



Fusion QbD[®]

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

S-Matrix: Transforming LC Method Development

**S-Matrix Introduces
LC Automation with
DoE for Method
Development**

2004

S-Matrix Presents to FDA:

- Concept of LC System as “Process in a Box”
- Integrated Monte Carlo Robustness Simulation

2009

**S-Matrix Integrates
Automated MS Spectra
Based Peak Tracking**

2018

2007

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

2009 - 2017

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

2019

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

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

2020


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

2022

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




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.



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

Printed on: Thu Mar 07 2024, 04:42:23 PM (EST) Status: Currently Official on 07-Mar-2024 DocId: GUID-35D7E47E-65E5-49B7-B4CC-4D96FA230821_2_en-US
 Printed by: George Cooney Official Date: Official as of 01-May-2022 Document Type: GENERAL CHAPTER @2024 USPC
 Do Not Distribute DOI Ref: 468ba DOI: https://doi.org/10.31003/USPNF_M10975_02_01 1

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.

https://online.uspnc.com/uspnfdocument/1_GUID-35D7E47E-65E5-49B7-B4CC-4D96FA230821_2_en-US 1/12

Printed on: Sat Mar 16 2024, 07:59:46 PM (EST) Status: Currently Official on 17-Mar-2024 DocId: GUID-13ED4BEB-4086-43B5-A7D7-994A02AF25C8_7_en-US
 Printed by: George Cooney Official Date: Official as of 01-May-2018 Document Type: GENERAL CHAPTER @2024 USPC
 Do Not Distribute DOI Ref: safim DOI: https://doi.org/10.31003/USPNF_M86846_07_01 1

Add the following:

▲(1210) STATISTICAL TOOLS FOR PROCEDURE VALIDATION

1. INTRODUCTION
2. CONSIDERATIONS PRIOR TO VALIDATION
3. ACCURACY AND PRECISION
 - 3.1 Methods for Estimating Accuracy and Precision
 - 3.2 Combined Validation of Accuracy and Precision
4. LIMITS OF DETECTION AND QUANTITATION
 - 4.1 Estimation of LOD
 - 4.2 Estimation of LOQ
5. CONCLUDING REMARKS

REFERENCES

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

https://online.uspnc.com/uspnfdocument/1_GUID-13ED4BEB-4086-43B5-A7D7-994A02AF25C8_7_en-US 1/12

Key Differentiators – Deployment



Deploy at any Scale



Deploy in any Install Environment

Install Environment

Fusion QbD

Standalone (Workstation)



WorkGroup / Network



Citrix Ready Certified

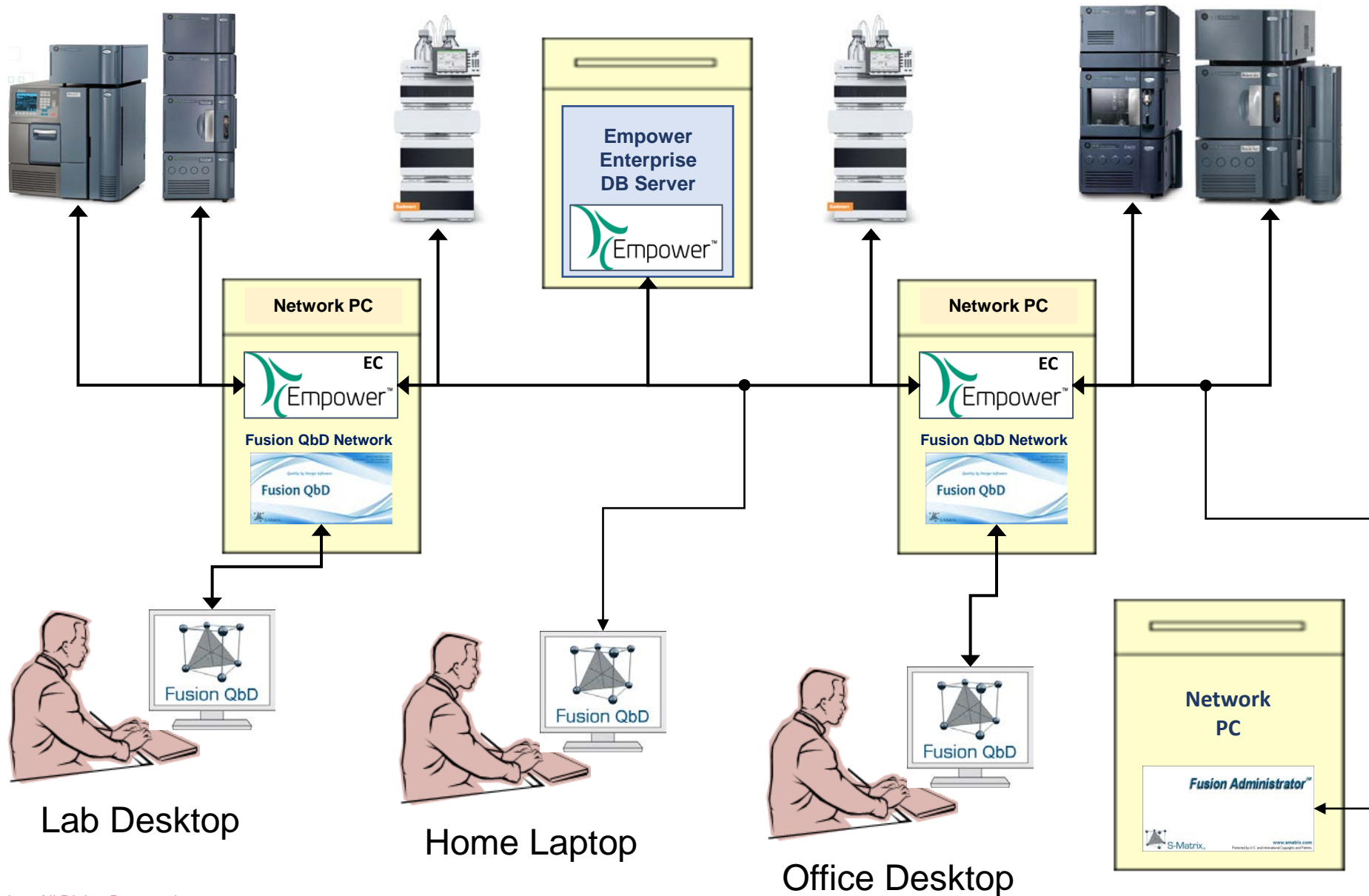


Fully Qualifiable for GxP*



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

Fusion QbD – Supports All Install Environments





✓ 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

Buffer Selector... pH Online Blending Mode: One Acid Base Pair pKa of Primary Compound

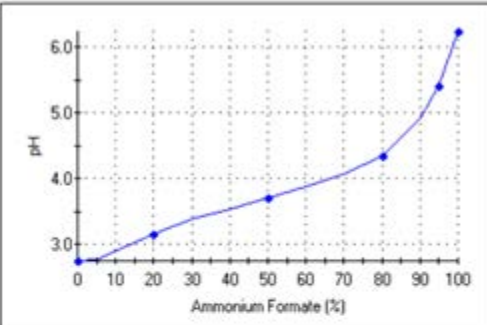
pH Buffer Settings: No. of Levels: 6

Buffer	Buffer Name	pH Level	Acid %	Base %
Acid	Formic Acid (20 mM)	2.75	100.0	0.0
Base	Ammonium Formate (20 mM)	3.16	80.0	20.0
		3.70	50.0	50.0
		4.34	20.0	80.0
		5.42	5.0	95.0
		6.24	0.0	100.0

Buffer Selector

Select Buffer System: pH 2.75 - 6.24 [Formate System (20 mM)]

Buffer Solutions: Formic Acid (20 mM), Ammonium Formate (20 mM)



Included	pH	Formic Acid (%)	Ammonium Formate (%)
<input checked="" type="checkbox"/>	2.75	100.00	0.00
<input checked="" type="checkbox"/>	2.78	95.00	5.00
<input checked="" type="checkbox"/>	2.89	90.00	10.00
<input checked="" type="checkbox"/>	3.16	80.00	20.00
<input checked="" type="checkbox"/>	3.38	70.00	30.00
<input checked="" type="checkbox"/>	3.54	60.00	40.00
<input checked="" type="checkbox"/>	3.70	50.00	50.00
<input checked="" type="checkbox"/>	3.88	40.00	60.00
<input checked="" type="checkbox"/>	4.06	30.00	70.00
<input checked="" type="checkbox"/>	4.34	20.00	80.00
<input checked="" type="checkbox"/>	4.91	10.00	90.00
<input checked="" type="checkbox"/>	5.42	5.00	95.00
<input checked="" type="checkbox"/>	6.24	0.00	100.00

OK Cancel

Built-in pH Titration Curves for Quaternary Pump Modules!

Or Use Your Own Buffer Curve.

Extremely Precise!





- ✓ 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





- ✓ Solvent Selection Valves
- ✓ Column Switching Valves

UltiMate LCs

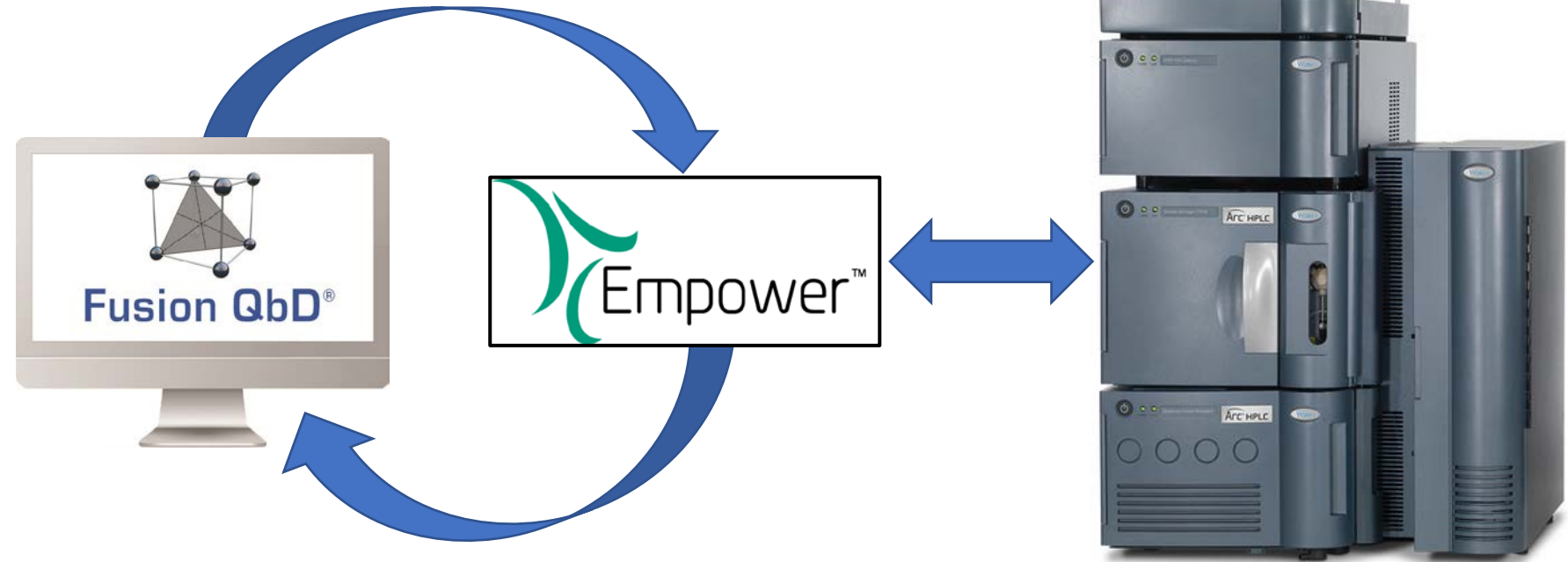


Vanquish Horizon And Flex LCs



Supports All These Separation Modes

Reversed Phase
Normal Phase
Chiral
HILIC
Ion Exchange
Size Exclusion
SFC



Full Support for Forced Degradation Studies

Forced Degradation Study

No. of Unique Degradation Path Samples:

No. of Injection Repeats Per Sample:

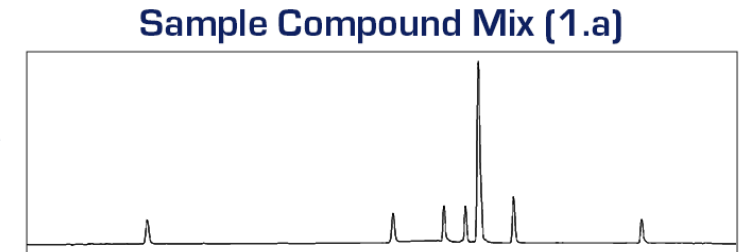
Path #	Degradation Path Description
	Sample Compound Mix
1	Oxidation

Experiment Design Matrix

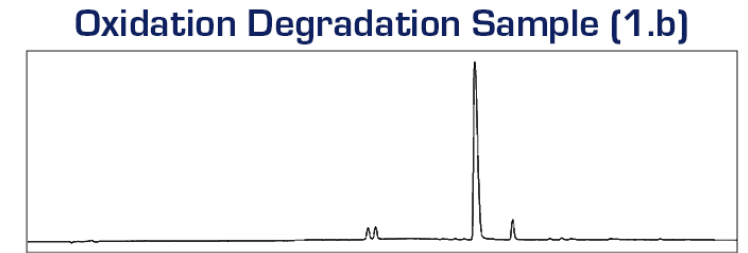
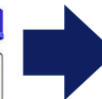
Run No.	Pump Flow Rate (mL/min)	Gradient Time (min)	Oven Temperature (°C)	pH
Conditioning_Run_1	0.400	2.0	30.0	4.60
1.a	0.300	8.0	30.0	4.60
1.b	0.300	8.0	30.0	4.60
2.a	0.500	8.0	30.0	4.60
2.b	0.500	8.0	30.0	4.60
3.a	0.300	16.0	30.0	4.60
3.b	0.300	16.0	30.0	4.60

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

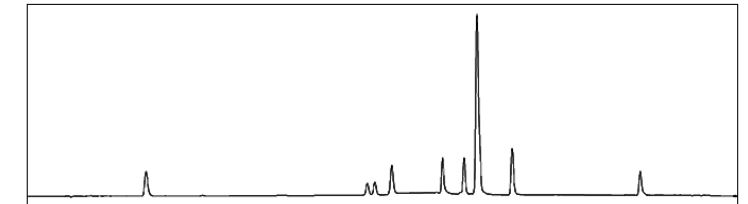
1.a Vial



1.b Vial



Composite Chromatogram - Run 1



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.

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



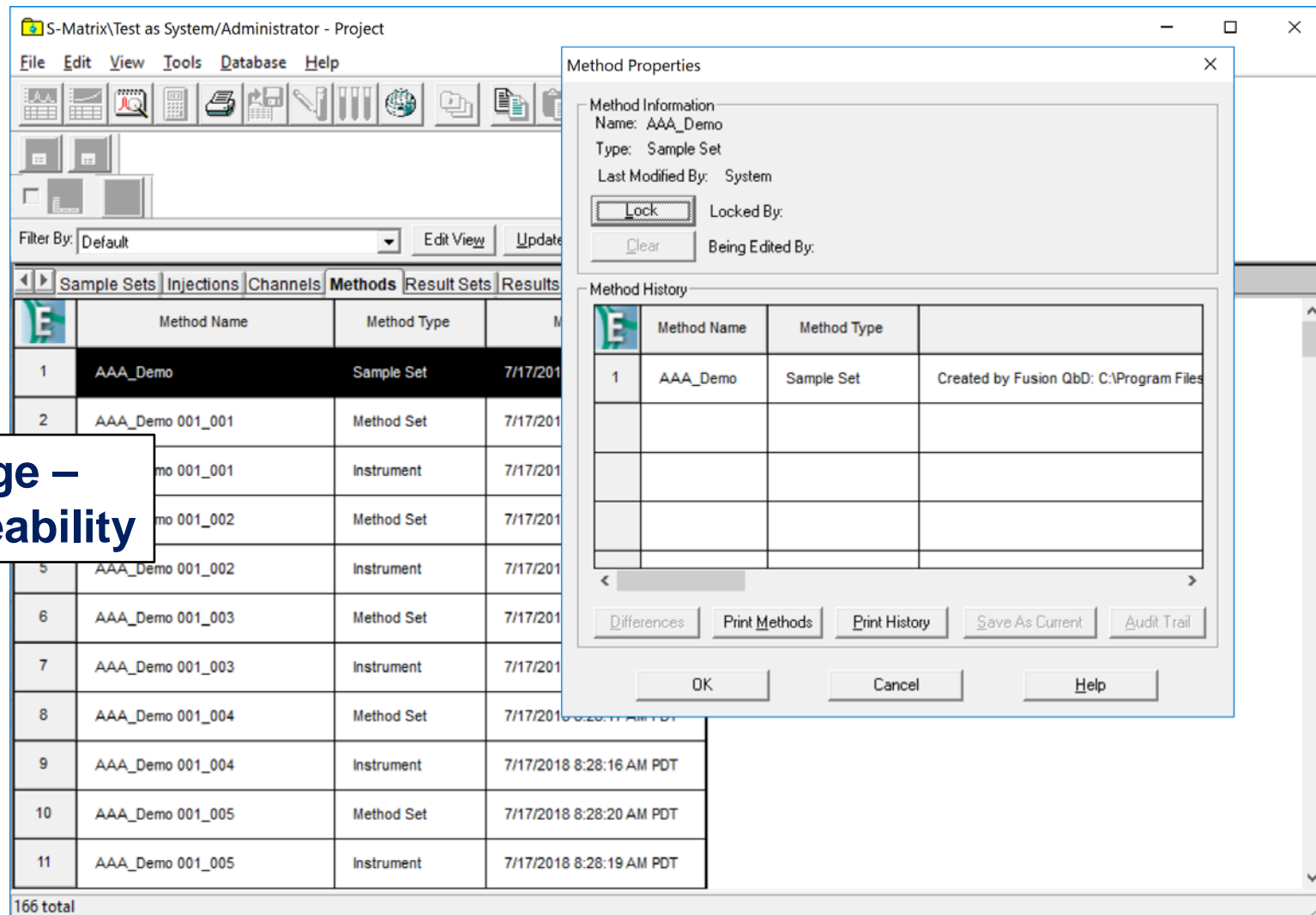
RD2 Optimization in S-Matrix\Internal Development\FMD\RD2 - Optimization - 9_9_0 as System/Administrator - Sample Set Method Editor

Plate/Vial	Inj Vol (uL)	# of Injs	Label	Sample Name	Level	Function	Method Set / Report or Export Method	Label Reference	Processing	Run Time (Minutes)	Data Start (Minutes)	Next Inj Delay (Minutes)	MS Tune Method	MS Calibration Method	Column Position	Auto Aestons	Sample Weight	Dilution
1						Condition Column	RD2 Optimization 001_031			6.00					Position 5			
2						Equilibrate	RD2 Optimization 001_001			4.00					No Change			
3	1A,1	2.0	1	Unk-001-001	1	Inject Samples	RD2 Optimization 001_001		Normal	17.00	0.00	3.50					1.00000	1.00000
4						Equilibrate	RD2 Optimization 001_002			4.00					No Change			
5	1A,1	2.0	1	Unk-001-002	2	Inject Samples	RD2 Optimization 001_002		Normal	9.00	0.00	3.50					1.00000	1.00000
6						Equilibrate	RD2 Optimization 001_003			4.00					No Change			
7	1A,1	2.0	1	Unk-001-003	3	Inject Samples	RD2 Optimization 001_003		Normal	17.00	0.00	3.50					1.00000	1.00000
8						Equilibrate	RD2 Optimization 001_004			4.00					No Change			
9	1A,1	2.0	1	Unk-001-004	4	Inject Samples	RD2 Optimization 001_004		Normal	9.00	0.00	3.50					1.00000	1.00000
10						Equilibrate	RD2 Optimization 001_005			4.00					No Change			
11	1A,1	2.0	1	Unk-001-005	5	Inject Samples	RD2 Optimization 001_005		Normal	9.00	0.00	3.50					1.00000	1.00000
12						Equilibrate	RD2 Optimization 001_006			4.00					No Change			
13	1A,1	2.0	1	Unk-001-006	6	Inject Samples	RD2 Optimization 001_006		Normal	9.00	0.00	3.50					1.00000	1.00000
14						Equilibrate	RD2 Optimization 001_007			4.00					No Change			
15	1A,1	2.0	1	Unk-001-007	7	Inject Samples	RD2 Optimization 001_007		Normal	17.00	0.00	3.50					1.00000	1.00000
16						Condition Column	RD2 Optimization 001_032			6.00					Position 5			
17						Equilibrate	RD2 Optimization 001_008			4.00					No Change			
18	1A,1	2.0	1	Unk-001-008	8	Inject Samples	RD2 Optimization 001_008		Normal	13.00	0.00	3.50					1.00000	1.00000
19						Condition Column	RD2 Optimization 001_033			6.00					Position 5			
20						Equilibrate	RD2 Optimization 001_009			4.00					No Change			
21	1A,1	2.0	1	Unk-001-009	9	Inject Samples	RD2 Optimization 001_009		Normal	9.00	0.00	3.50					1.00000	1.00000
22						Equilibrate	RD2 Optimization 001_010			4.00					No Change			
23	1A,1	2.0	1	Unk-001-010	10	Inject Samples	RD2 Optimization 001_010		Normal	9.00	0.00	3.50					1.00000	1.00000
24						Equilibrate	RD2 Optimization 001_011			4.00					No Change			
25	1A,1	2.0	1	Unk-001-011	11	Inject Samples	RD2 Optimization 001_011		Normal	17.00	0.00	3.50					1.00000	1.00000
26						Equilibrate	RD2 Optimization 001_012			4.00					No Change			
27	1A,1	2.0	1	Unk-001-012	12	Inject Samples	RD2 Optimization 001_012		Normal	17.00	0.00	3.50					1.00000	1.00000
28						Condition Column	RD2 Optimization 001_034			6.00					Position 5			
29						Equilibrate	RD2 Optimization 001_013			4.00					No Change			
30	1A,1	2.0	1	Unk-001-013	13	Inject Samples	RD2 Optimization 001_013		Normal	13.00	0.00	3.50					1.00000	1.00000
31						Condition Column	RD2 Optimization 001_035			6.00					Position 5			
32						Equilibrate	RD2 Optimization 001_014			4.00					No Change			
33	1A,1	2.0	1	Unk-001-014	14	Inject Samples	RD2 Optimization 001_014		Normal	13.00	0.00	3.50					1.00000	1.00000
34						Equilibrate	RD2 Optimization 001_015			4.00					No Change			
35	1A,1	2.0	1	Unk-001-015	15	Inject Samples	RD2 Optimization 001_015		Normal	13.00	0.00	3.50					1.00000	1.00000
36						Equilibrate	RD2 Optimization 001_016			4.00					No Change			
37	1A,1	2.0	1	Unk-001-016	16	Inject Samples	RD2 Optimization 001_016		Normal	9.00	0.00	3.50					1.00000	1.00000
38						Equilibrate	RD2 Optimization 001_017			4.00					No Change			

For Help, press F1

Auditing assures Data Integrity and Traceability

**Automated, Audited Data Exchange –
Preserves Data Integrity and Traceability**



The screenshot displays the S-Matrix software interface. The main window shows a table of methods under the 'Methods' tab. A 'Method Properties' dialog box is open, showing details for the 'AAA_Demo' method.

Method ID	Method Name	Method Type	Method Date
1	AAA_Demo	Sample Set	7/17/2018
2	AAA_Demo 001_001	Method Set	7/17/2018
3	AAA_Demo 001_001	Instrument	7/17/2018
4	AAA_Demo 001_002	Method Set	7/17/2018
5	AAA_Demo 001_002	Instrument	7/17/2018
6	AAA_Demo 001_003	Method Set	7/17/2018
7	AAA_Demo 001_003	Instrument	7/17/2018
8	AAA_Demo 001_004	Method Set	7/17/2018 8:28:16 AM PDT
9	AAA_Demo 001_004	Instrument	7/17/2018 8:28:16 AM PDT
10	AAA_Demo 001_005	Method Set	7/17/2018 8:28:20 AM PDT
11	AAA_Demo 001_005	Instrument	7/17/2018 8:28:19 AM PDT

The 'Method Properties' dialog box shows the following information:

- Method Information:**
 - Name: AAA_Demo
 - Type: Sample Set
 - Last Modified By: System
 - Buttons: Lock, Clear
 - Fields: Locked By, Being Edited By
- Method History:**

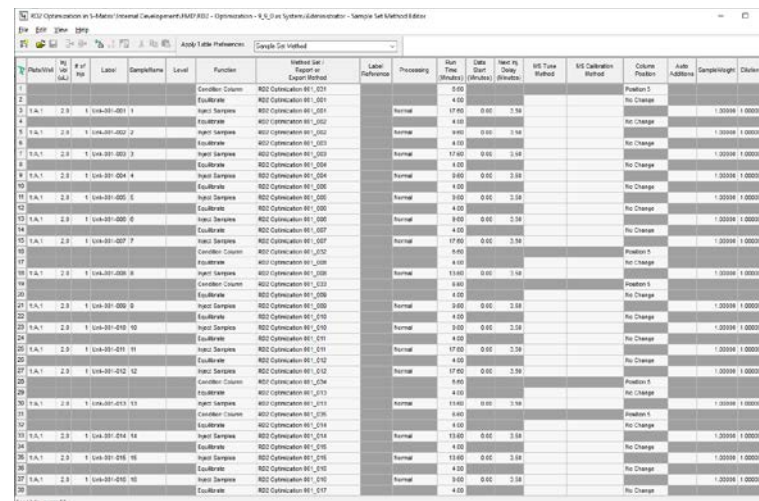
Method ID	Method Name	Method Type	Method Date
1	AAA_Demo	Sample Set	Created by Fusion QbD: C:\Program Files

Buttons at the bottom of the dialog: Differences, Print Methods, Print History, Save As Current, Audit Trail, OK, Cancel, Help.

Fusion QbD – Import from CDS



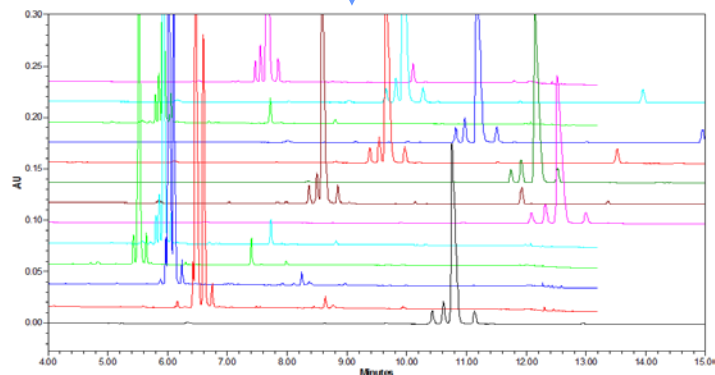
CDS Software

Run	Date	Time	Method	Run Time (Minutes)	Start (Minutes)	End (Minutes)	Method	Run Time (Minutes)	Start (Minutes)	End (Minutes)	Method	Run Time (Minutes)	Start (Minutes)	End (Minutes)	Method
11			Equilibration	4.00	0.00	4.00	Normal	4.00	0.00	4.00	Normal	4.00	0.00	4.00	Normal
12	1/11/1	2.0	1/11/1-01-001-1	4.00	0.00	4.00	Normal	4.00	0.00	4.00	Normal	4.00	0.00	4.00	Normal
13	1/11/1	2.0	1/11/1-01-002-2	4.00	0.00	4.00	Normal	4.00	0.00	4.00	Normal	4.00	0.00	4.00	Normal
14	1/11/1	2.0	1/11/1-01-003-3	4.00	0.00	4.00	Normal	4.00	0.00	4.00	Normal	4.00	0.00	4.00	Normal
15	1/11/1	2.0	1/11/1-01-004-4	4.00	0.00	4.00	Normal	4.00	0.00	4.00	Normal	4.00	0.00	4.00	Normal
16	1/11/1	2.0	1/11/1-01-005-5	4.00	0.00	4.00	Normal	4.00	0.00	4.00	Normal	4.00	0.00	4.00	Normal
17	1/11/1	2.0	1/11/1-01-006-6	4.00	0.00	4.00	Normal	4.00	0.00	4.00	Normal	4.00	0.00	4.00	Normal
18	1/11/1	2.0	1/11/1-01-007-7	4.00	0.00	4.00	Normal	4.00	0.00	4.00	Normal	4.00	0.00	4.00	Normal
19	1/11/1	2.0	1/11/1-01-008-8	4.00	0.00	4.00	Normal	4.00	0.00	4.00	Normal	4.00	0.00	4.00	Normal
20	1/11/1	2.0	1/11/1-01-009-9	4.00	0.00	4.00	Normal	4.00	0.00	4.00	Normal	4.00	0.00	4.00	Normal
21	1/11/1	2.0	1/11/1-01-010-10	4.00	0.00	4.00	Normal	4.00	0.00	4.00	Normal	4.00	0.00	4.00	Normal
22	1/11/1	2.0	1/11/1-01-011-11	4.00	0.00	4.00	Normal	4.00	0.00	4.00	Normal	4.00	0.00	4.00	Normal
23	1/11/1	2.0	1/11/1-01-012-12	4.00	0.00	4.00	Normal	4.00	0.00	4.00	Normal	4.00	0.00	4.00	Normal
24	1/11/1	2.0	1/11/1-01-013-13	4.00	0.00	4.00	Normal	4.00	0.00	4.00	Normal	4.00	0.00	4.00	Normal
25	1/11/1	2.0	1/11/1-01-014-14	4.00	0.00	4.00	Normal	4.00	0.00	4.00	Normal	4.00	0.00	4.00	Normal
26	1/11/1	2.0	1/11/1-01-015-15	4.00	0.00	4.00	Normal	4.00	0.00	4.00	Normal	4.00	0.00	4.00	Normal
27	1/11/1	2.0	1/11/1-01-016-16	4.00	0.00	4.00	Normal	4.00	0.00	4.00	Normal	4.00	0.00	4.00	Normal
28	1/11/1	2.0	1/11/1-01-017-17	4.00	0.00	4.00	Normal	4.00	0.00	4.00	Normal	4.00	0.00	4.00	Normal

Automatically Retrieves All Chromatogram Results Data

Automated analysis, graphing, and reporting.
Report formats:
RTF, DOC, HTML, PDF, XLSX, XML



**Automated, Audited Data Exchange –
Preserves Data Integrity and Traceability**

Method Development - FMD Tutorial - Optimization - Part 2 - 991 533.smae

File Edit Activity Tools Window Help

Generate Audit Log

Design of Experiments

- Create a Design
- Design Reports

Data Entry / Analysis

- Data Entry
- Data Analysis

Best Answer Searches

- Best Overall Answer
- Acceptable Performance Region
- Point Predictions


Visualization Graphics

- Single Response Series
- Multiple Response Series

Reporting Toolkit

- Fusion Reporter
- **Audit Log Reporter**

Name: Administrator
Company: S-Matrix Corporation
Project: Project 1
Date: 07 MAY 2022 15:13:56 PDT [UTC-07:00]



Audit Log

20 JUN 2021 10:13:51 PDT [UTC-07:00] - Administrator

Event Type: Import Responses

Import Response Settings

Setting	Value
Target CDS	EMPOWER
Empower Version	Empower 3 Software Build 3471 SPs Installed: Service Release 3 DB ID: 2484307300
Empower Database	(local)
Empower User	system
Project Name	RD2 - Optimization - 9_9_0
Result Set(ID)	RD2 Optimization (9001)
Processed Channel	PDA Ch1 225nm@4.8nm, Time offset by 0.020 mins.
Activate PeakTracker	Checked
Raw PDA Channel	Unchecked
Raw MS Channel	QDa Positive Scan
MS Time Offset(min)	0.02
MS Intensity Threshold	100000
Processed MS Channel	QDa Positive Scan MS TIC, Smoothed by 59 point Savitzky-Golay Filter. (QDa Positive(+)-Scan (100.00-1250.00)Da, Centroid, CV=15)
Track Non-absorbing Peaks	Checked
Auto-imported Response(s)	Height, RetentionTime, WidthAt50Pct, USP Tailing, WidthAtTangentUSPResolution, Area
Import Chromatogram Trace Data	Checked
Import Prediction Chromatogram Data	Checked
Total Import Time	00:06:42
Locale	English (United States)

Imported Data Source

Sample Name	ResultID	MS ResultID	TIC (ID/Type)	MS-Spectra (ID/Type)	UV-Spectra (ID/Type)
1	9155	9153	MS_TIC (9004/2D)	QDa Positive Scan (7036/3DMS)	
10	9048	9189	MS_TIC (9050/2D)	QDa Positive Scan (7063/3DMS)	
11	9191	9193	MS_TIC (9055/2D)	QDa Positive Scan (7066/3DMS)	
12	9058	9195	MS_TIC (9060/2D)	QDa Positive Scan (7069/3DMS)	

Ready

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?

Audit Log Filter Options

Enable

Starting Date:

March 2020						
Sun	Mon	Tue	Wed	Thu	Fri	Sat
23	24	25	26	27	28	29
1	2	3	4	5	6	7
8	9	10	11	12	13	14
15	16	17	18	19	20	21
22	23	24	25	26	27	28
29	30	31	1	2	3	4

Ending Date:

March 2020						
Sun	Mon	Tue	Wed	Thu	Fri	Sat
23	24	25	26	27	28	29
1	2	3	4	5	6	7
8	9	10	11	12	13	14
15	16	17	18	19	20	21
22	23	24	25	26	27	28
29	30	31	1	2	3	4

Enable

Available: Administrator

Included:

Enable

Available:

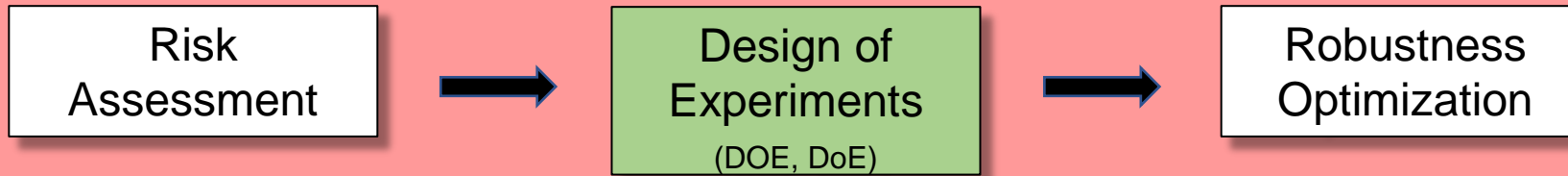
- Print Reports
- Experiment Setup
- Enable User Defined Option
- Generate Design
- Export Experiment Design
- Export Testing Design
- Matrix Master Wizard
- Edit Run No. Labels
- Robustness Simulator
- Create Testing Design
- Delete Testing Design
- Response Reductions

Included:

- Import Responses
- Create/Edit Response Data

OK Cancel ?

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.



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

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....

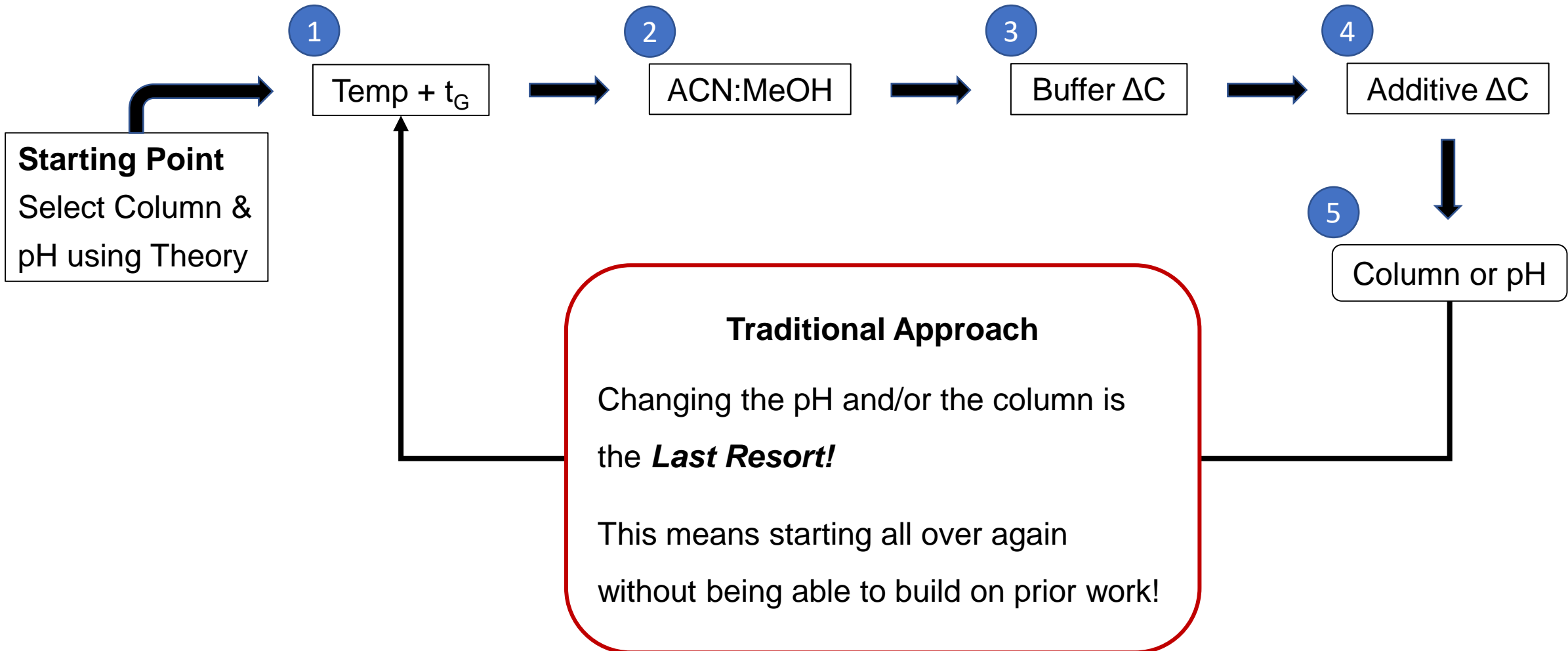
Experiment Automation Simplifies DoE!

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

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

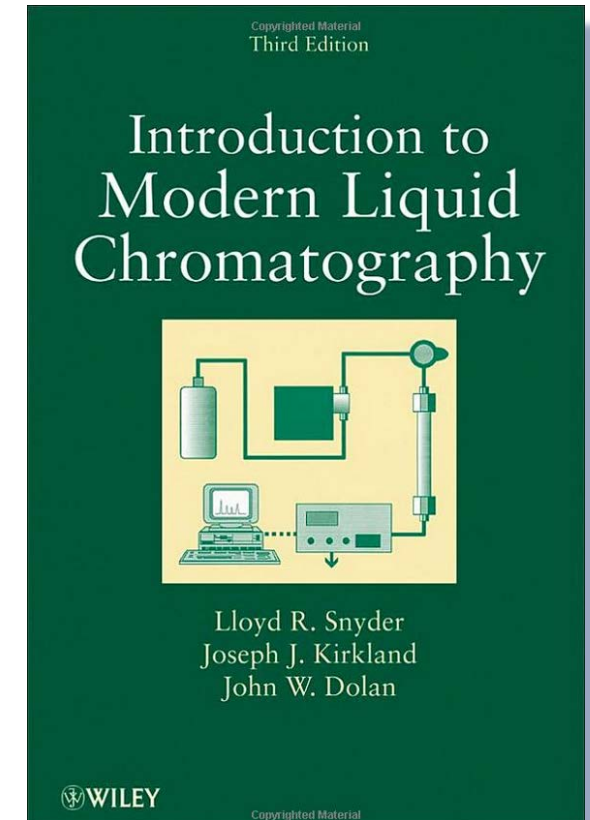
- Isocratic and Gradient Methods
- Strong Solvent Type
- Any pump program steps – e.g.
 - Equilibration Time & %
 - Isocratic Hold Time & %
 - Gradient Time / Slope
 - Initial / Final Hold Time & %
 - Re-equilibration Time & %
- Column Temperature
- Column Type
- Flow Rate
- Injection Volume
- pH
- Mobile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

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



“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:

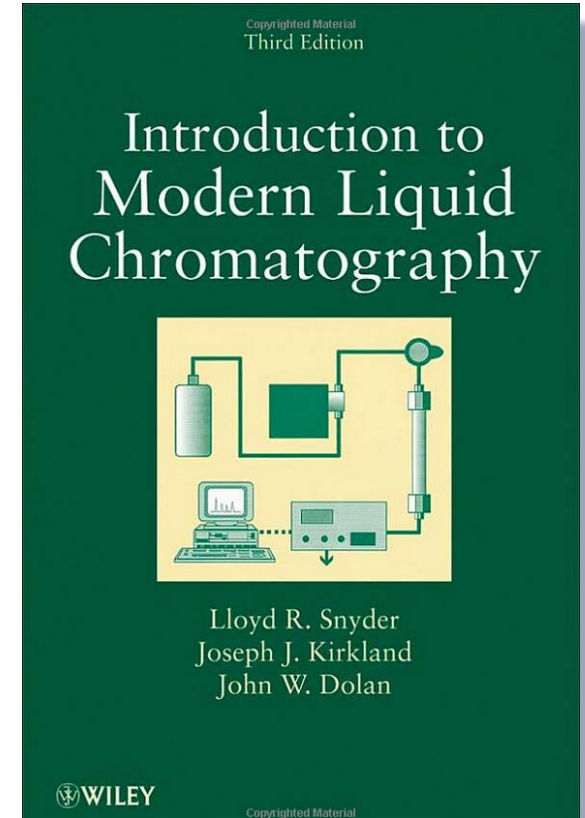
- Different **columns**
- Different **B-solvents**
- Variations in **pH** and **temperature**
- Use of **Gradient elution** during the experiments can help avoid the need to separately optimize values of %B for each variable studied.”



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

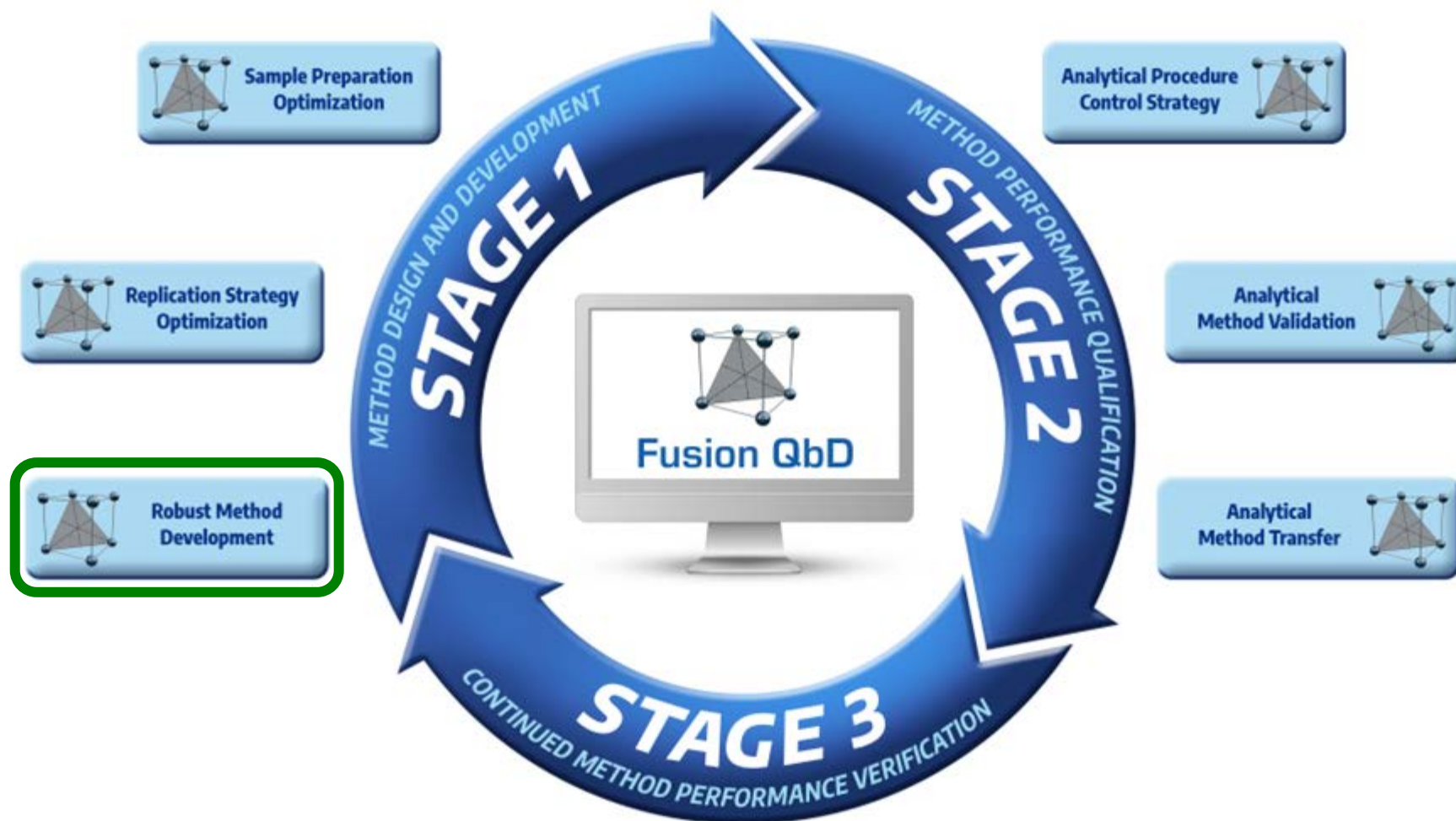
“Still another approach is to **search the literature** for separation of the same or similar sample. **Trial-and-error** modifications of conditions are then followed until an acceptable separation is achieved. ***We do not recommend this approach**** because possible deficiencies in literature methods 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





- ✓ **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**

Experiment Setup | Replication Settings

Method Type: Gradient

Available Variables: Gradient

Included Variables:

- Pump Flow Rate
- Injection Volume
- Oven Temperature
- Wavelength
- Column Type

Activate Online Preparation
 pH
 Buffer Concentration
 Additive Concentration

Solvent Type

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





Solvent Settings

No. of Strong Solvents: 2 | No. of Weak Solvents: 2

OK to Blend Strong Solvents |
 OK to Blend Weak Solvents |
 Mobile Phase Precision: 0.00 to 0.00

Mobile Phase Name	Solvent Type	Reservoir
Acetonitrile	Strong (Organic)	A
Methanol	Strong (Organic)	B
Acid	Weak (Aqueous)	---
Base	Weak (Aqueous)	---

Available Reservoirs

A 
 B 
 C 
 D 

D-1 | D-2 | D-3
 D-4 | D-5 | D-6

Chemistry System Screening

Multiple pH Levels

Buffer Selector... pH Online Blending Mode: One Acid:Base Pair pKa of Primary Compound

pH Buffer Settings

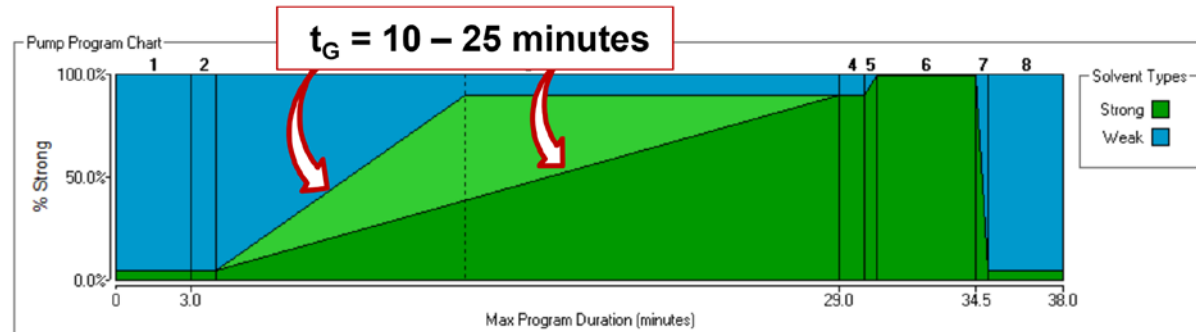
No. of Levels: 5

Buffer	Buffer Name	pH Level	Acid %	Base %
Acid	Formic Acid (20 mM)	2.73	100.0	0.0
Base	Ammonium Formate (20 mM)	3.20	75.0	25.0
		3.69	45.0	55.0
		4.27	20.0	80.0
		4.93	5.0	95.0

Clear Buffer System

Include recommended pH

Multiple Gradients (3-5 Levels Covering Range)



Include recommended column type

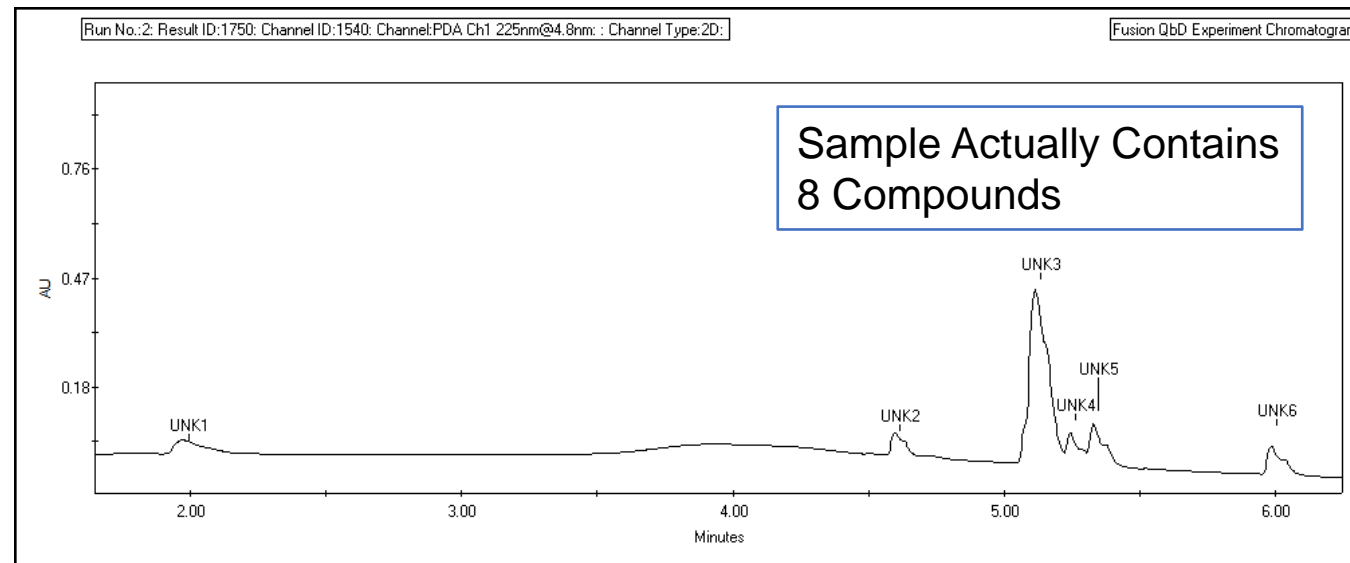
Multiple Columns

	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

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™

PDA Ch1 225nm@4.8nm, Time offset by 0.020 mins.

Trend Responses

Add Delete Undo Changes Restore

		Operator	Value	Response
1	<input checked="" type="checkbox"/>	No. of Peaks		
2	<input checked="" type="checkbox"/>	No. of Peaks >=	1.50	USPResolution
3	<input checked="" type="checkbox"/>	No. of Peaks >=	2.00	USPResolution
4	<input checked="" type="checkbox"/>	No. of Peaks <=	1.20	USPTailing
5	<input checked="" type="checkbox"/>	Max Peak	1	USPResolution
6	<input checked="" type="checkbox"/>	Max Peak	1	USPTailing

Select All Select None

I = Incomplete
D = Duplicate

Available

- 2ndDerivativeApex
- 2Sigma
- 3Sigma
- 4Sigma
- 5Sigma
- AboveIdentificationThreshold
- AboveMaximumThreshold
- AboveQualificationThreshold
- AboveReportingThreshold
- Asym
- AsymAt10
- AsymAt10Sqrd

Included

Auto-imported Responses... << Back Next >> Cancel ?

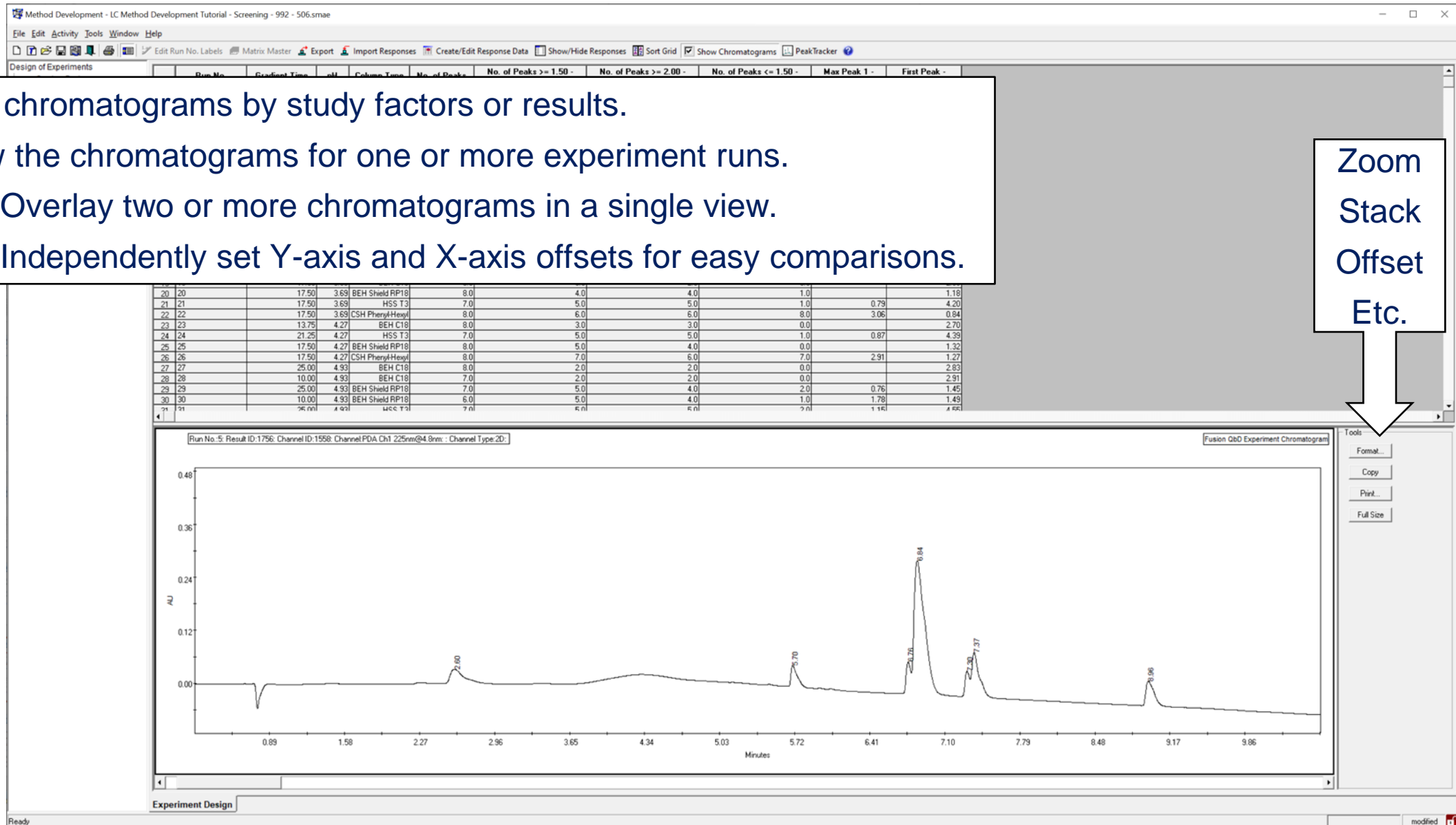
Support the Chromatographer's Screening Goals

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)!

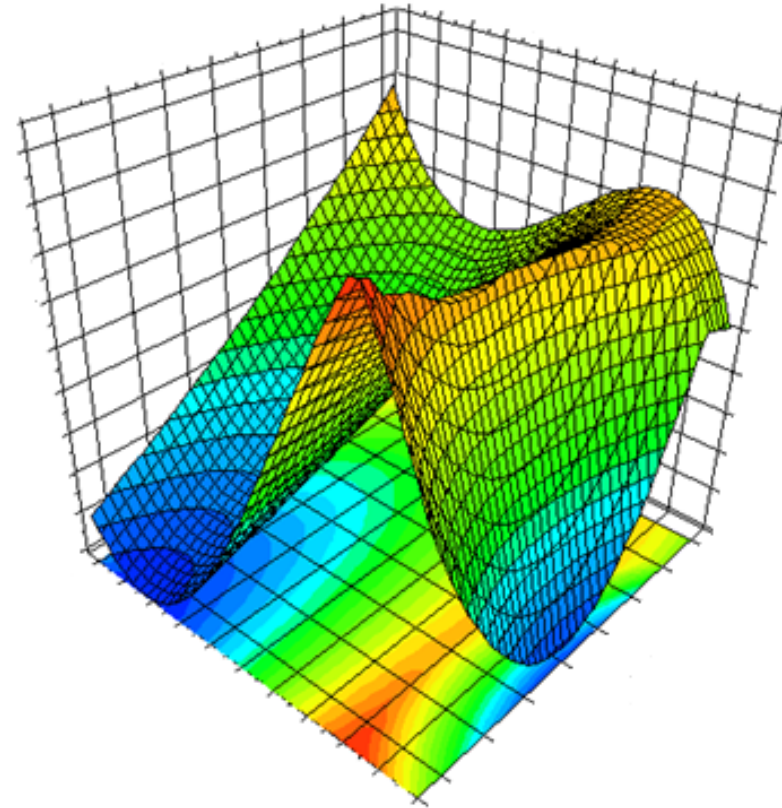
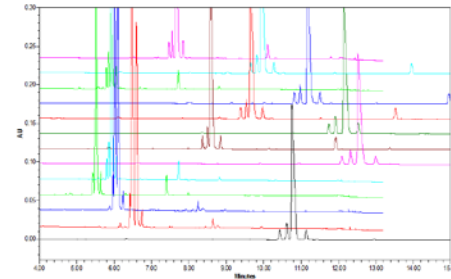
- Sort chromatograms by study factors or results.
- View the chromatograms for one or more experiment runs.
 - Overlay two or more chromatograms in a single view.
 - Independently set Y-axis and X-axis offsets for easy comparisons.

Zoom
Stack
Offset
Etc.



Turning Chromatograms into Knowledge

#	Run	Label	Component	Label	Function	Method	Column	Mobile Phase	Flow Rate	Temp	Wavelength	Injection Volume	Injection Concentration	Injection Time	Injection Position	Injection Temperature	Injection Pressure
1	1				Condition Column	Test Run pH_101_101			0.50	0.00	0.00	No Change					
2	1				Condition Column	Test Run pH_101_101			0.50	0.00	0.00	No Change					
3	1				Condition Column	Test Run pH_101_101			0.50	0.00	0.00	No Change					
4	1	1	1	1	Sample Injection	Test Run pH_101_101			0.50	0.00	0.00	No Change					
5	1	1	1	1	Sample Injection	Test Run pH_101_101			0.50	0.00	0.00	No Change					
6	1	1	1	1	Sample Injection	Test Run pH_101_101			0.50	0.00	0.00	No Change					
7	1	1	1	1	Sample Injection	Test Run pH_101_101			0.50	0.00	0.00	No Change					
8	1	1	1	1	Sample Injection	Test Run pH_101_101			0.50	0.00	0.00	No Change					
9	1	1	1	1	Sample Injection	Test Run pH_101_101			0.50	0.00	0.00	No Change					
10	1	1	1	1	Sample Injection	Test Run pH_101_101			0.50	0.00	0.00	No Change					
11	1	1	1	1	Sample Injection	Test Run pH_101_101			0.50	0.00	0.00	No Change					
12	1	1	1	1	Sample Injection	Test Run pH_101_101			0.50	0.00	0.00	No Change					
13	1	1	1	1	Sample Injection	Test Run pH_101_101			0.50	0.00	0.00	No Change					
14	1	1	1	1	Sample Injection	Test Run pH_101_101			0.50	0.00	0.00	No Change					
15	1	1	1	1	Sample Injection	Test Run pH_101_101			0.50	0.00	0.00	No Change					
16	1	1	1	1	Sample Injection	Test Run pH_101_101			0.50	0.00	0.00	No Change					
17	1	1	1	1	Sample Injection	Test Run pH_101_101			0.50	0.00	0.00	No Change					
18	1	1	1	1	Sample Injection	Test Run pH_101_101			0.50	0.00	0.00	No Change					
19	1	1	1	1	Sample Injection	Test Run pH_101_101			0.50	0.00	0.00	No Change					
20	1	1	1	1	Sample Injection	Test Run pH_101_101			0.50	0.00	0.00	No Change					
21	1	1	1	1	Sample Injection	Test Run pH_101_101			0.50	0.00	0.00	No Change					
22	1	1	1	1	Sample Injection	Test Run pH_101_101			0.50	0.00	0.00	No Change					
23	1	1	1	1	Sample Injection	Test Run pH_101_101			0.50	0.00	0.00	No Change					
24	1	1	1	1	Sample Injection	Test Run pH_101_101			0.50	0.00	0.00	No Change					
25	1	1	1	1	Sample Injection	Test Run pH_101_101			0.50	0.00	0.00	No Change					
26	1	1	1	1	Sample Injection	Test Run pH_101_101			0.50	0.00	0.00	No Change					
27	1	1	1	1	Sample Injection	Test Run pH_101_101			0.50	0.00	0.00	No Change					
28	1	1	1	1	Sample Injection	Test Run pH_101_101			0.50	0.00	0.00	No Change					
29	1	1	1	1	Sample Injection	Test Run pH_101_101			0.50	0.00	0.00	No Change					
30	1	1	1	1	Sample Injection	Test Run pH_101_101			0.50	0.00	0.00	No Change					



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

Linear Effect

Curvature Effect

Interaction Effects

Complex Effect

Reports

Best Overall Answer

View as Report

Graph Settings

Name	Units	Lower Bound	Upper Bound	Pointer Coordinate
X Gradient Time	min	20.00	70.00	45.00
Y pH	*	3.25	5.85	4.90

Column Type

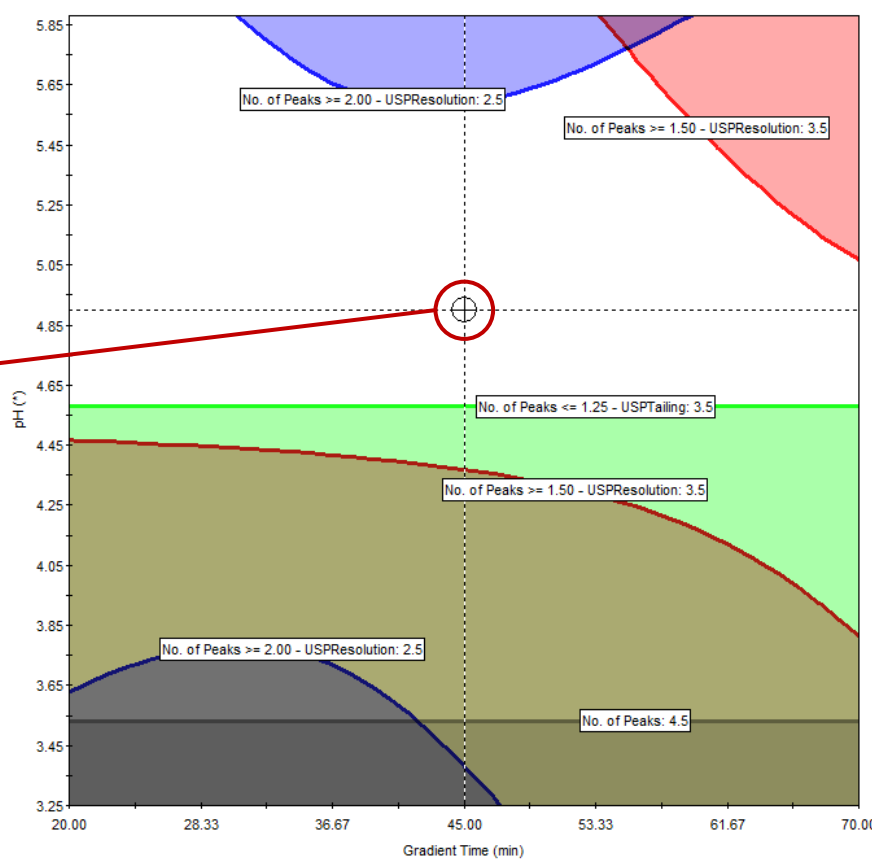
- Shield RP18
- BEH C8
- Shield RP18**
- BEH C18
- CSH Phenyl Hexyl

Verification Run Settings

Include Independently Adjustable Ranges Rectangle

Include Verification Runs

Graph



Fusion QbD Graph

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

Settings

Name	Units	Goal	Lower Bound	Upper Bound	Crosshair Prediction	Contour Label	Color
<input checked="" type="checkbox"/> No. of Peaks	*	Maximize	4.5		5.11		Gray
<input checked="" type="checkbox"/> No. of Peaks >= 1.50 - USPResolution		Maximize	3.5		3.74		Red
<input checked="" type="checkbox"/> No. of Peaks >= 2.00 - USPResolution		Maximize	2.5		2.86		Blue
<input checked="" type="checkbox"/> No. of Peaks <= 1.25 - USPTailing		Maximize	3.5		3.80		Green

Screening Study – Simple Analysis

Reports
Best Overall Answer
View as Report

Graph Settings

Name	Units	Lower Bound	Upper Bound	Pointer Coordinate
X Gradient Time	min	20.00	70.00	45.00
Y pH	*	3.25	5.88	4.90

Column Type

- BEH C18
- BEH C8
- Shield RP18
- BEH C18**
- CSH Phenyl Hexyl

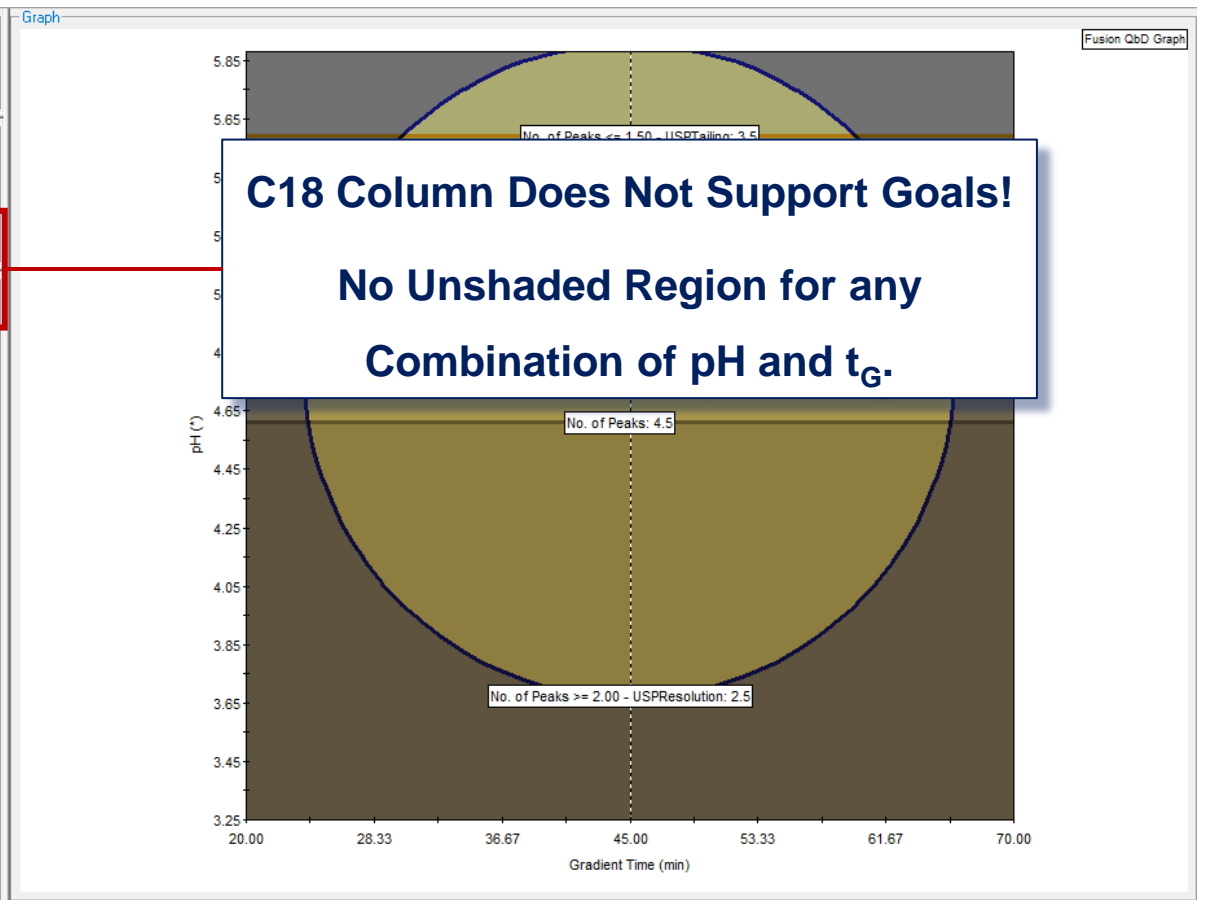
Verification Run Settings

Include Independently Adjustable Ranges Rectangle

Include Verification Runs

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

Graph



C18 Column Does Not Support Goals!

No Unshaded Region for any Combination of pH and t_G .

Overlay | Rs-Map

Response Settings

Name	Units	Goal	Lower Bound	Upper Bound	Crosshair Prediction	Contour Label	Color
<input checked="" type="checkbox"/> No. of Peaks	*	Maximize	4.5		5.11		Gray
<input checked="" type="checkbox"/> No. of Peaks >= 1.50 - USPResolution		Maximize	3.5		3.74		Red
<input checked="" type="checkbox"/> No. of Peaks >= 2.00 - USPResolution		Maximize	2.5		2.86		Blue
<input checked="" type="checkbox"/> No. of Peaks <= 1.25 - USPTailing		Maximize	3.5		3.80		Green
<input checked="" type="checkbox"/> No. of Peaks <= 1.50 - USPTailing		Maximize	3.5		3.9		Orange

Screening Study – Simple Analysis

Reports
Best Overall Answer
View as Report

Graph Settings

Name	Units	Lower Bound	Upper Bound	Pointer Coordinate
X Gradient Time	min	20.00	70.00	45.00
Y pH	*	3.25	5.88	4.90

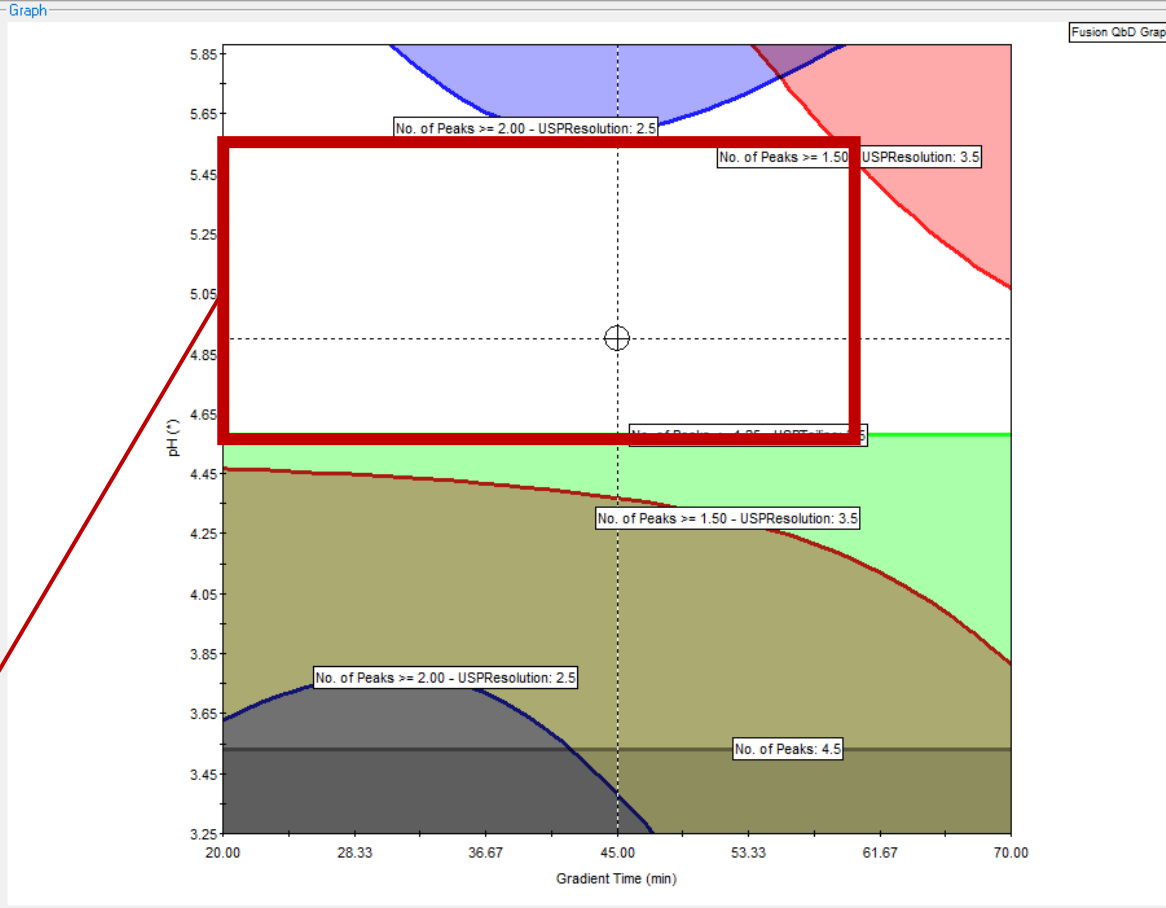
Column Type: Shield RP18

Verification Run Settings
 Include Independently Adjustable Ranges Rectangle
 Include Verification Runs

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

Graph



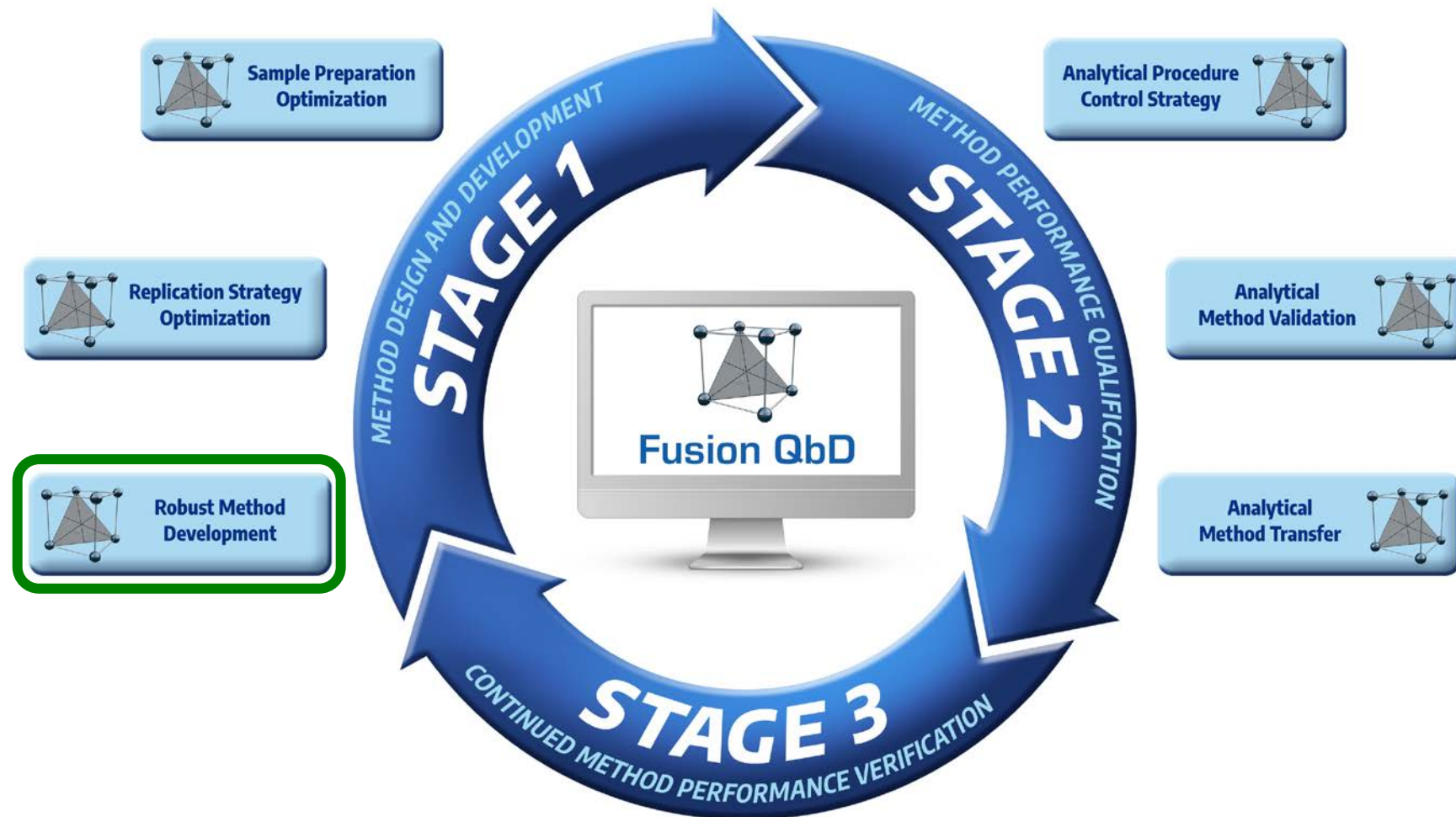
Fusion QbD Graph

Overlay | Fts-Map

Response Settings

Name	Units	Goal	Lower Bound	Upper Bound	Crosshair Prediction	Contour Label	Color
No. of Peaks	*	Maximize	4.5		5.11		Gray
No. of Peaks >= 1.50 - USPResolution		Maximize	3.5		3.74		Red
No. of Peaks >= 2.00 - USPResolution		Maximize	2.5		2.86		Blue
No. of Peaks <= 1.25 - USPTailing		Maximize	3.5		3.80		Green

LC and LC-MS Method Optimization



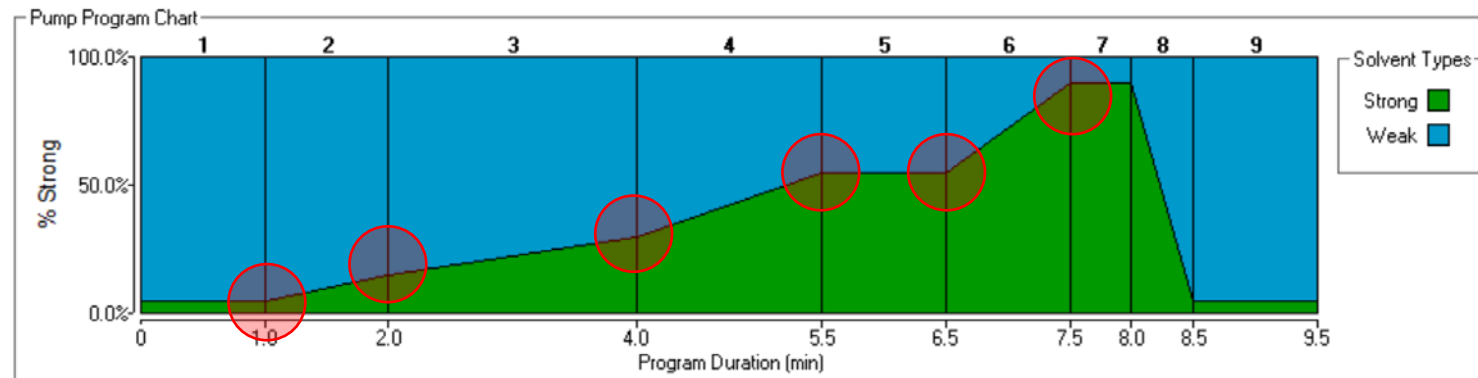


- ✓ **Ease of Experiment Setup**
- ✓ **Simple Chromatogram Integration**
- ✓ **Powerful UV & MS Based Peak Tracking**
- ✓ **Instant One-click Modeling – Any Results**
- ✓ **Complete Analysis Results Reporting**
- ✓ **Integrated Robustness Simulation**
- ✓ **Complete Multi-response Optimization**
- ✓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



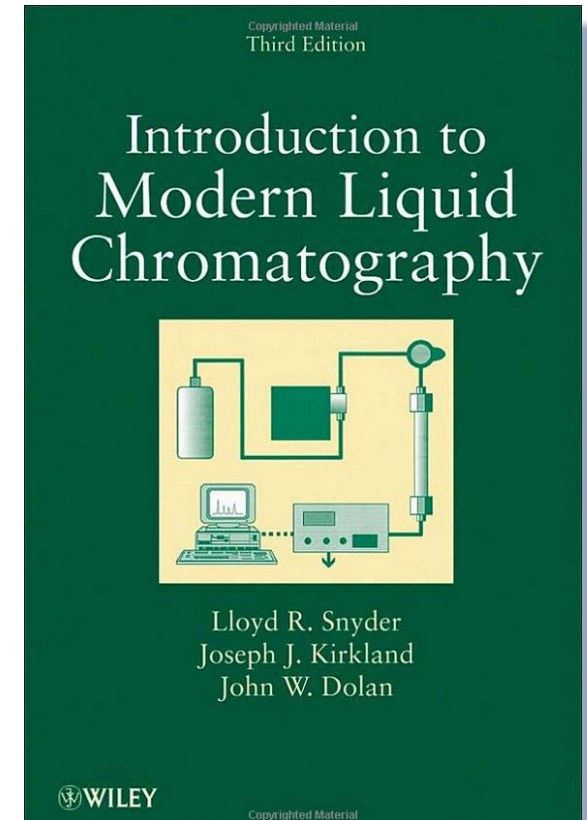
Localized Slope-Based Optimization is Now Recognized as High Risk.

Fusion QbD Does Not Rely on This Approach!

(See the Next Slide →)

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.**”



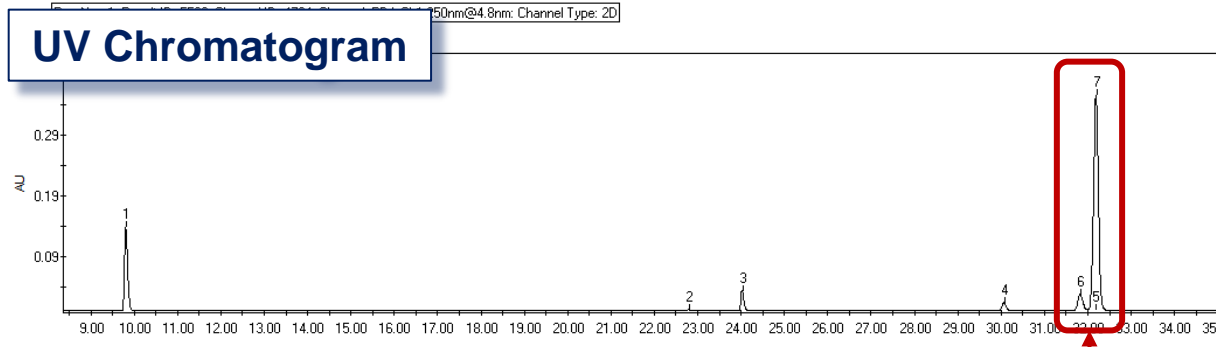
Snyder, Kirkland, and Dolan. (2010). *Introduction to Modern Liquid Chromatography*, 3rd Edition; John Wiley & Sons, Inc., Hoboken, New Jersey (p. 427-28)

Parameter Selection – Optimization Study

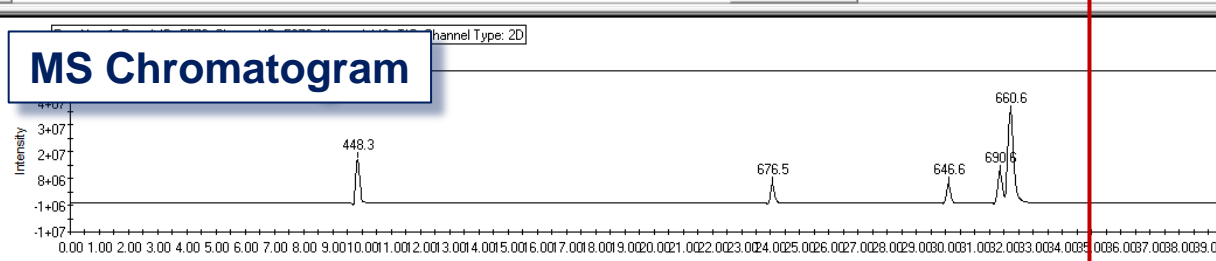
Method Parameter	Study Range
Pump Flow Rate (mL/min)	0.30 – 0.45
Column Oven Temperature (°C)	30.0 – 50.0
Gradient Time (min)*	25.0 – 40.0
pH	4.70 – 5.30
Column Type	BEH Shield RP18

Light green background color indicates result obtained from screening study.

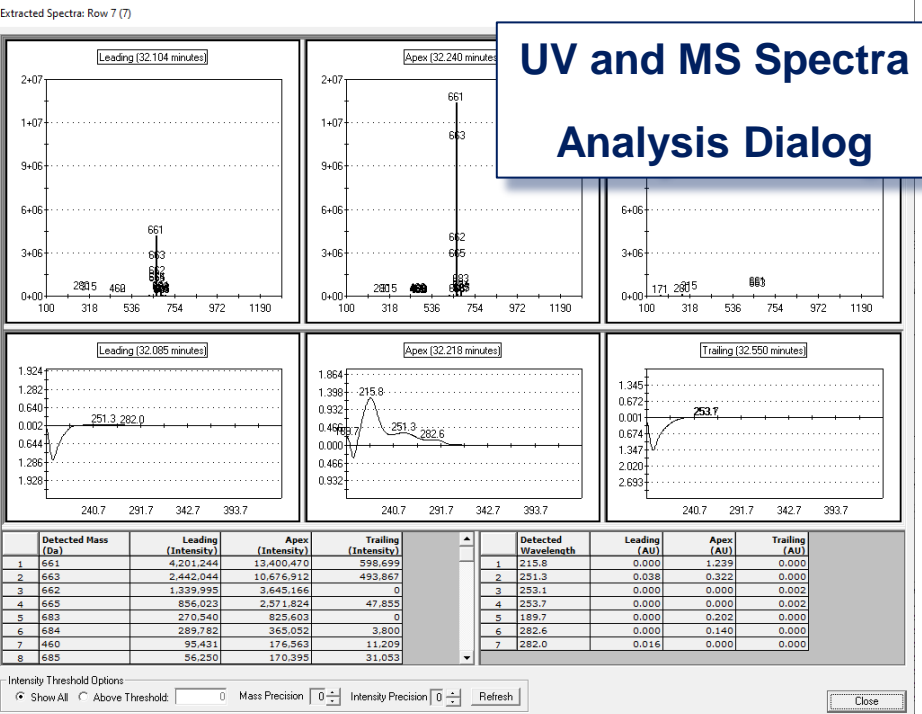
UV Chromatogram



MS Chromatogram



UV and MS Spectra Analysis Dialog



Detected Mass (Da)	Leading (Intensity)	Apex (Intensity)	Trailing (Intensity)
1	661	4,201,244	13,400,470
2	663	2,442,044	10,676,912
3	662	1,339,995	3,645,166
4	665	856,023	2,571,824
5	683	270,540	825,603
6	684	289,782	365,052
7	460	95,431	176,563
8	685	56,250	170,395

Peak Table - PDA Ch1 250nm@4.8nm - Run No. 1

Name	RT (min)	Base Peak (m/z)	AMV (m/z)	Area (uV*sec)	Height (uV)
1	9.830	448.3	448.3	677,862.4	137,118
2	22.815	108.2	108.2	7,239.8	1,295
3	24.054	676.5	676.5	156,751.3	33,167
4	30.097	646.6	646.6	87,734.4	12,988
5	31.850	690.6	690.6	216,408.1	26,976
6	32.200	450.0	450.0	1,060	1,060
7	32.219	660.6	660.6	2,768,651.5	354,216
8	39.122	122.3	122.3	13,879.6	3,345

Global Tracking Method (GTM): Generated from run 8

Mass | UV | Filters | Auto-name Peaks in GTM

Display Intensity Columns

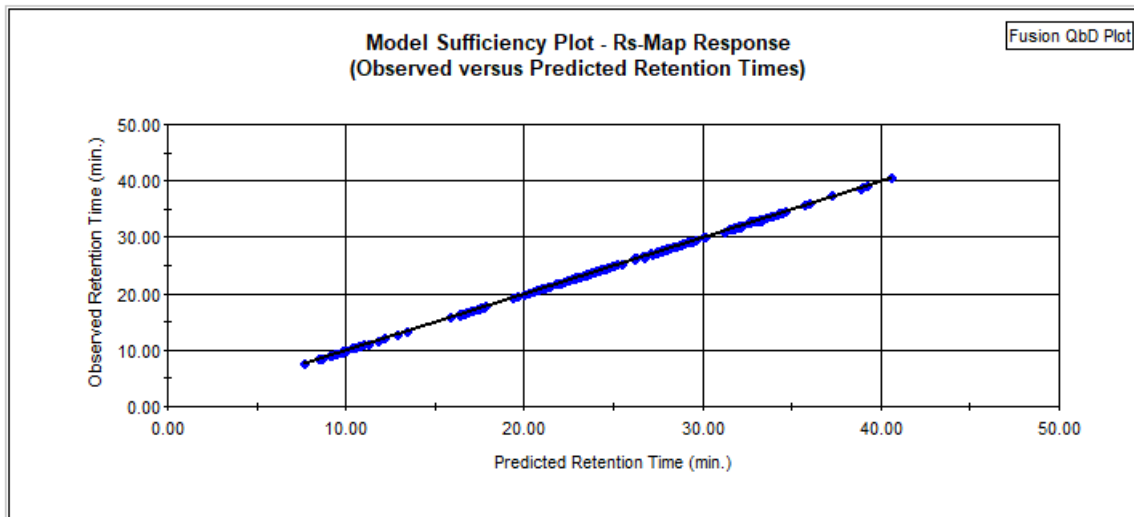
Run #	RT	Component Name	Expected Mass 1	Expected Mass 2	Expected Mass 3	Expected Mass 4	Expected Mass 5	
1	8	9.791	1	448.3	450.4	449.3	451.3	452.3
2	8	20.277	2	315.3	280.2	139.9	632.2	316.5
3	8	20.912	3	315.4	676.5	280.2	678.5	476.4
4	8	27.681	4	315.3	648.5	646.5	647.6	280.1
5	8	28.348	5	315.3	280.2	316.2	241.2	281.0
6	8	28.890	6	315.3	690.6	280.2	692.5	693.6
7	8	29.173	7	660.5	662.5	315.2	661.6	663.6
8	8	33.335	8					

Automatic Deconvolution of Peak 5 – which Coelutes with Peak 7 (API Peak) in this Run

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, ...

Analysis Summary Report - Rs-Map Response



Prediction Equation

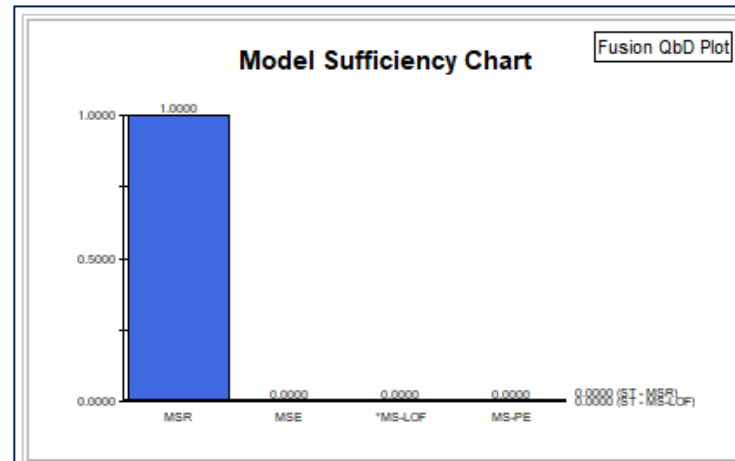
Observed Retention Time = -0.00068 + 1.00008 * Predicted Retention Time

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

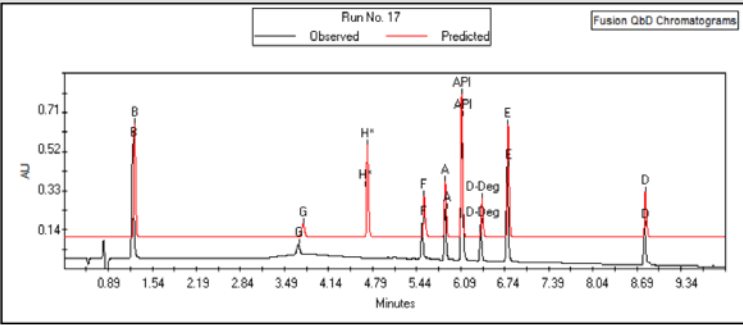
Name	Level Setting	Units
Pump Flow Rate	0.450	mL/min
Gradient Time	16.0	min
Oven Temperature	45.0	°C
pH	3.90	*

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



Run No. 17
— Observed — Predicted
 Fusion QbD Chromatograms

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

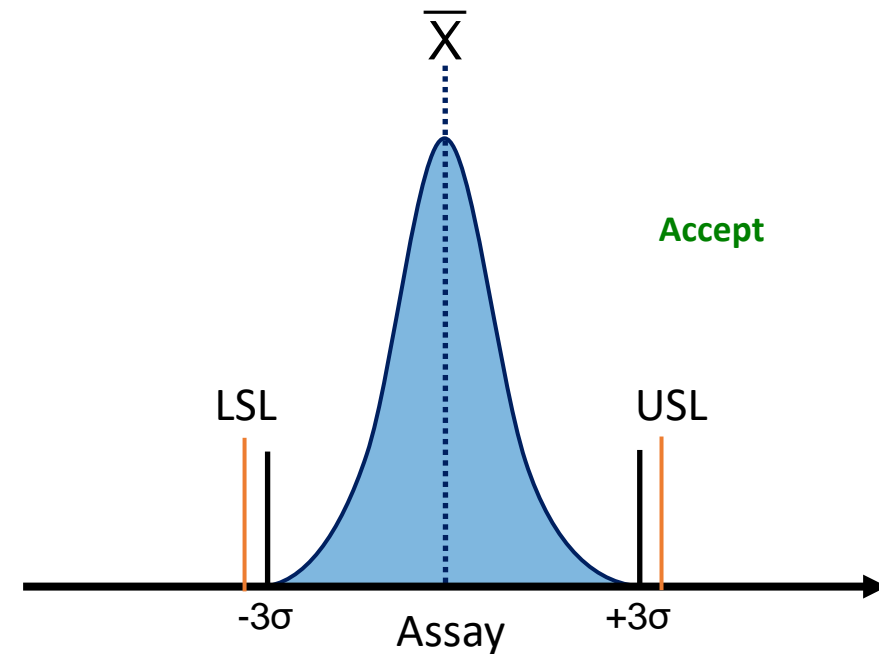
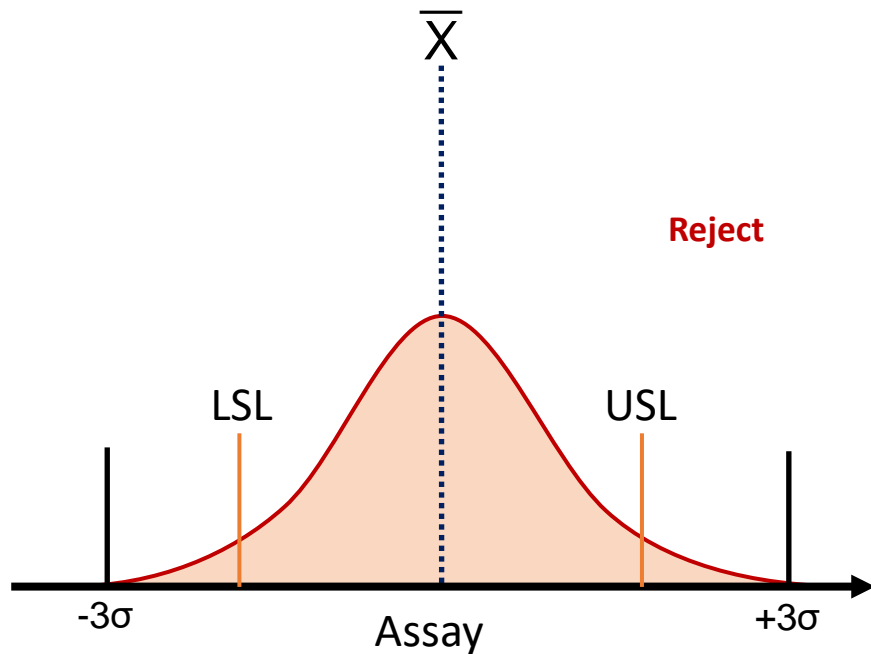
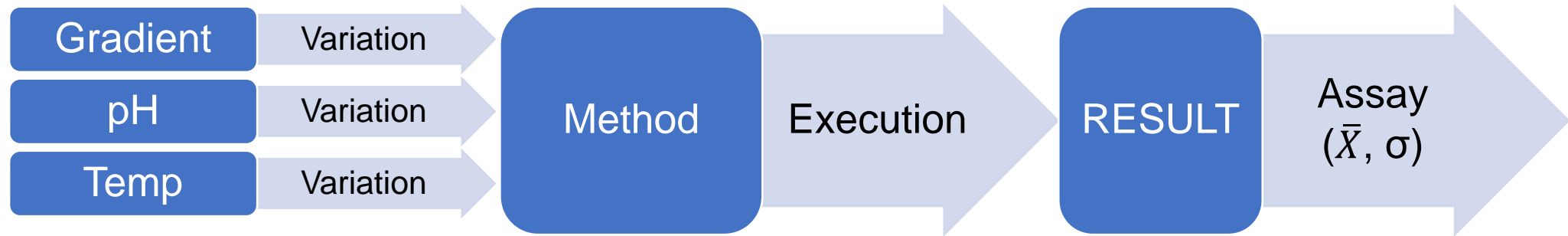
Data gained during the development studies (e.g., robustness data from a design of experiments (DoE) study) could be used as part of the validation data for the related analytical procedure performance characteristics and studies do not necessarily need to be repeated.

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

Method Development - LC Method Development Tutorial - Optimization - Part 2 - 992 - 653.smae

File Edit Activity Tools Window Help

Create Report Update Report Delete Report Restore Report Robustness Simulator Show Prediction Chromatogram

Design of Experiments

- Create a Design
- Design Reports

Data Management / Analysis

- Data Management
- Data Analysis

Best Answer Searches

- Best Overall Answer
- Robustness Performance Index
- Point Predictions

Visualization Graphics

- Single Response Series
- Multiple Response Series

Reporting Toolkit

- Fusion Reporter
- Audit Log Reporter

Repts

Robust Design Space 1

APR_2 Update Rs-Map

View as Report

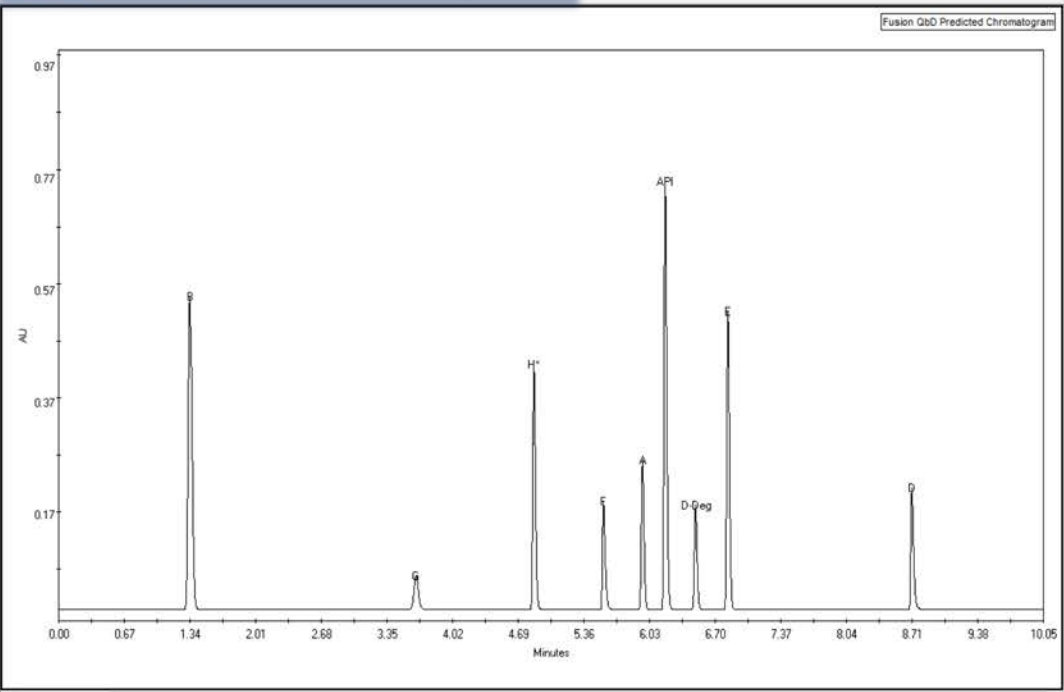
Graph Settings

Name	Units	Lower Bound	Upper Bound	Pointer Coordinate
X pH	*	3.60	4.20	3.90
Y Oven Temperature	°C	40.0	50.0	45.0

Pump Flow Rate: 0.400

Gradient Time: 15.0

Simulation Chromatogram



Fusion QsD Predicted Chromatogram

Overlay Rs-Map

Resolution Map Settings

Name	Pointer Predictions
1 B	...
2 API	4.118
3 D-Deg	5.670
4 E	6.218
5 A	7.784
6 F	13.326
7 H	17.583
8 G	22.760
9 C	25.128

View as 3D Reset

Graph Density:

- Low (Fast)
- Medium (Medium)
- High (Slow)

Color Mode:

- Color
- Greyscale
- B & W

3D Rs-Map Commands:

Print... Copy

Validation Status: Your settings are valid.

modified

3D Resolution Map View

Method Development - LC Method Development Tutorial - Optimization - Part 2 - 992 - 653.smae

File Edit Activity Tools Window Help

Create Report Update Report Delete Report Restore Report Robustness Simulator Show Prediction Chromatogram

Design of Experiments

- Create a Design
- Design Reports

Data Management / Analysis

- Data Management
- Data Analysis

Best Answer Searches

- Best Overall Answer
- Robustness Performance Index
- Point Predictions

Visualization Graphics

- Single Response Series
- Multiple Response Series

Reporting Toolkit

- Fusion Reporter
- Audit Log Reporter

Reports

Robust Design Space 1

APR_2 Update Ri-Map

View as Report

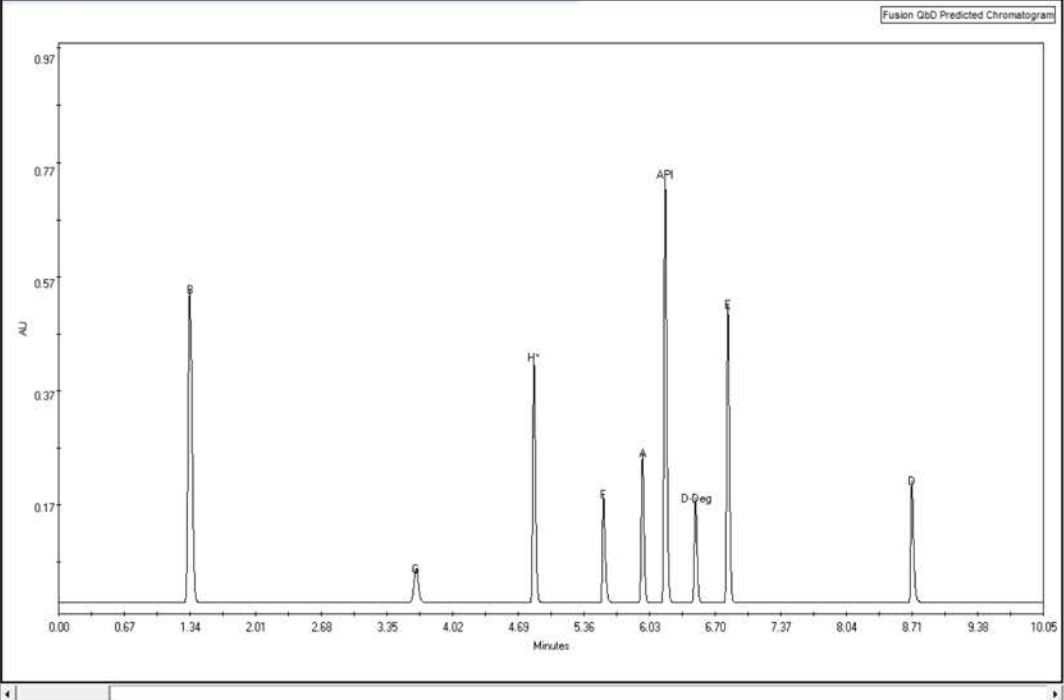
Graph Settings

Name	Units	Lower Bound	Upper Bound	Pointer Coordinate
X pH		3.60	4.20	3.90
Y Oven Temperature	°C	-40.0	50.0	45.0

Pump Flow Rate: 0.400

Gradient Time: 15.0

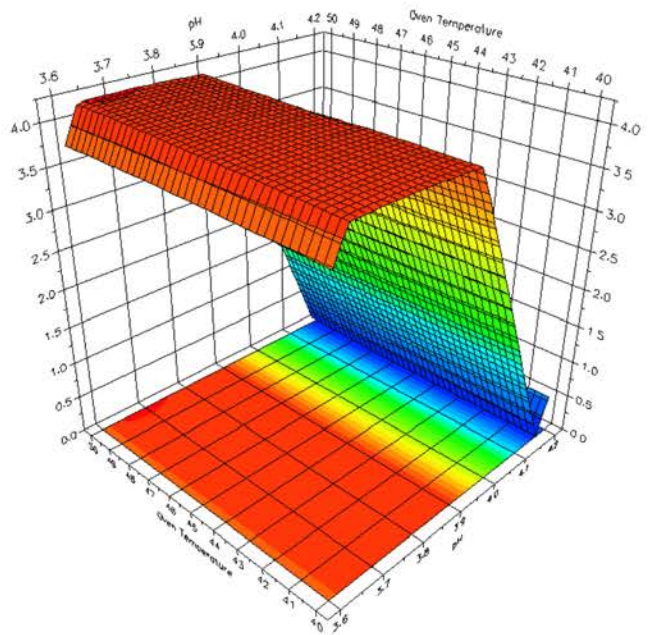
Simulation Chromatogram



Fusion QbD Predicted Chromatogram

Validation Status: Your settings are valid.

Graph



Legend

4.17
3.95
3.73
3.51
3.30
3.08
2.86
2.64
2.42
2.20
1.98
1.76
1.54
1.32
1.10
0.88
0.66
0.44
0.22
0.00

Overlay Ri-Map

Resolution Map Settings

Name	Pointer Predictions
1 B	...
2 API	4.118
3 D-Deg	5.670
4 E	6.218
5 A	7.784
6 F	13.326
7 H	17.583
8 G	32.760
9 D	35.128

View as 3D Reset

Graph Density:

- Low (Fast)
- Medium (Medium)
- High (Slow)

Color Mode:

- Color
- Greyscale
- B & W

3D Ri-Map Commands:

Print... Copy

Ready modified

Multi-Response Overlay View

Method Development - LC Method Development Tutorial - Optimization - Part 2 - 992 - 653.smae

File Edit Activity Tools Window Help

Create Report Update Report Delete Report Restore Report Robustness Simulator Show Prediction Chromatogram

Design of Experiments

- Create a Design
- Design Reports

Data Management / Analysis

- Data Management
- Data Analysis

Best Answer Searches

- Best Overall Answer
- Robustness Performance Region
- Point Predictions

Visualization Graphics

- Single Response Series
- Multiple Response Series

Reporting Toolkit

- Fusion Reporter
- Audit Log Reporter

Replots

Robust Design Space 1

APR_2 Update Rs-Map

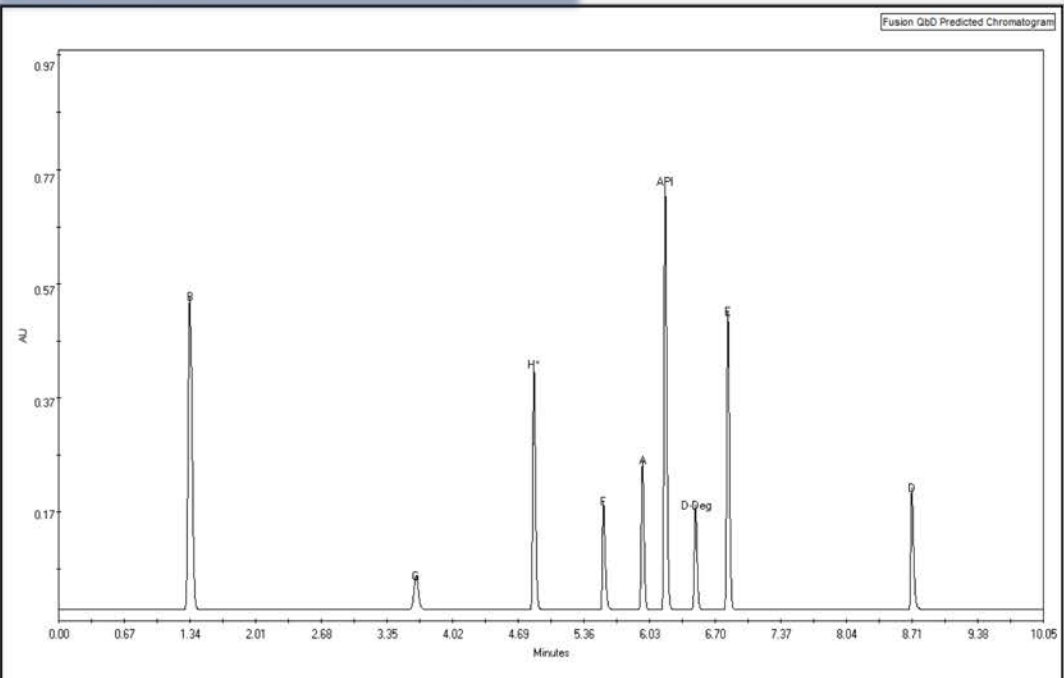
View as Report

Graph Settings

Name	Units	Lower Bound	Upper Bound	Pointer Coordinate
X pH	*	3.60	4.20	3.90
Y Oven Temperature	°C	40.0	50.0	45.0

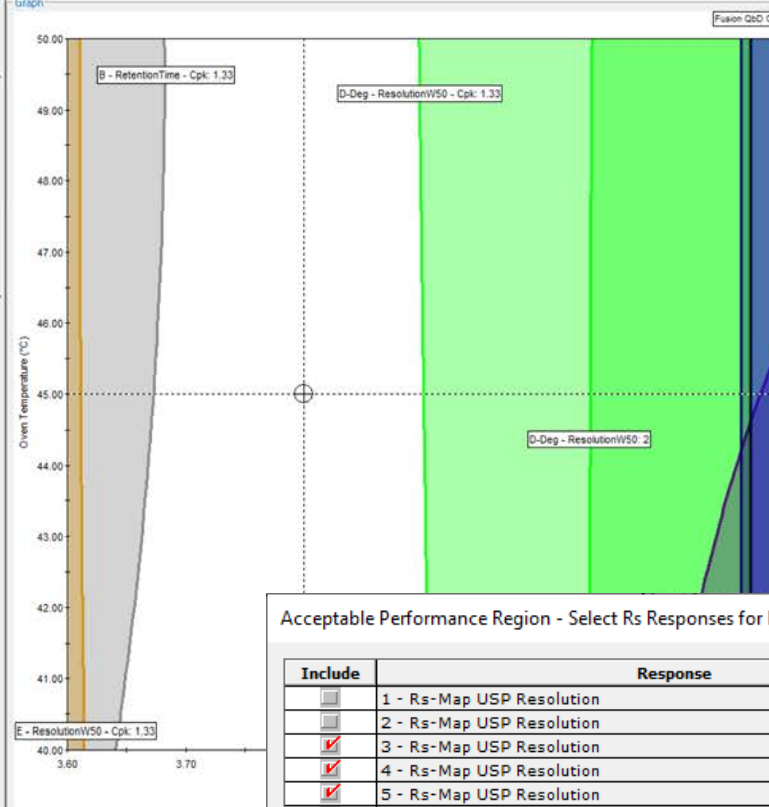
Pump Flow Rate: 0.400
Gradient Time: 15.0

Simulation Chromatogram



Validation Status: Your settings are valid.

Graph



Overlay: Rs-Map

Response Settings

Add Named Peak Rs Responses...

Name	Units	Goal
Rs-Map Response	*	
A - ResolutionW50	*	Maximize
API - ResolutionW50	*	Maximize
D-Deg - ResolutionW50	*	Maximize
E - ResolutionW50	*	Maximize
B - RetentionTime	*	Maximize
API - USPTailing		Minimize

Acceptable Performance Region - Select Rs Responses for Individual Compounds

Include	Response
<input type="checkbox"/>	1 - Rs-Map USP Resolution
<input type="checkbox"/>	2 - Rs-Map USP Resolution
<input checked="" type="checkbox"/>	3 - Rs-Map USP Resolution
<input checked="" type="checkbox"/>	4 - Rs-Map USP Resolution
<input checked="" type="checkbox"/>	5 - Rs-Map USP Resolution
<input checked="" type="checkbox"/>	6 - Rs-Map USP Resolution
<input checked="" type="checkbox"/>	7 - Rs-Map USP Resolution
<input type="checkbox"/>	8 - Rs-Map USP Resolution

Select All Select None OK Cancel

Reports
 Robust Design Space 1 [APR 2] Update Rs-Map
 View as Report

Graph Settings

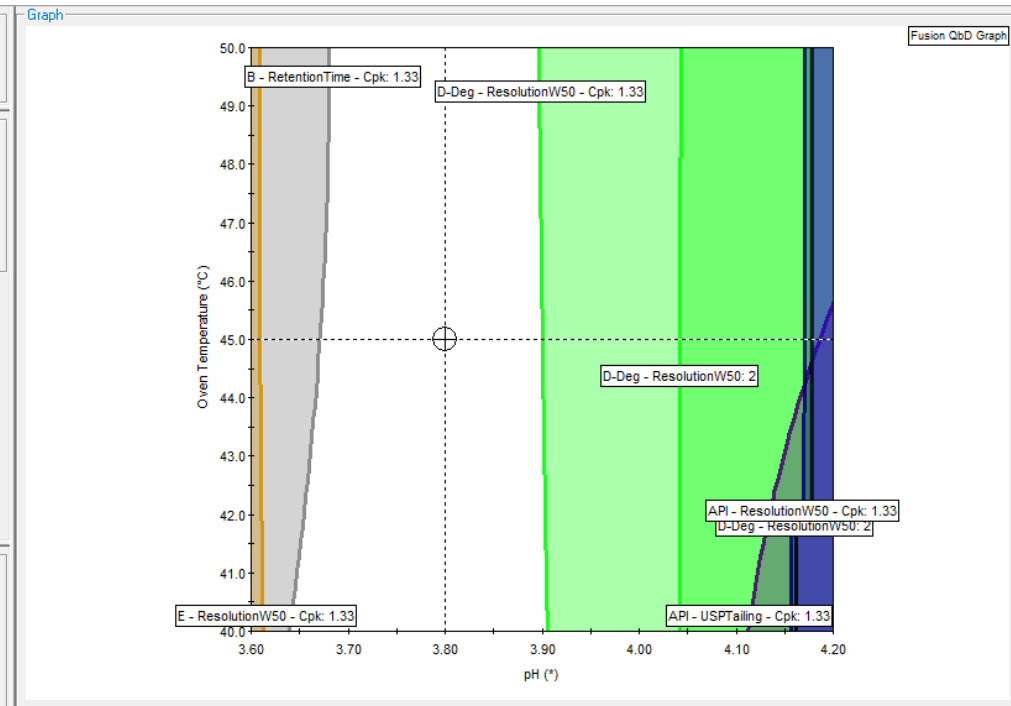
Name	Units	Lower Bound	Upper Bound	Pointer Coordinate
X pH	*	3.60	4.20	3.80
Y Oven Temperature	°C	40.0	50.0	45.0

Pump Flow Rate: 0.400
 Gradient Time: 15.0

Prediction Chromatogram Include in Report



Validation Status: Your settings are valid.



Overlay | Rs-Map

Response Settings

Add Named Peak Rs Responses...

Name	Units	Goal	Lower Bound	Upper Bound	Color	Crosshair Prediction	Contour Label
<input checked="" type="checkbox"/> A - ResolutionW50	*	Maximize	2,000		Red	7,784	<input checked="" type="checkbox"/>
<input checked="" type="checkbox"/> API - ResolutionW50	*	Maximize	2,000		Blue	4,118	<input checked="" type="checkbox"/>
<input checked="" type="checkbox"/> D-Deg - ResolutionW50	*	Maximize	2,000		Green	5,670	<input checked="" type="checkbox"/>
<input checked="" type="checkbox"/> E - ResolutionW50	*	Maximize	2,000		Orange	6,218	<input checked="" type="checkbox"/>
<input checked="" type="checkbox"/> B - RetentionTime	*	Maximize	1.00		Gray	1,341	<input checked="" type="checkbox"/>
<input checked="" type="checkbox"/> API - USPTailing	*	Maximize	2,000		Purple	2,845	<input checked="" type="checkbox"/>
<input checked="" type="checkbox"/> B - RetentionTime - Cpk	*	Maximize	1,330		Gray	2,2410	<input checked="" type="checkbox"/>
<input checked="" type="checkbox"/> API - USPTailing - Cpk	*	Maximize	1,330		Purple	4,8516	<input checked="" type="checkbox"/>
<input type="checkbox"/> Rs-Map Response - Cpk	*	---	---	---	---	---	<input type="checkbox"/>
<input checked="" type="checkbox"/> A - ResolutionW50 - Cpk	*	Maximize	1,330		Red	12,4820	<input checked="" type="checkbox"/>
<input checked="" type="checkbox"/> API - ResolutionW50 - Cpk	*	Maximize	1,330		Blue	22,2684	<input checked="" type="checkbox"/>
<input checked="" type="checkbox"/> D-Deg - ResolutionW50 - Cpk	*	Maximize	1,330		Green	2,4687	<input checked="" type="checkbox"/>
<input checked="" type="checkbox"/> E - ResolutionW50 - Cpk	*	Maximize	1,330		Orange	3,3400	<input checked="" type="checkbox"/>

Activate the Robustness Responses:
 Check the checkboxes in the grid.

4-Factor Robustness Trellis Graph View

Method Development - LC Method Development Tutorial - Optimization - Part 2

File Edit Activity Tools Window Help

Create Report Update Report Delete Report Restore Report Robustness Simulator Show Prediction Chromatogram

Design of Experiments

- Create a Design
- Design Reports
- Data Management / Analysis
- Data Management
- Data Analysis

Best Answer Searches

- Best Overall Answer
- Acceptable Performance Region
- Point Predictions

Visualization Graphics

- Single Response Series
- Multiple Response Series

Reporting Toolkit

- Fusion Reporter
- Audit Log Reporter

Reports

4-Factor MODR | APR 4 | Update Graph

View as Report

Axis Variable Units Lower Bound Upper Bound

X pH (D) * 3.60 4.00

Y Oven Temperature (C) °C 40.0 50.0

Horizontal Trellis Variable: Pump Flow Rate (A)

Vertical Trellis Variable: Gradient Time (B)

Low Middle High

mL/min min

0.300 14.0

0.350 15.0

0.400 16.0

Verification Run Settings

Include Independently Adjustable Ranges Rectangle

Variable	Lower Bound	Upper Bound	Center Point	Pointer Coordinate
pH	3.70	3.90	3.80	
Oven Temperature	42.0	48.0	45.0	

Verification Runs

Res IV: 8 Runs + CP

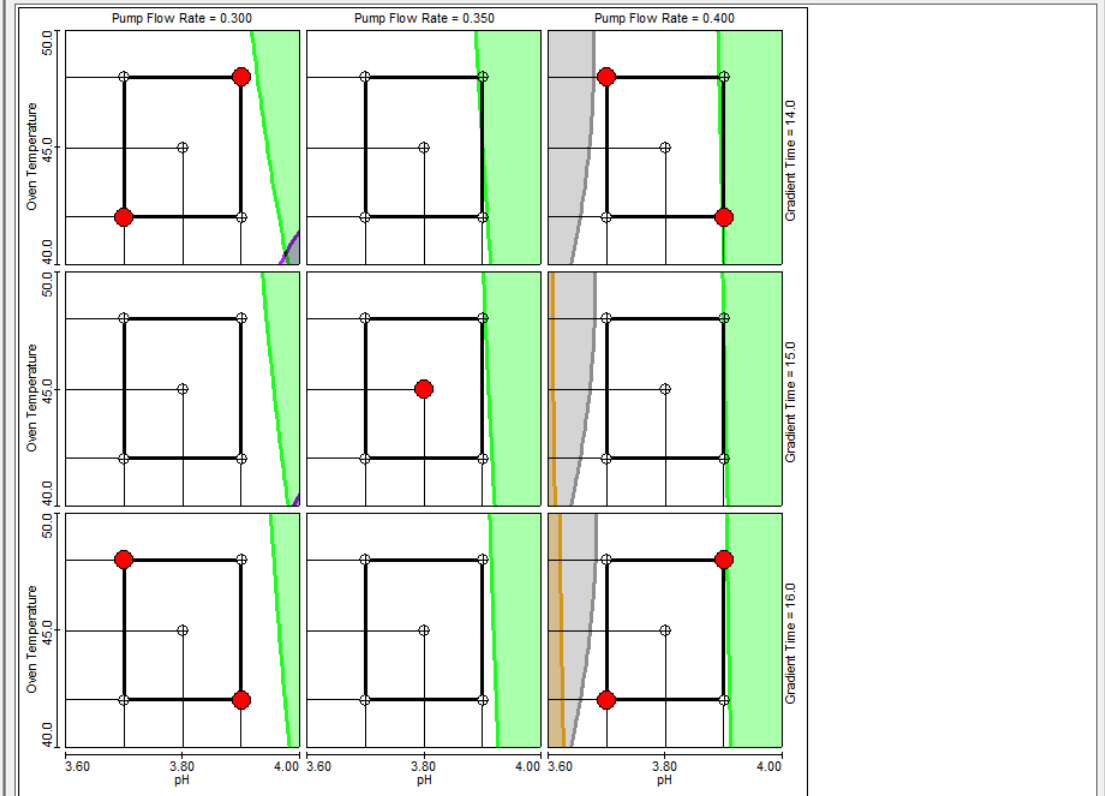
Show Verification Run Labels

Include Verification Runs in Report

Include Prediction Chromatograms in Report

None	Pump Flow Rate	Gradient Time	Oven Temperature	pH
All				
Res IV: 8 Runs + CP				
APR_4_A1_3	0.300	14.0	48.0	3.90
APR_4_A3_1	0.300	14.0	42.0	3.70
APR_4_A3_4	0.300	16.0	48.0	3.70
APR_4_A3_4	0.300	16.0	42.0	3.90
APR_4_B2_5	0.350	15.0	45.0	3.80
APR_4_C1_1	0.400	14.0	48.0	3.70
APR_4_C1_4	0.400	14.0	42.0	3.90
APR_4_C3_2	0.400	16.0	48.0	3.90
APR_4_C3_3	0.400	16.0	42.0	3.70

Graph



Overlay | Rs-Map

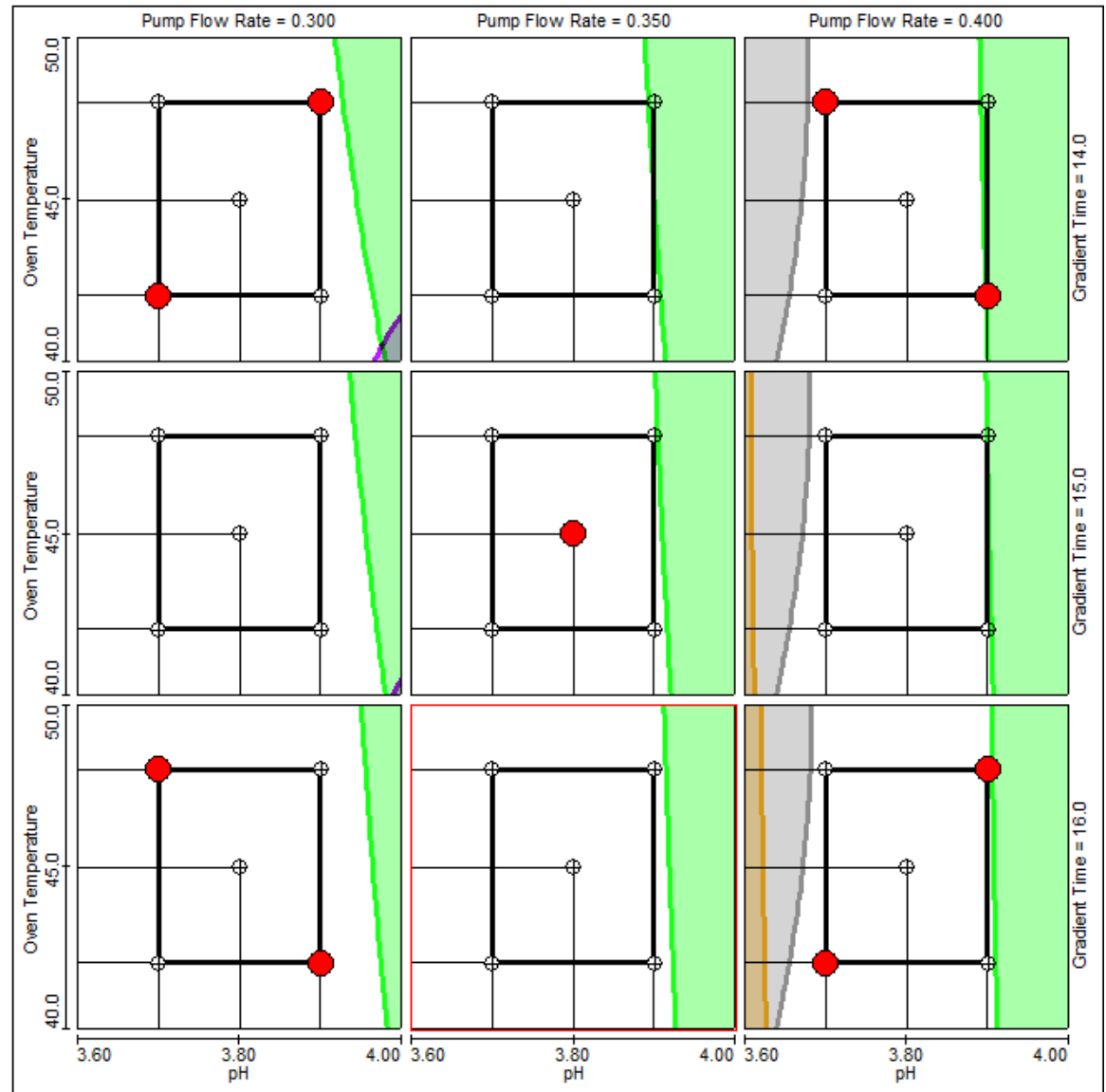
Response Settings

Name	Units	Goal	Lower Bound	Upper Bound	Color
Rs-Map Response	*	---	---	---	---
<input checked="" type="checkbox"/> A - ResolutionWS0	*	Maximize	2.000		Red
<input checked="" type="checkbox"/> API - ResolutionWS0	*	Maximize	2.000		Blue
<input checked="" type="checkbox"/> D-Deg - ResolutionWS0	*	Maximize	2.000		Green
<input checked="" type="checkbox"/> E - ResolutionWS0	*	Maximize	2.000		Orange
<input checked="" type="checkbox"/> R - RetentionTime	*	Maximize	1.00		Gray

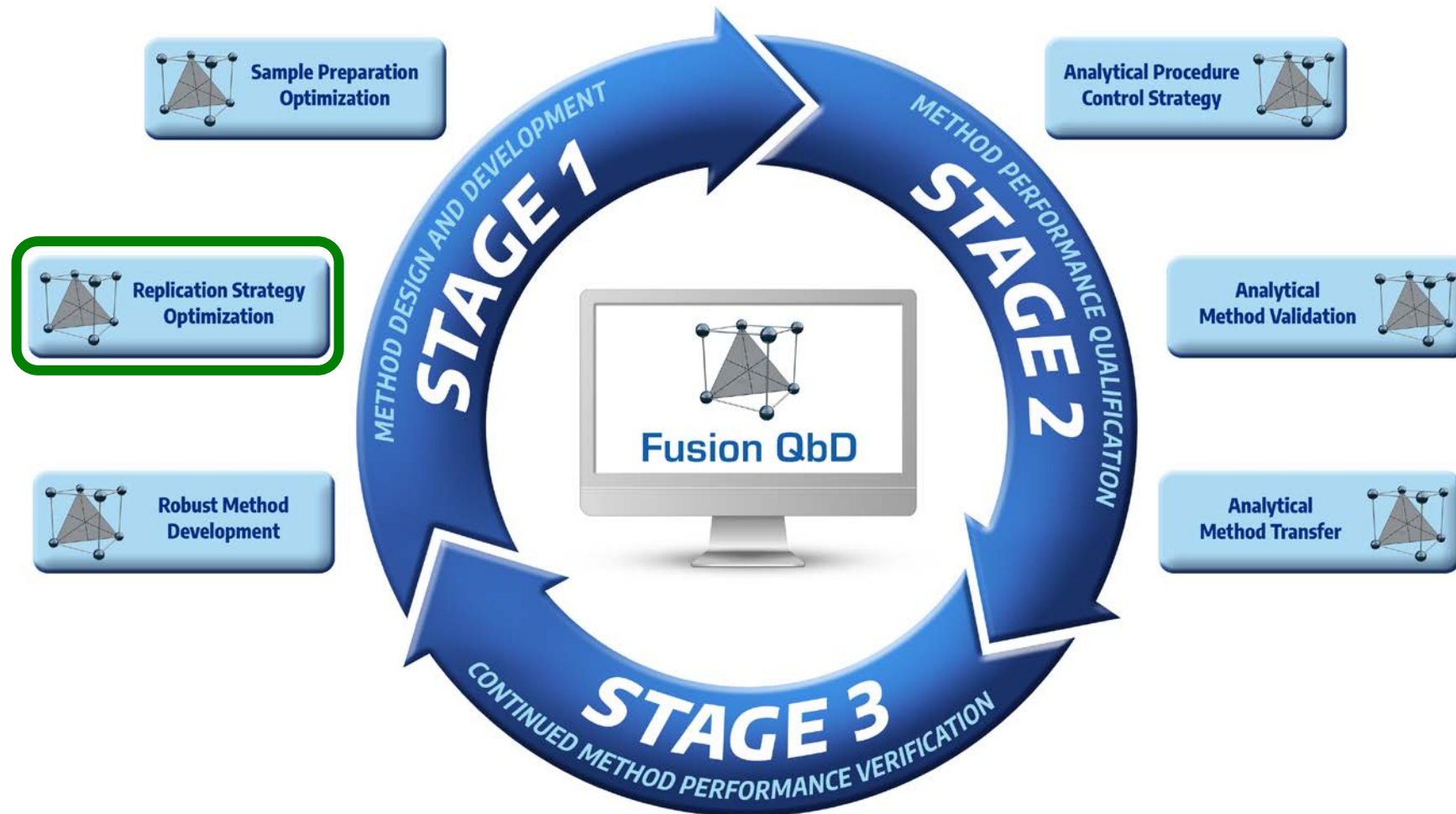
MODR Validation

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

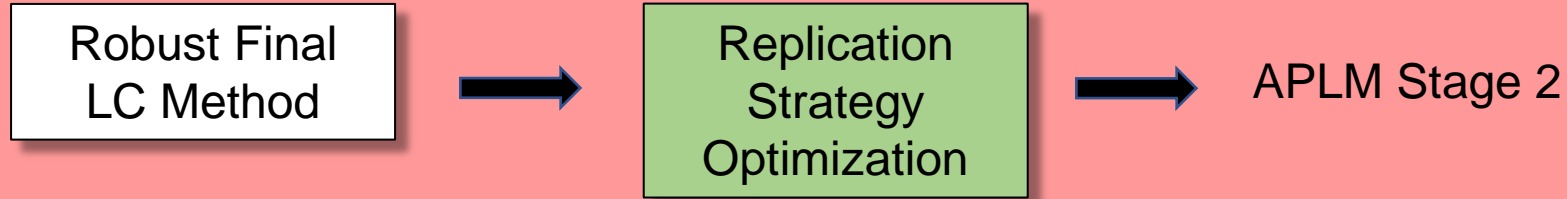
All Fusion QbD Reports, which can include 2D, 3D, and 4D Trellis graphics and prediction and verification chromatograms can be output in a variety of file formats, including MS Word and Acrobat PDF.



Replication Strategy Optimization



Replication Strategy Optimization



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})

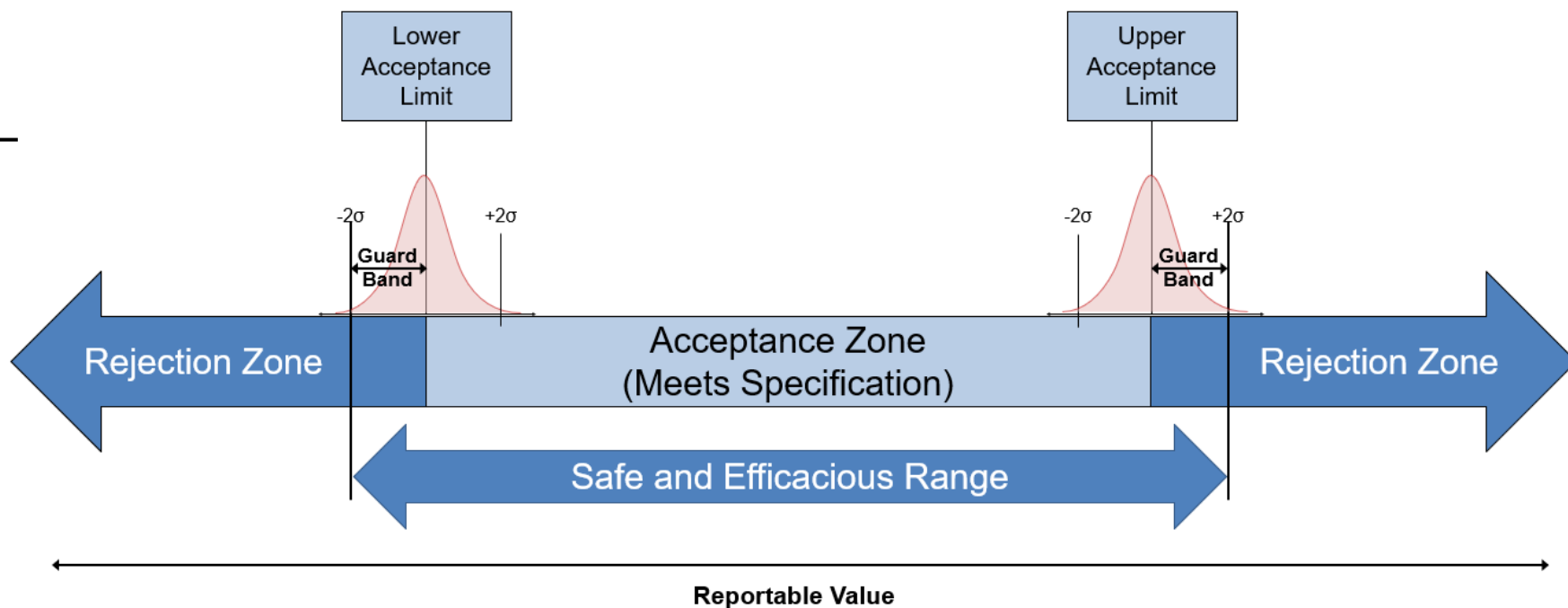
Tolerance Width = 4.00 (98.0 – 102.0)

Precision Width = $0.30 \times 4.00 = 1.20$

Split between LAL and UAL = 0.60

$0.60 = 2\sigma$ width for 95% Confidence

$$\sigma_{\max} = 0.60/2 = \pm 0.30$$



Replication Strategy for the Reportable Value

Method Development - Untitled1

File Edit Activity Tools Window Help

Select Autosampler Tray Update Setup Data Generate Design ?

Design of Experiments

- Create a Design
- Design Reports

Data Entry / Analysis

- Data Entry
- Data Analysis

Reporting Toolkit

- Fusion Reporter
- Audit Log Reporter

Project Name: Project 1 Experiment Name: Experiment 1 Instrument Name: Fusion QbD H_Class Experiment Phase: Method Development Experiment Type: Replication Strategy Separation Mode: Reversed Phase (RPC)

Experiment Setup

Global Sample Settings

Obtain all injection repeats from the same vial

Name: Preparation replicates per sample No. of Levels: 5

	Level setting
Level 1	P-1
Level 2	P-2
Level 3	P-3
Level 4	P-4
Level 5	P-5

Name: Injections per preparation replicate No. of Levels: 5

	Level setting
Level 1	I-1
Level 2	I-2
Level 3	I-3
Level 4	I-4
Level 5	I-5

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	0.38	11.61
Injection	0.14	0.37	20	2.0860	0.76	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 ($\pm\sigma$) and T.I Results for Replication Strategies from 1x1 to 10x10

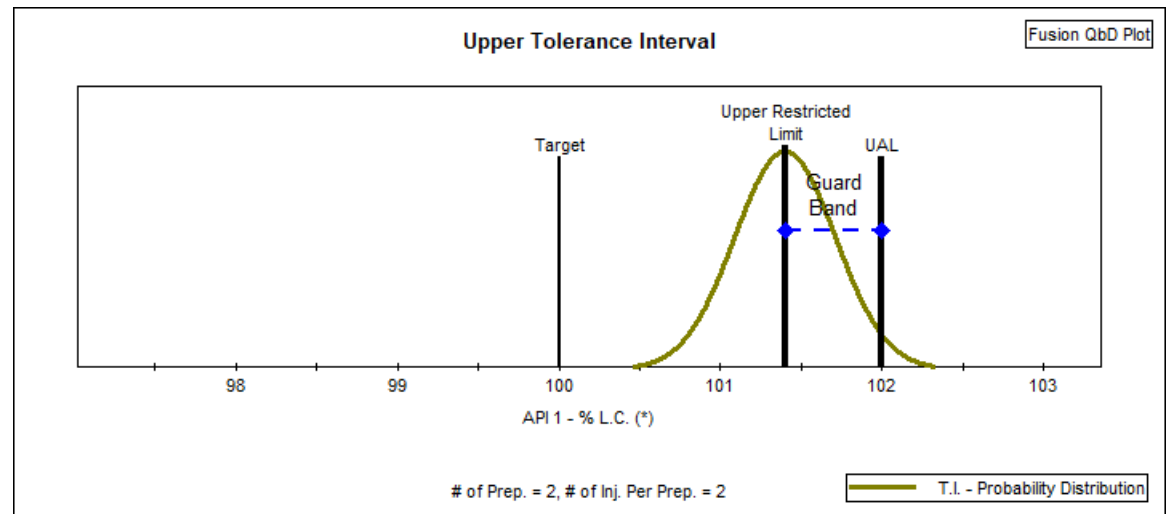
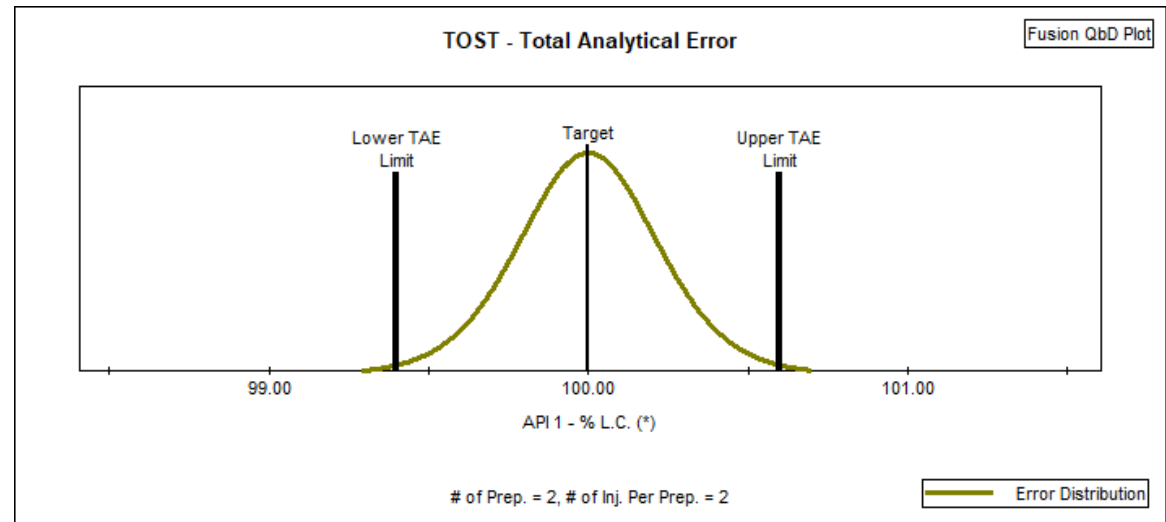
Replication Strategy Predicted TOST and Interval Results

No. of Injections		No. of Preparations									
		1	2	3	4	5	6	7	8	9	10
1	$\pm 2\sigma$	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	$\pm 2\sigma$	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	$\pm 2\sigma$	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	$\pm 2\sigma$	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	$\pm 2\sigma$	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	$\pm 2\sigma$	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	$\pm 2\sigma$	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	$\pm 2\sigma$	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	$\pm 2\sigma$	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	$\pm 2\sigma$	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

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

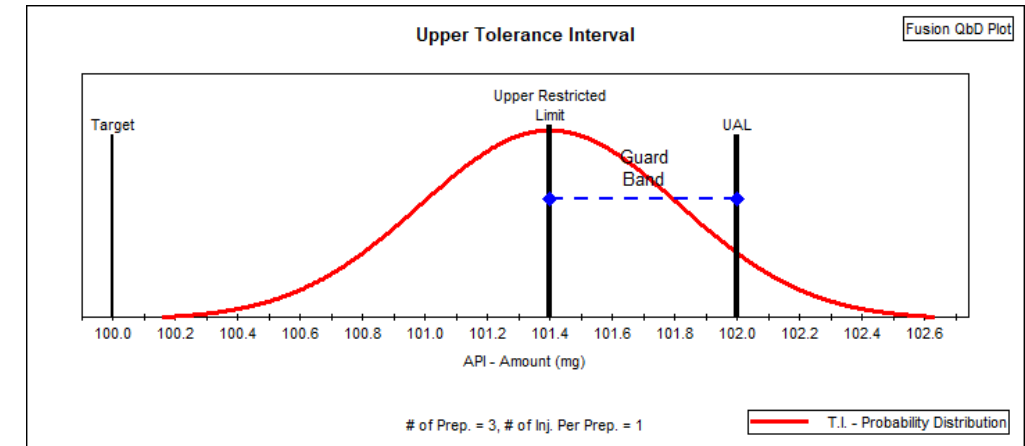
No. of Injections	No. of Preparations										
	1	2	3	4	5	6	7	8	9	10	
1	±2σ	0.7517	0.5311	0.4340	0.3759	0.3362	0.3069	0.2841	0.2658	0.2506	0.2377
	T.I.	1.7228	1.0551	0.8121	0.6810	0.5968	0.5372	0.4922	0.4568	0.4280	0.4040
2	±2σ	0.7428	0.5252	0.4288	0.3714	0.3322	0.3032	0.2807	0.2626	0.2476	0.2349
	T.I.	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

TOST Analysis Results Summary

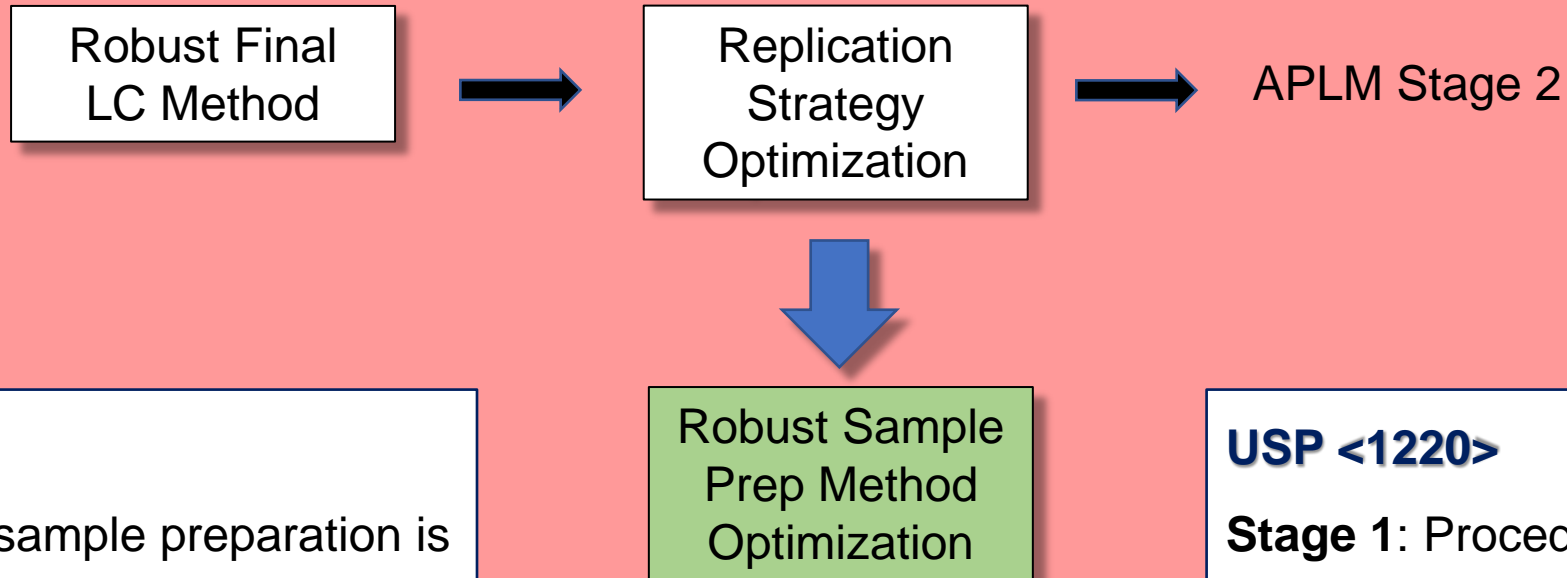
Statistic	Value	Pass/Fail
TAE Width (2σ) - Target	±0.600	
Computed TAE Width (2σ)	±0.434	Pass
FPT	<0.0001	
Cp	4.4075	
Variance	0.023	
Standard Deviation	0.151	
% RSD	0.15	
% CV	0.15	

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		



Sample Prep Method Optimization



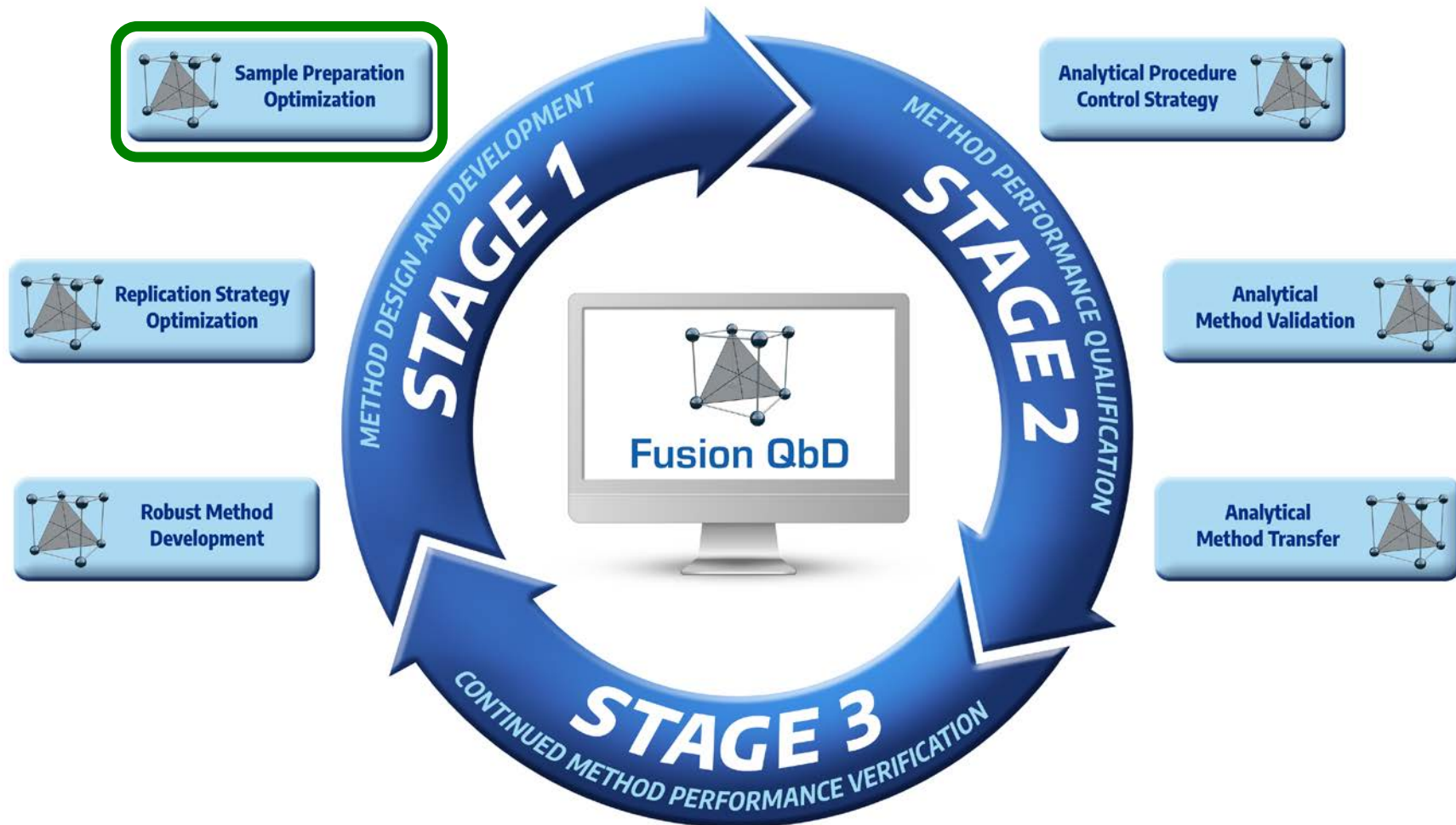
ICH Q14

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

 Experiment Type **Optimization**

Mixture Variable Settings

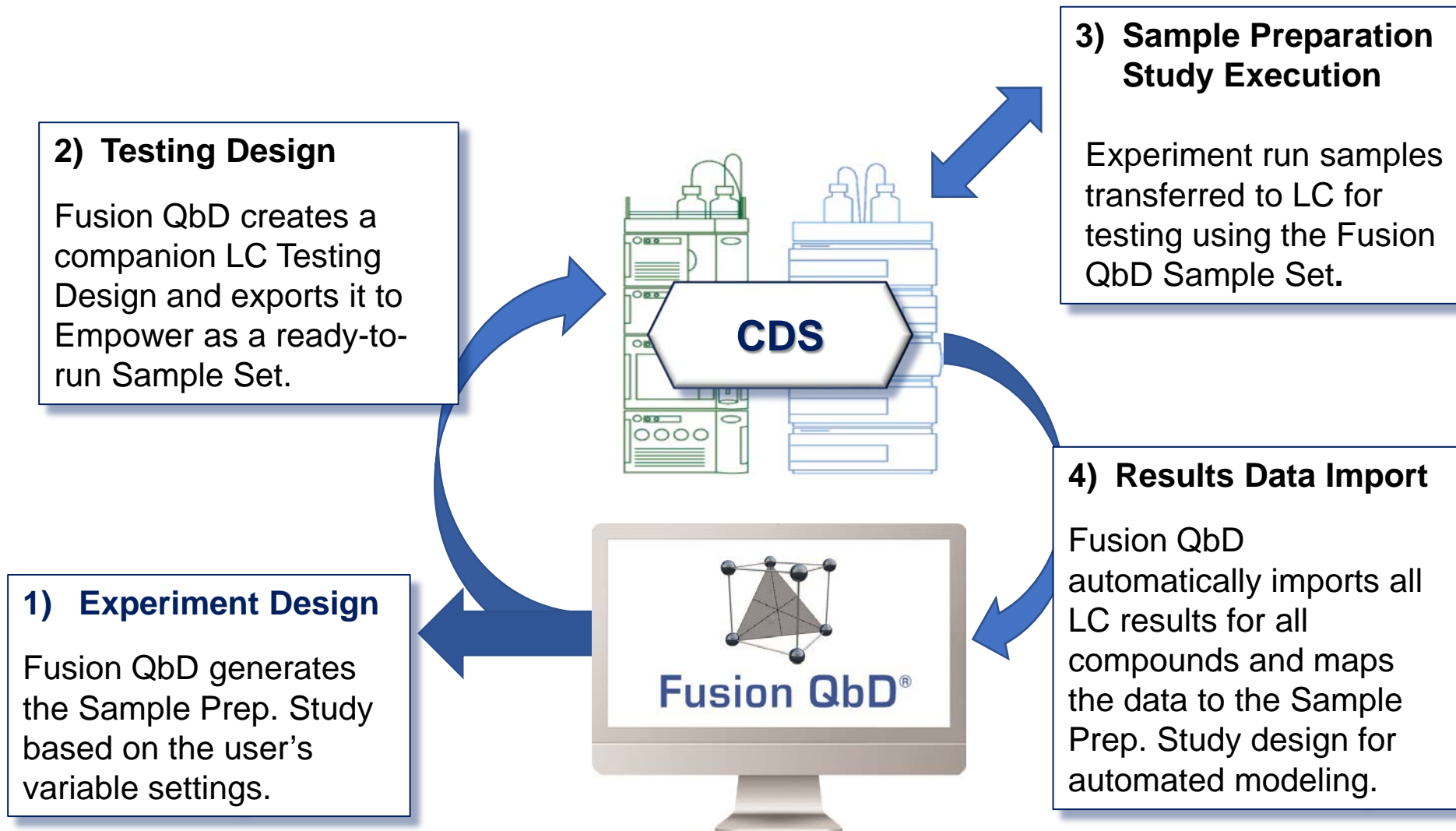
 No. of Mixture Variables **0**

Process Variable Settings

 No. of Process Variables **5**
 Split-plot Design (restriction on randomization)

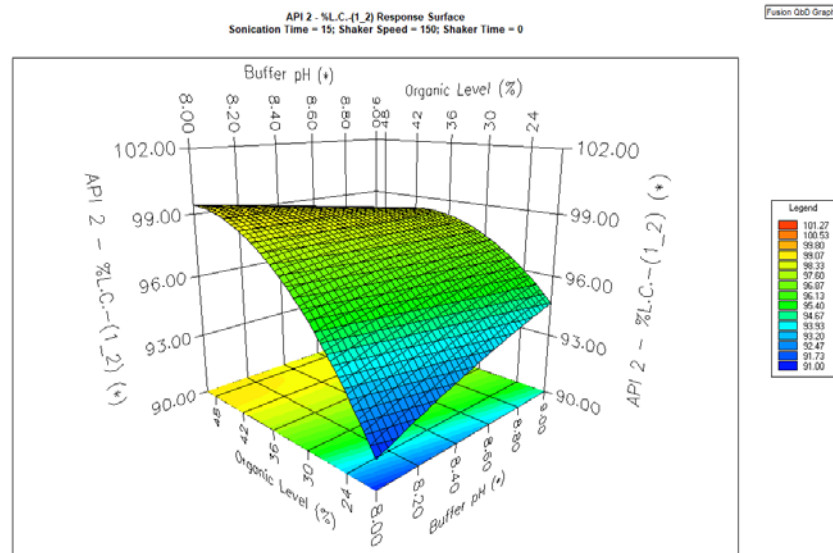
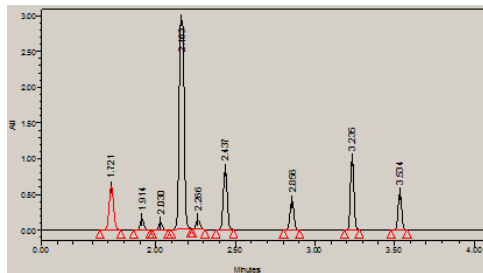
Name	Units	Type	Level Settings	
Buffer pH	x	Discrete Numeric	Level 1 <input type="text" value="8.00"/> Level 2 <input type="text" value="8.50"/> Level 3 <input type="text" value="9.00"/>	
State <input checked="" type="radio"/> Variable <input type="radio"/> Constant		No. of Levels 3		
Name	Units	Type	Lower Bound	Upper Bound
Organic Level	%	Continuous	<input type="text" value="20"/>	<input type="text" value="50"/>
State <input checked="" type="radio"/> Variable <input type="radio"/> Constant				
Name	Units	Type	Lower Bound	Upper Bound
Sonication Time	min	Continuous	<input type="text" value="0"/>	<input type="text" value="30"/>
State <input checked="" type="radio"/> Variable <input type="radio"/> Constant				
Name	Units	Type	Lower Bound	Upper Bound
Shaker Speed	rpm	Continuous	<input type="text" value="50"/>	<input type="text" value="250"/>
State <input checked="" type="radio"/> Variable <input type="radio"/> Constant				
Name	Units	Type	Lower Bound	Upper Bound
Shaker Time	min	Continuous	<input type="text" value="20"/>	<input type="text" value="120"/>
State <input checked="" type="radio"/> Variable <input type="radio"/> Constant				

Sample Preparation Experiment Dataflow



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

Run	Std	# of	Label	Sample	Level	Function	Method Set /	Label	Processing	Run	Data	Std	Column	Auto	Sample	Cloned
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1						Condition Column	Testfile per 001_001			8.75	0.00	0.00	No Change			
2						Condition Column	Testfile per 001_002			8.10	0.00	0.00	No Change			
3						Condition Column	Testfile per 001_003			3.00	0.00	7.00	No Change			
4	1	2.0	1	100.00	0.00	Inject Samples	Testfile per 001_004	Normal		10.00	0.00	1.00			1.00000	1.00000
5						Condition Column	Testfile per 001_005			8.10	0.00	0.00	No Change			
6						Condition Column	Testfile per 001_006			3.00	0.00	0.00	No Change			
7	2	2.0	1	100.00	0.00	Inject Samples	Testfile per 001_007	Normal		10.00	0.00	1.00			1.00000	1.00000
8						Condition Column	Testfile per 001_008			8.10	0.00	0.00	No Change			
9						Condition Column	Testfile per 001_009			3.00	0.00	0.00	No Change			
10	2	2.0	1	100.00	0.00	Inject Samples	Testfile per 001_010	Normal		10.00	0.00	1.00			1.00000	1.00000
11						Condition Column	Testfile per 001_011			8.75	0.00	0.00	No Change			
12						Condition Column	Testfile per 001_012			8.10	0.00	0.00	No Change			
13						Condition Column	Testfile per 001_013			3.00	0.00	0.00	No Change			
14	2	2.0	1	100.00	0.00	Inject Samples	Testfile per 001_014	Normal		10.00	0.00	1.00			1.00000	1.00000
15						Condition Column	Testfile per 001_015			8.75	0.00	0.00	No Change			
16						Condition Column	Testfile per 001_016			8.10	0.00	0.00	No Change			
17						Condition Column	Testfile per 001_017			3.00	0.00	0.00	No Change			
18	2	2.0	1	100.00	0.00	Inject Samples	Testfile per 001_018	Normal		10.00	0.00	1.00			1.00000	1.00000
19						Condition Column	Testfile per 001_019			8.10	0.00	0.00	No Change			
20						Condition Column	Testfile per 001_020			3.00	0.00	0.00	No Change			
21	2	2.0	1	100.00	0.00	Inject Samples	Testfile per 001_021	Normal		10.00	0.00	1.00			1.00000	1.00000
22						Condition Column	Testfile per 001_022			8.75	0.00	0.00	No Change			
23						Condition Column	Testfile per 001_023			8.10	0.00	0.00	No Change			
24						Condition Column	Testfile per 001_024			3.00	0.00	0.00	No Change			



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

Linear Effect

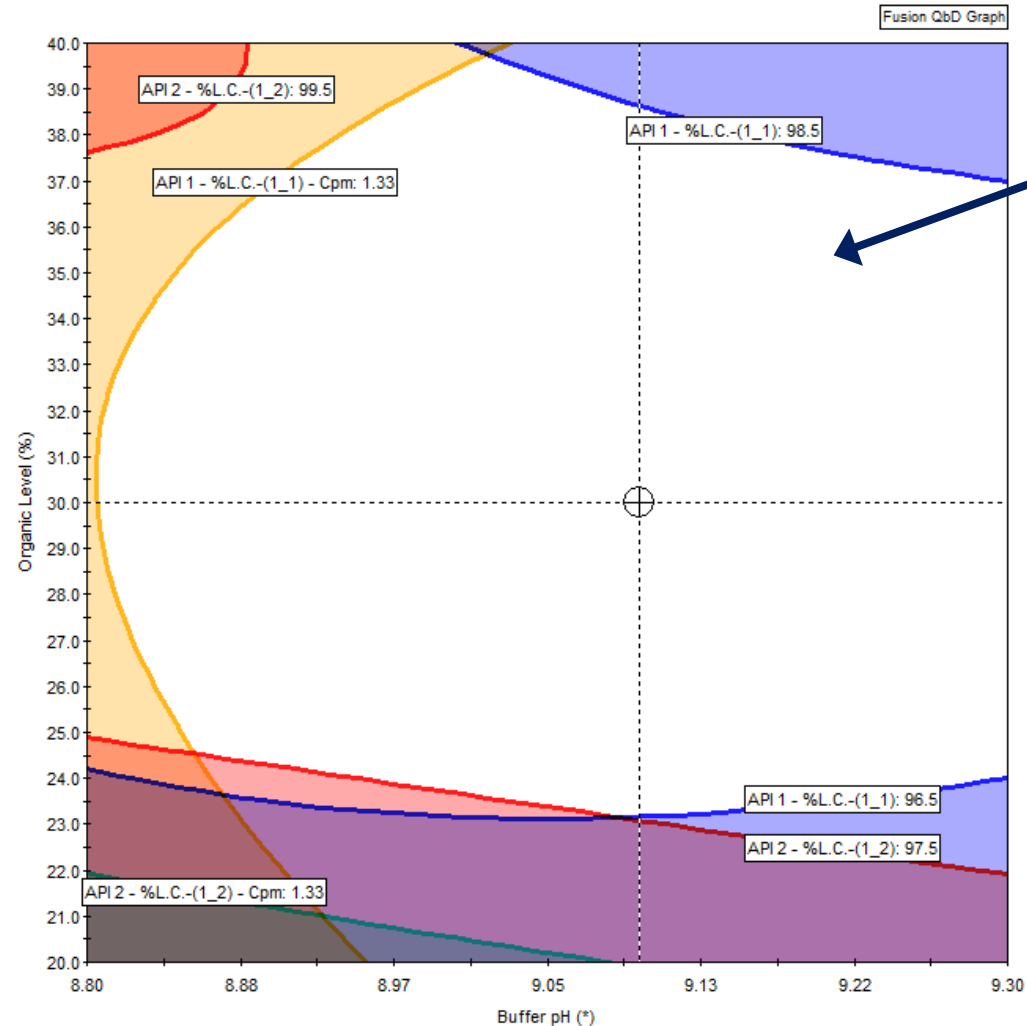
Curvature Effect

Interaction Effects

Complex Effect

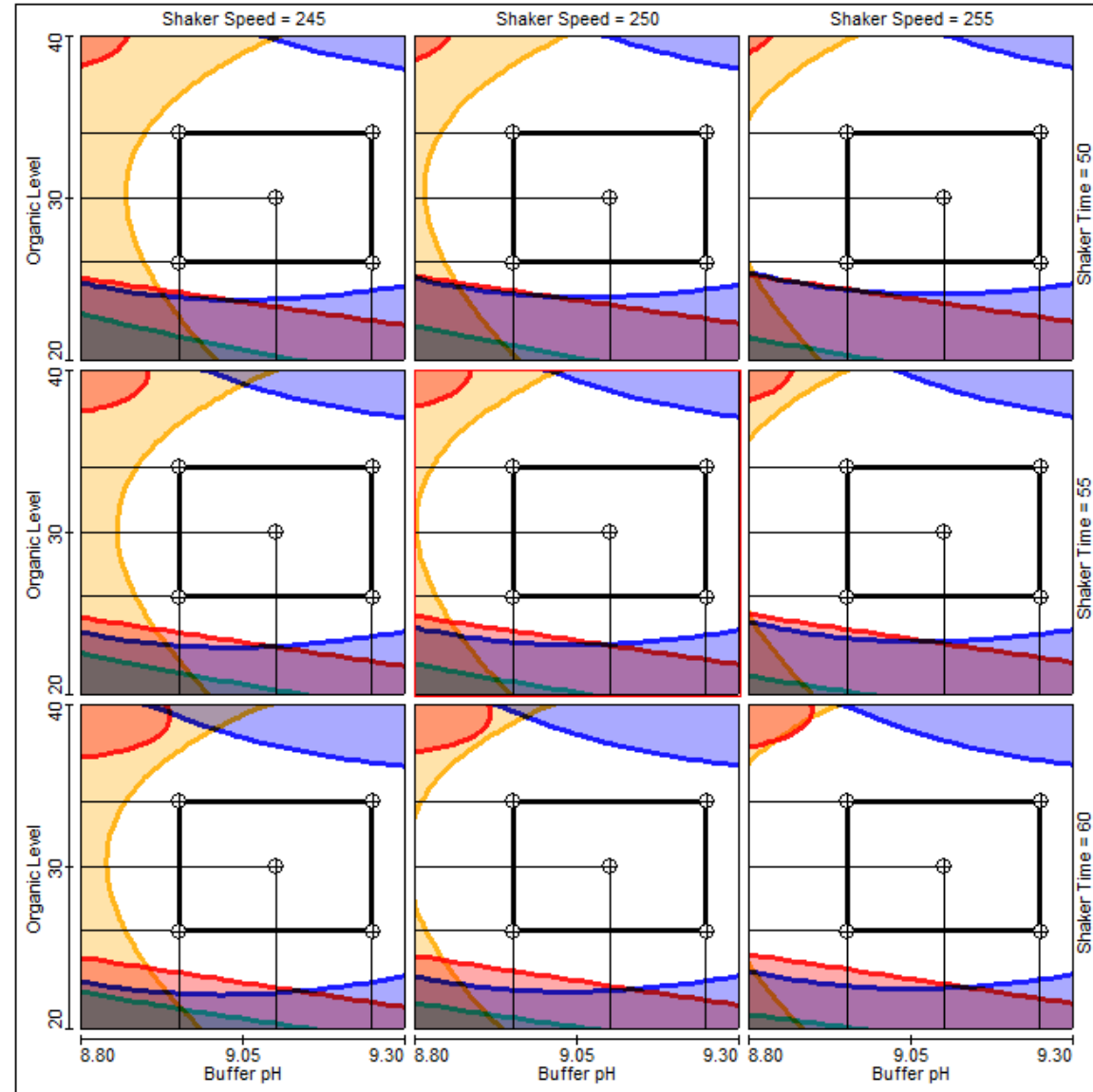
Multi-response Overlay Graph

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)

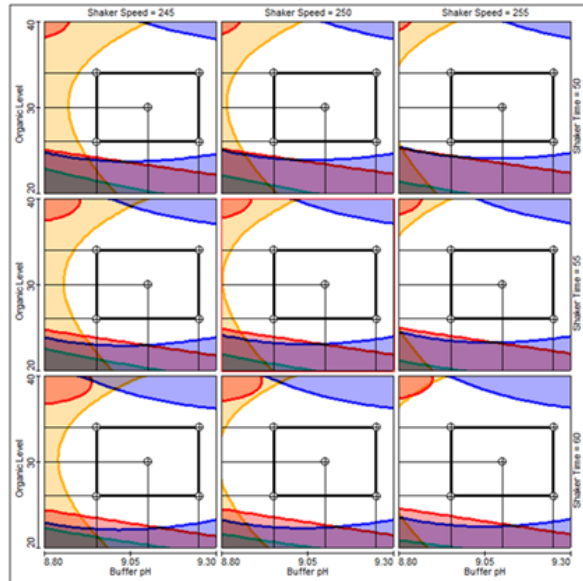
MODR Trellis Graph – 4 Study Factors



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



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

Report Output in Multiple Formats

- MS Excel



- MS Word

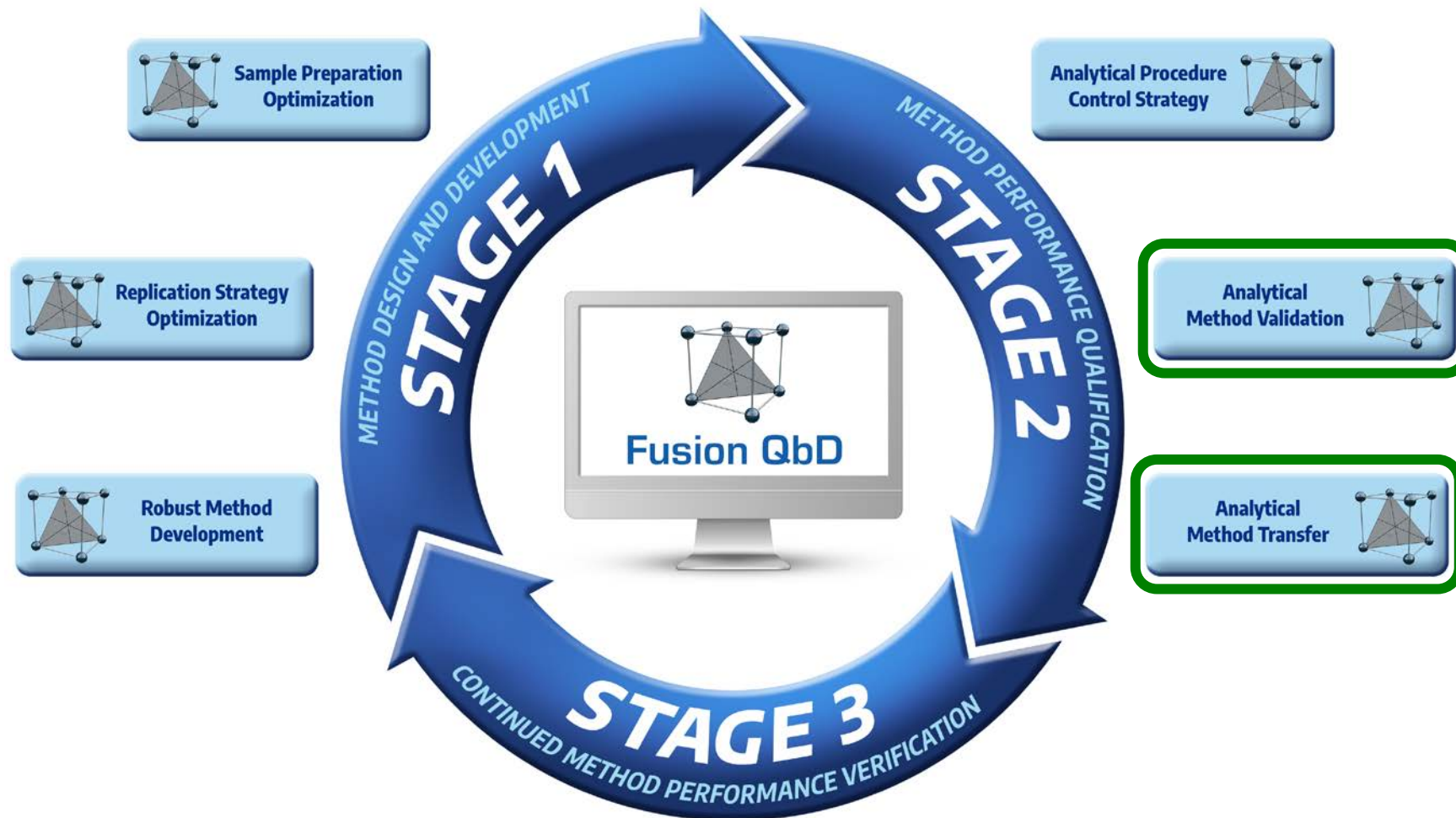


- PDF



- ...

Method Validation & Transfer



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

Method Validation - Small Molecule Data Analysis

Accuracy | Linearity | Repeatability

Select Response for Analysis
Amount

API

Perform Data Analysis

Response Treatment
 % Recovered (Relative) Difference from Mean (Absolute)

Compound-based Acceptance Criteria
 Tolerance / Prediction Interval

Interval Type
 Tolerance Prediction

Name	Value	Unit
Acceptance Limit <=	0.10	mg
Desired Probability	95.00	%
Tolerance Alpha	5.00	%

Level-based Acceptance Criteria

Level	Accuracy % Bias <=
1.000	15.00
2.000	10.00

Level	Individual Results Spec. Lower Limit	Individual Results Spec. Upper Limit
1.000	0.900	1.100
2.000	1.800	2.200

The settings are valid.

Back Finish Cancel

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

Interval Test (USP < 1210 >)

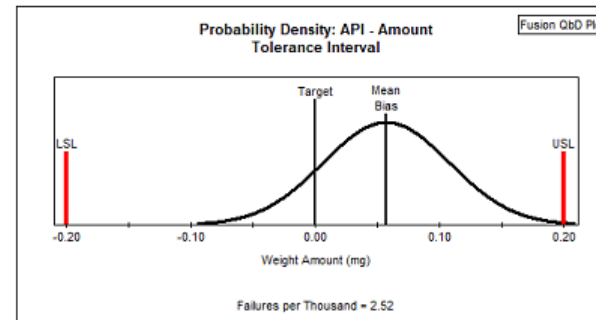
Tolerance Interval

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-Value
1	1.a	0.018	0.027	0.7368	0.5086
	1.b	0.072			
	1.c	0.051			
2	2.a	0.111	0.038	1.7550	0.2334
	2.b	0.109			
	2.c	0.044			
3	3.a	0.120	0.012	0.1267	0.8827
	3.b	0.097			
	3.c	0.102			
4	4.a	0.102	0.024	0.5602	0.5920
	4.b	0.092			
	4.c	0.056			
5	5.a	-0.074	0.043	2.5143	0.1422
	5.b	-0.047			
	5.c	0.010			



[\[Edit Graph\]](#)

Analytical Method Transfer

Transferring Lab



Fusion QbD
Sequence
Execution

Chromatography
Data Software

ALR
Design

Fusion QbD
Sequence
Execution

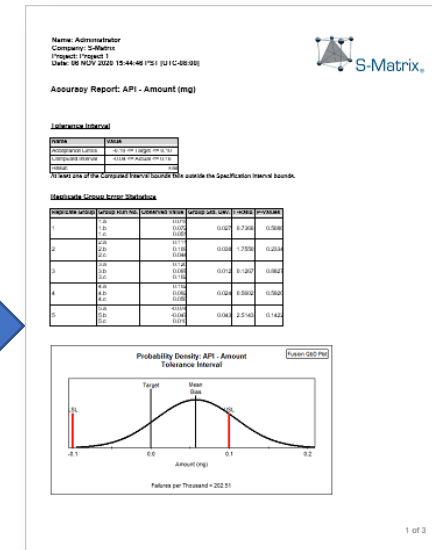
Chromatogram
Results Data



Receiving Lab



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

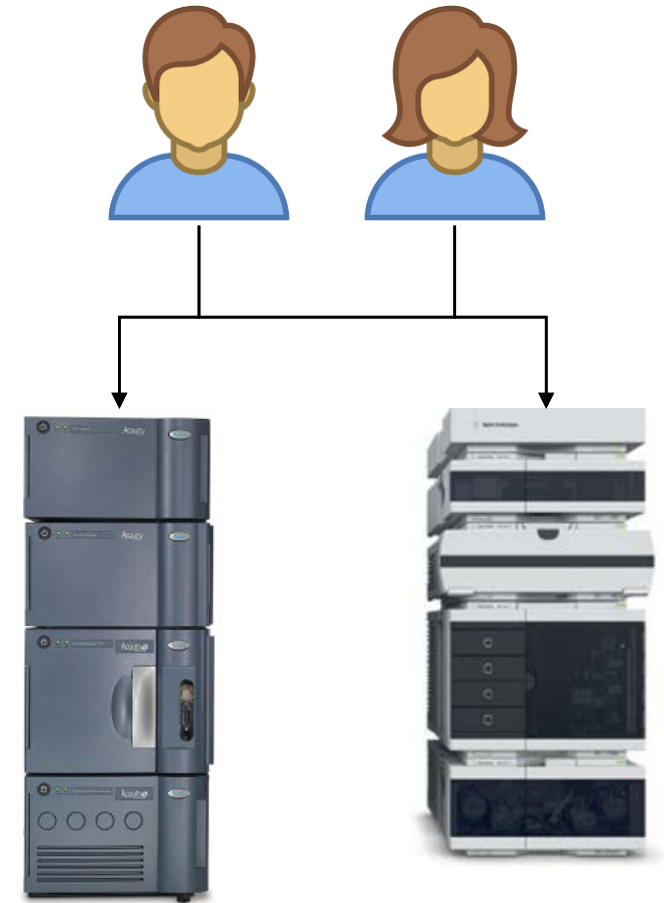
Analytical Method Transfer

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

