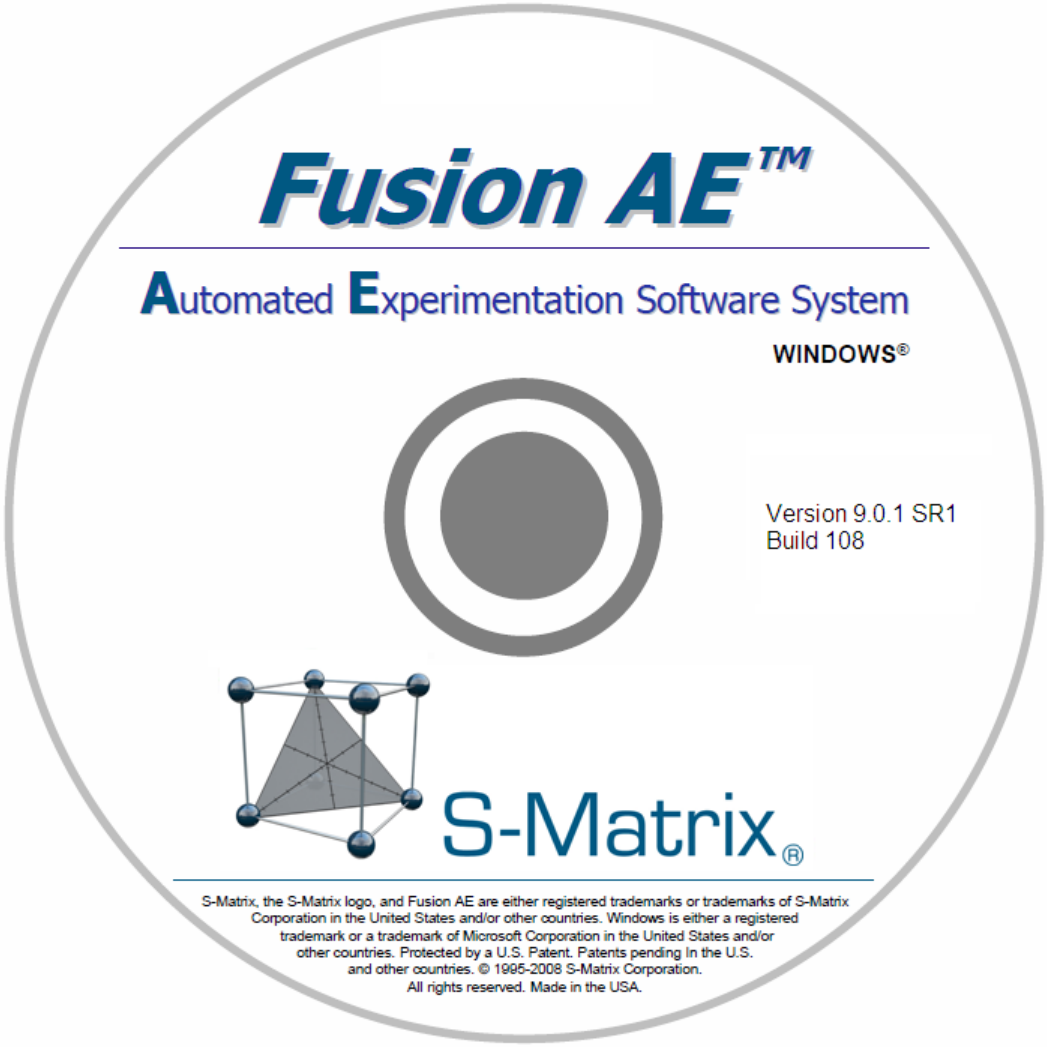
Fusion AE – LC Method Validation Module



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1594 Myrtle Avenue
Eureka, CA 95501
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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 identificattion 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.

Fusion AE Method Validation Experiment Suite

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

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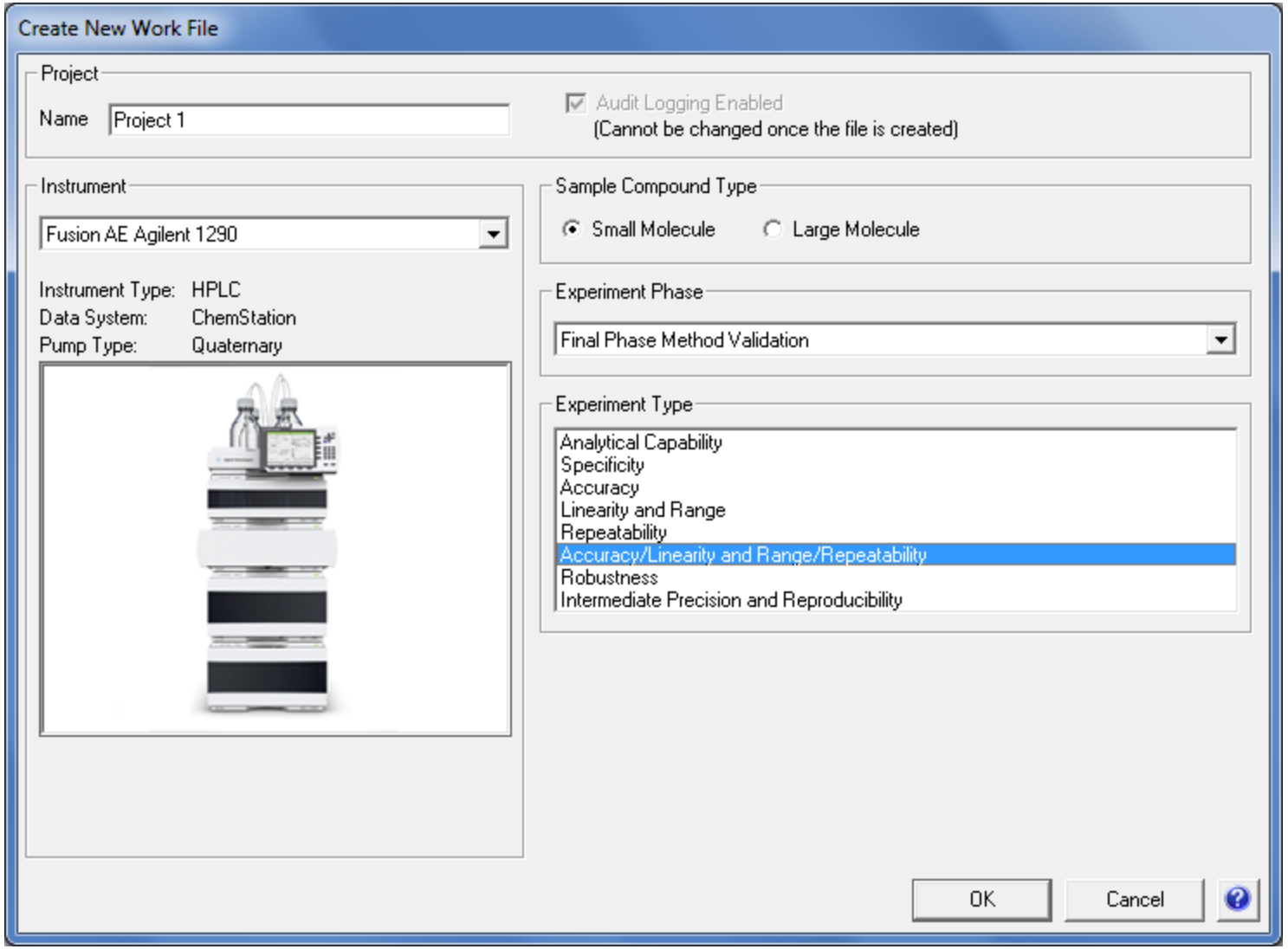
Method Validation Example – Experiment Type Selection



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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)

Automation Workflow

QbD-aligned Experimental Design

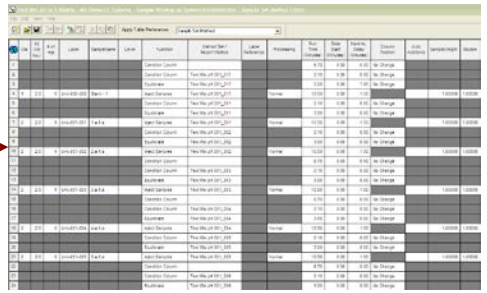
Automatically Builds Sequence and All Methods

Fusion AE

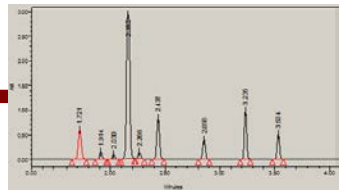
Automated, Audited Data Exchanges

Chromatography Data Software (CDS)

- ChemStation / OpenLab
- Empower 2 and 3



Fusion AE Automatically Retrieves All Chromatogram Results Data



Automated one-click analysis, graphing, and reporting.

Report formats: RTF, DOC, HTML, PDF.

- **Full Support for 21 CFR 11 Compliance**
- **Scalable from Standalone to Enterprise**
- **Citrix-Ready Certification**

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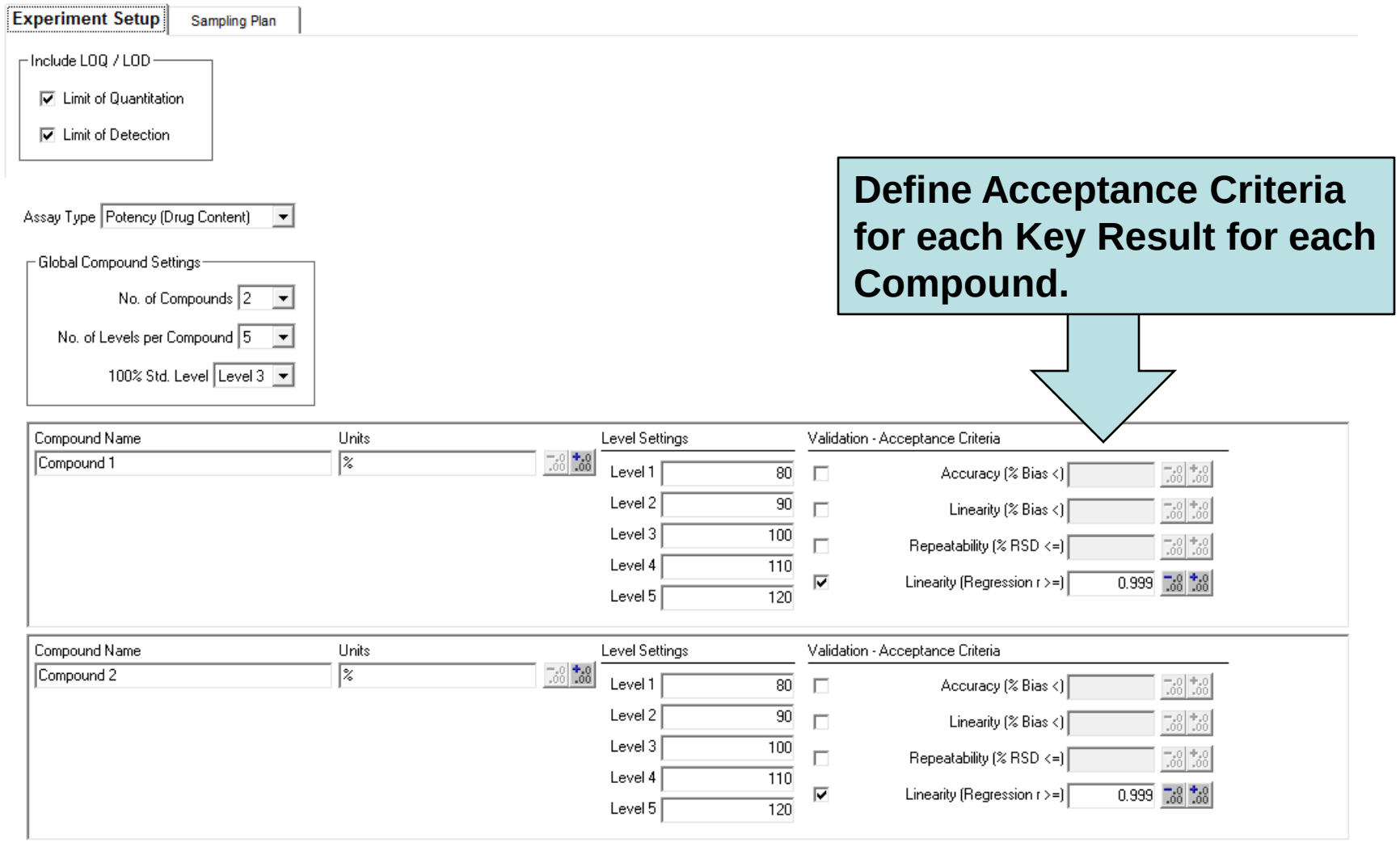
Linearity Example – Experiment Setup Template



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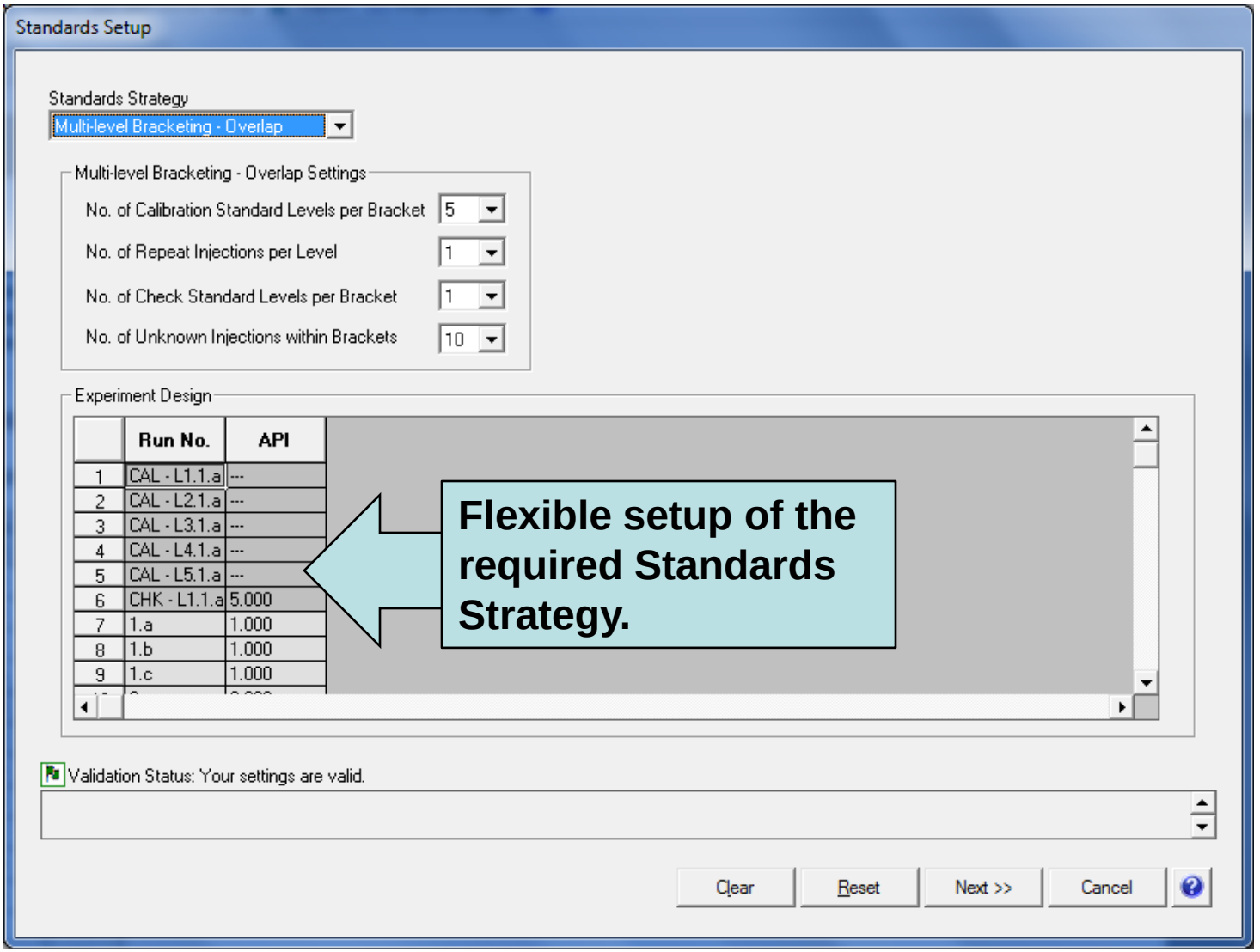
Linearity Example – Standards Setup Options



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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 8:51:17 PM PDT [GMT-07:00]

S-Matrix

Linearity and Range Report: API - Amount (mg)

Linearity and Range Data Table

Run No.	Target API (mg)	Actual Amount (mg)	API - Amount
1.a	1.000	1.000	1.091
1.b	1.000	1.01	1.092
1.c	1.000	1.012	1.109
2.a	2.000	1.995	2.109
2.b	2.000	1.99	2.099
2.c	2.000	2.004	2.049
3.a	4.000	3.999	4.119
3.b	4.000	4	4.097
3.c	4.000	3.997	4.099
4.a	5.000	5.005	5.107
4.b	5.000	4.992	5.094
4.c	5.000	5.009	5.095
5.a	6.000	6.004	5.93
5.b	6.000	6.003	5.929
5.c	6.000	5.997	6.007

General Regression Statistics Table

Regression Statistic Name	Statistic Value	Pass / Fail
r	0.9997	Pass
R Square	0.9993	---
Adj. R Square	0.9993	---
Residual MSE	0.00259	---
Standard Error (s)	0.05092	---
+/- 95% C.I.	0.11001	---
Intercept % Bias	2.49	---
Observations	15	---

Acceptance Criterion - Regression r: > 0.9990

Regression ANOVA Statistics Table

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F-Ratio	P-Value
Regression	49.60199	1	49.60199	19,213.2366	< 0.0001
Residual	0.03071	13	0.00236	---	---
Total	49.63270	14	---	---	---

Regression Coefficients Table

Variable Name	Coefficient Value	Coefficient Standard Error	Coefficient t Statistic	P-Value	Lower 95% Confidence Limit	Upper 95% Confidence Limit
Intercept	0.112532	0.02579	4.3630	0.0008	0.06685	0.16739
API	0.99321	0.00709	138.6119	< 0.0001	0.99768	0.99854

Natural Variable Model

API - Amount (pred) = 0.12532 + (0.99321 x API)

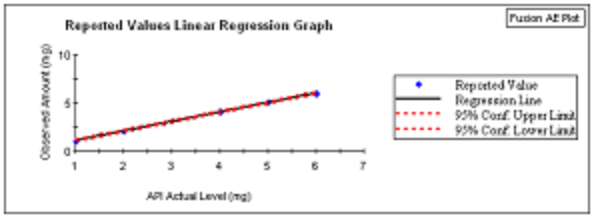
Range

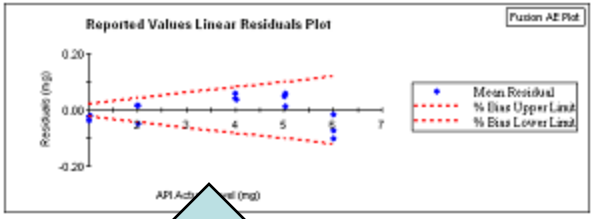
1.003 <= API <= 5.997

Residuals Table

Actual API - Amount (mg)	Predicted API - Amount	Observed API - Amount	Residuals	%Bias of Residuals	%Bias Pass / Fail
1.003	1.11149	1.091	-0.02049	-2.04	Fail
1.01	1.11857	1.092	-0.02957	-2.90	Fail
1.012	1.12059	1.103	-0.01733	-1.71	Pass
1.995	2.09993	2.109	0.01917	0.99	Pass
1.99	2.09191	2.099	0.01709	0.89	Pass
2.004	2.09399	2.049	-0.04799	-2.39	Fail
3.999	4.02921	4.119	0.09179	1.22	Pass
4	4.02917	4.097	0.03683	0.97	Pass
3.997	4.02533	4.099	0.04376	1.10	Pass
5.005	5.04930	5.107	0.05770	1.21	Pass
4.992	5.03652	5.094	0.05348	1.01	Pass
5.009	5.05034	5.065	0.01476	0.29	Pass
6.004	6.02823	5.930	-0.09823	-1.64	Pass
6.003	6.03795	5.929	-0.07122	-1.19	Pass
5.997	6.03166	6.007	-0.01466	-0.24	Pass

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





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

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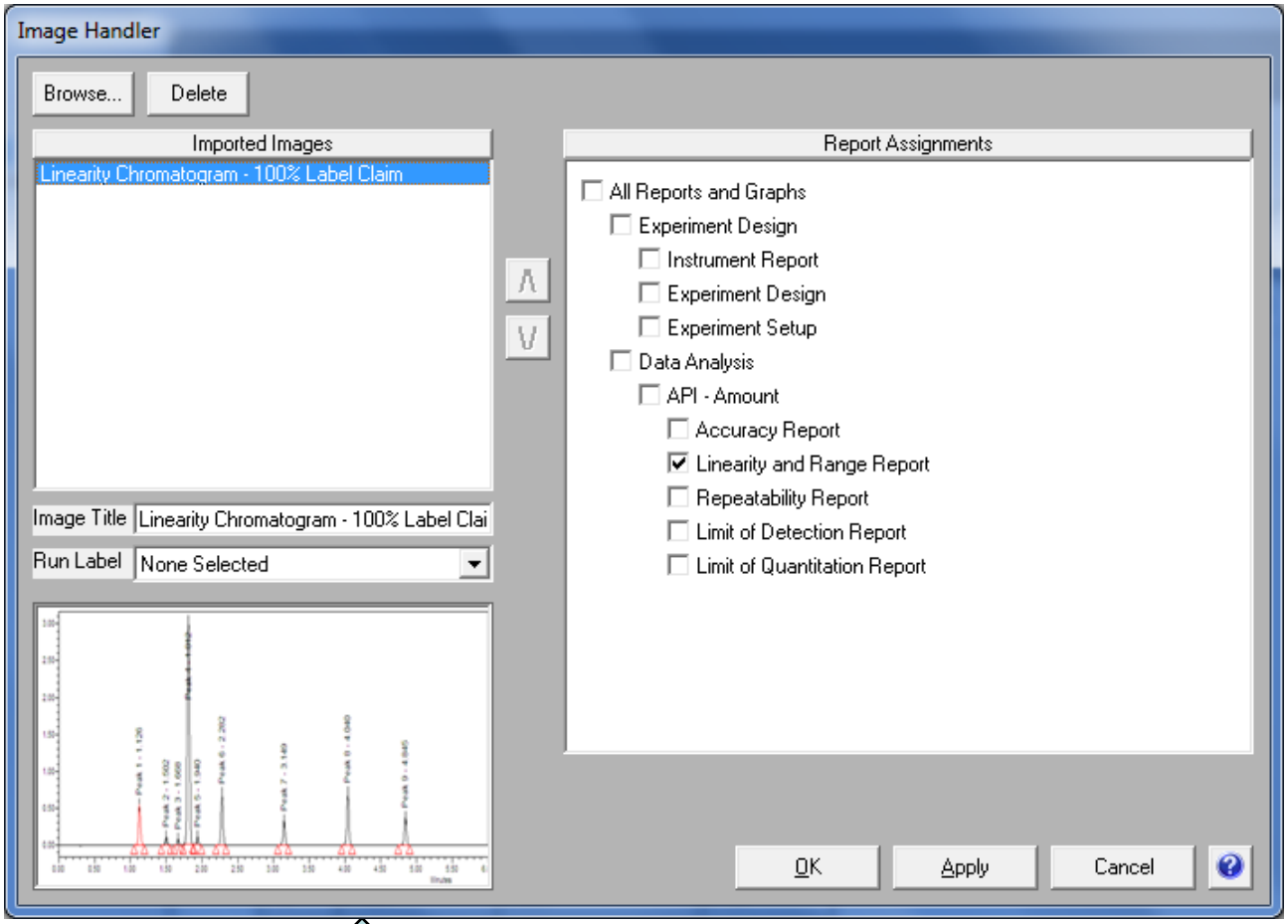
Method Validation – Linearity Example

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.



Reports can be augmented with images of relevant chromatograms.

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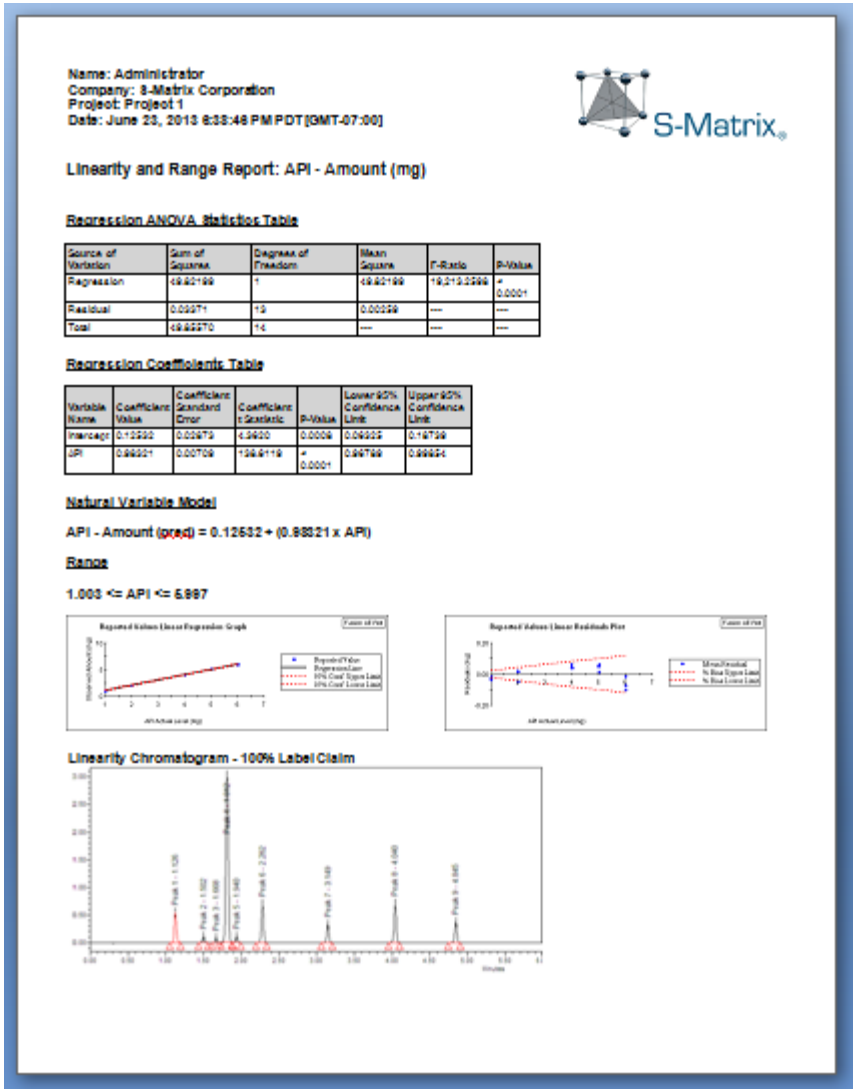
Method Validation – Linearity Example

Reports can be augmented with images of relevant chromatograms.



Name: Administrator
Company: S-Matrix Corporation
Project: Project 1
Date: June 23, 2013 6:38:48 PM PDT [GMT-07:00]

S-Matrix

Linearity and Range Report: API - Amount (mg)

Regression ANOVA Statistics Table

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F-Ratio	P-Value
Regression	49.82199	1	49.82199	19,213.2399	< 0.0001
Residual	0.03971	13	0.00259	---	---
Total	49.86170	14	---	---	---

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	0.02873	4.3620	0.0008	0.06325	0.18739
API	0.98321	0.00709	138.6119	< 0.0001	0.96789	0.99854

Natural Variable Model

API - Amount (pred) = 0.12532 + (0.98321 x API)

Range

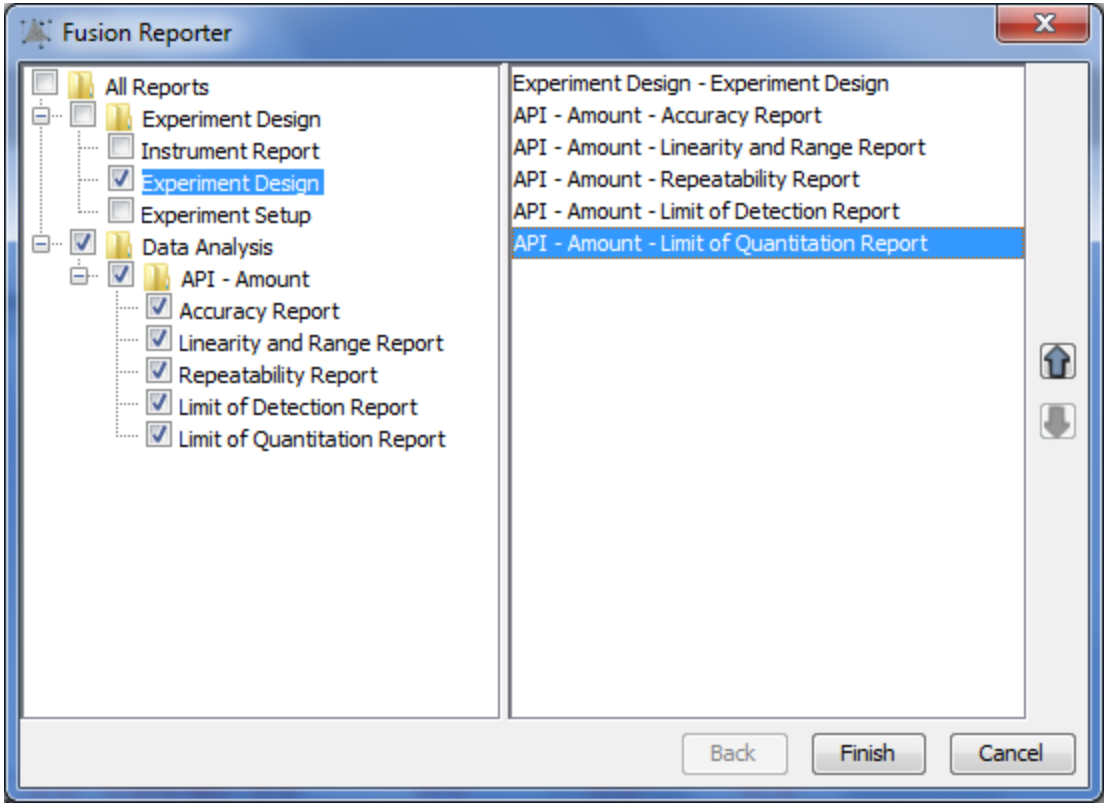
1.003 <= API <= 5.997

Linearity Chromatogram - 100% Label Claim

Linearity Example – Fusion AE Compiled Report Generator

Reports meet all output format requirements:

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



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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

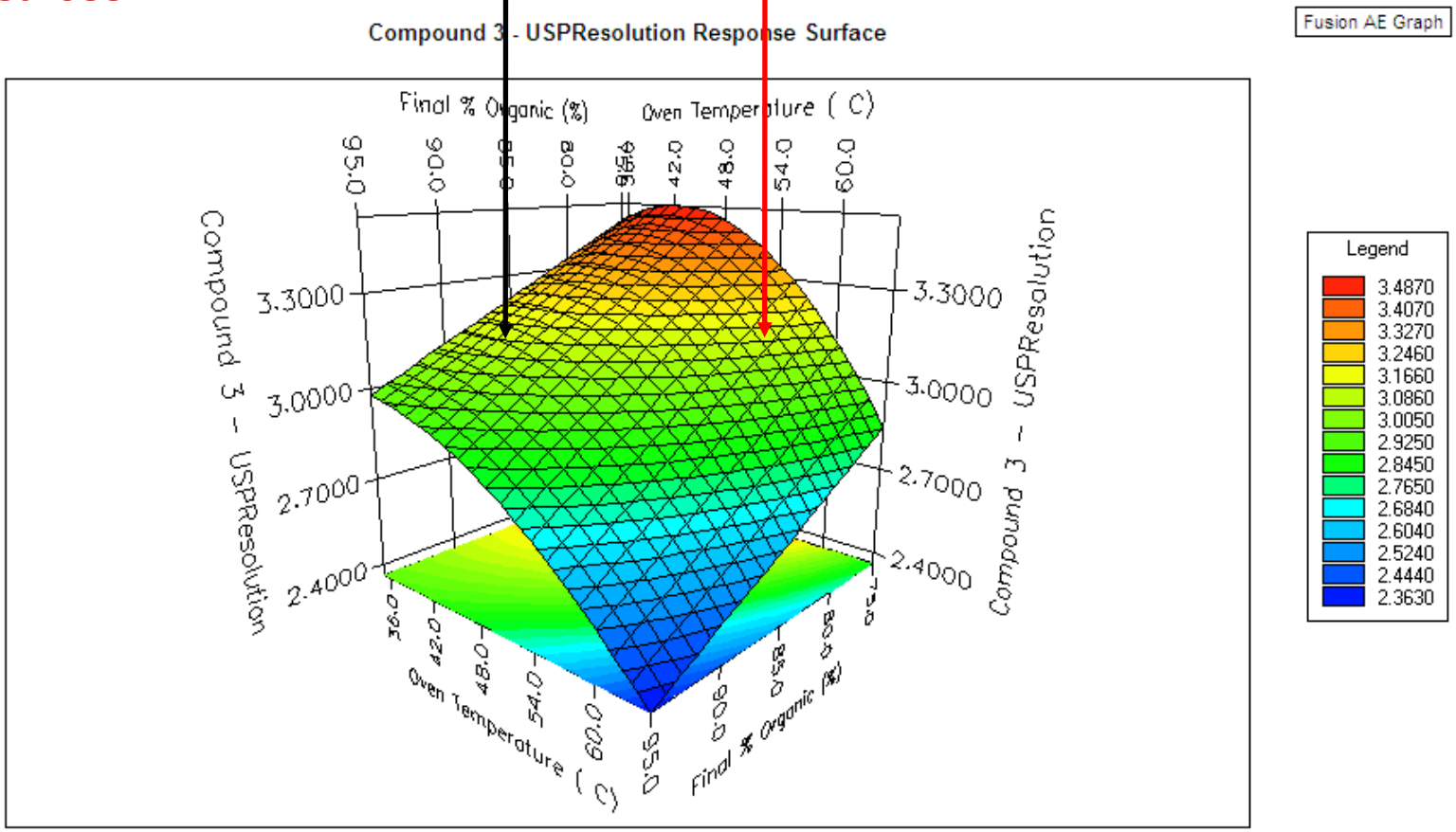
Methods A and B –

Identical Mean Performance –

Good mean performance ≠ good robustness

Method A – Good Robustness

Method B – Poor Robustness



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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 Robustness

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)

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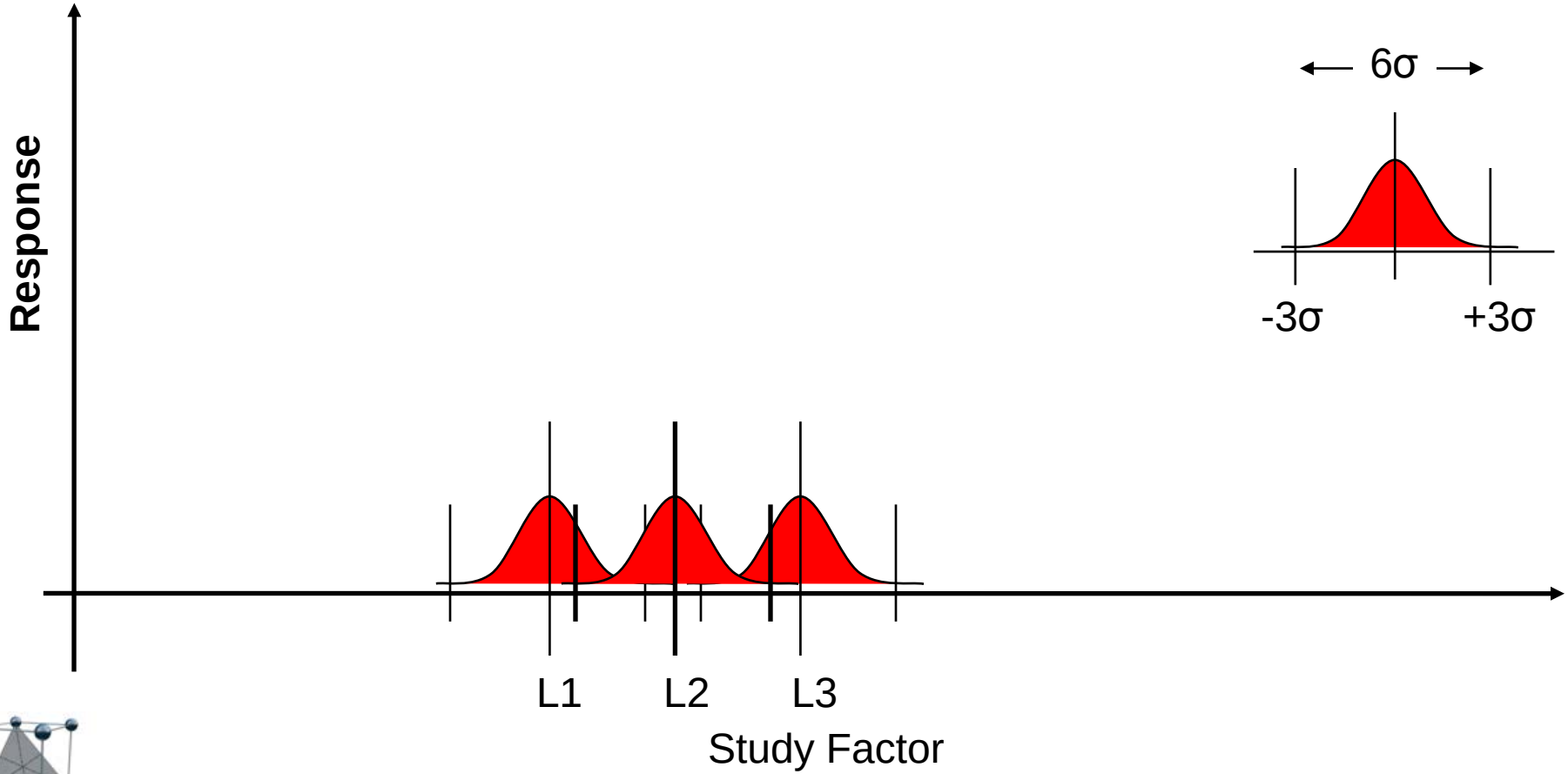
Small Range – Poor Effects Estimation

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.



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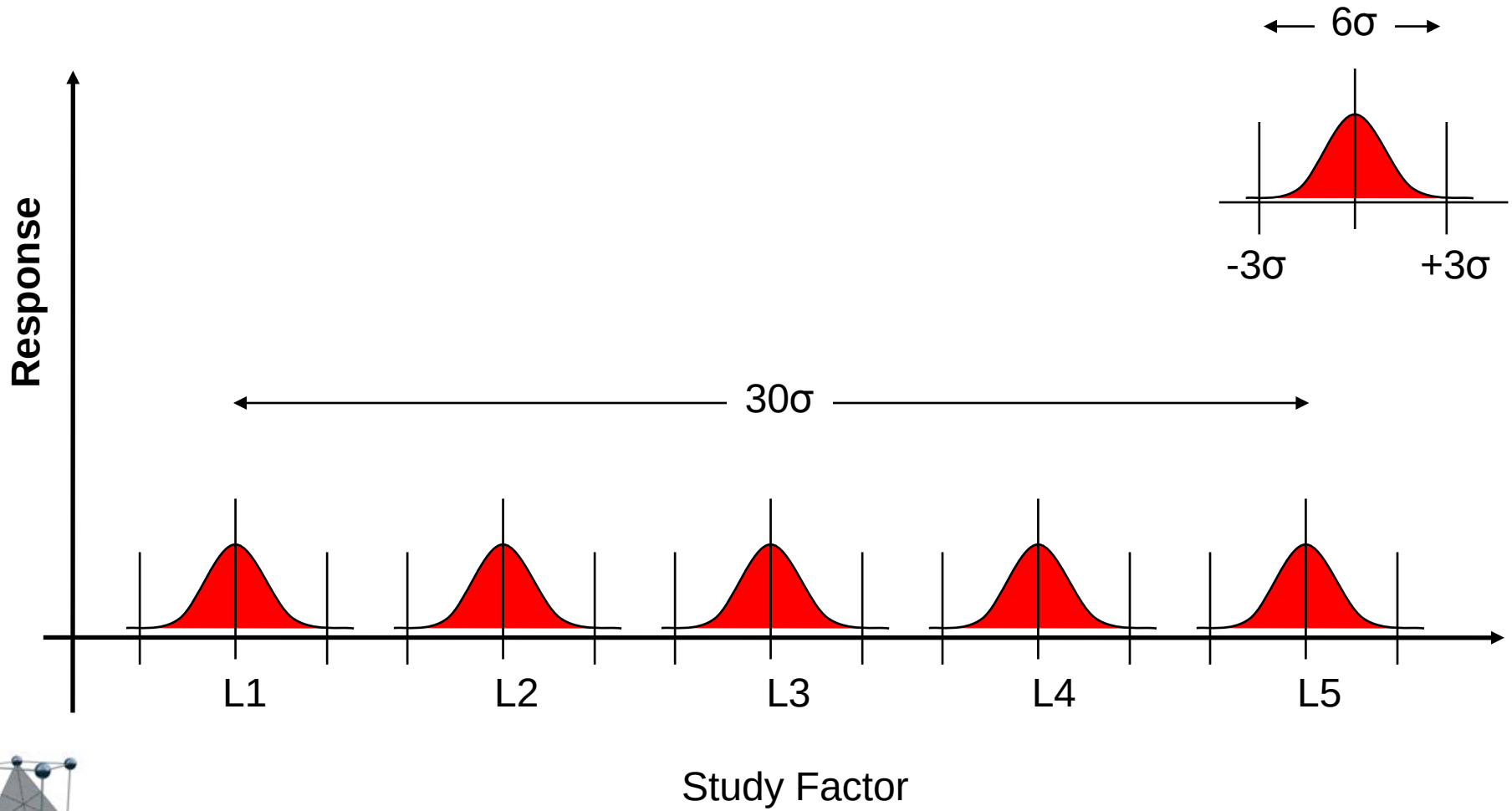
Best Practice – Large Ranges = High Signal/Noise

General Guideline: Minimum Study Range for 5 Level Designs Should be 30σ

(5 x 6σ interval width)



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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

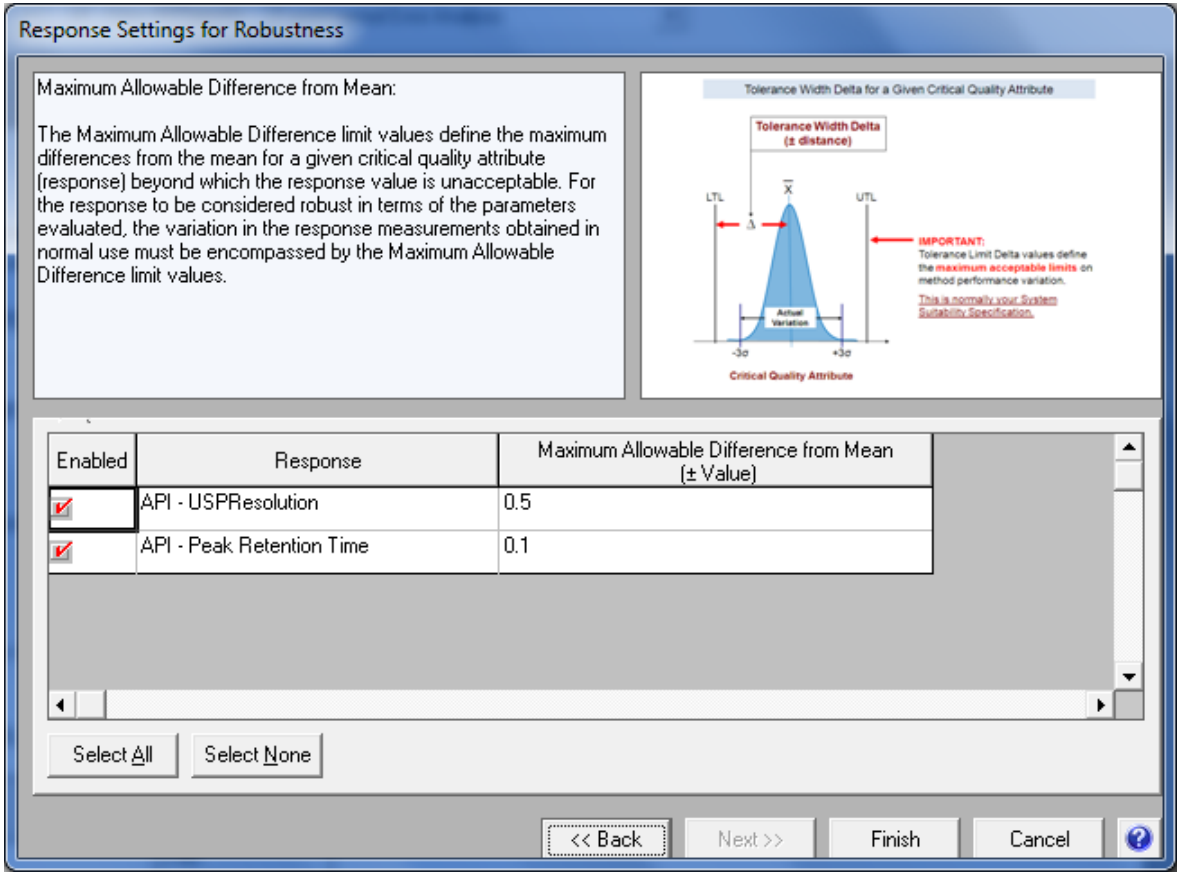
3. Performance requirements – a performance variation source of risk

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



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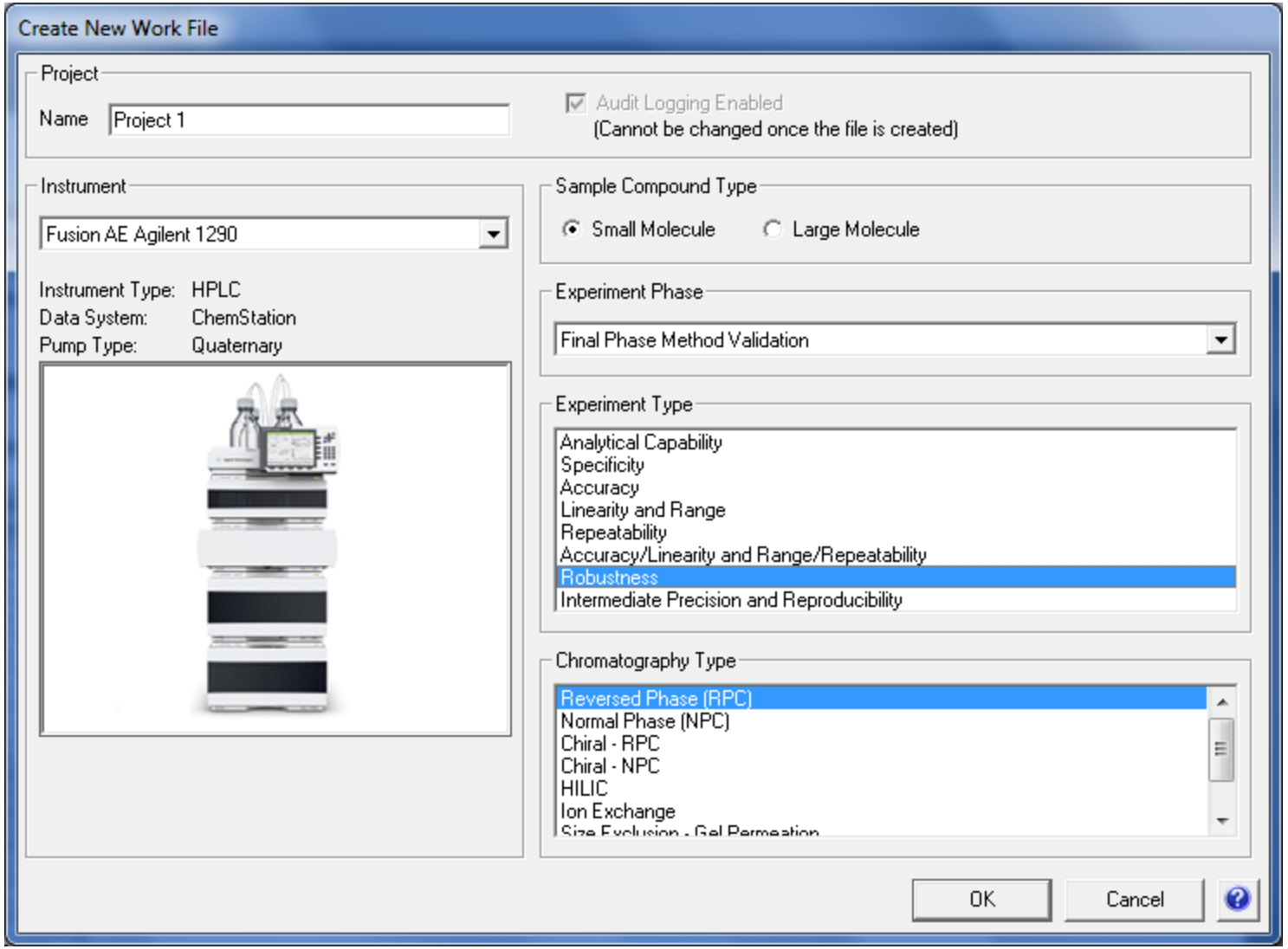
Demonstration Example – Experiment Type Selection



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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 (°C)	35.0	±2.0	±10.0
pH (*)	5.5	±0.15	±0.5

* – worst-case scenario considered.

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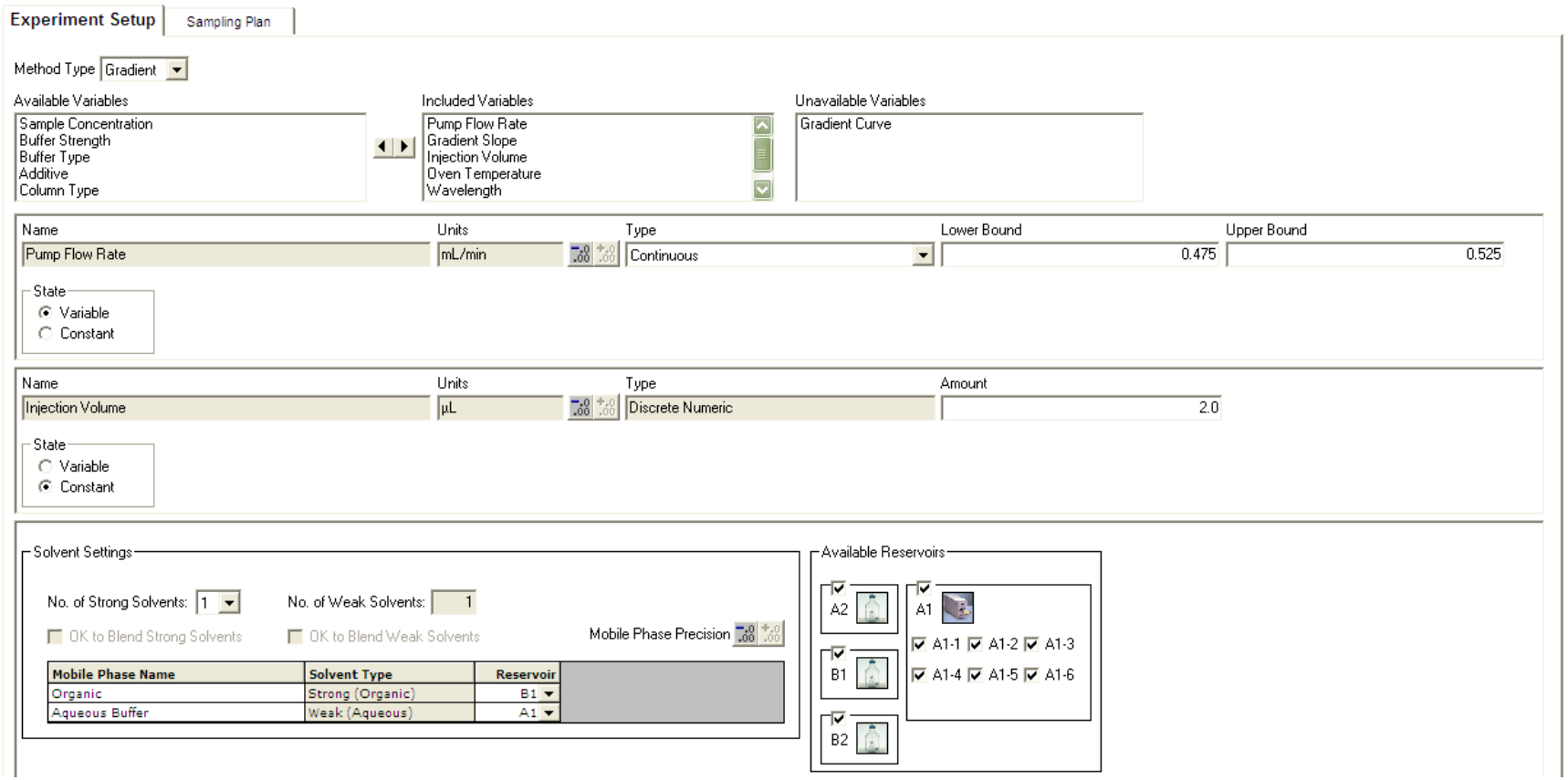
Experiment Setup Template



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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

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QbD-aligned Study Ranges

Peak Results Data Automatically Imported From the CDS

	Run No.	Pump Flow Rate	Initial % Organic	Oven Temperature	pH	Impurity B - USPResolution
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	

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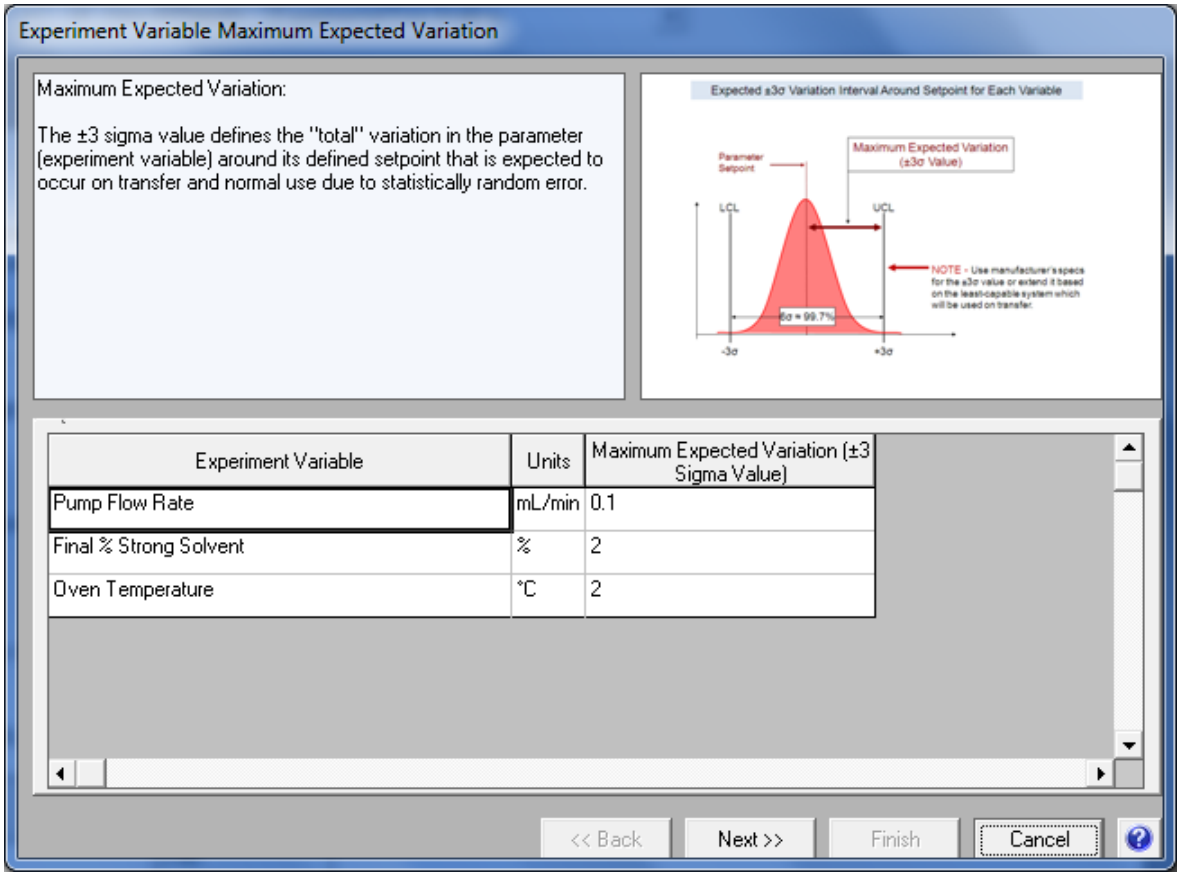
QbD-aligned Study Ranges

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



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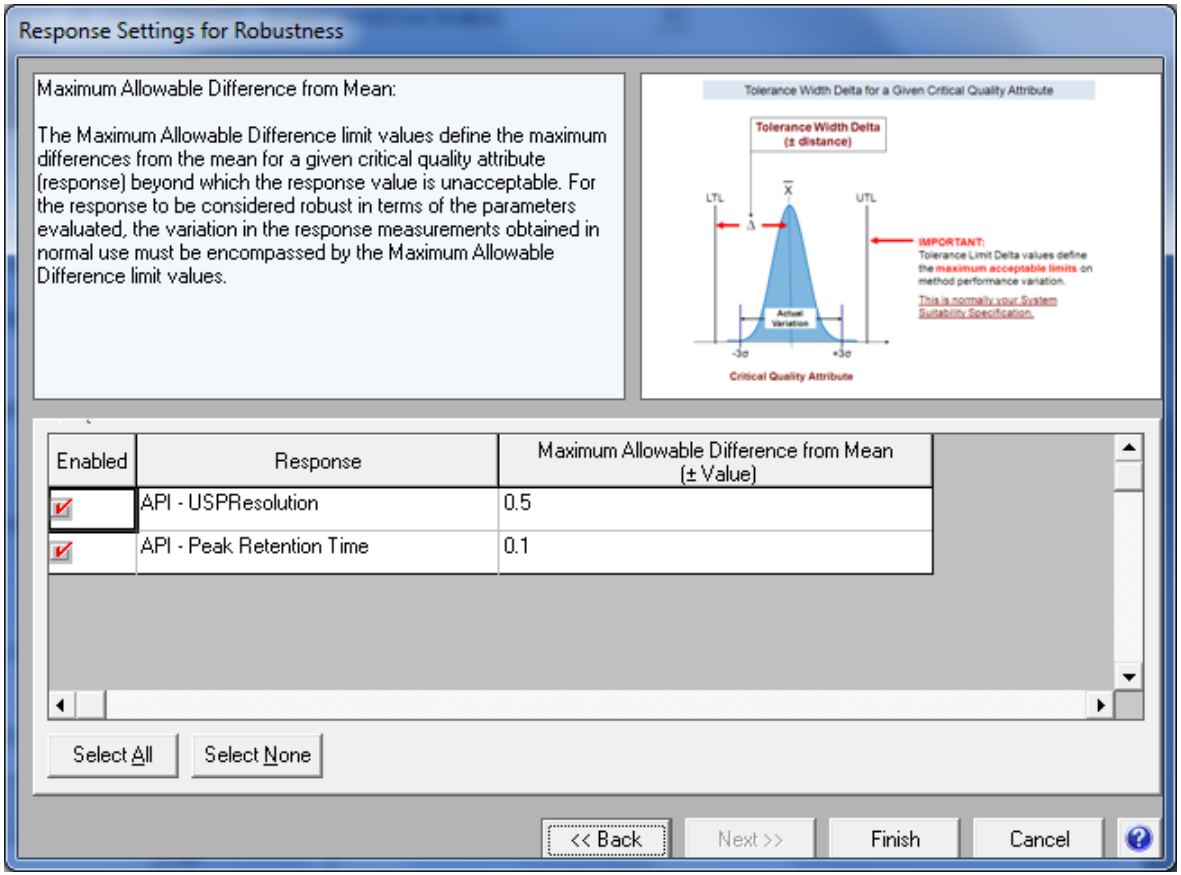
QbD-aligned Study Ranges

Response Performance Limits Required for Robust Method



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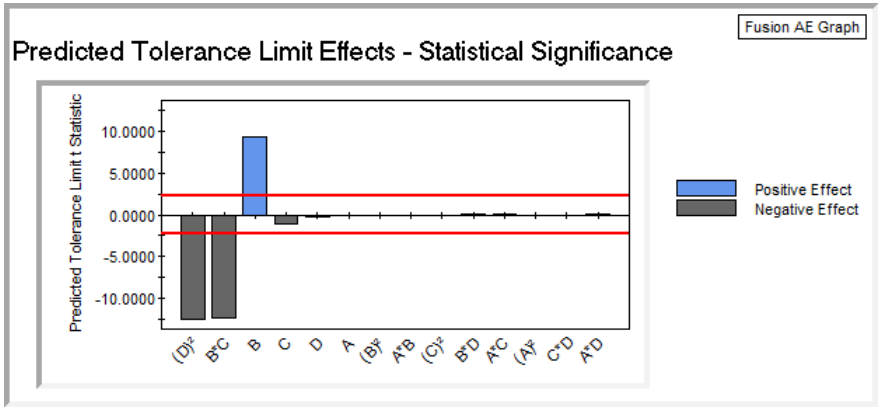
Demonstration Study – QbD-aligned Study Ranges

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.

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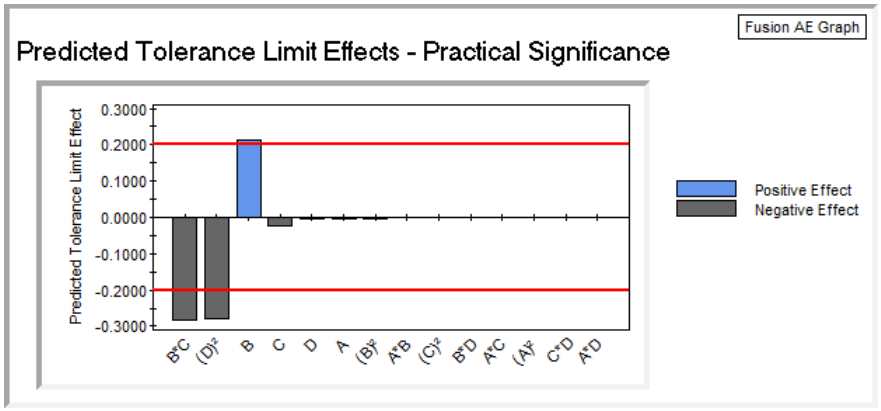
Demonstration Study – QbD-aligned Study Ranges

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.

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Fusion AE QbD Robustness – Summary

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.

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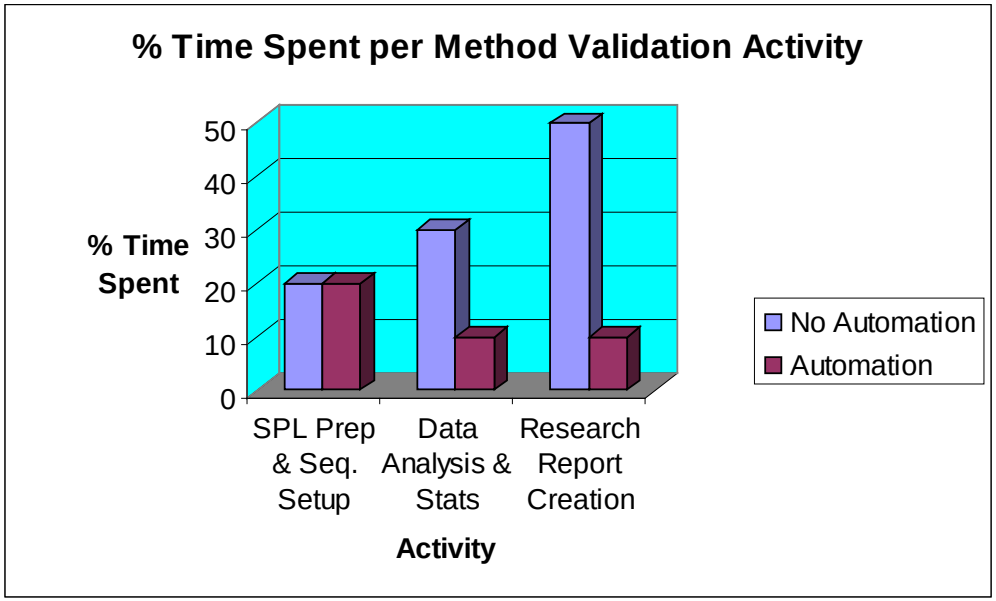
Method Validation – Fusion AE ROI

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.

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