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Meeting and Surpassing System Suitability for USP Fluconazole and Related Impurities Using Kinetex® Core-Shell HPLC/UHPLC Columns

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Introduction

Fluconazole is a third generation triazole antifungal drug with broad spectrum activity against systemic and superficial fungal infections. It is one of the most widely used antifungal agents on the market today. As a result, the development of a quick and efficient analysis of Fluconazole and its related impurities poses significant interest. For this report, we focused on Fluconazole and related impurities as identified in the US Pharmacopeia monograph. We were able to show better resolution for Fluconazole and the related compound impurities. The USP monograph requires that the resolution between Fluconazole related compound B and C be no less than 1.5 to meet system suitability; this was achieved here. In order to maximize performance and speed up analysis time, HPLC columns packed with core-shell (superficially porous) silica particles bonded with a C18 phase were used. The performance of the Kinetex core-shell columns used here was compared to that of the Inertsil® ODS-3 and all method parameters were consistent with the USP monograph for Fluconazole.

Conditions

LC Conditions	
Column:	Inertsil 3 µm ODS-3 Kinetex 5 µm C18 Kinetex 2.6 µm C18
Dimensions:	150 x 4.6 mm 100 x 4.6 mm (Kinetex 2.6 µm C18 only)
Pressure (bar):	96 bar (Inertsil 3 µm ODS-3 150 x 4.6 mm) 56 bar (Kinetex 5 µm C18 150 x 4.6 mm) 132 bar (Kinetex 2.6 µm C18 150 x 4.6 mm) 98 bar (Kinetex 2.6 µm C18 100 x 4.6 mm)
Mobile Phase:	Water/Acetonitrile (80:20) premixed
Flow Rate:	500 µL/min
Temperature:	40 °C
Detection:	UV @ 260 nm
Injection Volume:	20 µL
Instrument:	Agilent 1100 Quaternary HPLC system with a temperature-controlled column selector

Experimental Procedures

All reference solutions were obtained from USP and were prepared as indicated in the USP monograph for Fluconazole. Evaluation was performed with the Kinetex 5 µm and 2.6 µm 150 x 4.6 mm, and Kinetex 2.6 µm 100 x 4.6 mm C18 (Phenomenex, Torrance, California, USA) and the results from these columns were compared with the Inertsil 3 µm ODS-3 150 x 4.6 mm column (GL Sciences, Inc., Shinkjuku City, Tokyo, Japan).

To ensure that all results were comparable, all columns used in this study were tested using the same isocratic performance test conditions to confirm they were operating within the expected performance levels. The system used for this study was the Agilent® 1100 Quaternary HPLC system with a temperature-controlled column selector.

The sample solution of Fluconazole was diluted in mobile phase to a concentration of 3 mg/mL. For standard solutions, 10 µg/mL each of USP Fluconazole RS, USP Fluconazole Related Compound A RS, USP Fluconazole Related Compound B RS, and USP Fluconazole Related Compound C RS was dissolved in acetonitrile and then diluted in mobile phase. The mobile phase consisted of an 80:20 mixture of Water/Acetonitrile. System suitability was determined as a resolution no less than 1.5 between Fluconazole Related Compound B and Fluconazole Related Compound C, with a relative standard deviation no more than 5.0 % for each peak with 6 replicate injections, per the USP monograph for fluconazole. The LC conditions are listed above and were used to generate all of the data in this technical note.

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Results and Discussion

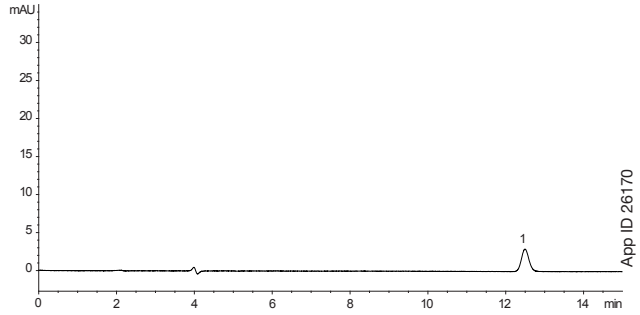
The standards were first run on the Inertsil® ODS-3 column in order to properly identify the compounds since this was the column that was referenced on the USP monograph. Each compound had a clearly defined peak and showed the elution order for Fluconazole and the three related compounds, A, B, and C using the conditions as published in the USP method for Fluconazole (**Figure 1**).

Next, a mixture of the standards was run on all four columns. As can be seen in **Figure 2**, all of the peaks were clearly defined and separated on the Inertsil column. All three of the Kinetex® columns showed clearly defined peaks with the same elution order as observed on the Inertsil column but showed shorter retention times. **Table 1** shows the summary of the resolution data between Fluconazole related compounds B and C. In order to meet system suitability as outlined in the USP monograph for Fluconazole, the resolution between compounds B and C must be no less than 1.5. As shown here, all four of the columns achieved this requirement. The percent relative standard deviation (%RSD) must be no more than 5 % for each peak, according to the USP monograph for Fluconazole. Again, all separations on all columns met this requirement (**Table 2**). As expected, the Kinetex columns showed higher resolution as compared to the Inertsil column, due to the inherent advantage of core-shell columns providing higher efficiency than corresponding fully porous columns.

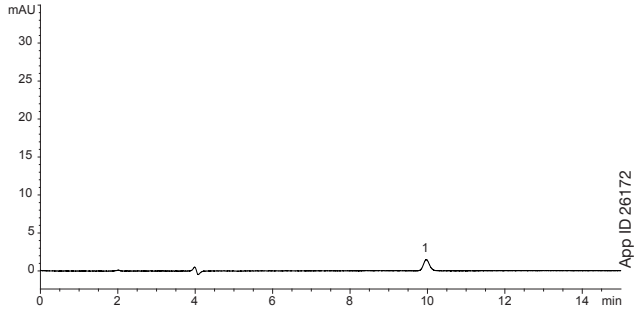
Figure 3 shows the chromatograms of the Inertsil ODS-3 column and three Kinetex columns run with a 3 mg/mL sample of Fluconazole. Because of the size of the Fluconazole peak, related compound C was not able to be separated on any of the columns used in this study. However, each of the Kinetex columns showed a faster run time without losing resolution as compared to the Inertsil ODS-3 column.

Figure 1
Peak identification, individual standards at 10 µg/mL on Inertsil 3 µm ODS-3 150 x 4.6 mm

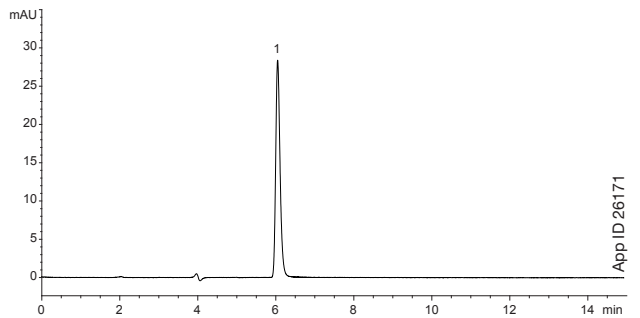
Fluconazole 10 µg/mL



Fluconazole Related Compound B 10 µg/mL



Fluconazole Related Compound A 10 µg/mL



Fluconazole Related Compound C 10 µg/mL

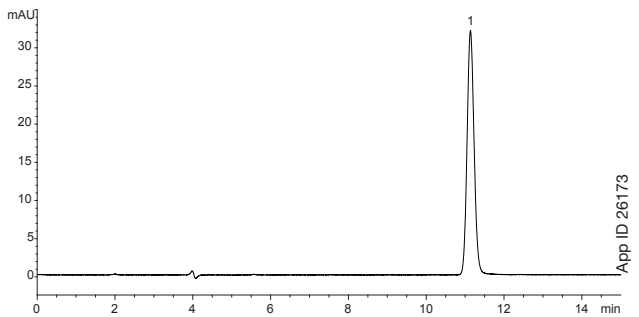


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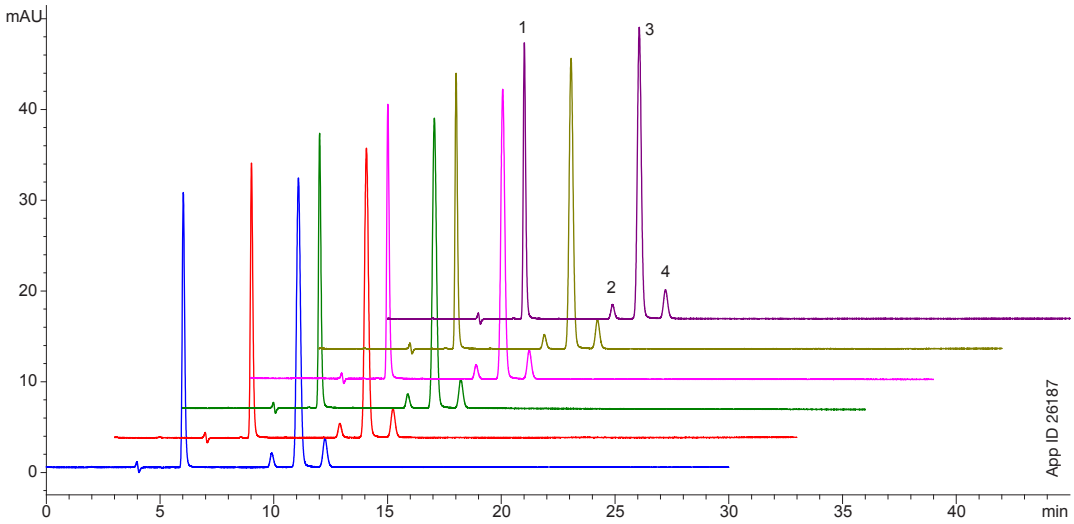
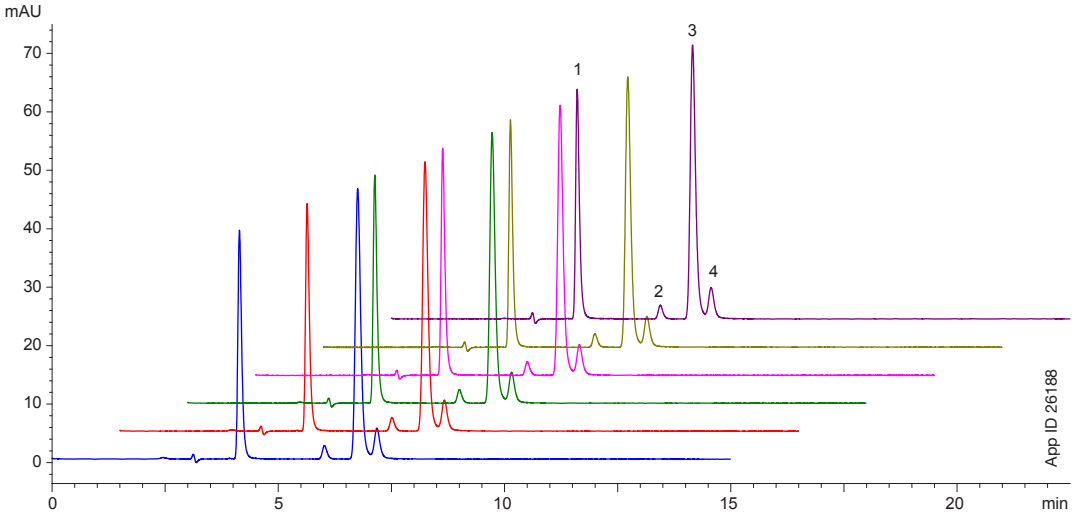
Figure 2
Standard Solution (Mix of all 4) 10 µg/mL of each; 6 replicates

Overlay of 6 injections for Inertsil® 3 µm ODS-3 150 x 4.6 mm



Peak No.	Analyte	Time	Area	Height	Width	Area%	USP Tailing Factor
1	Impurity A	6.013	236	30.4	0.1174	34.123	1.278
2	Impurity B	9.901	16.9	1.6	0.1801	2.444	1.115
3	Impurity C	11.065	397.2	32.1	0.1838	57.426	1.097
4	Fluconazole	12.222	41.5	3.2	0.1552	6.006	1.072

Overlay of 6 injections for Kinetex 5 µm C18 150 x 4.6 mm



Peak No.	Analyte	Time	Area	Height	Width	Area%	USP Tailing Factor
1	Impurity A	4.106	246.3	39.8	0.1031	34.787	1.479
2	Impurity B	5.943	17.7	2.3	0.1171	2.493	1.256
3	Impurity C	6.656	395	46.8	0.1304	55.782	1.332
4	Fluconazole	7.064	49.1	5.4	0.152	6.938	1.232

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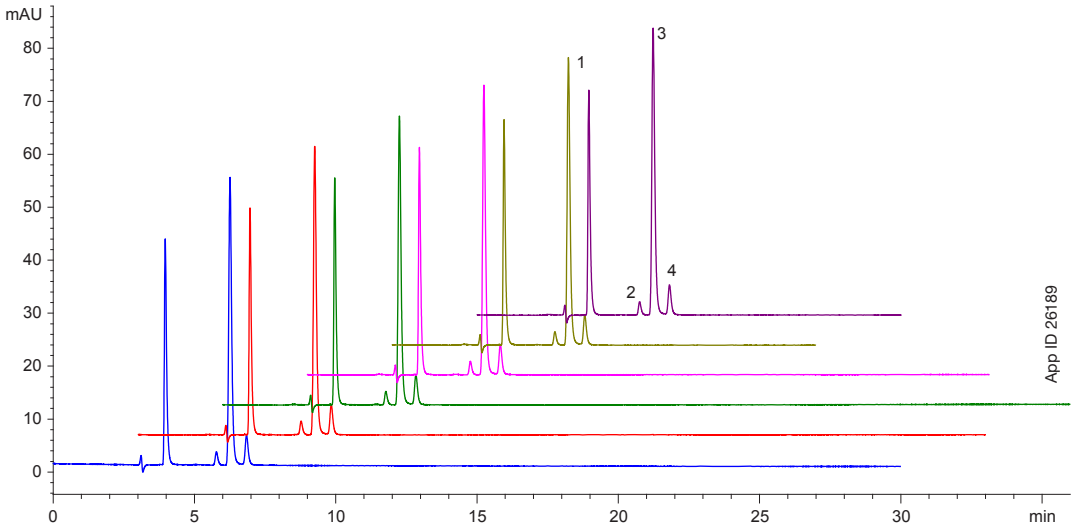
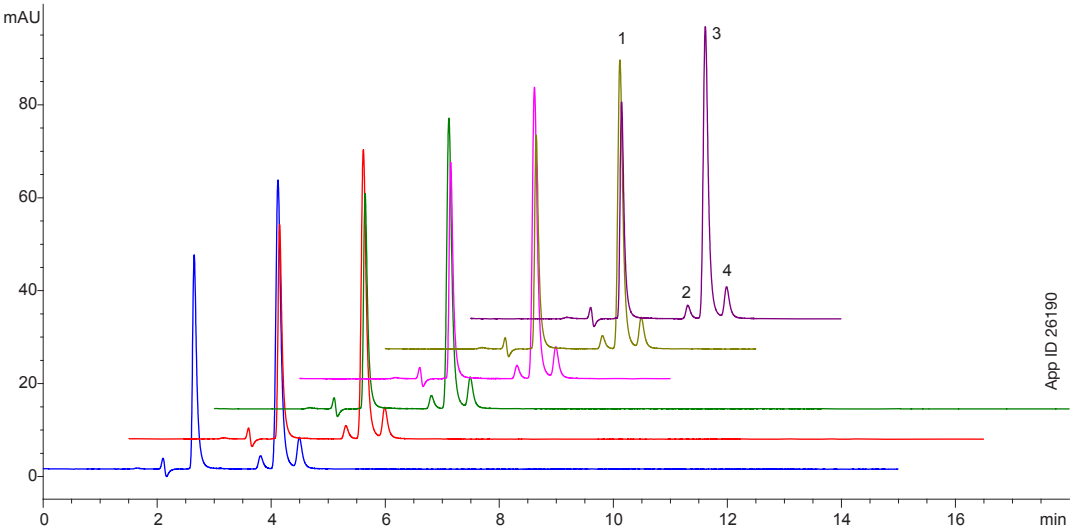
Overlay of 6 injections for Kinetex® 2.6µm C18 150 x 4.6mm



Peak No.	Analyte	Time	Area	Height	Width	Area%	USP Tailing Factor
1	Impurity A	3.962	235.7	43	0.0914	33.588	1.582
2	Impurity B	5.767	17.5	2.5	0.1065	2.487	1.395
3	Impurity C	6.246	398.4	54.5	0.1114	56.766	1.359
4	Fluconazole	6.827	41.5	5.6	0.116	5.913	1.234

Overlay of 6 injections for Kinetex® 2.6µm C18 100 x 4.6mm



Peak No.	Analyte	Time	Area	Height	Width	Area%	USP Tailing Factor
1	Impurity A	2.649	238.3	46.3	0.0858	34.062	1.734
2	Impurity B	3.814	16	2.8	0.0899	2.292	1.5
3	Impurity C	4.12	398.5	61.9	0.1013	56.959	1.515
4	Fluconazole	4.493	46.8	6.8	0.1149	6.686	1.363

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Table 1
Summary of the Resolution Data for Impurity B and C

Inj.	Inertsil® 3 µm ODS-3 150 x 4.6 mm	Kinetex® 5 µm C18 150 x 4.6 mm	Kinetex 2.6 µm 150 x 4.6 mm	Kinetex 2.6 µm 100 x 4.6 mm
1	3.84	3.6	2.76	2.03
2	3.84	3.62	2.7	2.03
3	3.85	3.55	2.69	2.02
4	3.79	3.59	2.67	2.035
5	3.83	3.57	2.68	2.04
6	3.93	3.58	2.7	2.05
Average	3.85	3.59	2.70	2.03
Std	0.05	0.02	0.03	0.01
%RSD	1.19	0.68	1.17	0.50

Table 2
Summary of Peak Area and %RSD for Compounds in Standard Solution

Compound		Inertsil® 3 µm ODS-3 150 x 4.6 mm	Kinetex® 5 µm C18 150 x 4.6 mm	Kinetex 2.6 µm 150 x 4.6 mm	Kinetex 2.6 µm 100 x 4.6 mm
Impurity A	**Average**	234.85	236.17	237.4	240.9
	STD	0.6348	0.4590	1.3565	10.556
	%RSD	0.2703	0.1943	0.5714	4.3819
Impurity B	**Average**	17.133	17.28	17.15	16.65
	STD	0.2503	0.1472	0.3619	0.3782
	%RSD	1.4611	0.8517	2.1104	2.2712
Impurity C	**Average**	397.05	393.93	401.55	400.13
	STD	0.7036	0.1506	0.4416	0.7607
	%RSD	0.1772	0.0382	0.11	0.1901
Fluconazole	**Average**	41.583	47.8167	43.9167	47.15
	STD	0.2858	0.4021	0.5154	0.5612
	%RSD	0.6872	0.8409	1.1737	1.1903

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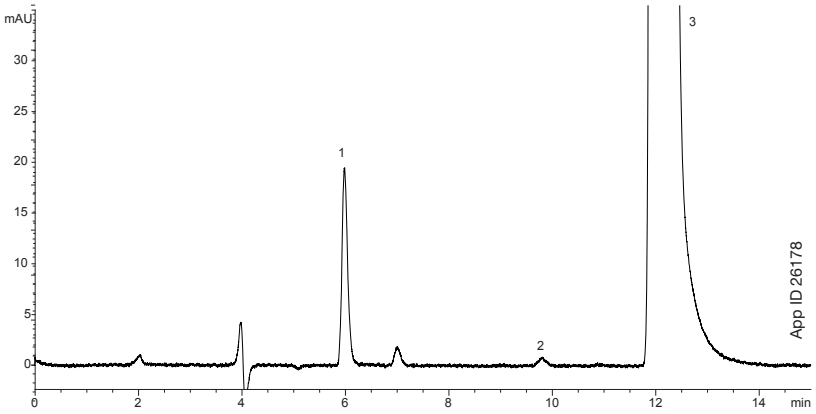
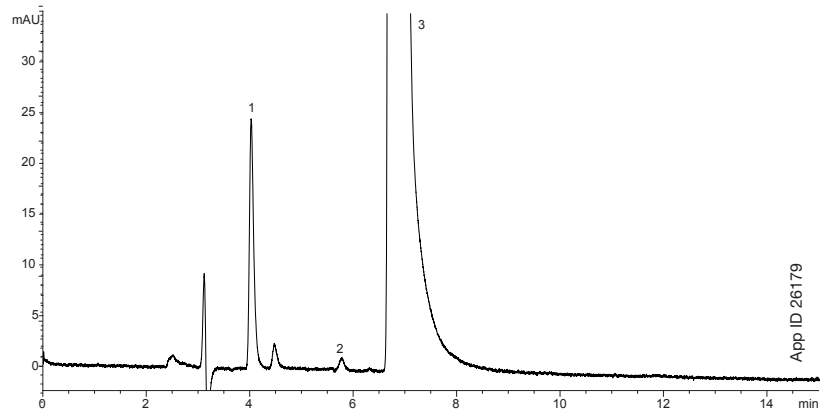
Figure 3
Sample Solution 3 mg/mL of Fluconazole

Inertsil 3 µm ODS-3 150 x 4.6 mm



Peak No.	Analyte	Time	Area	Height	Width	Area%	USP Tailing Factor
1	Impurity A	5.987	17.3	2.2	0.1204	0.253	1.253
2	Impurity B	9.798	1.2	9.9E-2	0.2073	0.018	1.176
3	Fluconazole	12.047	6806	479.5	0.2209	99.553	1.478

Kinetex 5 µm C18 150 x 4.6 mm



Peak No.	Analyte	Time	Area	Height	Width	Area%	USP Tailing Factor
1	Impurity A	4.017	16.9	2.9	0.0984	0.247	1.459
2	Impurity B	5.766	1.6	1.5E-1	0.1515	0.023	1.151
3	Fluconazole	6.758	6815.7	736.4	0.14	99.730	1.792

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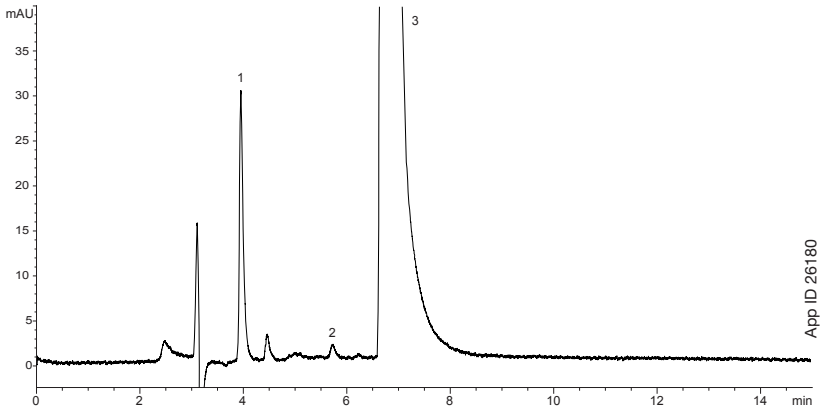
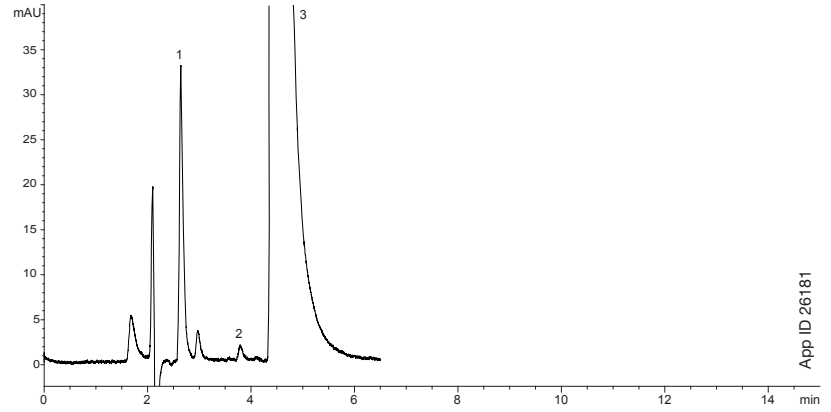
Figure 3 *(cont'd)*
Sample Solution 3 mg/mL of Fluconazole

Kinetex® 2.6 µm C18 150 x 4.6 mm



Peak No.	Analyte	Time	Area	Height	Width	Area%	USP Tailing Factor
1	Impurity A	3.952	16.8	3	0.0931	0.246	1.609
2	Impurity B	5.734	1.6	1.7E-1	0.1312	0.023	1.167
3	Fluconazole	6.739	6799.9	747.9	0.141	99.731	1.897

Kinetex 2.6 µm C18 100 x 4.6 mm



Peak No.	Analyte	Time	Area	Height	Width	Area%	USP Tailing Factor
1	Impurity A	2.642	16.7	3.3	0.0844	0.244	1.729
2	Impurity B	3.788	9.9E-1	1.7E-1	0.0992	0.014	1.561
3	Fluconazole	4.432	6826.5	886	0.1284	99.742	1.980

Conclusion

The results above clearly show that the resolution achieved on all the columns and replicate injections between Fluconazole related compound B and C met the minimum requirement of no less than 1.5. Also, the %RSD limits of no more than 5 % were met on all compounds used in this study. All conditions that were used were documented in the United States Pharmacopoeia monograph for Fluconazole. This would suggest that the Kinetex C18 columns meet the requirements for system suitability as set forth in the USP monograph for Fluconazole. Interestingly, in all cases each of the Kinetex C18 columns showed a faster run time. This is due to the nature of the core-shell particle. With a lower surface area, there is less C18 bonded phase leading to a decrease in retention. There was also higher peak heights and tighter peak widths as compared to the Inertsil® ODS-3, without losing resolution of the peaks. Additionally, the Kinetex 5 µm core-shell column provided a significant decrease in system backpressure.

Ordering Information

Kinetex® 5 µm Analytical Columns (mm)		SecurityGuard™ ULTRA Cartridges‡
Phases	150 x 4.6	3/pk
C18	00F-4601-E0	AJ0-8768
		for 4.6 mm ID

Kinetex 2.6 µm Analytical Columns (mm)			SecurityGuard ULTRA Cartridges‡
Phases	100 x 4.6	150 x 4.6	3/pk
C18	00D-4462-E0	00F-4462-E0	AJ0-8768
			for 4.6 mm ID

‡ SecurityGuard ULTRA Cartridges require holder, Part No.: AJ0-9000

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