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QUALITY BY DESIGN SOFTWARE

QbD-aligned LC Method Development with Fusion QbD

Introduction

This white paper describes the practical application of Quality by Design (QbD) principles and methods to the development of Liquid Chromatography (LC) methods using the Fusion QbD Software Platform. Fusion QbD is a modular software platform that is scalable from standalone (single workstation) to global enterprise network systems, has full support for 21 CFR Part 11 compliance, and is Citrix Ready certified. The platform can be configured with one or more application modules, such as LC Method Development and Method Validation, and also with modules which exchange data with one or more Chromatography Data Software (CDS) systems.

It is noteworthy that the "*FDA has approved some NDA applications applying QbD approach to analytical methods (e.g. HPLC and UV)*", and "*Regulatory flexibility has been granted for movements within the defined analytical method design space.*"[1] It should be understood that a regulatory submittal does not have to be declared as a Complete QbD Submittal in order to apply QbD principles and methods to the analytical method development components of the submittal. Companies can and do include 'QbD elements' in regulatory submittals which are not 100% QbD submittals.

Much has been written about QbD and its advancement within the pharmaceutical industry, and so this paper does not present a detailed overview of QbD. An excellent overview of the history of and regulatory framework for QbD within the pharmaceutical industry can be found in the book Quality by Design, Putting Theory into Practice.[2] Although many articles have been written about the successful application of QbD to analytical method development, there remains some uncertainty in the Analytical R&D community about the applicability of QbD tools and methods to analytical development. One reason for the continued uncertainty is that the ICH lexicon codified in the QbD guidances is process centric – a natural consequence of the fact that the introduction and first use of QbD tools and methods in pharma was in process development. However, the FDA has stated that "*ICHQ8(R2) doesn't explicitly discuss analytical method development. However, (the guidance) concepts apply:*

- *Application of Science and Risk based methodology*
- *Systematic approach that includes: risk assessment, defining a design space, control strategy and continual improvement to increases method robustness and understanding.*"[1]

The fact is that QbD maps perfectly to analytical method development. This mapping is pictured in Figure 1, which correctly represents the LC instrument system as a "process in a box".[3] This is a busy figure, so we describe the elements in the figure in some detail. First, in the QbD lexicon a "Critical Process Parameter", designated CPP, is a system component which exerts a consequential effect on a "Critical Quality Attribute", designated CQA. A CQA is an important quality or performance characteristic which usually has one or more acceptance limits. As Figure 1 shows, Gradient Slope, Column Oven Temperature, and pH are examples of CPPs for a liquid chromatographic (LC) method, and the resolution of a critical pair, designated R_s, is an example of an associated CQA. Each blue sphere in the figure represents the R_s result obtained from a single sample injection using the LC method (a single run), which is the same as one execution of a process script. The mean R_s result is the mathematical average of the R_s results obtained from the multiple runs, which corresponds to the expected average result at setpoint conditions, while the individual results differ due to the simultaneous variations of the CPP level settings about their individual setpoints, both within and between injections.
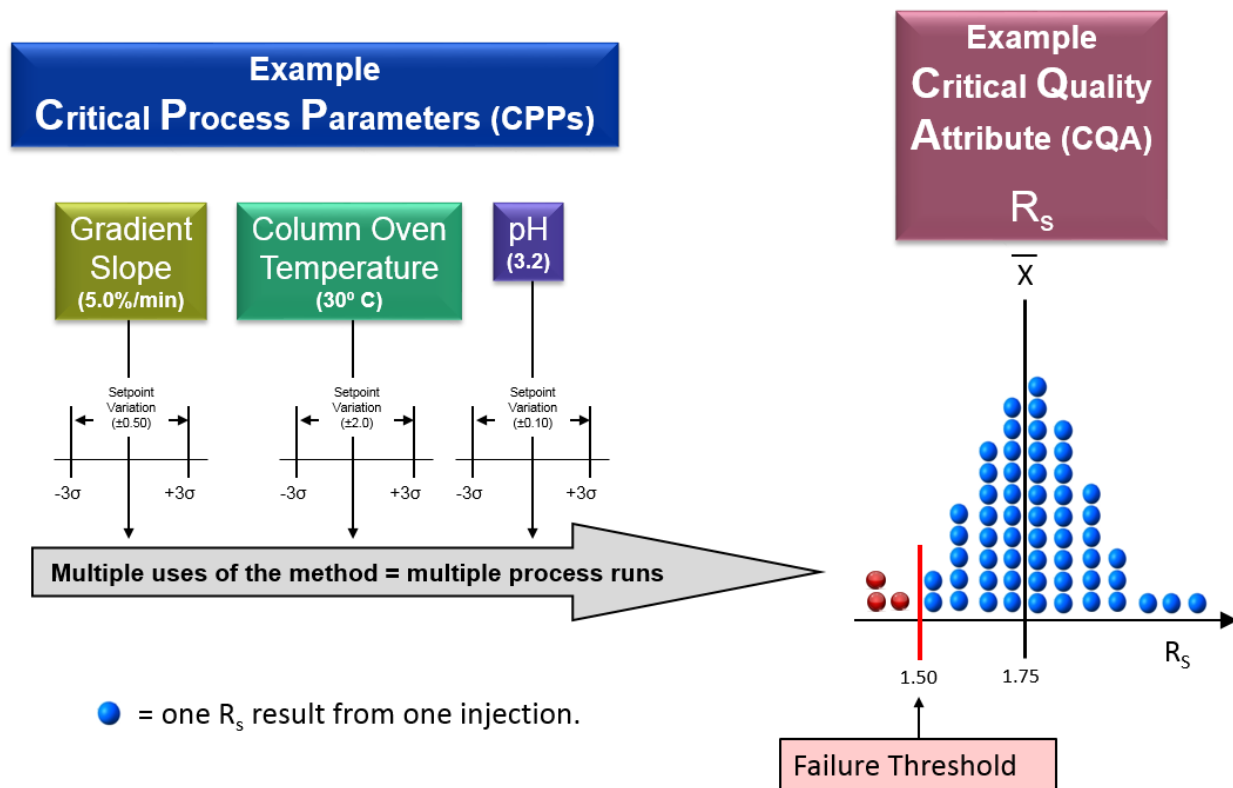

Figure 1. LC Instrument System – a "Process in a Box"

From an analytical research and development (AR&D) standpoint QbD moves beyond a one factor at a time (OFAT) approach to statistically based multi-parameter studies which enable both independent and interactive effects of the study parameters to be quantitatively characterized. In addition, it moves from qualitative visual inspection of experiment results to quantitative analysis and equation building (data modeling). QbD is therefore a data intensive methodology. For LC method development this means that a given study will (a) include multiple instrument parameters, and (b) apply numerical data analysis and modeling techniques to quantitative metrics of chromatogram quality. In the past, this additional work burden may have been seen as prohibitive to implementing QbD for LC method development. However, the good news is that Fusion QbD experiment automation and advanced analytics have dramatically reduced the work involved in implementing a QbD approach.

Integrated QbD Tools for LC Method Development

The three proven QbD tools listed and described below are all native technologies within Fusion QbD. These tools have been widely and successfully used across industries for over 50 years to develop and optimize processes and bring them into statistical control. Accepting the model of the LC system as a "process in a box" opens the door to using these proven QbD tools and methodologies to develop optimally performing and robust methods. This model is now accepted by the regulatory community, and is driving the regulatory expectation that AR&D work should employ the three QbD tools listed below.[1, 4, 5] In fact, some companies now apply statistical quality control charting to track method performance over time. The quantitative characterization of critical parameter effects, and the associated design space characterization, resulting from QbD method development provides the correct basis for method performance control charting and corrective action.[4, 5]

1. Design of Experiments (DOE)

2. Monte Carlo Robustness Simulation

3. Statistical Process Robustness Metrics

1.   Design of Experiments (DOE)

ICH Q8(R2) defines formal experimental design as "*a structured, organized method for determining the relationship between factors affecting a process and the output of that process. Also known as "Design of Experiments*".[6] This definition can be restated for analytical development as a structured, organized method for determining the relationship between instrument method parameters affecting method performance and the method's performance. In normal DOE terminology the "*relationship between instrument method parameters affecting method performance*" is called an interaction, which is a dependent relationship between the parameters. Given two CPPs A and B, an interaction between the two parameters, designated A*B, means that the observed effect on a CQA of changing the setpoint of A across a study range will be different at different levels of B. Figure 2 is a graph of such a two-parameter interaction involving Gradient Time (Δt_G) and pH.
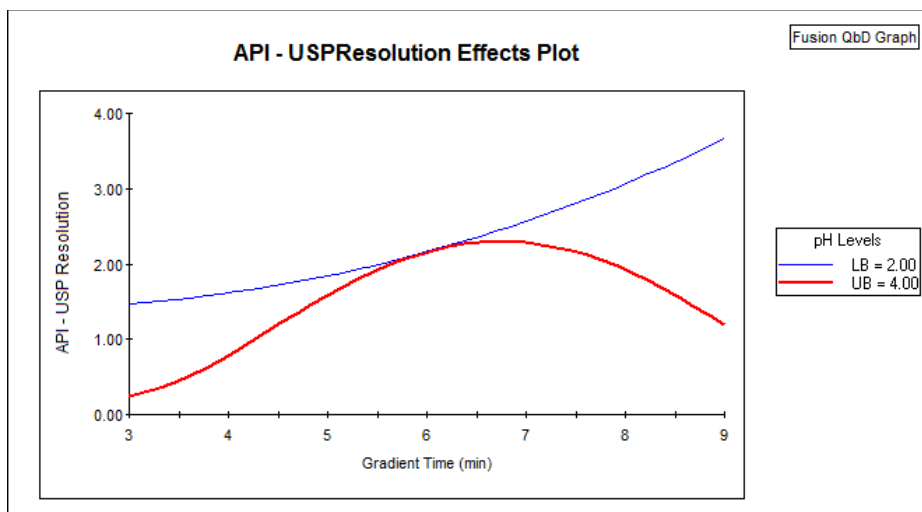
Figure 2. Effect of an Interaction Between pH and Gradient Time

Figure 2 shows that the effect of changing the gradient time on the resolution of the API is different at two different levels of the mobile phase pH: 2.00 (blue line) and 4.00 (red line). In other words, there is a pH-Gradient Time interaction effect. Interactions such as this are common in results data from chemistry system screening experiments. They can also be observed in method optimization experiments to have a significant effect on method robustness.

Figure 3 shows the flexible Experiment Setup window in Fusion QbD. Here the user can select any combination of parameters for a Gradient or Isocratic study, and also utilize the Online Preparation mode for study factors such as pH or Buffer Concentration. In addition, underlying Experiment Setup is a comprehensive DOE engine coupled with an expert system which diagnoses the level of study (screening or optimization), the parameters selected for study, and the user specified study ranges or levels for each parameter, and then automatically generates the most efficient experiment design to characterize the independent and interactive effects of the study parameters at the click of a button.
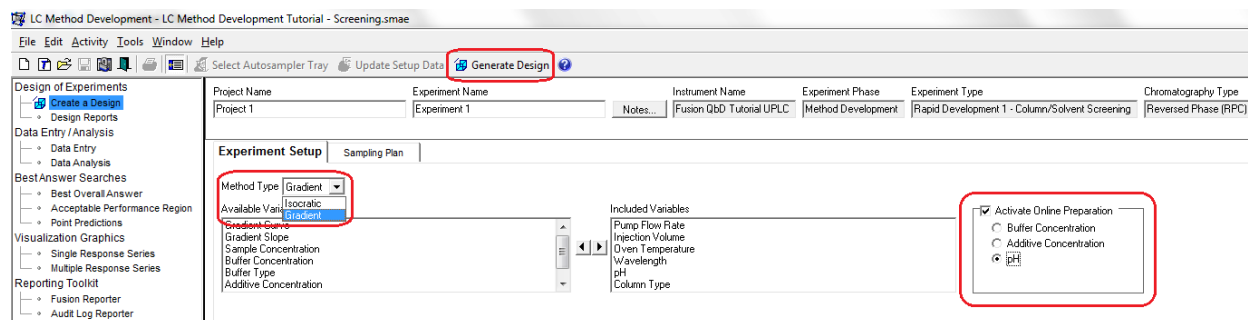

Figure 3. Flexible Experiment Setup within Fusion QbD

Fusion QbD can automatically reconstruct the DOE experiment within the chromatography data software (CDS) as ready-to-run methods and sequences, as shown in Figure 4.
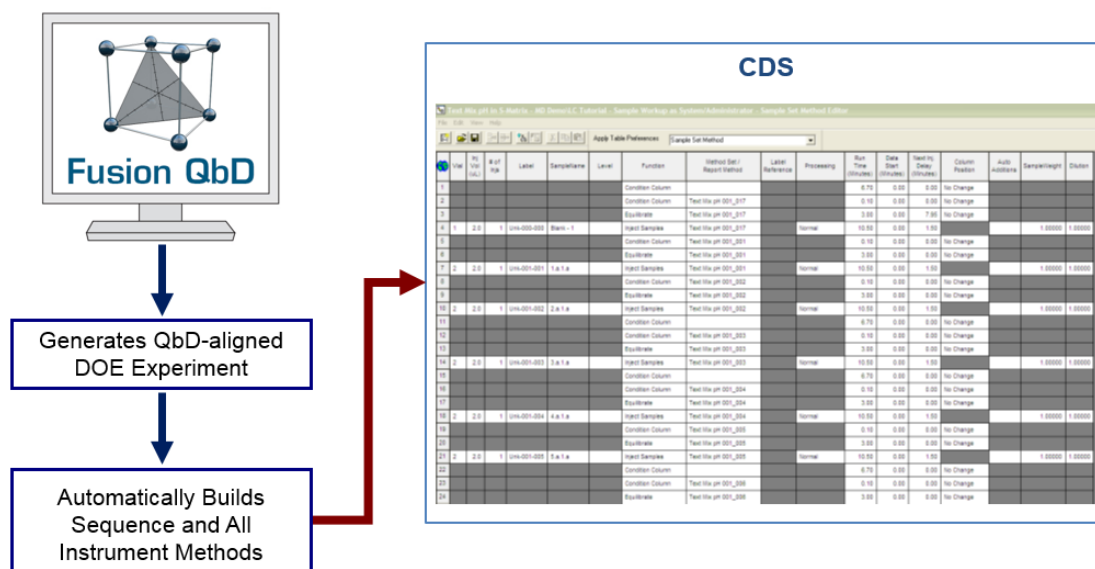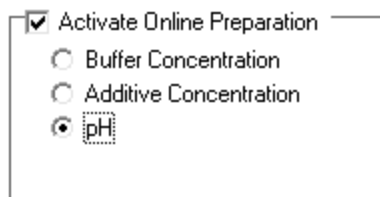

Figure 4. Experiment Design Exported to CDS as Ready to Run

Fusion QbD offers the most comprehensive and advanced capabilities available today for generating, automating, and analyzing QbD-aligned LC method development and method validation experiments on multiple Chromatography Data Software (CDS) platforms.**[7, 8]** Knowledge of the exact LC configuration is a key component of effectively utilizing this automation, and so the program enables the user to identify the exact configuration of the LC instrument system on which the experiment will be run, including the pump and column compartment modules and solvent and column selection valves. In this way the program can *understand* the walk-away automation capacity of the LC system for the user's current experiment. When the scope of the experiment exceeds the LC's automation capacity, the program will logically organize the experiment into the needed number of sequences, allowing the user to make the required configuration changes between running the sequences. For example, if the user's LC has a 2-position column switching valve, but has set up a 4-column screen, Fusion QbD will create two sequences of two columns each. The user can then run the first sequence, swap out the columns, and then run the second sequence with the remaining two columns. In addition, the program configures the experiment for proper execution given the users selected study parameters. For example, it includes conditioning runs for each column when the mobile phase chemistry changes between injections, and the program aggregates experiment methods with the same chemistry to minimize both overall run time and system perturbation.

Chemistry System Screening Made Simple

The DOE and experiment automation capabilities within Fusion QbD enable the user to combine major selectivity effectors into a single chemistry system screening study. For example, a reversed phase chemistry screening study can include multiple columns with very different chemistries in combination with different strong solvent types (e.g. methanol and acetonitrile), a broad range of pH, and gradient slope to characterize the combined (independent and interactive) effects of these parameters on overall method performance.

For LC instrument systems with quaternary pump modules Fusion QbD can even automatically prepare target levels of pH for experimenting. It does this by enabling the user to define an acid-base solvent pair and the solvent ratio needed to achieve each desired study level of pH. Fusion QbD 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.

Table 1 below contains acid-base solvent ratios and associated pH values for a six level pH screen covering a range of ~2.75 – 6.25 using Formic Acid and Ammonium Formate – each at a 20.0 mM concentration. Figure 5 shows the Fusion QbD Buffer Selector control used to select the buffer system and associated target pH levels, and the resulting pH setup for the screen within the Fusion QbD Experiment Setup window. The pH values in the Buffer Selector control were obtained by pumping the corresponding solvent ratios from two reservoirs of a quaternary pump and measuring the pH of the resulting output mobile phase.

Table 1, pH Screen: Range 2.75 – 6.25 (6-levels)

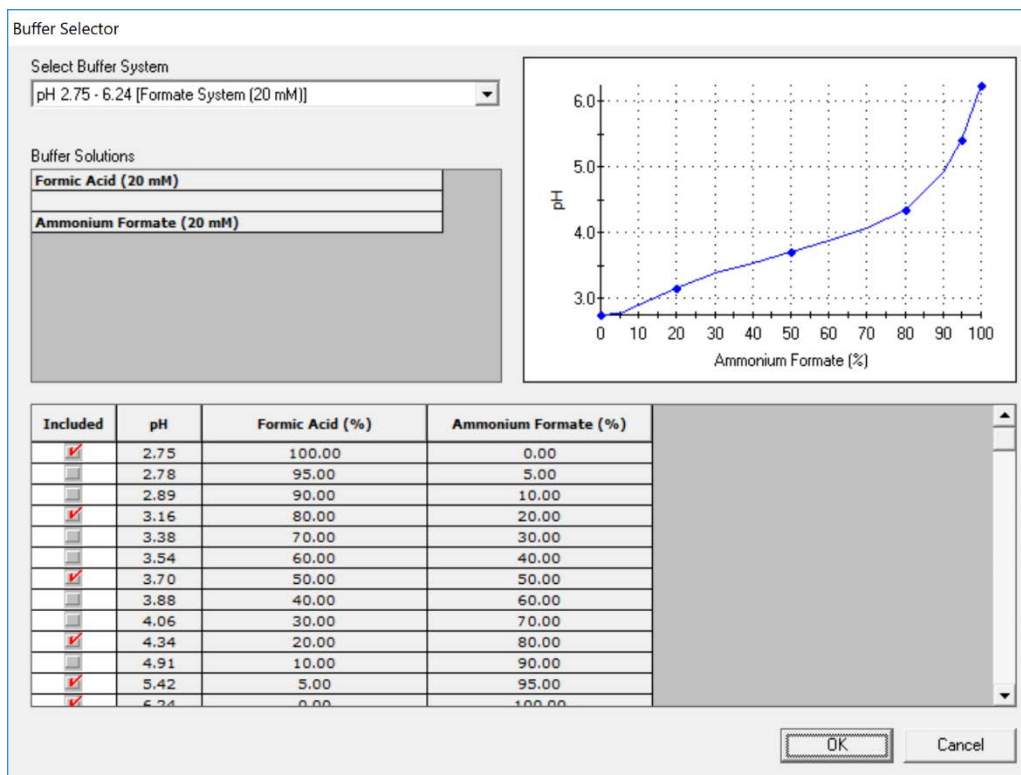| 20.0 mM Formic Acid (%)	20.0 mM Ammonium Formate (%)	pH
100	0	2.75
80	20	3.16
50	50	3.70
20	80	4.34
5	95	5.42
0	100	6.24

Figure 5. Online Preparation of pH for a broad Chemistry System Screening Study

Multifactor chemistry system screening studies, which in most cases can be run unattended overnight, eliminate the need to rely solely on a limited theoretical basis for selecting critical initial method conditions to use in the method development work such as column type, pH region, initial slope, and strong solvent type. However, multifactor chemistry system screening studies will generate chromatograms with large differences in the degree of co-elution and/or changes in elution order between experiment runs. Figure 6 illustrates these differences for five peaks in four chromatograms (A, B, C, and D) generated by such a study.
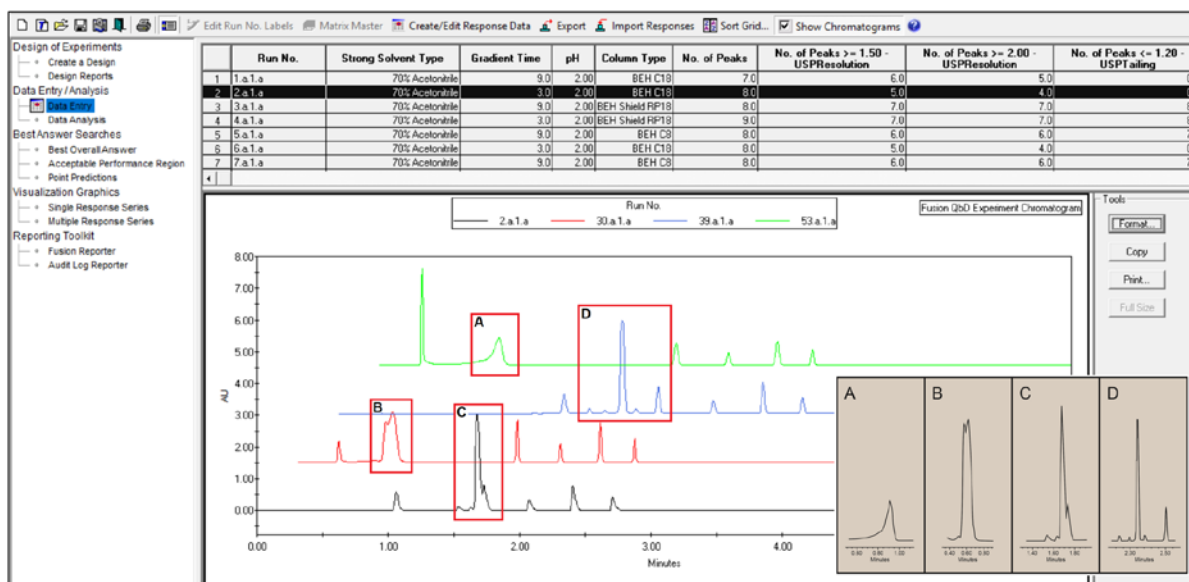

Figure 6. Selectivity Differences across Chemistry Screening Runs

The highly variable nature of these chromatograms makes it extremely difficult to correctly identify individual peaks in each experiment chromatogram (peak tracking). This problem is greatly amplified in early method development experiments in which the number and/or the identities of all sample compounds may not be known. Also, the unknown compounds may be related impurities or degradants with the same parent ion and/or very similar areas. Consequently, it is almost impossible to correctly identify all peaks, or even just the critical peaks of interest, in these experiments. Even when maximum effort is expended peaks are often misidentified, and data will be missing for co-eluting peaks, which has been shown to severely impact data analysis and modeling.

S-Matrix developed its patented Trend Response™ capability in Fusion QbD to specifically solve the peak identification challenges inherent in multifactor chemistry system screening studies. As shown in Figure 7, Fusion QbD displays all integrated chromatogram results data in a simple data import wizard. The patented Trend Response capability built into this wizard enables users to define specific metrics of chromatographic quality which the software will directly derive from each experiment chromatogram in the form of peak count based responses and peak property based responses.
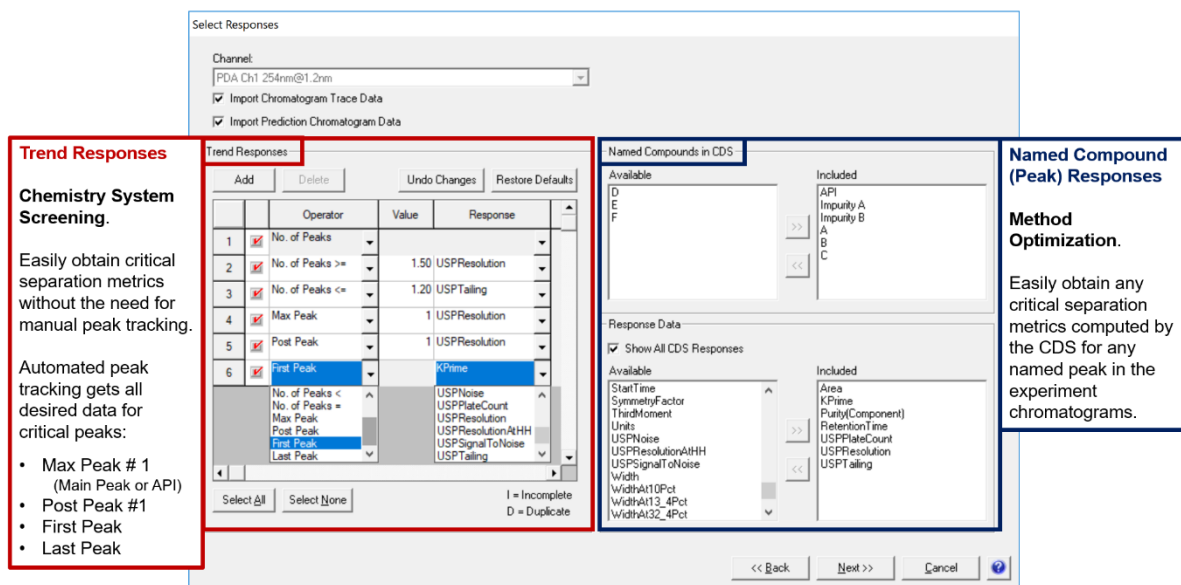

Figure 7. Response Data Import Wizard

The wizard lets users specify any chromatographic result for peak count based response data capture, including custom variables. Typical examples include the number of integrated peaks, the number of baseline resolved peaks, the number of peaks with acceptable tailing, the number of peaks with a minimum signal/noise ratio and/or minimum area (or % Area), etc.

Fusion QbD also automatically tracks specific key peaks in experiment chromatograms including the main peak(s), which are tracked by area (*Max Peak # operators*), their associated immediately later eluting peaks (*Post Peak # operators*), and the first and last peak in the chromatogram. Users can specify peak property based response data capture for any of these key peaks. Typical examples include resolution, retention time, area, and tailing for main peaks and their immediately later eluting peaks, and resolution, retention time, and retention factor (k') for the first and last peaks. As shown in Figure 8, once the user has specified the desired chromatogram results, Fusion QbD can automatically derive and import the results from the CDS in preparation for automated modeling.
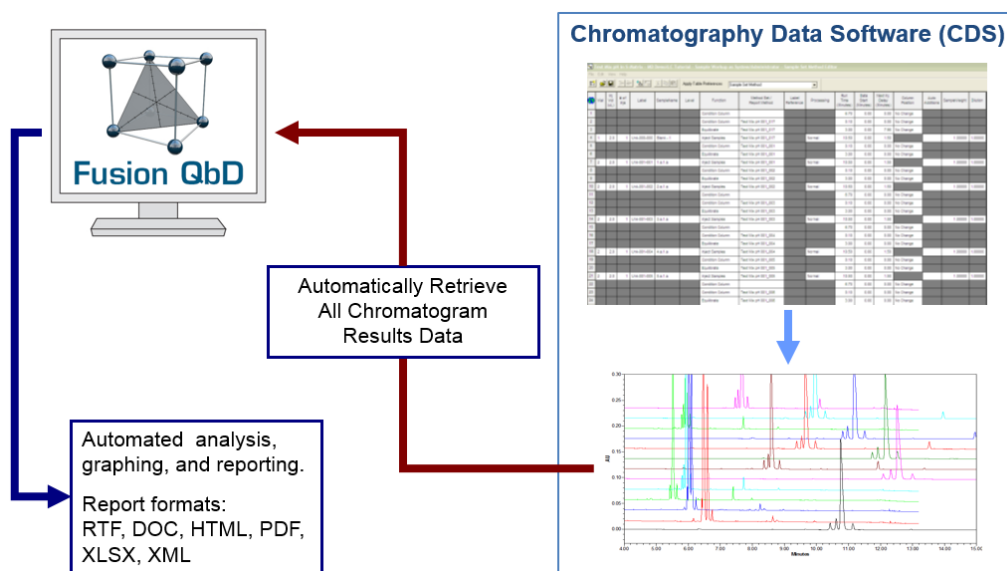

Figure 8. Chromatogram Results Automatically Imported from CDS for Analysis

Trend responses have consistently proven to be reliable and modelable, and to directly support the chemistry screening goal of rapidly identifying the specific combination of the study parameters which provides best overall method performance without the need for direct peak tracking. This is illustrated in the two comparison chromatograms presented in Figure 9. The left chromatogram in the figure was generated by a method in which the critical method parameter conditions were selected using first principles. The right chromatogram was generated using the predicted best chemistry settings determined by modeling trend response data from an overnight chemistry screening study which included the first principles based combination of these parameters as one of the trial runs. The results presented in Figure 9 are not unusual – these studies often identify the need for significant changes from first principles settings to at least one, and often all, of the study parameters given the variety of new column chemistries and the complexity of multi-compound samples. Studies like this are one reason that a QbD aligned approach can dramatically accelerate method development and lead to more robust methods overall.

- Column = C8
- pH = 2.30
- Gradient Time = 7.0 minutes
- Column Temperature = 40.0 °C

- Column = C18
- pH = 2.40
- Gradient Time = 11.0 minutes
- Column Temperature = 50.0 °C*
  * Follow on Optimization Experiment Result

Critical Peak Resolutions

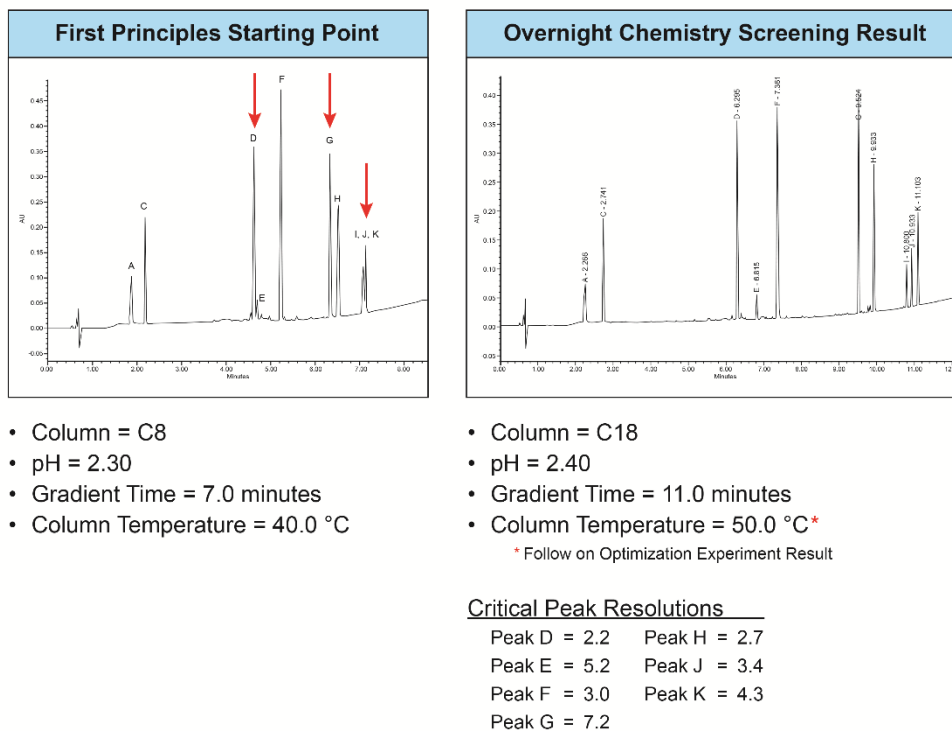|	
Peak D = 2.2	Peak H = 2.7
Peak E = 5.2	Peak J = 3.4
Peak F = 3.0	Peak K = 4.3
Peak G = 7.2	

Figure 9. "First Principles" versus Overnight Chemistry System Screening

Automated Data Modeling and Method Performance Visualization

Fusion QbD automatically generates prediction equations (models) which quantitatively characterize the independent and interactive effects of the study parameters on each CQA included in the study. These equations can then be used to predict the performance of any given method for all CQAs given any combination of CPP level settings within the experimental region. The equations can also be used to generate predictions for extrapolations based on observed trends which it can also export to the CDS for verification. Figure 10.a illustrates the automated modeling for one CQA – the resolution of the API from an earlier-eluting impurity – and the corresponding graphical visualization of the combined effects of two study factors on the response automatically generated from the equation. Figure 10.b illustrates the translation of the graphical visualization into an easy to interpret 2D visualization of the method conditions giving acceptable performance (unshaded region) and unacceptable performance (shaded region).
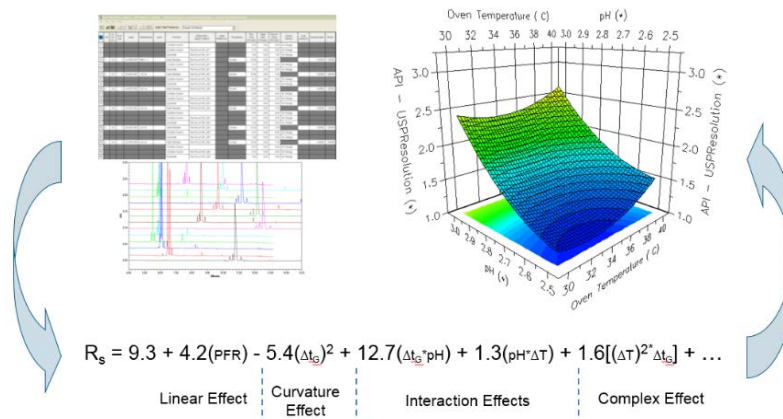

$$R_s = 9.3 + 4.2(\text{PFR}) - 5.4(\Delta t_G)^2 + 12.7(\Delta t_G \cdot pH) + 1.3(pH \cdot \Delta T) + 1.6[(\Delta T)^{2} \cdot \Delta t_G] + \ldots$$

| Linear Effect | Curvature Effect | Interaction Effects | Complex Effect |

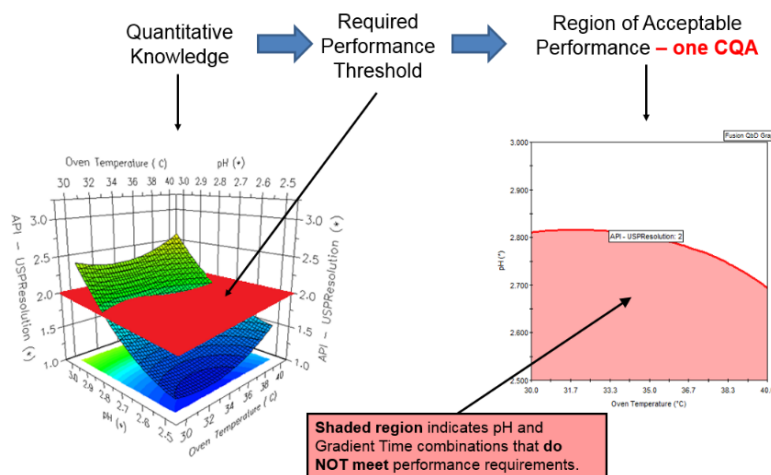**Figure 10.a. Automated Modeling of Chromatogram Results Data Sets**

Figure 10.b. Model-based Visualization of Acceptable Method Performance Conditions

Figure 10.c presents a Fusion QbD report detailing the observed and model predicted data for a given experiment run. As the figure shows, these results are presented in both tabular form and also as a stacked chromatogram.
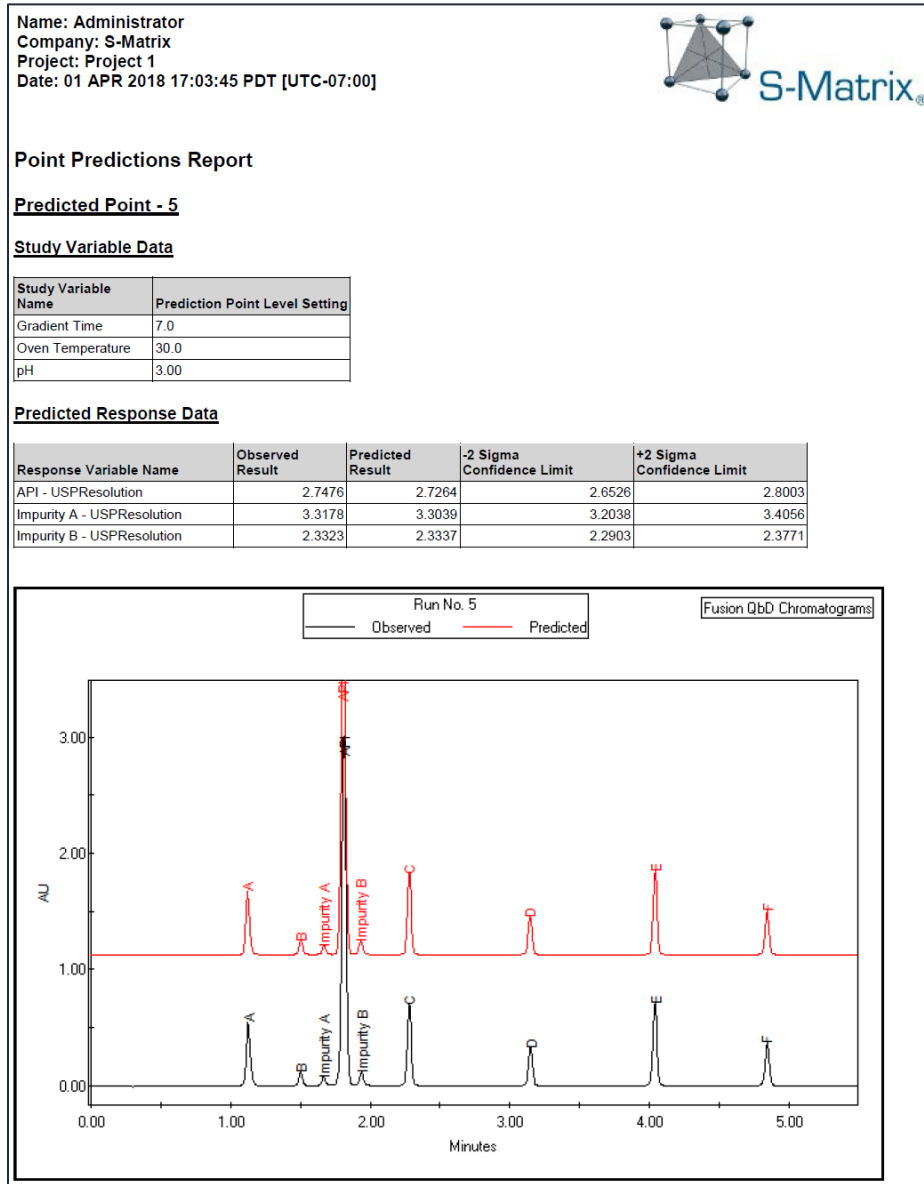

Figure 10.c. Fusion QbD Report of Observed and Predicted Results and Chromatogram

2.   Monte Carlo Robustness Simulation

The U.S. FDA final guidance on Analytical Procedures and Methods Validation for Drugs and Biologics states that "*During early stages of method development, the robustness of methods should be evaluated because this characteristic can help you decide which method you will submit for approval.*"[9] The guidance goes on to state that "*To fully understand the effect of changes in method parameters on an analytical procedure, you should adopt a systematic approach for a method robustness study (e.g., a design of experiments with method parameters). You should begin with an initial risk assessment and follow with multivariate experiments. Such approaches allow you to understand factorial parameter effects on method performance.*" However, it should be understood that a prediction equation for a given method performance characteristic obtained from a multivariate DOE experiment predicts the mean performance – the average expected performance – of the method. It does not predict the *variation* in the method's performance for the particular characteristic (response) that will be observed over multiple uses of the method. This is also true for so-called first principles equations.

Monte Carlo Simulation (MCS) is a well understood and accepted mathematical methodology for predicting the performance variation in a CQA given the joint variation in the CPPs expected during normal operation. ICH Q2(R1) defines the robustness of an analytical procedure as:

"*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*".

And further states:

"*The evaluation of robustness should be considered during the development phase and depends on the type of procedure under study. It should show the reliability of an analysis with respect to deliberate variations in method parameters.*".[10]

The FDA also states:

"*Statistical treatments (e.g., Monte Carlo simulations) can help evaluate the effects of uncertainty.*".[4]

Therefore, Fusion QbD has integrated a true Monte Carlo Simulation (MCS) engine into its software framework to fully integrate robustness analysis into method development.[11]

The MCS engine within Fusion QbD simulates variation in a given response by repeatedly solving the mean performance model obtained from the DOE study using a different level setting combination of the study parameters each time. The level setting of each study parameter used in each iteration is randomly selected from within a user defined expected variation range around the parameter's method setpoint. These thousands of predicted results translate directly into a predicted response variation distribution from which the $\pm 3\sigma$ method performance variation limits are calculated. The MCS engine correctly represents method variation in two ways. First, it represents setpoint variation in each study parameter as a Gaussian error distribution (a bell curve), and not as a uniform distribution in which small and very large setpoint deviations are represented as equally likely to occur. Second, the MCS engine incorporates the joint (cumulative) setpoint variation distributions of the study parameters, since the assumption that method variation can be represented by varying one parameter while holding all other parameters constant at their individual setpoints is erroneous.
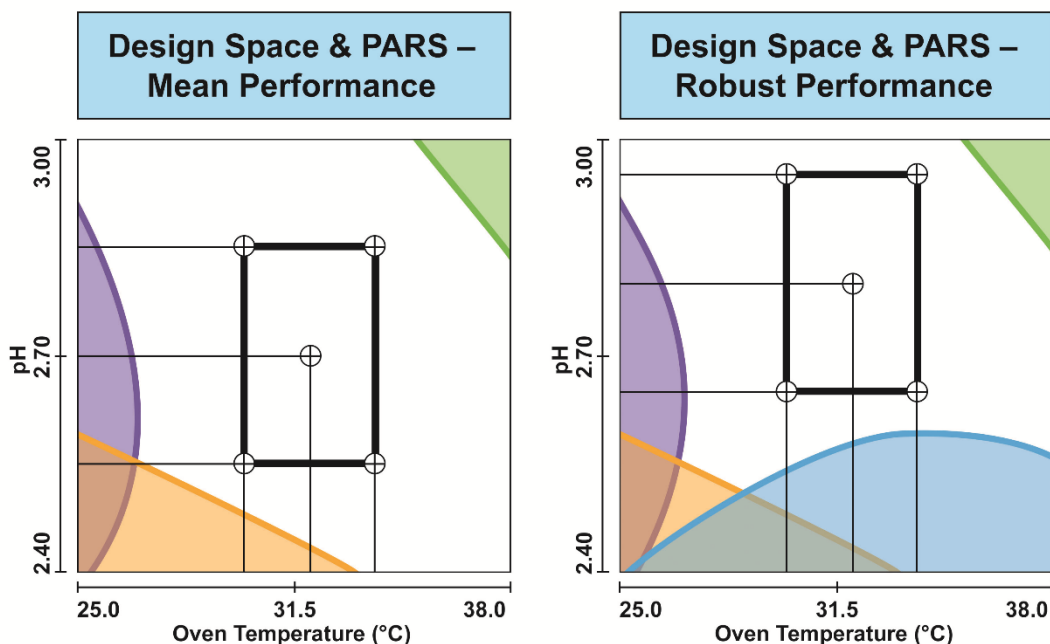
3.   Statistical Process Robustness Metrics

The FDA states that accepted process capability indices such as C_p, C_{pk}, C_{pm}, and C_{pkm} are also part of the QbD toolset.[5] The C_p, index is a quantitative metric of process robustness which is calculated for a given response as the ratio of the allowable specification width to the observed or computed response variation ±3σ width. The other indices are variations of the C_p index to accommodate cases in which the response has a specific result target, and whether or not the specification limits are symmetrical around the target. All these indices have the same result scale that translates directly into a risk of failure. For example, for a given method performance characteristic (response) such as resolution or tailing, a C_p index value of 1.00 indicates that at least one of the ±3σ performance variation limits is located exactly at an acceptance limit. Another way of stating this is that at least one acceptance limit is located at a distance of 3σ from the mean response. Therefore, a C_p index value of < 1.00 indicates a heightened risk of method performance failure for the response due to expected variation in one or more critical method parameters, while a C_p index value of > 1.00 indicates a reduced risk. The traditional C_p index value goal is ≥ 1.33, which indicates that the critical acceptance limit for the response is located at a distance of 4σ from the mean response.

Fusion QbD – the Integration of Mean Performance and Robustness

As stated, Fusion QbD can directly link the prediction equations obtained from the DOE experiment to its integrated MCS engine to obtain predictions of performance variation in the responses. It can also directly compute process capability indices from the predicted variation results, as well as more traditional metrics such as the variance and the 1σ, 2 σ, 3 σ, and 4 σ values. This means that we now have the capability to obtain predictions of mean performance directly from the DOE models and also coordinated quantitative predictions of method robustness for all responses included in a method development study. By linking mean performance prediction models and coordinated robustness simulation models, Fusion QbD enables the user to establish and visualize the robust design space in multiple dimensions.

Figure 11 presents two versions of a Design Space and Proven Acceptable Ranges (PARs) graph for the simplest case of two study parameters, Oven Temperature and pH. Each graph shows the combined effects of changes to pH and temperature on the eight method performance characteristics (responses) listed in the associated Method Performance Goals table. In these graphs each response is assigned a color, and the region of the graph shaded with that color represents pH-temperature combinations for which method performance will fail to meet the goal for that response. The region of the graph not shaded by that color therefore represents pH-temperature combinations which will meet or exceed the performance goal for the response. The left graph within the figure contains only the four mean performance responses, while the right graph also contains the four coordinated robustness responses.

Method Performance Goals					
NAME	GOAL	LOWER BOUND	UPPER BOUND	COLOR	
API - Resolution	Maximize ▼	2.00		Purple	▼
Impurity A - Resolution	Maximize ▼	2.00		Blue	▼
Impurity B - Resolution	Maximize ▼	2.00		Green	▼
API - Tailing	Target ▼	0.90	1.10	Orange	▼
API - Resolution - Cpk	Maximize ▼	1.33		Teal	▼
Impurity A - Resolution - Cpk	Maximize ▼	1.33		Red	▼
Impurity B - Resolution - Cpk	Maximize ▼	1.33		Lime	▼
API - Tailing - Cpm	Maximize ▼	1.33		Sky	▼

Figure 11. Integration of Mean Performance and Robustness

The desired operating flexibility of ±0.15 for pH and ±2.0°C for temperature is represented by the PAR rectangle around the central method (the central dot within the rectangle). Note that the PAR rectangle is within the joint unshaded region in the left graph when the method setpoints are 32.0°C and pH 2.70, which indicates that mean performance goals will always be met for the four responses. However, the right graph shows that the PAR rectangle will reside partly in the blue shaded region when robustness performance is also considered. This indicates that the method will sometimes fail to meet the API Tailing requirement due to lack of robustness ($C_{pm} <$ 1.33). But this graph also shows that the PARs can be supported by simply changing the method pH setpoint to 2.80, which demonstrates the power and value of the software's integrated robustness characterization methodology. With this powerful visualization capability you can easily create a 3x3 series of these graphs to establish and visualize the design space and PARs for four parameters at a time, and also output these reports in multiple document formats.

Figure 12 illustrates the Fusion QbD window displaying numerical and graphical predictions of the robust design space. The main display window includes a prediction chromatogram viewer which updates in real time as you move the mouse pointer within the graphed region. Both the design space graph and the prediction chromatogram also update in real time as you move the non-graphed variable slider control(s) to change the setpoints of these variables. You can also combine prediction chromatograms with the design space overlay graphs in output reports.
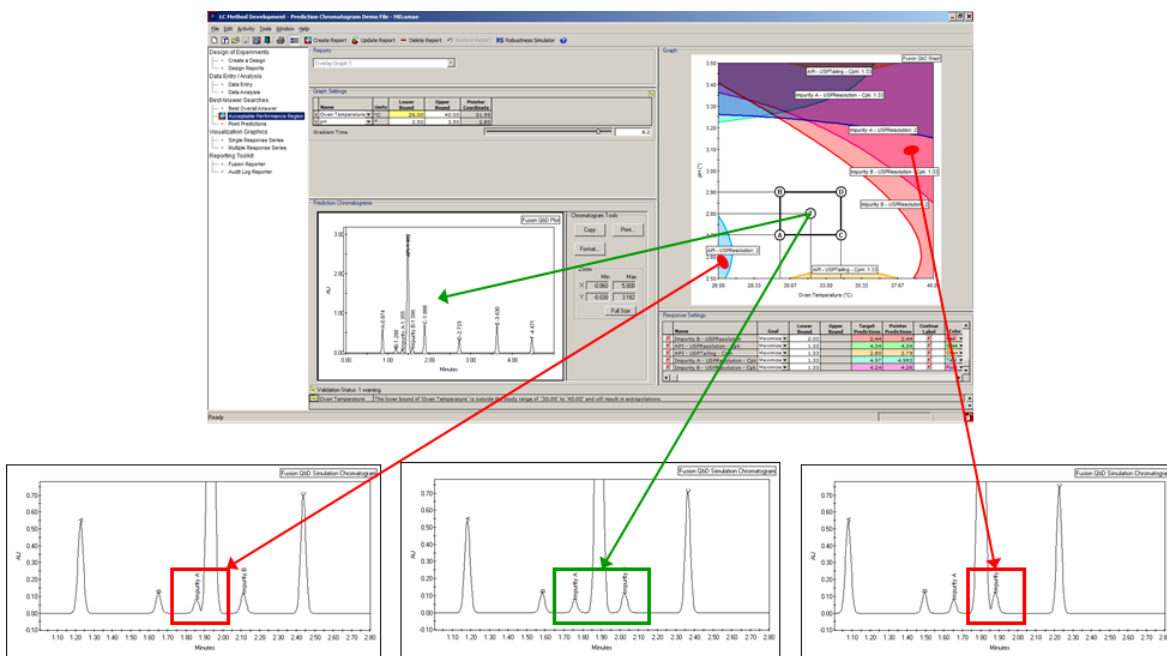

Figure 12. Integration of Overlay Graphics and Prediction Chromatogram Viewer

Conclusion

The three major advancements discussed herein can transform LC method development work from limited, manual one factor at a time or successive approximation based studies to automated, data rich studies which are fully aligned with QbD principles. Together they represent a best practices approach which is appropriate at any stage in the method development life cycle. The regulatory imperative for implementing QbD in analytical development is clear. However, there is also a solid business driver due to its real impact on the bottom line. One quantitative example of this impact was presented by a major international pharmaceutical company at a recent Pittsburgh Conference (Pittcon) workshop.[12] The company representative presented that using Fusion QbD saved an average of $47,000 in direct development costs per method, with average development time reduced from about 3.5 months per method to about 3 weeks. This represents an average 78% reduction in method development time and cost. But the representative also said that the most important savings were obtained when the methods were transferred to QC to support commercial production. Many of these methods have now been in use for several years, and not one of them required further development with the associated additional costs and downtime.

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