

Scaling of a USP Assay for Quetiapine Fumarate Across Different Liquid Chromatographic Systems

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Abstract

In this application note, the Waters Columns Calculator will be used to scale the USP monograph assay method for quetiapine fumarate, an anti-psychotic drug, to smaller particle sized columns.

Benefits

- The Waters Column Calculator enables users to scale a method by calculating operating parameters that give equivalent chromatographic performance.
- Improved USP method throughput is obtained by scaling HPLC column dimensions to columns with smaller particle sizes and shorter column lengths.
- The Quetiapine Fumarate assay method run time was reduced by 57% and 87% when analyzed on a 3.5 μ m column with a UHPLC system and on a 1.7 μ m column on a UPLC system, respectively.
- System suitability requirements as stated in USP monograph for the quetiapine fumarate assay are met when scaling down the column dimensions of the method.

Introduction

Users of compendial chromatographic procedures increasingly look to achieve cost savings through analytically equivalent procedures that decrease analysis time and solvent consumption. The United States Pharmacopeia (USP) compendial methods are often written for high performance liquid chromatography (HPLC) columns which use $>3\ \mu\text{m}$ particles resulting in long runtimes at higher flow rates. Although USP methods prescribe specific conditions, isocratic methods are capable of being scaled to columns which have a smaller particle size. This results in faster run times and lower solvent consumption. The USP has outlined in the General Chapter <621>¹ acceptable column and method adjustments to allow scaling of isocratic methods to provide the same if not improved performance of a method. These allowable adjustments include scaling particle size and column dimensions to maintain the L/dp ratio, where L is the length of the column and dp is the diameter of the particle size of the column packing material, as well as adjusting the flow rate and injection volumes accordingly. For these scaled methods it is important to consider the impact of dwell volume and extra-column dispersion on the chromatographic separation, and whether or not a lower dispersion, higher pressure system such as a UHPLC or a UPLC system is preferred.

In this study, the Waters Columns Calculator will be used to scale the USP monograph assay method for quetiapine fumarate,² an anti-psychotic drug, to smaller particle sized columns. These resulting methods will then be run on a variety of LC systems. The LC system platforms that will be included are an Alliance HPLC System, an ACQUITY Arc UHPLC System, and an ACQUITY UPLC H-Class PLUS System. We will demonstrate improvements to method performance, decreased run times and improved throughput while achieving the system suitability requirements as stated in the USP monograph for the quetiapine fumarate assay.

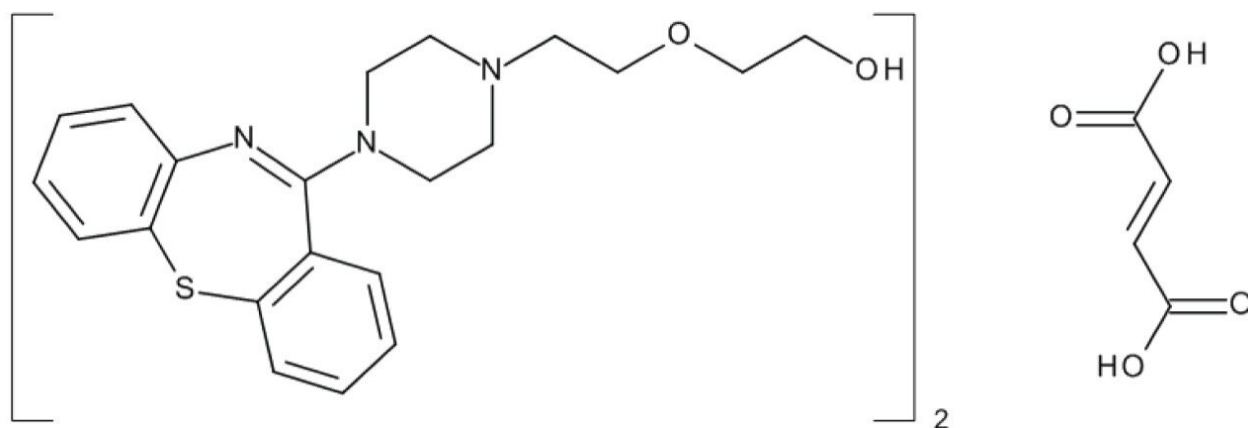


Figure 1. Structure of quetiapine fumarate.

Experimental

Sample description

Two reference standards were obtained from the USP: Quetiapine System Suitability (Catalog#: 1592715), and the Quetiapine Fumarate Standard (Catalog#: 1592704). The unknown sample was obtained from Alibaba.com. All samples were diluted in mobile phase to the following concentrations: 1.0 mg/mL for the system suitability solution and 0.08 mg/mL for the standard solution and sample solution.

LC conditions

Column temp.:	25 °C
Sample temp.:	4 °C
Mobile phase:	Methanol, acetonitrile, and buffer (54:7:39) Premixed and filtered using a 0.45 µm filter
Buffer:	2.6 g/L of dibasic ammonium phosphate adjusted to pH 6.5 with phosphoric acid

Gradient: Isocratic (premixed mobile phase)

PDA wavelength: 230 nm at 4.8 nm resolution

Individual system conditions

LC system 1: Alliance e2695 Separations Module with 100 μ L syringe, 2998 PDA Detector and CH-30 equipped with a passive preheater

Column: XBridge BEH C₈ Column, 5 μ m, 4.6 mm x 250 mm (p/n: 186003018)

Injection volume: 50 μ L

Flow rate: 1.3 mL/min

Run time: 15 minutes

Max. system pressure: 3850 psi

LC system 2: ACQUITY Arc System with active solvent preheating (CH-30A) and 2998 PDA Detector (Path 2)

Column: XBridge BEH C₈ Column, 3.5 μ m, 3.0 mm x 150 mm (p/n: 186003052)

Injection volume: 12.8 μ L

Flow rate: 0.8 mL/min

Run time: 7 minutes

Max. system pressure: 5200 psi

LC system 3:	ACQUITY UPLC H-Class PLUS System with active solvent preheating (CH-30A), 50 μ L extension loop, and ACQUITY UPLC PDA Detector
Column:	ACQUITY UPLC BEH C ₈ Column, 1.7 μ m, 2.1 mm x 75 mm (p/n: 186005606)
Injection volume:	3.1 μ L
Flow rate:	0.5 mL/min
Run time:	3 minutes
Max. system pressure:	14,000 psi

Data management

Empower 3 Chromatography Data Software, FR 3

Results and Discussion

The USP assay for quetiapine fumarate was initially run on the Alliance HPLC System following the column and method details described in the monograph. The column dimensions and method conditions were then scaled according to the USP guidelines¹ using the Waters Columns Calculator (Figure 2), and subsequently run on the ACQUITY Arc UHPLC System and the ACQUITY UPLC H-Class PLUS System. Both the column dimensions and the features of an LC system should be used to determine the optimal combination for running a method. Two key attributes of an LC system that should be considered are the dwell volume and the extra-column dispersion. The dwell volume of a system consists of the amount of volume from where the solvents are mixed until the mixed solvents contact the column inlet. While this may have a significant impact for gradient methods, dwell volume has very little effect on isocratic methods.⁴ Therefore, the extra-column dispersion is the main factor in determining the optimum LC system for a column with an isocratic method. It is important to reduce the extra-column dispersion when using smaller columns since it will affect the

resolution of the compounds, the broadening of peaks, and the efficiency of the column.⁵ Therefore, to achieve optimal performance, it is important to keep the extra-column dispersion as minimal as possible in comparison to the column volume. For a more in depth breakdown of dwell volume and extra-column dispersion refer to the white paper listed in reference #5.

To maintain the L/dp ratio the column dimensions and particle size were scaled from the original column dimensions to a column with a 3.5 μm particle size and 3.0 mm $\times$ 150 mm column dimensions (Figure 2). In order to match the lower column volume with a lower dispersion system, the analysis of the quetiapine fumarate was performed on the ACQUITY Arc UHPLC System. The L/dp ratio decreased by 14% but is within the USP criteria of -25% to +50% of the original method. When the particle size and diameter of a column are changed, the following equation is used to calculate the change in flow rate:

$$F_2 = F_1 \times [(dc_2^2 \times dp_1)/(dc_1^2 \times dp_2)]$$

where F_1 and F_2 are the flow rates for the original and scaled method, respectively; dc_1 and dc_2 are the column diameters for the original and scaled method, respectively, and dp_1 and dp_2 are the diameters of the particle sizes of the original and scaled methods, respectively.¹ The flow rate for this experiment was decreased from 1.3 mL/min in the original method to 0.8 mL/min for the 3.5 μm particle size column method. According to the USP general guidelines, "the injection volume for a method may be adjusted as long the value is within the accepted precision, linearity, and detection limits".¹ The injection volume was decreased from the original method of 50 μL to 12.8 μL for the scaled method conditions and was determined using the Waters Column Calculator (Figure 2).

From...
Describe your original method.

Column

Diameter (D): 4.600 mm

Length (L): 250 mm

Particle Size (dp): 5.0 μm

L/dp: 50,000

System

Dwell volume: 1.100 mL

Method

Injection volume: 50.0 μL

Temperature: 25 °C

Run time: 15.00 min

Time (min)	Flow Rate (mL/min)	%A Water	%B Acetonitrile	%C Methanol	%D Water	Column Volumes
0.00	1.300	39.0	0.0	61.0	0.0	0.00

3,238 psi
Maximum pressure

To...
Describe your target method.

Column

Diameter (D): 3.000 mm

Length (L): 150 mm

Particle Size (dp): 3.5 μm

L/dp: 42,857

System

Dwell volume: 1.100 mL

High pressure limit: 9,500 psi

Method

Flow rate: ☒ Scaled: (0.790 mL/min) ☐ Custom: 0.400 mL/min

Time (min)	Flow Rate (mL/min)	%A Water	%B Acetonitrile	%C Methanol	%D Water	Column Volumes
0.00	0.790	39.0	0.0	61.0	0.0	0.00

5,664 psi **12.8 μL** **6.30 min**
Maximum pressure Injection volume Run time

Figure 2. Waters Columns Calculator showing the original USP compendial method on the 5 μm particle size column method (left) scaled to the 3.5 μm particle size column method conditions (right).

In order to scale the quetiapine fumarate assay to a 1.7 μm particle column, the Waters Columns Calculator was utilized once again (Figure 3). The analysis of the quetiapine fumarate on the 2.1 mm × 75 mm column was performed on the ACQUITY UPLC H-Class PLUS System in order to decrease the extra-column dispersion that would be present on the Alliance HPLC System and ACQUITY Arc UHPLC System. The scaled column dimensions resulted in an L/dp ratio decrease of 12%, however, are within the USP criteria of -25% to +50% of the original method. The flow rate was decreased from 1.3 mL/min to 0.8 mL/min and the injection volume was decreased from 50 μL to 3.1 μL when scaling from the original method to the 1.7 μm particle size column. The flow rate was not run at 0.8 mL/min as stated in the Columns Calculator (Figure 3) because the ACQUITY UPLC H-Class PLUS System was over the 15,000 psi pressure limit at that flow rate. The final flow rate of 0.5 mL/min was used since the maximum system pressure achieved was 14,000 psi. This flow rate adjustment is acceptable under the USP conditions stated in the General Chapter <621> that once the flow rate is adjusted accordingly to the column, the flow rate can then be additionally adjusted

within $\pm 50\%$.¹

From...
Describe your original method.

Column Diameter (D): 4.600 mm
Length (L): 250 mm
Particle Size (dp): 5.0 µm
L/dp: 50,000

System Dwell volume: 1.100 mL ?

Method Injection volume: 50.0 µL
Temperature: 25 °C
Run time: 15.00 min

	Time (min)	Flow Rate (mL/min)	%A Water	%B Acetonitrile	%C Methanol	%D Water	Column Volumes
1	0.00	1.300	39.0	0.0	61.0	0.0	0.00

3,238 psi
Maximum pressure

To...
Describe your target method.

Column Diameter (D): 2.100 mm
Length (L): 75 mm
Particle Size (dp): 1.7 µm
L/dp: 44,118

System Dwell volume: 0.443 mL ?
High pressure limit: 15,000 psi

Method Flow rate: ☒ Scaled: (0.797 mL/min) ☐ Custom: 0.600 mL/min

	Time (min)	Flow Rate (mL/min)	%A Water	%B Acetonitrile	Column Volumes
1	0.00	0.797	39.0	0.0	0.00

24,713 psi **3.1 µL** **1.53 min**
Maximum pressure Injection volume Run time

Figure 3. Waters Columns Calculator showing the original USP compendial method on the 5 µm particle size column (left) scaled to the 1.7 µm particle size column method conditions (right).

All samples were prepared as described in the experimental conditions according to the USP assay with six replicate injections for each solution. In order to determine the successful performance of the scaled methods run on the appropriate chromatographic system, the system suitability requirements stated in the quetiapine fumarate USP monograph were evaluated. Additionally, an unknown sample was analyzed on each of the three systems to determine the reproducibility of the scaled methods.

System suitability tests are an important component of the USP monograph given that they determine whether or “not a chromatographic system is adequate for an intended analysis”.³ For the system suitability, the USP monograph requires that the resolution between the quetiapine desethoxy and the quetiapine peaks (Figure 4) must be not less than (NLT) 1.5, with peak identification based on relative retention times contained in the USP monograph. The standard solution requires the tailing factor be not more than (NMT)

2.0 and the relative standard deviation (RSD) be NMT 2.0%. The first peak of the chromatogram for all samples pertains to the fumaric acid and was not considered when evaluating the system suitability requirements for retention time, area, and tailing since it is not the main active pharmaceutical ingredient (API).

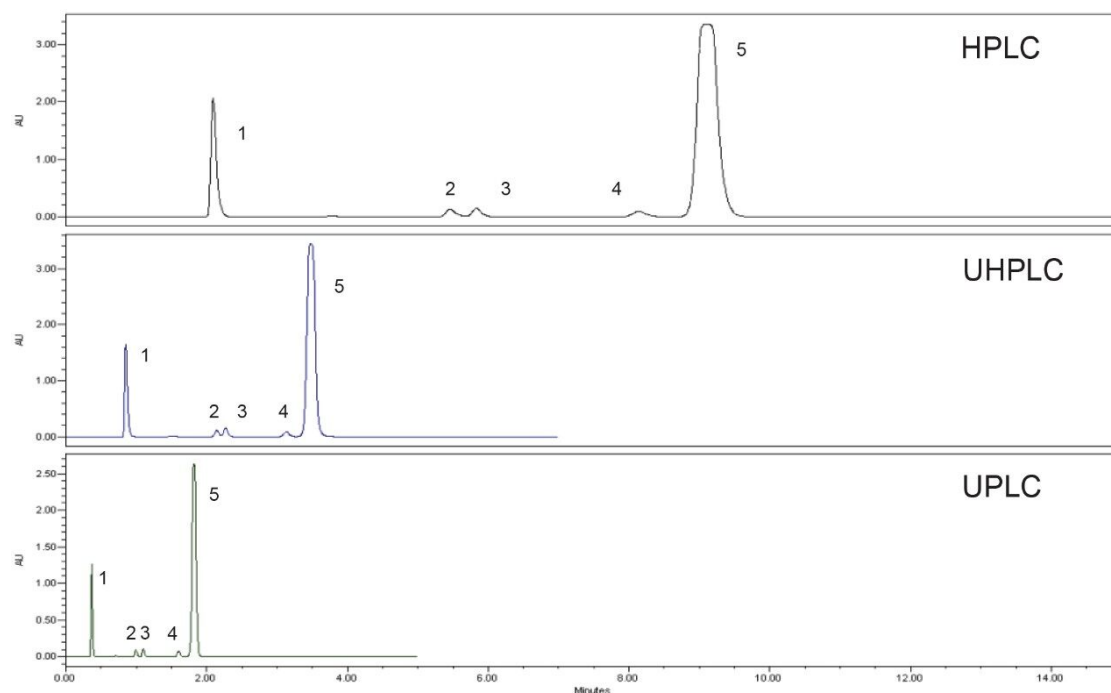


Figure 4. Comparison of the system suitability reference sample of quetiapine fumarate run on the 5 μ m particle column on the Alliance HPLC System (top) and scaled to a 3.5 μ m and a 1.7 μ m particle columns analyzed on the ACQUITY Arc UHPLC (middle) and ACQUITY UPLC H-Class PLUS (bottom) systems, respectively. The API (quetiapine) is saturated in the system suitability solution in order to get a high enough sensitivity of the impurity compounds. The compounds are identified as follows: Peak 1: fumaric acid, Peak 2: quetiapine related compound G, Peak 3: quetiapine related compound B, Peak 4: quetiapine desethoxy, and Peak 5: quetiapine.

The USP assay method for quetiapine fumarate was evaluated using the original and scaled methods run on the prescribed LC systems. Example chromatograms obtained using the three methods are shown in Figure 4 (system suitability) and Figure 5 (sample solution). All methods met the system suitability requirements for both the system suitability solution and the standard solution with the results located in Table 1.

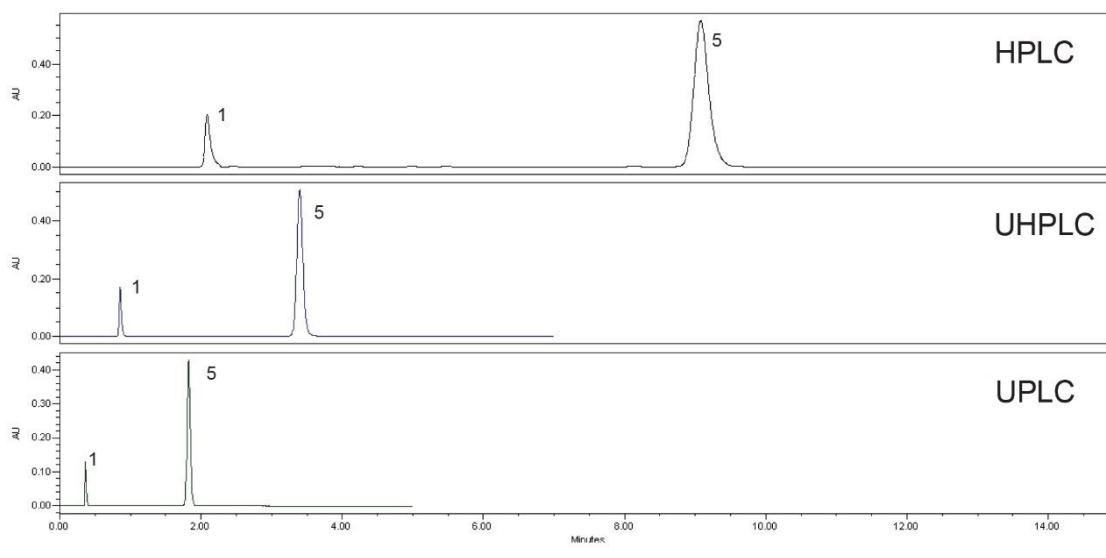


Figure 5. Comparison of the sample solution run on the 5 μm particle size column on the Alliance HPLC System (top), and scaled to a 3.5 μm and a 1.7 μm particle size columns analyzed on the ACQUITY Arc UHPLC System (middle), and the ACQUITY UPLC H-Class PLUS System (bottom), respectively. The compounds were identified based on the relative retention time provided in the USP method. The compounds are identified as follows: Peak 1: fumaric acid, Peak 5: quetiapine.

	Resolution (Peaks 4 & 5)	Quetiapine tailing	Quetiapine area %RSD	Quetiapine retention time %RSD	Run time (min)	Solvent consumption per sample (mL)
Alliance HPLC System	2.4	1.2	0.09	0.16	15	19.5
ACQUITY Arc UHPLC System	2.2	1.1	0.09	0.02	7	5.6
ACQUITY UPLC H-Class PLUS System	2.5	1.1	0.01	0.10	3	2.4
USP requirements	NLT 1.5 System suitability	NMT 2.0 Standard solution	NMT 2.0% Standard solution	NMT 2.0% Standard solution	N/A	N/A

Table 1. The USP quetiapine fumarate assay results for the system suitability and standard solutions on the Alliance HPLC System, the ACQUITY Arc UHPLC System, and the ACQUITY UPLC H-Class PLUS System compared to the USP requirements along with the run time and solvent consumption for each method.

In addition to providing similar or improved chromatographic performance, scaling the original method to a smaller particle column also significantly decreased the run time and solvent consumption. Scaling the original method to a 3.5 μm column decreased the run time from 15 minutes to 7 minutes, and decreased the solvent usage by 66%. Further scaling to a 1.7 μm column decreased the run time from 15 minutes to

approximately 2 minutes and reduced the solvent usage by almost 90%. Scaling USP methods to run on smaller particle columns can drastically increase throughput while decreasing cost per sample.

Also, an unknown sample was analyzed in order to determine the percent of quetiapine fumarate present. The standard solution and the sample solution data were used to calculate the percent as follows:

$$\text{Result} = (r_u/r_s) \times (C_s/C_u) \times 100$$

where r_u is the peak response from the sample solution, r_s is the peak response from the standard solution, C_s is the concentration of USP quetiapine fumarate standard in the standard solution (mg/mL), and C_u is the concentration of quetiapine fumarate in the sample solution (mg/mL).² The calculated percent of quetiapine fumarate generated using the different methods can be found in Table 2. These values do not fall within the USP acceptance criteria of 98.0% to 102.0%. If this sample were to be used for batch release, a further investigation would be required to determine the source of the over-estimation of the purity. This may include evaluation of peak purity or possibly analysis by an orthogonal detection mechanism such as mass spectrometry. However, since the aim of this application note is to demonstrate the scalability of the isocratic method, further evaluation is outside the scope of this work.

An important part of method scaling is the ability to generate the same results using either the original or the scaled method conditions. Scaling the USP quetiapine assay method across the different LC systems produced consistent results for the unknown sample (Table 2). This demonstrates that scaled methods can be used to generate reliable data.

Sample result (%)	
Alliance HPLC System	109.5
ACQUITY Arc UHPLC System	108.9
ACQUITY UPLC H-Class PLUS System	109.7

Table 2. The USP quetiapine fumarate assay calculated results for the percent of quetiapine fumarate in the sample solution on the 5 μm particle size column on the Alliance HPLC System, the 3.5 μm particle size column on the ACQUITY Arc UHPLC System and the 1.7 μm particle size column on the ACQUITY UPLC H-Class PLUS System.

Conclusion

Utilizing the method scaling guidelines within the USP General Chapter <621>,¹ traditional isocratic HPLC methods are capable of being scaled to columns with smaller particle sizes and shorter lengths. Scaling a USP method enables the use of modern chemistries and LC hardware to deliver improved throughput with decreased solvent consumption all while providing accurate and reproducible chromatographic data. The scaled modifications for the USP quetiapine fumarate assay method enabled the original method run time to be reduced by 57% for a 3 µm column on an ACQUITY Arc UHPLC System and 87% reduction for the 1.7 µm column on an ACQUITY UPLC H-Class PLUS System. The scaled methods maintained the same or improved chromatographic performance in terms of resolution, peak tailing, and retention time and peak area RSD.

References

1. Chapter <621> Chromatography. United States Pharmacopeia and National Formulary (USP 40-NF35 S1) Baltimore, MD: United Book Press, Inc.; 2017.
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4. Snyder, Lloyd R.; Kirkland, Joseph; Dolan, John W. *Introduction to Liquid Chromatography, 3rd Edition*. Hoboken: Wiley and Sons, 2010. Print. p. 112 (dwell volume).
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