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アプリケーションノート

A Scaled USP Method for Fluconazole Using an ACQUITY UPLC I-Class System and CORTECS T3 Column Chemistry

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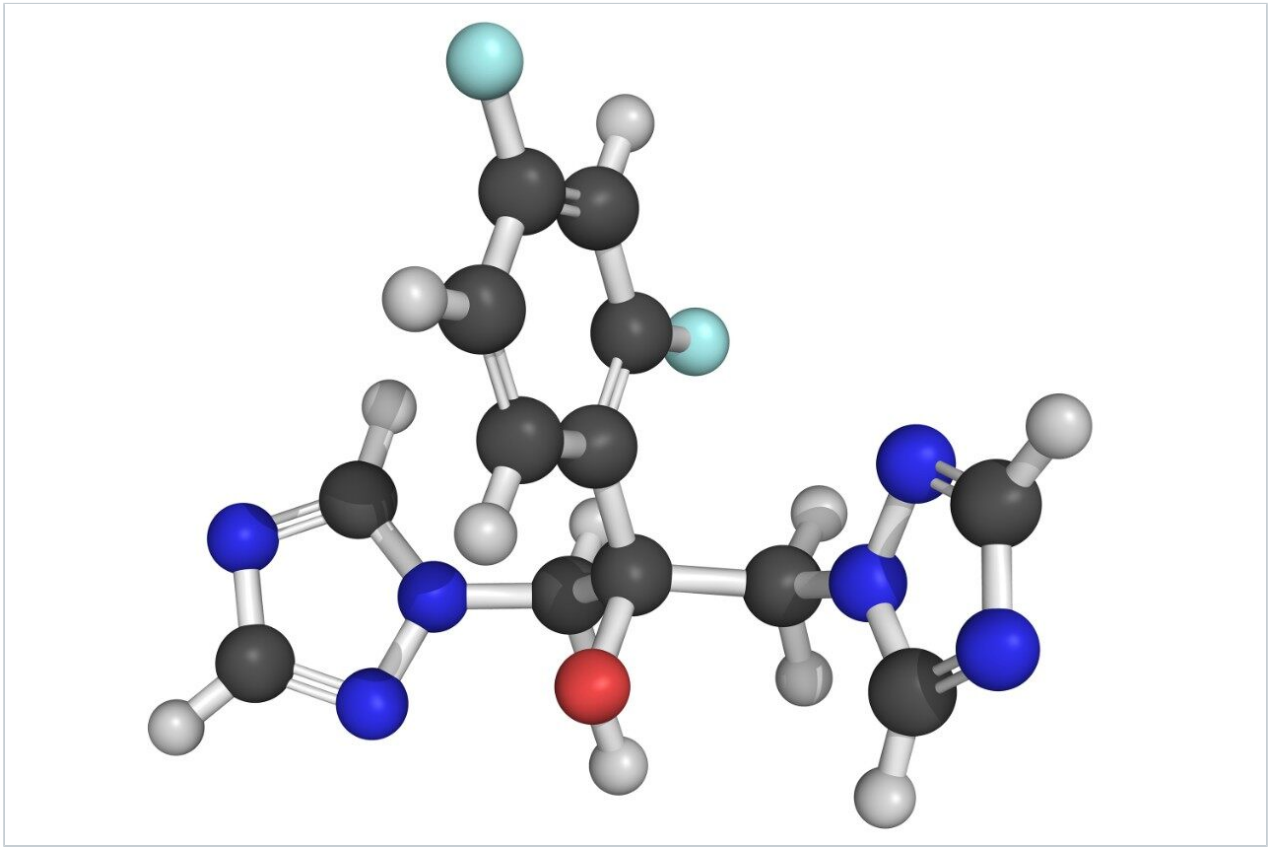

Abstract

This application note desbribes how we can increase throughput by scaling methods from HPLC to UPLC.

The Waters Columns Calculator is a useful tool when scaling methods by providing the user with scaled flow rates, injection volumes, and gradient tables (when applicable) while taking into account the particle porosity. Additionally, even within a specified 'L1' column packing, there are a number of C_{18} columns each differing in selectivity and retention. The CORTECS UPLC T3 Column chemistry proved to be a suitable column for the analysis of fluconazole and related compounds meeting all system suitability requirements of the USP monograph. The solid-core particle of the CORTECS Column provided narrower peak widths and improved efficiencies while reducing the total run time by approximately half when compared to a typical UPLC column and by more than eighty percent when compared to the original HPLC method.

Benefits

Increased throughput by scaling methods from HPLC to UPLC, additional selectivity with CORTECS T3 Columns

Introduction

Many current USP methods are designed for use with larger particle size (3 µm or greater) HPLC columns and instrumentation, which results in long run times and large volumes of hazardous solvents being consumed. It is possible to scale isocratic methods to sub-2-µm particle columns, which provides the same or improved performance with shorter run times resulting in increased throughput and lower solvent consumption. In order to scale methods appropriately, a number of USP guidelines must be followed.[1] For example, the L/d_p ratio, where L refers to the length of the column and d_p refers to the diameter of the particles packed within the column, must remain within -25% to +50% of the column specified in the original HPLC method. It is critical to be aware of these regulatory requirements when scaling from HPLC to UPLC methodologies.

In addition to method conditions and suitability requirements, USP monographs also specify the type of column packing material to be used for a particular analysis. For example an L1 packing refers to an octadecylsilane or C_{18} column packing material. However, there are numerous types of C_{18} columns, with differences in end-capping, the base particle, etc, which can all have an effect on the retention and selectivity of compounds. Therefore, it may be necessary to screen a number of columns when preparing to run a USP method to find one that meets system suitability requirements, which often includes a specified resolution between critical pairs.

Experimental

Sample description

Fluconazole and fluconazole related compounds A, B, and C were purchased from United States Pharmacopeia. Samples were initially dissolved in acetonitrile, then diluted with 80:20 (v:v) water: acetonitrile per the USP monograph for fluconazole. Samples were vortexed and sonicated to ensure complete dissolution. The final concentration of the sample was of 10 µg/mL for all reference compounds.

LC conditions

LC system:	ACQUITY UPLC I-Class System with CH-A
Detection:	ACQUITY UPLC PDA Detector
Sample:	Fluconazole and fluconazole related compounds A, B, and C (USP catalog numbers 1271700, 1271711, 1271722, and 1271733 respectively)
Columns:	CORTECS UPLC C_{18}+, 1.6 µm, 2.1 x 75 mm CORTECS UPLC T3, 1.6 µm, 2.1 x 75 mm ACQUITY UPLC HSS T3, 1.8 µm, 2.1 x 75 mm
Column temp.	40 °C
Mobile phase A:	Water
Mobile phase B:	Acetonitrile
Mobile phase composition:	80:20 Mobile phase A: Mobile phase B

For CORTECS UPLC C18+, 1.6 µm, 2.1 x 75 mm and CORTECS UPLC T3, 1.6 µm, 2.1 x 75 mm

Flow rate:	0.228 mL/min
Injection volume:	1.5 µL

For ACQUITY UPLC HSS T3, 1.8 µm, 2.1 x 75 mm

Flow rate	0.203 mL/min
Injection volume:	2.1 µL
Wavelength:	260 nm
Collection rate:	10 Hz
Needle wash:	90/10 methanol/water
Seal wash:	80/20 water/methanol
Data management:	Empower 3 FR2

Results and Discussion

The USP method for the analysis of fluconazole and related compounds (Organic Impurities, Procedure I[3]) was scaled to a UPLC method using the Waters Columns Calculator (Figure 1). Scaling from a 3.5 µm, 4.6 x 150 mm column to a 1.6 µm, 2.1 x 75 mm column results in a L/d_p ratio increase of 9.4%, which is well within the USP criteria (-25% to +50% of the original method L/d_p). The flow rate, scaled accounting for particle size, decreased from 0.500 mL/min in the original method to 0.228 mL/min in the updated method, while the injection volume decreased from the original value of 20 µL to 1.5 µL. Note that the calculator allows the user to specify the porosity factor (porous or superficially porous) to account for the differences in column volumes based on the porosity of the particle.

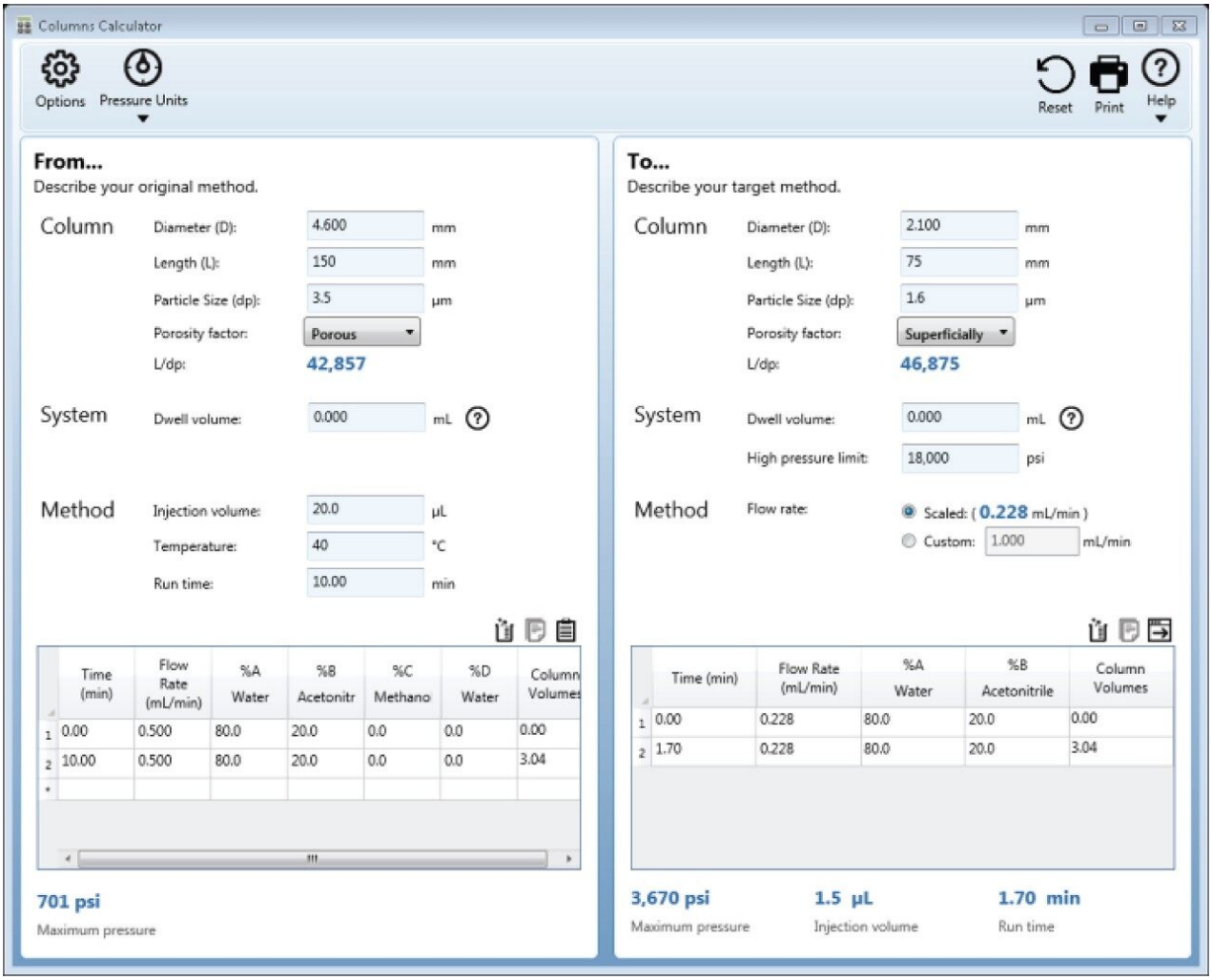

Figure 1. Waters Columns Calculator showing the original USP monograph HPLC method scaled to UPLC utilizing a CORTECS UPLC 1.6 µm, 2.1 x 75 mm Column.

The appropriately scaled method was then run on the ACQUITY I-Class UPLC System using both CORTECS UPLC C_{18}+ and CORTECS UPLC T3 chemistries. CORTECS Columns utilize solid-core particles (also sometimes referred to as superficially porous), which provide an increase in chromatographic performance and speed when compared to fully-porous particles. The CORTECS C_{18}+ Column has a charged surface, which in addition to giving exceptional peak shape also provides a unique selectivity. The CORTECS T3 Column is designed for improved retention of polar compounds. Both chemistries are classified as L1 packings, so either would be acceptable to use per the USP monograph. Example chromatograms of the separations acquired on each column are shown below (Figure 2). The CORTECS UPLC C_{18}+ Column did not provide baseline separation of fluconazole and related compound C under the scaled USP method conditions. The CORTECS UPLC T3 Column in comparison provided baseline separation for fluconazole and all related compounds A–C.

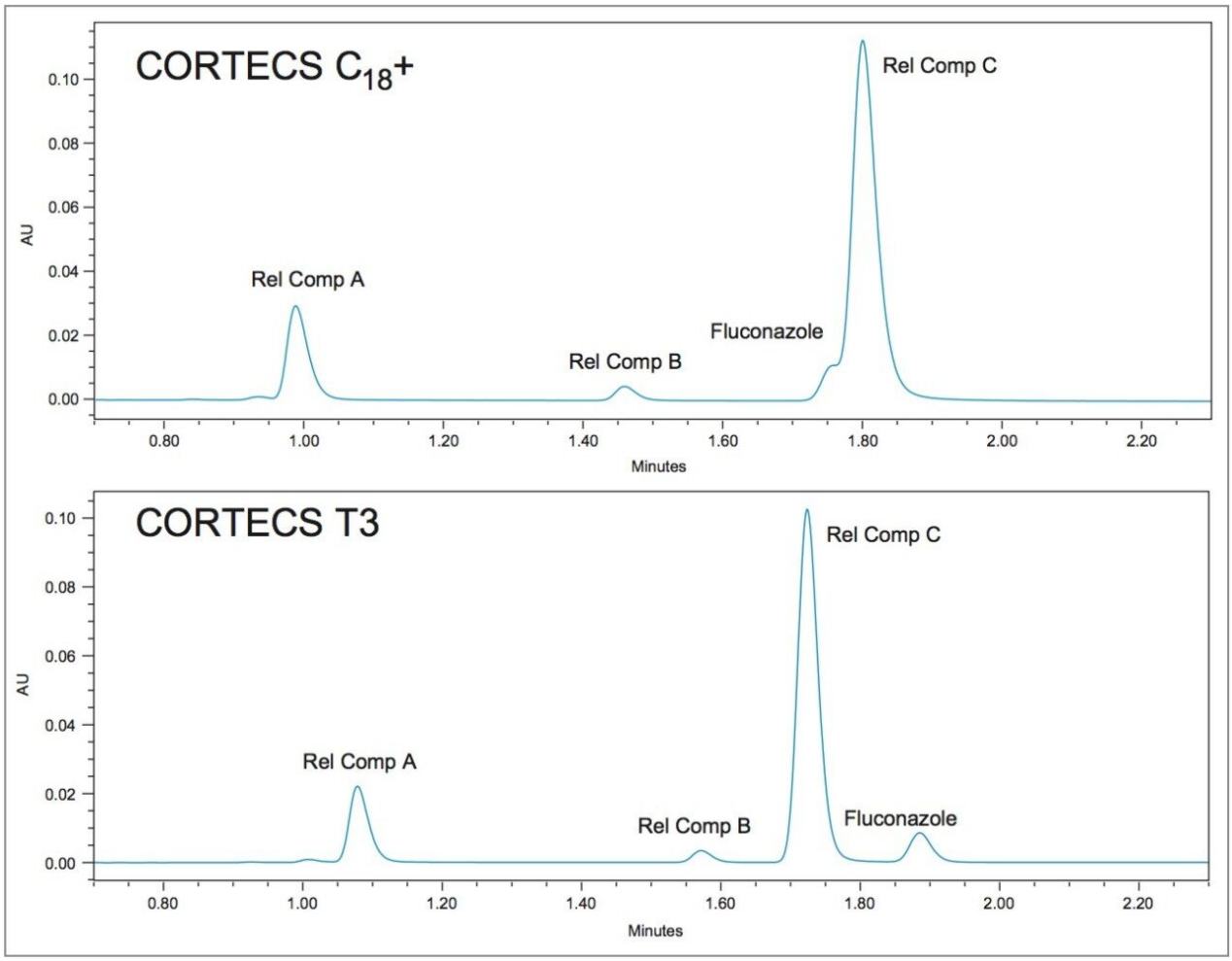

Figure 2. Comparison of fluconazole USP method run on a CORTECS UPLC C_{18}+ Column (p/n 186007115) (top) and a CORTECS UPLC T3 Column (p/n 186008498) (bottom).

System suitability requirements for fluconazole and fluconazole related compounds A-C state that resolution between related compound B and related compound C must be NLT (not less than) 1.5. Additionally, relative standard deviation must be NMT (not more than) 5.0% for both retention time and area. The results obtained on the CORTECS T3 are listed in Table 1.

	Retention time (min)	Retention time RSD	Area RSD	Resolution
System suitability requirement	N/A	≤5.0	≤5.0	≥1.5 for rel. comp B and rel. comp C
Related compound A	1.08	0.08	0.18	–
Related compound B	1.57	0.05	0.53	9.38
Related compound C	1.72	0.07	0.17	2.80
Fluconazole	1.88	0.05	0.27	2.83

Table 1. Chromatographic performance results for fluconazole and related compounds run on an ACQUITY UPLC I-Class System using a CORTECS UPLC T3 Column.

In addition to meeting the system suitability requirements using the CORTECS UPLC T3 chemistry, the run time was decreased from 10 minutes in the original HPLC method to approximately 2 minutes in the scaled UPLC method.

To further highlight the increased efficiency and throughput using solid-core particles, the method was also run on a fully-porous ACQUITY UPLC HSS T3 1.8 µm, 2.1 x 75 mm Column (p/n 186005614) (conditions were scaled accounting for particle size) and shown in Figure 3.

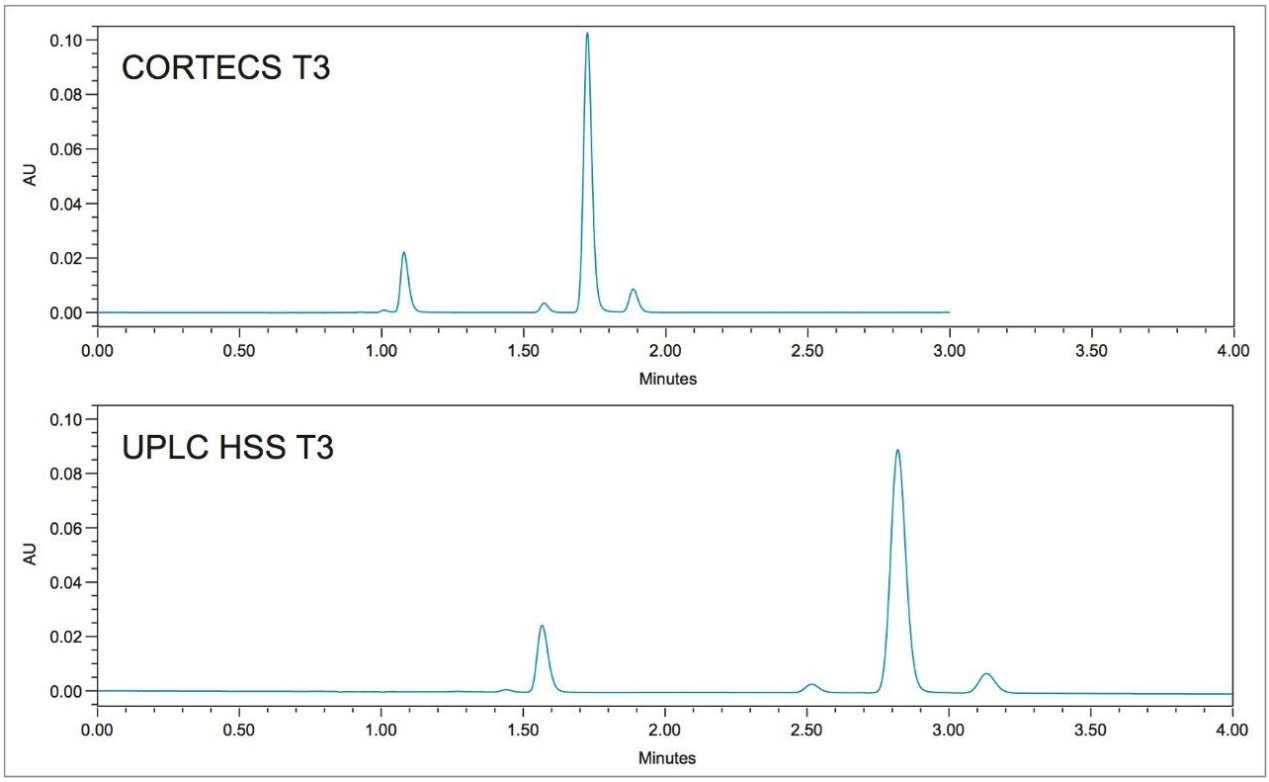

Figure 3. Comparison of a solid-core particle (CORTECS T3 Column – top) and a fully-porous particle (ACQUITY UPLC HSS T3 Column – bottom). ACQUITY UPLC HSS T3 Column used a flow rate of 0.203 mL/min and injection volume of 2.1 µL.

Despite the difference in particle size, use of the solid-core particle resulted in earlier elution of the compounds and a decrease in the run time by nearly 50%. In addition, all peak widths are narrower on the solid-core column. The equation used to calculate efficiency (N), states:

$$N = 5.54\,[t_R/W_{1/2}]^2$$

where t_R is the peak retention time, and W1/2 is the peak width at half height.[2] From this equation, the efficiency is dependent not only on the peak width, but also on the retention time of the peak. The earliest eluting peak (fluconazole related compound A) shows nearly conserved efficiency on the CORTECS UPLC T3 Column vs the ACQUITY UPLC HSS T3 Column. This is because the decrease in retention time effectively cancels out the gains from a decrease in peak width. All other peaks show an increase in column efficiency

due to the solid-core particle of the CORTECS Column. Decreased run time, narrower peak widths, and an increase in efficiency are some of the advantages of using a solid-core particle (Figure 4).

	Peak width at 50% (s)		Efficiency	
	CORTECS T3	UPLC HSS T3	CORTECS T3	UPLC HSS T3
RelComp A	1.80	2.57	7049	7216
RelCompB	1.81	3.27	14753	11603
RelComp C	1.92	3.55	15769	12303
Fluconazole	2.02	3.87	17180	12812

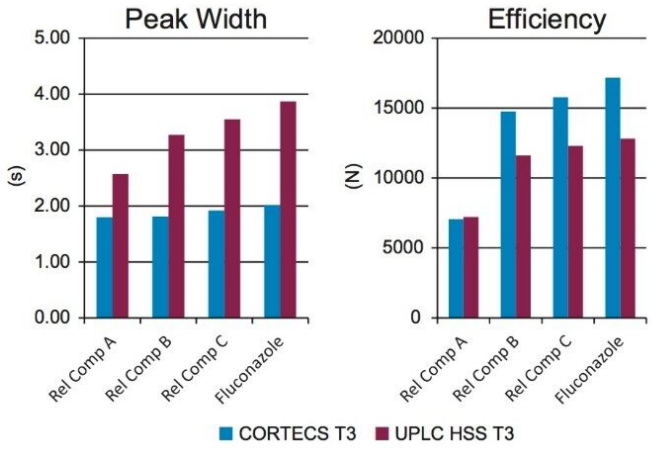

Figure 4. Comparison of results for fluconazole and related compounds obtained on a solid-core particle (CORTECS T3) and a fully-porous particle (ACQUITY UPLC HSS T3).

Conclusion

The ability to scale traditional HPLC methods to modern UPLC methods provides equivalent or enhanced performance while decreasing run time and solvent consumption. The Waters Columns Calculator is a useful tool when scaling methods by providing the user with scaled flow rates, injection volumes, and gradient tables (when applicable) while taking into account the particle porosity. Additionally, even within a specified 'L1' column packing, there are a number of C_{18} columns each differing in selectivity and retention. The CORTECS UPLC T3 Column chemistry proved to be a suitable column for the analysis of fluconazole and related compounds meeting all system suitability requirements of the USP monograph. The solid-core particle of the CORTECS Column provided narrower peak widths and improved efficiencies while reducing the total run time by approximately half when compared to a typical UPLC column and by more than eighty percent when compared to the original HPLC method.

References

1. Chapter <621> CHROMATOGRAPHY. United States Pharmacopeia and National Formulary (USP 37-NF 32 S2) Baltimore, MD: United Book Press, Inc.; 2014. p. 6376–85.
2. Snyder, Lloyd R., Kirkland, Joseph J., Dolan, John W., *Introduction to Liquid Chromatography*, 3rd Edition. Hoboken: Wiley & Sons, 2010. Print.
3. Official Monographs, USP 37 NF32 S2. United States Pharmacopeia and National Formulary (USP 37-NF 32 S2) Baltimore, MD: United Book Press, Inc.; 2014. p. 4968.

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