A Quality-by-Design Methodology for Rapid LC Method Development – Part 1

By:

Joseph Turpin
Associate Senior Scientist
Eli Lilly and Company, Inc.
Elanco Animal Health Division
Greenfield, IN

Patrick H. Lukulay, Ph.D.
Director, Drug Quality and Information
U.S. Pharmacopeia
Rockville, MD

Richard Verseput
President
S-Matrix Corporation
Eureka, CA

Abstract

Quality by Design (QbD) is a methodology which enables the practitioner to efficiently obtain quantitative ***process knowledge***. When applied to Liquid Chromatography (LC) method development work the LC instrument system is the ***process***; and quantitative definition of how controllable instrument parameters affect chromatographic performance is the ***knowledge***. This three-part article describes a QbD approach to the rapid development of robust LC methods. This approach provides quantitative process knowledge which can be used to identify the LC instrument parameter settings that provide optimum chromatographic performance, including method robustness. Most importantly, this knowledge can support the analyst's ability to modify the LC method as required to maintain acceptable performance.

This article describes how statistically rigorous QbD principles can be put into practice to accelerate each phase of LC instrument method development. The article is presented in three parts. Here in part one the authors examine the current approaches to column screening in terms of design space coverage – a key element of process knowledge. The second part presents novel data treatments to both accelerate and bring quantitation to the column screening effort. Part three of the article will focus on integrating quantitative method robustness estimation into formal method development.

Introduction

Development of analytical methods for Liquid Chromatography (LC) instrument systems is typically carried out in two phases. The first phase involves column screening, sometimes referred to as column scouting. Column screening is the experimental work done to identify the analytical column (stationary phase) with the best selectivity in terms of all compounds in the sample which must be adequately resolved. Formal method development, the second phase, involves experimenting with additional instrument parameters believed to strongly affect compound separation. The overall goal of the two phases is identification of the instrument parameter settings that provide optimum chromatographic performance.

This article describes how statistically rigorous Quality-by-Design (QbD) principles can be put into practice to accelerate each phase of LC instrument method development. The article is presented in three parts. In this first part we address column screening. We examine current method development approaches in terms of design space coverage – a key element of process knowledge. In part two we will present QbD data treatments to both accelerate and bring quantitation to the column screening effort. Part three of the article will focus on integrating quantitative method robustness estimation into formal method development. Moving robustness estimation upstream into the method development effort is consistent with both FDA and ICH guidances. It also enables the identification of instrument methods which simultaneously meet both mean performance and performance robustness requirements.

Traditional LC Method Development Practice

Reversed-phase liquid chromatography (RPC) is by far the most widely used liquid chromatography (LC) separation method in the pharmaceutical and biotechnology industries. RPC is therefore the basis of the discussions and examples used in this paper. However, the reader will recognize that the instrumentation, software, and Quality-by-Design (QbD) based methodologies presented here are applicable to other LC approaches such as normal-phase liquid chromatography (NPC) and hydrophilic interaction liquid chromatography (HILIC).

The traditional approach to LC method development is to systematically vary one factor across some experimental range while the level settings of all other controllable factors are held constant. This "**O**ne-**F**actor-**A**t-a-**T**ime" (OFAT) approach, still widely practiced today, is carried out by selecting one instrument parameter to study while holding all other parameters fixed. The "best" performing level of the study parameter is normally identified by visual inspection of the trial chromatograms; the parameter is then fixed at this level, and a new instrument parameter is selected for the next iteration. The OFAT process is repeated parameter by parameter until an adequately performing instrument method is obtained.

The OFAT approach is routinely carried out in two informal phases, with a specific set of instrument parameters relegated to each phase. The instrument parameters associated with the first phase are those which were historically "easy to adjust", meaning that new levels of the parameters could be set directly in the instrument method without the need to physically change the instrument configuration. The instrument parameters associated with the second informal phase were those for which changing a level normally required changing the instrument configuration; for example, switching a solvent reservoir or switching to a different analytical column. Table I lists the instrument parameters commonly utilized in the two informal OFAT phases of traditional method development.

Table I. Historical Phasing of Method Development Workflow

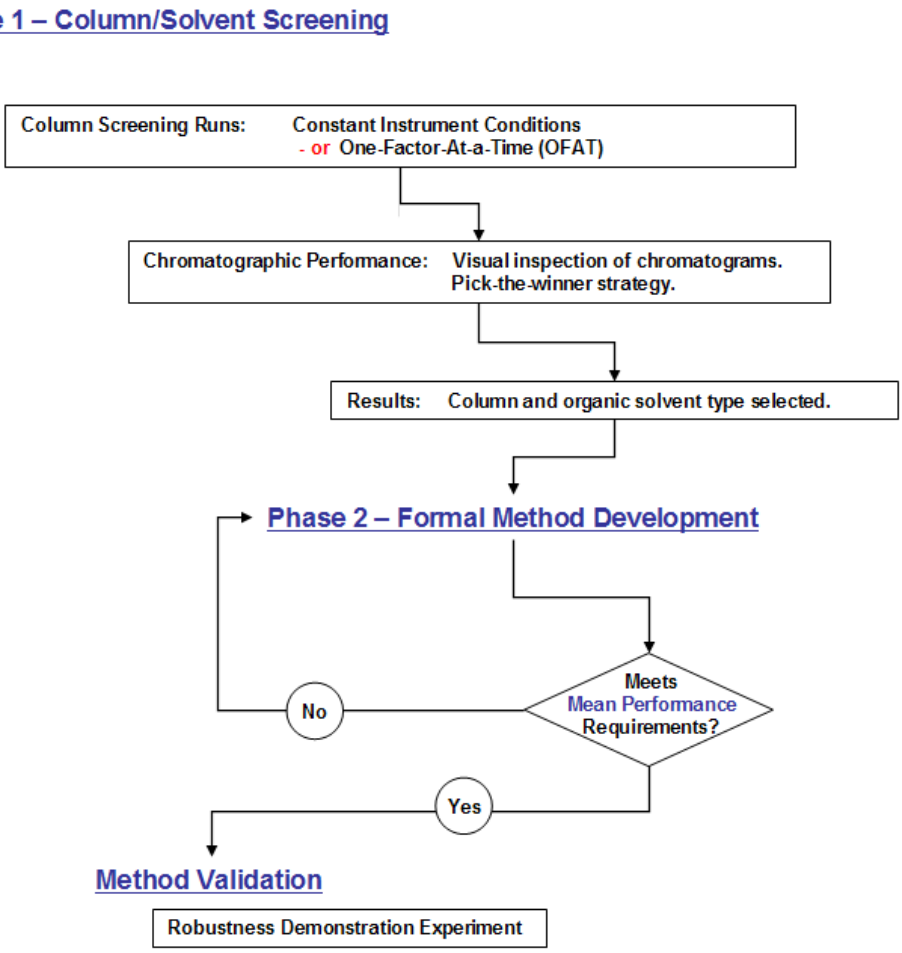
Phase 1 – *Continuous* Parameters (Easy to Adjust)	**Phase 2 – *Discontinuous* Parameters** (Hard to Adjust)
Solvent Strength (% Strong Solvent)	Column Type (cost, switching required)
Temperature	pH (online mixing not advised)
Gradient Conditions (e.g. Slope)	Organic Solvent Type (exclusive "or")
	Ion Pairing Agents (not universal, slow column equilibration)

The obvious limitation of the traditional OFAT approach is that the instrument parameters that often most dramatically affect selectivity, and therefore adequate separation, are the *hard-to-adjust* parameters relegated to the second informal development phase. This builds inefficiency into the approach as well as risk, since critical time may be spent studying secondary affecting parameters in the first phase without achieving much performance improvement.

However, modern LC instrument systems can support column and solvent selection valves. These new automation capabilities eliminate the traditional categories of easy to adjust and hard to adjust. The experimenter is now able to select instrument parameters for study based on relative potential to affect method performance. A direct consequence of the new automation capabilities is that LC method development protocols in many major pharmaceutical companies have promoted analytical column selection to "poll position" – i.e., analytical column screening, also referred to as column scouting, now routinely constitutes the first phase of LC method development.

Figure 1 is a diagram of the LC method development workflow as it is commonly practiced today. As the diagram indicates, the first phase is analytical column selection. Once the "best" column is identified, a second development phase is carried out that addresses the remaining important instrument parameters. The goal of this second phase is to identify the parameter settings that meet all critical method performance criteria, which normally include both compound separation and total assay time. Once these goals are met a separate experiment may be carried out to demonstrate the robustness of the resulting method. The robustness experiment is normally done as part of the method validation effort.

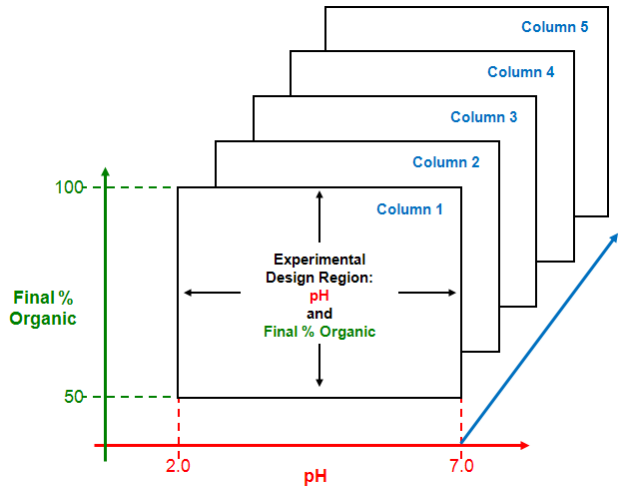
Figure 1. Current Method Development Workflow



This paper will apply the concept of ***design space*** in a comparison of current LC column screening approaches. The FDA defines design space as "*The multidimensional combination and interaction of input variables (e.g., material attributes) and process parameters that have been demonstrated to provide assurance of quality. Working within the design space is not considered as a change. Movement out of the design space is considered to be a change and would normally initiate a regulatory postapproval change process.*" [1].

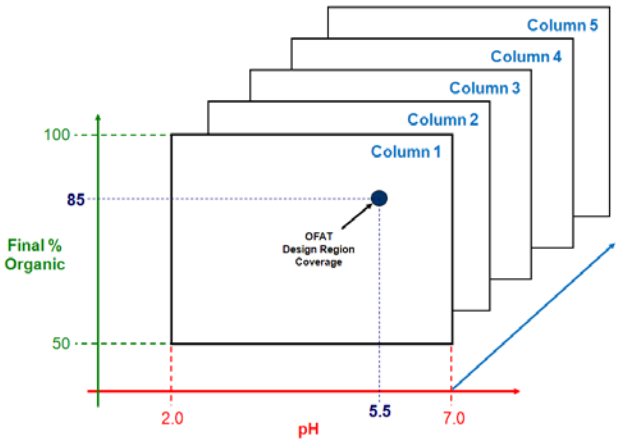
Screening analytical columns at fixed levels of all other instrument parameters – the OFAT approach – does not provide any process knowledge on how the candidate columns being screened would perform at other levels of instrument parameters that can also greatly affect column selectivity. To see this in terms of a potential design space, consider pH and gradient slope – two parameters which can strongly affect column selectivity. Figure 2 illustrates a two-parameter *experimental region* delineated by typical study ranges of these parameters. Here the gradient time is held constant, and so the gradient slope is set by the Final % Organic.

Figure 2. Experimental Region – pH and Final % Organic



As shown in Figure 2, the two-parameter experimental region must be duplicated for each column included in a column screening study (five columns in this example), since it can not be assumed that the parameter effects observed with one column will hold for other columns. Process knowledge of these parameters within a design space would be obtained by conducting an ***experimental design*** which statistically samples an experimental region encompassing a final design space by experimentally testing specifically defined pH/Slope combinations within the region [1]. However, as shown in Figure 3, the OFAT approach only evaluates each column at a single level setting combination of pH and Slope, i.e., screening the column at only one point within the region. The OFAT approach therefore does not provide any process knowledge for these parameters within this region.

Figure 3. Experimental Region Coverage – OFAT Column Screening



"First Principles" Equation Approach

The most common alternative to the OFAT approach is what is referred to as the "First Principles" equation approach. This approach employs pre-defined equations, also called *models*, which predict key method performance metrics such as retention time (t_R) of a compound as a function of one or a specific pair of LC instrument parameters within a defined range. For example, a *First Principles* equation may predict the retention time of a compound in reversed-phase chromatography as a function of multiple method and instrument parameters.

To understand how a *First Principles* equation relates to a design space we must understand the structure of the equation in terms of the included parameters. The simplest equation is the equation of a straight line relating the linear (straight-line) effect of one study variable to a response. Equation 1 is the general form of a linear equation in one variable: here y represents a response, x represents a study variable, β_1 is a *coefficient* which defines the slope of the line, and β_0 is the y-intercept (the value of the response, y, when the study variable, x, is set to zero). The magnitude and sign of the coefficient define the strength (steepness of the line) and direction of the variable's effect (positive or negative) on the response as it is adjusted from low to high through a defined range. The equation is therefore a model of variable behavior, since the equation can predict values of response, y, for different input values of the study variable, x. Equation 2 is an extension of Equation 1 into a full quadratic equation for two variables X_1 and X_2. Note that the equation contains a squared term [$\beta_{ii}(X_i)^2$] for each variable to model curved variable effects (i.e., the plot of the variable's effect on a response across its study range is a curved line), and a two-way interaction term [$\beta_{ij}(X_iX_j)$]. Equation 2 therefore enables modeling independent additive effects, interaction effects, and curvature effects of the two study variables. An equation of this form can be directly extended to include additional study variables.

Equation 1. General Linear Equation

$$y = \beta_0 + \beta_1(x_1)$$

Equation 2. Full Quadratic Equation in Two Parameters

$$y = \beta_0 + \beta_1(X_1) + \beta_2(X_2) + \beta_{12}(X_1 * X_2) + \beta_{11}(X_1)^2 + \beta_{22}(X_2)^2$$

Equation 3 is an example of a *First Principles* nonlinear equation which can be used to predict a compound's retention time (t_r) as a function of multiple method and instrument parameters. Solving this equation for two compounds (named peaks) in a chromatogram, and combining the results with the peak width data for the two compounds, enables the prediction of the resolution between the compounds.

Equation 3. Non-linear Equation of Retention Time [2]

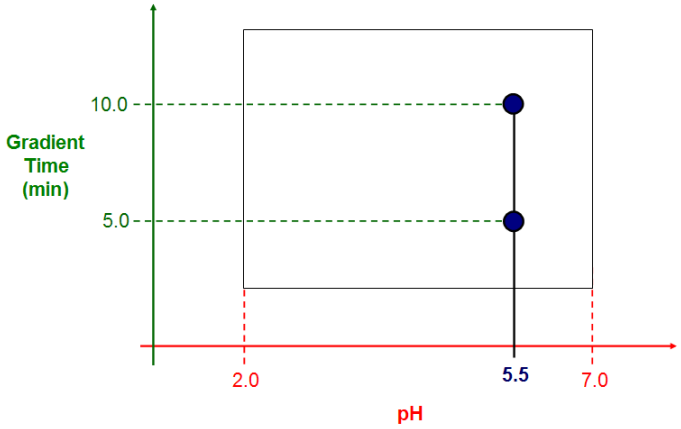
$$t_r = \left(\frac{t_0 F}{V_m \Delta\phi}\right) t_G S^{-1} \times \log\left[\left(\frac{2.3 V_m \Delta\phi}{F}\right) t_G^{-1} S k_0 + 1\right] + t_0 + t_D$$

First Principles equations such as the one presented above are hard wired in terms of their form and the complement of model terms (included method and instrument parameters). An equation such as this cannot be readily adapted to QbD approaches, which involve studying multiple variables at the same time within a single integrated experiment so that all variable effects can be fully characterized, including interaction effects.

Note that Equation 3 is not a fundamental function directly representing quantum mechanical or thermodynamic effects. In addition, Equation 3 utilizes real data from "tuning runs" to adjust the equation's theoretical coefficients to help the equation more accurately predict the current experimental conditions. In normal use Equation 3 is therefore more properly described as a semi-empirical model.

Figure 4 depicts an experimental region in two variables: pH and Gradient Time, along with two run conditions which can be used to normalize a *First Principles* equation such as Equation 3. As Figure 4 shows, an extremely limited amount of the region is addressed by the two normalizing runs. Since the form and parameter complement of such an equation cannot be modified, the equation must be independently solved at other levels of the other variable – pH in this example. The only alternative is to assume that pH and Gradient Time (which represents the gradient slope) do not interact, i.e., that their effects are strictly additive across the experimental region.

Figure 4. *First Principles* Equation Normalizing Runs



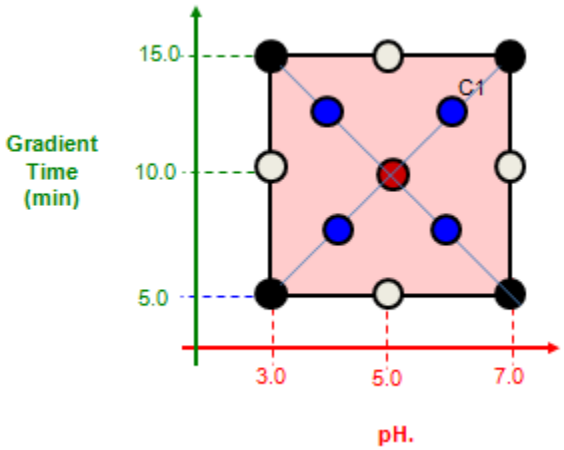
However, interaction effects are common among LC instrument parameters, and these effects can cause major deviations from expected behavior within a experimental region. In fact, the importance of interactions is implicit in the FDA definition of design space (*The multidimensional combination and interaction of input variables...*). The functional limitations in Equation 3 may therefore significantly limit the required scope of the method development work. What is needed is a methodology which enables visualization and quantitation of all variable effects which may be present in the experimental region, including interaction effects.

The Design of Experiments (DOE) Approach

Fortunately, QbD principles can be applied to the task of screening analytical columns to include factors such as pH, gradient slope, and organic solvent type, as these factors are recognized major effectors of column selectivity. In a QbD approach a statistical experiment design plan (Design of Experiments, or DOE) [1] would be used to systematically vary multiple study factors in combination through a series of experiment trials that, taken together, can comprehensively explore a multi-factor experimental region. A statistical experiment design can provide a data set from which interaction effects of the instrument parameters can be identified and quantified along with their linear additive effects, curvilinear effects, and even complex effects.

Figure 5 illustrates a DOE-based sampling plan for the two-factor experimental region previously discussed. The black dots correspond to a two-level factorial design used in traditional screening studies. Such a design can provide data from which both linear additive effects of the instrument parameters and two-way interaction effects can be estimated from the data analysis. The two-level design enables the terms in a partial quadratic model of the form presented in Equation 4 to be used in the analysis of the experiment results. The gray dots in Figure 5 correspond to the additional points which would be present in a classical three-level factorial design, also referred to as a response surface design. The addition of these points provides the data from which simple curvilinear effects can also be estimated from the data analysis. The blue dots in Figure 5 represent design runs which would be added in an advanced algorithm-based DOE design to support analysis of complex effects such as a sigmoidal response curve across a broad pH study range. The design in Figure 5 therefore enables all of the terms in an extended quadratic model of the form presented in Equation 5 to be used in the analysis of the experiment results, including the $\mathrm{Tan}(X_2)$ term used to model a sigmoidal pH effect.

Figure 5. Experimental Region Sampling – Advanced DOE Experiment Design



Equation 4. Partial Quadratic Equation in Two Parameters

$$y = \beta_0 + \beta_1(X_1) + \beta_2(X_2) + \beta_{12}(X_1 * X_2)$$

Equation 5. Extension of the Full Quadratic Equation in Two Parameters

$$y = \beta_0 + \beta_1(X_1) + \beta_2(X_2) + \beta_{12}(X_1 * X_2) + \beta_{11}(X_1)^2 + \beta_{22}(X_2)^2 + \beta_2'\mathrm{Tan}(X_2)$$

A factorial two-level design can accommodate non-numeric variables such as "Column Type" in which the two levels correspond to two different columns. However, the factorial-type three level designs, also referred to as Response Surface designs, which support the full quadratic equation, require numeric variables, and so can not be used for screening more than two columns at a time. Fortunately the advanced DOE algorithm designs can also construct and statistically sample more complex experimental regions such as those which include multiple levels of both numeric and categorical variables. These advanced DOE designs also support more complex analysis model forms that can estimate interactions between categorical and numeric variables as well as complex variable effects.

As previously mentioned, the new automation capabilities of most modern LC instrument systems have eliminated the traditional categories of easy to adjust and hard to adjust. Coupling advanced DOE designs with automation enables the new phased method development approach to include multiple analytical columns in combination with critical parameters such as pH and organic solvent type – parameters known or expected to have column-dependent effects. The second phase of method development then includes remaining instrument factors that can also affect separation. These factors are studied to further optimize method performance. Table II lists the instrument parameters commonly utilized in the new approach to phased method development.

Table II. Current Phasing of Method Development Workflow

Phase 1 – *Primary* Parameters (Primary Effectors of Separation)	**Phase 2 – *Secondary* Parameters** (Secondary Effectors of Separation)
Column Type (analytical column screening)	Temperature
pH	Pump Flow Rate
Organic Solvent Type	Gradient Conditions (refinement of time and slope)
Gradient Time (Controls Slope)	Ion Pairing Agents (when appropriate)

In practice column screening experiments, even those done using a Design of Experiments (DOE) approach, often have significant inherent data loss in critical results such as compound resolution. The data loss is due to both compound co-elution and also changes in compound elution order between experiment trials (peak exchange). These changes are due to the major effects that variables such as pH and organic solvent type can have on the selectivity of the various columns being screened. Switching columns between trials while simultaneously adjusting these factors dramatically affects compound elution and therefore the completeness of the resolution data obtained from the experiment trial chromatograms.

Part two of this article will describe the data loss inherent in most column screening experiments due to co-elution, peak exchange, and the general difficulty of accurately identifying peaks across the experiment trial chromatograms (peak tracking). Such data loss will be seen to make numerical analysis of the results very problematic. It often reduces data analysis to a manual exercise of viewing the experiment chromatograms and picking the one that looks the best in terms of overall chromatographic quality – a "pick-the-winner" strategy. Part two will then describe *Trend Responses*™, a novel type of results which can be directly derived from experiment chromatograms without peak tracking. Trend Responses overcome the data loss inherent in traditional column screening studies, and so enable accurate quantitative analysis of the experiment results.

Conclusions

Chromatographic analytical method development work normally begins with selection of the analytical column, the pH, and the organic solvent type. A major risk of using either a one-factor-at-a-time (OFAT) approach or a *First Principles* Equation approach in this phase is that these approaches provide extremely limited coverage of a potential design space. This limitation translates into little or no ability to visualize or understand the interaction effects usually present among these key instrument parameters.

Alternatively, a Quality-by-Design (QbD) based methodology employs a statistical experiment design to comprehensively address a potential design space and enable the experimenter to visualize and quantify all important variable effects. However, this approach often results in significant inherent data loss in key chromatographic performance indicators such as compound resolution due to the large amount of peak exchange and compound co-elution common in these experiments. Inherent loss makes it difficult or impossible to quantitatively analyze and model these data sets, reducing the analysis to a pick-the-winner strategy done by visual inspection of the chromatograms.

Part two of this article will describe the use of a statistical experimental design coupled with automatically computed *Trend Responses*™. This new methodology, implemented in a fully automated QbD-based software program, successfully replaces a pick-the-winner strategy with rigorous and quantitative column/solvent screening without the need for difficult, laborious, and error-prone peak tracking.

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Acronym Definitions:

21 CFR 11 –	Title 21, Part 11, of the Congressional Federal Register
API –	Active Pharmaceutical Ingredient
CDS –	Chromatography Data System
DOE –	Design of Experiments (also DOX)
FDA –	U.S. Food and Drug Administration
HILIC –	Hydrophilic Interaction LIquid Chromatography
LC –	Liquid Chromatography
ICH –	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
NPC –	Normal-phase (Liquid) Chromatography
PhRMA –	Pharmaceutical Research and Manufacturers of America
QbD –	Quality by Design
RPC –	Reversed-phase (Liquid) Chromatography
SDK –	Software Development Kit (third-party software development interface)
SOP –	Standard Operating Procedure
UHPLC –	Ultra High Performance Liquid Chromatography

Figures, Equations, and Tables:

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