Rapid Development and Validation of Chromatography Methods Using QbD Software

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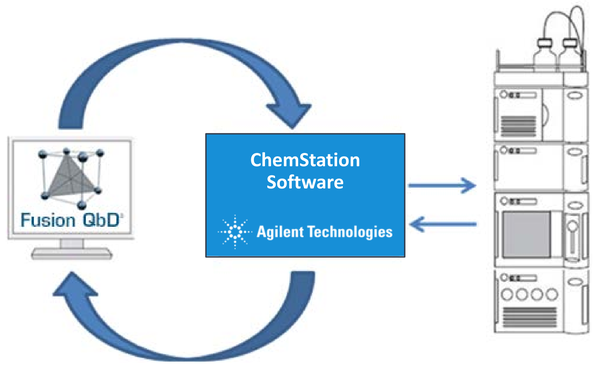
Summary

There is an urgent need to develop and validate chromatography methods to meet the FDA's expectation for post purification quality control of CDx (Companion Diagnostics) antibodies. Fusion QbD is a sophisticated software from S-Matrix that automates the method execution and analysis. The Quality by Design (QbD) software sped up the entire process from method develop, robustness, and validation by automating DOE set-up, quantifying the relationship between method parameters, and outputting an optimized HPLC method based on USP recommended chromatography attributes. The optimized method gave consistent quantitation and improved resolution, peak symmetry, and peak capacity. Using the Fusion QbD software a fully optimized chromatography method was developed and validated on an accelerated timeline.

Method Set Up and Output



The DOE methods and sequences were automated in the Fusion QbD software and then run on the HPLC using ChemStation. After the HPLC run, the processed chromatograms were exported back to the Fusion QbD software, which automatically calculated a plethora of peak attributes and performance measurements. Below are four attributes heavily used for the HPLC method development.

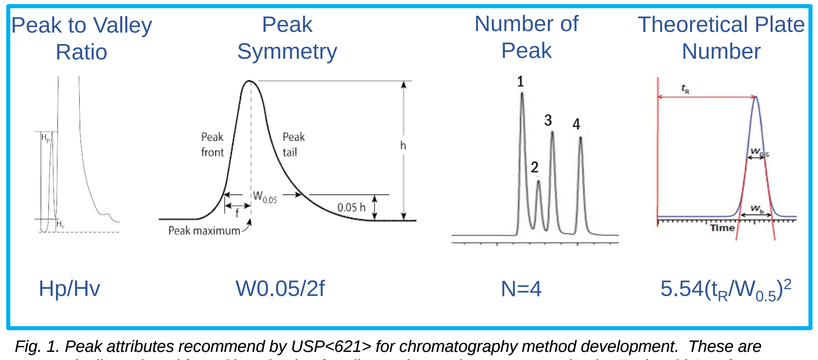


Fig. 1. Peak attributes recommend by USP<621> for chromatography method development. These are automatically retrieved from ChemStation for all experiment chromatograms by the Fusion QbD software.

QbD Optimization

Using DOE methodology, Fusion QbD set up automated method optimization experiments on an Agilent 1260 HPLC, and automatically modeled the imported experiment results. The software's visual output quickly highlighted the affect each parameter had on the chromatography.

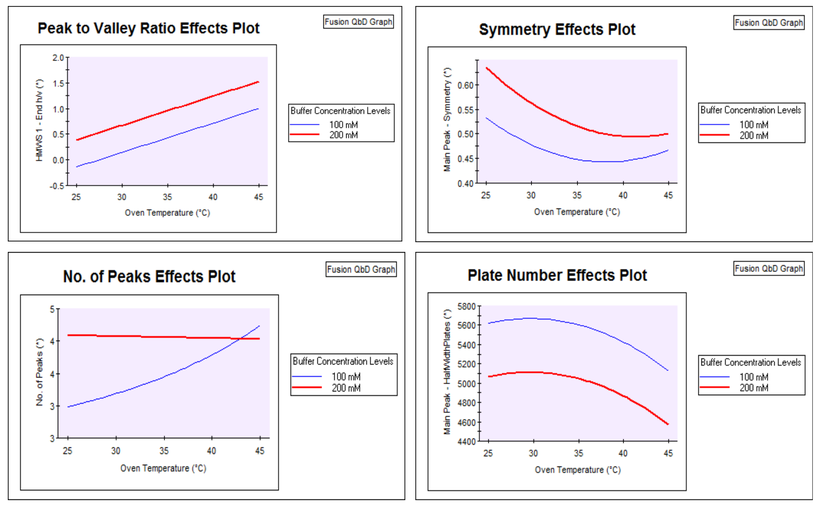


Fig. 2. Effect plots from the SEC study used to understand the effects of a combination of parameters.

Effects Plots (figure 2) and Pareto Charts (figure 3) made it easy to understand the relationships between parameters and quickly establish an optimized method using built-in searching and graphics. The Acceptable Performance Region (figure 4) visualized the design space to ensure a robust method.

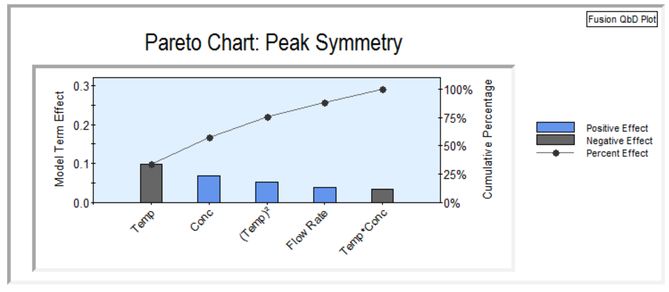


Fig. 3. A Pareto Chart of study factor effects from the SEC study, which highlights the relative impact of each of the parameters on peak symmetry.

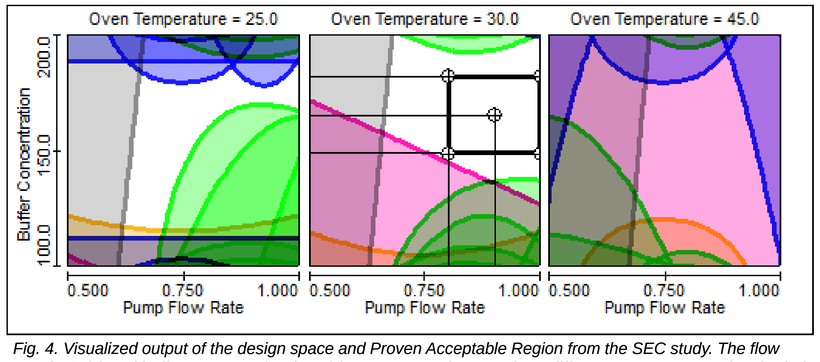


Fig. 4. Visualized output of the design space and Proven Acceptable Region from the SEC study. The flow rate (X-Axis) and buffer concentration (Y-axis) are compared across three different temperatures. The shaded regions are outside the design space, which is unshaded (white). The proven acceptable ranges are demarcated by the black box within the design space.

Results

The proven acceptable ranges from method development were used as limits for the robustness testing. Having these critical quality attributes calculated by Fusion QbD was essential for proving robustness (figure 4).

Robustness Parameters	Ranges tested	Number of Peak (=4)	Peak to Valley Ratio (≥ 0.2)	%CV peak area (<15%)	Results
pH	7.0 ± 0.2	4	>0.9	0.04%	✓
salt conc.	165 ± 17 mM	4	>1.2	0.05%	✓
column lots	3 lots	4	>0.9	0.02%	✓
temperature	30 ± 3°C	4	>0.8	0.04%	✓
flow rate	1.0 ± 0.1 mL/min	4	>0.9	0.04%	✓

The accuracy, precision, specificity, and linearity of the SEC method were validated using Fusion QbD, and all passed the acceptance criteria with ease. Different operators, on different days, using different buffers had less than 0.1% CV in quantitation of antibody X. These results show that the method development was fully optimized.

Conclusion

Using S-Matrix's Fusion QbD software made the method development and validation time three times faster (figure 5) and added confidence in the robustness in method.

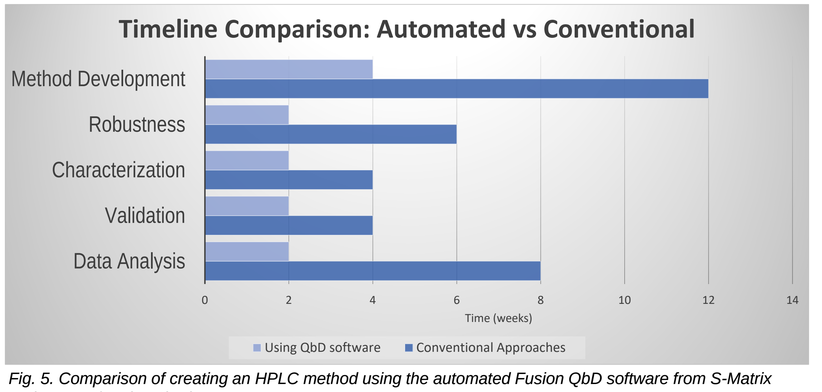


Fig. 5. Comparison of creating an HPLC method using the automated Fusion QbD software from S-Matrix and conventional methods.

Acknowledgements

Thank you Sima Nazemzadeh, Rachel Turner, Carolina Zapata, and Amanda Forsythe for supporting the validation. Thank you also Richard Verseput, Dave George and Ed Kallen of S-Matrix Corporation for demonstrating and trouble shooting the Fusion QbD software.