Quality-by-Design-Based Method Development Using an Agilent 1290 Infinity II LC

An Efficient Method Development Workflow Combined with ISET-mediated Method Transfer Under Waters Empower 3 CDS Control

Application Note

Pharmaceutical Developments and QA/QC

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Abstract

This Application Note demonstrates an UHPLC method for the separation of Amlodipine and its known EP impurities based on Quality by Design (QbD) principles. This method was translated and transferred in a second step for use on HPLC systems. Agilent Instrument Control Framework (ICF) was used as an interface to control the Agilent 1290 Infinity II LC by Waters Empower 3 chromatography data system (CDS). Fusion QbD (S-Matrix Corp, Eureka, CA) software was integrated to realize a QbD-based method development process.

The method, developed on a sub-2 µm column under UHPLC conditions, was translated using a freeware method translator tool into routine QA/QC workflows where HPLC systems are in use. For further optimization and evaluation processes, the performance characteristics of the target HPLC system was emulated using Agilent Intelligent System Emulation Technology (ISET) on an Agilent 1290 Infinity II method development system. After the transfer to the target system, all Critical Method Attributes (CMAs) were met, and the reproducibility was verified.

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Introduction

Quality by Design (QbD) based method development and method validation aligned with the ICH Q8 (R2) and ICH Q2 (R2) guidance is getting more attention in the pharmaceutical Analytical R&D community[2]. During the screening phase of different column chemistries, efficiency can dramatically be increased when using UHPLC methods on short, sub-2 µm columns. However, the final method may need to be transferred to QA/QC departments where most of the LC systems are conventional HPLC systems. Transferring a method from UHPLC to HPLC without compromising the critical method attributes (CMAs) is a challenging process[2]. A method developed on a UHPLC system, even when done using conventional HPLC columns, may not provide the same performance when transferred to an HPLC system due to differences in system delay volumes and gradient mixing precision. To overcome these issues, Agilent Intelligent System Emulation Technology (ISET) has been developed to emulate the properties of commonly used target systems[3].

This Application Note demonstrates the use of the Agilent 1290 Infinity II LC as a versatile UHPLC solution for robust QbD-based method development processes as well as the use of a third-party QbD software (Fusion QbD) with the 1290 Infinity II LC under Waters Empower 3 CDS. Finally, it demonstrates how Agilent ISET can be used to emulate the performance characteristics of different target LC systems that are frequently used in QA/QC environments under third-party software control.

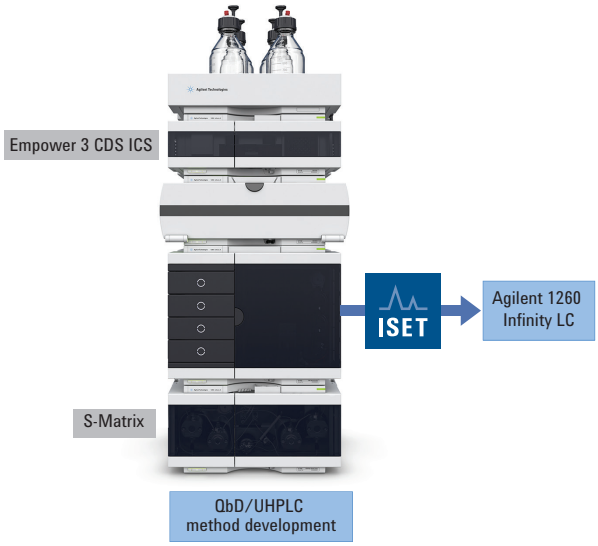

Figure 1. Agilent Intelligent System Emulation Technology-mediated method transfer under Waters Empower 3 CDS control.

Experimental

Instrumentation

An Agilent 1290 Infinity II LC method development system was used for method development. The individual modules and components of the 1290 Infinity II method development solution were:

- Agilent 1290 Infinity valve drive (G1170A) and 12 position/13-port solvent selection valve (G4235A)
- Agilent 1290 Infinity II high-speed pump (G7120A)
- Agilent 1290 Infinity II Multisampler (G7167B) maintained at 4 °C
- Agilent 1290 Infinity II Multicolumn Thermostat (G7116B) with 8 pos/18 port column selector valve (5067–4233)
- Agilent 1290 Infinity II Diode Array Detector (G7117B)

Minimum firmware requirements for all Agilent 1290 Infinity II modules are: B, C, and D.06.70

An Agilent 1260 Infinity LC was used to verify the reproducibility of transferred method. The individual modules of the 1260 Infinity LC were:

- Agilent 1260 Infinity Binary Pump (G1312B)
- Agilent 1260 Infinity Autosampler (G1367E)
- Agilent 1260 Infinity Thermostated Column Compartment (G1316A)
- Agilent 1260 Infinity Diode Array Detector (G4212B)

Software

- Fusion QbD Automated LC Method Development Software (S-Matrix Corp, Eureka, CA) (Version: 9.7.1, Build 458)
- Waters Empower Software (Version 3 build 3471) - with system suitability package.
- Waters Instrument Control Software (ICS) 2.1 HF1 includes Agilent ICF and driver package (A.02.03 DU1 HF2)
- ISET 4 (Driver Version A.02.11)

Reagents and samples

All solvents were HPLC grade (RCI Labscan Ltd, Thailand). Standards of Amlodipine Besylate (API) and the known EP impurities A, B, D, E, F, and G were obtained from Anant Pharmaceuticals Pvt Ltd, India. European pharmacopeia (EP) sample preparation protocol for Amlodipine Besylate was followed for the entire experiment, in which the API is spiked with known impurities[4].

Workflow

The method development workflow began with a screening process to determine the best chromatographic separation conditions using the Amlodipine Besylate standard and impurities on seven short sub-2 µm columns combined with two organic solvents and seven different pH levels (aqueous solvents) as liquid phases. This column chemistry screening experiment was performed using a 1290 Infinity II LC method development system and Fusion QbD Software under Empower 3 control (Figure 2). The chromatographic conditions found to be best (meeting the Analytical Target Profile (ATP) requirement of the screening phase) after the initial screening phase were further optimized by multivariate statistic experiments creating a design space according to QbD principles, creating a robust UHPLC method (satisfying the ATP requirement of the optimization phase).

The UHPLC method was transferred in a second step to two HPLC columns having different particle sizes. To mimic the performance characteristics of the target system, the Agilent 1290 Infinity II UHPLC was operated in emulation mode after activating the ISET tool. The gradient mixing behavior and autosampler delay volume of the Agilent 1260 Infinity system were emulated.

The performance results of the Agilent 1290 Infinity II LC in emulation mode were compared with the results from the target system. The reproducibility of RT, area, and resolution of system suitability impurities (impurities B and G) and API were determined.

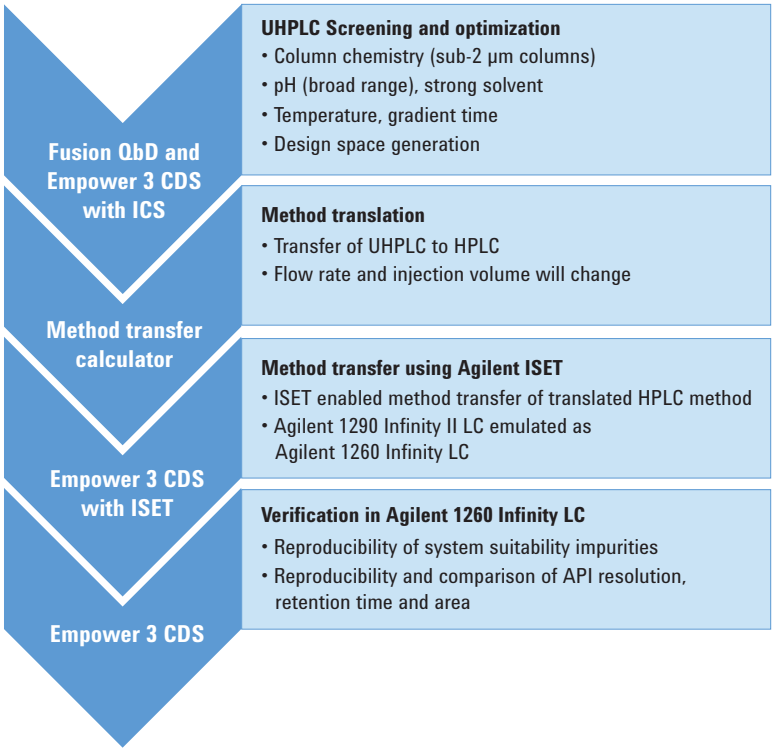

Figure 2. Overall workflow used for the study. The software packages used are shown on the left side of the flowchart, while detailed steps of the workflow are shown on the right side.

Results and Discussion

UHPLC Screening and optimization

The ATP of the screening phase was to develop a fast UHPLC method that meets the system suitability criteria of the EP method (resolution between Amlodipine impurities B and G should be greater than 2.0). Table 1 shows that, to achieve this, various sub-2 µm column chemistries, a broad range of pH, and organic solvents (ACN and MeOH) were screened. Table 2 shows the column chemistry screening experiment that provided the best overall chromatographic conditions. The chromatographic performance at this condition was found to be satisfactory, and all ATP criteria were met (Figure 3).

Table 1. The critical method parameters (CMPs) used in the screening phase experiments. Seven pH buffers, seven columns, three different flow rates, and two strong organic solvents were screened.

CMP	Range/Level(s)
Strong solvent type	Methanol, acetonitrile
Pump flow rate (mL/min)	0.8, 1.0, 1.2
pH	2.00 – 10 mM Trifluoroacetic acid 3.00 – 20 mM Formic acid 4.00 – 5 mM Formic acid + 10 mM ammonium formate 5.00 – 5 mM Acetic acid + 10 Mm ammonium acetate 7.00 – 10 mM Ammonium acetate 8.00 – 10 mM Ammonium hydrogen carbonate 9.00 – 10 mM Ammonium acetate+ 5 mM ammonia
Column type (3.0 × 50 mm, 1.8 µm)	Agilent ZORBAX Eclipse plus c18 Agilent ZORBAX Eclipse plus c8 Agilent ZORBAX SB Aq Agilent ZORBAX Eclipse Plus phenyl hexyl Agilent ZORBAX SB CN Agilent ZORBAX SB C18 Agilent ZORBAX Bonus RP*

*Column diameter used for Bonus RP column was 2.1 mm

Table 2. The best conditions for CMPs in screening phase experiments.

CMPs	Level setting
Strong solvent type	Acetonitrile
Pump flow rate (mL/min)	1.200
pH	2.00
Column type	Agilent ZORBAX Eclipse plus C8

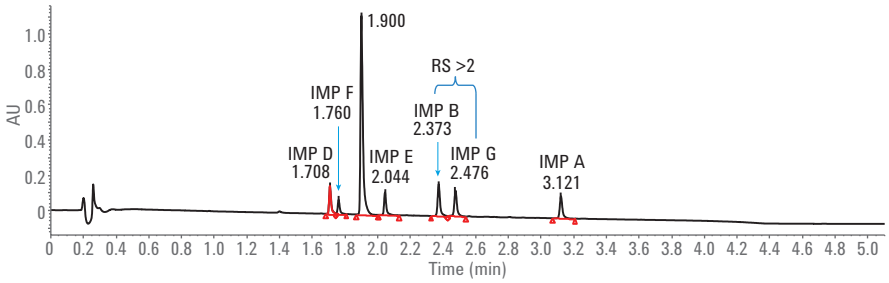

Figure 3. Resolution between impurities B and G was greater than 2, which met the ATP criteria of the screening phase.

The ATP for the optimization phase was to reduce the run time of the best condition of the screening phase, without compromising the system suitability criteria of the EP method and the resolution of API and other impurities. Critical method parameters (CMPs) such as pump flow rate, gradient time, and oven temperature were varied, as mentioned in Table 3. Data analysis of these experiments leads to a robust design space (Figure 4), which meets the previously established ATP criteria. The proven acceptable region (PAR), aligned with the ATP goal, was drawn in the design space. The resolution values of system suitability impurities were plotted for the five different conditions in the PAR (Table 4) and the respective chromatograms (Figure 5). The point prediction tool of Fusion QbD predicted the values of critical method attributes (CMAs), and compared and verified the experimental values (Table 5). The reproducibility of the final UHPLC method after optimization was verified, and an overlay of six replicates was plotted (Figure 6).

Table 3. CMPs varied in optimization phase.

CMP	Range/Level(s)
Pump flow rate (mL/min)	1.200–1.500
Gradient time (min)	1.0 ≤ Gradient time ≤ 4.0
Final hold time (min)	0.5 ≤ Final hold time ≤1.5
Oven temperature (°C)	25.0, 30.0, 35.0

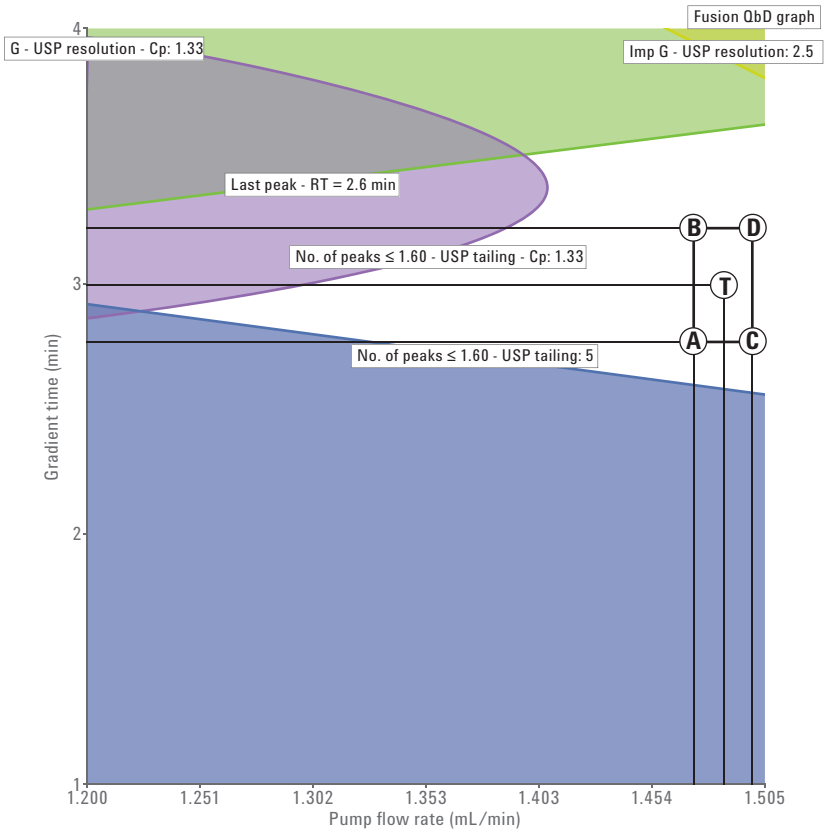

Figure 4. Final design space showing the PAR.

Table 4. CMPs and resolution of system suitability impurities reflecting five points of the PAR.

Conditions	Flow rate	Grad time	Final hold time	Oven temperature	Resolution b/w impurities B and G
A	1.47	2.76	0.5	30	3.28
B	1.47	3.08	0.5	30	3.16
T – Center point	1.48	2.92	0.5	30	3.20
C	1.50	3.08	0.5	30	3.22
D	1.50	2.92	0.5	30	3.10

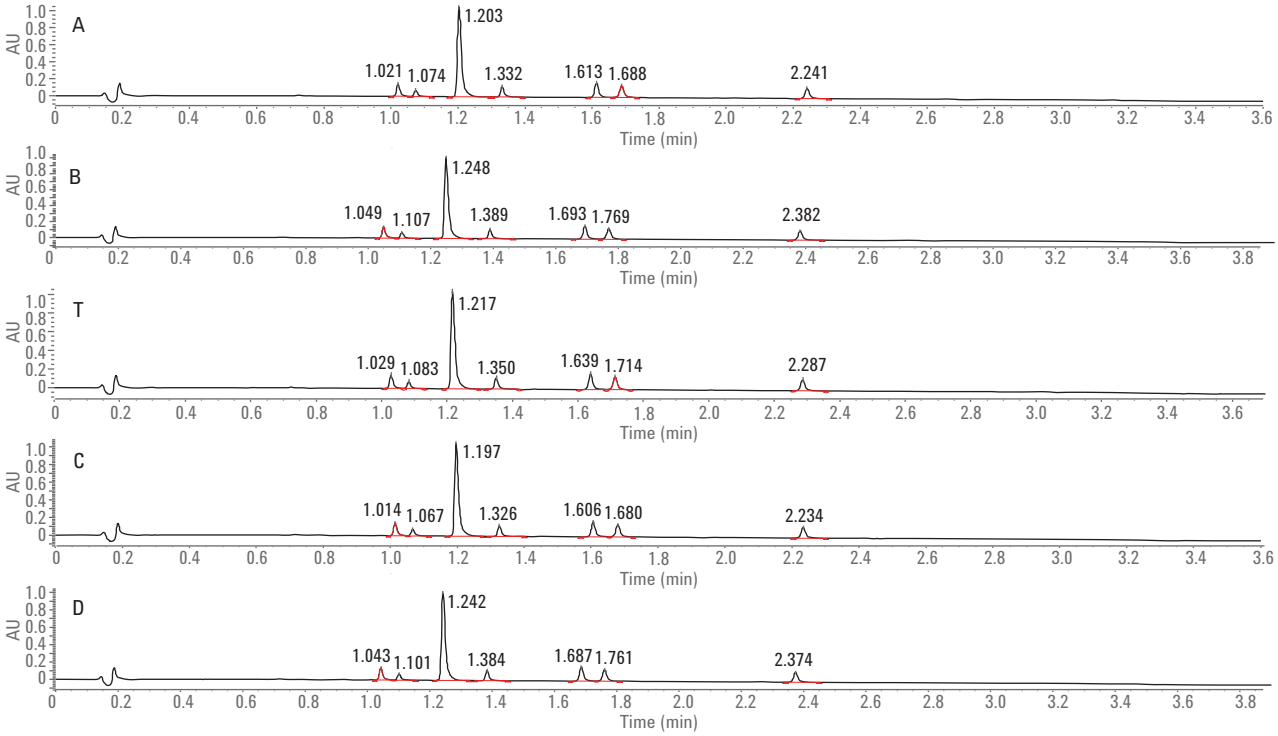

Figure 5. Chromatograms reflecting the conditions of five points (A, B, T, C, D) in PAR.

Table 5. Fusion QbD software-predicted response CMA values from the center point (T) of the PAR. The experimental results were compared with predicted values, and found to be within the Sigma confidence limit.

CMA	Predicted	Experimental	–2 Sigma confidence limit	+2 Sigma confidence limit
No. of peaks ≥2.00 – USP resolution	6.14	6.00	5.68	6.60
No. of peaks ≤ 1.60 – USP tailing	5.80	6.00	5.06	6.65
Last peak - retention time	2.28	2.28	2.28	2.29
USP Resolution b/w impurities B and G	2.97	3.20	2.90	3.20

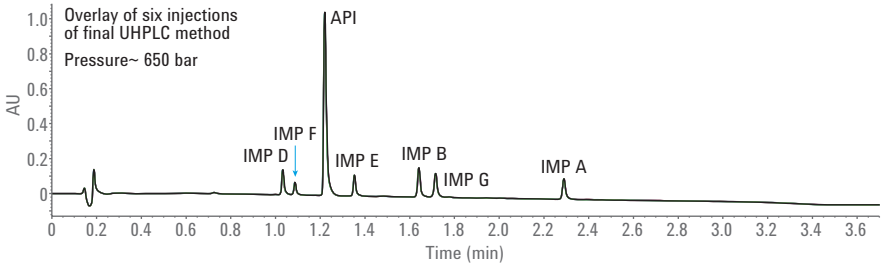

Figure 6. Reproducibility of final UHPLC method, using an overlay of six chromatograms.

Method translation and transfer

The UHPLC method developed on sub-2 µm columns was translated into three different HPLC methods using conventional particle sizes. The Microsoft excel-based method translation calculator from the University of Geneva was used for this purpose[5]. Initially, the UHPLC method was translated to the HPLC method (Agilent ZORBAX Eclipse Plus, 4.6 × 150 mm, 3.5 µm as column 1) with a reasonable run time of 27 minutes (Table 6). This method was evaluated on the 1290 Infinity II system using the ISET emulation mode of the target system (an Agilent 1260 Infinity LC), and later verified with the results of a 1260 Infinity LC system (Figure 7). The emulated solvent delivery module and autosampler were G1312B v1.0 and G1367E-100 µL syringe v1.0. The method transfer to column 1 (HPLC method 1) was achieved without compromising the ATP criteria, with a reasonable run time, however, the observed pressure range was approximately 300 bar (70 % of the pressure limit of conventional HPLC pumps). This might be a point of concern for users of legacy HPLC systems having pressure limitations. As a result, the UHPLC method was translated into HPLC method 2 using a column with larger particle sizes (column 2 = ZORBAX Eclipse Plus, 4.6 × 150 mm, 5 µm), reducing the backpressure (Table 6). The method translation calculator suggested a lower flow rate of 1.2 mL/min and a longer run time of 37 minutes. In HPLC method 3, the runtime was reduced by increasing the flow rate to 1.8 mL/min, without compromising the resolution. The results of HPLC method 3 were also verified with the results on a 1260 Infinity LC system (Figure 8), and could be used with systems having pressure limitations. Table 7 and Table 8 summarize the RT and resolution deviations of emulated and actual systems for the respective methods. Six replicates of HPLC methods 1 and 3 were performed to check the reproducibility of the respective methods and RSD values of resolution, RT, and area of API, and system suitability impurities were found to be ≤1.1 % (Table 9).

Table 6. The method parameters of UHPLC and all other translated HPLC methods.

Parameter	UHPLC method	HPLC method 1	HPLC method 2	HPLC method 3
Column	Agilent ZORBAX Eclipse Plus 3.0 × 50 mm, 1.8 µm	Agilent ZORBAX Eclipse Plus 4.6 × 150 mm, 3.5 µm	Agilent ZORBAX Eclipse Plus 4.6 × 150 mm, 5 µm	Agilent ZORBAX Eclipse Plus 4.6 × 150 mm, 5 µm
Flow rate (mL/min)	1.5	1.8	1.2	1.8
Injection volume (µL)	2	14	14	14
Gradient	Time %B 0.00 25 0.30 25 3.20 95 3.70 95 3.80 25 4.30 25	Time %B 0.00 25 2.87 25 19.79 95 22.71 95 23.29 25 26.21 25	Time %B 0.00 25 4.10 25 28.27 95 32.44 95 33.27 25 37.44 25	Time %B 0.00 25 2.87 25 19.78 95 22.70 95 23.28 25 26.20 25
Pressure (bar)	~650	~300	~130	~180

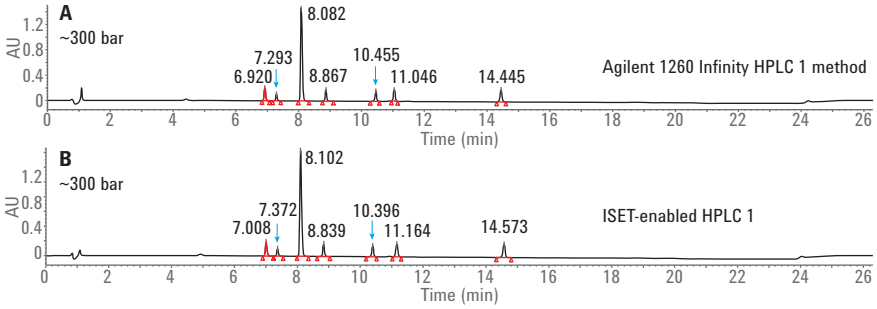

Figure 7. Overlaid chromatograms showing the similarity of the ISET-emulated method on the Agilent 1290 Infinity II system and the Agilent 1260 Infinity system for the HPLC method 1.

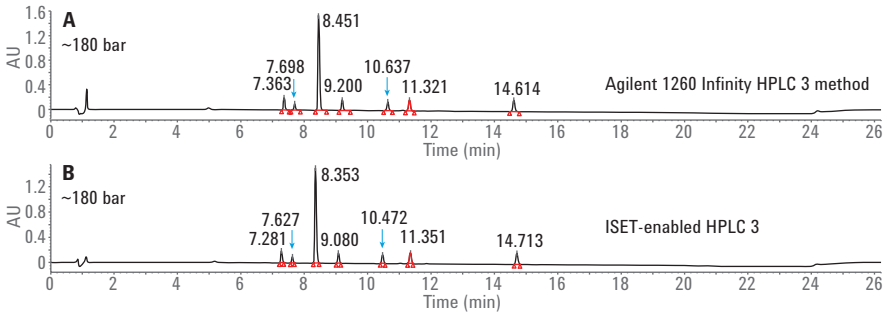

Figure 8. Overlaid chromatograms showing the similarity of ISET-emulated and an Agilent 1260 Infinity LC system for HPLC method 3.

Table 7. Calculated percentage deviations for HPLC method 1. All deviations were found to be within the allowed limit of acceptance criteria (resolution –5 % and retention time ±5 %).

	API Resolution	API RT (min)	Impurity B RT (min)	Impurity G RT (min)	Impurity G resolution
Agilent 1260 Infinity HPLC 1	8.01	8.08	10.45	11.05	5.63
ISET enabled HPLC 1	8.46	8.06	10.38	11.07	6.52
Percentage deviation (%)	+4.3	–0.24	–0.6	+0.1	+13.6

Table 8. Calculated percentage deviations for HPLC method 3. All deviations were found to be within the allowed limit of the acceptance criteria (resolution –5 % and retention time ±5 %).

	API Resolution	API RT (min)	Impurity B RT (min)	Impurity G RT (min)	Impurity G resolution
Agilent 1260 Infinity HPLC 3	7.63	8.45	10.63	11.35	5.9
ISET enabled HPLC 3	7.32	8.35	10.47	11.32	7.3
Percentage deviation (%)	–4.2	–0.24	–0.6	+0.1	+13.6

Table 9. RSD values showing the reproducibility of HPLC methods 1 and 3.

	Impurity G Rs	Impurity G RT	API RT	API Rs	Impurity G area	API Area
HPLC Method 1						
Average (min)	5.46	11.03	8.07	8.05	748,439	5,778,226
SD	0.05	0.008	0.008	0.01	2,951.619	5,852.5
RSD	0.96	0.07	0.10	0.14	0.39	0.10
HPLC Method 3						
Average (min)	5.52	11.04	8.07	8.07	752,041.8	5,784,162
SD	0.06	0.005	0.004	0.01	3,421.39	4,937.24
RSD	1.13	0.04	0.05	0.16	0.45	0.08

Conclusion

Agilent Instrument Control Framework (ICF) software was used as an interface to control the Agilent 1290 Infinity II LC by Waters Empower 3 chromatography data system, Waters ICS, and Fusion QbD software was used to develop a fast and robust UHPLC method. According to the QbD principles, a design space was generated after optimization. The five points of the design space were checked with the acceptance criteria, and found that all criteria were met. The predicted values of CMAs were found to match the experimental values. System suitability requirements (resolution > 2 for impurities B and G) were met, and all peaks were baseline-separated in a gradient time of 3.7 minutes. The final UHPLC method was reproducible (API and impurity G area RSD <0.5) and robust.

A seamless method transfer from an Agilent 1290 Infinity II UHPLC system to an Agilent 1260 Infinity system was achieved using Agilent ISET technology. The method was adapted to the pressure limits of the target system. The results in emulation mode and the results of the target system were compared. Thus, it was shown that method development, QbD principles, and method transfer can be achieved seamlessly by combining Agilent 1290 Infinity II LC, ISET, ICF, and third-party CDS and Fusion QbD software.

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