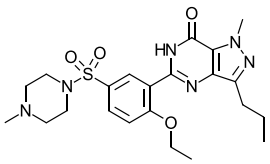
Sildenafil Comparison on HALO® 2, 2.7, and 5 µm

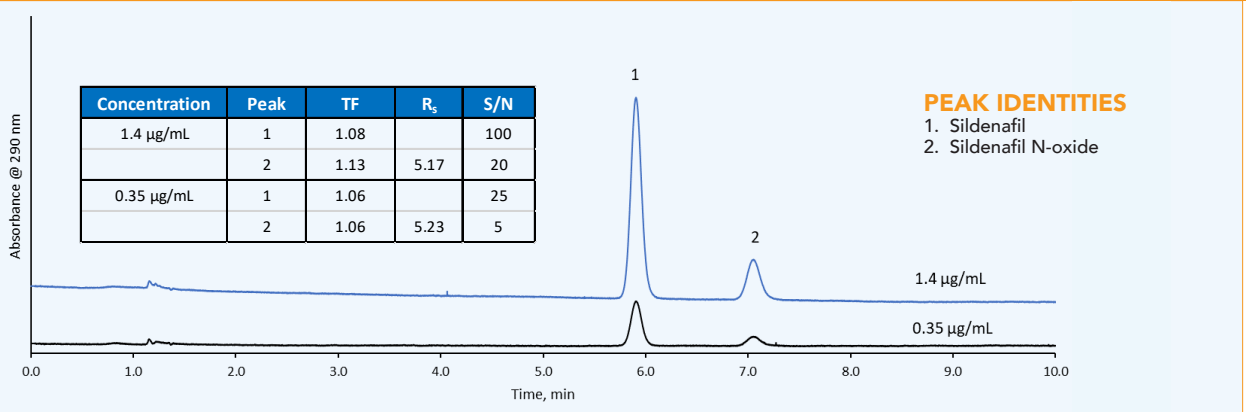
Sildenafil (better known as Viagra or Revatio) is a medication used to treat erectile dysfunction and pulmonary arterial hypertension. Pfizer filed a patent covering the use of sildenafil (published in 2002) which has expired since 2019. A chemical structure of sildenafil is shown in Figure 1 (below).



HALO® C18 HPLC columns (Advanced Materials Technology) can be used for the HPLC methods within the sildenafil citrate USP Monograph. (USP42-NF37) This chromatographic method includes an isocratic separation using a C18, 5 µm, 4.6 x 150 mm column. A sildenafil standard was reacted with a 2:1 ratio of hydrogen peroxide/formic acid in order to produce sildenafil N-oxide. The separation requires a three-part mobile phase including methanol, acetonitrile, and a buffer. The separation is shown in Figure 2.

Figure 2: A HALO® 5 µm C18 column is used for the HPLC methods specified within the sildenafil citrate USP Monograph. This includes the diluted sample solution (1.4 µg/mL) and the sensitivity solution (0.35 µg/mL). Tailing factor, resolution, and signal to noise ratio requirements are all met showing excellent column performance.

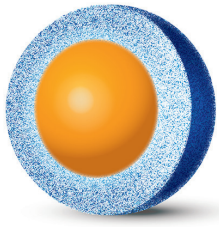
Concentration	Peak	TF	R_s	S/N
1.4 µg/mL	1	1.08		100
	2	1.13	5.17	20
0.35 µg/mL	1	1.06		25
	2	1.06	5.23	5



TEST CONDITIONS

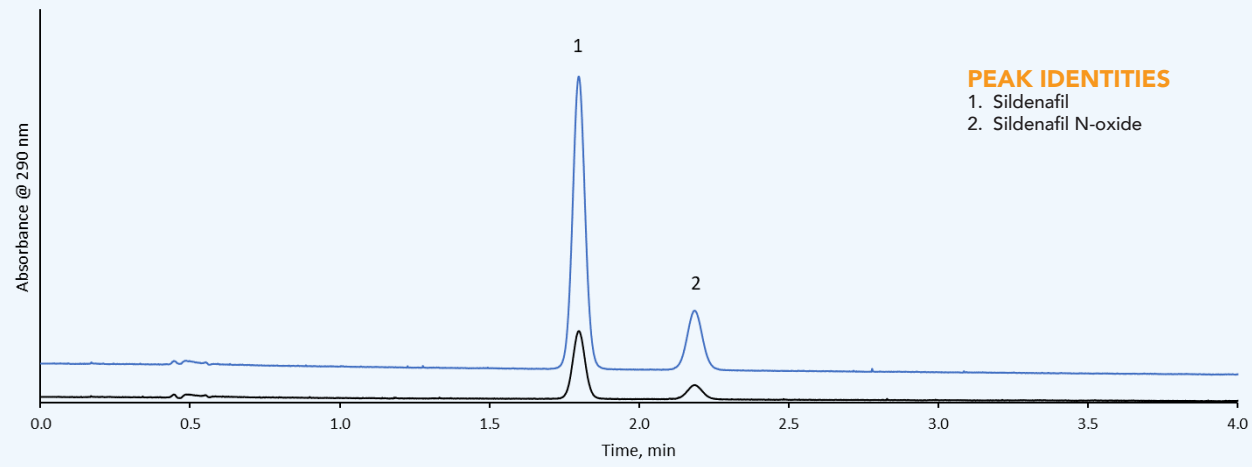
Column: HALO 90 Å C18 5 µm, 4.6 x 150mm
Part Number: 95814-702
Mobile Phase: 58/25/17 (v,v,v)
Buffer, Methanol, Acetonitrile
Buffer: 7 mL TEA in 1 L Water, adjusted to pH: 3 w/ phosphoric acid
Isocratic
Flow Rate: 1.0 mL/min
Initial Back Pressure: 193 bar

Temperature: 30 °C
Detection: 290 nm
Injection Volume: 10 µL
Sample Solvent: mobile phase buffer
Data Rate: 100 Hz
Response Time: 0.025 sec.
Flow Cell: 1 µL
LC System: Shimadzu Nexera X2



A high throughput environment constantly tests chromatographers to improve and optimize separations, while under increasing output demands. This optimization often manifests in faster run times, less solvent consumption, and improved resolution. The development of UHPLC instrumentation enabled labs to reduce their run times significantly due to the design to withstand higher back pressures and reduced extra-column volume contributions; this further spurred on the development of sub 2 micron particle sizes to further improve the chromatography. Superficially porous particles (SPP) are an alternative column technology that are excellent for high throughput laboratories, with an added advantage of being compatible with both UHPLC and HPLC systems. For example, a HALO 90 Å C18, 2.7 µm, 4.6 x 50 mm column is run under the same chromatographic conditions as the previous 5 µm sildenafil separation. The column shows 3x faster run times with similar back pressures resulting in less mobile phase consumption (<2.5x) and faster throughput. Figure 3 shows the chromatogram.

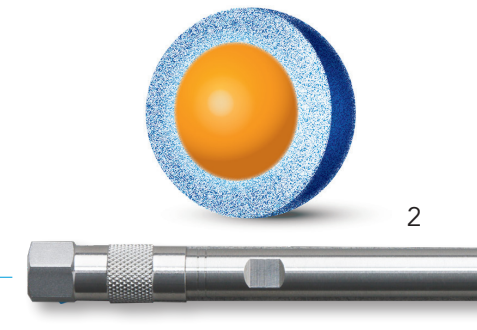
Figure 3. A HALO® 2.7 µm 4.6 x 50 mm C18 column is used for the HPLC methods specified within the sildenafil citrate USP Monograph using the same conditions as the 5 µm 4.6 x 150 mm. The 2.7 µm column is 3x faster than the 5 µm column saving mobile phase and increasing throughput.



TEST CONDITIONS

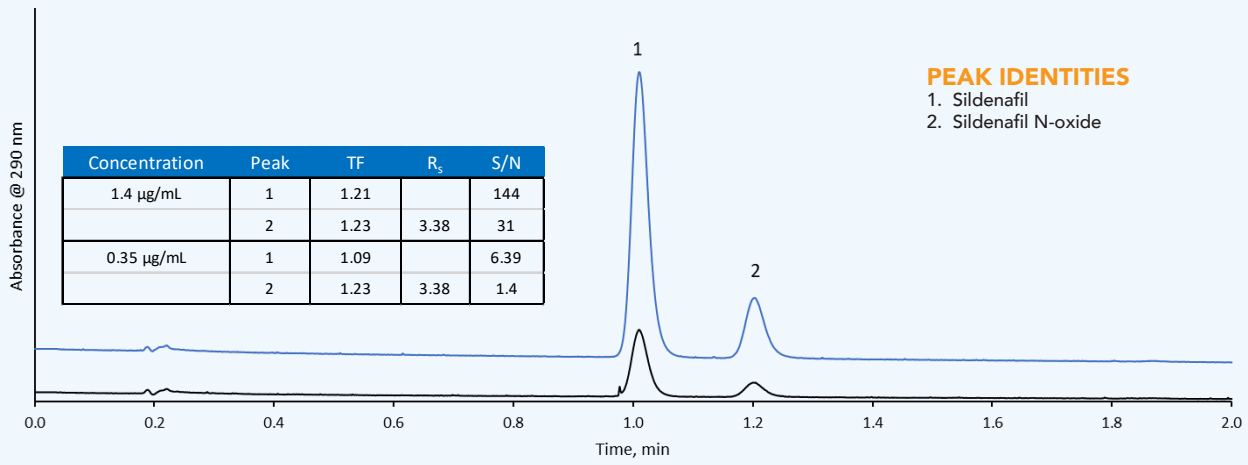
Column: HALO® 90 Å C18, 2.7 µm, 4.6 x 50mm
Part Number: 92814-402
Mobile Phase: 58/25/17 (v,v,v) buffer, Methanol, Acetonitrile
Buffer: 7mL TEA in 1 L Water, pH: 3 (adjust with phosphoric acid)
Isocratic
Flow Rate: 1.0 mL/min
Initial Back Pressure: 193 bar
Temperature: 30 °C
Detection: 290 nm
Injection Volume: 10 µL
Sample Solvent: mobile phase buffer
Data Rate: 100 Hz
Response Time: 0.025 sec
Flow Cell: 1 µL
Instrument: Shimadzu Nexera X2

Concentration	Peak	TF	R_s	S/N
1.4 µg/mL	1	1.03		180
	2	1.03	4.47	37
0.35 µg/mL	1	1.03		49
	2	1.03	4.44	11



Furthermore, method development utilizing SPP in a decreased particle diameter size to a 2 µm column is demonstrated (note: requires the use of a 600 bar HPLC system). Using the same method conditions, but moving the separation to a 2 µm, 3.0 x 50 mm column an even faster separation is observed. Figure 4 shows a separation 6x faster (<5x mobile phase consumption) compared to the 5 µm separation.

Figure 4. A HALO® 2 µm 3.0 x 50 mm C18 column is used for the HPLC methods specified within the sildenafil citrate USP Monograph using the same conditions as the 5 µm 4.6 x 150 mm. The 2 µm HALO® column is 6x faster than the 5 µm column saving mobile phase and increasing throughput.



Concentration	Peak	TF	R_s	S/N
1.4 µg/mL	1	1.21		144
	2	1.23	3.38	31
0.35 µg/mL	1	1.09		6.39
	2	1.23	3.38	1.4

TEST CONDITIONS

Column: HALO® 90 Å C18, 2 µm, 3.0 x 50mm
Part Number: 91813-402
Mobile Phase: 58/25/17 (v,v,v) buffer, Methanol, Acetonitrile
Buffer: 7mL TEA in 1 L Water, pH: 3 (adjust with phosphoric acid)
Isocratic
Flow Rate: 1.0 mL/min
Initial Back Pressure: 537 bar

Temperature: 30 °C
Detection: 290 nm
Injection Volume: 10 µL
Sample Solvent: mobile phase buffer
Data Rate: 100 Hz
Response Time: 0.025 sec
Flow Cell: 1 µL
Instrument: Shimadzu Nexera X2

CONCLUSIONS

HPLC chromatographic methods can be greatly improved upon by simply reducing the columns length and particle size. This not only saves time, but increases column throughput along with less mobile phase consumption and waste generation.