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. It is possible for pharmaceutical laboratories to reduce cost through analytically equivalent procedures 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 shorter run times while maintaining the same chromatographic performance.
Changes to the column include use of smaller particles. 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 columns using the Waters Columns Calculator and run on a single LC System. The scaled methods will then be compared to the original HPLC method to ensure no loss of chromatographic or quantitative performance.

METHODS

LC System	ACQUITY Arc UHPLC System (Path 2) with active solvent preheating (CH-30A) and 2998 PDA Detector
Solution A:	Acetonitrile and Buffer(25:75)
Solution B:	Acetonitrile
Buffer:	3.1 g/L of ammonium acetate in water. 2 mL of 25% ammonium hydroxide was added to each 1 liter of solution. Final pH is NLT 9.2
PDA:	250 nm at 4.8 nm resolution
Column Temp:	45 C
Sample Temp:	4 C

LC Conditions:
Gradient Table

Parameter	HPLC Column	UHPLC Column	UPLC Column
Column	XBridge BEH C8, 3.5 µm, 4.6 mm x 150 mm	XBridge BEH C8 XP, 2.5 µm, 3.0 mm x 100 mm	ACQUITY UPLC BEH C8, 1.7 µm, 2.1 mm x 75 mm
Inj. 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-Inj. Volume	NA	502 µL	627 µL
Run Time	70 minutes	34 minutes	17 minutes

Column Gradient Time (min)			Gradient Composition (%)	
HPLC	UHPLC	UPLC	Solution A	Solution B
0.0	0.0	0.0	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	34.0	17.00	100	0.0

RESULTS AND DISCUSSION

The quetiapine impurities USP method was first analyzed on the ACQUITY Arc UHPLC System using the prescribed monograph conditions3. The scaled column dimensions and particle sizes were determined by maintaining the L/dp ratio. Therefore, a 3.0 x 100 mm, 2.5 µm and a 2.1 x 75 mm, 1.7 µm column was chosen. To adjust the flow rate, injection volume, and gradient steps the Waters Columns Calculator was used

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

Table 1. Sample run time and solvent consumption for the HPLC, UHPLC and UPLC column/method conditions.

Scaling the original HPLC method to smaller particle columns significantly decreased 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..

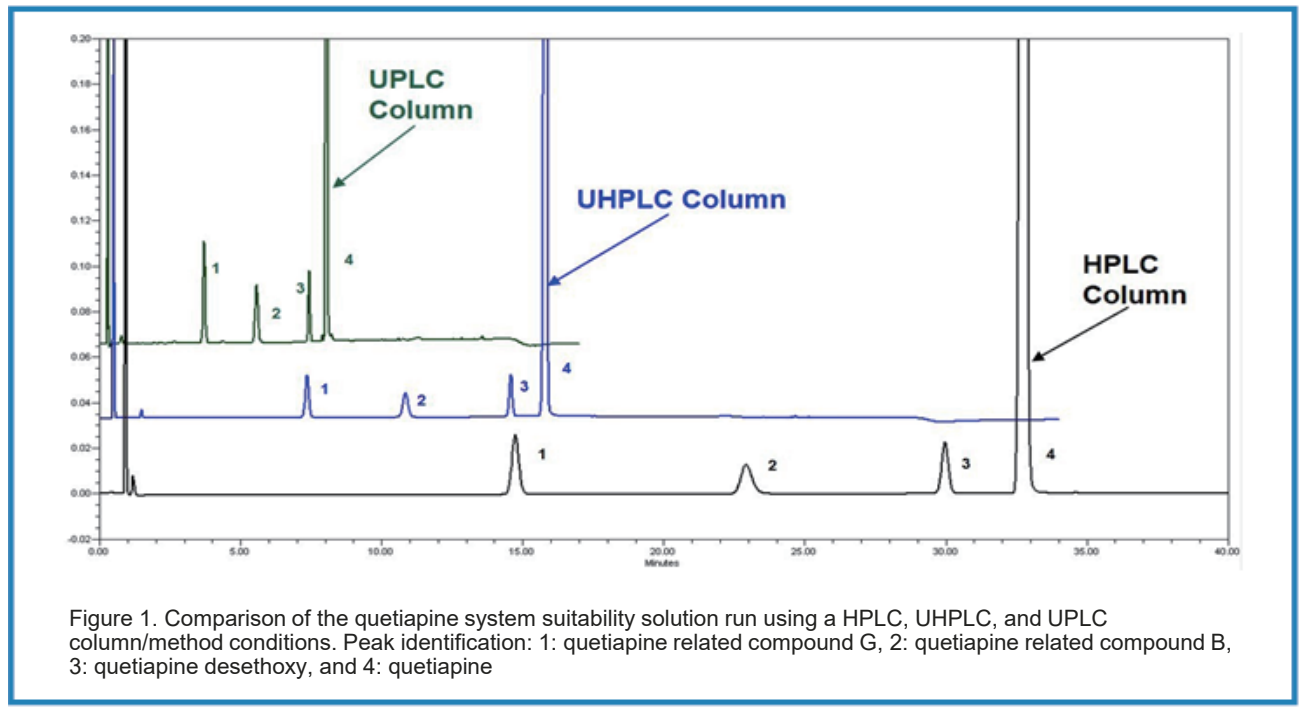

Figure 1. Comparison of the quetiapine system suitability solution run using a HPLC, UHPLC, and UPLC column/method conditions. Peak identification: 1: quetiapine related compound G, 2: quetiapine related compound B, 3: quetiapine desethoxy, and 4: quetiapine

Performance of the scaled methods was assessed using the system suitability requirements as outlined in the monograph, which include resolution, tailing, and peak area and retention time RSDs (Table 2). Based upon these results, the original HPLC method and the two scaled methods all show similar chromatographic performance. The resolution of peaks 1 and 2, was slightly lower for the UPLC method, likely due to the system dispersion5. 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 1.

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**
UHPL 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 chromatographic results obtained using HPLC, UHPLC, and UPLC column/ method conditions analyzed on the ACQUITY Arc UHPLC System.

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 quetiapine impurity methods method yielded similar chromatographic performance in terms of resolution, peak tailing and retention time and peak area RSD.**
- **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%.**

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