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Expanding the term "Design Space" in high performance liquid chromatography (I)

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ABSTRACT

The current article presents a novel approach to applying Quality by Design (QbD) principles to the development of high pressure reversed phase liquid chromatography (HPLC) methods. Four common critical parameters in HPLC – gradient time, temperature, pH of the aqueous eluent, and stationary phase – are evaluated within the Quality by Design framework by the means of computer modeling software and a column database, to a satisfactory degree. This work proposes the establishment of two mutually complimentary Design Spaces to fully depict a chromatographic method; one Column Design Space (CDS) and one Eluent Design Space (EDS) to describe the influence of the stationary phase and of the mobile phase on the separation selectivity, respectively. The merge of both Design Spaces into one is founded on the continuous nature of the mobile phase influence on retention and the great variety of the stationary phases available.

1. Introduction

The common practice in developing an HPLC method, for many years, has been a trial and error approach (pick the winner strategy). Often after the validation process was started, one found several surprising observations such as new peaks, or the disappearance of other peaks, changes in critical peak pairs, etc. The typical reaction was then to go back to the development process and to try to improve the separation by carrying out several test steps: trying to test quality into the method. This time consuming process can be avoided by applying Quality by Design (QbD) principles which plan or design quality into a method from the outset. A well established means of implementing QbD principles into HPLC method development is the use of modeling software [1].

Since the publication of the Quality Guidelines Q8, Q9, Q10 [2–4] the regulatory authorities FDA and ICH, as well as those in Europe, have been increasingly embracing and promoting QbD principles in the pharmaceutical environment [5–7]. This has sparked the publication of a number of works including the book of Ermer [8–13] proposing different ways in which to apply a systematic approach to analytical development based on understanding and sound science.

One of the steps in implementing QbD principles into the development of high pressure liquid chromatography methods is the elaboration of a so-called Design Space. A key benefit of defining a Design Space is a significant gain in flexibility, as working within this space is not considered as a change, and therefore would not initiate a regulatory post approval change process [2]. A Design Space, as defined by the ICH Q8(R2) [14] is the "multidimensional combination and interaction of input variables that have been demonstrated to provide assurance of quality". In chromatography terms, this means that all parameters (input variables) which have a strong influence on retention and selectivity (quality) should be studied in combination, thus defining a perfectly known multidimensional space.

A prerequisite to defining a Design Space for a HPLC method is the establishment of the most highly influencing parameters (critical parameters), the combined effect of which will be used to construct the Design Space. From all the influencing factors, the critical parameters in the overwhelming majority of HPLC separations are the gradient time, temperature, pH of the aqueous phase (eluent A) the composition of the organic modifier (eluent B), and the stationary phase. A reduced number of separations are highly influenced by the ionic strength of eluent A and/or additive concentrations [15], these cases will not, however be treated here. As indicated in the ICH Q8(R2), it is possible to either "establish independent Design Spaces for one or more unit operations, or to establish a single Design Space that spans multiple operations." This lead to the proposition that the Design Space in HPLC should be considered as two: one Column Design Space (CDS) and one Eluent Design Space (EDS).

The CDS, this is to say, a column for which equivalent columns exist, which has a robust phase, can be defined successfully with the aid of column databases, such as ColumnMatch®

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(Molnár-Institute, Berlin, Germany) a database of over 500 commercially available columns developed by Snyder and his team [16–19] or ACD/Column Selector® (Advanced Chemistry Development, Inc.). The EDS, describing the influence of the remainder of above mentioned critical parameters, is defined once a column has been selected, by the means of 3-D resolution cubes modeled with the software DryLab® [20].

In this article, expanding upon the work described by Molnár et al. [8], 3-D resolution models were generated for a constant sample on three different columns with the aim of finding a robust method with known tolerances with respect to the four critical parameters gradient time, temperature, pH of eluent A and stationary phase. Once resolution cubes were constructed and precision of the models was experimentally confirmed, working points were selected according to the triple criteria critical resolution, robustness ranges and run time. The resulting models were additionally analyzed and compared to selectivity predictions from the column database. Good correlation between predictions and experimental results were observed.

2. Experimental

2.1. Eluents

Methanol (gradient grade) and HPLC–water were purchased from Merck (Darmstadt, Germany). Eluent A was prepared by combining varying volumes of aqueous buffers of differing pH (A1 and A2): A1 was a solution of 25 mM phosphoric acid and A2 was a solution of 25 mM monobasic sodium monophosphate. For pH 2.0 we mixed 56.5% A1 and 43.5% A2 (V/V), for pH 2.6, 25% A1 and 75% A2 (V/V) and for pH 3.2, 5.5% A1 and 94.5% A2 (V/V) [21]. Eluent B was methanol. Gradient elution between 5% and 95% B was used at a flow rate of 0.8 ml/min unless indicated otherwise.

2.2. Sample

Model substances and reference materials used were phthalic acid, vanillic acid, isovanillic acid, aspirin, furosemide, doxepin, terbinafin, atorvastatin and clopidogrel, and were commercially available chemicals and prescription drugs. For systematic studies with 3-dimensional resolution models a stable sample mix of the model compounds as well as their decomposition products available over an extended period of time was needed. The sample was therefore kept in a frozen state and only small amounts were used to avoid rapid decomposition of the sample mixture.

2.3. Equipment

HPLC separations were performed on a Shimadzu LC-2010C with integrated 4-liquid gradient system, high-speed and cooled autosampler, temperature controlled column compartment and Shimadzu UV–VIS detector (Shimadzu Europe, Duisburg, Germany). UV detection was performed at 254 nm. The dwell volume was 1.06 ml and the extracolumn volume was 0.016 ml. ACE C18 columns (150 mm × 4.6 mm, 3 μm) were provided by HiChrom (Reading, United Kingdom) and HALO C18 columns (150 mm × 4.6 mm, 2.7 μm) and HALO RP-amide columns (100 mm × 4.6 mm, 2.7 μm) by MacMod Inc. (Chadds Ford, PA, USA).

2.4. Software

Column comparison was executed in the database ColumnMatch® (Molnár-Institute, Berlin, Germany). HPLC separations were generated using the automation option of DryLab®2010, which includes PeakMatch® V. 3.6.3 and DryLab® V.3.95 (Molnár-Institute, Berlin, Germany) coupled with Shimadzu's LCsolution integration software. Peaks were identified and aligned based on peak areas using user friendly tools, such as peak turnover and peak splitting functions of the software, reducing the usual problems of common misalignments between peaks. Modeling was performed in DryLab®2010 and predictions were compared with the original experiments to control the validity of the modeling process. Generation of 3-D resolution models was carried out with a proprietary algorithm in DryLab®2010.

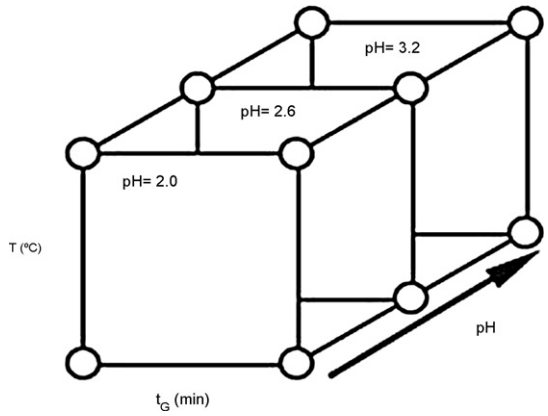

Fig. 1. Experimental design for a three-dimensional HPLC method optimization.

2.5. Experiments for modeling

Initial input data were acquired under the following conditions: gradient times of 20 min and 60 min (5–95%B) as recently reconfirmed by LoBrutto and his group at Novartis USA [22], temperatures of 30 °C and 60 °C and pH values of eluent A (25 mM phosphate buffer) of 2.0, 2.6 and 3.2 were selected. Eluent B (organic) was methanol. Twelve (4 × 3) experimental runs were performed on each column according to the design of experiments depicted in Fig. 1. All input data were run overnight automatically with the Shimadzu LC-2010C controlled from PeakMatch®. After the runs were finished, they were exported automatically to PeakMatch for the peak tracking process. Finally the data were transferred to DryLab®. The plate number was adjusted in various computer simulations of separation to the real column performance.

3. Results and discussion

3.1. Work flow

This project's work flow was constructed in accordance with QbD principles and can be divided into four steps:

3.1.1. Definition of goals

The goal of this project was to develop a good, robust method for the separation of nine model compounds of pharmaceutical interest in a multidimensional space comprised of four critical parameters: gradient time (t_G), temperature (T), pH of eluent A (pH_A) and stationary phase (sPh). The criteria of separation success (critical quality attributes) is three fold: maximum critical resolution, maximum robust tolerance windows and minimum run time.

3.1.2. Experimental design

Three identical sets of experiments, according to the design of experiments shown in Fig. 1, corresponding to a 3 parameter optimization for t_G, T, and pH_A were performed on the three different

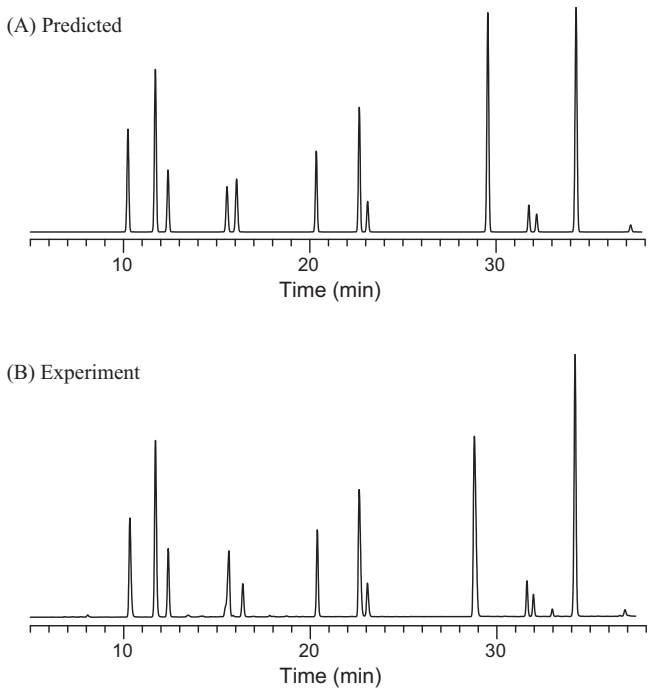

Fig. 2. Predicted (A) and experimental (B) chromatograms for gradient time 38 min, temperature 49 °C and pH of eluent A 2.8, on ACE C18 column (150 mm × 4.6 mm, 3 μm).

columns: (1) HALO C18 column (150 mm × 4.6 mm, 2.7 μm), (2) ACE C18 columns (150 mm × 4.6 mm, 3 μm) and (3) HALO RP-amide (100 mm × 4.6 mm, 2.7 μm).

3.1.3. Generation and analysis of data: Design Space generation

First the experimental data were generated overnight in an automated fashion, then peak tracking (matching of bands for the same compound between runs where conditions have been changed) was carried out and finally three different resolution cubes t_G–T–pH_A were constructed. The accuracy of the modeling was checked for each model before proceeding by running confirmation experiments, such as that shown in Fig. 2.

3.1.4. Definition of method and robustness

In light of the results obtained, working points were fixed with known tolerances with regards to the four critical parameters subject of this study.

3.2. Column Design Space

In order to adhere to Quality by Design principles, the selection of a column should be made with clear predefined objectives and based on quality risk assessment [23]. Predefined objectives can include – but are not limited to – the availability of equivalent or orthogonal columns and the robustness of the stationary phase. The benefit of establishing equivalent columns to the candidate column is first the flexibility to choose between columns and second that substitute columns may prove necessary if a particular column is discontinued. A robust stationary phase on the other hand, is not only important in the method development process, but all through the lifecycle of the method.

Equivalent (and/or orthogonal) columns can be determined from a column comparison database, eliminating the need for column screening technologies. Based on the "Hydrophobic-Subtraction Model" developed by Snyder and his associates, ColumnMatch characterizes the selectivity of reversed phase columns and provide a platform for generating a Column Design Space. The database allows the comparison between columns, based on "column selectivity function *Fs*" which can be used to quantitatively compare the selectivity of two columns. It is based upon the assumption that differences in selectivity for any two columns can be measured by the distance between the two points in a five parameter multi-dimensional space. Therefore, the smaller the distance (i.e. *Fs*), the more similar two columns are. In the extreme case when two columns are so close ($F \leq 3$), two columns can be considered essentially "equivalent". Conversely, columns with bigger *Fs* are more widely separated; therefore they are more different in terms of selectivity.

Table 1
Fs values for the three pairs of columns subject to this study, obtained from the column database ColumnMatch.

Fs value	HALO C18	ACE C18	HALO RP-amide
HALO C18	1	10.5	54.6
ACE C18	10.5	1	55.6
HALO RP-amide	54.6	55.6	1

In this work, three different columns were used: (1) HALO C18 column (150 mm × 4.6 mm, 2.7 μm), (2) ACE C18 columns (150 mm × 4.6 mm, 3 μm) and (3) HALO RP-amide (100 mm × 4.6 mm, 2.7 μm). The numerical comparison of these columns according to the ColumnMatch database can be found in Table 1 and can be interpreted in the following way: Selectivity is predicted to be similar on both C18 columns (low *Fs* value), and very different from that found on the amide column (high *Fs* values). A third observation is that, the HALO C18 column is predicted to be slightly more different to the HALO amide column than the ACE C18 column.

These predictions were evaluated in terms of 3-D resolution cubes (see Section 3.3).

3.3. Eluent Design Space

An Eluent Design Space was established for each column through 3-D resolution cubes with the aid of modeling software DryLab® 2010, and are shown in Fig. 3. Resolution cubes map the critical resolution (resolution between the least separated peak pair) for each combination of the three critical study parameters (i.e. t_G, *T*, pH). The value of the critical resolution ($R_{s,crit}$) is represented as a color so that warm colors show large $R_{s,crit}$ values and cold colors, low values. Specifically, red regions are above baseline resolution ($R_{s,crit} > 1.5$) and blue lines signalize peak overlaps ($R_{s,crit} = 0$). Each point within the cube corresponds to a precise modeled chromatogram, and each cube represent over a million virtual experiments. (For interpretation of the references to color in this text, the reader is referred to the web version of this article.)

Once the models had been experimentally verified, the three t_G–T–pH resolution cubes of the different column could be compared. Three 2-D planes of the resulting t_G–T–pH cubes are compared in Fig. 4.

The visual correlation between the resolution spaces generated on the two C18 columns is certainly apparent with both cubes showing large regions of identical coloring; contrastingly both are very different from the HALO amide cube. The same conclusion is drawn when comparing one point within the 3-D cubes, for instance the chromatogram t_G 38 min, *T* 49 °C and pH 2.8, shown in Fig. 2 for the ACE C18 column and Fig. 5 for the HALO C18 and amide columns. Upon comparing retention times of these three chromatograms (Table 2), it can be seen that the C18 stationary phase delivers a similar selectivity from both columns, indeed, other than an approximate 2 min delay shown in retention times of

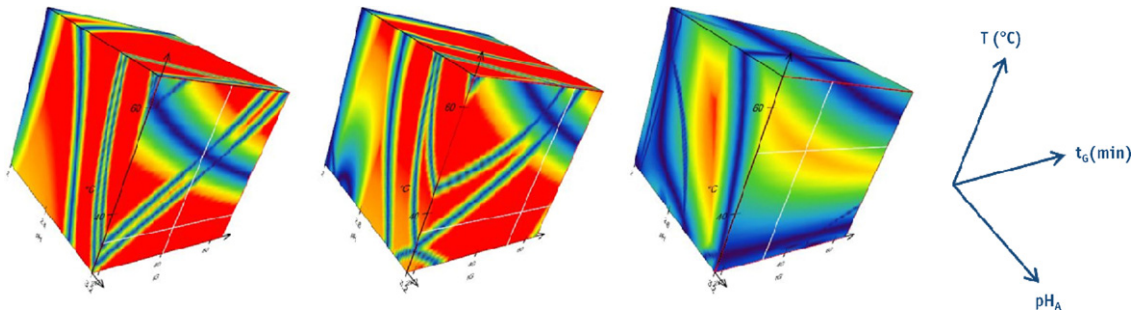

Fig. 3. 3-D resolution spaces modelning gradient time, temperature and pH of eluent A simultaneously for HALO C18 column (150 mm × 4.6 mm, 2.7 μm), ACE C18 column (150 mm × 4.6 mm, 3 μm) and HALO amide column (100 mm × 4.6 mm, 2.7 μm), from left to right.

Table 2
Comparison of retention times for the point t_G 38 min, T 49 °C, pH of eluent A 2.8.

Peak	Retention time (min)		
	ACE C18	HALO C18	HALO RP-amide
Phthalic acid	10.24	8.19	6.69
Isovanillic acid	11.71	9.57	9.76
Vanillic acid	12.39	10.27	9.49
Aspirin 1	15.55	13.13	11.38
Aspirin 2	16.07	14.24	13.62
Furosemid	20.35	18.81	18.01
Doxepin 1	22.66	20.38	17.20
Doxepin 2	23.10	20.92	17.67
Terbinafin	29.56	28.12	26.62
Atorvastatin 1	31.76	30.69	28.24
Atovastatin 2	32.18	31.09	28.56
Clopidogrel	34.29	33.31	28.75
Impurity	37.22	35.84	31.55

peaks using the ACE C18 (due to the larger surface coverage of the ACE column), there is very little difference in selectivity; both chromatograms present the same order of elution for all peaks and the critical peak pair is constant between chromatograms. When we move to compare the amide column with the C18 columns, however, there are a number of differences in selectivity, namely three peak turnovers, i.e. inversions in peak elution order and a change in critical peak pair.

In light of these results, it is reasonable to assume that columns presenting a lower predicted *Fs* value, and therefore classified as equivalent would present similar, sometimes nearly identical resolution spaces. This will be discussed further in future work.

3.4. Working point selection and robustness

There are a number of potential working points, within the now constructed Design Space to choose from. The first requisite for the method is above baseline separation for all peaks ($R_{s,crit} > 1.5$); therefore the working point should be set within one of the red geometric bodies of the resolution cubes. The next consideration is robustness tolerances; the larger the robust range of a given working point, the more durable and flexible it will be, and the more advantageous the working point. Finally, we can also take into consideration the run time of the potential working point.

First considering the cubes generated on the C18 stationary phase, one potential working point (WP1) is at t_G: 40 ± 3 min, T: 40 ± 3 °C, pH: 3.0 ± 0.1. Table 3 shows the robustness evaluation for this point in detail, the data of which was taken directly from the DryLab model. As demonstrated by the robustness test, the WP1

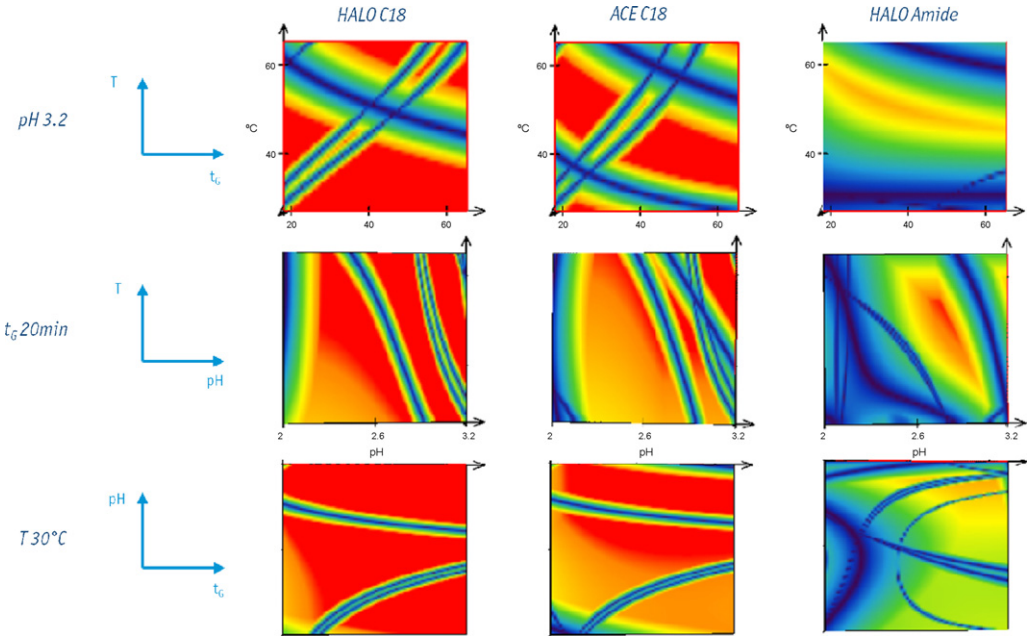

Fig. 4. Comparison of three different 2-D planes of the 3-D resolution spaces generated for each column.

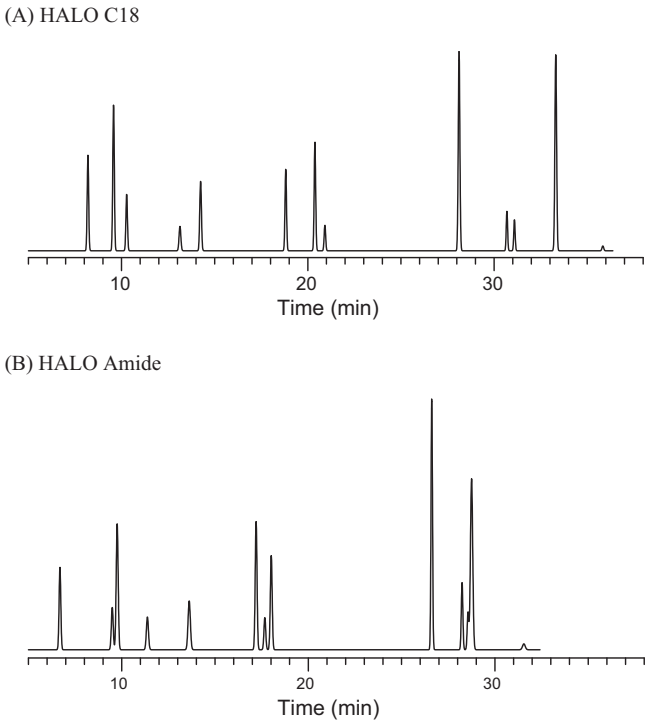

Fig. 5. Predicted chromatograms for gradient time 38 min, temperature 49 °C and pH of eluent A 2.8, on (A) HALO C18 column (150 mm × 4.6 mm, 2.7 μm) and (B) HALO amide column (100 mm × 4.6 mm, 2.7 μm).

robustness space has above baseline resolution in each point. Also included in Table 3 are the mean and standard deviation $R_{s,crit}$-values for the full factorial robustness test ($R_{s,crit} = 3.00 \pm 0.20$ for the HALO C18 column and $R_{s,crit} = 2.40 \pm 0.08$ for the ACE C18) which indicate that the robustness space is transferable from one C18 column to another, in other words, the WP1 robustness space includes tolerances for all four critical parameters.

Table 3
Full factorial robustness test for working point WP1 on HALO C18 and ACE C18 columns.

t_G (min)	T (°C)	pH_A	$R_{s,crit}$	
			HALO C18	ACE C18
37	37	2.9	2.60	2.26
37	37	3.0	2.81	2.26
37	37	3.1	3.07	2.25
37	40	2.9	2.70	2.30
37	40	3.0	2.92	2.30
37	40	3.1	3.14	2.30
37	43	2.9	2.79	2.35
37	43	3.0	3.02	2.34
37	43	3.1	3.20	2.34
40	37	2.9	2.67	2.35
40	37	3.0	2.89	2.35
40	37	3.1	3.15	2.34
40	40	2.9	2.77	2.40
40	40	3.0	3.00	2.39
40	40	3.1	3.27	2.39
40	43	2.9	2.87	2.44
40	43	3.0	3.11	2.43
40	43	3.1	3.33	2.43
43	37	2.9	2.73	2.44
43	37	3.0	2.96	2.43
43	37	3.1	3.23	2.42
43	40	2.9	2.84	2.48
43	40	3.0	3.07	2.47
43	40	3.1	3.35	2.47
43	43	2.9	2.93	2.52
43	43	3.0	3.18	2.51
43	43	3.1	3.46	2.51
			3.00 ± 0.23	2.39 ± 0.08

Another potential working point (WP2) is on the HALO amide column at t_G: 27 ± 1 min, T: 47 ± 1 °C, pH_A: 2.8 ± 0.1. Though WP2 has $R_{s,crit} = 1.65$ (above baseline separation) and has a shorter run time than WP1, when the WP2 robustness space is calculated, the critical resolution falls below baseline in some points. Indeed, the mean and standard deviation of the space were calculated as $R_{s,crit} = 1.51 \pm 0.14$. In fact it was not possible to construct a robustness space yielding baseline resolution with reasonable tolerances within the whole resolution cube constructed on the HALO amide column. Robustness issues will be dealt with in more detail in an upcoming work.

In conclusion, the goal here was not to find equivalent columns but to find equivalent selectivities and to find the best working positions within the Design Space for each column even with different column lengths and to show that within different columns we can find different unexpected robust regions. It was possible to compare different stationary phases with different column lengths to show the best selectivity be means of the resolution cube. Which column will be the best for a certain mixture may be different, depending on the sample. Some samples may have a robust separation on the HALO amide column, some will be best separated on the HALO C18 and others on the ACE C18 stationary phase.

4. Summary

An approach for applying Quality by Design principles to the HPLC method development process is presented in this article. A marriage of both HPLC Design Spaces is suggested: as the Column Design Space, in which the influence of the stationary phase on selectivity is collected, is discontinuous in selectivity. This lack can be compensated by knowledge of the Eluent Design Space, to describe mobile phase influences on selectivity. Design Space definitions were carried out with the aid of the column database ColumnMatch® and the modeling software DryLab®. An initial consultation of the database aids the selection of adequate stationary phase and once a robust column – preferably with known equivalent column – is found, the establishment of the Design Space of all other factors is carried out on the selected column.

It has been seen that two columns classified as similar in the column database present similar Eluent Design Spaces also, whereas two columns classified as different according to the column database exhibit dissimilar Eluent Design Spaces.

The retention mechanism is on a C18 phase different from that on an amide column. Whereas the C18 column has a fairly uniform behavior according to a solvophobic retention mechanism, the amide column has more an anion-exchanger character, resulting in different selectivities. It is not possible to predict, on which column a sample will have the largest robustness space – only the experiments and the resulting cube can impressively and simply answer this difficult question.

Additional experimentation would strengthen the assumption that the degree of similarity or dissimilarity stated in the column database between columns should be reflected in a comparison of Eluent Design Spaces. In part II of this article the approach is taken further, to examine the fact that a column with apparent insufficient separation can be turned into a good performing column with the knowledge of the Eluent Design Space. The visualization of the robust part of the Design Space is expected to reduce methodological difficulties in the industrial environment to a large degree, allowing the saving of valuable resources such as time and material in a more flexible production process of the future.

Acknowledgements

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