

Fusion AE – LC Method Validation Module

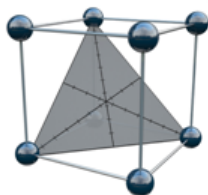
Fusion AE™

Automated **E**xperimentation Software System

WINDOWS®



Version 9.0.1 SR1
Build 108



S-Matrix®

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ICH – Q2A

The objective of Method Validation is to provide documented evidence and a high degree of assurance that an analytical method employed for a specific test is suitable for its intended use.

Method Validation is a regulatory requirement as much as a scientific necessity. A well executed method validation effort:

- provides scientific credence for the method.
(statistical confidence in the data)
- defines the limit of acceptable performance of the method.
(Low and high limits of identification and quantitation)

PhRMA's Analytical Technical Group

Recommends a phased approach to analytical method validation in which **early phase validation efforts** are done upstream on a reduced set of validation elements appropriate to the stage of method development.

Early Phase Validation – experiments are structured for internal consumption to support and guide method development.

Final Phase Validation – experiments are structured with the rigor and regulatory compliance overlay required of results that may be exported outside the lab.

Early Phase Specific Experiments (Performance Characterization)

- Specificity
- Filter Validation

Early Phase and Final Phase (FDA / ICH Submittal Quality)

- Accuracy
- Linearity and Range
- LOQ, LOD
- Repeatability* (intra-assay precision)
- Accuracy/Linearity and Range/Repeatability – Combined Design
(**ICH-Q2A** – *Accuracy, Linearity, and Repeatability can be done together as a single combined experiment*). Sample Solution Stability (stability for a given time period under prescribed conditions)
- Intermediate Precision and Reproducibility (USP Ruggedness)
- Robustness

Method Validation Example – Experiment Type Selection


Create New Work File

Project
Name:

Audit Logging Enabled
(Cannot be changed once the file is created)

Instrument

Instrument Type: HPLC
Data System: ChemStation
Pump Type: Quaternary



Sample Compound Type
 Small Molecule Large Molecule

Experiment Phase

Experiment Type

- Analytical Capability
- Specificity
- Accuracy
- Linearity and Range
- Repeatability
- Accuracy/Linearity and Range/Repeatability**
- Robustness
- Intermediate Precision and Reproducibility

OK Cancel ?

Fusion LC Method Validation – Automation Workflow

1. Complete the Fusion AE template with the relevant information
2. Fusion AE creates a Validation Experimental Design
3. Fusion AE exports the design to the CDS
 - The CDS runs the validation experiment sequence
4. Fusion AE imports and analyzes the CDS results
5. Fusion AE creates final reports and graphs

(See next slide)

Linearity Example – Experiment Setup Template

Experiment Setup

Sampling Plan

Include LOQ / LOD

- Limit of Quantitation
- Limit of Detection

Assay Type Potency (Drug Content)

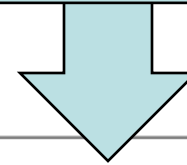
Global Compound Settings

No. of Compounds 2

No. of Levels per Compound 5

100% Std. Level Level 3

Define Acceptance Criteria for each Key Result for each Compound.



Compound Name	Units	Level Settings	Validation - Acceptance Criteria
Compound 1	%	Level 1 <input type="text" value="80"/> Level 2 <input type="text" value="90"/> Level 3 <input type="text" value="100"/> Level 4 <input type="text" value="110"/> Level 5 <input type="text" value="120"/>	<input type="checkbox"/> Accuracy (% Bias <) <input type="text" value=""/> <input type="checkbox"/> Linearity (% Bias <) <input type="text" value=""/> <input type="checkbox"/> Repeatability (% RSD <=) <input type="text" value=""/> <input checked="" type="checkbox"/> Linearity (Regression r >=) <input type="text" value="0.999"/>
Compound 2	%	Level 1 <input type="text" value="80"/> Level 2 <input type="text" value="90"/> Level 3 <input type="text" value="100"/> Level 4 <input type="text" value="110"/> Level 5 <input type="text" value="120"/>	<input type="checkbox"/> Accuracy (% Bias <) <input type="text" value=""/> <input type="checkbox"/> Linearity (% Bias <) <input type="text" value=""/> <input type="checkbox"/> Repeatability (% RSD <=) <input type="text" value=""/> <input checked="" type="checkbox"/> Linearity (Regression r >=) <input type="text" value="0.999"/>

Linearity Example – Standards Setup Options

Standards Setup

Standards Strategy
Multi-level Bracketing - Overlap

Multi-level Bracketing - Overlap Settings

No. of Calibration Standard Levels per Bracket 5

No. of Repeat Injections per Level 1

No. of Check Standard Levels per Bracket 1

No. of Unknown Injections within Brackets 10

Experiment Design

	Run No.	API
1	CAL - L1.1.a	---
2	CAL - L2.1.a	---
3	CAL - L3.1.a	---
4	CAL - L4.1.a	---
5	CAL - L5.1.a	---
6	CHK - L1.1.a	5.000
7	1.a	1.000
8	1.b	1.000
9	1.c	1.000

Flexible setup of the required Standards Strategy.

Validation Status: Your settings are valid.

Clear Reset Next >> Cancel ?

ICH Q2B. III. LINEARITY (2)

... If there is a linear relationship, test results should be evaluated by appropriate statistical methods, for example, by calculation of a regression line by the method of least squares... The correlation coefficient, y-intercept, slope of the regression line, and residual sum of squares should be submitted. A plot of the data should be included...

Calculation of a regression line by the method of least squares:

- correlation coefficient
- y-intercept
- slope of the regression line
- residual sum of squares
- plot of the data...

Linearity Example – Fusion AE Output Reports

Name: Administrator
 Company: S-Matrix Corporation
 Project: Project 1
 Date: October 13, 2012 8:11:17 PM PDT [GMT-07:00]



Linearity and Range Report: API - Amount (mg)

Linearity and Range Data Table

Run No.	Target API (mg)	Actual Amount (mg)	API-Amount
1a	1.000	1.009	1.001
1b	1.000	1.01	1.002
1c	1.000	1.012	1.103
2a	2.000	1.992	2.106
2b	2.000	1.99	2.099
2c	2.000	2.004	2.049
3a	4.000	3.999	4.116
3b	4.000	4	4.097
3c	4.000	3.997	4.099
4a	6.000	6.002	6.107
4b	6.000	6.002	6.061
4c	6.000	6.009	6.065
5a	9.000	9.004	9.09
5b	9.000	9.009	9.099
5c	9.000	8.997	9.007

General Regression Statistics Table

Regression Statistic Name	Statistic Value	Pass / Fail
r	0.9997	Pass
R Square	0.9993	---
Adj. R Square	0.9989	---
Residual SSE	0.00259	---
Standard Error (s)	0.05092	---
±2% C.I.	±1.1001	---
Intercept Bias	2.49	---
Observations	12	---

Acceptance Criterion - Regression r: > 0.9990

Regression ANOVA Statistics Table

Source of Variation	Sum of Squares	Degree of Freedom	Mean Square	F-Ratio	P-Value
Regression	49.82199	1	49.82199	19,212.2599	< 0.0001
Residual	0.00259	10	0.000259	---	---
Total	49.82458	11	---	---	---

Regression Coefficients Table

Variable Name	Coefficient Value	Coefficient Standard Error	Coefficient t-Statistic	P-Value	Lower 95% Confidence Limit	Upper 95% Confidence Limit
Intercept	0.12532	0.02673	4.3420	0.0008	0.06223	0.18739
API	0.99921	0.00709	140.9119	< 0.0001	0.98739	0.99951

Natural Variable Model

$$API - Amount (pred) = 0.12532 + (0.99921 \times API)$$

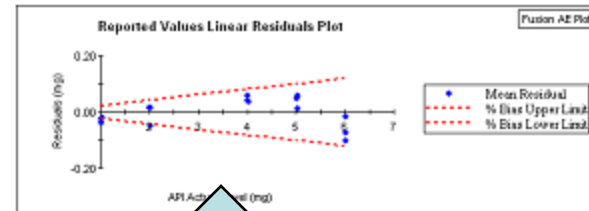
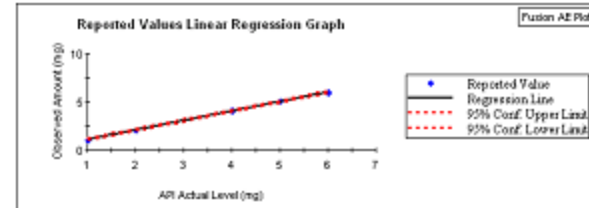
Range

$$1.000 \leq API \leq 9.000$$

Residuals Table

Actual API - Amount (mg)	Predicted API - Amount	Observed API - Amount	Residual	% Bias of Residual	% Bias Pass / Fail
1.002	1.11148	1.001	-0.00048	-2.01	Fail
1.01	1.11497	1.002	-0.00497	-2.92	Fail
1.012	1.12029	1.103	-0.01727	-1.71	Pass
1.992	2.04492	2.106	0.01917	0.99	Pass
1.99	2.04191	2.099	0.01709	0.89	Pass
2.004	2.04999	2.049	-0.00199	-0.39	Fail
3.999	4.05421	4.116	0.06179	1.22	Pass
4	4.05817	4.097	0.03889	0.97	Pass
3.997	4.05222	4.099	0.04379	1.10	Pass
4.002	4.04499	4.107	0.06200	1.21	Pass
4.992	5.03222	5.091	0.05899	1.01	Pass
5.999	5.95004	6.092	0.01479	0.29	Pass
6.004	6.03623	6.065	-0.00623	-1.04	Pass
6.002	6.02752	6.099	-0.07122	-1.19	Pass
8.997	8.02162	9.007	-0.01499	-0.21	Pass

Acceptance Criterion: % Bias < 2% for each concentration tested.



Fusion AE instantly creates formal reports with all required tables and graphs.



Method Validation – Linearity Example

ICH Q2B:

For chromatographic procedures, representative chromatograms should be used to demonstrate specificity, and individual components should be appropriately labeled.

If DL is determined based on visual evaluation or based on signal-to-noise ratio, the presentation of the relevant chromatograms is considered acceptable for justification.

Image Handler

Browse... Delete

Imported Images

Linearity Chromatogram - 100% Label Claim

Report Assignments

- All Reports and Graphs
 - Experiment Design
 - Instrument Report
 - Experiment Design
 - Experiment Setup
 - Data Analysis
 - API - Amount
 - Accuracy Report
 - Linearity and Range Report
 - Repeatability Report
 - Limit of Detection Report
 - Limit of Quantitation Report

Image Title: Linearity Chromatogram - 100% Label Clai

Run Label: None Selected

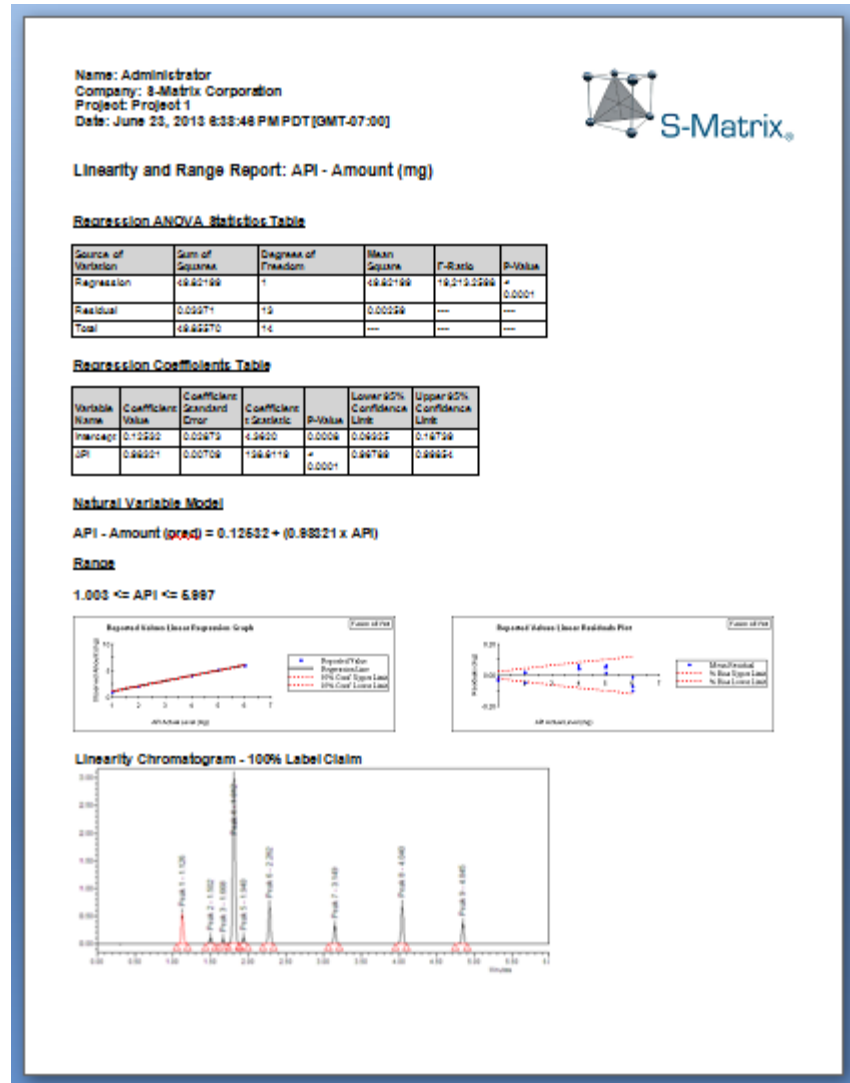
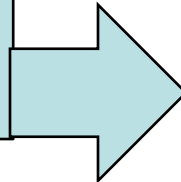
Chromatogram Plot: Peak 1 - 1.126, Peak 2 - 1.502, Peak 3 - 1.625, Peak 4 - 1.980, Peak 5 - 2.262, Peak 6 - 3.149, Peak 7 - 4.040, Peak 8 - 4.845

OK Apply Cancel

Reports can be augmented with images of relevant chromatograms.

Method Validation – Linearity Example

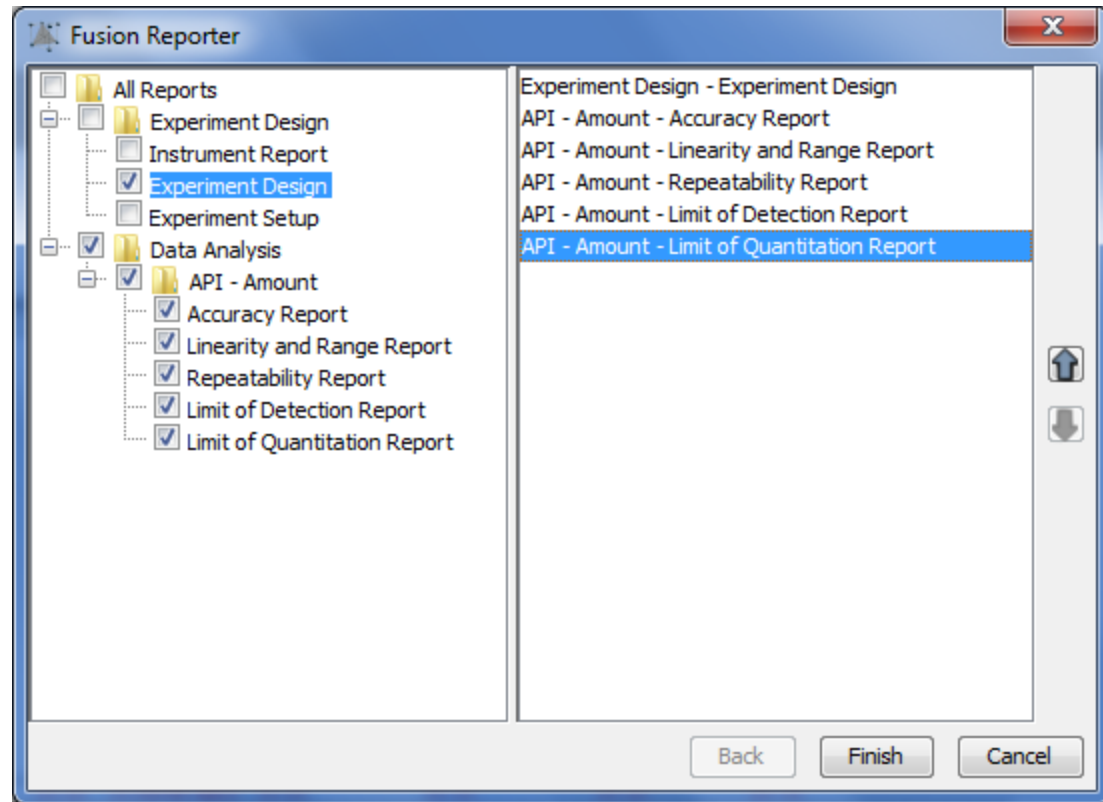
Reports can be augmented with images of relevant chromatograms.



Linearity Example – Fusion AE Compiled Report Generator

Reports meet
all output format
requirements:

.TXT
.RTF
.DOC
.PDF
.HTML
.XML



ICH Q2A / Q2B:

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, *but deliberate* variations in method parameters and provides an indication of its reliability during normal usage.

In the case of liquid chromatography, examples of typical variations are:

- Influence of variations of pH in a mobile phase
- Influence of variations in mobile phase composition
- Different columns (different lots and/or suppliers)
- Temperature
- Flow rate

Note – the text “*but deliberate*” refers to the deliberate perturbation of critical instrument parameters about their method setpoints done as part of a Validation-Robustness experiment.

Mean Performance Versus Robustness

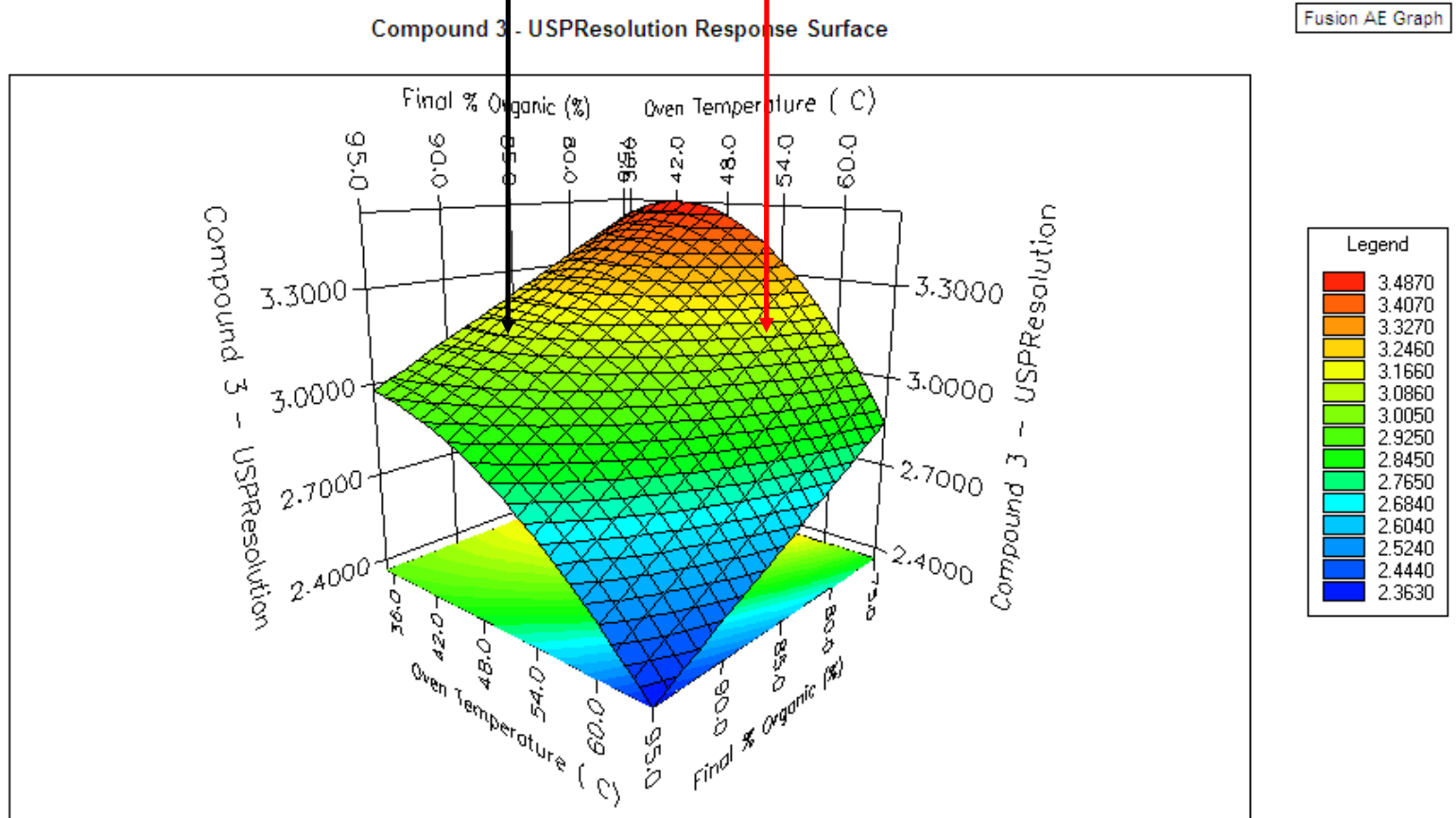
Methods A and B –

Identical Mean Performance –

Good mean performance \neq
good robustness

Method A – Good Robustness

Method B – Poor Robustness



I. Potential Sources of Risk in Current Practice

1. Experimental ranges – a “Signal/Noise” source of risk
2. Experimental design selection – an information content source of risk
3. Performance requirements – a performance variation source of risk

II. QbD-aligned strategy for validating method robustness

1. Define valid study ranges for critical instrument parameters (CPPs)
2. Select the right experimental design
3. Specify risk-based method performance requirements (CQAs)

I. Potential Sources of Risk in Current Practice

1. Experimental ranges – a “Signal/Noise” source of risk

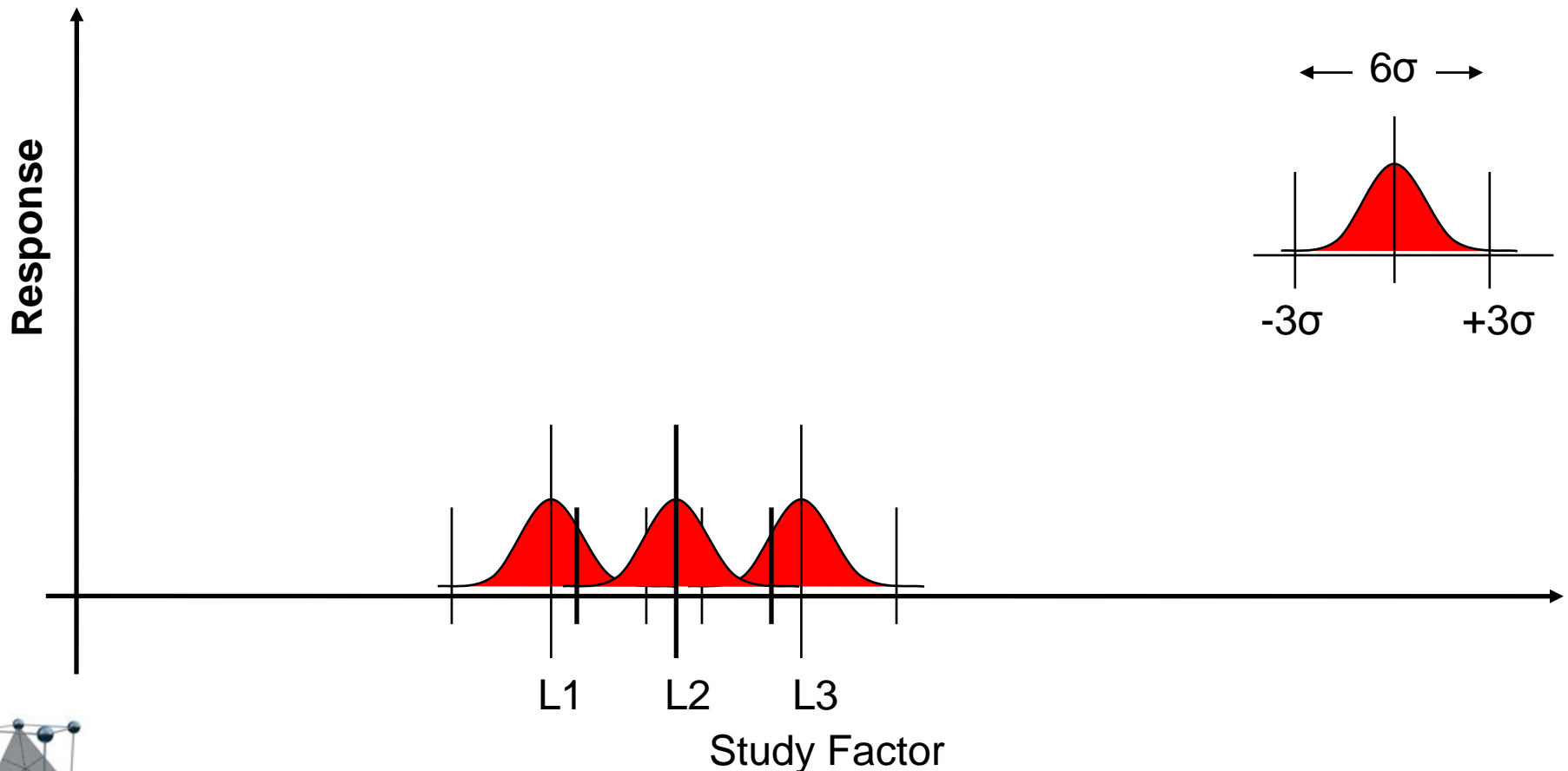
II. QbD-aligned strategy for validating method robustness

1. Define valid study ranges for critical instrument parameters (CPPs)

Small Range – Poor Effects Estimation

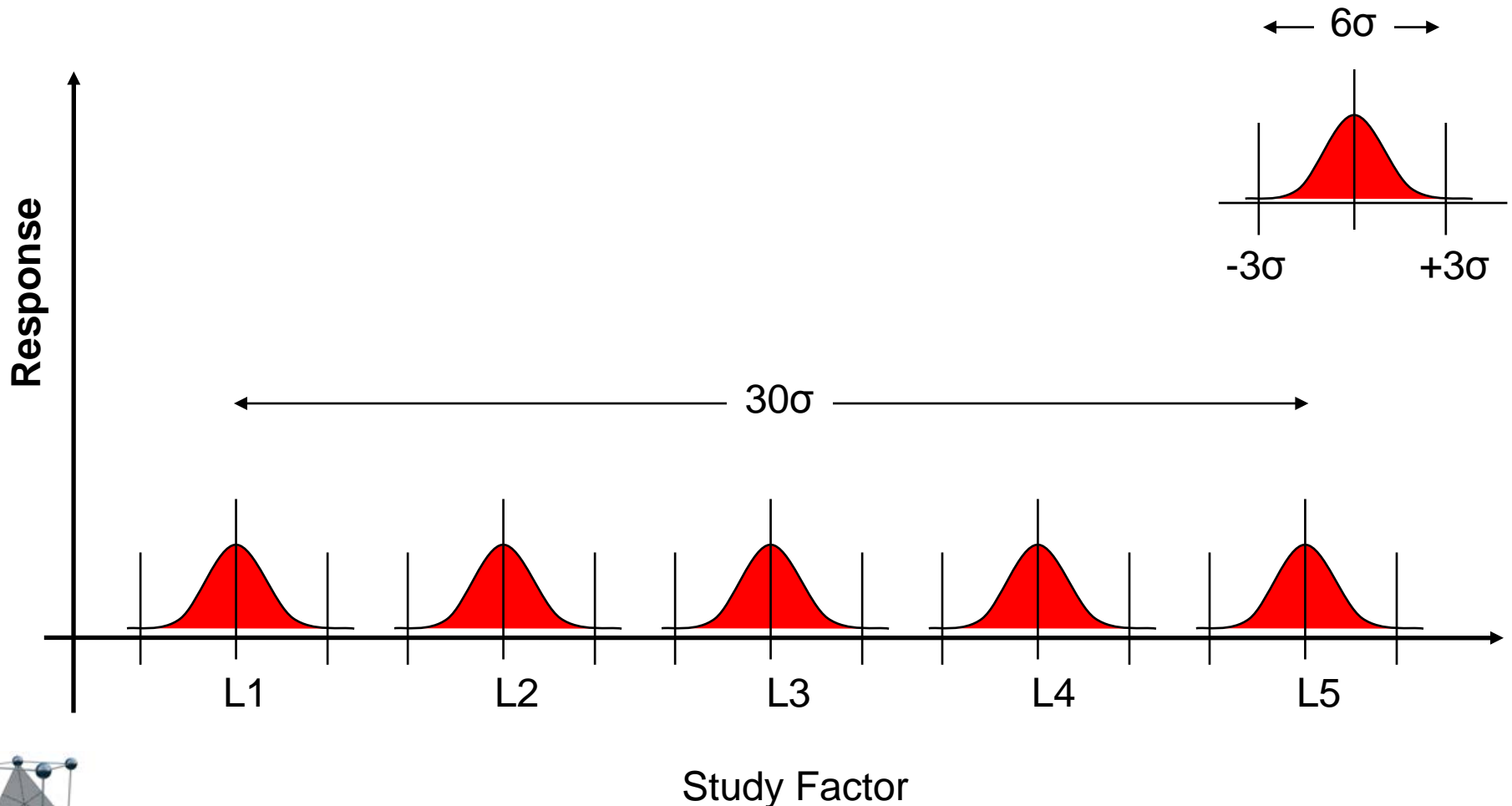
Traditional Range is Within Setpoint Error Range. The most likely result is that the study factor effects will be **UNDERESTIMATED**.

The Result – methods which are NOT robust will pass the robustness test.



Best Practice – Large Ranges = High Signal/Noise

General Guideline: Minimum Study Range for 5 Level Designs Should be 30σ
(5 x 6σ interval width)



I. Potential Sources of Risk in Current Practice

2. Experimental design selection – an information content source of risk

II. QbD-aligned strategy for validating method robustness

2. Select the right experimental design

II. QbD-aligned strategy for validating method robustness

- Fusion AE automatically selects the right experimental design for the included instrument parameters
- Fusion AE design is efficient and automated

Four variable Robustness Study – Efficiency Comparison

Full Factorial 3-Level Design = 81 Runs

Fusion AE Optimal* Design = 22 Runs

- * – Optimal designs can support studies with non-numeric factors (e.g. different columns) and factors that are not completely independent (e.g. mobile phase blends).

I. Potential Sources of Risk in Current Practice

3. Performance requirements – a performance variation source of risk

II. QbD-aligned strategy for validating method robustness

3. Specify risk-based method performance requirements (CQAs)

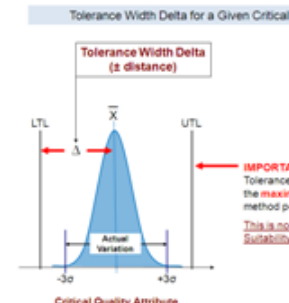
Method Robustness

Fusion AE lets you specify the Method's Required Performance Limits for Robustness Testing

Response Settings for Robustness

Maximum Allowable Difference from Mean:

The Maximum Allowable Difference limit values define the maximum differences from the mean for a given critical quality attribute (response) beyond which the response value is unacceptable. For the response to be considered robust in terms of the parameters evaluated, the variation in the response measurements obtained in normal use must be encompassed by the Maximum Allowable Difference limit values.



Tolerance Width Delta for a Given Critical Quality Attribute

IMPORTANT: Tolerance Limit Delta values define the maximum acceptable limits on method performance variation. This is normally your System Suitability Specification.

Enabled	Response	Maximum Allowable Difference from Mean (± Value)
<input checked="" type="checkbox"/>	API - USPResolution	0.5
<input checked="" type="checkbox"/>	API - Peak Retention Time	0.1

Select All Select None

<< Back Next >> Finish Cancel

Demonstration Example – Experiment Type Selection


Create New Work File

Project
Name:

Audit Logging Enabled
(Cannot be changed once the file is created)

Instrument

Instrument Type: HPLC
Data System: ChemStation
Pump Type: Quaternary



Sample Compound Type
 Small Molecule Large Molecule

Experiment Phase

Experiment Type

- Analytical Capability
- Specificity
- Accuracy
- Linearity and Range
- Repeatability
- Accuracy/Linearity and Range/Repeatability
- Robustness**
- Intermediate Precision and Reproducibility

Chromatography Type

- Reversed Phase (RPC)**
- Normal Phase (NPC)
- Chiral - RPC
- Chiral - NPC
- HILIC
- Ion Exchange
- Size Exclusion - Gel Permeation

OK Cancel ?

Comparative Study Ranges Around Method Setpoints

Factor	Method Nominal	Traditional Range*	QbD-aligned Range
Pump Flow Rate (mL/min)	1.0	± 0.025	± 0.125
% Strong Solvent (%)	80.0	± 2.0	± 5.0
Temperature ($^{\circ}\text{C}$)	35.0	± 2.0	± 10.0
pH (*)	5.5	± 0.15	± 0.5

* – worst-case scenario considered.

Experiment Setup Template

Experiment Setup

Sampling Plan

Method Type **Gradient**

Available Variables

Sample Concentration
Buffer Strength
Buffer Type
Additive
Column Type



Included Variables

Pump Flow Rate
Gradient Slope
Injection Volume
Oven Temperature
Wavelength



Unavailable Variables

Gradient Curve

Name	Units	Type	Lower Bound	Upper Bound
Pump Flow Rate	mL/min	Continuous	0.475	0.525

State:
 Variable
 Constant

Name	Units	Type	Amount
Injection Volume	µL	Discrete Numeric	2.0

State:
 Variable
 Constant

Solvent Settings

No. of Strong Solvents: **1**

No. of Weak Solvents: **1**

OK to Blend Strong Solvents

OK to Blend Weak Solvents

Mobile Phase Precision: **0.00**

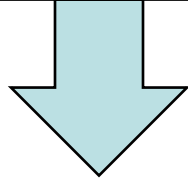
Mobile Phase Name	Solvent Type	Reservoir
Organic	Strong (Organic)	B1
Aqueous Buffer	Weak (Aqueous)	A1

Available Reservoirs

<input checked="" type="checkbox"/> A2	<input checked="" type="checkbox"/> A1
<input checked="" type="checkbox"/> B1	<input checked="" type="checkbox"/> A1-1 <input checked="" type="checkbox"/> A1-2 <input checked="" type="checkbox"/> A1-3
<input checked="" type="checkbox"/> B2	<input checked="" type="checkbox"/> A1-4 <input checked="" type="checkbox"/> A1-5 <input checked="" type="checkbox"/> A1-6

QbD-aligned Study Ranges

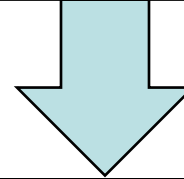
Fusion AE Optimization Design Formatted for Export to the CDS



	Run No.	Pump Flow Rate	Initial % Organic	Oven Temperature	pH
1	Condition Column - 1	0.5	65	40	6.5
2	1	0.375	65	40	6.5
3	2	0.625	55	40	6.5
4	3	0.375	65	40	6.5
5	4	0.625	55	40	6.5
6	Condition Column - 2	0.5	65	40	7
7	5	0.625	65	40	7
8	6	0.375	55	40	7
9	Condition Column - 3	0.5	55	40	7.5
10	7	0.5	55	40	7.5
11	8	0.375	65	40	7.5
12	9	0.625	60	40	7.5
13	Condition Column - 4	0.5	57.5	45	6.5
14	10	0.438	57.5	45	6.5
15	Condition Column - 5	0.5	57.5	45	7.5
16	11	0.438	57.5	45	7.5
17	Condition Column - 6	0.5	60	50	6.5
18	12	0.625	60	50	6.5
19	Condition Column - 7	0.5	60	50	7
20	13	0.5	60	50	7
21	14	0.5	60	50	7
22	Condition Column - 8	0.5	65	50	7.5
23	15	0.5	65	50	7.5
24	16	0.625	55	50	7.5
25	Condition Column - 9	0.5	57.5	55	6.5
26	17	0.563	57.5	55	6.5
27	18	0.438	62.5	55	6.5
28	19	0.375	55	60	6.5
29	20	0.625	65	60	6.5
30	Condition Column - 10	0.5	55	60	7
31	21	0.625	55	60	7
32	22	0.375	65	60	7
33	23	0.375	65	60	7
34	Condition Column - 11	0.5	55	60	7.5
35	24	0.375	55	60	7.5
36	25	0.5	60	60	7.5
37	26	0.625	65	60	7.5
38	Condition Column - 12	0.5	65	60	7.5

QbD-aligned Study Ranges

Peak Results Data Automatically Imported From the CDS



	Run No.	Pump Flow Rate	Initial % Organic	Oven Temperature	pH	Impurity B - USP Resolution
1	Condition Column - 1	0.5	65	40	6.5	
2	1	0.375	65	40	6.5	8.81
3	2	0.625	55	40	6.5	0.66
4	3	0.375	65	40	6.5	8.81
5	4	0.625	55	40	6.5	0.66
6	Condition Column - 2	0.5	65	40	7	
7	5	0.625	65	40	7	11.81
8	6	0.375	55	40	7	3.78
9	Condition Column - 3	0.5	55	40	7.5	
10	7	0.5	55	40	7.5	0.62
11	8	0.375	65	40	7.5	8.77
12	9	0.625	60	40	7.5	4.69
13	Condition Column - 4	0.5	57.5	45	6.5	
14	10	0.438	57.5	45	6.5	3.50
15	Condition Column - 5	0.5	57.5	45	7.5	
16	11	0.438	57.5	45	7.5	3.53
17	Condition Column - 6	0.5	60	50	6.5	
18	12	0.625	60	50	6.5	4.65
19	Condition Column - 7	0.5	60	50	7	
20	13	0.5	60	50	7	7.76
21	14	0.5	60	50	7	7.71
22	Condition Column - 8	0.5	65	50	7.5	
23	15	0.5	65	50	7.5	5.18
24	16	0.625	55	50	7.5	4.05
25	Condition Column - 9	0.5	57.5	55	6.5	
26	17	0.563	57.5	55	6.5	5.18
27	18	0.438	62.5	55	6.5	3.99
28	19	0.375	55	60	6.5	7.47
29	20	0.625	65	60	6.5	1.45
30	Condition Column - 10	0.5	55	60	7	
31	21	0.625	55	60	7	10.57
32	22	0.375	65	60	7	4.59
33	23	0.375	65	60	7	4.62
34	Condition Column - 11	0.5	55	60	7.5	
35	24	0.375	55	60	7.5	7.40
36	25	0.5	60	60	7.5	4.49
37	26	0.625	65	60	7.5	1.42
38	Condition Column - 12	0.5	65	60	7.5	

QbD-aligned Study Ranges

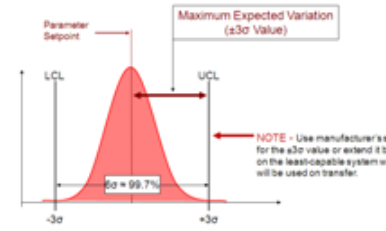
Robustness Assessment Limits – **Worst-case Scenario**

Experiment Variable Maximum Expected Variation

Maximum Expected Variation:

The ± 3 sigma value defines the "total" variation in the parameter (experiment variable) around its defined setpoint that is expected to occur on transfer and normal use due to statistically random error.

Expected $\pm 3\sigma$ Variation Interval Around Setpoint for Each Variable



NOTE - Use manufacturer's specs for the $\pm 3\sigma$ value or extend it based on the least-capable system which will be used on transfer.

Experiment Variable	Units	Maximum Expected Variation (± 3 Sigma Value)
Pump Flow Rate	mL/min	0.1
Final % Strong Solvent	%	2
Oven Temperature	°C	2

<< Back Next >> Finish Cancel ?

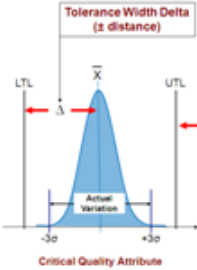
QbD-aligned Study Ranges

Response Performance Limits Required for Robust Method

Response Settings for Robustness

Maximum Allowable Difference from Mean:

The Maximum Allowable Difference limit values define the maximum differences from the mean for a given critical quality attribute (response) beyond which the response value is unacceptable. For the response to be considered robust in terms of the parameters evaluated, the variation in the response measurements obtained in normal use must be encompassed by the Maximum Allowable Difference limit values.



Tolerance Width Delta for a Given Critical Quality Attribute

IMPORTANT: Tolerance Limit Delta values define the maximum acceptable limits on method performance variation. This is normally your System Suitability Specification.

Enabled	Response	Maximum Allowable Difference from Mean (\pm Value)
<input checked="" type="checkbox"/>	API - USPResolution	0.5
<input checked="" type="checkbox"/>	API - Peak Retention Time	0.1

Select All Select None

<< Back Next >> Finish Cancel ?

Demonstration Study – QbD-aligned Study Ranges

Fusion AE Statistical Significance Testing – Model Coefficients

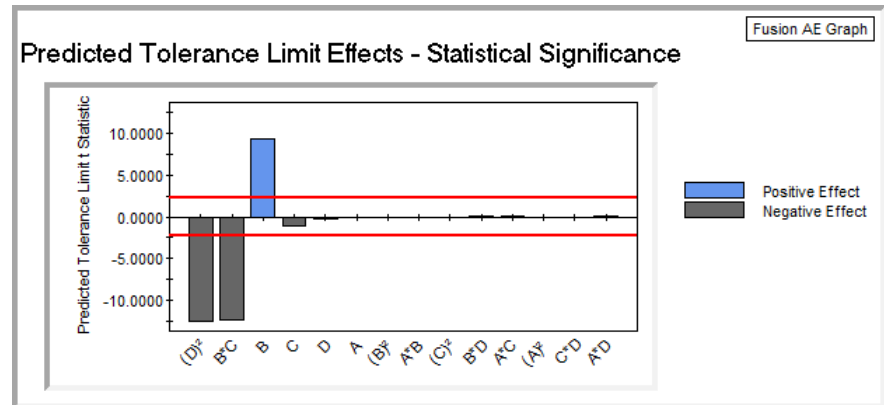
Robustness Report: Impurity B - USPResolution (*)

Coded Variable Name Key

Coded Variable Name	Actual Variable Name
A	Pump Flow Rate
B	Initial % Organic
C	Oven Temperature
D	pH

Variable Effects Table - Statistical Significance

Model Term Name	Robustness Testing Level (Coded)	Coefficient Value	Predicted Tolerance Limit Effect	Predicted Tolerance Limit Standard Error	Predicted Tolerance Limit t statistic	Pass/Fail
B*C	0.0800	-3.5200	-0.2816	0.0226	-12.4664	Fail
(D) ²	0.0900	-3.1080	-0.2797	0.0224	-12.5133	Fail
B	0.4000	0.5356	0.2142	0.0228	9.3845	Fail
C	0.2000	-0.1188	-0.0238	0.0227	-1.0479	Pass
D	0.3000	-0.0137	-0.0041	0.0224	-0.1837	Pass
A	0.2000	-0.0169	-0.0034	0.0228	-0.1484	Pass
(B) ²	0.1600	-0.0199	-0.0032	0.0228	-0.1394	Pass
A*B	0.0800	-0.0193	-0.0015	0.0229	-0.0676	Pass
(C) ²	0.0400	-0.0336	-0.0013	0.0227	-0.0593	Pass
B*D	0.1200	0.0068	0.0008	0.0224	0.0365	Pass
A*C	0.0400	0.0070	0.0003	0.0227	0.0123	Pass
(A) ²	0.0400	-0.0054	-0.0002	0.0228	-0.0095	Pass
C*D	0.0600	-0.0004	<±0.0001	0.0223	-0.0011	Pass
A*D	0.0600	0.0001	<±0.0001	0.0223	0.0003	Pass



Maximum Allowable Value: |Predicted Tolerance Limit t statistic| < 2.2010 for each variable studied.

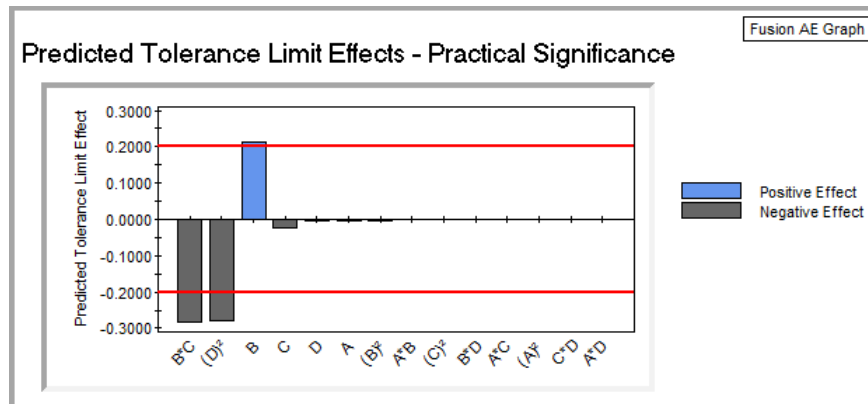


Demonstration Study – QbD-aligned Study Ranges

Fusion AE Practical Significance Testing – Effects Magnitude

Variable Effects Table - Practical Significance

Model Term Name	Robustness Testing Level (Coded)	Coefficient Value	Predicted Tolerance Limit Effect	Pass/Fail
B*C	0.0800	-3.5200	-0.2816	Fail
(D) ²	0.0900	-3.1080	-0.2797	Fail
B	0.4000	0.5356	0.2142	Fail
C	0.2000	-0.1188	-0.0238	Pass
D	0.3000	-0.0137	-0.0041	Pass
A	0.2000	-0.0169	-0.0034	Pass
(B) ²	0.1600	-0.0199	-0.0032	Pass
A*B	0.0800	-0.0193	-0.0015	Pass
(C) ²	0.0400	-0.0336	-0.0013	Pass
B*D	0.1200	0.0068	0.0008	Pass
A*C	0.0400	0.0070	0.0003	Pass
(A) ²	0.0400	-0.0054	-0.0002	Pass
C*D	0.0600	-0.0004	<±0.0001	Pass
A*D	0.0600	0.0001	<±0.0001	Pass



Maximum Allowable Difference from Mean: |Predicted Tolerance Limit Effect| < 0.2 for each variable studied.

I. Potential Sources of Risk in Current Practice

1. Experimental ranges – a “Signal/Noise” source of risk
2. Experimental design selection – an information content source of risk
3. Performance requirements – a performance variation source of risk

II. QbD-aligned strategy for validating method robustness

1. Define valid study ranges for critical instrument parameters (CPPs)
2. Select the right experimental design
3. Specify risk-based method performance requirements (CQAs)

Method Validation – Benefits of Fusion AE

- **Full automation – Phased Method Validation.**
Early Phase – performance characterization supports development.
Final Phase – aligned with FDA and ICH guidances.
- **21 CFR 11 compliance support toolset –**
Including E-records and E-signatures, Audit Logging.
Workflow Management with E-review and E-approve Loops.
- **Easy setup of experiments –**
Create standardized workflow templates.
Facilitate rigorous practice and defensibility.
- **Simple documentation review –** easy to defend and communicate.
- **Standardized reporting –** reports meet all FDA and ICH guidelines.
- **Method Robustness –** experimental approach is a reliable gatekeeper.

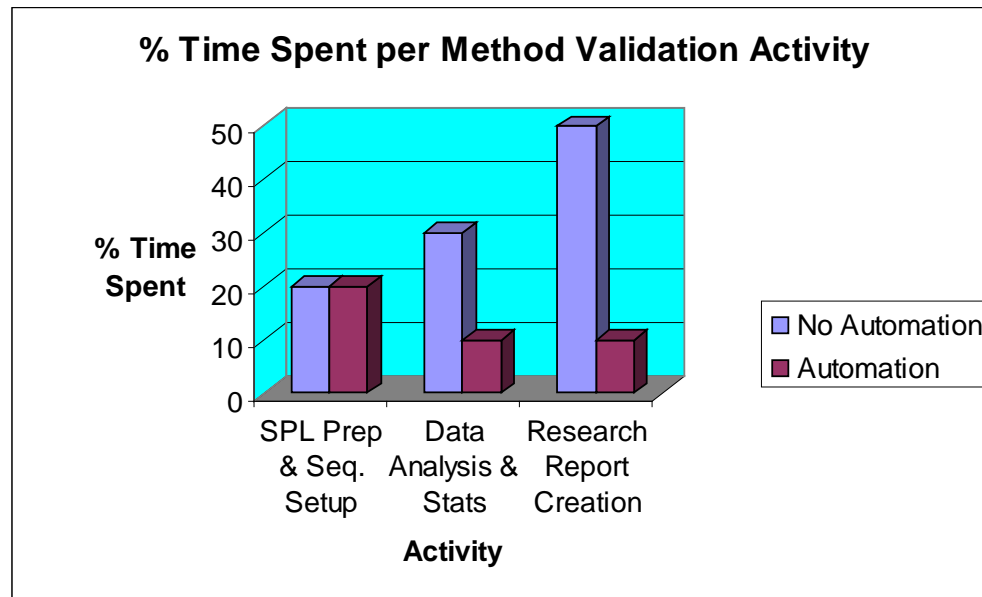
Method Validation – Fusion AE ROI

International Pharma Co. Benchmarking Project

Realized Time Savings = 85%.

Using historical records* and adjusting for project complexity

Minimum Expected Time Savings per Project = 60%.



* - on average 2.5 FTE equivalent years spent in method validation support work over 10 year life span of drug.