Modelling HPLC Method Robustness

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High Performance Liquid Chromatography (HPLC) is one of the most successful developments in analytical science of the last 50 years, and this article was written for young students of chromatography in order to assist their understanding of this important tool of modern science. Of the approximately 30.000 diseases described in medicine, only 100–150 are of such relevance that they qualify to become research fields of the pharmaceutical industry [1]. To address the uncured diseases, we must rethink the way in which we produce safe and effective drugs and develop an understanding of their biochemical background. We must develop drugs to treat even diseases which impact only small portions of the population, and HPLC is method key analytical tool by which we can contribute to achieve this.

Method validation with traditional compendial HPLC using long columns and wide diameters, large particle sizes 5 um are often painfully slow and costly. If we could reduce the timing of slow analytical processes in research and development and replace them with computer supported virtual modelling tools, then validated methods would be available in a more timely and effective way [2-8].

What are our most important goals in industrial production?

Goals for chromatographic analytical method development are three-fold. A method should

a. have the fastest separation,

b. select the most efficient column [9], and

c. find the most robust conditions for routine work (= critical resolution ($R_{s,crit}$) is maximised).

The achievement of all three goals is critical to the success of the method development process, but without a true understanding of the issue, they cannot be achieved in practice.

In order for a method to be robust, it must generate maximised peak distances by changing them and learn how they change. The goal is to get maximised critical resolution. i.e., baseline separation of all impurities and degradants deemed important in research process. If it does not, possible impurities could go unnoticed when they co-elute with other peaks. When a separation error occurs, chromatographic peaks may have moved with slight change in variables, and many users of HPLC do not understand why. In order to control the separation, we must develop an understanding of how HPLC functions and how methods were developed [8-11]. The treatment of different column chemistries is described in ref. [9].

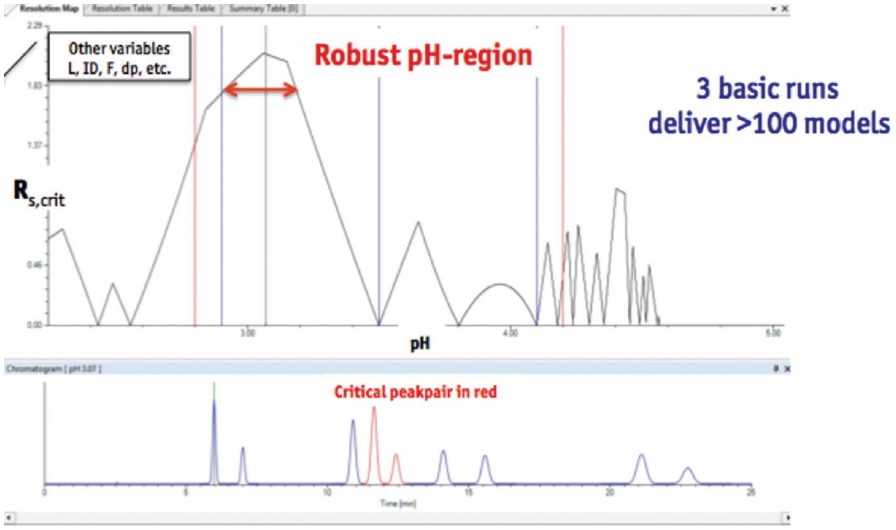

Figure 1: shows a chromatogram with peaks 4, and 5 marked as the critical peak pair in red. This critical peak pair is the closest pair of all components – at this pH. Below pH 3.1 the critical peak pair is 3 and 4. If this method came from a Pharmacopeia and the critical peak pair were to change from 4 and 5 to 3 and 4 by dropping the pH below 3.1, the inflexible method would be out of compliance and the drug it supported could not be sold despite the fact that the method is still robust. In a case such as this, a company, 'out of compliance', must start a 'change management process', which is expensive, because of the lost cost of development, and because of the time taken to come back within compliance.

The downfall of many otherwise robust methods is that they fall victim to regulatory control and outdated methods, latter often requiring columns no longer in production, or stating that critical peak pairs must be certain predefined pairs and that the method cannot be changed.

How to Understand Peak Movements: pH-Model

In order to gain meaningful control of all the peaks that emerge in a chromatogram, it is imperative to develop an understanding of which critical peak pairs are even possible. Figure 1 is an example of the pH-Model used in DryLab as a tool that offers this understanding. In the top half of Figure 1 a re-solution map where the y-axis is the critical resolution and the x-axis is the pH of the eluent is displayed. Within this top half there is a small robust region (red double-arrow) where a good baseline separation of $R_{s,crit} > 1.5$ is achieved. On the top left, other parameters such as the length- and inner diameter of the column, the particle size (dp) and the flow rate (F) can be optimised.

How to Understand Peak Movements: Gradient Time modelling

In the following Figure we show, how a Gradient Time Model and Design of Experiments are helping to understand the reasons behind why peaks move with different

gradient profiles.

For the creation of a gradient elution model in DryLab, only 2 runs are needed and using the model, a user can generate hundreds of precise chromatograms in a short time. Through the addition of steps in the gradient the resolution in certain areas can be improved. The model in the example above is a three-step gradient. However the robustness of a method might suffer under the influence of these steps. The most efficient Design of Experiments is using the 12 run cubic model described as follows.

The Cube

The 'Cube' (Figure 3) was developed because some time ago there was a major shortage of acetonitrile (ACN) – which is a by-product of a large scale chemical process. This affected the ability of certain companies – who were not supplied on a preferred basis - to perform methods which were validated. Method re-development in this situation using methanol was another option.

Using this model as reference, a three-dimensional colour-coded cube can be generated showing the robust zones in red. This tool can be invaluable to the visualisation and understanding of how a method operates, and provides an easy and intuitive way to develop the best method thus achieving goals number one and three discussed at the beginning of this article.

The Ternary Cube and Picking your Working Point

The three axes of this cube, tG, T, and tC are used to visualise regions affording suitable resolution within the design space. These (red) regions are the combination of approximately one million separate chromatograms each representing a single point and coordinated depending on changes in that individual chromatogram's variables. Areas in red are where baseline separation is possible, however these methods are not necessarily robust. In order for a method to be robust, it must have a 100% success rate in practical tests over many runs because practical tests require tolerance limits accounting for small changes in each of the variables. With access to the Cube, chromatographers can choose the most robust method for routine work and find the best separation quickly.

Case study: How to make 'Green HPLC'

Chromatographic modelling offers one of the most efficient ways to develop methods and can be considered a 'green' technology.

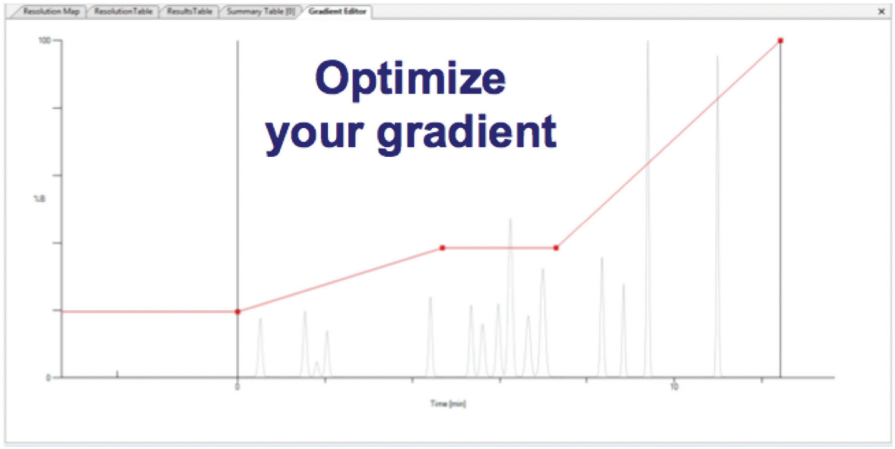

Figure 2: The Gradient Elution Model shown here was developed in 1987 to visualise the relationship between gradient time (T) on the x-axis and the critical resolution on the y-axis and is useful for understanding how the form of the gradient influences the separation [2]. Adding points to the gradient and moving them with the mouse allows users to see immediate changes in selectivity, which means changes in peak positions in the chromatogram. In this way, the time of development of a gradient method can be reduced from months to minutes.

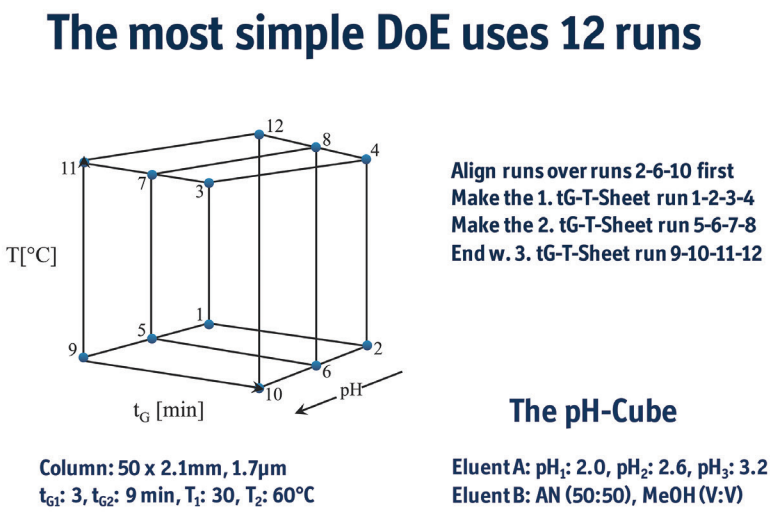

Figure 3: In the above Figure, a simple DoE or Design of Experiments is displayed showing 12 points with different combinations of variables representing individual chromatograms. In order to render this cube, three experiments, numbers 2, 6, and 10 are aligned by their gradient time, the x-axis of the cube above. Then three sets of four chromatograms are generated creating three layers, from which a three dimensional cube is calculated, where the y-axis always denoting temperature (T) and a z-axis denoting pH or ternary composition (pH in this case).

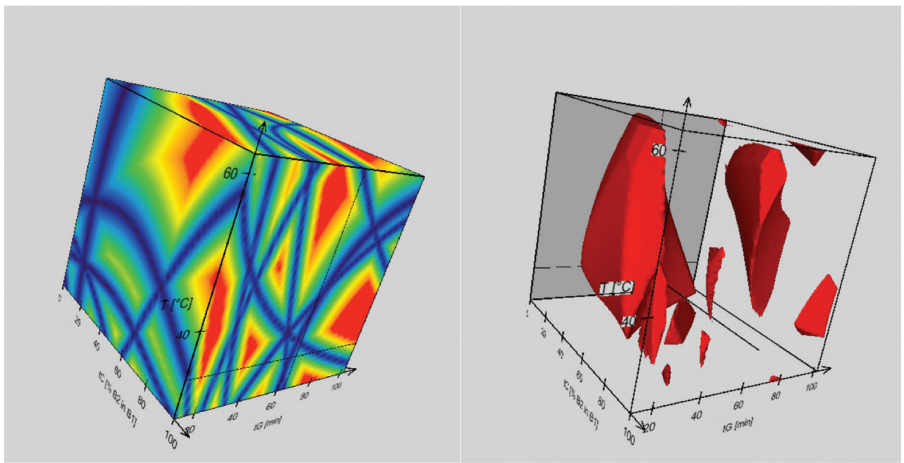

Figure 4: The Gradient-Time vs. Temperature vs. Ternary Composition Cube, (tG-T-tC-Cube) shows red areas where baseline separation is possible. Left: coelution is shown in blue, on the right red irregular bodies are showing the so-called baseline separation regions (design space regions). This gives in the routine work great flexibility.

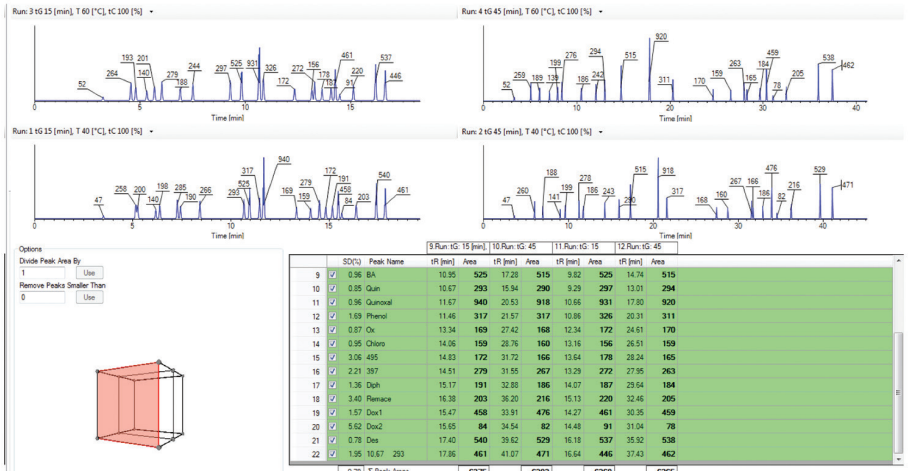

Figure 5: Peak tracking of a mixture of 22 drug substances, showing the wide variety of selectivities in the 4 chromatograms, changes which we have to understand before we can make any statements about robustness. The alignment of the peaks is done in a way, that the data of each component (tR + peak area) are positioned in a horizontal line. If the table has green lines, the peak tracking is deemed to be accurate in terms of peak area and retention time.

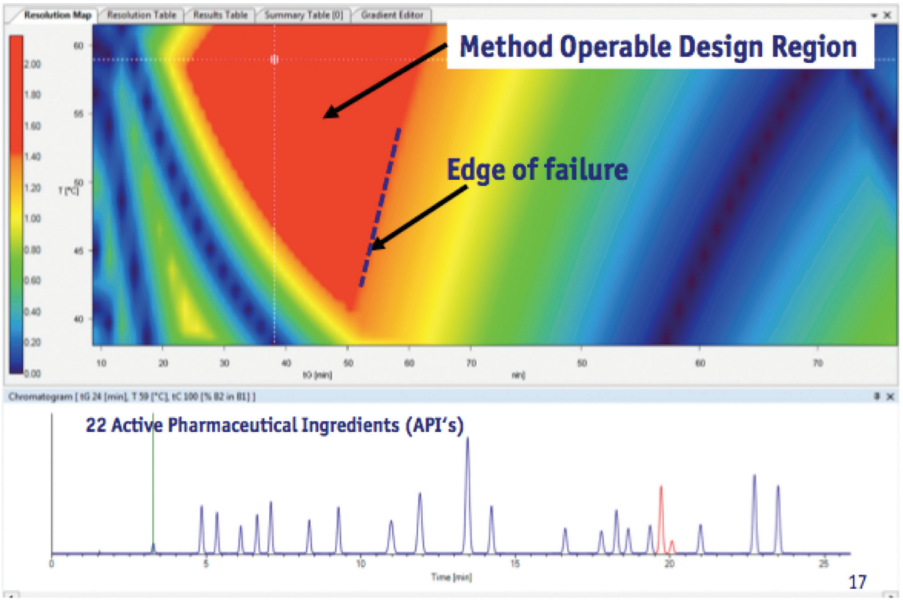

Figure 6: The red region allows separation of all components with baseline resolution. At the Edge of Failure (EoF) however this condition starts to become invalid. It is at the edge of failure that the risk of peak overlaps and insufficient separation begins to occur [13].

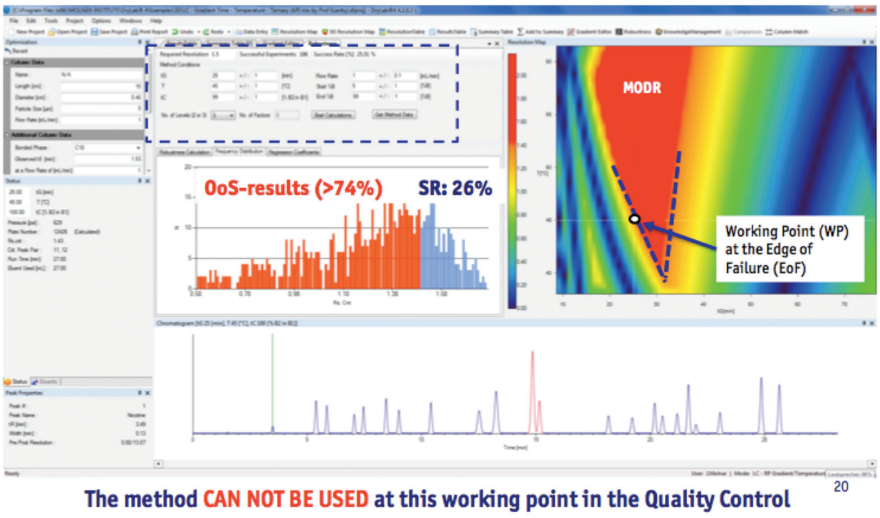

Figure 7: In the Figure, the working point has been selected at the indicated point, no DryLab optimisation was involved in the finding of this working point and there was no Method Operable Design Region (MODR - the red area in the map) established. As the workpoint is not in a robust red region, but instead between the yellow and the red region past the EoF, the method has an extremely high rate of failure, or Out of Specification (OoS) as indicated by the red bars to the left of the figure, which show, how many times a run was found with critical resolution less than 1.5 ('baseline resolution') out of 729 (3^6) experiments.

'Green' refers to the capability to create chromatograms of high precision without using acetonitrile or other potentially dangerous eluents. Only a few experiments are required to understand a large chromatographic space. So are for a Cube only 12 experimental runs required, however we can derive from them over one million different separations in-silico.

Here some steps of this process are shown – the peak tracking process.

Peak areas are relative to the mass of a substance injected. By injecting the same sample and the same amount (mL) the components can be identified based on the peak areas (although peak tracking via mass spectrometry offers additional confidence during this process).

After this process is finished, we can find the Method Operable Design Region (MODR), the red area in the resolution map. This is the place, where the method has greatest 'robustness' [7-12].

Specification (OoS) as indicated by the red bars to the left of the figure, which show, how many times a run was found with critical resolution less than 1.5 ('baseline resolution') out of 729 (36) experiments.

Recently a number of papers have demonstrated success of in-silico modelling using DryLab to achieve robust industrial HPLC methods for flexible quality control based on QbD principles [13-18].

Summary

In summary, HPLC virtual modelling can be a powerful tool for developing or understanding chromatographic methods, if used correctly by people who understand chromatography in general. DryLab specifically can be used to a great effect in the areas of robust method determination, column selection, and expedient method testing. The three goals introduced at the beginning of this article were once time consuming and laborious processes, but with HPLC modelling, they can be achieved efficiently and effectively [9].

Acknowledgement

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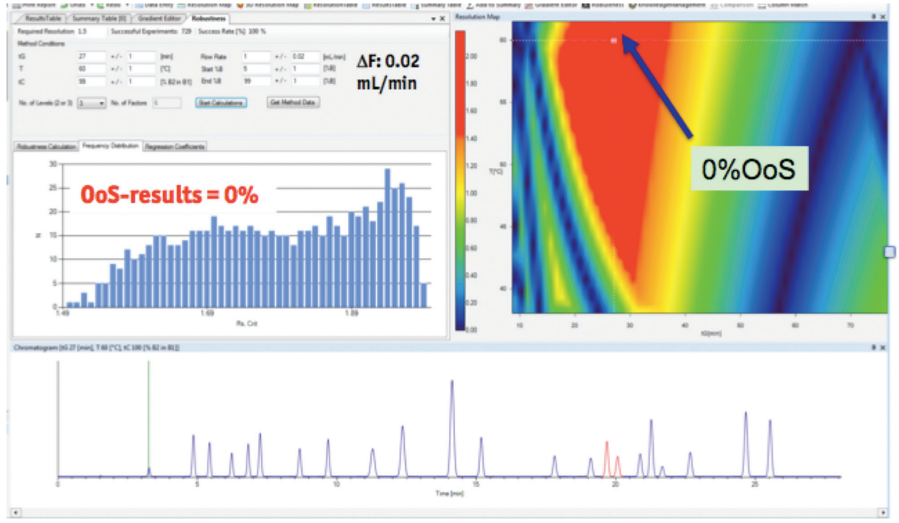

Figure 8: The success rate of the routine application improves to 100% after the working point is changed to a more robust region and after the tolerance limit of the pump is improved from 0.1 to 0.02 mL/min, which corresponds to a tolerance limit at 1.00 mL/min flowrate of 2%.

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