

# FUSION METHOD DEVELOPMENT SOFTWARE

## 12 Reasons Why **FMD** is the World's Best Quality by Design Software for LC Method Development



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## 1. Fully Scalable & Regulatory Compliance Ready

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Fusion LC Method Development (FMD) comes in two configurations to suit your individual or site needs. Each configuration is readily upgradable or extensible as needed, and each has all the features and tools required to support full 21 CFR 11 Compliance for closed systems.

Compliance in FMD is not just a simple named user logon feature, but full E-Record and E-Signature support, including full audit logging of all user executed operations and data events, and even the ability to interject E-Review and E-Approve work management loops. This extends to full auditing of all electronic data exchanges between FMD and chromatography data software

### **FMD – Workstation Configuration**

This single PC configuration of FMD contains a built-in 21 CFR 11 Compliance Support Toolset and associated Workflow Management System. The Workstation configuration enables you to create and manage multiple named users, create User Roles with specific access and use permissions and assign the roles to individual users, and incorporate e-Review and e-Approve management review loops.

### **FMD – Network/Citrix Configuration**

This configuration of FMD also contains the built-in 21 CFR 11 Compliance Support Toolset and associated Workflow Management System. The Network configuration enables you to operate FMD in a network environment to support concurrent use. FMD can be deployed in a traditional network environment where the user application is installed on multiple PCs or in a Citrix environment (Metaframe or XenApp) where the user application is installed on one or more Citrix server PCs.

## 2. Supports Risk Assessment

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When considering the FDA/ICH guidance documents it is important to understand that the regulatory agencies view an LC instrument as a “process in a box”, and therefore consider all of the statistical tools and approaches used for process development, optimization, and robustness characterization as directly applicable to LC method development. The Fusion LC Method Development software module (**FMD**) is expert system software which integrates the four “*Supporting Statistical Tools*” shown in **blue highlight** below which are relevant to LC method development experiments listed in the **ICH Q9 Quality Risk Management** guidance (refer to ICH Q9 Nov 9, 2005, Section I.9, reproduced below).

### ***I.9 Supporting Statistical Tools***

*“Statistical tools can support and facilitate quality risk management. They can enable effective data assessment, aid in determining the significance of the data set(s), and facilitate more reliable decision making. A listing of some of the principal statistical tools commonly used in the pharmaceutical industry is provided.”*

- Control Charts, for example: ...
- Design of Experiments (DOE)
- Histograms
- Pareto Charts
- Process Capability Analysis

As part of LC method development **Risk Assessment** includes identification of Critical Process Parameters (CPPs – in this case critical LC instrument parameters), and accurate characterization of all the types of effects that the CPPs have on the defined Critical Quality Attributes (CQAs – in this case critical method performance characteristics). This quantitative characterization leads to knowledge of the level of control of each CPP required for a robust method. FMD enables the user to study multiple parameters in combination, can characterize their independent, interactive, and complex effects, and can illustrate these effects in its graphical reporting of the Design Space and Proven Acceptable Ranges for the study parameters (see Item 9). FMD therefore provides strategic tools and capabilities which support Quality Risk Management (QRM) for LC method development as defined in the **ICH Q9 Quality Risk Management** guidance (refer to ICH Q9 Section II.3, reproduced on the following page).

### ***II.3 Quality Risk Management as Part of development***

To design a quality product and its manufacturing process to consistently deliver the intended performance of the product (see ICH Q8); (*see Items 2, 8, 10, and 11*)

To enhance knowledge of product performance over a wide range of material attributes (e.g., particle size distribution, moisture content, flow properties), processing options and process parameters; (*see Items 2, 3, 6, 8, 10, and 11*)

To assess the critical attributes of raw materials, solvents, Active Pharmaceutical Ingredient (API) starting materials, APIs, excipients, or packaging materials; (*see Items 2, 6, and 8*)

To establish appropriate specifications, identify critical process parameters and establish manufacturing controls ...; (*see Items 8, 10, 11, and 12*)

To decrease variability of quality attributes:

- reduce product and material defects;
- reduce manufacturing defects.

(*See Item 10*)

To assess the need for additional studies (e.g., bioequivalence, stability) relating to scale up and technology transfer; (*see Items 2, 8, and 10*)

To make use of the “design space” concept (see ICH Q8). (*see Items 10, 11, and 12*)

### 3. Facilitated LC Method Development Workflow

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A general Method Development Sequence is presented below. FMD can generate experiments to support each phase in this sequence.

Sample Preparation → Chemistry Screening → Method Optimization

#### Sample Preparation

This is a critical starting point to make sure that you will get meaningful data from experiment chromatograms. The variables studied here are discussed in the detail section on the next page. The end point of the Sample Preparation workup is a chromatogram with acceptable S/N Ratio for all peaks of interest, an acceptable  $k'$  value for the 1st eluted peak, and some confidence that most or all peaks are eluting during the gradient portion of the method.

#### Chemistry Screening

The primary goal of this phase is selection of the mobile phase and stationary phase chemistry to promote to optimization studies. Typical Reversed Phase study variables for this stage include:

- pH (very broad range can be done as two separate studies – e.g. 2-5 and 8-10)
- Strong Solvent Type (optional - e.g. ACN and MeOH)
- Gradient Time (use constant starting point and end point % Organic)
- Stationary Phase I (columns with differing chemistry – e.g. C8, C18, Phenyl, Amide, Cyano).
- Stationary Phase II (can follow up with different suppliers or lengths of the column identified as the best one from the Stationary Phase I experiment).

The end point of Chemistry Screening is the right range of pH (usually not the exact pH), the right strong solvent type, the right column, and generally the right Gradient Time to use in subsequent optimization studies. The screening endpoint method should yield a chromatogram with acceptable S/N Ratio for all peaks of interest, an acceptable  $k'$  value for the 1st eluted peak, and some confidence that most or all peaks are eluting during the gradient portion of the method.

## Method Optimization

The primary goal of this phase is a final method which meets all separation and chromatographic quality goals and has an acceptable assay time. Typical Reversed Phase study variables for this stage include those listed below. It should be noted that these instrument and chemistry parameters are not normally all studied in a single optimization experiment.

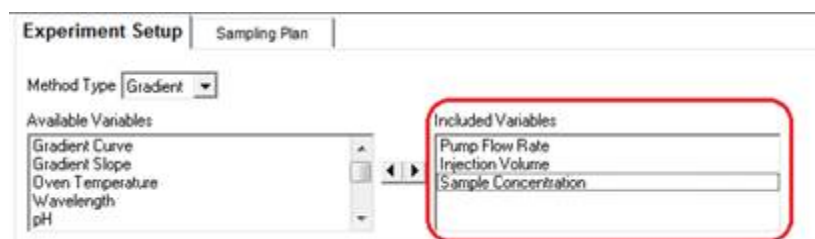
- Pump Flow Rate
- pH (narrow range with 5 levels - e.g. 2.0, 2.5, 3.0, 3.5, 4.0)
- Gradient Slope
- Oven Temperature
- Buffer Type and/or Concentration  
(optional - depends on chromatography type and sample compounds)
- Additive Type and/or Concentration  
(optional - depends on chromatography type and sample compounds)

The end point of the optimization phase is a well characterized Design Space. This includes a final method which demonstrates acceptable mean performance for all included CQAs and which performs robustly for all CPPs.

## Sample Preparation – Detailed Discussion

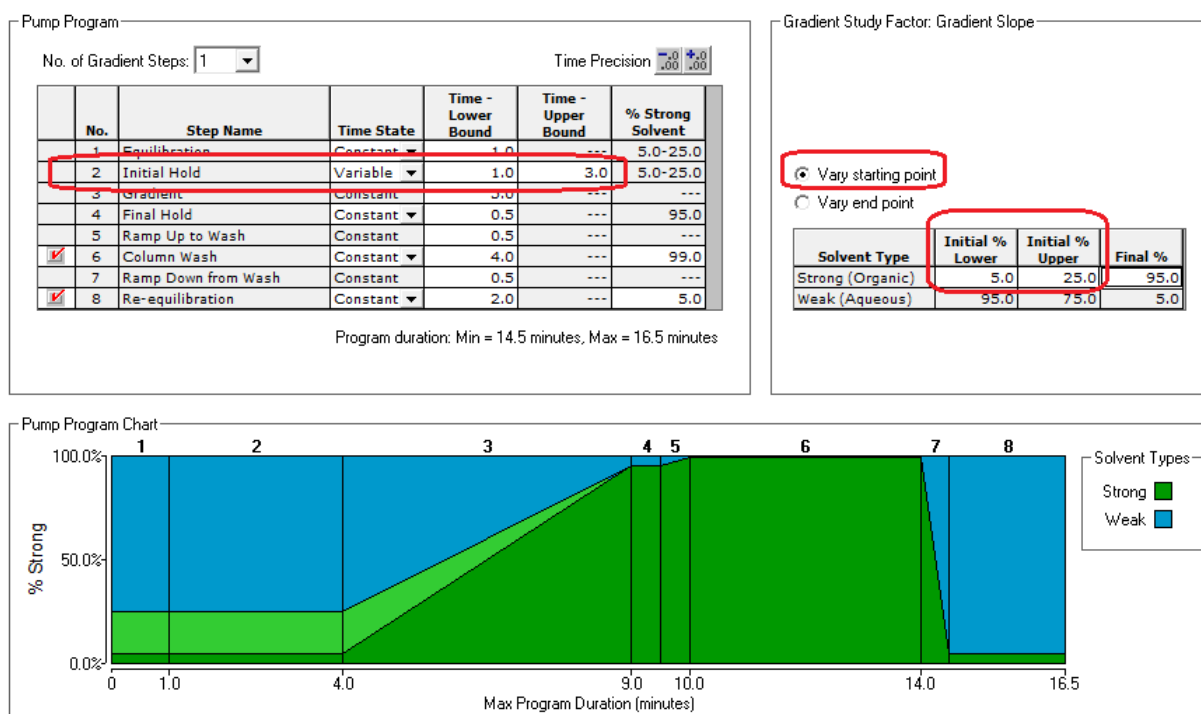
Customers use FMD to develop and run quick sample preparation screening studies with variables such as Pump Flow Rate, Injection Volume, and Sample Concentration (see Figure 1). The user can then either (1) visually inspect the resulting chromatograms to find acceptable starting-point conditions for more formal method development - for example check to see that the signal is in the right range and scale, or (2) use our patented Trend Response chromatogram results technology to obtain and model responses such as number of integrated peaks, number of peaks with S/N Ratio > a user specified value, and retention time of the main peak (usually the API). This type of study approach enables the user to quickly identify the best sample preparation conditions in terms of compound concentrations and injection volume.

**Figure 1**



Once the sample preparation is set, customers sometimes use FMD to develop and run a follow-on screening study to address “Dead Volume” considerations with variables such as Initial Hold Time (initial isocratic hold = post injection and prior to start of gradient) and Gradient Slope - Vary Starting Point % Organic (see Figure 2). The user typically identifies the 1st peak in each chromatogram and then uses the retention time of the 1st peak and/or the  $k'$  of the 1st peak as a result to import and model. The user can also again include Trend Responses such as the number of integrated peaks, number of peaks with S/N Ratio > a user specified value, and retention time of the main peak (usually the API) to make sure that these results are maintained at acceptable levels or further improved. Studying these two variables in combination enables the user to quickly optimize the initial part of the chromatogram in terms of when the 1st peaks elute, and also to make the method robust to variation in dead volume which the method will experience across multiple LCs as a QC method.

**Figure 2**





## 4. Simple Experiment Setup

As described under Reason 3, FMD has default template experiments for column/solvent screening and for method optimization for the Reversed Phase chemistry type. However, since each sample represents a different set of compounds, these templates are user editable, and users can create their own flexible templates for a given phase of method development.

Figures 3 and 4 show a part of the simple Experiment Setup workspace. Figure 3 shows that the Pump Flow Rate has been included in the study, but set to a Constant level of 0.500 mL/min. This convenient configuration tells FMD to build this flow rate into each experiment method. As shown in Figure 4, the pH variable and the Oven Temperature instrument parameters are also included, and in this case they are defined as State=Variable with multiple study levels.

**Figure 3**

The screenshot shows the 'Method Type' set to 'Gradient'. The 'Available Variables' list includes Sample Concentration, Buffer Concentration, Buffer Type, Additive Concentration, and Additive Type. The 'Included Variables' list includes Pump Flow Rate, Gradient Slope, Injection Volume, Oven Temperature, Wavelength, and pH. Below this, a table shows the configuration for 'Pump Flow Rate':

Name	Units	Type	Amount
Pump Flow Rate	mL/min	Discrete Numeric	0.500

Under the 'State' section, the 'Variable' radio button is selected and highlighted with a red box.

**Figure 4**

The screenshot shows the 'Buffer System Variables' section with 'pKa of Primary Compound' set to 4.8. The 'pH' section is expanded, showing 'No. of Levels' set to 6 and 'Units' set to °. Below this is a table for 'Level Settings':

Level Settings	Descripti
2.0	
3.0	
4.0	
5.0	
6.0	
7.0	

Below this, a table shows the configuration for 'Oven Temperature':

Name	Units	Type	Level Settings
Oven Temperature	°C	Discrete Numeric	

Under the 'State' section, the 'Variable' radio button is selected and highlighted with a red box. To the right, the 'Level Settings' for 'Oven Temperature' are shown:

Level	Value
Level 1	30.0
Level 2	40.0
Level 3	50.0

## Online Preparation

**Experiment Setup** | Sampling Plan

Method Type: Gradient

Available Variables:

- Gradient Curve
- Gradient Slope
- Sample Concentration
- Additive Concentration
- Additive Type
- Column Type

Included Variables:

- Pump Flow Rate
- Injection Volume
- Oven Temperature
- Detector Wavelength

Activate Online Preparation  
 Buffer Concentration  
 Additive Concentration  
 pH

### Buffer Concentration Example

As shown in Figure 5, the software will dilute the stock solution to each target level using the “Deionized Water” designated solvent.

NOTE – the highest target level of “Buffer Concentration” will restrict the allowable upper bound of the Strong solvent(s). Therefore, the stock solution should be prepared at a concentration which will minimize this restriction; for example, one which will require at most 10.0% of the mobile phase to achieve the highest target level. As shown below, no more than 10.0% of the Stock Solution mobile phase will be required to achieve the highest target concentration of 10.00 mM given a stock solution concentration of 100.00 mM.

**Figure 5**

Online Preparation Stock Solution

Stock Solution Name	Units	Amt/Conc.	Associated Solvent Type
Stock Solution	mM	100.00	Weak

Name: Buffer Concentration | Units: mM | Type: Discrete Numeric | Target Level:

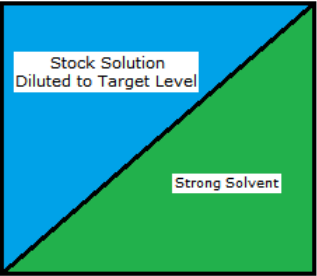
Level	Target Level
1	1.0
2	5.0
3	10.0

State:  Variable  Constant

No. of Levels: 3

Maintain target level in each run.

Include "Type" Variable



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
Strong Solvent 1	Strong	---
Stock Solution	Weak	---
Deionized Water	Weak	---

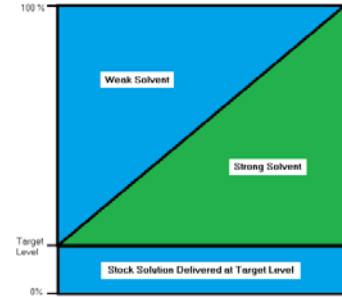
Available Reservoirs

- B
- A
  - A-1
  - A-2
  - A-3
  - A-4
  - A-5
  - A-6
- C
- D

## Maintain target level in each run:

**UNchecked:** as shown in the image above, the software will maintain the target buffer concentration in the Weak mobile phase within a given run (injection). However, the absolute concentration will change as the Strong/Weak solvent ratio changes across the gradient.

**Checked:** as shown in the image to the right, the software will maintain the target buffer concentration in a given run (injection) as an absolute constant within the mobile phase independent of the change in the Strong/Weak solvent ratio across the gradient.



## Include “Type” Variable:

As shown in Figure 6, this setting adds a linked Buffer Type variable to the Buffer Concentration variable. This is a Categorical variable which you include when you want to study different buffer types (or suppliers, etc.) across the concentration study range.

**Figure 6**

Online Preparation Stock Solution

Stock Solution Name	Units	Amt/Conc.	Associated Solvent Type
Stock Solution	mM	100.00	Weak

Name: Buffer Concentration | Units: mM | Type: Discrete Numeric | No. of Levels: 3 | Maintain target level in each run:

Target Level	Level	Value
1	1.0	1.0
2	5.0	5.0
3	10.0	10.0

State:  Variable  Constant

Include "Type" Variable

Name: Buffer Type | Units: \* | Type: Categorical | No. of Levels: 2

Level Settings	Level	Setting
1	Type 1	Type 1
2	Type 2	Type 2

## pH Example

For LC instrument systems with quaternary pump modules FMD can now automatically prepare target levels of pH for experimenting. It does this by enabling the user to define an acid-base solvent pair and the acid-base solvent ratio needed to achieve each desired study level of pH. FMD will then build the proportions required to maintain the target level of pH for the run into each step of the instrument method's pump program. To illustrate, Figure 7 presents a table of the acid-base solvent ratios for Formic Acid and Ammonium Formate which result in a six-level pH screen covering a range from ~ 2.3 to 6.2. The experiment setup for this pH range is shown in Figure 8 for the six target experimental levels of pH.

**Figure 7**

0.10 M Formic Acid (%)	0.10 M Ammonium Formate (%)	pH
100	0	2.31
80	20	2.99
60	40	3.39
40	60	3.75
5	95	4.80
0	100	6.16

**Figure 8**

pH Online Blending Mode: One Acid:Base Pair  pKa of Primary Compound: 4.80

pH Buffer Settings: No. of Levels: 6

pH Type	pH Buffer Name	pH Level	Acid %	Base %
Acid	Formic Acid - 0.10 M	2.31	100.0	0.0
Base	Ammonium Formate - 0.10 M	2.99	80.0	20.0
		3.39	60.0	40.0
		3.75	40.0	60.0
		4.80	5.0	95.0
		6.16	0.0	100.0

## 5. Automated Experimental Design Selection

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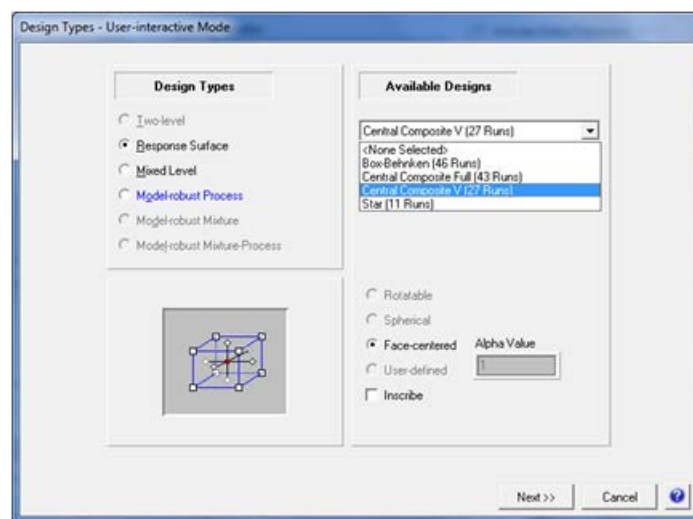
FMD has a built-in Design Wizard which can automatically select the most efficient DOE design based on your stage of work (e.g. chemistry screening or method optimization) and the LC parameters you selected for study.

FMD has a comprehensive experimental design library which includes the statistical experimental design types listed below. This assures the user that FMD can generate a statistically valid and defensible DOE design for any combination of instrument parameters selected for study. In the user-interactive mode of the built-in Design Wizard the user can select an alternative to the FMD recommended design, as shown in the wizard dialog pictured in Figure 9. However, FMD will disable DOE design options which are not valid for the user's selected instrument parameters.

FPD has built-in knowledge of each experiment design type in its DOE Design library.

- Full Factorial Designs
- Fractional Factorial Designs
- Plackett-Burman Designs
- Central Composite Designs
- Box-Behnken Designs
- Star Designs
- Mixed-level Designs
- Model Robust Designs (D-optimal, A+G Optimal)

**Figure 9**



## 6. Supports Column Selection

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Figure 10 is the Experiment Setup control within the Fusion LC Method Development software module (FMD) for the Column Type variable. When the LC has a column switching valve, FMD will set up the experiment to fully utilize the valve. When there is no valve, or when the number of columns included in the study exceeds the valve capacity, FMD will automatically build the experiment as multiple sequences in the chromatography data software. Column selection is normally done as part of a chemistry screening experiment which also includes Gradient Slope, pH (depending on the Chemistry Type – e.g. Reversed Phase), and strong solvent type. More detail on chemistry screening is provided in Item 8.

**Figure 10**

	Name	Valve Position	Flow Rate	Condition Column Time	pH Upper Bound
1	StableBond C8	1	1.0	5.0	14.00
2	Eclipse Plus C18	2	1.0	5.0	14.00
3	StableBond C18	3	1.0	5.0	14.00

### Flow Rate

When Pump Flow Rate is *not* set as a global constant or an experiment variable the settings fields in this column are enabled. This lets the user define a specific flow rate for each column included in the experiment. When Pump Flow Rate is set as a global constant or an experiment variable the settings fields in this column are disabled.

### Condition Column Time

FMD automatically inserts “Condition Column” methods to condition each column with the mobile phase chemistry which will be used in the next series of experiment runs. The user can define the time setting for each column which results in sufficient column volumes to acceptably condition the column for the next block of chemistry runs.

### pH Upper Bound

When pH is included as an experiment variable FMD will not use a study level of pH with a column when the study level is greater than or equal to the pH Upper Bound setting.

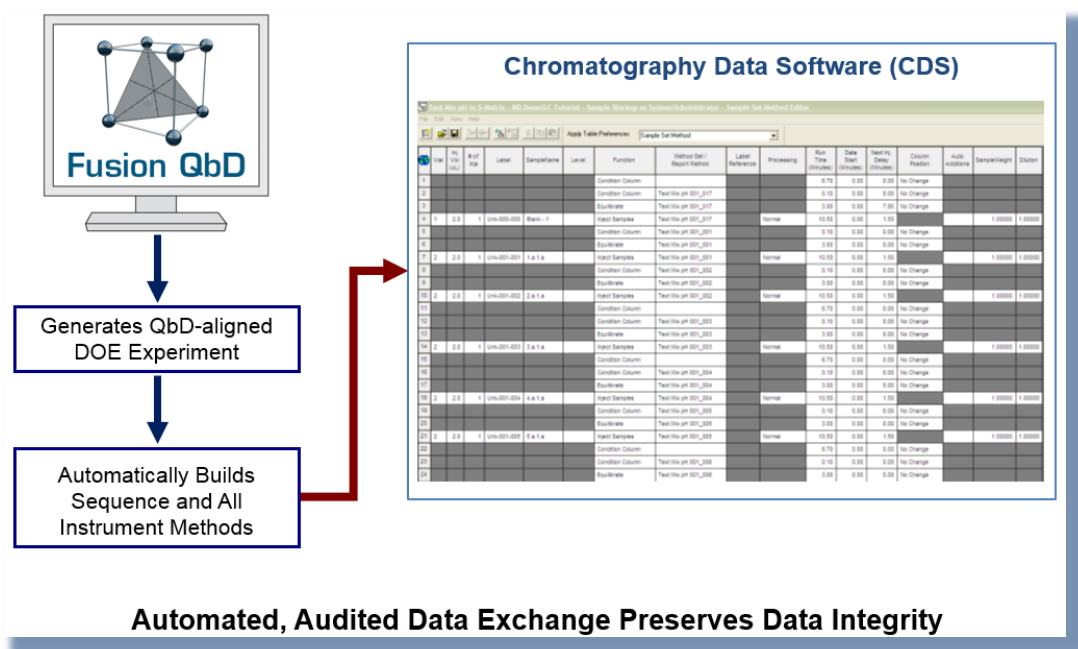
## 7. Automates QbD Experimentation on Your LC Instruments

FMD fully automates QbD experimentation on the Agilent ChemStation/OpenLab Chromatography Data Software (CDS) and the Waters Empower 2 and 3 CDS.

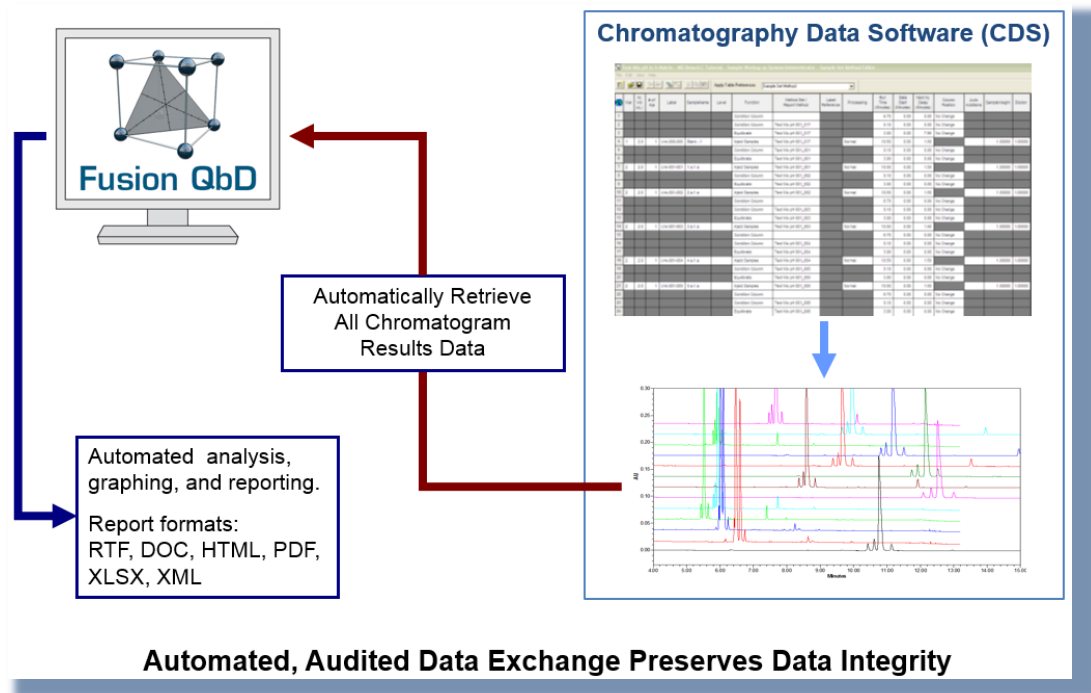
- **Fusion Export Wizard** automatically builds your experimental design in the CDS as ready to run methods and sequence.
- **Fusion Import Wizard** automatically imports all chromatogram results data from the CDS as analysis-ready data sets.

Figures 11.a and 11.b illustrate the automated DOE experiment dataflow between FMD and your CDS. Figures 11.c – 11.e are images of the LC modules and devices FMD supports for fully automated experimentation within Agilent ChemStation/OpenLab, ThermoFisher Chromeleon, and Waters Empower 2 and 3.

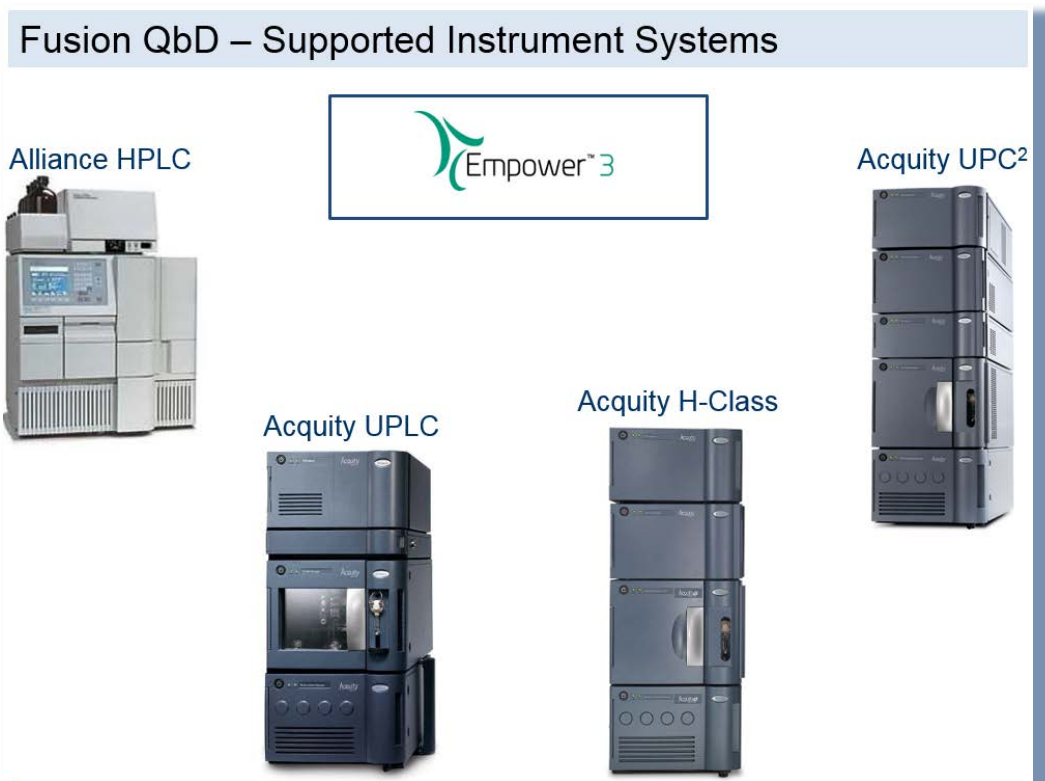
**Figure 11.a**



**Figure 11.b**

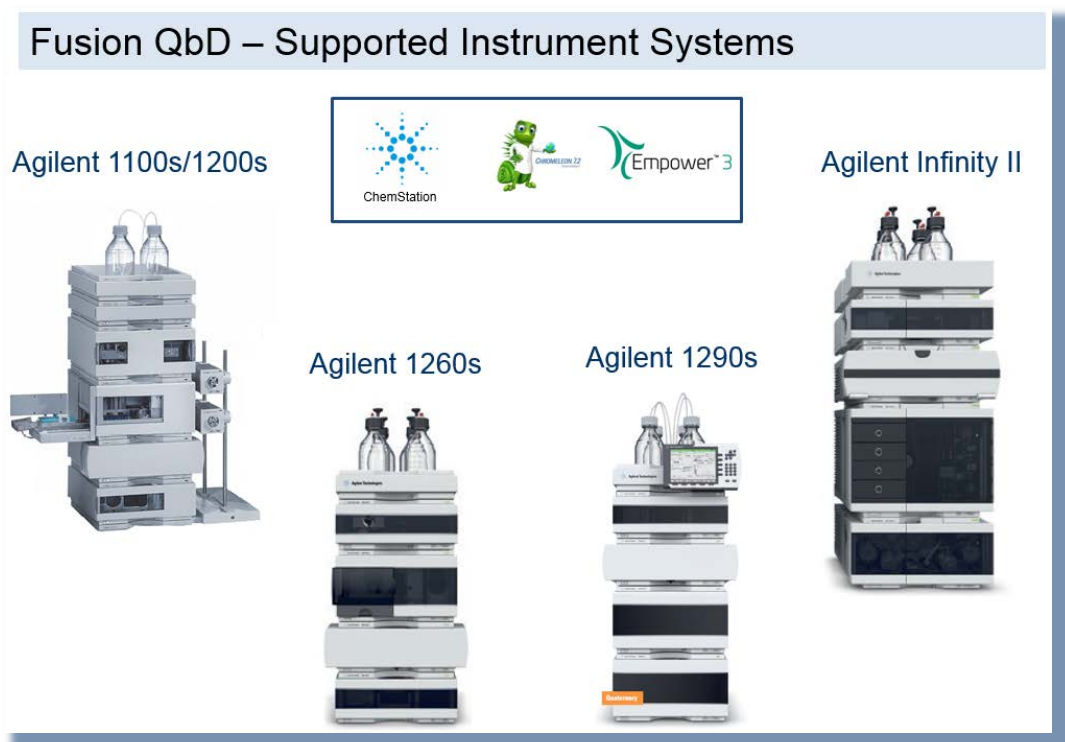


**Figure 11.c**

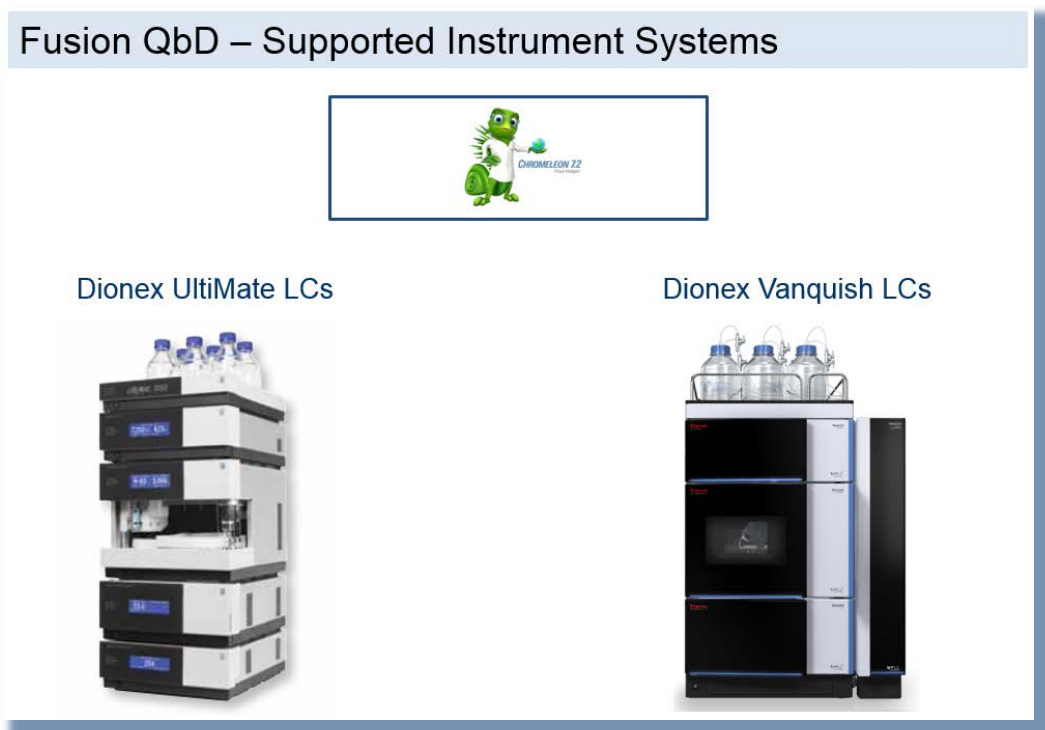




**Figure 11.d**



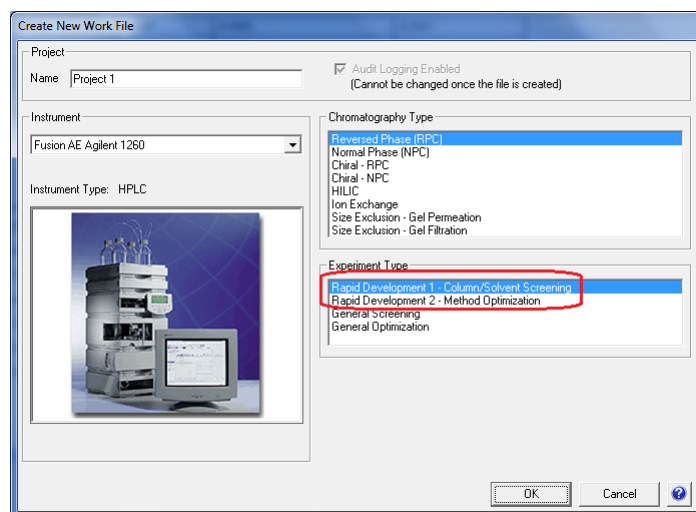
**Figure 11.e**



## 8. Automated Modeling & Critical Parameter Effects Ranking

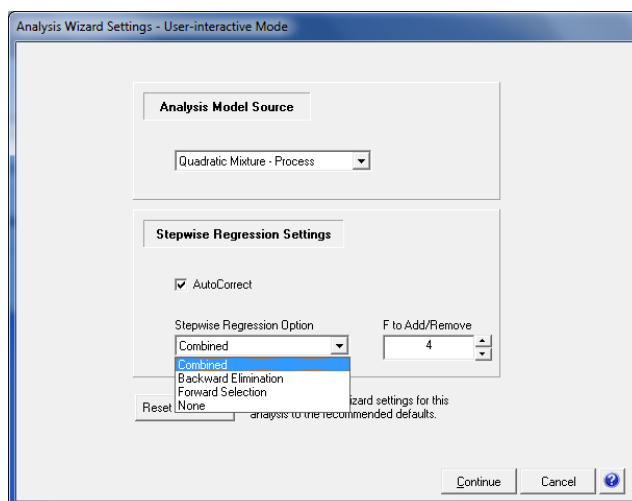
FMD has default template experiments for column/solvent screening and for method optimization for the Reversed Phase chemistry type, as shown in Figure 12. These templates preload recommended study variables into the Experiment Setup workspace. The templates are based on many years of successful use of these variables in screening and optimization studies. However, since each sample compound mix is unique, these templates can be directly edited to make them meaningful to the current sample. In addition, users can build their own templates. For example, many customers have created standardized chemistry screening protocol templates which define specific levels of pH, specific columns, and strong solvent types or composition.

**Figure 12**



FPD has a built-in Analysis Wizard with both Automated and User-interactive Modes. The Automated Mode will automatically derive the most statistically defensible prediction equation (model) from the experimental data for each response included in the study. The Automated mode is a state-of-the-art Multiple Linear Regression (MLR) technology which contains all the relevant component analysis operations required of a fully automated MLR routine, implemented in the correct operational sequence. The User-interactive mode gives the user complete control over the MLR configuration settings while showing its statistically defensible recommendations, as shown in the wizard dialog pictured in Figure 13.

**Figure 13**



The correctness of FPD’s automated MLR routine was rigorously verified by Douglas C. Montgomery, Ph.D., Regents' Professor of Industrial Engineering & Statistics at Arizona State University. Dr. Montgomery is the acknowledged world leader in the field of applied industrial statistics with special expertise in statistical experimental design, also referred to as Design of Experiments (DOE).

*“It is especially noteworthy that about a decade ago a major international corporation spent over \$1,000,000.00 attempting to develop an automated MLR analysis capability. They were unsuccessful. This validation exercise therefore demonstrates that FusionQbD’s automated MLR software is at once both correctly implemented and a major advance in the field of MLR analysis.”*

Douglas C. Montgomery, Ph.D.

FMD automatically generates a full statistical reporting of each equation derived from the experimental data. One of these reports is the Mean Effects Pareto Ranking Table and Plot report which is shown in Figure 14. This report was generated for the Peak 4 – USP Resolution response data obtained from a method optimization experiment. The ranking is based on the magnitude of the change in the response as the variables are changed across their individual experimental ranges. The ranking is applied to all effects, including interaction effects [e.g.  $(X_i * X_j)$ ], curvature effects [e.g.  $(X_i)^2$ ], and complex effects [e.g.  $(X_i)^2 * X_j$ ] as well as independent additive effects ( $X_i$ ).

An experiment variable is identified as a Critical Method Parameter (CMP) if one or more effects involving the variable are of *practical* significance. For example, although it is statistically significant, the effect of the nonlinear interaction between Initial % Organic and pH [(A)<sup>2</sup>\*C] only changes the Peak 4 Resolution by 0.059 units, and so it is not practically significant. However, both these study variables are CMPs for this response, since their independent additive effects (A and C) and their interaction effect (A\*C) are both statistically significant and practically significant.

**Figure 14**

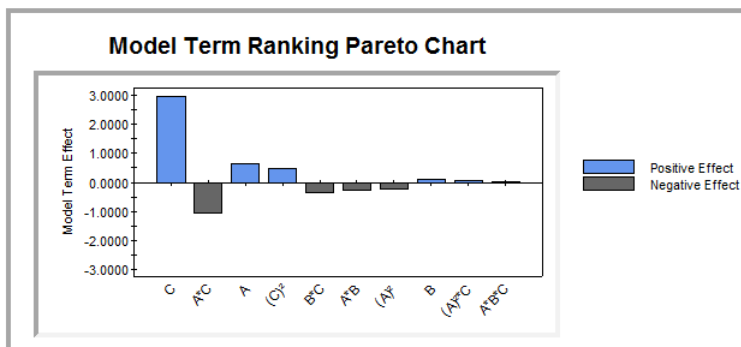
**Model Term Pareto Ranking: Peak 4 - USPResolution**

**Study Variable Code Name Key**

Study Variable	Code Name
Initial % Organic	A
Oven Temperature	B
pH	C

**Mean Effects Ranking Table**

Model Term	Model Term Range	Coefficient Value	Transformed Model Term Effect	Backtransformed Effect
C	2.0000	0.4989	0.9978	2.9635
A*C	2.0000	-0.1881	-0.3763	-1.0788
A	2.0000	0.1107	0.2213	0.6321
(C) <sup>2</sup>	1.0000	0.1571	0.1571	0.4848
B*C	2.0000	-0.0588	-0.1177	-0.3357
A*B	2.0000	-0.0447	-0.0895	-0.2551
(A) <sup>2</sup>	1.0000	-0.0808	-0.0808	-0.2211
B	2.0000	0.0208	0.0416	0.1185
(A) <sup>2</sup> *C	2.0000	0.0104	0.0208	0.0594
A*B*C	2.0000	0.0071	0.0143	0.0406



## 9. Support for Peak Tracking

Most LC method development software only works when every peak is accurately identified in each experiment chromatogram – an extremely difficult requirement for virtually all initial chemistry screening experiments and also for almost all early development experiments for drug product methods, stability-indicating methods, and cleaning methods in which the samples contain several impurity and/or degradant peaks. This is why S-Matrix developed our patented Trend Response™ technology in conjunction with several of our major international pharmaceutical company customers. Trend Responses provide quantitative metrics of chromatographic quality for early method development experiments such as column and solvent screening studies without the need for extremely difficult and error prone peak tracking.

Our Trend Response technology enables you to easily define their critical metrics of chromatographic quality for a given sample and stage of development. Example Trend Responses include the total number of integrated peaks in the chromatogram, the number of well resolved peaks, the number of peaks with S/N Ratio > a user specified value, etc. (see Figure 15). Once defined by the user, FMD will automatically import these responses from the experiment chromatogram data in the CDS and model these responses to identify the best column and chemistry conditions to use in subsequent method optimization studies.

**Figure 15**

**Trend Responses**

**Chemistry System Screening.**

Easily obtain critical separation metrics without the need for manual peak tracking.

Automated peak tracking gets all desired data for critical peaks:

- Main Peak (API)
- First Peak
- Last Peak

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

**Compounds Available**

Included: API, Impurity A, Impurity B

**Response Data Available**

Included: KPrime, RetentionTime, USPPlateCount, USPResolution, USPTailing

**Named Compound (Peak) Responses**

**Method Optimization.**

Easily obtain any critical separation metrics computed by the CDS for any named peak in the experiment chromatograms.

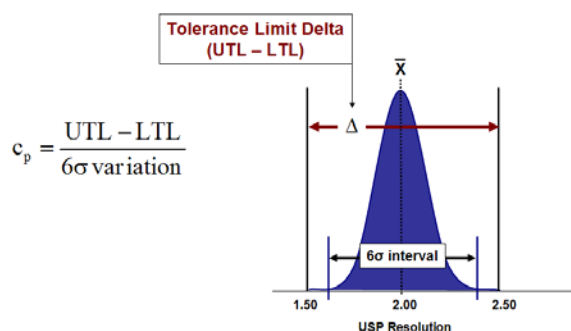
S-Matrix's patented Trend Response operators also enable Fusion QbD to track critical peaks in each experiment chromatogram such as the *First Peak* and *Last Peak*, and capture all chromatographic responses of interest for these peaks such as K-Prime and Retention Time. Also, the *Max Peak # operator* enables Fusion QbD to automatically track one or more main peak(s), and/or spiked peaks of interest, through the Peak Area response, and capture all chromatographic responses of interest for these peaks. For example, the *Max Peak 1* operator will locate the largest peak (in area) in each experiment chromatogram, and automatically capture the chromatographic result associated with it.

Fusion QbD can also utilize chromatographic results from peak tracking done in the CDS for critical pair peaks or group peaks in method optimization experiments (see Figure 15). However, this task is made easier by the column and chemistry screening experiments done earlier, since the correct stationary phase and strong solvent type selected in the screening experiments are now constant in optimization experiments. Also, other strong effectors such as pH are studied in a more limited range. As a result, the Fusion QbD strategy has made peak tracking much simpler in optimization experiments. When peak tracking help is needed, the user can set up FMD to generate paired repeat runs of each experiment method in which the two repeat injections use different sample vials. In this case one sample vial will contain all the compounds, while the other sample vial includes only the sample solvent spiked with one compound from each critical peak pair – each at a concentration which gives a different area. Since the same method is run using both sample vials, a simple comparison of the paired chromatograms lets the user easily identify the critical pair peaks using retention time.

## 10. Identifies Best Performing and Most Robust Methods

The robustness approach implemented in FMD uses Process Capability indices (e.g.  $C_p$ ,  $C_{pk}$ ) to quantify system robustness.  $C_p$  is a standard Statistical Process Control (SPC) metric widely used to quantify and evaluate method variation in critical quality and performance characteristics. For a given critical method performance characteristic,  $C_p$  is the ratio of the method's allowable tolerance width to its inherent variation, and is computed as shown in Figure 16.

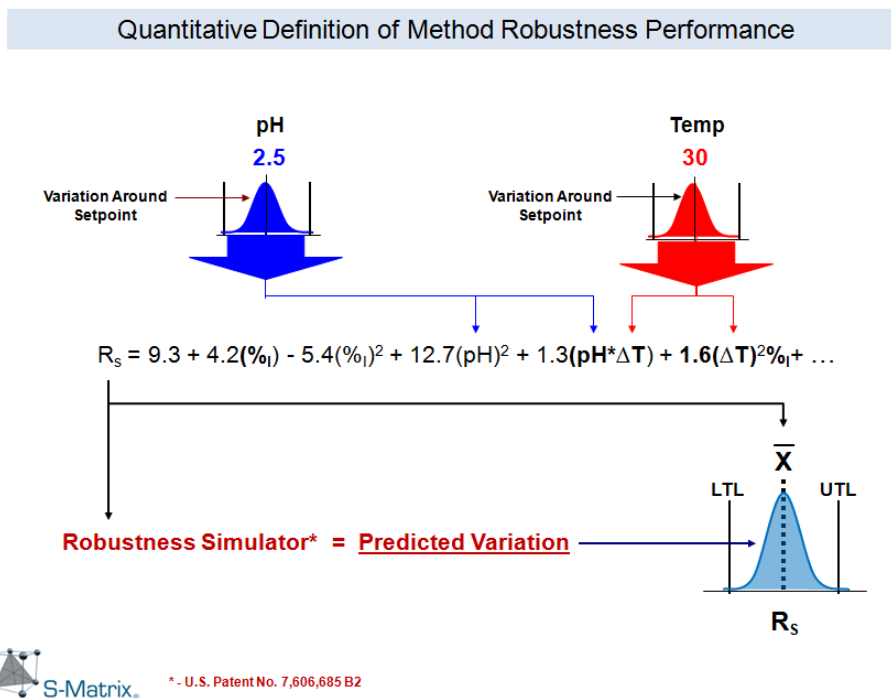
**Figure 16**



where **UTL** and **LTL** are the upper and lower response tolerance limits, and **6σ variation** is the amount of inherent variation about the mean response value bounded by the  $\pm 3\sigma$  confidence interval limits.  $C_p$  is therefore a direct measure of a method's inherent variation in a given critical performance characteristic relative to specified tolerances. **UTL** and **LTL** are symmetrical limits which define the smallest and/or largest acceptable values for the response. For a given instrument parameter, the **6σ variation** represents the total expected variation around the parameter's setpoint which will be expressed on method transfer and routine use over time. Figure 17 illustrates the  $C_p$  calculation elements for a critical method performance result – the Resolution of two drug component peaks as measured by liquid chromatography – given a mean resolution ( $\bar{X}$ ) of 2.00 and tolerance limits of  $\pm 0.50$ . Note that the lower tolerance limit of -0.50 assures that the resolution will never fall below 1.50 ( $2.00 - 0.50$ ). In classical SPC a process is deemed *capable* when its measured  $C_p$  is  $\geq 1.33$ . The value of 1.33 means that the inherent process variation, as defined by the  $6\sigma$  variation limits, is equal to 75% of the tolerance limits ( $4/3 = 1.33$ ). Conversely, a process is deemed *not capable* when its measured  $C_p$  is  $\leq 1.00$ , as the value of 1.00 means that the  $6\sigma$  variation limits are located at the tolerance limits.

Figure 17 illustrates how FMD uses a critical CQA response prediction model in a Monte Carlo simulation mode to obtain the prediction of the response variation for given candidate methods. First, 10,000 level settings are defined for each study variable using the normal distribution with mean value equal to a candidate method's target level setting and the  $6\sigma$  variation (the  $\pm 3.0\sigma$  limits) the user has defined for the variable. Next, the 10,000 combinations of the variable's variation distribution level settings are input into the CQA response prediction model, one combination at a time, which provides 10,000 response predictions. The distribution of these predictions correctly reflects the cumulative variation resulting from the combined variations of the individual study variables around their setpoints as the method executes. Statistically characterizing this distribution then provides the  $6\sigma$  variation value required in the Cp calculation.

**Figure 17**



FMD equations derived from the experiment data characterize the effects of the experiment variables on each analyzed response (CQA). FPD can combine these equations with the Monte Carlo Simulation equations for predicting robustness into its global Best Answer Search utility to find the best overall conditions from the current experiment.



The Best Answer Search operates based on the performance goals the user sets for the included responses. The search engine uses the equations to identify the level setting combination of the study variables which results in best overall method performance in terms of meeting all goals simultaneously. For example, the dialog pictured in Figure 18 shows the goal settings for each response data set included in the study. Figure 19 shows part of the answer report from the optimization search.

**Figure 18**

The screenshot shows a dialog box titled "Execute Search - Response Goals". At the top, there is a "Report Name" field containing "Numerical Search 2" and a "Model Prediction Error C.I. for Report" dropdown set to "2 Sigma". Below this is a table with the following columns: "Response Name", "Goal", "Lower Bound", "Upper Bound", and "Relative Rank". There are eight rows of response goals, each with a checked checkbox in the first column. The goals are: "Imp. A - USPRResolution" (Maximize, 2.00, 3.00, 1), "API - USPRResolution" (Maximize, 2.00, 3.00, 1), "Imp. B - USPRResolution" (Maximize, 2.00, 3.00, 1), "API - USPTailing" (Target, 0.90, 1.10, 1), "Imp. A - USPRResolution - Cp" (Maximize, 1.33, 2.00, 1), "API - USPRResolution - Cp" (Maximize, 1.33, 2.00, 1), "Imp. B - USPRResolution - Cp" (Maximize, 1.33, 2.00, 1), and "API - USPTailing - Cp" (Maximize, 1.33, 2.00, 1). At the bottom, there is a "Validation Status" section that says "Your settings are valid." and a row of buttons: "Modify Search Region...", "Restore Defaults", "<<Back", "Finish", "Cancel", and a help icon.

**Figure 19**

**Answer #1**

**Variable Settings**

Variable	Level Setting
Initial % Organic	11.05
Oven Temperature	34.3
pH	2.715

**Predicted Results**

Response	Goal	Predicted Result	Desirability	-2 Sigma Conf. Limit	+2 Sigma Conf. Limit
Imp. A - USPRResolution	Maximize	3.75	1.0000	3.64	3.87
API - USPRResolution	Maximize	2.33	0.3282	2.29	2.37
Imp. B - USPRResolution	Maximize	2.47	0.4702	2.31	2.63
API - USPTailing	1.00	1.02	0.8167	0.97	1.06
Imp. A - USPRResolution - Cp	Maximize	1.97	0.9517	1.89	2.04
API - USPRResolution - Cp	Maximize	2.00	0.9972	1.88	2.12
Imp. B - USPRResolution - Cp	Maximize	3.15	1.0000	2.59	3.72
API - USPTailing - Cp	Maximize	4.27	1.0000	3.76	4.81

Cumulative Desirability Target = 1.0000  
 Cumulative Desirability Result = 0.7671

## 11. Graphically Visualizes the Robust Design Space and PARs

The  $C_p$  metric is applied directly to critical responses to determine the relative robustness of a candidate method in terms of each Critical Quality Attribute (CQA – critical method performance characteristic). This enables you in a single plot to see the region where the graphed variable settings will yield acceptable results for all responses simultaneously, including robustness responses – i.e., the combined **Robust Design Space**. Such an overlay graph is presented in Figure 20.a, while Figure 20.b presents the same graph after scribing the **Proven Acceptable Ranges** (PARs) onto the design space within the graph.

Figure 20.a – Design Space

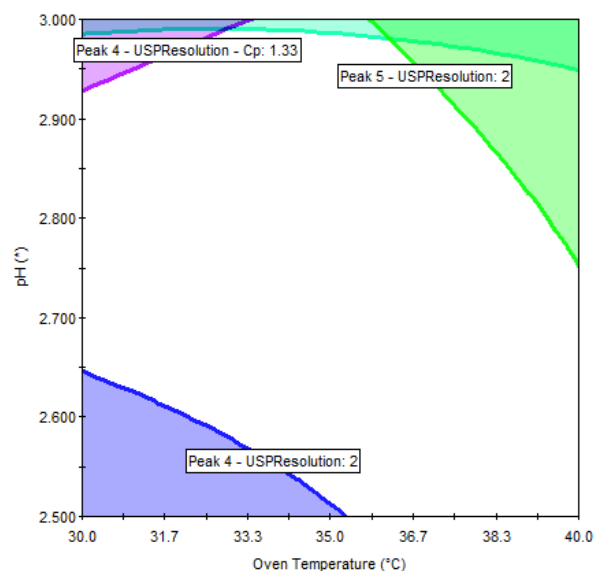
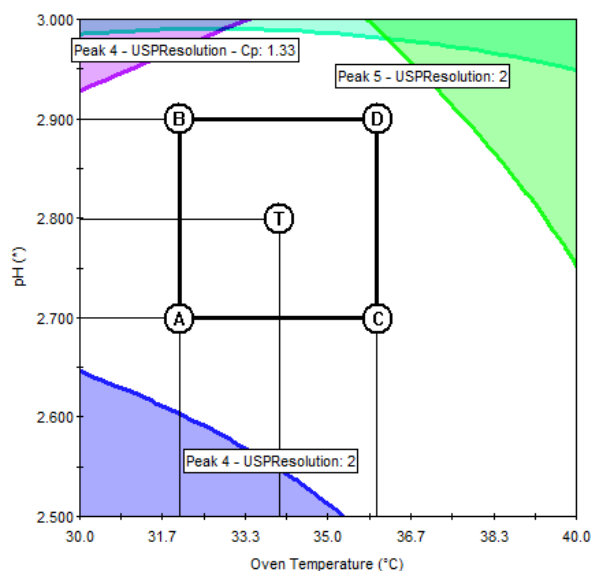
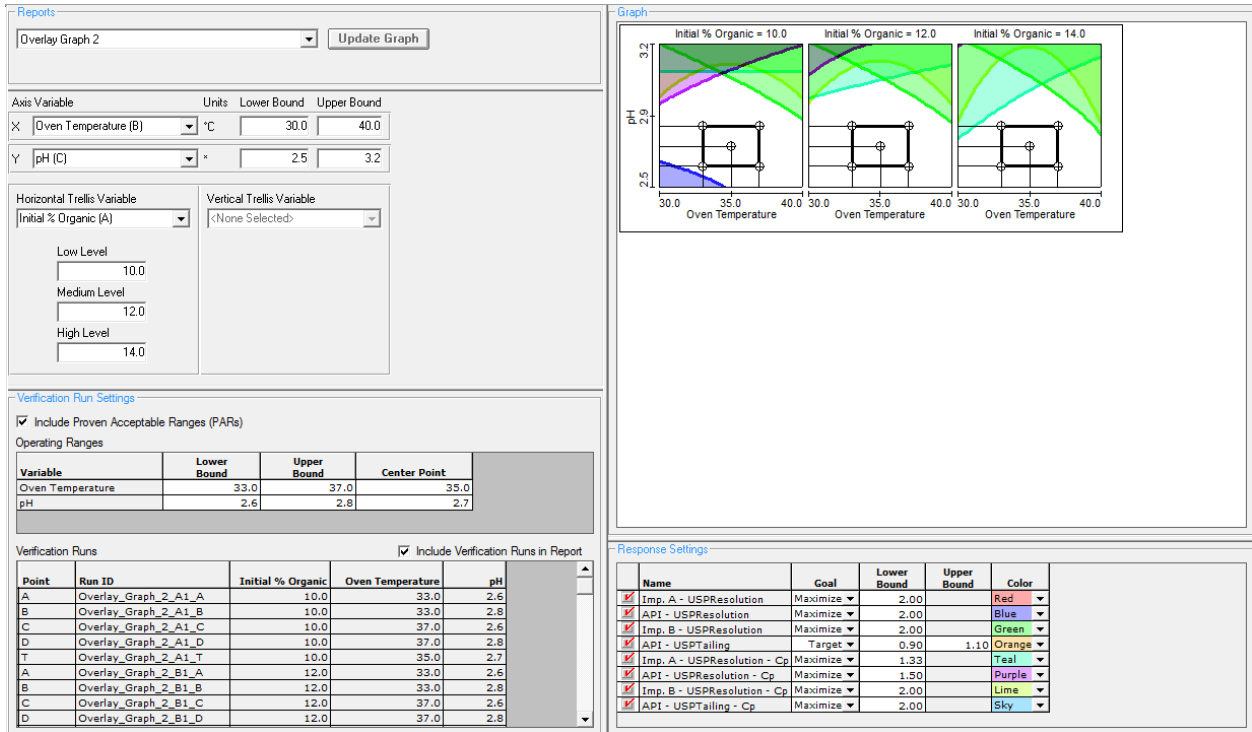


Figure 20.b – Design Space & PARs



FMD also has a 4D trellis graphing capability shown in Figure 21. This lets you visualize and report a graphical representation for up to 4-variable study variable combinations which result in **acceptable** method performance (*unshaded* regions) and **unacceptable** performance (*shaded* regions). The 4D trellis graphics also operate based on the performance goals the user sets for the included responses. Note that a specific color is associated with each response to denote where the performance for that specific response is unacceptable. This enables the 4D trellis feature to graphically display the 4-variable Robust Design Space and PARs in terms of meeting all user-defined goals simultaneously.

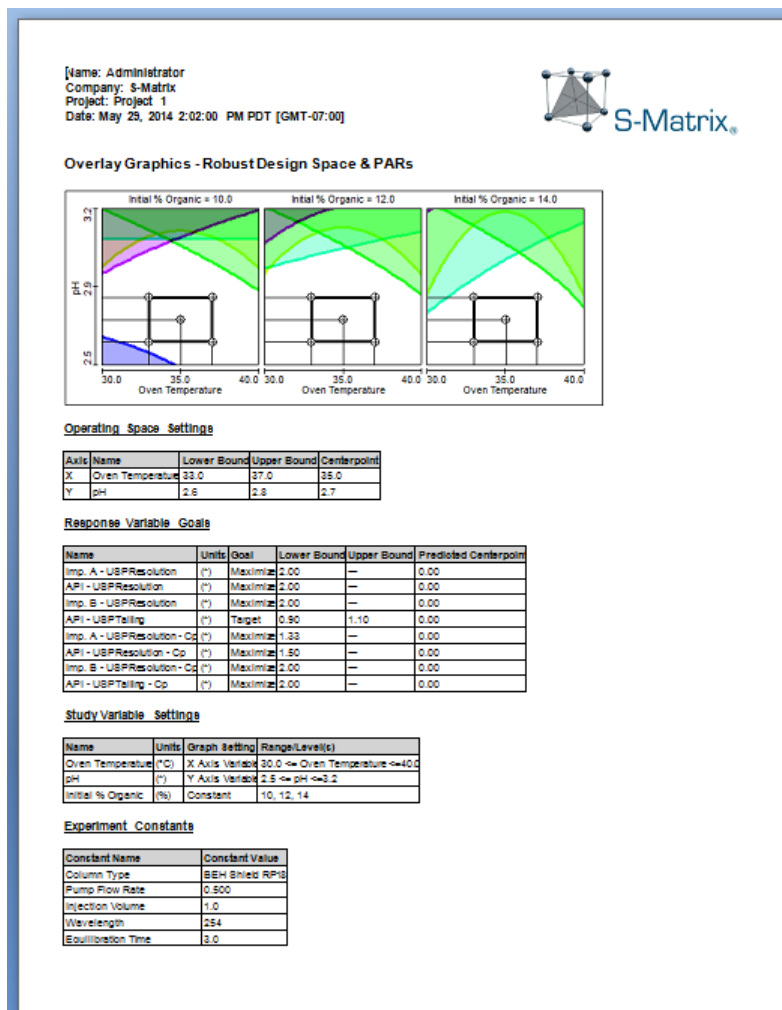
**Figure 21**



## 12. Generates Regulatory Submittal Quality Reports

FMD enables the user to create final reports of the Design Space and proven acceptable ranges (PARs – the “operating space”) which conform to FDA/ICH Guidances. Figure 22 is an example of one of these FMD reports, which can be compared directly to the guidelines presented in the ICH Q8(R2) Guidance. This is an ideal format for reporting QbD experiment results.

**Figure 22**



FMD’s comprehensive documentation also includes detailed statistical reports of the DOE design and the analysis results associated with each experiment. Any or all of these detail reports can be compiled into an internal report or an external report, depending on the target audience and the desired level of reporting detail.

For each response (chromatographic result) included in the experiment FMD generates a detail report of each of the interrelated analyses associated with the model (equation) it derives from the data. This includes an experimental error report, a transformation analysis report, a regression analysis report, a report of the statistical properties of the final model (equation), and a Pareto Ranking of Variable Effects report. In addition to these detail reports, FMD creates an Analysis Summary Report which contains a table and graph summarizing the key analysis results (Figure 23). The Analysis Summary Report defines the statistical quality of the model (model sufficiency), and is often used by customers as part of their analysis documentation.

**Figure 23**

