Importance of Proper HPLC Column Storage

How does a customer get the longest lifetime from their HPLC columns? The columns should be stored according to the Care and Use sheet that is included in each HALO® column box. Generally speaking, any buffer or acidic or basic additive should be rinsed from the column and the column should be stored in 100% organic, typically acetonitrile (ACN). Storage in ACN preserves the stationary phase and protects it from being exposed to water, which can hydrolyze the siloxane bonds of the stationary phase and cause reduced retention.

Three columns of HALO 90 Å C18, 5 µm were stored using different conditions: 95% Water/5% ACN/0.04% TFA, 5% Water/95% ACN/0.04% TFA, and 100% ACN. When the columns were not being run, they were capped and stored at 40 °C with no flow. The columns were periodically tested to investigate retention time and peak shape. After 28 days, the columns were then stored for 21 days at 70 °C to accelerate the aging process. Since not much change was observed the columns were stored at 70 °C for an additional 21 days. The test mix consisted of uracil, phenol, and reserpine. Method conditions are listed below:

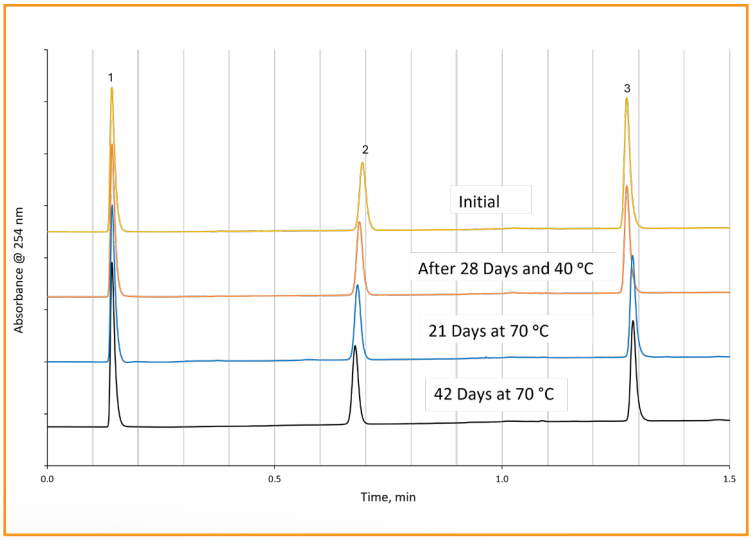

Figure 1. HALO® C18, 5 µm column stored in 95% Water/5% ACN/0.04% TFA

Columns: HALO 90 Å C18, 5 µm, 3.0 x 30 mm
Part Number: 95813-302
Mobile Phase A: Water/0.04% TFA
Mobile Phase B: Acetonitrile/0.04% TFA
Gradient:

Time	%B
0.0	5
2.5	95
3.0	95
3.01	5
3.50	5

Flow Rate: 1.0 mL/min
Back Pressure: 120 bar
Temperature: 40 °C
Injection Volume: 0.5 µL
Sample Solvent: Water/ACN
Detection: UV/PDA, 260 nm
Flow Cell: 1 µL
Data Rate: 40 Hz
Response Time: 0.05 s
LC System: Shimadzu Nexera X2

The retention time of peak 2/phenol, which is neutral shows decreased retention over the course of the stability study. This is in contrast to peak 3/reserpine, which is a basic compound and shows increased retention over time. As the column is stored in mostly water at low pH conditions, the endcapping is removed over time. Less endcapping translates to reduced retention for neutral phenol and increased retention for the basic reserpine. Positively charged reserpine interacts with free silanols that are present when endcapping is removed, thus increasing the retention. Figure 2 shows the results of the HALO® C18 column stored in 5% Water/95% ACN/0.04% TFA.

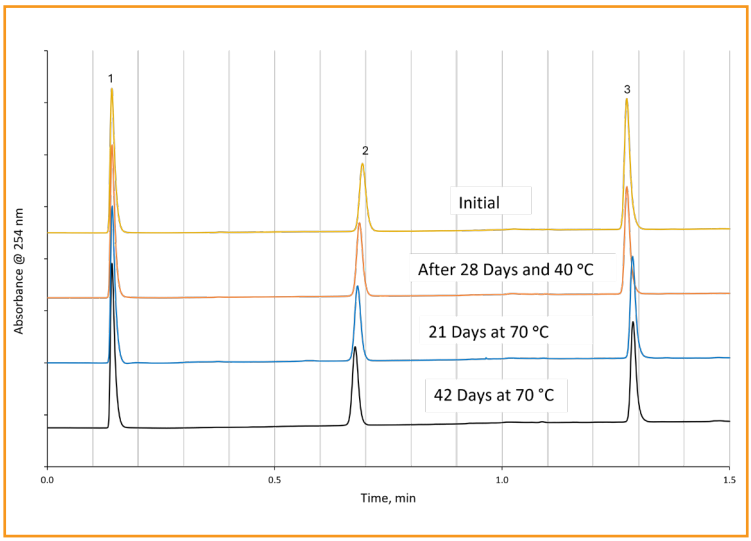

Figure 2. HALO® C18, 5 µm column stored in 5% Water/95% ACN/0.04% TFA

The retention time for both the neutral and the basic compound are maintained when the column is stored in mostly ACN, despite the presence of the 0.04% TFA. Since the environment is mostly organic, there is little water present to cause hydrolysis of the endcapping from the particles' surfaces. The retention time is essentially the same as the initial result compared to the result after storage at 70 °C. Figure 3 shows the results of the HALO® C18 column stored in 100% ACN with no TFA.

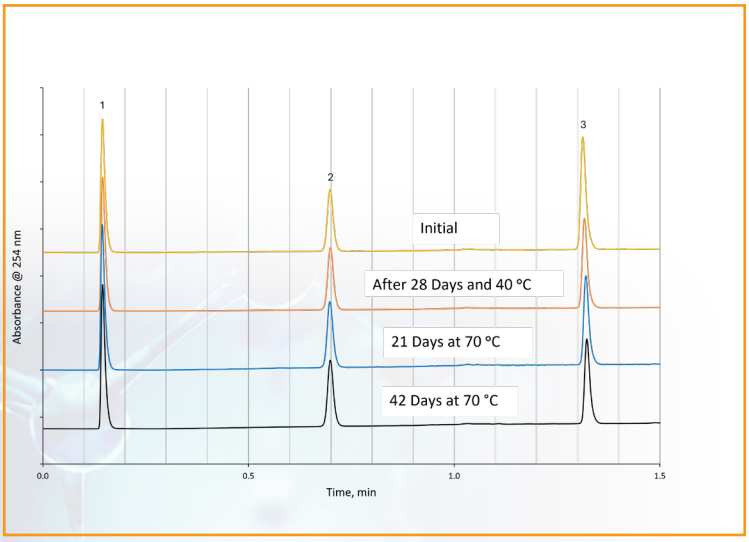

Figure 3. HALO® C18, 5 µm column stored in 100% ACN

Very similar to the column stored in 5% Water/95% ACN/0.04% TFA, the HALO® C18 column stored using 100% ACN demonstrated stable retention times for both the neutral and basic compounds over the course of the stability study. For the longest column lifetimes, HALO® C18 columns should be stored in 100% ACN, but if this is not possible, it is acceptable to store columns in 5% water/95% ACN even with small amounts of TFA, but this is not recommended for longest column life.

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Dr. Schuster earned her Ph.D. under the guidance of Prof. Joe P. Foley at Drexel University. She joined Advanced Materials Technology, Inc. (AMT) in June 2009 as a research scientist working with Dr. Jack Kirkland, providing contributions to the commercial development of products designed for peptide and protein separations. Most recently, as part of the Technical Support team at AMT, Stephanie has been providing customers support in optimizing and trouble-shooting their applications.