

Evaluation of Fusion AE system: A QbD Approach to Method Development and Robustness Studies

*Anna Ip
Drug Substance Department
Amgen Inc.*

Outline

- Fusion AE Background
- Case Study
 - SEC-HPLC Method Robustness
 - CEX-HPLC Preliminary Screening Study

Fusion AE's DOE Approach

Method Development & Robustness

Typical Approach

- **Screen One Factor at a Time:**
 - Column type
 - Column temp
 - Flow rate
 - Gradient
 - pH
 - etc.
- **Robustness tested at end of method development**
 - Failure=
redevelopment

Fusion AE Approach

- **Multiple variables tested simultaneously**
 - QbD approach using minimum number of runs to find optimal method parameters
- **Robustness included as part of method development**
 - Final Method is Robust

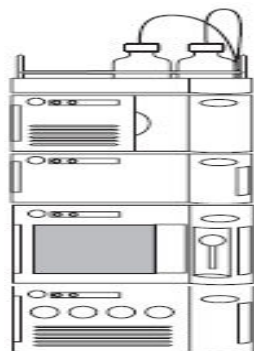
Fusion AE (Automated Experimentation): What is it?

- **Software**

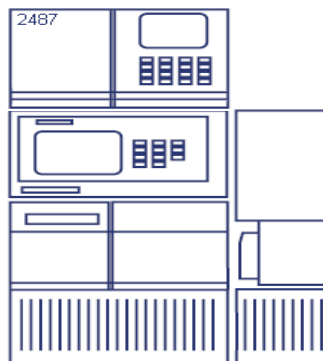
- Developed by S-Matrix Corporation (Eureka, CA)
- Automated set up of DOE studies for analytical method development, robustness testing and qualification
- Integrates with current CDS systems
(Varian's Galaxy, Waters' Empower, and Agilent's Chemstation)
- Dionex's Chromeleon –in progress

- **Hardware**

- Column heating compartment and switching valves
- Solvent switching valves



Waters Acquity UPLC



Waters Alliance

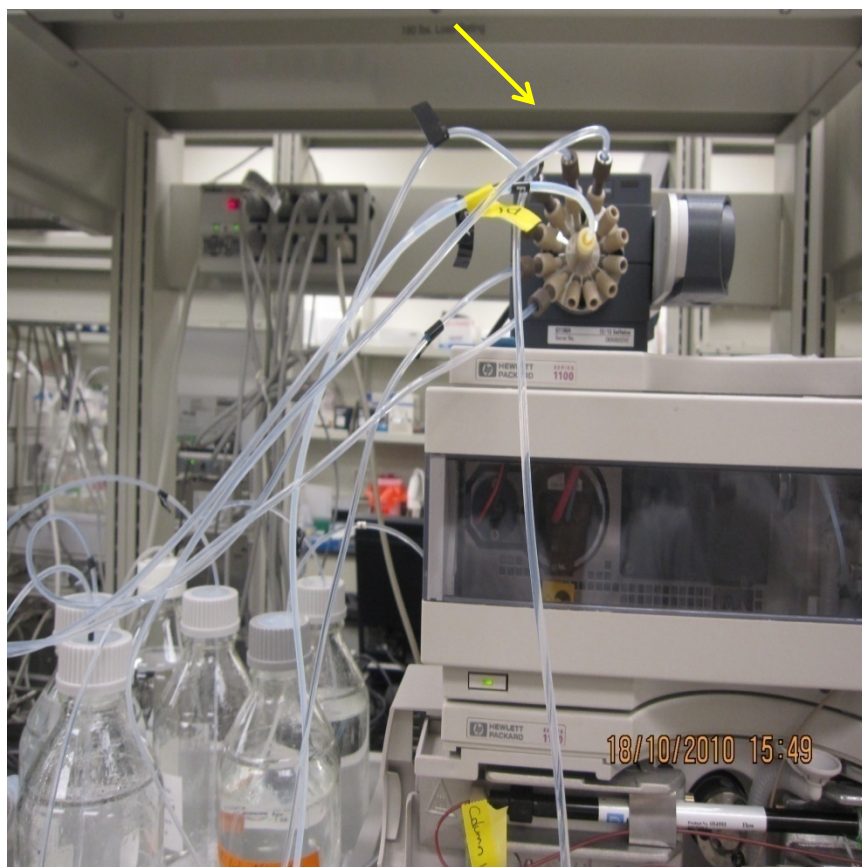


Agilent 1100/1200

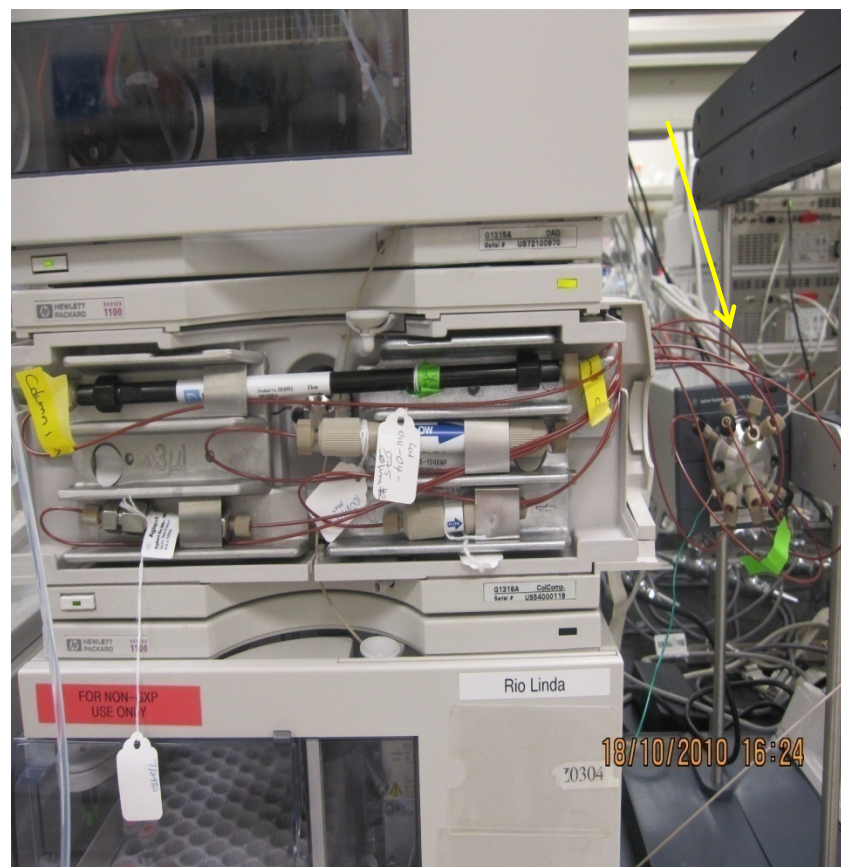
Hardware Requirements to Fully Utilize Fusion AE

Simultaneously test all the variables and be able to detect interactions between the variables.

Solvent Selection Valves

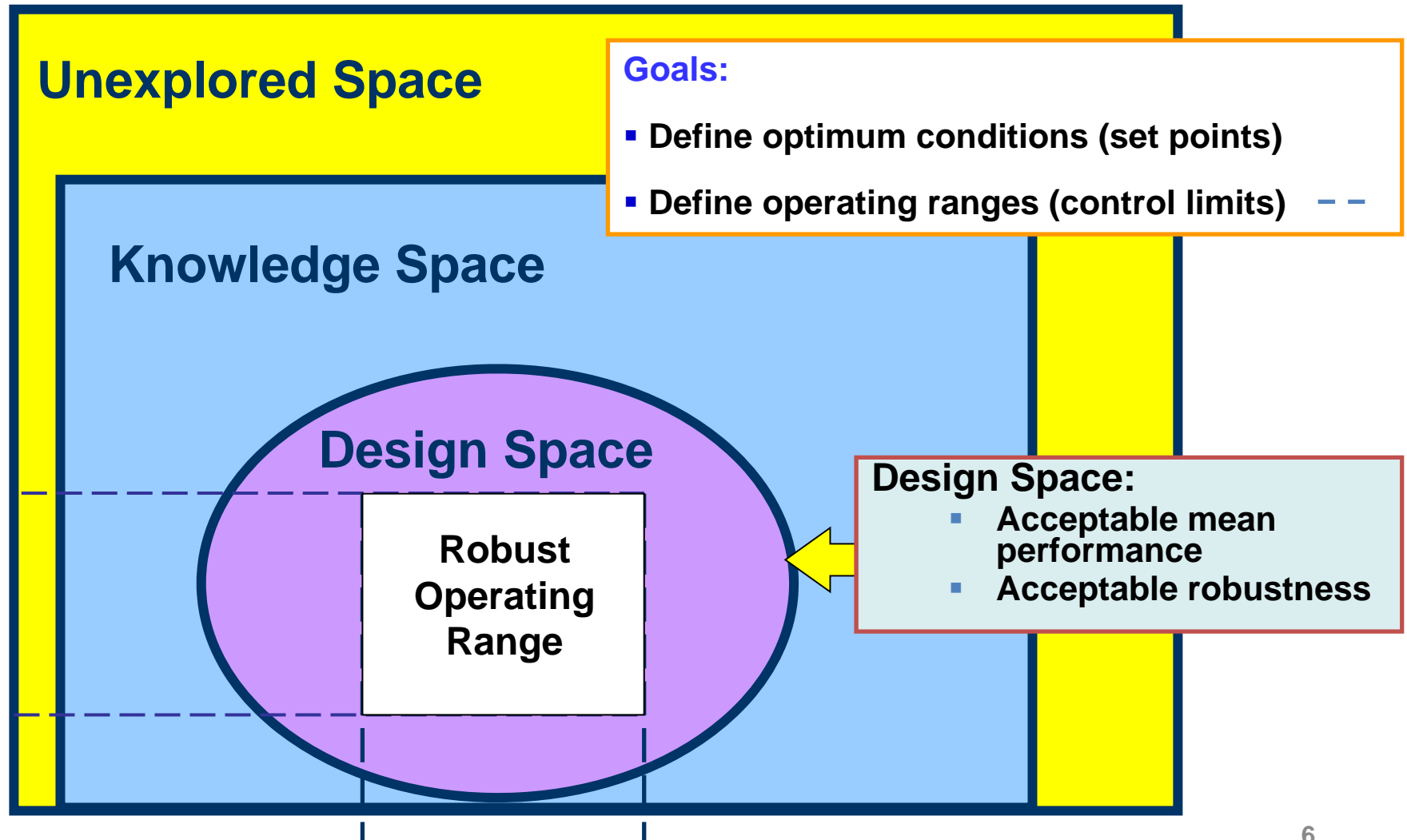


Column Selection Valves

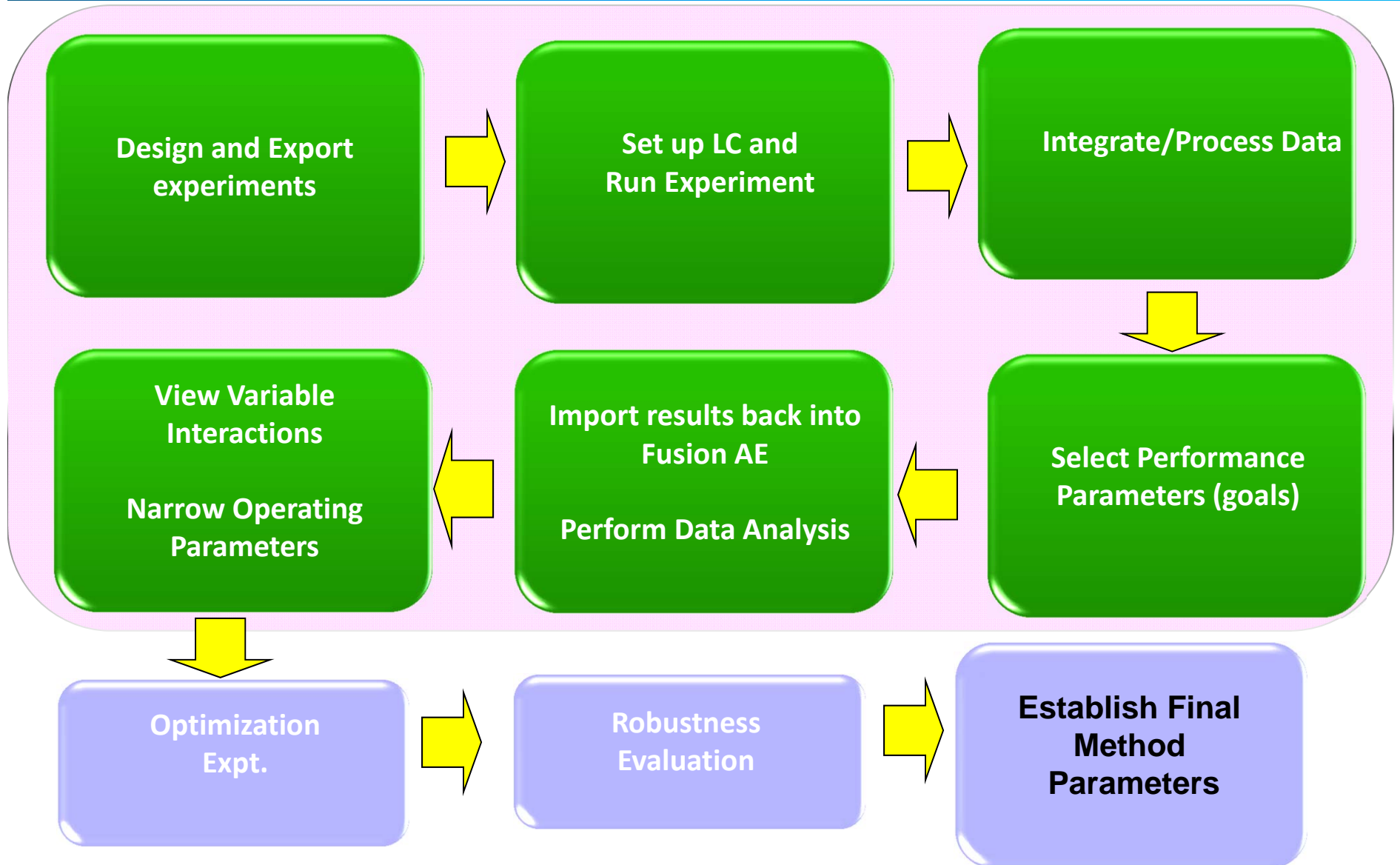


QbD –DOE Experimental Approach

Initial Screening Study will establish **knowledge space**



Fusion AE...Analytical Method Development



**Test Case I: Using Fusion AE to
test Size Exclusion HPLC Method Robustness**

Standard Fractional Factorial DOE Study

Category	Operating Parameters
Critical	2 column lots Buffer pH 0.3 [Salt] 10%
Less Critical*	Flow rate Column temperature

* From method development experience

- (1) Nominal pH, low salt: 10 mM NaPhosphate, 225 mM NaCl, pH 6.8
- (2) Nominal pH, nominal salt: 10 mM NaPhosphate, 250 mM NaCl, pH 6.8
- (3) Nominal pH, high salt: 10 mM NaPhosphate, 275 mM NaCl, pH 6.8
- (4) Low pH, nominal salt: 10 mM NaPhosphate, 250 mM NaCl, pH 6.5
- (5) High pH, nominal salt: 10 mM NaPhosphate, 250 mM NaCl, pH 7.1

8 sequence fractional factorial design with 4 center point runs

Robustness Testing of SE-HPLC Method using Fusion AE DOE

Operating Parameters	Number of Levels Studied	Range
Buffer pH	3	6.5, 6.8, 7.1
Flow rate (mL/min):	5	0.25, 0.38, 0.50, 0.63, 0.75
Column Temperature (°C):	5	20, 22.5, 25*, 27.5, 30
Salt Conc (mM):	3	225, 250, 275

*In current SE-HPLC method, column temperature setting is ambient

Four parameters evaluated:
(Critical and less critical variables studied simultaneously with Fusion AE)

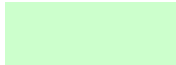
Preliminary study did not include column lot

26 experiments (for 1 column) or 41 experiments (for 2 columns) covering DOE combinations of method parameters are run in a single sequence

Run No.	Pump Flow Rate	Oven Temperature	pH	Buffer Strength
1.a.1.a	0.25	20	6.5	225
2.a.1.a	0.75	20	6.5	250
3.a.1.a	0.25	20	6.5	275
4.a.1.a	0.25	20	6.5	275
5.a.1.a	0.75	20	7.1	225
6.a.1.a	0.75	20	7.1	275
7.a.1.a	0.25	20	7.1	250
8.a.1.a	0.75	20	7.1	275
9.a.1.a	0.625	22.5	6.5	225
10.a.1.a	0.375	22.5	7.1	225
11.a.1.a	0.5	25	6.5	275
12.a.1.a	0.25	25	7.1	275
13.a.1.a	0.375	27.5	6.5	225
14.a.1.a	0.625	27.5	7.1	225
15.a.1.a	0.75	30	6.5	275
16.a.1.a	0.75	30	6.5	225
17.a.1.a	0.25	30	6.5	250
18.a.1.a	0.75	30	7.1	250
19.a.1.a	0.25	30	7.1	225
20.a.1.a	0.5	30	7.1	275
21.a.1.a	0.75	25	6.8	275
22.a.1.a	0.5	25	6.8	250
23.a.1.a	0.5	25	6.8	250
24.a.1.a	0.5	25	6.8	250
25.a.1.a	0.5	30	6.8	225
26.a.1.a	0.25	30	6.8	275

Fusion AE generated DOE Sequence Table using user input settings:

Includes center points and repeats of other variable combinations

 Center points (default conditions)

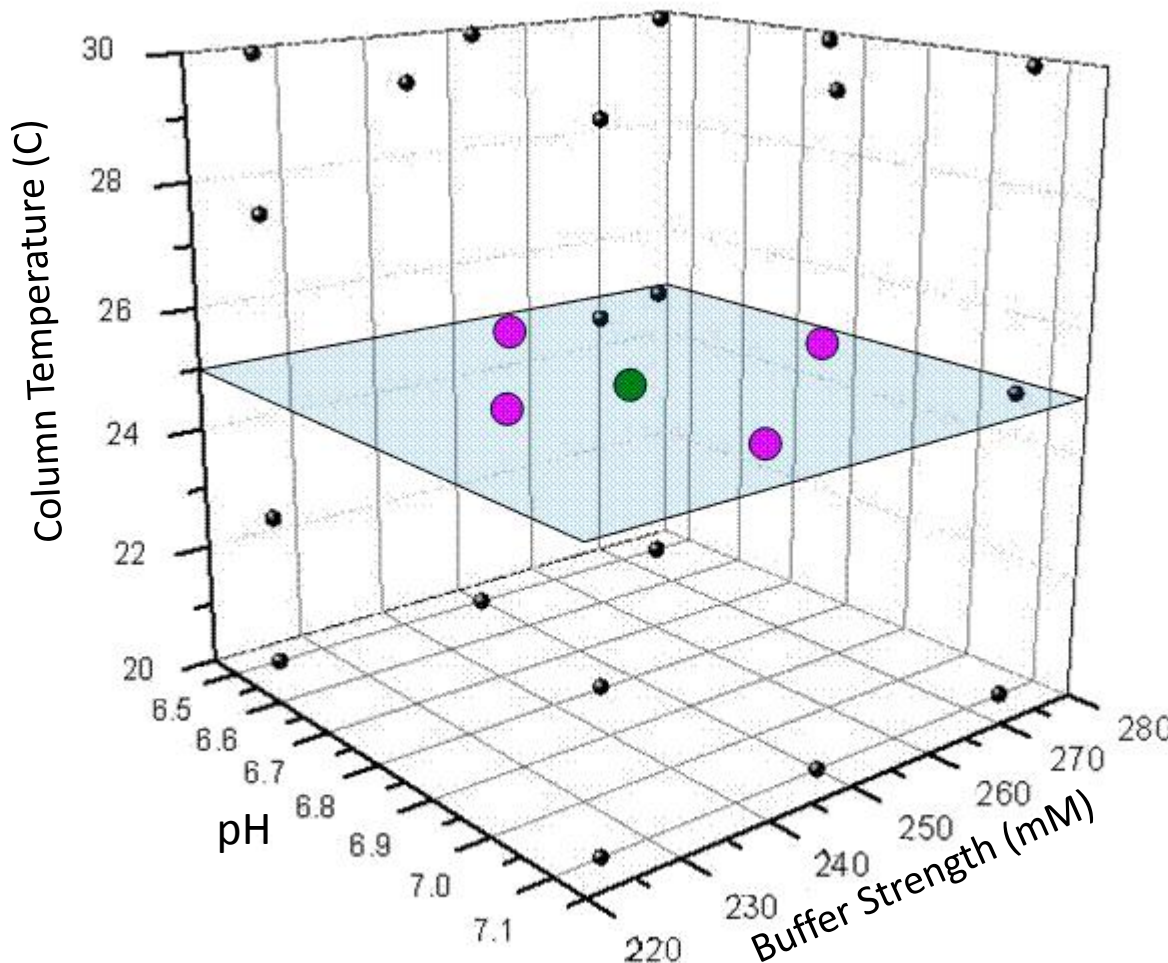
Design Type:
Model-Robust Quadratic
with three center point repeats

Total run time:
18.5 hrs

Design also includes 13 wash runs (15 minutes each)

Current SE-HPLC Robustness tests vs. more complete DOE generated design space using Fusion AE

3D representations of experimental points in test sequence

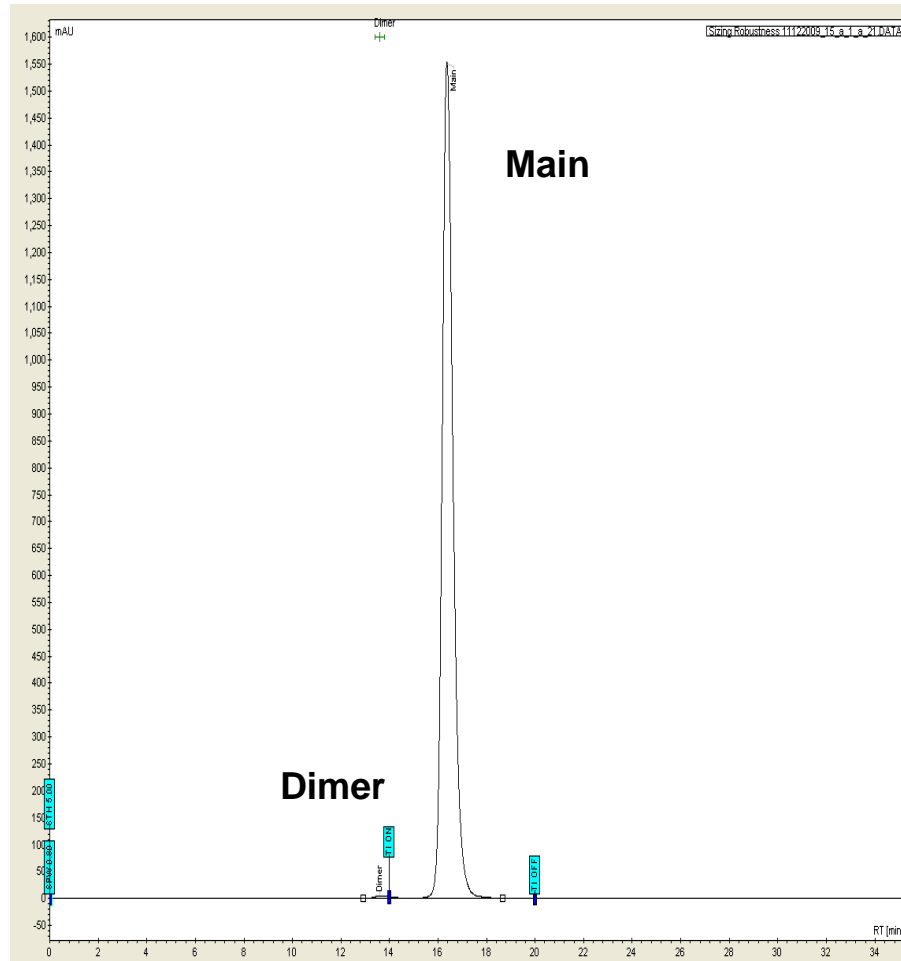


- Nominal Condition
- Buffer Variations
- Fusion AE's More complete Robustness Expt. points

Fusion AE Robustness Evaluation

- **Need user defined constraints to set Acceptance Criteria**
 - Precision
 - Resolution factor
 - Peak width
 - Retention time
 - Theoretical plates
 - etc.
- **Provides graphical and numerical output of robust and non-robust regions**
 - 3-D response surfaces
 - Overlay graphics
 - Optimized method parameters

Performance Parameters for Trending and Modeling: Select the Responses You Want to Track



For SE-HPLC Robustness:

*Performance Parameter
(Response) of interest:*

Performance Parameter	Acceptance Criteria
Main Peak Resolution (USP), R_s	$R_s > 1.5$
% Dimer	+/-5% of Centerpoint

Data source for modeling chromatographic performance

Results imported automatically into Fusion AE

Run No.	Pump Flow Rate	Oven Temperature	pH	Buffer Strength	Dimer - Area % [%]	Main Peak - Area % [%]	Main Peak - Res. USP
Wash - 1	0.5	20	6.5	225			
1.a.1.a	0.25	20	6.5	225	0.99167	99.00833	2.31
2.a.1.a	0.75	20	6.5	250	0.53964	99.46036	2.244
3.a.1.a	0.25	20	6.5	275	0.5253	99.4747	2.646
4.a.1.a	0.25	20	6.5	275	0.47	99.53	2.747
Wash - 2	0.5	20	7.1	225			
5.a.1.a	0.75	20	7.1	225	0.62214	99.37786	2.194
6.a.1.a	0.75	20	7.1	275	0.56432	99.43568	2.217
7.a.1.a	0.25	20	7.1	250	0.44768	99.55232	2.631
8.a.1.a	0.75	20	7.1	275	0.60318	99.39682	2.211
Wash - 3	0.5	22.5	6.5	225			
9.a.1.a	0.625	22.5	6.5	225	0.54938	99.45062	2.473
Wash - 4	0.5	22.5	7.1	225			
10.a.1.a	0.375	22.5	7.1	225	0.48056	99.51944	2.49
Wash - 5	0.5	25	6.5	275			
11.a.1.a	0.5	25	6.5	275	0.49101	99.50899	2.496
Wash - 6	0.5	25	7.1	275			
12.a.1.a	0.25	25	7.1	275	0.50148	99.49852	2.659
Wash - 7	0.5	27.5	6.5	225			
13.a.1.a	0.375	27.5	6.5	225	0.49687	99.50313	2.508
Wash - 8	0.5	27.5	7.1	225			
14.a.1.a	0.625	27.5	7.1	225	0.52839	99.47161	2.39
Wash - 9	0.5	30	6.5	275			
15.a.1.a	0.75	30	6.5	275	0.55574	99.44426	2.325
16.a.1.a	0.75	30	6.5	225	0.548	99.452	2.302
17.a.1.a	0.25	30	6.5	250	0.42554	99.57446	2.759
Wash - 10	0.5	30	7.1	250			
18.a.1.a	0.75	30	7.1	250	1.25881	98.74119	2.078
19.a.1.a	0.25	30	7.1	225	0.46267		
20.a.1.a	0.5	30	7.1	275	0.49434	99.50566	2.383
Wash - 11	0.5	30	7.1	275			
Wash - 12	0.5	25	6.8	275			
21.a.1.a	0.75	25	6.8	275	0.7746	99.09031	2.116
22.a.1.a	0.5	25	6.8	250	0.43852	99.56148	2.483
23.a.1.a	0.5	25	6.8	250	0.52645	99.47355	2.332
24.a.1.a	0.5	25	6.8	250	0.52548	99.47452	2.309
25.a.1.a	0.5	30	6.8	225	0.52261	99.47739	2.335
26.a.1.a	0.25	30	6.8	275	0.67693	99.32307	2.373
Wash - 13	0.5	30	6.8	275			

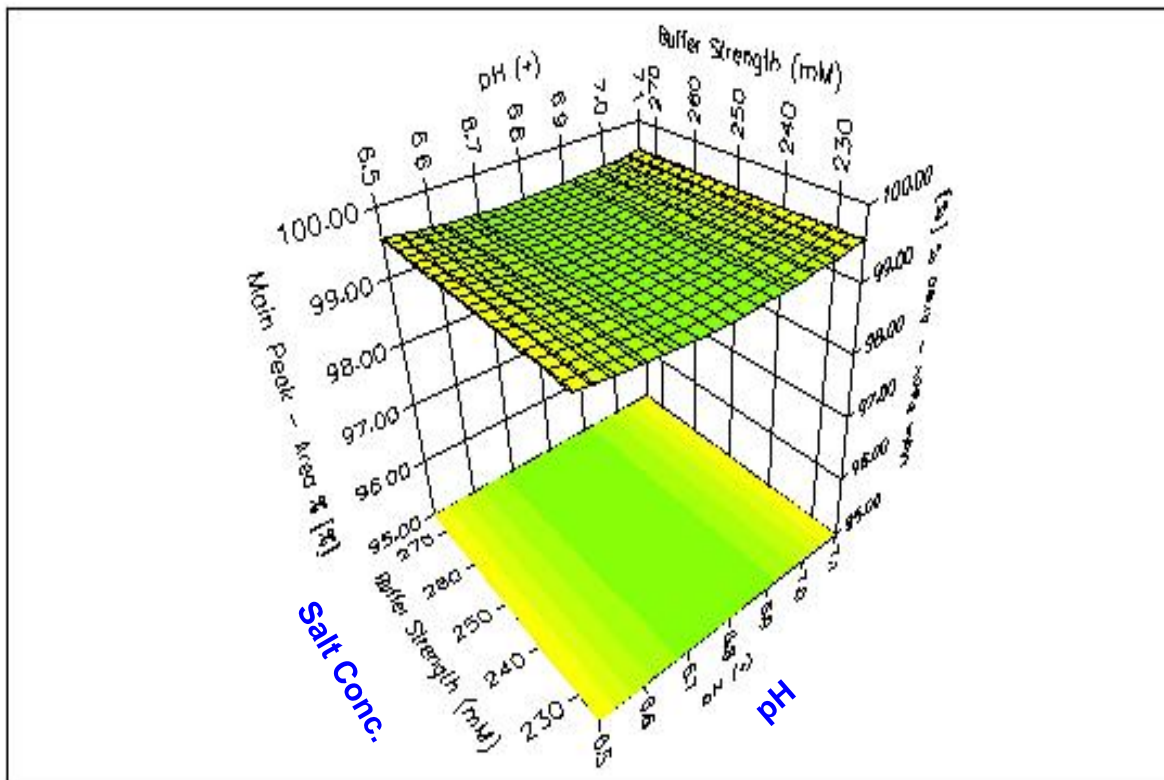
- (1) %Dimer
- (2) Main Peak Resolution, Rs

Fusion Results: 3D Response Surfaces for Method Robustness: % Main Peak

pH vs. Salt conc.

Main Peak - Area % [%] Response Surface
Pump Flow Rate = 0.500; Oven Temperature = 25.0

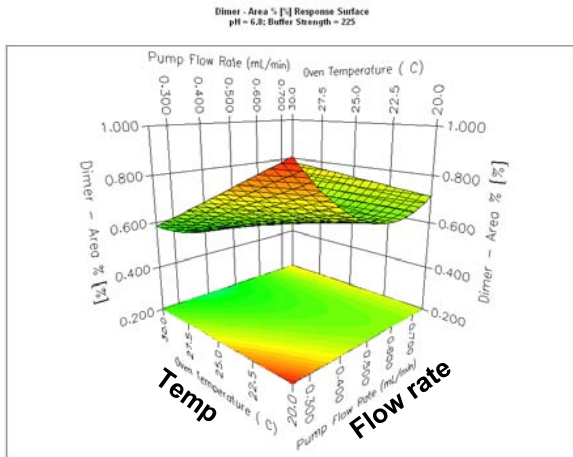
Fusion .AE Graph



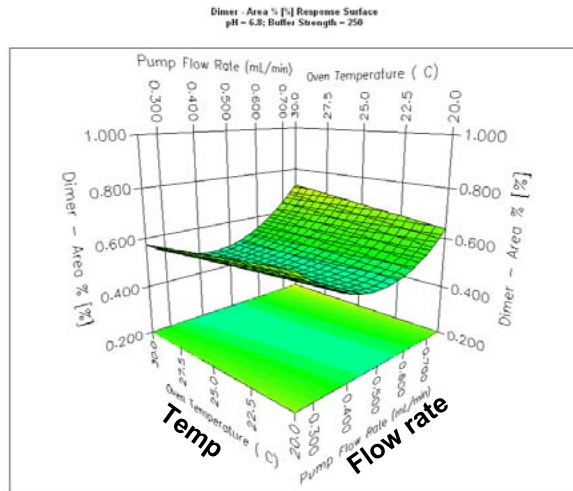
Curvature in %Main Peak response surface – shows minimal variation
%Main Peak is robust for range of pH and Salt Conc. tested

Fusion Results: 3D Response Surfaces for Method Robustness: % Dimer as Acceptance Criteria

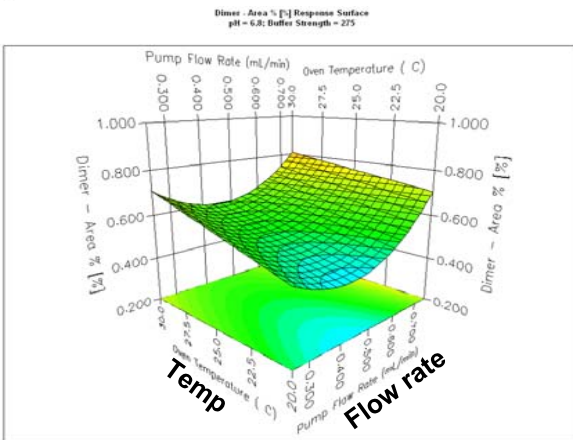
pH 6.8
225 mM



pH 6.8
250mM



pH 6.8
275mM



Complex interaction

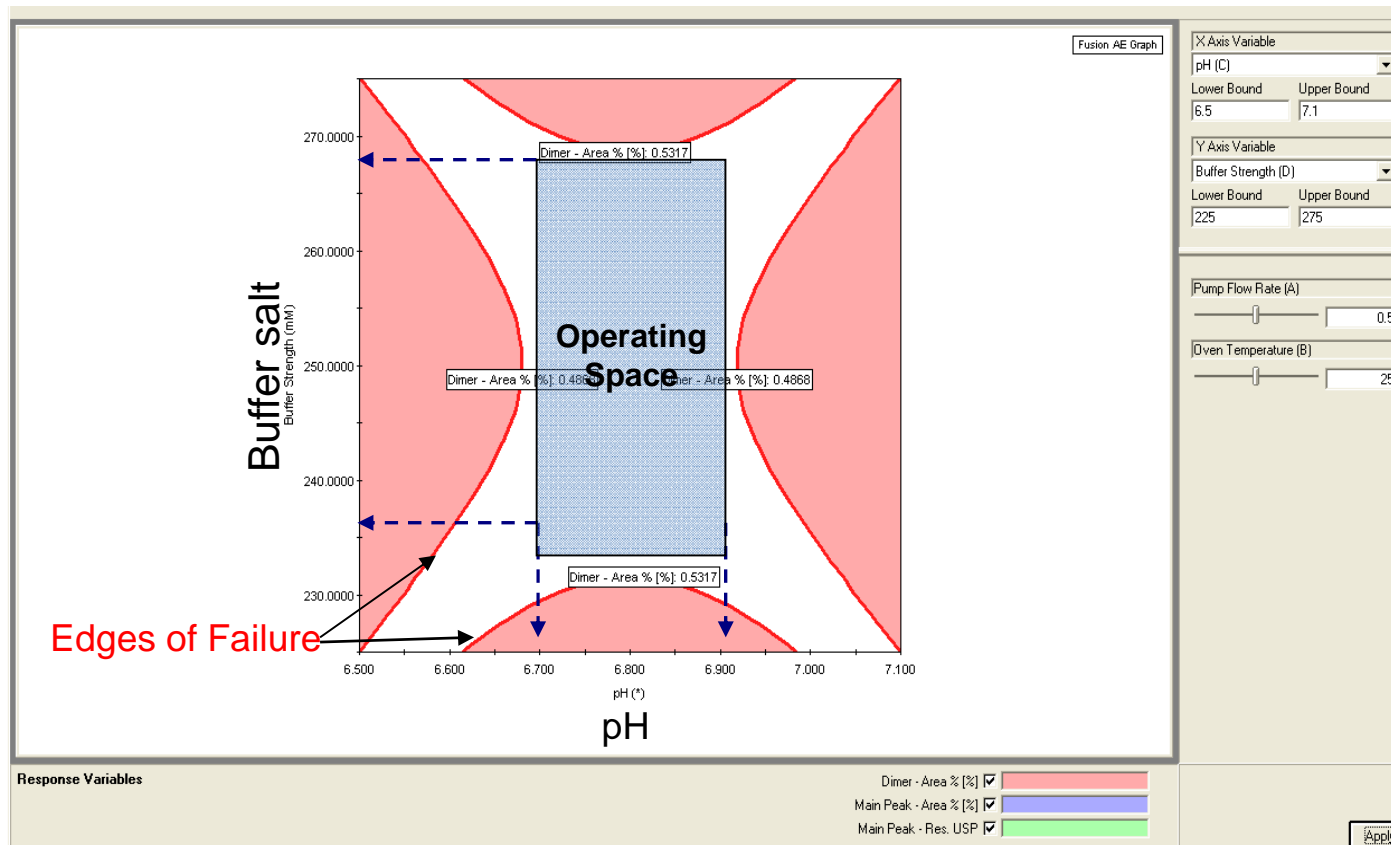
Simpler curvature interaction

Complex interaction

In this DOE, study variable interactions are identified.

Overlay Graphics

With both criteria (% Dimer & Main Peak Rs)

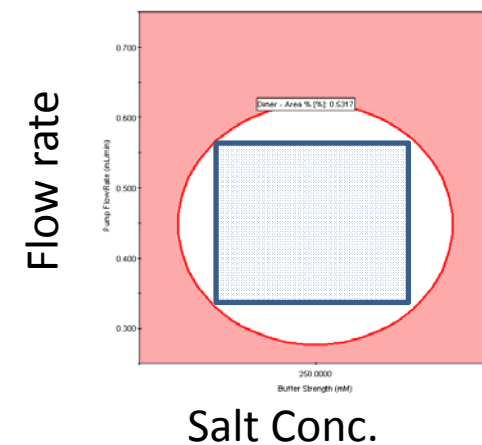
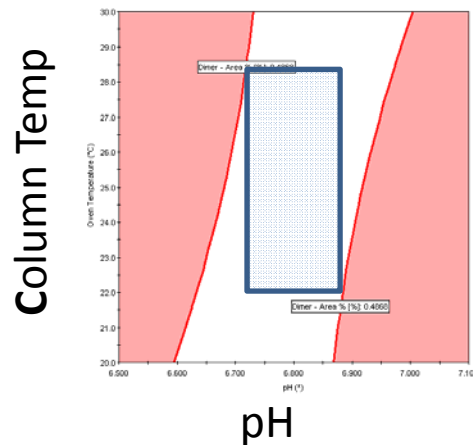
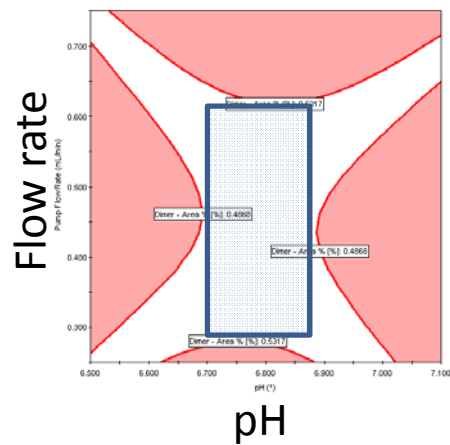
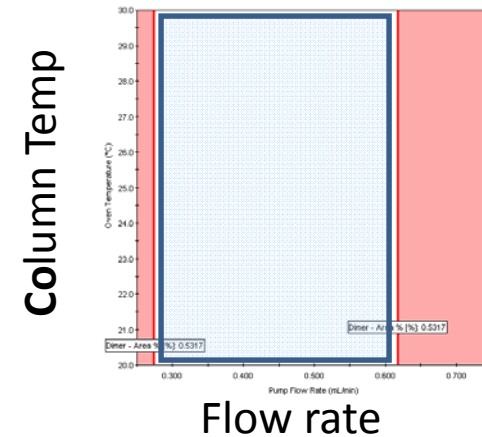
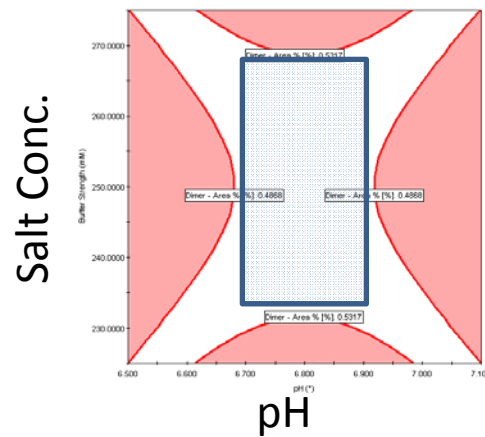
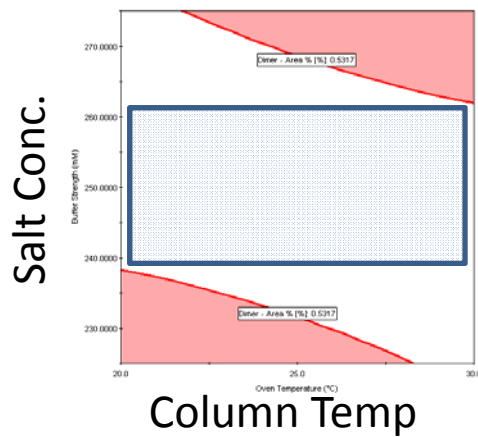


Can select
What variables to
Plot
X-axis: pH
Y-axis: Buffer Salt

Visualize the Operating Space within Design Space, then set operating ranges

Overlay Graphics

SEC-HPLC Method Robustness



Set operating ranges for each pair of variable interaction overlays

Robustness Evaluation Output:

Operating Parameters	Method Operating Range	Fusion AE Robust Operating Range*
Buffer pH	6.8 ± 0.1	6.7 - 6.9
Flow rate (mL/min):	0.5	0.34 - 0.56
Column Temperature (°C):	Ambient*	22.0 – 27.9°C
Salt Conc (mM):	250	238 - 263

* Consider 25°C set point based on Fusion AE recommendation

Test Case II: DOE Approach to CEX-HPLC Method Development

IEX-HPLC Method Parameters in DOE Design using Fusion AE

Method Parameters	Range /Type Tested	Type of Variable
Columns	Column A: Column B: Column C: Column D:	Categorical
Buffer pH (Weak Solvent)	5.5 6.0 6.5 7.0 7.5	Discrete
Gradient (Strong Solvent 0.5 M NaCl)	0.8% B/min 1.3% B/min 1.7% B/min 2.1% B/min 2.5% B/min	Continuous
Column Temperature	25°C, 30°C and 35°C	Discrete

Fusion AE Design Report (49 Experiment DOE) in one sequence

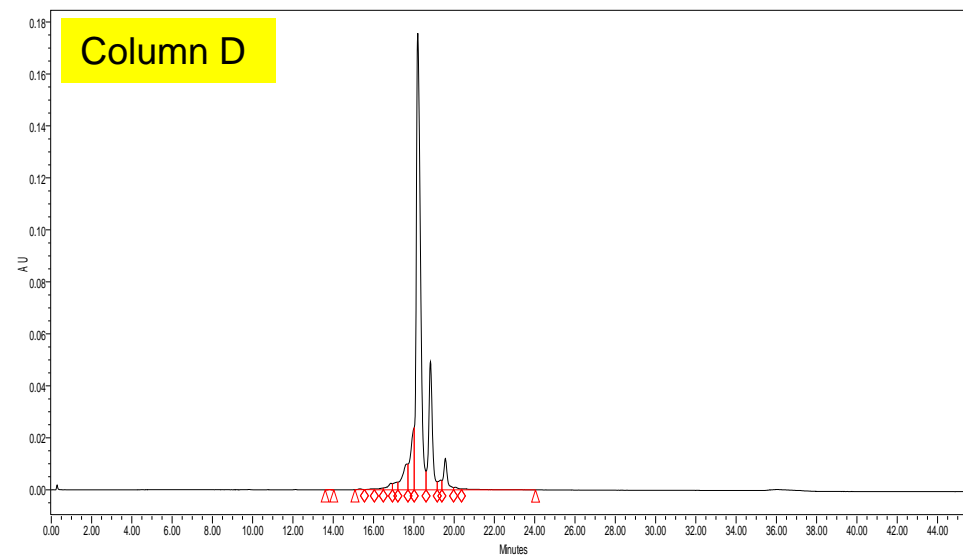
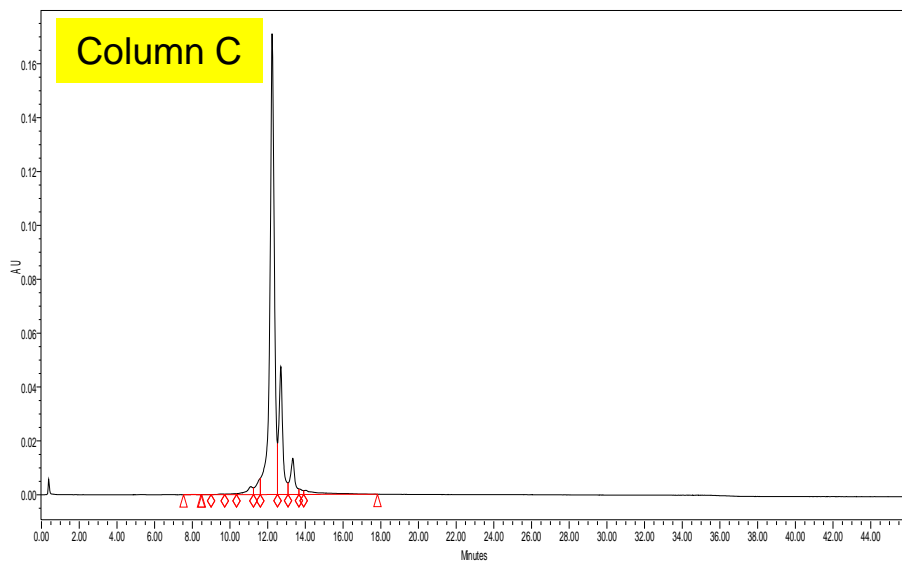
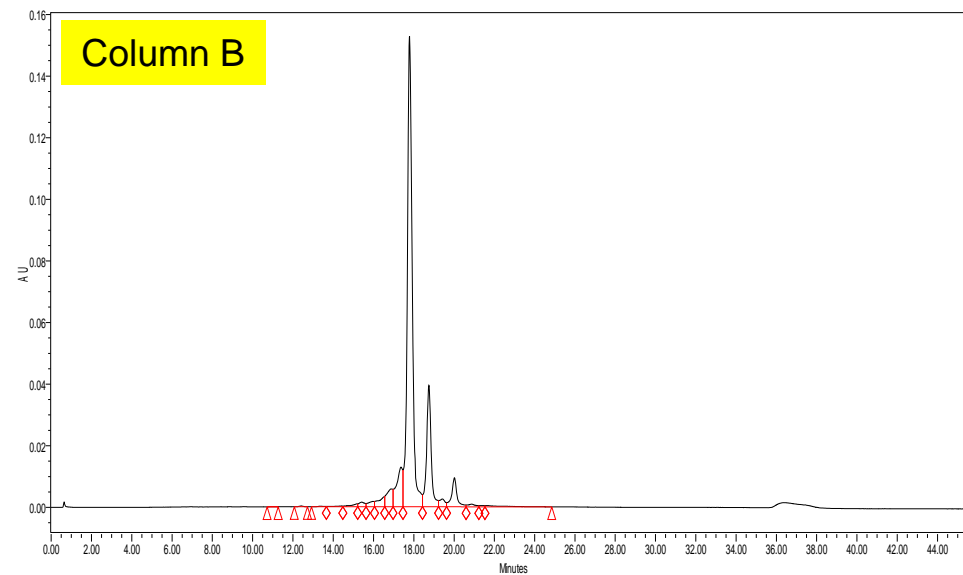
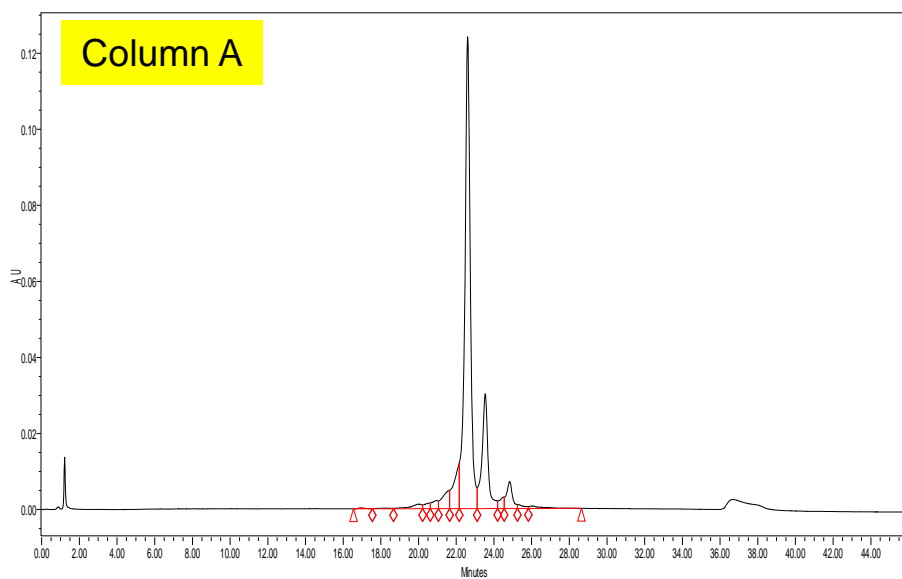
Run #	Final % Strong Solvent	Column Temp	Buffer pH	Column
Wash -1	80	25	5.5	A
Wash -2	80	25	5.5	B
Wash -3	80	25	5.5	C
Wash -4	80	25	5.5	D
1.a.1.a	80	25	5.5	A
2.a.1.a	80	25	5.5	B
3.a.1.a	30	25	5.5	B
4.a.1.a	80	25	5.5	D
5.a.1.a	55	25	5.5	C
6.a.1.a	80	25	5.5	D
Wash -5	30	25	6.5	A
Wash -6	30	25	6.5	D
46.a.1.a	30	35	7.5	A
47.a.1.a	80	35	7.5	B
48.a.1.a	80	35	7.5	C
49.a.1.a	30	35	7.5	C
Wash-37	30	35	7.5	A
Wash-38	30	35	7.5	B

Fusion AE writes the LC methods, as well as column Conditioning (wash methods) between different test buffers

Less than 48 hours to run this set!

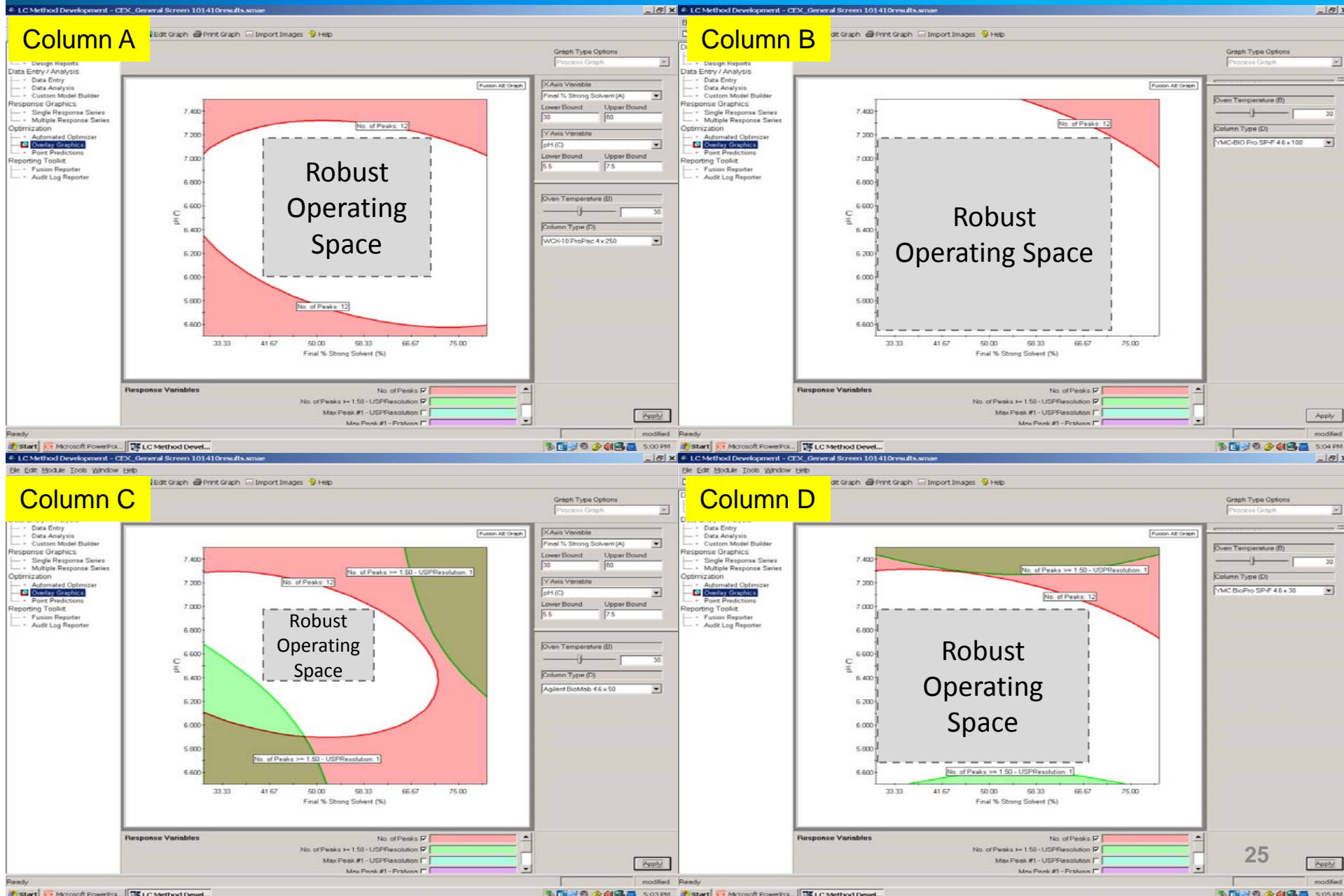
40 wash runs included

Example of CEX-HPLC Peak Profiles from Fusion AE Screening Runs



Run conditions are similar but not identical due to DOE

Optimized Method Selected from 1 DOE sequence with 49 runs



Fusion AE's Automatic Optimizer Output

File Edit Module Tools Window Help

Optimize Responses Export Optimizer Answers Import Images Help

Design of Experiments

- Create a Design
- Design Reports

Data Entry / Analysis

- Data Entry
- Data Analysis
- Custom Model Builder

Response Graphics

- Single Response Series
- Multiple Response Series

Optimization

- Automated Optimizer**
- Overlay Graphics
- Point Predictions

Reporting Toolkit

- Fusion Reporter
- Audit Log Reporter

Optimization Reports

Optimization Search Results

No. of Peaks	Maximize	18.37	18.17	20.57	1.0
No. of Peaks >= 1.50 - USPResolution	Maximize	5.02	3.05	7.49	1.0

Desirability Target = 1.0000
Desirability Result = 0.9549

Optimizer Answer #6: 4 of 41 Solution Searches

Study Variable Data

Study Variable Name	Optimizer Answer Level Setting
Final % Strong Solvent	30.00
Oven Temperature	33.0
pH	6.831
Column Type	4.6 x 100

Predicted Response Data

Response Variable Name	Target	Optimizer Answer Predicted Response	-2 Sigma Confidence Limit	+2 Sigma Confidence Limit	Relative Rank
No. of Peaks	Maximize	18.35	16.24	20.47	1.0
No. of Peaks >= 1.50 - USPResolution	Maximize	5.03	3.06	7.50	1.0

Desirability Target = 1.0000
Desirability Result = 0.9540

Optimizer Answer #7: 7 of 41 Solution Searches

Study Variable Data

Study Variable Name	Optimizer Answer Level Setting
Final % Strong Solvent	30.00
Oven Temperature	33.4
pH	7.139
Column Type	4.6 x 100

Ready

Microsoft PowerPo... LC Method Devel... modified

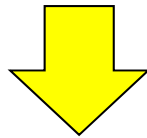
5:18 PM

5:18 PM

Column B: 4.6 x 100 mm

Fusion AE: Refinement/Optimization Experiments

- Select Buffer Strength
- Select Flow Rate
- Compare Detection Wavelengths
- Test Effects of Additives
- Test Narrower Buffer pH screen
- Test Narrower Column Temperature

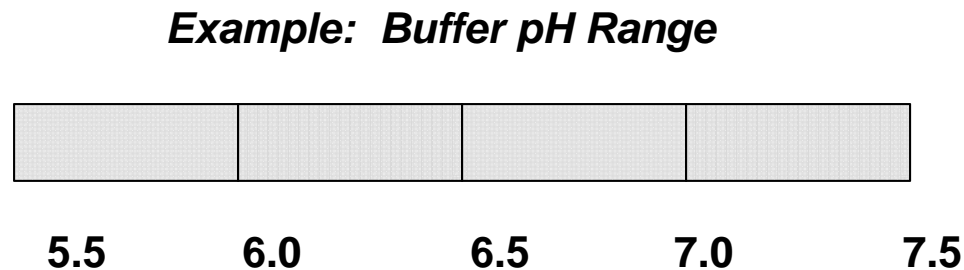


**Robustness Evaluation with
Selected Method Parameters**

Fusion AE: From Screening Design to Optimization Design

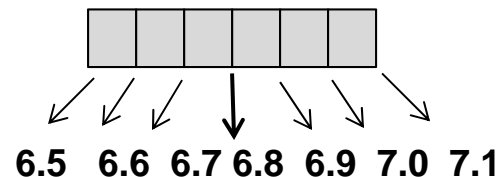
Screening experiments do not have the data density required to develop models which can predict a design space and corresponding safe operating ranges with the required precision

Screening Design
•Wider Range
•Less Intervals



pH 6.78 defined as optimum pH from initial screening

Optimization Design
•Narrow Range
•More Intervals



Data Density Drives Model Precision

- Design of Experiments
 - Create a Design
 - Design Reports
- Data Entry / Analysis
 - Data Entry
 - Data Analysis
- Response Graphics
 - Single Response Series
 - Multiple Response Series
- Optimization
 - Numerical Optimizer
 - **Graphical Optimizer**
 - Point Predictions
- Reporting Toolkit
 - Fusion Reporter
 - Audit Log Reporter

Reports Delete Report

Process Graph 1

Graph Settings

Name	Units	Lower Bound	Upper Bound	Pointer Coordinate
X Final % Strong Solvent	%	30.0	80.0	
Y Oven Temperature	°C	25.0	35.0	

pH

Column **Operating Ranges** Column A

Operating Space Settings

Operating Ranges

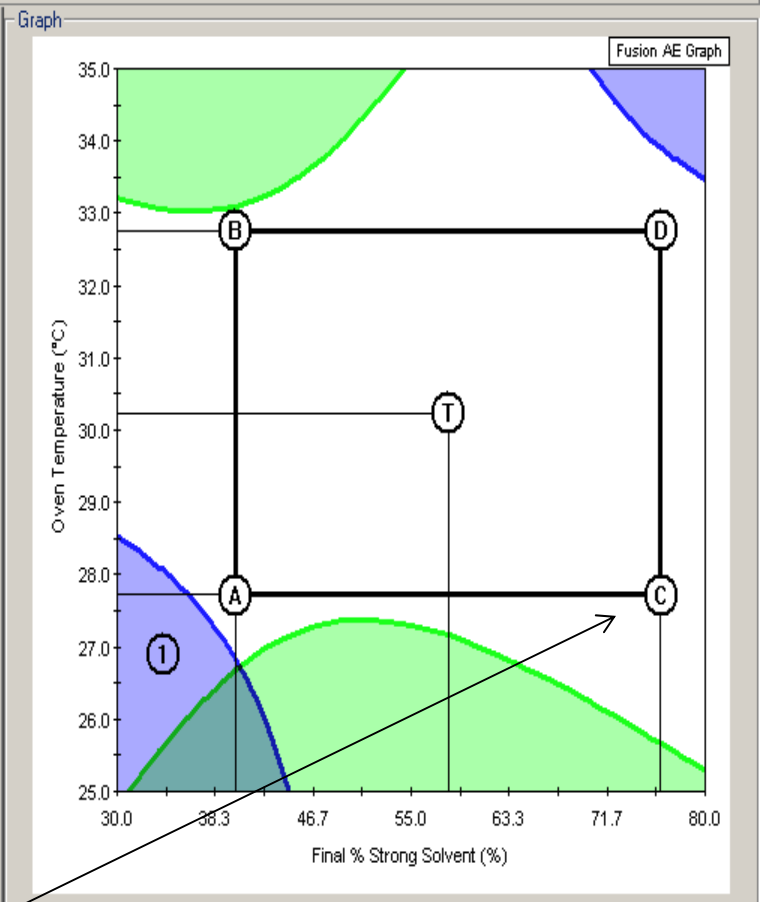
Variable	Lower Bound	Upper Bound	Center Point
Final % Strong Solvent	40.1	76.2	58.1
Oven Temperature	27.7	32.8	30.2

Verification Runs Show Verification Run Labels

	Point	Run ID	Final % Strong Solvent	Oven Temperature	pH	Column Type
1	A	Process_Graph_	40.1	27.7	6.8	
2	B	Process_Graph_	40.1	32.8	6.8	
3	C	Process_Graph_	76.2	27.7	6.8	
4	D	Process_Graph_	76.2	32.8	6.8	
5	T	Process_Graph_	58.1	30.2	6.8	
6	1	Process_Graph_	34.0	26.9	6.8	

Verification Runs

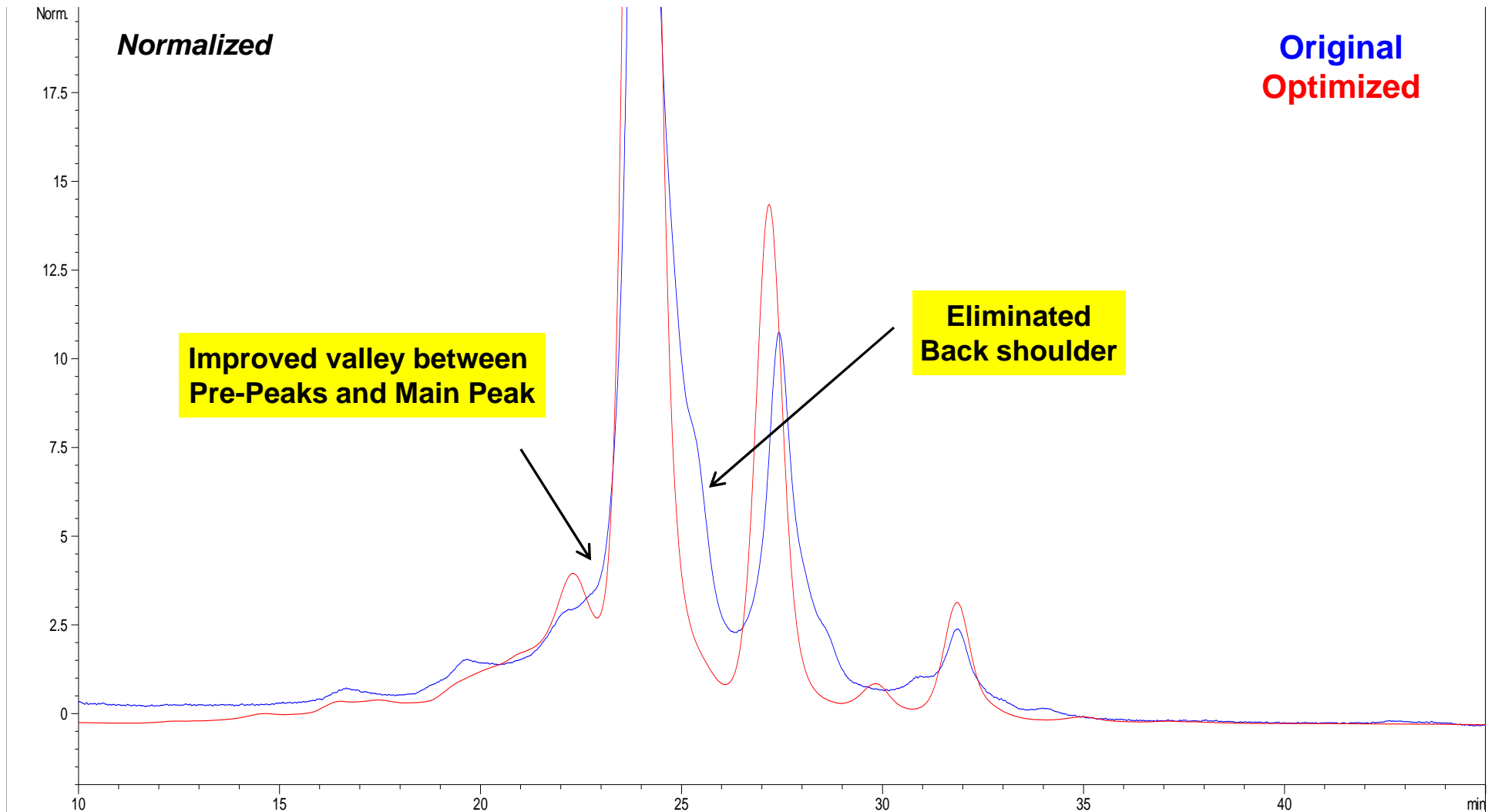
Validation Status: Your settings are valid.



Response Settings

Name	Goal	Lower Bound	Upper Bound	Target Predictions	Poin Predic
<input checked="" type="checkbox"/> No. of Peaks	Maximize	10		11	
<input type="checkbox"/> Max Peak #1 - RetentionTime	---	---	---	---	---
<input checked="" type="checkbox"/> Max Peak #1 - PctArea	Maximize	68.0		73.7	

CEX-HPLC Method Improvement Using Fusion AE System



Initial and Final Method Operating Parameters' Settings

Operating Parameter	Preliminary Screening Recommendation	Final Optimized Method Settings
Gradient: % B/min	0.80% B/min	0.75% B/min
Column Temp(°C)	32	30
Buffer pH	6.78	6.80

Fusion AE System Evaluation: Conclusions

Advantages:

- Fast approach to method development/test method robustness evaluations
- Automated- Set up design and walk away
- Establish knowledge base of variable interactions
- Experiments performed following DOE and QbD principles-improvement over One-Factor-at-a time analytical method development approach
- Visual results for variable interactions and robustness studies
- Visual results of operating space
- Generates statistical results to assess method robustness

Disadvantages:

- New software to learn
- Software/hardware costs

Chromatographers generally are unwilling to become statisticians!

Acknowledgements

Amgen Thousand Oaks

Jennifer Liu

Tamer Eris

Oleg Borisov

Izydor Apostol

S-Matrix

Graham Shelver

Richard Verseput