A Quality-by-Design Methodology for Rapid LC Method Development – Part 2

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Abstract

Quality by Design (QbD) is a methodology which enables the practitioner to efficiently obtain quantitative ***process knowledge***. When applied to chromatographic method development work the liquid chromatograph (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. Part one examined the current approaches to column screening in terms of experimental region, knowledge space, and design space coverage – a key element of process knowledge. In this second part we present 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

Part 1 of this article described the application of Quality-by-Design (QbD) principles to the task of screening analytical columns. In a QbD approach a statistical experiment design plan (Design of Experiments, or DOE) [1] is 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 which potentially encompasses a final design space. Such an experimental design can provide a data set from which the experimenter can identify and quantify interaction effects of the factors along with their linear additive effects, curvilinear effects, and even non-linear effects. This quantitation translates the experimental region into a knowledge space.

As also described in part 1, in a traditional method development approach one would first study "easy to adjust" instrument parameters, meaning those for which no equipment configuration change was required. However, categorizing instrument parameters as easy versus hard to control is no longer valid in many cases due to the availability of multi-column and multi-solvent switching capabilities for most modern LC instrument systems. Therefore one can now address up front the instrument parameters which are understood or expected to have the strongest affects on separation of the sample compounds. This is consistent with a QbD approach in which a Pareto analysis would first be carried out to rank instrument parameters in terms of their *expected* ability to affect compound separation; a manageable number of the top-ranked parameters are then included in the first phase of method development. Table I presents a phased method development approach using a rank-based partitioning of instrument parameters consistent with QbD-based practice.

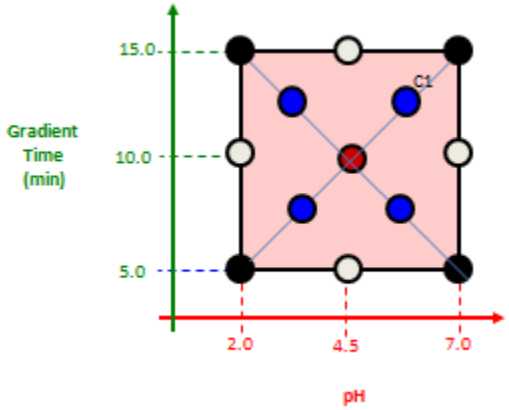
Table I. Rank-based Phasing of Method Development Workflow

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

An experimenter defines the experimental region for a given method development study by selecting instrument parameters (study factors) and defining the range or level settings of each. A DOE-based experiment then defines the specific combinations of the study factors which together provide a statistically valid sampling of the region. Figure 1 illustrates a DOE-based sampling plan for the two-factor experimental region previously discussed in Part 1 of this white paper. The black dots in the figure 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 1 to be used in the analysis of the experiment results. The gray dots in Figure 1 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 curvilinear effects can also be estimated from the data analysis.

The blue dots in Figure 1 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 1 therefore enables all of the terms in an extended quadratic model of the form presented in Equation 2 to be used in the analysis of the experiment results, including the $Tan(X_2)$ term used to model a sigmoidal pH effect.

Figure 1. Experimental Region Sampling – Advanced DOE Experiment Design



Equation 1. Partial Quadratic Equation in Two Parameters

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

Equation 2. Extension of the Full Quadratic Equation in Two Parameters

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

Classical two-level DOE designs support a categorical (non-numeric) factor such as Column Type. However, classical three-level and mixed-level designs do not - these designs require the variables to be numeric. Fortunately advanced DOE algorithm designs enable the experimenter to study greater than two columns at a time in combination with factors such as pH, gradient slope, and organic solvent type – factors which are major effectors of column selectivity. This is important for two reasons. First, it enables the experimenter to include a larger portion of the column selectivity space in the experimental region. Second, the effects of factors such as pH and organic solvent makeup on the separation of a given set of compounds can be substantially different for different columns. Expanding the experimental region and characterizing interaction effects therefore provide a solid opportunity for knowledge-based selection of the stationary and mobile phases.

A DOE-based experiment is a sound statistical platform for LC method development consistent with QbD best practice. However, compound co-elution and changes in compound elution order between experiment trials (peak exchange) are common in early column and solvent screening experiments. Co-elution and peak exchanges 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. These circumstances cause inherent data loss and inconsistency in critical results such as compound resolution which can substantially reduce the ability to translate the experimental region into a true knowledge space.

This second part of the article first illustrates the data loss inherent in most early method development experiments due to co-elution, peak exchange, and the general difficulty of accurately identifying peaks across the experiment trial chromatograms (peak tracking). It then shows the affect such data loss can have on numerical analysis of experiment results – often reducing data analysis to manual inspection of the experiment chromatograms, a "pick-the-winner" strategy. This article then describes *Trend Responses*™, novel interpretations of results which are directly derived from experiment chromatograms without peak tracking and are more robust to co-elution and peak exchange. Trend Responses overcome the data loss inherent in traditional column screening studies, and so enable accurate quantitative analysis of the experiment results.

Inherent Data Loss in Early Method Development Experiments

Inherent data loss due to co-elution and peak exchange in column screening experiments can be seen by comparing the chromatograms in Figures 2 and 3, obtained from a column/solvent screening experiment which included three columns, pH, and Gradient Time as study factors. In Figure 2 the resolution data value for Impurity C obtained from trial 13 is a measure of its separation from the main active pharmaceutical ingredient (API 1), the immediately prior eluting compound. However, in Figure 3 the instrument parameter settings associated with trial 19 have caused Impurity G to migrate away from API 1 so that the two compounds no longer co-elute. As a result, the Impurity C resolution data obtained from trial 19 now measures its separation from Impurity G. Therefore, the Impurity C resolution data in the two chromatograms are not measuring the separation of the same peak pair. Additionally, the co-elution of Impurity G with API 1 under the trial 13 conditions means that trial 13 will have missing data for this impurity.

Figure 2. Chromatogram from Trial 13

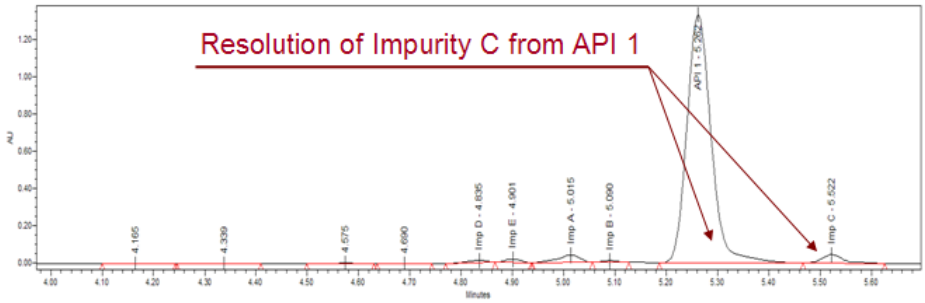


Figure 3. Chromatogram from Trial 19

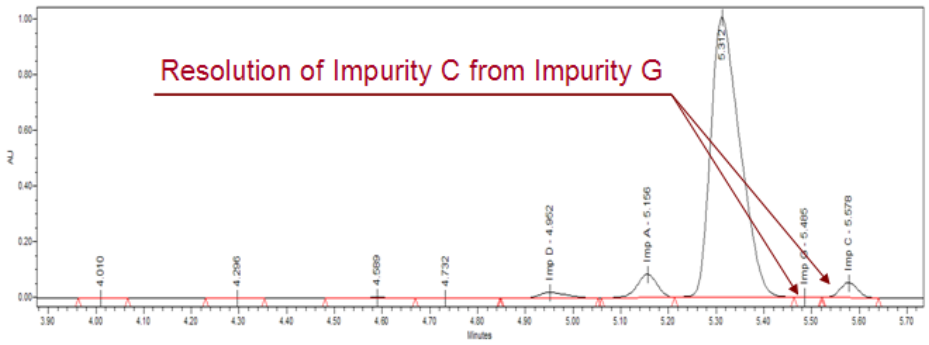


Table II presents the experiment design used in the column/solvent screening experiment from which the chromatograms in Figures 2 and 3 were obtained, along with resolution results for three of the impurities in the experiment sample. These data were generated by tracking the peaks across the trial chromatograms. One can see the two common problems associated with the Table II data: the large number of missing resolution results overall, and the huge disparity in the resolution results present for a given compound. These problems are due to the major differences in the degree of co-elution and peak exchange between the DOE experiment runs. The result of this inherent data loss, which is typical in multi-factor column/solvent screening experiments, is that the data may not accurately represent a compound's actual chemistry-based behavior, and so provide no basis for legitimate modeling and interpretation of the results.

Table II. Example Data Set - Current Practice Data

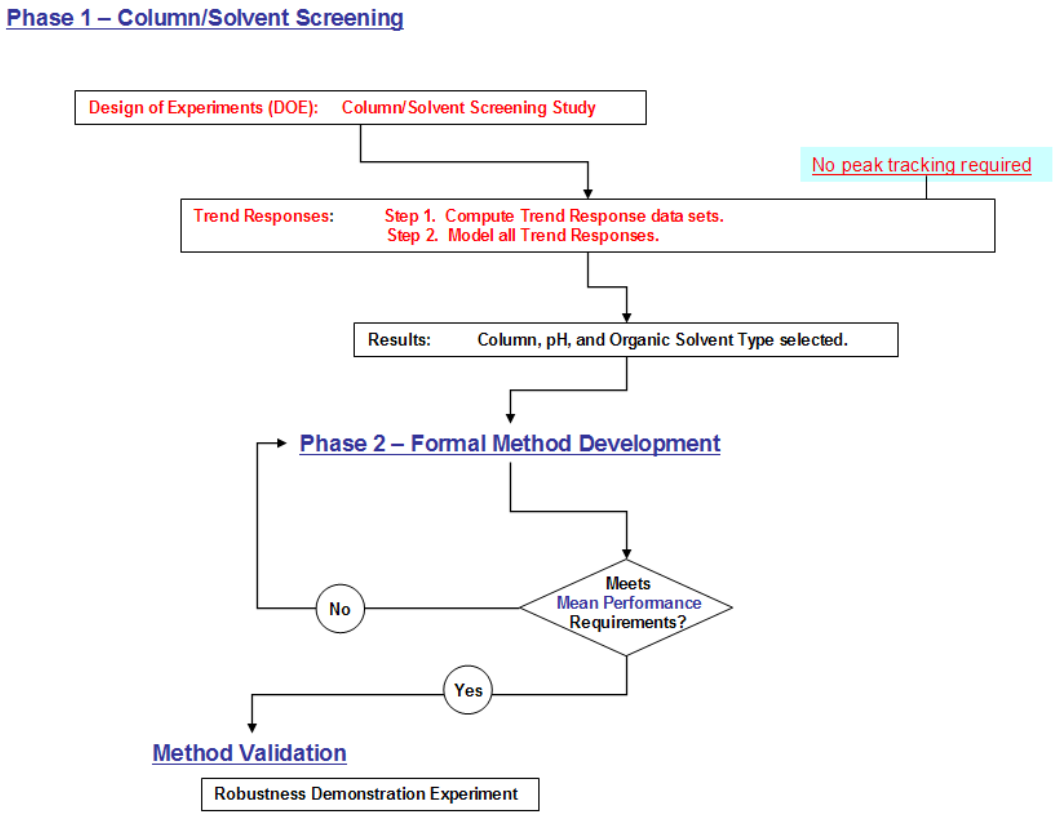
Run No.	Gradient Time	pH	Column Type	Imp E - USPResolution	Imp F - USPResolution	Imp G - USPResolution
1.a.1.a	8.8	2	C18	1.4		2.3
2.a.1.a	6.3	2	Phenyl	1.78		
3.a.1.a	10	2	C18		1.45	2.36
4.a.1.a	5	2	C18		1.15	
5.a.1.a	10	2	Phenyl		1.06	2.25
6.a.1.a	5	2	Phenyl		1.66	
7.a.1.a	10	2	RP			
8.a.1.a	5	2	RP			
9.a.1.a	7.5	2	RP			2.95
10.a.1.a	7.5	4.5	C18	0.97	1.18	3.8
11.a.1.a	7.5	4.5	Phenyl	1.24		2.27
12.a.1.a	7.5	4.5	RP		2.08	2.15
13.a.1.a	5	4.5	RP	0.98		
14.a.1.a	7.5	4.5	C18	1	2.63	3.85
15.a.1.a	7.5	4.5	Phenyl	1.29		2.26
16.a.1.a	7.5	4.5	RP		2.08	
17.a.1.a	5	7	C18		2.35	
18.a.1.a	10	7	Phenyl	1.45	1.08	2.45
19.a.1.a	5	7	Phenyl			2.03
20.a.1.a	10	7	RP			3.05
21.a.1.a	5	7	RP	1.02		
22.a.1.a	8.8	7	Phenyl		1.42	2.34
23.a.1.a	6.3	7	RP		1.54	
24.a.1.a	10	7	C18	1.89	2.99	2.82
25.a.1.a	10	7	C18	1.87	2.95	2.81
26.a.1.a	5	7	C18			

Peak tracking in early column/solvent screening experiments is laborious and error-prone. And as seen in Table II, even when it is done the data may not be sufficient to accurately define the effects of changing chromatographic conditions on the resolution of all critical peak pairs. However, this does not mean that data analysis must be reduced to examining chromatograms with no opportunity for quantitative knowledge. As described below, the inherent data loss problems can be solved by using DOE methods in combination with unique *Trend Responses*™. These responses provide data from which the variable effects on chromatographic performance can be quantitatively determined without direct peak tracking.

A New Quality-by-Design Based Methodology

Figure 4 is a flowchart of a new QbD-based method development workflow. The new methodology harmonizes with the current SOPs of many leading Pharma labs in that it is a phased approach to method development. However, as shown in Red text within the figure, a solid DOE approach to column/solvent screening is utilized in Phase 1, along with novel Trend Response data treatments, which together enable the qualitative elements of the current approach to be transformed into statistically rigorous quantitative practice without the need for peak tracking. DOE is also used in the new Phase 2 workflow; in this phase novel data treatments have been developed to derive and integrate quantitative method robustness metrics into formal method development and optimization. The details of the new Phase 2 technologies and methods will be presented in part three of this article.

Figure 4. New Method Development Practice Workflow



The new QbD-based methodology has been implemented in Fusion AE™, a commercial software package, and successfully demonstrated in "live" studies carried out at many pharmaceutical laboratories. These studies involved either (1) test mixes of active ingredients and impurities designed to challenge the methodology, or (2) current method development projects in which obtaining an acceptably performing method proved resistant to all current attempts. The next two sub-sections of this article present the new QbD-based methodology and the novel Trend Response data treatments used in Phase 1, and describe one of the successful proof-of-technology experiments.

QbD-based Phase 1 Workflow

The new QbD methodology executes the column/solvent screening phase using statistical design of experiments (DOE). The experimental workflow involves the following five steps which are greatly facilitated by software and hardware automation.

1. **Define the experimental region.** Table IV presents experiment variables and settings appropriate to a *Phase 1 - Column/Solvent Screening* experiment to be conducted on a traditional LC. The template can be modified as required by the target instrument platform (e.g. gradient time range for an ultra-high pressure LC) and/or the nature of the sample compounds.

2. **Generate a statistically designed experiment.** The experiment design defines a variety of different study factor level setting combinations (different instrument methods) to be run on the LC. The use of a statistical experimental design assures that all important study factor effects will be expressed in the experiment data. The software does this step automatically.

3. **Transform the experiment into instrument control settings.** This requires constructing a sample set and building the instrument methods required by the DOE design within the chromatography data software (CDS). The software does this step automatically.

4. **Run the various design conditions on the instrument.** This requires running the sample set and processing the resulting chromatograms. The CDS runs the sample set automatically. Processing the chromatograms is done manually using native CDS features.

5. **Derive predictive models of the Response data sets.** The results data within each experiment chromatogram are retrieved from the CDS, and unique Trend Responses (defined in Table V) are derived from these results and modeled. The software does this step automatically.

   The experimenter then enters chromatographic performance goals which the software uses to identify the study parameter settings that provide the best method performance.

Table IV. QbD – Phase 1 Experiment Template

Experiment Variable	Range or Level Settings
Gradient Time (min)	15.0 – 40.0
pH	2.5, 5.0, 7.0
Column Type	Five Columns: C18 C8 Phenyl Nitrile Polar Embedded (e.g. Amide)
Gradient Slope (% Organic range)	5.0 – 95.0
Organic Solvent Type	Acetonitrile, Methanol, Blend

Table V defines the unique Trend Responses which are utilized in the data analysis. These responses are of two general types: peak count based and peak results based. As the name implies, a *peak count* based response is obtained by counting the number of integrated peaks in each chromatogram that meet a certain criterion. A *peak result* based response is a way of obtaining a result such as resolution or retention time for a specific peak using *indirect* peak tracking. For example, setting the *Max Peak # – RESPONSE* operator to *Max Peak 2 – USP Resolution* will find the 2nd largest peak in each chromatogram and obtain the resolution result for the peak. It is important to note that obtaining Trend Responses does not require any assignments of sample compounds to peaks in the experiment chromatograms, i.e., no peak tracking. And as opposed to a pick-the-winner strategy these responses are statistically analyzed and modeled. The models quantify the effects of changing the study factors on important chromatographic trends such as the number of visualized and separated compounds in addition to the critical peak-pair specific information provided by the peak result trend responses.

Table V. Novel Trend Response Data Sets

Response Name	Definition
Peak Count Based Responses	
No. of Peaks - Number of integrated peaks in the chromatogram.	This is a measure of overall chromatographic quality in terms of overall separation and also the ability to visualize all compounds (peaks) in the sample.
No. of Peaks ≥ X - X is a user-definable value.	This is the "Number of Resolved Peaks" trend response. This is a measure of overall chromatographic quality in terms of minimum "acceptable" separation.
No. of Peaks > X – *RESPONSE* - Operators include: >, <, ≥, ≤, =. - X is a user-definable value. - *RESPONSE* is any chromatogram result.	These flexible operators let the user set other count-based trend responses. As an example, many assay conditions that are required for difficult separations can cause peak tailing, which further complicates separation. A user could therefore define a trend response such as "*No. of Peaks > 1.2 – Asymmetry USP*", which could track this element of chromatographic quality overall.
Peak Result Based Responses	
Max Peak # – *RESPONSE* - *RESPONSE is any chromatogram result.*	This is the ith largest peak – the 1st largest peak when # = 1, the 2nd largest peak when # = 2, etc. Normally the largest peaks are the APIs.
e.g. - Max Peak # – USP Resolution	Resolution is sensitive to "shoulder" peaks that can co-elute with the API, and so the Resolution response enables to focus on peak pairs that are critical to resolve.
e.g. - Max Peak # – Area (or Area %)	Area is also sensitive to "shoulder" peaks that can co-elute with the API, and so the Area response is an indirect measure of peak purity.
Last Peak – *RESPONSE* - *RESPONSE is any chromatogram result.*	This is the last eluted peak.
e.g. - Last Peak – Retention Time	This represents the retention time of the last eluted peak, and so indirectly represents assay time.
e.g. - Last Peak – USP Resolution	This is the resolution of the last eluted peak. This can be used in conjunction with the "Last Peak – Retention Time" response, since one could inadvertently reduce assay time without realizing that the reduced elution time has caused the last peak to co-elute with the immediately previous peak.

Proof-of-Technology Experiment

A column/solvent screening experiment was conducted to validate the new QbD methodology, as implemented in Fusion AE™, and demonstrate the utility of Trend Responses. The ultimate goal was the development of a stability-indicating method. To seriously challenge both the approach and the software a current product was selected as the target sample. This product contains two APIs, a minimum of nine impurities which are structurally related to the APIs (same parent ion), two degradants, and one process impurity.

The instrument platform used in this work was a Waters® ACUITY UPLC® System (UPLC) equipped with a 4-position internal solvent selection valve. The UPLC was controlled by the Waters Empower™ 2 CDS. To accommodate the UPLC the *Phase 1 - Column/Solvent Screening* experiment template was modified as shown in Table VI in terms of the gradient time, the organic mobile phase, and the number of analytical columns evaluated (three were used in this study).

Table VI. Modified Phase 1 Experiment Template

Experiment Variable	Range or Level Settings
Gradient Time (min)	5.0 – 10.0
pH	2.0, 4.5, 7.0
Column Type	Three Columns (2.1 x 100): BEH C18 BEH Shield RP18 BEH Phenyl
Mobile Phases	Mobile Phase A1-1: 0.05% TFA Buffer, pH 2.00 Mobile Phase A1-2: 20 mM Ammonium Acetate Buffer, pH 4.50 Mobile Phase A1-3: 10 mM Sodium Phosphate Dibasic Buffer, pH 7.00 Mobile Phase B1: 50% Acetonitrile, 50% Methanol
Gradient Slope (% Organic)	5.0 — 95.0
Important Constants	Pump Flow Rate = 0.50 mL/min Temperature = 70 °C Wavelength = 250 nm

Prior to running the screening experiment an experimental region qualification trial was run at *center point* conditions of the numeric experiment variables (Gradient Time = 7.5 minutes, pH = 4.50). The BEH C18 column was used in this trial, since it has the most central position in the selectivity space of the three candidate columns. Figure 5 presents the chromatogram obtained from the qualification trial. As the chromatogram shows, all sample compounds eluted after five minutes, i.e., after the mobile phase reached 50% organic. The initial % organic value for the Gradient Slope variable in the QbD screening experiment template was therefore changed from 5.0% to 50.0%.

Figure 5. Design Qualification Trial Chromatogram

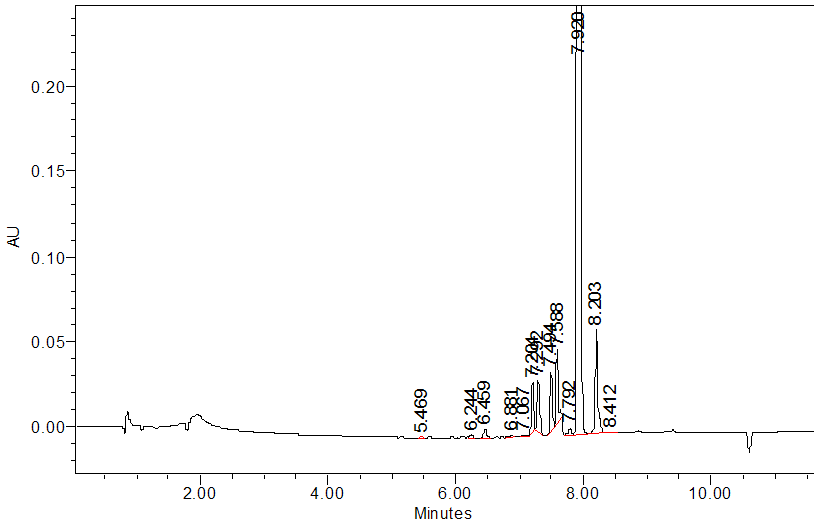


Table VII presents the column/solvent screening experiment design generated from the template along with the Trend Response results computed directly from the chromatogram data. Table VIII presents the modeling results for the No. of Peaks Trend Response in the form of a variable effects ranking table. The strongest effect, i.e., the largest effect in absolute magnitude, is assigned a rank of 1.0000; the strength of each other statistically significant variable effect is proportionately ranked relative to that of the strongest effector. The table contains two critical results worth describing in detail. First, the C18 column occupied Valve Position 1, and so is used by the software as the performance standard by which the other two columns are compared. As the yellow highlighted results in Table VIII show, switching from the C18 column to either the Phenyl or the RP column has a strong negative effect on the No. of Peaks Trend Response. Second, pH and Gradient Time express significant column-type dependent interaction effects. In fact, the relative rank of 0.960 associated with the complex (*Gradient Time*pH*)**RP* interaction term identifies this as the second largest observed effect.

Table VII. Column/Solvent Screening Experiment Data Set

Run No.	Gradient Time	pH	Column Type	No. of Peaks	No. of Peaks >= 1.50 - USP Resolution	Max Peak #1 - USP Resolution	Max Peak #1 - Area	Max Peak #2 - USP Resolution	Max Peak #2 - Area
1.a.1.a	8.8	2	C18	10	7	0.71	4,564,869	2.56	149,171
2.a.1.a	6.3	2	Phenyl	11	9	1.29	4,767,790	1.82	156,280
3.a.1.a	10	2	C18	11	7	0.74	4,686,715	2.6	152,904
4.a.1.a	5	2	C18	9	6	1.14	4,484,994	2.44	193,620
5.a.1.a	10	2	Phenyl	11	5	0.7	4,577,109	2.92	50,770
6.a.1.a	5	2	Phenyl	7	5	1.19	4,698,297	1.66	149,715
7.a.1.a	10	2	RP	11	6	1.57	4,789,602	3.36	174,213
8.a.1.a	5	2	RP	7	4	1.16	4,520,810	1.17	153,710
9.a.1.a	7.5	2	RP	8	5	1.44	4,603,548	2.95	165,678
10.a.1.a	7.5	4.5	C18	15	7	2.79	4,460,964	1.31	122,509
11.a.1.a	7.5	4.5	Phenyl	10	6	1.21	4,532,870	1.59	235,296
12.a.1.a	7.5	4.5	RP	12	7	1.65	4,428,354	4.25	107,326
13.a.1.a	5	4.5	RP	11	8	2.37	4,383,250	3.33	127,947
14.a.1.a	7.5	4.5	C18	16	7	1.66	4,434,727	1.33	121,956
15.a.1.a	7.5	4.5	Phenyl	9	5	1.26	4,585,177	1.81	240,296
16.a.1.a	7.5	4.5	RP	12	7	1.6	4,687,179	4.22	112,074
17.a.1.a	5	7	C18	14	10	1.34	4,202,020	1.5	154,970
18.a.1.a	10	7	Phenyl	13	7	1.07	4,460,211	1.26	142,240
19.a.1.a	5	7	Phenyl	11	10	1.56	4,300,750	2.03	250,944
20.a.1.a	10	7	RP	10	7	2.77	4,472,905	2.56	201,024
21.a.1.a	5	7	RP	11	8	1.76	4,270,430	3.28	125,334
22.a.1.a	8.8	7	Phenyl	10	5	1.28	4,427,427	1.94	231,908
23.a.1.a	6.3	7	RP	11	6	1.44	4,340,568	3.88	101,561
24.a.1.a	10	7	C18	17	13	1.98	4,342,349	1.82	119,099
25.a.1.a	10	7	C18	19	12	1.42	4,457,981	1.81	122,525
26.a.1.a	5	7	C18	10	7	1.32	4,166,825	2.65	150,747

Table VIII. Effects Ranking – No. of Peaks

Model Term Name	Model Term Effect	Model Term Rank
pH	5.5801096640	1.000
(Gradient Time*pH)*RP	-5.3592053833	0.960
Phenyl	-5.0278439810	0.901
pH*Phenyl	-4.3356390558	0.777
pH*RP	-3.6147651548	0.648
(Gradient Time*pH)*Phenyl	-3.4961644292	0.627
(Gradient Time)^2*Phenyl	3.2409705954	0.581
RP	-3.0110998129	0.540

Once the software derives an equation for each Trend Response, the equations can be linked to a best answer search engine that identifies the best-performing study variable settings according to user-definable goals. Table IX presents the response goal settings which a multiple response search algorithm uses to gauge the acceptability of any given solution. The settings for the *Max_Peak_1 – Peak Area* response illustrate the power and flexibility of the trend responses. This response is used as an indirect measure of the purity of the primary active pharmaceutical ingredient (API), since a co-eluting compound will increase the integrated area of the API peak. The response goal is therefore set to ***Minimize***, and the Upper Bound value is set to 4,700,000, since several chromatograms demonstrated that API areas of greater than this value occurred when the API co-eluted with an impurity. Table X presents the numerical "best answer" obtained for the goal settings in Table IX. The answer identifies the best performing level settings of the experiment variables within the experimental region studied.

Table IX. Best Answer Search Settings

Response Variable Name	Relative Importance	Target	Lower Bound	Upper Bound
No. of Peaks	1	Maximize	13.00	16.00
No. of Peaks >= 1.50 – USP Resolution	1	Maximize	10.00	13.00
Max Peak #1 – USP Resolution	1	Maximize	1.50	2.50
Max Peak #1 - Area	1	Minimize	4,166,825	4,700,000
Max Peak #2 – USP Resolution	1	Maximize	1.50	2.50
Max Peak #2 - Area	1	Minimize	50,000	120,000

Table X. Numerical Search Result – Best Overall Answer

Study Variable Name	Optimizer Answer Level Setting
Gradient Time	9.33
pH	7.0
Column Type	BEH C18

A graphical search capability can also be used to visualize acceptable performing methods within an experimental region. Figure 6 presents three 2D contour graphs of the *No. of Peaks* Trend Response – one for each column evaluated. The graphs show the combined effects of pH and Gradient Time on this key response. Figure 7 is a simplified version of the second contour graph in Figure 6. In this graph a goal of ***Maximize*** is applied to the No. of Peaks response with the minimum acceptability value (Lower Bound) of 14 visualized peaks. The graph is interpreted as follows:

- The Pink shaded region corresponds to parameter settings that *do not meet* the minimum acceptability goal (equation predicts less than 14 peaks).
- The Dark Pink line demarcating the shaded and unshaded regions defines parameter settings that *exactly meet* the response goal (equation predicts exactly 14 peaks).
- The unshaded region corresponds to parameter settings that *exceed* the goal (equation predicts greater than 14 peaks).

Figure 6. Contour Graphs: No. of Peaks Trend Response

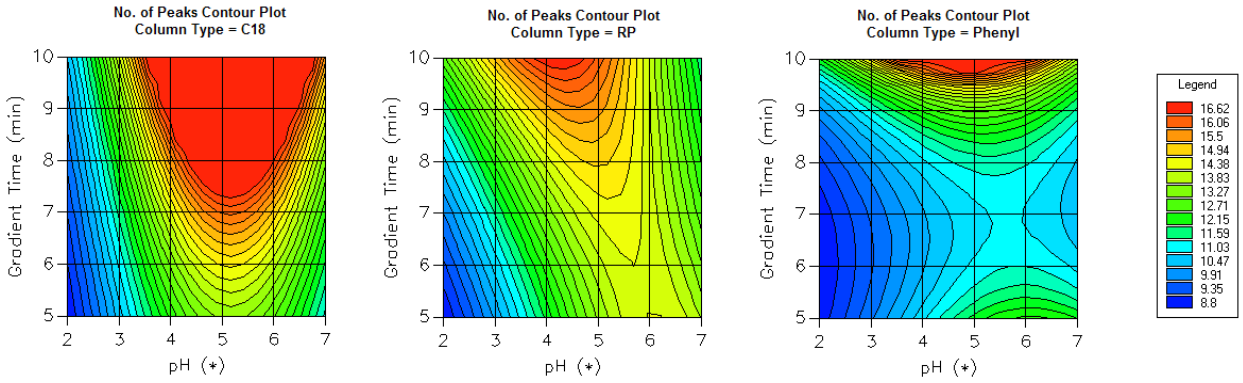


Figure 7 Graphical Answer Search Result – One Response Goal

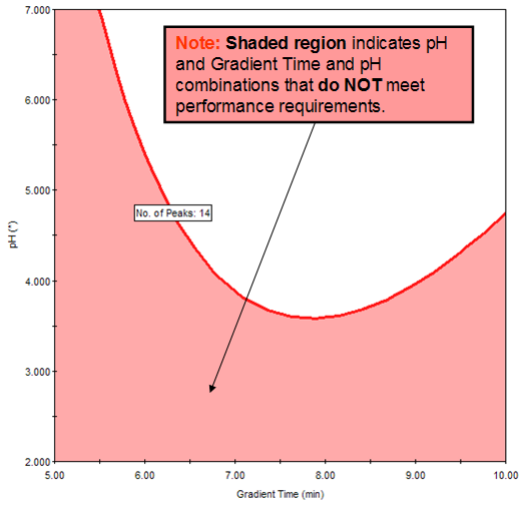


Figure 7 is called a Response Overlay graph, since multiple response goals can be displayed (overlaid) on such a graph. This is shown in Figure 8, which contains shading for each of the six key Trend Responses analyzed in the Column/Solvent screening study. Note that six individual graphs would have to be generated and visually compared to determine the same information contained in this one response overlay graph in terms of level setting combinations that meet/do not meet all Trend Response goals.

Figure 8 Graphical Answer Search Result – All Response Goals

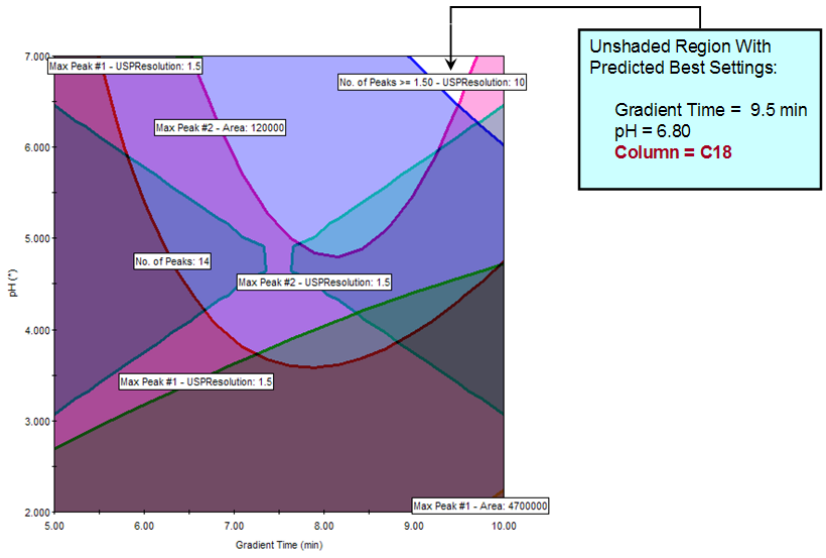
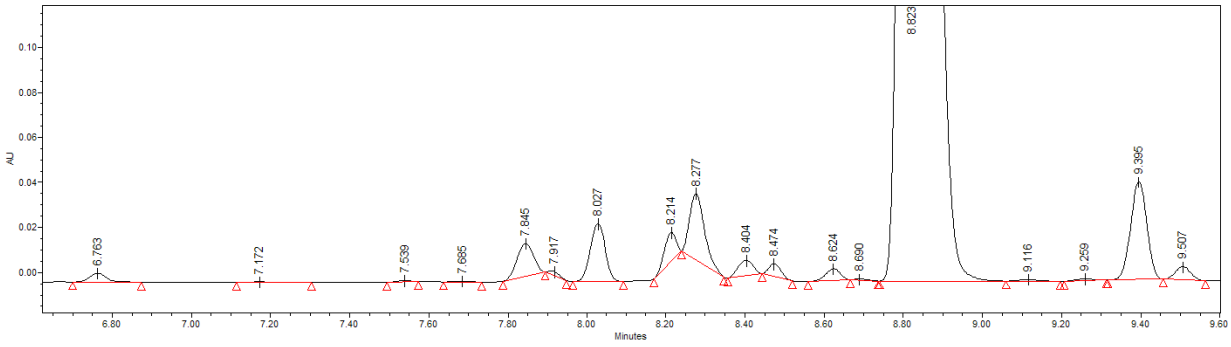


Figure 9 presents the chromatogram obtained by analyzing the sample with the UPLC system set at the numerical answer search result settings presented in Table X – the best result obtainable within this experimental region. It is important to note that the resolutions of the two APIs are both below 2.00. This means that an optimization experiment should be performed to further optimize separation for both mean performance and method robustness. In this second experiment the Column Type, pH, and Gradient Time will be fixed at best conditions, and other parameters will be brought into play.

Figure 9. Best Overall Answer Chromatogram



In practice the Trend Response approach will not always yield the optimum LC method (instrument parameter settings) in a single experiment, and indeed it is not meant to. The Trend Response approach is part of a phased workflow in which these responses enable the experimenter to identify the best settings of parameters such as Column Type, pH, and mobile phase organic type; parameters that normally have the greatest effect on separation, and therefore cause the most inherent data loss. Once these settings are identified, these parameters are then held constant to minimize co-elution and peak exchange. This simplifies any peak tracking which may be required in a subsequent optimization experiment.

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 an experimental region encompassing a potential final 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 the experimental region, 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 peak exchange and compound co-elution. The inherent data loss common in these screening experiments makes it difficult or impossible to quantitatively analyze and model these data sets, often reducing the analysis to manual inspection of the chromatograms – a pick-the-winner strategy. The limitations of obtaining a complete data set during screening experiments and the lack of quantitative performance metrics have been successfully overcome by 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. Part three of this article will describe the integration of quantitative method robustness estimation into method optimization – the second phase of LC method development.

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Figures, Equations, and Tables:

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