Quality-by-Design Approach to Stability Indicating Method Development for Linagliptin Drug Product

Application Note

Pharmaceutical QA/QC

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Abstract

A traditional approach to method development could fail to meet desired separation during validation, transfer, or out of specification studies.
A quality-by-design (QbD) approach to method development can potentially lead to a more robust/rugged method due to the emphasis on risk management. In a QbD approach, the impact and interactions between critical method variables are understood using a Design of Experiments (DOE) approach, which incorporates statistical multi-variate analysis and modeling. This study applied a QbD approach to linagliptin stability indicating method development using Fusion AE automated QbD method development software (S-Matrix) and an Agilent 1200 Infinity Series Method Development Solution. The allowed deviations of the method variables are determined within the design space – the Proven Acceptable Ranges (PARs). The critical method variables in a linagliptin stability-indicating method are percent organic 90.5 ± 1.5 and pH 7.7 ± 0.1 at a column temperature of 45 °C. The potential interference of method variables in terms of desirable method responses was determined, leading to a better understanding of the method, and achieving desirable method quality.

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Introduction

QbD is defined in ICH guidelines Q8(R2) as "A systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management"[1]. ICH guidelines suggest quality in the design to eliminate risk or failures of the process and thus the product. In this approach, the quality associated process variables are defined, their interactions studied, control strategy implemented, and finally, the method is continually monitored.

The analytical method development for a drug is also a process, and quality principles in the ICH guidelines can be implemented in the design of the method development[2,3]. The goal of Analytical QbD is to achieve quality in measurement leading to consistent quality of drug product. While there are several similarities in approaching method development in a QbD environment, it may be difficult to imagine a single approach. Figure 1 represents a typical workflow for QbD-based analytical method development with the desired state having a reliable (robust and rugged) method through its life-cycle.

Analytical QbD begins with defining goals and identifying potential method variables and responses that affect method quality (Stage I). The Analytical Target Profile (ATP) states the intended purpose of the method[4]. The method-specific related information is tabulated in the Quality Target Method Profile (QTMP), which helps to identify potential method variables. Examination of potential variables is performed in this definition phase, prior to experiments. This helps to focus on specific variables and their ranges. The potential variables that can impact method quality can be identified using an Ishikawa (fishbone) diagram (Figure 2).

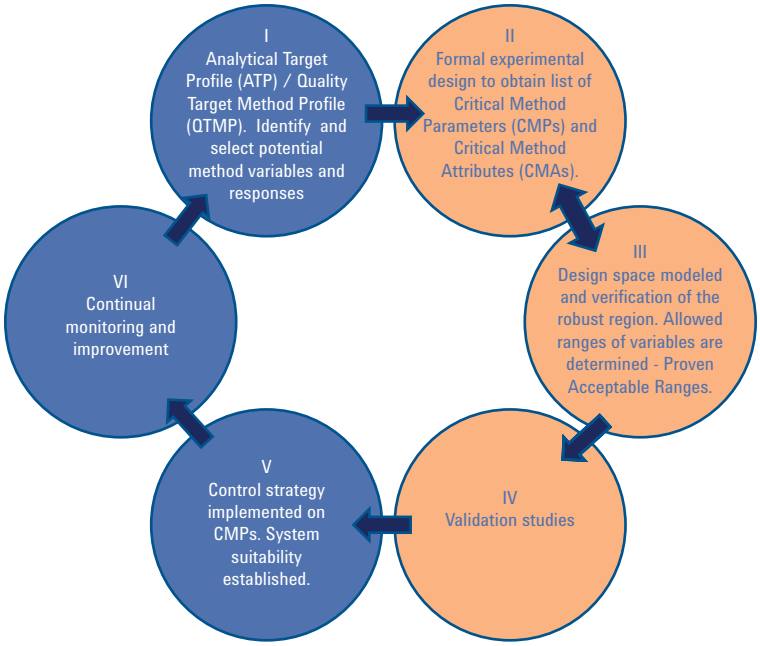

Figure 1. Typical workflow for QbD based analytical method development. The II, III, and IV steps represent software assisted sections.

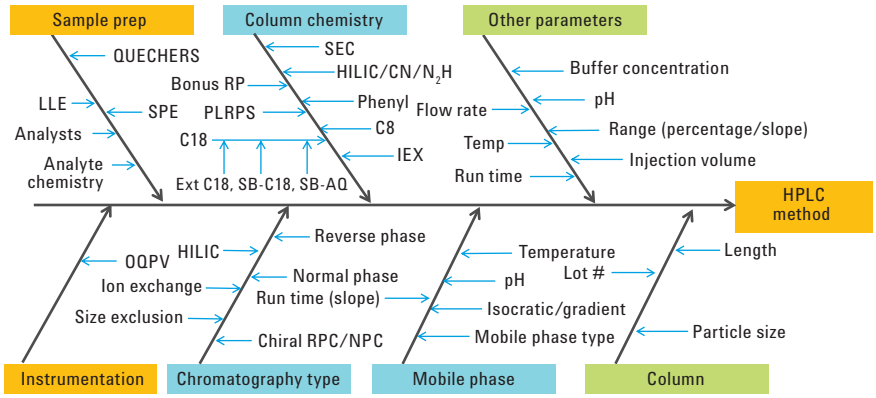

Figure 2. The ishikawa or fishbone diagram to identify potential variables in HPLC method development. Liquid-liquid extraction (LLE), solid phase extraction (SPE), reverse phase (RP), reverse phase chromatography (RPC), normal phase chromatography (NPC), size exclusion chromatography (SEC), ion exchange chromatography (IEX).

After defining the method variables, formal experimental designs such as statistical Design of Experiments (DOE) were applied to the selected method variables leading to process and method understanding (Stage II). The DOE helps eliminate the need for performing a large number of runs and achieves desirable results from a limited number of experiments. Since multivariate interactions of variables and process parameters have been studied, we have increased understanding of method variability, thus there is greater understanding of the method. There is a better understanding of the specific levels of control required for critical method parameters (CMP) to maintain the allowable response ranges, that is, the critical method attributes (CMA)[2].

The experimentally measured responses were then modeled to determine the design space (Stage III). ICH Q8(R2) defines the design space as, "The multidimensional combination and interaction of input variables (for example, material attributes) and process parameters that have been demonstrated to provide assurance of quality". The allowed deviations of the variables were determined within the design space, the Proven Acceptable Ranges (PARs). The PARs form the robust regions where the deliberate variations in the method parameters do not change the CMA. This ensures that the method does not fail downstream during validation testing. Thus, the risk is minimized and quality is assured. If the modeling experiments do not lead to desired responses, method variables can be adjusted and new experiments performed.

A verified method is used to perform validation experiments to validate the developed method (Stage IV). An understanding of the method robustness/ method variability can be useful in risk management and risk reduction. A validation experiment verifies that the method served its intended goal.

After the method is validated, a control strategy is implemented (Stage V). Here the appropriate system suitability is implemented in this method.

Continual monitoring of the method performance forms the last stage of Analytical QbD (Stage VI). Here, continual improvement can be implemented to redefine the ATP.

This Application Note describes the analytical QbD approach to method development of a stability-indicating method for linagliptin drug product. The intended purpose was described, the method variables were selected, and their interactions studied to define appropriate ranges that gave the desired responses. The modeled data were then verified. Studying the variety and combination of variables in chromatographic separations was facilitated by the Analytical R&D LC Method Development feature of Fusion AE (S-Matrix), which controls the instrument unaided, generates and performs the DOE, tracks components, and models the responses. Although validation, control strategy, and continuous monitoring (Stages IV, V, and VI) would complete the QbD process, these steps are not demonstrated here.

Experimental

Instrument

Table 1 shows the instrument configuration used in the automated method development. The Agilent 1290 Infinity Binary Pump was connected to an Agilent 1290 Infinity Valve Drive (but not clustered during instrument configuration). The valve drive was equipped with a 12-position/13-port valve and attached to A1 solvent tubing of the 1290 Infinity Binary pump. Since the connection was made prior to the degasser, all 12 solvents connected to channel A1 were degassed. The two Agilent 1290 Infinity Thermostatted Column Compartments (TCCs) were configured as clustered; having two 8-position/9-port valves allowed eight 50-mm columns to be coupled. The TCCs were clustered such that the 1,200 bar 8-position/9-port valve was in the first TCC, where all the inlet tubings were connected. The second TCC had 600 bar with all outlet connection. The autosampler was equipped with a 100-vial tray.

Table 1. Instrument configuration used in QbD approach for analytical method development.

Agilent 1200 Series Infinity method development solution	Features	Details
Agilent 1290 Infinity Binary Pump		G4220A
Agilent 1290 Infinity Valve Drive and solvent selection valve	12-position/13-port valve	G1170A and G4235A
Agilent 1290 Infinity Autosampler maintained at 5 °C using a thermostat	100-vial tray	G4226A with G1330B
Two Agilent 1290 Infinity TCCs		G1316C
Two 8-position/9-port valves		G4230B
Solvent Selection Tubing Kit	Four solvents	p/n 5067-4601
Agilent 1290 Infinity DAD		G4212A
OpenLAB CDS ChemStation Edition Workstation	For data acquisition and data analysis	M8301AA; Rev. C.01.05[36]
Fusion AE – Automated Experimentation Software (S-Matrix)	Ver: 9.6.22 Build 42	

Software

The LC Method Development module of the Fusion AE software Platform (FusionAE) is used for process understanding and modeling. The instrument name assigned in OpenLab Chemstation is the same as the name assigned in Fusion AE for appropriate communication. The OpenLab Chemstation modules are selected from the comprehensive list of supported modules and their clustered/valve position in the Fusion AE Administrator application. The user specifies the operating pH range of the column, and Fusion AE only generates runs with the appropriate mobile phases based on the operating pH range. The software suggests statistical DOE for the selected variables and generates a sequence table that can be run by OpenLab Chemstation. The sequence constructed by Fusion AE can automatically include blank runs before each injection, column conditioning runs, and repeat injections.

The method development was performed sequentially in two phases, screening and optimization. This is a two-part rapid method development strategy proven highly successful in practice[5,6,7]. The screening phase selects method parameters that have direct impact on selectivity and capacity factor. All columns used were sub-2 µm, for increased theoretical plates. The optimization phase further improved separation. For the screening phase, peak integration was performed in OpenLab Chemstation while peak labeling was performed for optimization. The default model-robust algorithm design suggested by Fusion AE was used in the screening experiments. The default face-centered central composite design used for optimization had three center points and three non-center points as suggested by Fusion AE. Data analysis used the default automatic mode in the Fusion AE software. Fusion AE's Robustness Simulator performed a Process Capability (C_p) analysis to generate direct quantitative measures of process robustness. This was done by theoretical Monte Carlo simulations using the DOE-derived models on the joint proposed variations in the method. The point prediction forecasts the theoretically expected results that are then verified.

Reagents and Materials

Preparation of linagliptin degradation samples

Two linagliptin formulated tablets (5 mg) were crushed and weighted accurately to 150 mg formulated powder. A 1,000 µL solution of 3 % hydrogen peroxide was added, vortexed, and incubated for 30 minutes at room temperature, in darkness. Afterwards, the solution was kept in a rotatory evaporator for 30 minutes to evaporate any residual peroxide. A 1,000 µL solution of diluent (50 % acetonitrile/50 % water) was added and vortexed, and the solution was centrifuged for 5 minutes at 13,000 rpm. The supernatant was filtered using a glass microfiber filter. A filtered solution was mixed with an equal amount of diluent and centrifuged for 5 minutes at 13,000 rpm before being injected into the HPLC. No significant secondary degradation is observed over time.

All mobile phases used were HPLC grade (RCI Labscan Ltd, Thailand). Linagliptin formulation was purchased from a local drug store (Bangalore, India).

Results and Discussion

QbD: definition/goal

Analytical target profile (ATP)

ATP suggests that the stability-indicating method accurately measures linagliptin without interferences from degradants in stability samples. The method should separate linagliptin from degradants formed from forced degradation. Mass Spectrometer (MS) compatible mobile phase for possible MS-based identification of degradants can be used during the method development.

Quality target method profile

Log P values or molecular structure suggests possible hydrophobic chromatography. pKa values of 1.9 and 8.6 were reported and the compound was readily soluble in 50:50 acetonitrile and water. A preliminary chromatographic run of linagliptin on an Eclipse Plus C18 column, and 5 to 95 % gradient using water and acetonitrile with acetic acid additive suggested that a gradient elution mechanism is preferred. The UV max was 292 nm and the sample did not saturate the UV signal. A 10 % degradation of the main peak was seen after oxidative degradation (Figure 3).

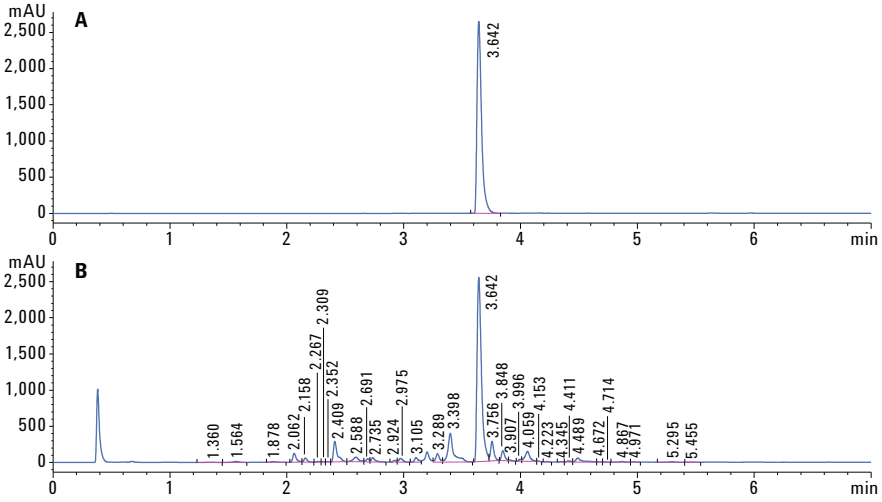

Figure 3. A preliminary chromatographic run on linagliptin before (A) and after degradation (B).

Preliminary method variables

Mobile phase type, column chemistry, gradient, and run time.

Preliminary method attributes

Resolution of linaglitpin from nearest degradants (Rs), peak purity of linagliptin (purity value), peak capacity, and peak tailing.

QbD: process (method) understanding/risk assessment

Screening

The screening phase of method development is based on early risk assessment test variables: mobile phase type, pH, column chemistry, and run time (Table 2). For some columns, the pH of the mobile phase was not compatible, but Fusion AE chose the appropriate mobile phase pH based on the operating pH range of the column. The statistical design of experiments using full factorial design or other default designs can be used. After peak integrations, the data were exported to Fusion AE, which was used to model the data. The critical method attributes (CMA) of number of peaks, resolution, and peaks having peak tailing less than 1.2 was maximized, and the software modeled the contour plot for various columns.

Table 2. The column, solvent, gradient conditions used in screening experiments. The text in bold are the variables.

Columns	
Agilent ZORBAX RRHD StableBond C18, 3.0 × 50 mm, 1.8 µm (p/n 857700-302)	
Agilent ZORBAX RRHD Bonus-RP 2.1 × 50 mm, 1.8 µm (p/n 857768-901)	
Agilent ZORBAX RRHD Eclipse Plus C8, 3.0 × 50 mm, 1.8 µm (p/n 959757-306)	
Agilent ZORBAX RRHD StableBond Phenyl 3.0 × 50 mm, 1.8 µm (p/n 857700-312)	
Agilent PLRP-S 4.0 × 50 mm, 3.0 µm (p/n PL1512-1300)	
Agilent ZORBAX RRHD Extend-C18, 3.0 × 50 mm, 1.8 µm (p/n 757700-302)	
Solvents	
Mobile phase A1	**pH 2.0, 10 mM TFA in water**
Mobile phase A2	**pH 5.0, 10 mM ammonium acetate and 5 mM acetic acid in water**
Mobile phase A3	**pH 6.4, 10 mM ammonium acetate in water**
Mobile phase A4	**pH 8.0, 10 mM ammonium hydrogencarbonate in water**
Mobile phase A5	**pH 11.0, 10 mM ammonia in water***
Mobile phase B1	**Acetonitrile**
Mobile phase B2	**Methanol**
Gradient	
Initial hold	0.6 minutes, 5 % B
Gradient time	**Condition 1, 5 minutes - 5 % B to 95 % B** **Condition 2, 10 minutes - 5 % B to 95 % B**
Hold	1 minute at 95 % B
Re-equilibrate	3 minutes at 5 % B
Experimental details (constants)	
Pump flow	0.6 mL/min
Injection volume	1 µL
Oven temperature	40 °C
Wavelength	292 nm ± 4 nm (ref 400 ± 20 nm)

The contour plot of the modeled data for the Agilent ZORBAX RRHD Eclipse Plus C8 in Figure 4A shows the unshaded region as the acceptable region where all the CQA limits are met. The surface plot in Figure 4B shows the region where gradient time greater than 8 minutes and pH greater than 5.5 leads to maximum number of integrated peaks. In setting up the models in Fusion AE, minimizing the resolution of the largest peak (API) can also provide optimal screening conditions that show peaks that elute near the main peak. This method, in general, was found to be sensitive to pH and gradient time/slope within a range, thereby indicating a stringent control on these method parameters

Of the columns selected, which included C18, Bonus-RP, and Phenyl Hexyl, the ZORBAX RRHD Eclipse Plus C8 provided the optimal resolution for the next stage of experiments. The best overall answer for the screening experiments, as shown in Table 3, suggests that the ZORBAX RRHD Eclipse Plus C8, pH 7, with a gradient time of 10 minutes to be best among the screening experiments. During the next optimization steps, some parameters were varied further in smaller increments.

Table 3. The best overall answer from the screening experiments.

Best overall answer	
Strong solvent	Methanol
Gradient time	10 minutes
pH	7
Column type	Agilent ZORBAX RRHD Eclipse Plus C8 3 × 50 mm, 1.8 µm

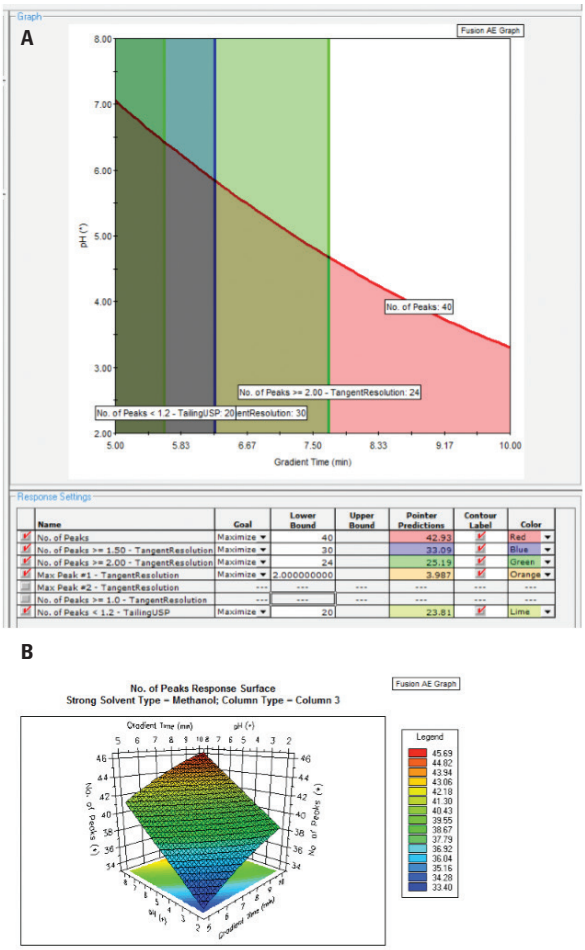

Figure 4. Contour plot (A) of pH as a function of gradient time. The surface plot (B) shows the maximum number of peaks based on gradient time and pH of the mobile phase.

Optimization (mean method performance)

The method was further optimized by studying the gradient endpoint percent strong solvent in combination with narrow pH and temperature ranges around the best values identified from the screening experiments (Table 4). This stage optimized mean method performance, with the analysis modeling and Best Overall Answer feature (Table 5) identifying the best conditions as pH 7.7, temperature 45 °C, and final percentage strong solvent 90.5 % at the gradient time of 15 minutes. At this point, the critical method parameters (CMPs) and critical method attributes/responses (CMAs) were determined.

Table 4. The variables tested during the optimization phase of the method development. The text in bold are the variables.

Columns	
Agilent ZORBAX RRHD Eclipse Plus C8 3.0 × 50 mm 1.8 µm (p/n 959757-306)	
Solvents	
Mobile phase A	**pH 7.0**, 10 mM ammonium acetate in water*
Mobile phase A	**pH 7.5**, 10 mM ammonium acetate in water*
Mobile phase A	**pH 8.0**, 10 mM ammonium acetate in water*
Mobile phase B	Methanol
Gradient	
Initial hold	0.6 minutes, 5% B
Gradient % organic/slope	
Condition 1	**5 % B to 95 % B in 15 minutes**
Condition 2	**5 % B to 80 % B in 15 minutes**
Hold	1 minute at 95 % B
Re-equilibrate	2 minutes at 5 % B
Experimental details	
Pump flow	0.6 mL/min
Injection volume	1 µL
Oven temperature	**30 °C, 40 °C, and 45 °C**
Wavelength	292 nm ± 4 nm (ref 400 ± 20 nm)

*pH adjusted by dilute ammonia

Table 5. The best overall answer from the optimization study.

Best overall answer	
Gradient time	15 minutes
Final % strong solvent	90.5 %
pH	7.7
Oven temperature	45 °C

Design space

A preliminary design space is the multidimensional combination of the CMAs in terms of CMPs. Any small variations in the method parameters could alter the desired attribute. A maximum attribute does not necessary occur in the most robust region since the maximum values may lie in the steep slope region of the response surface plot. It is, therefore, important to characterize the process capability (C_p), which defines the combined effects of method parameter set point variations on the method variability. Robustness stimulator generation and modeling of the C_p values associated with each response[8,9] was performed on the robust region (Figure 5). After the robustness stimulation, the design space now incorporated both the mean method performance and method robustness. Table 6 summarizes the findings based on the fixed temperature setting of 45 °C. The deviations of the variables within the design space represents the allowed deviations where we can still expect that the acceptable method performance of resolution and peak tailing will be met.

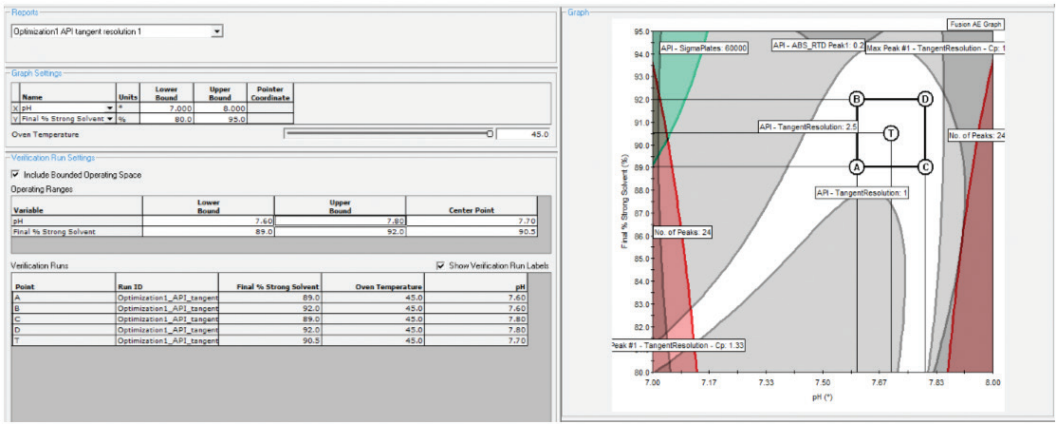

Figure 5. The model of the final percentage organic versus pH. The nonshaded region is the design space for percent strong solvent ranges and mobile phase pH, composed of the overlapping regions of acceptable performance for all critical responses in terms of mean performance and robustness. The square marks the variable regions at the fixed oven temperature of 45 °C.

Table 6. The various critical method parameters, the proven acceptable ranges for some of the critical method parameters and the critical method attributes.

Critical Method Parameters (CMPs)		Proven Acceptable Range (PARs)	Critical Method Attributes (CMAs)
Column	Agilent ZORBAX RRHD Eclipse Plus C8, 3.0 × 50 mm, 1.8 µm	–	No. of peaks (> 40) API resolution (> 1.5) Peak purity (≥ 98 %) Peak tailing (< 1.5)
Strong solvent	Methanol	–	
% Strong solvent	90.5 %	± 1.5 %	
Aqueous solvent pH	7.7	± 0.1	
Gradient range	5 % to 90.5 %	–	
Oven temperature	45 °C	–	
Gradient time	15 minutes	–	
Flow rate	0.6 mL/min	–	
Wavelength	292 nm	–	

Verification by point predication

The Point Predictions feature of Fusion AE predicts the responses in the robust region and allows for verification of chromatographic runs to be performed. Figure 6 shows the experimentally verified chromatographic runs at target pH ± 0.1 and target percent organic ± 1.5 % with resulting resolution > 1.5 for linagliptin. The predicted results and experimental results are tabulated in Table 7.

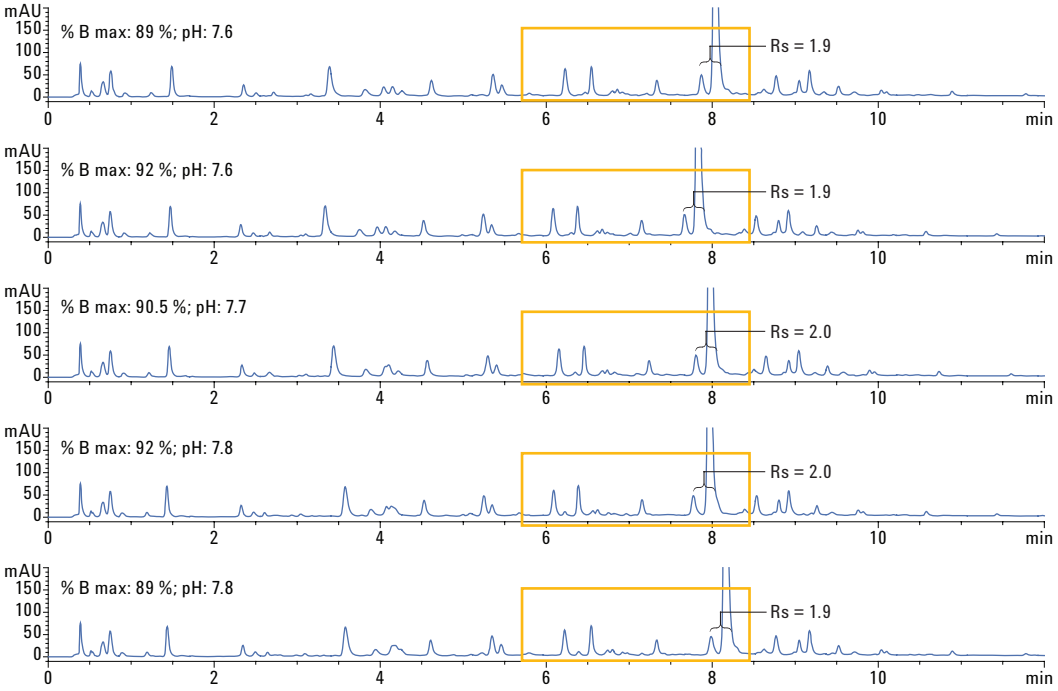

Figure 6. The overlay of the center and four robustness points.

Table 7. The critical method attributes of the predicted and experimental results.

	Center point of robust region – prediction	Center point of robust region – experiment
API-Symmetry	0.71 ± 0.04	0.66
API-Tailing USP	1.4 ± 0.07	1.4
API-Tangent width	0.081 ± 0.004	0.083
API-Resolution API	2.3 ± 0.3	2.0

Conclusions

QbD principles were applied to stability-indicating method development for linagliptin using Fusion AE automated method development software on an Agilent 1200 Infinity Series Method Development Solution. Statistical Design of Experiments (DOE) was applied to the study of column chemistry, temperature, mobile phase type, and gradient slope for both selectivity and capacity factor. Short columns were screened to achieve reasonable run time, and small particle sized columns were used to increase the theoretical plates. Performance of the resulting method could be further improved by increasing the column length and run time. Multivariate analysis of several critical method parameters including column and solvent type, % mobile phase, pH, and column temperature was used to determine the best performing chemistry system and the final Design Space. A robust final method was obtained with a column temperature of 45 °C, percent strong solvent of 90.5 % ± 1.5, and pH 7.7 ± 0.1. QbD approach to method development has helped to better understand the method variables, leading to less chance of failure during method validation and transfer. The automated QbD method development approach using FusionAE software has provided a better performing and more robust method in less time compared to manual method development.

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