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A workflow for column interchangeability in liquid chromatography using modeling software and quality-by-design principles

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ABSTRACT

The goal of the present study was to develop a generic workflow to evaluate the chromatographic resolution in a large design space and easily find some replacement column for the method. To attain this objective from a limited number of initial experiments, modern LC modeling software (Drylab) was employed to study the behaviour of the compounds and visually compare the parts of design spaces obtained with different columns, where a given criterion of critical resolution is fullfilled. A zone of robust space can then easily be found by overlapping design spaces. By using 50 × 2.1 mm columns packed with sub–2 μm fully porous particles (UHPLC), the resolution in the entire design space can be modeled on the basis of only 2–3 h experimental work *per* column.

To demonstrate the applicability of the developed procedure, amlodipine and its related pharmacopeia impurities were selected as a case study. It was demonstrated that two columns from different providers (Waters Acquity HSS C18, Thermo Hypersil Gold C18) can be interchanged, providing a sufficient resolution at the same working point and a high degree of robustness around this condition.

1. Introduction

Nowadays thousands of liquid chromatographic columns are available. If only octadecyl (C18) phases are taken into account, then we have the possibility to choose from more than 500 products. On one hand, this can make the method development easier since the chromatographer can select the most suitable stationary phase for a given separation. On the other hand, it can be a heavy task to find an appropriate replacement (alternative) column, which provides a very similar separation as the original column. Today, it is indeed required to suggest an alternative column in pharmaceutical analytical laboratories, and to prove its equivalency during the method validation process. In fact, the pharmaceutical regulatory guidelines mention that method robustness has to be checked on columns from different batches and also on other manufacturer's column providing similar separation quality [1].

The column interchangeability in the U.S. Pharmacopeia Convention is quite straightforward. The liquid chromatography columns are classified in 'L' groups according to their chemical modification [2]. All the columns with C18 bonding belong to the L1 group, which is defined as: "octadecyl silane chemically bonded to porous silica or ceramic micro-particles, 1.5–10 μm in diameter, or a monolithic rod". This definition is quite broad, since it contains all the phases with irregular silica particles, high metal ion content and low surface coverage as well as the widely used hybrid silica endcapped phases with high surface coverage, showing significant differences in retention properties. These phases are clearly not interchangeable and it can even occur that C18 and C8 ligands that are attached to the same silica particle show more similar retention properties than C18 ligands bonded to different silica particles [3].

To compare different reversed phase (RP) materials, various tests have been proposed in the literature [4–7]. Available databases are also based on these tests. The limitation of such tests is that they provide information only on a limited number of compounds, measured under "one constant set" particular conditions. Those tests cannot predict the applicability of columns for impurity profiling or assays, which are the most common applications in pharmaceutical analysis. One of the most popular databases is based on the "hydrophobic-subtraction model" proposed by Snyder and coworkers in the early 2000 s [8]. This model takes the hydrophobicity (*H*), hydrogen bond basicity (*B*), ionic interactions at two pH (*C(2.8)* and *C(7.0)*), hydrogen bond acidity (*A*) and steric selectivity (*S*) into account. These parameters are used for the calculation of similarity factor (*Fs*) between columns. $Fs < 3$ corresponds to an excellent selectivity similarity between the compared columns; between $3 < Fs < 5$, the selectivity similarity is moderate;

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between $5 < Fs < 10$, there is a questionable but still fair comparability of selectivity, and for $Fs > 10$, the selectivity is considered as different [9]. This approximation gives a scientific based comparison against the USP classification, but it does not consider peak shape and peak width, which are crucial for pharmaceutical impurity profiling.

Modern silica gels (Type B silica) have low metal ion content, and acidic pK_a value of surface silanol groups typically ranges between 3 and 5. The amount of residual silanol groups is around 8 μmol/m^2, half of which being covered after alkyl modification. The number of residual silanol groups can be further reduced by endcapping, but unreacted silanol groups are always present. Depending on the pH conditions, these acidic silanols can be ionized and are then able to offer strong electrostatic interaction with basic compounds which are also ionized. To demonstrate the differences between C18 phases, it is therefore important to select chromatographic conditions where the residual silanol groups are ionized and the sample should contain some basic, acidic and neutral-like compounds as well. This is for example the case when using a mobile phase pH comprised between 3 and 6 [10–12].

In previous studies, the simulated robustness testing, included within commercial modeling softwares, was systematically studied and compared to experimental measurements and DoE based predictions [13,14]. The reliability of this "early stage" simulated robustness approach was critically evaluated for real-life separations applying short narrow bore columns (50 × 2.1 mm) and fast separations. Moreover, as a continuation of robustness study, the column interchangeability was further investigated, using four different C18 columns packed with sub–2 μm particles. By properly varying the method variables, the separation was feasible on all columns within the same timescale (less than 4 min). This work demonstrates the accuracy of simulated robustness testing and shows that nearly the same quality of separation can be achieved on different stationary phases.

The novelty of the present work is the practical use of the recently introduced Column Comparison module in DryLab modeling software. In this module, various 3D resolution maps can be compared by overlapping two or more cubes, which can help studying the measured points – in a design space – of the different phases and find a common zone where the sample components are all separated with sufficient selectivity and resolution. For this illustration, amlodipine and related impurities have been selected as model samples. The pharmacopeia suggests a 60 min long conventional separation for amlodipine impurity profiling which has already been shortened drastically in our previous study [15].

2. Experimental

2.1. Chemicals, columns

The mobile phase used in this work was a mixture of acetonitrile and water buffered with 10 mM ammonium-acetate buffer. Acetonitrile (gradient grade), acetic acid, ammonium hydroxide and standard reference buffers (pH 2.00, 4.01 and 7.00) were purchased from Merck (Darmstadt, Germany). For the measurements, water was prepared freshly using ELGA Purelab UHQ water (ELGA, Lane End, UK).

Sample was prepared from amlodipine API (0.5 mg/mL) and spiked with all the impurities at 0.5% level. Amlodipine and its impurities [15] were purchased from European Directorate for the Quality of Medicines & HealthCare (EDQM, Strasbourg, France). Sample solvent was acetonitrile:water 30:70 (v/v).

The columns used in this study were selected on the basis of the following criteria: all of them should be based on porous silica gel (to neglect differences in morphology), with similar particle size (to have comparable specific surface area and efficiency). We focused on differences and effects of accessible free silanols.

The Acquity HSS C18 and HSS C18 SB columns (50 × 2.1 mm, 1.7 μm) were purchased from Waters (Milford, USA), Hypersil GOLD C18 column (50 × 2.1 mm, 1.9 μm) was purchased from Thermo Scientific (Waltham, USA), Titan C18 column (50 × 2.1 mm, 1.9 μm) was purchased from Sigma-Aldrich (St. Louis, USA). Acquity HSS C18 and Hypersil GOLD C18 colums have relatively high surface coverage with endcapping, Titan C18 column has medium surface coverage with endcapping and Acquity HSS C18 SB possesses low surface coverage without endcapping (see Table 1).

2.2. Equipment and software

UHPLC experiments were performed on a Waters Acquity UPLC I-Class system (Milford, USA) equipped with binary solvent delivery pump, autosampler, photodiode array detector and Empower 3 software. This UHPLC system had flow-through-needle (FTN) sample injector and 500 nL flow cell. The dwell volume of the system was measured as 0.1 mL.

The MP 225 pH-meter was purchased from Mettler-Toledo (Mettler-Toledo, Greifensee, Switzerland).

UHPLC method development and modeling was performed by using DryLab® 4, v.4.3.1 optimization software (Molnár-Institute, Berlin, Germany).

3. Results and discussion

3.1. Preliminary experiments

As previously mentioned, the goal of this study was to introduce a strategy where – beside method optimization – a substitution (alternative) column can be offered as part of the robustness testing. About robustness the ICH Q2 (R1) guideline contains the following "*The evaluation of robustness should be considered during the development phase and depends on the type of procedure under study. It should show the reliability of an analysis with respect to deliberate variations in method parameters. . . . In the case of liquid chromatography, examples of typical variations are . . . rent columns (different lots and/or suppliers)*" [1].

Based on former experiments, amlodipine and its impurities were found to be relatively lipophilic, so the starting mobile phase composition was set as 30% acetonitrile. However, the ImpA compound was highly lipophilic, so high acetonitrile content (90%) is required at the end of the gradient to elute this substance. In addition, it is also important to mention that there is structural similarity between amlodipine, ImpD, ImpE and ImpF and all of them contain a primary amino group (p$K_{a>}$ 10). Therefore, all these substances will be ionized under common RP conditions. The ImpH impurity has acidic character, due to the carboxylic acid group attached to an aromatic structure (p$K_a \sim 4$), so depending on the RP conditions, it can be either fully ionized or neutral [3,14,15].

During the preliminary experiments, four C18 columns belonging to the USP L1 group were chosen. The reference column was the Acquity HSS C18 and our goal was to find the appropriate replacement column. During the initial experiments at pH = 4.5, it occurred that Acquity HSS SB C18 column showed high silanol activity under these conditions, since the peaks of the basic substances were broad and tailed, with a significant increase in retention (Fig. 1*b*), below). For all these reasons, this column was excluded.

In the case of Titan C18 column, which has medium surface coverage and endcapping, the peak shapes of basic compounds were more asymmetrical than the peaks of acidic or neutral compounds, but they could be evaluated during method optimization (Fig. 1*d*), below).

Table 1
Characteristics of the columns packed with sub–2 μm partciles tested in this study.

	HSS C18	HSS C18 SB	Hypersil GOLD C18	Titan C18
Pore Size (Å)	130	130	175	80
Surface Area (m^2/g)	230	230	220	410
Surface Coverage ($\mu mol/m^2$)	3,2	1,6	NA	NA
Carbon Load (%)	15	8	11	13
Endcapping	Yes	No	Yes	Yes

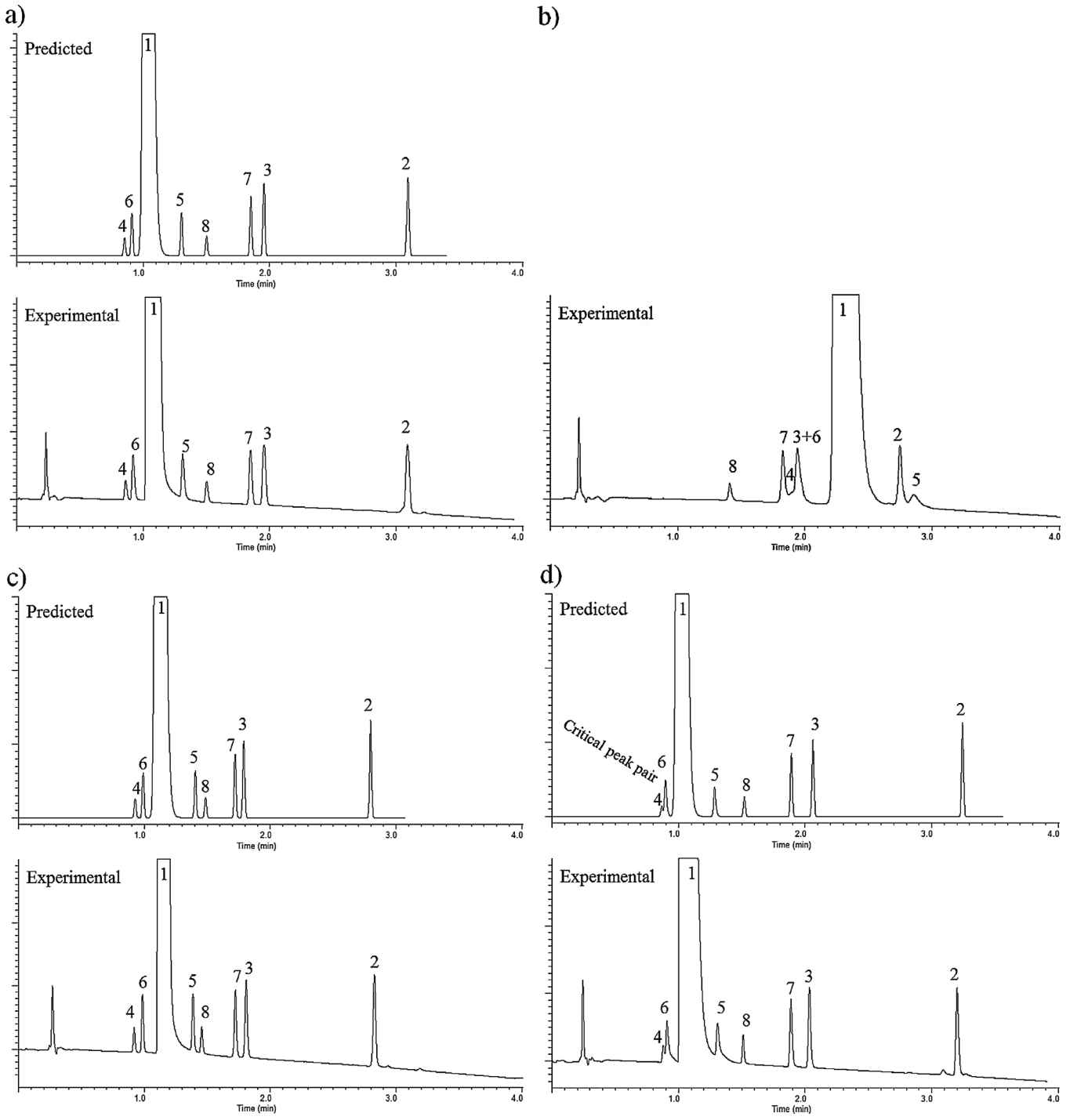

Fig. 1. Predicted (top) and experimental (bottom) chromatograms of the four tested 50 × 2.1 mm C18 columns packed with sub–2 μm particle. Acquity HSS C18 a), Acquity HSS C18 SB b), Hypersil GOLD C18 c) and Titan C18 d). Amlodipine (1), ImpD (4), ImpE (5) and ImpF (6) contain free amino groups. ImpH (8) contains free carboxylic group. There is a movement of ImpH with increasing pH to shorter retention times, which has a strong influence on the elution order. ImpA (2), ImpB (3) and ImpG (7) are netural in the tested chromatographic conditions.

With the Acquity HSS C18 (Fig. 1*a*), below) and Hypersil GOLD C18 (Fig. 1*c*), below) columns, which have both high surface coverage and endcapping, the peak shapes of all compounds were symmetrical.

3.2. Design of experiments (DoE)

The selected example describes a fast and efficient method development for the determination of amlodipine and its impurities, using the high separation power of state-of-the-art RP columns. A general methodology consists in simultaneously model the effect of temperature and gradient steepness on selectivity for a given RP column. Thanks to the current developments in chromatographic modeling softwares, it is now possible to model simultaneously the effect of three variables for a given separation. In our case, gradient steepness (t_G), temperature (T) and mobile phase pH were selected as model variables to create a critical resolution cube, showing the critical resolution of the peaks to be separated against the three factors. Probably, these selected variables have the most significant effect on selectivity and resolution for such analytes. In most cases, the retention can be described as a function of gradient steepness, with the linear solvent strength (LSS) theory and its temperature dependence following a van't Hoff type relationship. Both relationships can be transformed to linear dependencies. When separating ionizable compounds, strong pH-related changes in retention occur for pH values within ± 1.5 units of the pK_a value. Outside this range, the compound is considered as mostly ionized or non-ionized, and its retention is not significantly altered with pH. In a relatively small pH range – within the ± 1.5 units of the pK_a value –, the dependence of retention on the mobile phase pH can generally be described using quadratic polynomials [14].

Therefore, in our proposed final model, two variables (t_G and T) were set at two levels (t_{G1} = 3 min, t_{G2} = 9 min and T_1 = 20 °C and T_2 = 50 °C), while the third factor (pH) was set at 3 levels (pH_1 = 4.0, pH_2 = 4.5 and pH_3 = 5.0). This full factorial experimental design required 12 initial experiments (2 × 2 x 3) on a given column. These experiments have been performed on the selected three columns.

3.3. Calculation of a 3D-Critical resolution space (CRS) also called method operable design region (MODR)

As illustrated in Fig. 2*a*), at low temperature and short gradient time (the left bottom side of the resolution cube) the design space has a range where the resolution ($R_{s,crit}$) is larger than 1.5. At intermediate temperature (and intermediate gradient time), the separation is not acceptable. However, at high temperature and long gradient time (the right top side of resolution cube) the $R_{s,crit} > 1.5$ criterion is also fulfilled, but probably column life time would be shorter at high temperature conditions. For these reasons, the best working point was selected as: t_G = 4 min (30–90%B), T = 25 °C, pH = 4.2. The working point is indicated as the intercept of horizontal and vertical black lines.

Fig. 1*a*) shows the predicted and measured chromatograms on Acquity HSS C18 column at the selected working point. The correlation between calculated and measured retention times was excellent. The average deviation of the retention times between model and measured data was 0.5 s.

3.4. Column interchangeability

Our 12 experiments based approach seems to be a reliable procedure when comparing the achievable analysis time, resolution and working point. By applying 50 × 2.1 mm columns, it takes approximately only 2–3 h of experimental work for one given column. The advantage of this column screening approach is that the suitability of a column – for a given application – can be evaluated at the very early stage of the method development. In addition, the column interchangeability can also be estimated during the method development. Therefore, our column screening approach seems to be a promising method development strategy, as it consists in performing initial runs and building up 3 dimensional models using different columns at the early phase of method development.

The same procedure as described in Sections 3.2 and 3.3 was then applied on Hypersil GOLD C18 and Titan C18. In Fig. 2, the resolution cubes were compared for these two additional columns, and the reference one. When comparing Fig. 2*c*) and *a*), it is clear that the Titan C18 column cannot be considered as a suitable replacement column under the conditions described in Section 3.3, since it provides suitable separation in the opposite part of the design space, as the Acquity HSS C18 column does. Even if appropriate separation is feasible on the Titan C18 column, it is not comparable to the one obtained on the reference column. When comparing Fig. 2*a*) and *b*), some differences can be observed in the low temperature range in the resolution cube, due to the acidic ImpH impurity, which has variable ionic characteristics at pH between 4 and 5. Nevertheless, under the conditions described in Section 3.3, the Hypersil GOLD C18 seems to be an appropriate replacement column, as it also provides $R_{s,crit} > 1.5$.

To help selecting the most interesting alternative column, the new version of DryLab software allows the user to compare the parts of the resolution cubes where the $R_{s,crit} > 1.5$ criteria is fulfilled. It has to be mentioned that the retention order has to be checked carefully (change in elution order may happen).

By comparing the two resolution cubes (Fig. 3*b*)), it can be established that the working points obtained on the Acquity HSS C18 and Hypersil GOLD columns at t_G = 4 min (30–90%B), T = 25 °C, pH = 4.2, are interchangeable for the measurement of amlodipine and its related impurities, as they share a relatively large zone in the design space around the selected working point, while the Titan C18 is clearly inappropriate (Fig. 3*a*)).

Using the above mentioned analytical strategy, it is therefore possible to quickly develop a robust method and easily find out an appropriate replacement column for the method.

3.5. Robustness testing

From the design space, as defined in a resolution map or cube, it is possible to get robustness information for the measured parameters, including gradient time (t_G), mobile phase temperature (T) and mobile phase pH. In addition, based on the models included in the software, the retention time variation of any compound can also be evaluated when varying the mobile phase flow rate as well as start- and end-% B of the gradient. Consequently, the impact of these 6 different parameters on the resolution can be assessed using simulated three levels (3^6) type factorial designs (including 729 simulated experiments). The possible deviations from the nominal values have simply to be defined (for example by instrument qualification protocolls of the HPLC-instrument manufacturers) and then the software makes the calculations for all the simulated conditions. At the end, a 'frequency distribution graph' showing how often (N) a certain critical resolution occurs under any combination of possible parameters is provided. This graph clearly shows the failure rate, i.e. percentage of experiments that could fall outside the required critical resolution. On the other hand, 'regression coefficients' can also be obtained to show the effect of each individual parameter, related to the selected deviation from the nominal value, for the critical resolution.

In this study, the robustness of the method around the working point was compared for the Acquity HSS C18 and Hypersil GOLD C18 columns. Nominal deviations from the working point were set as: T = 25 ± 1 °C, mobile phase pH = 4.2 ± 0.1, gradient

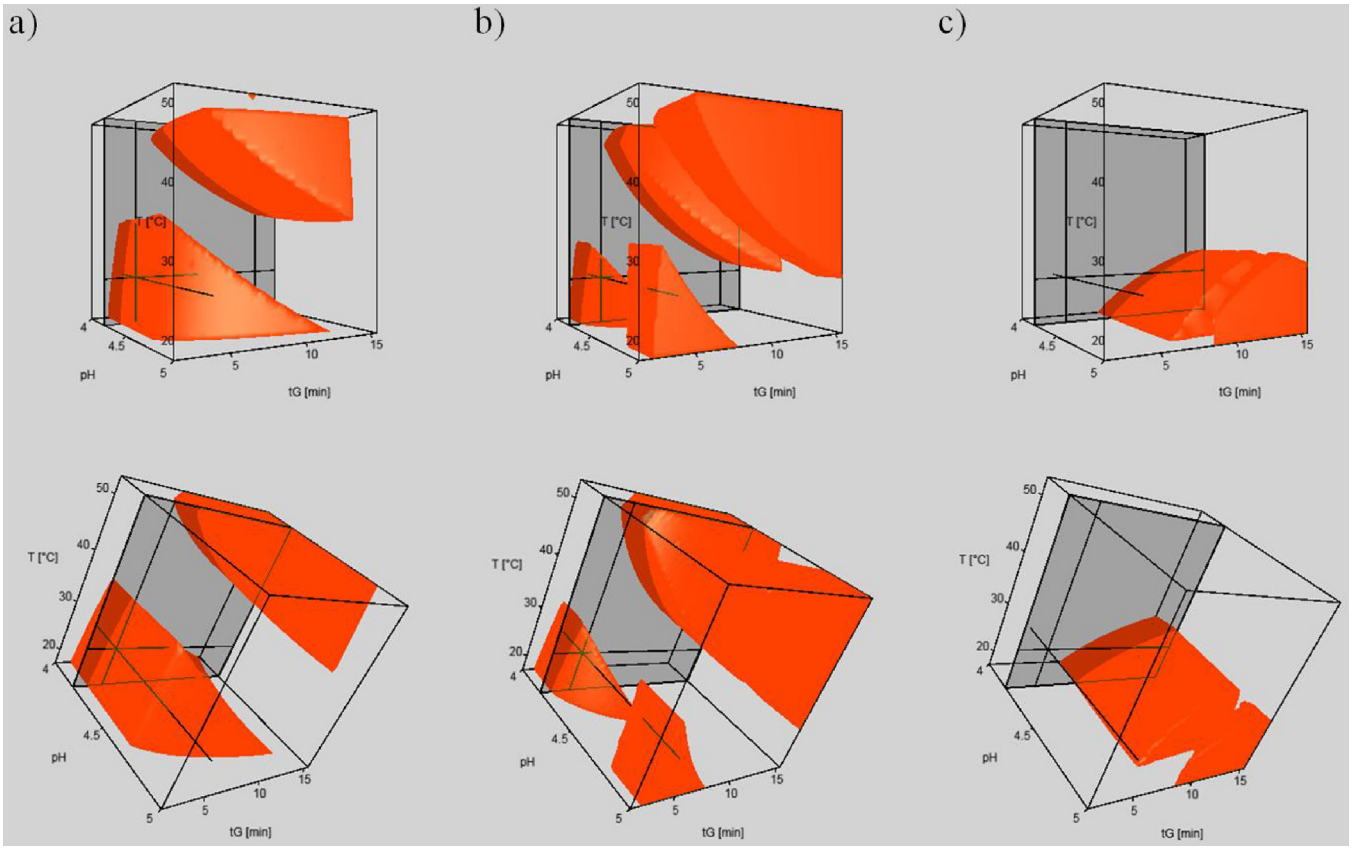

Fig. 2. DryLab 3D models of different columns, Acquity HSS C18 a), Hypersil GOLD C18 b), and Titan C18 c). Baseline resolution regions are shown in red. The different geometric bodies form a Design Space, which allow altering the position of the set point (working point, WP) without the need for a new validation, as the alteration of the WP inside the Design Space is not considered as a "change", so far no change management is necessary. The robustness of the individual WP's is different between the different red regions. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

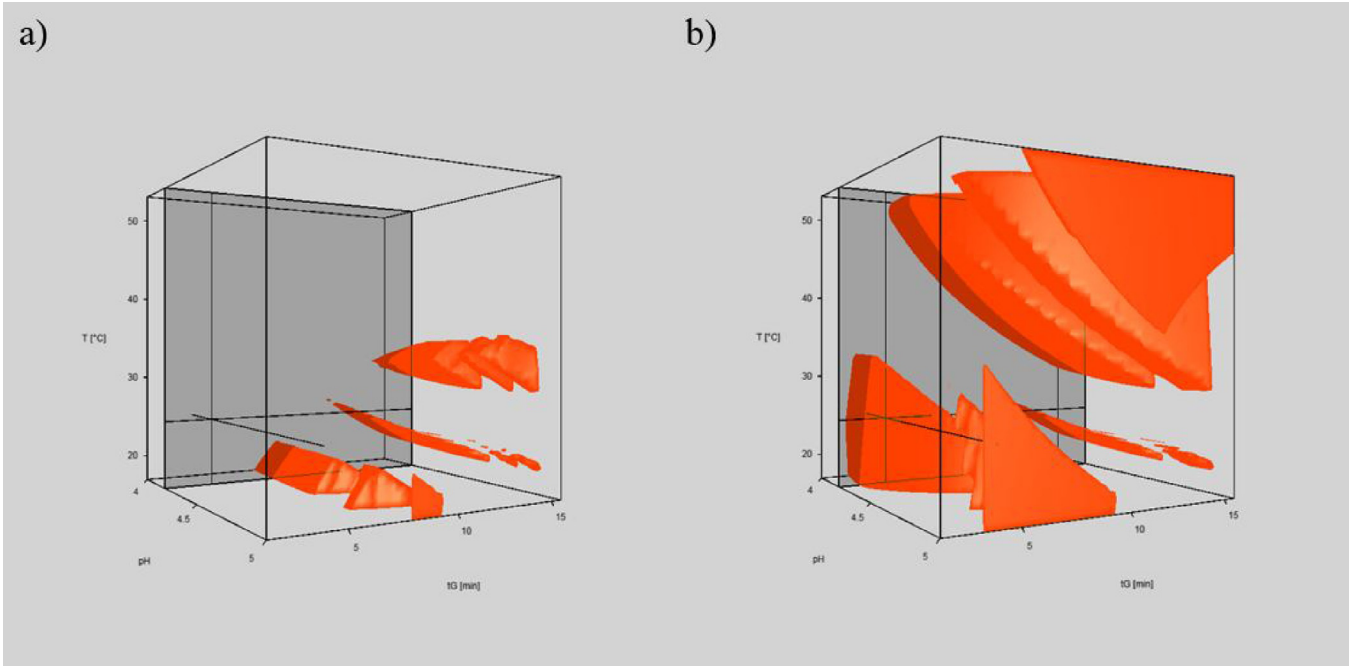

Fig. 3. Column Comparison: Common rubust space of Acquity HSS C18, Hypersil GOLD C18 and Titan C18 columns a) and common rubust space of Acquity HSS C18 and Hypersil GOLD C18 columns b).

time $t_G = 4 \pm 0.1$ min, initial mobile phase composition: $30 \pm 1\%$ B, final mobile phase composition: $90 \pm 1\%$ B and flow rate $F = 0.5 \pm 0.1$ mL/min. A required resolution of $R_{s,crit} > 1.5$ was considered. Performing the 729 virtual experiments resulted in 100% and 96.3% success rate using the Acquity HSS C18 and Hypersil GOLD C18 columns, respectively. The lowest resolution ($R_{s,crit}$) was equal to 1.4 with this latter column, which occurs when five parameters of the six were set on its + levels. In real life experiments, this

situation has low probability to occur. The most influencing parameters on the Hypersil GOLD column were the mobile phase pH and flow rate, while on the Acquity HSS C18 these were t_G and flow rate. To conclude, the two stationary phases showed some minor differences, but in overall they both can be considered as robust around the same working point. Later on, adjustments may be done, if the retention-model was a part of the Drug Master File. With this, flexibility in routine QC work can be increased.

4. Conclusion

A workflow was proposed for the first time to compare the resolution of an impurity profiling method in a large design space of 3 measured and 3 calculates variables and to find some possible replacement columns for the method. This strategy is based on the use of state-of-the-art LC modeling software, allowing to visually compare the parts of design spaces obtained with different columns, where the analytical target profile (ATP) for a selected critical resolution is fulfilled. A section of robust spaces can then easily be found by overlapping design spaces.

In this work, several C18 columns packed with particles of similar morphology (sub–2 μm fully porous) were compared to separate amlodipine and its main impurities (including basic, acidic and neutral like compounds). The selected four stationary phases (Acquity HSS C18, Acquity HSS C18 SB, Hypersil Gold C18 and Titan C18) have some obvious differences in terms of surface area, coverage, carbon load and endcapping, but three of them could provide baseline separation in the same design space. At the end, by using LC modeling software, it was found that two of the four columns share the same working point and are robust around this condition. Therefore, these two columns from different providers (Acquity HSS C18, Hypersil Gold C18) can be interchanged.

The advantage of this approach is the mapping of the retention behaviour of the compounds of interest (and not common test solutes) in an entire 3D design space, instead of some selected conditions (as suggested by earlier column tests). By using 50 × 2.1 mm columns, the resolution in the entire design space can be modeled on the basis of only 2–3 h experimental work.

By applying this approach, a replacement column can easily be proposed at the early stage of method development.

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