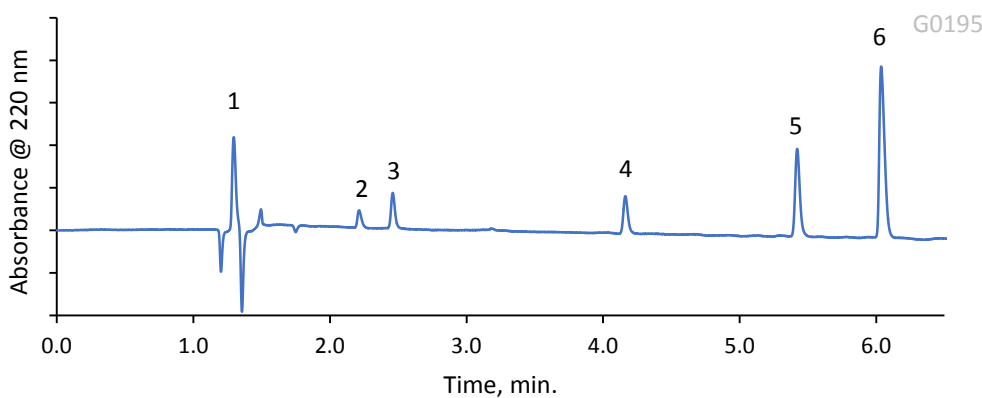


Amine Medications Separated Using HALO® C18, 5µm



PEAK IDENTITIES:

1. Maleic Acid
2. Pseudoephedrine
3. Scopolamine
4. Doxylamine
5. Chlorpheniramine
6. Diphenhydramine

TEST CONDITIONS:

Column: HALO 90 Å C18, 5 µm, 4.6 x 150mm

Part Number: 95814-702

Mobile Phase A: 50mM Ammonium Formate/ 0.1% Formic Acid

Mobile Phase B: 50/50 MeOH:Acetonitrile/ 0.1% Formic Acid

Gradient: Time (min.) %B

0.0 20

6.5 60

Flow Rate: 1.0 mL/min

Initial Back Pressure: 190 bar

Temperature: 30°C

Detection: 220 nm, PDA

Injection Volume: 3 µL

Sample Solvent: 80/20 Mobile Phase A/B

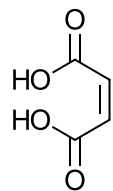
Data Rate: 40 Hz

Response Time: 0.025 sec.

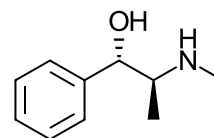
Flow Cell: 1 µL

LC System: Shimadzu Nexera X2

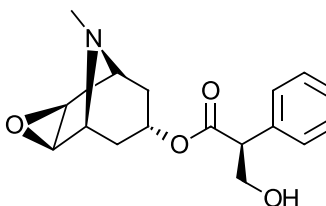
STRUCTURES



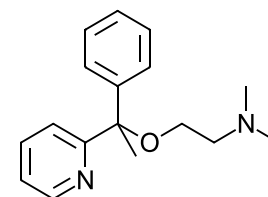
Maleic Acid



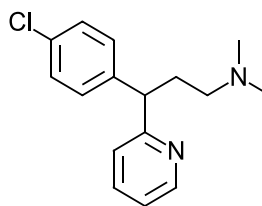
Pseudoephedrine



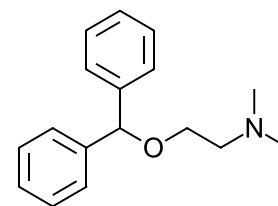
Scopolamine



Doxylamine



Chlorpheniramine

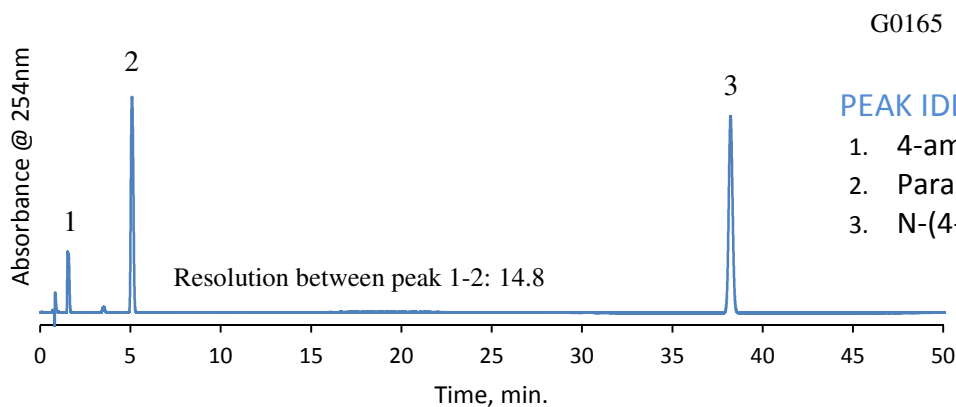


Diphenhydramine

A mixture of amines including antihistamines, decongestants, and other medications is separated on a HALO® 5µm C18 column. The column shows excellent peak shapes for basic compounds using an ammonium formate buffer at low pH.

Application Note: 171-EP

Separation of Paracetamol and Impurities According to EP 9.4



PEAK IDENTITIES:

1. 4-aminophenol (impurity K)
2. Paracetamol
3. N-(4-chlorophenyl) acetamide (impurity J)

TEST CONDITIONS:

Column: HALO 90Å C18, 2.7 µm, 2.1 x 100mm

Part Number: 92812-602

Mobile Phase A: 20mM Potassium Phosphate Buffer

Mobile Phase B: Methanol

Gradient: 0-1 min. hold at 5%

1-10 min. 5-10% B

10-20 min. hold at 10% B

20-40 min. 10-34% B

40-50 min. hold at 34% B

Flow Rate: 0.3 mL/min

Initial Pressure: 171 bar

Temperature: 30°C

Detection: UV 254 nm, PDA

Injection Volume: 5 µL

Sample Solvent: methanol:water, 5:95

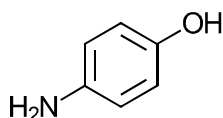
Data Rate: 40 Hz

Response Time: 0.005 sec

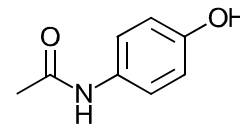
Flow Cell: 2 µL

LC System: Agilent 1200 SL

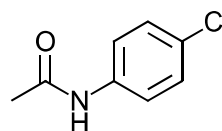
STRUCTURES:



4-aminophenol



Paracetamol



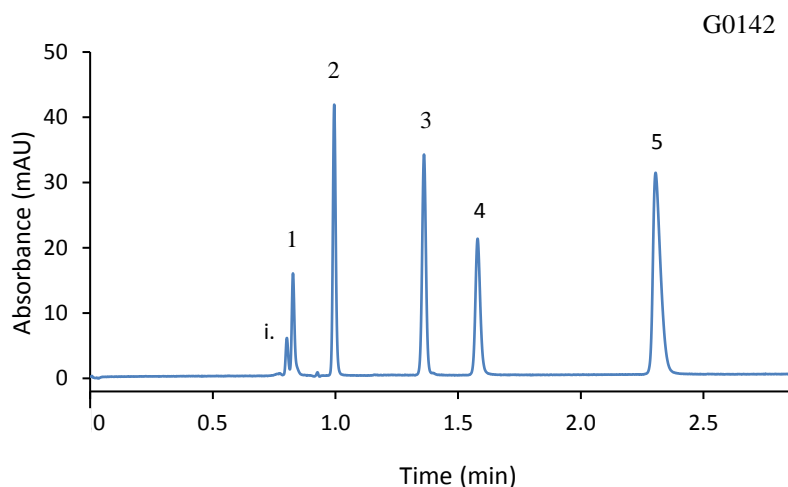
N-(4-chlorophenyl) acetamide

A HALO C18 column is used to separate paracetamol and two of its impurities following the European Pharmacopoeia 9.4 monograph for paracetamol. This method is used to examine several paracetamol impurities providing high resolution between peaks while leaving sufficient separation in the baseline for any other impurity or degradant peaks that may be present in a sample.

HALO | Fused-Core® Particle Technology

Application Note: 152-CM

Separation of OTC Common Cold Medicinal Compounds



PEAK IDENTITIES:

1. Maleic Acid
2. Acetaminophen
3. Guaifenesin
4. Chlorpheniramine Maleate
5. Dextromethorphan HBr
- i. Impurity from Dextromethorphan HBr

TEST CONDITIONS:

Column: HALO 90Å, C18, 2.7 μ m, 4.6 x 150mm
Part Number: 92814-702

Mobile Phase:

A= 50mM Potassium Phosphate buffer, pH: 2.5
B= Acetonitrile

Isocratic: 30% B

Flow Rate: 1.5 mL/min

Pressure: 266 bar

Temperature: 45°C

Detection: UV 220 nm, PDA

Injection Volume: 0.5 μ L

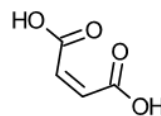
Acquisition Rate: 40 Hz

Flow Cell: 2.5 μ L semi-micro

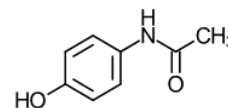
LC System: Agilent 1200 SL

Acetaminophen (analgesic), guaifenesin (expectorant), chlorpheniramine maleate (antihistamine), and dextromethorphan (cough suppressant) are common compounds found in many over-the-counter (OTC) cold medicines. A HALO 90Å, C18 2.7 μ m column is used to separate these compounds quickly and accurately under isocratic conditions.

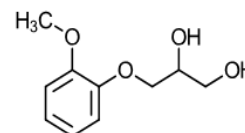
STRUCTURES:



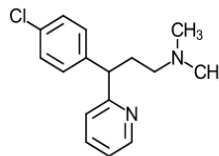
Maleic Acid



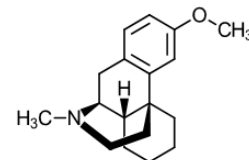
Acetaminophen



Guaifenesin

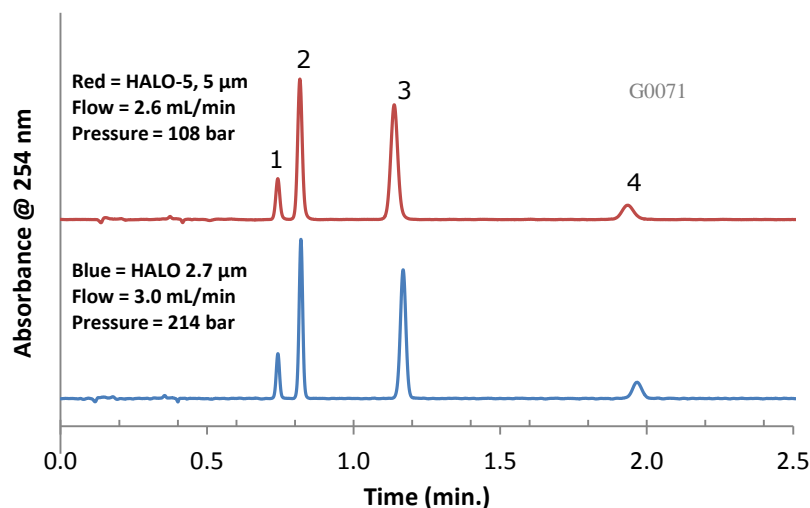


Chlorpheniramine Maleate



Dextromethorphan HBr

Comparable Selectivity between HALO-5 and HALO 2.7 µm HILIC Phases



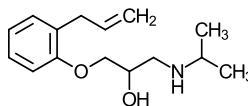
PEAK IDENTITIES:

1. Alprenolol
2. Pindolol
3. Acebutolol
4. Atenolol

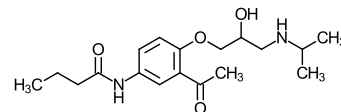
TEST CONDITIONS:

Column 1: 4.6 x 100 mm, HALO 5 µm HILIC
 Part Number: 95814-601
 Column 2: 4.6 x 100 mm, HALO 2.7 µm HILIC
 Part Number: 92814-601
 Mobile Phase: 11/89: A/B
 A = 0.1 M Ammonium formate, pH=3 (adj.)
 B = Acetonitrile
 Flow Rate: See chart
 Pressure: See chart
 Temperature: 30°C
 Detection: UV 254 nm, VWD
 Injection Volume: 2.0 µL
 Sample Solvent: mobile phase
 Response Time: 0.02 sec.
 Flow Cell: 2.5 µL semi-micro
 LC System: Shimadzu Prominence UFLC XR
 ECV: ~14 µL

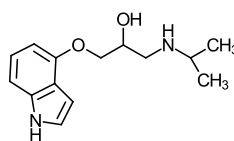
STRUCTURES:



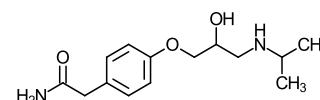
Alprenolol



Acebutolol



Pindolol

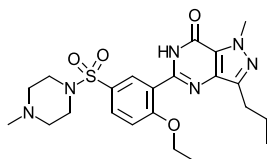


Atenolol

Because of similar selectivities, easy method transfer is accomplished between the 5 µm and 2.7 µm HALO HILIC phases with small changes in flow rate.

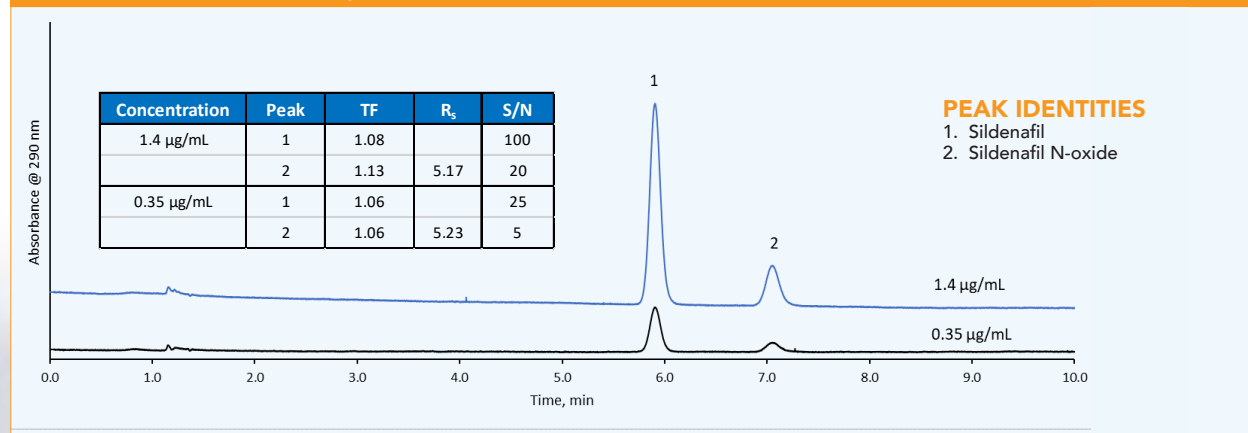
Sildenafil Comparison on HALO® 2, 2.7, and 5 µm

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.

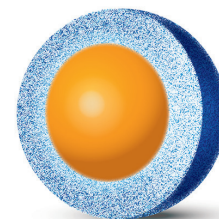
Figure 2: A HALO® 5 µm C18 column is used for the HPLC methods specified within the sildenafil citrate USP Monograph. This includes the diluted sample solution (1.4 µg/mL) and the sensitivity solution (0.35 µg/mL). Tailing factor, resolution, and signal to noise ratio requirements are all met showing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



TEST CONDITIONS

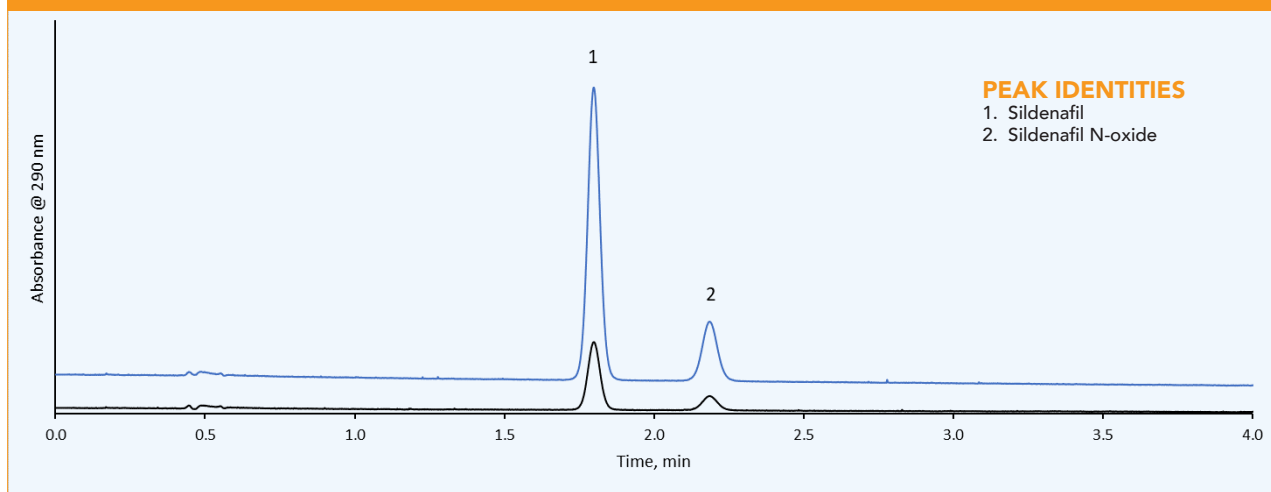
Column: HALO 90 Å C18 5 µm, 4.6 x 150mm
 Part Number: 95814-702
 Mobile Phase: 58/25/17 (v,v,v)
 Buffer: Methanol, Acetonitrile
 Buffer: 7 mL TEA in 1 L Water, adjusted to pH: 3 w/ phosphoric acid
 Isocratic
 Flow Rate: 1.0 mL/min
 Initial Back Pressure: 193 bar

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.

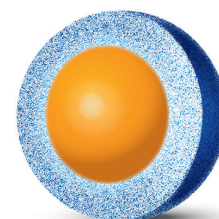
Figure 3. A HALO® 2.7 µm 4.6 x 50 mm C18 column is used for the HPLC methods specified within the sildenafil citrate USP Monograph using the same conditions as the 5 µm 4.6 x 150 mm. The 2.7 µm column is 3x faster than the 5 µm column saving mobile phase and increasing throughput.



TEST CONDITIONS

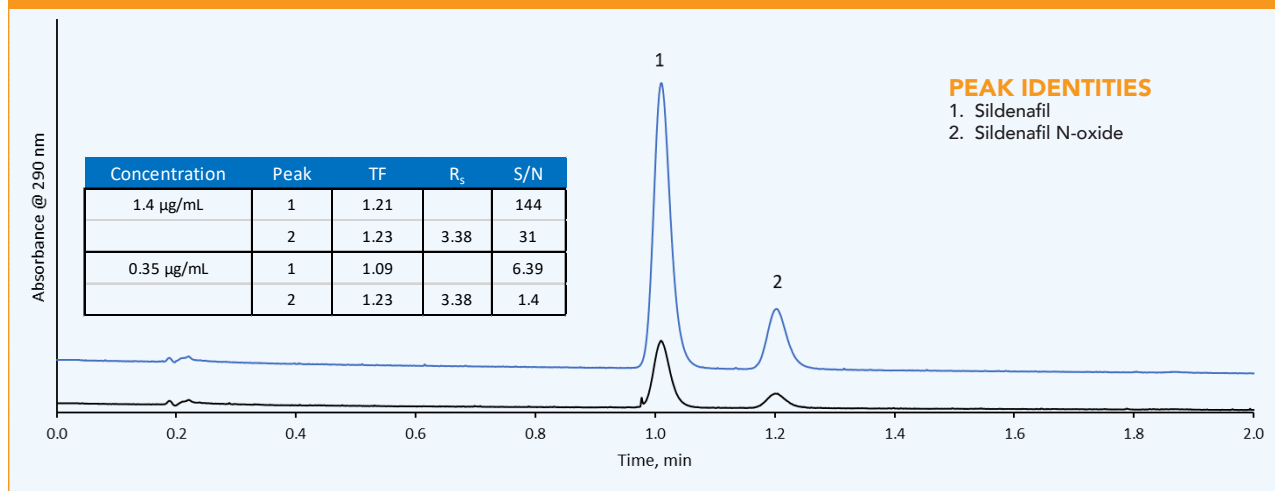
Column: HALO® 90 Å C18, 2.7 µm, 4.6 x 50mm
 Part Number: 92814-402
 Mobile Phase: 58/25/17 (v,v,v) buffer, Methanol, Acetonitrile
 Buffer: 7mL TEA in 1 L Water, pH: 3 (adjust with phosphoric acid)
 Isocratic
 Flow Rate: 1.0 mL/min
 Initial Back Pressure: 193 bar
 Temperature: 30 °C
 Detection: 290 nm
 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.



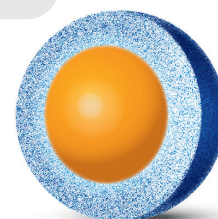
TEST CONDITIONS

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

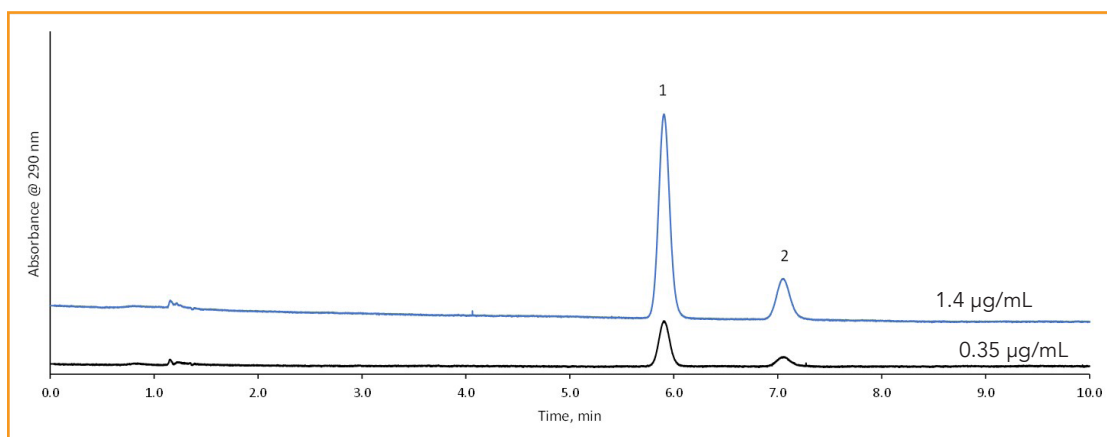
HPLC chromatographic methods can be greatly improved upon by simply reducing the columns length and particle size. This not only saves time, but increases column throughput along with less mobile phase consumption and waste generation.





Sildenafil Citrate on HALO® C18, 5 µm

234-P



PEAK IDENTITIES

1. Sildenafil
2. Sildenafil N-oxide

TEST CONDITIONS:

Column: HALO 90 Å C18, 5 µm, 4.6 x 150 mm
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
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

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

Sildenafil (better known as Viagra) is a medication used to treat erectile dysfunction. The drug came off patent in 2019. 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.

