EMPLOYING MODERN LIQUID CHROMATOGRAPHY TECHNOLOGY TO SCALE A USP GRADIENT METHOD ON A SINGLE LIQUID CHROMATOGRAPHIC SYSTEM

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INTRODUCTION

The continual development and modernization of pharmaceutical procedures helps to ensure product quality and safety as part of lifecycle management that is supported in the International Committee of Harmonization (ICH). In lifecycle management, pharmaceutical laboratories are looking to achieve cost savings through analytically equivalent procedures by using modern equipment and columns. When modernizing a gradient method, there are method attributes that can be adjusted, including changes to the column dimensions, flow rate, injection volume, and programmed gradient. Properly adjusting these method conditions on the same liquid chromatograph (LC) system may provide a shorter run time while maintaining the same chromatographic performance. Changes to the column may include use of smaller particle size, while maintaining the L/dp ratio, where L is the length of the column and dp is the diameter of the particle size. When the column dimensions are changed, adjustments must also be made to the flow rate, the injection volume and the programmed gradient. In this study, the USP monograph gradient impurities method for quetiapine fumarate will be scaled to smaller particle sized columns using the Waters Columns Calculator and analyzed on the ACQUITY Arc UHPLC System. The scaled methods will then be compared to the original HPLC method to ensure no loss of chromatographic or quantitative performance.

Samples:

- 1.0 mg/mL USP Quetiapine System Suitability in diluent (Catalog#: 1592715)
- 0.001 mg/mL USP Quetiapine Fumarate Standard in diluent (Catalog#: 1592704)
- 1.0 mg/mL unknown sample in Solution A (Alibaba.com)
- Diluent: Solution A: Solution B (86:14)

LC System:

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

Figure 1. ACQUITY Arc UHPLC System.

METHODS

USP Monograph Impurities LC Conditions:

Column (HPLC)	XBridge BEH C8 Column,3 .5 µm, 4.6 mm x 150 mm (p/n:186003055)
Column Temp.	45° C
Sample Temp.	12° C
Injection Volume	20 µL
Flow Rate	1.5 mL/min
Mobile Phase	Solution A: Acetonitrile: Buffer (25:75)
	Solution B: Acetonitrile
Buffer	3.1 g/L of dibasic ammonium acetate in water. Add 2 mL of 25 % ammonium hydroxide to each 1 L of solution. The pH of the resulting solution is NLT 9.2
Run Time	70 minutes
PDA wavelength	250 nm at 4.8 nm resolution
Gradient:	

Time (min)	Solution A (%)	Solution B (%)
0.0	100	0.0
25.0	100	0.0
60.0	29.3	70.7
60.1	100	0.0
68.0	100	0.0
70.0	100	0.0

Scaled Column Conditions:

Column (UHPLC)	XBridge BEH C8 XP Column, 2.5 µm, 3 mm x 100 mm (p/n: 186006047)
Injection Volume	5.7 µL
Flow Rate	0.893 mL/min
Run Time	34 minutes
Column (UPLC)	ACQUITY UPLC BEH C8 Column, 1.7 µm, 2.1 mm x 75 mm (p/n: 186005606)
Injection Volume	2.1 µL
Flow Rate	0.644 mL/min
Run Time	17 minutes
Scaled Gradient:	

Scaled Column Gradient Time		Gradient Composition	
UHPLC Time (min)	**UPLC Time (min)**	**Solution A (%)**	**Solution B (%)**
0.0	0.0	100	0.0
11.90	6.07	100	0.0
28.57	14.57	29.3	70.7
28.62	14.60	100	0.0
32.38	16.51	100	0.0
34.0	17.0	100	0.0

The quetiapine impurities USP method was first analyzed on the ACQUITY Arc UHPLC System using the prescribed monograph conditions[3]. The geometrically scaled column dimensions and particle sizes were determined by maintaining the L/dp ratio, where L is the length of the column and dp is the diameter of the particle size. To adjust the flow rate, injection volume, and gradient steps the Waters Columns Calculator (Figure 2) was used.

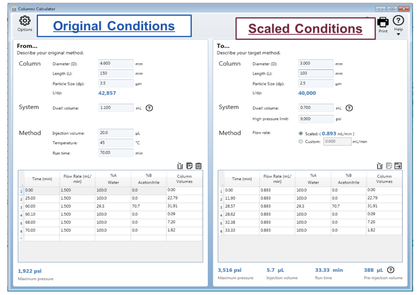

Figure 2. The Waters Column Calculator was used to scale a 4.6 x 150 mm, 3.5 µm particle size HPLC column to a 3.0 x 100 mm, 2.5 µm particle size UHPLC column and to a 2.1 x 75 mm, 1.7 µm particle size UPLC column .

Scaling the original HPLC method to a smaller particle column was able to significantly decrease the run time and solvent consumption (Table 1). For example, scaling the HPLC method to a 2.5 µm column decreased the run time by 51 % and the solvent usage by 71%. Scaling the method to a 1.7 µm column decreased the run time by 75 % and reduced the solvent usage by 89% compared to the original method.

ACQUITY Arc UHPLC System	Flow Rate (mL/min)	Run Time (minutes)	Solvent Consumption per Sample (mL)
HPLC Column	1.500	70	105
UHPLC Column	0.893	34	30
UPLC Column	0.644	17	11

Table 1. The run times and solvent consumption for an individual sample analyzed on the ACQUITY Arc UHPLC System using an HPLC, UHPLC and UPLC column/method conditions.

RESULTS AND DISCUSSION

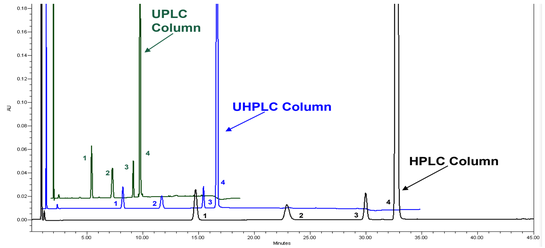

Figure 3. Comparison of the quetiapine system suitability solution run using a HPLC, UHPLC, and UPLC column/method conditions. Peak identification: 1: quetiapine related compound G, 2: quetiapine related compound B, 3: quetiapine desethoxy, and 4: quetiapine .

The performance of the original and scaled methods were assessed based on the system suitability requirements as outlined in the monograph. These requirements include resolution, tailing, and RSD for peak retention time and area (Table 2). Based on these parameters the original HPLC method and the two scaled methods all show similar chromatographic performance. It was determined that because the mobile phase for the UPLC column was prepared on a different day, the resolution of the first critical pair is effected.

ACQUITY Arc UHPLC System	Resolution (peak 1 & 2)	Resolution (peak 3 & 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 2. Quetiapine performance results obtained using HPLC, UHPLC, and UPLC column/method conditions analyzed on the ACQUITY Arc UHPLC System.

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

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

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].

ACQUITY Arc UHPLC 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 on the ACQUITY Arc UHPLC System.

Two impurity peaks were found in the unknown sample (Figure 4), quetiapine desthoxy and an unknown impurity. The quantitative results for the impurities contained in the active pharmaceutical ingredient (API) sample were consistent regardless of which method/column was used on the ACQUITY Arc UHPLC System. Whenever a method is adjusted, producing consistent reliable results is a critical factor to ensure reproducibility from the original method to the scaled methods.

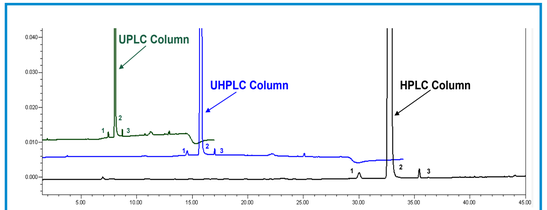

Figure 4. Comparison of the unknown sample solution analyzed with an HPLC, UHPLC and UPLC column/method conditions. Peak identification: 1: quetiapine desethoxy, 2: quetiapine, and 3: unknown impurity.

CONCLUSION

- **It is possible to scale traditional HPLC methods to columns with a smaller particle size and length on the ACQUITY Arc UHPLC System.**
- **The Waters Columns Calculator is an easy to use tool for scaling gradient methods.**
- **The HPLC, UHPLC and UPLC column methods for the quetiapine impurity method maintained similar chromatographic performance in terms of resolution, peak tailing and retention time and peak area RSD.**
- **Quantitative results for impurities contained in the API sample were consistent regardless of which method/column was used.**
- **Scaling the HPLC method to a 2.5 µm column decreased the run time by 51 % and the solvent usage by 71%.**
- **Scaling the HPLC method to a 1.7 µm column decreased the run time by 75 % and reduced the solvent usage by 89%.**

References

1. Fountain, Kenneth. Transferring Compendial HPLC Methods to UPLC Technology for Routine Generic Drug Analysis. Waters Application Note. 720004251en. 2012
2. Guidance for Industry Q10 Pharmaceutical Quality System. ICH, 2008
3. Official Monographs, Quetiapine Fumarate USP 40 NF35 S1, United States Pharmacopeia and National Formulary (USP 40-NF35 S1) Baltimore, MD: United Book Press, Inc.; 2017. p. 5939.
4. Neue Uwe D, McCabe Doug, Ramesh Vijaya, Pappa Horacio, DeMuth Jim. Transfer of HPLC Procedures to Suitable Columns of Reduced Dimensions. Pharmacopeial Forum 2009 Nov--Dec. 35(6):1622.
5. Columns Calculator Online Help. Waters Columns Calculator, version 2.0

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