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UHPLC Method Development and Modelling in the Framework of Quality by Design

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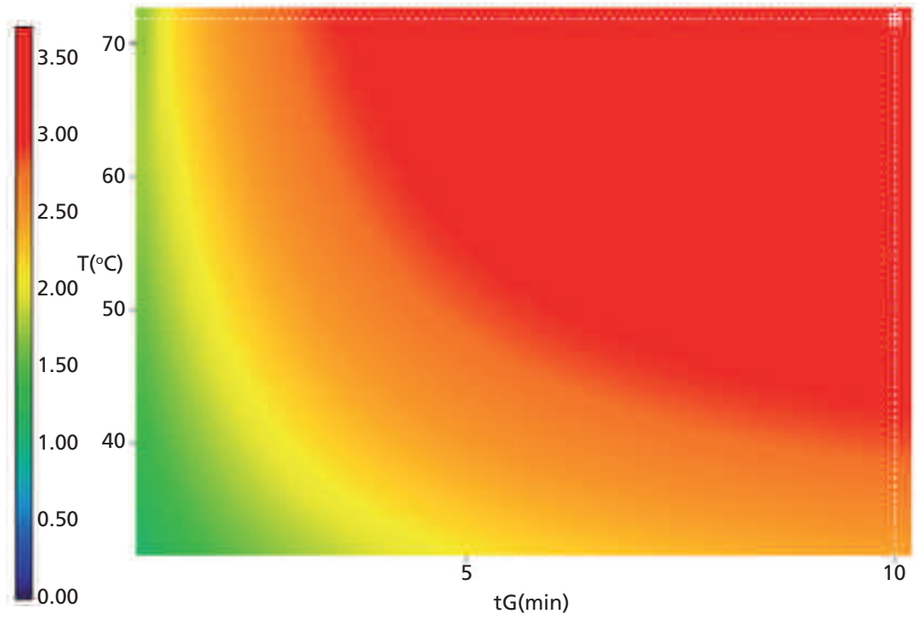
The goals in ultrahigh-pressure liquid chromatography (UHPLC) method development are to first find the best separation, second find the best column, and third find the most robust method in a multifactorial Design Space. Trial and error methods are not sufficient anymore and solid science based on Quality by Design (QbD) principles is required. Control strategies and continual improvements of the methods are necessary, which offer much greater flexibility in the quality control laboratories than before. Modelling tools based on QbD principles used in method development give transparency to the control of multivariable influences and achieve faster drug applications and commercial authorization by the regulatory agencies.

In the last decade, high performance liquid chromatography (HPLC) method development was advanced to a great degree by a) modelling software; b) development of very high pressure instruments with reduced dwell- and extra-column volumina; and c) new particle technologies and new stationary phase chemistries.

The use of modelling software in HPLC method development led many scientists in the pharmaceutical industry away from a trial-and-error approach to methods using a systematic, risk-oriented procedure based on solid science. Although the influence of experimental factors (such as gradient time, pH, and temperature) were well-known in reversed-phase chromatography in 1976,[1,2] regulatory control on methods was extremely tight. This continued until 2002, when the pharmaceutical industry submitted a strong protest note to the Food and Drug Administration (FDA). As a result, the FDA introduced a paradigm change, and adopted the Quality by Design (QbD) framework, well known in pharmaceutical manufacturing also for analytical work, and laid down recommendations in various International Conference on Harmonization (ICH) guidelines. Understanding of the underlying phenomena was supported by the development of modelling tools by the group of Lloyd R. Snyder and many other researchers.[3] Studies on further investigations using modelling tools were published in a number of important papers.[4–14]

Photo Credit: Image by Catherine MacBride/Getty Images

Figure 1: Gradient time (tG) versus temperature (T) map of the critical resolution. In the red area (MODR = Method Operating Design Region) the critical resolution is > 1.5, and here is a chance to work at any point with baseline resolution. The edge from red to yellow colour is called the "Edge of Failure" of the method. The inclusion of a third measured parameter, such as the ternary eluent composition from acetonitrile (AN=B1) and (AN:2-propanol(40:60) =B2), allows the chromatographic selectivity to be changed and a Design Space (DS) formed, as shown in Figure 2.



The key steps for QbD-related method developments are:

- Clearly defining the method goals, where the QbD term is the Analytical Target Profile (ATP). In HPLC method development one important method goal will almost always be a sufficient — mostly baseline — resolution ($R_{s,crit} > 1.5$) between the critical peaks of the sample.
- Performing a risk assessment, which means evaluating which variables may have a negative influence on the defined method goals. In this instance the method parameters affect the resolution of peaks in the chromatogram in a negative way.
- Experimental evaluation of how the critical variables affect the method goals.

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EVENT OVERVIEW:

The introduction of columns packed with solid-core particles has been embraced by pharmaceutical and other analysts to speed up or improve the analysis of compounds of low molecular mass. The same benefits, summarized as 'faster or better," are now also available to bioanalytical scientists for the analysis of proteins, peptides, and other biopolymers.

This presentation will first discuss how the benefits of solid-core technology, particularly the BIOshell Fused-Core® HPLC columns, apply to compounds of larger molecular mass with emphasis on the analysis of proteins and peptides as they relate to the development of biotherapeutics.

The second part of this webcast will show the application of LC-MS-MS to assess the pharmacokinetic (PK) properties of biotherapeutic antibodies using a stable labeled antibody internal standard (SILUMab). This standard universally supports preclinical quantification of monoclonal antibodies as well as Fc-fusion therapeutics. SILUMab can be introduced early in the workflow to reduce variability and improve accuracy.

Who Should Attend:

- Bench biochemists and managers interested in reducing HPLC analysis time.
- Analysts and managers interested in separating more peptides and proteins per unit time.
- Pharmaceutical researchers studying the pharmacokinetics of monoclonal antibody therapeutics (PK).

Key Learning Objectives:

- The benefits of fused-core technology apply to peptide and protein analysis.
- BIOshell™ fused-Core columns are stable at high temperature, as required for the analysis of hydrophobic antibodies.
- The universal peptide strategy supports bioanalysis of a broad range of preclinical monoclonal antibody candidates by LC-MS.
- The SILUMab universal antibody standard improves reproducibility and accuracy by accounting for variability throughout the entire analytical workflow.

Presenters

Dr. Hillel Brandes
Principal Applications Chemist
Analytical Research Services
Sigma-Aldrich

Dr. Kevin Ray
Manager of Analytical
Research & Development
Sigma-Aldrich

Moderator

Laura Bush
Editorial Director
LCGC

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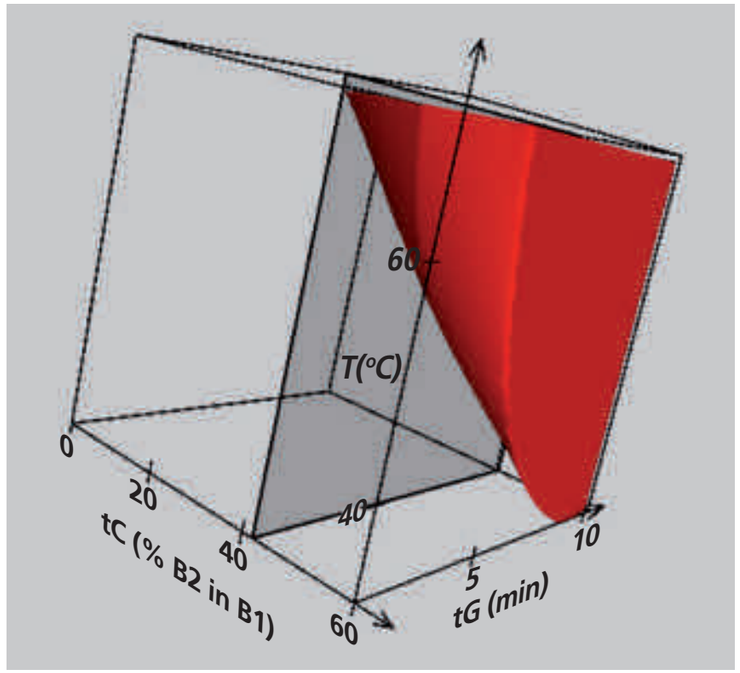
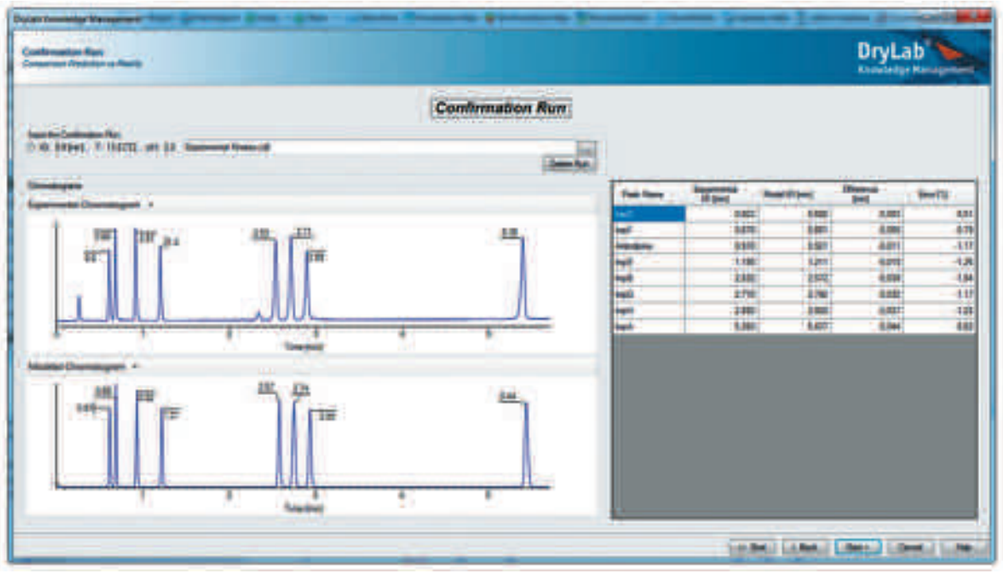
Figure 2: The tG-T-tC-Cube shows the Design Space as a red volume, in which the Working Point ("Set Point") can be altered without a new validation. B1 is acetonitrile, B2 is (acetonitrile:2-propanol) (40:60) (V:V). It is obvious that a robust work is not possible with eluent B1 (pure acetonitrile) (no red area), but possible with eluent B2 (front part of the Cube, large red region). Although the critical resolution is > 1.5, in a multivariable space the robust area has to be calculated, including tolerance limits of the individual variables. So far, flow rate, temperature, gradient time, and ternary eluent composition might reduce the robustness of methods, leading to "Out of Specification"(OoS) results.



This should be done in a systematic, multifactorial way using a scientifically based Design of Experiments (DoE). As a result of the experimentation a Design Space can be created, which describes the range of parameters in which the method goals are fulfilled.

- If a final method has been found in the steps described above, a robustness study should be performed to evaluate how the method goals (for example the critical resolution of the chromatogram) are influenced by small unintentional deviations from the defined method parameters.

Figure 3: Comparison of the predicted with the real experiment approves the applicability and reliability of the final position of the set point in the multifactorial Design Space. The table lists the deviations between predicted and real experimental retention times of on average under 0.04 min (< 3 s) (12).



- The results of the robustness study will help to set up a control strategy for the method or for any of the critical separation variables (the next important step in QbD-related method development).
- As the basic experiments are well defined, adjustments are easily possible by repeating the experiments and finding the actual Design Space again. Normally only small differences will be found. Altering the variables of a method (the set point) within the calculated Design Space is not considered as a "change" by the regulatory authorities and therefore can be done without a revalidation of the method. This allows a previously unknown flexibility in many laboratories.

Experimental

According to an older method of the European Pharmacopoeia (EP), the analysis of a drug sample took 160 min.[8,9] A new synthesis route of the drug substance has now been discovered and should be used. The substance was produced in one

day, but the analysis, according to the EP method, took five to six days. A new analytical method had to be developed, which could separate all impurities from the drug substance in a much shorter time. At the end of the process the analysis time could be reduced to less than 3 min using modelling software.

The Analytical Target Profile (ATP) was formulated as follows: Separate the drug substance from all the impurities with a minimum critical resolution of $R_{s,crit} > 1.5$ (baseline separation).

The risk assessment could be based on previous experiences and in this case has been established as follows: Based on prior knowledge and experience with similar projects, the gradient time tG (2–6 min), pH, temperature T (35–70 °C), and ternary composition, tC, of the eluent B are the most important, potentially critical separation parameters among the three other variables: Flow rate, %B(start), and %B(end) of the gradient.

The pH 6.2 (10mM) acetate buffer and the separation column (50 × 2.1 mm, 1.7 µm Acquity BEH C18 [Waters]) were selected based on preliminary experience.

From the risk assessment it was concluded that the variables tG, T, and tC should be modelled to evaluate how they influence the resolution of the critical peak pair in the variable space. Furthermore, the other three variables — flow rate, %B(start), and %B(end) — should also be tested in the robustness evaluation.

Peak tracking was carried out according to the description in references 6–9, either with the help of peak areas or with molecular weights of an MS detector. The sensitivity of the MS detection can be increased if ternary (acetonitrile:methanol)-cubes are established, because methanol provides much more sensitive signals than acetonitrile.

Column comparison, the establishment of the best column performance, and the selection of equivalent columns was carried out with ternary and pH-Cubes.[9,10,12]

Results and Discussion

The modelling software used to build the separation model required a Design of Experiment: 3 tG-T-sheets, which consisted of 12 experiments of all possible combinations; 2 gradient times; 2 temperatures; and 3 different organic eluents (acetonitrile, 2-Propanol-mix).[9] The experiments were performed and the resulting chromatograms were integrated. After importing the data (tR and peak areas) of the 12 experiments into the modelling software and performing peak tracking, the software calculated 97 other tG-T-sheets, leading to a Cube, in which the Design Space of the separation could be visualized in the form of irregular geometric bodies. A cross-section is shown in Figure 1 (see also Figure 2).

There is a problem however, if the variables (also called factors or parameters) might change their value simultaneously within their so called "tolerance limits". These limits are in many cases instrument dependent. In this case the Design Space is reduced to a smaller volume with multivariable tolerance limits around the set point and the critical resolution is reduced to a smaller value than the preset required critical resolution limit. In quality control (QC) this often leads to "Out of Specification" (OoS) and to a disqualification of production batches, which causes big losses in the pharmaceutical industry. About 10% of produced batches today have to be burned, which corresponds to a loss of drugs with a value of around $85 billion per year worldwide.

After the model has been created, a robustness test can be performed with the modeling software. In this step variables tG, T, and tC, and 3 calculated parameters (flow rate, %B[start] and %B[end]) were simultaneously varied, so that 3^6 = 729 experiments were evaluated in approximately 30 s and graphically visualized in the form of chromatograms. The robustness testing calculates a "success rate", which can provide a percentage of how many experiments in a 100 will be successful and how many will be OoS; in this instance, how many tests will be unable to qualify a certain production batch for commercial authorization.

Each of the 729 chromatograms can be visually observed and investigated in a table, and, by clicking on the corresponding line. the chromatogram can be studied. The calculation of the 729 chromatograms takes only 30 s. In case of too many OoS runs, the errors in the six variables can be examined and modified, according to the manufacturer specifications. After measuring these values and corresponding adjustments, the calculation can be performed again.

It was observed that in many instances the flow rate was responsible for the OoS, because the tightness of the seals around the plungers of the pump was wearing out. The seals should therefore be replaced at regular intervals. In addition, the oven temperature precision contributes to method performance. With the correct tolerance limits it is possible to establish results free from OoS and successfully produce pharmaceutical products fast to

help patients who are desperately waiting for treatment.

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Imre Molnár has a distinguished background in the study, research, and applications of HPLC and the development of DryLab and PeakMatch software. He recently integrated PeakMatch into the software DryLab 4.

Hans-Jürgen Rieger has worked with the Molnár-Institute since 1999 as a chemist specializing in software programming. In close cooperation with Dr Molnár, he is responsible for the development of new versions of DryLab, PeakMatch, and the Robustness module.

Alexander Schmidt is quality control director at Steiner Pharmaceuticals in Berlin, Germany. He is also head of analytical development of an R&D and contract analysis lab and supervises 35 lab assistants and chemists. Over the years, he has published numerous articles on HPLC and UHPLC method development for pharmaceuticals and complex natural compound mixtures. He is also a guest lecturer at the Beuth University of Applied Sciences, in Berlin, Germany. In addition, he is currently writing his doctoral thesis at the Institute of Pharmacy at Freie Universität Berlin in Germany.

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Róbert Kormány is a chemist with a Master of Science degree from the University of Debrecen in Hungary. He completed his education with an additional degree in chromatography and analytical chemistry from the Institute of Analytical Chemistry of the Technical University Budapest in the department of Prof. Dr. Jenö Fekete. Mr.Kormány specializes in UHPLC method developments for the separation of pharmaceutical compounds using computer simulation.

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