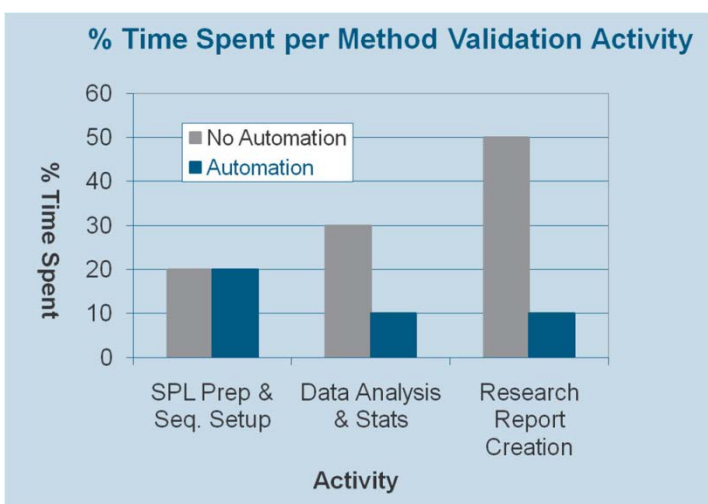


## Fusion Method Validation



### The Only Software That Has It All!

- Fully automates all your method validation experiments on multiple instruments and CDS systems!
- Contains statistically rigorous and defensible automated robustness testing!
- Handles multiple compounds and creates complete reports for each.
- Can shorten your LC method validation time by as much as 75%!



# Automated LC Method Validation Experiments

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
- defines the limit of acceptable performance of the method (LOQ, LOD)

## Key Benefits

- **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, full audit logging
  - Workflow management system with E-review and E-approve loops
- **Easy setup of experiments** –
  - Create standardized workflow templates
  - Facilitate rigorous practice and defensibility
- **Simple documentation review and reporting** –
  - Easy to defend and communicate
  - Reports meet all FDA and ICH guidelines

## Early Phase Method Validation (Performance Characterization)

Analytical Capability and System Suitability  
Specificity  
Filter Validation  
Accuracy  
Linearity and Range  
LOQ, LOD  
Repeatability\* (intra-assay precision)  
Sample Solution Stability (stability for a given time period under prescribed conditions)

## Final Phase Method Validation (FDA and ICH Submittal Quality)

Analytical Capability and System Suitability  
Specificity  
Accuracy/Linearity and Range/Repeatability – Combined Design  
(ICH-Q2A – Accuracy, Linearity, and Repeatability can be done together as a single combined experiment)  
LOQ, LOD  
Intermediate Precision and Reproducibility (USP Ruggedness)  
Robustness – done the right way!

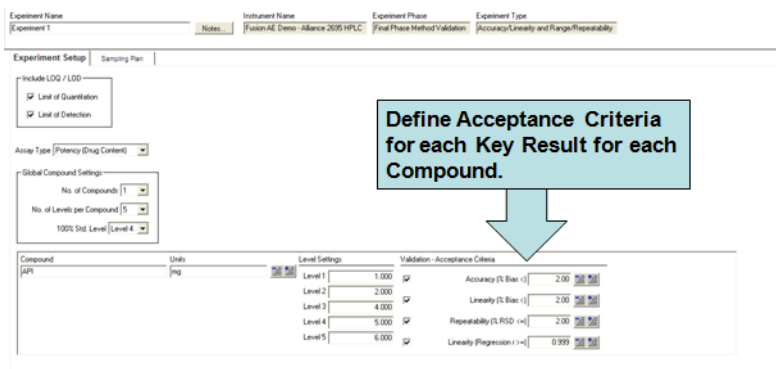
## Automated LC Method Validation – Five Step Workflow

1. You complete a simple experiment setup template.
2. Fusion QbD creates the Validation Experimental Design and exports it to the CDS.
3. The CDS runs the validation experiment sequence.
4. Fusion QbD imports and analyzes the results.
5. Fusion QbD automatically creates final reports and graphs.

## Automated Workflow Illustrated – Combined Accuracy / Linearity / Repeatability

### Step 1 – You Complete the Template

**Fusion LC Method Validation Software (FMV)** has simple experiment setup templates for each type of validation experiment. The simple Linearity and Range template is shown below with user definable settings:



Compound	Units	Level Settings	Validation - Acceptance Criteria
API	mg	Level 1 1.000	Accuracy (% Bias <=) 2.00
		Level 2 2.000	Linearity (% Bias <=) 2.00
		Level 3 4.000	Repeatability (% RSD <=) 2.00
		Level 4 5.000	Linearity (Regression r >=) 0.999
		Level 5 6.000	

### User-definable Settings – Basic Setup

- Include Limit of Quantitation
- Include Limit of Detection
- No. of Compounds
- No. of Levels per Compound
- 100% Standard Level
- No. of Injections of 100% Level

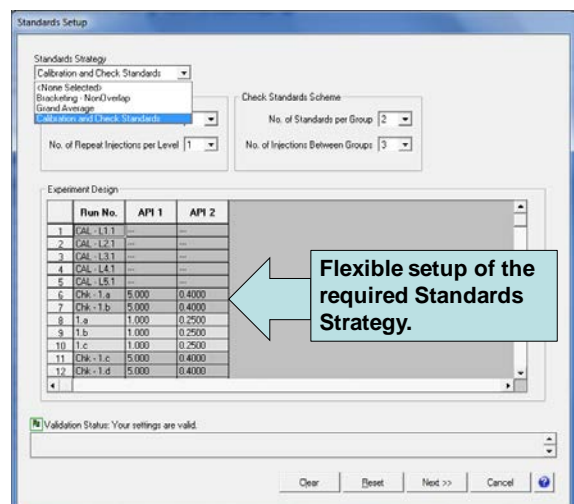
### User-definable Settings – Method Performance Acceptance Criteria

- Linearity (% Bias <=)
- Accuracy (% Bias <=)
- Linearity (Regression r >=)
- Repeatability (% RSD <=)

### User-definable Settings – Standards Setup

**FMV** has a flexible Standards Setup wizard which enables you to select your desired standards strategy for results quantitation within the CDS:

- Bracketing – Overlap
- Bracketing – Non-overlap
- Grand Average
- Calibration and Check Standards




Run No.	API 1	API 2
1	CAL - L3.1	...
2	CAL - L2.1	...
3	CAL - L3.1	...
4	CAL - L4.1	...
5	CAL - L5.1	...
6	Chk - 1 a	5.000 0.4000
7	Chk - 1 b	5.000 0.4000
8	1 a	1.000 0.2500
9	1 b	1.000 0.2500
10	1 c	1.000 0.2500
11	Chk - 1 c	5.000 0.4000
12	Chk - 1 d	5.000 0.4000

## Step 2 – Fusion QbD Creates the Validation Experimental Design and Exports it to the CDS

**FMV** automatically constructs the validation experiment designs within the CDS as ready-to-run sequences/sample with the proper Vial No. and Injection Type designations for Samples, Standards, and Blanks.

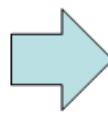
Name: Administrator  
 Company: S-Matrix  
 Project: Project 1  
 Date: October 17, 2012 2:11:07 AM PDT [GMT-07:00]



**Experiment Design - Experiment 1**

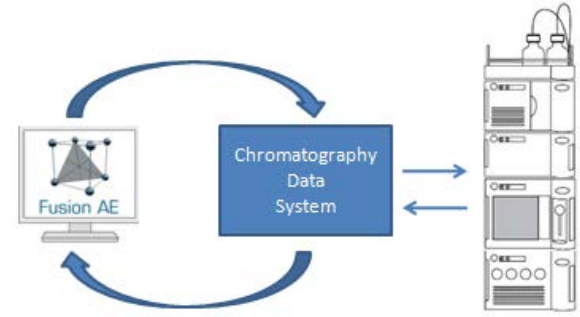
**Experiment Design Matrix**

Run No.	Sample Set No.	API1 (mg)	API2 (%)
1.a	1	1.000	0.2500
1.b	1	1.000	0.2500
1.c	1	1.000	0.2500
2.a	1	2.000	0.3500
2.b	1	2.000	0.3500
2.c	1	2.000	0.3500
3.a	1	4.000	0.4000
3.b	1	4.000	0.4000
3.c	1	4.000	0.4000
4.a	1	6.000	0.4500
4.b	1	6.000	0.4500
4.c	1	6.000	0.4500



## Step 3 – CDS runs the Validation Experiment

**FMV** sequences run automatically on the CDS. **FMV** even enables you to include a Shutdown method as the last method run so that you can execute **FMV** sequences overnight while you sleep!



## Step 4 – Fusion QbD Imports and Analyzes the Chromatogram Results

**FMV** automatically imports the required peak result data from the CDS, and re-maps the results to the design for automated analysis, graphing, and reporting. This is a key feature ensuring quality, as manual transcription is a common source of error and risk.

Enter Sample Preparation Data

Response Name: \_\_\_\_\_ Response Units: [mg]

Run	API 1 Target	API 1 Actual	API 2 Target	API 2 Actual
1	1.a	1.000	1.003	0.2500
2	1.b	1.000	1.011	0.2500
3	1.c	1.000	1.012	0.2500
4	2.a	2.000	1.995	0.3500
5	2.b	2.000	1.999	0.3500
6	2.c	2.000	2.004	0.3500
7	3.a	4.000	3.998	0.3600
8	3.b	4.000	4.002	0.3600
9	3.c	4.000	3.997	0.3600
10	4.a	5.000	5.005	0.4000
11	4.b	5.000	4.992	0.4000
12	4.c	5.000	5.009	0.4000
13	5.a	6.000	6.004	0.4500
14	5.b	6.000	6.003	0.4500
15	5.c	6.000	5.997	0.4500

OK Cancel

S-Matrix/FIT Tutorial 1/FIT Tutorial 1

Channel: FDA Ch1 273nm@4.8nm Compound 1

Compounds Available

[Empty list]

Compounds Included

Compound 1

Response Data Available

4Sigma  
5Sigma  
Amount  
Area  
Asym  
AsymA10  
AsymA10sqrd  
AsymA14\_4  
AsymA14\_4sqrd  
BaselineEnd  
BaselineStart  
ControlValue

Response Data Included

Concentration

<< Back Next >> Cancel ?

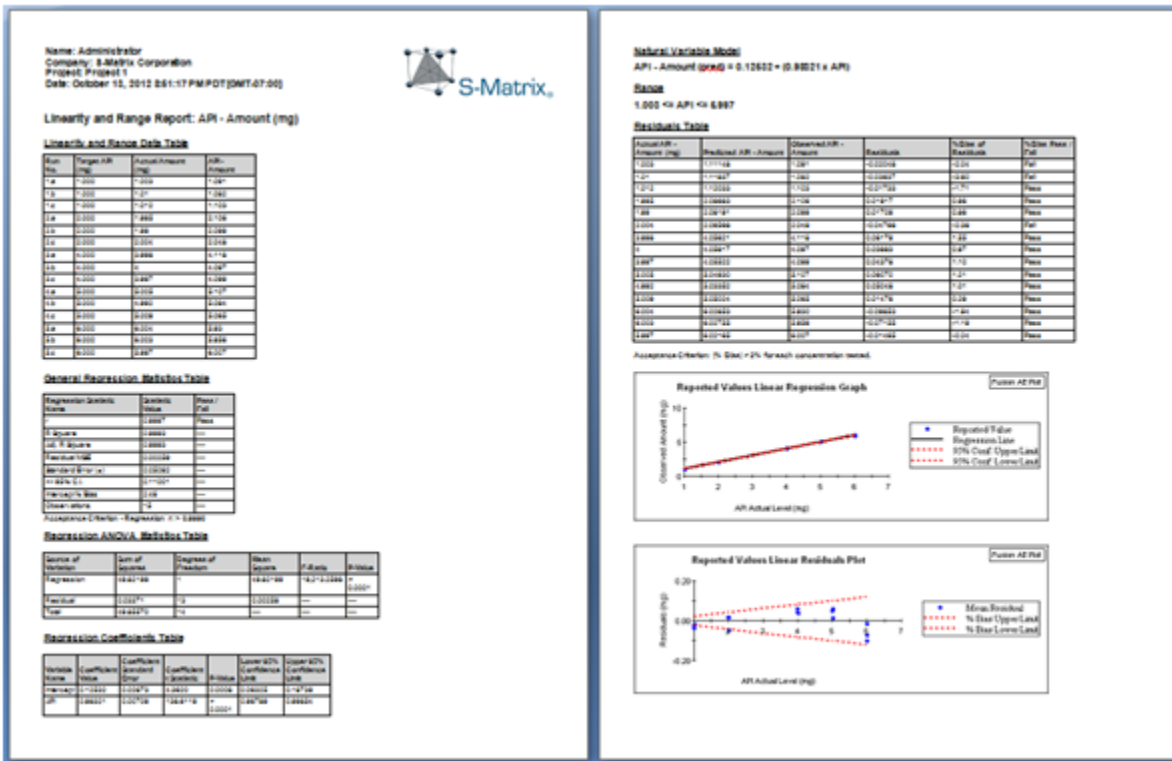
## Step 5 – Fusion QbD Automatically Creates Final Reports and Graphs

### 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....:

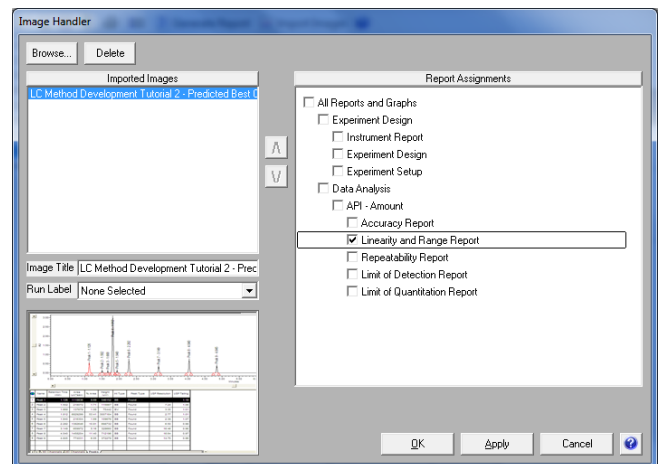
- Correlation Coefficient
- Y Intercept
- Slope of the Regression Line
- Residual Sum of Squares
- Linear Regression Plot
- Residuals Data Table and Plot



**FMV** enables you to include images of representative chromatograms into your final reports. You can associate these chromatogram images with any of the individual results reports which **FMV** automatically generates,

### 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.



# Robustness Validation – DONE RIGHT!

## 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.

You select the parameters to include in the automated **FMV** robustness experiment. **FMV** will automatically generate the robustness design, re-construct it in the CDS as ready-to-run methods and sequence, import the chromatogram results directly from the CDS, re-map them to the robustness study, and instantly analyze, graph, and report the results.

Method Type Gradient

Available Variables

Injection Volume  
Sample Concentration  
Detector Wavelength



Included Variables

Pump Flow Rate  
Gradient Slope  
Oven Temperature  
pH  
Column Type

Activate Online Preparation

Buffer Concentration

Additive Concentration

## The FMV Difference Lowers your Field Failure Risk:

**FMV** robustness experiments let you use *valid experiment ranges for accurate, defensible estimates of parameter effects.*

This avoids the risks associated with setting ranges equal to the expected variation ranges of your instrument parameters.

**FMV** robustness analysis wizard lets you set:

- expected parameter variation ranges
- acceptable performance limits for each key response

The wizard then accurately determines and reports the method's true robustness.

Experiment Variable Maximum Expected Variation

Maximum Expected Variation:  
The  $\pm 3$  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.

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

Navigation: << Back, Next >>, Finish, Cancel

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.

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

Buttons: Select All, Select None

Navigation: << Back, Next >>, Finish, Cancel

# Robustness Validation – Statistical Significance Testing – Model Coefficients

## Robustness Report: API - Area (%)

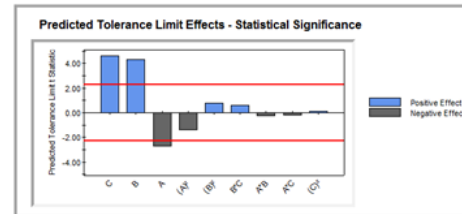
### Coded Variable Name Key

Coded Variable Name	Actual Variable Name
A	Initial % Organic
B	Oven Temperature
C	pH

### Variable Effects Table - Statistical Significance

Model Term	Robustness Testing Range (Coded)	Coefficient Value	Predicted Effect	Effect Standard Error	Effect t statistic	Pass/Fail
C	0.4000	161,391.4753	64,556.59	13,911.0838	4.6407	Fail
B	0.8000	74,520.8782	59,616.70	13,794.1618	4.3219	Fail
A	0.8000	-47,297.1750	-37,837.74	14,136.9455	-2.6765	Fail
(A) <sup>2</sup>	0.1600	-124,093.0600	-19,854.89	14,136.9455	-1.4045	Pass
(B) <sup>2</sup>	0.1600	64,847.5165	10,375.60	13,794.1618	0.7522	Pass
B <sup>2</sup> C	0.1600	50,247.7248	8,039.64	13,714.4961	0.5862	Pass
A <sup>2</sup> B	0.3200	-9,783.1120	-3,130.60	13,874.0259	-0.2256	Pass
A <sup>2</sup> C	0.1600	-13,383.4646	-2,141.35	14,022.6463	-0.1527	Pass
(C) <sup>2</sup>	0.0400	32,821.4015	1,312.86	13,911.0838	0.0944	Pass

Maximum Allowable Value: |Predicted Tolerance Limit t statistic| < 2.2622 for each variable studied.



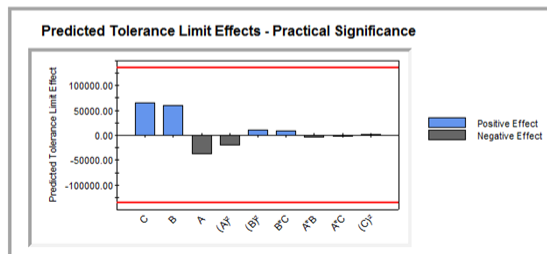
Absolute Predicted Tolerance Limit t statistic: < 2.2622

# Robustness Validation – Practical Significance Testing – Effects Magnitude

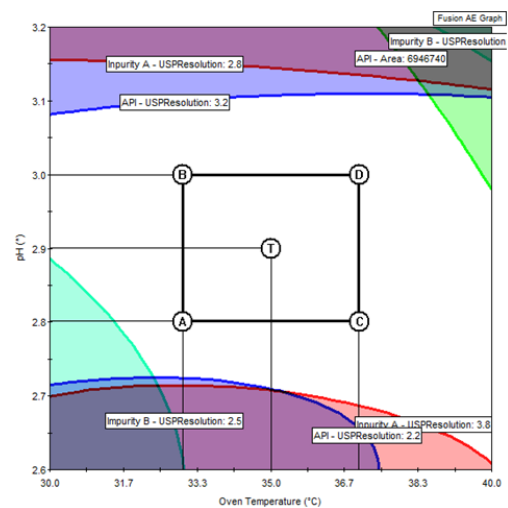
### Variable Effects Table - Practical Significance

Model Term	Robustness Testing Range (Coded)	Coefficient Value	Predicted Effect	Pass/Fail
C	0.4000	161,391.4753	64,556.5901	Pass
B	0.8000	74,520.8782	59,616.7026	Pass
A	0.8000	-47,297.1750	-37,837.7400	Pass
(A) <sup>2</sup>	0.1600	-124,093.0600	-19,854.8896	Pass
(B) <sup>2</sup>	0.1600	64,847.5165	10,375.6026	Pass
B <sup>2</sup> C	0.1600	50,247.7248	8,039.6300	Pass
A <sup>2</sup> B	0.3200	-9,783.1120	-3,130.5958	Pass
A <sup>2</sup> C	0.1600	-13,383.4646	-2,141.3543	Pass
(C) <sup>2</sup>	0.0400	32,821.4015	1,312.8561	Pass

Maximum Allowable Difference from Mean: |Predicted Tolerance Limit Effect| < 136,200 for each variable studied.



Absolute Predicted Tolerance Limit Effect: < 136,200



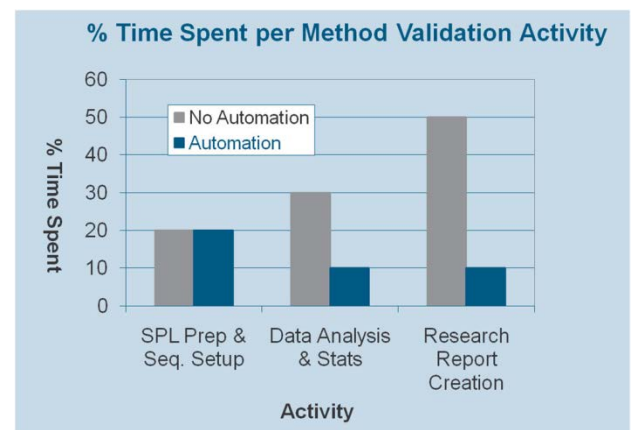
# Fusion Method Validation – Proven Value – Rapid ROI

International Pharma Co. Benchmarking Project

Realized Time Savings = 85%.

Using historical records\* and adjusting for project complexity

Minimum Expected Time Savings = 60%.



# S-Matrix Software Products and Support

S-Matrix Corporation develops advanced Design of Experiment based-software that automates R&D experimental work according to Quality-by-Design principles and methodologies. S-Matrix's Fusion QbD platform automates and redefines experimentation in Analytical R&D, Chemical and Process R&D, Formulation, and Product R&D.

## Fusion QbD Software System Product Suite

### ■ Fusion LC Method Development

Rapidly develop and optimize robust LC methods on instruments from multiple vendors.

### ■ Fusion LC Method Validation

Meet regulatory guidelines with a best-practices approach toward LC method validation with comprehensive reporting.

### ■ Fusion Inhaler Testing

Create sampling plans, export and import data from your CDS via validated data exchange and calculate particle size distribution results, and generate reports according to USP 601, Ph.Eur. 2.9.18, and ISO 27427.

### ■ Fusion Product Development

The perfect QbD software for formulation & product development – automated experimental design selection, sophisticated analysis tools, including automated modeling and simulation, comprehensive reporting, with a full 21 CFR 11 compliance toolset.

## Sales and Support

Sales: Tel: 800-336-8428 (Outside the USA: 707-441-0406). Email: [Sales@smatrix.com](mailto:Sales@smatrix.com)  
Customer Support: Tel: 707-441-0407. Fax: 707-441-0410. Email: [Support@smatrix.com](mailto:Support@smatrix.com)

## On-site and Web Training

S-Matrix offers on-site training programs for installed systems. Training includes experiment strategies, experimental design (DOE), data analysis, graphical visualization and ranking of effects, numerical and graphical optimization, and QbD Reporting.

S-Matrix also offers interactive web training which covers software features and operation, along with general principles of DOE and QbD. Web training programs can be tailored to suit your individual focus and information requirements.

To arrange an on-site or web-based training program, call 707-441-0406.

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