Application Note

Scaling a USP Gradient Method on the ACQUITY Arc System in Support of Lifecycle Management

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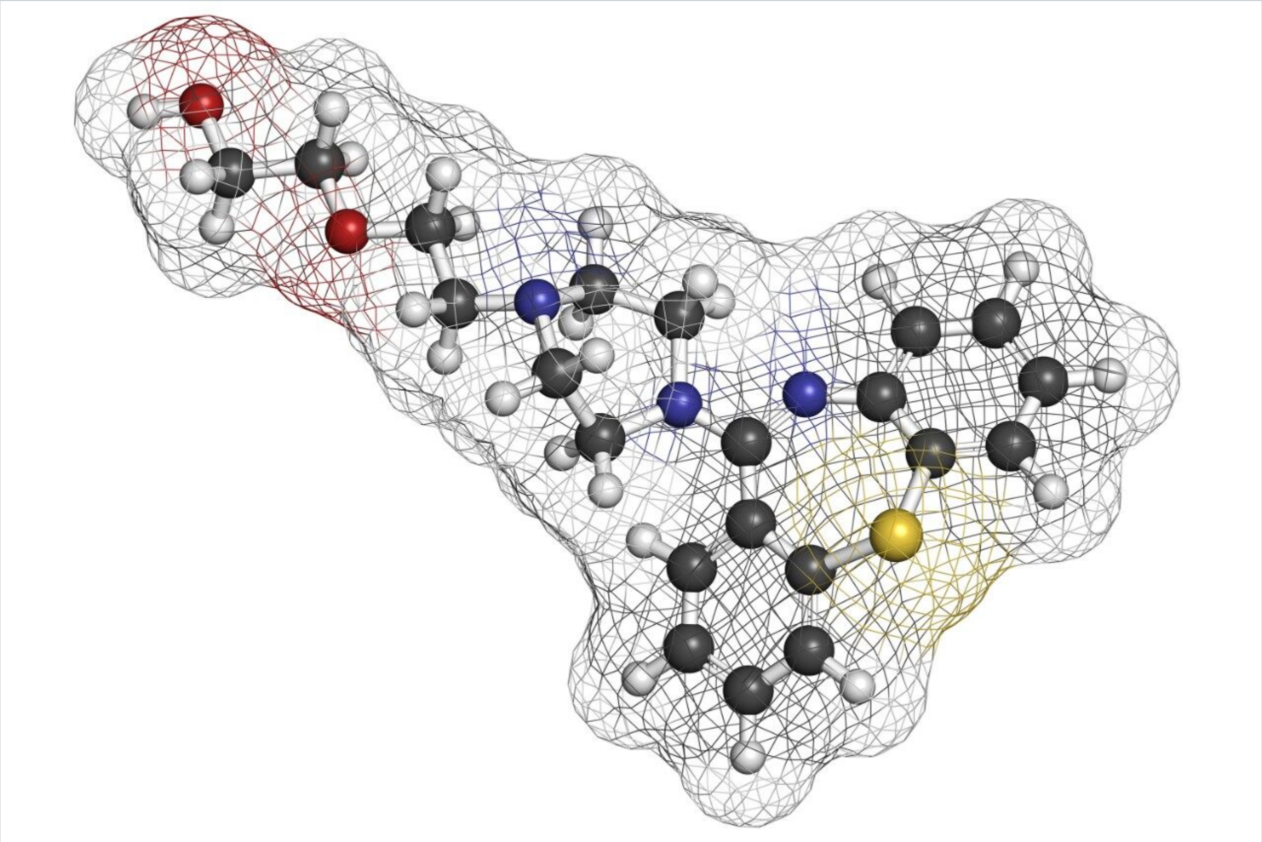


Abstract

In this application note, the USP impurity monograph for quetiapine fumarate will be analyzed on the ACQUITY Arc System and then scaled to smaller particle sized columns using the Waters Columns Calculator.

Benefits

- Demonstration of geometrically scaling a USP gradient method from a 3.5 µm particle column to a 2.5 and 1.7 µm particle column on the ACQUITY Arc System.
- The Waters Columns Calculator is an easy-to-use tool for scaling gradient methods.
- Scaling a method to smaller particle size columns reduces run time and solvent consumption.

Introduction

Modernization of older high performance liquid chromatography (HPLC) methods, including scaling or transferring[1] a method to new column or liquid chromatographic (LC) technologies can be considered a part of pharmaceutical lifecycle management.[2] Many of these methods use >3 µm particle columns combined with high flow rates, which can result in long runtimes and high solvent consumption. Geometrically scaling HPLC methods to columns with smaller particle sizes can increase throughput while maintaining analytical performance. System dispersion and pressure limit are two factors to consider when scaling a method on a single instrument. Specifically, the LC system dispersion may impact the overall method performance and the LC system operating pressure may impose a physical limitation. Once the column dimensions and particle size are adjusted, the method requires the proper scaling of various method parameters including flow rate, injection volume, and gradient timing.

In this study, the USP impurity monograph for quetiapine fumarate[3] will be analyzed on the ACQUITY Arc System and then scaled to smaller particle sized columns using the Waters Columns Calculator. Using the resolution, tailing factor, percent RSDs and the percent impurity calculation specified in the monograph,[3] the scaled methods will be compared to the original HPLC method to ensure no loss of chromatographic or quantitative performance. The scaled methods provide decreased run times, lower solvent consumption, and overall increased sample throughput without changing the LC system.

Experimental

Sample description

The quetiapine fumarate standard (catalog #1592704), and the quetiapine system suitability standard (catalog #1592715) were purchased from the United States Pharmacopeia (USP). The unknown quetiapine fumarate sample was purchased from Alibaba.com.

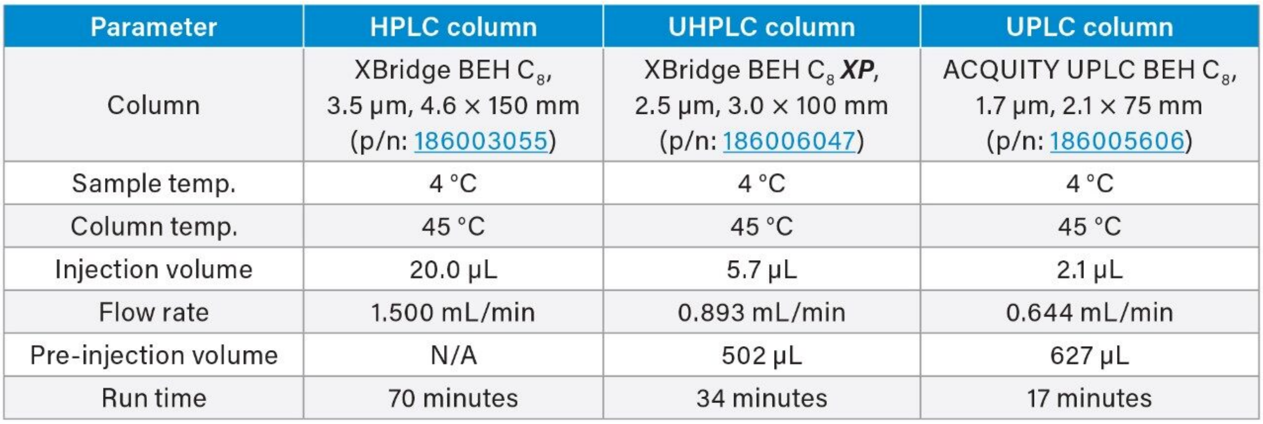
All solutions were prepared according to the USP monograph.[3] The system suitability and standard solutions were prepared in diluent comprised of Solution A and Solution B (86:14). The unknown sample solution was prepared in Solution A.

The concentrations of the solutions are 1.0 mg/mL for the system suitability solution, 0.001 mg/mL for the standard solution, and 1.0 mg/mL for the unknown sample solution.

LC conditions

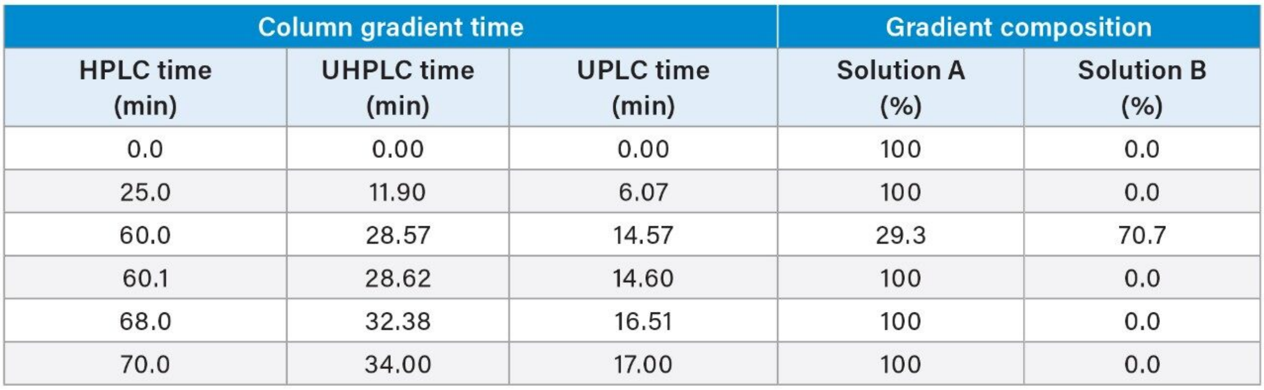
System:	ACQUITY Arc (Path 2) with active solvent preheating (CH-30A) and 2998 PDA Detector
Mobile phase A:	Solution A: Acetonitrile and buffer (25:75)
Mobile phase B:	Solution B: Acetonitrile
Buffer:	3.1 g/L of ammonium acetate in water. Two mL of 25% ammonium hydroxide was added to each 1 liter of solution. The final pH is not less than (NLT) 9.2
PDA wavelength:	250 nm at 4.8 nm resolution

Columns:



Parameter	HPLC column	UHPLC column	UPLC column
Column	XBridge BEH C_8, 3.5 µm, 4.6 × 150 mm (p/n: 186003055)	XBridge BEH C_8 ***XP***, 2.5 µm, 3.0 × 100 mm (p/n: 186006047)	ACQUITY UPLC BEH C_8, 1.7 µm, 2.1 × 75 mm (p/n: 186005606)
Sample temp.	4 °C	4 °C	4 °C
Column temp.	45 °C	45 °C	45 °C
Injection volume	20.0 µL	5.7 µL	2.1 µL
Flow rate	1.500 mL/min	0.893 mL/min	0.644 mL/min
Pre-injection volume	N/A	502 µL	627 µL
Run time	70 minutes	34 minutes	17 minutes

Gradient table:



Column gradient time			Gradient composition	
HPLC time (min)	**UHPLC time (min)**	**UPLC time (min)**	**Solution A (%)**	**Solution B (%)**
0.0	0.00	0.00	100	0.0
25.0	11.90	6.07	100	0.0
60.0	28.57	14.57	29.3	70.7
60.1	28.62	14.60	100	0.0
68.0	32.38	16.51	100	0.0
70.0	34.00	17.00	100	0.0

Data management

Chromatography data software: Empower 3 FR 3

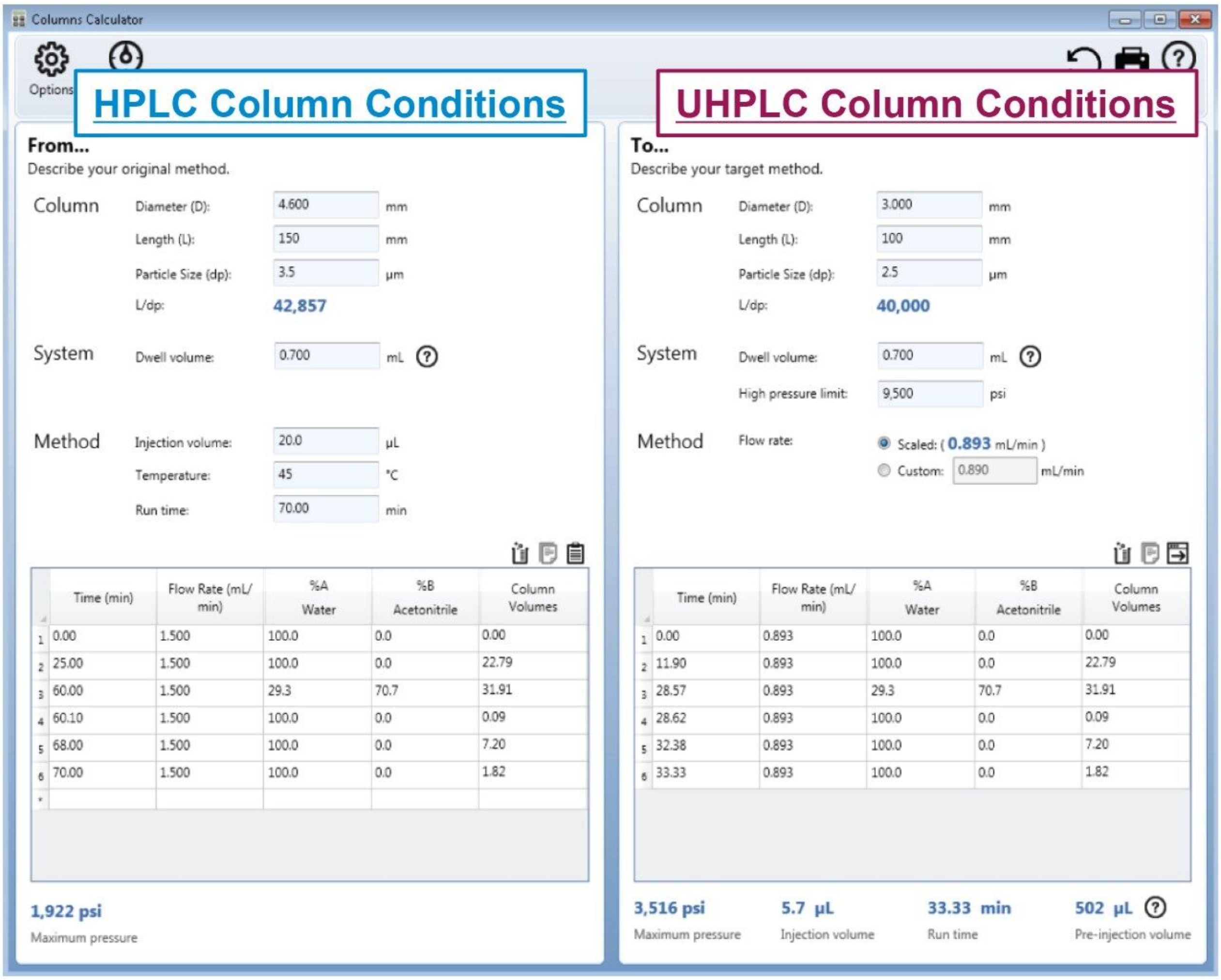
Results and Discussion

When scaling a method on a single instrument, the extra-column dispersion can impact the performance of the method. To maximize column efficiency, it should be paired with an LC system whose dispersion is small enough that it minimizes the extra-column dispersion or band broadening effects. However, optimal column performance, in terms of narrowest peak widths and highest peak capacity, may not be required for an analysis. It may be possible to use a small particle size column on a UHPLC, or even an HPLC system, and still meet method requirements. For example, the dispersion of the ACQUITY Arc System allows for suitable UHPLC analysis of a range of column particle sizes while maintaining the method requirements for the gradient quetiapine impurity method.

In addition to extra-column dispersion, it is important to consider the operating pressure limit of the LC system. When column particle size is decreased, the resulting backpressure generated is increased on the LC system. Therefore, geometrically scaling some methods may not be possible due to the pressure limits of the LC system. The Waters Columns Calculator[4] can provide an estimated system pressure for a scaled method, but the actual pressure will be determined when running the scaled method on the LC system.

The quetiapine fumarate impurities USP method was first analyzed on the ACQUITY Arc System using the described monograph conditions.[3] Performance was assessed based on the system suitability requirements as outlined in the monograph, which include resolution, tailing, and RSD for peak retention time and area. Then the column dimensions and method conditions were geometrically scaled to 2.5 and 1.7 µm particle columns.[5]

The scaled UHPLC and UPLC column dimensions were determined by maintaining the L/d_p ratio of the original column, where L is the length of the column and d_p is the diameter of the particle size. Therefore, a 2.5 µm, 3.0 × 100 mm, and a 1.7 µm, 2.1 × 75 mm column was chosen. To adjust the columns flow rate, injection volume, and gradient steps, the Waters Columns Calculator was used (Figure 1). When analyzed on the LC system, the scaled conditions for both methods did not exceed the pressure limits of the ACQUITY Arc System.



From...
Describe your original method.

Column
Diameter (D): 4.600 mm
Length (L): 150 mm
Particle Size (dp): 3.5 µm
L/dp: 42,857

System
Dwell volume: 0.700 mL

Method
Injection volume: 20.0 µL
Temperature: 45 °C
Run time: 70.00 min

	Time (min)	Flow Rate (mL/min)	%A Water	%B Acetonitrile	Column Volumes
1	0.00	1.500	100.0	0.0	0.00
2	25.00	1.500	100.0	0.0	22.79
3	60.00	1.500	29.3	70.7	31.91
4	60.10	1.500	100.0	0.0	0.09
5	68.00	1.500	100.0	0.0	7.20
6	70.00	1.500	100.0	0.0	1.82
*					

1,922 psi
Maximum pressure

To...
Describe your target method.

Column
Diameter (D): 3.000 mm
Length (L): 100 mm
Particle Size (dp): 2.5 µm
L/dp: 40,000

System
Dwell volume: 0.700 mL
High pressure limit: 9,500 psi

Method
Flow rate:
● Scaled: (0.893 mL/min)
○ Custom: 0.890 mL/min

	Time (min)	Flow Rate (mL/min)	%A Water	%B Acetonitrile	Column Volumes
1	0.00	0.893	100.0	0.0	0.00
2	11.90	0.893	100.0	0.0	22.79
3	28.57	0.893	29.3	70.7	31.91
4	28.62	0.893	100.0	0.0	0.09
5	32.38	0.893	100.0	0.0	7.20
6	33.33	0.893	100.0	0.0	1.82

3,516 psi
Maximum pressure

5.7 µL
Injection volume

33.33 min
Run time

502 µL
Pre-injection volume

Figure 1. The Waters Column Calculator was used to scale from a 3.5 µm, 4.6 × 150 mm HPLC column to a 2.5 µm, 3.0 × 100 mm UHPLC column.

During a gradient method there is a delay between the time the mobile-phase composition is changed at the pump to when it reaches the head of the column. When scaling gradient methods, it is important to maintain this delay so that there is no change to selectivity of early eluting compounds. To account for this, the Waters Columns Calculator determines the "Pre-Inject" volume based upon the entered system dwell volume and the column dimensions. The "Pre-Inject" volume is calculated to be 502 µL for the 2.5 µm particle column method and 627 µL for the 1.7 µm particle column method[6,7] (Figure 1).

The two standard solutions and the unknown sample were prepared as described above with six replicate injections performed for each solution. The original HPLC method and the two scaled methods all show similar chromatographic performance (Table 1) in terms of resolution, tailing, and peak area and retention time RSDs. The resolution of peaks 1 and 2, was slightly lower for the UPLC method, likely due to the system dispersion which is not optimal for 1.7 µm particle columns. Although there is a small decrease, the resolution is still well above the method requirements of 1.5. Chromatograms of the system suitability solution are shown in Figure 2.

ACQUITY Arc System	Resolution (peaks 1 and 2)	Resolution (peaks 3 and 4)	Quetiapine tailing	Quetiapine area %RSD	Quetiapine retention time %RSD
HPLC column	13.3	7.4	1.0	1.14	0.14
UHPLC column	13.2	6.7	0.95	0.57	0.02
UPLC column	10.8	6.6	0.95	1.25	0.04

Table 1. A 3.5 µm (original), 2.5 µm, and 1.7 µm column/method conditions were analyzed on the ACQUITY Arc System.

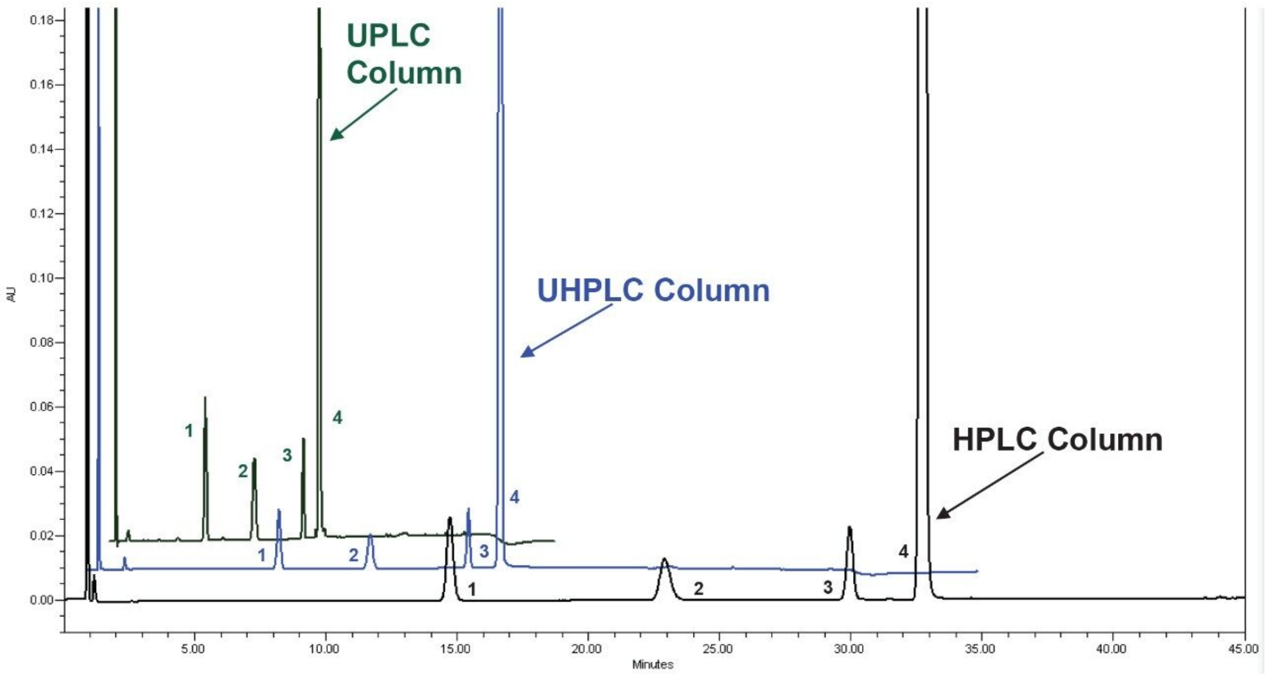


Figure 2. Quetiapine fumarate system suitability solution analyzed on the ACQUITY Arc System using HPLC, UHPLC, and UPLC column/method conditions for the quetiapine impurity method. Peak identification: (1) quetiapine related compound G, (2) quetiapine related compound B, (3) quetiapine desethoxy, and (4) quetiapine.

Scaling the original method to a smaller particle column significantly decreased the run time and solvent consumption (Table 2). For example, scaling the HPLC method to a 2.5 µm particle column decreased the run time by 51% and the solvent usage by 71%. Scaling the method to a 1.7 µm particle column decreased the run time by 75% and reduced the solvent usage by 89%.

ACQUITY Arc System	Flow rate (mL/min)	Run time (min)	Solvent consumption per sample (mL)
HPLC column	1.500	70	105
UHPLC column	0.893	34	30
UPLC column	0.644	17	11

Table 2. The run times and solvent consumption for an individual sample analyzed on the ACQUITY Arc System using an HPLC, UHPLC, and UPLC column/method conditions.

To evaluate the quantitative reproducibility of the impurity method, the impurity content of the unknown sample was determined (Figure 3) using:

$$\text{Result} = (r_u/r_s) \text{ x } (C_s/C_u) \times (1/F) \times 100$$

where r_u is the peak response of each impurity from the sample solution, r_s is the peak response of quetiapine from the standard solution, C_s is the concentration of USP quetiapine fumarate standard in the standard solution (mg/mL), C_u is the concentration of quetiapine fumarate in the sample solution (mg/mL), and F is the relative response factor for the impurity peak provided in the monograph.[3]

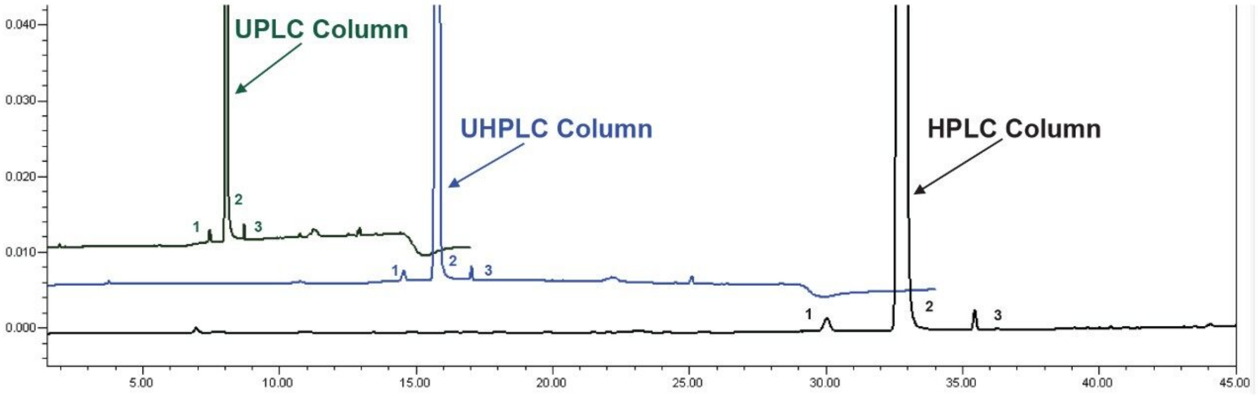


Figure 3. The unknown sample solution analyzed on the ACQUITY Arc System with an HPLC, UHPLC, and UPLC column/method conditions for the quetiapine impurity method. Peak identification: (1) quetiapine desethoxy, (2) quetiapine, and (3) unknown impurity.

Two impurity peaks were found in the unknown sample, quetiapine desethoxy and an unknown impurity. The calculated percent of each impurity, as well as the total amount of impurities in the sample, can be found in Table 3. The quantitative results for the impurities contained in the active pharmaceutical ingredient (API) sample were consistent regardless of which method/column was employed on the ACQUITY Arc System.

ACQUITY Arc System	Quetiapine desethoxy	Unknown impurity	Total impurities
HPLC column	0.13%	0.08%	0.21%
UHPLC column	0.09%	0.06%	0.17%
UPLC column	0.11%	0.08%	0.19%

Table 3. Calculated impurity results obtained for all three column/methods used on the ACQUITY Arc System.

When a method is adjusted, it is important that the same analytical results are generated using the new method conditions. Scaling the USP quetiapine fumarate impurities method across the different column categories produced equivalent quantification of impurities contained within an unknown sample of the API.

Conclusion

When a laboratory has limited LC systems, it is possible to modernize methods by scaling traditional HPLC methods to columns with a smaller particle size and length. This was demonstrated on the ACQUITY Arc System using the USP quetiapine fumarate impurity gradient method. The original method was scaled from a 3.5 µm particle column to a 2.5 and 1.7 µm particle column using the Waters Columns Calculator. Without changing the ACQUITY Arc System, the HPLC, UHPLC, and UPLC column impurity methods provided similar chromatographic performance in terms of resolution, peak tailing, and retention time and peak area RSD. Additionally, quantitative results for impurities contained within an unknown API sample were consistent regardless of which method/column was used for analysis on the ACQUITY Arc System.

References

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