Application note | 001631

Pharma

Development of a robust LC method for metolazone and related impurities using analytical quality by design best practices

Authors

Sylvia Grosse[1], Soo Hyun Park[1], Richard Verseput[2], Frank Steiner[1]

[1]Thermo Fisher Scientific, Germering, Germany

[2]S-Matrix Corporation, Eureka, CA, USA

Keywords

Quality by design, AQbD, system suitability, SST, Vanquish Flex, UHPLC, robustness, MODR, verification

Application benefits

- Thermo Scientific™ Vanquish™ UHPLC Method Development System in combination with Fusion QbD™ software enables automated and unattended method development.
- The workflow presented herein significantly reduces the number of experiments, method development time, and related costs.

Analytical QbD benefits

- Statistically valid study protocols
- Independently verifiable, data-driven decisions
- Quantitative characterization of the effects of all study parameters on critical performance characteristics, i.e., mean (average) performance and robustness

Goal

Demonstrate the benefits of the Analytical Quality-by-Design (AQbD) approach by developing a fast and robust UHPLC method for metolazone and related impurities.

Introduction

The development of analytical methods is a critical component of the drug substance and drug product development process in every laboratory and market. Traditionally, method development has been done by trial and error, with the endpoint sometimes even determined by limitations of time and resources rather than by documented successful development of a robust and transferrable method. In the case of liquid chromatography (LC) methods, an additional limitation arose from early method development software, which supported method development primarily through gradient slope optimization to separate critical peak pairs. This approach most often drives final development to multi-segment gradient methods, the risks of which are now widely understood and accepted.[1] The Fusion QbD software platform[2] overcomes these limitations with a complete analytical quality by design (AQbD) solution toolset for LC method development, validation, and transfer that includes full closed-loop experiment automation support for Thermo Scientific™ Chromeleon™ CDS (Chromatography Data System) and Thermo Scientific™ Vanquish™ LC systems.

In this application note the AQbD approach is described for the development of a fast and robust UHPLC method for metolazone and its related impurities. Fusion QbD software combined with a Vanquish Flex UHPLC system enables automated method scouting, optimization, and robustness evaluation as well as enhanced data visualization and reporting (Stage 1 of Analytical Product Lifecycle Management Workflow[2]). A flow scheme for the Vanquish Flex quaternary system is shown in Figure 1. The online pH buffer preparation automation feature within Fusion QbD was used for pH screening in the scouting step. This minimizes the manual preparation of solvents. Only stock solutions of acidic and basic buffers need to be prepared. Fusion QbD then automatically determines the buffer composition for individual pH values. In this way, a wide pH range can be covered with minimal solvent preparation.

Experimental

Chemicals

- Deionized water, 18.2 MΩ·cm at 25 °C, Thermo Scientific™ Barnstead™ GenPure™ xCAD Plus Ultrapure Water Purification System (P/N 50136149)
- Acetonitrile, Optima™ LC-MS grade, Fisher Chemical™ (P/N A955)
- Methanol, Optima™ LC-MS grade, Fisher Chemical™ (P/N A456-212)
- Ammonium acetate, LC-MS Ultra, Honeywell™ (P/N 15691400)
- Formic acid, Optima™ LC-MS grade, Fisher Chemical™ (P/N A117)
- Ph. Eur. reference standard: Metolazone for system suitability (SST) CRS batch[3] (P/N Y00007022)

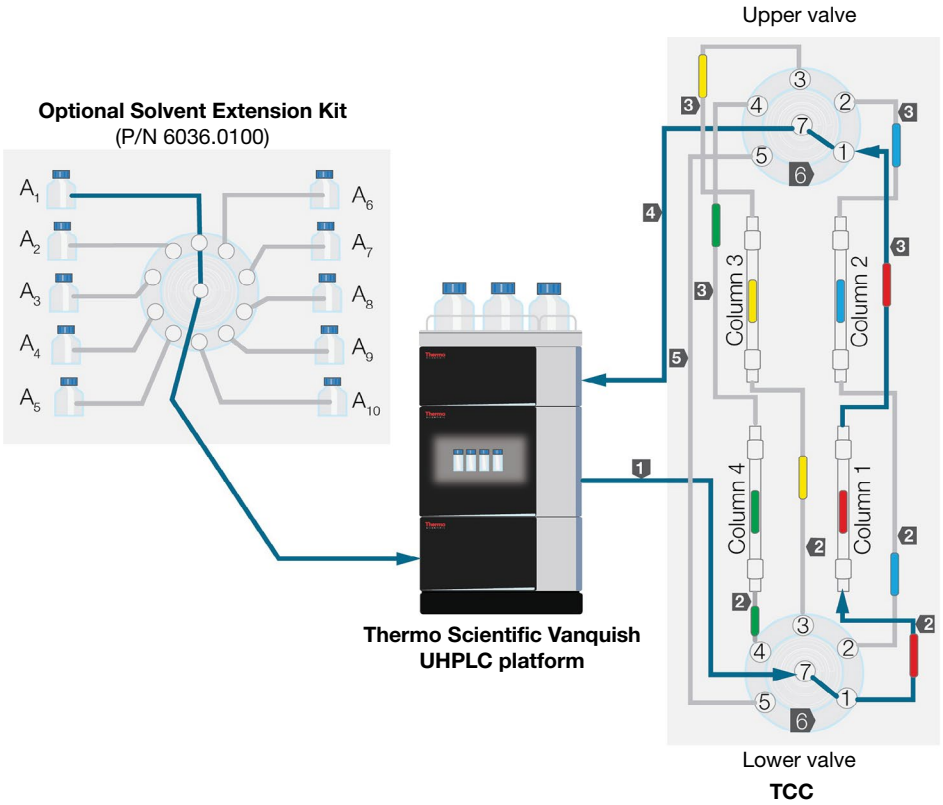


Figure 1. Scheme of the Vanquish Flex Quaternary UHPLC system with the Automated Method Scouting Option for Vanquish Systems

Sample handling

- Thermo Scientific™ Orion™ 3 Star pH Benchtop Meter (P/N 13-644-928)
- Fisherbrand™ Mini Vortex Mixer (P/N 14-955-152)
- Vials (amber, 2 mL), Fisher Scientific™ (P/N 15508760)
- Snap Cap with Septum (Silicone/PTFE), Thermo Scientific™ (P/N 10547445)

Instrumentation

Thermo Scientific™ Vanquish™ Quaternary Flex UHPLC system consisting of:

- System Base Vanquish Horizon/ Flex (P/N VF-S01-A)
- Vanquish Quaternary Pump F (P/N VF-P20-A)
- Vanquish Split Sampler FT (P/N VF-A10-A)
- Vanquish Column Compartment H (P/N VH-C10-A-02)
- Vanquish Diode Array Detector FG (P/N VF-D11-A)
- Standard flow cell 13 µL (P/N 6083.0510)
- Thermo Scientific™ Automated Viper™ Method Scouting Kit for Vanquish Systems (P/N 6036.2807)
- Vanquish Switching Valve with 6-position/7-port (2×) (P/N 6036.2530)
- Extension Kit for Automated Method Scouting, Vanquish Systems (P/N 6036.0100)

Preparation of standards

To obtain a working standard solution containing metolazone and impurities A, B, C, D, and E, 3.00 mg of the reference standard for system suitability was dissolved in 1.000 mL of methanol.

Experiment study factors – Chemistry system screening (method scouting)

Table 1 lists the instrument and chemistry system parameters included in the chemistry system screening (method scouting) experiment.

Experiment study factors – Method optimization

Table 2 lists the instrument and chemistry system parameters included in the method optimization experiment.

Table 1. Method parameters used for method scouting

Study factor	Study range/levels
Column type	• Thermo Scientific™ Hypersil GOLD™, 100 × 2.1 mm; 1.9 µm (P/N 25002-102130) • Thermo Scientific™ Acclaim™ Polar Advantage II, 100 × 2.1 mm, 2.2 µm (P/N 068990) • Thermo Scientific™ Accucore™ PhenylHexyl, 100 × 2.1 mm; 2.6 µm (P/N 17926-102130) • Thermo Scientific™ Accucore™ Biphenyl, 100 × 2.1 mm; 2.6 µm (P/N 17826-102130)
pH of aqueous solvent	2.90–6.42 pH online blending mode. Acid-base pair: A1: 0.1 M Formic acid A2: 0.1 M Ammonium formate
Organic solvent	B: 90/10 Acetonitrile/water (v/v) C: 90/10 Methanol/water (v/v)
Gradient slope (using t_G)	5–100% B t_G Range = 10–25 min = 3.6–9.0%/min
Constant factor	**Level setting**
Flow rate	0.600 mL/min
Column temperature	40.0 °C (passive pre-heater)
Autosampler temperature	8.0 °C
Injection volume	0.8 µL
UV detector parameters	Detection at 230 nm Data collection rate: 10 Hz Response time: 0.5 s

Table 2. Method parameters used for method optimization

Study factor	Study range/levels
Aqueous mobile phase and pH	• A1: 10 mM Ammonium acetate pH 5.4 (adjusted with formic acid) • A2: 10 mM Ammonium acetate pH 5.9 (adjusted with formic acid) • A3: 10 mM Ammonium acetate pH 6.4 (adjusted with formic acid)
Gradient conditions	Starting Point %B: 15–30% t_G: 16–30 min
Flow rate	0.400–0.600 mL/min
Column temperature	35–45 °C (passive pre-heater)
Constant factor	**Level setting**
Column	Hypersil GOLD, 100 x 2.1 mm; 1.9 µm
Organic solvent	B: 90/10 Methanol/water (v/v)
Final % strong solvent in gradient	70% B
Autosampler temperature	8.0 °C
Injection volume	0.8 µL
UV detector parameters	Detection at 230 nm Data collection rate: 10 Hz Response time: 0.5 s

Final robust method

Table 3 lists the chromatographic parameters of the final method performed for SST verification.

Table 3. Chromatographic parameters of the final method

Parameter	Value
Column	Hypersil GOLD, 100 × 2.1 mm; 1.9 µm
Mobile phase	A: 10.0 mM Ammonium acetate, pH 5.50 (adjusted with formic acid) B: 90/10 Methanol/water (v/v)
Flow rate	0.500 mL/min
Gradient	Time (min) %B -8.0 25.0 0.0 25.0 1.0 25.0 16.0 47.5 17.0 47.5 17.5 100.0 19.5 100.0 20.0 25.0 22.0 25.0
Column temperature	40.0 °C (passive pre-heater and still air)
Autosampler temperature	8.0 °C
Injection volume	0.8 µL
Detection	Wavelength: 230 nm Data collection rate: 10 Hz Response time: 0.5 s

Chromatography Data System/software

Chromeleon 7.3.1 Chromatography Data System (CDS) was used for data acquisition and basic data processing, such as peak integration.

Fusion QbD Software, version 9.9.2, was used for experimental set-up, experimental design automation, and data evaluation.

Fusion QbD and Chromeleon CDS work together flawlessly. The experimental design is created in the Fusion QbD software, which then automatically transfers the data to Chromeleon CDS, transforming the experimental design into a ready-to-run sequence (Figure 2). All relevant peaks are then integrated in Chromeleon CDS, and all results required for response data modeling, Monte Carlo robustness simulation, and chromatogram simulation are automatically imported into Fusion QbD. It is important to note that Fusion QbD supports full data integrity by internally auditing data transfers with Chromeleon CDS as well as incorporating audit information on the transfers within Chromeleon CDS.

A

#	UV_VIS_1	Name	Type	Level	Position	Volume	Instrument Method
1		Conditioning_Run_1	Blank		R:A5	0.0100	Conditioning_Run_1
2		Conditioning_Run_2	Blank		R:A5	0.0100	Conditioning_Run_2
3		Conditioning_Run_3	Blank		R:A5	0.0100	Conditioning_Run_3
4		Conditioning_Run_4	Blank		R:A5	0.0100	Conditioning_Run_4
5		Blank - 1	Unknown		R:A1	2.0000	Blank - 1
6		Blank - 2	Unknown		R:A1	2.0000	Blank - 2
7		Blank - 3	Unknown		R:A1	2.0000	Blank - 3
8		Blank - 4	Unknown		R:A1	2.0000	Blank - 4
9		1	Unknown		R:A2	2.0000	1
10		2	Unknown		R:A2	2.0000	2
11		3	Unknown		R:A2	2.0000	3
12		4	Unknown		R:A2	2.0000	4
13		5	Unknown		R:A2	2.0000	5
14		Conditioning_Run_5	Blank		R:A5	0.0100	Conditioning_Run_5
15		Conditioning_Run_6	Blank		R:A5	0.0100	Conditioning_Run_6
16		Conditioning_Run_7	Blank		R:A5	0.0100	Conditioning_Run_7
17		Conditioning_Run_8	Blank		R:A5	0.0100	Conditioning_Run_8
18		6	Unknown		R:A2	2.0000	6
19		7	Unknown		R:A2	2.0000	7
20		8	Unknown		R:A2	2.0000	8
21		9	Unknown		R:A2	2.0000	9

B

#	UV_VIS_1	Name	Type	Level	Position	Volume	Instrument Method
1		Conditioning_Run_1	Blank		R:A4	0.0100	Conditioning_Run_1
2		Blank - 1	Unknown		R:A1	0.8000	Blank - 1
3		1	Unknown		R:A2	0.8000	1
4		2	Unknown		R:A2	0.8000	2
5		3	Unknown		R:A2	0.8000	3
6		4	Unknown		R:A2	0.8000	4
7		5	Unknown		R:A2	0.8000	5
8		6	Unknown		R:A2	0.8000	6
9		Conditioning_Run_2	Blank		R:A4	0.0100	Conditioning_Run_2
10		7	Unknown		R:A2	0.8000	7
11		Conditioning_Run_3	Blank		R:A4	0.0100	Conditioning_Run_3
12		8	Unknown		R:A2	0.8000	8
13		9	Unknown		R:A2	0.8000	9
14		10	Unknown		R:A2	0.8000	10
15		11	Unknown		R:A2	0.8000	11
16		12	Unknown		R:A2	0.8000	12
17		Conditioning_Run_4	Blank		R:A4	0.0100	Conditioning_Run_4
18		13	Unknown		R:A2	0.8000	13
19		Conditioning_Run_5	Blank		R:A4	0.0100	Conditioning_Run_5
20		14	Unknown		R:A2	0.8000	14
21		15	Unknown		R:A2	0.8000	15
22		16	Unknown		R:A2	0.8000	16

Figure 2. Example injection lists (A) screening and (B) optimization after the experimental design has been created in Fusion QbD and automatically created within the Chromeleon CDS as a ready-to-run sequence

Results and discussion

The AQbD approach involves the following general workflow steps, which are illustrated in Figure 3.

1. Define the Analytical Target Profile (ATP).
2. Perform Risk Analysis – select study variables and define study ranges.
3. Conduct a formal experimental design.
4. Model all significant variable effects on all included responses.
5. Determine best method conditions for mean performance and robustness.
6. Establish final method and Method Operable Design Region (MODR); experimentally validate predictions of final method performance and MODR.

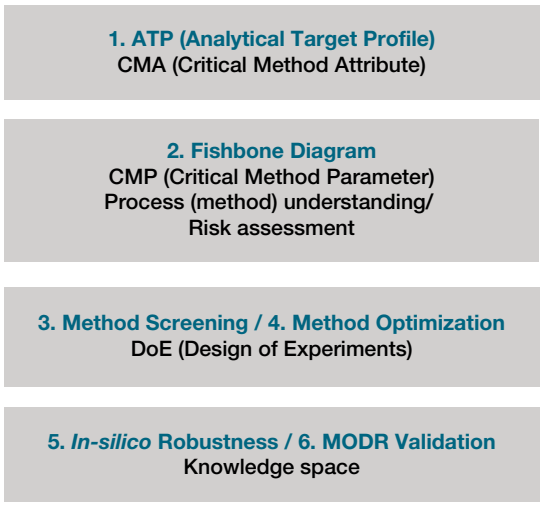


Figure 3. AQbD workflow steps

Analytical Target Profile

In a traditional method, the goal for precision of a drug substance is to quantify the API through a range of 98% to 102% of label claim in the presence of the related impurities so that the reportable value falls within ±1.00% of the true value with ≥90% probability and 95% confidence. In this application note, this quantitation goal has been translated into the following surrogate response goals for the method development effort.

The critical method performance characteristics, and the associated performance goals, specified for chemistry system screening and robust method optimization are as follows.

Screening goals:

a) Separate the API from the known impurities: Goal: $R_s \geq 1.50$

b) All peaks visualized: Goal = 6

c) Maximize the number of peaks with $R_s > 1.50$: Goal = 5

d) Minimize the asymmetry of all peaks: Goal ≤ 2.00

Optimization goals:

a) EP Resolution – Mean Performance: all peaks ≥ 2.00

b) EP Resolution – Robustness: C_{pk} for all critical pairs ≥ 1.33 (see Figure 11 for details)

c) Asymmetry: all peaks ≤ 1.20

d) API Area % RSD: ≤ 2.00

Critical Method Parameters

The fishbone diagram in Figure 4 contains the reduced set of method parameters selected for chemistry system screening and/or the robust method optimization experiments.

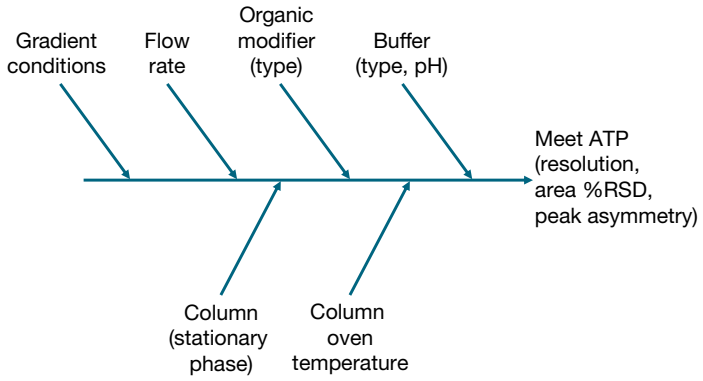


Figure 4. Fishbone diagram with studied method variables for the critical attributes

Design of Experiments (DoE)

The unique feature of Fusion QbD software is that it uses statistical sampling procedures to generate comprehensive and efficient experimental designs. In this way, a robust MODR can be predicted that takes into account several different parameters without measuring all possible study factor combinations. In Phase I - Screening - the most appropriate stationary phase, aqueous and organic solvents, pH range, and initial gradient conditions were determined by examining the main effectors of separation. To investigate all possible combinations of this large number of variables would require 200 analysis runs. However, the Fusion QbD statistical sampling protocol required only 54 runs, which were conducted completely unattended and without any user interaction.

Table 4 presents the results obtained for the screening goal responses. As the table shows, all previously defined screening design goals have been met or exceeded. Figure 5A is an example chromatogram from the current EU Monograph method[4]. Figure 5B is the example chromatogram obtained using the predicted best method conditions obtained from the screening experiment. It is noteworthy that the EP resolution of impurity C (peak 2) to impurity E (peak 1) has increased from the monograph method – it is 4.63 in the best method after screening compared to the 2.60 obtained for the EP method. In addition, the EP resolution of impurity B (peak 5) from impurity A (peak 4) as the critical pair is 1.73. Although this critical pair is baseline resolved (resolution is ≥1.50), the optimization goal will be to obtain a final method with mean performance (average result) of ≥2.00 and resolution robustness such that all individual results will be ≥1.50.

Table 4. Response goals achieved in Phase I - Screening

Response	Goal	Result
No. of peaks	6	6
No. of peaks ≥ 1.50 – Resolution	5	5
No. of peaks ≤ 1.20 – Asymmetry	6	6
API – Resolution	≥ 1.50	9.06

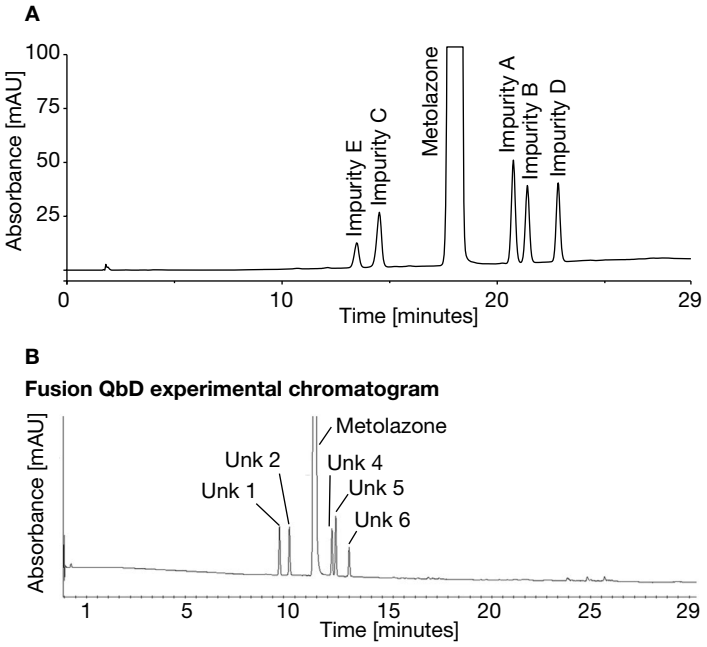


Figure 5. (A) EP Monograph method[4] chromatogram; (B) screening predicted best method chromatogram

Using a multi-factor DoE-based screening experiment enabled quantitative characterization of all interactions between column type, strong solvent type, and gradient time. The screening study showed that these interaction effects have the greatest influence on separation. Therefore, characterizing these effects enabled identification of the column type and organic solvent combination to use for the optimization study along with the workable regions of both pH and t_G. To illustrate, only methods using the Hypersil GOLD column were able to achieve all of the required screening goals, but only when used in combination with the methanol strong solvent, and only when both pH and t_G were at or near the upper end of their combined study ranges.

Three test runs were conducted at different initial organic start conditions (%B) after the screening experiment to see if the significant time between injection and elution of the first peak could be reduced. The results from these runs indicated the value of including the initial % strong solvent factor (gradient starting point %B) in the optimization experiment. Therefore, the Phase II - Optimization experiment was performed using the optimal combination of column and organic solvent derived from the screening experiment along with the initial % strong solvent factor, the workable ranges of pH and t_G and two additional optimization-level study factors: column oven temperature and pump flow rate. Fusion QbD generated a 32-run optimization experiment design for these five study factors.

The Fusion QbD retention models derived from the optimization experiment results are extremely precise – the overall prediction error across all peaks in all runs in this experiment was ±0.04 minutes (±2.4 seconds). These models, and the associated Fusion QbD models for peak width, were used to automatically calculate and predict resolution (R_s) for all sample peaks. In the data processing workflow, these models are first used to predict the mean (average) R_s of all peaks for all possible methods within the multi-factor experiment region. A numerical "Best Overall Answer" (BOA) search is then carried out to determine if one or more methods can meet the defined mean performance R_s goal – in this case an average R_s of ≥2.00 for all peaks, along with the specified goal for peak asymmetry (≤1.20 for all peaks). However, in this study the asymmetry results indicated that, apart from the API, all peaks were symmetrical. The API demonstrated minor fronting, with a mean result of 0.81, with no significant run-to-run variation (Std. Dev. = 0.04). All other peaks had average asymmetry results of 1.00, with again very small run-to-run variations (Std. Dev. = 0.02 for four peaks and 0.10 for one peak). Therefore, it was not necessary to include peak asymmetry goals in the final optimization search.

The BOA search identified a method within the experimental region that would achieve the defined goal: average R_s of ≥2.00 for all peaks. An overlay of the predicted and observed chromatograms for optimization Run 17, the run with conditions closest to those defined in the BOA method, are shown in Figure 6. The predicted and observed retention times corresponding to Run 17 can be found in Table 5. The BOA method is considered a valid starting point for the final method optimization search, which includes quantitative Monte-Carlo robustness simulation.

The resolution maps (2D and 3D) in Figure 7 were generated using pH 5.50 (BOA method), oven temperature 40 °C (BOA method), but with a pump flow rate of 0.500 mL/min (adjusted from the BOA method of 0.560 mL/min). It can be concluded from Figure 7, that a gradient time of 30 minutes together with an initial % strong solvent composition of 25% results in an optimal resolution for the critical peak pair. Note that the upper bounds of the variable study ranges have been extended to 32% and 32 minutes for the X-axis and Y-axis graphed variables, respectively, to make it easier to see the graph crosshair which is positioned at the method setpoints for the graphed variables. Figure 8 presents the predicted (simulated) chromatogram generated by Fusion QbD for these new optimal conditions.

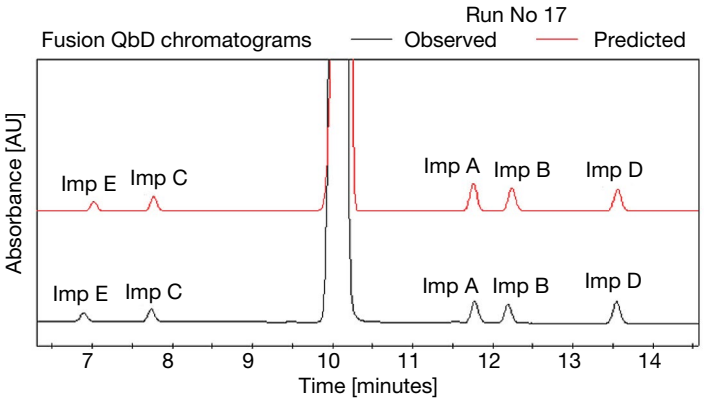


Figure 6. Overlay of predicted (red) and observed (black) chromatograms of Run 17 – the run closest to the BOA conditions. BOA conditions are as follows: flow rate: 0.560 mL/min, gradient time: 30 minutes, initial % strong solvent: 22.5%, oven temperature: 40 °C, pH (aqueous solvent): 5.50. Run 17 conditions are the same as BOA, except the flow rate which is 0.500 mL/min.

Table 5. Comparison of predicted and observed retention times for the analyte peaks in Run 17

Response name	Predicted result	Observed result
Imp E – retention time	7.02	6.90
Imp C – retention time	7.77	7.74
API – retention time	10.13	10.11
Imp A – retention time	11.76	11.78
Imp B – retention time	12.24	12.19
Imp D – retention time	13.56	13.55

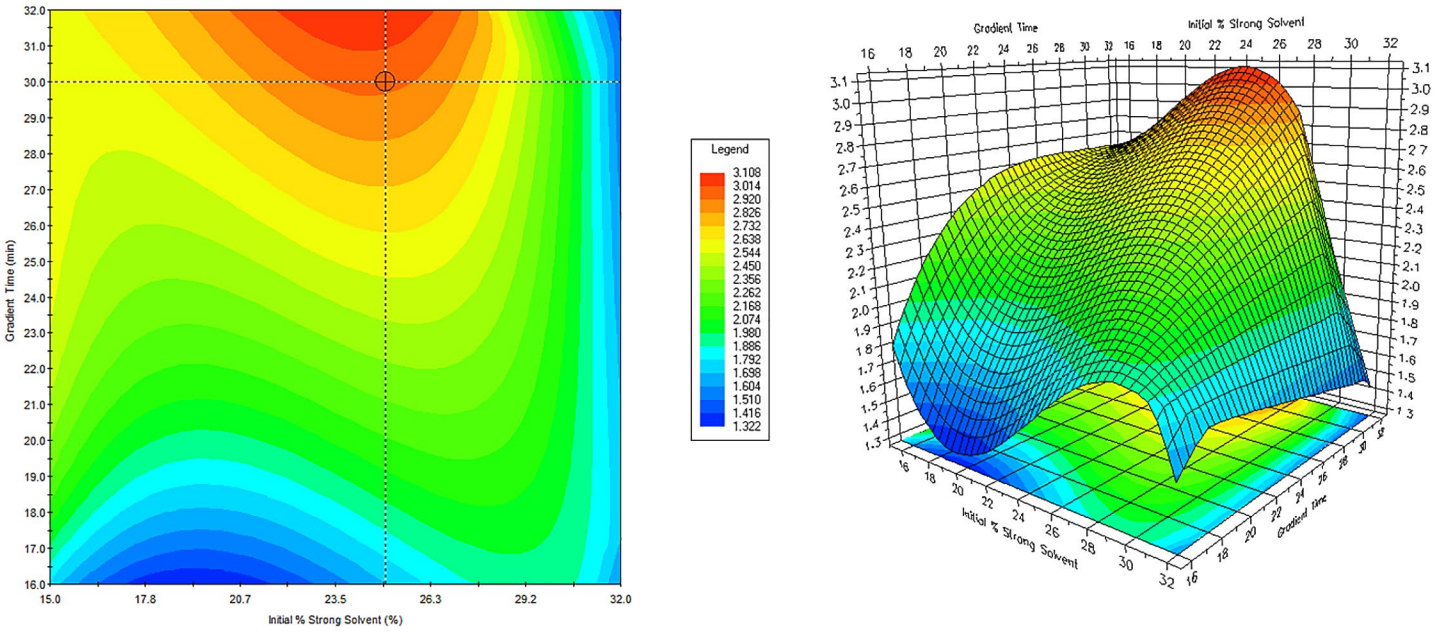


Figure 7. Resolution map – 2D contour and 3D overlay for the critical peak pair (here always impurities A and B). The color represents the resolution.

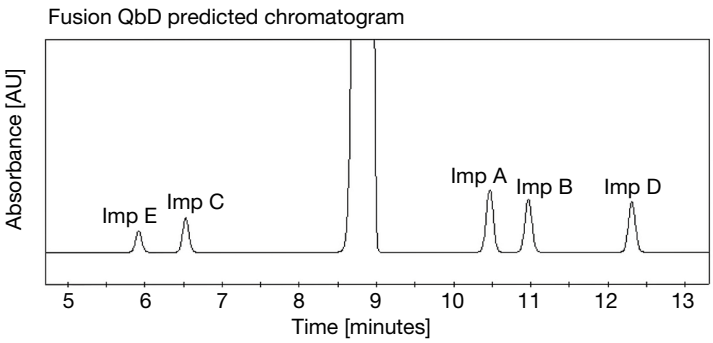


Figure 8. Simulated chromatogram corresponding to new optimum condition with flow rate of 0.500 mL/min, initial % strong solvent of 25%, pH of aqueous mobile phase of 5.50, a gradient time of 30 minutes, and an oven temperature of 40 °C

Robustness and Method Operable Design Region (MODR)

It should be understood that the prediction models obtained from all chromatography modeling software can only directly predict the mean performance of a given method for a given modeled critical method performance characteristic. The models cannot directly predict method performance variation due to fluctuating method parameters (i.e., method robustness). To put this in real-world terms, consider that system suitability testing normally yields two results for each tested performance characteristic: the mean (average) result of the repeated injections and the variation of the individual injections about the mean result – usually reported as the % RSD. However, models that can predict both components of system suitability – mean performance and robustness – are required to establish a true, robust MODR. In addition, the robustness simulation modeling must be able to reflect the setpoint variations in the critical parameters beyond the single LC system used for the method´s development to the real-world variations that the method will experience in a QC lab over time (i.e., different days, LC systems, labs, personnel).

Fusion QbD has built-in Monte Carlo Robustness Simulation, which uniquely enables it to simultaneously predict both mean performance and performance variation, i.e., method robustness, for all included critical method performance characteristics across the entire multi-factor experimental region. To do this you only need to define 1) the maximum variation in each included critical method parameter expected across instruments, personnel, days, etc., on transfer and normal use in a QC lab (worst case scenario), and 2) the performance specification limits for the included critical method performance characteristics. Fusion QbD can then automatically generate graphs that show the MODR, including the performance boundaries (edges of failure) in terms of both mean performance and robustness for all performance characteristics.

Figure 9 presents the maximum expected variations in the study parameters used in the robustness simulation. It should be understood that these settings do not represent the precision of the Vanquish LC system on which the experiments were run. As stated, these variation range settings have been broadened to represent the possible variation on transfer and normal use across instruments and personnel in a QC lab over time. In particular, the expected variation in oven temperature is set higher because the actual column temperature has a greater impact compared to the effect of pre-heater or column compartment temperature. However, the column temperature itself is difficult to determine or control. Furthermore, it is important to note that variation in gradient time actually represents slope variation, which is in turn variation in mobile phase composition due to pump precision. This is why, as seen in Figure 9, Fusion QbD has automatically converted the gradient time study factor into mobile phase composition for the robustness simulation setup. Users can therefore input the expected pump precision variation directly, and Fusion QbD will automatically do the necessary conversions for robustness modeling.

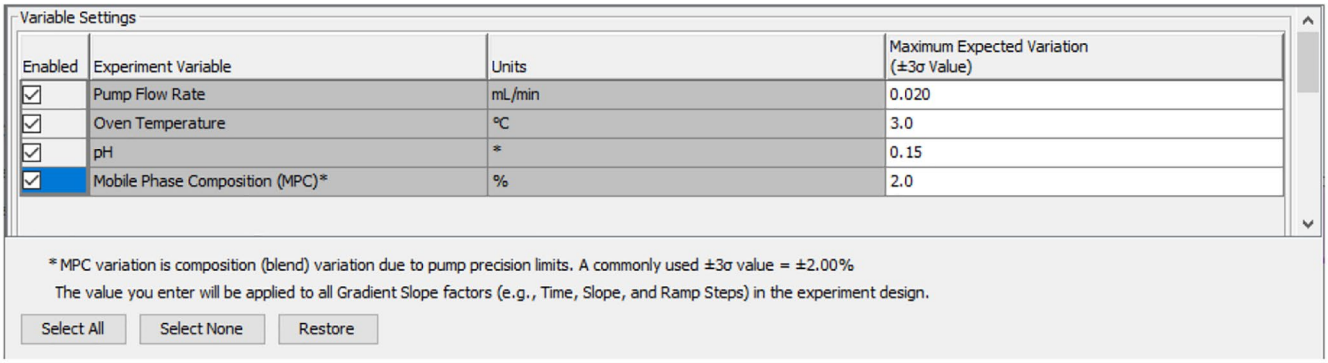


Figure 9. Study variable settings for robustness simulator

The overlay graphs presented in Figure 10 illustrate the difference between a mean performance only solution and a true robust method development solution. In these graphs, each included critical method performance characteristic is assigned a color, and the region(s) in the graph shaded with that color identify method conditions which fail to meet the defined performance requirements for that characteristic. The performance characteristics included in the left graph – Mean Performance Only – include the mean R_s performance of the impurity C–E critical pair, which is assigned the red color, and the mean R_s performance of the impurity B–A critical pair, which is assigned the blue color (for color coding refer to Figure 10 – tables below the graphs). In both graphs the mean R_s performance requirement for each critical pair has been set to ≥2.00, and both graphs correspond to the non-graphed variable settings of 40 °C for column oven temperature, 0.500 mL/min for flow rate, and 5.50 for pH. The left graph indicates that the target method (gradient time = 30.0 minutes, 25.0% initial % strong solvent) will meet or exceed the mean R_s performance requirement for both critical pairs at the target setpoint conditions of pump flow rate, oven temperature, and pH. The graph also indicates that mean performance will be maintained within the combined ranges of 21.0–30.0 minutes for gradient time and 15.0–30.0% for initial % strong solvent.

However, in addition to the mean performance R_s requirements, there are also R_s robustness performance requirements for the two critical pairs, as well as a % RSD robustness requirement for the API area response. The Robustness Simulator within Fusion QbD is used to automatically model the variation in method performance for each included performance characteristic resulting from simultaneous variations in the method parameters entered by the user in the setup dialog shown in Figure 9. The integrated Monte Carlo simulation then characterizes the robustness of thousands of methods within the experimental region for each response in terms of the user-specified metric for the response. In this case, we selected the C_{pk} metric for the R_s responses and the % RSD metric for the Area. The C_{pk} metric is illustrated in Figure 11.

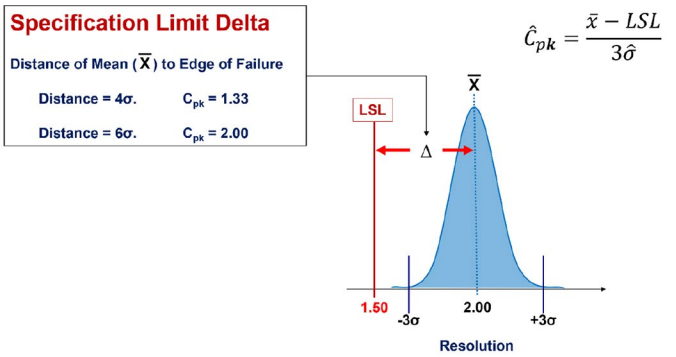
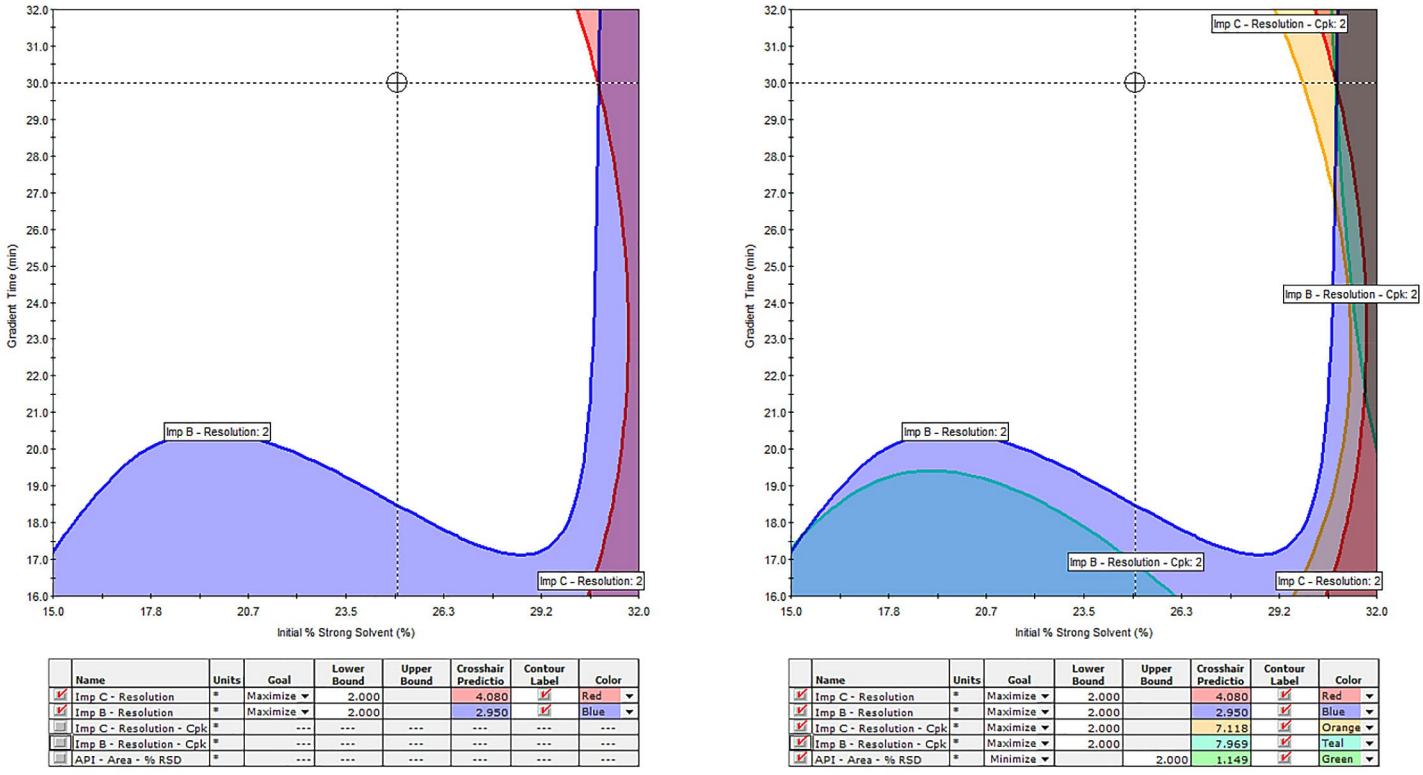


Figure 11. C_{pk} Robustness – Lower Specification Limit



Name	Units	Goal	Lower Bound	Upper Bound	Crosshair Predictio	Contour Label	Color
Imp C - Resolution	*	Maximize	2.000		4.080	☑	Red
Imp B - Resolution	*	Maximize	2.000		2.950	☑	Blue
Imp C - Resolution - Cpk	*	---	---	---	---	---	---
Imp B - Resolution - Cpk	*	---	---	---	---	---	---
API - Area - % RSD	*	---	---	---	---	---	---

Name	Units	Goal	Lower Bound	Upper Bound	Crosshair Predictio	Contour Label	Color
Imp C - Resolution	*	Maximize	2.000		4.080	☑	Red
Imp B - Resolution	*	Maximize	2.000		2.950	☑	Blue
Imp C - Resolution - Cpk	*	Maximize	2.000		7.118	☑	Orange
Imp B - Resolution - Cpk	*	Maximize	2.000		7.969	☑	Teal
API - Area - % RSD	*	Minimize		2.000	1.149	☑	Green

Figure 10. Overlay graphs – Mean Performance Only (left) and Mean Performance + Robustness (right). Color shaded regions identify method conditions that fail to meet the defined performance requirements for that characteristic.

As Figure 11 shows, the C_{pk} metric is equal to the distance of the predicted mean performance for the response to the specification limit (the acceptable performance threshold) divided by the predicted 3σ variation in individual results due to the specified simultaneous variations in the method parameters.

The right graph – MODR: Mean Performance + Robustness – in Figure 10 therefore also includes the associated R_s robustness requirement for each critical pair that no individual injection generated in the QC environment over time will have a critical pair R_s result of ≤1.50 (orange color for the impurity C–E critical pair and teal color for the impurity B–A critical pair). The C_{pk} robustness metric applied to these requirements has a minimum threshold value of 1.33, which is the traditional goal for a robust process. However, due to the excellent R_s robustness performance achieved for this method, we increased the minimum C_{pk} requirement to ≥2.00, which corresponds to a six-sigma method for resolution of these critical pairs. The graph on the right also includes the robustness requirement for the API area response that no suitability injection series will provide a % RSD result of >2.00 (green color). Furthermore, it indicates that the target method (gradient time = 30.0 minutes, 25.0% initial % strong solvent) will also have excellent robustness performance in terms of the resolution of the critical pairs and the % RSD of the API at the target setpoint conditions of pump flow rate, oven temperature, and pH. The graph also indicates that the robustness performance will also be maintained within the combined ranges of 21.0–30.0 minutes for gradient time and 15.0–30.0% for initial % strong solvent.

It is important to note that, in the absence of robustness simulation analysis, any robustness issues with the target method, or with the proposed operable ranges, would not be visualized and understood. In this case these issues could also go undetected during validation, and only show up after transfer. Conversely, the robustness simulation analysis supports a risk-based approach, and provides a high level of confidence that the developed method will not only pass validation, but will perform according to requirements in a QC setting over time.

In addition to individual 2D overlay graphs that can contain multiple mean performance and robustness responses, Fusion QbD can generate 3D and 4D overlay trellis graphics of these responses. Figure 12 presents a 4D overlay trellis graph series in which each of the nine individual graphs contains the same graphed variables: X-axis = Initial % strong solvent, Y-axis = Gradient time. Columns A, B, and C represent the 3rd dimension of this trellis, here corresponding to oven temperature levels of 35.0, 40.0, and 45.0 °C, respectively, while rows 1, 2, and 3 represent the 4th dimension of this trellis, here corresponding to pump flow rate levels of 0.450 mL/min, 0.500 mL/min, and 0.550 mL/min, respectively. The trellis graph shows at a glance how the 2D MODR of the graphed variables changes at different level setting combinations of a 3rd and 4th study variable. It is clear that the robust MODR of the final method (temp = 40.0 °C, flow rate = 0.500 mL/min), represented by the unshaded region, will be maintained across a broader range of temperature and flow rate level setting combinations than is expected on transfer and normal use in a QC lab.

As previously mentioned, the peak asymmetry results data were extremely consistent across all experiment runs. For example, the mean asymmetry result for each peak other than the API was 1.00, with the largest corresponding standard deviation of 0.10. The mean asymmetry result for the API across all experiment runs was 0.81, with a corresponding standard deviation of 0.03. Therefore, the peak asymmetry results were not included in either the mean performance or the robustness optimization searches.

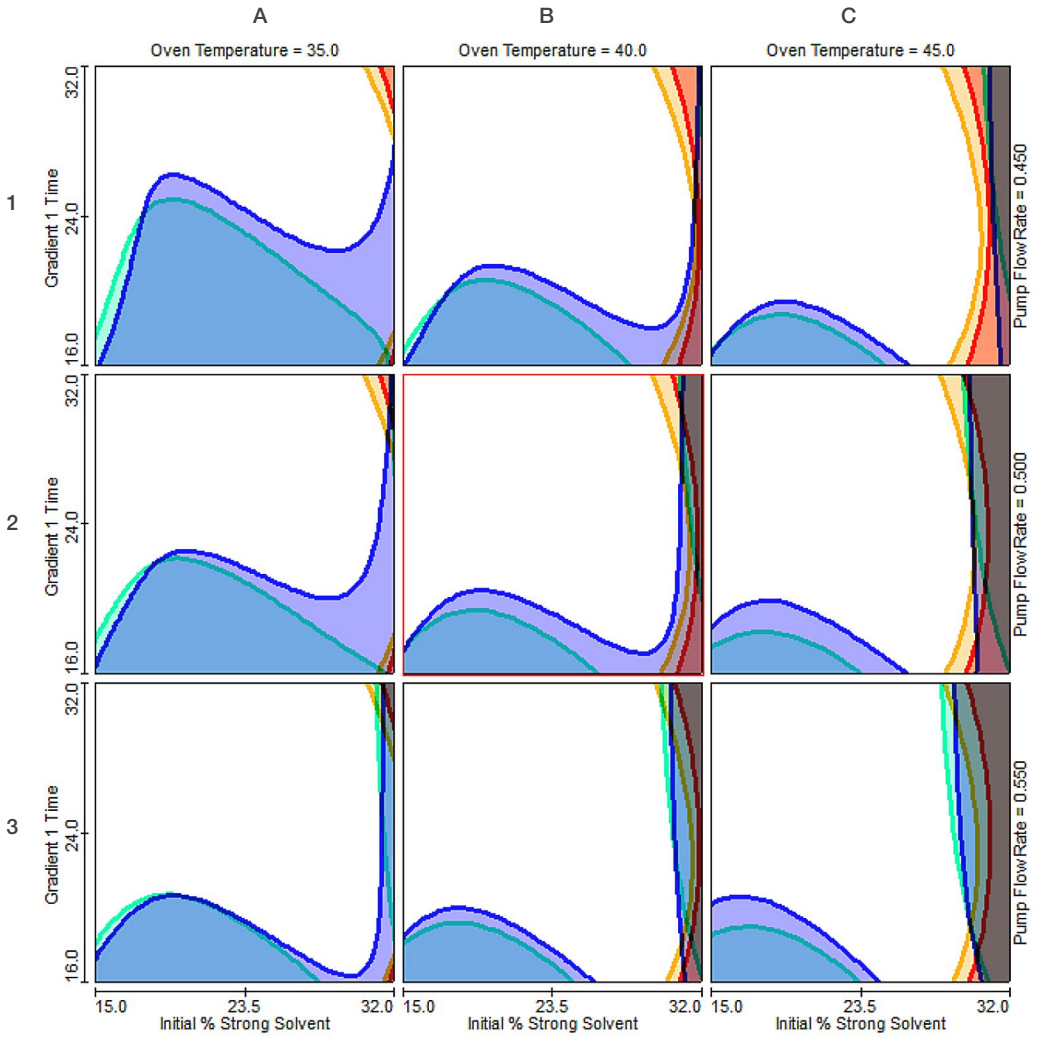
Method efficiency

The final method run time, referred to here as the efficiency of the method, can be addressed once we have achieved robust method performance for all critical requirements. We can easily do this by translating the predicted optimal run conditions of initial % strong solvent (25%), gradient time (30.0 min), and constant final % strong solvent of 70.0% into a gradient slope of 1.50%/min [(70.0% – 25%)/30.0 min = 1.50%/min]. Since the last peak elutes at 12.3 minutes (Figure 8), which corresponds to ~42.0% strong solvent, we could use the final method conditions of 25.0% for initial % strong solvent, the gradient slope of 1.50%/min, and a new final % strong solvent of 47.5% to define a new gradient time of 15 minutes. This maintains the slope of 1.50%/min without stopping the run too soon after the elution of the last peak. Therefore, the new minimum run time would be 22.0 minutes according to the final method pump program presented in Table 3.

Final method performance verification

Verification of final method performance at the new efficient conditions was obtained by running a SST using the method identified by Fusion QbD optimization and robustness simulation. For these runs, a new column was used and fresh mobile phase and sample were prepared. The sample was injected six times consecutively. The resulting 6-injection overlay chromatogram is shown in Figure 13, annotated with the critical method performance results, which demonstrate the excellent mean performance and repeatability (robustness) of the final method.

The optimized method resulted in a faster total run time than the method described in the EP Monograph method, which is now 22 minutes versus 48 minutes for the EP method. This represents a 54% reduction in overall run time relative to the current EP method. In addition, the resolutions between impurities E & C and impurities A & B are significantly improved.



	Name	Units	Goal	Lower Bound	Upper Bound	Color
☑	Imp C - Resolution	*	Maximize	2.000		Red
☑	Imp B - Resolution	*	Maximize	2.000		Blue
☑	Imp C - Resolution - Cpk	*	Maximize	2.000		Orange
☑	Imp B - Resolution - Cpk	*	Maximize	2.000		Teal
☑	API - Area - % RSD	*	Minimize		2.000	Green

Figure 12. Four parameter robust method operable design region

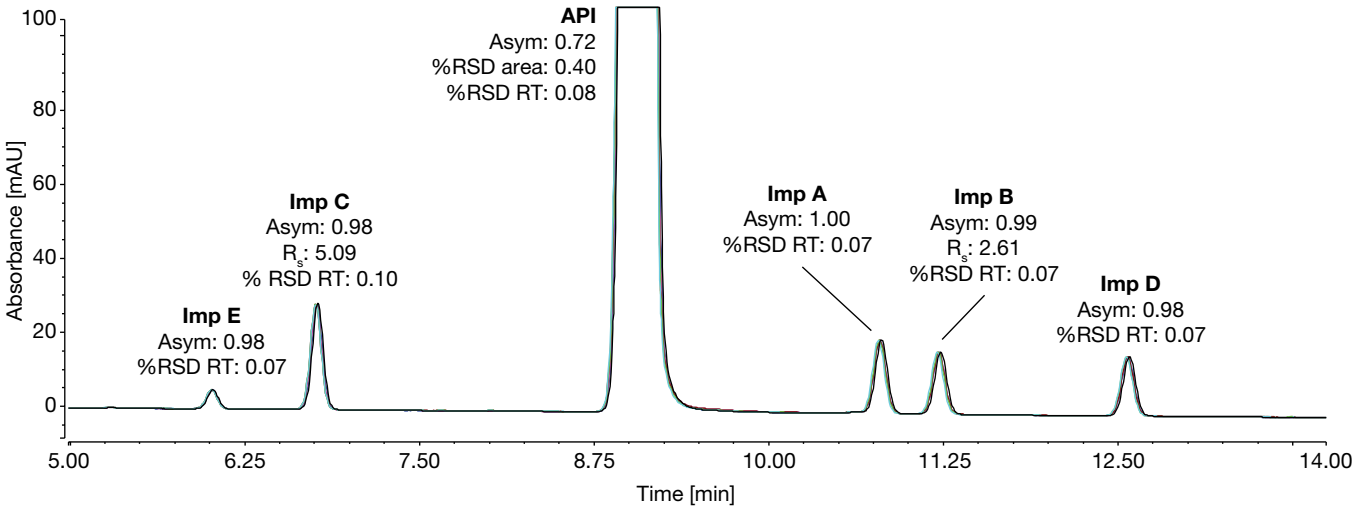


Figure 13. Overlay of six consecutive injections for a SST sample on the final method after optimization and robustness simulation

Conclusion

Thermo Scientific Vanquish UHPLC Method Development system in combination with Chromeleon CDS and Fusion QbD software enabled rapid, successful modernization of the current EP monograph method for metolazone and related impurities. The seamless connectivity between the Chromeleon CDS and Fusion QbD, which included full QbD experiment automation support, enabled quick execution of a best practices AQbD approach to method development. As the results below present, the project yielded a fast and robust final method for separation of metolazone and five related known impurities which met all the project's method performance goals:

- Critical peak pair resolutions:
  - Development goal: ≥ 2.00
  - Final method result: ≥ 2.61
- Area % RSD of all peaks:
  - Development goal: ≤ 2.00
  - Final method result: ≤ 0.40
- Peak asymmetry of all peaks:
  - Development goal: ≤ 1.20
  - Final method result: close to 1.00 for impurity peaks and 0.81 for the API

Development of a fit-for-purpose robust method makes good business sense for three important reasons. First, it minimizes the time, effort, and cost associated with a method that fails on validation or transfer, and so must be re-developed. Second, it minimizes or eliminates out-of-specification (OOS) results, and therefore the extremely costly and time-consuming activities associated with OOS investigations. Third, and most importantly, it provides accurate and precise results that support both good business decisions and patient health and safety.[5,6]

Acknowledgement

The authors are very thankful to Ingo Green for the familiarization of Fusion QbD software and his great support during the study.

References:

1. Snyder, Kirkland, and Dolan. (2010). *Introduction to Modern Liquid Chromatography,* 3rd Edition; John Wiley & Sons, Inc., Hoboken, New Jersey
2. Fusion QbD Software Platform: Fusion QbD Software - Quality by Design - DOE - Automated Data Analysis - Chromatography Modeling Software (smatrix.com)
3. European Directorate for the Quality of Medicines & HealthCare; European Pharmacopoeia (Ph. Eur.); 7, Allée Kastner CS 30026, F-67081 Strasbourg (France).
4. European Directorate for the Quality of Medicines & HealthCare; European Pharmacopoeia (Ph. Eur.) Online, 9th edition 2018 (9.0), monograph 1757: Metolazone.
5. United States Pharmacopeia, chapter <1220>, Analytical Life Cycle.
6. International Conference on Harmonization (ICH), Q14, Analytical Procedure Development.

Learn more at **thermofisher.com/vanquish**

AN001631-EN 0123S