Quality by Design (QbD) Based Development of a Stability Indicating HPLC Method for Drug and Impurities

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Abstract

In this paper, an application of Quality by Design (QbD) concepts to the development of a stability indicating HPLC method for a complex pain management drug product containing drug substance, two preservatives, and their degradants is described. The QbD approach consisted of (*i*) developing a full understanding of the intended purpose, (*ii*) developing predictive solutions, (*iii*) designing a meaningful system suitability solution that helps to identify failure modes, and (*iv*) following design of experiments (DOE) approach. The starting method lacked any resolution among drug degradant and preservative oxidative degradant peaks, and peaks for preservative and another drug degradant. The method optimization was accomplished using Fusion AE™ software (S-Matrix Corporation, Eureka, CA) that follows a DOE approach. Column temperature (50 ± 5°C), mobile phase buffer pH (2.9 ± 0.2), initial % acetonitrile (ACN, 2 ± 1%), and initial hold time (2.5, 5, or 10 min) of the HPLC method were simultaneously studied to optimize separation of the unresolved peaks. The optimized HPLC conditions (column temperature of 50°C, buffer pH of 3.1, 3% initial ACN with 2.5 min initial hold) resulted in fully resolved peaks in the two critical pairs. The QbD based method development helped in generating a design space and operating space with knowledge of all method performance characteristics and limitations and successful method robustness within the operating space.

Introduction

International conference on harmonization (ICH) document Q8 (R2) describes the suggested contents for the 3.2.P.2 (pharmaceutical development) section of a regulatory submission in the ICH M4 Common Technical Document (CTD) format (1). The Pharmaceutical Development section is intended to provide a comprehensive understanding of the product and manufacturing process for reviewers and inspectors. The document underscores one of the basic tenets of QbD that quality cannot be tested into the products, (i.e., quality should be built in by design). The information and knowledge gained from pharmaceutical development studies and manufacturing experience provide scientific understanding to support the establishment of the design space, specifications, and manufacturing controls. Working within the design space is not considered a change, while movement outside the design space is considered a change that would normally initiate a regulatory post approval change process. When the design space is expanded with enhanced knowledge of product performance over a wider range of material attributes, processing options, and process parameters, opportunities exist for more flexible regulatory approaches, (e.g., risk-based regulatory decisions, manufacturing process improvements within the approved design space without further regulatory review and reduction of post-approval supplements (1). As such, both industry and regulators have recognized the benefits of adopting a QbD approach to drug development and manufacture) (2–4).

Although QbD concepts have not been established for analytical development, the concepts such as quality included by design and not tested into the product (the product being a developed analytical method with meaningful system suitability criteria that help to identify failure modes) and working within a design space (i.e., knowledge of method parameters affecting its performance) without having to seek a regulatory approval of the modified method are quite appealing to the analytical community. As such, the QbD approach including application of design of experiments concepts for analytical development have recently gained momentum in the literature (2–8). Although the compendia have viewed design space as the acceptability of changes in method conditions outlined in the guidance (9) the analyst still needs to demonstrate that the method performance within the allowable changes is acceptable for its intended purpose.

In this paper, a conceptual approach for QbD based development (Figure 1) with four essential elements is presented; a full understanding of the intended purpose, developing predictive solutions, designing meaningful system suitability criteria, and "design of experiments" approach to the method development.

A full understanding of the intended purpose of the method must be developed (10) from an understanding of drug, its impurities and the degradation pathways, drug sensitivity to various

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factors such as pH, light, oxygen, and temperature, excipients and their impurities, product pH, container enclosure, processing conditions (sterile fill or terminally sterilized), storage conditions, desired shelf life, and finally the critical quality attributes (CQAs) for the product. ICH Q8 defines CQA as a physical, chemical, biological, or microbiological property or characteristic that should be within an appropriate limit, range, or distribution to ensure the desired product quality. The method performance (e.g., specificity, accuracy, precision, linearity, range, and quantitation limits for impurities) should be targeted such that the method is suitable for demonstrating measurable control of the critical quality attribute in the manufacturing process and stability testing. For example, acceptance criteria for method accuracy and precision will need to be tighter for a product with assay limit of 100.0 ± 5.0% than for a product with assay limit of 100.0 ± 10.0%. Recently, Analytical Target Profile (ATP) term has been discussed by Pohl et al. (3) and Schweitzer et al. (4) to describe the full understanding of the intended purpose of the method.

It is worthwhile noting that analytical development and formulation development are most often on a parallel path, and the analyst has to predict drug degradation through the end of the

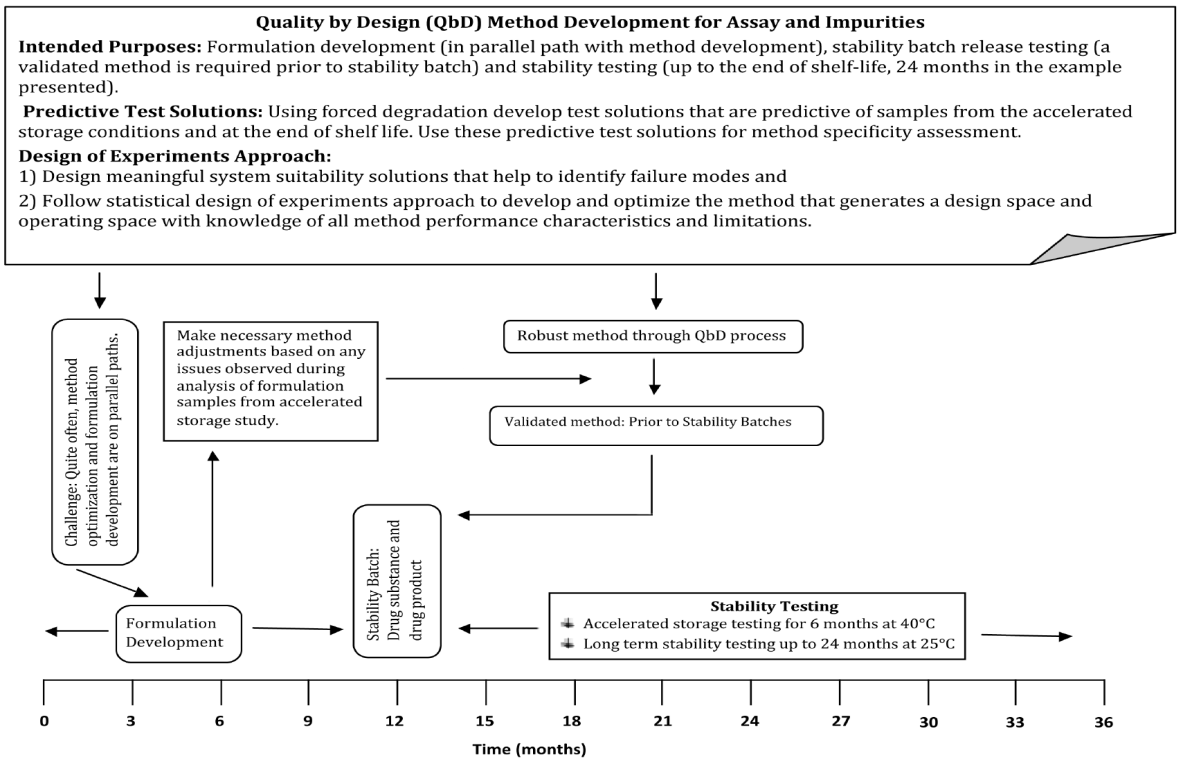

Figure 1. Method development following QbD process.

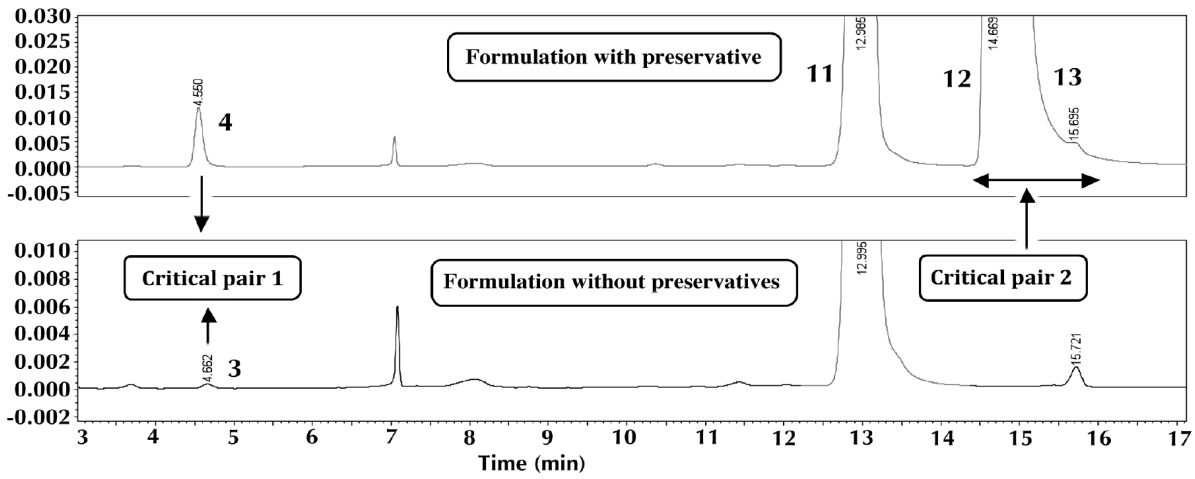

Figure 2. Chromatograms obtained with the initial method for formulations with or without preservatives. Only portions of chromatograms from approximately 3 to 17 min are presented for clarity purposes. Chromatographic conditions as stated in Table I. Peak annotations are described in Figure 6. Note that preservative degradant (peak 4) and drug degradant (peak 3) are not resolved and another drug degradant (peak 13) is only partially resolved from the tail end of a preservative peak (peak 12).

shelf life well before formal registration stability batches are manufactured. This formidable task can be accomplished by forced degradation of test solutions with a goal of generating solutions that are predictive of drug degradation through the end of the product shelf life. These solutions should be used in evaluating method specificity. The next key element is developing a meaningful system suitability solution. As noted by Gavin and Olsen (2), system suitability solution should be developed such that it can help identify failure modes and prevent the generation of erroneous results. Lastly, the method development should be completed using a statistical design of experiments approach to generate a design space and operating space with knowledge of all method performance characteristics and limitations. The information would lead to how critical process controls of the method (e.g., column chemistry and mobile phase pH needed to attain the desired separation) affect the method performance. Traditionally performed HPLC method development, using trial and error technique or more sophisticated techniques such as one factor at a time and first principles approach, cannot predict additive or interactive effects of instrumental parameters on performance of a method intended for the analyses of a complex matrix. Therefore, it is essential to follow a design of experiments approach with systematic and multivariate strategy. As a final note, Figure 1 also shows time scale in months starting with formulation development in parallel path with the analytical development, manufacturing of registration batches and stability testing for a drug product.

Stability indicating assay is defined as a validated quantitative analytical procedure that can detect the changes with time in the pertinent properties (e.g., active ingredient, preservative level) of the drug substance and drug product. A stability-indicating assay accurately measures the active ingredients without interferences from degradation products, process impurities, excipients, or other potential impurities (11). The concept has been well documented in the literature (12–14). In the work presented here, the development of a stability indicating method for the determinations of drug substance and its degradants, along with two preservatives and their related degradants in a complex pain management drug product following the QbD principles is reported. It was necessary to know the concentrations of drug and excipients, and the amounts of drug and excipient degradants for assessing drug product shelf-life. Although the proprietary drug product cannot be fully described, drug and excipient information provided under Materials section along with information on inadequate separation obtained with the initial HPLC method conditions (Figure 2), are considered sufficient in describing the implementation of a successful QbD approach.

Table I. A Comparison of Initial HPLC Method, Optimized HPLC Method from Fusion AE Experiments, and Geometrically Scaled down HPLC Method

Parameter	Initial method	Optimized method	Scaled down method*
Column	Waters Symmetry C8 (3.9 × 150 mm, 5 µm)		Waters Symmetry C8 (2.1 mm i.d. x 150 mm length, 3.5 µm packing)
Flow rate	1.5 mL/min		0.5 mL/min
Injection volume	30 µL	20 µL	5 µL
Column Temp.	50°C		50°C
Detection	UV at 280 nm		UV at 280 nm
Mobile Phase A	6:94 ACN† pH 2.9	3:97 ACN–buffer pH 3.1	3:97 ACN–buffer pH 3.1
Mobile Phase B	80:20 ACN–buffer pH 2.9	80:20 ACN–buffer pH 3.1	80:20 ACN–buffer pH 3.1
Gradient	100% A up to 2.5 min, linear gradient to 60% B at 35 min, 100% B at 36 min to 46 min, 100% A at 48 min to 60 min	100% A up to 2.5 min, linear gradient to 60% B at 35 min, 100% B at 36 min to 46 min, 100% A at 48 min to 60 min	100% A up to 2.2 min, linear gradient to 60% B at 30.4 min, 100% B at 31.3 min to 40 min, 100% A at 41 min to 52 min
Run Time	60 min	60 min	52 min

* Geometrically scaled from column with 3.9 mm i.d. packed with 5 µm particles to column with 2.1 mm i.d. packed with 3.5 µm particles.
† ACN = acetonitrile

Experimental

HPLC system, materials, and optimization software

Experiments were performed on an Agilent 1100 HPLC System (Agilent Technologies, Palo Alto, CA) equipped with an autosampler, a quaternary or a dual binary gradient pump (both pump types were used in the study), a temperature controlled column compartment, and a variable wavelength UV–vis detector, along with Waters photo-diode array detector (Waters Corporation, Milford, MA). Instrument control and data acquisition were performed on Empower 2 Chromatography Software (Waters). Initially, the optimization experiments were performed using a Waters Symmetry C8 (3.9 mm i.d. × 150 mm length) column packed with 5 µm material (Waters Catalog # WAT046970). Further optimization to reduce the ACN consumption was achieved by replacing the Symmetry C8 column dimensions to 2.1 × 150 mm column with particle size reduced to 3.5 µm material (Waters Catalog # WAT106011).

Eighty-five percent phosphoric acid (ACS grade), acetonitrile (ACS grade), Triethylamine (purity of > 99.5%), sodium 1-octanesulfonate (1-octane sulfonic acid sodium salt, purity of > 98.0%), and ammonium dihydrogen phosphate [$(NH_4)H_2PO_4$, ACS grade] of the specified grade or purity were used as components of mobile phase. To prepare mobile phase buffer 4.9 g of $(NH_4)H_2PO_4$ and 1.15 g of sodium 1-Octanesulfonate were dissolved in ~750 mL water and 1.05 mL of triethylamine were added to it. The optimization experiment was conducted at pH levels of 2.9, 3.0, and 3.1. The buffer solution was then filtered and degassed using a 0.2 µm filter. The optimized mobile phase for the gradient separation consisted of mobile phase A:

3:97 (v/v) acetonitrile–pH 3.1 buffer, and mobile phase B: 80:20 (v/v) acetonitrile–pH 3.1 buffer. To prepare the resolution solution, a matrix solution was first prepared that consisted of preservatives and compendial grade buffer components at their nominal concentrations in the drug product. Drug A material, also of compendial grade, in the amount of 100 mg was weighed and dissolved in about 25 mL matrix solution, and the final volume was brought to 50 mL using the matrix solution. The resulting solution, comprised of drug A and matrix components at their nominal concentrations in the drug product, was filled in either sealable and autoclavable ampoules or vials, and autoclaved at 121°C for 45 min. Alternatively in cases where an autoclave is not readily available, it was experimentally determined that the solution heated at 90°C for 15 h yielded a similar degradation profile as autoclaved solution for use as a resolution solution. The solution was allowed to cool to ambient temperature and stored at 5°C for its use as long as no visible precipitate appeared in ampoules or vials.

The HPLC method optimization experiments were performed using Fusion AE software (S-Matrix Corporation, Eureka, CA). In this case, the test solutions were laboratory scale aqueous drug formulations (at nominal concentrations) consisting of an organic base (amine) drug substance (pKa of 8.2) in an organic acid buffering system prepared with and without two organic preservatives (pKa of 8.4 for each of them). The drug and preservatives have very similar UV chromophores. Aliquots of these samples were transferred into multiple ampoules of appropriate size and the ampoules were flame sealed. These ampoules were then autoclaved at 121°C for 22 or 44 min in a bench-top autoclave, model AS12 (VWR, West Chester, PA) to represent 1X and 2X sterilization cycles, respectively. The samples from 2X sterilization cycle generated several degradant peaks, and therefore, 2X sterilized sample was used for the HPLC method optimization experiments using Fusion AE software.

Table II. Example of a Test Plan Illustrating Multivariate Analysis*

Column Temp (°C)	Buffer pH	Hold time (min)	Mobile Phase A (% ACN)
45	2.7	2.5	1
45	3.1	2.5	3
55	2.7	2.5	3
55	3.1	2.5	3
55	2.7	2.5	1
50	2.9	5.0	2
50	3.1	5.0	2
55	3.1	2.5	1
45	2.7	5.0	3
45	3.1	5.0	1

* A total of 25 such experiments were performed.

Table III. Response Variable Goals and Relative Rank for Fusion AE Optimization

Response variable*	Goal	Lower bound†	Upper bound†	Relative rank
Retention time of preservative degradant (peak 4) (min)	Minimize	4.82	10.27	1
Unknown-Retention time (peak 3) (min)	Minimize	5.06	8.78	1
Drug peak retention time (peak 11) (min)	Minimize	13.93	24.19	1
Resolution among drug and preservative peaks (peaks 11 and 12, respectively)	Maximize	1.50	3.31	1
Resolution among preservative and RRT 1.2 peaks (peaks 12 and 13, respectively)	Maximize	1.50	7.44	1

* In italics: peak numbers as identified in Figure 6.
† Experimentally obtained data were used in setting the lower and upper bounds.

Test solution for robustness experiments

For robustness experiments, a mixture of known drug impurities was prepared to contain each impurity at 0.2 mg/mL. The mixture solution in the amount of 0.5 mL was then combined to 20.0 mL final dilution with heat stressed (2X sterilized) sample from a preservative containing formulation (with 2 mg/mL of drug). The resulting solution contained each known drug impurity at 0.005 mg/mL or 0.25% with respect to the drug concentration of 2 mg/mL.

Results and Discussion

HPLC method optimization using Fusion AE software

The initially developed method is described in Table I, and the obtained chromatograms for the analyses of formulation sam-

Table IV. The optimized Method, Report Card

Response variable*	Goal	Initial Method	Optimized Method† Predicted by Fusion AE	Optimized Method† Actual
Retention time of preservative degradant (peak 4), min	Minimize	4.6	5.8	5.9
Unknown-Retention time (peak 3), min	Minimize	4.7	4.8	5.0
Drug peak retention time (peak 11), min	Minimize	13.0	14.9	15.4
Resolution among drug and preservative peaks (peaks 11 and 12, respectively)	2	4.3	2.8	4.1
Resolution among preservative and RRT 1.2 peaks (peaks 12 and 13, respectively)	2	Not Resolved	3.6	3.5

* In italics: peak numbers as identified in Figure 5.
† Chromatographic conditions as listed in Table I for method prior to the method being geometrically scaled down.

ples with or without preservatives are presented in Figure 2. As could be seen from the chromatograms, the drug degradant peak (peak 3) and preservative degradant peak (peak 4) in the critical pair 1 are unresolved. Also, the drug degradant peak (peak 13) is only partially resolved from the tail end of preservative peak (peak 12) in the critical pair 2. The Fusion AE experiments were designed to resolve the peaks in these two critical pairs.

In the optimization experiments, effects of four study parameters; column temperature (45, 50, and 55°C), mobile phase pH (2.7, 2.9, and 3.1 pH units), % ACN at initial conditions (1, 2, and 3%), and initial hold time (2.5, 5.0, and 10.0 min); were simultaneously evaluated to assess the effects of these parameters on each of the five response variables. An example test plan is shown in Table II using 10 of the total 25 combinations of the four parameters evaluated in the study. The five response variables are listed in Table III. Test solution from the 2X sterilization cycle was analyzed using each of these conditions with a runtime of 60 min. The obtained results for retention times of peaks and the necessary resolution values were then transcribed back into Fusion AE software for modeling purposes.

The effects of study parameters on each of the five response variables were visually evaluated using Fusion AE generated three dimensional plots (e.g., effect of oven temperature and pH on retention time of the drug degradant in critical pair 1). As expected, peak retention times were inversely proportional to the column temperature. Mobile phase buffer pH had a strong influence on the retention time of drug degradant peak in critical pair 1 (e.g., retention time of 7.8 and 12.1 min at buffer pH of 3.1 and 2.7, respectively, when the column temperature, % ACN and initial hold time were held constant at 45°C, 1%, and 2.5 min, respectively), but only marginal effect on retention time of the preservative degradant peak. The other study parameters also affected the response variables in varying degrees (data not presented).

In the next step of optimization, experimentally obtained data were used in setting the lower and upper bounds for each of the response variables (Table III). Using the set goals (minimize or maximize), assigned lower and upper bounds for each value, and relative rank of 1 (desirable rank set to the highest value) for each of the response variables, the software performed an iterative

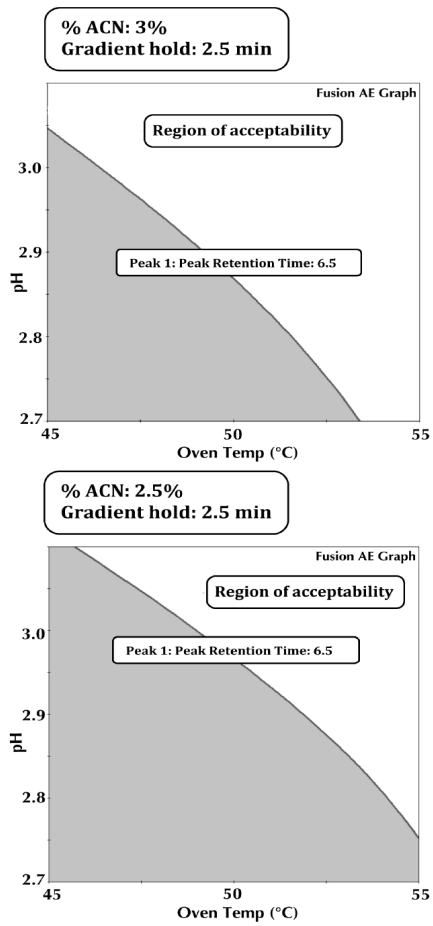

Figure 3. Response graph showing interactions among pH and oven temperature (°C) with 3% ACN (top graph) or 2.5% ACN (bottom graph) with initial gradient held at 2.5 min on a) retention time (number 6.5 next to retention time is the target retention time) of peak 1 (drug degradant, peak 3 in Figure 2) eluting at about 4.6 min. The white area, marked as region of acceptability, corresponds to Fusion AE predicted method conditions where the target goals listed in Table IV can be achieved.

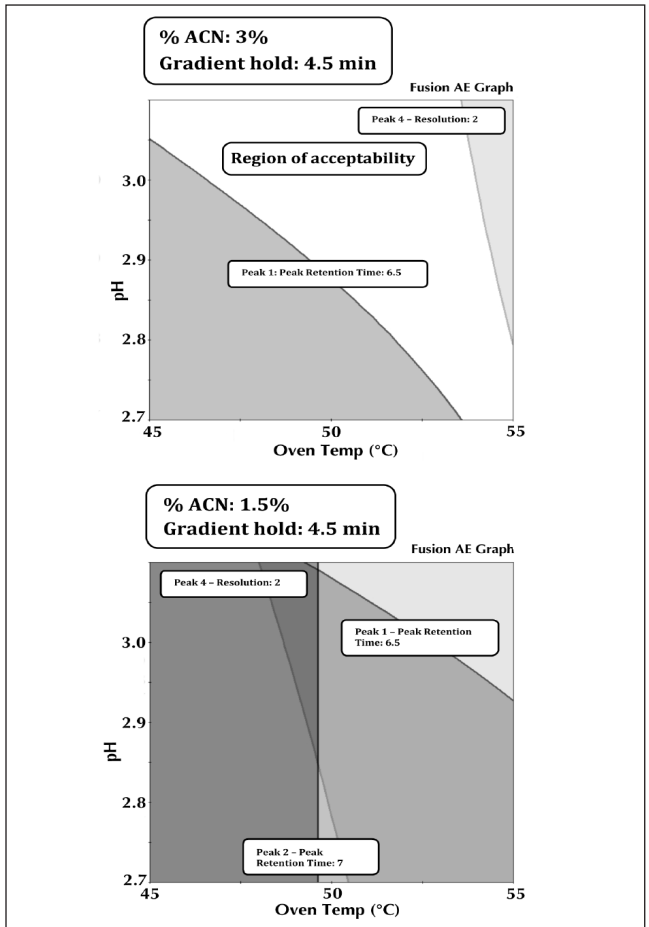

Figure 4. Response graph showing interactions among pH and oven temperature (°C) with 3% ACN (top graph) or 1.5% ACN (bottom graph) with gradient hold kept at 4.5 min on the response variables. Peaks 1 and 2 in the Fusion AE plots are peaks 3 and 4, respectively, in Figure 2, while Peak 4 resolution implies resolution among peaks 12 and 13 in Figure 2. A number next to the response variable (e.g., resolution 2) is the target goal. The white area, marked as region of acceptability, corresponds to Fusion AE predicted method conditions where the target goals listed in Table IV can be achieved.

search to find the optimum conditions with less error. The optimum HPLC conditions predicted by the software and the predicted response values are presented in Table I and Table IV, respectively. Using the modeled data, visual inspections of interactive effects were performed from multiple overlay graphs plotting two parameters at a time (e.g., oven temperature vs. pH with varying values of % ACN and initial gradient hold time). Representative graphs are shown in Figures 3 and 4. As could be seen from top plot in Figures 3 showing effects of pH vs. oven temperature (with % ACN and initial gradient time were held at 3% and 2.5 min, respectively), a region of acceptability was observed in the upper right hand corner (seen as white space) where all conditions were met. The region of acceptability shrunk when % ACN was changed to 2.5% (bottom plot, Figure 3), and a further reduction in acceptability region was observed (top plot in Figure 4) when % ACN and gradient hold were changed to 3% and 4.5 minutes, respectively. A further illustration of interactions among the study parameters can be seen from the absence of acceptability region (bottom plot in Figure 4) when % ACN and gradient hold were changed to 1.5% and 4.5 min.

The obtained desirability result of 0.7144 (expressed as a geometric mean of desirability value for each response variable) matched very well with a desirability target of 1.0 (calculated using a relative rank of 1 for each response variable). The software predicted retention times values of 4.8 and 5.8 min for peaks in the critical pair 1 (Table IV) suggested that these peaks would be fully resolved. Using the software predicted optimized conditions, chromatograms were generated for the analysis of 2X sterilized formulation samples with or without preservatives. The obtained chromatograms (Figure 5) indeed demonstrated baseline resolution among drug degradant (peak 3) and preservative degradant (peak 4) peaks in the critical pair 1. The predicted value for the unknown peak retention time of 4.8 min (experimentally obtained value of 5.0 min) being slightly outside of the stated lower bound of 5.06 (Table III) seems to have no bearing on the obtained resolution of critical pair 1 peaks. Also the drug (peak 11) and preservative (peak 12) peaks and preservative and drug degradant (peak 13) peaks in the critical pair 2 are now fully resolved with resolution values of 4.1 and 3.5, respectively. The "report card" for the optimized method is presented in Table IV. For each of the response variables studied, predicted values by Fusion AE software matched well with the actual values.

Further method optimization was achieved by geometrically scaling down the HPLC method (Table I). As could be seen from comparison of the chromatographic performance of Figure 5 (3.9 mm i.d. column) and Figure 6 (2.1 mm i.d. column), the geometrically scaled down method not only maintained the chromatographic resolution, it also reduced the solvent consumption, and associated disposal costs, with a reduced flow rate of 0.5 mL/min (vs. 1.5 mL/min) and shortened runtime of 52 min (vs. 60 min). The drug degradant peak (peak 3) elutes slightly early with the scaled down method (retention times of 5.0 and 4.2 min, respectively, with optimized and scaled down methods), probably because the gradient ramp starts slightly earlier at 2.2 min (2.5 min with the optimized method) for the scaled down method.

Robustness

Robustness of the scaled down method was demonstrated at the following conditions assessed during method optimization; % ACN of 3 ± 0.2% (range narrowed from that used in the optimization experiments), buffer pH of 3.1 ± 0.1 pH units, and column temperature of 50 ± 5°C (Table V). Additionally, the robustness was assessed at triethylamine volume of 2.1 ± 0.1 mL per 2 L and sodium octane sulfonate amount of 2.30 ± 0.1 g/2 L to evaluate if potential errors in the preparation of ion-pair reagent could affect the method performance. At each condition,

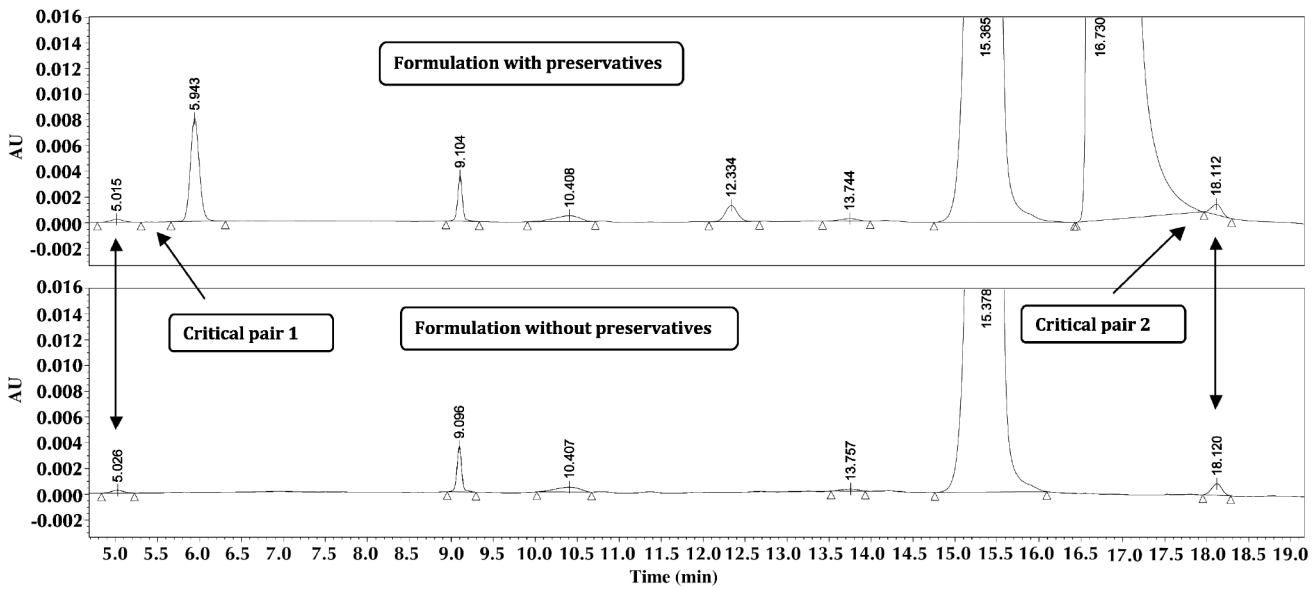

Figure 5. Chromatograms obtained for 2X sterilized formulation samples with (Top) or without (Bottom) preservatives using Fusion AE optimized method (not geometrically scaled down). Chromatographic conditions as stated in Table I. Chromatographic region from only about 4.6 min to about 19.2 min is shown to demonstrate separation of peaks 3 and 4 (drug and preservative degradants, respectively), peaks 11 and 12 (drug and preservative peaks, respectively), and peaks 12 and 13 (preservative and drug degradant peaks, respectively) achieved using the optimized method. The remaining peaks in the chromatograms are as described in Figure 6.

system suitability was performed (except % difference for 2 standards) and test solution for robustness was injected in triplicate. The same analytical column was used throughout the analytical runs and the nominal conditions bracketed the varied experimental conditions to verify that any changes observed were due to system variation and not to a change in the chromatographic performance of the column. The obtained results (results not presented) met the predefined acceptance criteria (Table V).

The geometrically scaled down method was also evaluated in terms of method specificity by analyzing predictive test solutions. The laboratory prepared formulation samples, with or without preservatives, were subjected to forced degradation with respect to acid, base, light (daylight and UV light), oxygen, and temperature (1X and 2X sterilized samples) to obtain the predictive test solutions. The obtained chromatographic peaks for drug and preservatives were spectrally pure as judged from the Empower 2 peak purity analysis. Also the known impurities peaks were well resolved among themselves and also from the drug and preservatives peaks in the chromatograms obtained for these predictive test solutions. Subsequently the method has been fully validated per ICH Q2 (R1) guidance document (15) using columns of different lots and transferred to the Quality Control laboratory for batch release and stability testing (data not presented).

Resolution solution and stability testing

Appropriateness of the resolution solution is illustrated from stacked chromatograms in Figure 6 comparing chromatograms obtained for the resolution solution and a sample from the accelerated storage conditions. Stability testing includes analysis of samples stored at accelerated storage conditions for 6 months at 40°C (16), and the obtained results may be used as one of the early predictors of formulation stability from long term storage for 24 months at 25°C. The comparison demonstrates that the system suitability in terms of resolution among the critical pairs (resolution criteria of NLT 1.5 among each pair) was designed in a meaningful fashion since resolution of a small peak from the tail end of preservative peak afforded by the system suitability helped in achieving resolution of the same small peak found in the accelerated storage sample. The accelerated storage sample also contained a couple of drug degradant peaks (peaks 7 and 9 in Figure 6) on either side of a preservative degradant peak (peak 8) in the chromatographic region from 12 to 13 min. This chromatographic separation was most likely a result of the optimized HPLC method that gave resolution of peaks prior to and after the 12 to 13 min region (i.e., resolution among drug and preservative degradant peaks from 4 to 7 min and resolution of peaks from approximately 14.5 to 17.9 min).

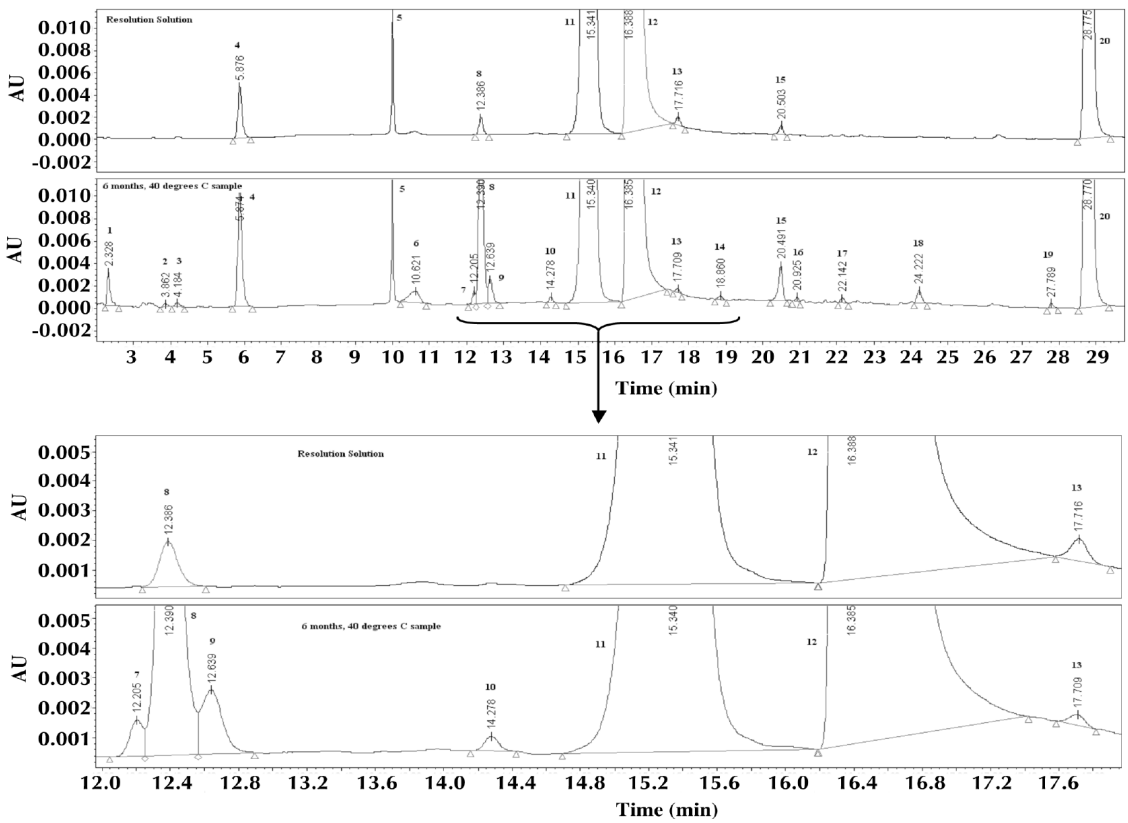

Figure 6. Chromatograms for resolution sample and accelerated storage sample (6 months at 40°C). The bottom chromatograms are for the expanded view of upper chromatograms showing separation of peaks 7 through 13. Critical pairs in the resolution were, peaks 11 (drug peak) and 12 (preservative peak) and peaks 12 and 13 (drug degradant). The requirements for resolution values between peaks 11 and 12 and that between peaks 12 and 13 per the system suitability criteria were NLT 1.5 for each, while the obtained values were 3.5 and 5.0, respectively. Resolution of peaks in the critical pairs enabled separation of peaks 7, 8, and 9. The HPLC conditions were as described in Table I for scaled down optimized method. The other peaks are numbered as follows: peak 5 (diluent related peak), peak 20 (preservative peak), peaks 8 (preservative degradant peak), and all other peaks are drug related peaks.

Table V. Robustness Experiments Summary

Condition	Mobile Phase A, % Acetonitrile	Ion-pair Reagent per 2 Liters		Buffer pH	Column Temp. (°C)
		TEA*	NaOct†		
Nominal	3	2.1 mL	2.30 g	3.1	50
1	3	2.1 mL	2.30 g	3.0	50
2	3	2.1 mL	2.30 g	3.2	50
3	2.8	2.1 mL	2.30 g	3.1	50
4	3.2	2.1 mL	2.30 g	3.1	50
5	3	2.0 mL	2.20 g	3.1	50
6	3	2.2 mL	2.40 g	3.1	50
7	3	2.1 mL	2.30 g	3.1	45
8	3	2.1 mL	2.30 g	3.1	55

Criteria

1. Meet system suitability requirements at each condition.
2. Recovery 98.0–102.0% for drug A vs. the mean result for the bracketing nominal conditions.
3. Recovery 95.0–105.0% for each preservative vs mean result for the bracketing nominal conditions
4. Absolute difference in any individual impurity NMT 0.05%. If an impurity is above the LOQ (0.05% w/w) in one condition and < LOQ in the other, the levels ≤ 0.10% satisfy this criterion.

* TEA, Triethylamine
† NaOct, sodium 1-octanesulfonate

Conclusion

In this paper, a QbD approach for analytical method development that consists of (*i*) developing a full understanding of the intended purpose, (*ii*) developing predictive solutions, (*iii*) designing a meaningful system suitability solution that helps to identify failure modes, and (*iv*) following design of experiments approach to the method development has been presented. These concepts were successfully applied to the development and optimization of a stability indicating HPLC method for drug, preservatives, and their degradant peaks. A full understanding of the product was gathered to develop method performance expectations including critical pairs in the chromatographic separation and also in developing predictive test solutions. The developed system suitability solution was meaningful since it helped in ensuring chromatographic separation of peaks in samples from stability studies. The method was optimized using the Fusion AE software with multivariate experiments. The QbD based method development helped in generating a design space and operating space with knowledge of all method performance characteristics and limitations and successful method robustness within the operating space. The method has been successfully applied for the analyses of accelerated storage samples and registration stability batches.

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