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应用纪要

Scaling USP Methods to Smaller Particle Columns on the Alliance HPLC System

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Abstract

In this application note, the USP assay and impurity methods for quetiapine fumarate is analyzed on the Alliance HPLC System and then scaled to a smaller particle size column using the Waters Columns Calculator. The scaled method is then compared to the original HPLC method to ensure no loss of chromatographic or quantitative performance.

Benefits

- Reduce run time and solvent consumption by scaling an isocratic method from a 5.0 µm, 4.6 mm x 250 mm column to a 3.5 µm, 3.0 mm x 150 mm column and scaling a gradient method from a 3.5 µm, 4.6 mm x 150 mm column to a 2.5 µm, 3.0 mm x 100 mm column on the Alliance HPLC System.
- The Waters Columns Calculator is an easy to use tool for scaling isocratic and gradient methods.

Introduction

The continual development and modernization of pharmaceutical procedures helps to ensure product quality and safety while taking advantage of new instrument and column technologies. Specifically, high performance liquid chromatography (HPLC) methods can be scaled to smaller particle columns to increase throughput while maintaining the analytical performance of the method. When scaling a method, it is important to consider characteristics of the liquid chromatography (LC) system, such as dispersion and operating pressure, and how these might impact the chromatographic results. The LC system dispersion may impact the overall method performance including resolution, peak capacity and the separation efficiency. Also, the LC system operating pressure may impose a physical limitation on the ability to scale some methods.

In this study, the USP assay and impurity methods for quetiapine fumarate[1] will be analyzed on the Alliance HPLC System and then scaled to a smaller particle size column using the Waters Columns Calculator. The scaled method will then be compared to the original HPLC method to ensure no loss of chromatographic or quantitative performance. The chromatographic assessment is based upon the resolution, tailing factor, percent RSDs of area and retention time specified for each method in the monograph.[1] The quantitative performance is assessed by the calculated active pharmaceutical ingredient (API) and the calculated impurities of an unknown sample. 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. The unknown quetiapine fumarate sample was purchased from Alibaba.com.

Method conditions

LC System: Alliance e2695 Separations Module with 100 µL syringe (p/n: 700000564), 2998 PDA Detector and CH-30 equipped with a passive preheater.

Assay (Isocratic) method

All samples were diluted in mobile phase to the following concentrations: 1.00 mg/mL for the system suitability solution and 0.08 mg/mL for the standard and sample solutions.

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
PDA wavelength:	230 nm at 4.8 nm resolution

LC conditions:

Parameter	5 µm HPLC Column	3.5 µm UHPLC Column
Column:	**XBridge BEH C_8, 5 µm, 4.6 mm × 250 mm (p/n: 186003018)**	**XBridge BEH C_8 *XP*, 3.5 µm, 3.0 mm × 150 mm (p/n: 186003052)**
Sample temp.:	4 °C	4 °C
Column temp.:	25 °C	25 °C
Injection volume:	50.0 µL	12.8 µL
Flow rate:	1.30 mL/min	0.60 mL/min
Run time:	15 minutes	7 minutes

Impurities (Gradient) method

The system suitability and the standard solutions were prepared in diluent (Solution A: Solution B, 86:14). The concentrations were 1.0 mg/mL for the system suitability solution and 0.001 mg/mL for the standard solution. The unknown sample solution was prepared in Solution A at a concentration of 1.0 mg/mL.

Mobile phase:	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. The final pH is not less than (NLT) 9.2
PDA wavelength:	250 nm at 4.8 nm resolution

LC conditions:

Parameter	3.5 µm HPLC Column	2.5 µm UHPLC Column
Column:	XBridge BEH C_8, 3.5 µm, 4.6 mm × 150 mm (p/n: 186003055)	XBridge BEH C_8 XP, 2.5 µm, 3.0 mm × 100 mm (p/n: 186006047)
Sample temp.:	4 °C	4 °C
Column temp.:	45 °C	45 °C
Injection volume:	20.0 µL	5.7 µL
Flow rate:	1.50 mL/min	0.89 mL/min
Pre-injection volume:	NA	502 µL
Run time:	70 min	34 min

Gradient table:

Column gradient time		Gradient composition	
3.5 µm, 4.6 × 150 mm HPLC (min)	2.5 µm, 3.0 × 100 mm UHPLC (min)	Solution A (%)	Solution B (%)
0.0	0.0	100	0.0
25.0	11.90	100	0.0
60.0	28.57	29.3	70.7
60.1	28.62	100	0.0
68.0	32.38	100	0.0
70.0	34.0	100	0.0

Data management

Chromatography data software:	Empower 3 FR 4

Results and Discussion

Geometrically scaling the column and method conditions on a single instrument can impact the performance of the method. To maximize the column efficiency, the scaled column can be paired with an LC system[2] whose system dispersion is sufficient to minimize the effects of extra-column dispersion.[3] However, optimal column performance, in terms of highest efficiency, may not be required for an analysis. It may be possible to use a smaller particle size column on an HPLC system and still meet the method requirements. For example, the dispersion of the Alliance HPLC System allows for suitable analysis of a range of column particle sizes, from 5 µm to 2 µm, while maintaining the method requirements for the isocratic quetiapine assay method and the gradient quetiapine impurity method.

In addition to dispersion, it is important to consider the operating pressure limit of the LC system. When column particle size and column dimensions are scaled to smaller particles and ID, the resulting backpressure also typically increases. Therefore, geometrically scaling some methods may not be possible due to the pressure limits of the LC system. The Waters Columns Calculator tool can provide an estimated system pressure for a scaled method.[4] If necessary, certain parameters, such as the flow rate, may be altered to decrease the overall system pressure.

The quetiapine assay and impurities methods were first analyzed on the Alliance HPLC System with the prescribed monograph conditions.[1] The column dimensions and method conditions were then geometrically scaled to a UHPLC column with smaller particle size, inside diameter (I.D.), and length[4] using the Waters Column Calculator. Resolution, tailing, and RSDs for peak area and retention time were used to assess the chromatographic performance of the scaled methods. To verify the quantitative performance of the scaled methods, the amount of API and impurity in an unknown sample was determined.

Scaling of the isocratic assay method

To maintain the L/d_p ratio, the USP monograph isocratic assay method was scaled to a 3.0 × 150

mm, 3.5 µm particle column. The Waters Columns Calculator was used to determine the scaled flow rate of 0.79 mL/min and injection volume of 12.8 µL (Figure 1). However, the scaled flow rate 0.79 mL/min had to be decreased to 0.60 mL/min to avoid over-pressurizing the LC system.

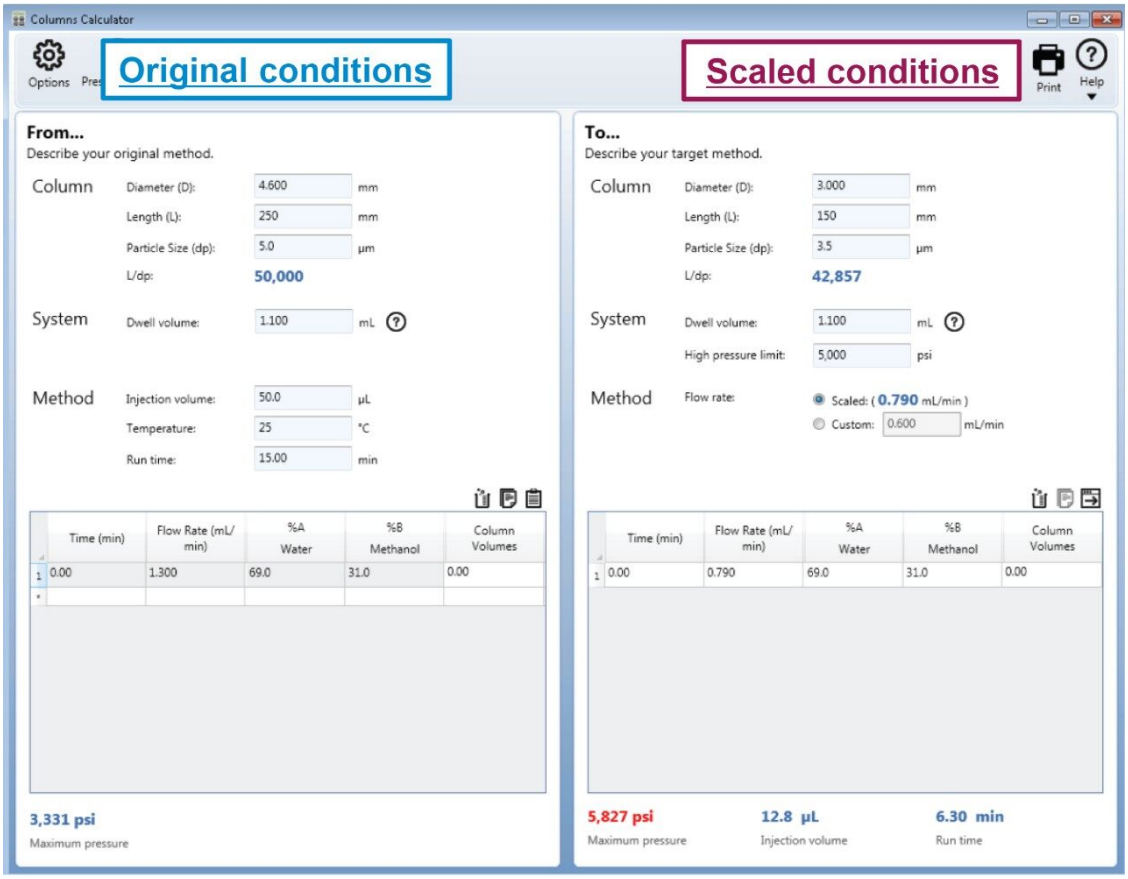

Figure 1. Waters Columns Calculator for scaling the isocratic assay method from an HPLC to UHPLC column. In this instance, the scaled flow rate was decreased because of the system pressure being too high while running at the scaled flow rate, as can be seen in the Calculator with the estimated maximum system pressure highlighted in red.

Results for six replicate injections of the standard solution and the system suitability solution are shown in Table 1. The resolution, tailing, and RSDs for peak area and retention time for the original HPLC method and the scaled UHPLC method showed similar chromatographic performance. The small decrease in the resolution of peaks 3 and 4 may be a result of the Alliance HPLC System dispersion, the decrease in L/d_p from the HPLC to UHPLC column, or the decrease in flow rate from 0.79 to 0.60 mL/min, all of which result in a less favorable relationship between the extra-column volume and peak volumes. Scaling the original assay method decreased the run time by 57% and the

solvent consumption by 66%. Chromatograms of the system suitability solution for the assay method are shown in Figure 2.

Alliance HPLC System	Resolution (peak 3 and 4)	Quetiapine tailing	Quetiapine area %RSD	Quetiapine retention time %RSD	Run time (minutes)	Solvent consumption per sample (mL)
HPLC Column	2.4	1.2	0.09	0.16	15	19.5
UHPLC Column	1.9	1.2	0.27	0.05	7	5.6

Table 1. Quetiapine assay method results on the Alliance HPLC System.

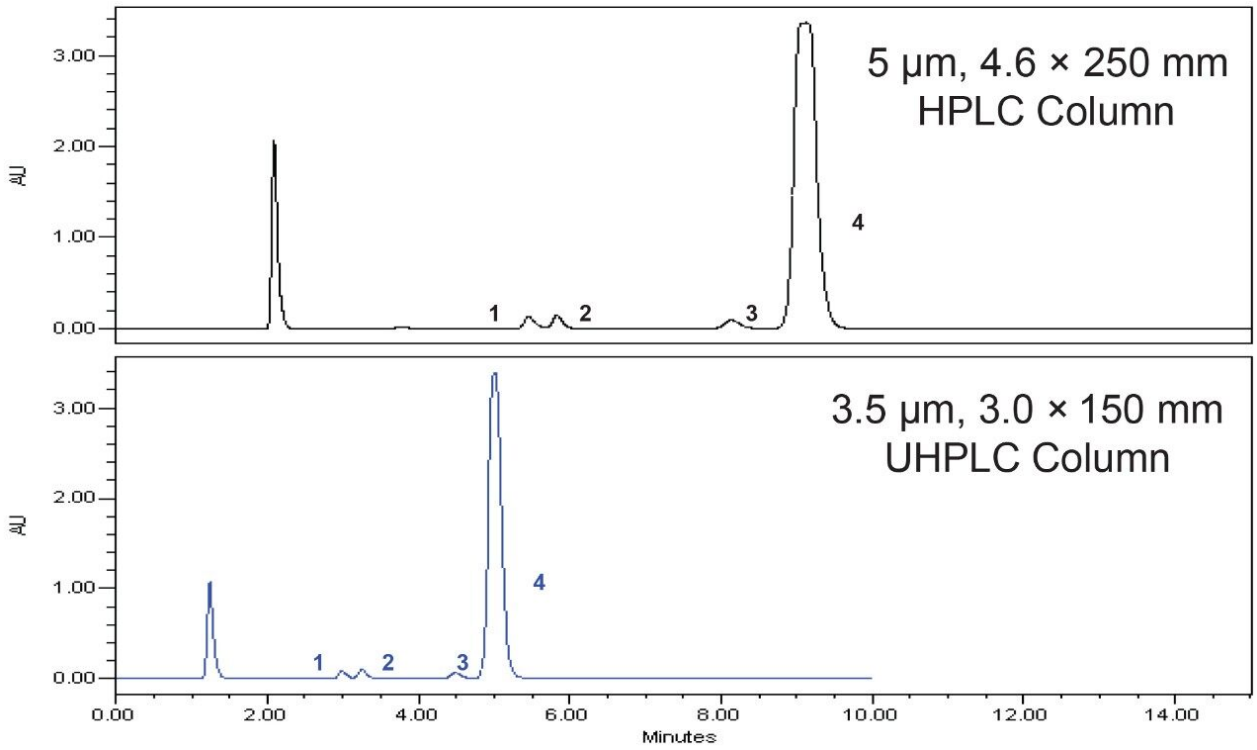

Figure 2. Comparison of the system suitability solution for the isocratic assay method. Peak identification: 1: quetiapine related compound G, 2: quetiapine related compound B, 3: quetiapine desethoxy, and 4: quetiapine.

To assess the quantitative performance of the scaled method, the percent of quetiapine fumarate present in an unknown sample (Table 2) was calculated 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).

Using the original HPLC method and the scaled method, the amount of API in the unknown sample was measured to be 109.5% and 110.1%, respectively. The similar results demonstrate the ability to scale the assay method with no impact on the quantitative results.

Alliance HPLC System	Unknown sample API result (%)
HPLC Column	109.5
UHPLC Column	110.1

Table 2. Quetiapine assay method results for an unknown sample on the Alliance HPLC System.

Scaling of the gradient impurities method

The quetiapine impurities method was scaled from the prescribed HPLC column to a 3.0 × 100 mm, 2.5 µm particle column. Again, the column adjustment maintains the L/d_p ratio. Using the Waters Columns Calculator, the scaled flow rate is 0.89 mL/min with an injection volume of 5.7 µL. The Waters Column Calculator calculates the gradient timing steps according to column volumes.[5]

The results for six replicate injections of the standard solutions are shown in Table 3. The original HPLC method and the scaled UHPLC method showed comparable chromatographic performance in terms of resolution, tailing, and peak area and retention time RSDs. The small decrease in resolution between peaks 1 and 2 may again be due to the Alliance HPLC System extra-column dispersion or the decrease in column dimensions going from the HPLC to UHPLC column. Scaling the original USP impurity method to a 2.5 µm particle column decreased the run time by 51% and the solvent usage by 71%. Chromatograms of the system suitability solution for the impurity method are shown in Figure 3.

Alliance HPLC System	Resolution (peak 1 and 2)	Resolution (peak 3 and 4)	Quetiapine tailing	Quetiapine area %RSD	Quetiapine retention time %RSD	Run time (min)	Solvent consumption per Sample (mL)
HPLC Column	11.0	6.6	1.00	0.84	0.14	70	105
UHPLC Column	9.0	6.2	0.97	0.93	0.03	34	30

Table 3. Quetiapine impurity method scaling results on the Alliance HPLC System.

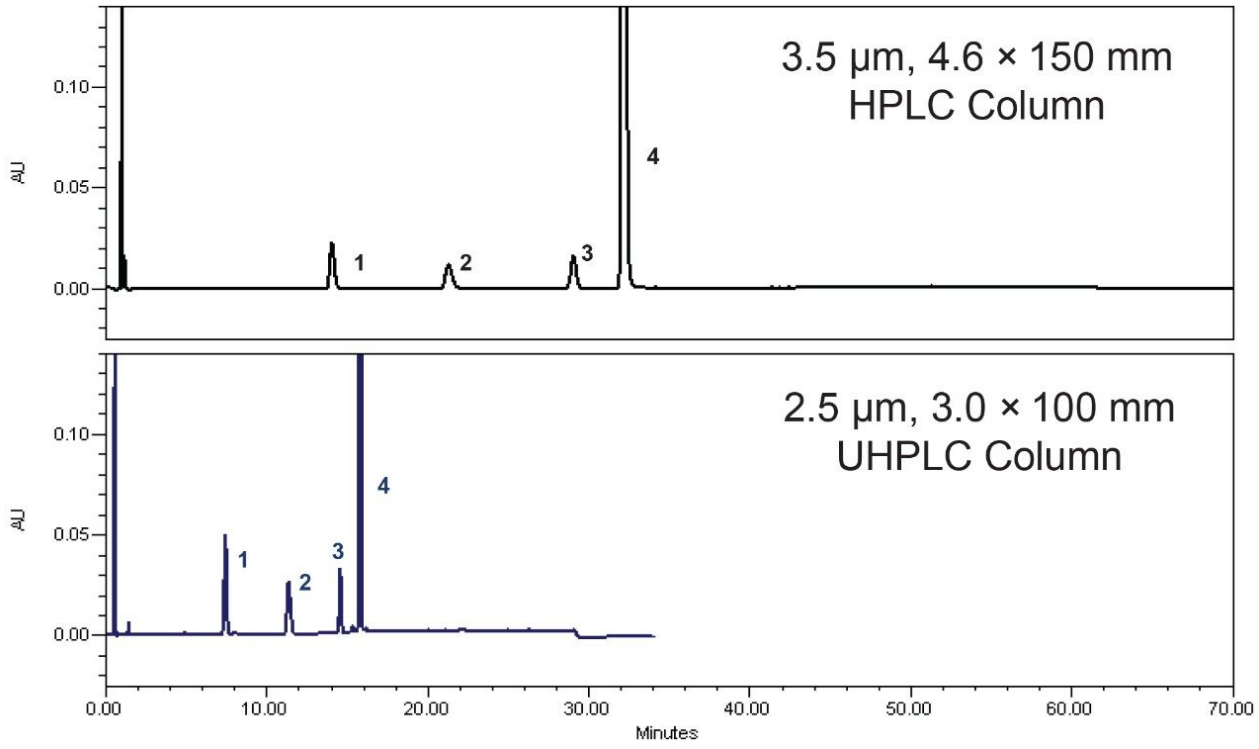

Figure 3. Comparison of the system suitability solution for the USP gradient impurity method. Peak identification: 1: quetiapine related compound G, 2: quetiapine related compound B, 3: quetiapine desethoxy, and 4: quetiapine.

The quantitative performance of the scaled impurity method was assessed from the calculated impurity content of an unknown sample (Figure 4) using the equation:

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

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.[1]

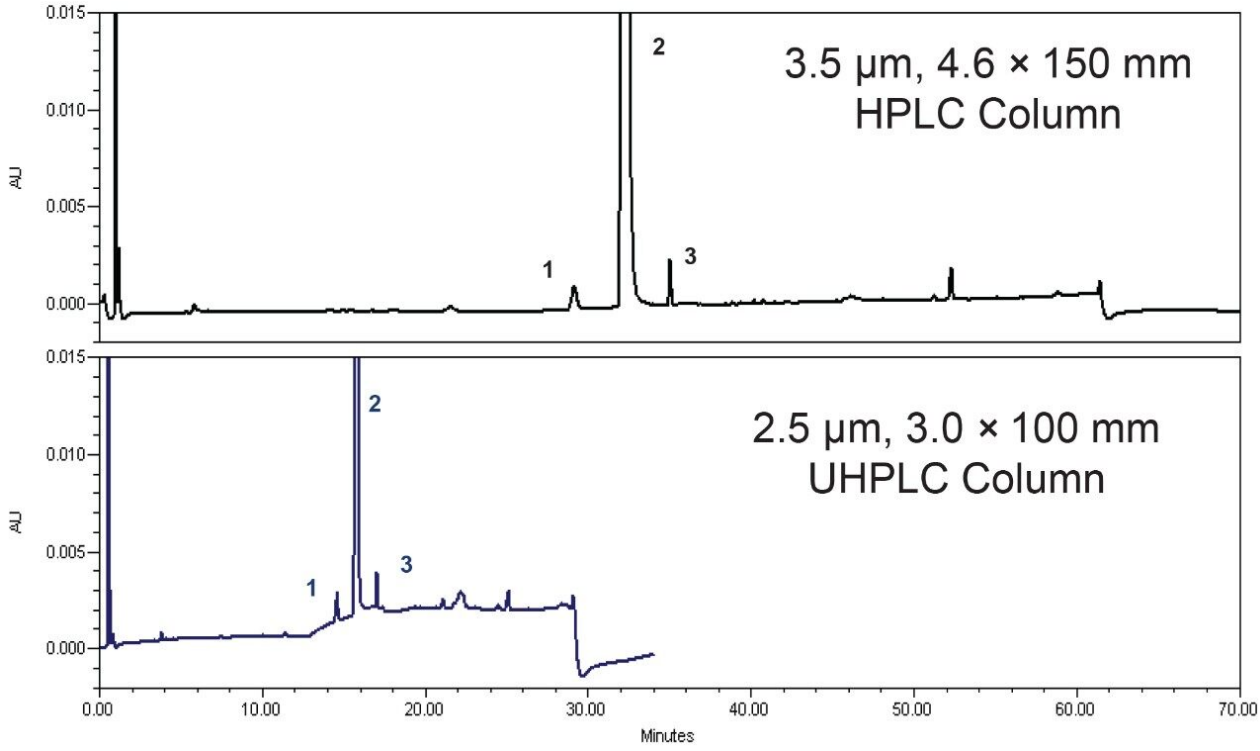

Figure 4. Comparison of the unknown sample for the impurity method on the Alliance HPLC System. Peak identification: 1: quetiapine desethoxy, 2: quetiapine, and 3: unknown impurity.

The unknown sample contained two impurity peaks, quetiapine desthoxy and an unknown impurity. The calculated results for the original method and the scaled method are in Table 4.

Unknown sample	Quetiapine desethoxy	Unknown impurity	Total impurities
HPLC Column	0.10%	0.07%	0.17%
UHPLC Column	0.12%	0.08%	0.20%

Table 4. Quetiapine impurity method results for the unknown sample analyzed on the Alliance HPLC System.

Using the original HPLC method and the scaled method, the amount of total impurities in the

unknown sample was calculated to be 0.17% and 0.20%, respectively. Scaling the USP quetiapine fumarate impurities method on the Alliance HPLC System produced equivalent quantification of impurities contained within a sample of the API.

Conclusion

It is possible to scale HPLC isocratic and gradient methods to smaller particle size columns on an Alliance HPLC System. As a result of scaling to smaller particles, the resolution of critical pairs may decrease, but if the resolution stays within the method requirements the changes are acceptable. Both the isocratic assay method and the gradient impurities method were successfully scaled to smaller particle size columns using the Waters Columns Calculator. Without changing the LC System, the run time of the assay method was decreased by 57% and the impurity method run time was decreased by 51%. Additionally, the scaled methods produced similar chromatographic performance in terms of resolution, peak tailing, and retention time and peak area RSD when compared to the original HPLC method.

References

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