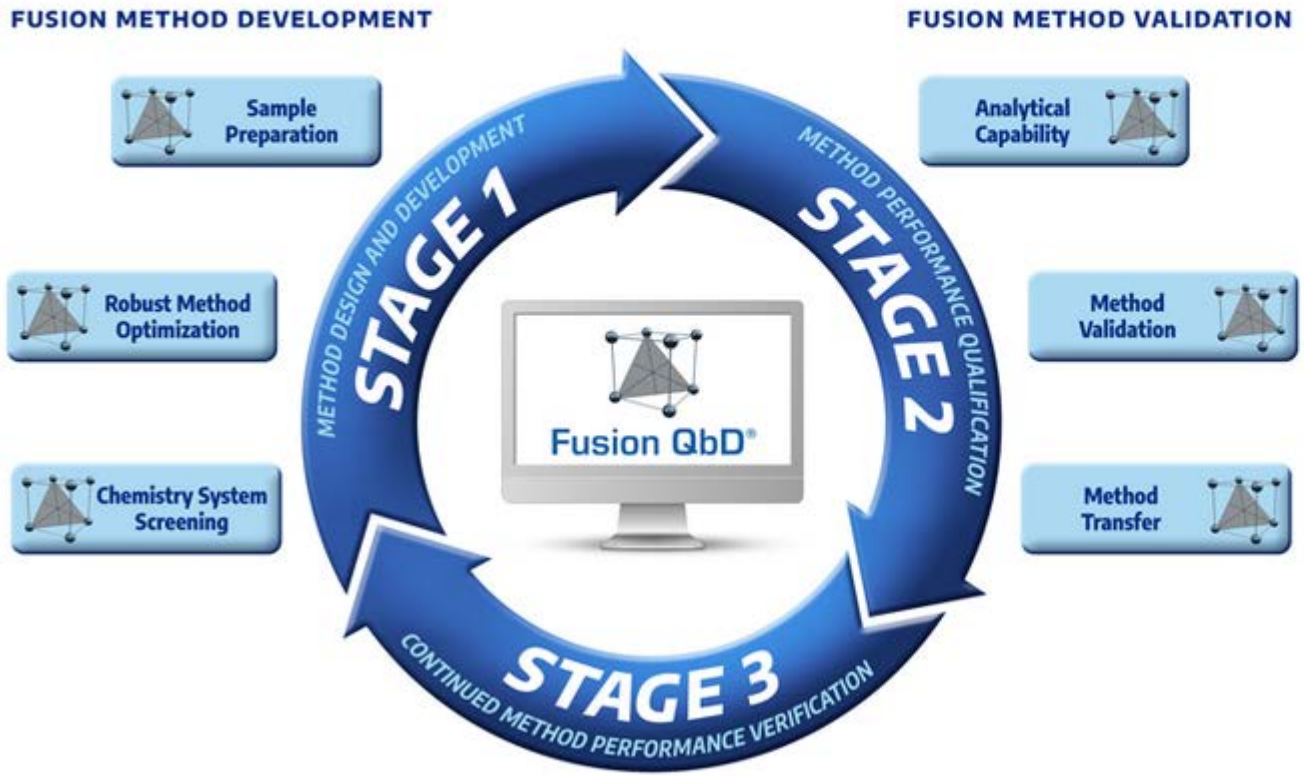
Fusion QbD – Advanced LC & LC-MS Method Development Software

Fusion Method Development™

Chromatography-centric QbD Software for LC, LC-MS, and SFC Method Development



S-Matrix – Software Solution Partner of:

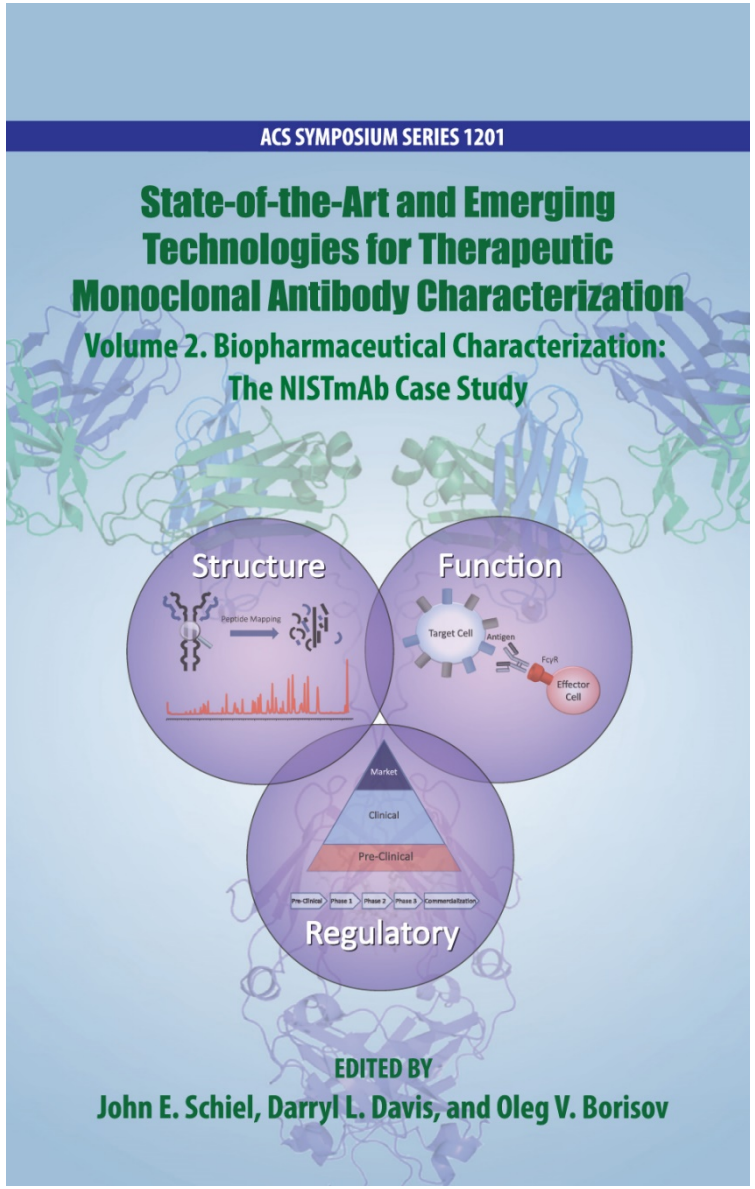
Agilent Technologies

ThermoFisher SCIENTIFIC

Waters

Protein and Peptide Application Examples

NIST – mAb Case Study



Only Fusion QbD –
Supports Small and Large Molecule Method Development

NIST mAb Case Study

Work was done at Amgen using Fusion QbD

John Schiel, Darryl Davis, Oleg Borisov. ed. (2015), *State-of-the-Art and Emerging Technologies for Therapeutic Monoclonal Antibody Characterization Volume 2. Biopharmaceutical Characterization: The NIST mAb Case Study*, American Chemical Society

Automated Method Screening for Biotherapeutics

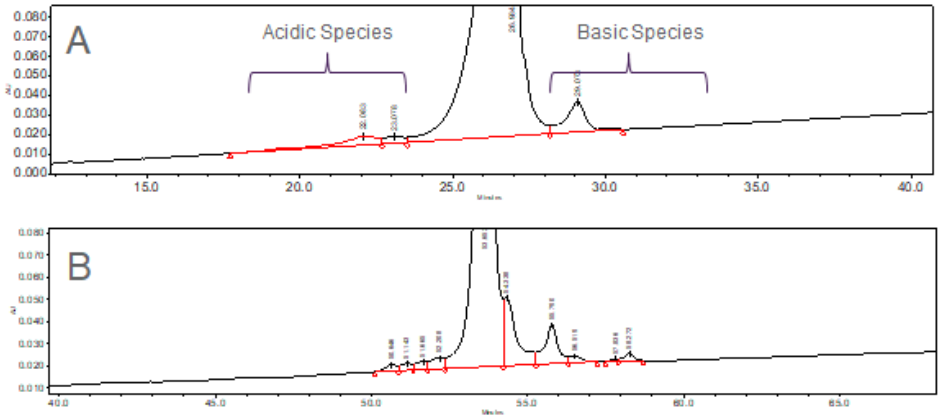
Use of Fusion QbD for Automated Method Screening for Biotherapeutics

Joshua Woods [1], Marguerite Arechederra [2], Barbara Kelly [1], and Justin Sperry [1]
[1]Analytical R&D, Pfizer Inc. Chesterfield MO 63017
[2] Waters, Milford MA 01757

Case Study 1 - WCX Development

Fusion QbD Screening and Optimization

- Variables: pH, gradient time, mobile phase composition, organic additive, salt concentration, and column temperature.
- Resulting method showed no fronting, better resolution of acidic species, and better resolution of basic species.



Productivity Gain

Resulting method comparable to method developed in 5 months prior to use of Fusion QbD.

Automated Method Screening for Biotherapeutics

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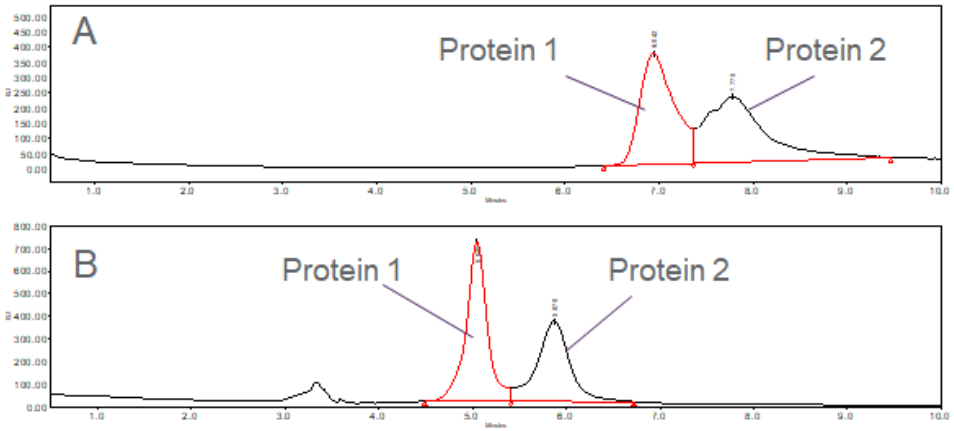
Case Study 2 - HILIC Development

Fusion QbD Screening

- Variables in DOE: pH, Column, Temp., t_G.
- Resulting method shows increased resolution between Protein 1 and Protein 2 in addition to less tailing of both protein peaks.
- 5 Full time employee (FTE) hours, 120 instrument hours.



Overall Productivity Gain –

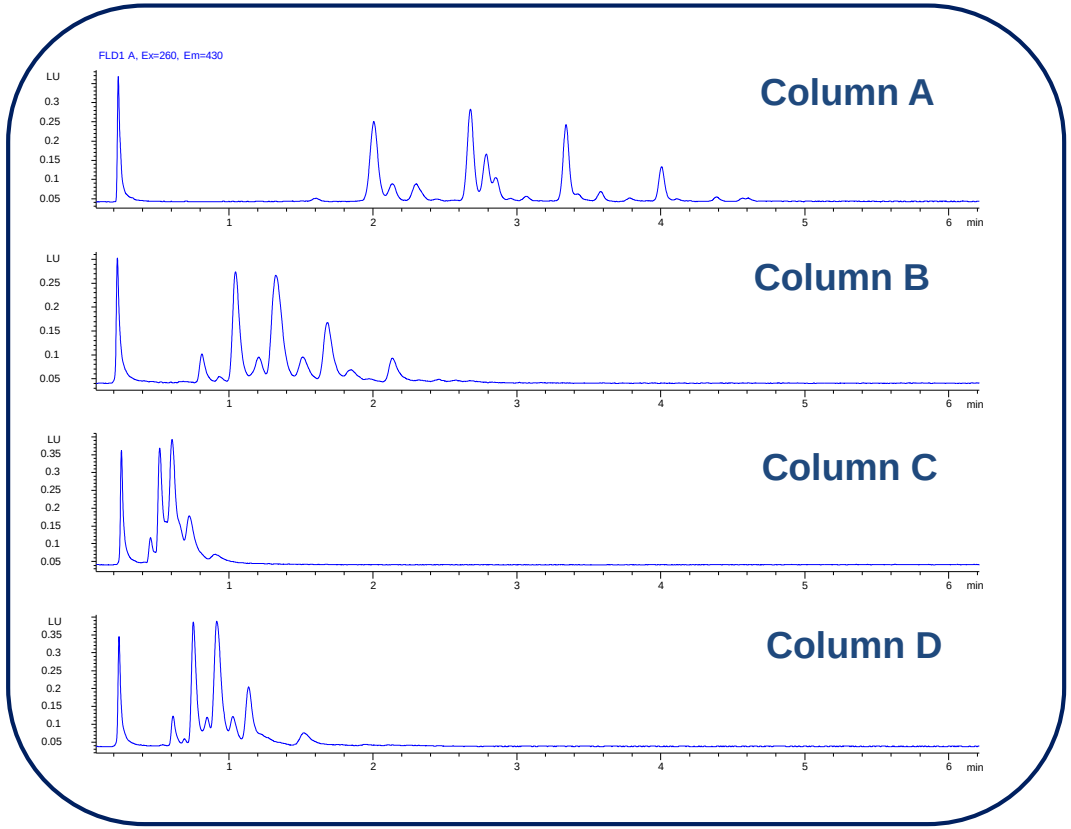
Both Case Studies:

The amount of time saved using Fusion QbD is estimated at 2.5 full time employees (FTE's) over the course of a month.

Separation of a Critical Glycan Pair

Agilent Technologies Case Study

Chemistry Screening Study – Example Results



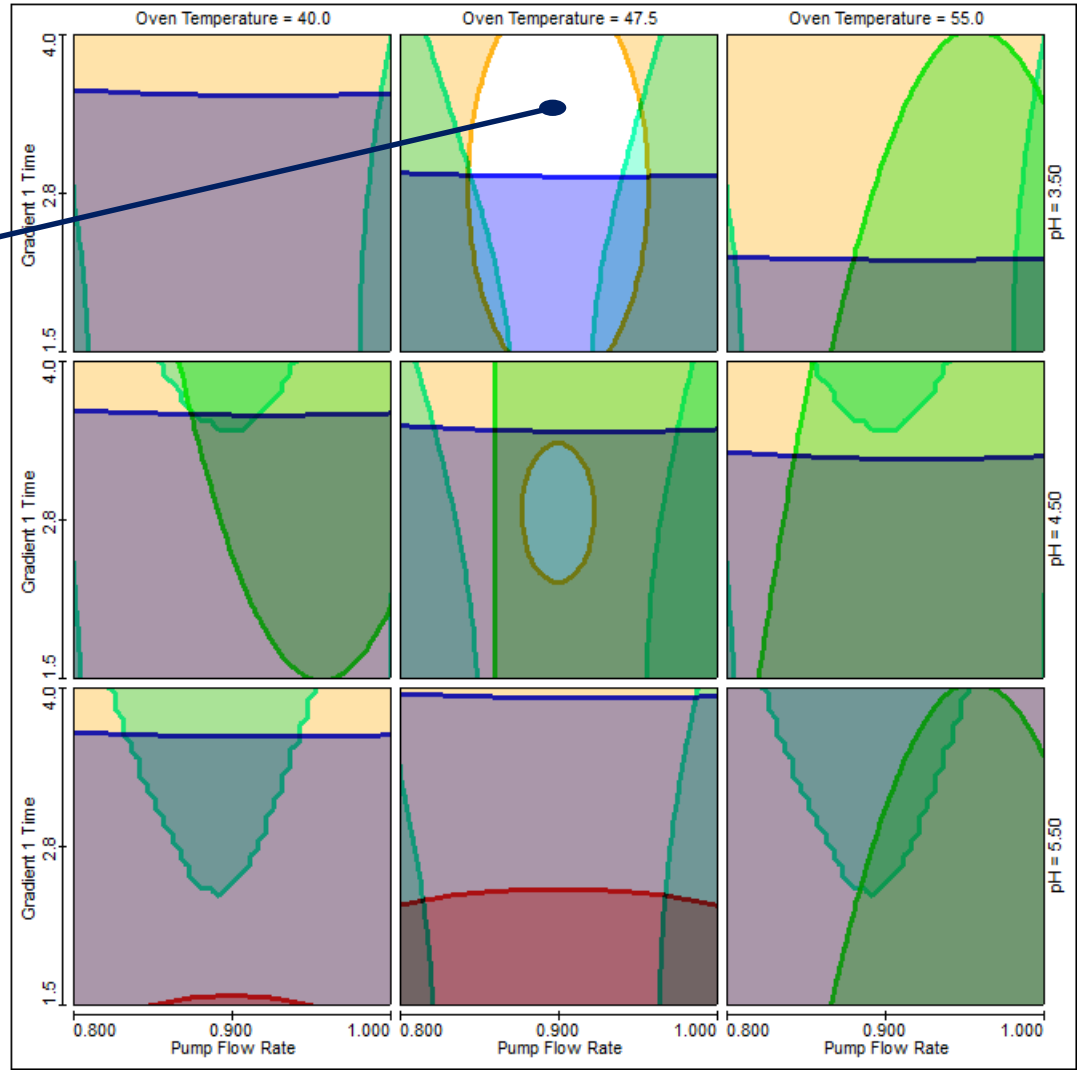
Separation of a Critical Glycan Pair

Optimization Study Results

Best Performing Method

Variable	Level Setting
Pump Flow Rate	0.85
Gradient 1 Time	3.3
Methanol	2.8
Oven Temperature	47.5
pH	3.50

Trellis Graphs Cover Full Experiment Ranges



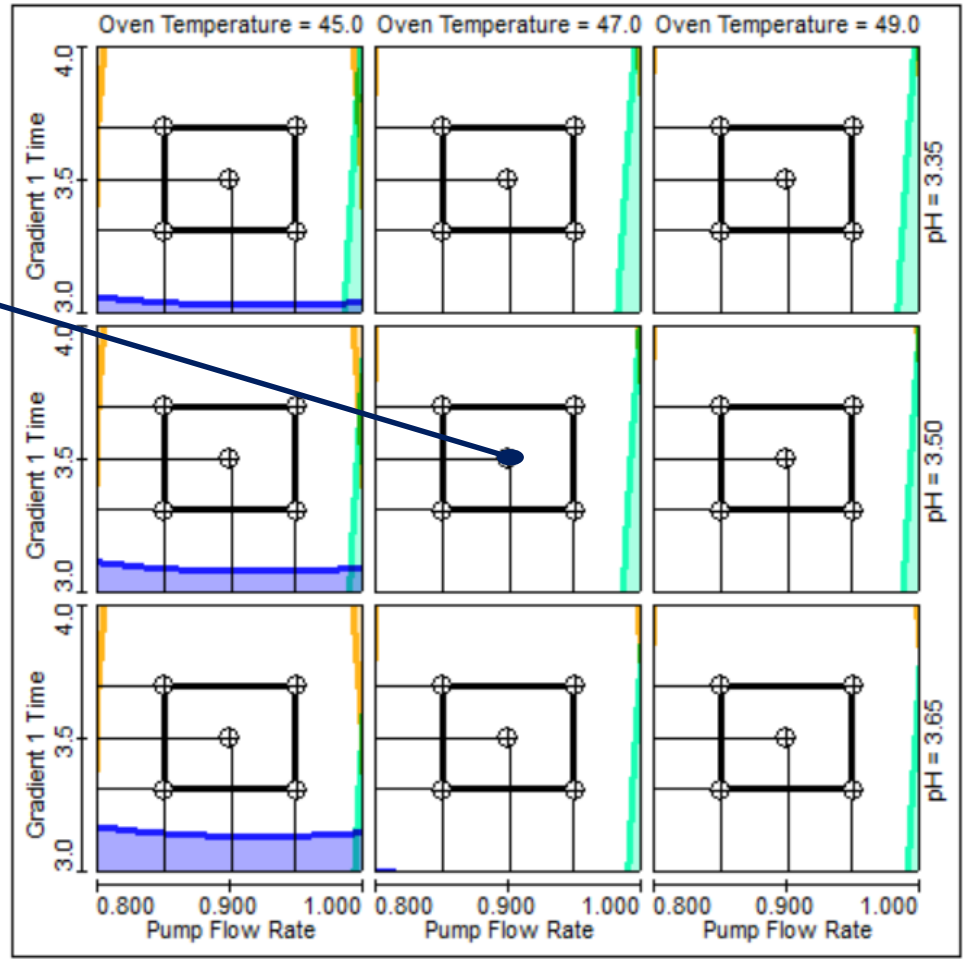
Separation of a Critical Glycan Pair

Optimization Study Results

Best Performing Method

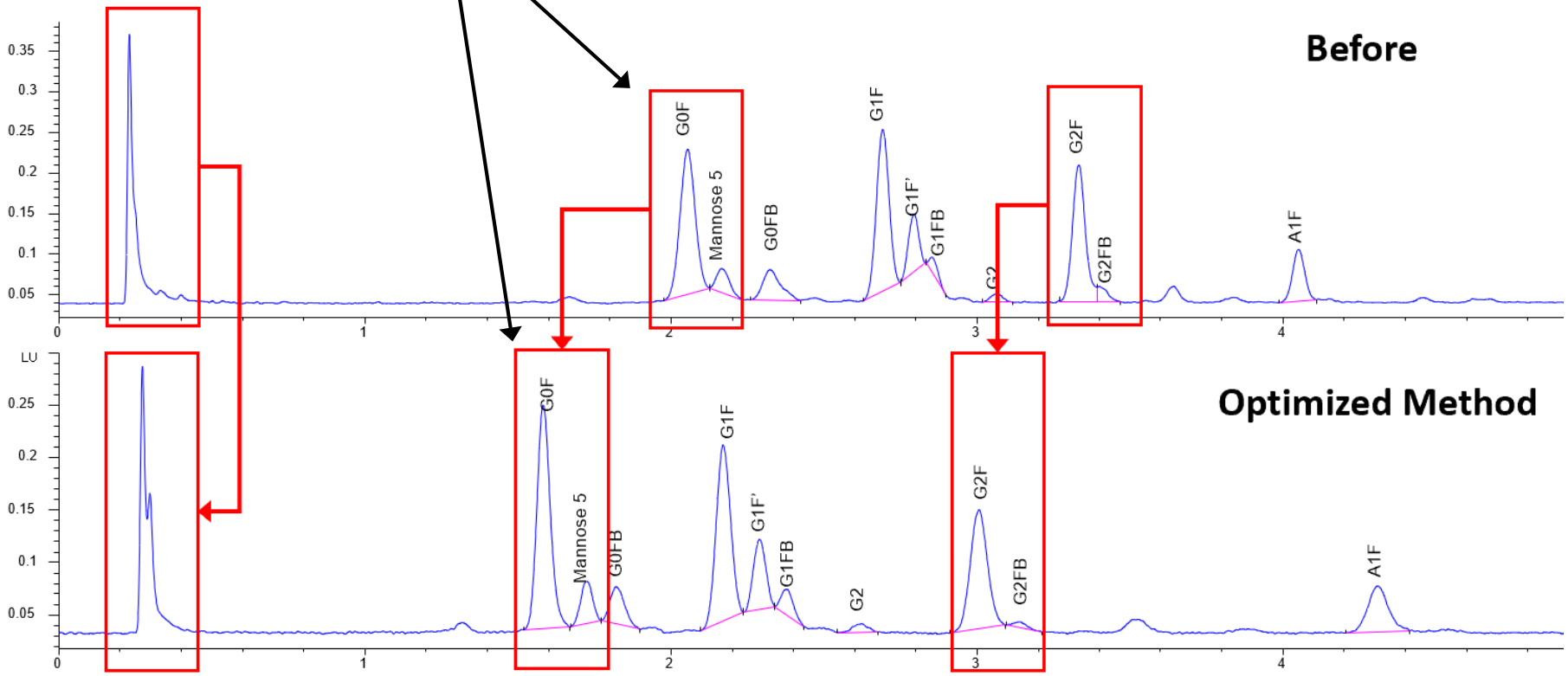
Variable	Level Setting
Pump Flow Rate	0.85
Gradient 1 Time	3.3
Methanol	2.8
Oven Temperature	47.5
pH	3.50

Trellis Graphs Zoomed in on Robustness Ranges



Separation of a Critical Glycan Pair

Critical Pair Resolution	Initial Method	Optimized Method
	1.30	2.04



Modernization of the USP Monograph method for Human serum albumin

How To Use QbD Software To Improve An Existing Identification Method

Source: Pfizer CentreOne

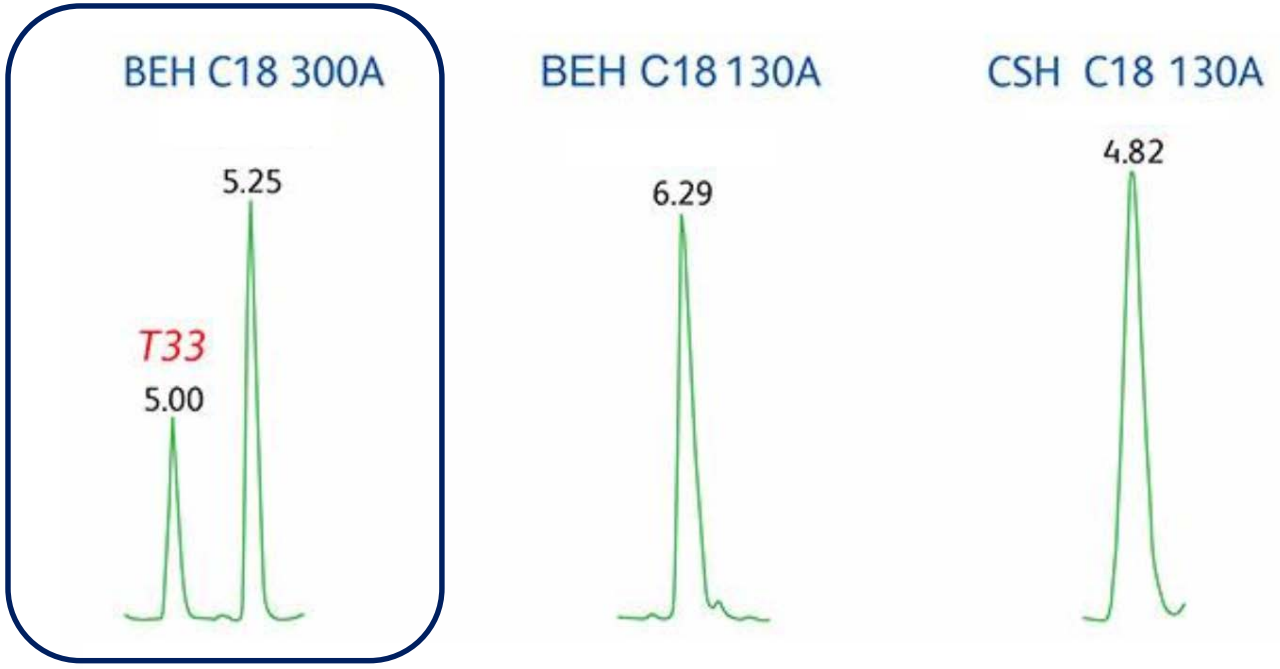
By Ashraf Madian, Ph.D., Sr Group Leader, Pfizer Global Technology Services Biomanufacturing Sciences and Shen Chen, Ph.D., Director, R&D, Lisa Cherry, Ph.D., Pharmaceutical Sciences Manager, Irish Gibson, Ph.D., Associate Research Scientist, all three from Pfizer CentreOne

Pharmaceutical Online, November 15, 2017

Modernization of the USP Monograph method for Human serum albumin

Chemistry Screening Study – Example Results

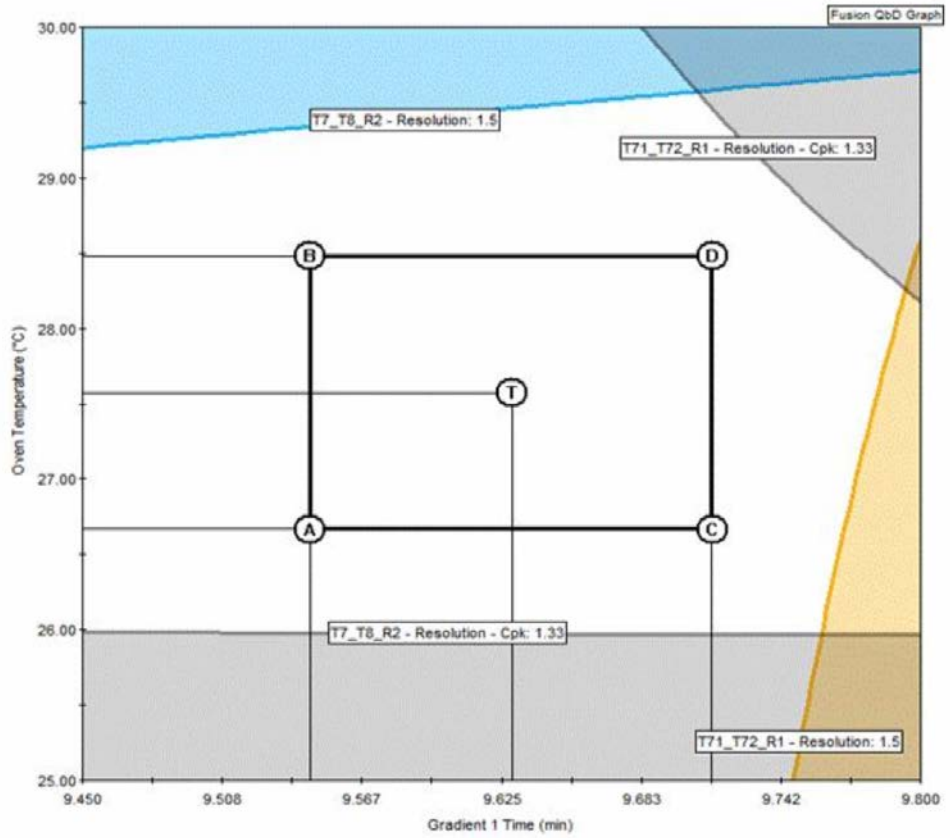
BEH C18 Column separates a critical marker peak from a co-eluting peak.



Modernization of the USP Monograph method for Human serum albumin

Optimization Study Results

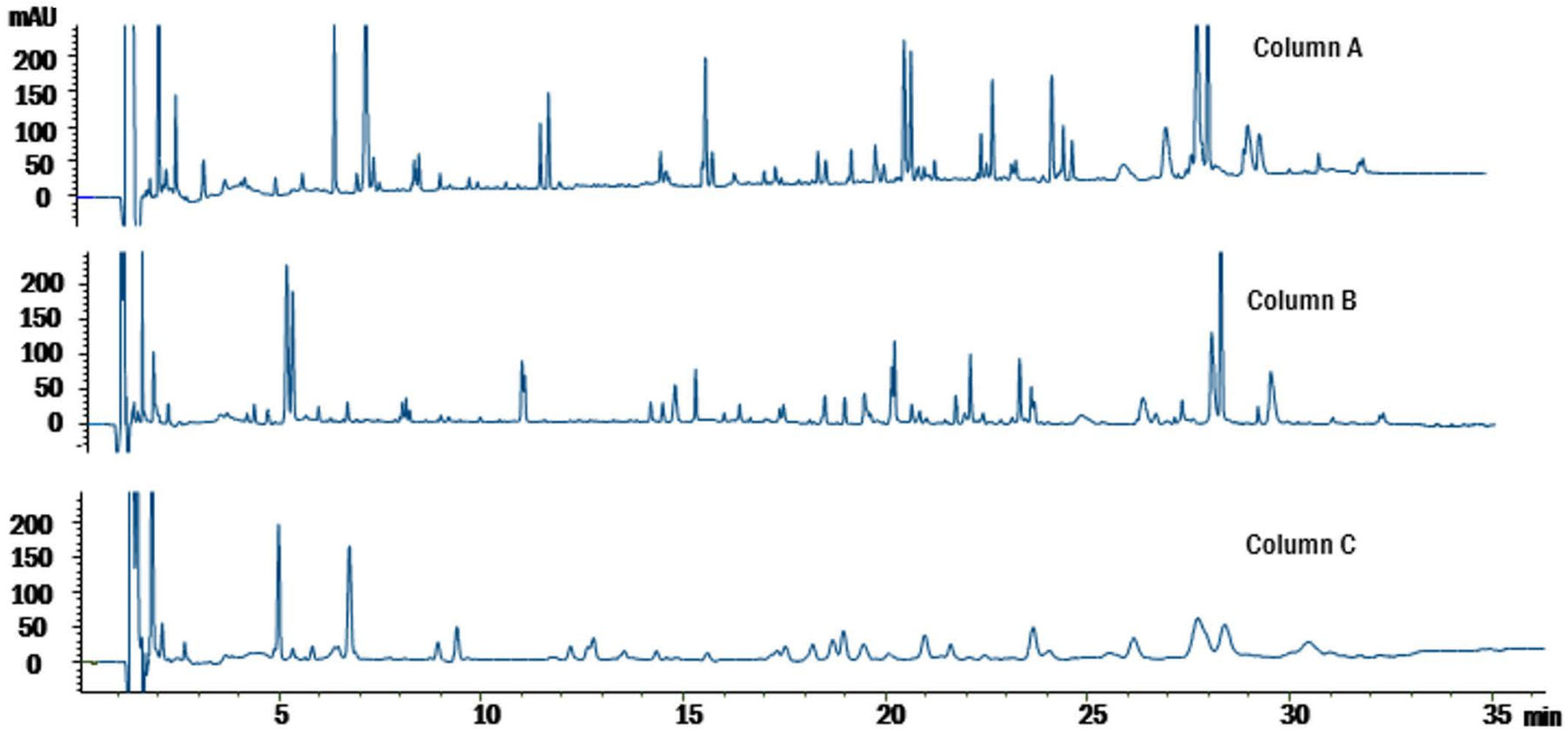
- Optimized column selection, column temperature, gradient time, and Mobile Phase Composition.
- Reduced Gradient Time from 120 minutes to < 15 minutes
- Achieved a Final Method with Robust Resolution (C_{pk} ≥ 1.33) and Excellent Peak Shape for All Seven Marker Peaks.



Peptide Mapping Case Study

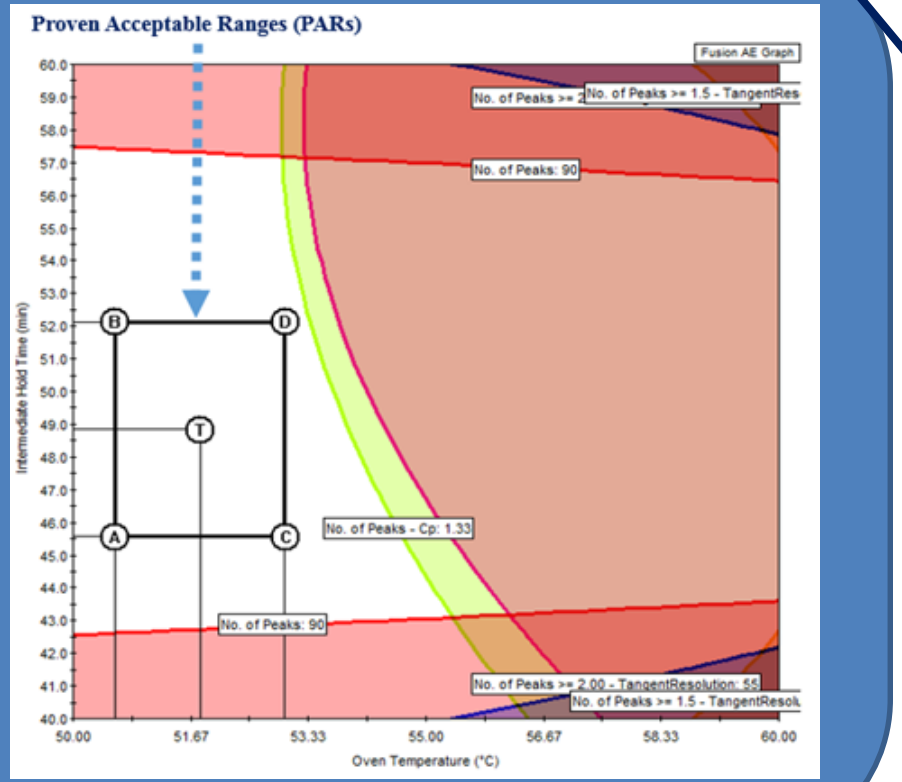
Agilent Technologies Case Study

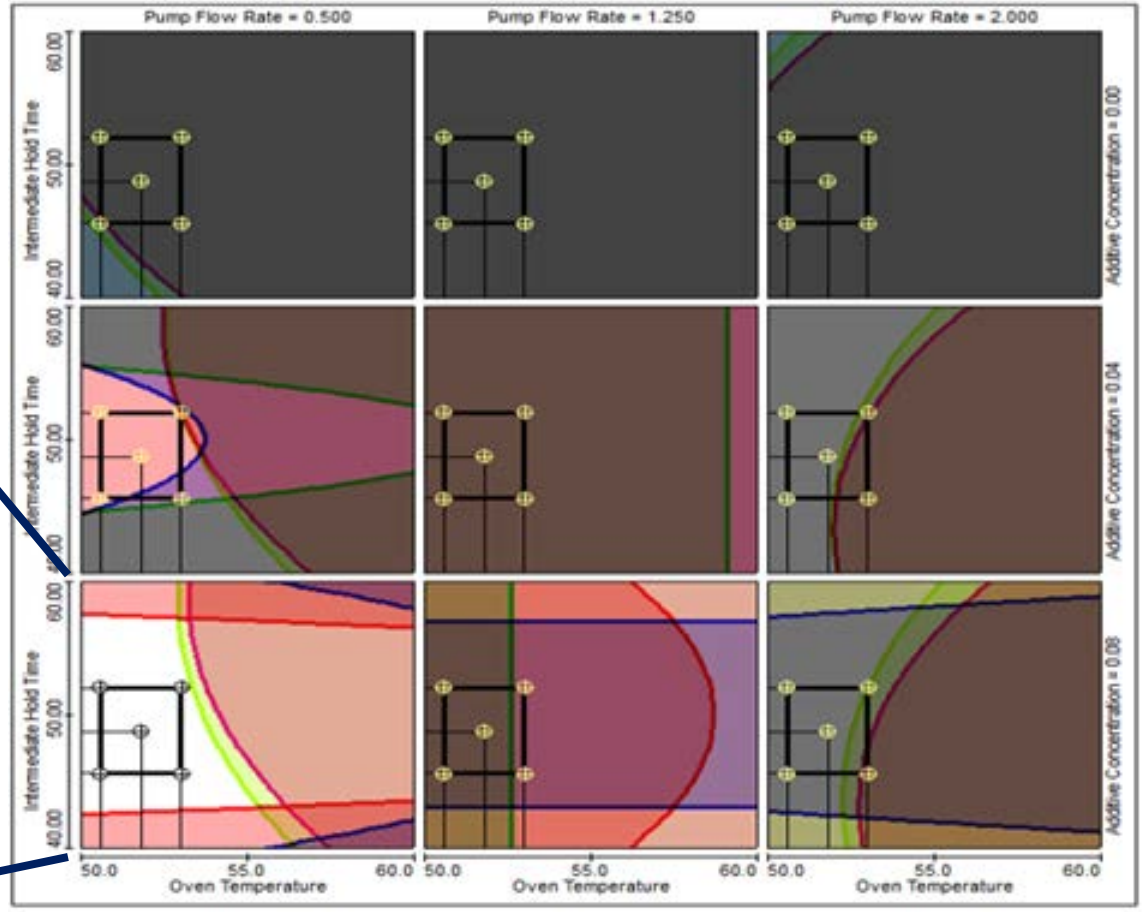
Chemistry Screening Study – Example Results



Peptide Mapping Case Study

Optimization Study Results



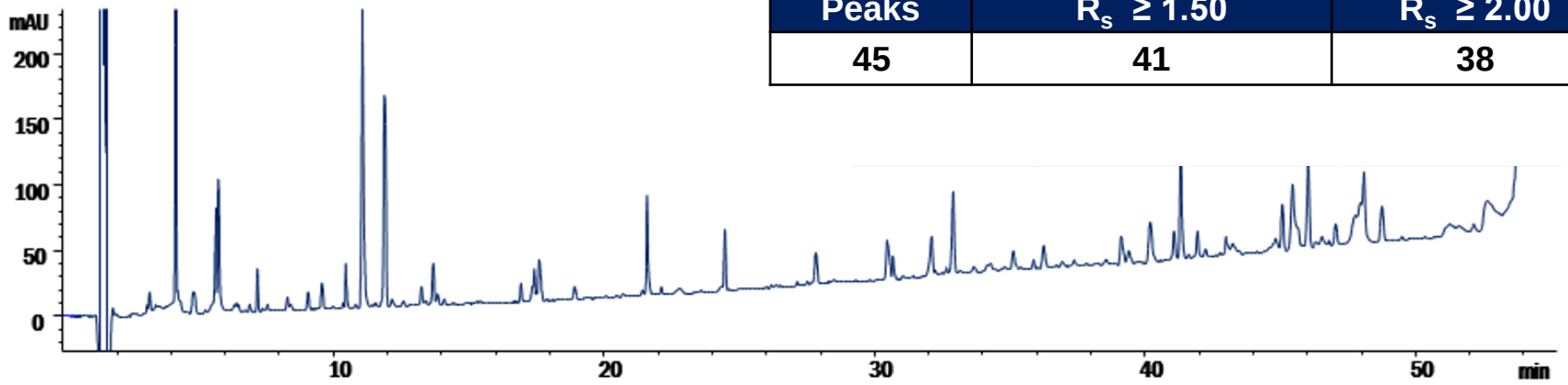


Peptide Mapping Case Study

Optimization Study Results

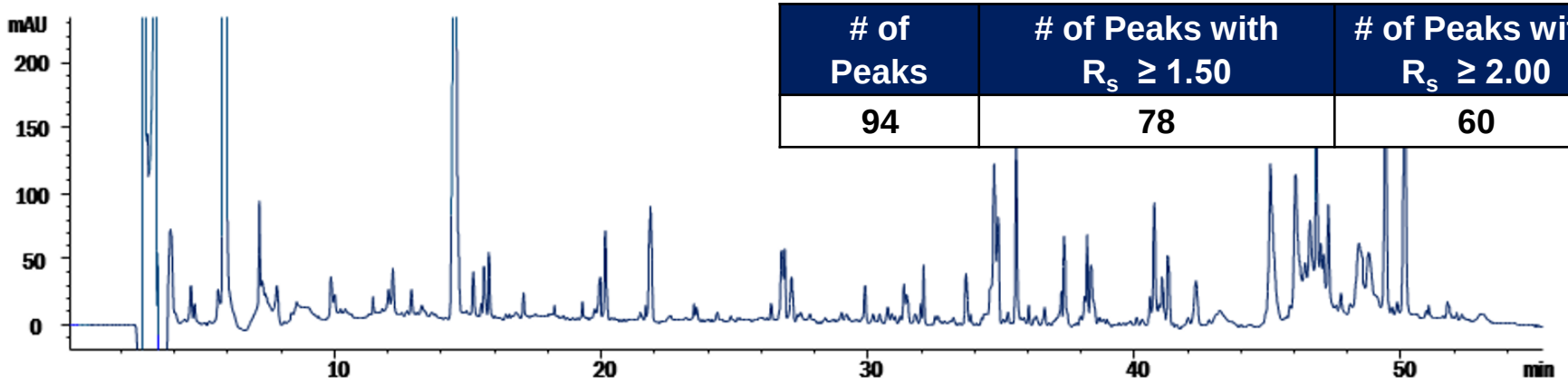
Before

# of Peaks	# of Peaks with $R_s \geq 1.50$	# of Peaks with $R_s \geq 2.00$
45	41	38

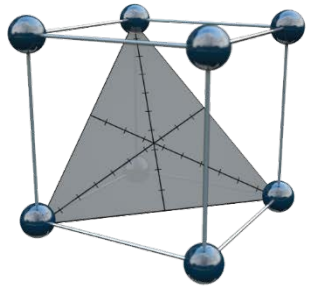


Optimized Method

# of Peaks	# of Peaks with $R_s \geq 1.50$	# of Peaks with $R_s \geq 2.00$
94	78	60



End of Presentation



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