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European Pharmacopeia Monograph 2287 Fluconazole: Efficient Separation on Fully Porous and Core-Shell C18 Columns Within the Allowable Adjustments

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Introduction

Fluconazole [2-(2,4-Difluorophenyl)-1,3-bis(1H-1,2,4-triazol-1-yl)propan-2-ol] is a triazole based systemic antifungal medication. It belongs to the first-generation antifungals used for oral treatment of Candida infections. The desirable pharmacologic properties of Fluconazole include a relatively long half-life, and the ability to be administered orally or parentally¹. The monograph for related substances of Fluconazole specifies three main impurities (A [2(RS)-2-(2,4-Difluorophenyl)-1-(1H-1,2,4-triazol-1-yl)-3-(4H-1,2,4-triazol-4-yl)propan-2-ol], B {2-[2-fluoro-4-(1H-1,2,4-triazol-1-yl)phenyl]-1,3-bis(1H-1,2,4-triazol-1-yl)propan-2-ol}, and C [1,1'-(1,3-phenylene)di-1H-1,2,4-triazole]) which need to be separated and detected to demonstrate compliance².

Conditions

LC-UV Conditions	
Columns:	Luna [®] 5 µm C18(2) 150 x 4.6 mm (00F-4252-E0) Kinetex [®] 2.6 µm C18 100 x 4.6 mm (00D-4462-E0)
Mobile Phase:	Isocratic (84:16) A: 0.63g/L Ammonium formate in water B: Acetonitrile
Flow Rate:	1.0 mL/min
Temperature:	40 °C
Detection:	UV @ 260 nm
Injection Volume:	20 µL
HPLC System:	Agilent [®] 1260 (Agilent Technologies [®] , Santa Clara, CA, USA)

Table 1

Preparation of Test and Reference Solutions

Solution	Step 1	Step 2	Step 3	Final Conc.
Test Solution	100mg Fluconazole	dissolve in mobile phase	sonicate if necessary and dilute to 10.0mL with mobile phase	10 mg/mL Fluconazole
Ref a	dilute 5.0mL Test Solution with mobile phase to 100.0mL	take 1.0mL	dilute with mobile phase to 10.0mL	0.05 mg/mL Fluconazole
Ref b	5mg Fluconazole for Peak Identification	dissolve in mobile phase	sonicate if necessary and dilute to 10.0mL with mobile phase	0.5 mg/mL Fluconazole
Ref c	3.0mg Fluconazole impurity B	dissolve in mobile phase	sonicate if necessary and dilute with mobile phase to 100.0mL	0.03 mg/mL impurity B
Ref d	3.0mg Fluconazole impurity C	dissolve in mobile phase	sonicate if necessary and dilute with mobile phase to 20.0mL	0.15 mg/mL impurity C
Ref e	1.0mL Ref d	add 1.0mL Test Solution	dilute with mobile phase to 10.0mL	0.015 mg/mL impurity C 1 mg/mL Fluconazole
Ref f	1.0mL Ref d	dilute with mobile phase to 10.0mL		0.015 mg/mL impurity C

Results

The test solution and reference solutions (a) to (f) have been prepared as indicated in the monograph for Fluconazole² and detailed in **Table 1**. **Figures 2a** and **2b** show the chromatograms for the peak identification of the Fluconazole peak. The respective retention times achieved are 12.86 min on Luna 5 µm C18(2) and 5.12 min on Kinetex 2.6 µm C18. The peak identification of impurity A and Fluconazole is shown in **Figures 3a** and **3b**. The retention time for impurity A is 6.33 min on Luna 5 µm C18(2) and

2.71 min on Kinetex 2.6 µm C18. Impurity B is identified through injection of reference solution (c) shown in **Figures 4a** and **4b**. The retention time for this impurity is 5.58 min on Luna 5 µm C18(2) and 2.49 min on Kinetex 2.6 µm C18. The last impurity – impurity C – is identified through injection of reference solution (f) shown in **Figures 7a** and **7b**. The respective retention times for impurity C are 10.69 min on Luna 5 µm C18(2) and 8.34 min on Kinetex 2.6 µm C18.

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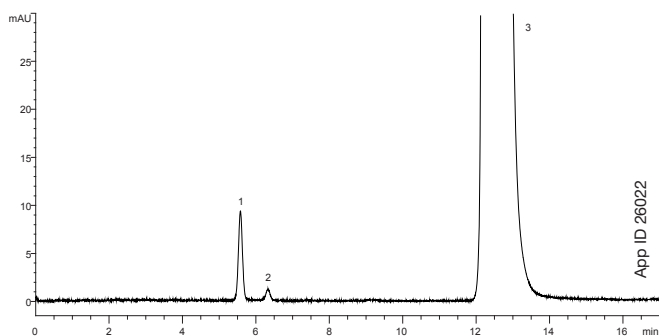
Test and Reference solutions

Test solution

100 mg of Fluconazole was dissolved in mobile phase, sonicated and diluted to 10 mL with mobile phase – Injection: 20 μ L

Figure 1a

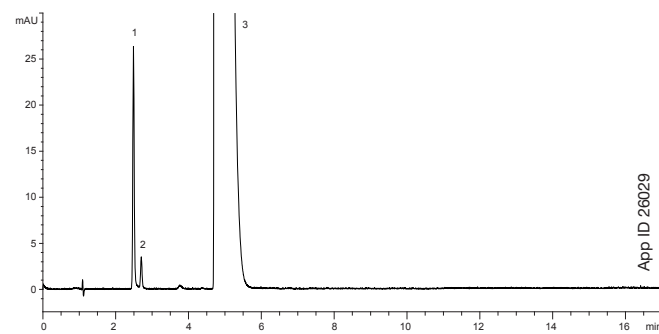
Test Solution (10 mg/mL of Fluconazole CRS) on Luna[®] 5 μ m C18(2)
150 x 4.6 mm



Peak No.	Analyte	Time	Area	Height	Width	Area%	Symmetry
1	Impurity B	5.579	74.2	9.3	0.1209	0.525	0.935
2	Impurity A	6.33	11	1.2	0.1408	0.078	0.841
3	Fluconazole	12.256	14052.7	506.8	0.3875	99.397	0.214

Figure 1b

Test Solution (10 mg/mL of Fluconazole CRS) on Kinetex[®] 2.6 μ m C18
100 x 4.6 mm



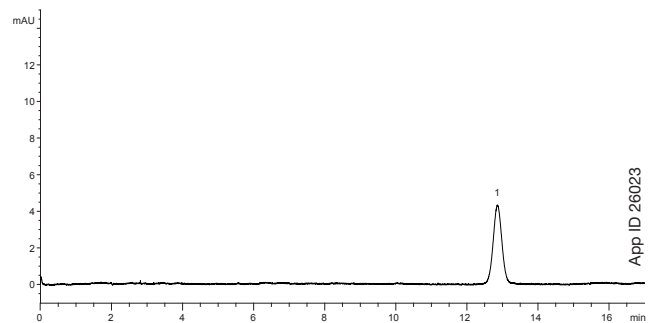
Peak No.	Analyte	Time	Area	Height	Width	Area%	Symmetry
1	Impurity B	2.489	73.8	25.7	0.0456	0.521	0.814
2	Impurity A	2.703	11.3	3.4	0.0506	0.080	0.868
3	Fluconazole	4.744	14075.9	912.3	0.208	99.399	0.146

Reference solution (a)

5 mL of the test solution was diluted to 100 mL with the mobile phase. 1 mL of this solution was then diluted to 10 mL with the mobile phase – Injection: 20 μ L.

Figure 2a

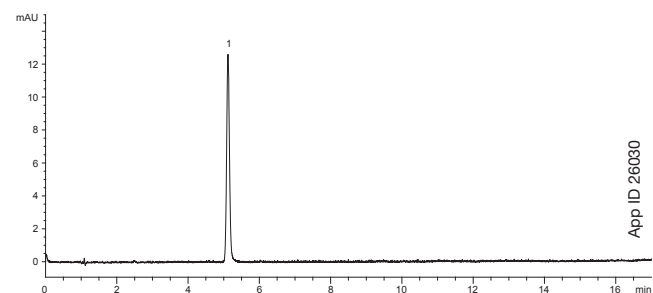
Reference solution (a) on Luna 5 μ m C18(2) on Agilent[®] 1260



Peak No.	Analyte	Time	Area	Height	Width	Area%	Symmetry
1	Fluconazole	12.86	77.4	4.3	0.2719	100.000	0.93

Figure 2b

Reference solution (a) on Kinetex 2.6 μ m C18 on Agilent 1260

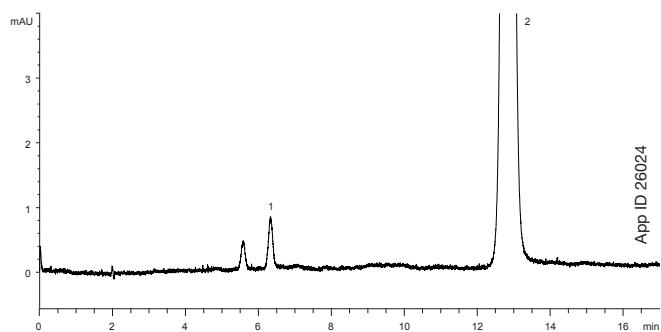


Peak No.	Analyte	Time	Area	Height	Width	Area%	Symmetry
1	Fluconazole	5.12	72.8	12.6	0.0915	100.000	0.812

Reference solution (b)

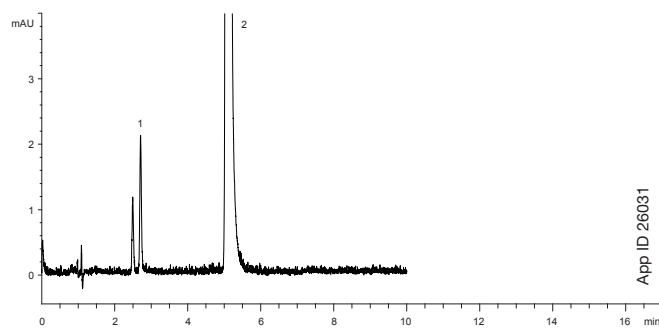
5 mg of Fluconazole for peak identification CRS (containing impurity A) was dissolved in mobile phase, sonicated and diluted to 10 mL with the mobile phase—Injection: 20 µL.

Figure 3a
Reference solution (b) on Luna[®] 5 µm C18(2) on Agilent[®] 1260



Peak No.	Analyte	Time	Area	Height	Width	Area%	Symmetry
1	Impurity A	6.332	7.4	8.3E-1	0.1477	1.307	0.987
2	Fluconazole	12.826	558.4	32.7	0.2633	98.693	0.857

Figure 3b
Reference solution (b) on Kinetex[®] 2.6 µm C18 on Agilent 1260

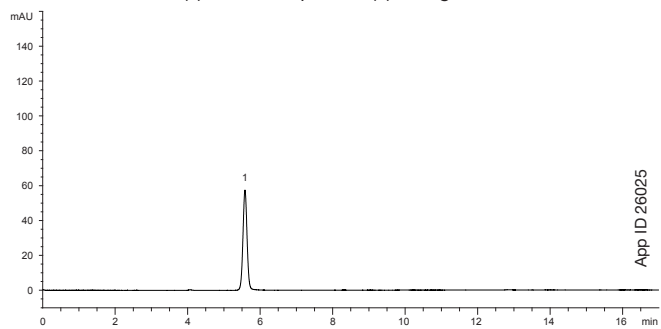


Peak No.	Analyte	Time	Area	Height	Width	Area%	Symmetry
1	Impurity A	2.709	6.5	2.1	0.052	1.149	1.191
2	Fluconazole	5.088	558.5	94.2	0.0892	98.851	0.623

Reference solution (c)

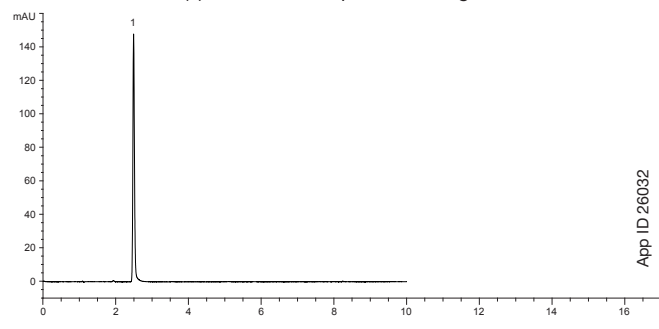
3.0 mg of Fluconazole impurity B CRS was dissolved in the mobile phase, sonicated and then diluted to 100 mL with mobile phase—Injection: 20 µL.

Figure 4a
Reference solution (c) on Luna 5 µm C18(2) on Agilent 1260



Peak No.	Analyte	Time	Area	Height	Width	Area%	Symmetry
1	Impurity B	5.582	458.8	57.4	0.1213	100.000	0.918

Figure 4b
Reference solution (c) on Kinetex 2.6 µm C18 on Agilent 1260



Peak No.	Analyte	Time	Area	Height	Width	Area%	Symmetry
1	Impurity B	2.493	457.7	143.3	0.0533	100.000	0.762

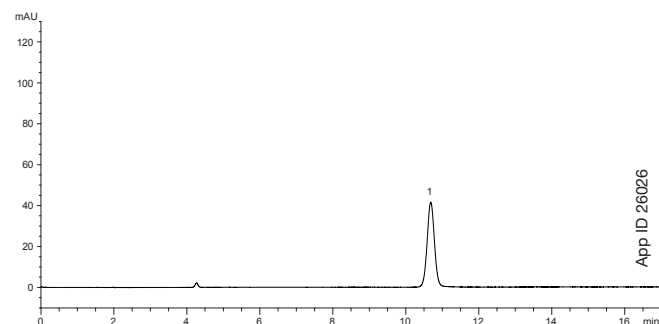
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Reference solution (d)

3.0 mg of Fluconazole impurity C CRS was dissolved in the mobile phase, sonicated and then diluted to 20 mL with mobile phase—
Injection: 20 μ L

Figure 5a

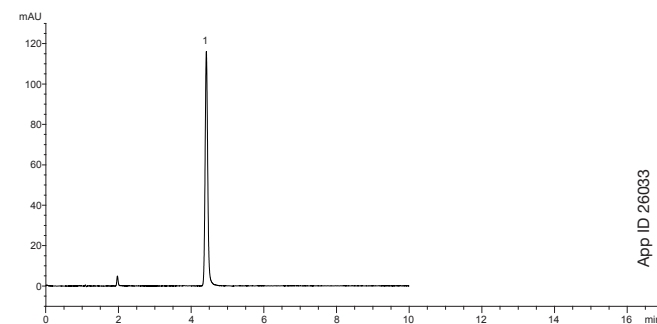
Reference solution (d) on Luna[®] 5 μ m C18(2) on Agilent[®] 1260



Peak No.	Analyte	Time	Area	Height	Width	Area%	Symmetry
1	Impurity C	10.684	582.2	41.6	0.2153	100.000	0.935

Figure 5b

Reference solution (d) on Kinetex[®] 2.6 μ m C18 on Agilent 1260



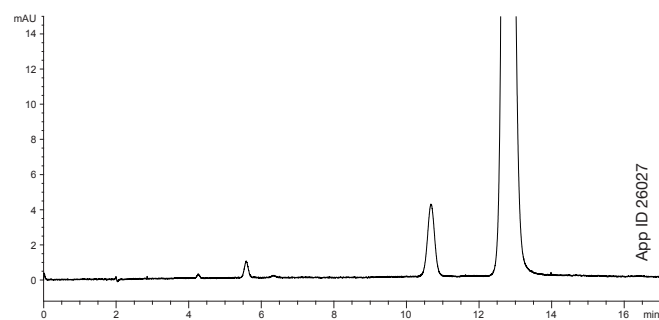
Peak No.	Analyte	Time	Area	Height	Width	Area%	Symmetry
1	Impurity C	4.418	580.3	115	0.0789	100.000	0.824

Reference solution (e)

System suitability: A mixture of 1 mL of reference solution (d) and 1 mL of test solution were diluted to 10 mL with the solvent mixture.
Injection: 20 μ L.

Figure 6a

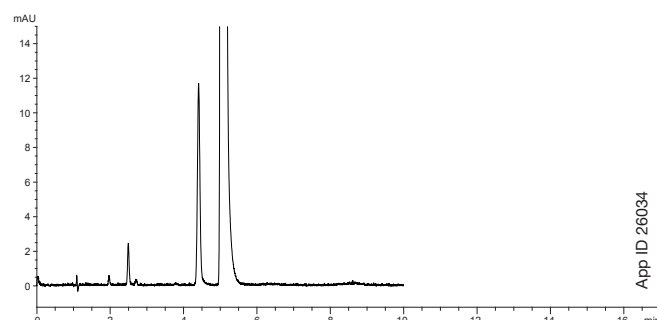
Reference solution (e) on Luna 5 μ m C18(2) on Agilent 1260



Peak No.	Analyte	Time	Area	Height	Width	Area%	Symmetry
1	Impurity C	10.678	59.1	4.1	0.2227	3.998	0.932
2	Fluconazole	12.769	1418.5	81.4	0.2674	96.002	0.697

Figure 6b

Reference solution (e) on Kinetex 2.6 μ m C18 on Agilent 1260



Peak No.	Analyte	Time	Area	Height	Width	Area%	Symmetry
1	Impurity C	4.411	58.6	11.6	0.0791	3.977	0.821
2	Fluconazole	5.04	1414.2	210.8	0.0983	96.023	0.409

Table 2

Reference solution (e) on Luna 5 μ m C18(2) 150 x 4.6 mm

Inj. #	Analyte	Time	Area	Height	Width	Resolution	
1	1 Impurity C	10.678	59.1	4.1	0.2227		
	2 Fluconazole	12.769	1418.5	81.4	0.2674	5.15	
2	1 Impurity C	10.695	59.7	4.1	0.2245		
	2 Fluconazole	12.785	1416.7	81.3	0.2714	5.12	
3	1 Impurity C	10.68	58.2	4.1	0.2216		
	2 Fluconazole	12.776	1415.2	81.2	0.2674	5.15	
4	1 Impurity C	10.683	58.8	4.1	0.2175		
	2 Fluconazole	12.774	1413.7	81.3	0.2668	5.13	
5	1 Impurity C	10.695	62.1	4.2	0.23		
	2 Fluconazole	12.785	1414.9	81.3	0.271	5.11	
						Average	5.132
						STD	0.0178885
						%RSD	0.3485687

Table 3

Reference solution (e) on Kinetex 2.6 μ m C18 100 x 4.6 mm

Inj. #	Analyte	Time	Area	Height	Width	Resolution	
1	1 Impurity C	4.411	58.6	11.6	0.0791		
	2 Fluconazole	5.04	1414.2	210.8	0.0983	4.22	
2	1 Impurity C	4.413	58.7	11.5	0.0754		
	2 Fluconazole	5.043	1411.7	204.2	0.1007	4.22	
3	1 Impurity C	4.413	56.2	11.6	0.0708		
	2 Fluconazole	5.038	1409.3	209	0.0967	4.2	
4	1 Impurity C	4.413	56.4	11.7	0.0709		
	2 Fluconazole	5.038	1406.2	209.1	0.0985	4.21	
5	1 Impurity C	4.414	56.6	11.7	0.0736		
	2 Fluconazole	5.038	1408.5	209.3	0.0955	4.21	
						Average	4.212
						STD	0.0083666
						%RSD	0.1986372

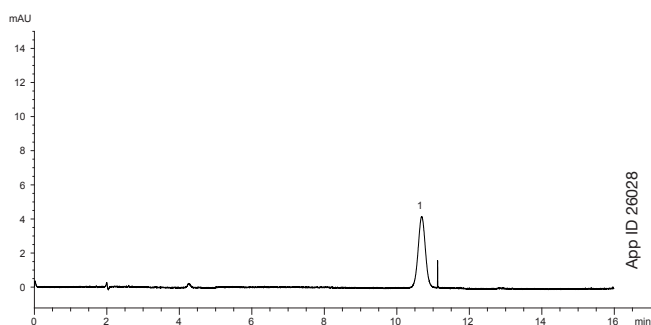
The Luna[®] 5 μ m C18(2) column and the Kinetex[®] 2.6 μ m C18 column were able to provide a resolution factor between Fluconazole and impurity C above the system suitability requirement of 3.0. The resolution on Luna 5 μ m C18(2) was 5.13 (**Table 2**). The resolution on Kinetex 2.6 μ m C18 was 4.21 (**Table 3**).

Reference solution (f)

A mixture of 1 mL of reference solution (d) was diluted to 10 mL with the mobile phase. Injection: 20 μ L.

Figure 7a

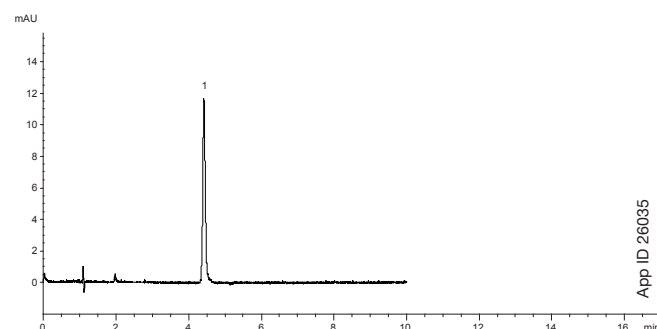
Reference solution (f) on Luna 5 μ m C18(2) on Agilent[®] 1260



Peak No.	Analyte	Time	Area	Height	Width	Area%	Symmetry
1	Impurity C	10.687	62.3	4.2	0.224	100.000	0.914

Figure 7b

Reference solution (f) on Kinetex 2.6 μ m C18 on Agilent 1260



Analyte	Time	Area	Height	Width	Area%	Symmetry
Impurity C	8.338	60.4	5	0.1842	100.000	0.837

Conclusion

The above experiments show both Luna 5 μ m C18(2) and Kinetex 2.6 μ m C18 are suitable under the conditions outlined in the monograph for Fluconazole². With the Kinetex 2.6 μ m C18 column we demonstrated the possibility to reduce the total analysis time significantly by reducing the retention time for Fluconazole to 5.0 min within the allowable adjustments of compendial methods³. Therefore, Luna 5 μ m C18(2) and Kinetex 2.6 μ m C18 are reliable solutions for the analysis of Fluconazole in routine laboratories following the Ph. Eur. regulations.

Sources of Standards

Standard	Source
Fluconazole CRS	EDQM, Y0000557
Fluconazole for Peak Identification CRS (contains Impurity A)	EDQM, Y0000558
Fluconazole impurity B CRS	EDQM, Y0000573
Fluconazole impurity C CRS	EDQM, Y0000574

References

- Wallace, J. E.; Harris, S. C.; Gallegos, J.; Foulds, G.; Chen, T. J. H.; Rinaldi, M. G., *Antimicrobial Agents and Chemotherapy*, 1992, 36(3), 603-606
- European Pharmacopoeia; Supplement 10 – Monograph 2287
- European Pharmacopoeia; Supplement 10 – Chapter 2.2.46 Chromatographic Separation Techniques

Ordering Information

Luna [®] 3 µm MidBore™ and Analytical Columns (mm)									SecurityGuard™ Cartridges (mm)	
Phases	30 x 3.0	50 x 3.0	150 x 3.0	30 x 4.6	50 x 4.6	75 x 4.6	100 x 4.6	150 x 4.6	4 x 2.0*	4 x 3.0*
C18(2)	00A-4251-Y0	00B-4251-Y0	00F-4251-Y0	00A-4251-E0	00B-4251-E0	00C-4251-E0	00D-4251-E0	00F-4251-E0	/10pk AJ0-4286	/10pk AJ0-4287
									for ID: 2.0-3.0 mm 3.2-8.0 mm	

Luna [®] 5 µm MidBore and Analytical Columns (mm)								SecurityGuard™ Cartridges (mm)	
Phases	30 x 3.0	50 x 3.0	150 x 3.0	250 x 3.0	30 x 4.6	50 x 4.6	75 x 4.6	4 x 2.0*	4 x 3.0*
Luna C18(2)	00A-4252-Y0	00B-4252-Y0	00F-4252-Y0	00G-4252-Y0	00A-4252-E0	00B-4252-E0	00C-4252-E0	/10pk AJ0-4286	/10pk AJ0-4287
								for ID: 2.0-3.0 mm 3.2-8.0 mm	

Luna [®] 5 µm Analytical and Semi-Prep Columns (mm)				SecurityGuard™ Cartridges (mm)		
Phases	100 x 4.6	150 x 4.6	250 x 4.6	250 x 10	4 x 3.0*	10 x 10**
Luna C18(2)	00D-4252-E0	00F-4252-E0	00G-4252-E0	00G-4252-N0	/10pk AJ0-4287	/3pk AJ0-7221
				for ID: 3.2-8.0 mm 9-16 mm		

Kinetex [®] 2.6 µm Analytical Columns (mm)							SecurityGuard™ ULTRA Cartridges [‡]
Phases	30 x 4.6	50 x 4.6	75 x 4.6	100 x 4.6	150 x 4.6	250 x 4.6	3/pk
C18	00A-4462-E0	00B-4462-E0	00C-4462-E0	00D-4462-E0	00F-4462-E0	00G-4462-E0	AJ0-8768
							for 4.6 mm ID

‡ SecurityGuard ULTRA Cartridges require holder, Part No.: [AJ0-9000](#)

* SecurityGuard Analytical Cartridges require holder, Part No.: [KJ0-4282](#)

** SemiPREP SecurityGuard Cartridges require holder, Part No.: [AJ0-9281](#)

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