QbD Based Method Development on an Agilent 1290 Infinity UHPLC System Combined with a Seamless Method Transfer to HPLC Using Intelligent System Emulation Technology

Application Note

Pharmaceutical QA/QC

Author

Vinayak A.K
Agilent Technologies
Bangalore, India

Abstract

In this Application Note, a method was developed on sub-2 µm particle columns and transferred to a conventional HPLC method using Quality by Design (QbD) principles. A seamless method transfer between the different chromatography systems was achieved using the Agilent Intelligent System Emulation Technology (ISET). Initial method development work on the Agilent Infinity 1290 system included automated chemistry screening of different sub-2 µm particle columns under different chromatographic conditions involving multiple combinations of eluents, flow rates, gradient slopes, and temperatures. Subsequent optimization of the best performing chemistry system was carried out using a Design of Experiments (DOE) approach to establish the ICH Design Space. The Agilent Method Translator was used for the transfer from sub-2 µm column material to conventional column material. An Agilent 1290 Infinity UHPLC system was used in combination with ISET to emulate an Agilent 1260 Infinity HPLC system. This enabled establishing a Design Space for the HPLC method. Reproducibility and resolution of the transferred method was then verified on an Agilent 1260 Infinity HPLC system. This successfully demonstrates that ISET in combination with Fusion QbD Method Development and Validation Software (S-Matrix) enables an efficient transfer of UHPLC methods to HPLC methods within the QbD approach.

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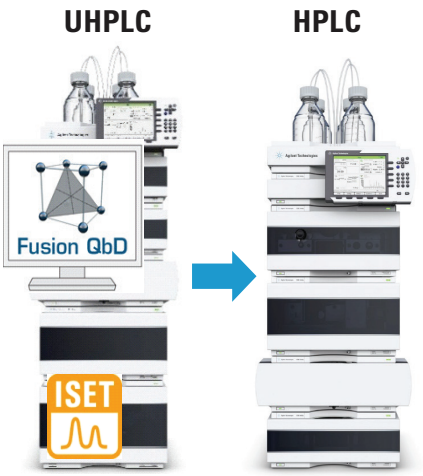

Introduction

Quality by Design (QbD) based method development and validation aligned with the ICH Q8 (R2) and ICH Q2 (R2) guidances is now a hot topic in the analytical R&D community within the pharmaceutical industry. In the QbD approach, Critical Method Attributes (CMAs) are established, and the effects of interactions between Critical Method Parameters (CMPs) on these CMAs are characterized using statistical multivariate analysis and modeling. The goal of QbD-aligned LC method development is establishing a Robust Design Space, defined as the multidimensional (joint) operating ranges of the CMPs in which changes to method parameters will not cause an unacceptable result in one or more CMAs. Design of Experiments (DOE), experiment automation and multivariate analysis can be used to establish such a robust design space. This leads to reductions of method failures in the field, and fewer out-of-specification studies[1]. This approach incorporates robustness to the method during method development.

Method development, validation, and transfer to QA/QC laboratories is routinely performed in the pharmaceutical industry[2]. Chemistry screening and formal method development using conventional, long HPLC methods under QbD principles are time-consuming processes.

In chemistry screening, efficiency can be dramatically increased using UHPLC methods on short, sub-2 µm columns[3]. However, the final method may need to be transferred to QA/QC departments where the majority of the LC systems are HPLC systems. Transferring a method from UHPLC to HPLC without compromising the CMAs is a challenging process. A method developed on a UHPLC system, even when done using conventional columns, may not provide the same method performance upon transfer to an HPLC system due to differences in system delay volumes and gradient mixing precision. To overcome these issues, ISET has been applied, emulating the properties of the target system during the method development process.

Experimental

Instrumentation

An Agilent 1290 Infinity LC Method Development system consisting of the following modules and components:

- Agilent 1290 Infinity Binary Pump (G4220A)
- 1290 Infinity Valve Drive (G1170A) and 12-position/13-port solvent selection valve (G4235A)
- Agilent 1290 Infinity Autosampler (G4226A) maintained at 4 °C using a thermostat (G1330B)
- Agilent 1290 Infinity TCC (G1316C) cluster with 8 position/9 port valve (G4230B)
- Solvent Selection Tubing Kit for four solvent (p/n 5067-4601)
- Agilent 1290 Infinity DAD (G4212A)

An Agilent 1260 Infinity system was used to verify the reproducibility of transferred method. The system consisted of the following modules:

- Agilent 1260 Infinity Binary Pump (G1312B)
- Agilent 1260 Infinity Autosampler (G1367E)
- Agilent 1260 Infinity TCC (G1316A)
- Agilent 1260 Infinity DAD (G4212B)

Software

- Fusion QbD Automated LC Method Development Software (S-Matrix) (Version: 9.6.22, Build 42)
- Agilent OpenLAB CDS ChemStation Edition Workstation (C.01.05, [38])
- ISET 3 (driver version A.02.09)

Reagents and samples

All solvents were HPLC grade (RCI Labscan Ltd, Thailand). Linagliptin formulation was purchased from a local drug store, and hydrogen peroxide was purchased from a local supplier (Bangalore, India).

Degradation procedure

Linagliptin formulated tablets were crushed and weighed 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. The solution was then placed 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.

Workflow

The method development workflow began with screening six short sub-2 µm columns in combination with two organic solvents and five different levels of pH (aqueous solvents). This chemistry system screening experiment was carried out on an Agilent 1290 Infinity LC Method Development system using the Fusion QbD Software (Figure 1). The chromatographic conditions found to be best after the initial screening phase were then further optimized to achieve a robust UHPLC method and establish the robust design space for the method[3].

The newly created UHPLC method was then transferred into an HPLC method using the Agilent Method Translator. For all further steps, the 1290 Infinity system was operated in emulation mode as a 1260 Infinity system by ISET. The transferred method was optimized using a DOE experiment design, which included temperature, gradient slope, and pH. After optimizing the HPLC method, the resulting design space was compared with the previously generated UHPLC design space. The Proven Acceptable Ranges (PARs) within the design space were verified in both systems. The final HPLC method was then run on an Agilent 1260 Infinity HPLC system, and its performance was verified for reproducibility of API area, RT, and resolution.

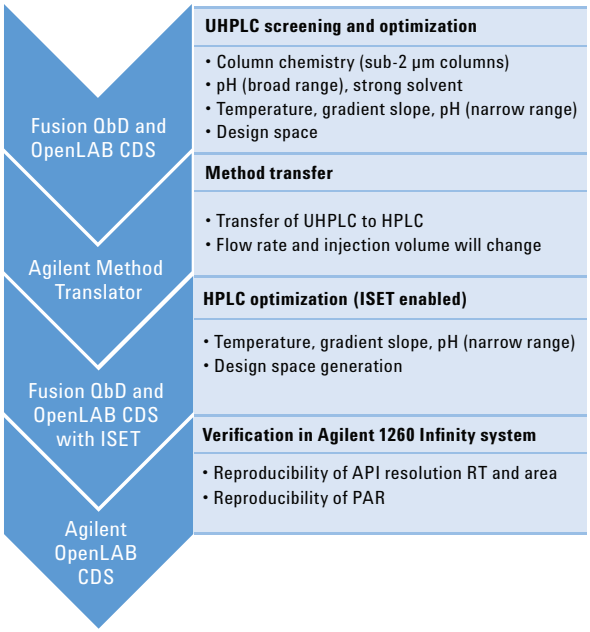

Figure 1. Overall workflow used for the study. Software packages used are shown on left side of the flow chart, while detailed steps of the workflow are shown on the right side.

Results and Discussion

UHPLC screening and optimization

In this step, multiple column chemistries, a broad range of pH, and organic solvents (ACN and MeOH) were screened using fast methods on sub-2 µm columns. The chemistry system screening experiment identified the best overall chromatographic conditions as an Agilent ZORBAX RRHD Eclipse Plus C8 column, pH 7, and a gradient time of 10 minutes. Additional chromatographic parameters such as gradient endpoint percent strong solvent, a narrower range of pH, and column temperature were combined in a DOE-based method optimization experiment. The best overall answer of optimization conditions were pH 7.7, 45 °C, a final percent strong solvent of 90.5 %, and a gradient time of 15 minutes. The design space for these CMPs was established around this method. The Point Prediction feature within Fusion QbD was used to automate execution of the verification runs on the LC. Further details of this study are described in an earlier publication[3].

Method transfer using Agilent Method Translator

The fast UHPLC method was transferred to an HPLC method using the Agilent Method Translator software. The new HPLC parameters are tabulated in Table 1. The method translator predicts a new gradient time, injection volume, and flow rate, which are scaled up according to the HPLC column dimensions. QbD principles were then applied by applying a DOE-based optimization experiment to this translated method.

Table 1. UHPLC and transferred HPLC method parameters.

Parameter	UHPLC		HPLC	
Flow rate	0.6 mL/min		1.4 mL/min	
Column	Agilent ZORBAX Eclipse Plus C8, 3.0 × 50 mm, 1.8 µm		Agilent ZORBAX Eclipse Plus C8, 4.6 × 150 mm, 3.5 µm	
Injection volume	1 µL		5 µL	
Gradient	Time	%B	Time	%B
	0	5	0	5
	0.1	5	0.21	5
	15.1	90.5	45.3	90.5
	16.3	95	48.9	95
	16.4	5	49.2	5
	18.4	5	55.2	5

HPLC method optimization (ISET enabled)

The 1290 Infinity system was operated in emulation mode as a 1260 Infinity system. The base HPLC method obtained from the method translator was further optimized using a DOE-based experiment design. The CMPs were varied, and the HPLC design space was established in terms of the previously defined CMAs (Table 2). Figure 2 shows graphs of the joint experimental ranges of pH and final % organic used in the optimization experiments, the left graph for the UHPLC and the right for the HPLC. In these graphs, each CMA is associated with a color, and the part of the graph region shaded with that color corresponds to methods that fail to meet the performance requirements for that CMA. The design space in Figure 2 corresponds to the unshaded region (the white space) where performance requirements are met for all CMAs. Note that the design space is larger for the HPLC method relative to the UHPLC method. This shows the overall improvement in method performance resulting from the HPLC method optimization. The expanded safe operating ranges for the CMAs in the HPLC method enable the imposition of more stringent method performance requirements. This helps to improve the chromatographic performance without compromising the robustness of the method. For example, as Table 3 shows, we can now require API resolution of > 4.0 (previously > 1.5), and peak tailing of < 1.3 (previously < 1.5).

Table 2. Critical Method Attributes and other parameters used in the UHPLC design space.

Critical Method Parameters (CMPs)	Value	Proven Acceptable Ranges (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		
Optimized gradient	Time %B 0 5 0.1 5 15.1 90.5 16.3 95 16.4 5 18.4 5		

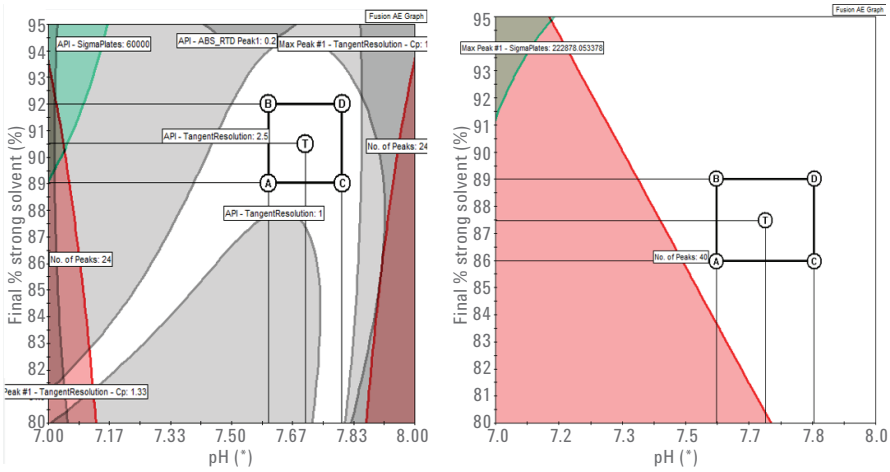

Figure 2. UHPLC design space and HPLC design space with the same CMA performance requirements are plotted. The design space (shown as the unshaded region within the graphs, the white space), is expanded for the HPLC relative to the UHPLC.

New design space graphs were generated in Fusion QbD with more stringent CMA performance requirements (Figure 3). The PARs for pH and final % organic are illustrated as rectangles drawn within the design space graphs in Figure 3. The four corner points and center point of each PAR rectangle represent methods that can be run to experimentally verify the PARs. The verification runs shown in the center graph (temperature = 37 °C) were carried out, and the CMA performance requirements were met in all these verification method runs. The chromatograms corresponding to these verification runs are shown in Figure 4. The chromatograms shows extremely small variations in resolution and retention time of the critical peak pairs when keeping the CMPs within the design space, indicating that the method is highly stable.

Table 3. Critical Method Attributes and other parameters used in modified HPLC design space. The optimized gradient for the HPLC method is also shown.

Critical Method Parameters (CMPs)	Value	Proven Acceptable Ranges (PARs)	Critical Method Attributes (CMAs)
Column	Agilent ZORBAX Eclipse Plus C8, 4.6 × 150 mm, 3.5 µm		No. of peaks > 40 API resolution > 4.0 Peak purity ≥ 98 % Peak tailing < 1.3
Strong solvent	Methanol		
% Strong solvent	87.5 %	± 1.5 %	
Aqueous solvent pH	7.7	± 0.1	
Gradient range	5 % to 87.5 %		
Oven temperature	37 °C	± 4 %	
Gradient time	45 minutes		
Flow rate	1.4 mL/min		
Wavelength	292 nm		
Optimized gradient	Time %B 0.3 5 45.6 87.5 49.2 87.5 49.5 95 49.8 95 50.1 5 53.1 5		

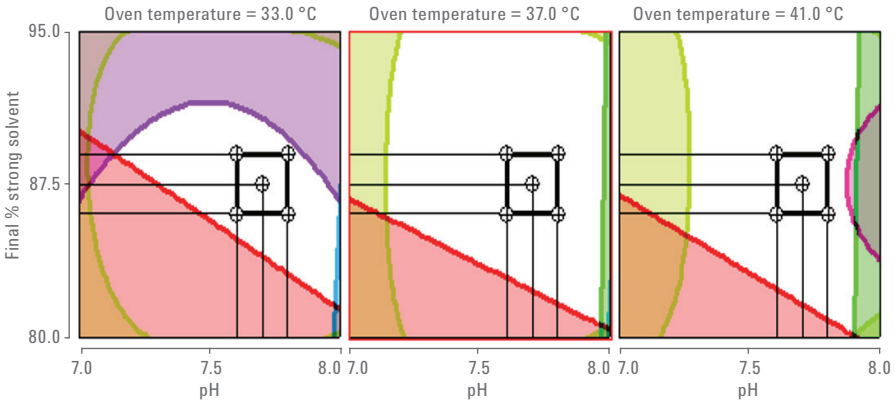

Figure 3. The HPLC design space in all three graphs reveals that temperature can be varied from 33 °C to 41 °C. The graphs reflect more stringent method performance requirements (CMAs). The rectangles drawn in the graphed design spaces show the PARs of the graph variables across the PAR for temperature. The four corner points and center point of each PAR rectangle are methods that can be run to experimentally verify the PARs. In this case, the temperature range was not incorporated into the PAR verification.

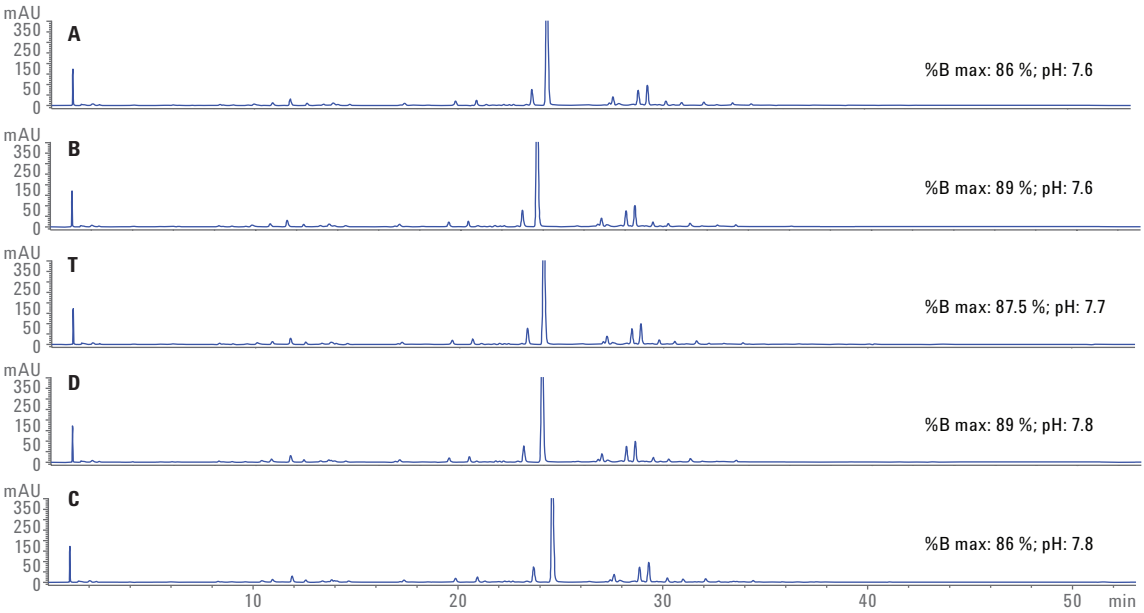

Figure 4. Proven acceptable range (PAR) verification chromatograms. The PAR rectangle contains four corner points (A, B, D, and C) and the center point (T) corresponding to the method conditions generating the chromatograms. The CMA performance requirements (API Rs > 4 and API tailing < 1.3) was met in all chromatograms.

Verification on an Agilent 1260 Infinity system

The optimized HPLC method was replicated on a 1260 Infinity system. The differences in API retention time (RT) and resolution between the two chromatograms were within the acceptance limits. (Figure 5). The reproducibility of method was verified by six replicates, and the RSDs of API area, RT, and resolution were all within acceptance limits (Figure 6). The PARs were replicated in the 1260 Infinity system to confirm whether the CMA performance requirements were met. CMA performance requirements were all met, and were extremely similar to the emulated 1260 Infinity results (Table 4).

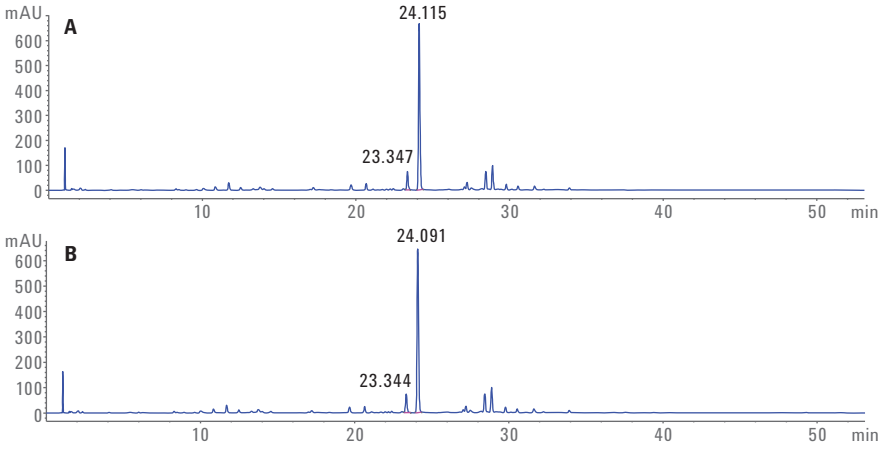

Figure 5. Chromatograms showing the optimized method in the Agilent 1290 Infinity system emulated as an Agilent 1260 Infinity system (A) and in the Agilent 1260 Infinity system (B). The percent deviations of API Resolution and RT were 4.2 and 0.1, respectively, which were within the acceptance deviation limits.

Advantage of using sub-2 um columns in screening

The total time required for the screening and optimization experiments is shown in Table 5. The UHPLC optimization step could be skipped if the conventional long columns were used in the screening experiment, but a screening phase with conventional columns will have long run times and large solvent consumption. Hence, short sub-2 µm columns are the best choice for chemistry system screening. An extra HPLC optimization step would be required in a workflow with sub-2 µm column screening, but there will still be a reduction of 19 hours in method development experiment run time.

Conclusion

An efficient method development process has been demonstrated using an Agilent 1290 Infinity Method Development system, short sub-2 µm columns in chemistry system screening, and the application of QbD principles with the Fusion QbD Software Platform. The final UHPLC method was then transferred to a conventional HPLC system and column. The method was then optimized for CMA performance and robustness, first using the 1290 Infinity UHPLC system in ISET emulation mode, and next on an Agilent 1260 Infinity system. Nineteen hours could be saved by using sub-2 µm columns in the screening step.

The final realized design space was expanded in the HPLC method compared to the UHPLC method while achieving improved performance for all CMAs. This shows that the HPLC method is more robust to variations in CMPs. API resolution and tailing of > 4 and < 1.3, respectively, were achieved in the final optimized HPLC method. Replicate runs showed % RSD values for API area and RT of ≤ 1.5 %, and a % RSD value for API resolution of ≤ 2 %. The final method was transferred for verification to an Agilent 1260 Infinity routine QA/QC system. The results showed extremely reproducible performance. The percent deviation of API resolution and RT between the two systems was found to be well within allowed limits.

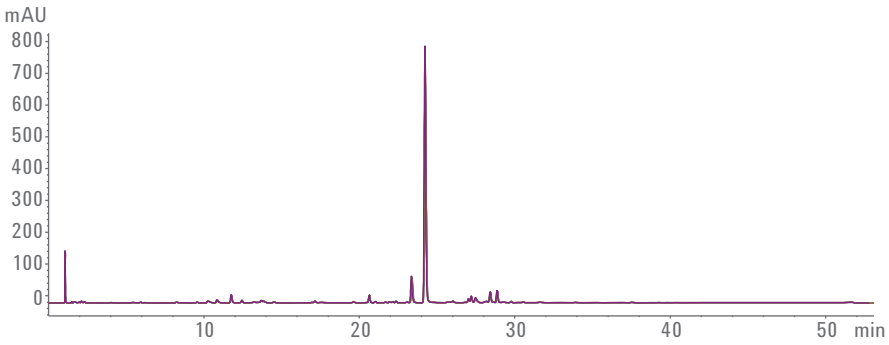

Figure 6. Overlay of six runs of the optimized method for the Agilent 1260 Infinity system. API area, RT, and resolution RSDs are 1.55, 0.03, and 2.09 %, respectively.

Table 4. Replication of CMA performance for the Agilent 1260 Infinity system. The emulated 1260 Infinity met the CMA performance requirements (API Rs > 4 and API tailing < 1.3), and the actual 1260 Infinity system also met these requirements.

	Proven performance	
CMA	Emulated Agilent 1260 Infinity HPLC	Agilent 1260 Infinity HPLC
Resolution	> 4	> 4
USP tailing	1.2	1.2

Table 5. Comparison of time consumption between a workflow using sub-2 µm columns and conventional columns are described.

Experiments	Sub-2-µm columns (hours)	Conventional columns (hours)
Screening	20	47
Optimization*	28*	20
Total	48	67

*An extra HPLC optimization time is added into the workflow with sub-2 µm columns.

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