S-Matrix®

Fusion QbD®

Advanced Chromatography Modeling and Robust Method Optimization

S-Matrix Corporation
www.smatrix.com

Key Differentiators – Method Optimization

- ✓ Ease of Experiment Setup
- ✓ Simple Chromatogram Integration
- ✓ Powerful UV & MS Based Peak Tracking
- ✓ Instant One-click Modeling – Any Results
- ✓ Complete Analysis Results Reporting
- ✓ Integrated Robustness Simulation
- ✓ Complete Multi-response Optimization
- ✓ Multi-dimensional Visualization Graphics

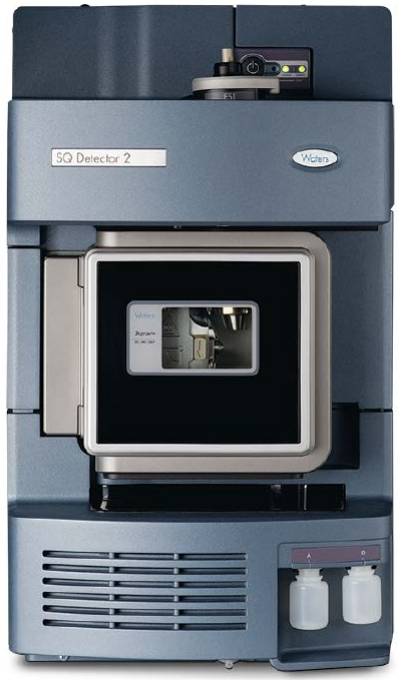
Flexible Data Modeling

Flexible Modeling of **ALL** important chromatographic performance properties **for each peak in the chromatogram**: Examples include, but not limited to:

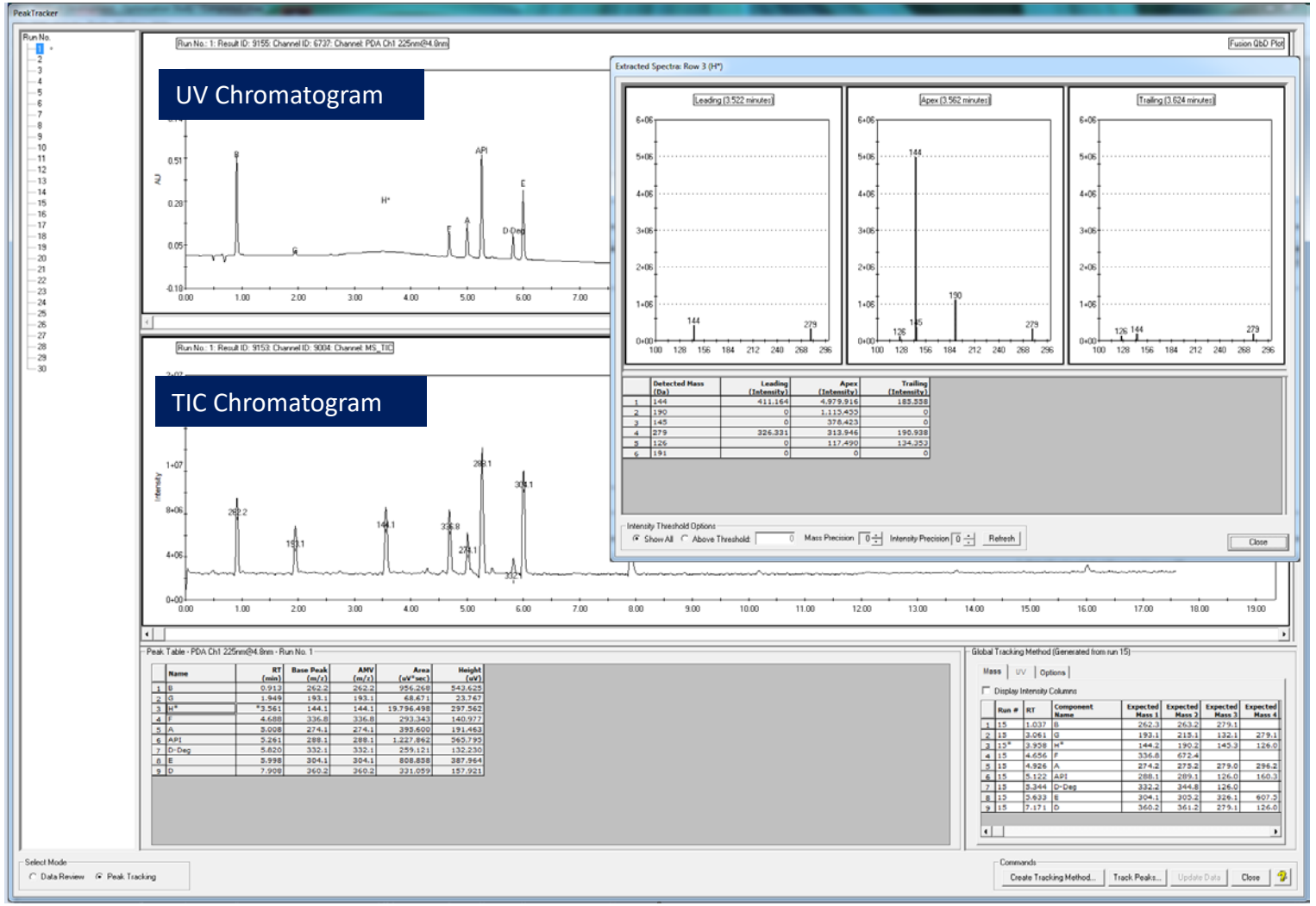
- Retention Time
- K Prime
- Resolution
- Tailing
- Area, Area %, % RSD, etc.
- S/N Ratio
- Large Molecule Metrics – e.g., Retention Time Difference, P/V Ratio, …

PeakTracker™ – UV & MS Data Based Tracking

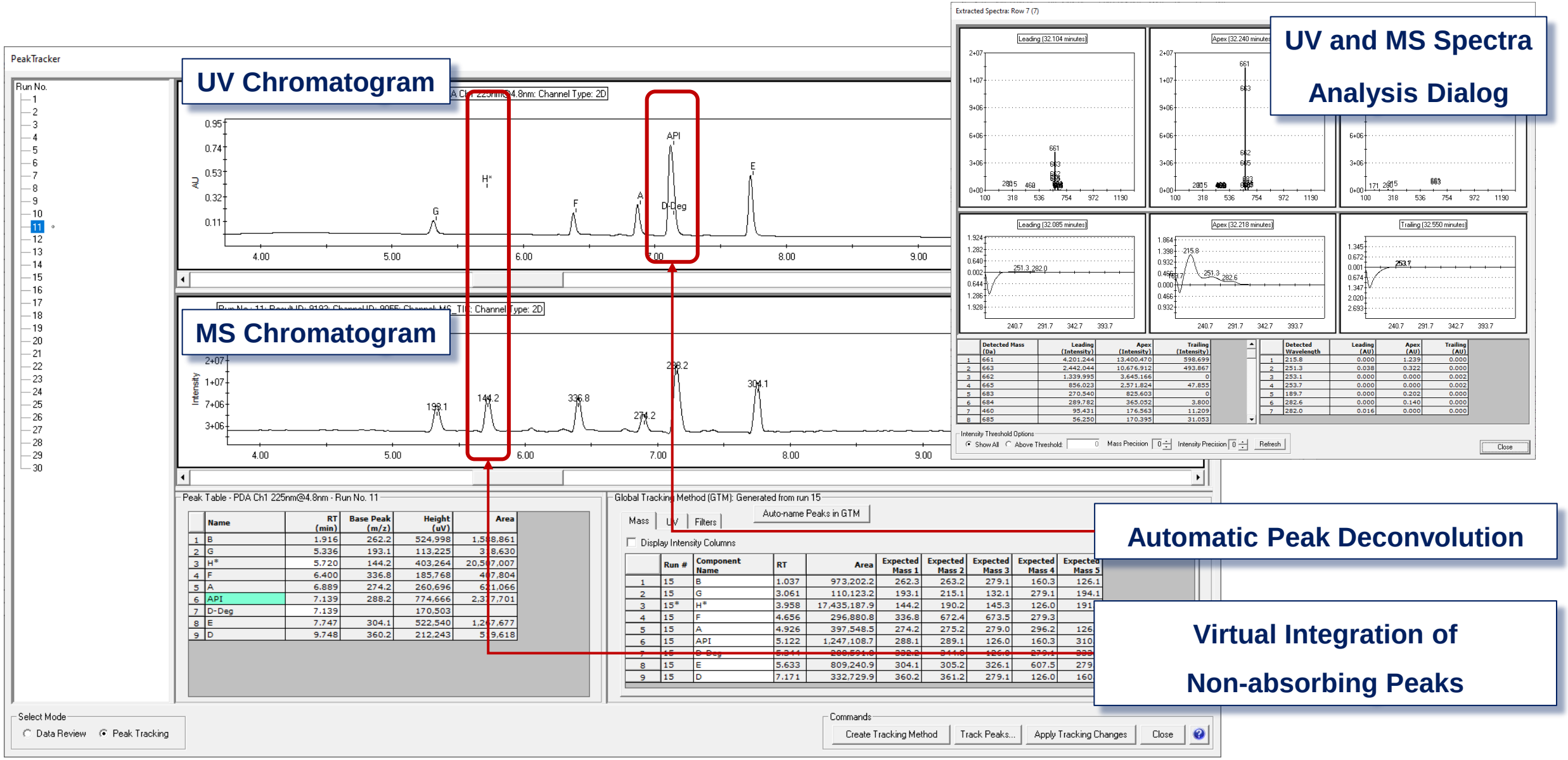
QDa



SQD & SQD 2

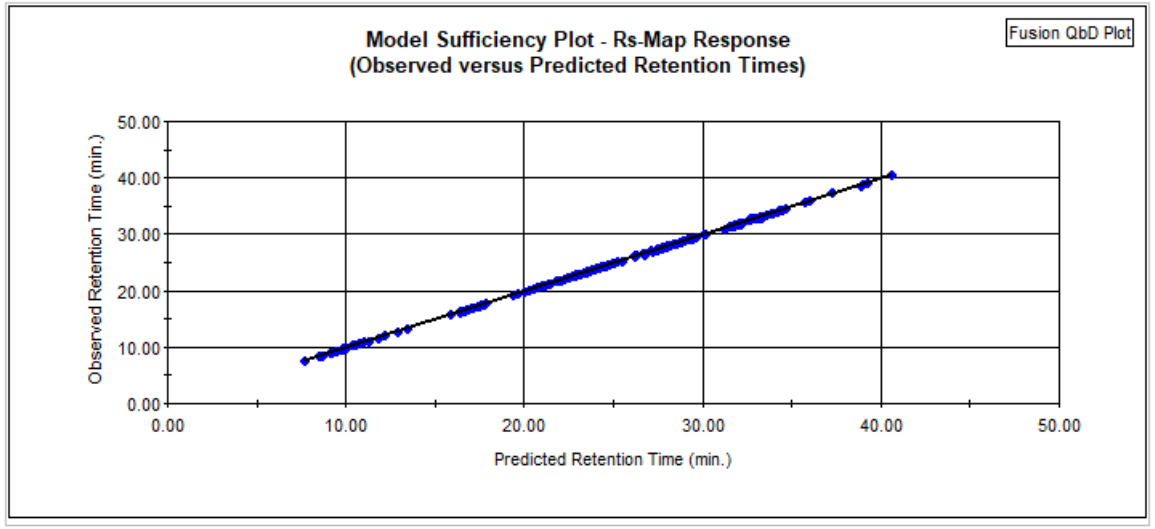


PeakTracker™ – UV & MS Data Based Tracking



Model Validation – Model Fit Metrics

Analysis Summary Report - Rs-Map Response



Prediction Equation

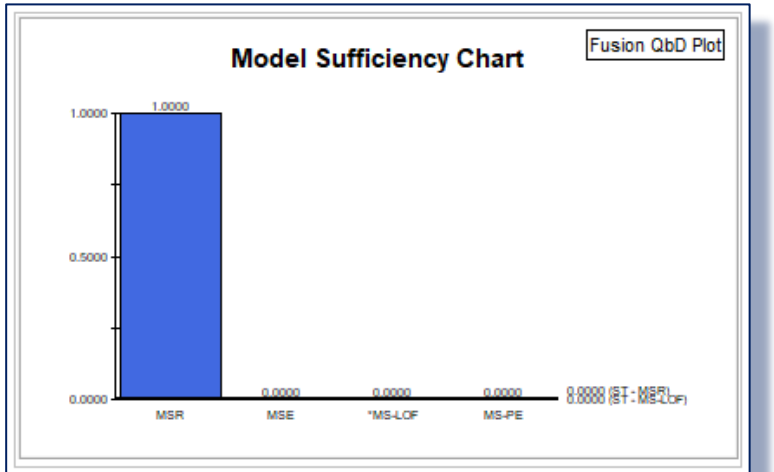
Observed Retention Time = -0.00068 + 1.00008 * Predicted Retention Time

General Regression Statistics

Error Statistic	Computed Value
R Square	0.9999
Adj. R Square	0.9999
Residual MSE	0.0033
Standard Error (+/-)	0.0572

Outlier Analysis Results

Run No.	Compound Name	R.T. Observed (min)	R.T. Predicted (min)	Residual
17	5	32.907	32.642	0.264



*** - The model LOF is statistically significant (P-value < 0.0500)**

Regression Statistic	Computed Value	Scaled Value
R Square	1.0000	---
Adj. R Square	1.0000	---
Model Error (+/- 1 Std. Dev.)	0.0000	---
Error %	0.0000	---
Untransformed Model Error (+/- 1 Std. Dev.)	0.0208	---
Expt. Error (+/- 1 Std. Dev.)	0.0000	---
Untransformed Expt. Error (+/- 1 Std. Dev.)	0.0026	---
MSR	0.0001	1.0000
MSE	0.0000	0.0000
MSR/MSE F-ratio	140,913.8456	---
MSR Significance Threshold	0.0000	0.0000
*MS-LOF	0.0000	0.0000
MS-PE	0.0000	0.0000
MS-LOF Significance Threshold	0.0000	0.0000

Model Validation – Predicted Best Conditions

Name: Administrator
Company: S-Matrix Corporation
Project: Project 1
Date: 26 MAR 2024 07:43:20 PDT [UTC-07:00]

Point Predictions: Point Predictions 1

Predicted Point - 17

Variable Settings

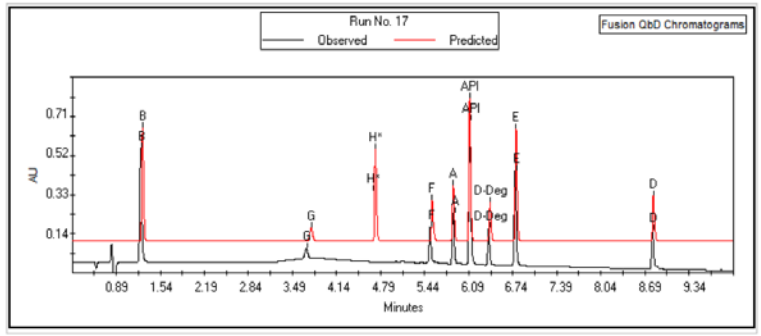
Name	Level Setting	Units
Pump Flow Rate	0.450	mL/min
Gradient Time	16.0	min
Oven Temperature	45.0	°C
pH	3.90	*

Rs-Map Predicted Results

Response Name	Predicted Result	Calculated Result
A - ResolutionW50	6.079	6.630
API - ResolutionW50	4.358	4.392
D-Deg - ResolutionW50	5.502	5.172
E - ResolutionW50	7.046	7.294

Predicted Results

Response Name	Predicted Result	Observed Result
B - RetentionTime	1.27	1.26
API - USPTailing	1.35	1.36

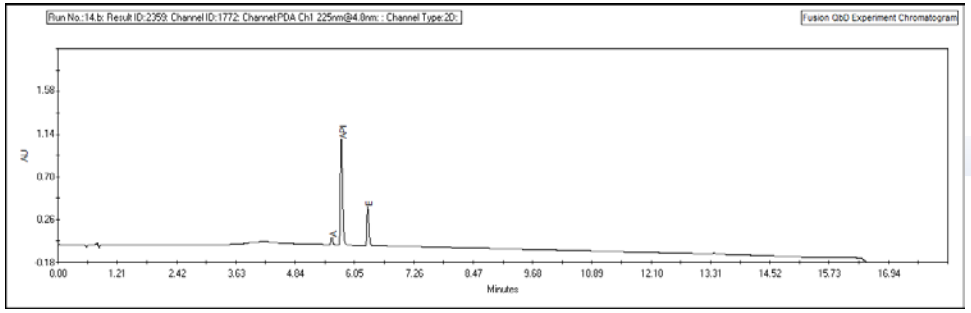


1 of 5

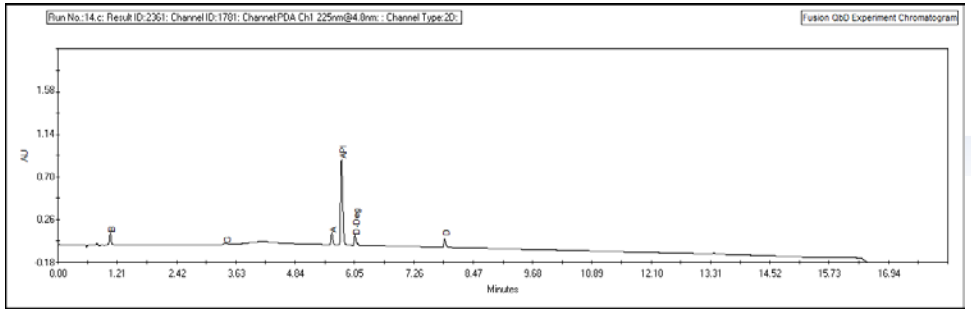
Observed and Predicted Results and Chromatogram for Run #17 – the experiment run with level settings closest to the predicted optimum conditions.

Full Support for Forced Degradation Studies

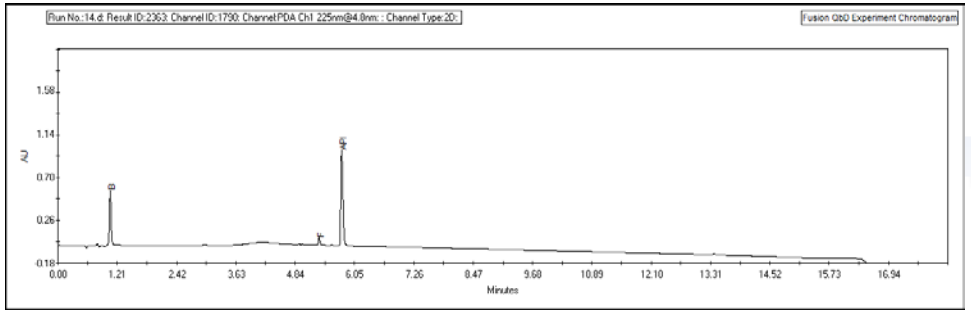
Acid Degradation Path

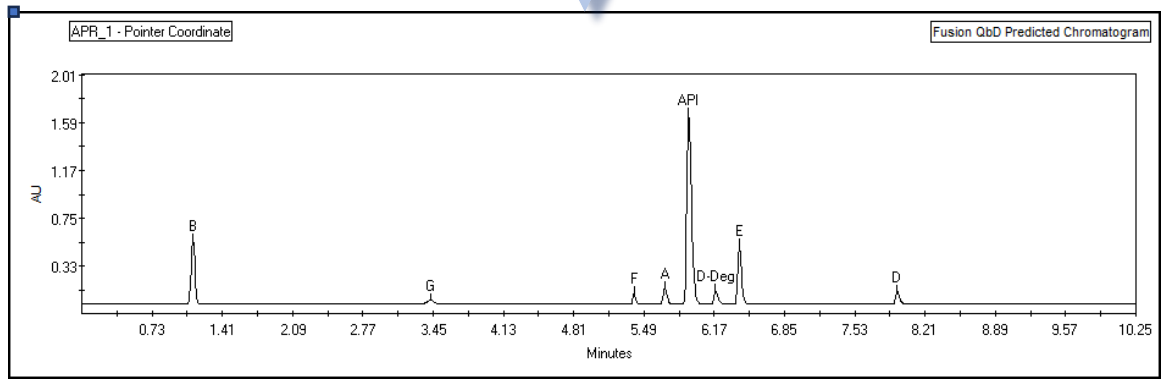


Base Degradation Path



Peroxide Degradation Path



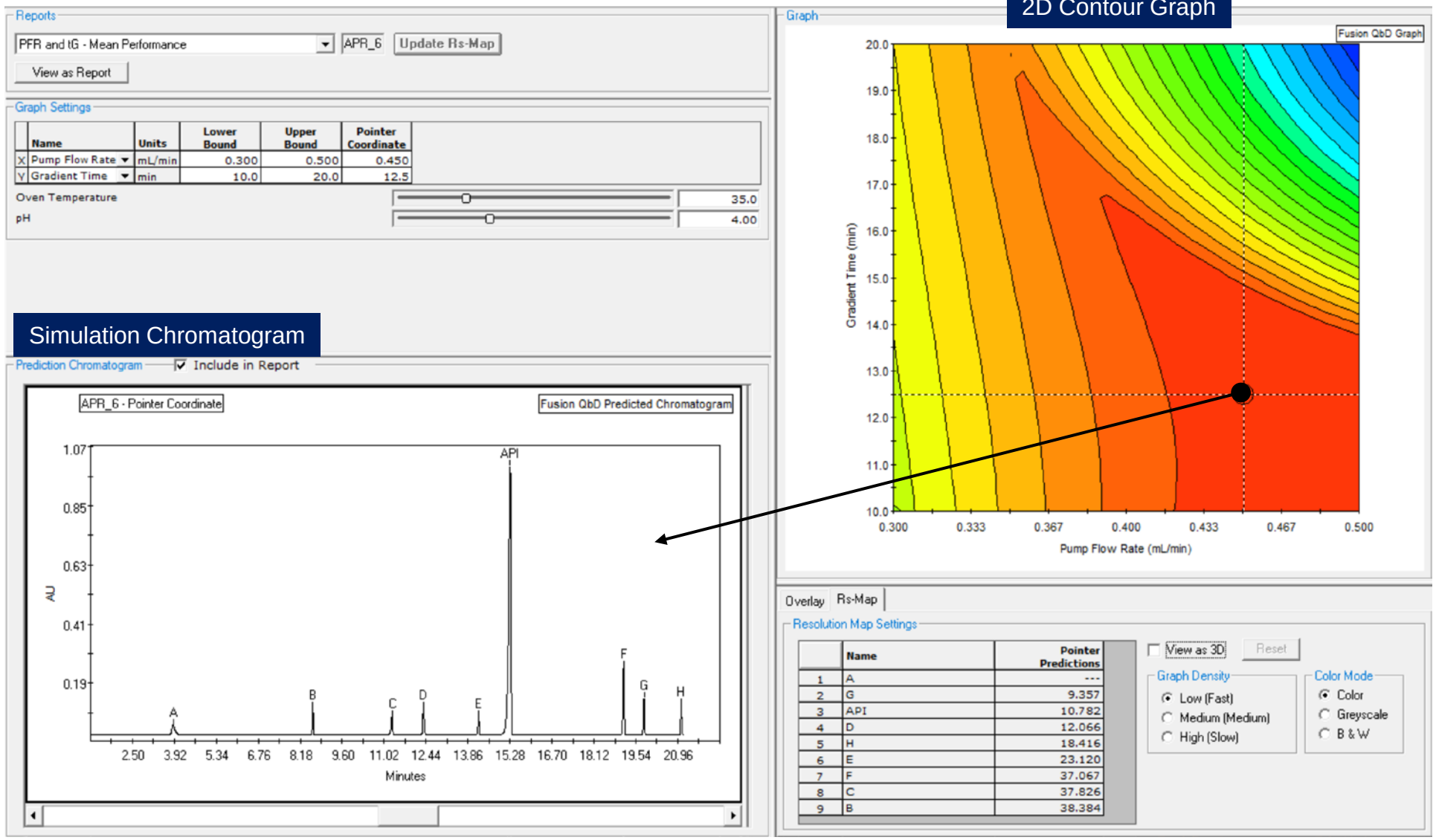


Fusion QbD Composite Chromatogram

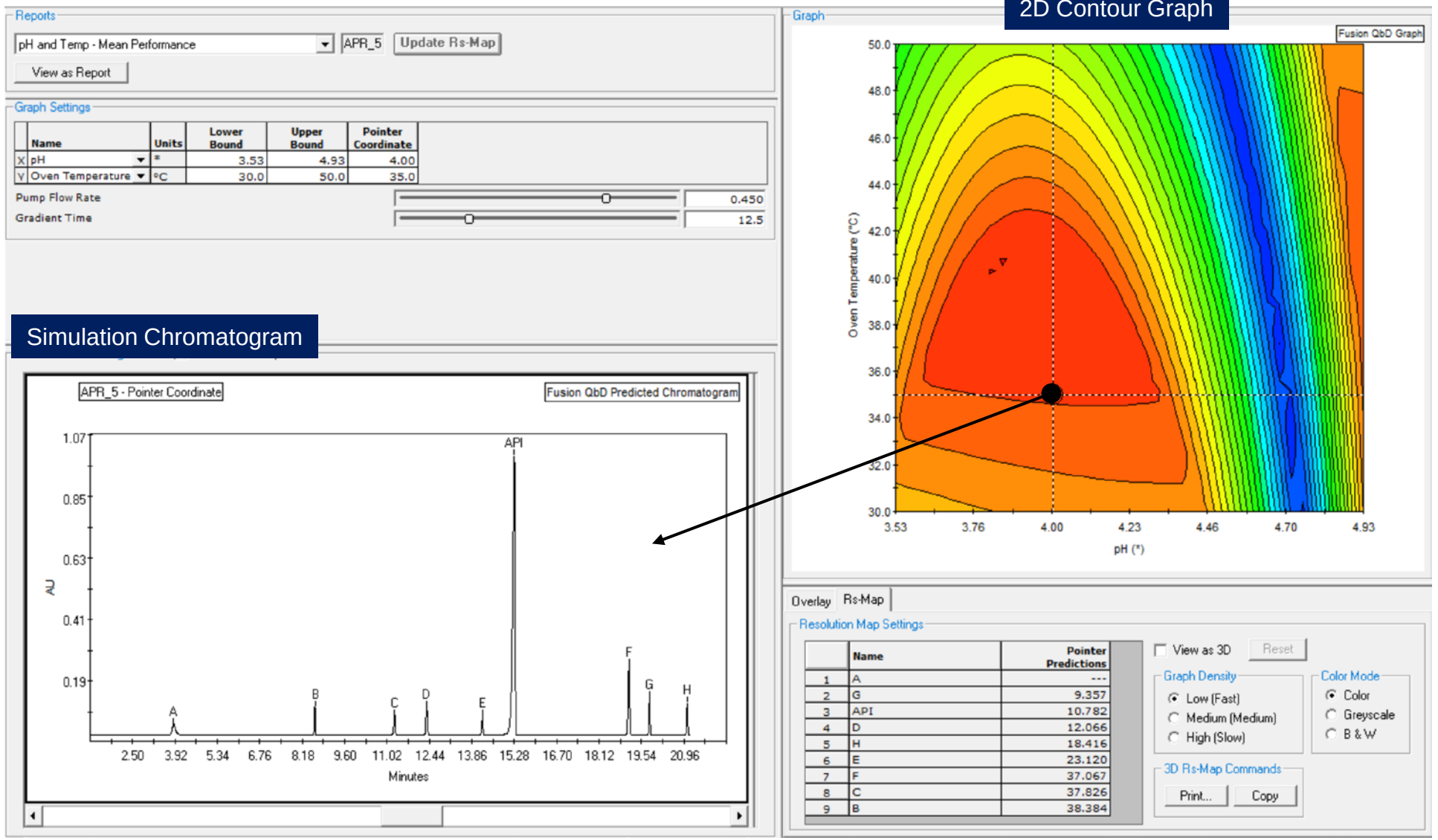
2D Resolution Map – Flow Rate and t_G

2D Contour Graph

Simulation Chromatogram



Traditional 2D Resolution Map – pH and Temp

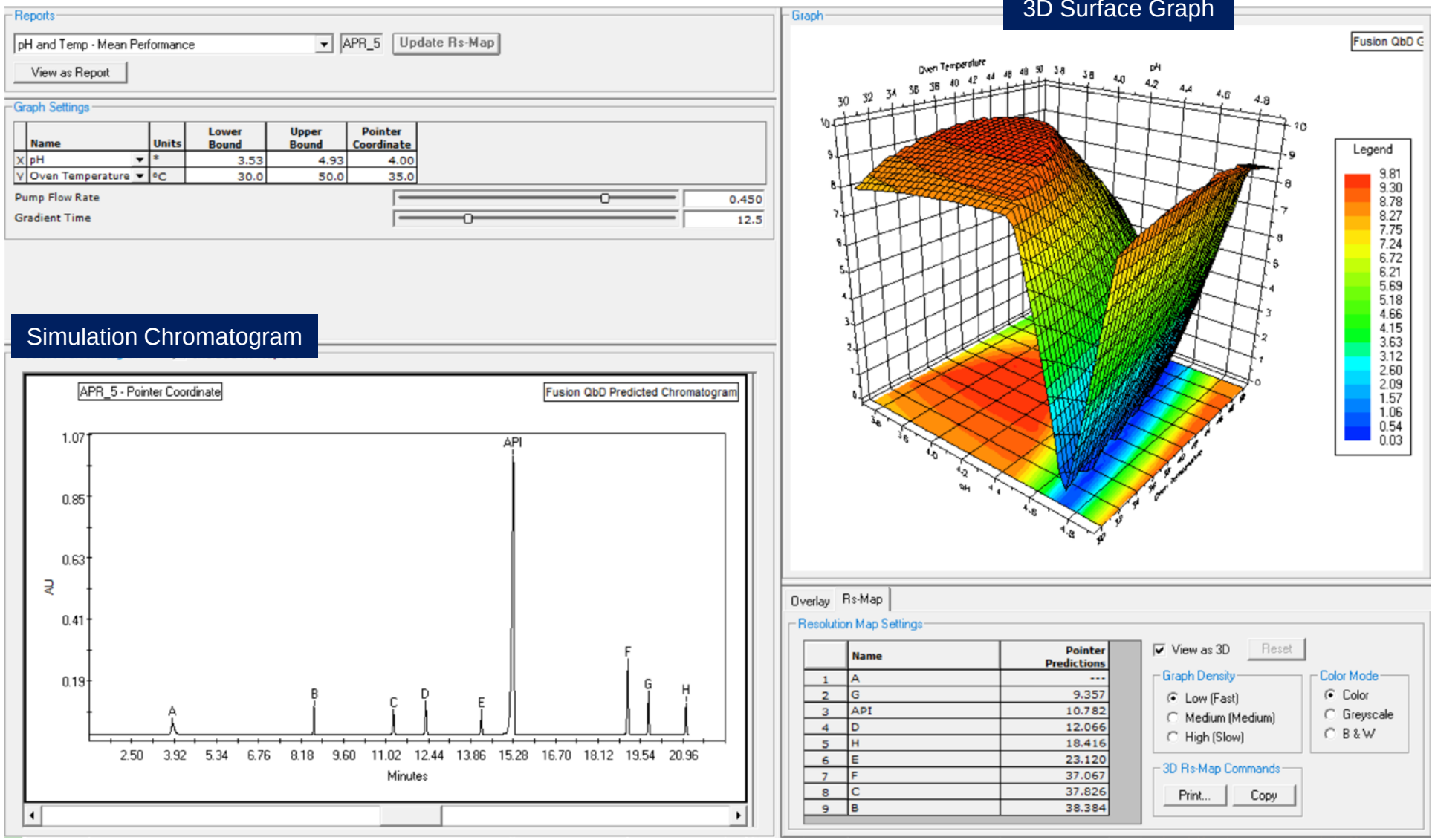


	Name	Pointer Predictions
1	A	---
2	G	9.357
3	API	10.782
4	D	12.066
5	H	18.416
6	E	23.120
7	F	37.067
8	C	37.826
9	B	38.384

Rotatable 3D Resolution Surface – pH and Temp

3D Surface Graph

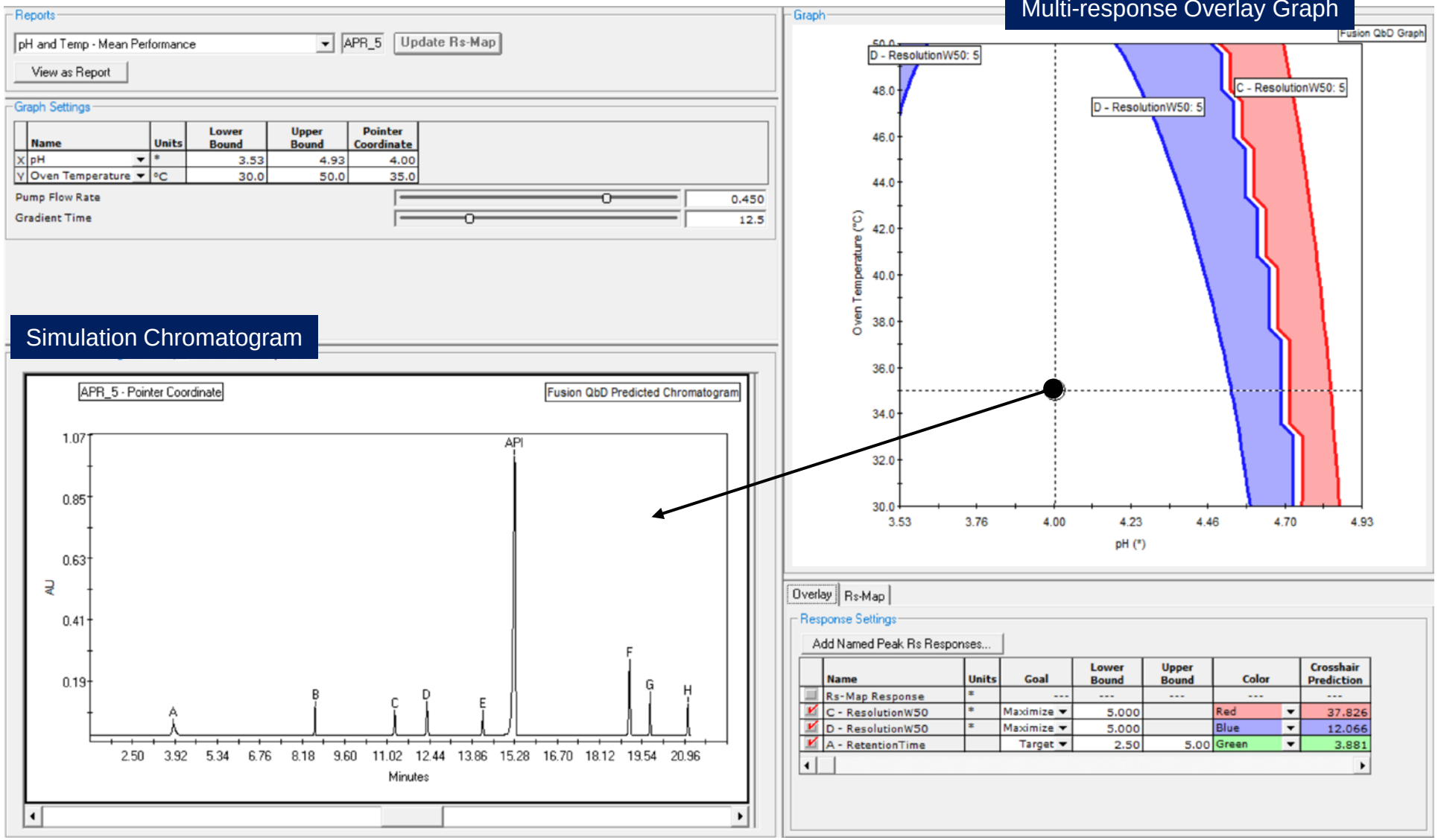
Simulation Chromatogram



Mean Performance Overlay – pH and Temp

Multi-response Overlay Graph

Simulation Chromatogram

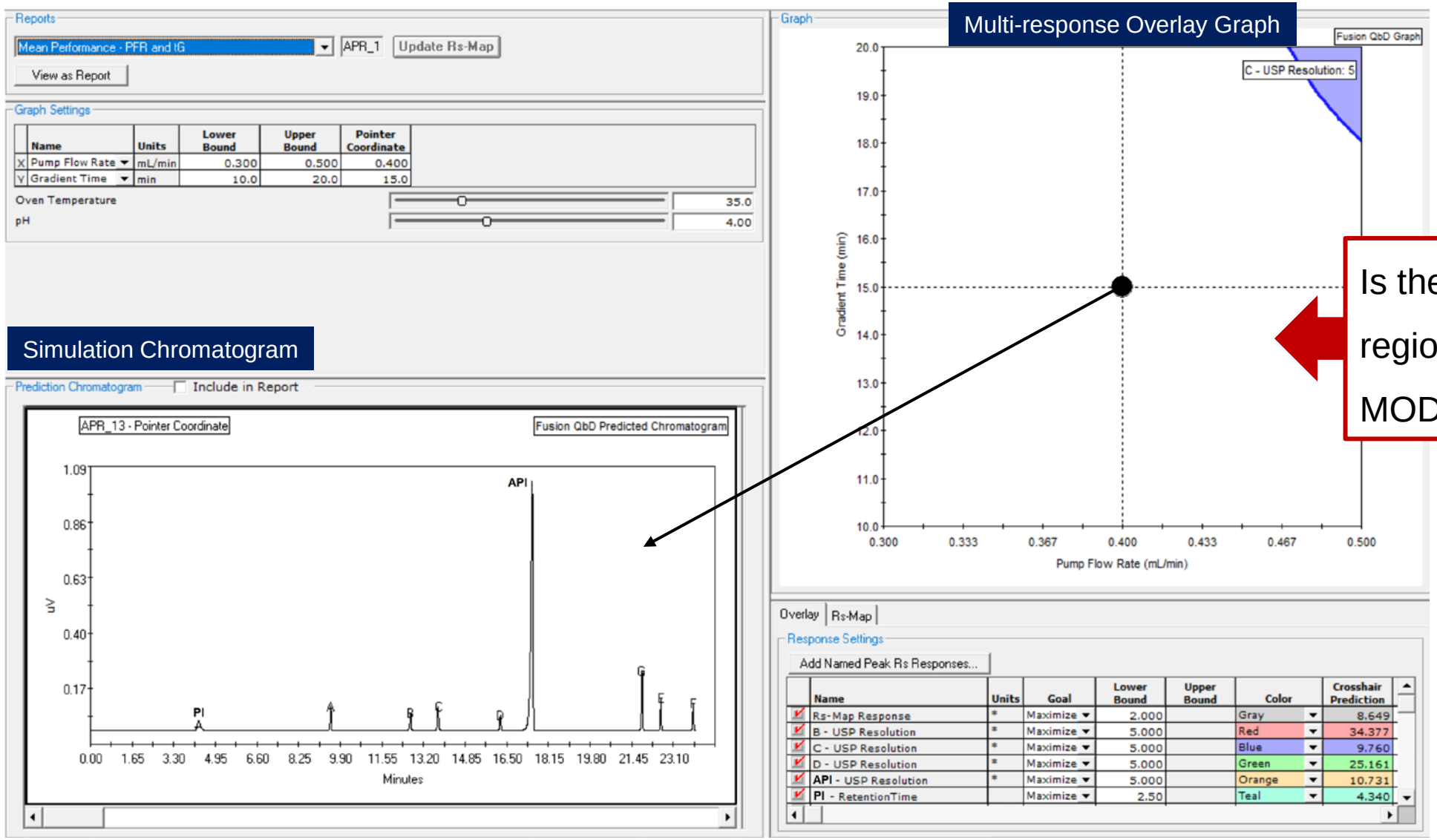


Mean Performance Overlay – Flow Rate and t_G

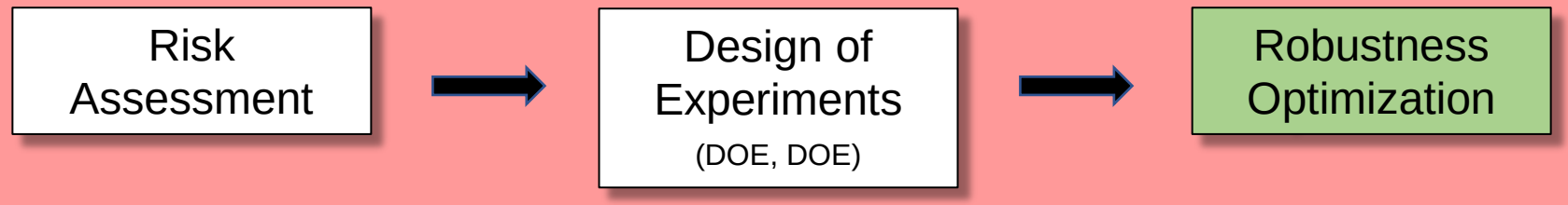
Multi-response Overlay Graph

Simulation Chromatogram

Is the unshaded region a robust MODR?



Method Operable Design Region



ICH Q14

For most procedures, robustness evaluation is conducted during development. **If the evaluation of robustness was already conducted during development, it does not need to be repeated during validation** as discussed in ICH Q2. (*Pg. 5*)

USP <1220>

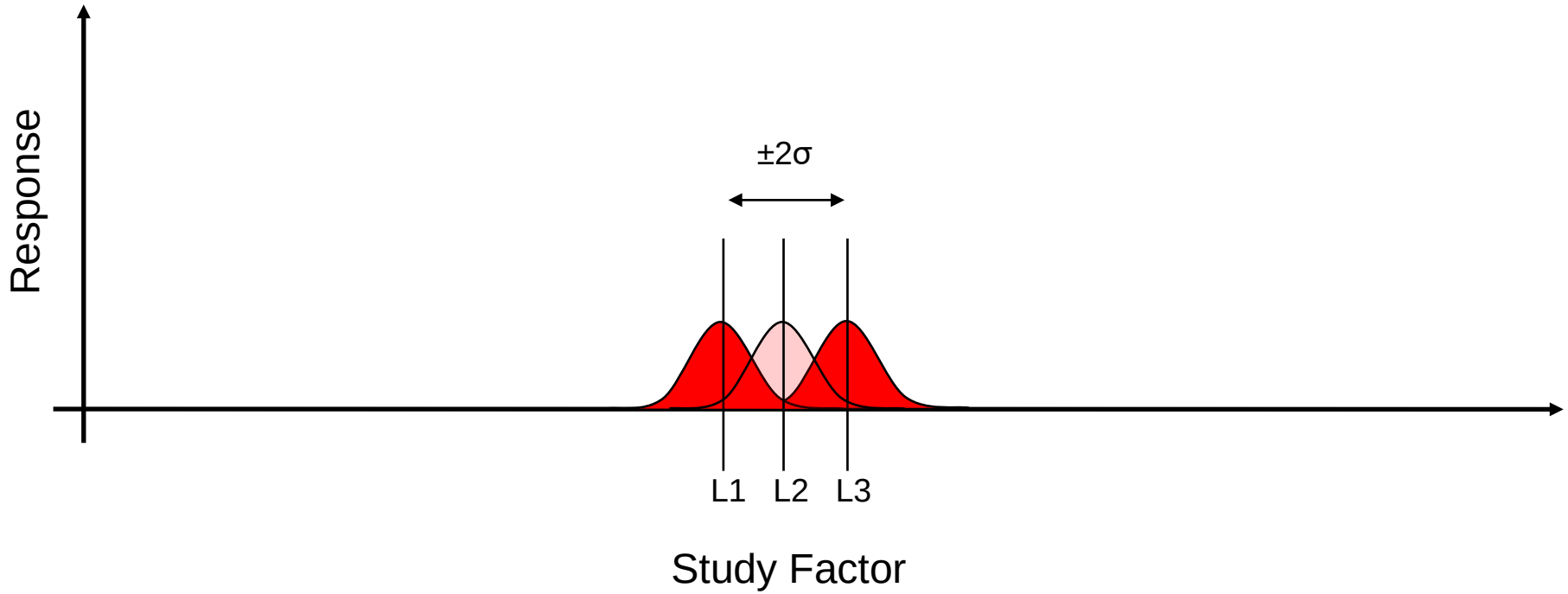
In some cases, it is helpful to demonstrate robustness of the procedure by developing models that describe the effect of parameters on the performance of the procedure, … **This knowledge also enables the determination of robust operation regions for procedure parameters and, if desired, a method operable design region (MODR)**. (*Pg. 8*)

Hidden Flaw #1 in Traditional Robustness

- Traditional Range is Within Setpoint Error Range (Driven by Traditional Data Analytics*).

Small Range = Poor Effects Estimation

- The most likely result is that the study factor effects will be **UNDERESTIMATED**.
- **The Result** – methods which are NOT robust will pass the robustness test.

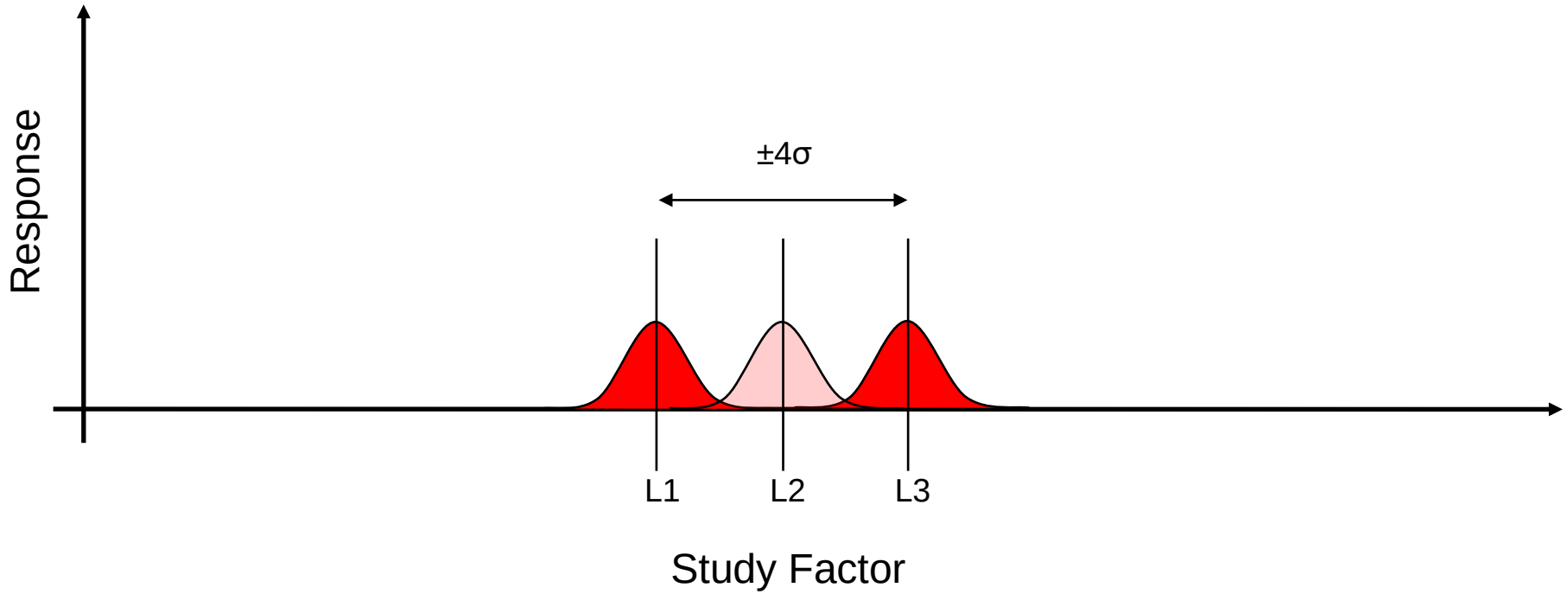


* – Effects Estimation by Method of Averaging.

Large Range = Good Effects Estimation

Minimum Effective Range Between Study Levels is 4σ

(**6σ Recommended**).



Comparative Study Ranges

Factor	Method Setpoint	Traditional Range	QbD-aligned Range
Pump Flow Rate (mL/min)	1.00	±0.05	±0.20
Gradient Time (min) *	15.00	±0.70	±5.00
Temperature (°C)	37.00	±2.00	±5.00
pH (*)	6.80	±0.15	±0.50

Traditional Approach *	
MPC Variation (±2.0%)	Time Variation (±0.70 min)
90.0%/15.0 min = 6.0%/min. • 94.0%/(15.0%/min) = 6.3%/min. • 86.0%/(15.0%/min) = 5.7%/min.	• 90.0%/(6.3%/min) = 14.3 min. • 90.0%/(5.7%/min) = 15.8%/min

Hidden Flaw #2 in Traditional Robustness

True for ALL Software!

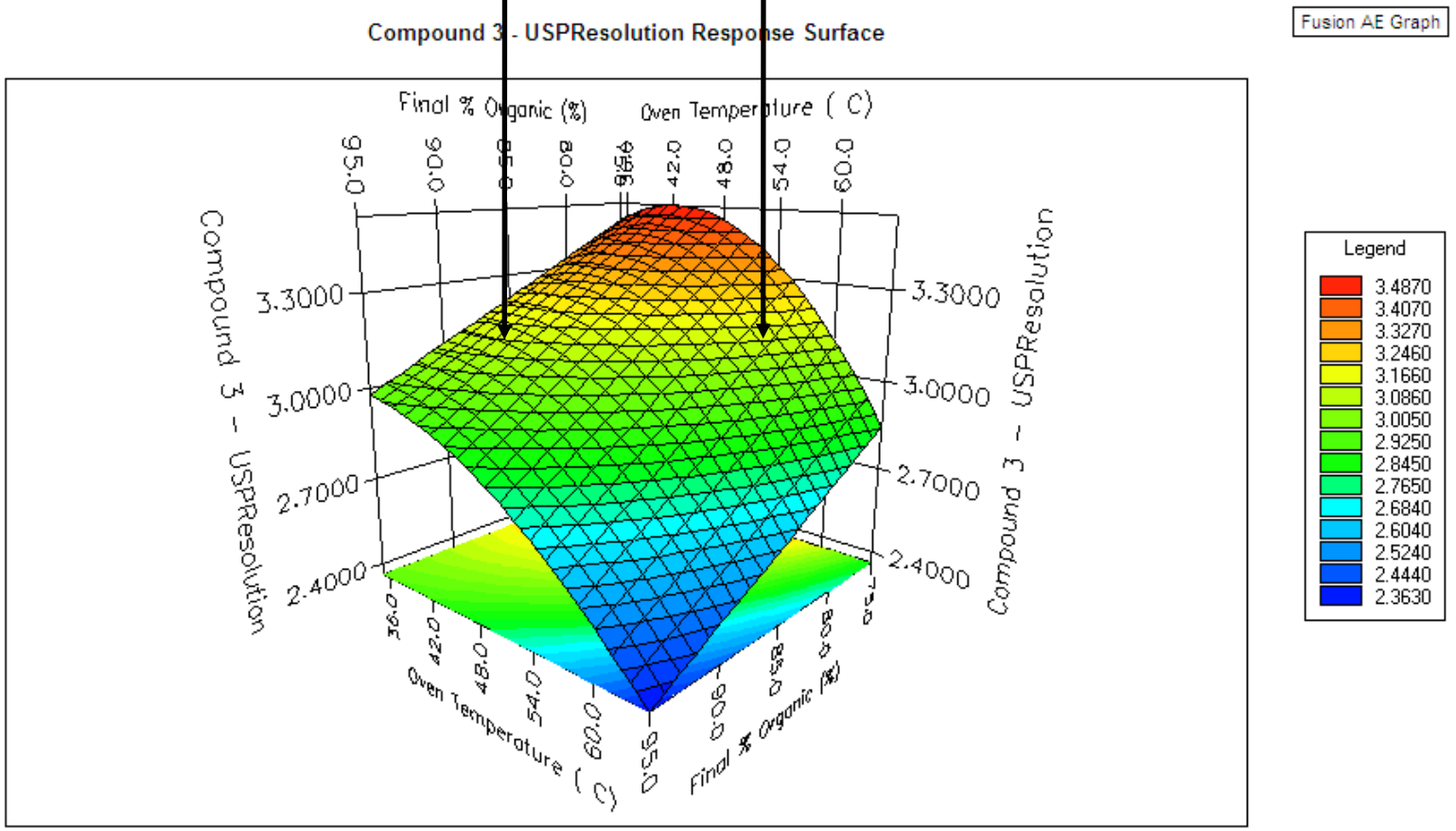
Software models can only directly predict Mean Performance – they cannot predict the robustness of a given method.

Methods A and B:

- **Identical Mean Performance.**
- **Good mean performance ≠ good robustness.**

Method A – Good Robustness

Method B – Poor Robustness



System Suitability and QbD Robustness

System Suitability has two requirements:

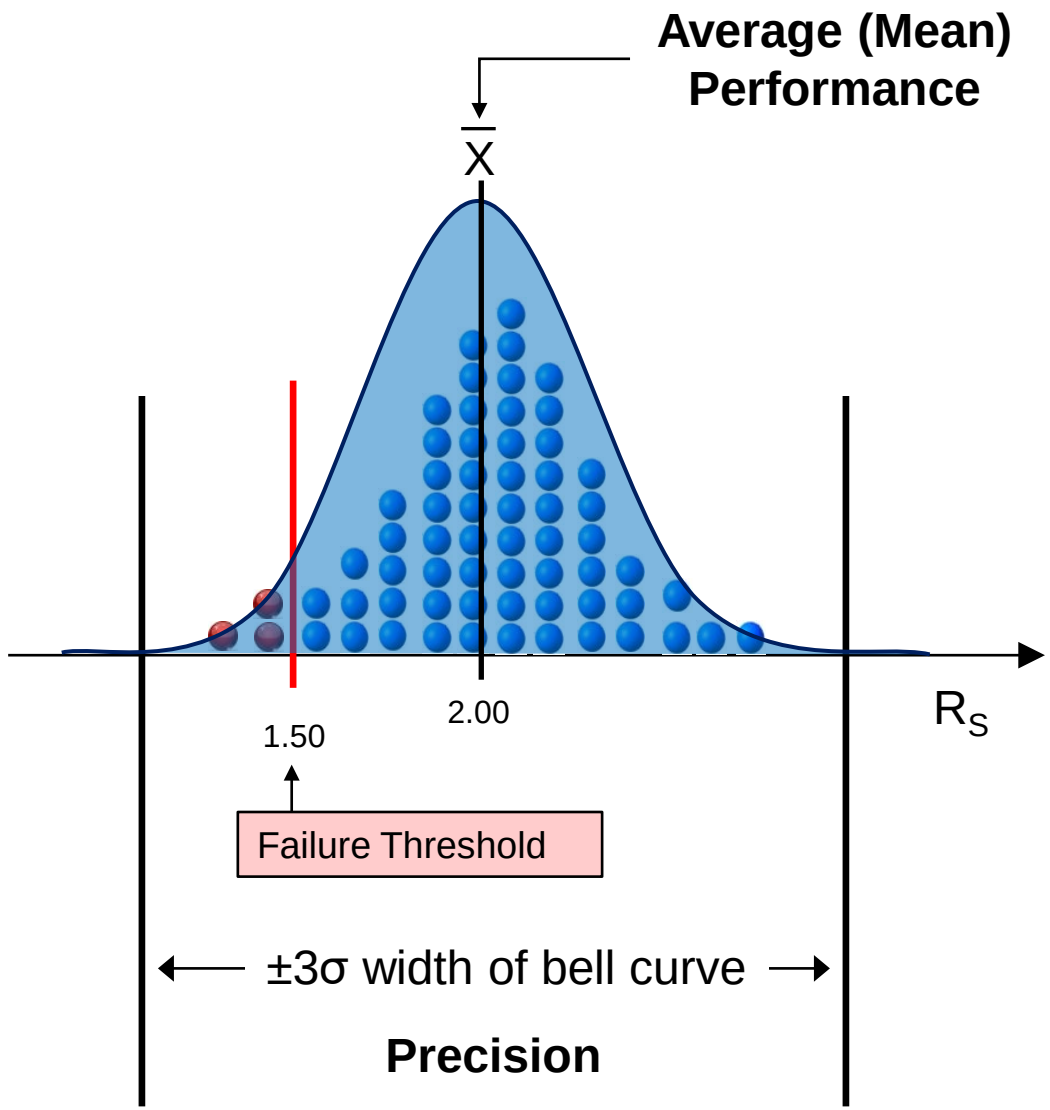
1. Mean (Average) Performance ($\overline{X}$)
2. Precision – % RSD = ($\sigma/\overline{X}$)*100%

Precision is a Robustness Specification

But Models ONLY Predict the Mean Result

NOTE: % RSD is a ±1σ value.

The ±1σ value is defined by the requirement that edges of failure be a minimum distance of ±3σ from the $\overline{X}$ value.



Monte Carlo Robustness Simulation

Wizard Page 1 – Define Maximum Expected Variation in Study Parameters

Variable Settings

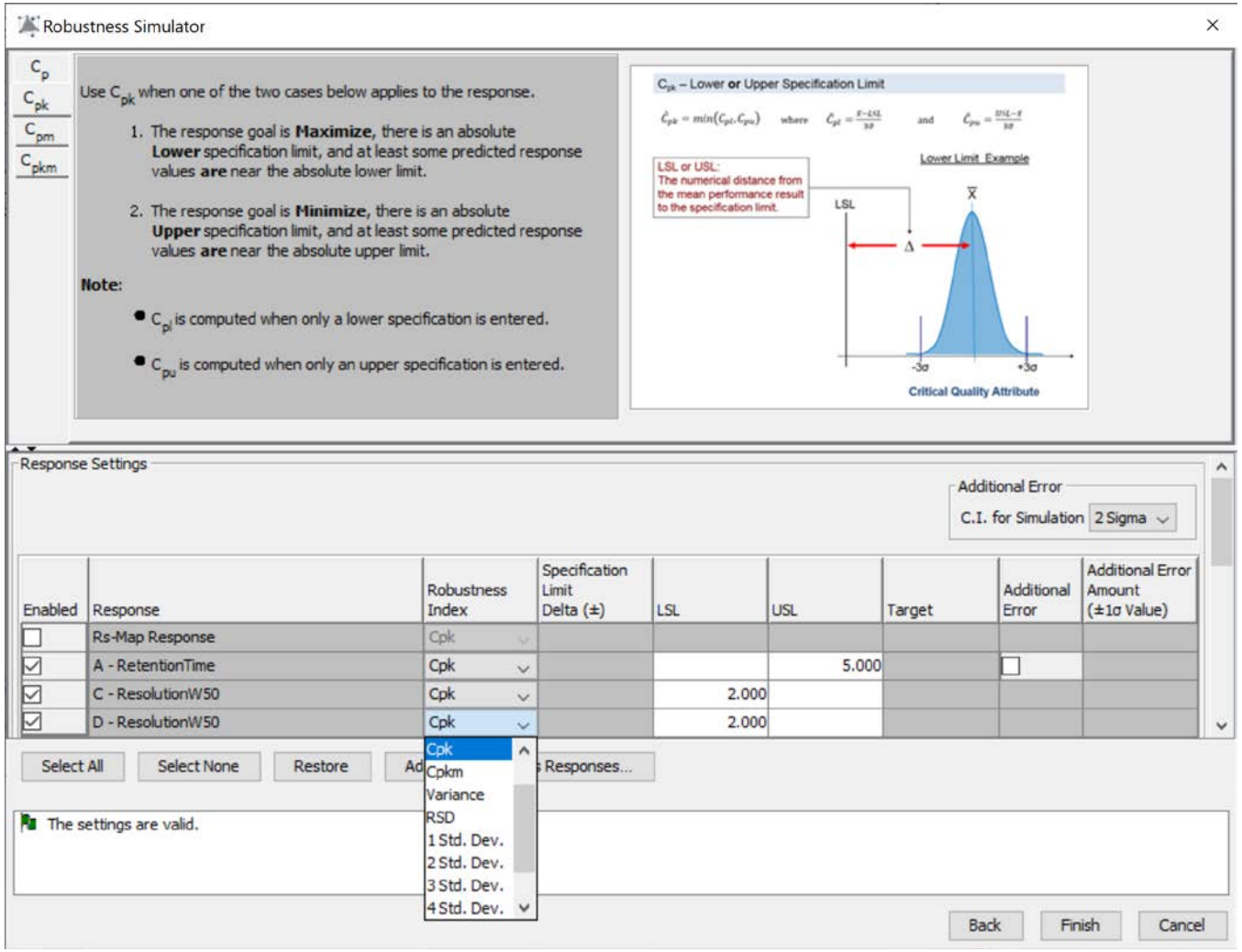
Enabled	Experiment Variable	Units	Maximum Expected Variation (±3σ Value)
☑	Pump Flow Rate	mL/min	0.025
☑	Oven Temperature	°C	3.0
☑	pH	*	0.20
☑	Mobile Phase Composition (MPC)*	%	2.0

Go beyond development LC system

expected variation in QC lab across LC systems during routine use.

Monte Carlo Robustness Simulation

Wizard Page 2 – Define Failure Mode and Spec Limits

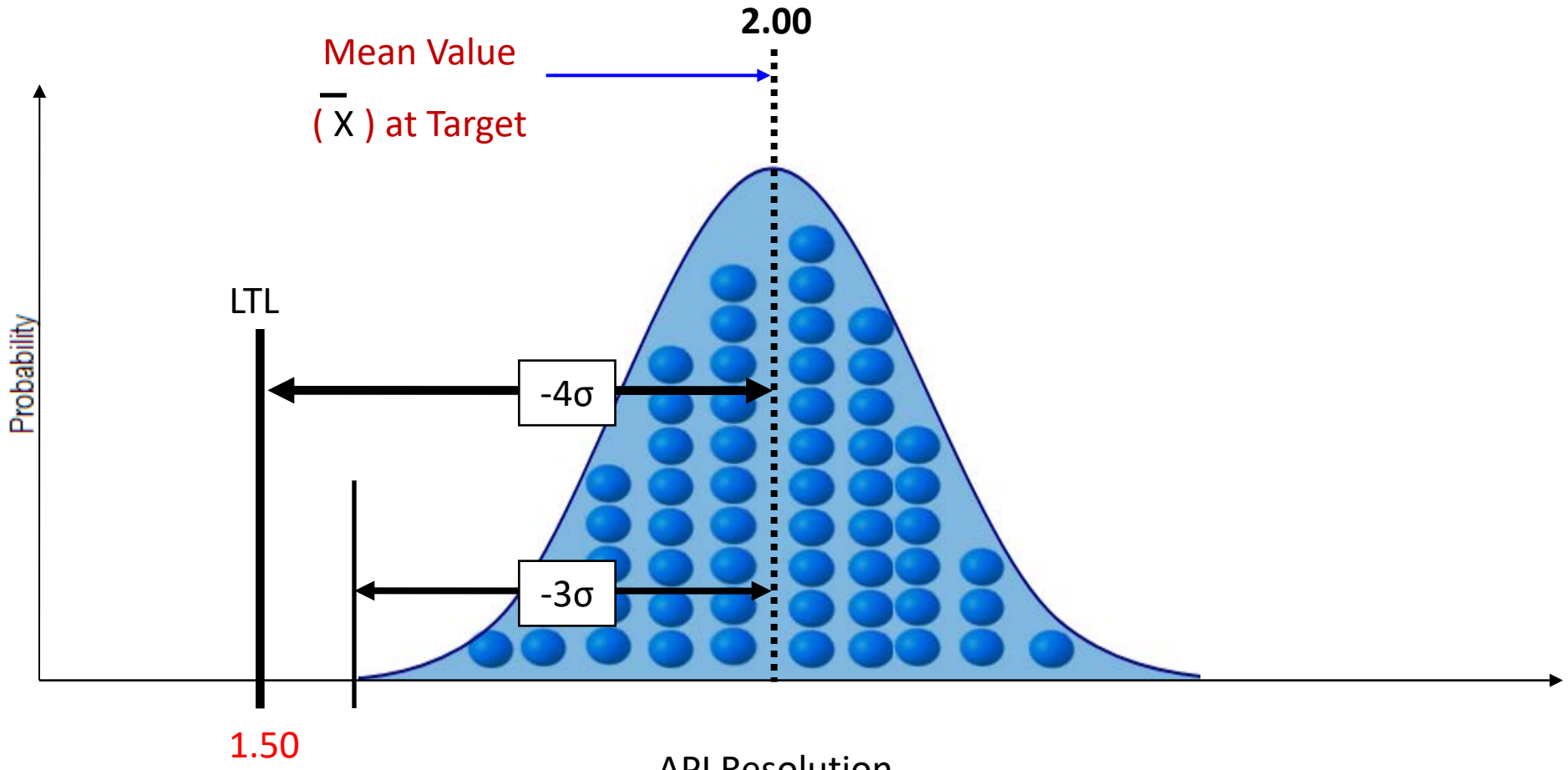


Process Capability: C_{pk} – Single-sided Failure

$$C_{pk} = 1.33$$

Edge of Failure is 4σ from Predicted Mean Result.

±3σ Variation = 75% of Maximum Allowable Variation Limit.

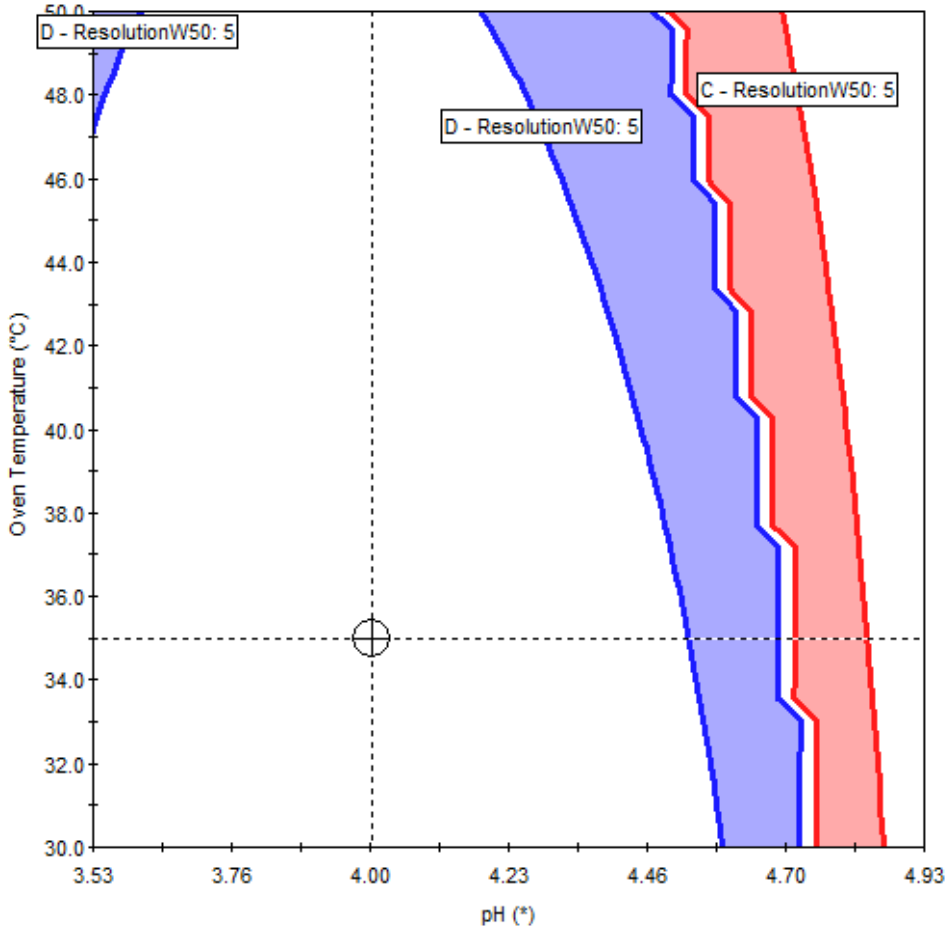


$\overline{X}$ and Robustness MODR – Overlay

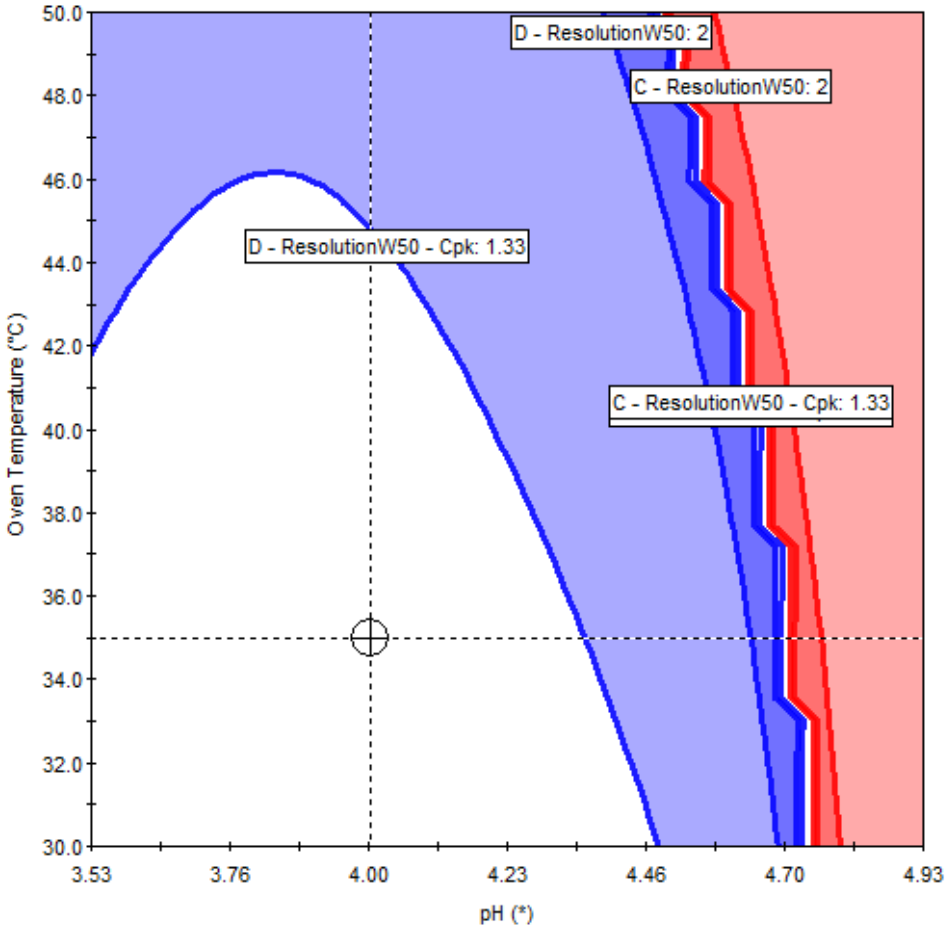
Mean Performance and Robustness for any Combination of Responses:

- Resolution
- K-Prime
- Tailing
- Area %RSD
- Etc.

Acceptable Mean Performance



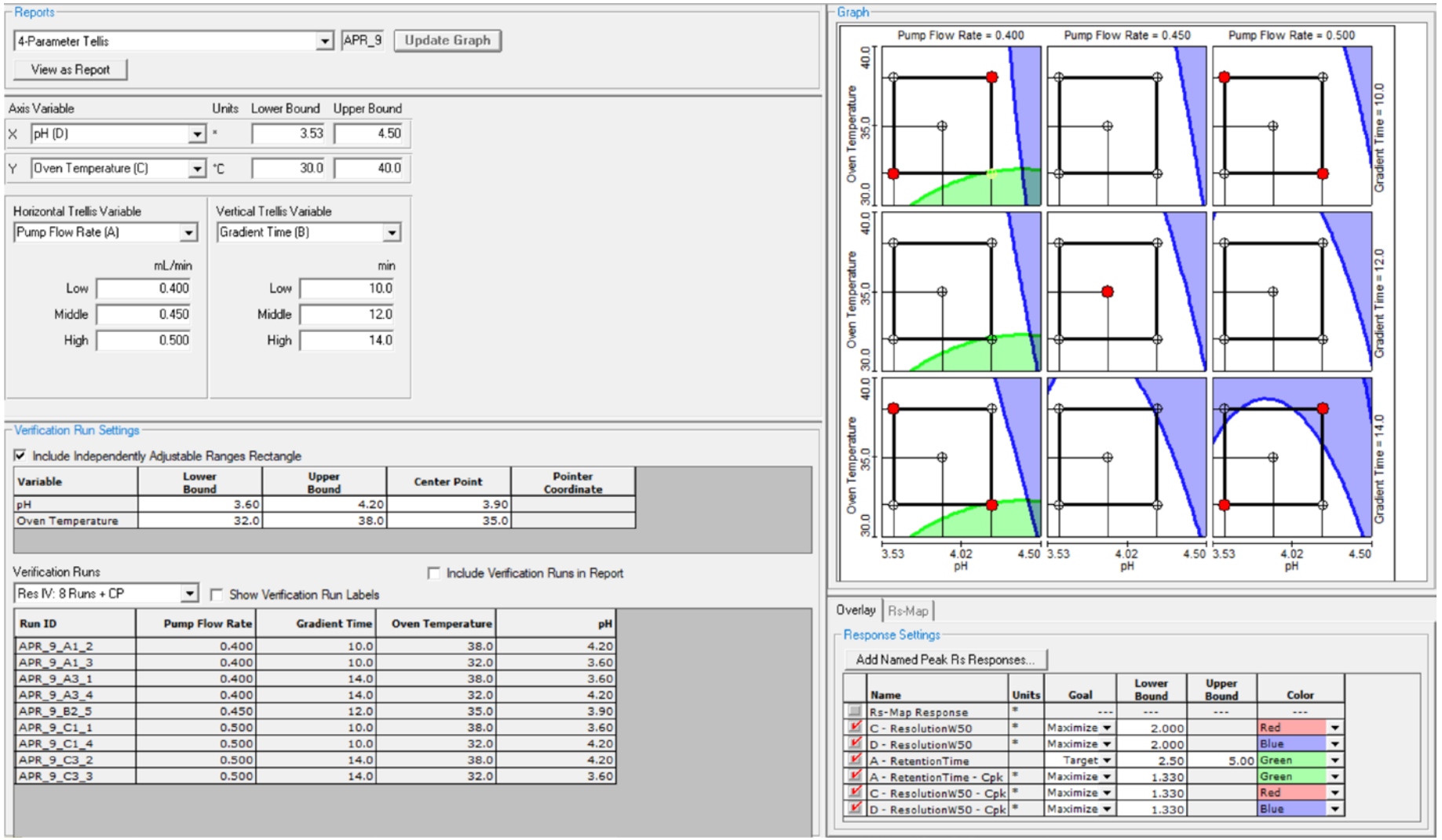
Acceptable Robustness



Robust MODR – 4-factor Trellis

Red Dots Designate Minimally Sufficient Verification Run Sampling of MODR –

- **Final method (Center Point)**
- **Extreme Level Setting Combinations**



Reports

4-Parameter Tellis | APR_9 | Update Graph | View as Report

Axis	Variable	Units	Lower Bound	Upper Bound
X	pH (D)	*	3.53	4.50
Y	Oven Temperature (C)	°C	30.0	40.0

Horizontal Trellis Variable	mL/min	Vertical Trellis Variable	min
Pump Flow Rate (A)		Gradient Time (B)	
Low	0.400	Low	10.0
Middle	0.450	Middle	12.0
High	0.500	High	14.0

Verification Run Settings

Include Independently Adjustable Ranges Rectangle

Variable	Lower Bound	Upper Bound	Center Point	Pointer Coordinate
pH	3.60	4.20	3.90	
Oven Temperature	32.0	38.0	35.0	

Verification Runs: Res IV: 8 Runs + CP | Show Verification Run Labels | Include Verification Runs in Report

Run ID	Pump Flow Rate	Gradient Time	Oven Temperature	pH
APR_9_A1_2	0.400	10.0	38.0	4.20
APR_9_A1_3	0.400	10.0	32.0	3.60
APR_9_A3_1	0.400	14.0	38.0	3.60
APR_9_A3_4	0.400	14.0	32.0	4.20
APR_9_B2_5	0.450	12.0	35.0	3.90
APR_9_C1_1	0.500	10.0	38.0	3.60
APR_9_C1_4	0.500	10.0	32.0	4.20
APR_9_C3_2	0.500	14.0	38.0	4.20
APR_9_C3_3	0.500	14.0	32.0	3.60

Overlay | Rs-Map

Response Settings

Add Named Peak Rs Responses...

Name	Units	Goal	Lower Bound	Upper Bound	Color
Rs-Map Response	*	---	---	---	---
C - ResolutionW50	*	Maximize	2.000		Red
D - ResolutionW50	*	Maximize	2.000		Blue
A - RetentionTime		Target	2.50	5.00	Green
A - RetentionTime - Cpk	*	Maximize	1.330		Green
C - ResolutionW50 - Cpk	*	Maximize	1.330		Red
D - ResolutionW50 - Cpk	*	Maximize	1.330		Blue

Overcoming the Most Common Objection to the Value of the MODR

Currently Represented Value Proposition:

Minimal or no post-approval changes needed.

Standard Objection to the Value Proposition:

"***Once I have a final method, I will never need to change it.***"

But: How many times has a method's performance changed (degraded) due to:

New column lot? New Peak? Transfer to a new site?

Overcoming the Most Common Objection to the Value of the MODR

"Once I have a final method, I will never need to change it" ?

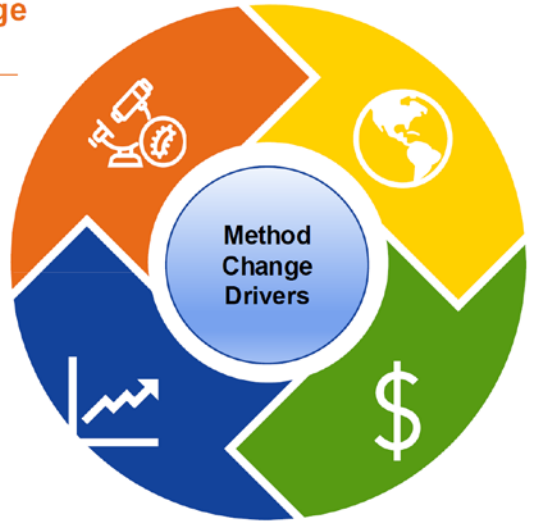
Drivers for Analytical Method Changes

Technology and Product Knowledge Advancements

- Monitoring new CQAs
- Regulatory expectations
- New assay platforms
- Obsolete equipment

Assay Optimization Opportunities

- Improve precision and accuracy
- Increase throughput
- Reduce invalid assay rate



Global Supply Chain Support

- New Manufacturing Sites
- Backup Testing Sites
- In-country Testing Requirements

Business / Financial Decisions

- Acquisitions / Divesting Programs
- Insourcing / Outsourcing Testing
- Reducing Testing Costs and Required Resources

ICH Q14: Analytical Procedure Development:

Section VII: Life Cycle Management and Post Approval Changes of Analytical Procedures

Changes to analytical procedures can occur throughout the product life cycle and could involve modification of existing procedures or a complete replacement including introduction of a new technology. Major changes in the performance characteristics or additional information on quality attributes could lead to reevaluation of the ATP and/or a new procedure. Typically, process knowledge, analytical procedure knowledge, and continual improvement are drivers for change. If possible, changes should lead to improved analytical procedures in line with best practices and instrumentation.

Vir Biotechnology™

Dan Willingmyre, Associate Director, Stability & Data Analytics

Robust MODR – The True Benefits

Most Important Benefits of Establishing a Robust MODR:

Correct, Efficient, Pro-active Analytical Control Strategy (ACS):

1. Identifies absolute setpoint control limits for critical instrument parameters – pre-qualifies QC instruments for the method.
2. Establishes the edges of failure for all CQAs.

   IMPORTANT: CQAs can be a chromatographic performance metrics and/or quantitation performance requirements for **both** components of System Suitability – $\bar{X}$ and % RSD.
3. Defines the appropriate corrective actions for all critical chromatographic performance metrics and CQAs expressing performance problems **before** the method generates OOS results.

And of Course:

4. Minimal or no post-approval changes needed when a method requires modification.

Knowledge Transfer to Stages 2 and 3

ICH Q14

The outcome of development studies should provide an understanding of the relationships between analytical procedure parameters (inputs) and the responses of the analytical procedure (outputs). **Based on the results, fixed set-points may be defined for some parameters. For others, PARs could be defined while still others could be included into an MODR**. … (*Pg. 5*)

Analytical Control Strategy

Robust Final LC Method → Analytical Control Strategy → APLM Stage 3

ICH Q14

The analytical procedure control strategy includes **analytical procedure parameters needing control** and the system suitability test (SST) which is part of the analytical procedure...

This **can include (but is not limited to) …, the number of replicates, use of the formulae for the calculation of the reportable results** and other necessary steps. (*Pg. 6*)

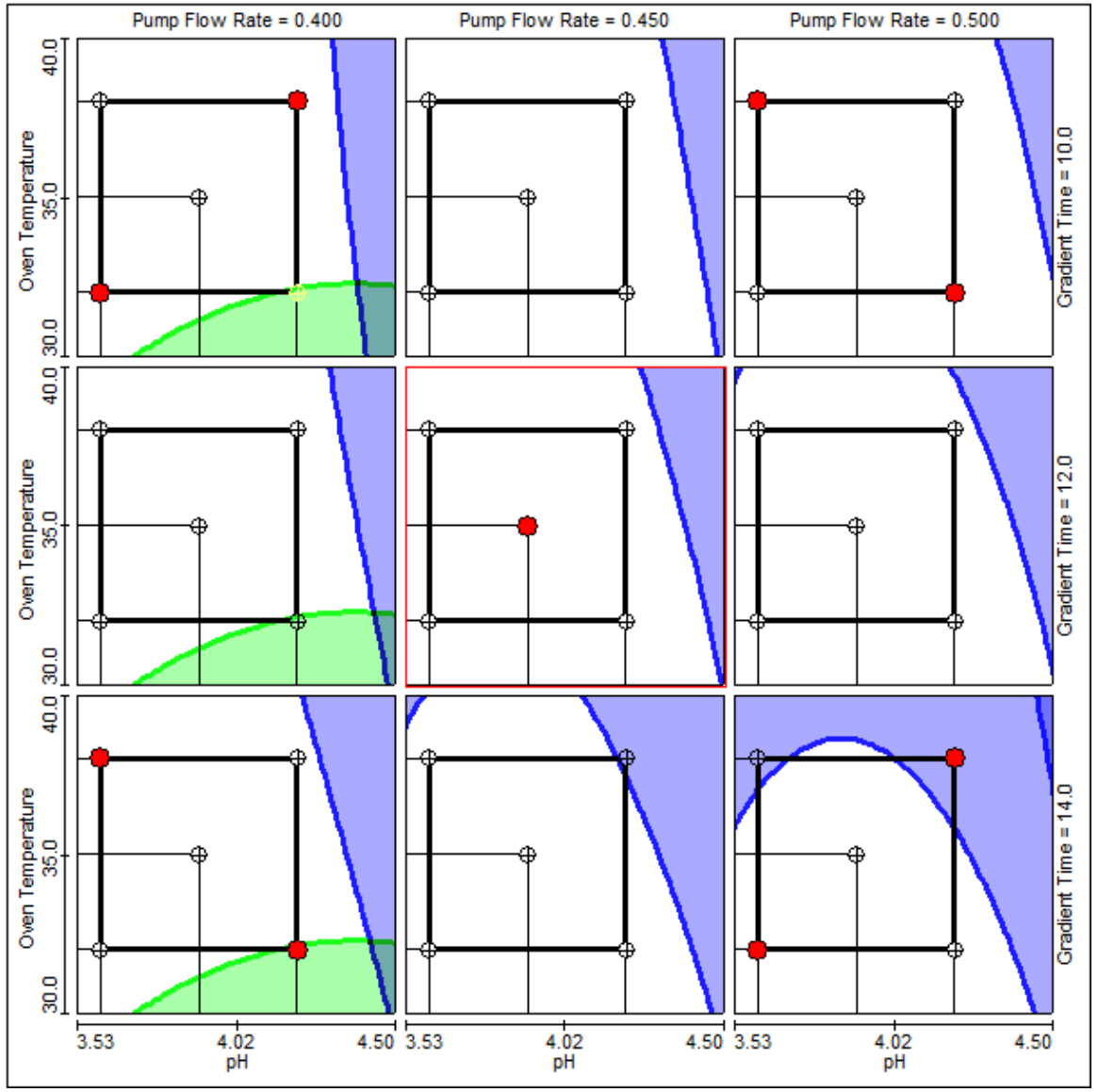
USP <1220>

The ACS is a set of controls needed to ensure the procedure performs as expected and plays a key role in ensuring that the ATP is realized throughout the life cycle. **The preliminary ACS is identified during the procedure development process in Stage 1**, … (*Pg. 9*)

Analytical Control Strategy (ACS)

Enabled	Experiment Variable	Units	Maximum Expected Variation (±3σ Value)
☑	Pump Flow Rate	mL/min	0.025
☑	Oven Temperature	°C	3.0
☑	pH	*	0.20
☑	Mobile Phase Composition (MPC)*	%	2.0

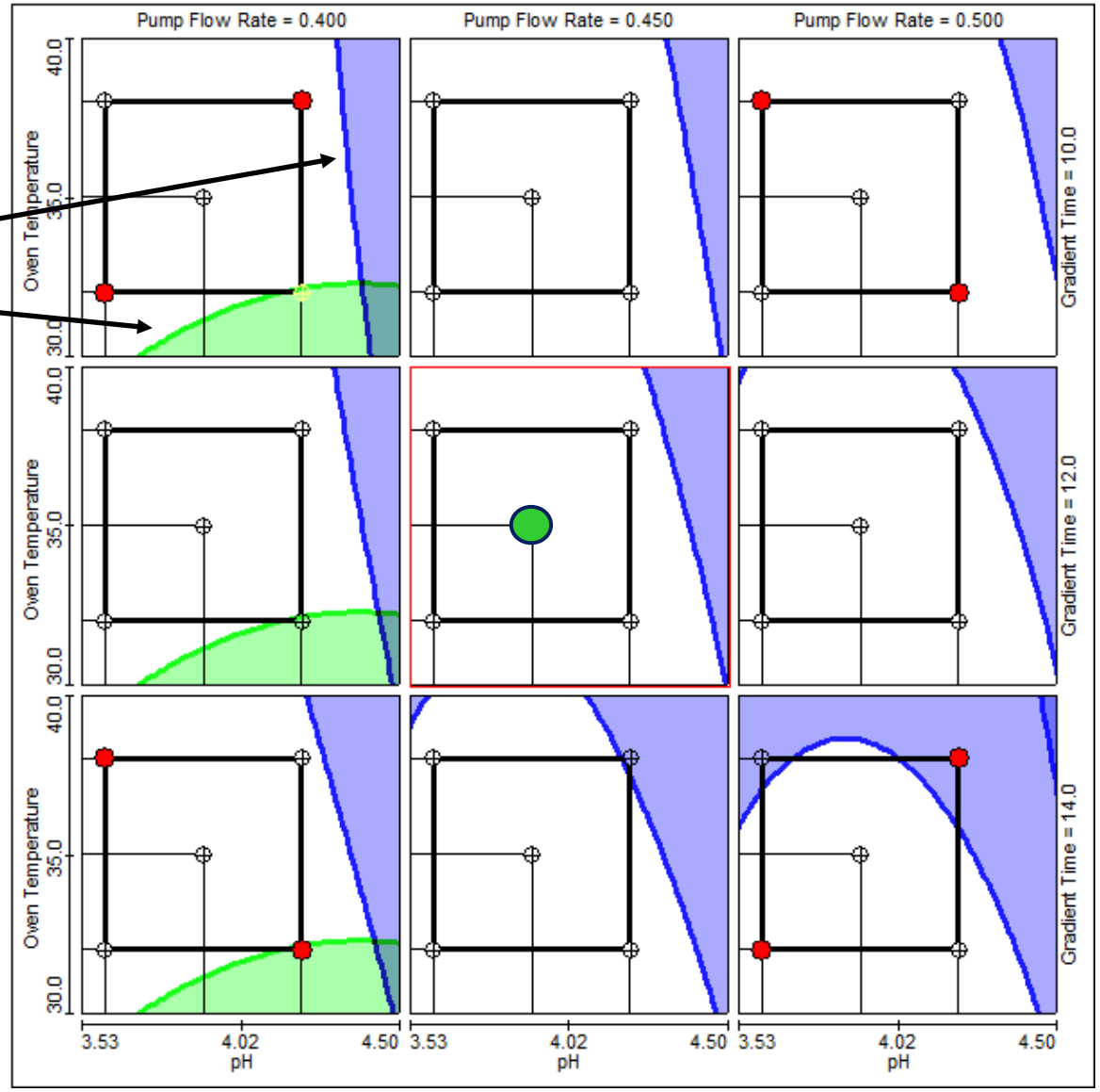
LC System Control Specifications



Analytical Control Strategy (ACS)

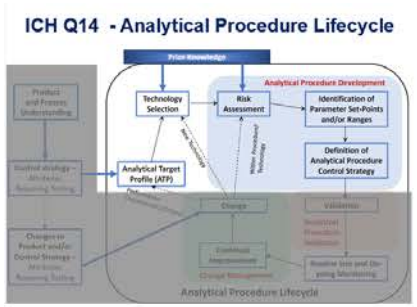
	Name	Units	Goal	Lower Bound	Upper Bound	Color
☐	Rs-Map Response	*	---	---	---	---
☑	C - ResolutionW50	*	Maximize	2.000		Red
☑	D - ResolutionW50	*	Maximize	2.000		Blue
☑	A - RetentionTime		Target	2.50	5.00	Green
☑	A - RetentionTime - Cpk	*	Maximize	1.330		Green
☑	C - ResolutionW50 - Cpk	*	Maximize	1.330		Red
☑	D - ResolutionW50 - Cpk	*	Maximize	1.330		Blue

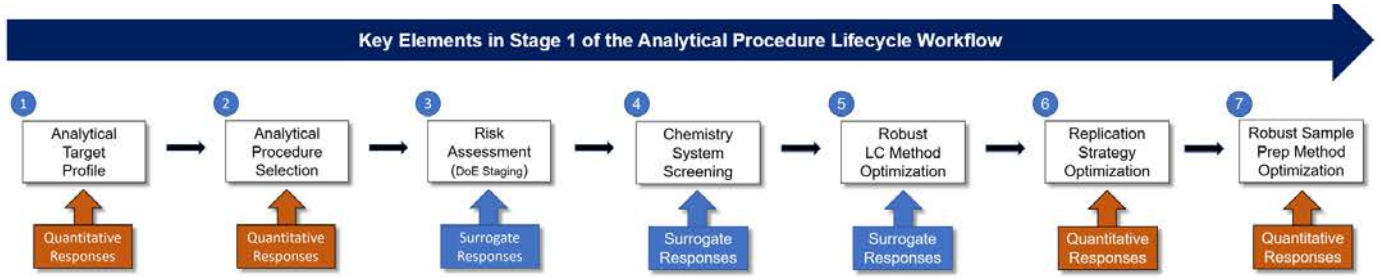
Routine Monitoring – Control Charts

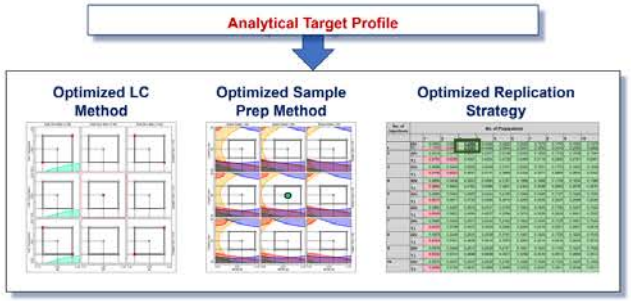


End of Presentation

Fusion QbD is the Only LC Method Development Software Which Completely Supports the AQbD / APLM Workflow in the Regulatory Guidances







ICH Q2(R2) / ICH Q14 / USP <1210> / USP <1220> / EP 11.60

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