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Automated Method Validation & Transfer

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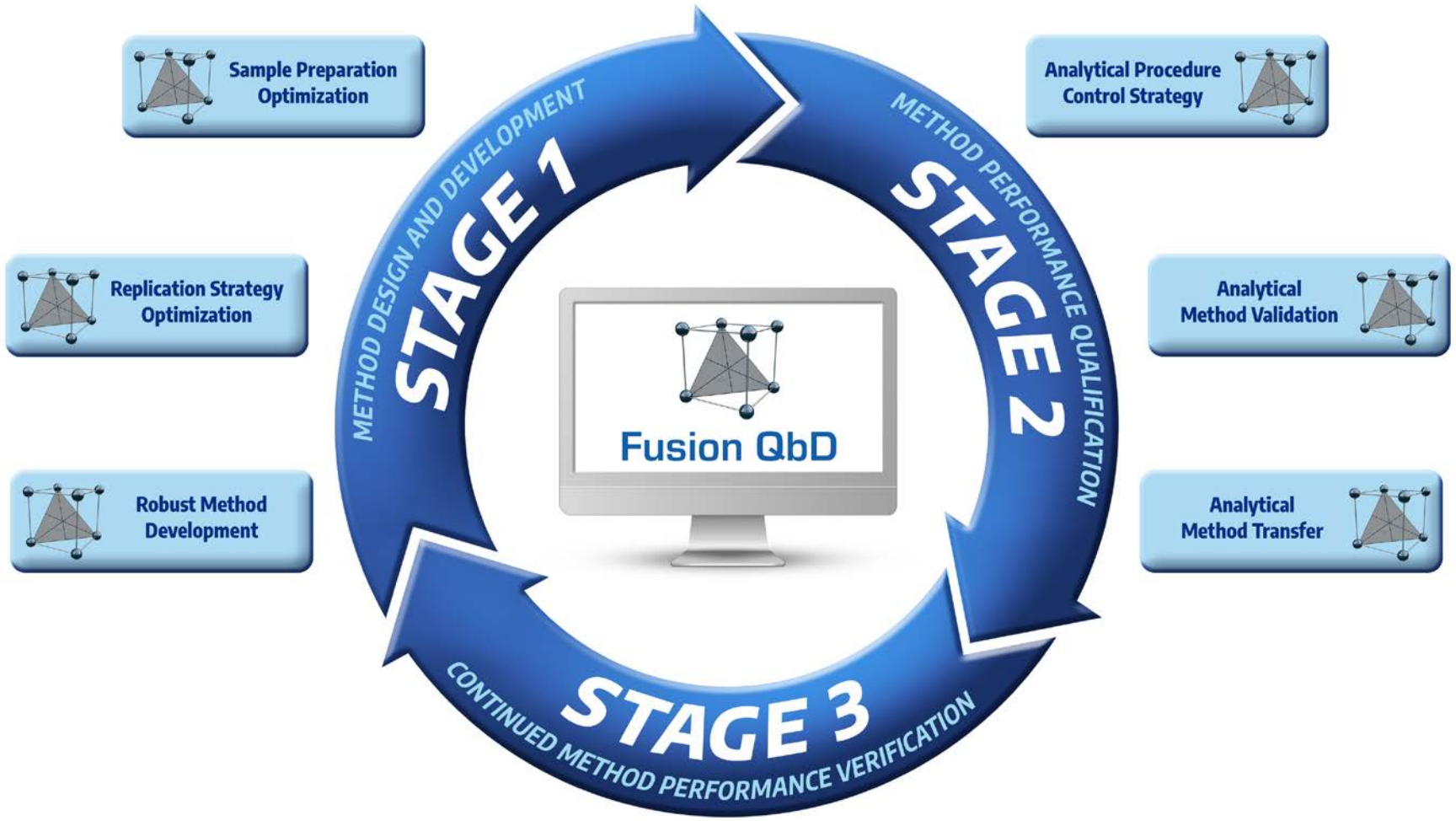
A Complete Solution for APLM Stages 1 and 2

Analytical Procedure Lifecycle Management Workflow



Referenced Guidance Documents – ICH / EP

ICH
harmonisation for better health

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

ICH HARMONISED GUIDELINE

ANALYTICAL PROCEDURE DEVELOPMENT Q14

Final Version

Adopted on 1 November 2023

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

ICH
harmonisation for better health

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

ICH HARMONISED GUIDELINE

VALIDATION OF ANALYTICAL PROCEDURES Q2(R2)

Final Version

Adopted on 1 November 2023

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

01/2025:20246

2. Methods of analysis

2.2.46. CHROMATOGRAPHIC SEPARATION TECHNIQUES(1)

INTRODUCTION

Chromatographic separation techniques are multi-stage separation methods in which the components of a sample are distributed between 2 phases, one of which is stationary, while the other is mobile. The stationary phase may be a solid or a liquid supported on a solid or a gel. The stationary phase may be packed in a column, spread as a layer, or distributed as a film, etc. The mobile phase may be gaseous or liquid. The separation may be based on adsorption, mass distribution (partition), ion exchange, etc., or may be based on differences in the physico-chemical properties of the molecules such as size, mass, volume, etc.

This chapter contains definitions and calculations of common parameters and generally applicable requirements for system suitability. Principles of separation, equipment and methods are given in the corresponding general chapters:

◊– paper chromatography (*2.2.26*);
– thin-layer chromatography (*2.2.27*);
– gas chromatography (*2.2.28*);
– liquid chromatography (*2.2.29*);
– size-exclusion chromatography (*2.2.30*).◊

DEFINITIONS

The system suitability and acceptance criteria in monographs have been set using parameters as defined below. With some equipment, certain parameters such as signal-to-noise ratio and resolution can be calculated using software provided by the manufacturer. It is the responsibility of the user to ensure that the calculation methods used in the software are equivalent to the requirements of the European Pharmacopoeia and to make any necessary corrections if this is not the case.

Chromatogram

A graphical or other representation of detector response, effluent concentration or other quantity used as a measure of effluent concentration, versus time or volume. Ideally, chromatograms are represented as a sequence of Gaussian peaks on a baseline (Figure 2.2.46.-1).

V_M = hold-up volume;
t_M = hold-up time;
V_{R1} = retention volume of peak 1;
t_{R1} = retention time of peak 1;
V_{R2} = retention volume of peak 2;
t_{R2} = retention time of peak 2;
W_h = peak width at half-height;
W_i = peak width at the inflexion point;
h = height of the peak;
$h/2$ = half-height of the peak.

Distribution constant (K_0)

In size-exclusion chromatography, the elution characteristics of a component in a particular column may be given by the distribution constant (also referred to as distribution coefficient), which is calculated using the following equation:

$$K_0 = \frac{t_R - t_0}{t_t - t_0}$$

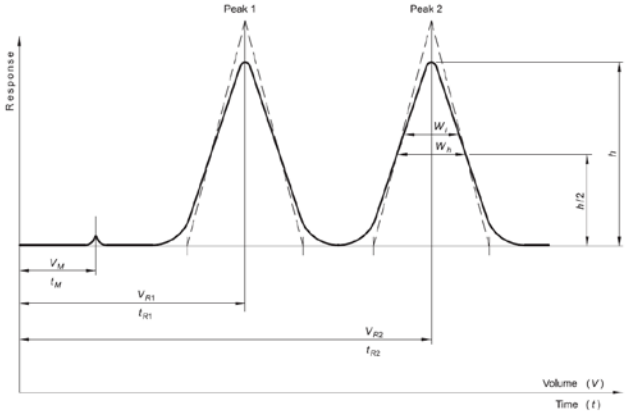
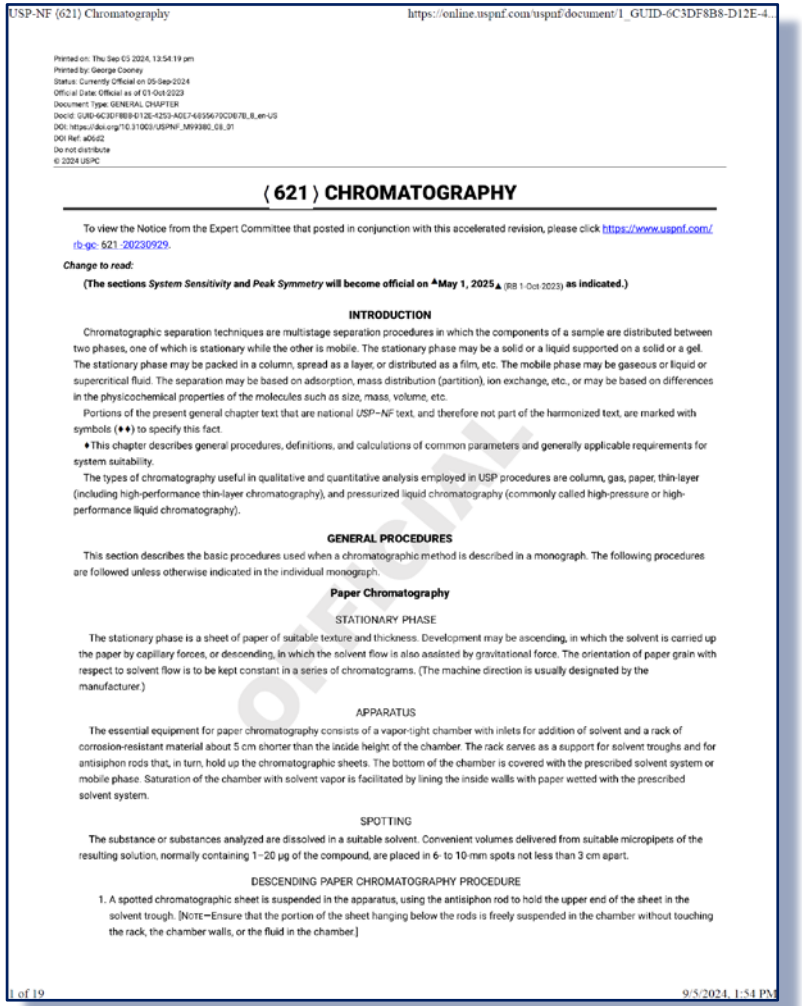


Figure 2.2.46.-1.

(1) This chapter has undergone pharmacopoeial harmonisation. See chapter *5.8. Pharmacopoeial harmonisation*.

Referenced Guidance Documents – USP



Printed on: Thu Sep 05 2024, 13:54:19 pm
Printed by: George Cooney
Status: Currently Official on 05-Sep-2024
Official Date: Official as of 01-Oct-2023
Document Type: GENERAL CHAPTER
DocId: GUID-6C3DF8B8-D12E-4253-AD17-65556705D67B_8_en-US
DOI: https://doi.org/10.31003/USPNF_M9938C_08_01
DOI Ref: a06d2
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⟨621⟩ CHROMATOGRAPHY

To view the Notice from the Expert Committee that posted in conjunction with this accelerated revision, please click https://www.uspnf.com/rb-gc: 621 :20230929.

Change to read:

(The sections *System Sensitivity* and *Peak Symmetry* will become official on ▲May 1, 2025▲ (RB 1-Oct-2023) as indicated.)

INTRODUCTION

Chromatographic separation techniques are multistage separation procedures in which the components of a sample are distributed between two phases, one of which is stationary while the other is mobile. The stationary phase may be a solid or a liquid supported on a solid or a gel. The stationary phase may be packed in a column, spread as a layer, or distributed as a film, etc. The mobile phase may be gaseous or liquid or supercritical fluid. The separation may be based on adsorption, mass distribution (partition), ion exchange, etc., or may be based on differences in the physicochemical properties of the molecules such as size, mass, volume, etc.

Portions of the present general chapter text that are national *USP–NF* text, and therefore not part of the harmonized text, are marked with symbols (♦♦) to specify this fact.

♦This chapter describes general procedures, definitions, and calculations of common parameters and generally applicable requirements for system suitability.

The types of chromatography useful in qualitative and quantitative analysis employed in USP procedures are column, gas, paper, thin-layer (including high-performance thin-layer chromatography), and pressurized liquid chromatography (commonly called high-pressure or high-performance liquid chromatography).

GENERAL PROCEDURES

This section describes the basic procedures used when a chromatographic method is described in a monograph. The following procedures are followed unless otherwise indicated in the individual monograph.

Paper Chromatography

STATIONARY PHASE

The stationary phase is a sheet of paper of suitable texture and thickness. Development may be ascending, in which the solvent is carried up the paper by capillary forces, or descending, in which the solvent flow is also assisted by gravitational force. The orientation of paper grain with respect to solvent flow is to be kept constant in a series of chromatograms. (The machine direction is usually designated by the manufacturer.)

APPARATUS

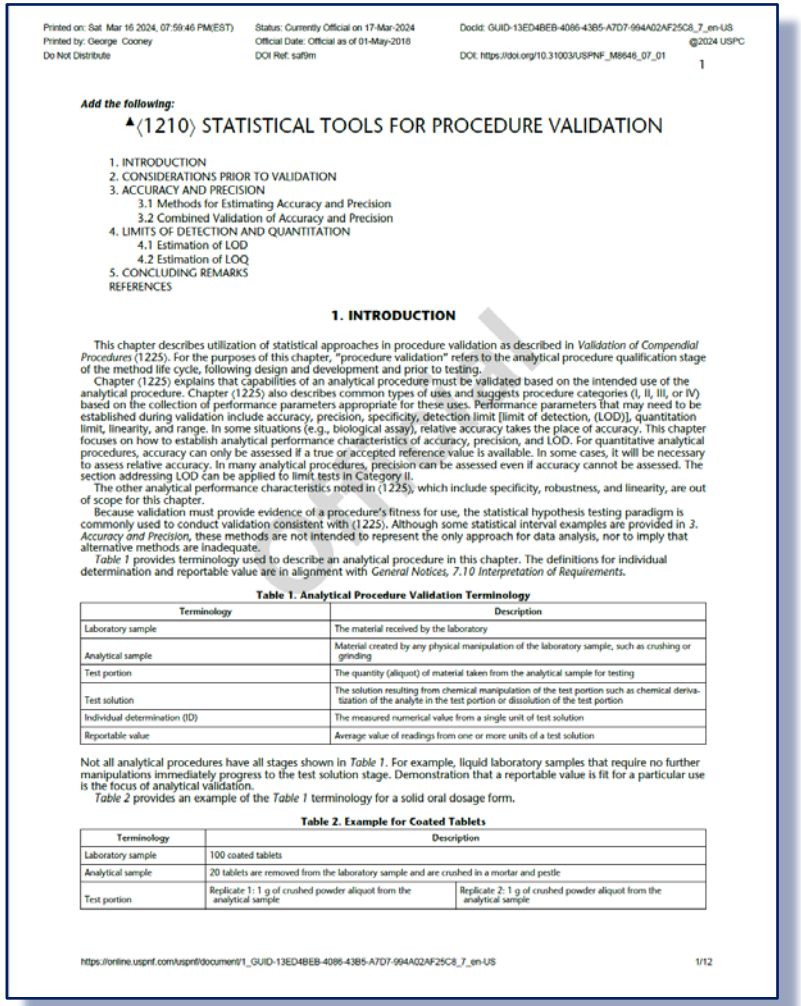
The essential equipment for paper chromatography consists of a vapor-tight chamber with inlets for addition of solvent and a rack of corrosion-resistant material about 5 cm shorter than the inside height of the chamber. The rack serves as a support for solvent troughs and for antisiphon rods that, in turn, hold up the chromatographic sheets. The bottom of the chamber is covered with the prescribed solvent system or mobile phase. Saturation of the chamber with solvent vapor is facilitated by lining the inside walls with paper wetted with the prescribed solvent system.

SPOTTING

The substance or substances analyzed are dissolved in a suitable solvent. Convenient volumes delivered from suitable micropipets of the resulting solution, normally containing 1–20 µg of the compound, are placed in 6- to 10-mm spots not less than 3 cm apart.

DESCENDING PAPER CHROMATOGRAPHY PROCEDURE

1. A spotted chromatographic sheet is suspended in the apparatus, using the antisiphon rod to hold the upper end of the sheet in the solvent trough. [NOTE—Ensure that the portion of the sheet hanging below the rods is freely suspended in the chamber without touching the rack, the chamber walls, or the fluid in the chamber.]



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Printed by: George Cooney Official Date: Official as of 01-May-2018 @2024 USPC
Do Not Distribute DOI Ref: saf9m DOI: https://doi.org/10.31003/USPNF_M8646_07_01

Add the following:

▲⟨1210⟩ STATISTICAL TOOLS FOR PROCEDURE VALIDATION

1. INTRODUCTION

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

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

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

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

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

Table 1. Analytical Procedure Validation Terminology

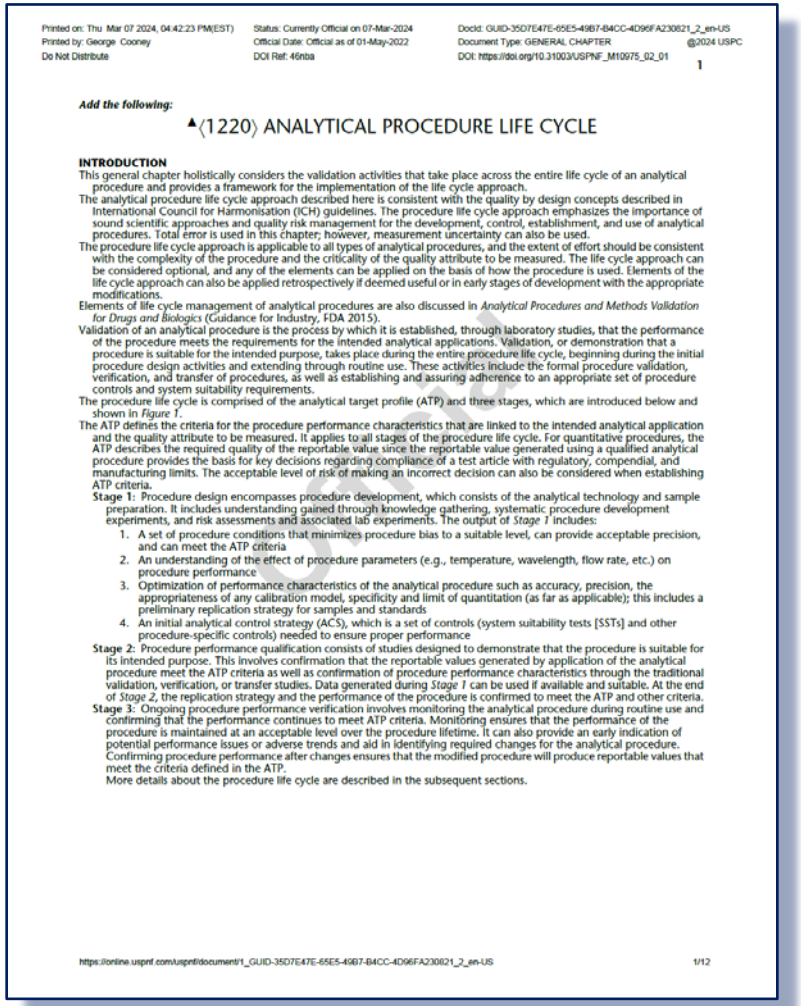
Terminology	Description
Laboratory sample	The material received by the laboratory
Analytical sample	Material created by any physical manipulation of the laboratory sample, such as crushing or grinding
Test portion	The quantity (aliquot) of material taken from the analytical sample for testing
Test solution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

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

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

Table 2. Example for Coated Tablets

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



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Do Not Distribute DOI Ref: 46nba DOI: https://doi.org/10.31003/USPNF_M10975_02_01

Add the following:

▲⟨1220⟩ ANALYTICAL PROCEDURE LIFE CYCLE

INTRODUCTION

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

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

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

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

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

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

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

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

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

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

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

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

A Complete Solution for APLM Stage 2

METHOD VALIDATION MODULE

- Full Validation Experiment Suite
- Instant Analysis and Reporting
- Advanced Method Transfer Support
- Meets all Regulatory Requirements

All the Critical QbD Capabilities You Need

Critical QbD Capability	FMV
Complete Method Validation Experiment Suite	✓
Simple Experiment Workflows	✓
Full LC Experiment Automation	✓
USP 1210> Tolerance and Prediction Interval Metrics	✓

- Replication Strategy and Total Analytical Error
- Accuracy and Repeatability
- Analytical Method Transfer

All the Critical QbD Capabilities You Need

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Simple Experiment Workflows	✓
Full LC Experiment Automation	✓
USP 1210> Tolerance and Prediction Interval Metrics	✓

- Replication Strategy and Total Analytical Error
- Accuracy and Repeatability
- Analytical Method 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, Dissolution]
- Method Transfer Study Support*

* – integration of USP <1210> Tolerance & Prediction Intervals]

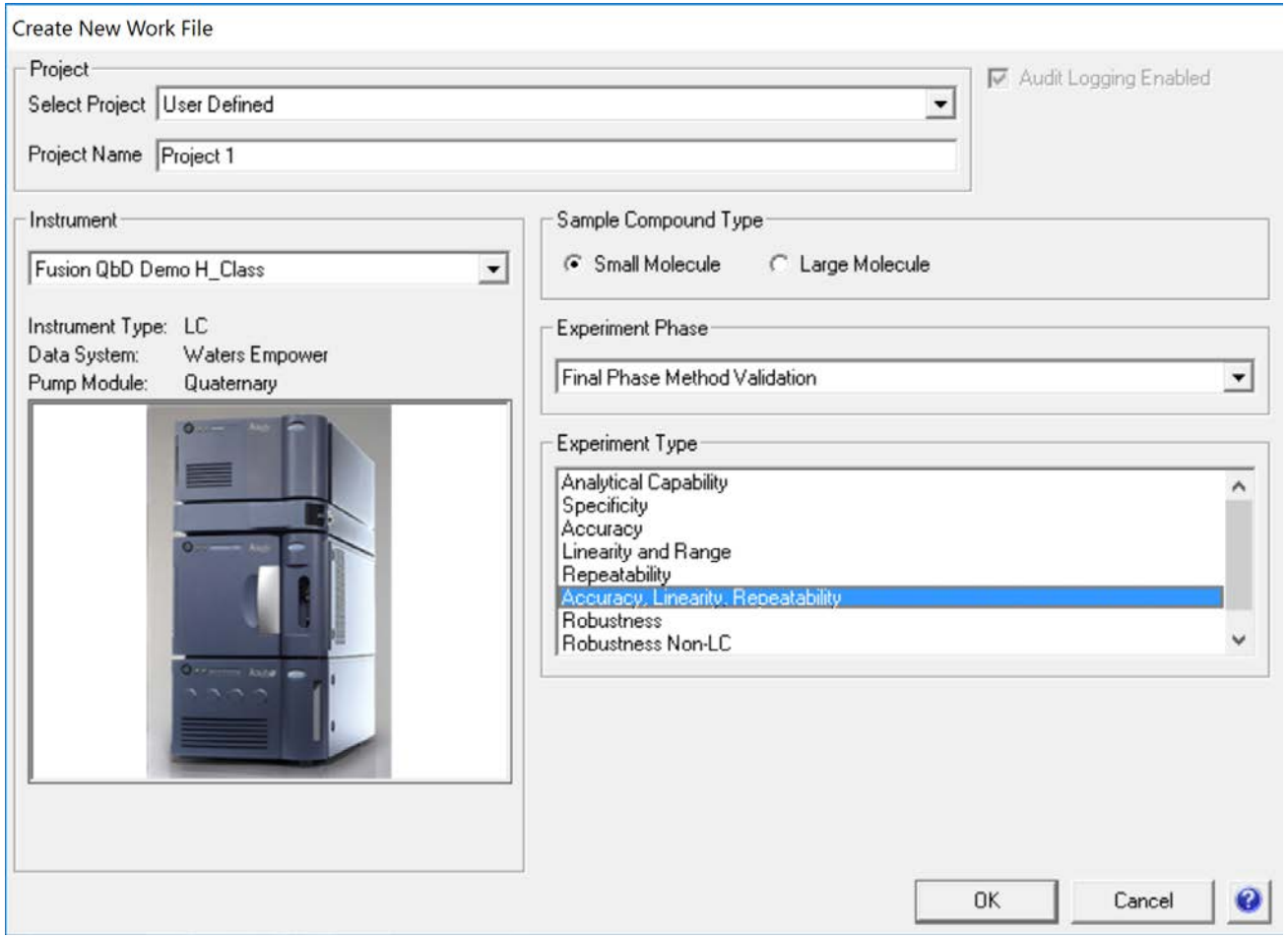
All the Critical QbD Capabilities You Need

Critical QbD Capability	FMV
Complete Method Validation Experiment Suite	✓
Simple Experiment Workflows	✓
Full LC Experiment Automation	✓
USP 1210> Tolerance and Prediction Interval Metrics	✓

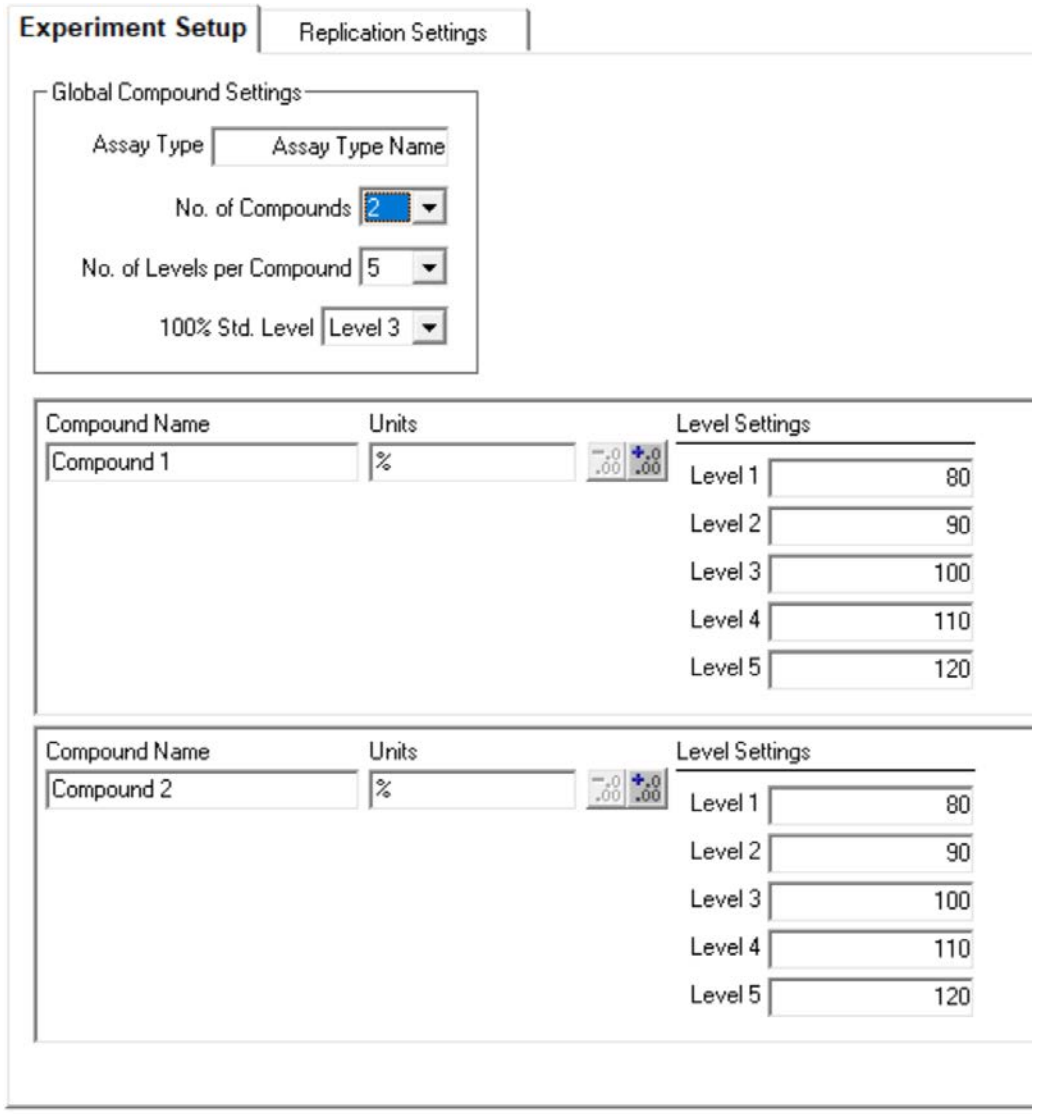
- Replication Strategy and Total Analytical Error
- Accuracy and Repeatability
- Analytical Method Transfer

Simple Workflow with Complete QbD Reporting

Example: Accuracy / Linearity / Repeatability – Combined Experiment



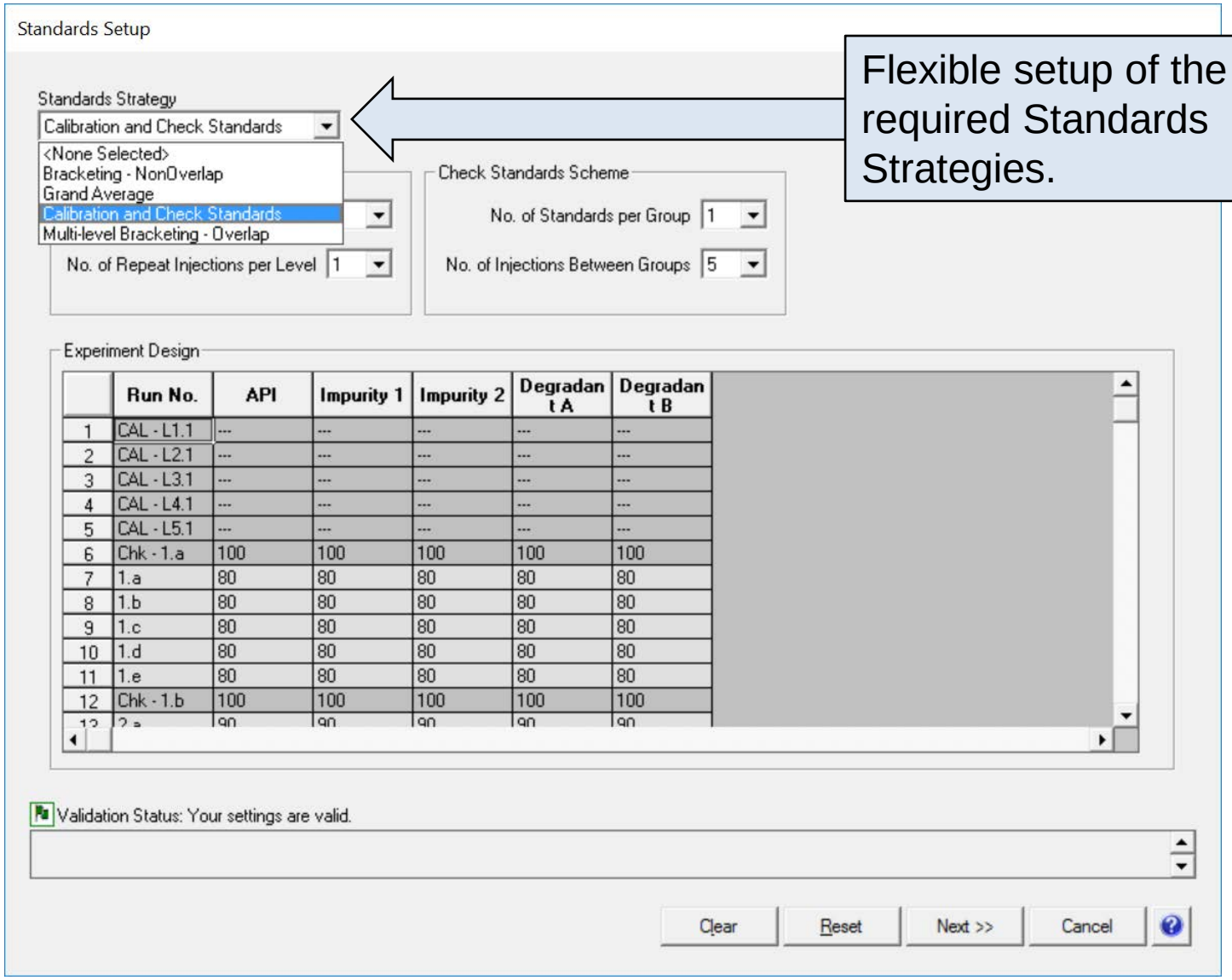
1. Simple Experiment Setup Template



Create and Maintain Templates.

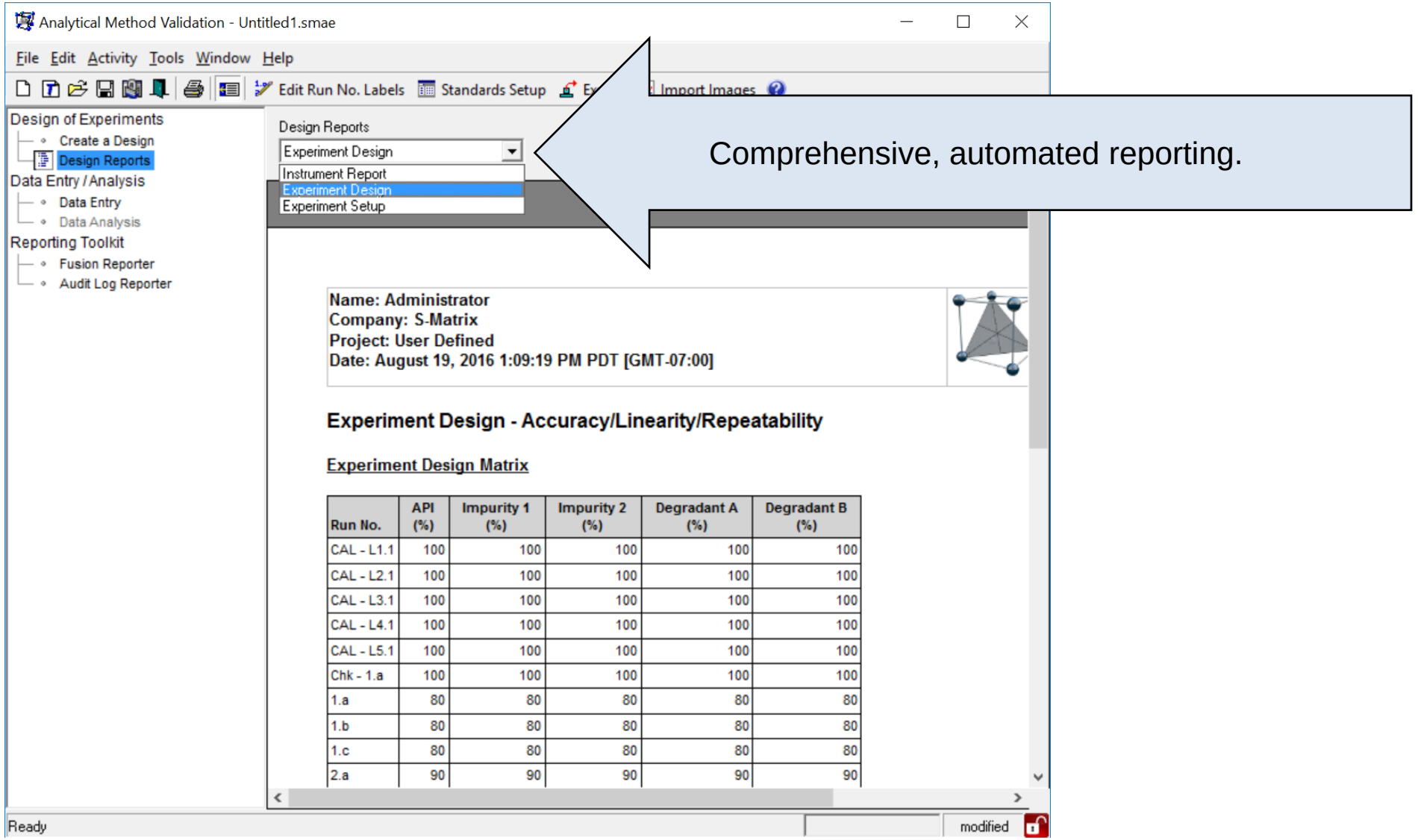
Set Automatic E-Review and E-Approve Loops.

2. Standards Setup Options

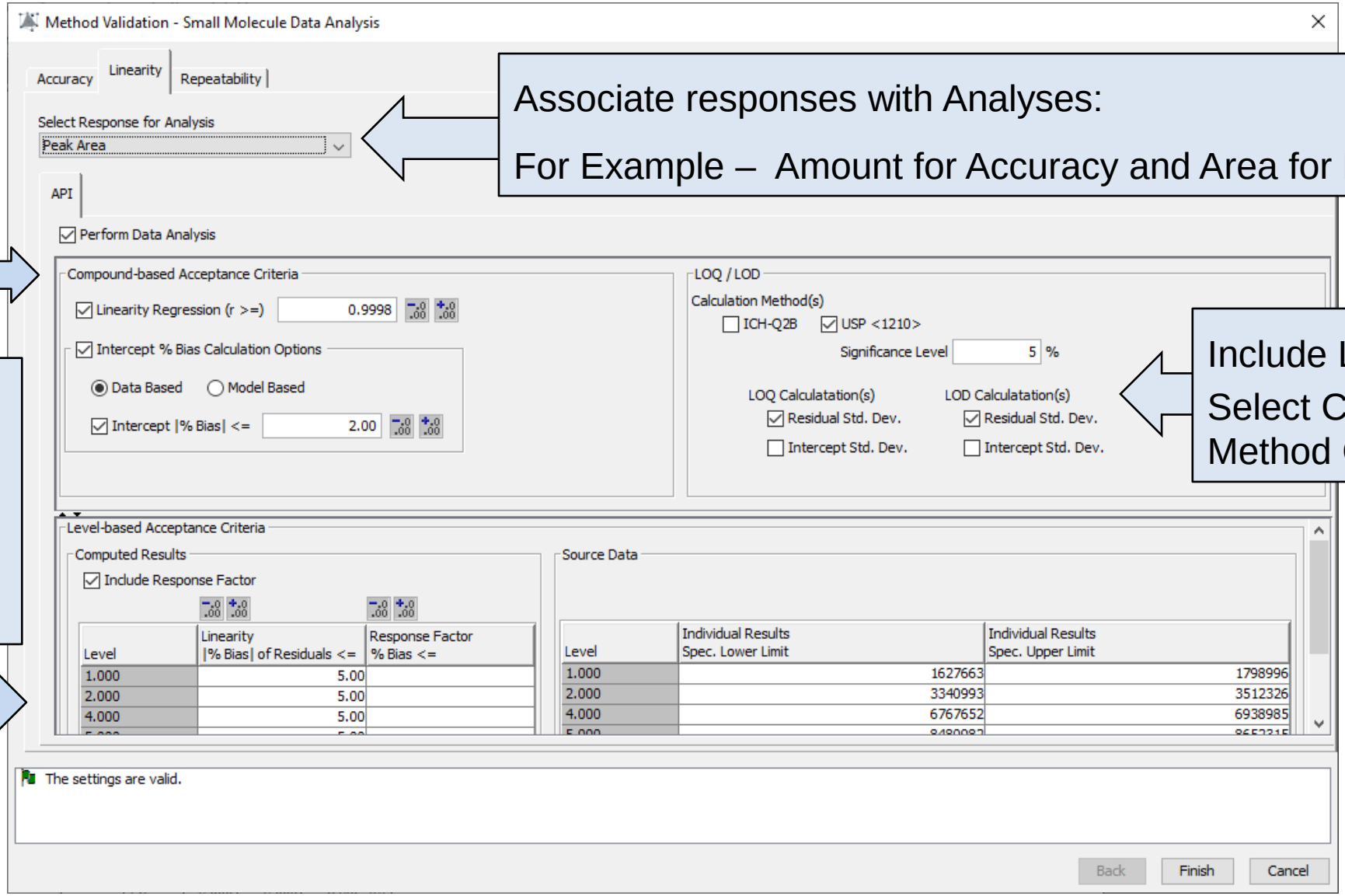


Flexible setup of the required Standards Strategies.

3. Auto-generated Experiment Design



4. Analysis Wizard for CDS Imported Results



Associate responses with Analyses:

For Example – Amount for Accuracy and Area for Linearity.

Set Global and Level-specific Acceptance Criteria, including Spec Limits for Data

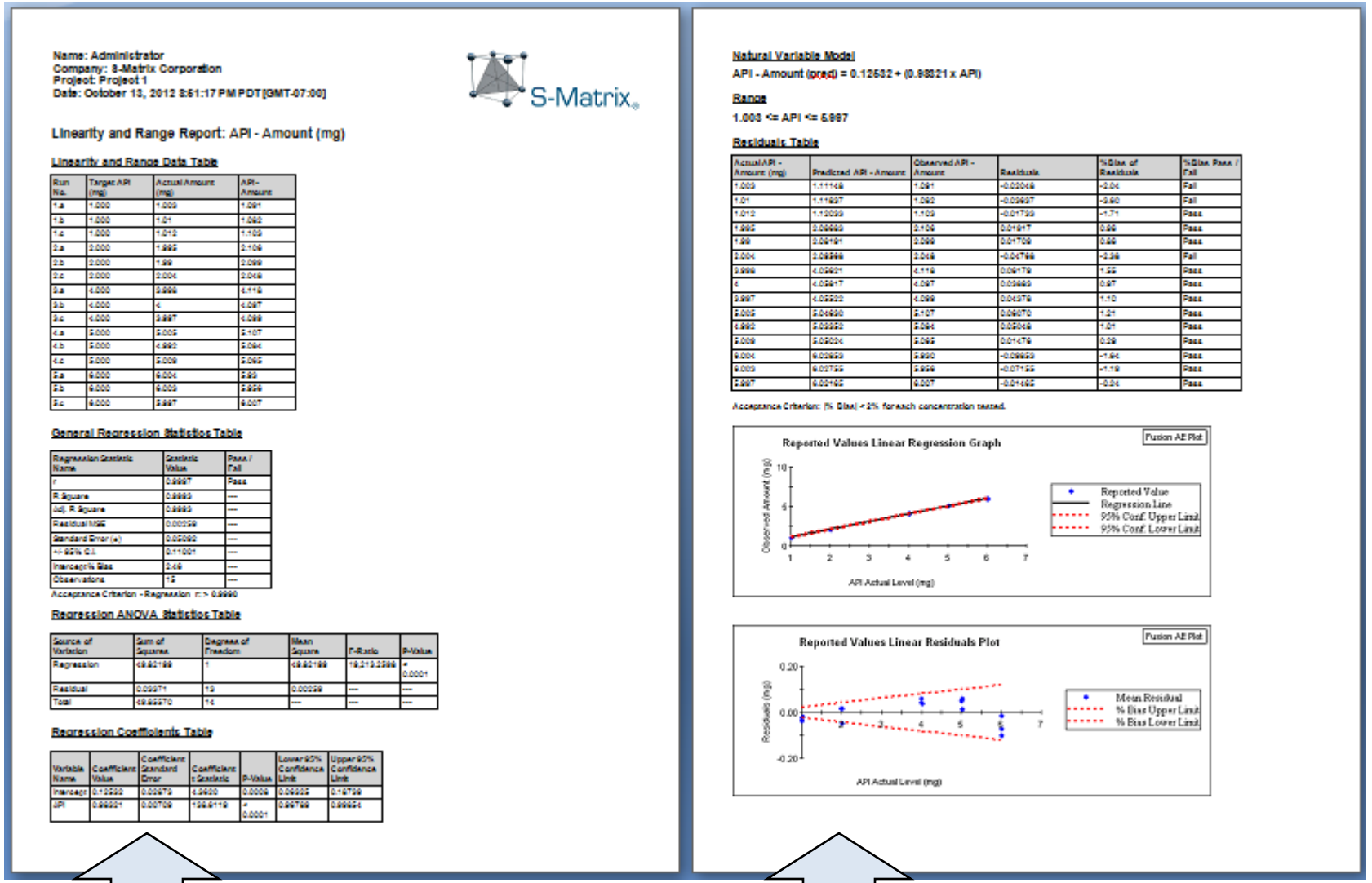
Include LOQ and LOD.

Select Calculation Method Options.

5. Instant Analysis, Graphing, and Reporting

ICH Q2(R2):

Data derived from the regression line may help to provide mathematical estimates of the linearity. A plot of the data, the correlation coefficient or coefficient of determination, y-intercept and slope of the regression line should be provided. An analysis of the deviation of the actual data points from the regression line is helpful for evaluating linearity (e.g., for a linear response, the impact of any non-random pattern in the residuals plot from the regression analysis should be assessed).



Name: Administrator
Company: S-Matrix Corporation
Project: Project 1
Date: October 13, 2012 8:51:17 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
1.a	1.000	1.009	1.091
1.b	1.000	1.01	1.092
1.c	1.000	1.012	1.103
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	3.996	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	5.092
5.a	6.000	6.004	5.93
5.b	6.000	6.003	5.936
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	49.92199	1	49.92199	19,213.2399	< 0.0001
Residual	0.03371	13	0.00259	---	---
Total	49.95570	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	0.03879	4.3920	0.0008	0.04325	0.16739
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

1.000 <= API <= 5.987

Residuals Table

Actual API - Amount (mg)	Predicted API - Amount	Observed API - Amount	Residuals	%Bias of Residuals	%Bias Pass / Fail
1.009	1.11149	1.091	-0.02049	-2.04	Fail
1.01	1.11257	1.092	-0.02057	-2.60	Fail
1.012	1.12093	1.103	-0.01793	-1.71	Pass
1.995	2.09883	2.109	0.01017	0.99	Pass
1.99	2.09191	2.099	0.01709	0.86	Pass
2.004	2.09599	2.049	-0.04799	-2.39	Fail
3.996	4.03921	4.119	0.08179	1.23	Pass
4	4.03917	4.097	0.05883	0.97	Pass
3.997	4.05522	4.099	0.04378	1.10	Pass
5.005	5.04930	5.107	0.05770	1.21	Pass
4.992	5.03932	5.094	0.05048	1.01	Pass
5.009	5.05004	5.092	0.04179	0.59	Pass
6.004	6.03929	5.930	-0.09929	-1.64	Pass
6.003	6.03722	5.939	-0.07122	-1.19	Pass
5.997	6.02162	6.007	-0.01462	-0.24	Pass

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

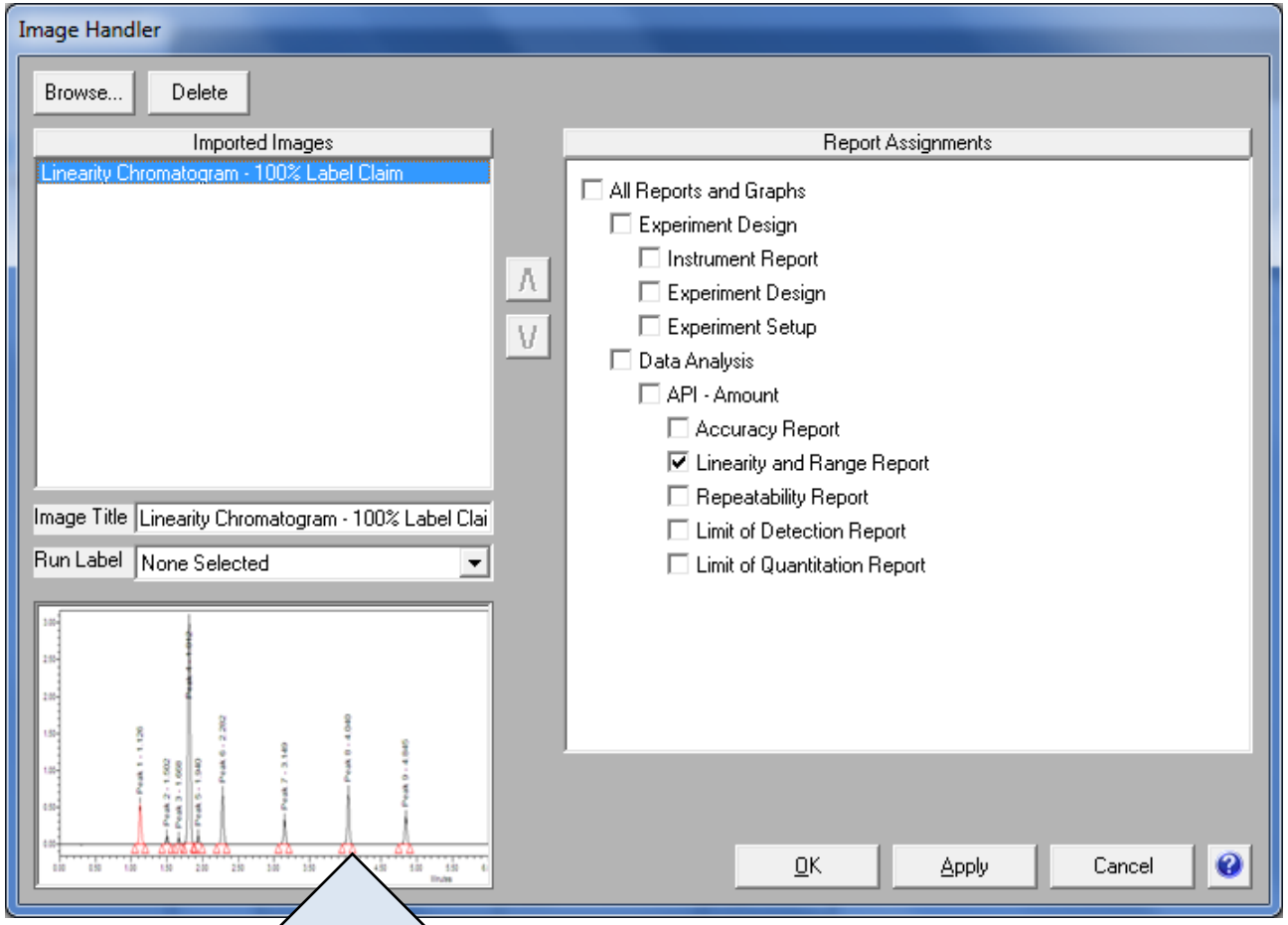
Fusion QbD instantly creates formal reports with all required tables and graphs.

5. Instant Analysis, Graphing, and Reporting

ICH Q2(R2):

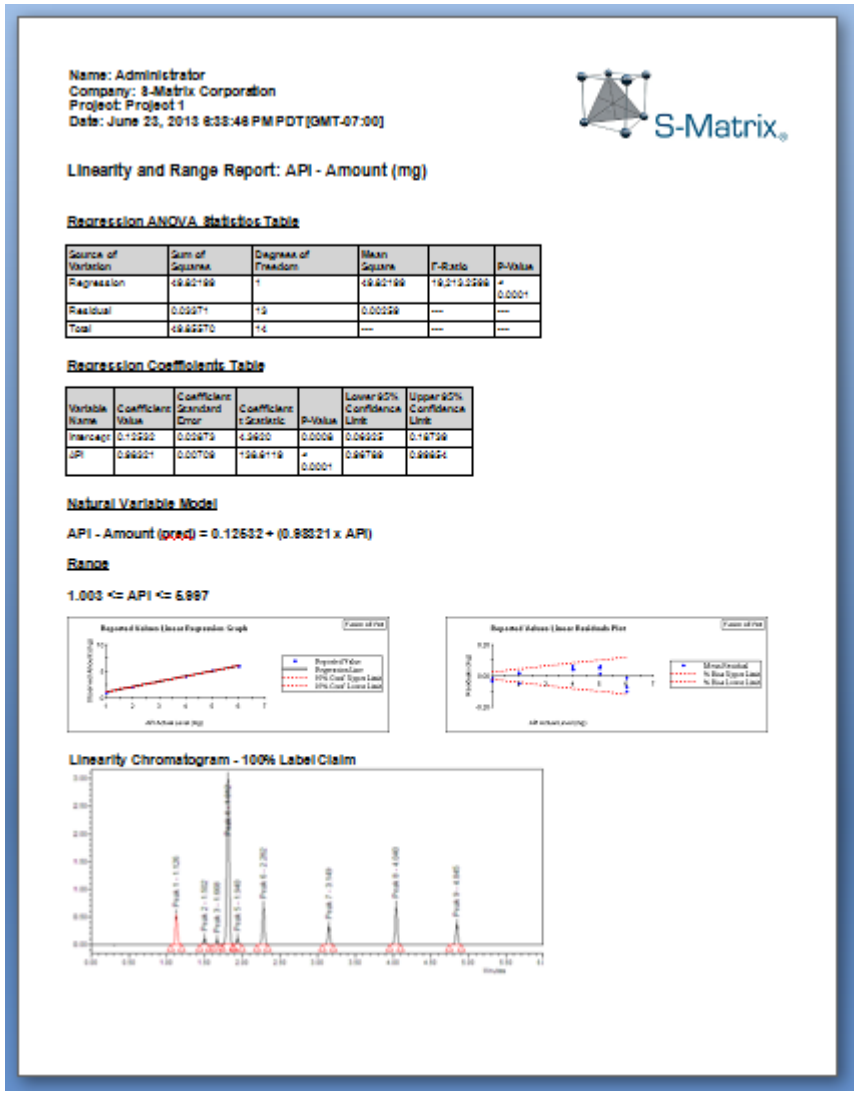
Representative data (e.g., chromatograms, electropherograms, spectra, biological response) should be used to demonstrate specificity and relevant components should be labelled, if appropriate.

For a purity or impurity test, discrimination can be established by stressing or spiking product to achieve appropriate levels of impurities or related substances and demonstrating the absence of interference.



Reports can be augmented with images of relevant chromatograms.

5. Instant Analysis, Graphing, and Reporting



Name: Administrator
Company: S-Matrix Corporation
Project: Project 1
Date: June 23, 2013 8:33:48 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	19,210.5396	< 0.0001
Residual	0.03971	13	0.00306	---	---
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	0.01973	6.3520	0.0000	0.08268	0.16796
API	0.88321	0.00709	138.6119	< 0.0001	0.86788	0.89854

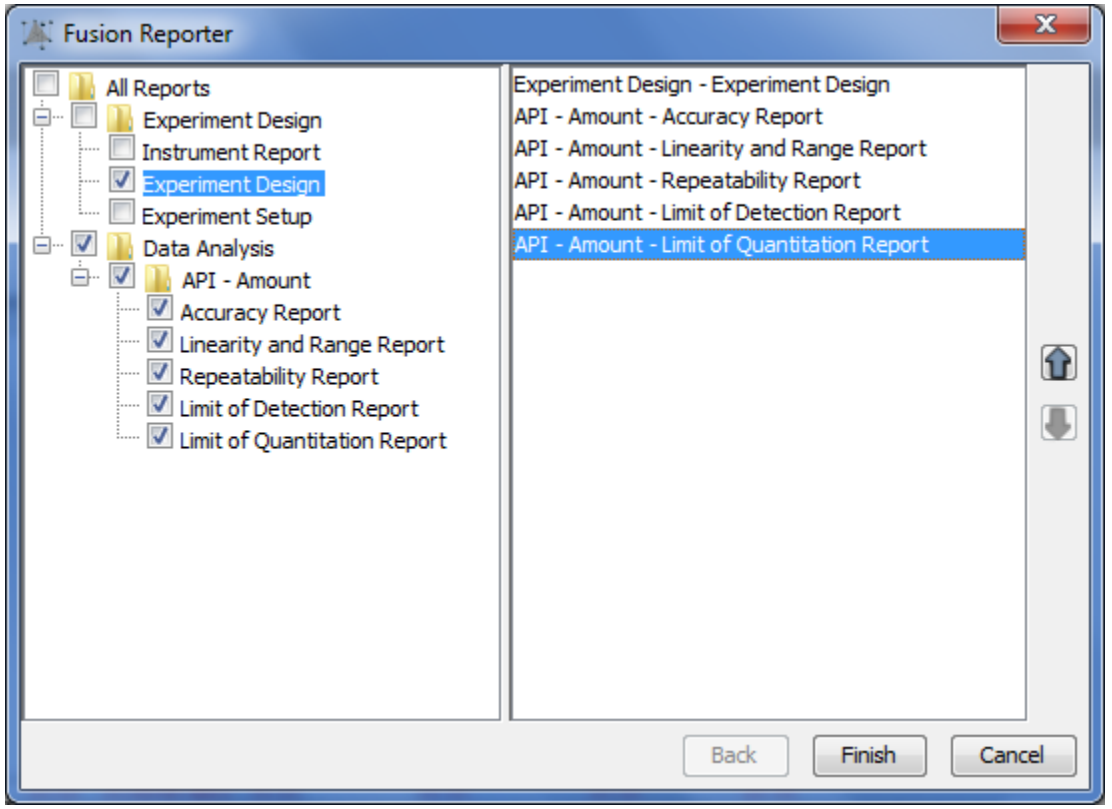
Natural Variable Model

API - Amount (pred) = 0.12532 + (0.88321 x API)

Range

1.003 <= API <= 5.997

Linearity Chromatogram - 100% Label Claim



Reports meet all output format requirements:

.TXT / .RTF / .DOC / .PDF / .HTML / XLSX

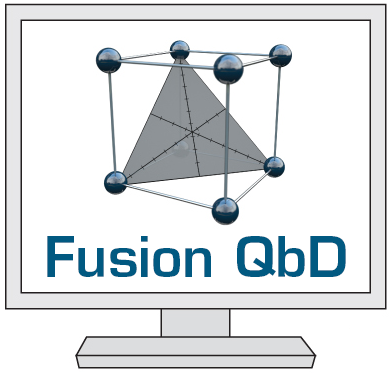
All the Critical QbD Capabilities You Need

Critical QbD Capability	FMV
Complete Method Validation Experiment Suite	✓
Simple Experiment Workflows	✓
Full LC Experiment Automation	✓
USP 1210> Tolerance and Prediction Interval Metrics	✓

- Replication Strategy and Total Analytical Error
- Accuracy and Repeatability
- Analytical Method Transfer

Automated Experiment Workflow

Steps 1 and 2

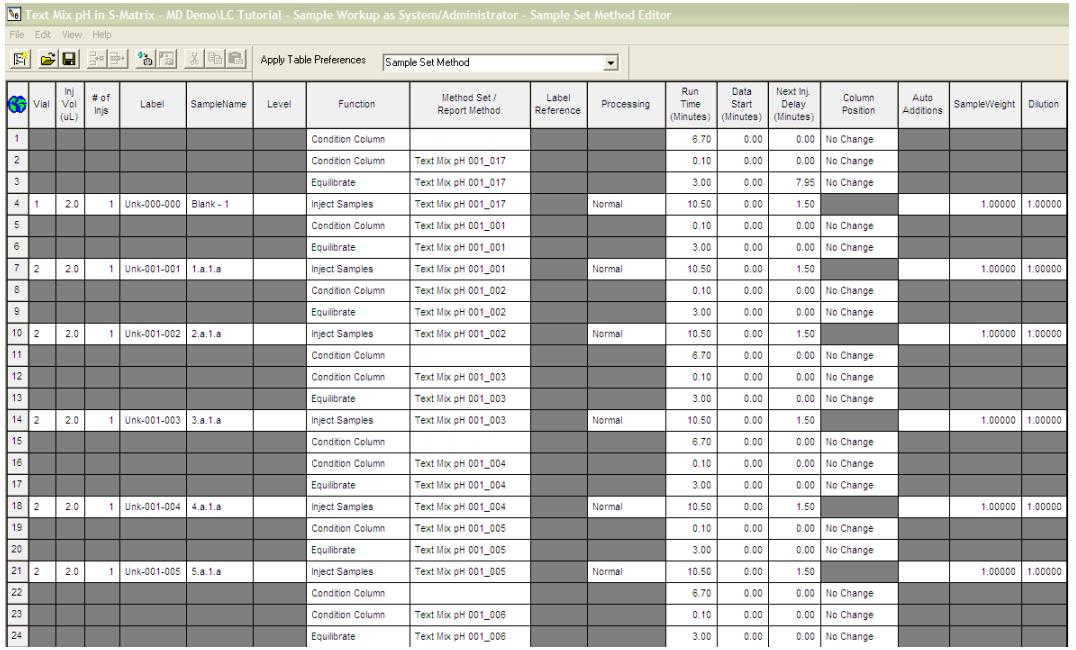


Generates Selected Validation Experiment

Automatically Builds Sequence with Standards Protocol and Assigns Method

Step 3

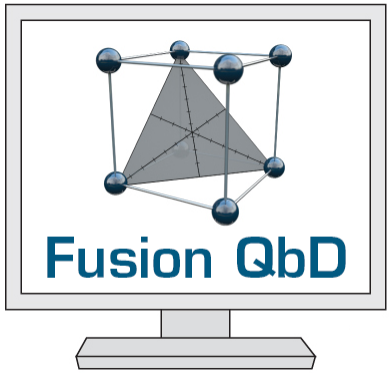
Chromatography Data Software (CDS)



	Vial	Inj Vol (uL)	# of Injs	Label	SampleName	Level	Function	Method Set / Report Method	Label Reference	Processing	Run Time (Minutes)	Data Start (Minutes)	Next Inj. Delay (Minutes)	Column Position	Auto Additions	SampleWeight	Dilution
1							Condition Column				6.70	0.00	0.00	No Change			
2							Condition Column	Text Mix pH 001_017			0.10	0.00	0.00	No Change			
3							Equilibrate	Text Mix pH 001_017			3.00	0.00	7.95	No Change			
4	1	2.0	1	Unk-000-000	Blank - 1		Inject Samples	Text Mix pH 001_017		Normal	10.50	0.00	1.50			1.00000	1.00000
5							Condition Column	Text Mix pH 001_001			0.10	0.00	0.00	No Change			
6							Equilibrate	Text Mix pH 001_001			3.00	0.00	0.00	No Change			
7	2	2.0	1	Unk-001-001	1.a.1.a		Inject Samples	Text Mix pH 001_001		Normal	10.50	0.00	1.50			1.00000	1.00000
8							Condition Column	Text Mix pH 001_002			0.10	0.00	0.00	No Change			
9							Equilibrate	Text Mix pH 001_002			3.00	0.00	0.00	No Change			
10	2	2.0	1	Unk-001-002	2.a.1.a		Inject Samples	Text Mix pH 001_002		Normal	10.50	0.00	1.50			1.00000	1.00000
11							Condition Column				6.70	0.00	0.00	No Change			
12							Condition Column	Text Mix pH 001_003			0.10	0.00	0.00	No Change			
13							Equilibrate	Text Mix pH 001_003			3.00	0.00	0.00	No Change			
14	2	2.0	1	Unk-001-003	3.a.1.a		Inject Samples	Text Mix pH 001_003		Normal	10.50	0.00	1.50			1.00000	1.00000
15							Condition Column				6.70	0.00	0.00	No Change			
16							Condition Column	Text Mix pH 001_004			0.10	0.00	0.00	No Change			
17							Equilibrate	Text Mix pH 001_004			3.00	0.00	0.00	No Change			
18	2	2.0	1	Unk-001-004	4.a.1.a		Inject Samples	Text Mix pH 001_004		Normal	10.50	0.00	1.50			1.00000	1.00000
19							Condition Column	Text Mix pH 001_005			0.10	0.00	0.00	No Change			
20							Equilibrate	Text Mix pH 001_005			3.00	0.00	0.00	No Change			
21	2	2.0	1	Unk-001-005	5.a.1.a		Inject Samples	Text Mix pH 001_005		Normal	10.50	0.00	1.50			1.00000	1.00000
22							Condition Column				6.70	0.00	0.00	No Change			
23							Condition Column	Text Mix pH 001_006			0.10	0.00	0.00	No Change			
24							Equilibrate	Text Mix pH 001_006			3.00	0.00	0.00	No Change			

Automated, Audited Data Exchange Preserves Data Integrity

Automated Experiment Workflow



Step 4

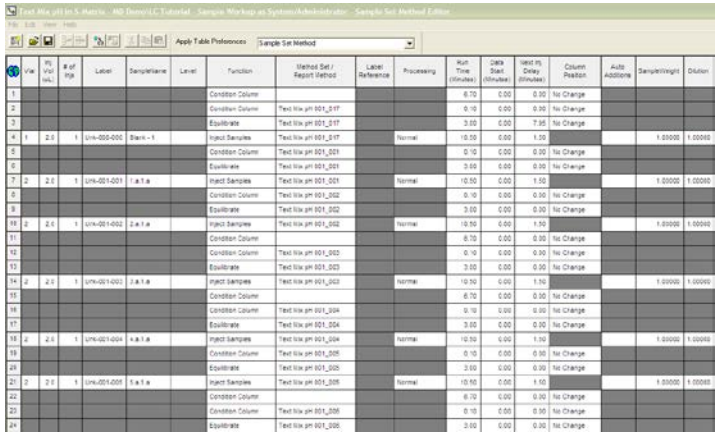
Automatically Retrieve All Chromatogram Results Data

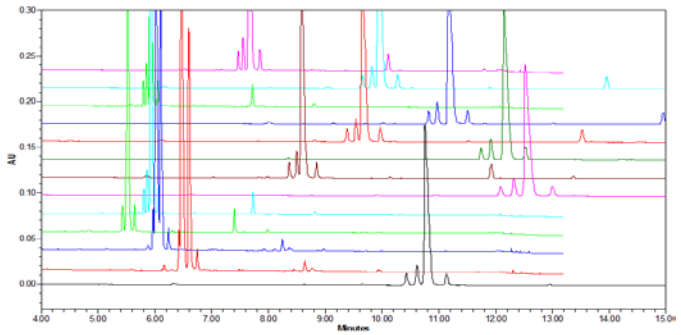
Step 5

Automated analysis, graphing, and reporting.

Multiple Report Formats: RTF, DOC, HTML, PDF, XLSX

Chromatography Data Software (CDS)





Automated, Audited Data Exchange Preserves Data Integrity

Full Automation for Robustness Studies

Empower™

- ✓ Solvent Selection Valves
- ✓ Column Switching Valves

Alliance HPLC

Alliance iS HPLC

Acquity Binary

Acquity H-Class

Acquity Arc

Acquity UPC²

Full Automation for Robustness Studies

OpenLab – ChemStation Edition

CHROMELEON 7.2

Empower™

- ✓ 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

Full Automation for Robustness Studies

- ✓ Solvent Selection Valves
- ✓ Column Switching Valves

UltiMate LCs

Vanquish Horizon And Flex LCs

All the Critical QbD Capabilities You Need

Critical QbD Capability	FMV
Complete Method Validation Experiment Suite	✓
Simple Experiment Workflows	✓
Full LC Experiment Automation	✓
USP 1210> Tolerance and Prediction Interval Metrics	✓

- Replication Strategy and Total Analytical Error
- Accuracy and Repeatability
- Analytical Method Transfer

<1210> Statistical Tools for Procedure Validation

2. CONSIDERATIONS PRIOR TO VALIDATION

How many individual determinations will compose the reportable value, and how will they be aggregated?

- To answer this question, it is necessary to understand the contributors to the procedure variance and the ultimate purpose of the procedure. **Estimation of variance components** during pre-validation provides useful information for making this decision.

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Full 21 CFR Part 11 Compliance Support	✓
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- **Replication Strategy and Total Analytical Error**
- Accuracy and Repeatability
- Analytical Method Transfer

Replication Strategy Optimization

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

ICH Q14

Reportable Result: the result as generated by the analytical procedure after calculation or processing and applying the described sample replication. (ICH Q2). (*Pg. 19*)

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. *(Pg. 2)*

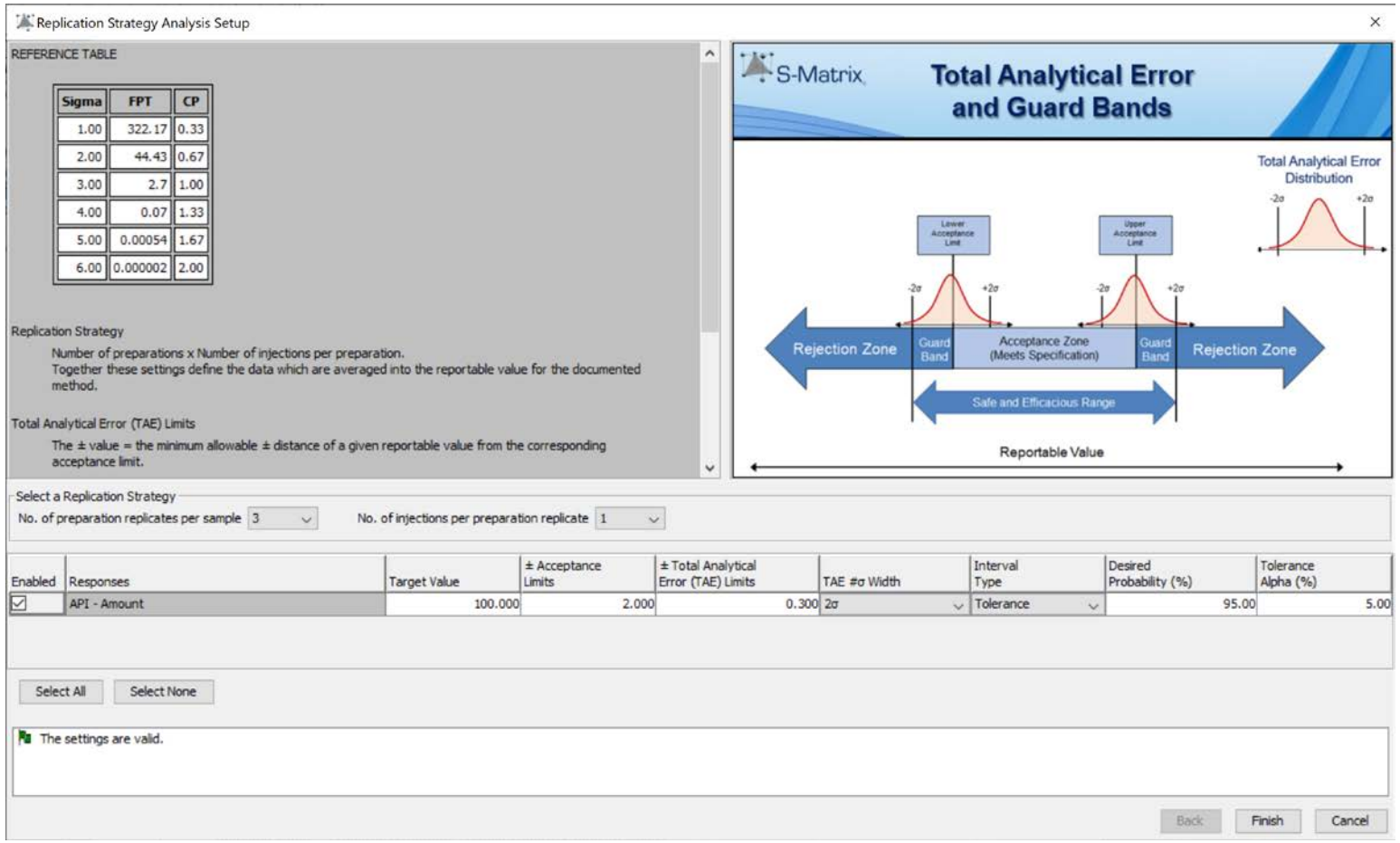
USP <1220>

Stage 1:

Optimization of performance characteristics of the analytical procedure such as accuracy, precision, …; this includes a preliminary replication strategy for samples and standards. (*Pg. 1*)

Replication Strategy Experiment

Define your Proposed Replication Strategy, Target Result Value, Acceptance Limits, Desired TAE Limits, and your Desired Probability and Tolerance (Confidence Interval).



Replication Strategy for the Reportable Value

Between Variables Components of Variation

Variable Name	Variance	Standard Deviation	Degrees of Freedom	t-table Value	(+/-) 95% Confidence Limits	Error Contribution (%)
Sample Preparation	0.008	0.092	4	2.7764	0.25	94.17
Injection	0.001	0.023	20	2.0860	0.04	5.83

Overall Error in a Single Determination

Statistic	Value
Mean	100.051
Variance	0.009
Standard Deviation	0.094
% RSD	0.094

TOST Analysis Results Summary

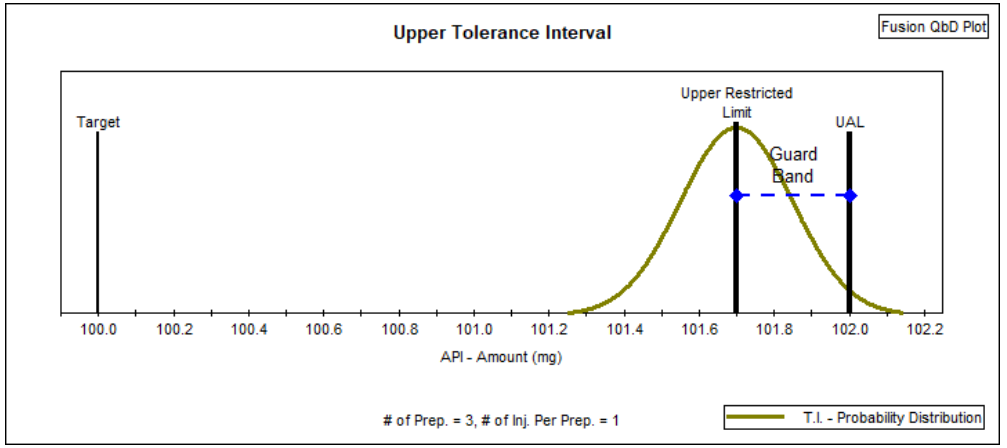
Statistic	Value	Pass/Fail
TAE Width (2σ) - Target	±0.300	
Computed TAE Width (2σ)	±0.156	Pass
FPT	<0.0001	
Cp	12.2271	
Variance	0.003	
Standard Deviation	0.055	
% RSD	0.05	
% CV	0.05	

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	100.051		
Computed Tolerance Interval	±0.293	Pass	
Required Guard Band Width	±0.300		

The computed Tolerance Interval falls within the defined Total Analytical Error Limits.

No. of Injections		No. of Preparations									
		1	2	3	4	5	6	7	8	9	10
1	±2σ	0.2710	0.1910	**0.1564**	0.1355	0.1212	0.1106	0.1024	0.0958	0.0903	0.0857
	T.I.	0.6210	0.3801	**0.2927**	0.2455	0.2151	0.1936	0.1774	0.1647	0.1543	0.1456
2	±2σ	0.2670	0.1888	0.1541	0.1335	0.1194	0.1090	0.1009	0.0944	0.0890	0.0844
	T.I.	0.5299	0.3421	0.2698	0.2295	0.2029	0.1838	0.1693	0.1577	0.1482	0.1402
3	±2σ	0.2657	0.1878	0.1534	0.1328	0.1188	0.1085	0.1004	0.0939	0.0886	0.0840
	T.I.	0.4971	0.3288	0.2620	0.2240	0.1988	0.1806	0.1665	0.1553	0.1461	0.1384
4	±2σ	0.2650	0.1874	0.1530	0.1325	0.1185	0.1082	0.1002	0.0937	0.0883	0.0838
	T.I.	0.4801	0.3221	0.2580	0.2213	0.1968	0.1789	0.1652	0.1542	0.1451	0.1375
5	±2σ	0.2646	0.1871	0.1528	0.1323	0.1183	0.1080	0.1000	0.0935	0.0882	0.0837
	T.I.	0.4697	0.3180	0.2557	0.2197	0.1955	0.1779	0.1644	0.1535	0.1445	0.1369
6	±2σ	0.2643	0.1869	0.1526	0.1322	0.1182	0.1079	0.0999	0.0934	0.0881	0.0836
	T.I.	0.4626	0.3152	0.2541	0.2186	0.1947	0.1773	0.1638	0.1530	0.1441	0.1366
7	±2σ	0.2641	0.1868	0.1525	0.1321	0.1181	0.1078	0.0998	0.0934	0.0880	0.0835
	T.I.	0.4576	0.3133	0.2529	0.2178	0.1941	0.1768	0.1634	0.1527	0.1438	0.1363
8	±2σ	0.2640	0.1867	0.1524	0.1320	0.1181	0.1078	0.0998	0.0933	0.0880	0.0835
	T.I.	0.4537	0.3118	0.2521	0.2172	0.1937	0.1764	0.1631	0.1524	0.1436	0.1361
9	±2σ	0.2639	0.1866	0.1523	0.1319	0.1180	0.1077	0.0997	0.0933	0.0880	0.0834
	T.I.	0.4507	0.3106	0.2514	0.2167	0.1933	0.1762	0.1629	0.1522	0.1434	0.1360
10	±2σ	0.2638	0.1865	0.1523	0.1319	0.1180	0.1077	0.0997	0.0933	0.0879	0.0834
	T.I.	0.4483	0.3097	0.2509	0.2164	0.1931	0.1759	0.1627	0.1521	0.1433	0.1358



Replication Strategy for the Reportable Value

Fusion QbD reports the Components of Variation and the Corresponding % Contributions to Total Analytical Error.

Between Variables Components of Variation

Variable Name	Variance	Standard Deviation	Degrees of Freedom	t-table Value	(+/-) 95% Confidence Limits	Error Contribution (%)
Sample Preparation	0.008	0.092	4	2.7764	0.254	94.17
Injection	0.001	0.023	20	2.0860	0.048	5.83

Overall Error in a Single Determination

Statistic	Value
Mean	100.051
Variance	0.009
Standard Deviation	0.094
% RSD	0.094

Fusion QbD also reports the TOST Results (Traditional Precision Only) and the USP <1210> Interval Results (Combined Precision + Bias).

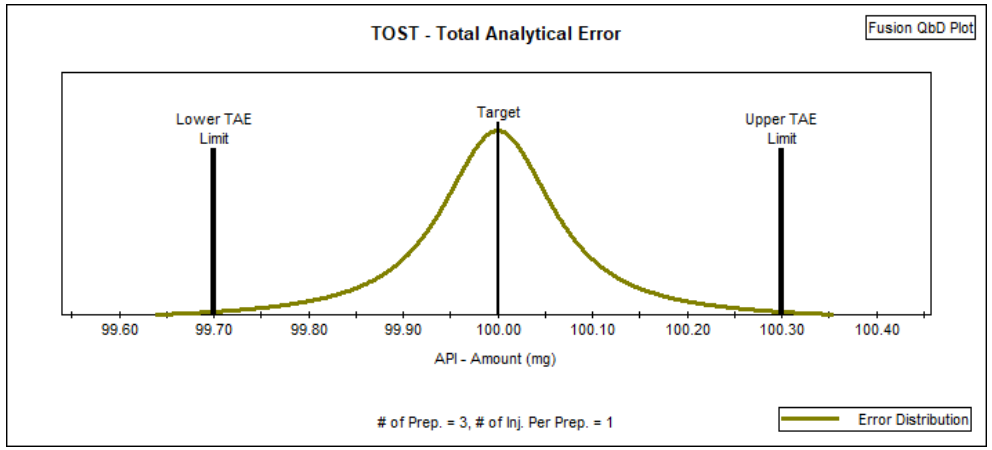
TOST Analysis Results Summary

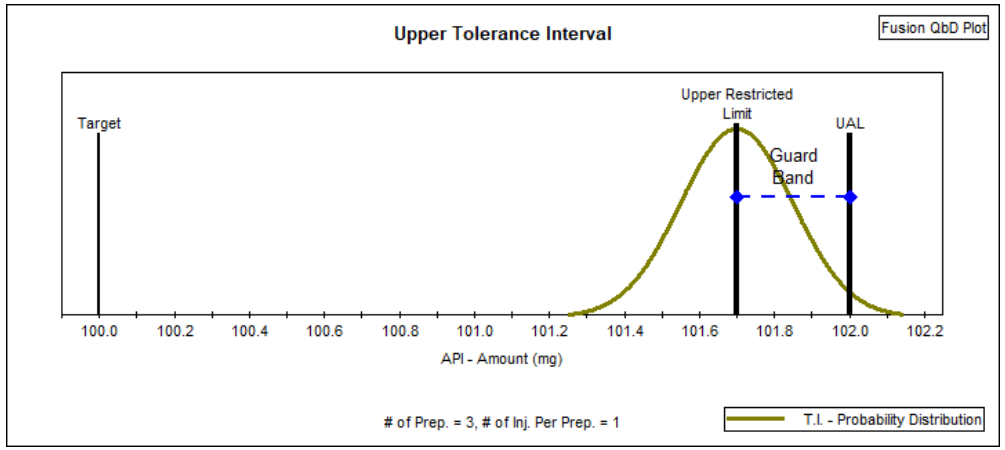
Statistic	Value	Pass/Fail
TAE Width (2σ) - Target	±0.300	
Computed TAE Width (2σ)	±0.156	Pass
FPT	<0.0001	
Cp	12.2271	
Variance	0.003	
Standard Deviation	0.055	
% RSD	0.05	
% CV	0.05	

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	100.051		
Computed Tolerance Interval	±0.293	Pass	
Required Guard Band Width	±0.300		

The computed Tolerance Interval falls within the defined Total Analytical Error Limits.





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Critical QbD Capability	FMV
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Full 21 CFR Part 11 Compliance Support	✓
Complete Method Validation Experiment Suite	✓
Simple Experiment Workflows	✓
Full LC Experiment Automation	✓
USP 1210> Tolerance and Prediction Interval Metrics	✓

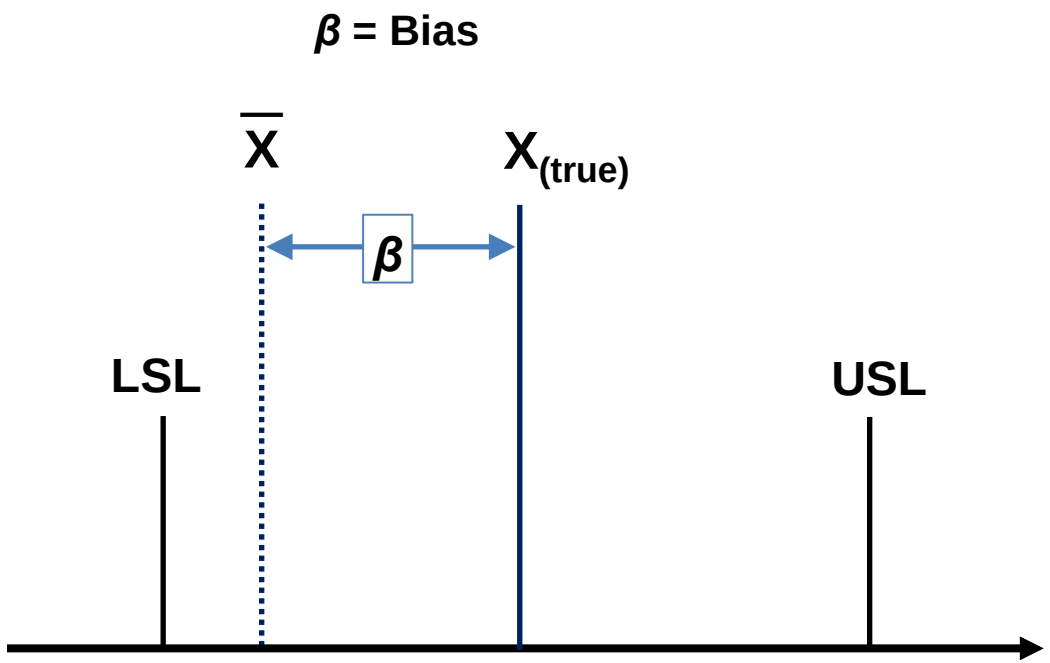
- Replication Strategy and Total Analytical Error
- **Accuracy and Repeatability**
- Analytical Method Transfer

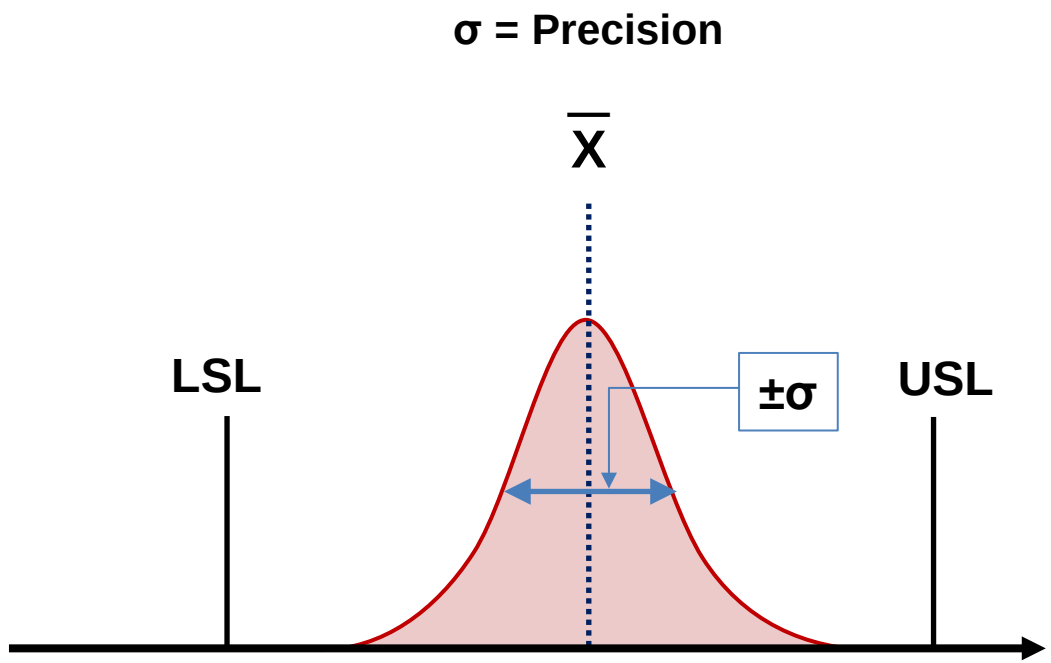
<1210> Statistical Tools for Procedure Validation

3. ACCURACY AND PRECISION

3.2 Combined Validation of Accuracy and Precision

The illustration below shows that the method will pass System Suitability performance for the Critical Quality Attribute (CQA) being tested SST when Accuracy (***β* – bias estimate**) and Precision (**σ – variation estimate**) **are assessed independently (= High Risk Approach).**



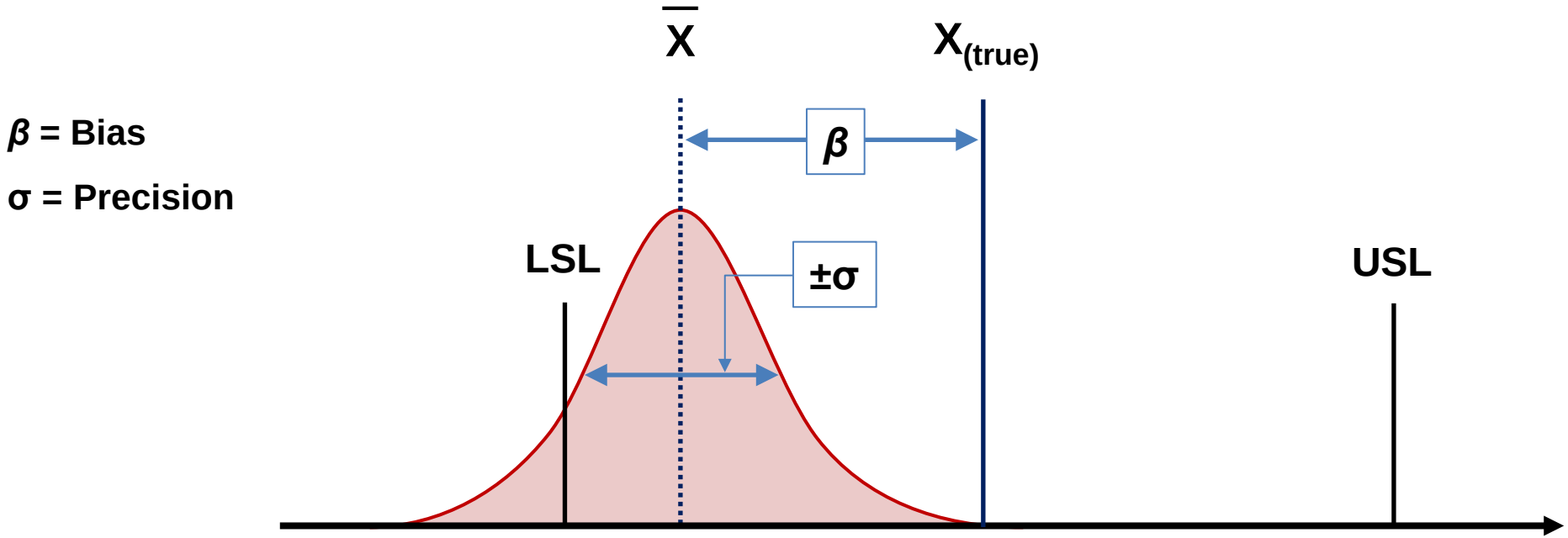


<1210> Statistical Tools for Procedure Validation

3. ACCURACY AND PRECISION

3.2 Combined Validation of Accuracy and Precision

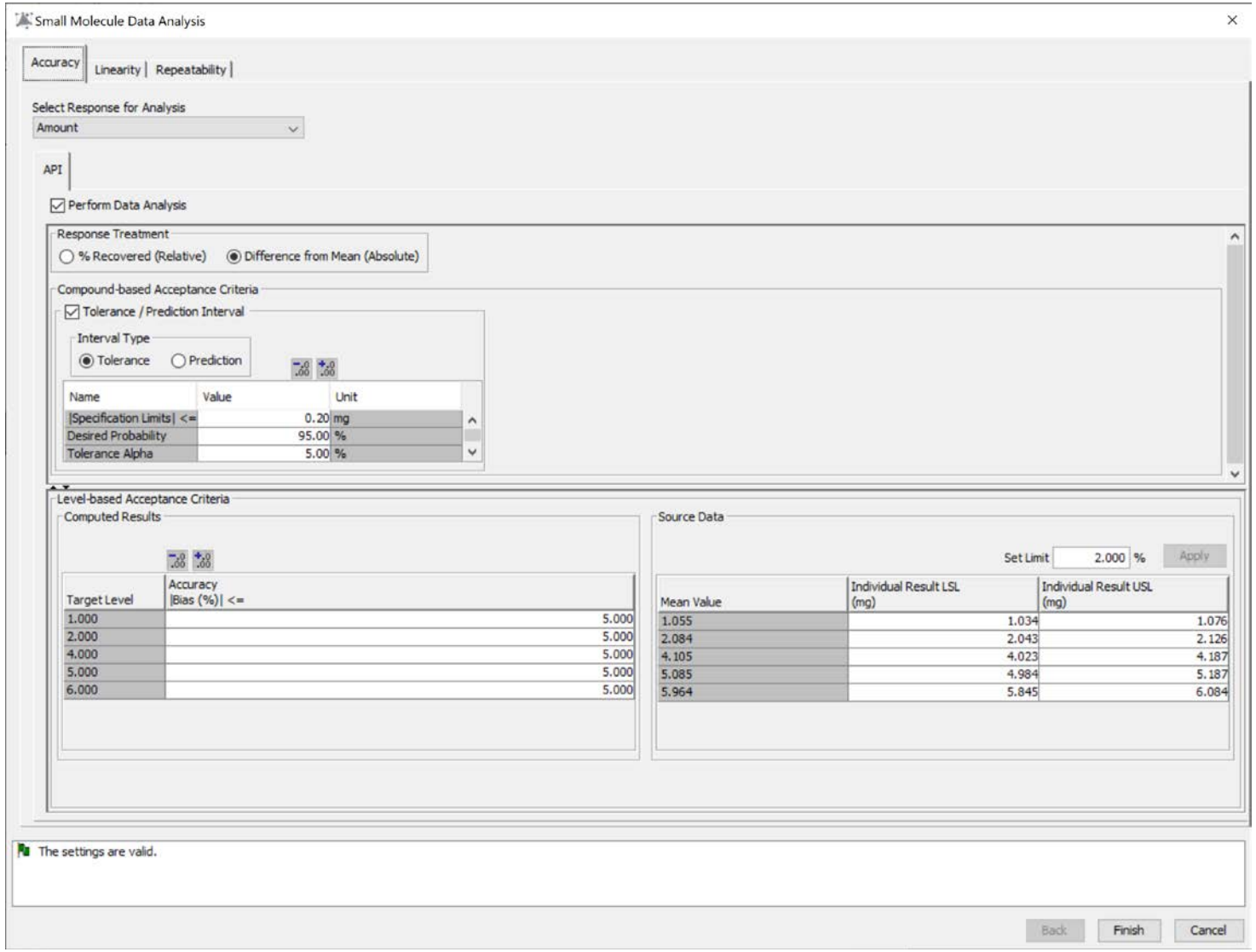
However, as the illustration below shows – the method does not have acceptable System Suitability performance for the Critical Quality Attribute (CQA) being tested when both Accuracy (*β* – bias estimation) and Precision (σ – variation estimation) **are assessed together (= Low Risk Approach).**



Simple Analysis Setup Wizard

Define your Acceptance Limits:

- **Compound-based – USP <1210>**
- **Computed Results**
- **Source Data**



Accuracy, Linearity, Repeatability, Intervals

Automated Reporting – all Results and Graphs for Accuracy, Linearity, Repeatability, and USP <1210> Intervals.

General Regression Statistics

Regression Statistic Name	Statistic Value
R Square	0.9999
Adj. R Square	0.9999
Residual MSE	682,072,000
Standard Error (±)	26,117
+/- 95% C.I.	56,421
Observations	15

General Validation Acceptance Criteria

Regression Statistic Name	Statistic Value	Validation	Pass / Fail
R	1.0000	0.9998	Pass
Intercept % Bias - Data Based	-0.17	2.00	Pass

Regression Coefficients

Variable Name	Coefficient Value	Coefficient Standard Error	Coefficient t Statistic	P-Value	Lower 95% Confidence Limit	Upper 95% Confidence Limit
Intercept	-11,549	14,735	< 0.0001	0.4472	-43,382	20,283
API	1,715,593	3,638	471.5873	< 0.0001	1,707,734	1,723,452

Natural Variable Model

API - Area (pred) = -11,549 + (1,715,593 x API - Weight Amount)

Range

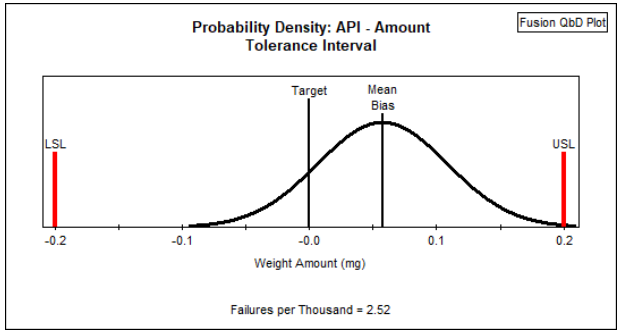
1.003 (mg) <= API - Weight Amount <= 6.004 (mg)

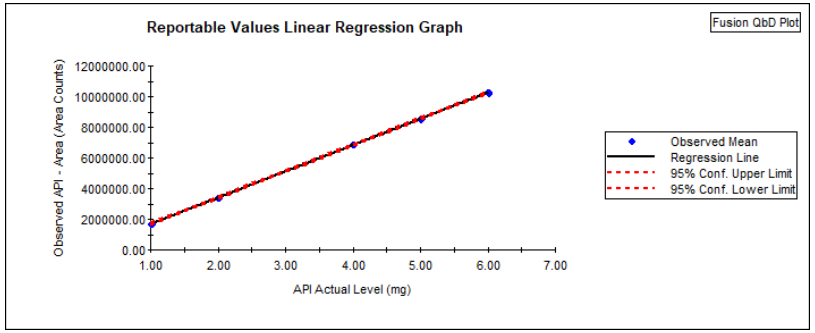
Accuracy Results

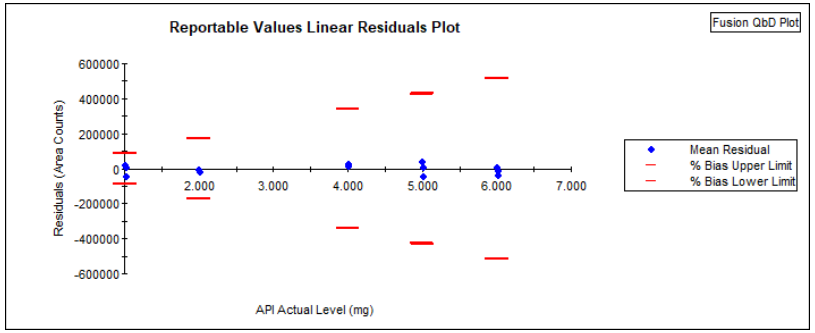
Target API (mg)	Mean Observed API - Amount (mg)	Standard Deviation	Lower 95% Confidence Limit	Upper 95% Confidence Limit	RSD (%)	Mean % Bias	Accuracy |% Bias| <=	% Bias Pass/Fail
1.000	1.055	0.031	0.921	1.190	2.96	4.654	5.000	Pass
2.000	2.084	0.032	1.948	2.221	1.52	4.412	5.000	Pass
4.000	4.105	0.012	4.055	4.155	0.28	2.659	5.000	Pass
5.000	5.085	0.021	4.995	5.176	0.41	1.666	5.000	Pass
6.000	5.964	0.039	5.796	6.133	0.66	-0.616	5.000	Pass

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







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USP 1210> Tolerance and Prediction Interval Metrics	✓

- Replication Strategy and Total Analytical Error
- Accuracy and Repeatability
- **Analytical Method Transfer**

USP ⟨ 1224⟩ – Transfer of Analytical Procedures

Comparative Testing

Comparative testing requires the analysis of a predetermined number of samples of the same lot by both the sending and the receiving units. Other approaches may be valid, e.g., if the receiving unit meets a predetermined acceptance criterion for the recovery of an impurity in a spiked product. Such analysis is based on a preapproved transfer protocol that stipulates the details of the procedure, the samples that will be used, **and the predetermined acceptance criteria, including acceptable variability**. Meeting the predetermined acceptance criteria is necessary to assure that the receiving unit is qualified to run the procedure.

Analytical Method Transfer Example

Transferring Lab

Fusion QbD Sequence Execution

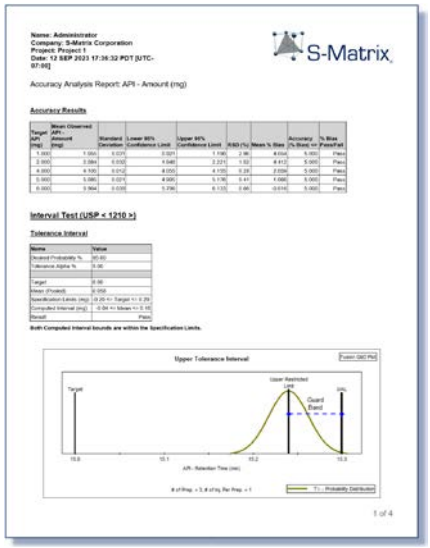
Fusion QbD Sequence Execution

Receiving Lab

Chromatography Data Software

ALR Design

Chromatogram Results Data



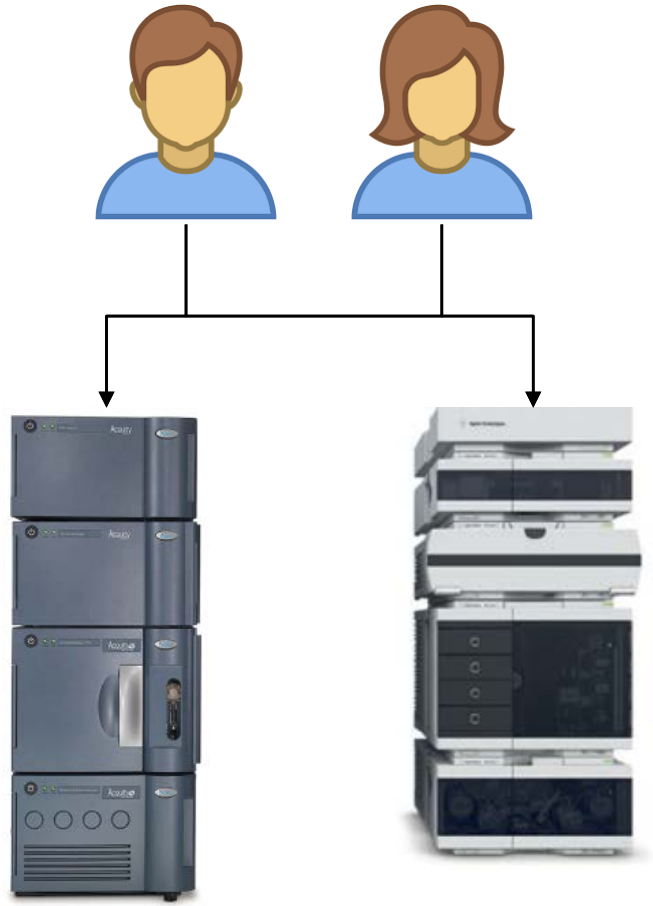
1. Fusion QbD – Exports experiment to the CDS as Ready-to-Run sequence, methods, standards
2. Sequence is run at both labs.
3. Fusion QbD – Imports results for instant and complete analysis and reporting.

Accuracy
Linearity
Repeatability
Tolerance Interval
Pass/Fail 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 o**n each LC on each Day.**

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

Key Benefits of FMV

1. **Consistency – Workflow and Reporting.**

   Work is standardized – done the same way every time. Reporting is standardized, complete, easy to communicate.

2. **Simplicity**

   Tremendous ease of use. Very brief learning curve. Clearly defined templatable workflows with built-in workflow management.

3. **Speed (Productivity)**

   Automation and simplified workflows dramatically increase productivity. Review process is minimized and simplified.

4. **Regulatory Alignment and Completeness**

   All required validation experiment types are supported. Reporting meets regulatory requirements. Reports can be attached to Project specific narrative documents.

Key Benefits of FMV

5. Platform Independence

Support for Empower, ChemStation, and Chromeleon means that the standardized workflows and reporting can be easily extended to users of other platforms at other sites or other companies (e.g. CMOs).

6. Customer Support

Our support is top-rated worldwide. S-Matrix and our local distributors have a multi-year history of proven ability to meet all our customer's support needs.

End of Presentation

Analytical Procedure Lifecycle Management Workflow