HIGH PERFORMANCE LIQUID CHROMATOGRAPHY METHOD DEVELOPMENT STRATEGY FOR PHARMACEUTICAL APPLICATIONS WITHIN A QUALITY BY DESIGN FRAMEWORK

Dissertation

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Abstract

This work presents the investigation and evaluation of reversed-phase high pressure liquid chromatography (HPLC) method development strategies in accordance with Quality by Design (QbD) principles for a number of practical applications of pharmaceutical interest. Workflow schemes were established targeting the current needs of the pharmaceutical industry to develop robust, reliable, durable and flexible HPLC methods with limited investment of resources, time and money.

A fundamental tool employed throughout such schemes is chromatography modeling software which is shown to greatly aid the understanding and visualization of chromatographic behavior *in silico* and therefore significantly reduces the experimental burden typically associated with attaining good robust methods.

Introduction

The use of chromatography modeling software in the development of robust reversed phase high performance liquid chromatography (HPLC) methods is well documented, particularly within the field of pharmaceutical analysis [1,2,3,4,5,6]. Over the past decades, these computer programs have continually progressed and adapted to meet the changing needs of the industry, and in the current increasingly Quality by Design (QbD) orientated climate, this modern technology is arguably more relevant than ever [7,8,9,10,11,12,13,14].

QbD methodology is now actively promoted and encouraged by regulatory pharmaceutical agencies both in manufacturing and analytical development. From a QbD perspective, development should be carried out in a systematic manner, beginning with predefined objectives, emphasizing understanding and control and based on sound science and quality risk management [15].

The industrial benefits of implementing this approach are multifold; through enhanced scientific insight method quality can be assured; failures and costs can be reduced; and flexibility to innovate and continually improve without excessive regulatory oversight can be attained [16,17]. A flourish of recent works have responded to the QbD initiative by proposing practical ways in which these principles may be applied to analytical [18 , 19] and predominantly, HPLC method development [20,21,22,23,24,25,26].

The workflow adhered to in most QbD-based HPLC method development studies can typically be described as consisting of four key steps:

- Definition of method intent
- Method design and selection
- Method evaluation
- Method control

Ways in which the chromatography software DryLab® and 3D resolution modeling can be implemented into this scheme have been investigated for a number of different applications [8-13]; these shall be highlighted and briefly discussed in the following text.

A Stepwise Strategy to Quality by Design HPLC Method Development

A generic step-by-step guide to developing an HPLC method in accordance with QbD principles is presented below.

1. Method intent

According to the ICH Q8(R2) guidelines [14], pharmaceutical development should begin with the end in mind, therefore the first step in each HPLC method development project is to clearly establish the objectives for the final method in accordance with all known information. Possible aspects to be taken into account may be:

- *Method requirements*: accuracy, precision, specificity, range, robustness, ruggedness; regulatory requirements; formal validation;
- *Business requirements*: costs of analysis (equipment, maintenance of equipment analysis time, solvents); available resource; voice of the customer;
- *Practical requirements*: location where is the method required (method transfer), equipment and expertise available, development time available.

Though the precise targets of each separation problem will differ from project to project, a common goal can be summarized as: the robust separation of the components of interest within a reasonable analysis time and at an acceptable cost.

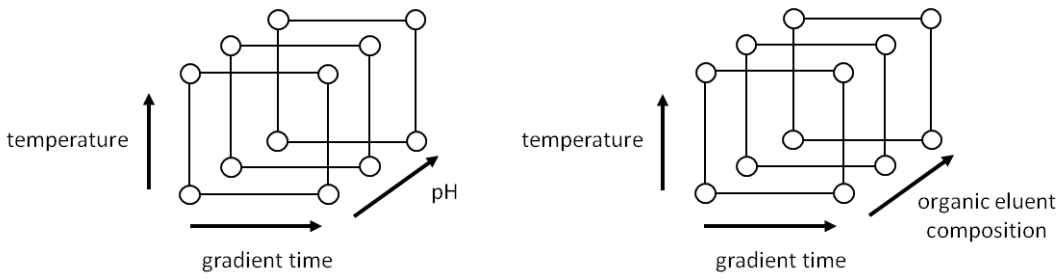
2. Method design

An effective technique for developing a method capable of fulfilling all predefined targets is to systematically evaluate the influence of all critical separation parameters on results in a multifactorial and simultaneous fashion. This procedure of evaluation is also known as exploring the Design Space [14] of the analysis. Critical separation parameters are those experimental factors most strongly impacting the outcome of the analysis and, in reversed phase HPLC, are thoroughly well known [27,28,29,30]. In the majority of cases, they are gradient time or slope, temperature, pH, ternary eluent composition, and stationary phase. Notwithstanding, other parameters such as flow rate, gradient shape, dwell volume, etc, may also be important in gradient elution.

Design Space generation and investigation can be carried out with minimum experimental requirement using computer modeling [31,32] and column databases [33,34,35,36,37].

2.1. Design of experiments

Multifactorial models characterizing the chromatographic behavior of a given sample with regards to changes in the mobile phase are constructed from empirical data (retention times and peak areas) generated according to experimental designs (see Fig. 1). Parameter settings and ranges for these designs are typically selected based on: the DryLab® software's recommendations, established on the work of Snyder et al.; the results of a scouting experiment; and on prior knowledge about the sample, where available.



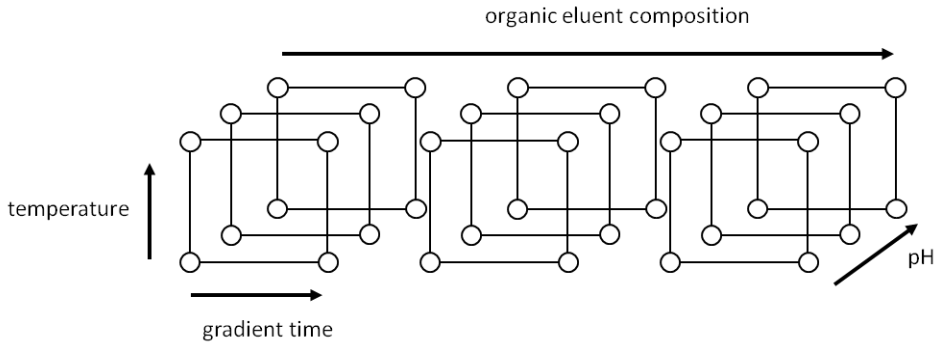


Fig. 1 Typical experimental designs

2.2. Design Space generation

Once the appropriate design of experiments has been chosen and the corresponding experiments have been conducted, the resulting data is analyzed and entered into the chromatography modeling software. The program then generates resolution models which map the measured critical separation parameters against the critical resolution ($R_{s,crit}$) - resolution between the least well separated peak pair - of the separation.

From within the Design Spaces, favorable working regions can be easily and visually located (see Fig. 2).

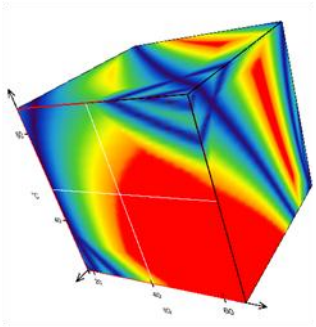


Fig. 2 Representation of a Design Space. A 3D resolution space mapping the influence of gradient time (tG), temperature (T) and ternary eluent composition on critical resolution (color) is shown. Regions yielding above base-line resolution ($R_{s,crit} > 1.5$) are colored red

2.3. Method selection

The final choice for method conditions is based on the initial targets of the analysis. Generally favorable methods have high values of critical resolution, short run times and are contained within a large robustness region.

3. Method evaluation

Once promising method conditions have been determined, it must be assured that the developed method is capable of consistently meeting its design intent (acceptance criteria). Multifactorial robustness studies resulting in the definition of a control space (also known as Method Operable Design Region), are an integral part of this process, as is formal validation [38,39,40,41,42]. Evaluation should be based on the results generated in the previous stage captured within the Design Spaces or resolution models (see Fig. 3). If acceptance criteria are not met, another candidate method from the method design step may be selected and reviewed for evaluation.

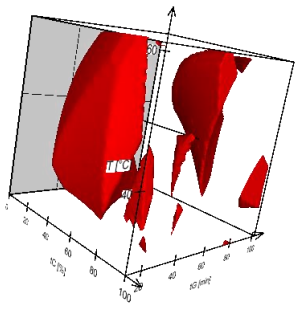


Fig. 3 Representation of robustness spaces. Regions of the space yielding above base-line resolution ($R_{s,crit} > 1.5$) are colored red

4. Method control

The final stage is the continued verification of method performance. Ongoing schemes should be put into place to monitor, document and understand method performance, established on the wealth of information generated during design, evaluation and control stages (lifecycle management). Based on a deep scientific understanding and knowledge base, the impact of any proposed future changes to the method may be assessed, justified and more easily implemented with limited regulatory oversight.

Case studies

The following section reviews a series of practical examples implementing modern 3D resolution modeling technology to the QbD based HPLC method development strategy. More details of these works can be found in the Appendix.

Case study 1

Aspects of the "Design Space" in high pressure liquid chromatography method development

The focus of this study was the generation, visualization and evaluation of a four dimensional (4D) Design Space, describing the simultaneous influence of four measured critical separation parameters (factors) on selectivity (results).

Highly accurate multidimensional resolution spaces modeling gradient time, temperature, pH and ternary eluent composition against critical resolution, were successfully constructed for a sample mixture comprising of 14 peaks. From these models, favorable working conditions (combinations of the four measured parameters) with high values of critical resolution were selected. It was also shown how further optimization to shorten run time with retention of selectivity could be achieved within the software without the need for additional experimentation.

A novel feature of this work was the introduction of DryLab® resolution cubes with full rotation and scrolling functionality. This technology enabled for the first time a 3D visualization and assessment of the HPLC Design Space, until that time limited to 2D.

Case study 2

From Csaba Horváth to Quality by Design: Visualizing Design Space in Selectivity Exploration of HPLC Separations

In this study, a part of the Design Space for the separation of a sample of 9 model compounds was generated by means of a 3D resolution model. From 12 initial experiments, the joint influence of gradient time, temperature and ternary eluent composition were evaluated in terms of selectivity. The effect of transferring separation conditions from one organic eluent to another was also assessed.

Case study 3

Experimental Combination of Method Development Strategies in a Working Environment of Different Instrumental Set-ups

In an industrial setting method transfer is a common activity and should be considered during method evaluation. The aim of this study was to investigate how chromatography modeling software DryLab® may be used to predict the results of method transfer from one experimental set-up to another.

For this purpose, multidimensional resolution models (2D and 3D Design Spaces) were generated on three different set-ups with different instrument type, flow rate and column dimensions for a constant sample of toxicological interest. Results (retention times and resolution values) from one set-up were used to predict *in silico* the results on another.

The suitability of the software to mitigate method transfer was successfully verified.

Case study 4

Expanding the term "Design Space" in high pressure liquid chromatography

Building on the work presented in case studies 1 and 2, the focus of this investigation was to examine ways in which the influence of different stationary phase chemistry may also be included into the representation of the Design Space.

In the first part of this two part study, it was proposed that the HPLC Design Space be considered as the product of two complimentary Design Spaces: one Column Design Space (CDS) and one Eluent Design Space (EDS). CDS and EDS definitions were carried out with the aid of the column database ColumnMatch® and the modeling software DryLab®, respectively.

3D resolution models were constructed for a constant sample on different stationary phases and it was shown that similar columns exhibit similar EDS whereas dissimilar columns give dissimilar EDS. This was exploited to successfully define robustness spaces (control spaces) with regards to all critical parameters from both mobile and stationary phase.

The second part of the work investigated an approach for contrasting columns over the Eluent Design Space. It was shown on the one hand how an apparently poorly performing column can be turned into a well performing column through knowledge of the EDS and on the other hand how the suitability of a stationary phase for a given separation problem can be easily and visually judged by means of comparing relative sizes of robustness spaces.

Case study 5

Quality by Design: Multidimensional Exploration of the Design Space in High Performance Liquid Chromatography Method Development for better Robustness before Validation

The aim of this project was to develop a fully validated assay of two APIs and impurities for an eye drop sample, providing a fast and robust stability indicating analysis, according to QbD principles.

3D resolution models were constructed in order to describe and explore the Design Space of the separation. Final working conditions were selected from within the models based on triple criteria: highest critical resolution; largest robustness region; and shortest run time.

The robustness of the selected method was evaluated prior to validation, with respect to 6 critical separation parameters within the DryLab® software without need for further experimentation using a novel robustness testing interface. Formal validation confirmed that the developed method fulfilled all predefined performance criteria within the investigated robustness region.

Summary

A number of practical examples of pharmaceutical interest have shown how with the appropriate work flow and tools, Quality by Design principles can be easily applied to the development of HPLC methods. Design Spaces could be generated with the aid of chromatography modeling software, and were explored to afford a systematic and science-based selection of method conditions.

Candidate methods were evaluated *in silico* and underwent in depth robustness testing and simulated method transfer, giving a realistic reflection of method performance and reliability over the lifecycle of a method. Given the large amount of chromatographic information contained in generated models, these offer a sound foundation for method troubleshooting and to justify continuous future improvement.

Zusammenfassung

An einer Reihe praktischer Beispiele von pharmazeutischen Interesse konnte gezeigt werden, wir mit Hilfe geeigneter Arbeitsabläufe und passender Werkzeuge Prinzipien des Quality by Design Konzepts auf die Entwicklung von HPLC Methoden angewandt werden können.

Unter Verwendung einer Chromatographie Modellierungssoftware konnten verschiedene Design Spaces berechnet und ausgewertet werden, um auf diese Weise eine systematische und wissenschaftlich fundierte Auswahl der Methodenbedingungen zu erhalten.

Exemplarisch wurden einige HPLC Methoden ausgewertet und ausführlichen Robustheitstests und simulierten Methodentransfers unterzogen, um eine realistische Einschätzung über die Leistungsfähigkeit und Vertrauenswürdigkeit der Methode über ihren gesamten Anwendungszeitraum hinweg zu erhalten. Aufgrund der zahlreichen chromatographischen Information, die die berechneten Modelle enthalten, können diese hervorragend für Methodenanpassungen und zur Entwicklung kontinuierlicher Methodenoptimierungen verwendet werden.

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K.E. Monks, I. Molnár, H.-J. Rieger. ***Expanding the term "Design Space" in high pressure liquid chromatography (II)***

Keywords

Quality by Design (QbD); Design space; 3D Resolution modeling; Column selection criteria; Column comparison

Abstract

In this work multidimensional resolution maps - which model the simultaneous influence of the highly critical parameters gradient time (t_G), temperature (T) and ternary eluent composition (t_C) on the selectivity of an HPLC separation - were generated on three stationary phases of different column chemistry for a constant sample mixture. The constructed models were employed first to explore the Eluent Design Space (EDS) of the analysis to find optimum working conditions on each column, and then the models were compared to one another in terms of robustness in order to determine the relative suitability of each column for the separation problem subject to investigation.

1. Introduction

Extensive literature reports how resolution modeling can greatly aid in the task of ascertaining optimal conditions for HPLC methods [i,ii,iii,iv,v,vi]. These models have furthermore been applied to the evaluation of robustness [vii,viii,ix,x,xi] and, within the current Quality by Design (QbD) climate, have also been employed to describe and explore HPLC Design Spaces, such as the Eluent Design Space and the Column Design Space [xii,xiii,xiv].

The application of Quality by Design principles to the development of analytical and particularly HPLC methods is notably on the increase [xv,xvi,xvii,xviii,xix,xx]. These principles promote a systematic development based on a solid scientific foundation and resulting in the fulfillment of predefined targets [xxi]. For the development of HPLC methods a number of studies [16-18] have interpreted and translated these requisites into a workflow consisting of four key steps: (1) Definition of Method Intent, (2) Method Design and Selection, (3) Method Evaluation and (4) Method Control. A important factor which must be considered in each of these steps is the column.

Indeed, as part of every HPLC method development project, an appropriate stationary phase must be selected. At a time when hundreds of reversed-phase columns are commercially available, it is increasingly difficult for an analyst to select “the best one” for the separation problem at hand [xxii,xxiii]. One reported strategy is screening different stationary phases, typically in combination with a number of different mobile phase compositions, such as ternary eluent composition and/or pH [xxiv]. The experimental workload, however, of such scouting processes is

considerable and analysis of the generated data remains a substantial challenge, generally being reduced to a pick the winner approach.

The focus of this work is to investigate how multidimensional resolution mapping may be implemented in the selection of an appropriate stationary phase, in a fashion accordant with QbD principles.

2. Experimental

2.1. Eluents

Acetonitrile (AN) and methanol (MeOH) (gradient grade), HPLC–water and all chemicals were purchased from Merck (Darmstadt, Germany). Eluent A was prepared by mixing 25% A1 and 75% A2 (V/V) where A1 was a solution 25mM phosphoric acid and A2 was a solution 25mM monobasic sodium monophosphate, for a pH of 2.6 [1]. Eluent B was varied between AN and MeOH. Gradient elution between 0 and 100% B was used at a flow rate of 0.8 mL/min.

2.2. Sample

Model substances and reference materials used were phthalic acid (1), vanillic acid (2), isovanillic acid (3), anthranilic acid (4), vanillin (5), syringaldehyde (6), ferulic acid (7), ortho vanillin (8), benzoic acid (9).

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. HALO C18 (100mmx4.6mm, 2.7µm), HALO phenyl-hexyl (100mmx4.6mm, 2.7µm) and HALO amide (100mmx4.6mm, 2.7µm) fused-core columns provided by HiChrom (Reading, United Kingdom) and MacMod Inc. (Chadds Ford, USA) were used.

2.4. Software

HPLC separations were generated using the automation option of DryLab®2010 V 4.0 (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 V. 4.0 and predictions were compared with the original experiments to control the validity of the modeling process. The plate number was adjusted in various computer simulations of separation to the real column performance.

2.5. Experiments for modeling

3D resolution spaces modeling the simultaneous influence of gradient time (t_G), temperature (T) and ternary eluent composition (t_C) on the selectivity of a constant sample mixture, were generated on each of the three aforementioned columns. Initial input data, largely in compliance with recommendations from Snyder et al. [1], were acquired under the following conditions: gradient times of 20 min (t_{G1}) and 60 min (t_{G2}), temperatures of 30 °C (T_1) and 60 °C (T_2), and ternary eluent compositions of 100% AN (t_{C1}), AN:MeOH (50:50 V/V) (t_{C2}), and 100% MeOH (t_{C3}). These conditions were combined to afford a design of experiments (DoE) comprising of 12 (2x2x3)

experimental runs (Fig. 1). All chromatographic data were generated overnight unattended in an automated fashion.

3. Results and discussion

3.1. Generation of Eluent Design Spaces

An identical set of experiments was designed and run on each of the three fused-core columns as described in Section 2.5 and schematically shown in Fig. 1.

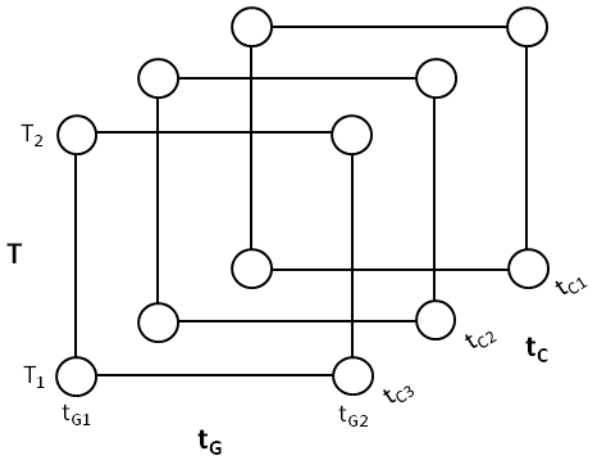


Fig. 1 Design of experiments for the construction of 3D gradient time (t_G), temperature (T) and ternary eluent composition (t_C) resolution spaces

From the chromatographic data generated (retention times and peak areas) on each column, 3D resolution spaces representing gradient time, temperature, ternary eluent composition in function of critical resolution (color) were constructed (Fig. 2). Resolution models map the critical resolution - resolution between the least well separated peak pair - for each combination of the study parameters (i.e. t_G, T, t_C). The value of the critical resolution ($R_{s,crit}$) is represented in color so that warm colors show large $R_{s,crit}$ values and cold colors show low values corresponding to inefficient separations. Specifically, in red regions the resolution is baseline or above ($R_{s,crit} \geq 1.5$) and dark blue lines signalize peak overlaps ($R_{s,crit} = 0$).

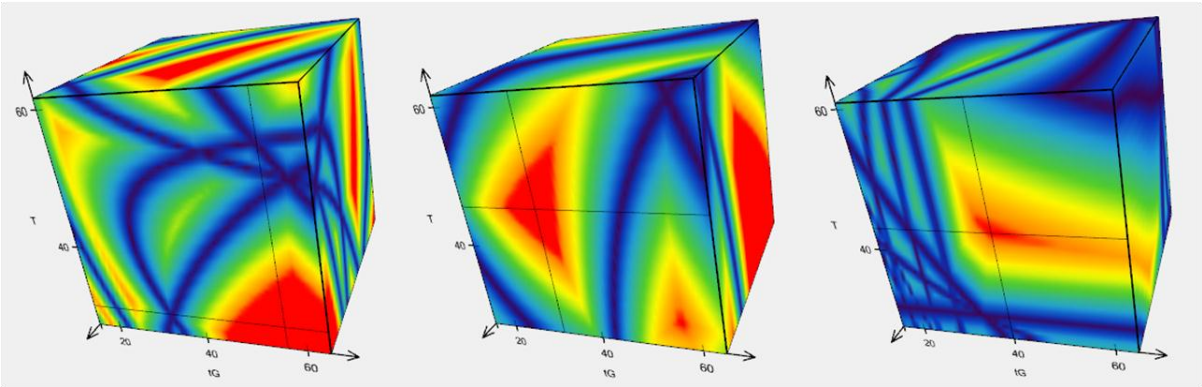


Fig. 2 3D resolution spaces mapping the simultaneous influence of gradient time (t_G), temperature (T) and ternary eluent composition (t_C) on critical resolution (color) generated on (from left to right) HALO C18, HALO phenyl-hexyl and HALO amide columns. Regions yielding above baseline resolution ($R_{s,crit} \geq 1.5$) are colored red.

3.2. Evaluation of Eluent Design Spaces

The three resolution spaces (Eluent Design Spaces), each representing over a million highly precise simulated chromatograms, differ significantly from one another visually. This result was perhaps foreseeable given the different chemical nature of the three stationary phases. The point highest $R_{s,crit}$ - in other words the combination of conditions affording the highest $R_{s,crit}$ - within each resolution cube also considerably varies, as summarized in Table 1.

Table 1 Summary of optimal conditions in terms of critical resolution for each of the three EDS

	t_G (min)	T (°C)	t_C (%)		$R_{s,crit}$	run time (min)
			%AN	%MeOH		
HALO C18	48	27	94	6	3.2	15
HALO phenyl-hexyl	56	28	48	52	2.7	19
HALO amide	40	43	7	93	1.6	16

According to the data presented in Table 1, the most favorable separation conditions with the highest value of critical resolution and the shortest run time, appears to be afforded by the HALO C18 column. However, when robustness of the method are also taken into account, as discussed below, this afirmation is not necessarily applicable.

In order to evaluate robustness, all working points with a critical resolution below the threshold of 1.5 ($R_{s,crit}$ < 1.5) were removed from the resolution spaces, leaving behind irregular geometric bodies representing regions of baseline robustness (robustness spaces). These robustness spaces (Fig. 3) consist of method conditions for which the $R_{s,crit}$ remains above 1.5, therefore the larger the size of the space, the more robust the region is.

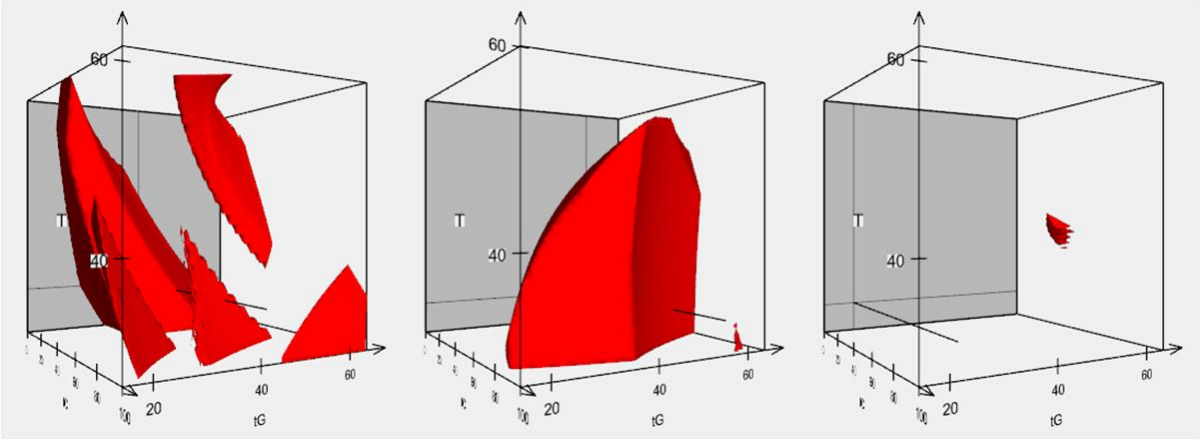


Fig. 3 Robustness spaces for (from left to right): HALO C18, HALO phenyl-hexyl and HALO amide columns. Red regions represent robust above baseline separations.

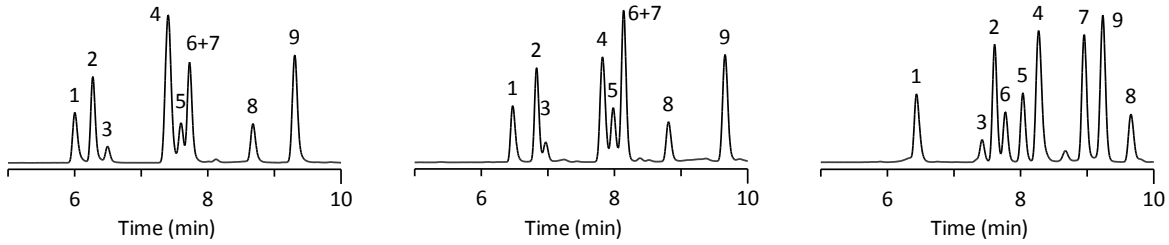
Given that the size of the red region is an indication of how robust the separation conditions are to change, the robustness spaces for the three HALO columns shown in Fig. 3 can serve as a means for column comparison. It can be observed that the HALO phenyl-hexyl affords the largest robustness space of the three whereas the

HALO amide column, has the smallest and interestingly, the HALO C18 displays five separate robust regions showing that under a variety of different working conditions a robust separation could be achieved. In line with these observations, the HALO phenyl-hexyl would appear to be the most suitable stationary phase for this separation problem, with the HALO C18 also delivering good separation within a smaller robust region. Even the HALO amide can perform satisfactorily , albeit within a reduced region of the Eluent Design Space. It is also worth noting that on each column, the point of highest critical resolution (see Table 1) is contained within the largest robustness space.

3.3. Single chromatograms vs. multidimensional column comparison

A common way of contrasting column performance and/or suitability is via chromatogram comparison under a single set of conditions. Fig. 4 shows two such comparisons at the points (A) t_{G1}, T_1, t_{C1} and (B) t_{G1}, T_1, t_{C3} (see Section 2.5 for details). In (A) it can be observed that an acceptable separation of all peaks can only be achieved on the amide phase, whereas in (B) the C18 (one double peak) and phenyl-hexyl (one double peak) phases appear to be a better choice than the amide phase (one quadruple peak). In light of these data, a satisfactory statement about which of the three phases is the most suited to this particular separation problem is not easy to draw. This is perhaps not surprising when one imagines these single chromatograms as discrete points within the Eluent Design Space. Depending on where within the space the selected comparison conditions are, one column may appear to perform better than another, and this may or - more likely - may not correctly indicate the overall performance and/or sutibility of the stationary phase.

(A)



(B)

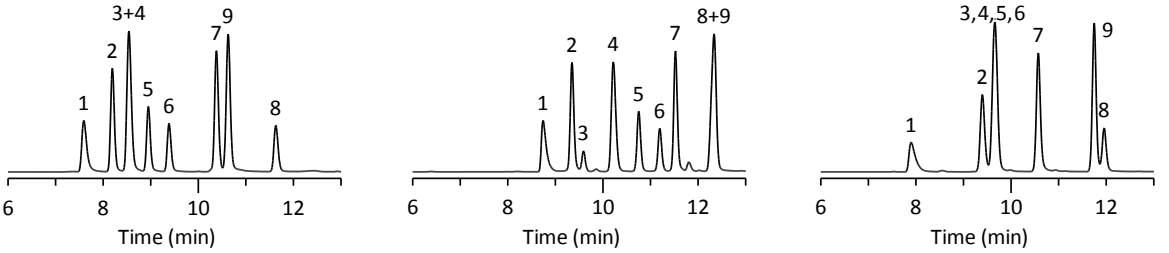


Fig. 4 Single working condition column comparison (from left to right) HALO C18, HALO phenyl-hexyl and HALO amide columns, at (A) t_G: 20 min, T: 30 °C, t_C: AN and (B) t_G: 20 min, T: 30 °C, t_C: MeOH

4. Summary

Part I of this two part study proposed the description of the HPLC Design Space as two complimentary Design Spaces: one describing the influence of the critical separation parameters present in the mobile phase (Eluent Design Space, EDS) and the second describing the influence of the stationary phase chemistry (Column Design Space, CDS). This second part explores the EDS further and examines how 3D resolution spaces may be applied in evaluating the suitability of stationary phases for a given separation problem. This approach for column comparison was contrasted with another method for the identification of an appropriate stationary phase, that of column comparison under a single set of working conditions. It is shown how, by exploring a larger experimental region, acceptable separation conditions could be

achieved on each of the three columns and how robustness could be effectively used as criteria for comparing columns.

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