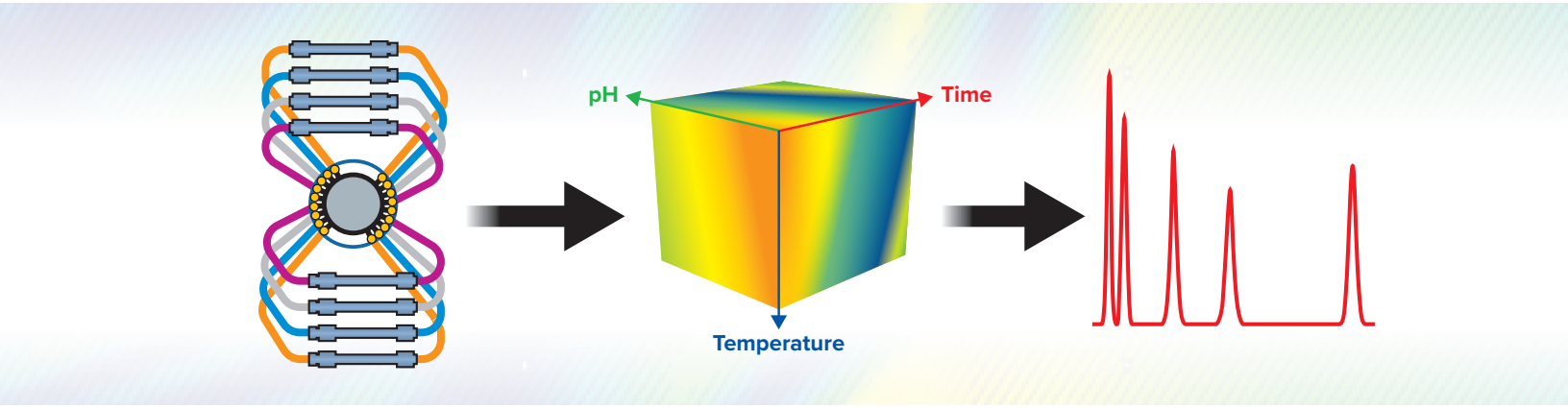
Case Study

Merck Simplifies and Streamlines Method Development with *In Silico* Modeling

The number of biopharmaceuticals sold on the market has quadrupled since 2006 and this trend is expected to continue. Biopharmaceuticals are structurally complex, which poses several challenges in drug development as they require complicated analytical assays and multistep purifications. To overcome these challenges, companies are developing strategies to simplify method development for large molecules.



A Strategy to Simplify Method Development

Luca Losacco and his colleagues on the method screening and purification group at Merck use LC Simulator, a module within ACD/Method Selection Suite to manage their screening process. They have introduced a dual strategy which combines their column screening platform with *in silico* modeling to dramatically accelerate their method development. Using the software, they screen and optimize a comprehensive set of conditions to determine the best combinations of columns, stationary phases, and chromatographic techniques most suited for the sample.

> "We're starting to rely more on the utilization of modeling tools to dramatically reduce the number of experiments, to quickly identify the best set of combinations that give us the final separation, and the baseline resolution of all our peaks and components." – Luca Losacco

Goals of the combined automated screening and *in silico* modeling workflow:

- Save time
- Minimal manual intervention
- Minimize # of experiments

Benefits of the workflow:

- Simple
- Practical
- Versatile
- Robust

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Losacco and his team combine automated method development strategies for various separation techniques including reverse phase, normal phase, ion exchange chromatography (IEX) and chiral ultra-high performance liquid chromatography (UHPLC) with *in silico* modeling to accelerate their method development process for small and large molecules. The strategy implemented by the team has proved to be simple, practical, and versatile as they have been able to apply it successfully to different techniques.

Streamlining Purification of Biopharmaceuticals with Minimal Manual Intervention

As part of Merck's workflow, and to reduce laborious method development, the team conducts multicolumn screening in conjunction with computer-assisted simulation. With the aim to develop methods and purify biopharmaceuticals efficiently. This practical workflow successfully separates, analyzes, and purifies nucleotides, peptides, and proteins.

Experimental parameters:

- A total of 12 different columns and 24 mobile phases were sequentially operated for cation and anion exchange modes (CEX and AEX).
- Three different classes of multicomponent mixtures (nucleotides, peptides, and proteins) were screened and tested under AEX and CEX conditions, through all the columns at different mobile phase pHs.
- The appropriate factors for elution profiles (gradient, pH, and different types and concentrations of salts) were determined.
- Automation allowed the team to perform 96 injections (for each mixture) overnight.

Using the IEX workflow led to rapid identification of optimal and robust conditions with the best separation, without requiring any manual intervention.

To save time and effort in method optimization, the team uses the LC Simulator module to perform *in silico* modeling. They use the software to conduct multifactorial optimization of the IEX methods, minimizing the number of experiments, and analyzing the impact of multiple chromatographic parameters. The LC Simulator module allows visualization of a resolution map cube modeling pH, time, and temperature, showing the condition(s) of maximal resolution and minimal analysis time. Experimental verification shows excellent correlation between the simulated and experimental chromatograms, with a difference in retention time of less than 0.5%. Implementation of this workflow successfully enables more efficient analysis and purification of different nucleotides, proteins, and peptides.

> "The final method was deployed to verify the correlation of the experimental retention times with those obtained *in silico*, demonstrating excellent accuracy with differences of less than 0.5%."[1]

Automating Screening to Reduce the Time Spent Developing Ultrafast Chiral Separation Methods

Chiral separations are some of the most challenging for chromatographers, requiring extensive screening and a comprehensive evaluation of all possible column and mobile phase combinations. There is also a critical need for these separation assays to be delivered quickly. The versatility of the workflow means the team can also apply it to develop ultrafast chiral separation methods.

> "This chiral UHPLC screening platform not only provides the possibility to quickly identify the best analytical conditions needed to efficiently separate enantiomers, but it also lays the foundation for streamlined development of fast-paced enantiopurity assays."[2]

Experimental parameters:

- A multicolumn UHPLC screening workflow is automated to run overnight operating under various conditions with minimal manual intervention, taking approx. 6 hours per compound.
- A comprehensive set of experimental conditions was obtained.
- To verify the workflow, automated screening was then set up for 18 chiral compounds (mostly classic synthetic APIs and amino acids).
- Each racemate was tested on all 14 columns using different combinations of chromatographic conditions.

The team screens the experimental conditions in a reduced timeframe, identifying at least one stationary and mobile phase combination for baseline resolution. Next, the team performs *in silico* modeling for method optimization. A crucial step in the optimization process for large biomolecules is to ensure that the correct regression retention model is being deployed. The team uses the LC Simulator module to generate a second-degree regression model and creates a resolution map using 9 combinations of the gradient slope and column temperatures for the enantiomeric mixtures. From this they obtain optimal experimental conditions. The results show an excellent correlation of retention time between experimental and simulated experiments, with a difference of less than 0.5%. Using the workflow helps to reduce the amount of time the team spends developing enantiopurity assays.

> We have introduced an automated enantioselective UHPLC screening workflow that dramatically reduces the time spent developing enantiopurity assays.[2]

Successful Development of Robust Methods

Losacco and his team have shown how method development can be simplified and streamlined by efficiently integrating automated screening platforms with computer-assisted *in silico* modeling in different applications. The workflow has helped to reduce the time spent developing enantiopurity assays and successfully enabled more efficient analysis and purification of different nucleotides, proteins, and peptides. In both applications, the results consistently show an excellent correlation of retention time between experimental and simulated experiments.

Automation of screening platforms has significantly reduced the amount of manual intervention required while also saving time. *In silico* modeling with the LC Simulator module has proven to be an effective tool to conduct fewer experiments and identify optimal conditions, improving the overall screening outcome, and generating robust LC methods faster.

> Using *in silico* modeling, we were able to optimize three parameters at the same time, in a single morning. Normally, with other approaches, this takes days. We were able to reduce the entire method development from days to just half a day." – Luca Losacco

References

1. Losacco, G. L., Hicks, M. B., DaSilva, J. O., Wang, H., Potapenko, M., Tsay, F.-R., Ahmad, I. A. H., Mangion, I., Guillarme, D., & Regalado, E. L. (2022, April 20). *Automated ion exchange chromatography screening combined with in silico multifactorial simulation for efficient method development and purification of biopharmaceutical targets - analytical and bioanalytical chemistry*. SpringerLink. Retrieved March 15, 2023, from https://link.springer.com/article/10.1007/s00216-022-03982-z

2. Losacco, G. L., Wang, H., Haidar Ahmad, I. A., DaSilva, J., Makarov, A. A., Mangion, I., Gasparini, F., Lämmerhofer, M., Armstrong, D. W., & Regalado, E. L. (n.d.). *Enantioselective UHPLC screening combined with - ACS publications*. Analytical Chemistry. Retrieved March 15, 2023, from https://pubs.acs.org/doi/10.1021/acs.analchem.1c04585

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