8 Modeling of HPLC Methods Using QbD Principles in HPLC

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8.1 INTRODUCTION

The increasing demand for quality by design (QbD) in analytical science is a logical consequence of the often chaotic method development practices in high-performance liquid chromatography (HPLC) that result from a trial and error approach, during which stumbling over new or disappearing peaks in the ultra high-performance liquid

chromatography (UHPLC) validation process is a common pitfall. To ensure a higher standard of method quality, in 2002, the International Conference on Harmonization (ICH) and Food and Drug Administration (FDA) started demanding solid and scientific work using Design of Experiments (DoE).

The Molnár-Institute has been promoting this type of approach for almost 30 years by contributing to the development of DryLab® software through cooperation with LC resources, under the leadership of Lloyd R. Snyder. Using DryLab 4, the systematic and accurate preparation of experiments was initiated, achieving useful and reproducible results. Better peak tracking in DryLab ensured safe and precise data entry before the model was built.

Designed by UHPLC experts, DryLab 4 offers chromatographers unprecedented insight into how a substance can best be separated and efficiently support the success of their chromatographic work.

DryLab is the world standard for chromatography modeling in both method development and training applications. The following time schedule shows the long and well-documented development history of DryLab—from the very beginning in 1986 to the essential UHPLC method development tool we have today.

8.2 HISTORY OF DRYLAB DEVELOPMENT

Modeling HPLC started with Csaba Horváth at Yale University in 1975 by systematic measurements of retention phenomena in reversed-phase chromatography (RPC) [1–3].

In 1986, Lloyd Snyder, John Dolan, Tom Jupille, and Imre Molnár started a modeling tool by first learning how to optimize and model isocratic HPLC, and then creating software with several extensions called DryLab 1–5 [4, 5, 6]. It was first programmed by John Dolan in Basic language, under Microsoft *DOS*, for modeling capacity factors, calculating flow rate changes, and critical resolution values as well. Learning the influence of column dimensions led to a first column optimization part (DryLab 1). The software was then extended to RP-%B optimization (DryLab 2) and to normal phase HPLC (DryLab 3), followed by a module for ion pair-RPC (DryLab 4). The first calculations of retention in gradient modeling created DryLab 5. In the very first versions, chromatograms were plotted with stars*.

In 1987, DryLab I (I for isocratic) was born as a combination of DryLab 1,2,3, and 4 which were programmed all in isocratic modeling; this means, the peak widths were increasing with the retention time. With the addition of new graphics in DOS, where chromatograms could be plotted, one could create chromatograms similar to well-known ones from real instruments. This step of visualization of chromatographic science was a unique property of DryLab already as early as in 1988 and it is still a revolutionary approach today.

The next extension was a combination of DryLab 1,2,3 and DryLab 5 to DryLab G—*G* for gradient modeling.

In 1989, DryLab I/plus, DryLab G/plus came out and were the first versions of DryLab programmed in *C*, which included a number of new features, such as

peak name options, zoom and scale of chromatograms, resolution maps for partial peak sets, ASCII files for data input and storage, and the ability to import data system files.

- 1992 DryLab I/mp: Isocratic multiparameter version was born with a wholly graphical interface for the Windows® 1.0 operating system, including functions for the mouse control of the program.
- 1998 DryLab version 2.0 was launched, a first version with two-dimensional (2D) modeling capabilities, incorporating simultaneous modeling of two separation parameters, for example, gradient time (tG) versus temperature (*T*) or %B versus *T*. It was also capable of modeling six to seven additional method parameters as in all other versions included new features, such as automated peak matching for maximum eight peaks at that time.
- In year 2000 "DryLab 2000", version 3.0 was launched.
- In 2002 DryLab 2000 plus v. 3.1 was adjusted to Windows® 3.1 and NT for network applications. It was also the first version to be released in C++. It could do 2D modeling for any combination of variables called *anything versus anything*.
- In 2005 DryLab was acquired by the Molnár-Institute and further developed in Berlin. The first addition of a new module—called *PeakMatch* v.1.0—was the first peak tracking software introduced for DryLab for easier alignment of peaks in four to six different chromatograms, and was running on Windows XP.
- In 2006 DryLab and PeakMatch v. 2.0 came out and allowed the automated generation of experiments with Agilent 1100.
- 2007 DryLab 2000 plus v. 3.5 included DryLab v. 3.9 and PeakMatch v. 3.5, running three tG-*T*-models running on Windows Vista.
- 2009 DryLab v. 3.9—introduction of the revolutionary *3D Cube* at the HPLC meeting in Dresden: a 3D resolution map allowing and simultaneously optimizing three critical parameters and calculating further seven factors, which was truly a leapfrog step in the development toward more flexible methods. It was also the first modeling tool, which was programmed in C# (C-sharp) with new user-friendly windows management and the amazing 3D Cube, compatible from Windows® XP through Windows 8 [7, 8].
- 2010 Followed the introduction of DryLab 2010—a combination of the DryLab Core and PeakMatch.
- 2012 DryLab 4.0 was launched with a completely new windows management and an automated data acquisition procedure for the Shimadzu HPLC-line in Europe.
- 2013 DryLab 4.1—Introduction of the robustness module, which could calculate six factors at three levels 3^6 = 729 runs in 20 s.
- 2014 Introduction of the knowledge management protocol, a complete documentation of a method development process including all input data, the peak tracking, the model validation, the robustness study all together in a pdf document.

8.3 KNOWLEDGE MANAGEMENT DOCUMENT IS MAINLY USED FOR POST-APPROVAL CHANGES

The actual DryLab version is 4.2, in which the robustness module was extended also to step gradients with 4 points: this means 6 + 2 + 2 additional factors at 3 levels = 3^10 ca. 60,000 runs in ca. 3 min.

DryLab is trying to answer the following questions: What are our most important goals in separation science?

1. Get the *best and fastest separation*
2. Find the *most robust conditions for routine work* that is, get critical resolution ($R_{s,\,crit}$) maximized
3. Select the *best column* for the application, based on multifactorial studies for the creation of *maximum robustness and flexibility* by working inside of the design space

The most efficient DoE is using 12 experiments and predicts more than 10^6 precise model chromatograms with a precision in retention times of better than 99% compared to the corresponding real runs [9, 10, 11, 12].

8.4 ISOCRATIC MODELS

This type of chromatography was used in the 1970–1980s in quality control, where only a few peaks were analyzed. The peak width in isocratic work is linearly proportional to the retention time. This means, that peaks, which are strongly retarded, become very wide and therefore very flat and cannot be recognized anymore, so that the analysis is *de facto* incomplete. In gradient elution the late retarded peaks are just as visible as any other peak, so in the meantime, gradient elution dominates in the field.

8.4.1 Case Study 1: pH Model

This example shows the dependence of the critical resolution (y-axis) on the pH in the range of 2–5 (Figure 8.1). In RPC, this is the most common pH range, simply because here the silanol groups are protonated and therefore fairly homogeneous. This means, band spreading due to heterogeneous silanols is at a minimum; the peaks are sharp, instead to be broader and/or having tailing. The model is based on only 3 runs at pH values of 2.9, 3.5, and 4.1. The *y*-axis is showing the critical resolution values. Baseline resolution is above $R_{s,\,crit} > 1.5$.

We can see, that there is only a small pH interval from 2.9 to 3.1 (shown by the arrow), where we have a high critical resolution, $R_{s,\,crit}$ with baseline separation 1.5. At all other pH values we would have problems with more or less peak overlaps, where $R_{s,\,crit}$ is approaching the value 0, leading even to missing peaks. In this way, one can reduce a possible pH range *screening* from 2.0 to 5.0 to the range of 2.9–3.1.

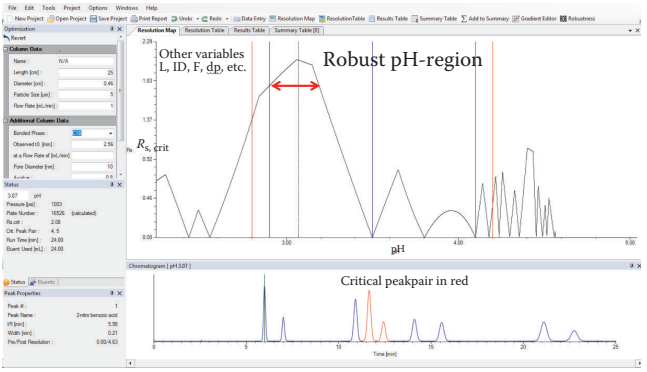


FIGURE 8.1 The case study is showing a model for the separation of nine organic acids. The critical resolution map (top right) is exhibiting the dependence of the critical resolution from the pH in the first place. However, other variables (top left) indicate other possibilities to change f.e. the column length, ID, particle size and flow rate and see, how the chromatogram, ($R_{s,\,crit}$), retention times, and peak elution orders are changing.

Outside of the range 2.9–3.1 the screening does not make sense at all and would only keep the instrument and the lab worker busy without satisfactory results.

8.5 GRADIENT MODELS

8.5.1 Case Study 2: Gradient Time (tG) Model and Development of Step Gradients

In this study the influence of the gradient steepness (or tG) is shown. The critical band spacing is depending on the gradient time also strongly as we can see this on the resolution map [12]. There are three peak overlaps ($R_{s,\,crit} = 0$) shown. The best separation is possible not only at ca. tG: 100 min, but also in the region of tG = 20 min with the same quality. This is another proof that *screening* without a scientific basis is a waste of time (Figure 8.2).

Step gradients are a common way of trying to improve a separation. However, due to misinterpretation of the timescale of the gradient program, retention times in the resulting chromatograms are often very different from the expected results, even for experienced chromatographers (Figure 8.3).

8.6 SIMULTANEOUS CHANGE OF TWO FACTORS—2D MODELS

8.6.1 Case Study 3

This example was generated in the development for the separation of 15 compounds in a pharmaceutical company. One worked on it for quite a long period of several months, without any success. Using DryLab 2D design, the individual peak movements could be understood and the final method allowed a robust control of the product as shown in Figure 8.4.

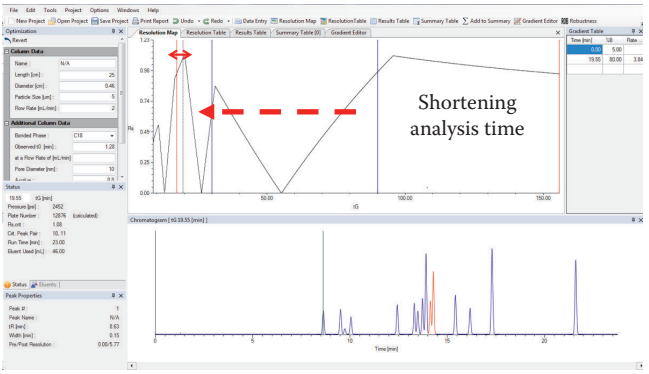


FIGURE 8.2 Reduction of *screening* to the meaningful values of gradient time. Based on only two experimental gradient runs the selection of the best gradient time can be rapidly achieved. The time for ca. 100 potential, but unnecessary screening runs can be saved.

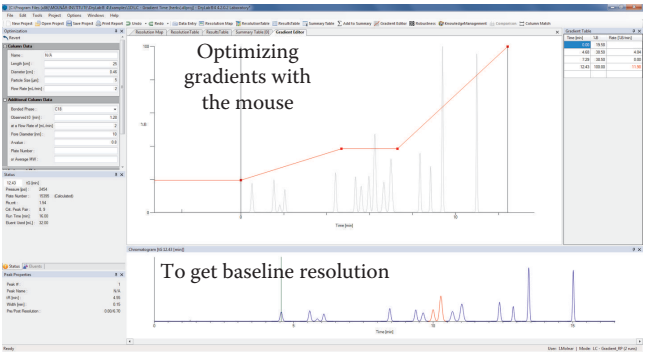


FIGURE 8.3 The timescale of the chromatogram and the timescale of the gradient program are different. In the top chromatogram the gradient line shows the composition of the *eluent* in the detector cell, while the *sample* composition is registered in the lower chromatogram. This important detail enables development of a robust gradient method in a short time. *Screening experiments* with step gradients are *trial and error* and they cannot be justified with *statistics* in analytical Quality by Design (AQbD).

A presentation of a great number of scientific papers on DryLab modeling is compiled in Molnár [13].

8.7 WHICH MODEL IS THE MOST EFFICIENT?

In statistical DoE there are a great number of the so-called Designs which are rather confusing for a chromatographer. In HPLC we reduced these many different designs to a reasonable set of experiments and recommend the following steps:

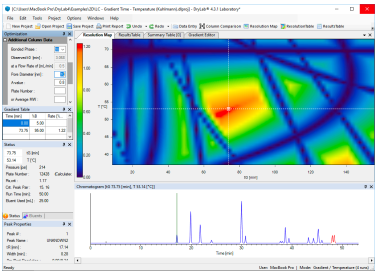


FIGURE 8.4 2D Resolution map, gradient time, tG (*x*-axis) versus temperature (*y*-axis) showing the best separation at the red area. The map is based on only 4 runs and it corresponds to ca. 10,000 model experiments and the optimum is found in seconds instead of running screening experiments in weeks.

- Start with the tG-*T*-model (4 runs only)
- Continue to form a tG-*T*-pH-Cube (12 runs) for polar compounds or form a tG-*T*-tC-Cube (12 runs) for neutral compounds
- Test the robustness of the separation for variabilities using 6 factors at 3 levels => 3^6 = 729 runs in 30 s

The most efficient design is certainly the Cube. It needs only 12 runs and produces more than 10^6 different chromatograms with varying selectivities [13–15].

8.8 WHICH MODELING TOOLS TO SELECT?

Three different types of modeling tools are available.

1. Tools for drug design, where molecular structures play an important role, are the following tools available: Pallas (Budapest, Hungary), ChromSword (S. Galushko, Darmstadt, Germany), and ACD-Lab (Toronto, Canada)
2. Tools for separation understanding, mainly in QC:
   DryLab 4 (Molnár-Institute) based on gradient elution, works with complex unknown mixtures, helping to visualize peak movements:
   1. Eluent (pH-, ternary-, temperature-, tG, etc.) influences are all measured
   2. Influences of column dimensions (L, ID), dp, flow rate, in gradient elution are calculated
   3. Instrument influences (Vd, Vext.col.) are considered
   4. 3D resolution maps based on only 12 runs to find the best separation out of >1 million choices with visualized design space are increasing flexibility with Out of Specification (OoS)
   5. Robustness metrics allows safe industrial QC work
3. Tools based on statistics: Fusion (S-Matrix, USA) is fixed to an instrument (Waters) and automates the creation of runs with different possible experimental designs

8.9 MULTIFACTORIAL MODELING: THE 3D RESOLUTION CUBE

8.9.1 Case Study 4

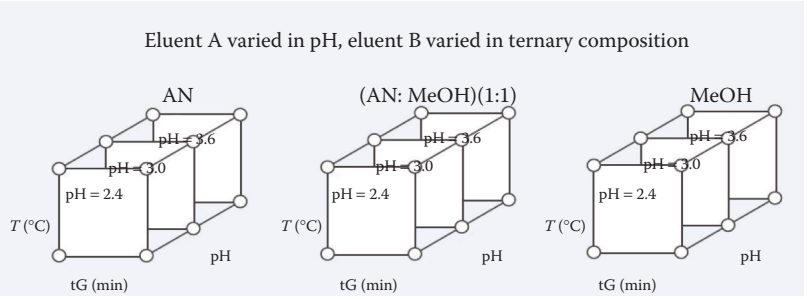
In 2009 there was a shortage of acetonitrile in the market and everyone feared what to do with all the QC work, if acetonitrile as the organic mobile phase was not available anymore. We thought, we would try out a different concept of changing the organic eluent and replace the acetonitrile amount in the eluent by HPLC-grade methanol. It turned out, it was not just a success to save acetonitrile (and money) but also to improve critical resolution and at the same time develop a design space according to QbD requirements. At the same time the pH influence on selectivity was also investigated between 2.4 and 3.6 as shown in Figures 8.5 and 8.6.

The first ternary Cube (the *original Cube*) was calculated out of 3 tG-*T*-sheets with three different eluent B mobile phases: AcN, (50 : 50) mix, and MeOH. Then DryLab calculated another 97 sheets in between the measured ones and so the Cube could be filled out and allow to visualize over 10^6 different selectivities. The best one with the highest robustness (= highest critical resolution) could be found by one mouse click. The method operable design region (MODR) is visualized as one or more irregular geometric bodies shown in Figure 8.6 on the very right.

8.10 HOW TO FIND THE BEST COLUMN?

8.10.1 Case Study 5

There are several ways how to select a column. The most universal columns are certainly the C18 or octadecylsilica reversed-phase materials. However, we have ca. 500 different types of them and other RP-column variants on the market; some are as



Multifactorial optimization strategy of 4 measured critical HPLC method parameters: Gradient time (tG), temperature (*T*), pH, and ternary composition (B1:B2), based on 12 × 3 = 36 experiments.

FIGURE 8.5 Multifactorial design of experiments to study the influence of four measured and seven calculated factors on chromatographic selectivity for higher method robustness. The measured factors were tG, *T*, pH, ternary composition tC (AcN: MeOH ratio). Calculated influences are by the column length, inner diameter, particle size, flow rate, dwell volume, extra column volume, starting and final %B. (From Molnár, I. et al., *J. Chromatogr. A*, 1217, 3193–3200, 2010.)

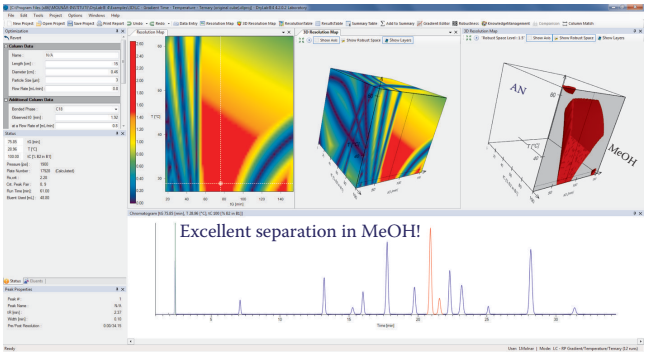


FIGURE 8.6 The ternary Cube from the original paper shows in the further developed version DryLab 4 with a new windows management, where baseline resolution is occuring (red body in the right top figure). It turned out, that baseline separation was not possible in acetonitrile, as the back side of the cube remained white, as long as the front side of the Cube (eluent B is MeOH) was largely red and was offering excellent baseline separation of all peaks involved, as shown in the corresponding chromatogram (below).

old as 30+ years and some are very new. As the stationary phase geometry, the reaction of the chemical modifications is all developing further year by year. Snyder and Dolan tried to classify this great variety on columns in their hydrophobicity subtraction database, which is also included in DryLab 4. This database has the advantage that all the many columns are comparable based on a scientific experimental comparison [16,17]. So it is easy to find a reference column, a replacement column, or find columns with very different selectivities. The comparison is based on an isocratic experiment at a fixed temperature and fixed eluent.

Snyder started to use another approach to look at column selectivity by using gradient elution, variable temperature, and pH or ternary composition of the eluent B [18]. In this way, column selectivity might be optimized better than in isocratic conditions. In the following we are reporting about an old Pharmacopoeia method, which caused a lot of trouble in routine industrial applications, producing permanent OoS results, such as missing peaks, false order of elution, and critical resolution out of compliance [19]. The very long analysis time took over 50 min, so one hesitated to make a new time-consuming validation. The plan was to reestablish the understanding of peak movements and reduce the analysis time below 10 min. One assumed that the pH would be the main reason for the confusion, so a pH Cube was created with 12 runs.

After data generation for a pH Cube and data import, peak tracking was carried out to align peaks in a table so that each peak was put in a horizontal line. Based on the data in the table (retention times and peak area pairs) the Cube could be calculated.

In the finished Cube the best working point for each column could be selected. The robustness of the method could be challenged using the robustness module of DryLab. The best column was found by comparisons of the best $R_{s,\,\mathrm{crit}}$ values for all columns (Table 8.1). The technique was used to select the best column for each different sample.

TABLE 8.1

Comparison of 25 Different Column Chemistries and the Accuracy between Predicted and Real Retention Times, Compared Over the Cube Evaluation Method Showing Excellent Predictability of the Average Retention Time with a Precision of Typically MT 99%

	Properties of the Columns				Predicted Parameters					Average of Retention Time	
Columns	Silica Type	Pore Size (Å)	Surface Area (m^2/g)	Surface Coverage ($\mu mol/m^2$)	pH	T (°C)	tG (min)	$R_{s,\,crit}$	Critical Imp. Peak Pair	Difference[a] (min)	% Error[b]
1. Acquity BEH C18	Hybrid	130	185	3.0	2.1	13.5	8.1	2.54	G–H	0.007	0.23
2. Acquity BEH Shield RP 18	Hybrid	130	185	3.3	2.0	38.3	9.8	2.16	B–G	–0.017	–0.79
3. Acquity BEH C8	Hybrid	130	185	3.3	2.5	33.0	9.8	2.27	D–F	–0.018	–0.85
4. Acquity BEH Phenyl	Hybrid	130	185	3.0	2.0	29.3	9.8	2.32	G–B	–0.001	0.41
5. Acquity CSH C18	Hybrid	130	185	2.3	3.0	13.5	9.8	3.13	D–F	0.017	0.88
6. Acquity CSH Phenyl-Hexyl	Hybrid	130	185	2.1	2.1	13.5	2.9	1.92	D–F	0.005	0.60
7. Acquity CSH Fluoro-Phenyl	Hybrid	130	185	2.4	3.0	13.5	2.7	1.22	D–F	–0.002	–0.55
8. Triart C18	Hybrid	110	370	1.5	3.0	13.5	7.4	2.49	D–F	0.011	0.57
9. Acquity HSS C18	Fully porous	100	230	3.2	2.1	24.0	9.8	2.50	G–H	–0.038	–1.95
10. Acquity HSS C18 SB	Fully porous	100	230	1.8	2.0	30.0	9.8	2.04	D–F	–0.014	–0.37
11. Acquity HSS T3	Fully porous	100	230	1.7	2.0	31.5	9.6	2.16	G–H	–0.023	–0.94
12. Acquity HSS PFP	Fully porous	100	230	3.2	2.0	19.5	9.8	1.58	D–F	–0.005	–0.27
13. Acquity HSS CN	Fully porous	100	230	2.0	3.0	13.5	7.9	1.95	D–F	0.000	–0.15
14. Hypersil GOLD C18	Fully porous	175	220		3.0	41.3	9.8	2.72	D–F	–0.003	–0.10
15. Hypersil GOLD C8	Fully porous	175	220		2.7	42.0	9.8	2.55	D–F	–0.009	–0.24
16. Hypersil GOLD CN	Fully porous	175	220		2.9	27.8	9.0	1.67	G–B	0.002	0.56
17. Zorbax SB-C18	Fully porous	80	180	1.8	2.2	29.3	9.8	2.13	G–H	–0.016	–0.36

(*Continued*)

TABLE 8.1 (*Continued*)
Comparison of 25 Different Column Chemistries and the Accuracy between Predicted and Real Retention Times, Compared Over the Cube Evaluation Method Showing Excellent Predictability of the Average Retention Time with a Precision of Typically MT 99%

	Properties of the Columns				Predicted Parameters					Average of Retention Time	
Columns	Silica Type	Pore Size (Å)	Surface Area (m^2/g)	Surface Coverage ($\mu mol/m^2$)	pH	T (°C)	tG (min)	$R_{s,\,crit}$	Critical Imp. Peak Pair	Difference[a] (min)	% Error[b]
18. Zorbax SB-C8	Fully porous	80	180	1.6	2.8	13.5	6.1	2.03	D–F	0.013	1.09
19. Zorbax SB-Phenyl	Fully porous	80	180	2.1	2.0	13.5	8.9	1.52	D–F	−0.022	−2.42
20. Kinetex XB-C18	Core shell	100	200	1.8	2.2	13.5	9.8	2.24	D–F	0.013	0.81
21. Aeris XB-C18	Core shell	100	200	1.7	3.0	15.0	9.8	2.50	G–H	0.001	0.35
22. Kinetex C18	Core shell	100	200	3.0	2.5	20.3	9.8	2.38	D–F	−0.011	−0.54
23. Kinetex C8	Core shell	100	200	3.5	2.4	13.5	9.8	2.52	D–F	0.002	0.14
24. Kinetex Phenyl-Hexyl	Core shell	100	200	3.4	2.2	33.8	9.8	2.22	D–F	−0.010	−0.28
25. Kinetex PFP	Core shell	100	200	3.2	2.4	16.5	9.8	2.44	G–H	0.019	1.67

Source: Kormány, R. et al., *J. Pharm. Biomed. Anal.*, 80, 79–88, 2013. With permission.

Note: The table shows furthermore 3 different critical peak pairs (G–H, D–F, B–G), meaning, that it is important not to fix only one critical peak pair, leading often to noncompliance in inspections (23). The best results are achieved with column number 1, 5, 14, 21, and 23. These columns are the *alternative* columns for the method.

[a] Difference (min): Predicted Retention Time – Experimental Retention Time.

[b] % Error: ([Predicted Retention Time – Experimental Retention Time] / Experimental Retention Time) × 100 Columns: 50 × 2.1 mm with sub-2 µm particles (porous shell 0.23 µm on core shell types).

We are trying to demonstrate this on the following industrial example:

- To have comparable experimental conditions, all columns were having the same geometry: 50 mm long, 2.1 mm ID, 1.7–1.8 µm.
- To find the best gradient slopes scouting gradients were carried out, first with MeOH, followed by AcN. DoE was based on two gradient time values (tG1 and tG2) with a factor 3 difference, that is, tG2 = 3 × tG1 (3 and 9 min from 0%B to 100%B) at two different temperatures T_1 and T_2 (T_1: 30°C, T_2: 70°C).
- Eluent A was varied with three different pH1, pH2, and pH3 distances of 0.5–0.6 pH units. The design is carried out as follows:
  - Running six experiments first at the low temperature T_1 (1, 2, 5, 6, 9, 10), followed by running six experiments at the high temperature T_2 (3, 4, 7, 8, 11, 12).
  - The next step is to import the 12 experimental data in AIA-format (*.cdf) and go to peak tracking.
  - In peak tracking, the reduction of peak areas in mV × second might help to have a better visual control on how peaks are moving, as they are used as labels over the peaks (Figures 8.7 and 8.8).

This observation did not change, if the pH was changed in the investigated region, so in the study the pH could be excluded as the reason for lack of robustness. It turned out that the reasons were rather small changes in the temperature or in the gradient slope. DryLab could show the MODR as the space, where robust routine work could be performed as a red irregular body, in which the working point might be moving [20–22]. However, the robustness of the working point in a multifactorial space might be changing. Also different columns might have different best working point parameters, but differently robust methods (Figure 8.9).

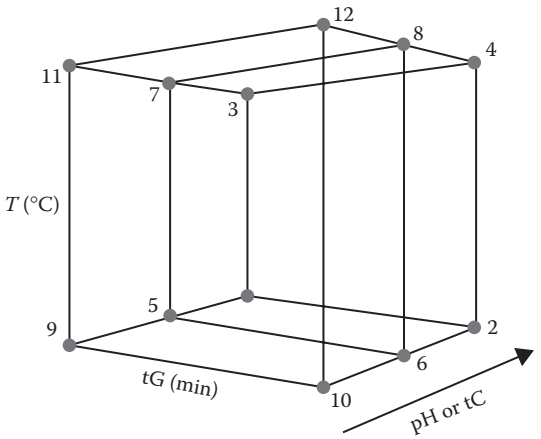


FIGURE 8.7 A revolutionary design of experiments (DoE) for the simultaneous optimization of gradient time (tG), temperature (*T*), and ternary composition (tC) of the eluent B or the pH of the eluent A. (From Kormány, R. et al., *J. Pharm. Biomed. Anal.,* 80, 79–88, 2013. With permission.)

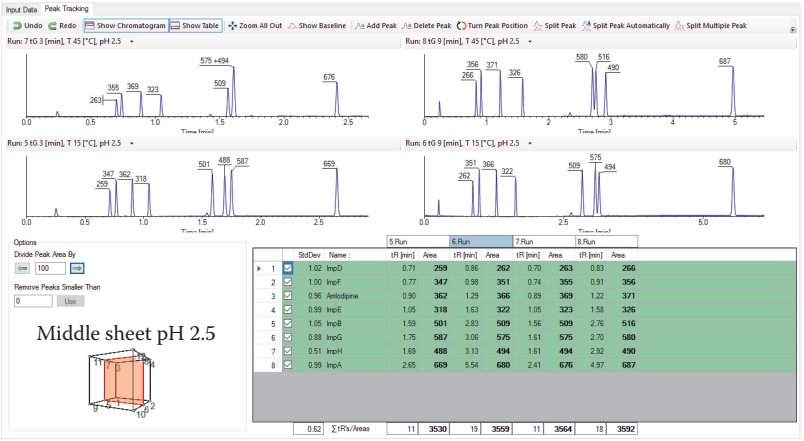


FIGURE 8.8 Peak tracking: the goal here is to align individual peaks in a horizontal line and to separate double or triple peaks, do turn over peaks, if necessary. It is important to consider peak areas not as quantitation tools, but more as peak-identification tools, like spectral data. In the middle group of three compounds the largest peak is in the first run (lower left), the last one in the middle group; in the second run (lower right) it is in the middle position; in the third run (top left) it coelutes with another peak; and in the fourth run (top right) it is the first peak. So this substance is moving around quite a bit and if we do not understand these movements, we will always have OoS results in the QC work. (From Kormány, R. et al., *J. Pharm. Biomed. Anal.,* 80, 79–88, 2013. With permission.)

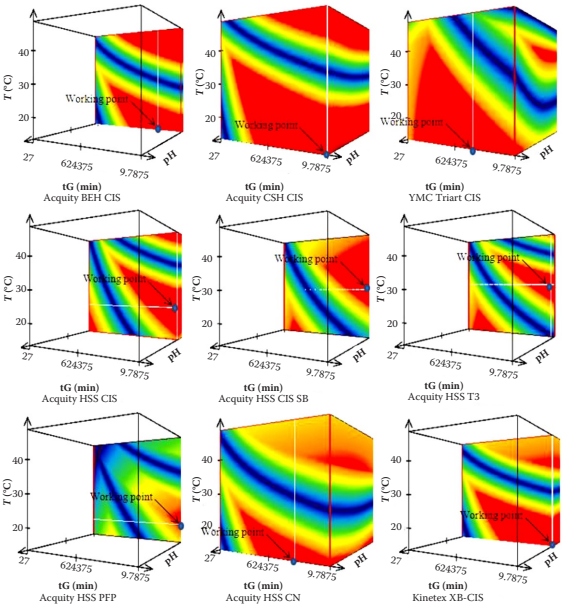


FIGURE 8.9 Selection of the best working point for different column chemistries. As it can be seen, the best working point with the highest critical resolution is different from column to column. Blue regions mean sample overlap; red areas are showing where baseline separation can be achieved. (From Kormány, R. et al., *J. Pharm. Biomed. Anal.,* 80, 79–88, 2013. With permission.)

8.11 ROBUSTNESS MODELING

8.11.1 Case Study 6

There are a number of papers discussing robustness issues [23–26]. In 2011 the breakthrough Robustness Tool was started, which is based on modeling of 3^n (n = number of factors) experiments using DryLab 4. In the first version the following six factors were evaluated in three levels (−1, 0, +1): Gradient time tG, temperature T, pH, as measured factors and flow rate, starting %B, and end %B as calculated factors. In the meantime this model was extended to step gradients with three steps, including the dwell volume also as an important factor in method transfer.

The advantage of this technology is to model every one of the 3^6 = 729 experiments, collect them in an Excel-like table, sort them, and additionally by clicking on any of them with the mouse one can see each of them as a chromatogram. Furthermore, the so-called % success rate (100% failure rate) is calculated with the analytical target profile, which is the critical resolution $R_{s,\,\mathrm{crit}}$,—typically as baseline resolution with the value of 1.5. In this way we can calculate in advance, how a method will perform in routine analysis and how much *OoS* results will it produce (Figure 8.10).

It is possible to see also which factors are influencing the results in the strongest way, so one can act to reduce that influence. This means, if a method produces OoS data, one can see, if the instrument is under control or not, because the accuracy in gradient mixing, temperature, flow rate, etc., are included. Therefore, it is easy to find in a case of OoS the reason for declining instrument performance and it is easy to correct it (Figure 8.11). Generally, modeled experiments are highly precise and allow in this way great amount of time saving and faster development of new drugs [27,28].

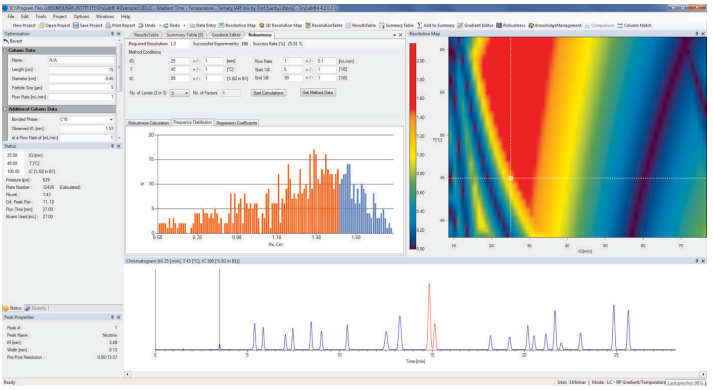


FIGURE 8.10 The fact to have a baseline resolution $R_{s,\,\mathrm{crit}} > 1.5$ does not mean that we have a robust method. If we work at the edge of failure (EoF) (top right) (tG: 25 min, T: 45°C), that is, at the border (edge) of the baseline-separation (red) region, we might have only ca. 25% runs with $R_{s,\,\mathrm{crit}} > 1.5$ (blue lines at top left) as long ca. 75% runs are $R_{s,\,\mathrm{crit}} < 1.5$ (red lines top left) (OoS results).

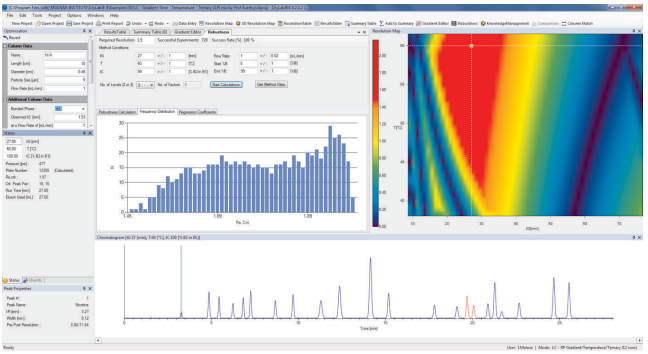


FIGURE 8.11 Moving away from the EoF to the middle of the red area (tG: 30 min, *T*: 60°C) and increase the accuracy of the pump from 0.1 to 0.02 mL/min tolerance limit, we have no OoS results, but 100% success rate (blue lines in top left) in routine QC operations.

The investigations were further extended as it is shown in Tables 8.1 and 8.2 for 25 commercially available columns.

We can see in the table, 8 columns with $R_{s,\,crit} > 2.5$, at different combinations of pH, temperature, and gradient time tG. These are the best columns, delivering the most robust methods. Twenty columns are at the working point better than baseline resolution $R_{s,\,crit} > 1.5$ and only one column shows a weak performance with $R_{s,\,crit} = 1.22$ (Acquity CSH Fluoro-Phenyl). The critical peak pairs at the four best columns are D–F and G–H. The average retention time error is mostly under 1%.

However, these results do not equal to a 100% performance in routine QC operation, where multifactorial changes might reduce the success rate. Therefore we were looking at those columns, where we can assume—based on modeling multifactorial changes of six factors—that the failure rate is 0% (or success rate is 100%), which is shown in Table 8.2. The five equivalent columns with 0% failure rate are Acquity BEH C18, Acquity CSH C18, Hypersil Gold C18, Kinetex C18, and Kinetex C8.

8.12 MODELING PROTEIN SEPARATIONS

As presented in this chapter, HPLC or UHPLC modeling using DryLab 4 provides several advantages on various fields of pharmaceutical analysis including assays, impurity profiling, protein analysis, or even charge heterogeneity analysis of mAbs in IEX chromatography [29,30]. The time spent for method development can drastically be shortened and with the resolution maps in our hands, further adjustment and transfer of the methods is easier and more flexible.

In 2013, the Molnár-Institute launched the DryLab HPLC-Knowledge Management Document, which offers comprehensive method documentation for better knowledge sharing. It automatically collects all relevant method data directly from DryLab 4 and offers a platform for comments and the justification of method criteria. DryLab Knowledge Management is a new reporting tool for documenting and archiving an HPLC method. It encourages a QbD approach to method development and ensures that the method conforms to these standards by providing a comprehensive method

TABLE 8.2
With Each Column Robustness, Studies Were Carried Out and a Failure Rate Was Established with the DryLab Robustness Module

Best Columns for the Sample	Predicted Parameters					Robustness				Average of Retention Time	
Columns	PH	T (°C)	tG (min)	$R_{s,\,crit}$	Critical Peak Pair	Failed Experiments		Failure Rate (%)		Difference[c] (min)	% Error[d]
						a	b	a	b		
Acquity BEH C18	2.1	13.5	8.1	2.54	ImpG–ImpH	0	0	0.00	0.00	0.007	0.23
Acquity BEH Shield RP 18	2.0	38.3	9.8	2.16	ImpB–ImpG	0	228	0.00	31.28	−0.017	−0.79
Acquity BEN C8	2.5	33.0	9.8	2.27	ImpD–ImpF	0	0	0.00	0.00	−0.018	−0.85
Acquity BEH Phenyl	2.0	29.3	9.8	2.32	ImpG–ImpB	0	0	0.00	0.00	−0.001	0.41
Acquity CSH C18	3.0	13.5	9.8	3.13	ImpD–ImpF	0	0	0.00	0.00	0.017	0.88
Acquity CSH Phenyl-Hexyl	2.1	13.5	2.9	1.92	ImpD–ImpF	0	81	0.00	11.11	0.005	0.60
Acquity CSH fluoro-Phenyl	3.0	13.5	2.7	1.22	ImpD–ImpF	729	729	100.00	100.00	−0.002	−0.55
Triart C18	3.0	13.5	7.4	2.49	ImpD–ImpF	0	0	0.00	0.00	0.011	0.57
Acquity HSS C18	2.1	24.0	9.8	2.50	ImpG–ImpH	0	0	0.00	0.00	−0.038	−1.95
Acquity HSS C18 SB	2.0	30.0	9.8	2.04	ImpD–ImpF	0	0	0.00	0.00	−0.014	−0.37
Acquity HSS T3	2.0	31.5	9.8	2.16	ImpG–ImpH	0	149	0.00	20.47	−0.023	−0.94
Acquity HSS PFP	2.0	19.5	9.8	1.58	ImpD–ImpF	489	570	67.17	78.30	−0.005	−0.27
Acquity HSS CN	3.0	13.5	7.9	1.95	ImpD–ImpF	0	39	0.00	5.36	0.000	−0.15
Hypersil GOLD C18	3.0	41.3	9.8	2.72	ImpD–ImpF	0	0	0.00	0.00	−0.003	−0.10
Hypersil GOLD C8	2.7	42.0	9.8	2.55	ImpD–ImpF	0	0	0.00	0.00	−0.009	−0.24
Hypersil GOLD CN	2.9	27.8	9.0	1.67	ImpG–ImpB	567	646	77.78	88.61	0.002	0.56
Zorbax SB-C18	2.2	29.3	9.8	2.13	ImpG–ImpH	62	211	8.50	28.94	−0.016	−0.36
Zorbax SB-C8	2.8	13.5	6.1	2.03	ImpD–ImpF	0	243	0.00	33.33	0.013	1.09

(*Continued*)

TABLE 8.2 (*Continued*)
With Each Column Robustness, Studies Were Carried Out and a Failure Rate Was Established with the DryLab Robustness Module

Best Columns for the Sample	Predicted Parameters					Robustness				Average of Retention Time	
Columns	PH	T (°C)	tG (min)	$R_{s,\,crit}$	Critical Peak Pair	Failed Experiments		Failure Rate (%)		Difference[c] (min)	% Error[d]
						a	b	a	b		
Zorbax SB-Phenyl	2.0	13.5	8.9	1.52	ImpD–ImpF	299	345	41.02	47.33	−0.022	−2.42
Kinetex XB-C18	2.2	13.5	9.8	2.24	ImpD–ImpF	0	0	0.00	0.00	0.013	0.81
Aeris XB-C18	3.0	15.0	9.8	2.50	ImpG–ImpH	0	0	0.00	0.00	0.001	0.35
Kinetex C18	2.5	20.3	9.8	2.38	ImpD–ImpF	0	0	0.00	0.00	−0.011	−0.54
Kinetex C8	2.4	13.5	9.8	2.52	ImpD–ImpF	0	0	0.00	0.00	0.002	0.14
Kinetex Phenyl-Hexyl	2.2	33.8	9.8	2.22	ImpD–ImpF	0	84	0.00	11.52	−0.010	−0.28
Kinetex PFP	2.4	16.5	9.8	2.44	ImpG–ImpH	0	242	0.00	33.20	0.019	1.67

Source: For another sample other columns might be the best ones.

Note: The results from 729 virtual experiments of 6 factors at 3 levels are shown in the table under column *Robustness*. The columns a and b under *Robustness* have different tolerance limits, in column *a* smaller, in column *b* twice as large. As visible, the tighter the tolerance limits can be maintained, the more robust the method is.

[a] Robust parameter ± tolerances: tG ± 0.1 min, T ± 1°C, pH ± 0.1, Flow Rate ± 0.005 mL/min, Start%B ± 0.5, End%B ± 0.5.

[b] Robust parameter ± tolerances: tG ± 0.2 min, T ± 2°C, pH ± 0.2, Flow Rate ± 0.010 mL/min, Start%B ± 1.0, End%B ± 1.0.

[c] Difference (min): Predicted Retention Time − Experimental Retention Time.

[d] % Error: ([Predicted Retention Time − Experimental Retention Time/Experimental Retention Time) × 100.

report, including a platform for the step-by-step justification of method choices. By implementing DryLab Knowledge Management, one can achieve excellent good manufacturing practice (GMP) documentation of a method, but one can also more easily and effectively collaborate between departments, and support analytical method transfer during development and manufacturing. DryLab Knowledge Management Document provides an analytical method summary to be signed and dated by the author and supervisor, making it GMP compliant and to be the perfect and safe documentation for inspections.

8.13 SUMMARY

HPLC method modeling is becoming a powerful tool to be used in the communication about method quality in HPLC between different labs, different companies, and between companies and regulatory agencies. The understanding of simple rules of peak movements will facilitate the development of new drugs, which are badly needed for smaller patient populations.

The new features of HPLC modeling software, such as 3D resolution map, the modeled robustness testing, a practicable method transfer, or a method knowledge management offer a closed loop of all information about the birth and practical use of a method, and it further suggests the use of such software solutions in regulated laboratories to make analyst's life easier—especially in the pharmaceutical industry.

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