For quantitation purposes, again CS1–CS4 (with CS2 least preferred) can best be used to start further method development of the impurity profile (Figure 3B). The additional difficulty in a further optimization is the separation of 2C and AS2, which, given the results observed on all systems, will not be evident.

For impurity profile 3, the substances that seem most difficult to separate are 3F,

3G, 3H, and 3I, and especially 3G and 3H (Figures 1C and 2C). Further on, many changes in elution order are present (Figure 1C). CS16, CS10, CS6, CS4, and CS1 exhibit the best separation conditions (Figures 1C, 2C, and 3C). Their chromatograms each contain only one co-elution. The systems were ranked according to decreasing separation quality. Evaluation of Figure 1C and 2C is of limited help in the selection of the best systems, which thus mainly is based on the evaluation of the chromatograms. CS16 was preferred because 3F is completely resolved from peaks 3G, 3H, and 3I, whereas the peaks 3G-3H-3I are quasi baseline separated, and in general the highest resolution is encountered for the other substances. In a further optimization of CS16, the separation between 3C, 3D, and AS3 will be the main concern, while occasionally the separation between 3I, 3H, and 3G can also be improved for quantitation purposes.

Concerning impurity profile 4, several changes in elution order and peak shifts occur (Figure 1D) between the different CS. The best separations of the impurity profile are seen on CS16, CS3, CS10, CS11, and CS6 (Figures 1D, 2D, and 3D). No co-elution occurs. CS16 was ranked first, because overall separation is best. During further method optimization it could be tried to reduce analysis times by eluting the first peak earlier, while the separation between some peak pairs also might be improved.

It can be concluded that, except for one impurity in profile 2, all were at least partially separated from the active substance and from each other on a number of systems from the orthogonal set. The fact that for each profile several selectivity changes occur and that different systems are suited for further optimization could be expected if the set indeed contained dissimilar systems. The separation of the different profiles emphasizes the generic applicability of the dissimilar set. For each impurity profile, there is a good indication that further method optimization may lead to methods allowing quantitation of the impurities.

The systems are delivering complementary information: peak pairs that are not separated on one system are on another. This means that for an unknown mixture all peaks can be revealed and in case optimization on the selected system fails, another can be further developed.

The profiles examined indicate several systems interesting towards achieving separation. The results also show that for a

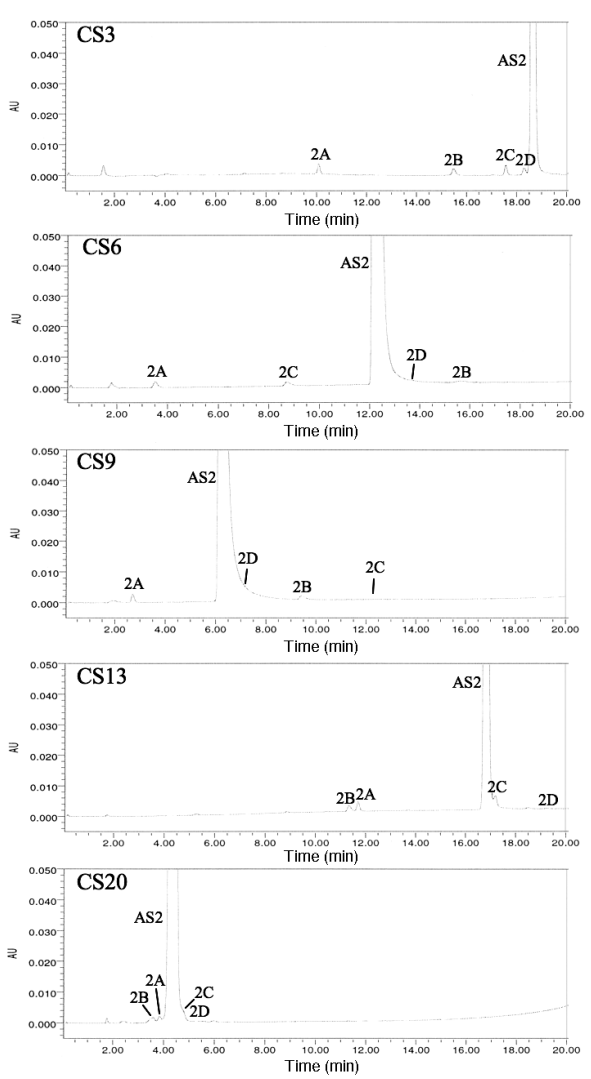

Figure 6B. Impurity profile 2 (on CS3, CS6, CS9, CS13, and CS20).

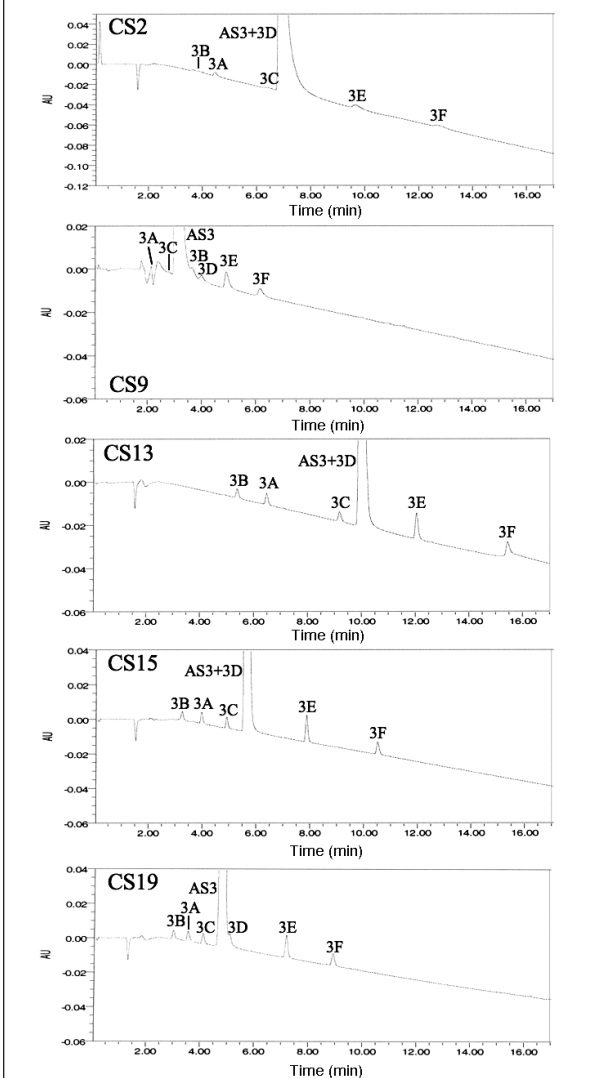

Figure 6C. Impurity profile 3 (on CS2, CS9, CS13, CS15, and CS19).

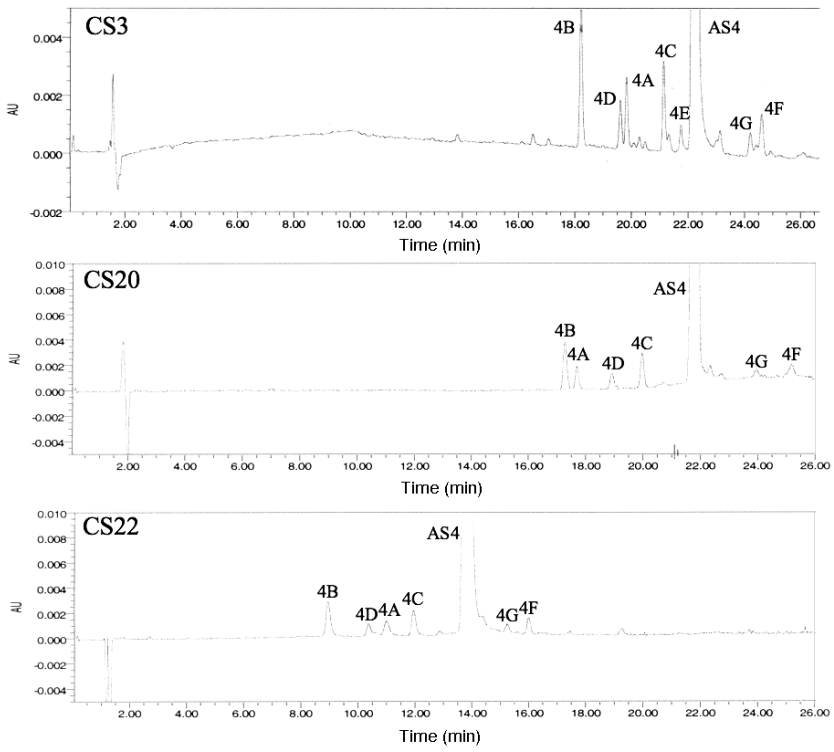

Figure 6D. Impurity profile 4 (on CS3, CS20 and CS22).

given impurity profile only a limited number is important as could be expected.

Suggestions to further optimize methods can consist of fine-tuning the pH, the organic modifier composition, and the buffer concentration, possibly combined with adjusting the gradient steepness, the gradient limits, the flow rate and/or the column temperature. As already mentioned, it also could consist in changing from the gradient system to an isocratic (16–19), after which the above-mentioned factors (except for those related to the gradient) can be further optimized.

Evaluation of the set of 17 systems

In the parallel axes plots (Figures 4 and 5), the systems were ranked so that the most similar were next to each other, and the most dissimilar furthest, based on the results of the literature (7). In Figure 4, the normalized retention times on the 17 systems are visualized for the four impurity profiles. Figure 5 represents the resolutions between consecutive peak pairs on the 17 systems, and Figure 6 shows the chromatograms of the preferred systems for the four impurity profiles.

The selectivity differences are the largest comparing the systems at the left of Figure 4 with those at the right, which is in accordance with the findings in the literature (7). The above-mentioned rules again were used to select and rank the systems exhibiting the best separation properties. Here more attention was paid to the peak shape to determine the final ranking, as the larger diversity in the systems leads to larger differences in tailing.

For impurity profile 1, several selectivity differences occur. Whereas the previous set only showed peak shifts, with this set the elution order changed completely, with separation properties scaling from co-elution to baseline resolution (Figures 4A, 5A, and 6A). This indicates the larger diversity of the latter set.

The systems exhibiting the best separations are CS1, CS19, CS35, CS15, CS2, CS22, and CS3 (Figure 5A). The chromatograms however revealed that CS15, CS22, and CS35 suffered from a larger tailing, which occasionally led to a severe peak overlap. The remaining four systems were ranked as CS1, CS2, CS19, and CS3. CS1 was preferred, because all peaks are baseline separated, and 1A elutes in front of AS1.

For impurity profile 2, CS3, CS6, CS9, CS13, and CS20 provide the best chances to separate the substances, as these are the systems without co-elution (Figure 5B). Again several selectivity differences with changing elution order were obtained (Figure 4B). The peak pair AS2-2C, which could not be separated with the previous set, can now clearly be baseline resolved (CS3, CS6, and CS9, see Figure 6B). Again, large differences in tailing between the systems were observed. Taking this into account, CS3 was ranked first, because all impurities are eluting in front of the active substance. Only 2D is slightly overlapping with the front of AS2. As second and third, CS13 and CS20, respectively, were chosen. Both systems exhibit the same elution order, but the overall separation is better on CS13. CS6 and CS9 were ranked fourth and fifth, respectively, since they both tail. In CS6, the tailing is less pronounced than in CS9, and 2A and 2C are eluting in front of AS2, whereas in CS9, only 2A is.

For impurity profile 3, again switches in elution order are observed (Figure 4C). CS9 and CS19 only show peak overlap and no co-elution; while CS2, CS5, CS6, CS13, CS15, CS16, CS41, and CS45 all have one co-elution (Figure 5C). Again, because of differences in tailing, the ranking of the systems depended partly on the visual inspection. It was observed that CS5 and CS6 showed a rather large tailing, while CS16, CS41 and CS45 have an undesirable overall separation. As a result, five systems were ranked as CS19, CS9, CS15, CS13, and CS2 (Figure 6C). The chromatograms show that CS13, CS15, and CS19 exhibit narrow peaks, whereas CS2 and CS9 show some tailing. Overall, CS19 was considered the top choice system.

For impurity profile 4, only on CS4 and CS6 one co-elution occurs (Figure 5D), and the most critical peak pair is 4F and 4G (Figure 4D and Figure 5D). Three systems exhibit better separation conditions (i.e., CS20, CS22, and CS3). For these systems all resolutions are above 1.5, the other systems show at least one overlap or co-elution. The systems were ranked in the order CS22, CS20, and CS3 (Figures 4D, 5D, and 6D). CS22 is preferred, because of its best overall separation with uniform peak distribution.

The larger diversity in the systems compared to the previous

set is reflected by larger changes in selectivity, which already led to the baseline separation of more substances (e.g., of AS2 and 2C in the second profile).

Again, for the different impurity profiles, several systems could be selected for further method development, and the preferences differ for the four profiles. To further optimize the methods, the same suggestions can be made as for the former set of 16 systems.

Conclusion

Two sets of orthogonal/dissimilar reversed-phase chromatographic systems were evaluated for their potential use in the method development of drug/impurity profiles. It was demonstrated that for each profile, several systems could be selected for further optimization. As expected from their dissimilar character, the systems showed selectivity differences also for the impurity profiles.

Because of the larger diversity in the chromatographic conditions, the dissimilarity of the impurity profiles between systems was more pronounced for the second set.

In general, this study demonstrated systems selected as dissimilar based on the retention of a set of diverse test compounds, also show selectivity differences (i.e., are dissimilar) for impurity profiles, where the different substances are much more similar.

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