S-Matrix®

Fusion QbD®

Advanced Chromatography Software for LC and LC-MS Method Development, Validation, and Transfer

S-Matrix Corporation
www.smatrix.com

S-Matrix: Transforming LC Method Development

2004

S-Matrix Introduces LC Automation with DoE for Method Development

2007

S-Matrix Introduces Trend Responses to Simplify Chemistry System Screening (Method Scouting)

2009

S-Matrix Presents to FDA:

- **Concept of LC System as "Process in a Box"**
- **Integrated Monte Carlo Robustness Simulation**

2009 - 2017

S-Matrix Continually Advances LC Data Modeling and Extends Automation Support to SFC, Multiple LC Systems and CDS Software

2018

S-Matrix Integrates Automated MS Spectra Based Peak Tracking

2019

S-Matrix Integrates USP <1210> Tolerance & Prediction Interval Metrics to Support Method Validation and Transfer

2020

S-Matrix Integrates Full Experiment Automation and Peak Tracking Support for Forced Degradation Studies

2022

S-Matrix Integrates Replication Strategy Optimization into Fusion QbD for Method Development

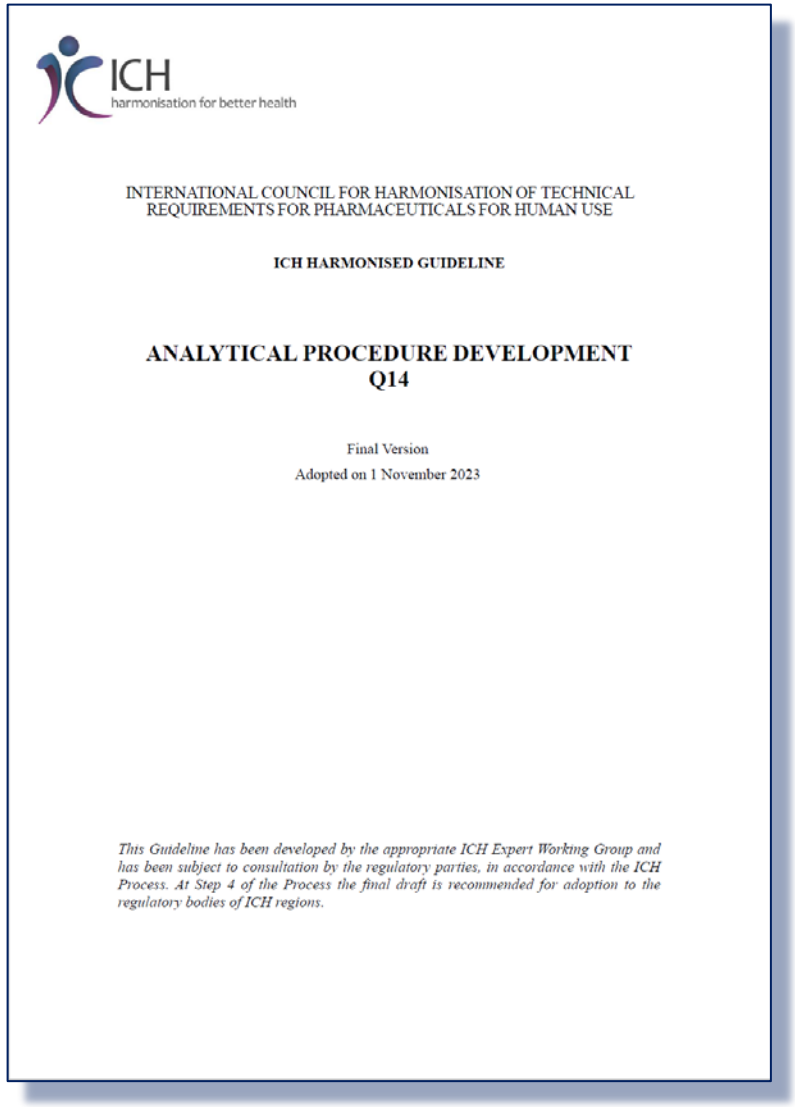
Presentation Overview

Fusion QbD is the Only LC and LC-MS Method Development Software Which Brings All These Strategic Analytical Quality-by-Design (AQbD) Tools to Support Your Experimental Workflows for APLM* Method Development, Validation, and Transfer.

- Support for All Install Environments
- Fusion QbD – Automation & Compliance
- Fusion QbD – Design of Experiments (DoE)
- Chemistry System Screening
- LC Method Optimization
- Sample Preparation Method Optimization
- Replication Strategy Optimization
- Method Validation & Transfer

* – Analytical Procedure Lifecycle Management

Referenced Guidance Documents – ICH



ICH
harmonisation for better health

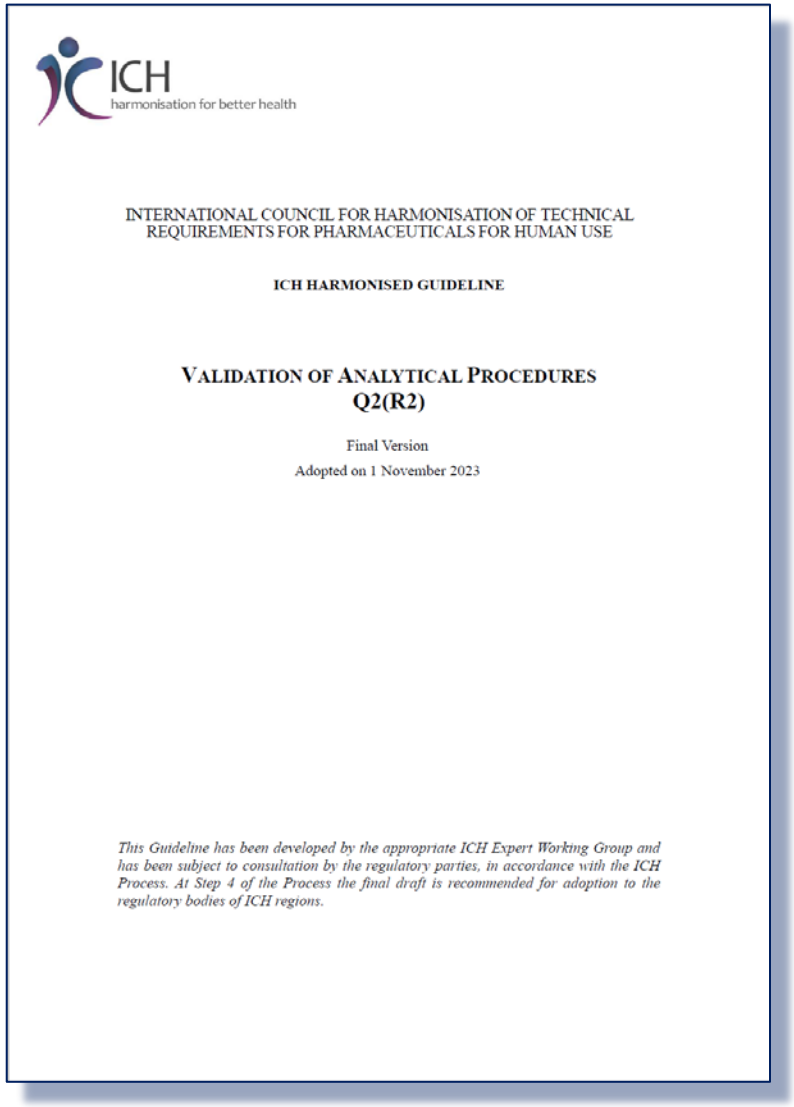
INTERNATIONAL COUNCIL FOR HARMONISATION OF TECHNICAL REQUIREMENTS FOR PHARMACEUTICALS FOR HUMAN USE

ICH HARMONISED GUIDELINE

ANALYTICAL PROCEDURE DEVELOPMENT
Q14

Final Version
Adopted on 1 November 2023

This Guideline has been developed by the appropriate ICH Expert Working Group and has been subject to consultation by the regulatory parties, in accordance with the ICH Process. At Step 4 of the Process the final draft is recommended for adoption to the regulatory bodies of ICH regions.



ICH
harmonisation for better health

INTERNATIONAL COUNCIL FOR HARMONISATION OF TECHNICAL REQUIREMENTS FOR PHARMACEUTICALS FOR HUMAN USE

ICH HARMONISED GUIDELINE

VALIDATION OF ANALYTICAL PROCEDURES
Q2(R2)

Final Version
Adopted on 1 November 2023

This Guideline has been developed by the appropriate ICH Expert Working Group and has been subject to consultation by the regulatory parties, in accordance with the ICH Process. At Step 4 of the Process the final draft is recommended for adoption to the regulatory bodies of ICH regions.

Referenced Guidance Documents – USP

Status: Currently Official on 07-Mar-2024
Official Date: Official as of 01-May-2022
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Document Type: GENERAL CHAPTER

DOI: https://doi.org/10.31003/USPNF_M10975_02_01

Add the following:

▲⟨1220⟩ ANALYTICAL PROCEDURE LIFE CYCLE

INTRODUCTION

This general chapter holistically considers the validation activities that take place across the entire life cycle of an analytical procedure and provides a framework for the implementation of the life cycle approach.

The analytical procedure life cycle approach described here is consistent with the quality by design concepts described in International Council for Harmonisation (ICH) guidelines. The procedure life cycle approach emphasizes the importance of sound scientific approaches and quality risk management for the development, control, establishment, and use of analytical procedures. Total error is used in this chapter; however, measurement uncertainty can also be used.

The procedure life cycle approach is applicable to all types of analytical procedures, and the extent of effort should be consistent with the complexity of the procedure and the criticality of the quality attribute to be measured. The life cycle approach can be considered optional, and any of the elements can be applied on the basis of how the procedure is used. Elements of the life cycle approach can also be applied retrospectively if deemed useful or in early stages of development with the appropriate modifications.

Elements of life cycle management of analytical procedures are also discussed in *Analytical Procedures and Methods Validation for Drugs and Biologics* (Guidance for Industry, FDA 2015).

Validation of an analytical procedure is the process by which it is established, through laboratory studies, that the performance of the procedure meets the requirements for the intended analytical applications. Validation, or demonstration that a procedure is suitable for the intended purpose, takes place during the entire procedure life cycle, beginning during the initial procedure design activities and extending through routine use. These activities include the formal procedure validation, verification, and transfer of procedures, as well as establishing and assuring adherence to an appropriate set of procedure controls and system suitability requirements.

The procedure life cycle is comprised of the analytical target profile (ATP) and three stages, which are introduced below and shown in *Figure 1*.

The ATP defines the criteria for the procedure performance characteristics that are linked to the intended analytical application and the quality attribute to be measured. It applies to all stages of the procedure life cycle. For quantitative procedures, the ATP describes the required quality of the reportable value since the reportable value generated using a qualified analytical procedure provides the basis for key decisions regarding compliance of a test article with regulatory, compendial, and manufacturing limits. The acceptable level of risk of making an incorrect decision can also be considered when establishing ATP criteria.

Stage 1: Procedure design encompasses procedure development, which consists of the analytical technology and sample preparation. It includes understanding gained through knowledge gathering, systematic procedure development experiments, and risk assessments and associated lab experiments. The output of *Stage 1* includes:

1. A set of procedure conditions that minimizes procedure bias to a suitable level, can provide acceptable precision, and can meet the ATP criteria
2. An understanding of the effect of procedure parameters (e.g., temperature, wavelength, flow rate, etc.) on procedure performance
3. Optimization of performance characteristics of the analytical procedure such as accuracy, precision, the appropriateness of any calibration model, specificity and limit of quantitation (as far as applicable); this includes a preliminary replication strategy for samples and standards
4. An initial analytical control strategy (ACS), which is a set of controls (system suitability tests [SSTs] and other procedure-specific controls) needed to ensure proper performance

Stage 2: Procedure performance qualification consists of studies designed to demonstrate that the procedure is suitable for its intended purpose. This involves confirmation that the reportable values generated by application of the analytical procedure meet the ATP criteria as well as confirmation of procedure performance characteristics through the traditional validation, verification, or transfer studies. Data generated during *Stage 1* can be used if available and suitable. At the end of *Stage 2*, the replication strategy and the performance of the procedure is confirmed to meet the ATP and other criteria.

Stage 3: Ongoing procedure performance verification involves monitoring the analytical procedure during routine use and confirming that the performance continues to meet ATP criteria. Monitoring ensures that the performance of the procedure is maintained at an acceptable level over the procedure lifetime. It can also provide an early indication of potential performance issues or adverse trends and aid in identifying required changes for the analytical procedure. Confirming procedure performance after changes ensures that the modified procedure will produce reportable values that meet the criteria defined in the ATP.

More details about the procedure life cycle are described in the subsequent sections.

Status: Currently Official on 17-Mar-2024
Official Date: Official as of 01-May-2018
DOI Ref: saf9m
DocId: GUID-13ED4BEB-4086-43B5-A7D7-994A02AF25C8_7_en-US

DOI: https://doi.org/10.31003/USPNF_M8646_07_01

Add the following:

▲⟨1210⟩ STATISTICAL TOOLS FOR PROCEDURE VALIDATION

1. INTRODUCTION

This chapter describes utilization of statistical approaches in procedure validation as described in *Validation of Compendial Procedures* ⟨1225⟩. For the purposes of this chapter, "procedure validation" refers to the analytical procedure qualification stage of the method life cycle, following design and development and prior to testing.

Chapter ⟨1225⟩ explains that capabilities of an analytical procedure must be validated based on the intended use of the analytical procedure. Chapter ⟨1225⟩ also describes common types of uses and suggests procedure categories (I, II, III, or IV) based on the collection of performance parameters appropriate for these uses. Performance parameters that may need to be established during validation include accuracy, precision, specificity, detection limit [limit of detection, (LOD)], quantitation limit, linearity, and range. In some situations (e.g., biological assay), relative accuracy takes the place of accuracy. This chapter focuses on how to establish analytical performance characteristics of accuracy, precision, and LOD. For quantitative analytical procedures, accuracy can only be assessed if a true or accepted reference value is available. In some cases, it will be necessary to assess relative accuracy. In many analytical procedures, precision can be assessed even if accuracy cannot be assessed. The section addressing LOD can be applied to limit tests in Category II.

The other analytical performance characteristics noted in ⟨1225⟩, which include specificity, robustness, and linearity, are out of scope for this chapter.

Because validation must provide evidence of a procedure's fitness for use, the statistical hypothesis testing paradigm is commonly used to conduct validation consistent with ⟨1225⟩. Although some statistical interval examples are provided in *3. Accuracy and Precision*, these methods are not intended to represent the only approach for data analysis, nor to imply that alternative methods are inadequate.

Table 1 provides terminology used to describe an analytical procedure in this chapter. The definitions for individual determination and reportable value are in alignment with *General Notices, 7.10 Interpretation of Requirements*.

Table 1. Analytical Procedure Validation Terminology

Terminology	Description
Laboratory sample	The material received by the laboratory
Analytical sample	Material created by any physical manipulation of the laboratory sample, such as crushing or grinding
Test portion	The quantity (aliquot) of material taken from the analytical sample for testing
Test solution	The solution resulting from chemical manipulation of the test portion such as chemical derivatization of the analyte in the test portion or dissolution of the test portion
Individual determination (ID)	The measured numerical value from a single unit of test solution
Reportable value	Average value of readings from one or more units of a test solution

Not all analytical procedures have all stages shown in *Table 1*. For example, liquid laboratory samples that require no further manipulations immediately progress to the test solution stage. Demonstration that a reportable value is fit for a particular use is the focus of analytical validation.

Table 2 provides an example of the *Table 1* terminology for a solid oral dosage form.

Table 2. Example for Coated Tablets

Terminology	Description	
Laboratory sample	100 coated tablets	
Analytical sample	20 tablets are removed from the laboratory sample and are crushed in a mortar and pestle	
Test portion	Replicate 1: 1 g of crushed powder aliquot from the analytical sample	Replicate 2: 1 g of crushed powder aliquot from the analytical sample

Key Differentiators – Deployment

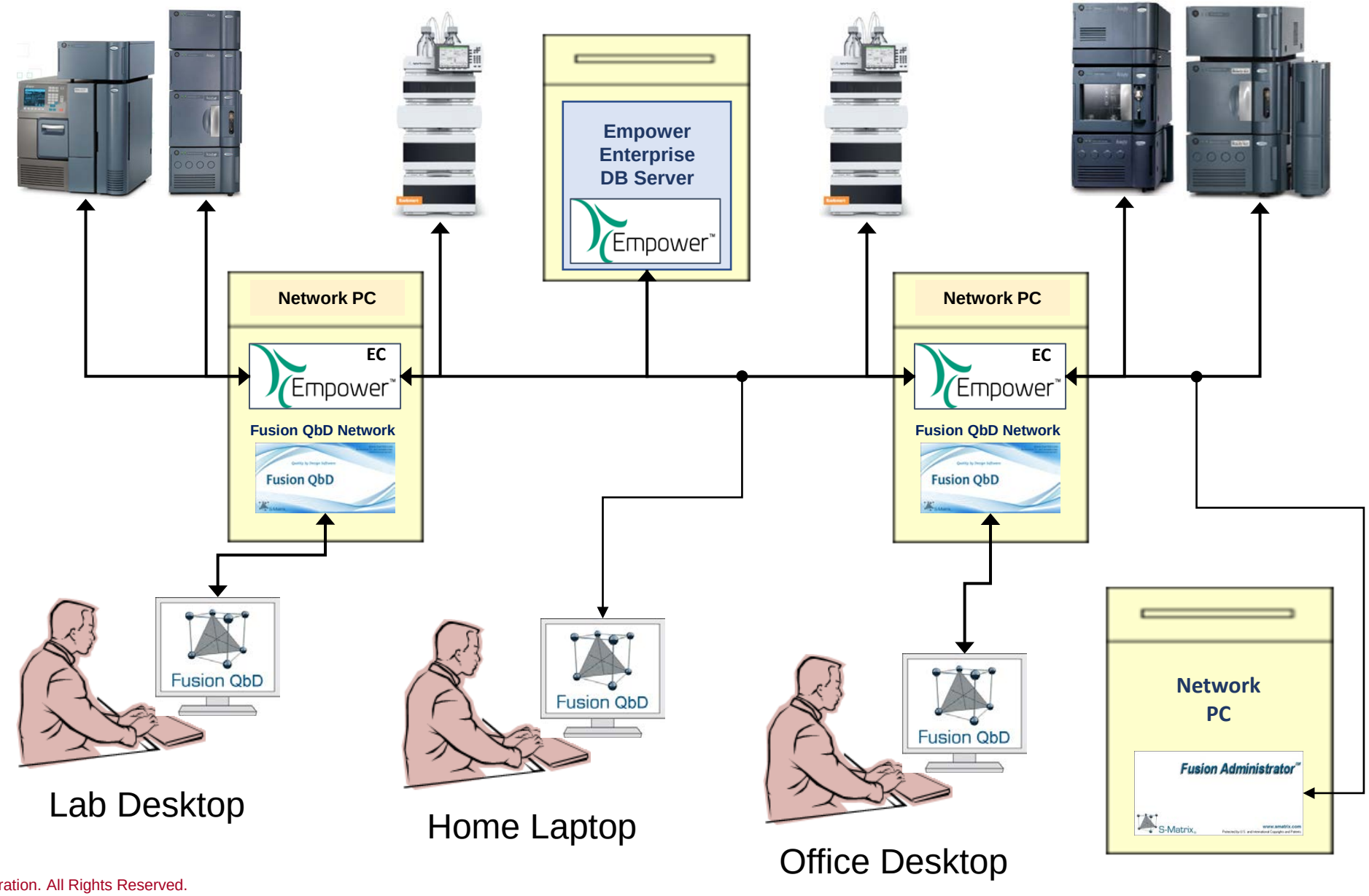
- ✓ **Deploy at any Scale**
- ✓ **Deploy in any Install Environment**

Fusion QbD – Supports All Install Environments

Install Environment	Fusion QbD
Standalone (Workstation)	✓
WorkGroup / Network	✓
Citrix Ready Certified	✓
Fully Qualifiable for GxP*	✓

* – Fusion QbD is operating in the GxP environments of international pharmaceutical companies worldwide.

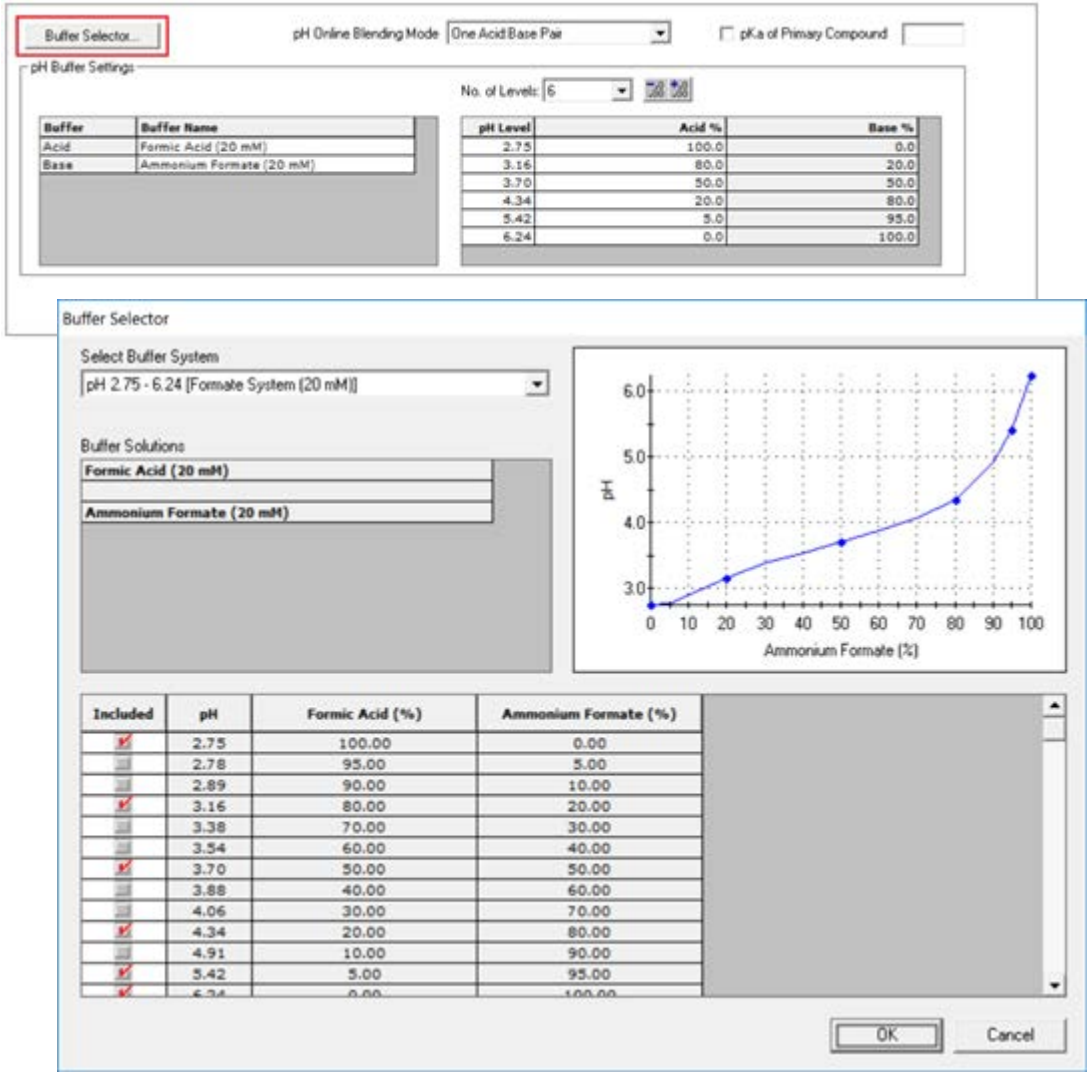
Fusion QbD – Supports All Install Environments



Key Differentiators – Automation & Compliance

- ✓ **Full Experiment Automation Support**
  - LC Systems
  - Column/Solvent Valves
  - Separation Modes
  - Automation Supports Data Quality
- ✓ **Forced Degradation Studies**
- ✓ **Bi-directional Audit Trail Support**
  - Automation/Auditing Support Data Integrity

Online Prep – pH, Salt ΔC, Buffer ΔC, Additive ΔC



Built-in pH Titration Curves for Quaternary Pump Modules!

Or Use Your Own Buffer Curve.

Extremely Precise!

Fusion QbD – LC System Automation

Empower™

- ✓ Solvent Selection Valves
- ✓ Column Switching Valves

Alliance HPLC

Alliance iS HPLC

Acquity Binary

Acquity H-Class

Acquity Arc

Acquity UPC²

Fusion QbD – LC System Automation

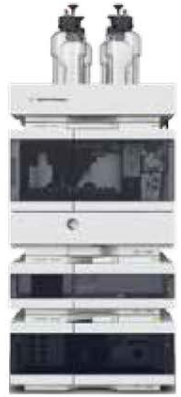
ChemStation
OpenLab

- ✓ Solvent Selection Valves
- ✓ Column Switching Valves

Agilent 1100s And 1200s

Agilent 1260 Infinity Series

Agilent 1260 Infinity II Series



Agilent 1290 Infinity Series

Agilent 1290 Infinity II Series

Fusion QbD – LC System Automation

- ✓ Solvent Selection Valves
- ✓ Column Switching Valves

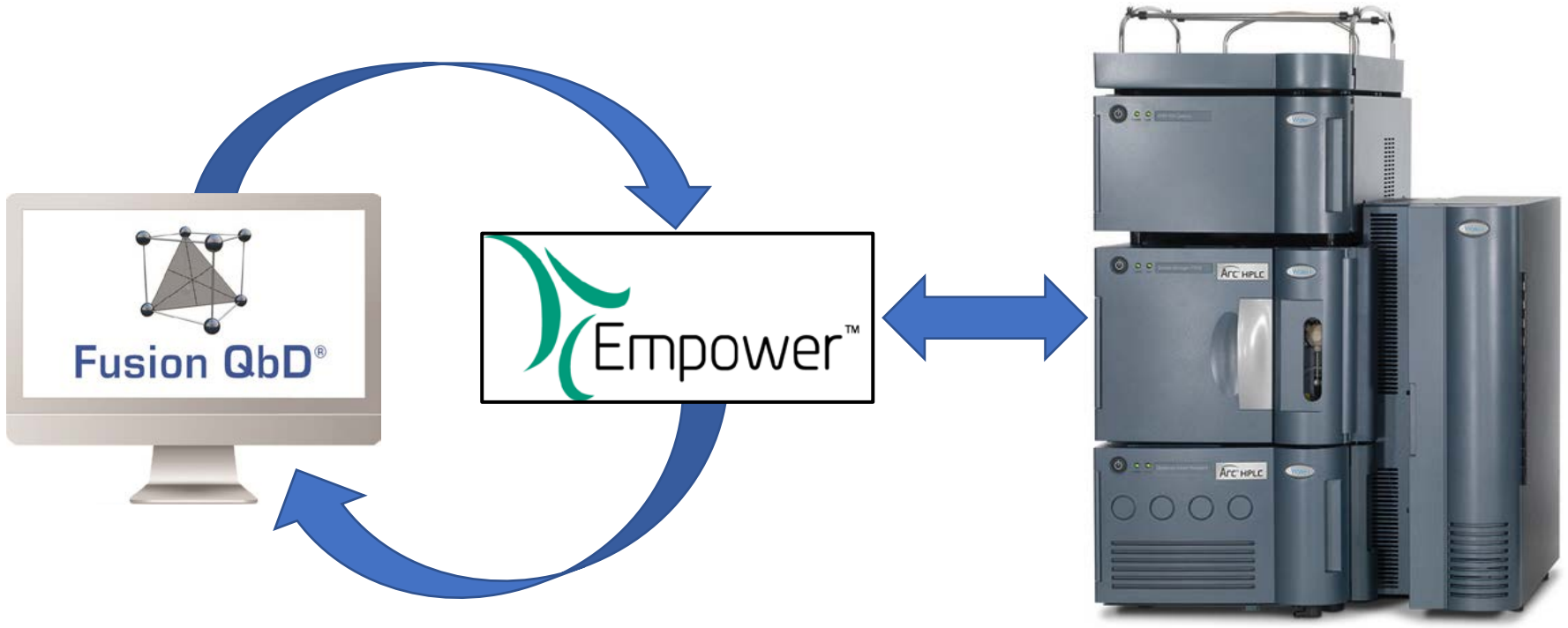
UltiMate LCs

Vanquish Horizon And Flex LCs

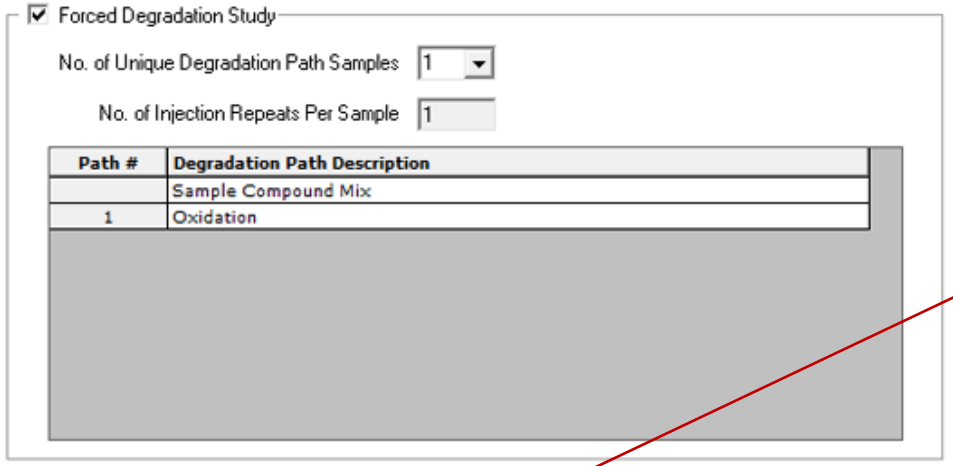
Fusion QbD Automation with Empower

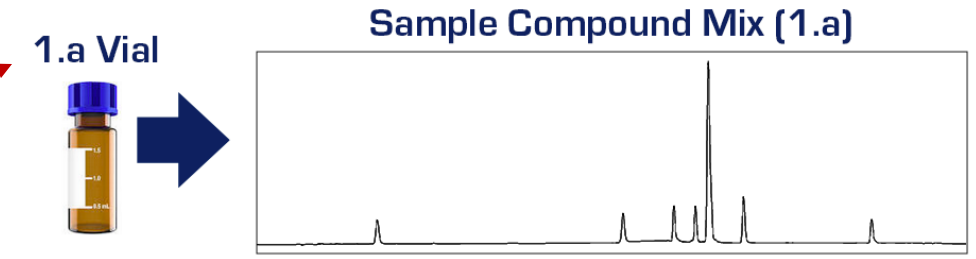
Supports All These Separation Modes

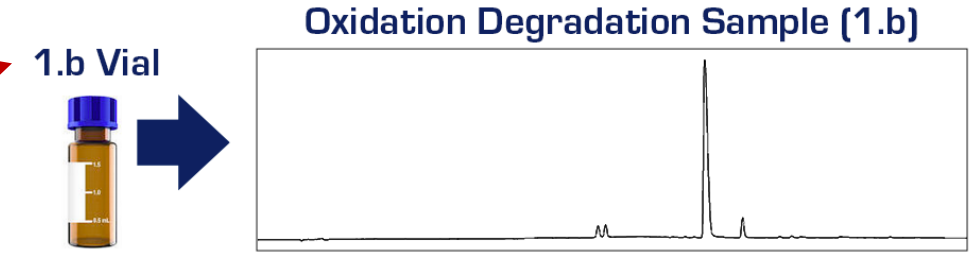
- Reversed Phase
- Normal Phase
- Chiral
- HILIC
- Ion Exchange
- Size Exclusion
- SFC

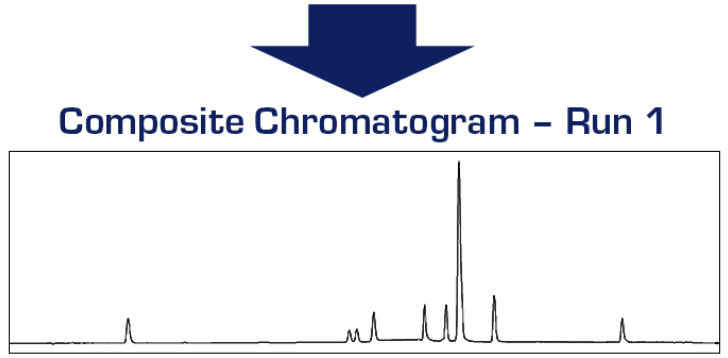


Full Support for Forced Degradation Studies









Experiment Design Matrix

Run No.	Pump Flow Rate (mL/min)	Gradient Time (min)	Oven Temperature (°C)	pH
Conditioning_Run_1	0.400	2.0	30.0	4.60
1.a	0.300	8.0	30.0	4.60
1.b	0.300	8.0	30.0	4.60
2.a	0.500	8.0	30.0	4.60
2.b	0.500	8.0	30.0	4.60
3.a	0.300	16.0	30.0	4.60
3.b	0.300	16.0	30.0	4.60

Simple Setup integrates the replication scheme into the DoE Study, and automatically assigns a separate vial position to each replicate injection.

Fusion QbD tracks all peaks in all replicate chromatograms for each run and generates a *composite chromatogram* for each run containing all unique peaks from all replicate injections.

Experiment Automation Intelligence

Maximum Efficiency + Maximum Data Quality:

- ✓ Automates Mobile Phase Preparation.
- ✓ Maximizes use of reservoirs and solvent selection valves.
- ✓ Incorporates column conditioning.
- ✓ Ramps on pH.
- ✓ Ramps on Temperature.

Fusion QbD – Export to CDS

Generates QbD-aligned DOE Experiment

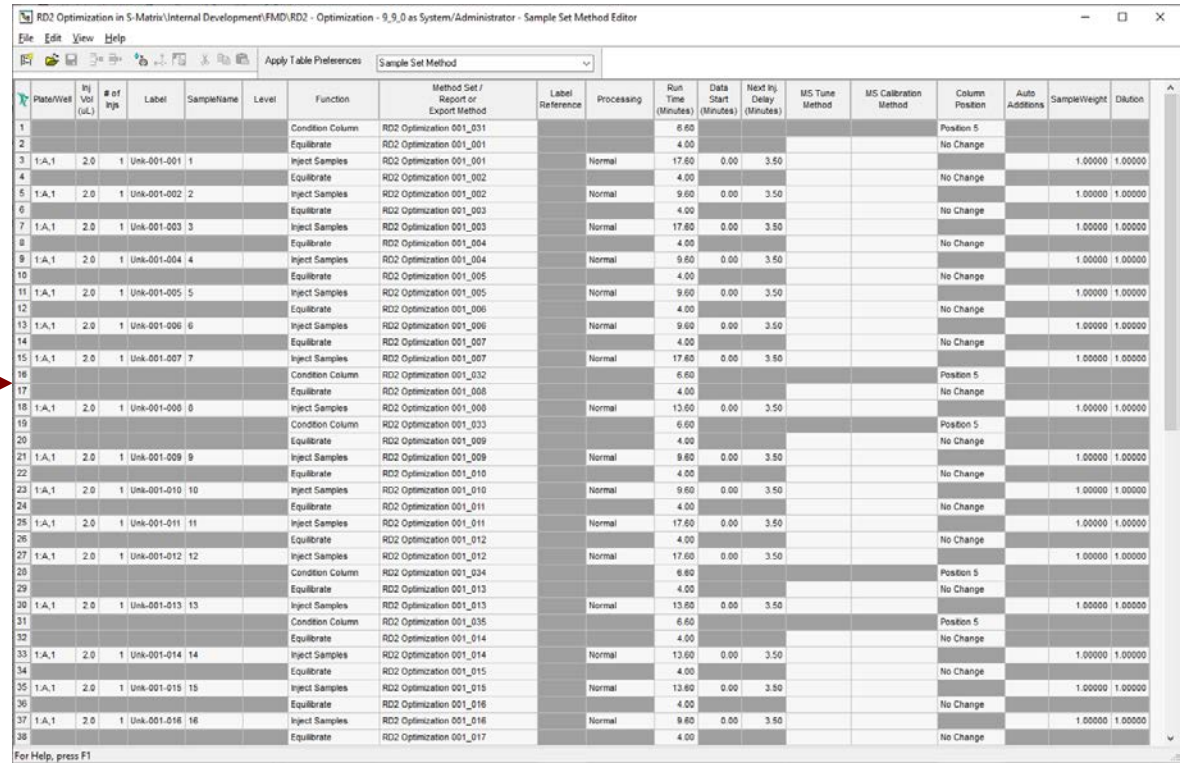
Automatically Builds Sequence and All Instrument Methods

CDS Software

ChemStation
OpenLab

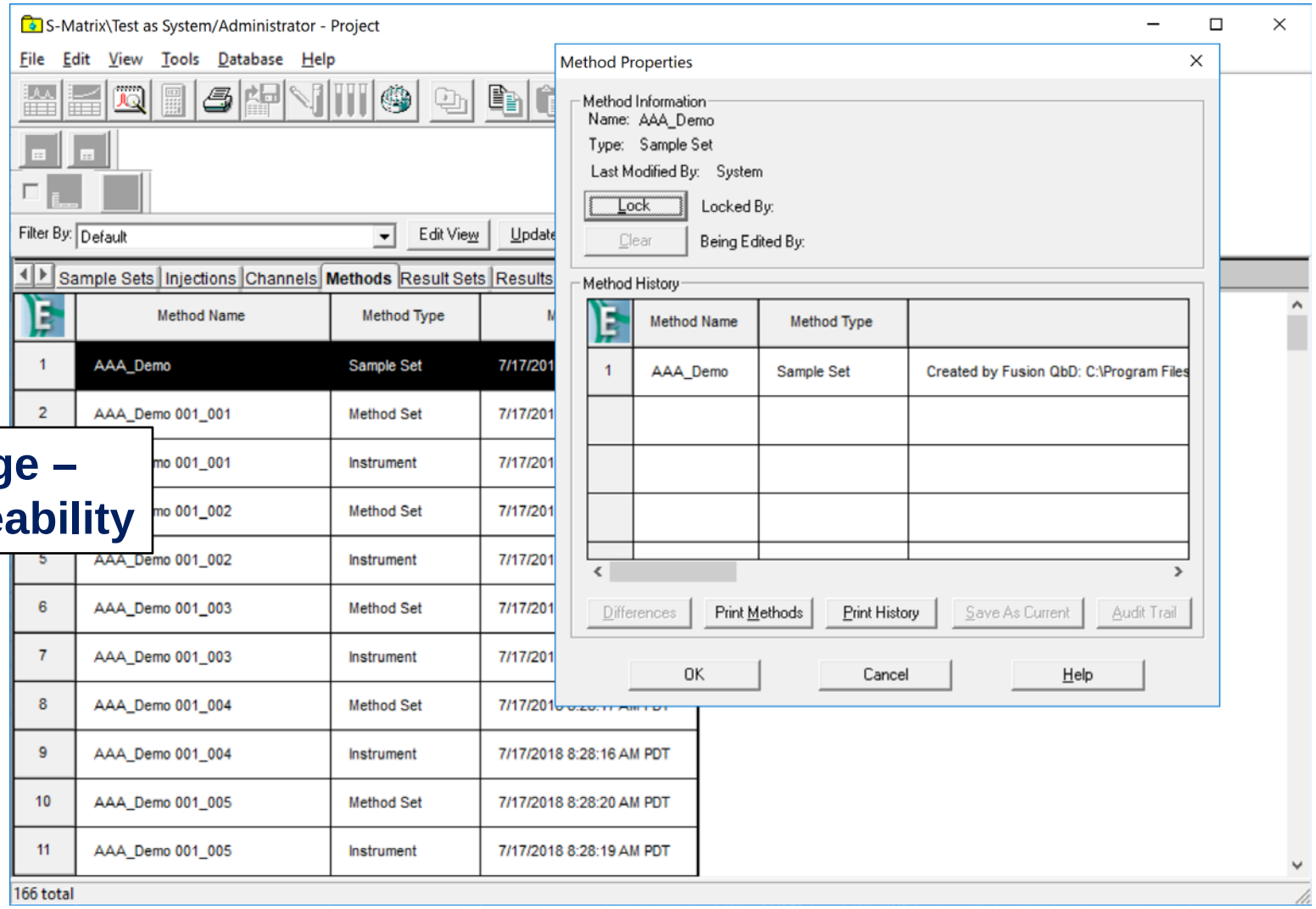
CHROMELEON 7.2

Empower™



Auditing assures Data Integrity and Traceability

Automated, Audited Data Exchange – Preserves Data Integrity and Traceability



Fusion QbD – Import from CDS

Automatically Retrieves All Chromatogram Results Data

Automated analysis, graphing, and reporting.

Report formats:

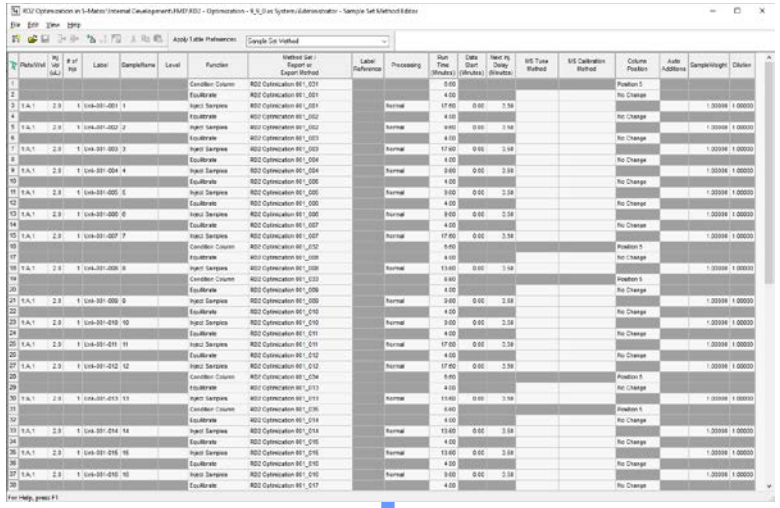
RTF, DOC, HTML, PDF, XLSX, XML

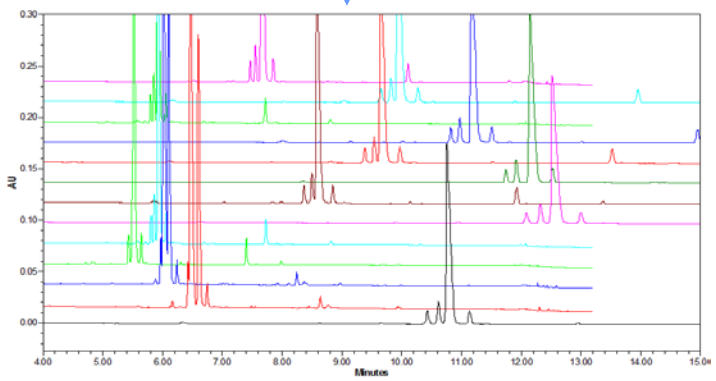
CDS Software

ChemStation
OpenLab

CHROMELEON 7.2

Empower™





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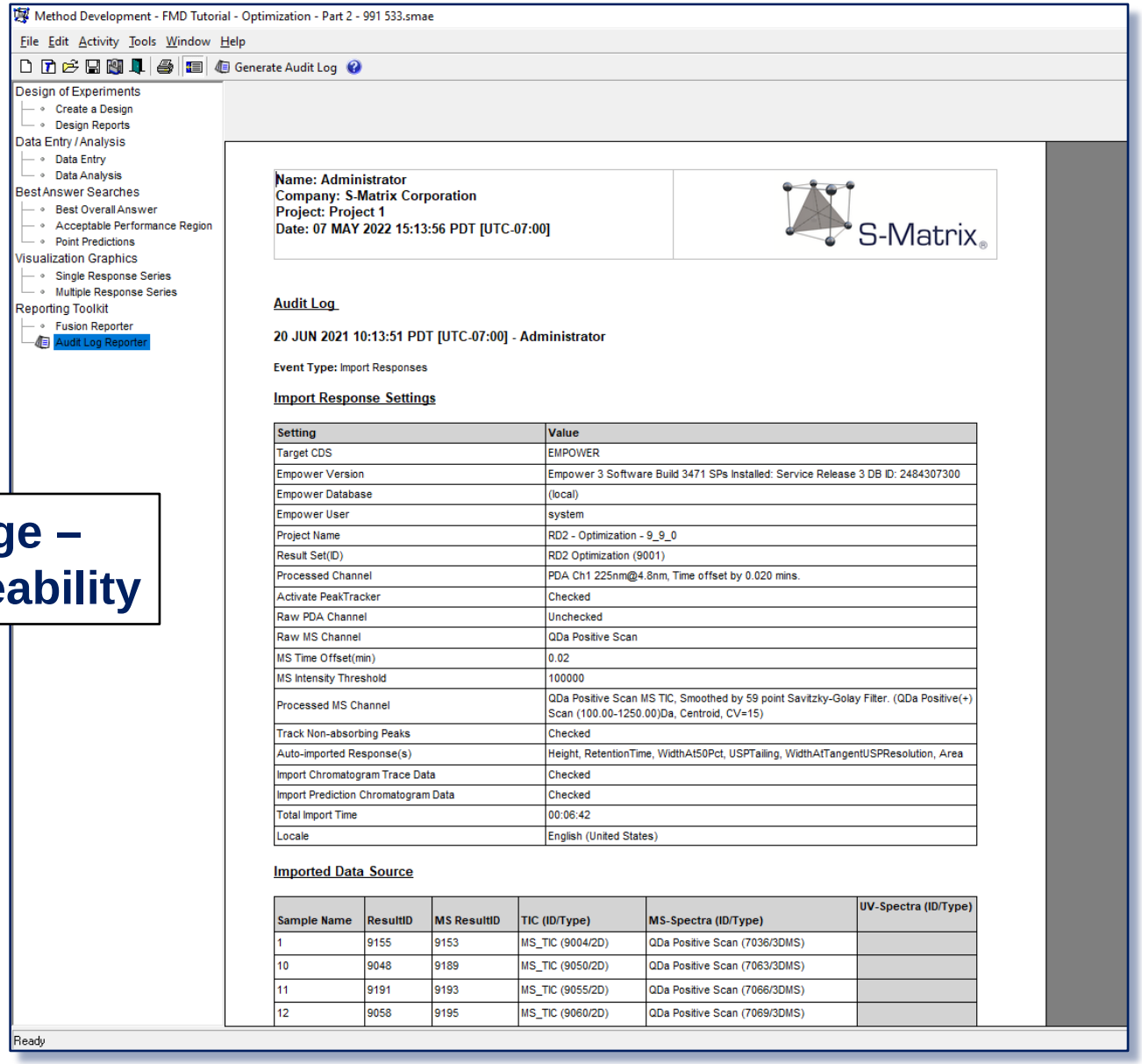
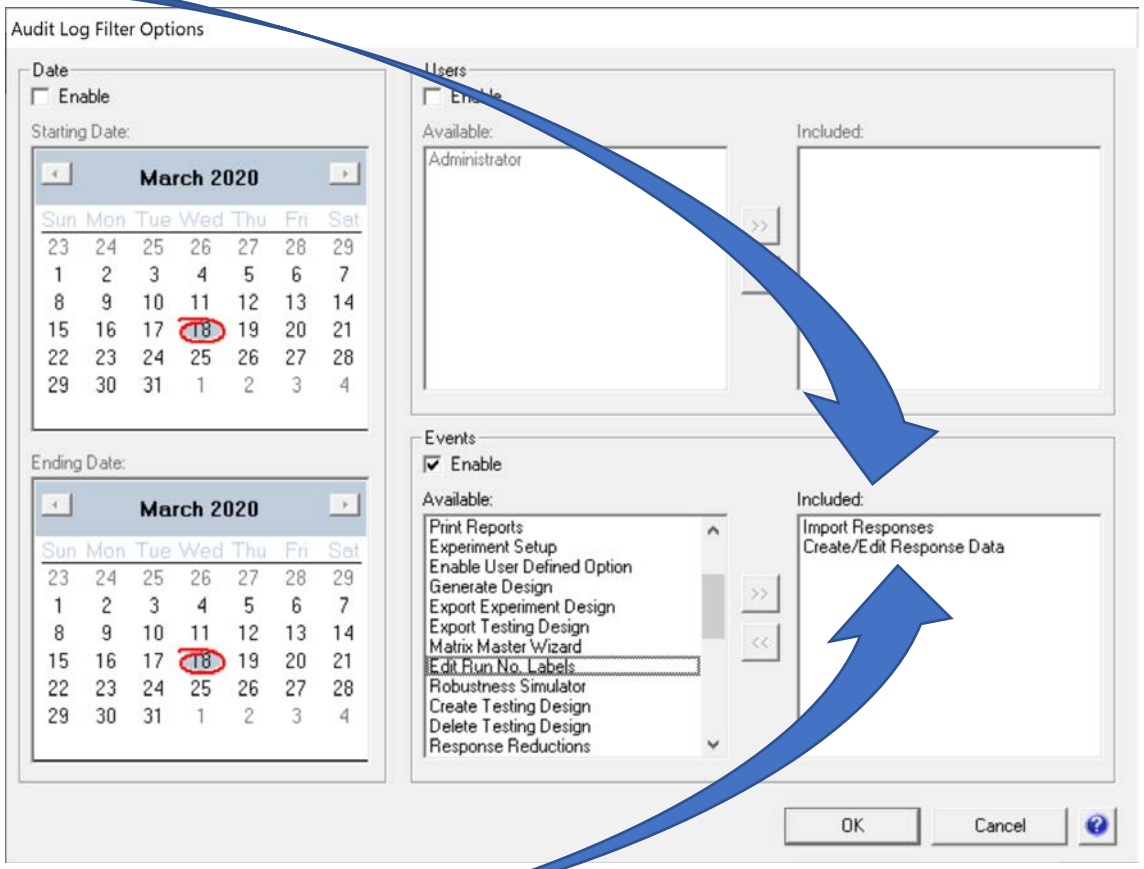


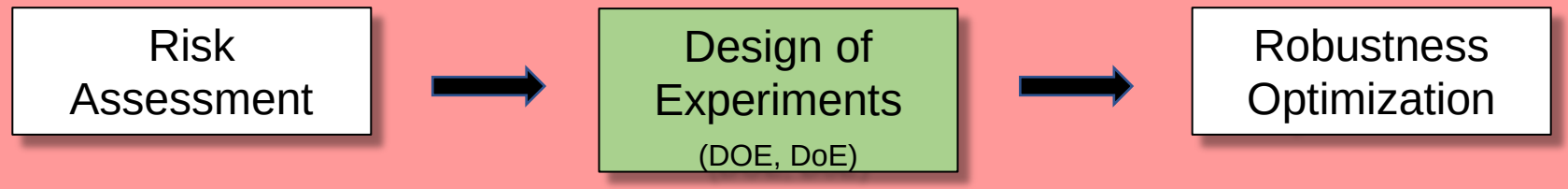
Why Audit Trail is Important !

Where did this data come from?
Which Project?
Which Results Set?
Which Chromatograms?

Who imported this data – was the data modified?



Design of Experiments (DOE, DoE)



ICH Q14

In an enhanced approach, the ranges for the relevant parameters and their interactions can be investigated in multivariate experiments (DoE).

USP <1220>

Experimentation is a direct way of generating data that can be used to assess the impact of procedure parameters on performance, and the use of statistical design of experiments (DOE) is an effective way to do this.

Key Differentiators – Design of Experiments

- ✓ **Full Design of Experiments Support**
  - **Chemistry and Instrument Parameters**
  - **Separation Modes**
- ✓ **Built-in Expert System Wizards**
- ✓ **Beyond Trial and Error**

Fusion QbD – Design of Experiments (DoE)

Design of Experiments (DoE, DOE) is discussed extensively in the current and proposed guidances (FDA, USP, ICH)

Discussed as a Core QbD Tool for Many Applications, Including:

- Robust Method Optimization to Establish a MODR
- Sample Preparation Method Optimization
- Replication Strategy Optimization
- etc.…

Fusion QbD Automation → DoE Flexibility

Experiment Automation Simplifies DoE!

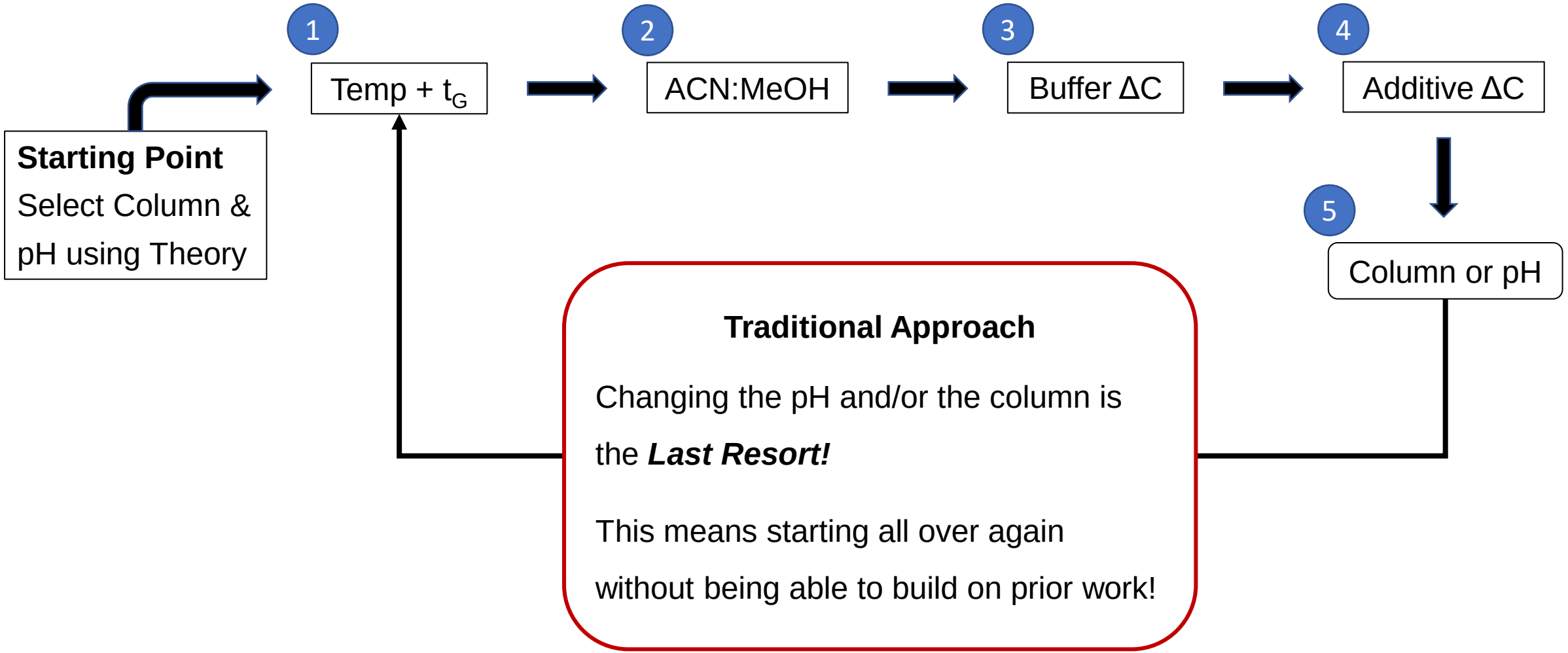
Full utilization of Quaternary Pumps, Solvent Selection Valves, and Column Switching Valves

Study **any combination** of LC parameters which can **interactively effect** method performance!

- Isocratic and Gradient Methods
- Strong Solvent Type
- Any pump program steps – e.g.
  - Equilibration Time & %
  - Isocratic Hold Time & %
  - Gradient Time / Slope
  - Initial / Final Hold Time & %
  - Re-equilibration Time & %
- Column Temperature
- Column Type
- Flow Rate
- Injection Volume
- pH
- Mobile Phase Blends
- Salt, Buffer, Additive – Type & ΔC
- Wavelength

Before Fusion QbD

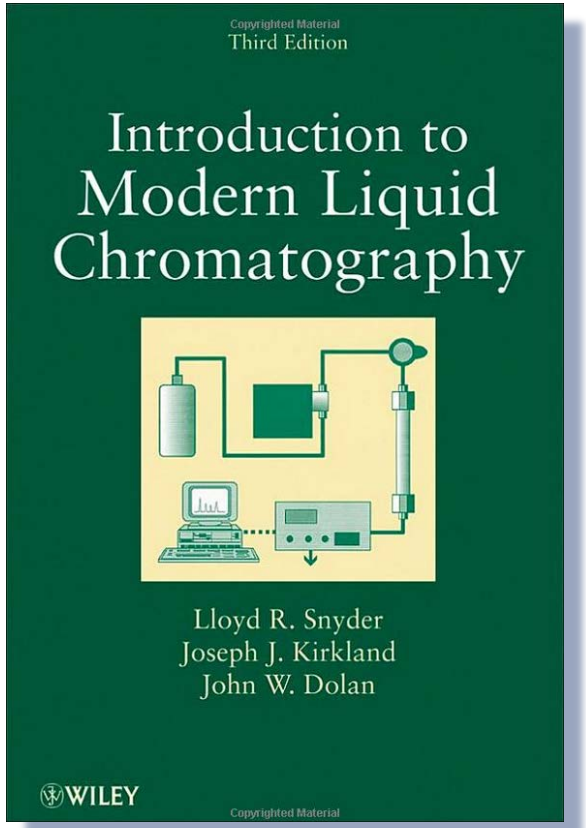
Before Fusion QbD: One-Factor-At-a-Time (OFAT) Approach



Modern Thinking by Recognized Experts

"For methods involving a large number of samples, and where adequate resolution must be combined with run times that are as short as possible, **it can be profitable to spend more time initially on "scouting" experiments**. The experimentation may be with:

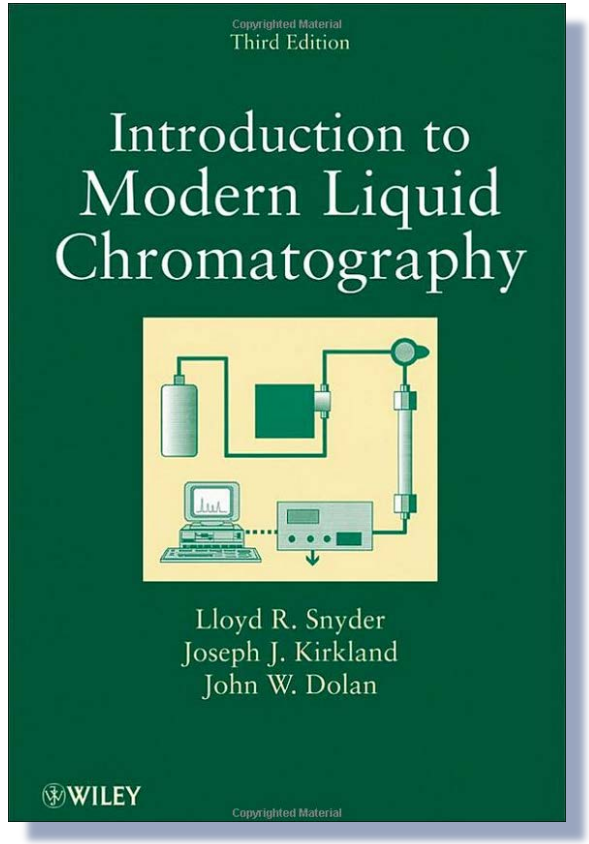
- Different **columns**
- Different **B-solvents**
- Variations in **pH** and **temperature**
- Use of **Gradient elution** during the experiments can help avoid the need to separately optimize values of %B for each variable studied."



Snyder, Kirkland, and Dolan. (2010). *Introduction to Modern Liquid Chromatography*, 3rd Edition; John Wiley & Sons, Inc., Hoboken, New Jersey (p. 67)

Modern Thinking by Recognized Experts

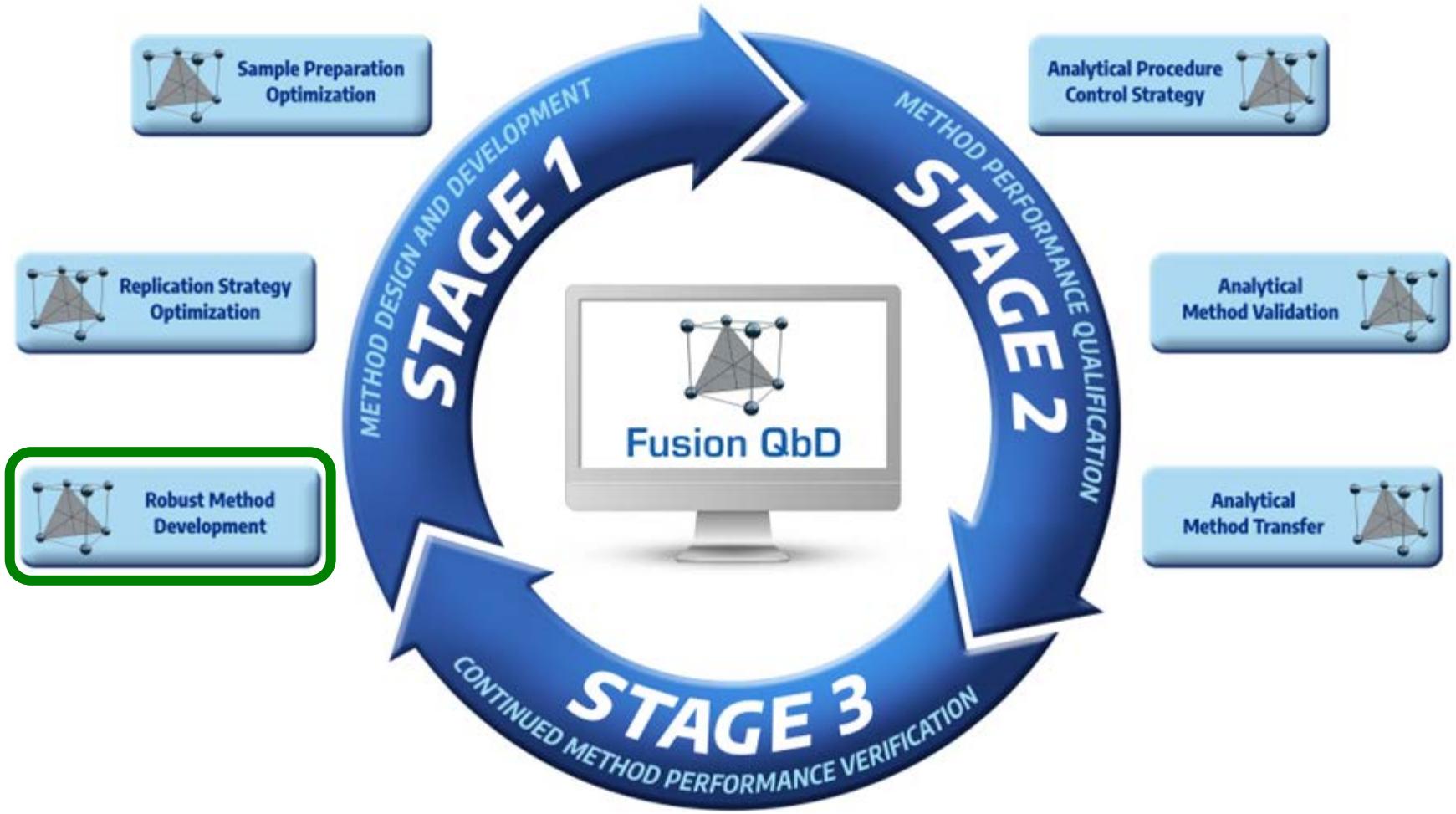
"Still another approach is to **search the literature** for separation of the same or similar sample. **Trial-and-error** modifications of conditions are then followed until an acceptable separation is achieved. ***We do not recommend this approach**** because possible deficiencies in literature methods can delay subsequent attempts at achieving a final, acceptable separation."



***** – italics added by Snyder, Kirkland, and Dolan in book text to emphasize the point.

Snyder, Kirkland, and Dolan. (2010). *Introduction to Modern Liquid Chromatography*, 3rd Edition; John Wiley & Sons, Inc., Hoboken, New Jersey (p. 67)

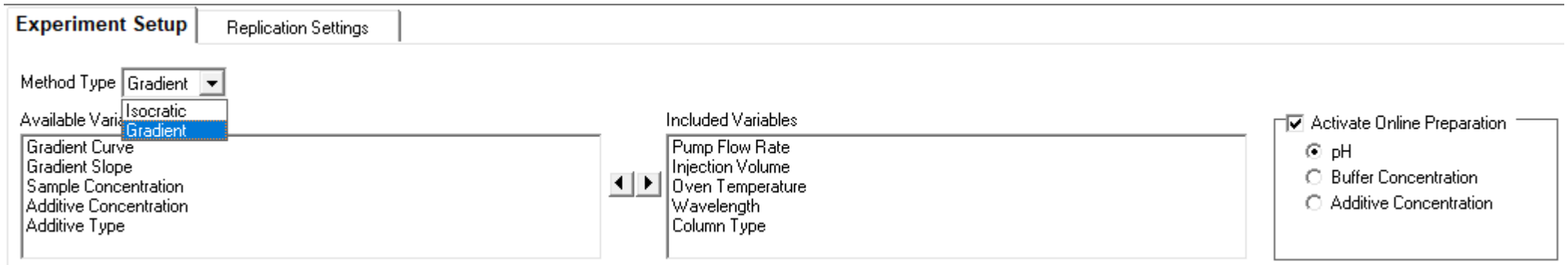
Chemistry System Screening



Key Differentiators – Chemistry Screening

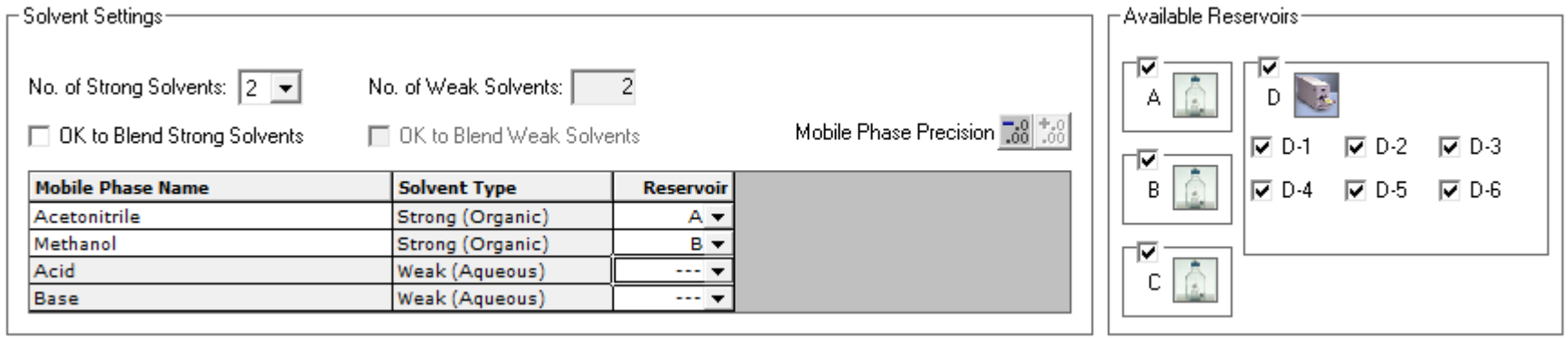
- ✓ Ease of Experiment Setup
- ✓ Simple Chromatogram Integration
  - No Peak Tracking Needed
  - Trend Responses Keep it Simple
- ✓ Fast Data/Chromatogram Review
- ✓ Instant One-click Modeling – Any Results
- ✓ Great Best Answer Visualization Graphics

Chemistry System Screening



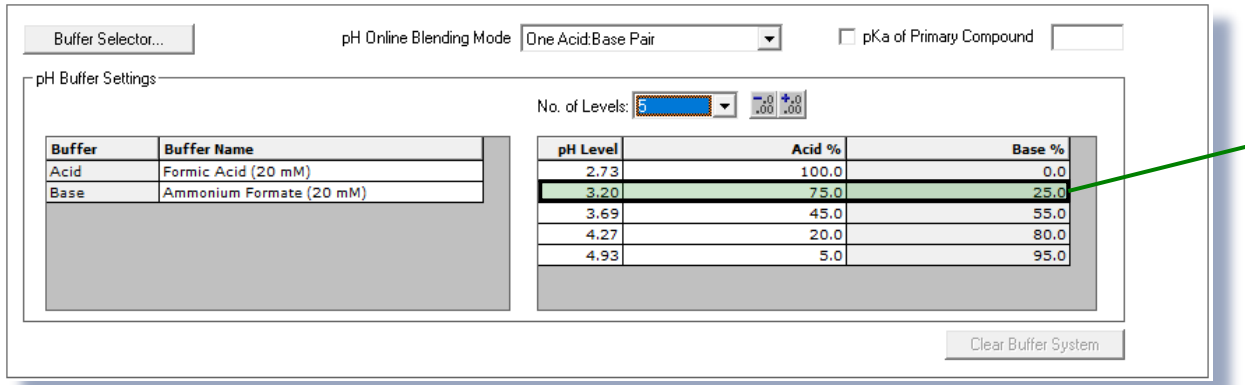
Solvent Type

Include Strong Solvent Alternatives – e.g., Acetonitrile and Methanol.



Chemistry System Screening

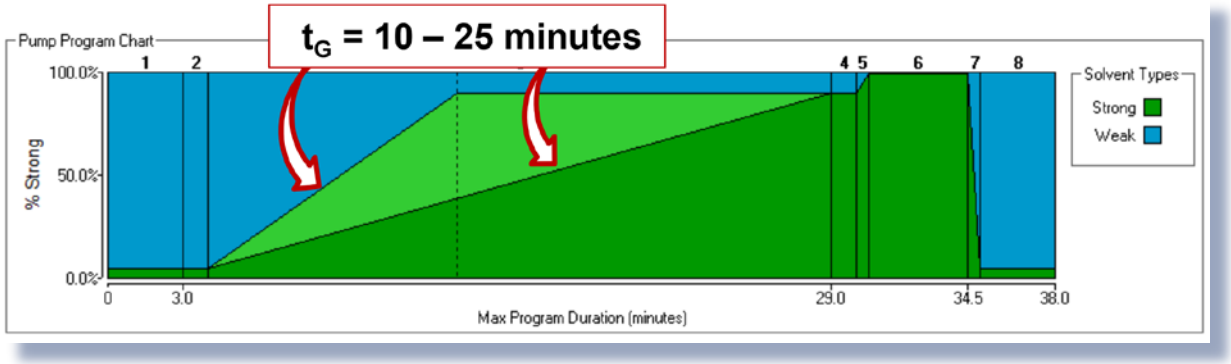
Multiple pH Levels



Include recommended pH

Multiple Gradients

(3-5 Levels Covering Range)



Multiple Columns

Include recommended column type

	Name	Valve Position	pH Upper Limit	Flow Rate	Diameter (mm)	Length (mm)	Time Required for One Column Volume (min)	Conditioning Time
1	BEH C18	Position 1	14.00	0.400	2.10	100.00	0.9	9.00
2	BEH Shield RP18	Position 2	14.00	0.400	2.10	100.00	0.9	9.00
3	HSS T3	Position 3	14.00	0.400	2.10	100.00	0.9	9.00
4	CSH Phenyl-Hexyl	Position 4	14.00	0.400	2.10	100.00	0.9	9.00

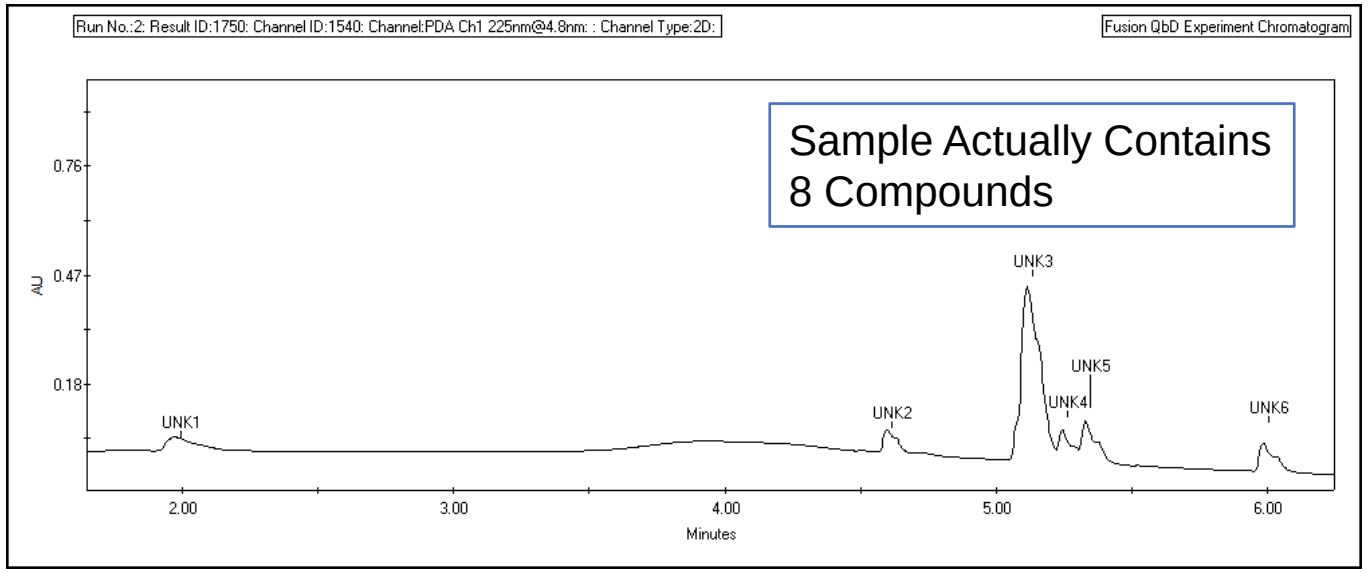
Trend Response* Automation

* – U.S. Patents No. 7,613,574 B2 and No. 8,219,328 B2

Flexible Trend Response Data Modeling Requires no Peak Tracking – Just Consistent Integration

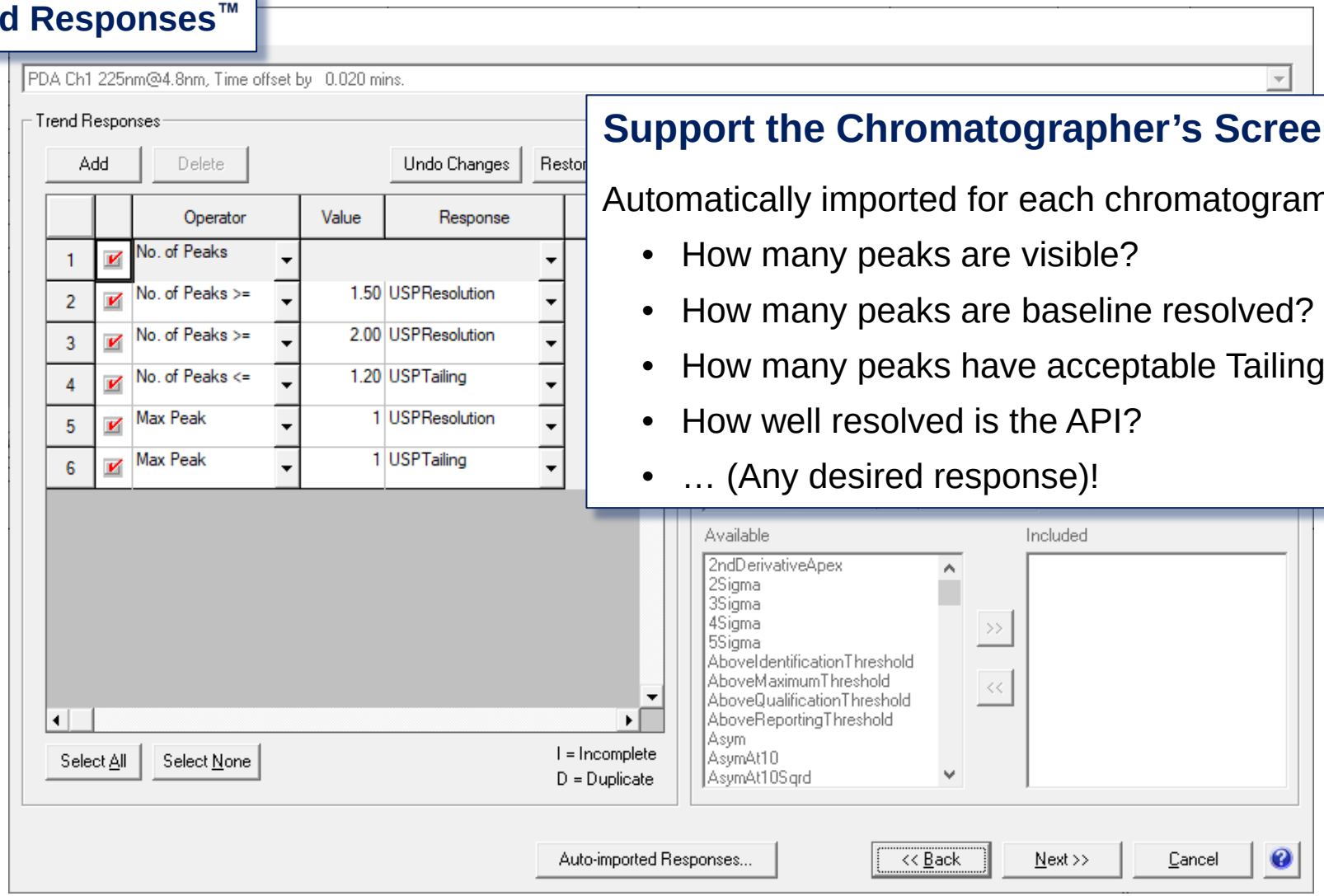
Consistent Integration Means:

- All integratable peaks of interest are integrated in each chromatogram.
- Baseline noise and artifact peaks of no interest are not integrated.



Screening Study – Simple Analysis

Trend Responses™



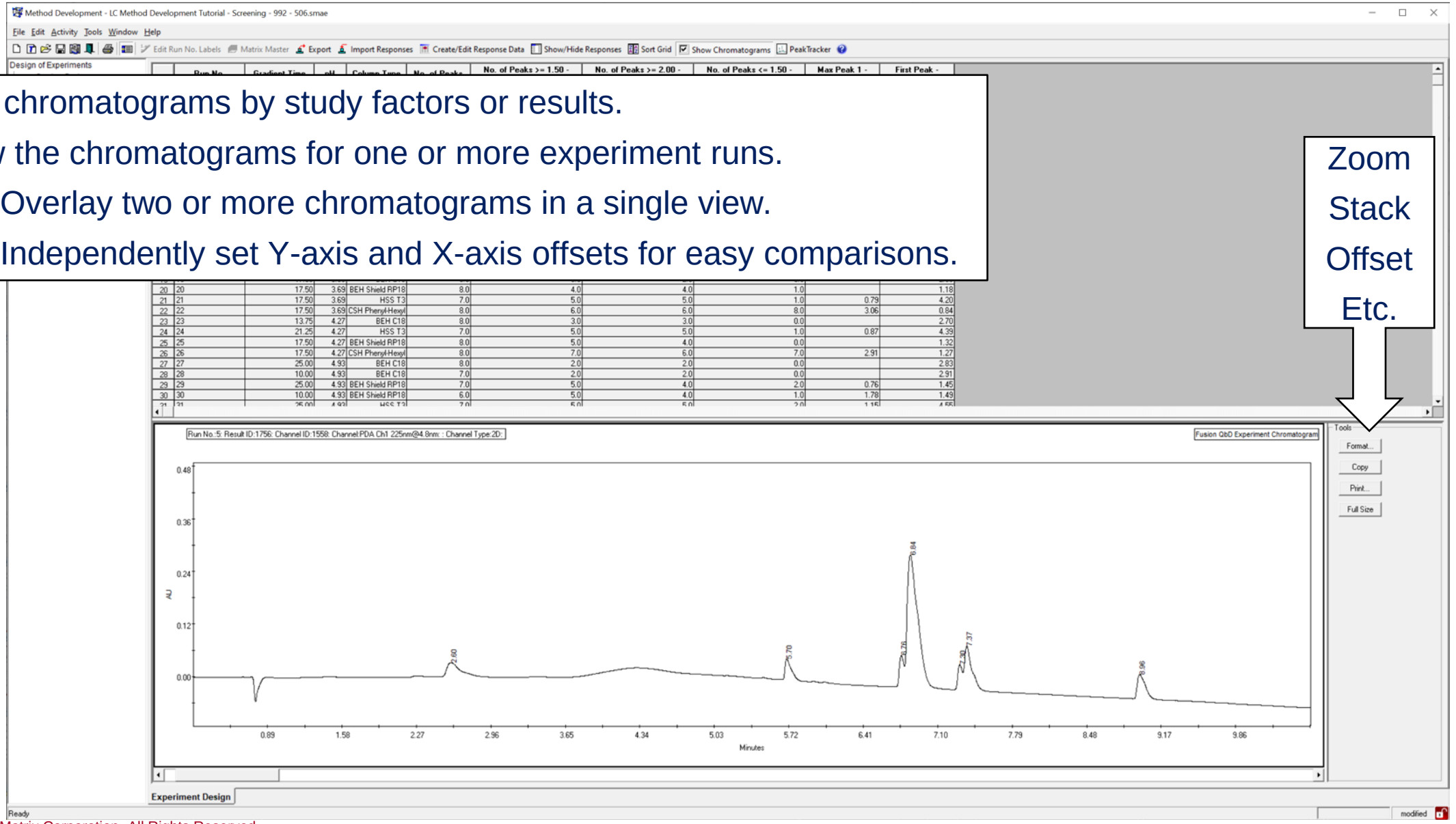
Support the Chromatographer's Screening Goals

Automatically imported for each chromatogram:

- How many peaks are visible?
- How many peaks are baseline resolved?
- How many peaks have acceptable Tailing?
- How well resolved is the API?
- … (Any desired response)!

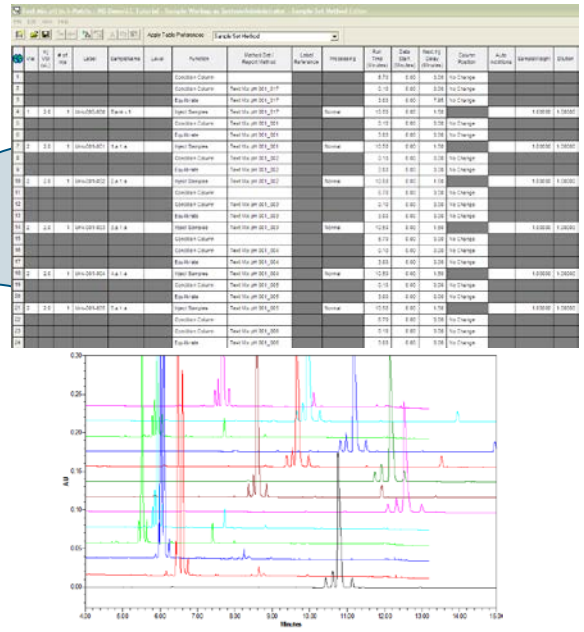
Automated Results Import & Data Review

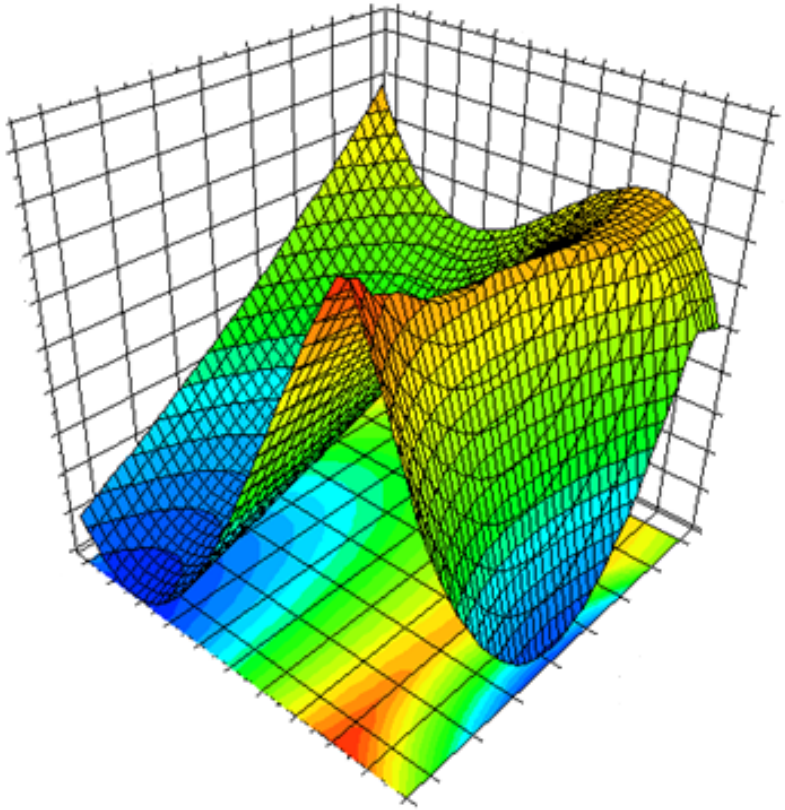
- Sort chromatograms by study factors or results.
- View the chromatograms for one or more experiment runs.
  - Overlay two or more chromatograms in a single view.
  - Independently set Y-axis and X-axis offsets for easy comparisons.



DoE (DOE) – A Model Building Methodology

Turning Chromatograms into Knowledge

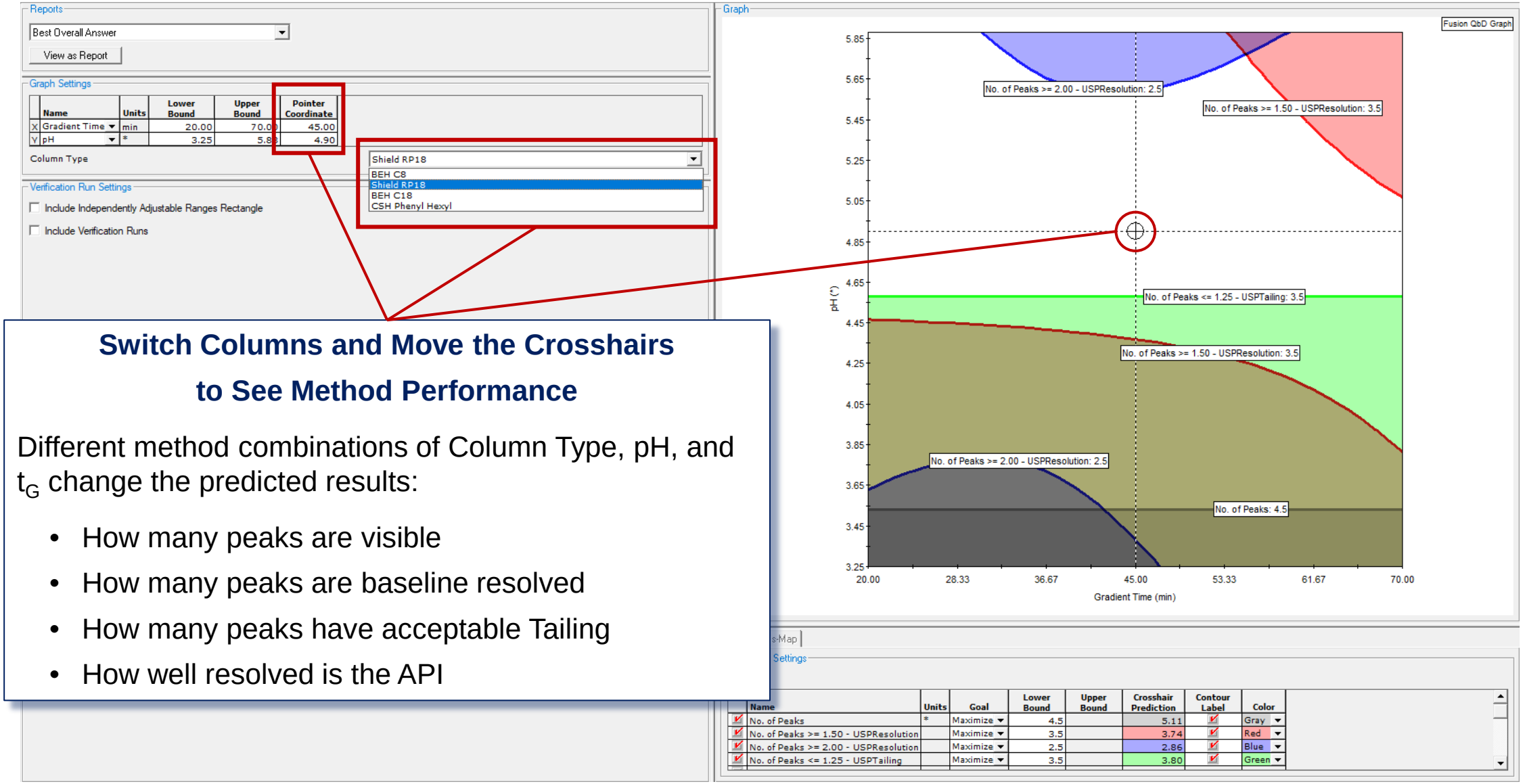




$$\#-R_s \geq 2.00 = 9.3 + 4.2(PFR) - 5.4(\Delta t_G)^2 + 12.7(\Delta t_G * pH) + 1.3(pH * \Delta T) + 1.6[(\Delta T)^{2*} \Delta t_G] + \ldots$$

Linear Effect | Curvature Effect | Interaction Effects | Complex Effect

Screening Study – Simple Analysis

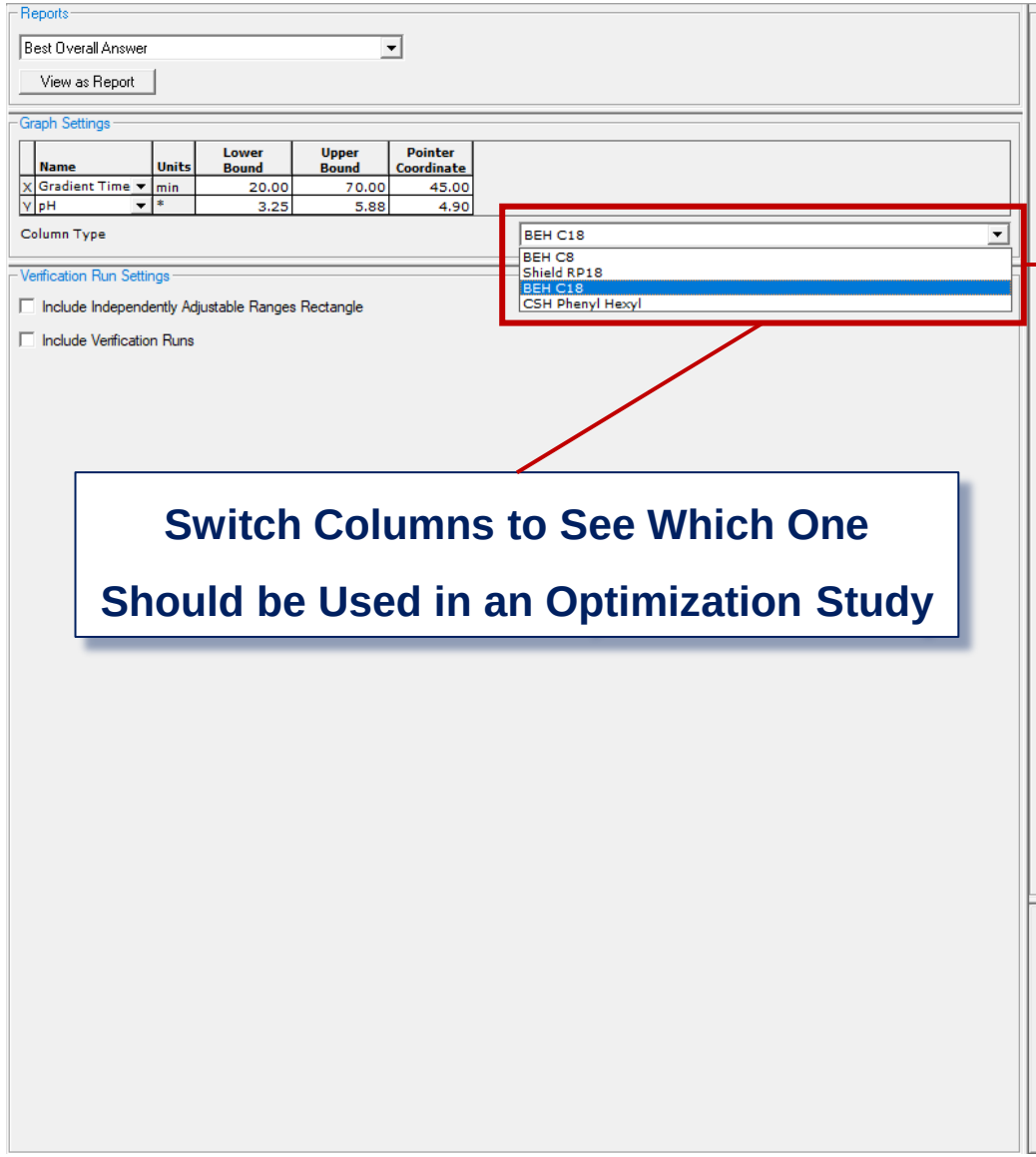


Switch Columns and Move the Crosshairs to See Method Performance

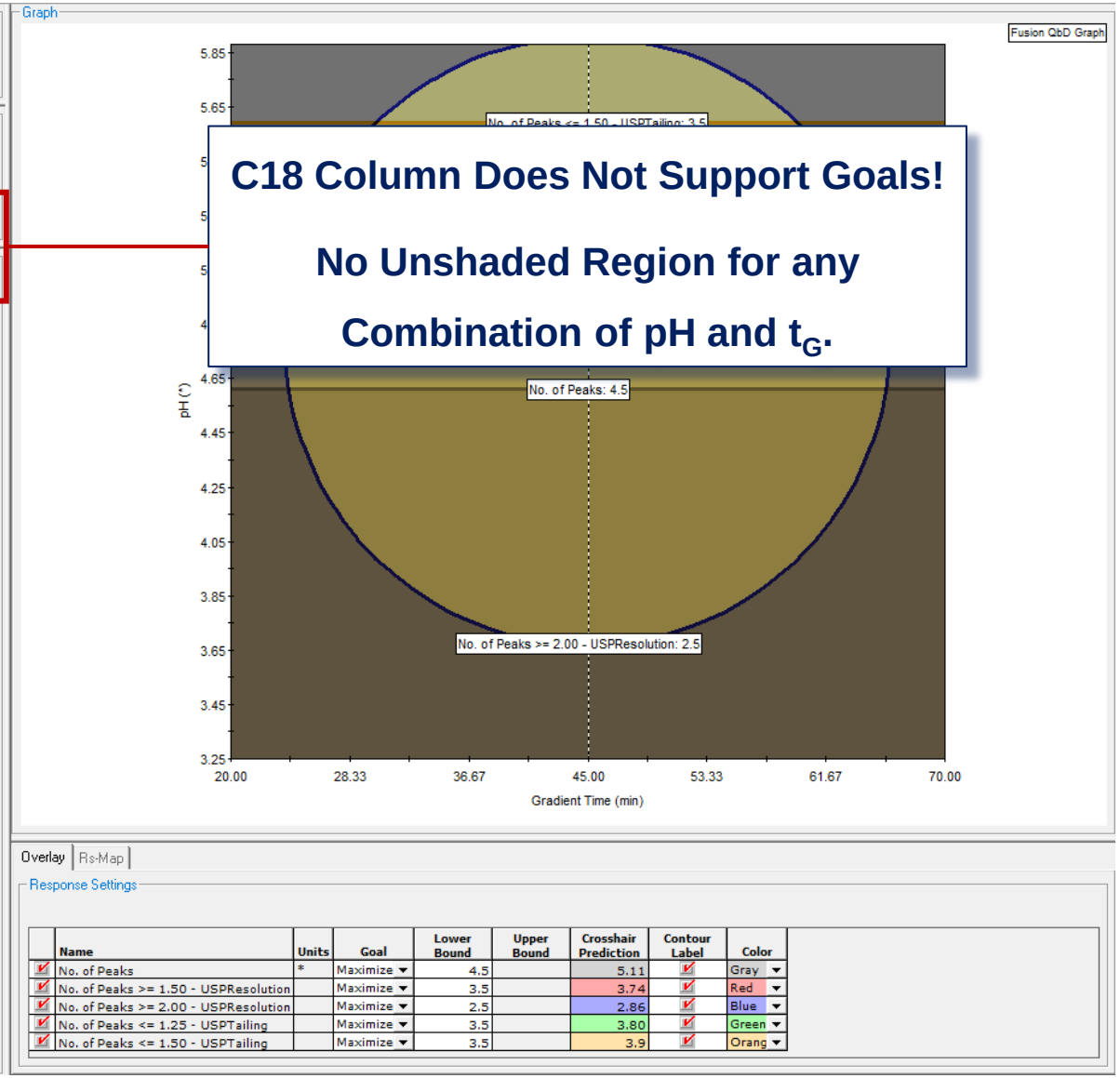
Different method combinations of Column Type, pH, and t_G change the predicted results:

- How many peaks are visible
- How many peaks are baseline resolved
- How many peaks have acceptable Tailing
- How well resolved is the API

Screening Study – Simple Analysis



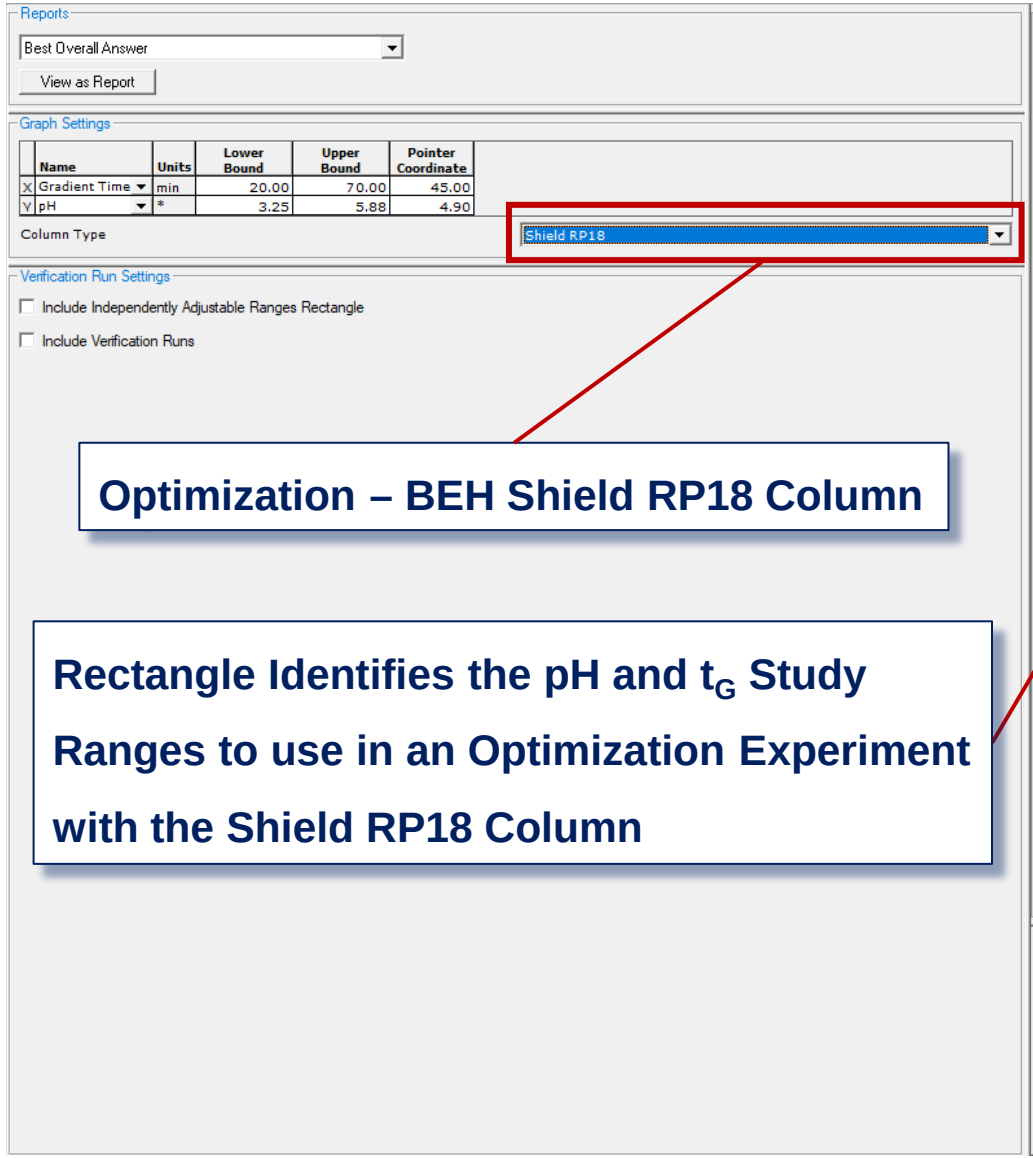
Switch Columns to See Which One Should be Used in an Optimization Study



C18 Column Does Not Support Goals!

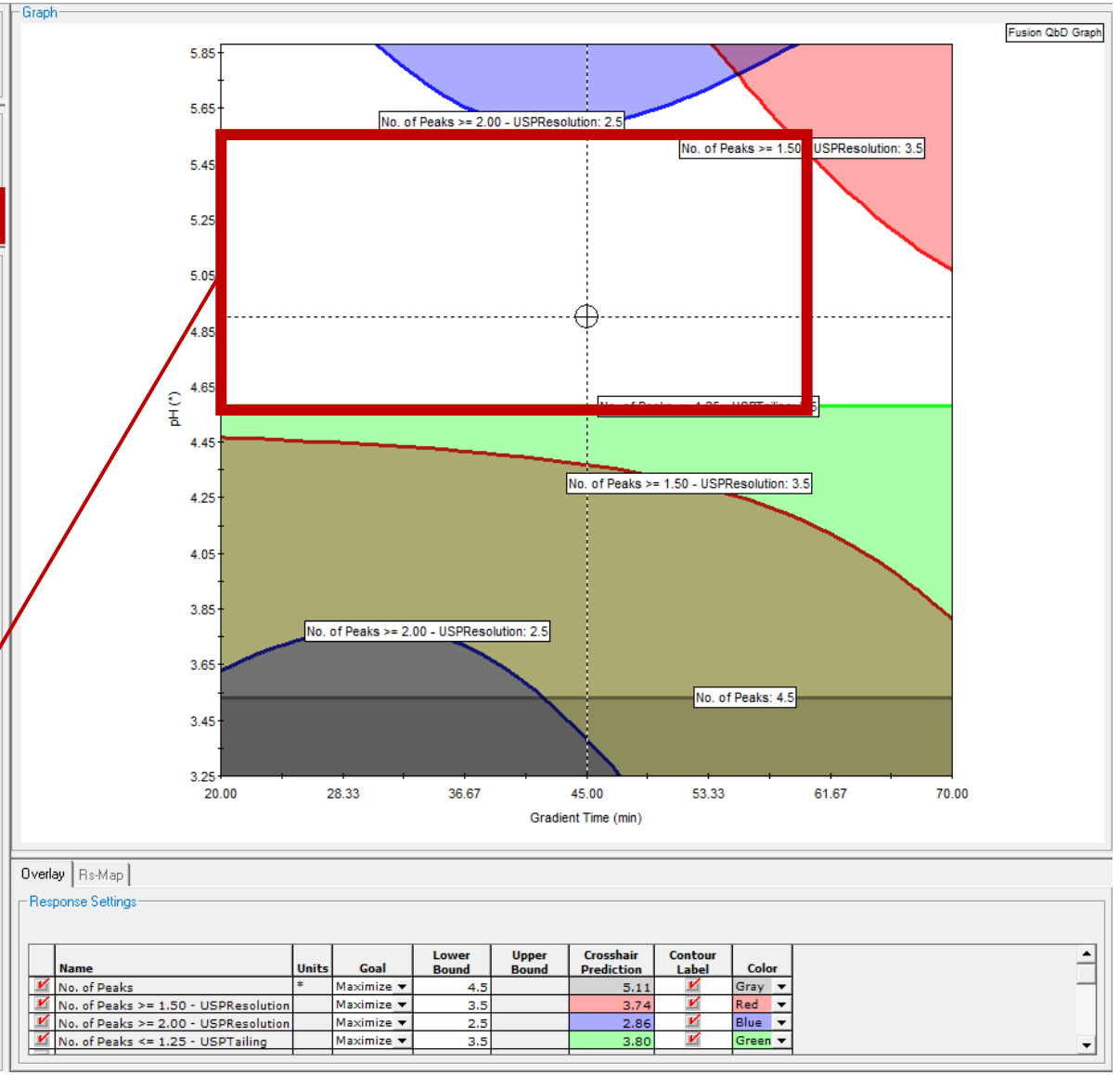
No Unshaded Region for any Combination of pH and t_G.

Screening Study – Simple Analysis

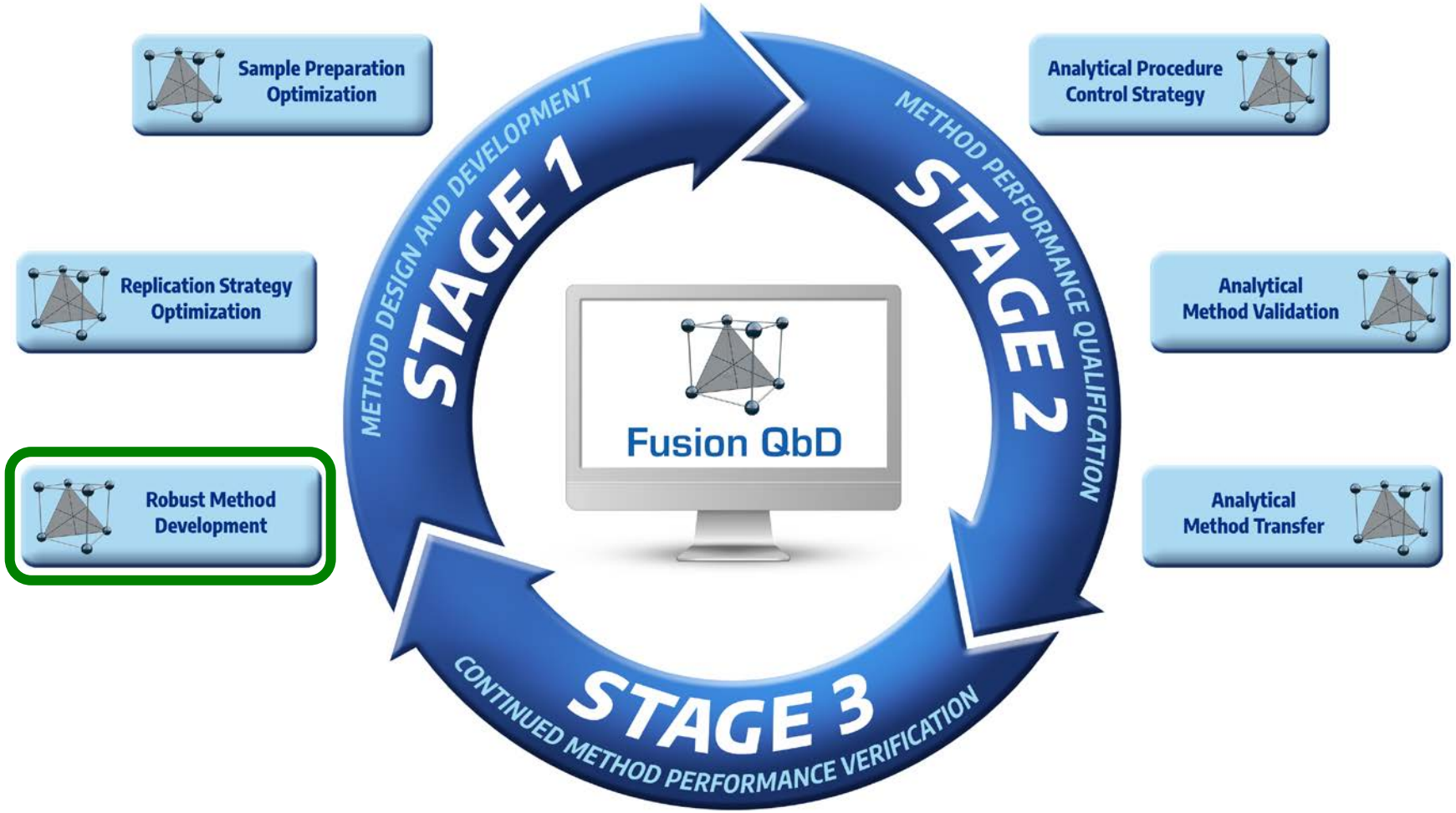


Optimization – BEH Shield RP18 Column

Rectangle Identifies the pH and t_G Study Ranges to use in an Optimization Experiment with the Shield RP18 Column



LC and LC-MS Method Optimization



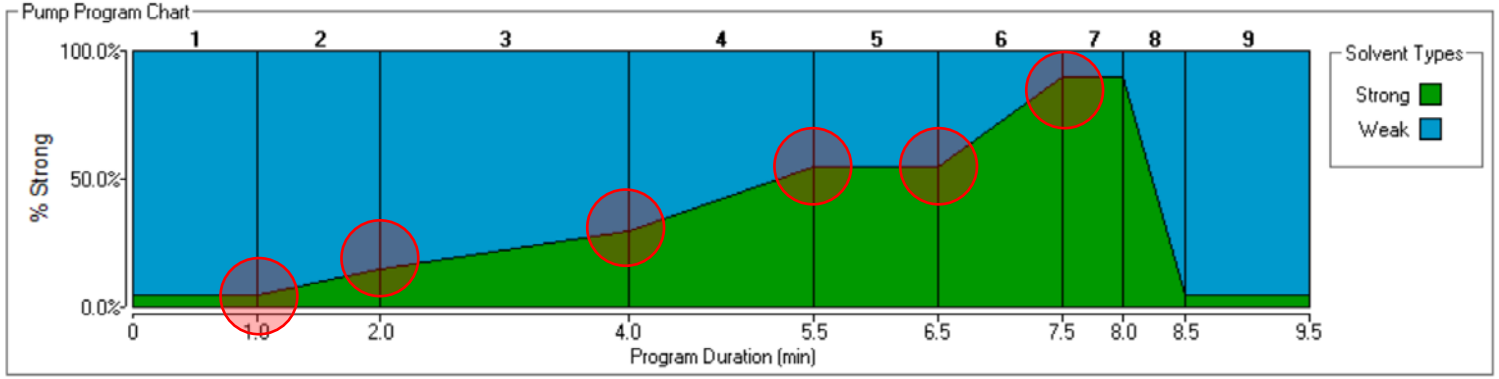
Key Differentiators – Method Optimization

- ✓ 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

Issues with Multi-segment Gradient Optimization

Most LC Method Development software relies primarily on localized gradient slope-based optimization. This drives the user to a multi-segment gradient method.

Multi-segment Gradients = Multiple Regions of POOR Robustness!



Localized Slope-Based Optimization is Now Recognized as High Risk.

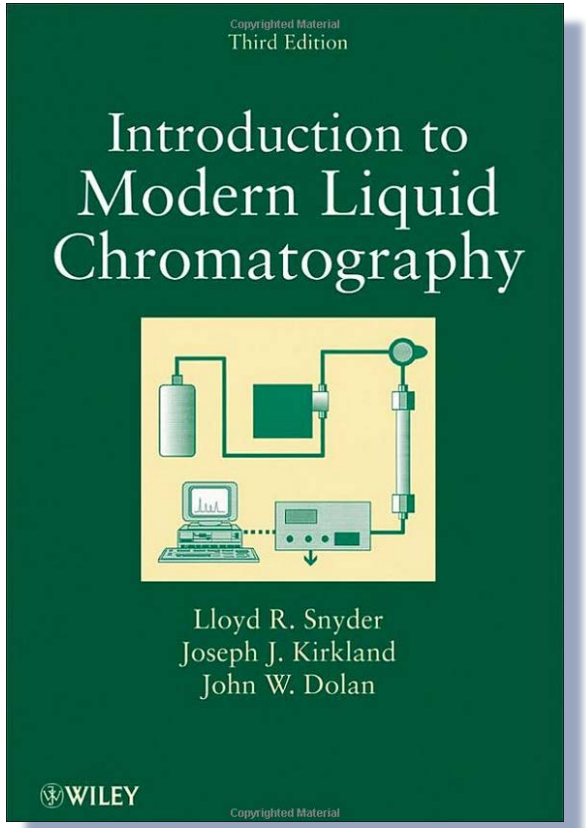
Fusion QbD Does Not Rely on This Approach!

(See the Next Slide →)

Modern Thinking by Recognized Experts

Issues with a Multi-step Gradient Approach to Method Optimization

"Increasing resolution by adjusting selectivity for different parts of the chromatogram can sometimes be achieved with a segmented gradient; … **Segmented gradients are not often used for improving resolution … because their ability to enhance resolution without increasing run time is usually limited**… However, there are other – generally more useful – means of optimizing resolution by changing selectivity and relative retention. **Also, separations that use segmented gradients to improve resolution are likely to be less reproducible when transferred to another piece of equipment**."



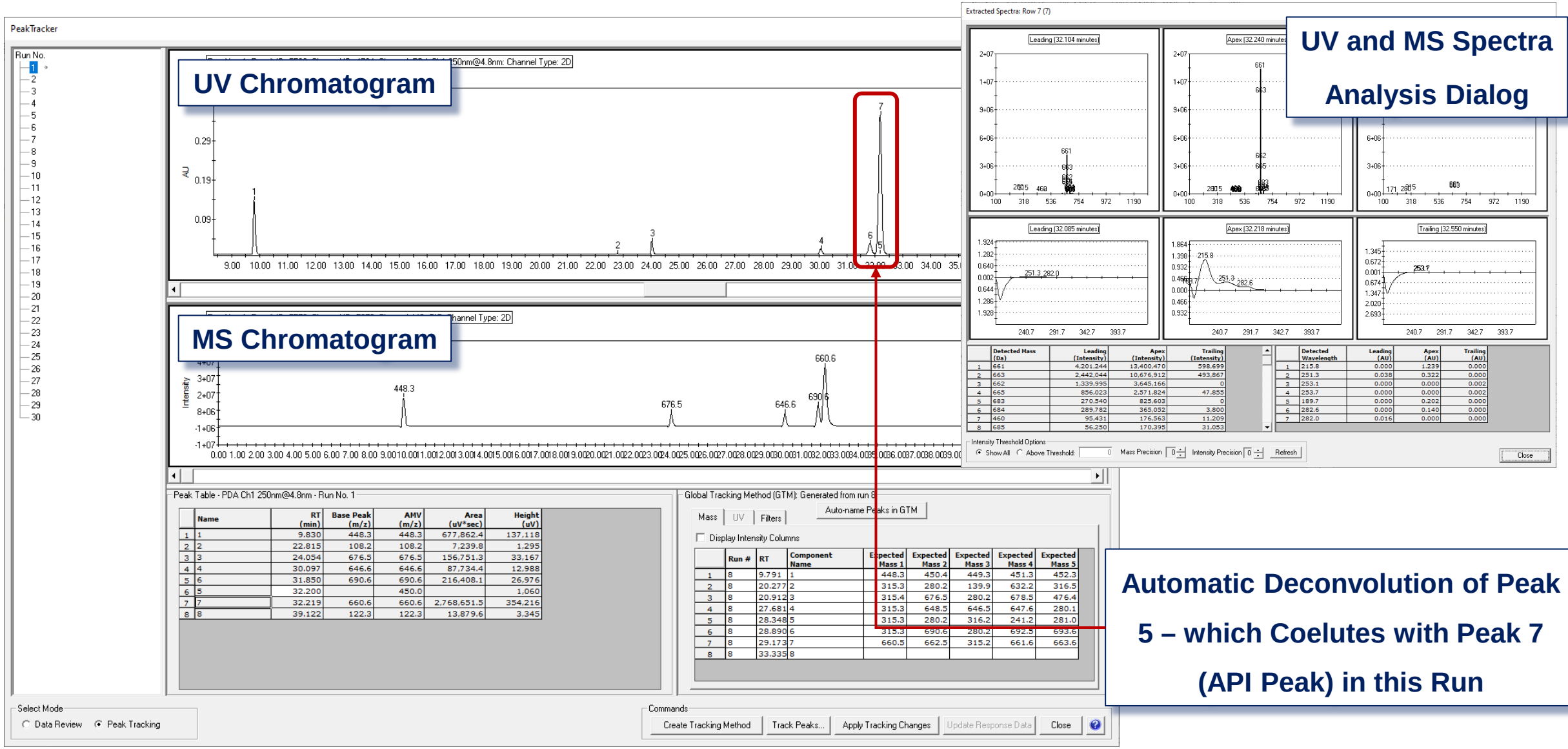
Snyder, Kirkland, and Dolan. (2010). *Introduction to Modern Liquid Chromatography*, 3rd Edition; John Wiley & Sons, Inc., Hoboken, New Jersey (p. 427-28)

Parameter Selection – Optimization Study

Method Parameter	Study Range
Pump Flow Rate (mL/min)	0.30 – 0.45
Column Oven Temperature (°C)	30.0 – 50.0
Gradient Time (min)*	25.0 – 40.0
pH	4.70 – 5.30
Column Type	BEH Shield RP18

Light green background color indicates result obtained from screening study.

PeakTracker™ – UV & MS Data Based Tracking



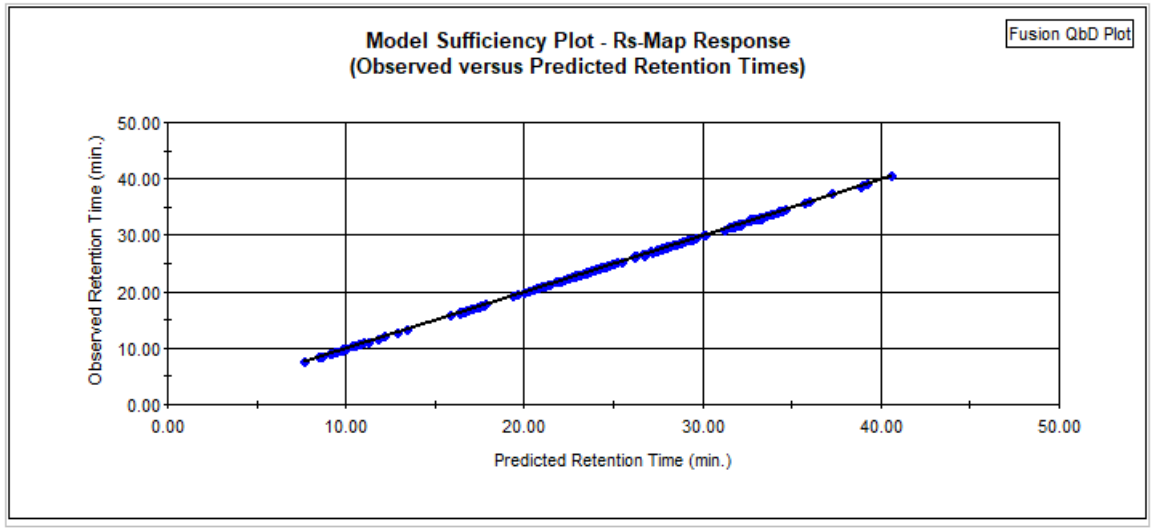
Flexible Data Modeling

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, …

Model Validation – Model Fit Metrics

Analysis Summary Report - Rs-Map Response



Prediction Equation

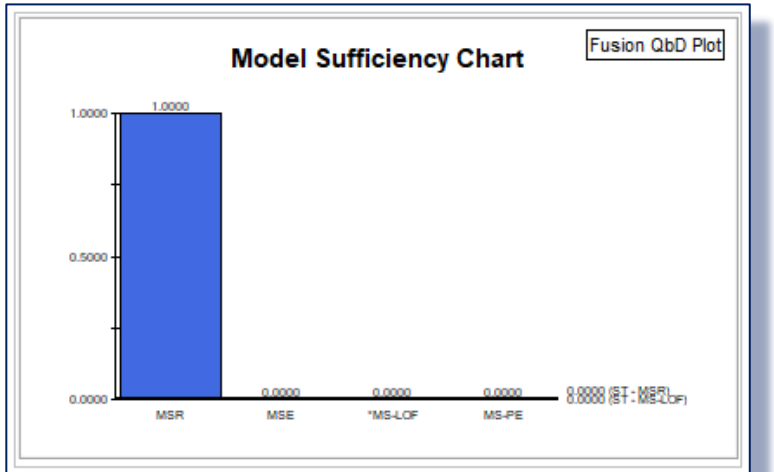
Observed Retention Time = -0.00068 + 1.00008 * Predicted Retention Time

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

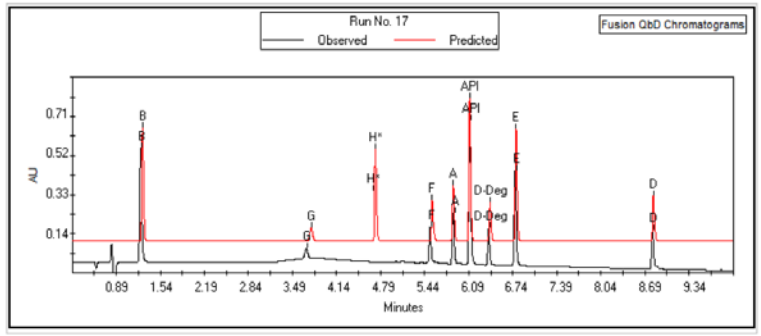
Name	Level Setting	Units
Pump Flow Rate	0.450	mL/min
Gradient Time	16.0	min
Oven Temperature	45.0	°C
pH	3.90	

Rs-Map Predicted Results

Response Name	Predicted Result	Calculated Result
A - ResolutionW50	6.079	6.630
API - ResolutionW50	4.356	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.

Integrated Robustness Simulation

ICH Q14

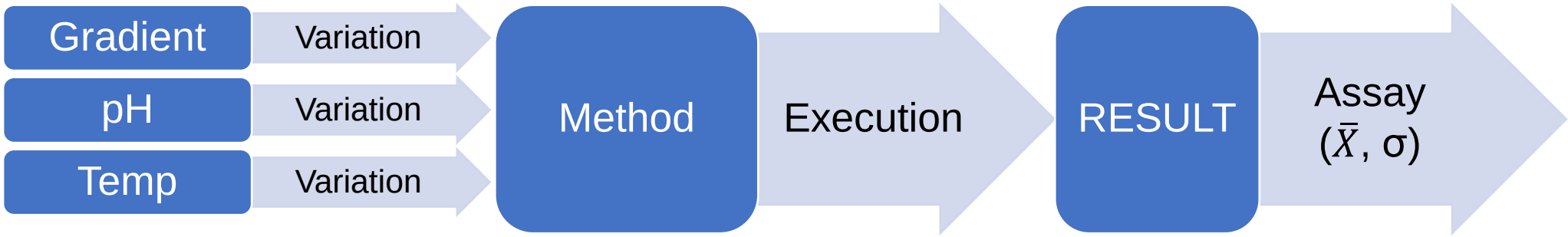
Data gained during the development studies (e.g., robustness data from a design of experiments (DoE) study) could be used as part of the validation data for the related analytical procedure performance characteristics and studies do not necessarily need to be repeated.

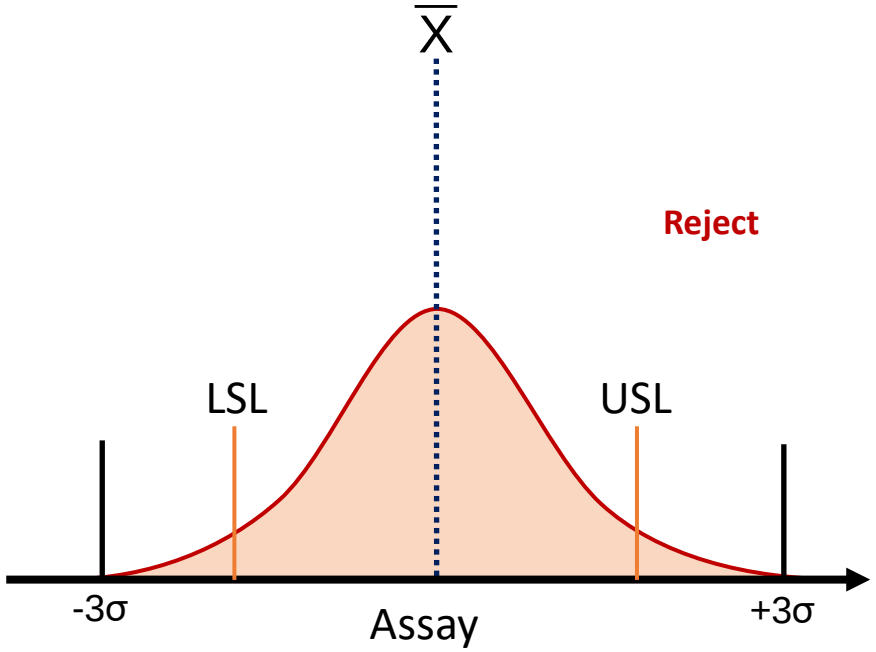
USP <1220>

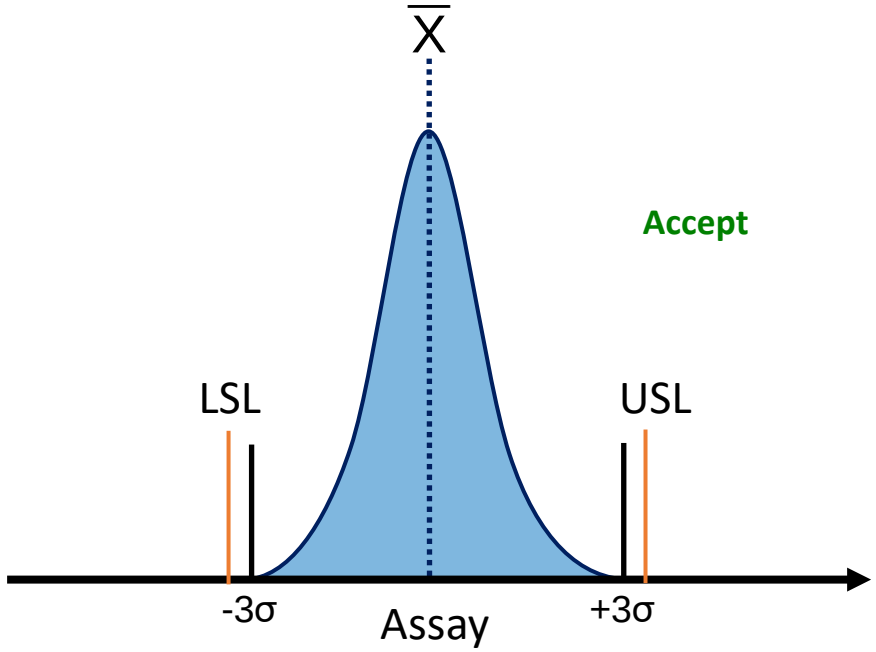
In some cases, it is helpful to demonstrate robustness of the procedure by developing models that describe the effect of parameters on the performance of the procedure, … This knowledge also enables the determination of robust operation regions for procedure parameters and, if desired, a method operable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

Monte Carlo Robustness Simulation

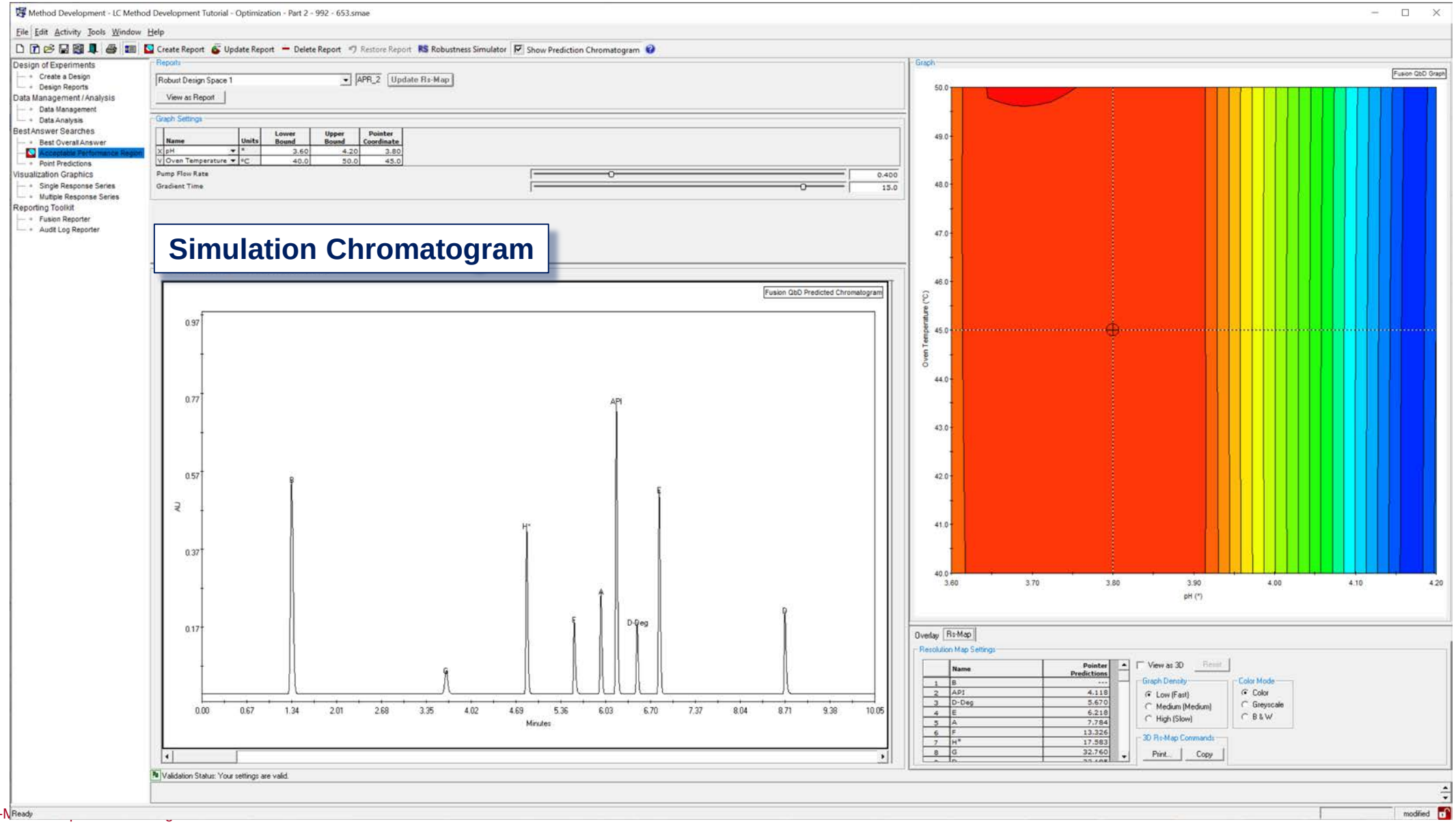
Example Study Parameters –
Expected Variation on Transfer



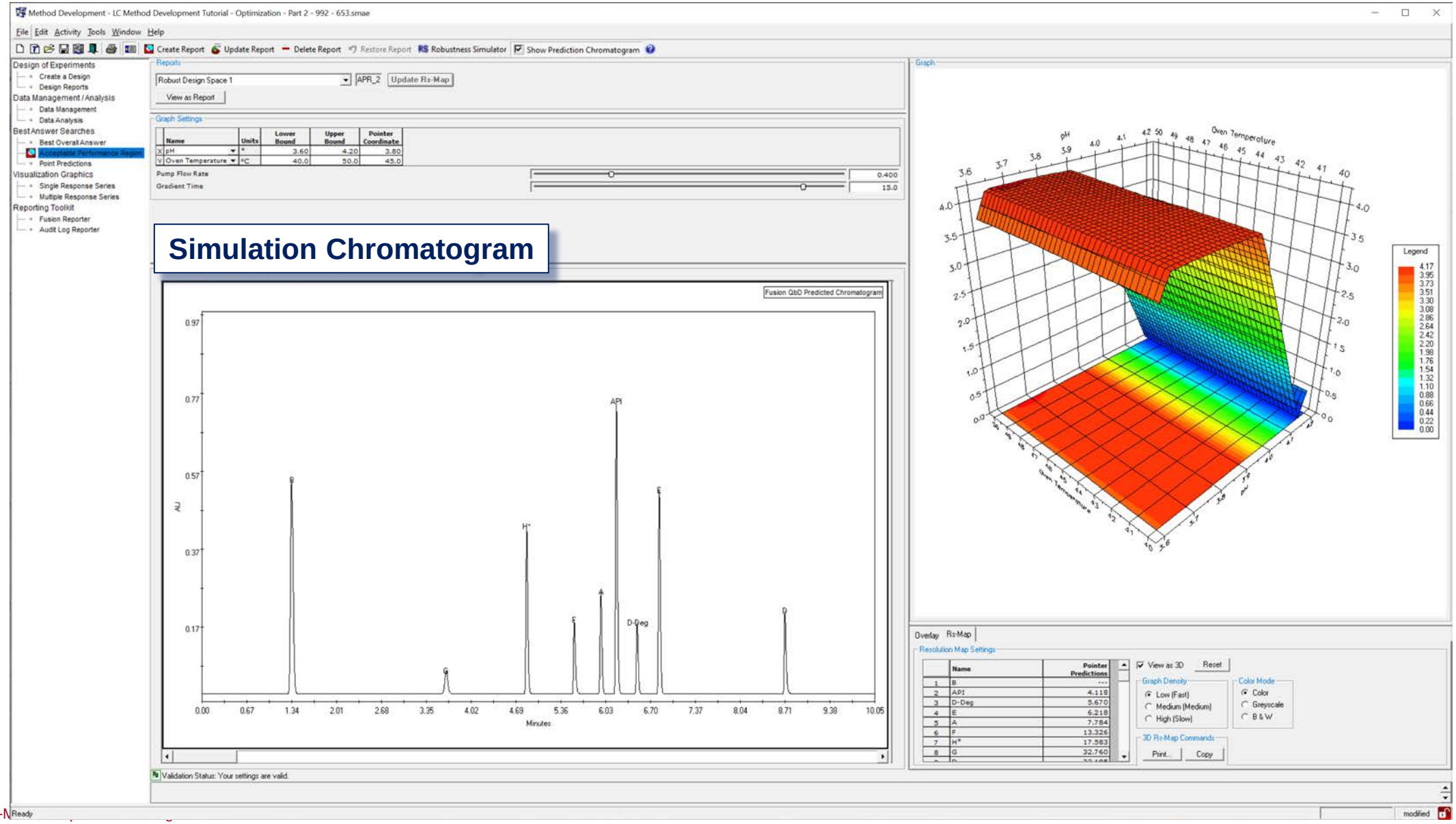




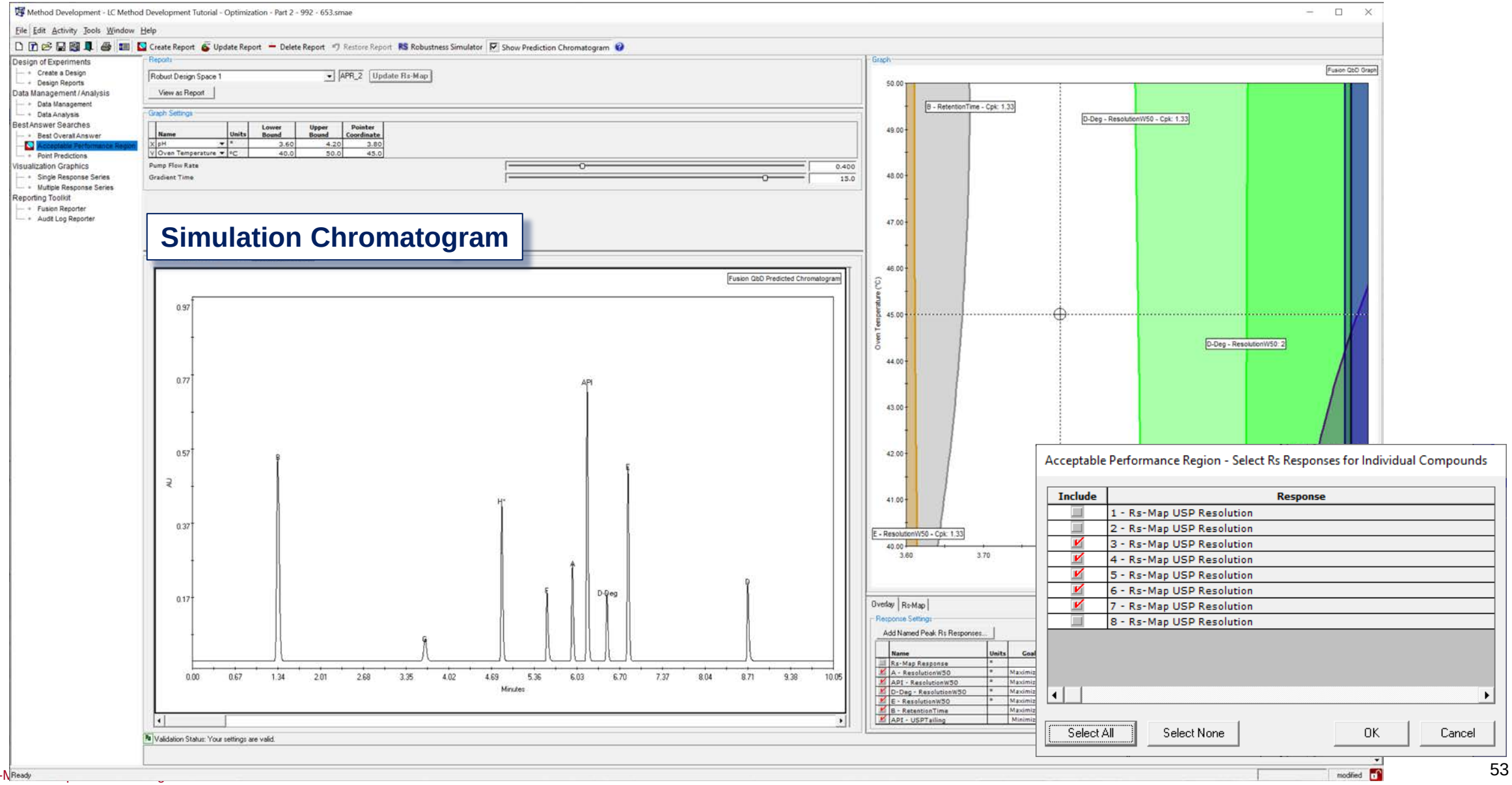
2D Resolution Map View



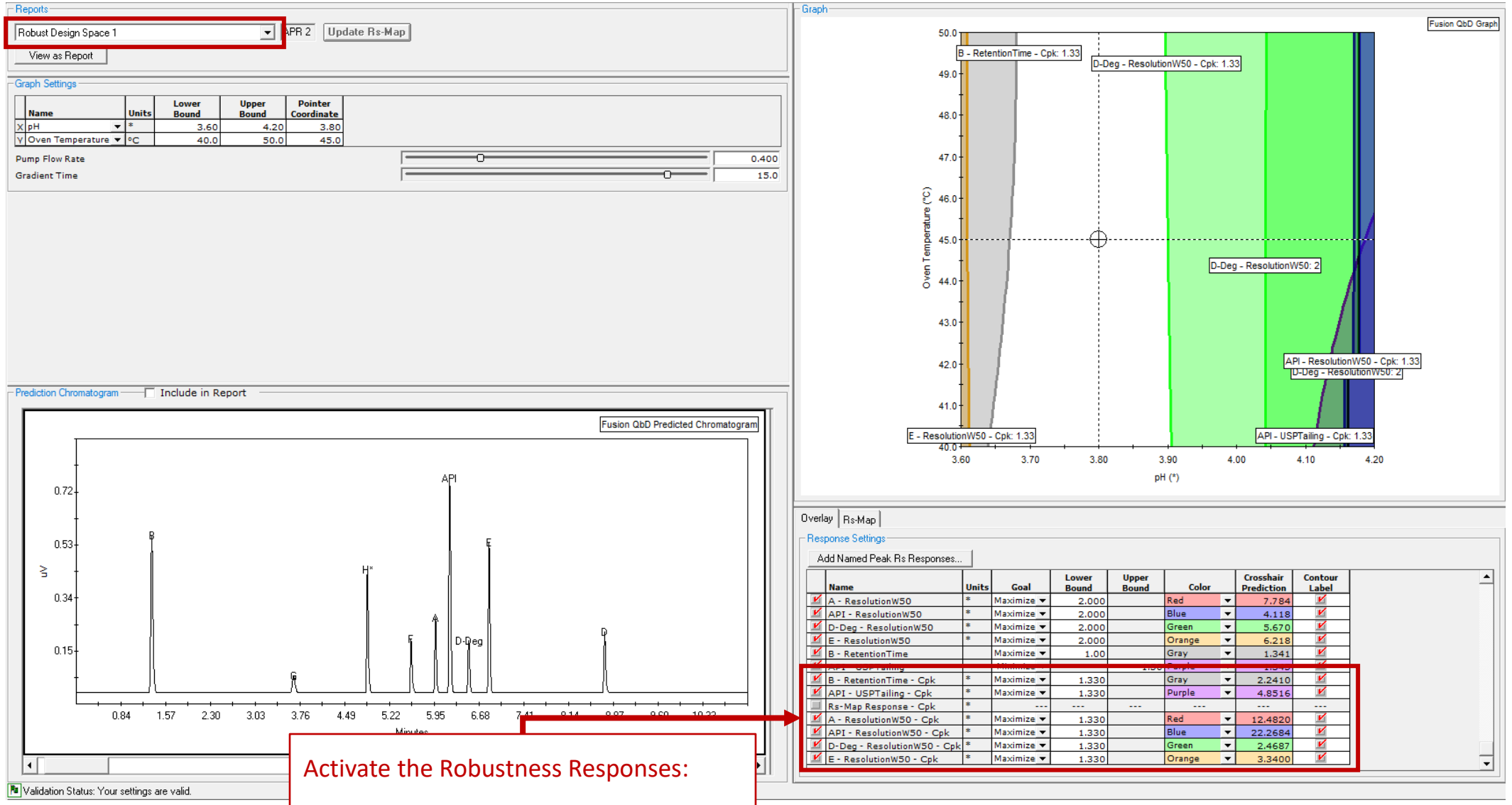
3D Resolution Map View



Multi-Response Overlay View



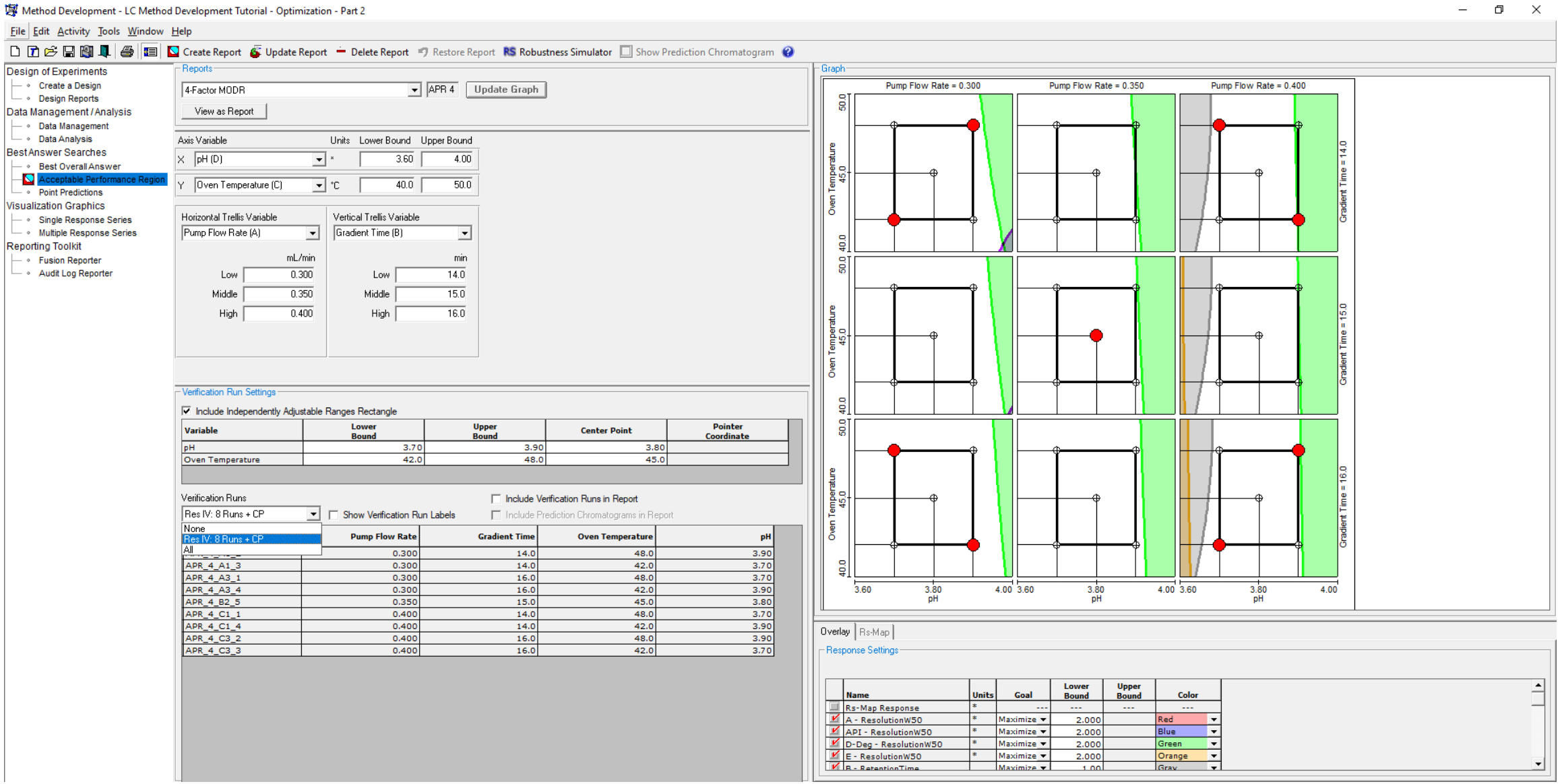
Final Design Space – Mean Performance + Robustness



Activate the Robustness Responses:

Check the checkboxes in the grid.

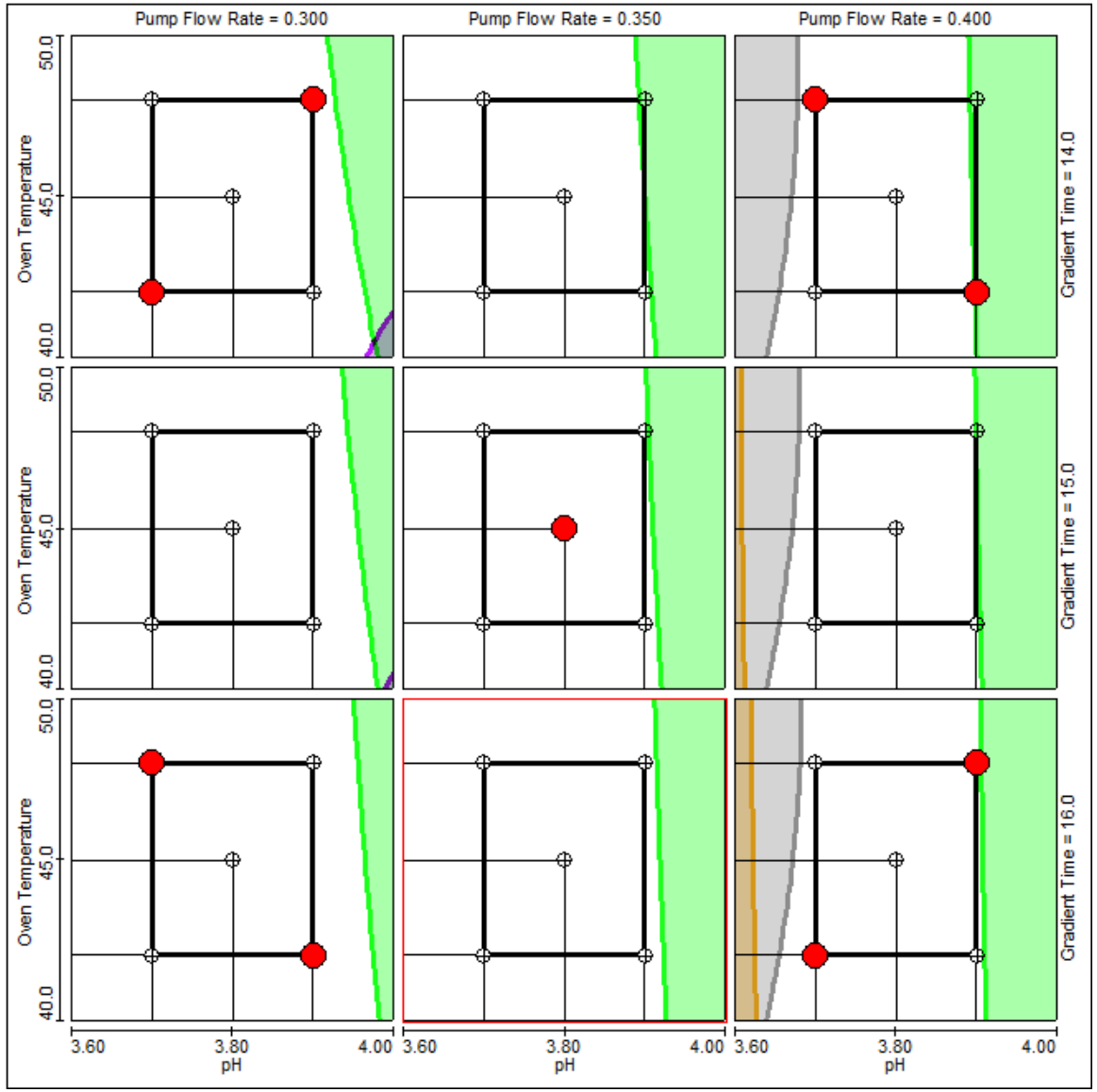
4-Factor Robustness Trellis Graph View



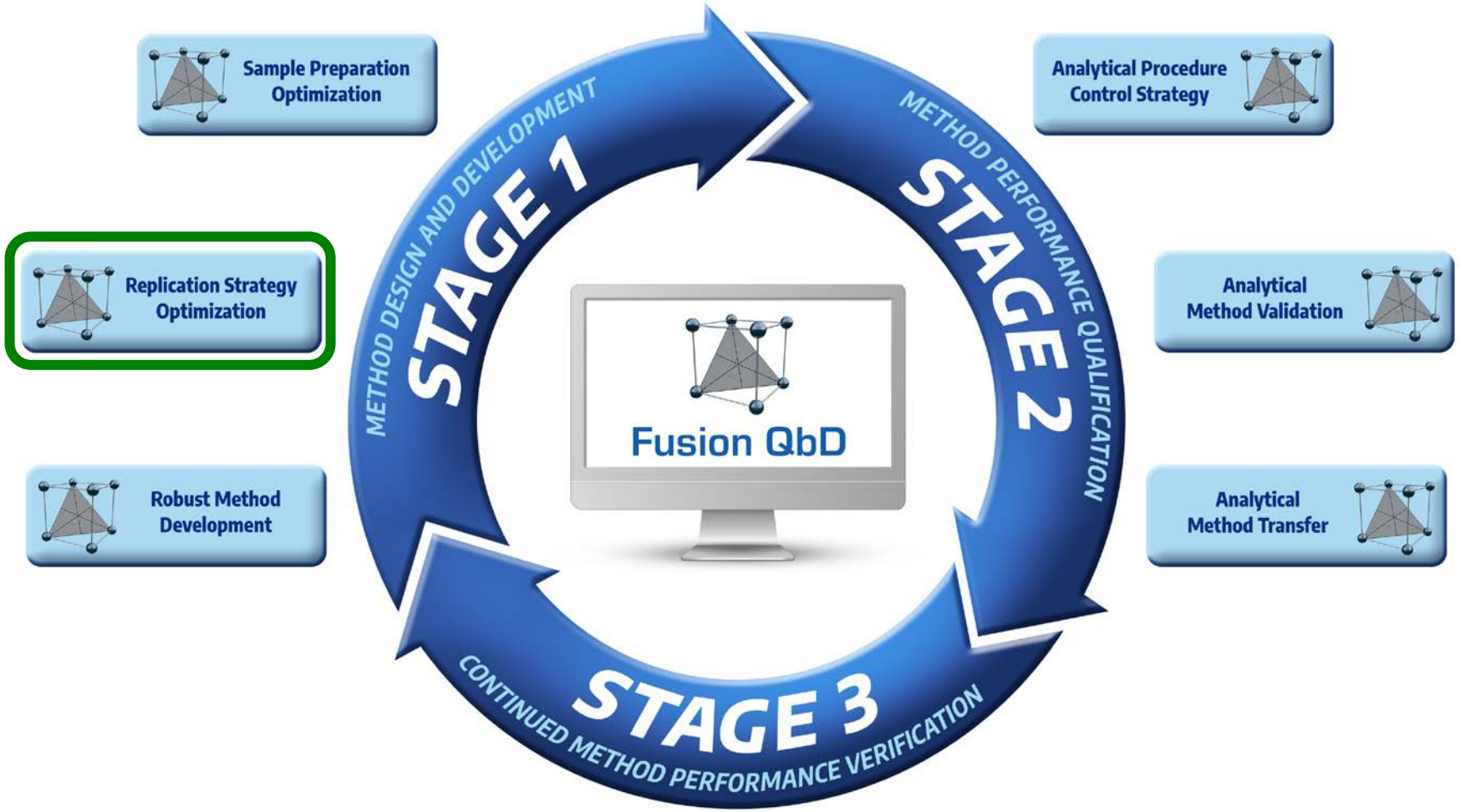
MODR Validation

Fusion QbD can generate Trellis graphs which display the mean performance and robustness MODR for 4 Factors Simultaneously.

All Fusion QbD Reports, which can include 2D, 3D, and 4D Trellis graphics and prediction and verification chromatograms can be output in a variety of file formats, including MS Word and Acrobat PDF.



Replication Strategy Optimization



Replication Strategy Optimization

Robust Final LC Method → Replication Strategy Optimization → APLM Stage 2

ICH Q14

Reportable Result: the result as generated by the analytical procedure after calculation or processing and applying the described sample replication. *(ICH Q2)*

ICH Q2(R2)

The experimental design of the validation study should reflect the number of replicates used in routine analysis to generate a reportable result.

USP <1220>

Stage 1:

Optimization of performance characteristics of the analytical procedure such as accuracy, precision, …; this includes a preliminary replication strategy for samples and standards.

Key Differentiator – Replication Strategy Optimization

✓ Quantifies Method Precision

- Defines the relative contribution of sample preparation error and sample injection error to overall method precision

✓ Optimizes Your Reportable Value

- Defines the *Preparation x Injection* combination which most efficiently and cost effectively meets the precision requirements of your method

Analytical Target Profile → Guard Bands

Negotiated with Production: Amount of Precision-to-Tolerance Ratio Available for the Analytical Method

- API method has a tolerance range of 4.0% (i.e., 98.0% to 102.0%)
- Analytical method may take up to 30% of the precision-to-tolerance ratio using a 95% confidence interval.

Determining Required Precision (σ_{max})

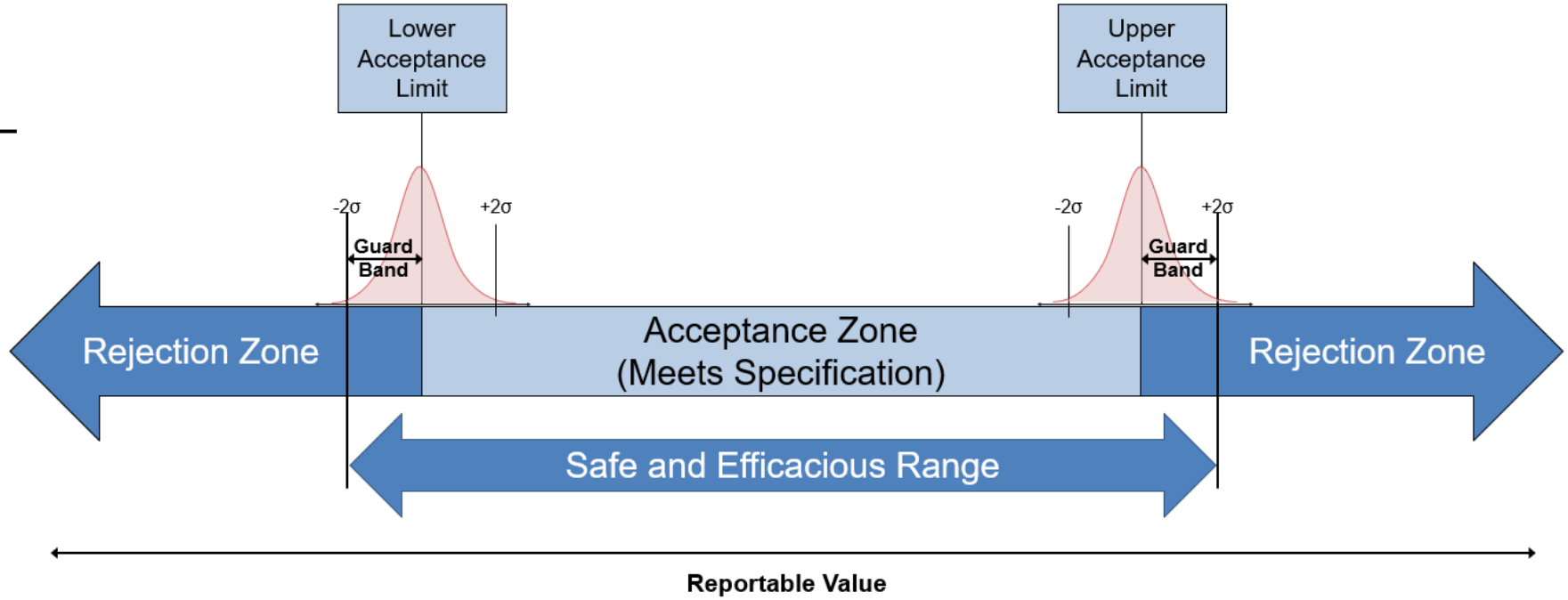
Tolerance Width = 4.00 (98.0 – 102.0)

Precision Width = 0.30*4.00 = 1.20

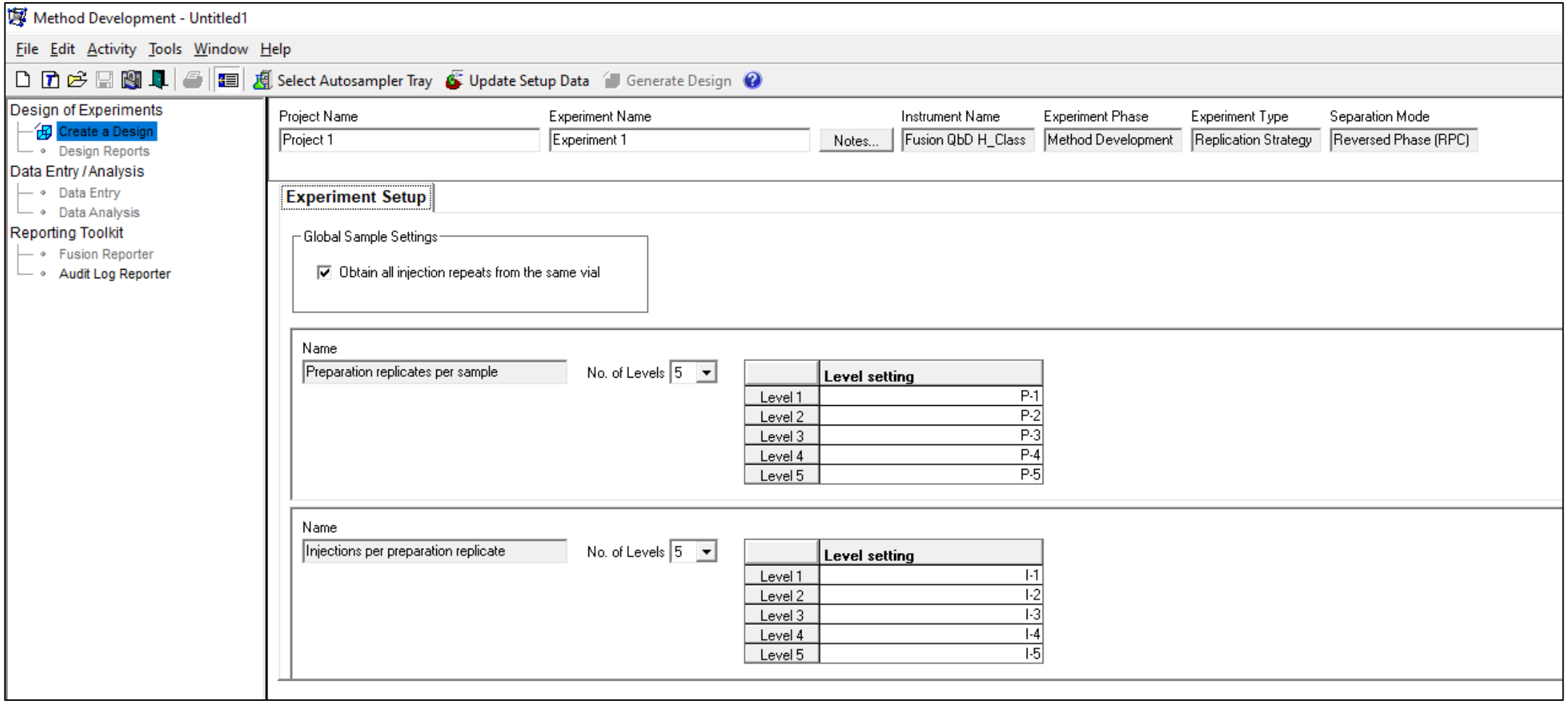
Split between LAL and UAL = 0.60

0.60 = 2σ width for 95% Confidence

σ_{max} = 0.60/2 = ±0.30



Replication Strategy for the Reportable Value



Replication Strategy for the Reportable Value

Fusion QbD reports the Components of Variation and the Corresponding % Contributions to method precision.

ANOVA

Variable Name	Sum of Squares	Degrees of Freedom	Mean Square	F-ratio	P-value
Sample Preparation	0.93	4	0.23	1.6566	0.1995
Injection	2.81	20	0.14		
Overall	3.74	24			

Between Variables Components of Variation

Variable Name	Variance	Standard Deviation	Degrees of Freedom	t-table Value	(+/-) 95% Confidence Limits	Error Contribution (%)
Sample Preparation	0.02	0.14	4	2.7764	0.3	11.61
Injection	0.14	0.37	20	2.0860	0.7	88.39

Overall Error in a Single Determination

Statistic	Value
Mean	99.88
Variance	0.16
Standard Deviation	0.40
% RSD	0.40

Fusion QbD also reports the TOST (±σ) and T.I Results for Replication Strategies from 1x1 to 10x10

Replication Strategy Predicted TOST and Interval Results

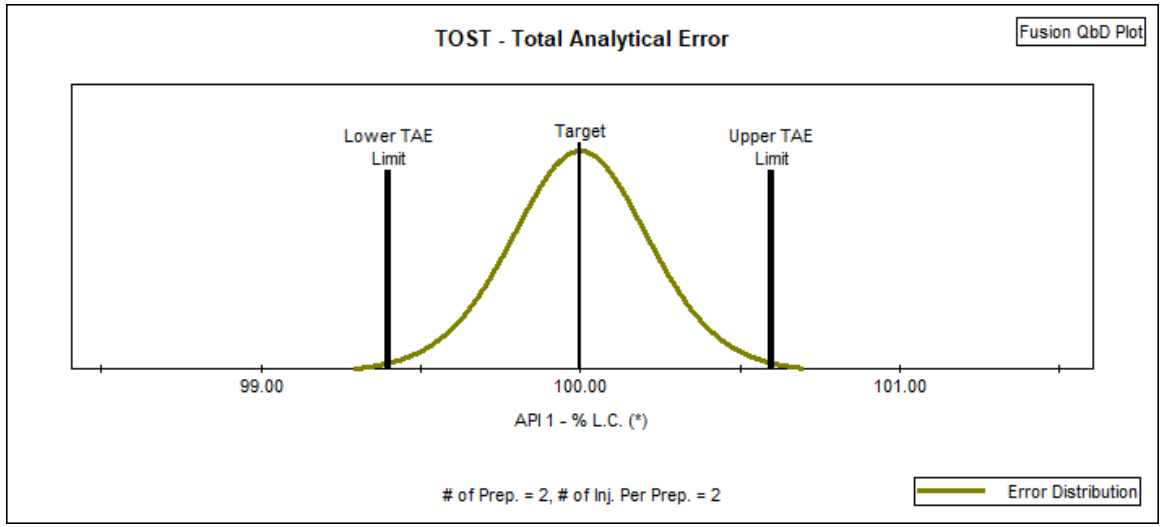
No. of Injections		No. of Preparations									
		1	2	3	4	5	6	7	8	9	10
1	±2σ	0.8426	0.5958	0.4865	0.4213	0.3768	0.3440	0.3185	0.2979	0.2809	0.2665
	T.I.	1.2286	0.7524	0.5792	0.4857	0.4256	0.3831	0.3510	0.3258	0.3053	0.2881
2	±2σ	0.6295	**0.4451**	0.3634	0.3147	0.2815	0.2570	0.2379	0.2225	0.2098	0.1991
	T.I.	0.7948	**0.5131**	0.4047	0.3442	0.3044	0.2758	0.2539	0.2365	0.2223	0.2103
3	±2σ	0.5400	0.3819	0.3118	0.2700	0.2415	0.2205	0.2041	0.1909	0.1800	0.1708
	T.I.	0.6429	0.4252	0.3388	0.2897	0.2572	0.2335	0.2154	0.2009	0.1890	0.1790
4	±2σ	0.4892	0.3459	0.2824	0.2446	0.2188	0.1997	0.1849	0.1730	0.1631	0.1547
	T.I.	0.5639	0.3783	0.3031	0.2599	0.2311	0.2102	0.1940	0.1811	0.1704	0.1615
5	±2σ	0.4560	0.3224	0.2633	0.2280	0.2039	0.1862	0.1724	0.1612	0.1520	0.1442
	T.I.	0.5150	0.3487	0.2803	0.2409	0.2144	0.1951	0.1802	0.1683	0.1584	0.1502
6	±2σ	0.4325	0.3058	0.2497	0.2162	0.1934	0.1766	0.1635	0.1529	0.1442	0.1368
	T.I.	0.4816	0.3281	0.2645	0.2275	0.2027	0.1845	0.1705	0.1593	0.1500	0.1422
7	±2σ	0.4148	0.2933	0.2395	0.2074	0.1855	0.1694	0.1568	0.1467	0.1383	0.1312
	T.I.	0.4572	0.3130	0.2527	0.2176	0.1940	0.1767	0.1633	0.1526	0.1437	0.1362
8	±2σ	0.4011	0.2836	0.2316	0.2005	0.1794	0.1637	0.1516	0.1418	0.1337	0.1268
	T.I.	0.4386	0.3014	0.2437	0.2100	0.1872	0.1706	0.1577	0.1473	0.1388	0.1316
9	±2σ	0.3901	0.2758	0.2252	0.1950	0.1744	0.1592	0.1474	0.1379	0.1300	0.1234
	T.I.	0.4239	0.2922	0.2364	0.2039	0.1818	0.1657	0.1532	0.1432	0.1349	0.1279
10	±2σ	0.3810	0.2694	0.2200	0.1905	0.1704	0.1556	0.1440	0.1347	0.1270	0.1205
	T.I.	0.4120	0.2846	0.2306	0.1989	0.1774	0.1617	0.1495	0.1398	0.1317	0.1248

Replication Strategy for the Reportable Value

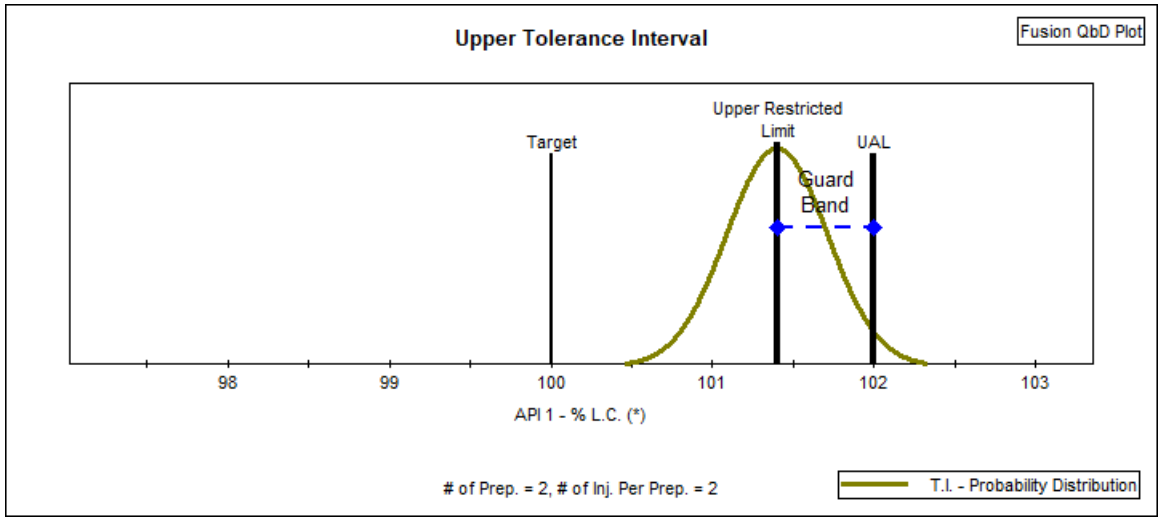
Tolerance Interval Analysis Results

Interval Setting	Value	Number of Preparations	Number of Injections per Preparation
Target	100.00	2	2
Acceptance Limits	±2.00		
Desired Probability %	90.00		
Tolerance Alpha %	5.00		
Grand Mean	99.88		
Computed Tolerance Interval	±0.51	Pass	
Required Guard Band Width	±0.60		

The computed Tolerance Interval falls within the defined Acceptance Limits.



The Final Replication Strategy is Transferred to APLM Stage 2



Replication Strategy Fails → Sample Prep Study

Between Variables Components of Variation

Variable Name	Variance	Standard Deviation	Degrees of Freedom	t-table Value	(+/-) 95% Confidence Limits	Error Contribution (%)
Sample Preparation	0.065	0.256	4	2.7764	0.71	95.27
Injection	0.003	0.057	20	2.0860	0.11	4.73

Overall Error in a Single Determination

Statistic	Value
Mean	100.142
Variance	0.069
Standard Deviation	0.262
% RSD	0.262

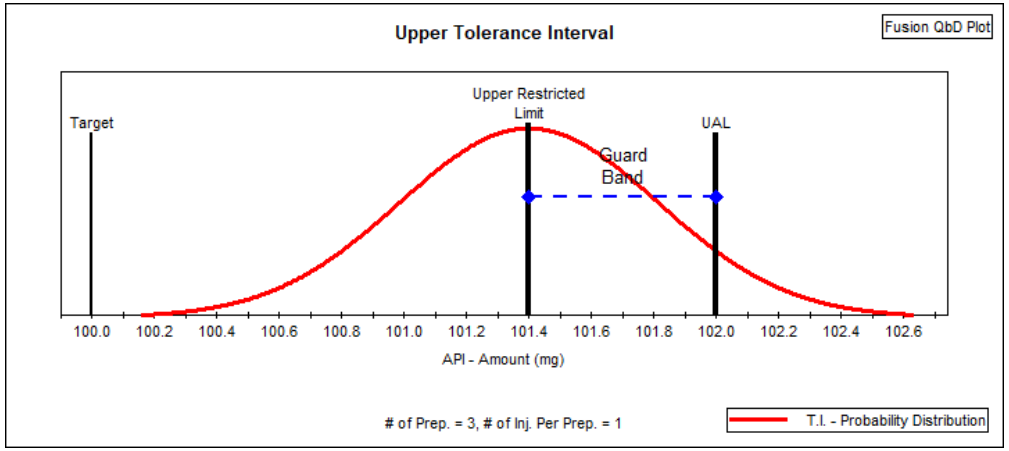
TOST Analysis Results Summary

Statistic	Value	Pass/Fail
TAE Width (2σ) - Target	±0.600	
Computed TAE Width (2σ)	±0.434	Pass
FPT	<0.0001	
Cp	4.4075	
Variance	0.023	
Standard Deviation	0.151	
% RSD	0.15	
% CV	0.15	

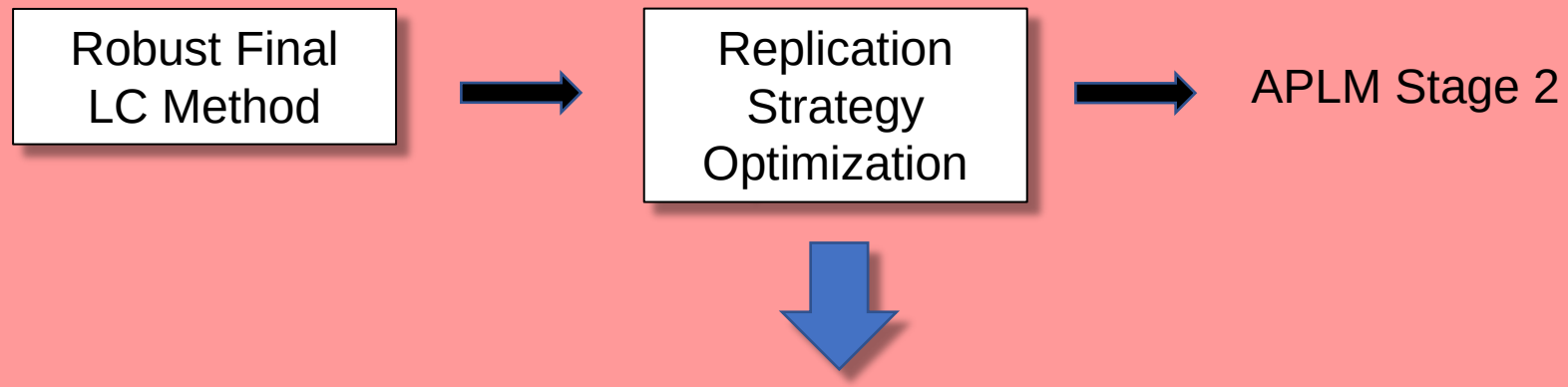
Tolerance Interval Analysis Results

Interval Setting	Value	Number of Preparations	Number of Injections per Preparation
Target	100.000	3	1
Acceptance Limits	±2.000		
Desired Probability %	95.00		
Tolerance Alpha %	5.00		
Grand Mean	100.142		
Computed Tolerance Interval	±0.812	Fail	
Required Guard Band Width	±0.600		

No. of Injections		No. of Preparations									
		1	2	3	4	5	6	7	8	9	10
	±2σ	0.7517	0.5313	**0.4340**	0.3759	0.3362	0.3069	0.2841	0.2658	0.2506	0.2377
1	T.I.	1.7228	1.0550	**0.8121**	0.6810	0.5968	0.5372	0.4922	0.4568	0.4280	0.4040
2	±2σ	0.7428	0.5252	0.4288	0.3714	0.3322	0.3032	0.2807	0.2626	0.2476	0.2349
	T.I.	1.4742	0.9516	0.7506	0.6383	0.5646	0.5115	0.4709	0.4387	0.4122	0.3900
3	±2σ	0.7398	0.5231	0.4271	0.3699	0.3308	0.3020	0.2796	0.2615	0.2466	0.2339
	T.I.	1.3843	0.9156	0.7296	0.6239	0.5537	0.5028	0.4638	0.4326	0.4069	0.3854
4	±2σ	0.7383	0.5220	0.4262	0.3691	0.3302	0.3014	0.2790	0.2610	0.2461	0.2335
	T.I.	1.3376	0.8973	0.7189	0.6166	0.5482	0.4985	0.4602	0.4295	0.4043	0.3830
5	±2σ	0.7374	0.5214	0.4257	0.3687	0.3298	0.3010	0.2787	0.2607	0.2458	0.2332
	T.I.	1.3089	0.8862	0.7125	0.6122	0.5450	0.4959	0.4580	0.4277	0.4027	0.3816
6	±2σ	0.7368	0.5210	0.4254	0.3684	0.3295	0.3008	0.2785	0.2605	0.2456	0.2330
	T.I.	1.2896	0.8787	0.7082	0.6093	0.5428	0.4941	0.4566	0.4265	0.4016	0.3807
7	±2σ	0.7363	0.5207	0.4251	0.3682	0.3293	0.3006	0.2783	0.2603	0.2454	0.2328
	T.I.	1.2756	0.8733	0.7051	0.6072	0.5412	0.4929	0.4556	0.4256	0.4009	0.3800
8	±2σ	0.7360	0.5204	0.4249	0.3680	0.3291	0.3005	0.2782	0.2602	0.2453	0.2327
	T.I.	1.2650	0.8693	0.7028	0.6056	0.5400	0.4920	0.4548	0.4250	0.4003	0.3795
9	±2σ	0.7357	0.5202	0.4248	0.3679	0.3290	0.3004	0.2781	0.2601	0.2452	0.2327
	T.I.	1.2568	0.8662	0.7010	0.6044	0.5391	0.4912	0.4542	0.4244	0.3999	0.3791
10	±2σ	0.7355	0.5201	0.4247	0.3678	0.3289	0.3003	0.2780	0.2601	0.2452	0.2326
	T.I.	1.2501	0.8636	0.6995	0.6034	0.5384	0.4906	0.4537	0.4240	0.3995	0.3788



Sample Prep Method Optimization



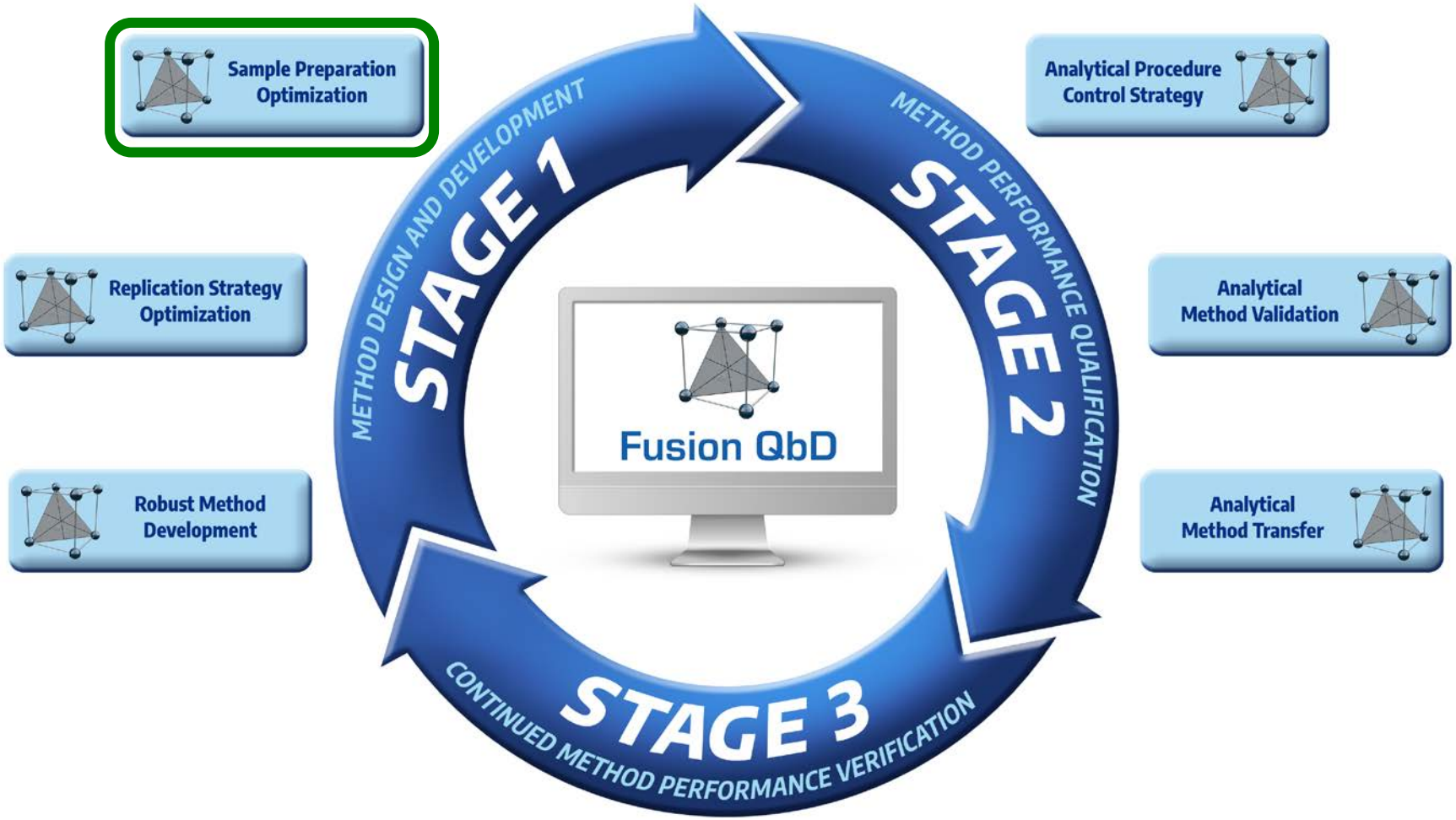
ICH Q14

A sample and/or sample preparation is considered suitable if the measurement response of the sample satisfies pre-defined acceptance criteria for the analytical procedure attributes that have been developed for the validated analytical procedure.

USP <1220>

Stage 1: Procedure design encompasses procedure development, which consists of the analytical technology and sample preparation.

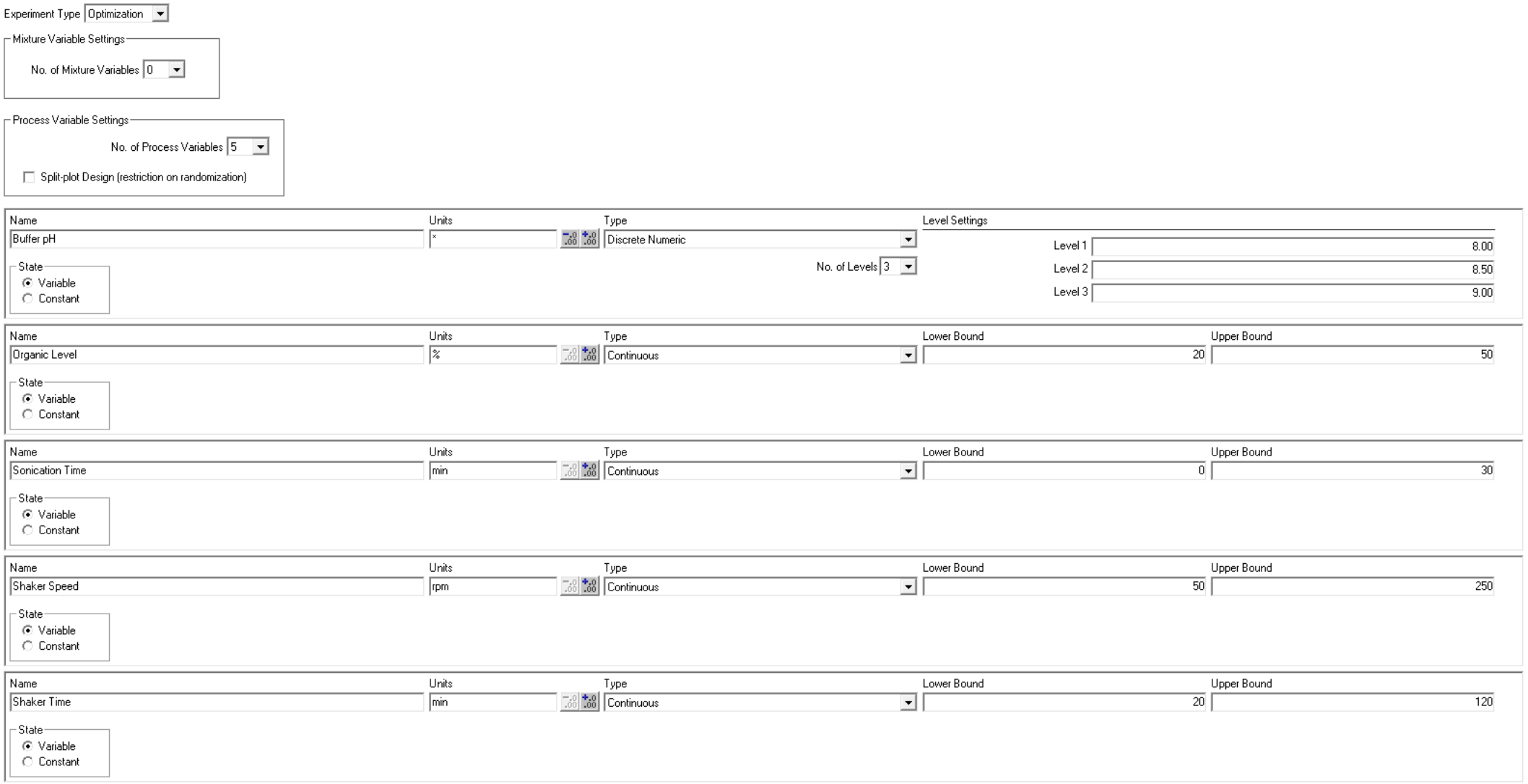
Sample Preparation Method Optimization



Key Differentiator – Sample Preparation Optimization

- ✓ **Support for Sample Preparation Studies**
- ✓ **Full CDS Testing Automation**
- ✓ **Same Powerful Modeling, Optimization, and Visualization Tool Suite:**
  - **Instant One-click Modeling – Any Results**
  - **Complete Analysis Results Reporting**
  - **Integrated Robustness Simulation**
  - **Complete Multi-response Optimization**
  - **Multi-dimensional Visualization Graphics**

Flexible Experiment Setup



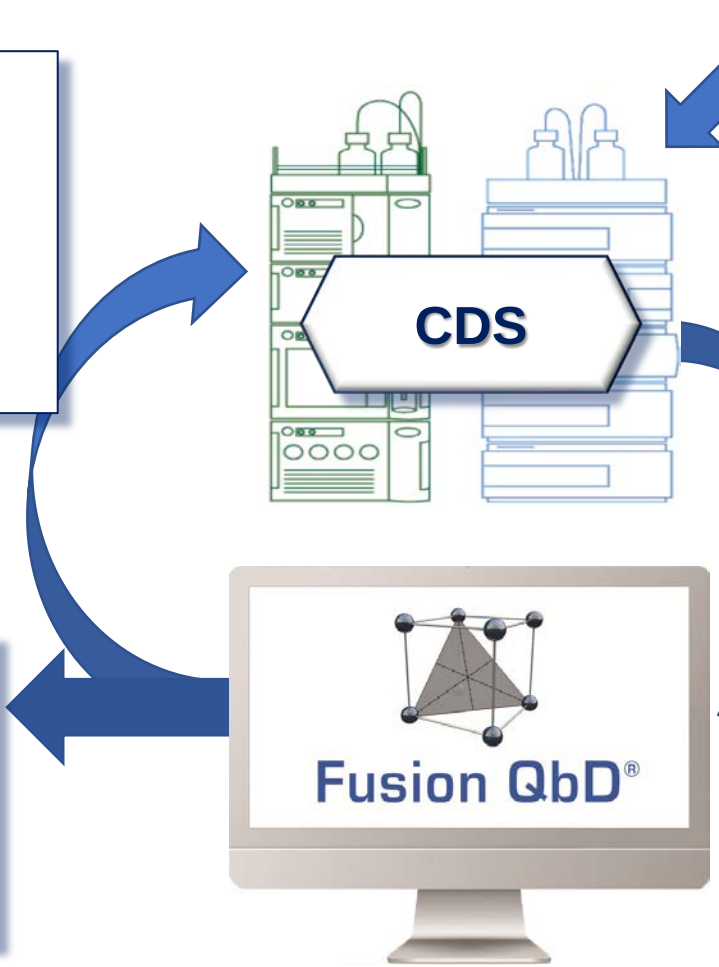
Sample Preparation Experiment Dataflow

1) Experiment Design

Fusion QbD generates the Sample Prep. Study based on the user's variable settings.

2) Testing Design

Fusion QbD creates a companion LC Testing Design and exports it to Empower as a ready-to-run Sample Set.



3) Sample Preparation Study Execution

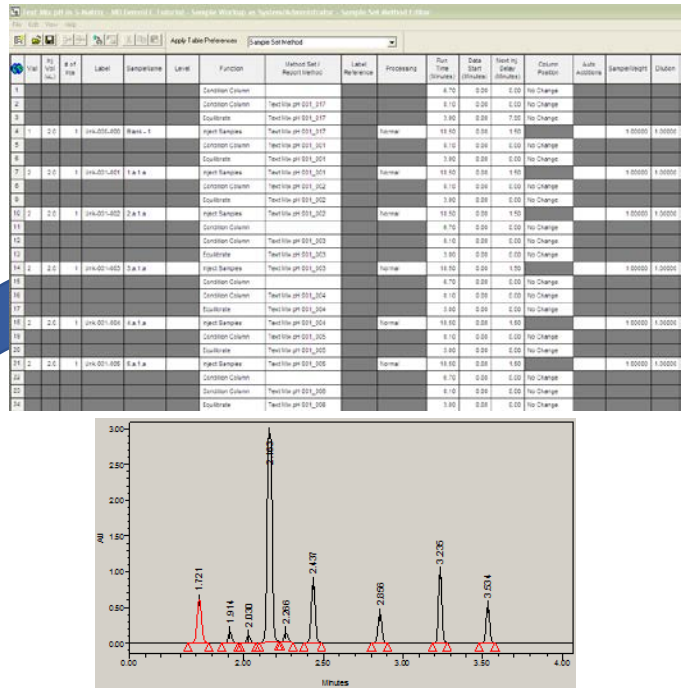
Experiment run samples transferred to LC for testing using the Fusion QbD Sample Set.

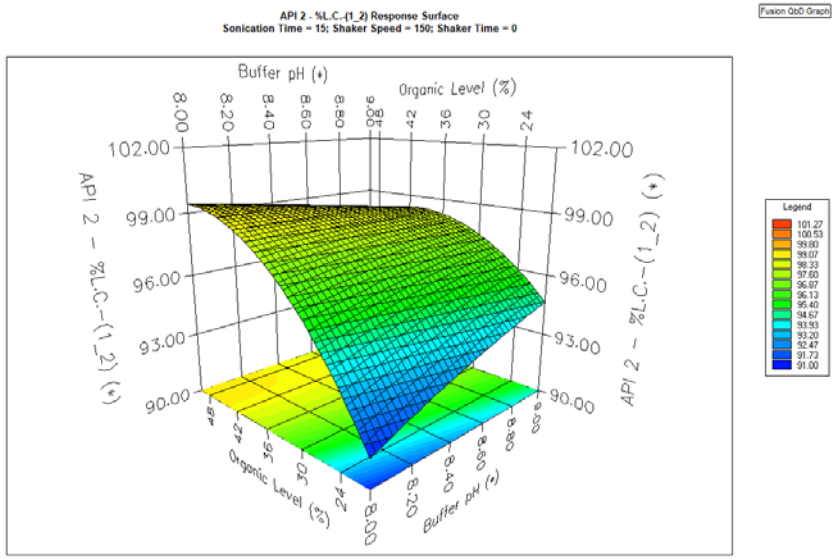
4) Results Data Import

Fusion QbD automatically imports all LC results for all compounds and maps the data to the Sample Prep. Study design for automated modeling.

Automated Import and Data Modeling

Multivariate DOE Study – goal is characterizing all significant effects of the study parameters on all Critical Quality Attributes (CQAs)



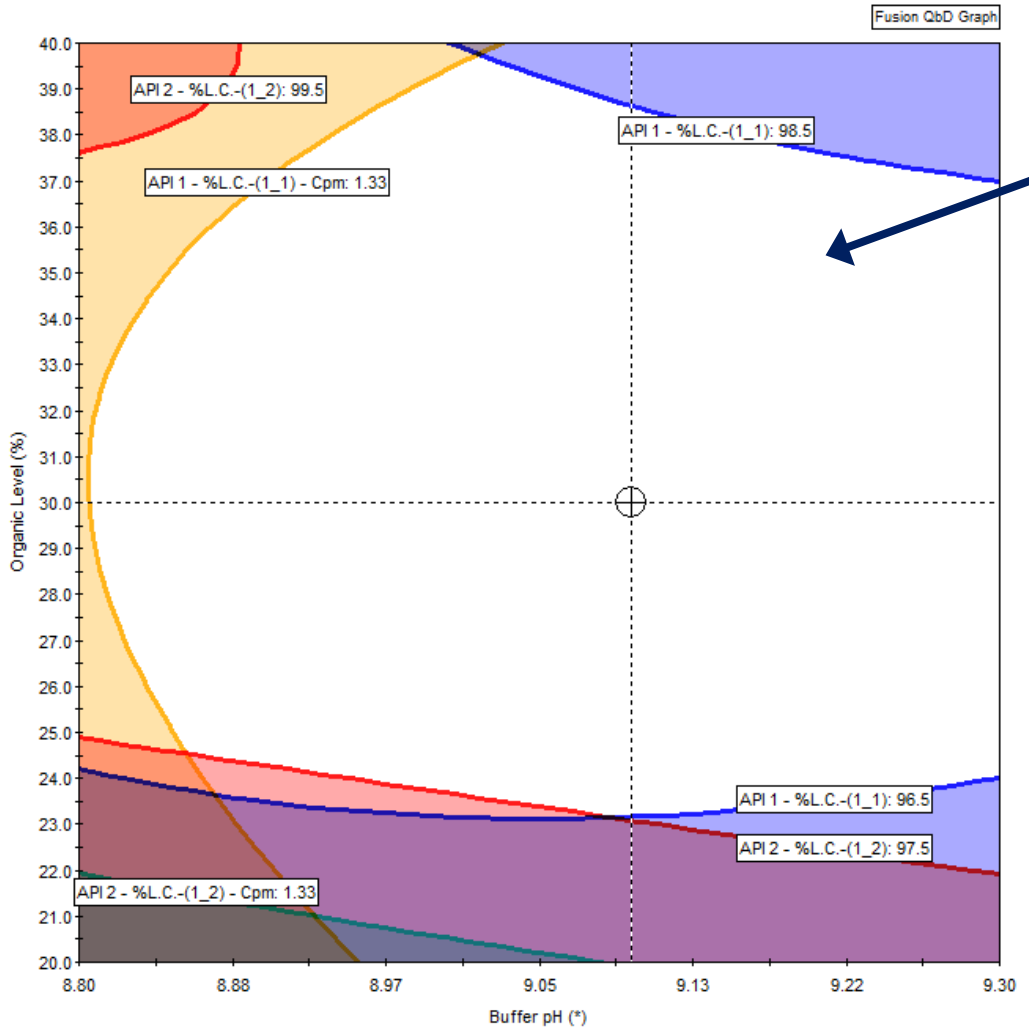


$$CQA = 9.3 + 4.2(pH) - 5.4(Add.)^2 + 12.7(Add*SolvAmt) + 1.3(SolvAmt*Sonic\Delta t) + 1.6[(\Delta pH)^2(Add.)] + \ldots$$

Linear Effect | Curvature Effect | Interaction Effects | Complex Effect

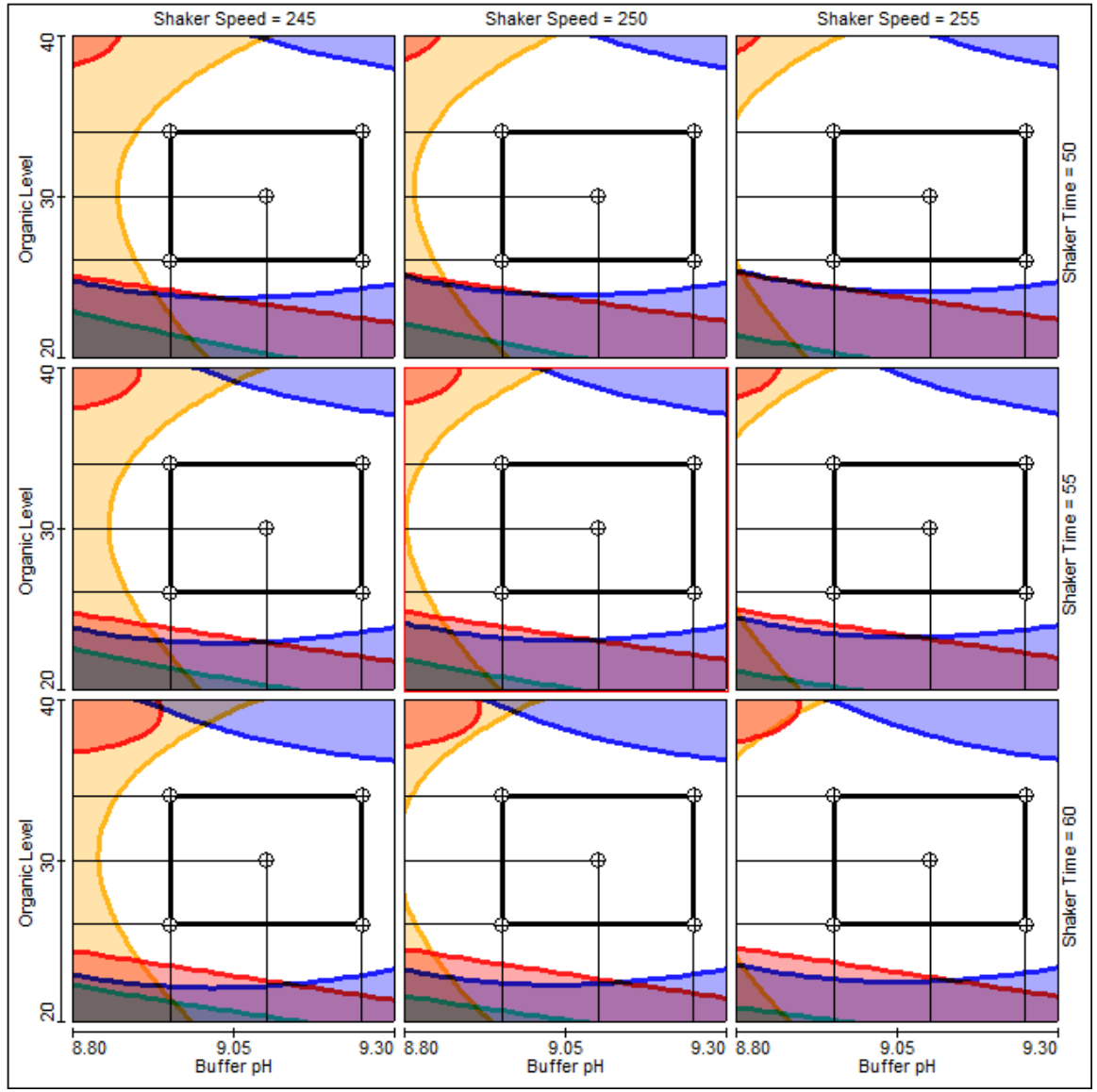
Multi-response Overlay Graph

Below is the ***Final Robust MODR*** in which methods meet or exceed all critical **mean performance and robustness** goals simultaneously.

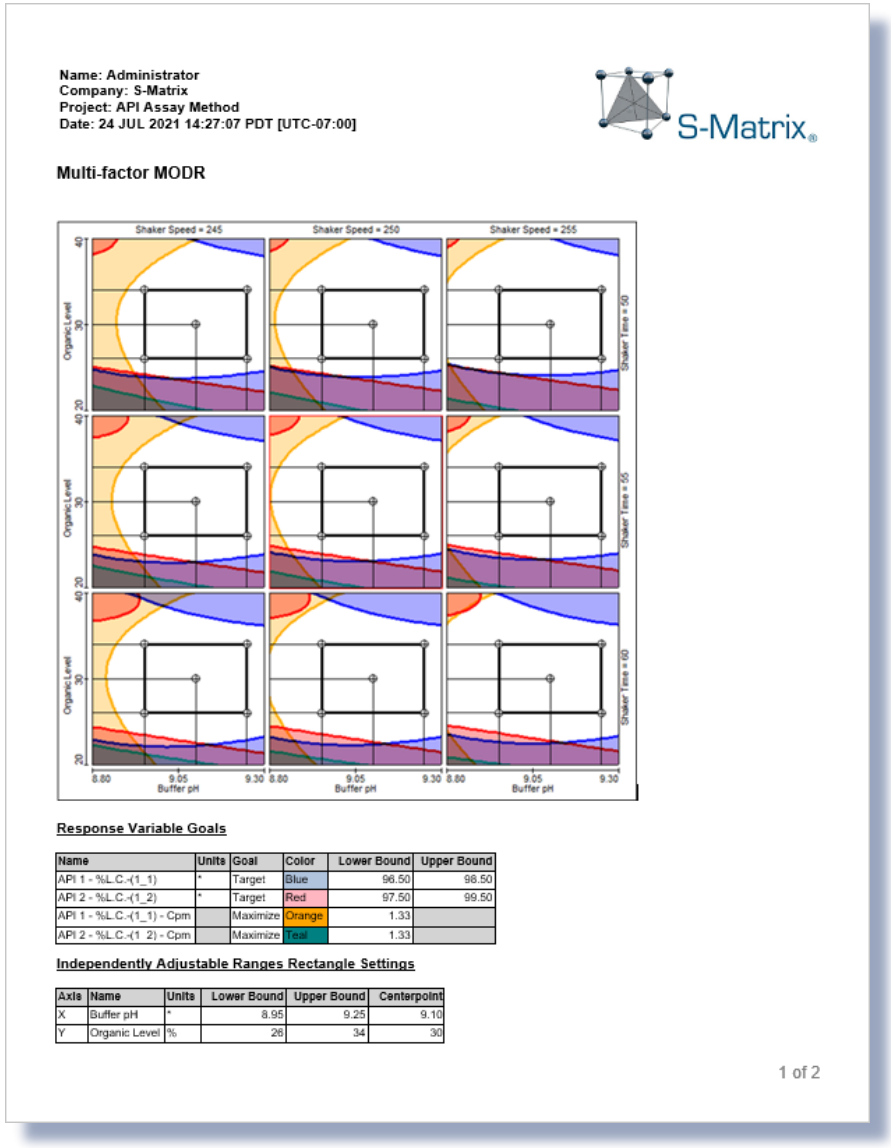


UNshaded Region in the graph is the Method Operable Design Region (MODR)

MODR Trellis Graph – 4 Study Factors



Complete QbD Reporting



Name: Administrator
Company: S-Matrix
Project: API Assay Method
Date: 24 JUL 2021 14:27:07 PDT [UTC-07:00]

S-Matrix®

Multi-factor MODR

Response Variable Goals

Name	Units	Goal	Color	Lower Bound	Upper Bound
API 1 - %L.C.-(1_1)	%	Target	Blue	96.50	98.50
API 2 - %L.C.-(1_2)	%	Target	Red	97.50	99.50
API 1 - %L.C.-(1_1) - Cpm		Maximize	Orange	1.33	
API 2 - %L.C.-(1_2) - Cpm		Maximize	Teal	1.33	

Independently Adjustable Ranges Rectangle Settings

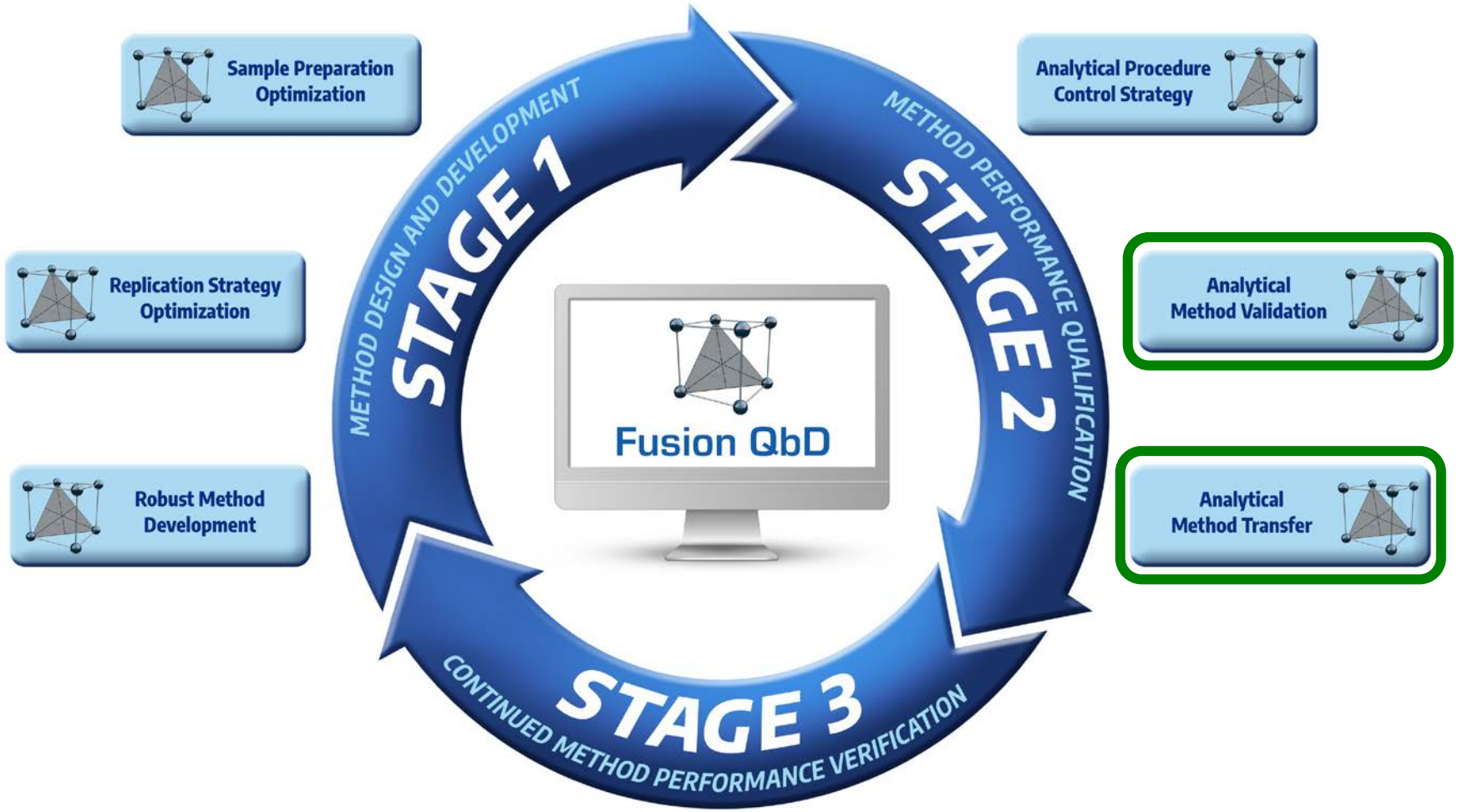
Axis	Name	Units	Lower Bound	Upper Bound	Centerpoint
X	Buffer pH		8.95	9.25	9.10
Y	Organic Level	%	26	34	30

1 of 2

Report Output in Multiple Formats

- MS Excel
- MS Word
- PDF
- …

Method Validation & Transfer



Analytical Method Validation Suite

Complete Method Validation Experiment Suite

- Replication Strategy
- Specificity
- Filter Validation
- Sample Solution Stability
- Accuracy
- Linearity & Range
- Repeatability
- Accuracy / Linearity / Repeatability

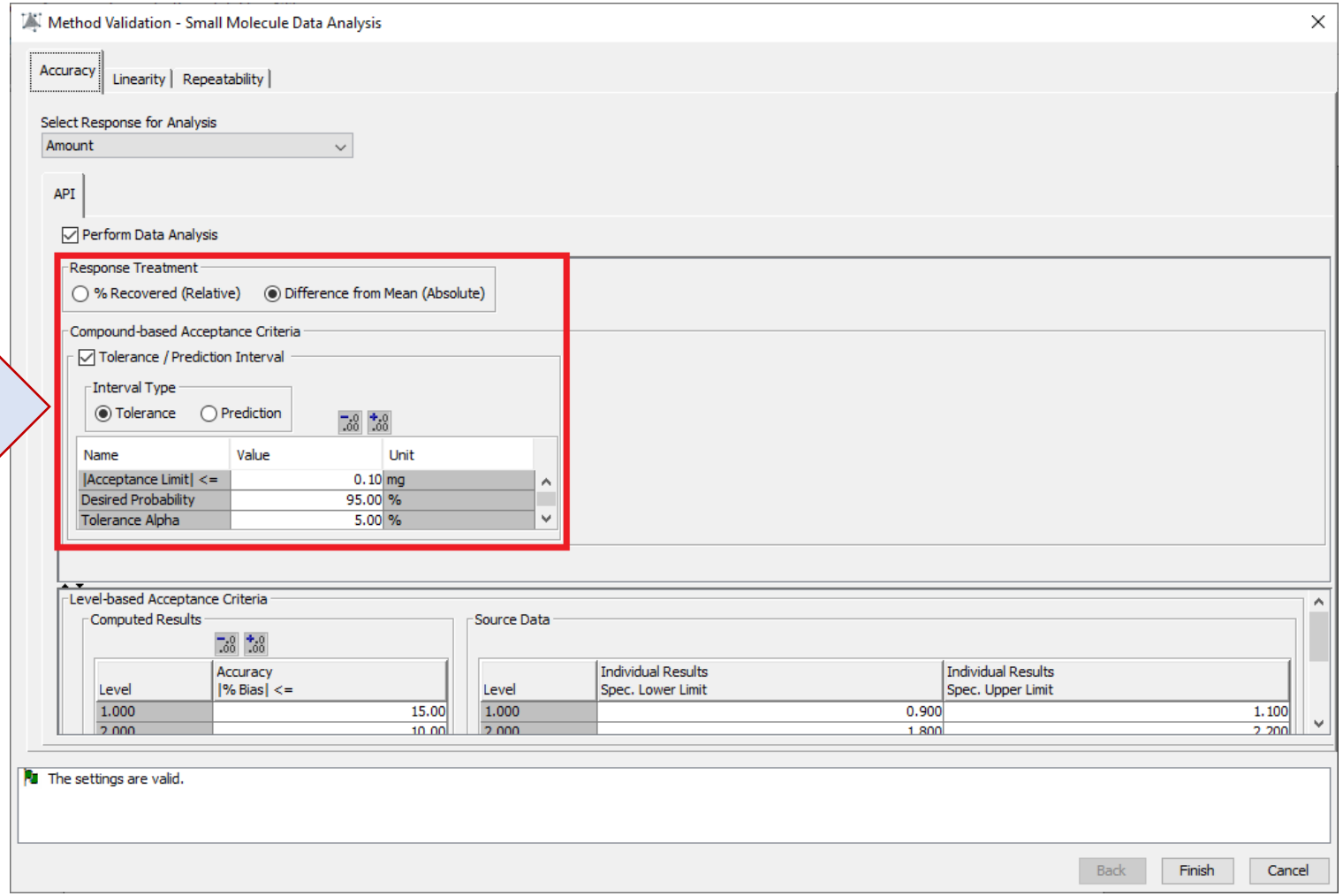
  [Combined as per ICH Q2(R1)]
- LOQ, LOD
- Intermediate Precision and Reproducibility
- Validation Robustness – LC
- Validation Robustness – Non-LC

  [e.g., Sample Preparation, GC, CE, Dissolution]

Accuracy, Linearity, and Repeatability with USP <1210> Metrics

Define your Acceptance Limits and Associated Estimation Precision Requirements for the Determination.

USP <1210> Interval Metrics Integrated within the Accuracy and Repeatability Analysis and Reporting



Accuracy Report – Tolerance Interval Results

Report Includes Results of Required Verification Test for Validity of Data Compilation for Tolerance Interval Analysis.

Interval Test (USP < 1210 >)

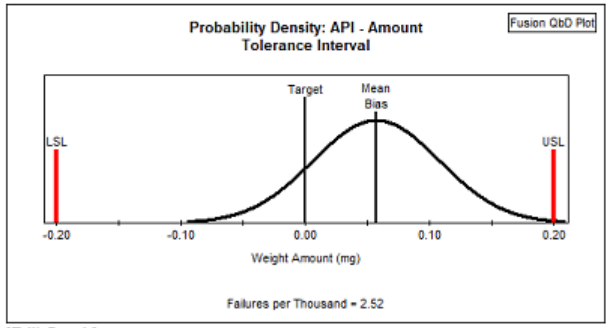
Tolerance Interval

Name	Value
Desired Probability %	95.00
Tolerance Alpha %	5.00
Target	0.00
Mean (Pooled)	0.058
Specification Limits (mg)	-0.20 <= Target <= 0.20
Computed Interval (mg)	-0.04 <= Mean <= 0.16
Result	Pass

Both Computed Interval bounds are within the Specification Limits.

Replicate Group Error Statistics

Replicate Group	Group Run No.	Difference from Mean	Group Std. Dev.	F-Ratio	P-Values
1	1.a 1.b 1.c	0.018 0.072 0.051	0.027	0.7366	0.5086
2	2.a 2.b 2.c	0.111 0.109 0.044	0.038	1.7550	0.2334
3	3.a 3.b 3.c	0.120 0.097 0.102	0.012	0.1267	0.8827
4	4.a 4.b 4.c	0.102 0.092 0.056	0.024	0.5602	0.5920
5	5.a 5.b 5.c	-0.074 -0.047 0.010	0.043	2.5143	0.1422



[Edit Graph]

3 of 4

Analytical Method Transfer

Transferring Lab

Fusion QbD Sequence Execution

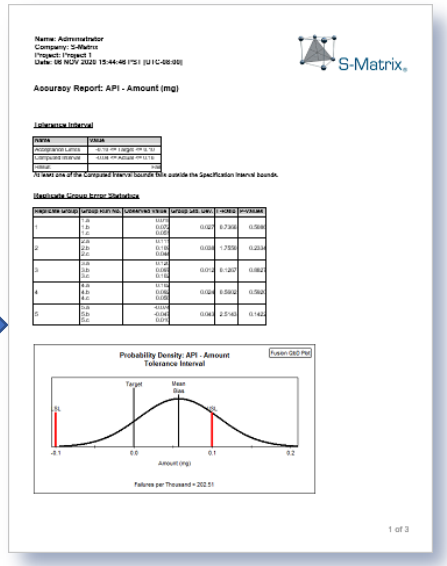
Fusion QbD Sequence Execution

Receiving Lab

Chromatography Data Software

ALR Design

Chromatogram Results Data



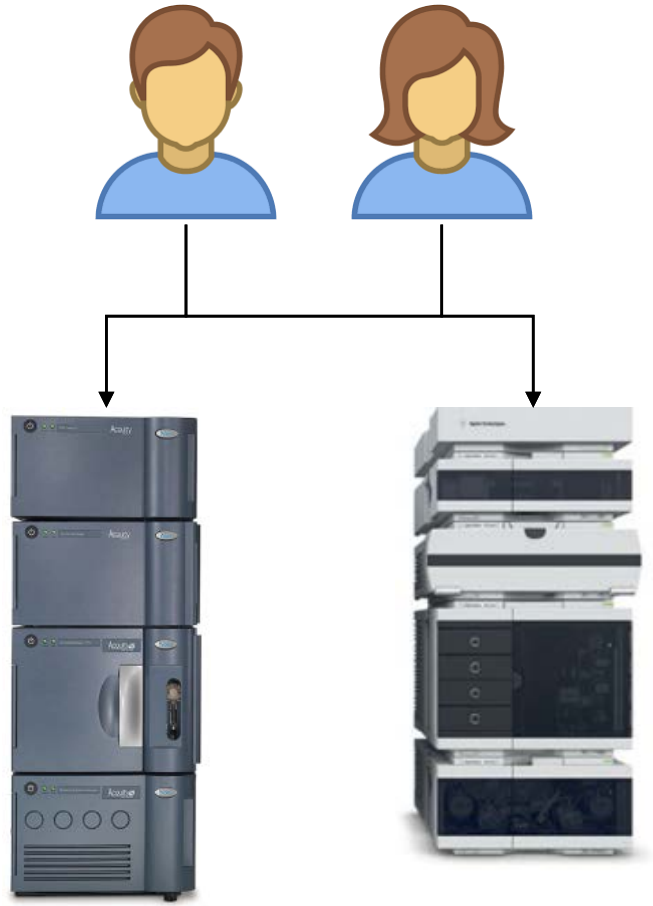
1. Fusion QbD – Exports Experiment to the CDS as Ready-to Run methods and sequence.
2. Sequence is Run at Both Labs.
3. Fusion QbD – Imports Results for Instant and Complete Analysis and Reporting.

Accuracy
Linearity
Repeatability
Tolerance or
Prediction Interval
Pass/Fail Criteria
Results

Analytical Method Transfer

Automation Makes it Easy to Extend the Analysis to Address Bias Concerns:

- Analyst
- Equipment
- Day
- Etc.



For example, each analyst could run the sequence on each LC on each Day.

Each results set could then be imported into Fusion QbD for direct analysis and comparison.

Important References

1. Snyder, Kirkland, and Dolan. (2010). *Introduction to Modern Liquid Chromatography*, 3rd Edition; John Wiley & Sons, Inc., Hoboken, New Jersey

2. *Lifecycle Management of Analytical Procedures: Method Development, Procedure Performance Qualification, and Procedure Performance Verification;* Pharmacopeial Forum 39(5) 2013

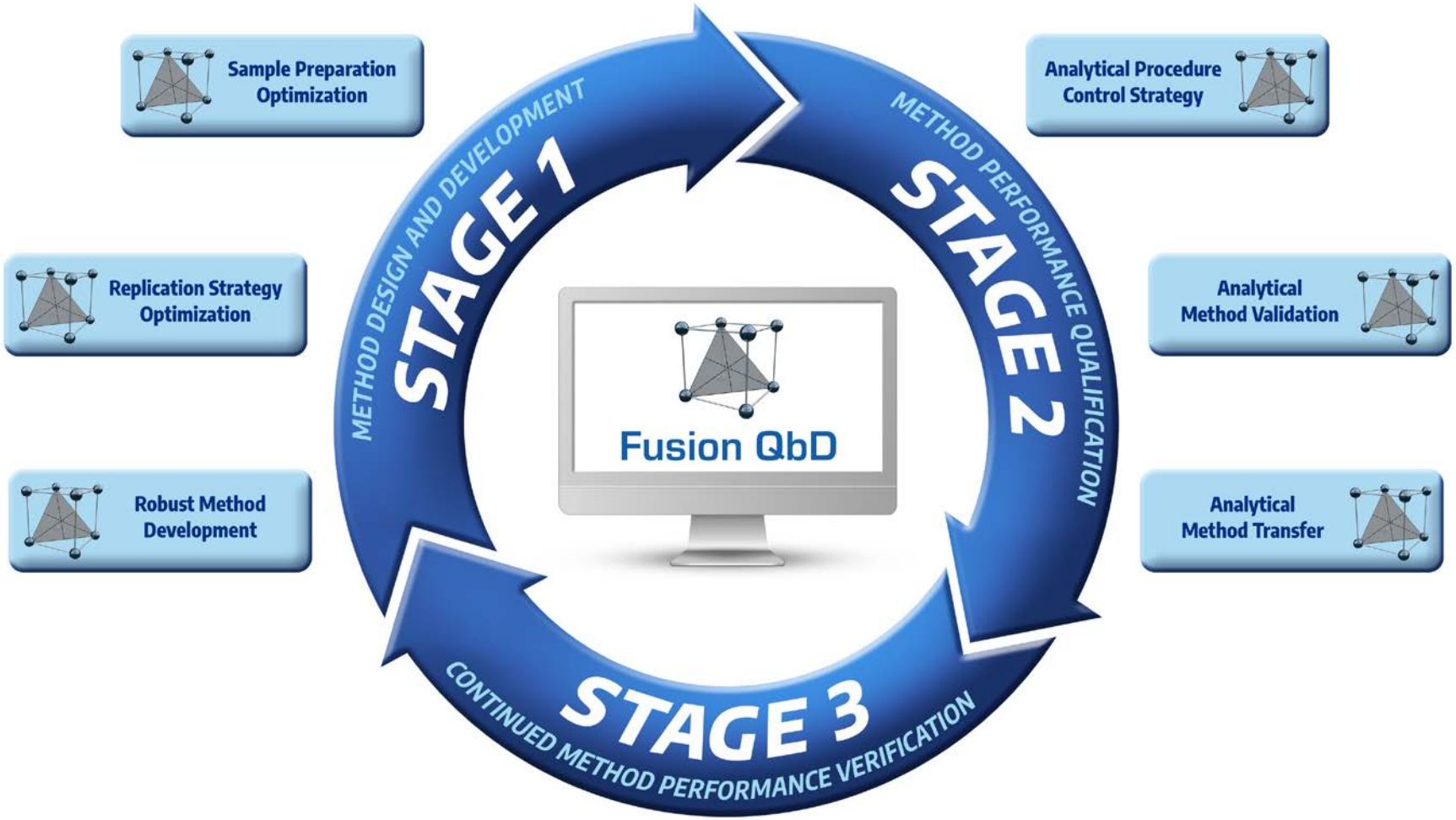
3. USP <1210> *Statistical Tools for Procedure Validation*, The United States Pharmacopeial Convention, May 2018

4. USP <1220> *Analytical Procedure Lifecycle Management*, The United States Pharmacopeial Convention, May 2022

5. ICH Q14, *Analytical Procedure Development (Draft Version)*, March 2022

6. ICH Q2(R2), *Validation of Analytical Procedures (Draft Version)*, March 2022

End of Presentation



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