

An Executive Summary

How to Manage Analytical Methods Across all Stages of their Lifecycle Using One Chromatographic Modeling Tool



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Visual chromatography modeling and optimization software.

Introduction

Chromatographic modeling using DryLab®4 software from Molnár Institute saves time and resources across all three stages of the lifecycle of an analytical method. Method run time can easily be reduced 40-fold with improved selectivity¹ when redesigning an old method, simultaneously reducing the development time by a factor of 12.² *US Pharmacopeia (USP)* methods that frequently fail can be fixed in accordance with allowed changes (*USP 621*) using DryLab4. Chromatographic modeling can safeguard method robustness across the lifecycle for compendial methods, new method development, method transfers, and methods that fail in routine use (trading/continuous improvement).

Lifecycle Management of Analytical Methods

The lifecycle of analytical methods: has three stages: Design, Qualification, and Continued Verification. Ideally, a design space is defined during the early stages of the method development. Methods design is a range of parameters within which the method performs as intended and within which the method is fit for use. *ICH Q8(R2)* states that “working within the design space is not considered as a change.” In addition, *ICH Q12* (draft) introduces a post-approval management plan to be initiated at submission (Post-Approval Change Management Protocol or PACMP). It integrates flexibility and lowers the need for new validation when changes are made to the method in Stage 3 (i.e., continued verification).

When one runs into problems with a method in Stage 3, it may be necessary to return to design and development (Stage 1) to resolve the problems. This task can be an arduous one, especially when production is ongoing. Indeed, one department typically handles both Stage 1 method development and design, while the Stage 3 routine method use is handled by a different department, with tight timelines for production of results.

DryLab4 software from Molnár Institute helps with this challenge because it can optimize the performance of an existing method in three simple steps: input runs, peak tracking, and creation of the model, which helps find the most robust separation within the existing design space. The input runs bracket the existing method conditions about the allowed variations for the multiple variables of the method. Peak tracking is the identification of peaks across input runs. DryLab then creates the model.

Using just a few experimental runs, DryLab visualizes the chromatographic process interactively, running hundreds of simulations in a few moments to find the best separation

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conditions, highest critical resolution, shortest run time and the highest robustness. Furthermore, evaluating method robustness during method development, while defining the analytical target profile (ATP), ensures a working design space for post-approval management and flexibility in dealing with out of specification (OoS) results in routine work. DryLab can be used at all stages, as shown in **Figure 1**.

Case Study 1: Ebastine

Compendial methods are Stage 3 methods by definition and do not require further method development and validation. In some cases, however, the method may need to be modernized. Such was the case with ebastine from a description in the *European Pharmacopeia*. The method was not fit for purpose because it was too long. The analytical work could not keep up with production. DryLab was used to reduce

the analysis time from 160 minutes to less than three minutes, the development of the new method took up to two weeks.

Based on prior knowledge and experience with similar projects, the parameters: gradient time tG (3-9 min), pH

Figure 1: Lifecycle Management of Analytical Methods, showing DryLab should be used in all phases to enhance a better chromatographic method understanding.

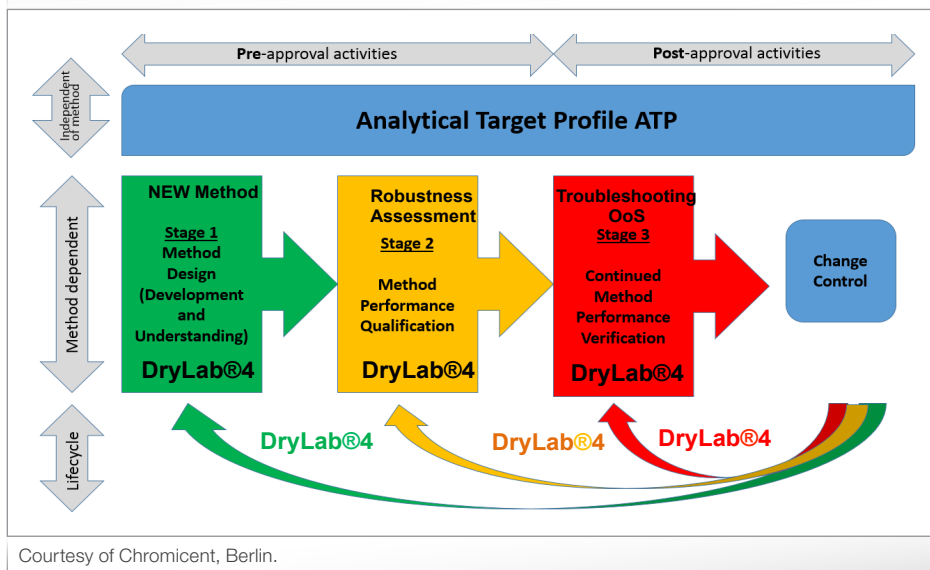
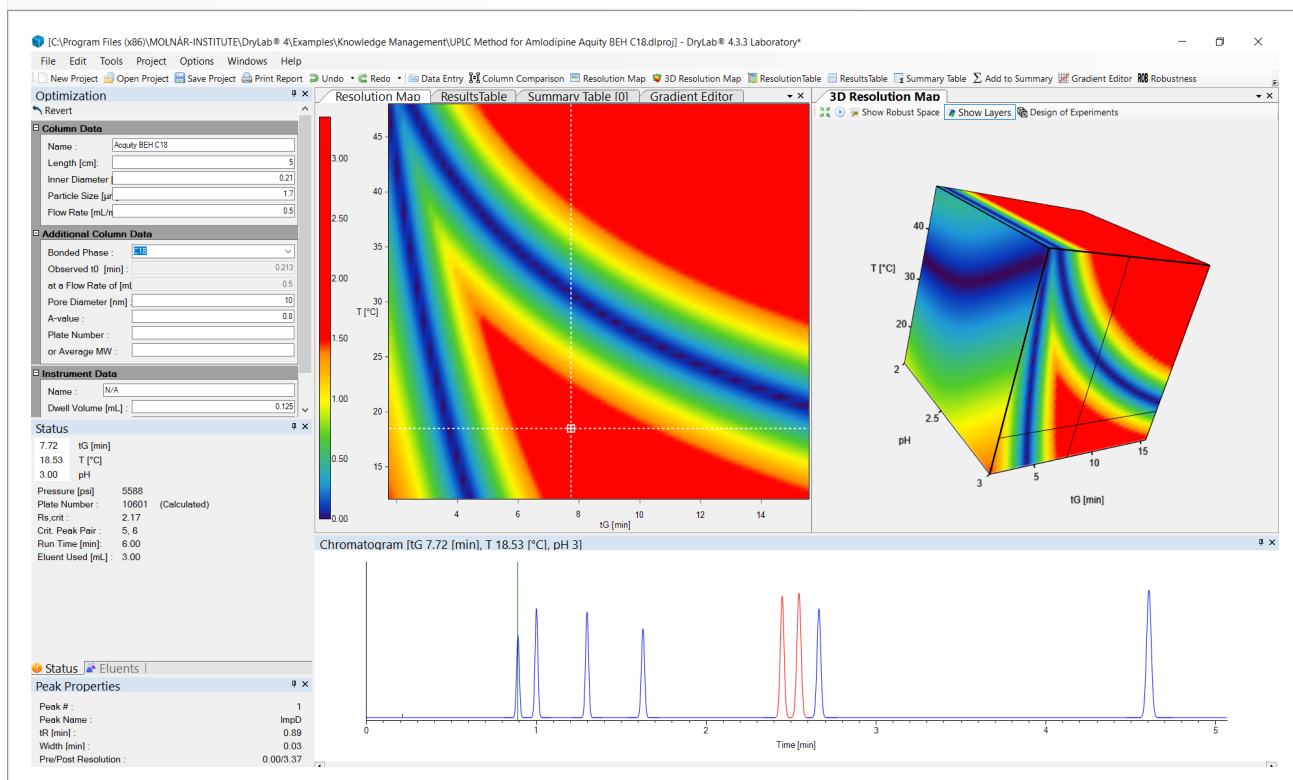


Figure 2: DryLab model of Amlodipine. The original EuPharm method could be reduced from 50 minutes to less than 6 minutes and the chromatographic understanding could be improved.



(2–3), temperature T (15–45 °C), and ternary composition of the eluent B were considered the most probable potentially critical separation parameters for the method. DryLab also produced the Knowledge Management Document, complete with the original chromatograms, peak tracking, resolution maps, robustness testing, and confirmation of predictions. This improved method was accepted in five countries and is described in the literature.

Case Study 2: Amlodipine

In another case, the routine method for amlodipine in the *European Pharmacopeia* was found to be not fit for purpose. Not only was it a long 45–60-minute isocratic method, but it also had coelutions and peak turnovers. Again, using only 12 experimental runs, and calculation of the model as shown in **Figure 2**. It became clear that the separation in the middle group of three peaks was not based on pH-changes, but much more based on temperature fluctuations and had to do also with the gradient time selection. The model enabled researchers to evaluate the resolution and robustness across the range of critical separation parameters. *US Pharmacopeia* <621> states, “multiple adjustments can have a cumulative effect on the performance of the system and are to be considered carefully before implementation.” Using DryLab to troubleshoot and understand the method does this using a virtual model that combines the multiple parameters in a 3D cube.

In **Figure 2**, the cross-hatch is set to the optimum area. When the user moves the cross-hatch around different areas of the cube, the chromatogram shows the predicted result as a chromatogram. The red area indicates areas of robustness. The gradient of color strips from orange to deep blue show how the critical resolution as the most important method parameter most likely result in method

Figure 3: Separation of Pramipexol and its impurities. The last two peaks did often change their positions causing Out of Specifications (OoS) results.

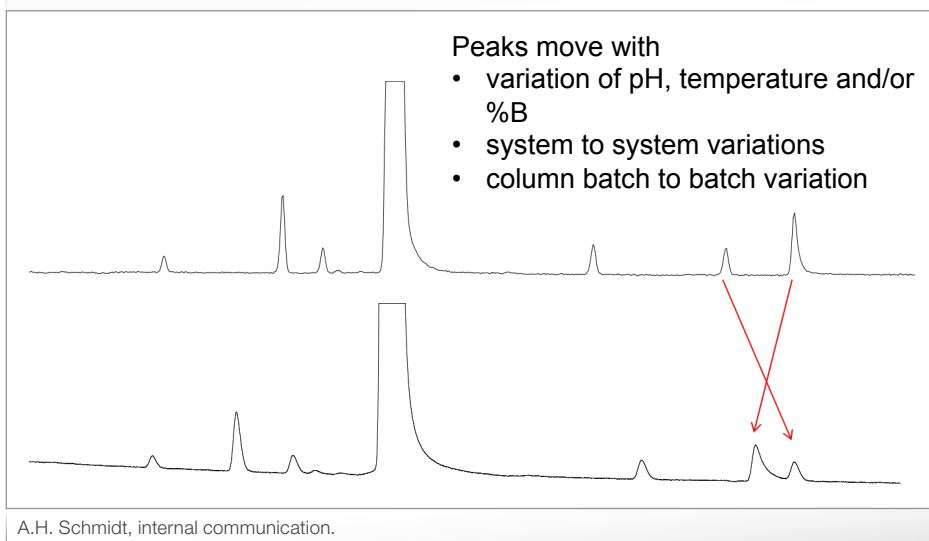
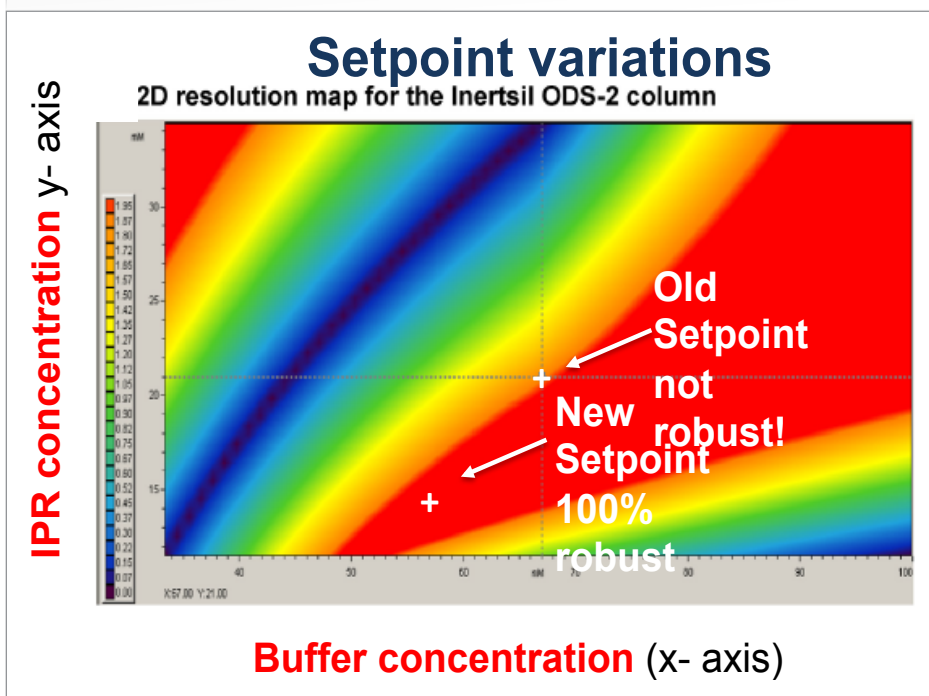


Figure 4: With DryLab the setpoint of the method can be altered without the need for a new validation.



failure and OoS results. Working near the boundary of the red zone results in poor method robustness. The critical pair for resolution is shown in red. As method parameters change, the critical peak pair may change also, which is reflected in the virtual chromatogram in the model. DryLab makes it very straightforward to take advantage of modern ultrahigh pressure liquid chromatography equipment and to optimize a robust method with a less than six-minute run time.

Using DryLab to Safeguard Method Robustness

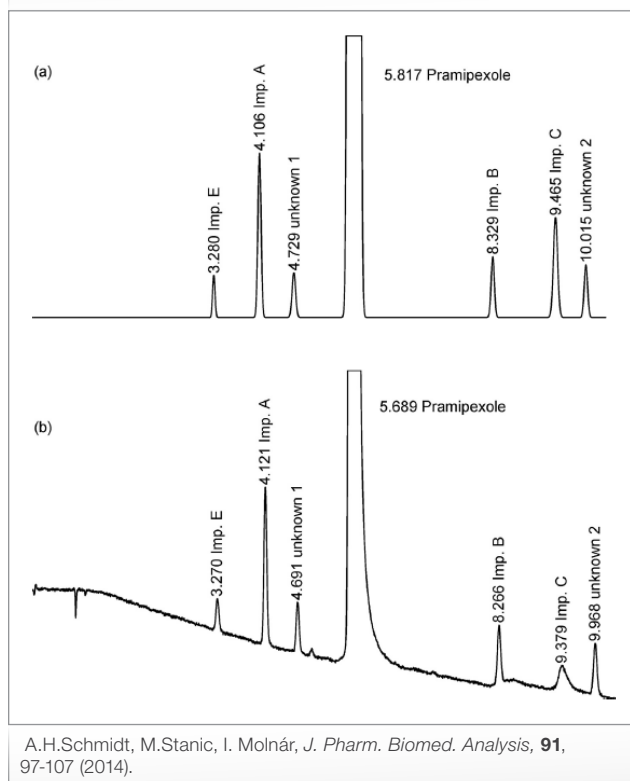
Building robustness into the method as early as possible in method development will help safeguard against later OoS results. A method is robust if it remains unaffected by small deliberate variations in method parameters. A robust method provides reliability when used in normal daily work with multiple operators, multiple instruments, and slight changes in columns from batch to batch. This was not often done in the past, perhaps because of the complexity of designing an experimental matrix that takes into consideration the cumulative effect of multiple factors. If the intended purpose of the method is quantification, then the first requirement for robustness is good resolution. The value of the critical resolution should be at least 1.5 (= baseline separation). A critical resolution of 2 for the critical peak pair is even better. Good resolution means one has a baseline separated chromatogram, which means one can more reliably integrate and quantify the peaks.

Pramipexole is a drug substance used to treat Parkinson's disease or restless leg syndrome. The separation of pramipexole from its known impurities is described in the *European Pharmacopeia* as well as in the *US Pharmacopeia*. The method calls for a C18 stationary phase; recommended columns are Inertsil or Symmetry. The mobile phase is a pH 3 phosphate buffer with an ion pair reagent. The organic eluent is acetonitrile and the gradient range is from 20–40%B in 15 minutes at a temperature of 40 °C. This method was used in the laboratory for a long time, but reliability has since declined. As shown in **Figure 3**, appeared in approximately 25% of the runs.

Thus, researchers wanted to adjust the method to improve the robustness and to comply again with the system suitability testing. DryLab was used to create a model about the design space for the conditions observed in the official method to see if they were at the optimum working point. Researchers ran 12 experiments across the range of the official method with the combinations of gradient times of 8 minutes, 16 minutes, and 24 minutes; pH values of slightly higher than 3.3, and slightly lower than 3; and temperatures of 27 °C, 40 °C, and 54 °C. Data were imported into DryLab, which helped with peak tracking. DryLab calculated the model and used the input data to run several hundred thousands of virtual experiments to create the model and generate the resolution map (see **Figure 4**).

As mentioned, the red zone in the resolution map is the area of well-separated peaks. The old setpoint for the official method is right at the edge of the red zone, at the “edge of failure.” Thus, the original method was not nearly as robust as one might have thought. Using DryLab, researchers could move the cross hatch in the red zone and find the spot with the best resolution for any critical pair. Interestingly, areas of decent robustness and decent resolution were further to the left of the original method conditions on the model, which also reduced run time. Using DryLab, researchers were able to work

Figure 5: Predicted vs. experimental verification of the pramipexol method.



within the method design space, to be compliant with both the system suitability test and the defined ATP method expectations. Thus, work could be continued with the improved method. It is important to note that DryLab also enabled the team to maintain and update its acquired method understanding to its LCM-compliant Knowledge Management Documentation. The model was verified, with the results shown in **Figure 5**.

Conclusion

In summary, DryLab reliably models analytical methods, with good agreement between the predicted and experimental chromatograms. The Knowledge Management Document supported the continued pramipexol analysis using the redesigned robust method. Furthermore, researchers were able to modernize methods for use with the latest technology, both instruments and columns, as in another case with amlodipine. The use of Visual Chromatography Modeling is well received, with the ebastine method accepted at regulatory agencies in five countries. Finally, DryLab enables production laboratories to readily adjust methods at the end of their lifecycle to eliminate OoS results and improve productivity.

References

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2. J. Kochling, W. Wu, Y. Hua, Q. Gua, J. Castaneda-Merced, J. Pharm. Biomed. Analysis **125**, 130-139 (2016).