Acid/Base Stability of Silica Based C8, C18 and Amide HPLC Columns

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Introduction

- **Data is often presented regarding HPLC column stability in which efficiency and retention values are presented with respect to the number of days the column has been running. The weakness of such data is that the mobile phases are frequently not aggressive, and the test mixes are trivial.**
- **In this study we wanted to truly evaluate the stability of the Ascentis C8, C18 and RP-Amide columns, which are based upon a pure silica platform, under aggressive mobile phase conditions. We further wanted to see the effects that column aging has on analytes that are more challenging and hence a better probe of the column's mechanical integrity, main phase stability and end-capping.**

Experimental

1. Each column was subjected to an initial battery of tests. The analytes and conditions in these tests probe the surface chemistry and integrity of the phase. Two of those tests are shown in this presentation.
2. The initial battery tests were followed by continuous flow experiments using aggressive mobile phases (either pH = 1.5 or pH = 10) for 10,000 column volumes. Hourly injections were made with a test mixture which measures column aging. Efficiency, k' and asymmetry were monitored as key indicators of aging.
3. The battery tests were then repeated after each 10,000 column volumes. With the pH = 10 mobile phase, the experiments were continued until a 20% loss in either efficiency or k' occurred. At pH 1.5, the experiments were stopped at 100,000 column volumes with less than 10% loss of efficiency in all cases.

Stability at pH = 1.5 Stability Test Mix

column:	Ascentis C18, Ascentis C8 or Ascentis RP-Amide, 5 cm x 3.0 mm I.D., 5 µm particles
mobile phase:	40:60, water:methanol with 0.5% TFA (w/v)
flow rate:	0.9 mL/min.
temp.:	25 °C
det.:	UV at 220 nm

The acidic conditions provided by the TFA can accelerate the loss of phase via hydrolysis of the linkages of the silanes to the surface silanols. Usual running conditions for TFA mobile phases is 0.1% (w/v). The 0.5% (w/v) in this case is a much harsher condition.

Stability Test Mix:

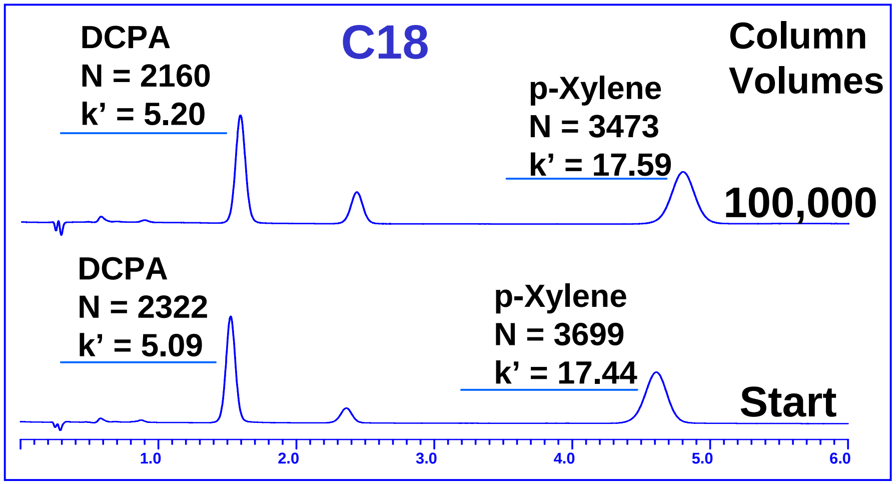
Uracil was used as a void marker.

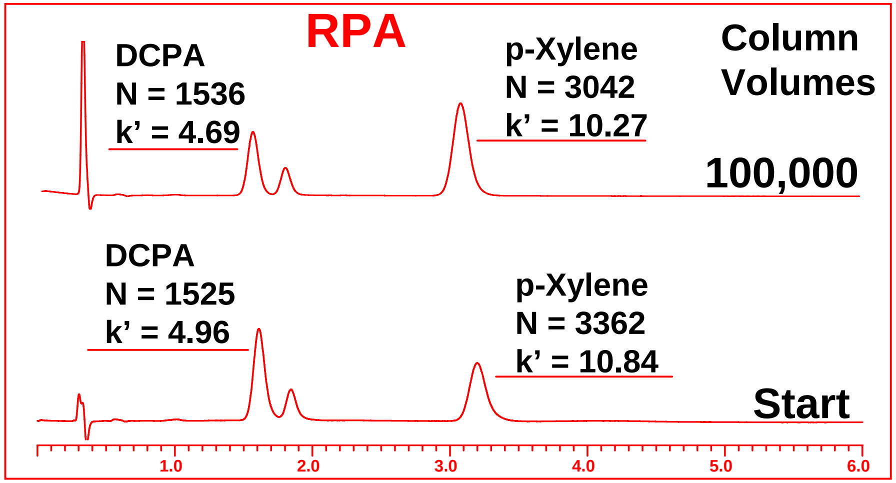
2,4-Dichlorophenoxyacetic Acid (DCPA) was used as a polar acidic analyte that is sensitive to phase loss and silanol activity. As end-capping is lost, peak shape and efficiency should suffer.

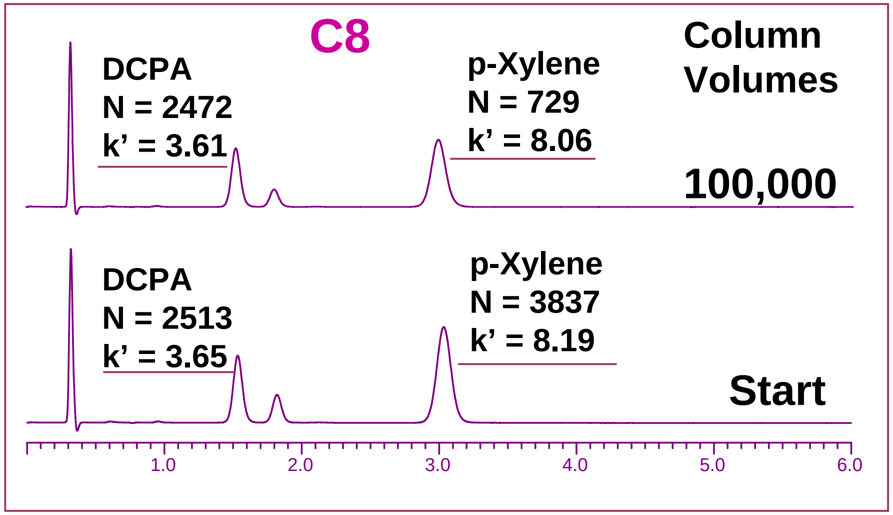
Toluene provided neutral k', efficiency and asymmetry marker.

p-Xylene is a highly retained neutral efficiency marker that is very sensitive to loss of main phase due to its high k'.

C18, RPA, C8 Stability at pH = 1.5







Note: at a k’ = 9, 100,000 column volumes represent 10,000 injections

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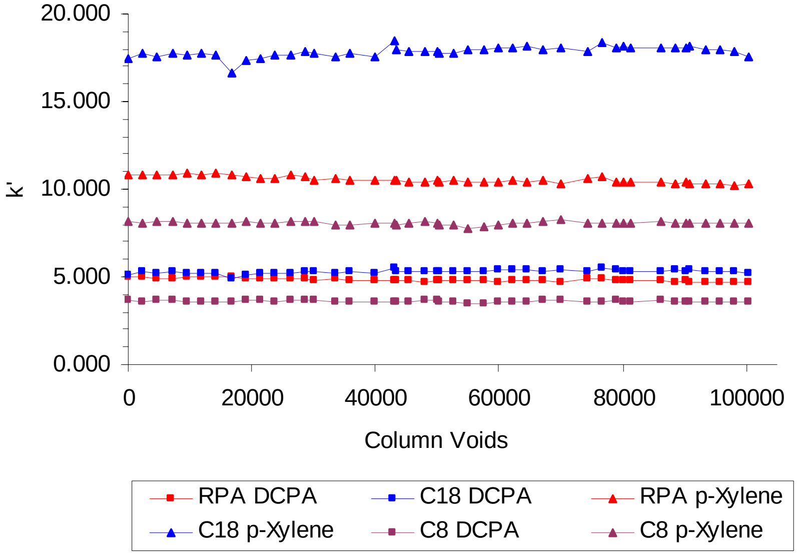
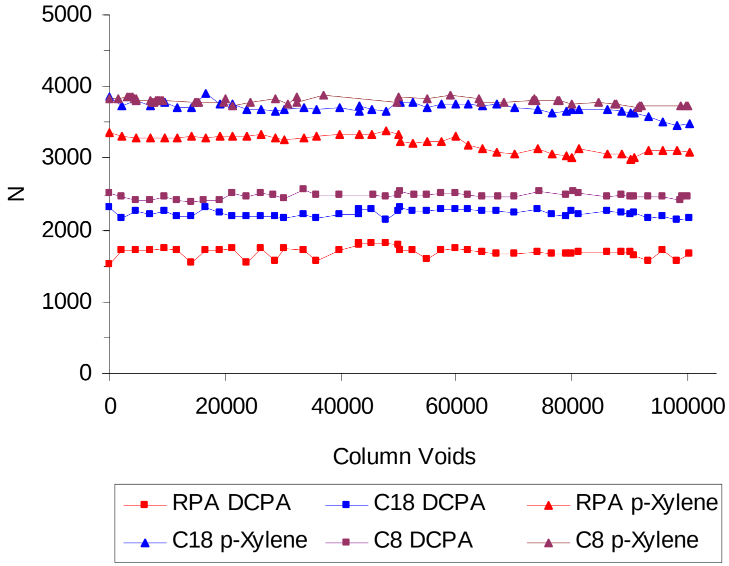
C18, C8, RP-Amide Stability at pH = 1.5





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Stability at pH = 10 Stability Test Mix

column:	Ascentis C18, Ascentis C8 or Ascentis RP-Amide, 5 cm x 3.0 mm I.D., 5 µm particles
mobile phase:	40:60, 10 mM ammonium phosphate (pH 10):methanol
flow rate:	0.9 mL/min.
temp.:	25 °C
det.:	UV at 220 nm

Phosphate buffers are aggressive at high pH and can cause dissolution of silica, stripping of phase and voiding in columns and usually should be avoided at high pH conditions.

Stability Test Mix:

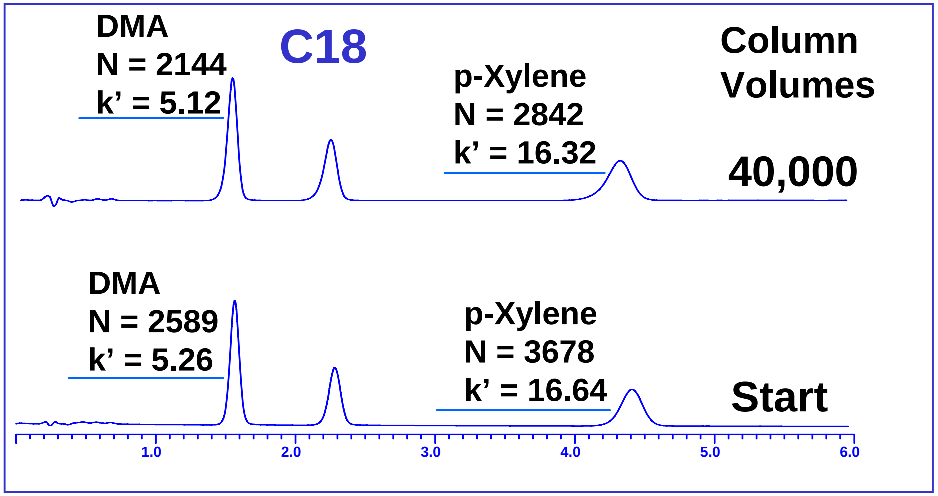
Uracil was used as a void marker.

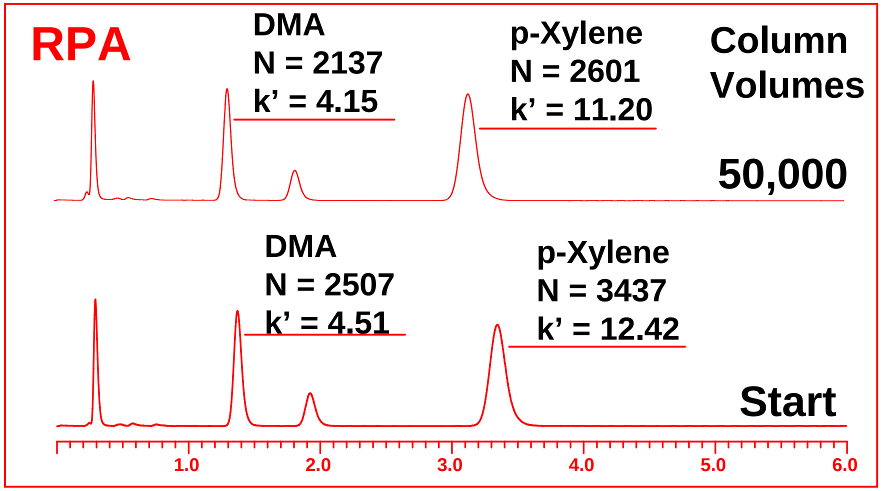
Dimethylaniline (DMA) was used as a polar basic analyte that is sensitive to phase loss and silanol activity. As end-capping is lost, peak shape and efficiency should suffer.

Toluene provided neutral k', efficiency and asymmetry marker.

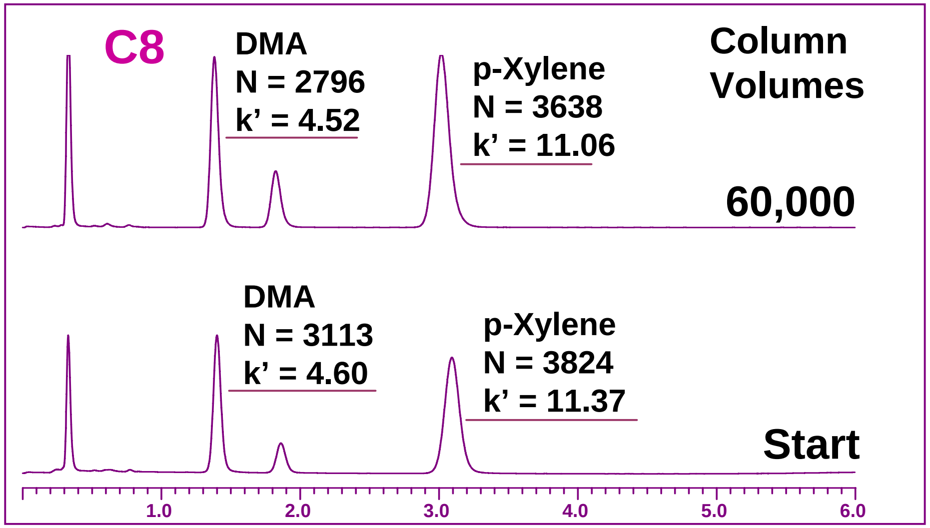
p-Xylene is a highly retained neutral efficiency marker that is very sensitive to loss of main phase due to its high k'.

C18, RPA, C8 Stability at pH = 10





Note: at a k'=9, 40,000 column volumes represent 4,000 injections



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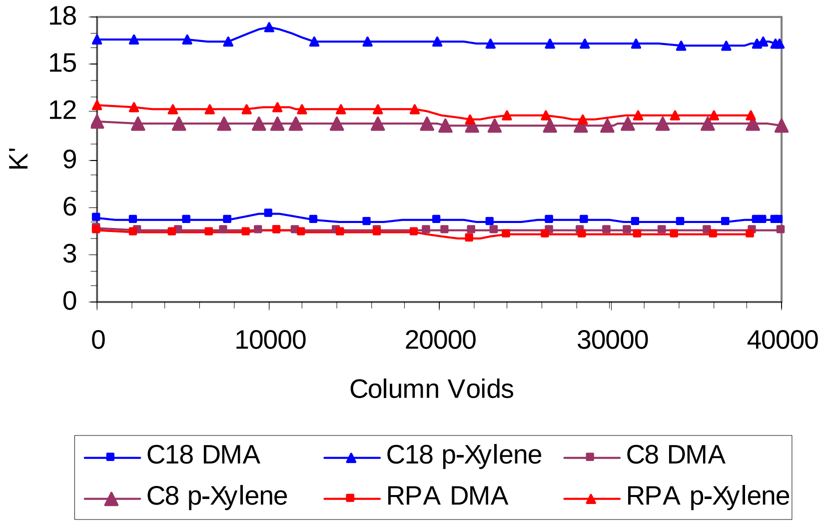
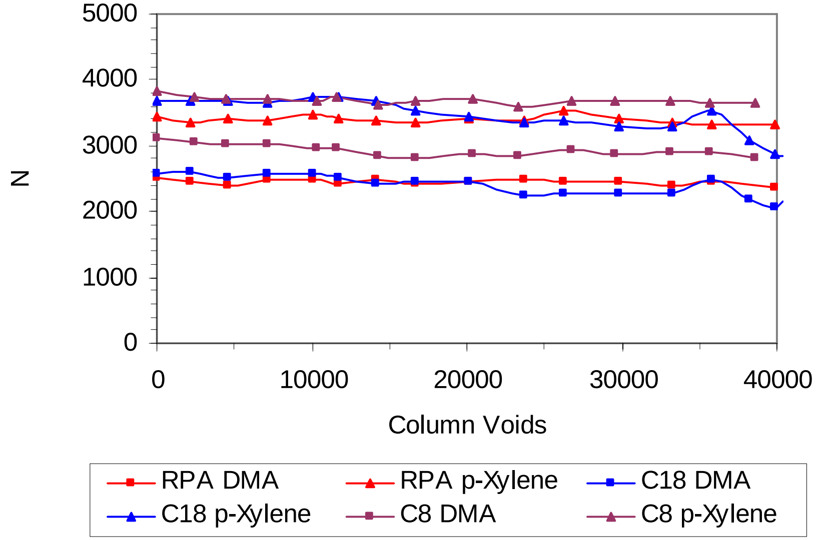
C18, C8, RP-Amide Stability at pH = 10





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Intermediate Stability at pH = 1.5 and 10   DFQ Test Mix

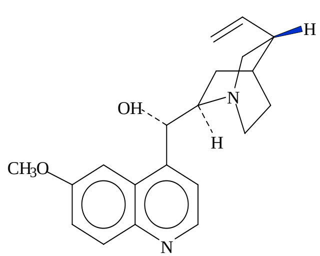
This test was carried out every 10,000 column volumes

DFQ Test Mix:

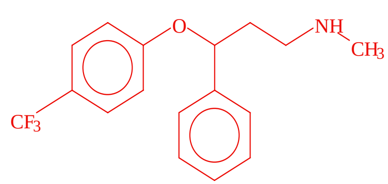
Diphenhydramine, fluoxetine and quinidine provide a sensitive test of end-capping and surface silanol activity. Even on a well end-capped column, these basic molecules can show tailing. Fluoxetine is the most sensitive of the three. As the column ages, loss of end-cap or main phase, or dis-solution of underlying silica will increase number of active silanols and degrade peak shape.

Test Conditions for DFQ Test

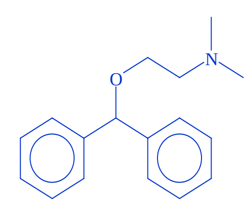
mobile phase:	60% methanol 40% 100 mM ammonium acetate (pH 6.8)
flow rate:	0.43 mL/min.
temp.:	25 °C
det.:	UV at 230 nm



1. Quinidine



2. Fluoxetine



3. Diphenhydramine

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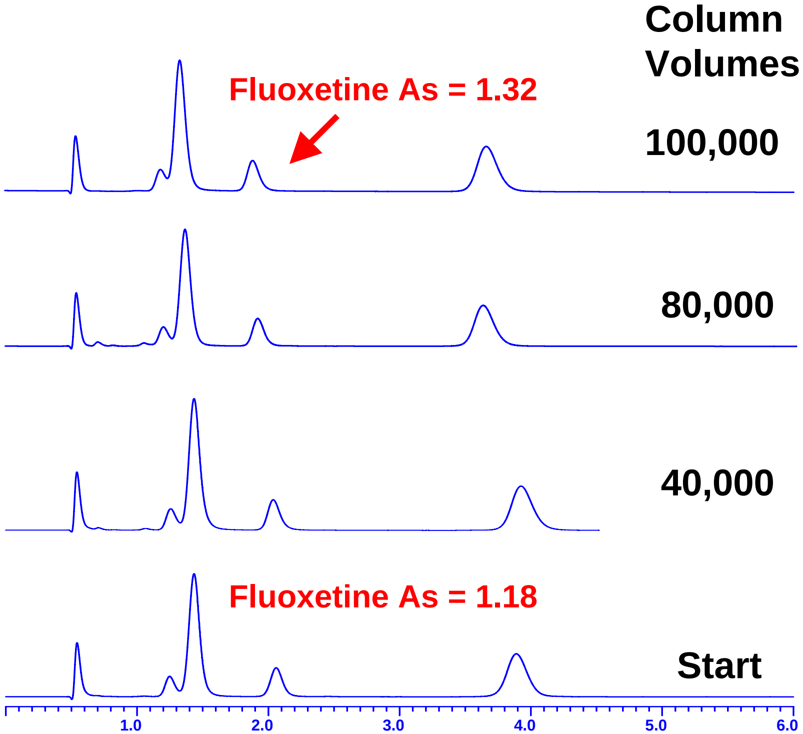
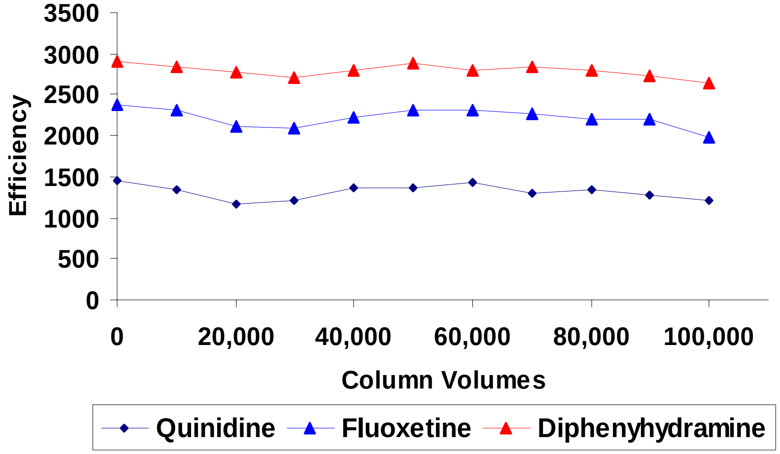
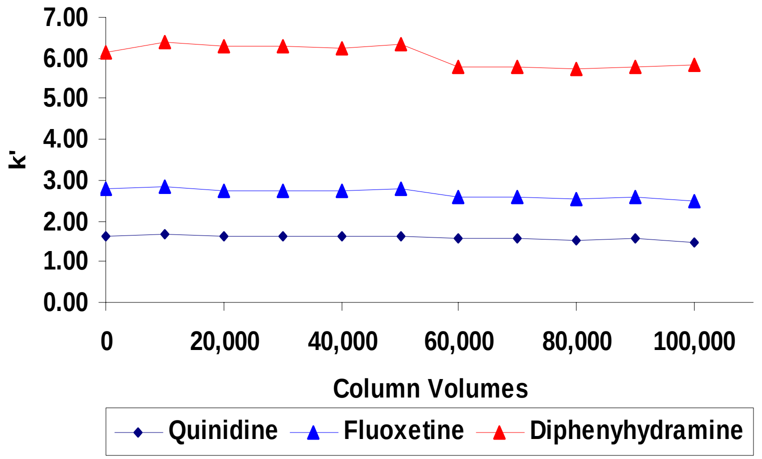
C18 Stability at pH = 1.5 DFQ Test Mix

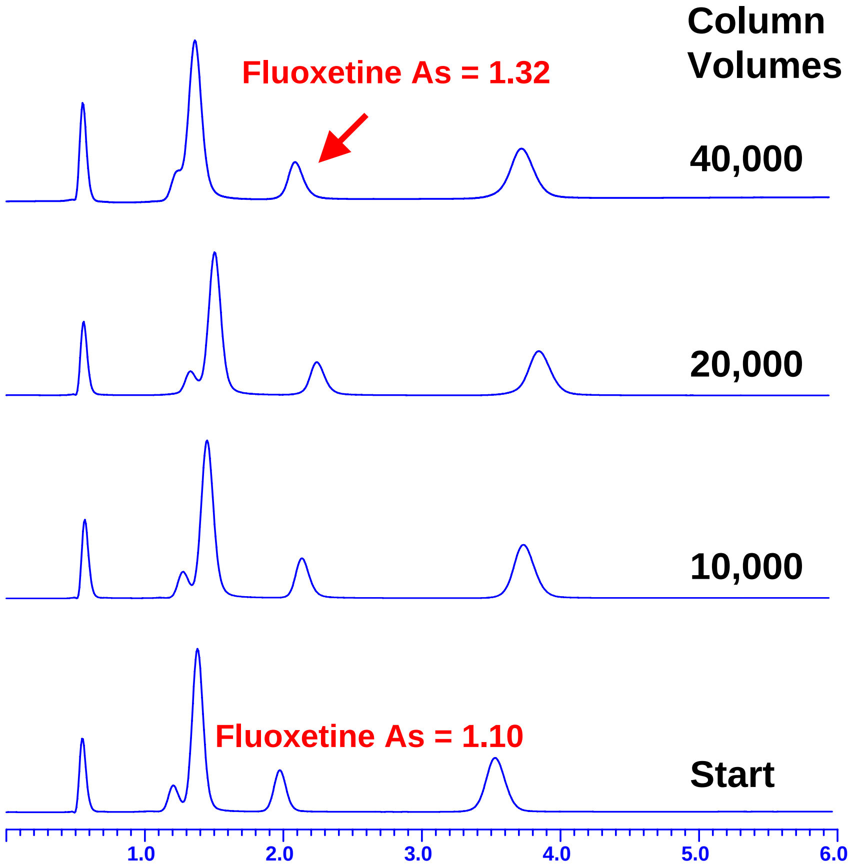


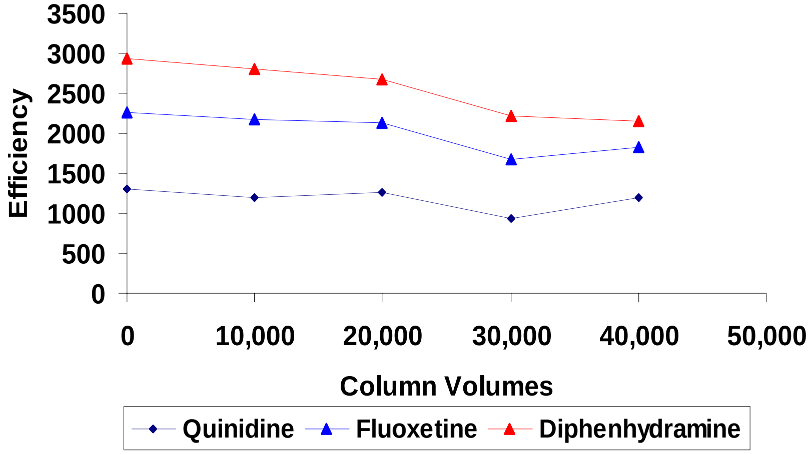


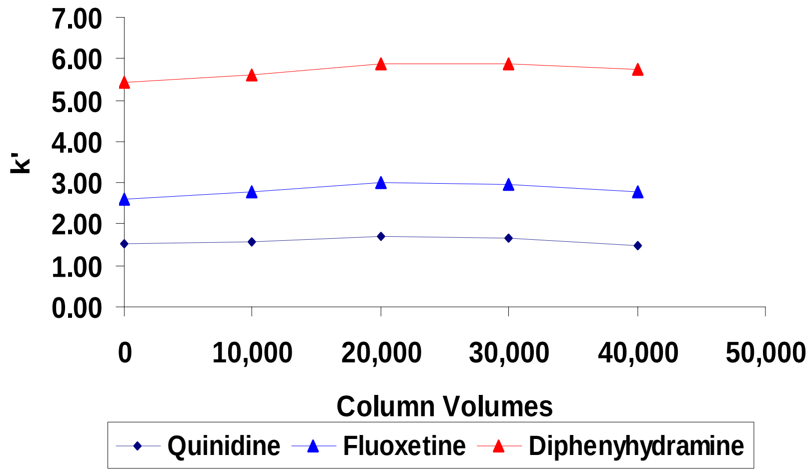


Efficiency and retention were stable for this test mix up to 100,000 column volumes at pH 1.5 and up to 40,000 column volumes at pH 10. Asymmetry also did not change to a large degree. The phase did not significantly bleed from the silica during the course of the experiment and the column bed was stable.

C18 Stability at pH = 10 DFQ Test Mix







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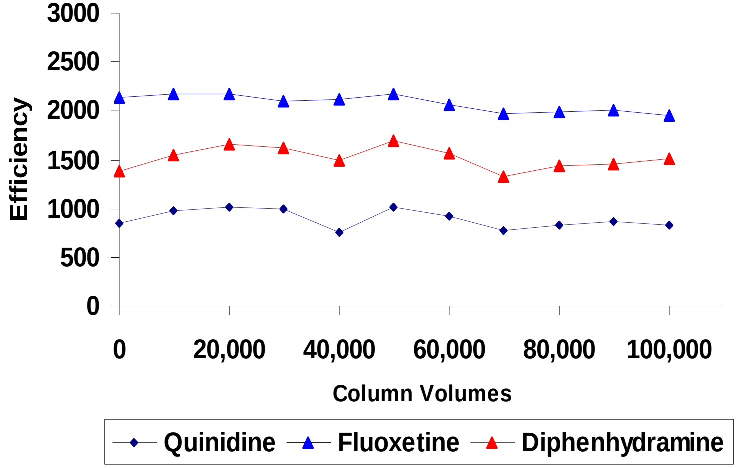
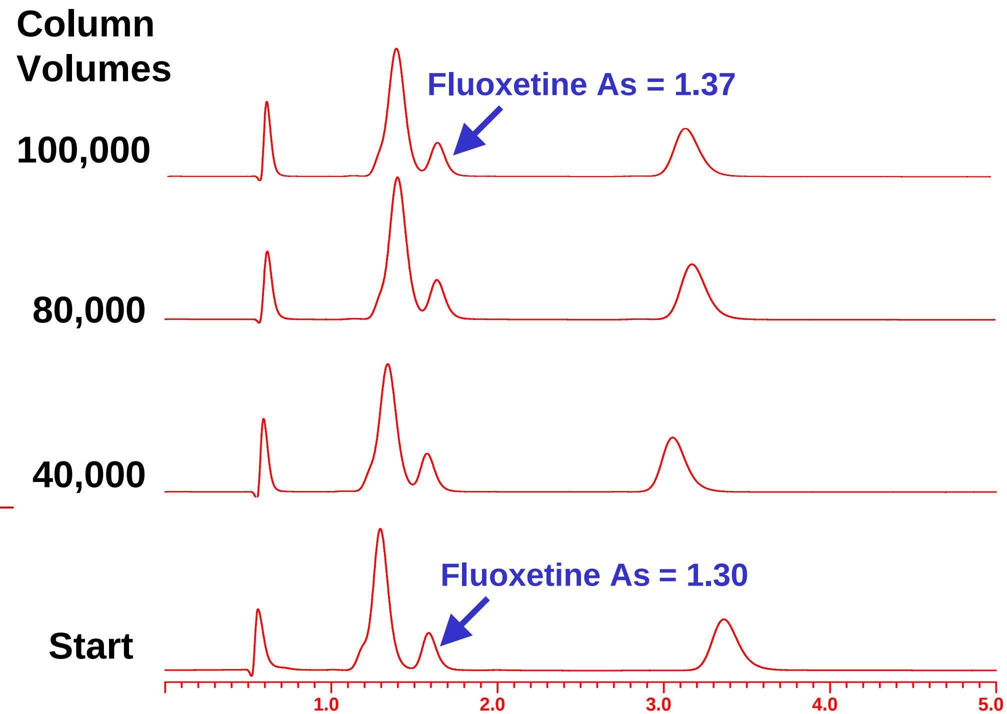
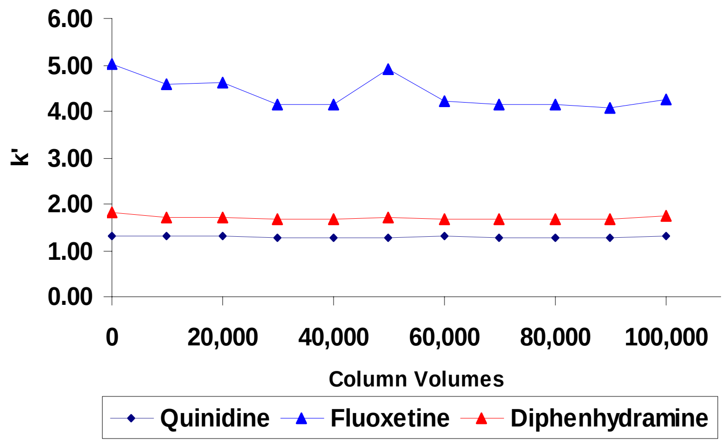
RP-Amide Stability at pH = 1.5 DFQ Test Mix







Efficiency and retention were stable for the RPA up to 100,000 column volumes at pH 1.5 and up to 60,000 column volumes at pH 10. Asymmetry again did not change to a large degree. The phase did not significantly bleed from the silica during the course of the experiment and the column bed was stable.

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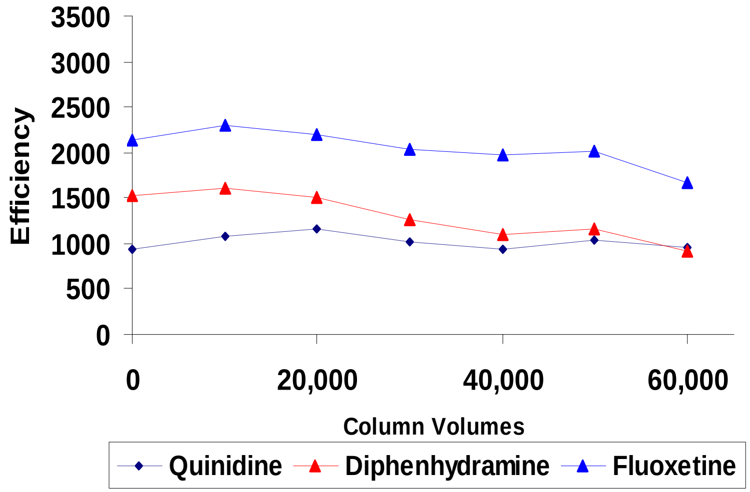
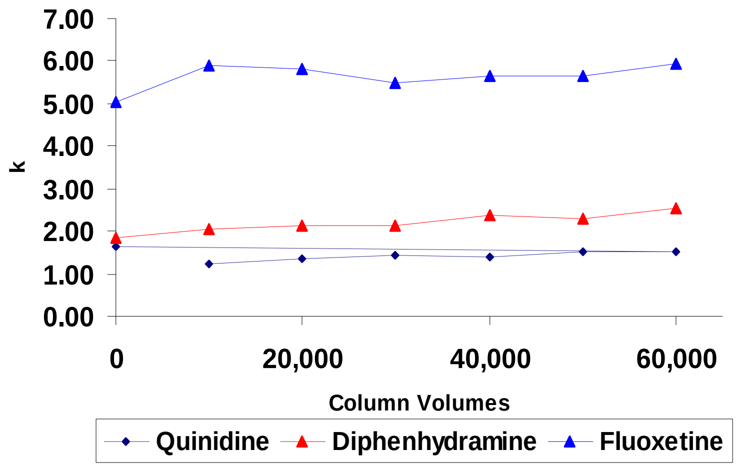
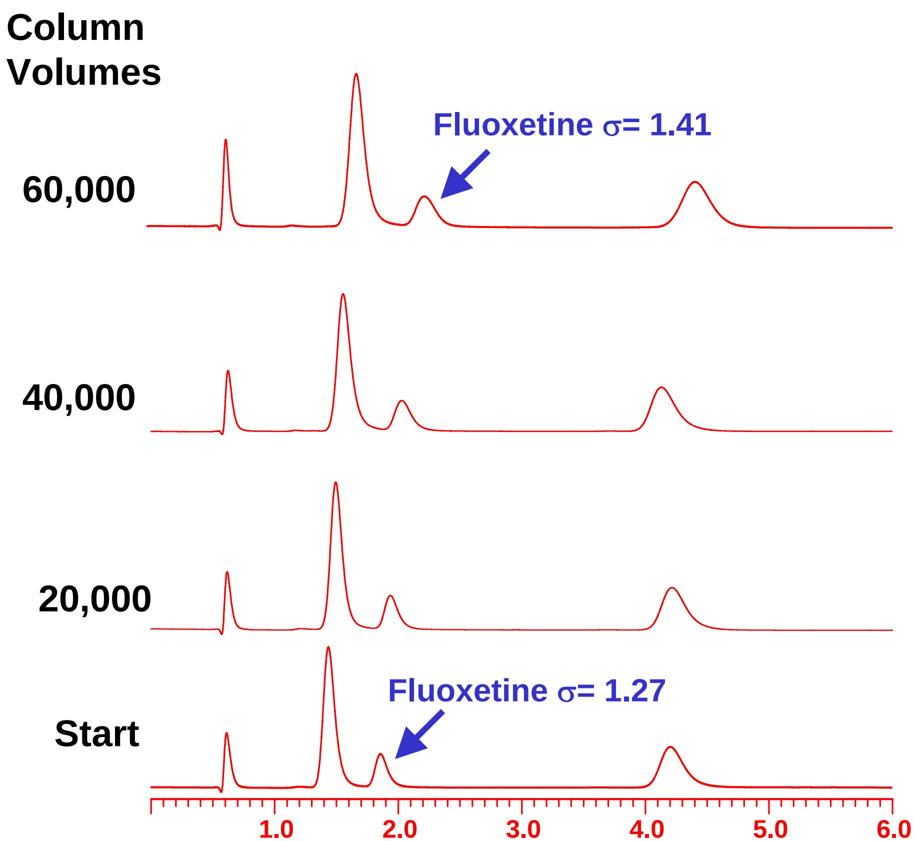
RP-Amide Stability at pH = 10 DFQ Test Mix







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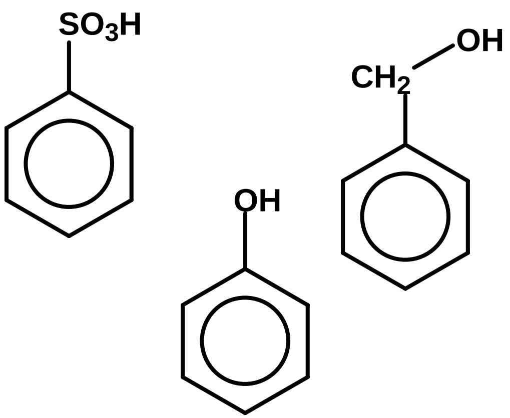
Intermediate Stability at pH = 1.5 and 10 Phenol Test

This test was carried out every 10,000 column volumes

- **Intermediate test B – Phenol Test: Benzenesulfonic Acid (BSA), Benzyl Alcohol (BA) and Phenol (PhOH) serve to probe changes in surface activity under acidic conditions.**
- **Benzenesulfonic acid is a strong acid and will interact strongly with available non-bonded electron pairs in either the phase or the silica surface. Note with the C18 phase, the Benzenesulfonic acid does not interact and therefore is not retained. On the RP-Amide, the polar portion of the phase interacts strongly with BSA and position and peak shape begin to vary with column age.**
- **Benzyl Alcohol / Phenol will co-elute on a well deactivated phase. On the RP-Amide, strong interaction with the phenolic moiety extends the retention of phenol, so the two do not co-elute. In either case, the stability of the retention characteristics and peak shape show the columns performed very well through out the study.**

Test Conditions for Acidic Test

mobile phase:	70% Methanol 30% 10 mM phosphoric acid
flow rate:	0.43 mL/min.
temp.:	25 °C
det.:	UV at 210 nm



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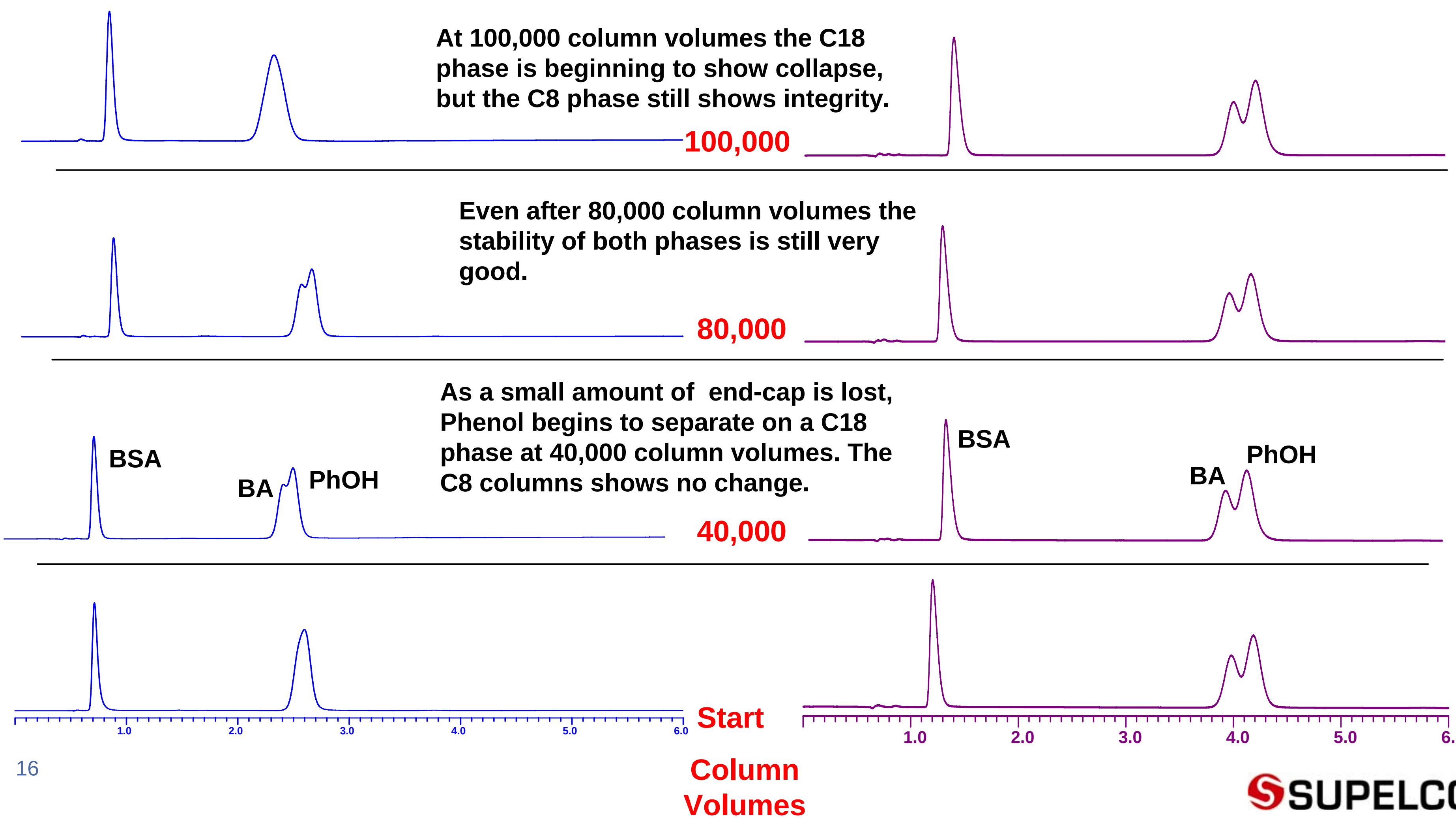
C18 Stability at pH = 1.5

C8 Stability at pH = 1.5



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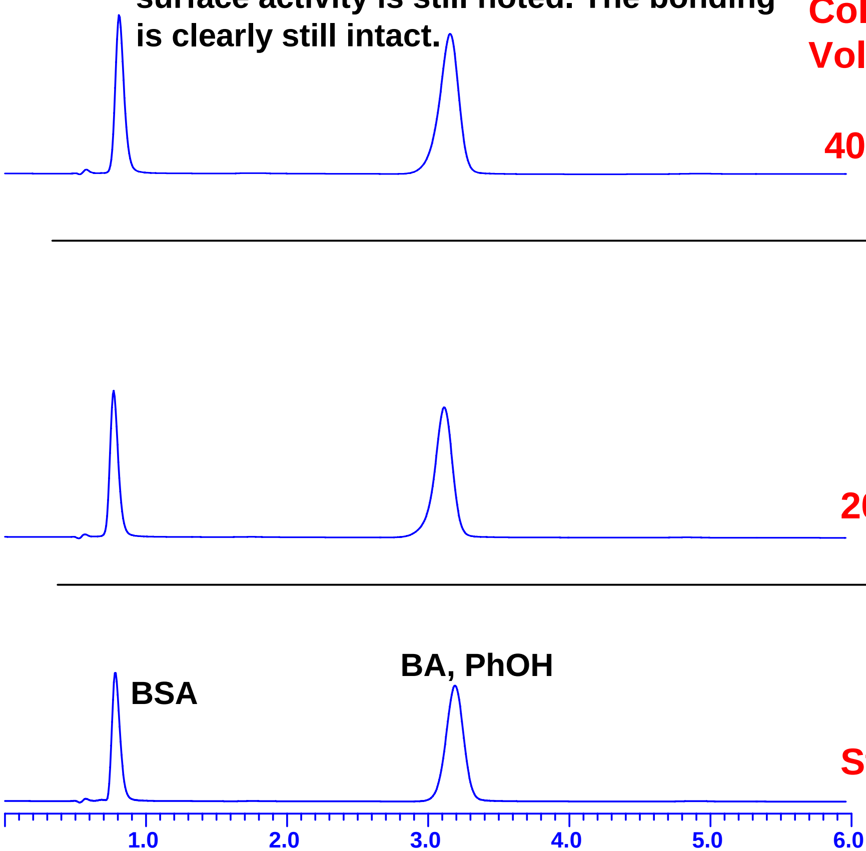
C18 Stability at pH = 10

After 40,000 column volumes at pH=10 low surface activity is still noted. The bonding is clearly still intact.



Column Volumes

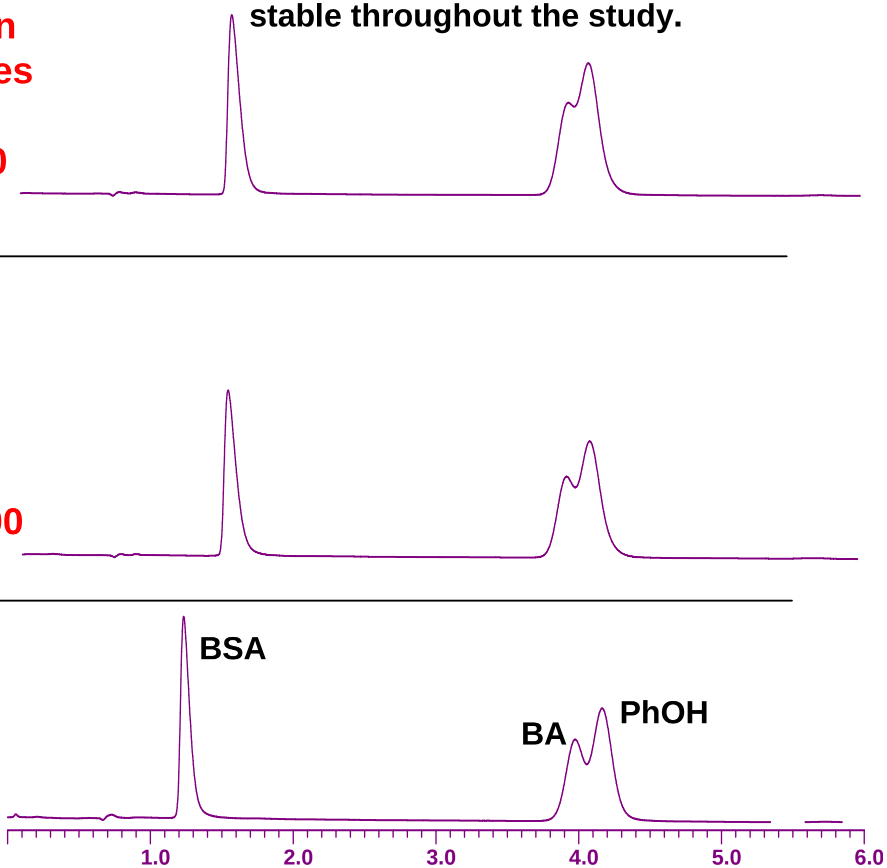
40,000

20,000

Start

C8 Stability at pH = 10

The separation of PhOH and BA were stable throughout the study.



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Conclusion

- The Ascentis C18, C8 and RPA showed strong overall stability when subjected to continuous flow of mobile phase at pH 1.5 and pH 10, even with aggressive mobile phase conditions with phosphate and methanol.
- Hybrid silica particles are not necessary for high phase and bed stability when excellent bonding techniques are employed even on pure silica. Retention characteristics for neutral, basic and polar analytes showed little change in the nature of the bonded phase even at high pH usage.
- Although all Ascentis HPLC phases show extremely high stability at both low and high pH, even with aggressive additives, Ascentis C8 showed remarkable stability. This can most likely be attributed to higher main phase bonding density resulting from smaller C8 reagent size relative to packing pore size.