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Meeting and Surpassing System Suitability for USP Sildenafil Tablets Assay and Organic Impurities Using Kinetex® Core-Shell and Luna® Omega HPLC/UHPLC Columns

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Introduction

Sildenafil Citrate is a specific inhibitor of phosphodiesterase-5 (PDE5), sold in the market under the brand name Viagra®. This present study was conducted to compare the column performance of three different 5 µm L1 columns. We used Waters® Symmetry® C18, Luna Omega C18, and Kinetex C18 in identifying the Sildenafil Citrate compound and its related impurity according to the USP monograph for Sildenafil Citrate Tablets. The USP monograph requires that the resolution between Sildenafil Citrate and Sildenafil N-Oxide be not less than 2.6 to meet system suitability; this was achieved here for all columns. Relative standard deviation (RSD), symmetry factor and signal-to-noise ratio are other system suitability parameters tested for and passed for all columns in this study. All method parameters were in accordance with the USP monograph for Sildenafil Citrate Tablets.

Experimental Procedures

A 100 mg Sildenafil tablet was ground and diluted in 100 mL of ammonium hydroxide and water (10:90), then a 20 µL sample of that solution was mixed in 980 mL of acetonitrile and water (90:10), to give a concentration of 0.02 mg/mL of Sildenafil Citrate. A standard solution of 0.028 mg/mL Sildenafil Citrate RS was prepared, and from that the sensitivity solution (0.35 µg/mL) was prepared.

The system suitability solution, a mixture of Sildenafil and Sildenafil N-Oxide in mobile phase, was prepared by dissolving 70 mg of USP Sildenafil Citrate RS in 1 mL of a solution of hydrogen peroxide and formic acid (2:1), allowing it to stand for not more than 10 minutes to produce the Sildenafil N-Oxide. It was then diluted with mobile phase to a final volume of 250.0 mL.

All reference standards were obtained from USP and all solutions were prepared according to the USP monograph for Sildenafil Citrate Tablets. The original column dimension called for in the monograph was 150 x 3.9 mm. We ran the comparison with Luna Omega 5 µm C18 and Kinetex 5 µm C18 columns on a 150 x 4.6 mm dimension. The flow rate was scaled accordingly to maintain the same linear velocity in accordance with the recommendations of USP General Chapter <621>. The injection volume was also scaled to account for change in column internal diameter.

LC Conditions

Column:	Symmetry 5 µm C18
Dimensions:	150 x 3.9 mm
Pressure (bar):	160 bar
Mobile Phase:	Buffer (dilute 7 mL of triethylamine with water to 1 L. Stir and adjust with phosphoric acid to a pH of 3.0 ± 0.1), Methanol and Acetonitrile (58:25:17)
Flow Rate:	1 mL/min
Injection Volume:	20 µL
Temperature:	30 °C
Detection:	UV @ 290 nm
Instrument:	Agilent® 1100 Quaternary HPLC system with a temperature-controlled column selector

LC Conditions

Columns:	Luna Omega 5 µm C18 Kinetex 5 µm C18
Part No.:	00F-4785-E0 00F-4601-E0
Dimensions:	150 x 4.6 mm
Pressure (bar):	160 bar (Luna Omega) 159 bar (Kinetex)
Mobile Phase:	Buffer (dilute 7 mL of triethylamine with water to 1 L. Stir and adjust with phosphoric acid to a pH of 3.0 ± 0.1), Methanol and Acetonitrile (58:25:17)
Flow Rate:	1.4 mL/min
Injection Volume:	28 µL
Temperature:	30 °C
Detection:	UV @ 290 nm
Instrument:	Agilent 1100 Quaternary HPLC system with a temperature-controlled column selector

Results and Discussions

The standard solution for both the assay and the organic impurities was first run on all three columns to check for symmetry factor and percent relative standard deviation (%RSD). For both the assay and the organic impurities, all three columns produced a result of less than 1.3 for symmetry factor, meeting system suitability criteria. % RSD results for all the columns also met system suitability (**Figure 1**).

The system suitability solution was run to determine resolution between Sildenafil and its impurity Sildenafil N-Oxide. As shown in **Figure 2**, all columns responded with good resolution results surpassing the minimum threshold of 2.6 specified in the USP monograph for Sildenafil Citrate Tablets. The chromatogram

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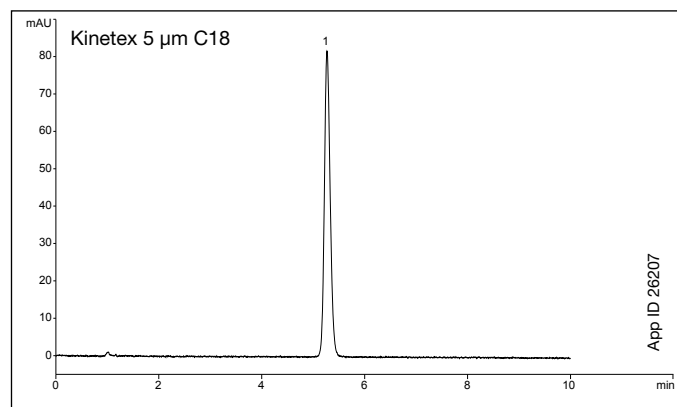
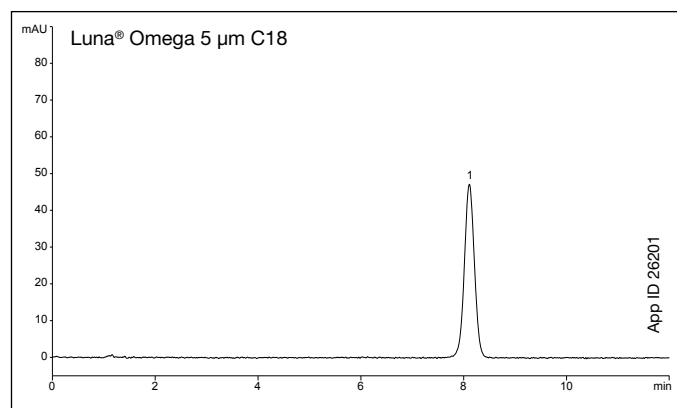
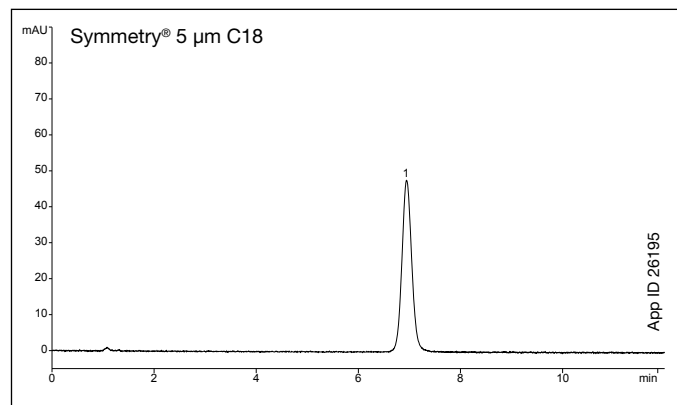
Zeshan loves to collect watches and the Back to the Future Trilogy. He has twin boys which drive him crazy! He is an Apple Fanboy for life and he likes being in the lab more than anywhere else.



for the Kinetex® column, compared to the two other columns, displayed a taller and narrower peak with a higher resolution factor (**Figure 2**) as expected due to the higher efficiency expressed by the core-shell particle. Retention time is also observed to be significantly shorter for the Kinetex column, which is typical given the lower surface area and % carbon load for the Kinetex core-shell column.

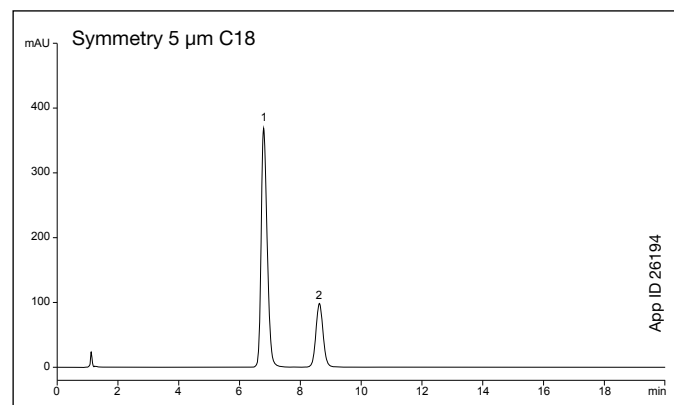
The sensitivity sample solution (0.35 µg/mL), was used to test for signal-to-noise (S/N) ratio. USP requires a S/N ratio of not less than (NLT) 10, and as shown in **Figure 3**, all columns had a result of over 10, meeting system suitability.

Figure 1.
Sildenafil Citrate Tablets Assay System Suitability Solution
0.02 mg/mL

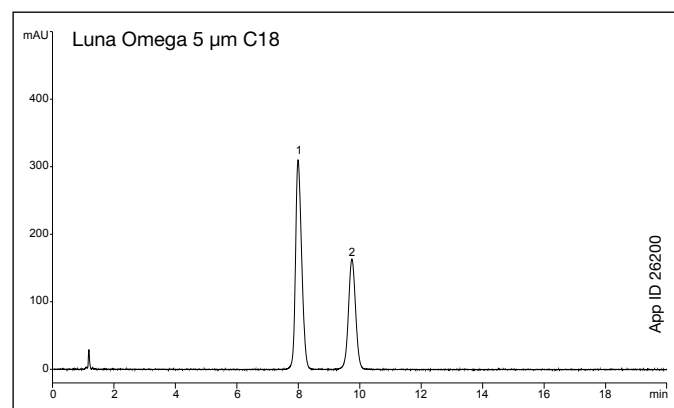


Column	Symmetry Factor (NMT 1.3)	Peak Area	
		STD	%RSD (n = 5)
Symmetry 5 µm C18	1.08	2.42	0.4
Luna Omega 5 µm C18	1.01	2.74	0.4
Kinetex 5 µm C18	1.16	2.30	0.4

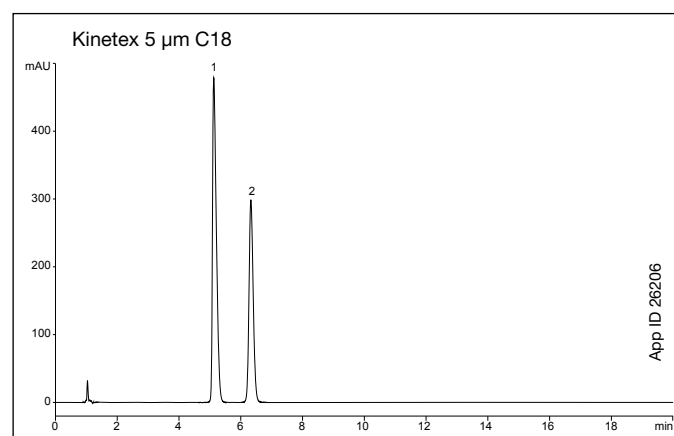
Figure 2.
USP Sildenafil Citrate RS + Sildenafil N-Oxide System Suitability
Solution 0.028 mg/mL



#	Compound	Time	Area	Height	Width	Resolution
1	Sildenafil	6.793	5059.4	368.5	0.2102	4.7
2	Sildenafil N-Oxide	8.628	1612.1	98.3	0.2473	



#	Compound	Time	Area	Height	Width	Resolution
1	Sildenafil	7.993	4310.9	310	0.2112	4.5
2	Sildenafil N-Oxide	9.746	2659.2	163.8	0.2514	

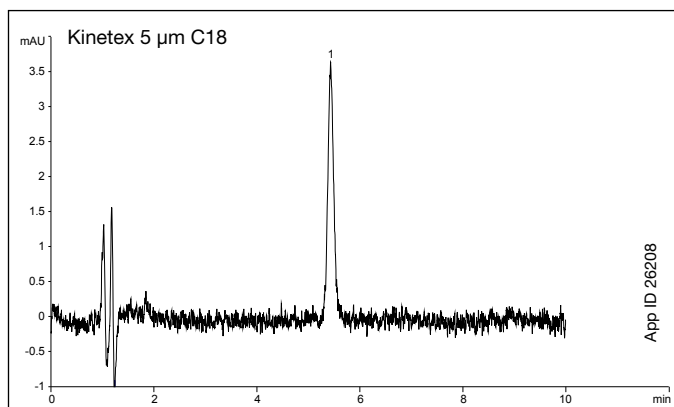
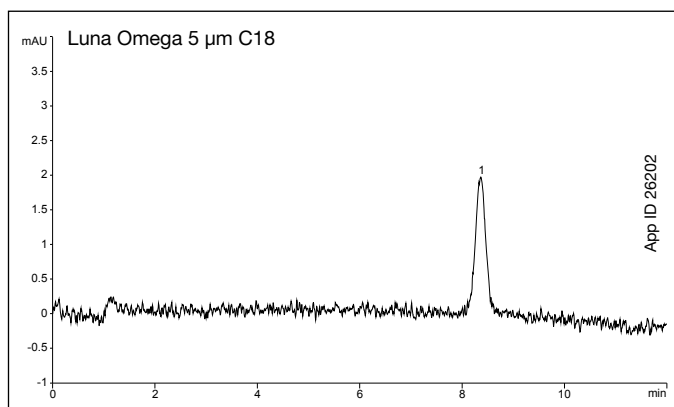
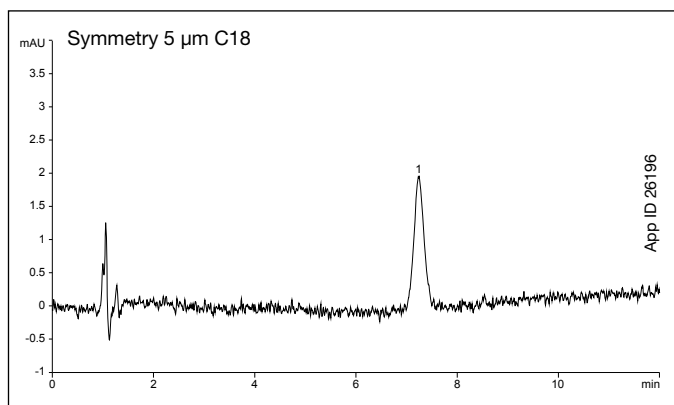


#	Compound	Time	Area	Height	Width	Resolution
1	Sildenafil	5.126	4172.9	478.8	0.1317	5.1
2	Sildenafil N-Oxide	6.327	2814.2	298.8	0.1469	



Figure 3.

Sildenafil Citrate Tablets Organic Impurities Sensitivity Solution
0.35 µg/mL



Column	S/N Ratio
Symmetry 5 µm C18	11.23
Luna Omega 5 µm C18	10.63
Kinetex 5 µm C18	14.23

Conclusion

The results above show that all three columns: Symmetry® C18, Luna® Omega C18 and Kinetex® C18 met USP system suitability requirements for the analysis of USP Sildenafil Citrate Tablets.

There is an impressive enhancement in resolution and sensitivity when observing the results for the Kinetex C18 core-shell column compared to the traditional fully porous column alternatives with the same particle size. This is largely due to the much higher efficiencies expressed by the core-shell particle column when compared to fully porous particle columns. The Kinetex column also showed better performance in regard to peak response, peak shape and enhanced signal-to-noise ratio.



Kinetex® Ordering Information

5 µm Analytical Columns (mm)					SecurityGuard™ ULTRA Cartridges†
Phases	50 x 4.6	100 x 4.6	150 x 4.6	250 x 4.6	3/pk
C18	00B-4601-E0	00D-4601-E0	00F-4601-E0	00G-4601-E0	AJO-8768

for 4.6 mm ID

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

Luna® Omega Ordering Information

5 µm Analytical Columns (mm)					SecurityGuard Cartridges (mm)
Phases	50 x 4.6	100 x 4.6	150 x 4.6	250 x 4.6	4 x 3.0* /10 pk
C18	00B-4785-E0	00D-4785-E0	00F-4785-E0	00G-4785-E0	AJO-7612

for ID: 3.2-8.0 mm

*SecurityGuard Analytical Cartridges require holder, Part No.: [KJO-4282](#)BE-HAPPY™
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