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

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Introduction

Sildenafil Citrate is a phosphodiesterase-5 (PDE5) inhibitor, which plays a role in the contraction and relaxation of smooth muscle cells that line blood vessels. It is one of the most widely used PDE5 inhibitors on the market today. As a result, the development of a quick and efficient analysis of Sildenafil Citrate and its related impurities is of significant interest for the manufacturers of this drug substance. For this report, we focused on Sildenafil Citrate assay and organic impurities as identified in the US Pharmacopeia (USP) monograph. The system suitability requirement as outlined in the USP monograph requires that the resolution between Sildenafil Citrate and Sildenafil N-Oxide be no less than 2.5; this was achieved here on all of the columns evaluated. In order to maximize performance and speed up analysis time, HPLC columns packed with core-shell (superficially porous) and fully porous silica particles bonded with a C18 phase were used. The performance of the Kinetex core-shell columns and Luna Omega fully porous columns used here were compared to that of the Waters® Symmetry® C18 column and all method parameters were consistent with the USP monograph for Sildenafil Citrate.

Experimental Procedures

All reference standards were obtained from USP and solutions were prepared as indicated in the USP monograph for Sildenafil Citrate. Evaluation was performed with the Kinetex 5 µm 150 x 4.6 mm and Luna Omega 5 µm 100 x 4.6 mm C18 (Phenomenex, Torrance, California, USA) and the results from these columns were compared with the Symmetry 5 µm C18 150 x 3.9 mm column (Waters Technologies Corporation, Millford, Massachusetts, USA).

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 standard solution and the sample solution of USP Sildenafil Citrate RS was diluted in mobile phase to a concentration of 0.028 mg/mL. The mobile phase consisted of a 58:25:17 mixture of Buffer/Water/Acetonitrile. The system suitability solution for the impurities was made by dissolving 70 mg of Sidenafil Citrate in 1 mL of a solution of hydrogen peroxide and anhydrous formic acid (2:1). This solution was allowed to stand for at least 10 minutes to generate Sildenafil N-oxide, then diluted with mobile phase to 250 mL. System suitability for the assay was determined as symmetry factor for sildenafil of no more than 1.5 and relative standard deviation (%RSD) of not more than 0.85 % for six replicate injections of the standard solution. The system suitability for the organic impurities was determined as a resolution no less than 2.5 between Sildenafil Citrate and Sildenafil N-oxide for the system suitability solution, with a tailing factor no more than 1.5 for the Sildenafil Citrate peak (diluted sample solution), and a signal to noise (S/N) ratio of no less than 10 for sildenafil citrate in the sensitivity solution. The LC conditions are listed below for each column used and were used to generate all of the data in this technical note.

The original column dimension called for in the monograph was 150 by 3.9 mm. We ran the comparison with Luna Omega 5 µm C18 and Kinetex 5 µm C18 columns on a 150 by 4.6 mm dimension. The flow rate was scaled accordingly to maintain the same linear velocity in accordance with the recommendations of the USP General Chapter <621>. The injection volume was also adjusted to account for the change in column internal diameter.

LC Conditions – Symmetry Column

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
Temperature:	30 °C
Detection:	UV @ 290 nm
Injection Volume:	20 µL
Instrument:	Agilent® 1100 Quaternary HPLC system with a temperature-controlled column selector

LC Conditions – Luna Omega and Kinetex Column

Column:	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
Temperature:	30 °C
Detection:	UV @ 290 nm
Injection Volume:	28 µL
Instrument:	Agilent 1100 Quaternary HPLC system with a temperature-controlled column selector.

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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.

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Results and Discussion

For the assay of sildenafil citrate, the percent relative standard deviation (% RSD) for peak area and tailing factor for the three columns was determined for the standard solution of 0.028 mg/mL of Sildenafil Citrate as per the USP monograph. All columns had a peak area % RSD lower than the required 0.85 % for six replicate injections, meeting system suitability. As displayed in **Figure 1**, all three columns had an average tailing factor less than the required 1.5, meeting the system suitability requirements for tailing factor.

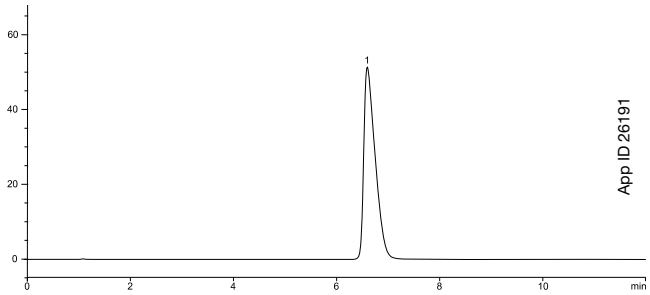
System suitability test for organic impurities for tailing factor was performed with a diluted sample solution (0.0014 mg/mL) as per the USP method. The result for the three columns, as shown in **Figure 2**, was under 1.5, meeting this system suitability requirement.

The results for the analysis of the identification solution, which had a concentration of 0.0075 mg/mL of Sildenafil Citrate, to test for the identification of Sildenafil related compound A. As shown in **Figure 3**, all columns were positively able to identify sildenafil related compound A from the solution.

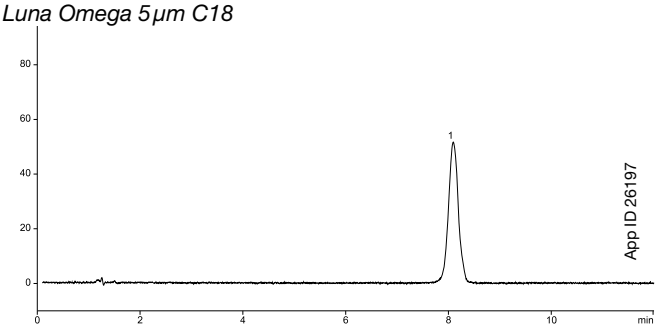
Resolution between Sildenafil and the impurity Sildenafil N-Oxide was determined by running the standard solution (0.028 mg/mL). USP required a resolution of at least 2.5 for system suitability. As displayed in **Figure 4**, all three columns, met system suitability.

Figure 1.
USP Sildenafil Citrate RS Standard Solution 0.028 mg/mL

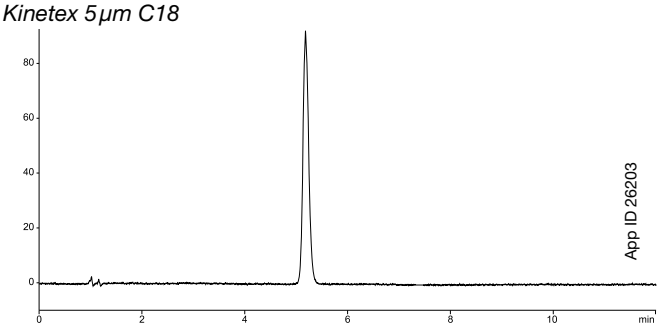
Symmetry 5 µm C18



Luna Omega 5 µm C18



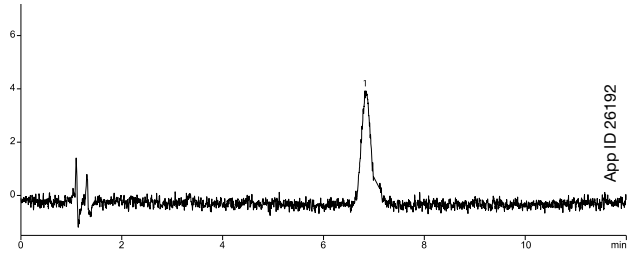
Kinetex 5 µm C18



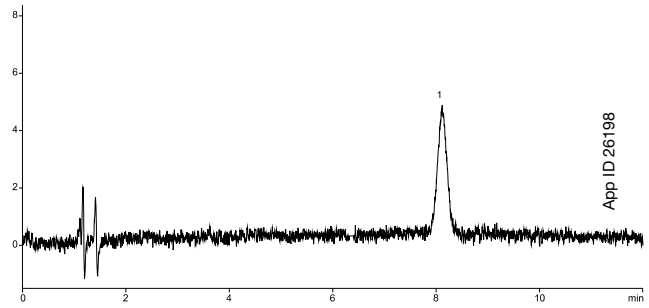
Column	Symmetry Factor	Peak Area	
		St. Dev.	%RSD
Symmetry® 5 µm C18	1.10	5.47	0.8
Luna® Omega 5 µm C18	1.00	3.88	0.6
Kinetex® 5 µm C18	1.16	2.17	0.3

Figure 2.
USP Sildenafil Citrate RS Standard Solution 0.0014 mg/mL

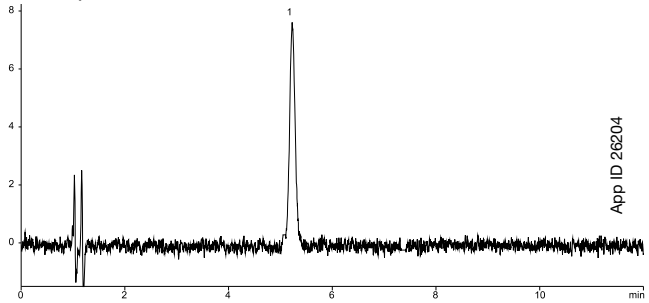
Symmetry 5 µm C18



Luna Omega 5 µm C18



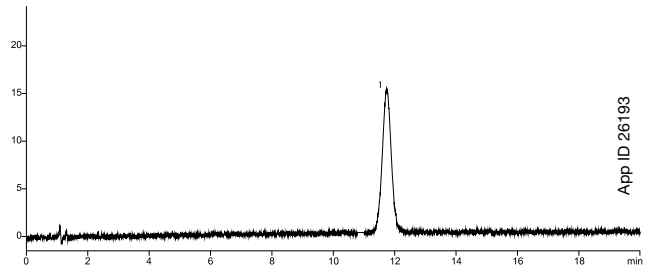
Kinetex 5 µm C18



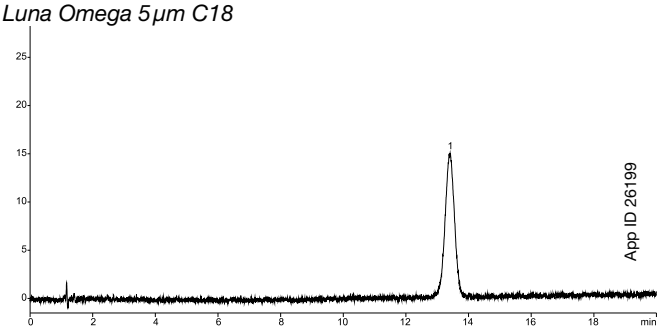
Column	Symmetry Factor
Symmetry 5 µm C18 Average	1.09
Luna Omega 5 µm C18 Average	0.97
Kinetex 5 µm C18 Average	1.07

Figure 3.
USP Sildenafil Related Compound A RS Identification Solution 0.0075 mg/mL

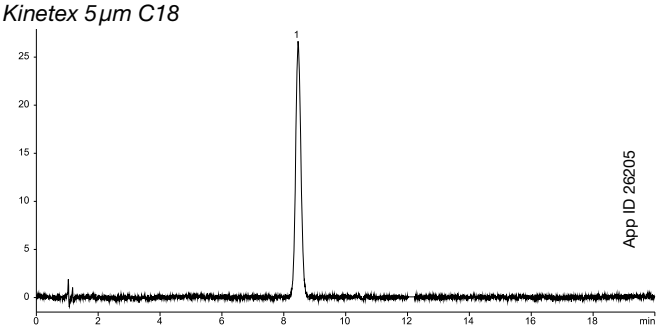
Symmetry 5 µm C18



Luna Omega 5 µm C18



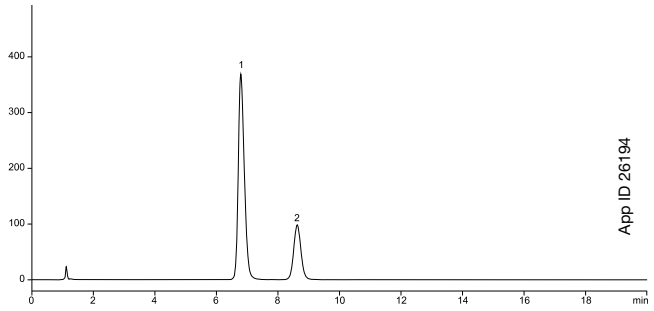
Kinetex 5 µm C18



Column	Symmetry Factor
Symmetry 5 µm C18 Average	1.03
Luna Omega 5 µm C18 Average	1.01
Kinetex 5 µm C18 Average	1.08

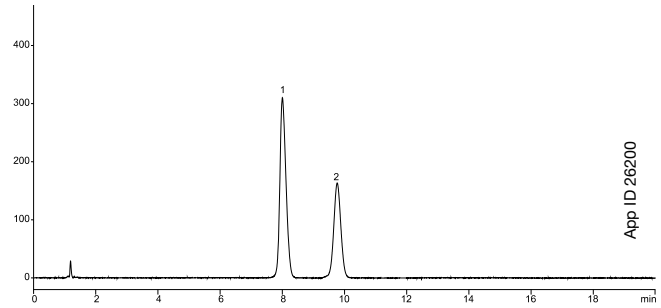
Figure 4.
USP Sildenafil Citrate RS + Sildenafil N-Oxide System Suitability Solution 0.028 mg/mL

Symmetry 5 µm C18



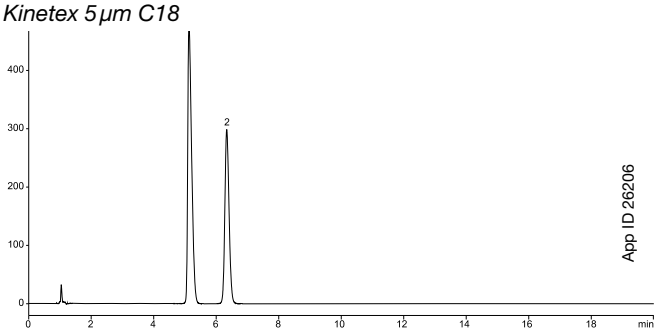
#	Compound	Time	Area	Height	Width	Resolution
1	Sildenafil	6.793	5059.4	368.5	0.2102	4.7
2	Sildenafil N-Oxide	8.628	1612.1	98.3	0.2473	

Luna Omega 5 µm C18



#	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	

Kinetex 5 µm C18



#	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	

Conclusions

The results obtained for the assay and organic impurities analysis of sildenafil citrate highlight a significant advantage of the Kinetex C18 column. While all three columns met all of the USP system suitability requirements for sildenafil citrate, the Kinetex core-shell column provided overall superior performance indicated by improved peak response, narrower peaks, and greater resolution. This advantage can be attributed to the morphology of the core-shell Kinetex C18. An added benefit to the Kinetex column is the reduction in overall analysis time.

Ordering Information

Kinetex® 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	AJ0-8768
					for 4.6 mm ID

Luna® Omega 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	AJ0-7612
					for ID: 3.2-8.0 mm

†SecurityGuard ULTRA cartridges require holder, Part No.: AJ0-9000

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