The Impact of Methanol on Hydrophilic Interaction Liquid Chromatography (HILIC) Retention Mechanisms – A Systematic Approach

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Outline

Quick HILIC system review

Choice of organic solvents

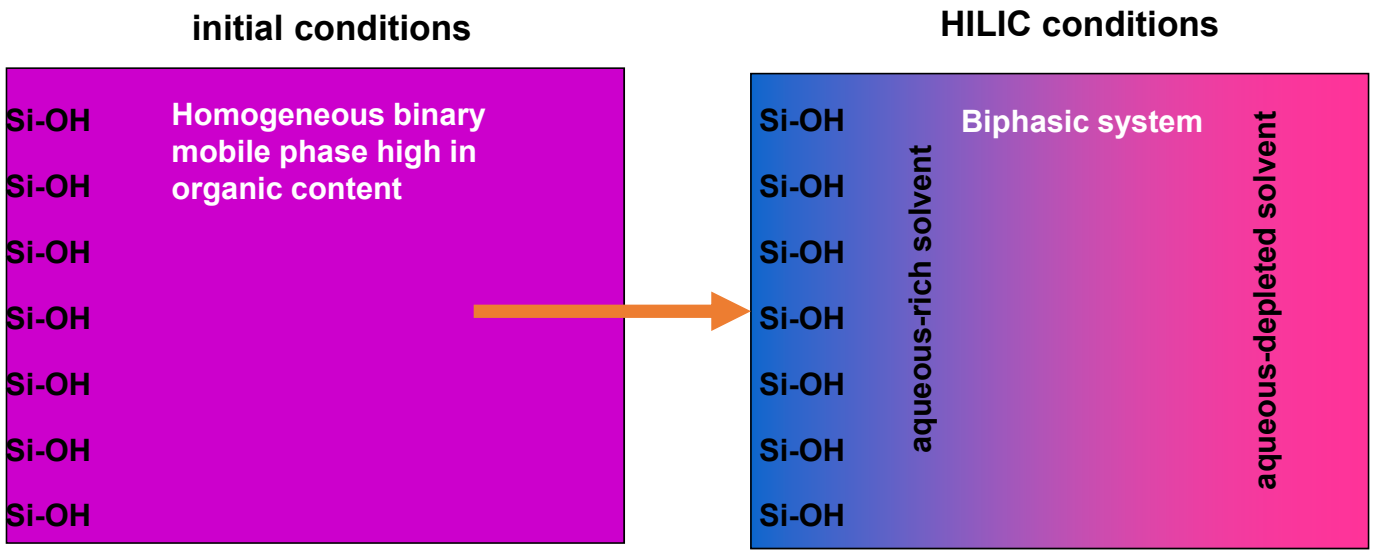
Systematic investigation of methanol incorporation and its impact on retention and selectivity

- bare silica
- positively charged HILIC phase

One additional sidebar

Summary/Conclusions

Biphasic Solvent Distribution at HILIC Phase Surface



Simplistic cartoon showing the preferential solvation of water on the polar surface. Upon equilibration a biphasic system develops whereby polar solute can partition from the primarily organic mobile phase to the aqueous rich layer adsorbed on the surface

D. Bell, *LCGC North America, 33 (2)*

More to Consider than Just Partitioning

- By definition, polar molecules are interactive
- Often strong ion-exchange and adsorption mechanisms

In HILIC, these highly interactive molecules are being drawn very close to another polar, highly interactive surface making ion-exchange and polar interactions *highly probable*.

Tools for Retention and Selectivity Manipulations in HILIC

- Stationary Phase
- Organic/Aqueous Ratio
- pH and Buffer Type/Concentration
- *Organic Modifier*

Column	IEX	Partition
Waters BEH Amide	Low	High
Tosoh TSK Amide	Moderate	High
Cogent Diamond Hydride	Moderate	Moderate
Ascentis Express F5 (pentafluorophenyl)	High	Low
Ascentis Express HILIC (bare silica)	Moderate	Moderate
Ascentis Express OH5 (pentahydroxyl)	Low	High
Ascentis Express ES-Cyano	High	Low
Waters BEH HILIC (hybridized silica)	Low	Moderate

D. Bell, Investigations of equilibration dynamics in hydrophilic interaction liquid chromatography (HILIC), Paper, HPLC 2017, Prague

D. Bell, *LCGC North America, 33 (2)*

Organic Solvent as a Variable

Acetonitrile = most common organic solvent used in HILIC

Acetonitrile + Water = 

Mountain, R. D., J. Phys. Chem. A 1999, 103, 10744-10748

Methanol is also a common additive found in the literature and in applications

Methanol + Water = 

Very little information on what the incorporation of methanol into HILIC systems accomplishes

Motivation for this work…..

- **Study the incorporation of methanol into standard HILIC mobile phase system using a select set of probes and systematic introduction of methanol as a modifier using two different HILIC stationary phases**

- **Interpret data based on potential molecular interactions that are impacted by the presence of methanol**

Experimental

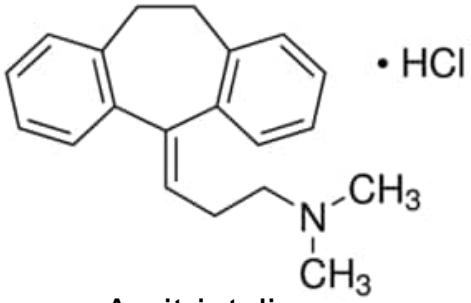
Introduce methanol into system by substituting for acetonitrile volume fractions

Mobile phase A – 5 mM ammonium formate (pH unadjusted) in 5:95 water:acetonitrile
Mobile phase B – 5 mM ammonium formate (pH unadjusted) in 5:75:20 water:acetonitrile:methanol
Mix (on-line) [A:B] 100:0, 90:10, 80:20, ….10:90, 0:100

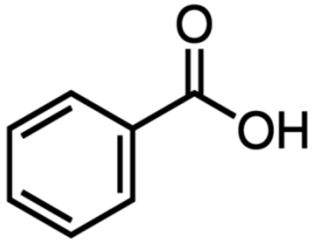
Raptor Polar X [proprietary polar ligand with positive charge]
Raptor HILIC [bare silica]

- Columns: 100 mm x 2.1 mm, 2.7 µm (superficially porous particles)
- Instrument: Shimadzu 20A
- Flow Rate: 0.3 mL/min
- Detection: PDA, various wavelengths monitored (220 nm shown within)
- Injection: 2 µL (50 µg/mL, MPA)
- Temperature: 35°C

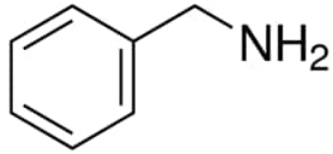
Probes



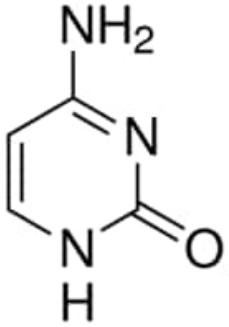
Amitriptyline
LogP 4.79, pKa 9.4



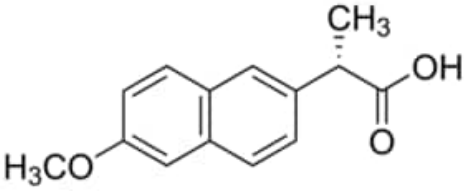
Benzoic Acid
LogP 1.87, pKa 4.2



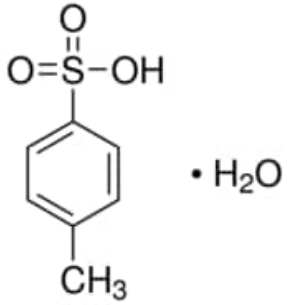
Benzylamine
LogP 1.09, pKa 9.34



Cytosine
LogP -0.469, pKa -



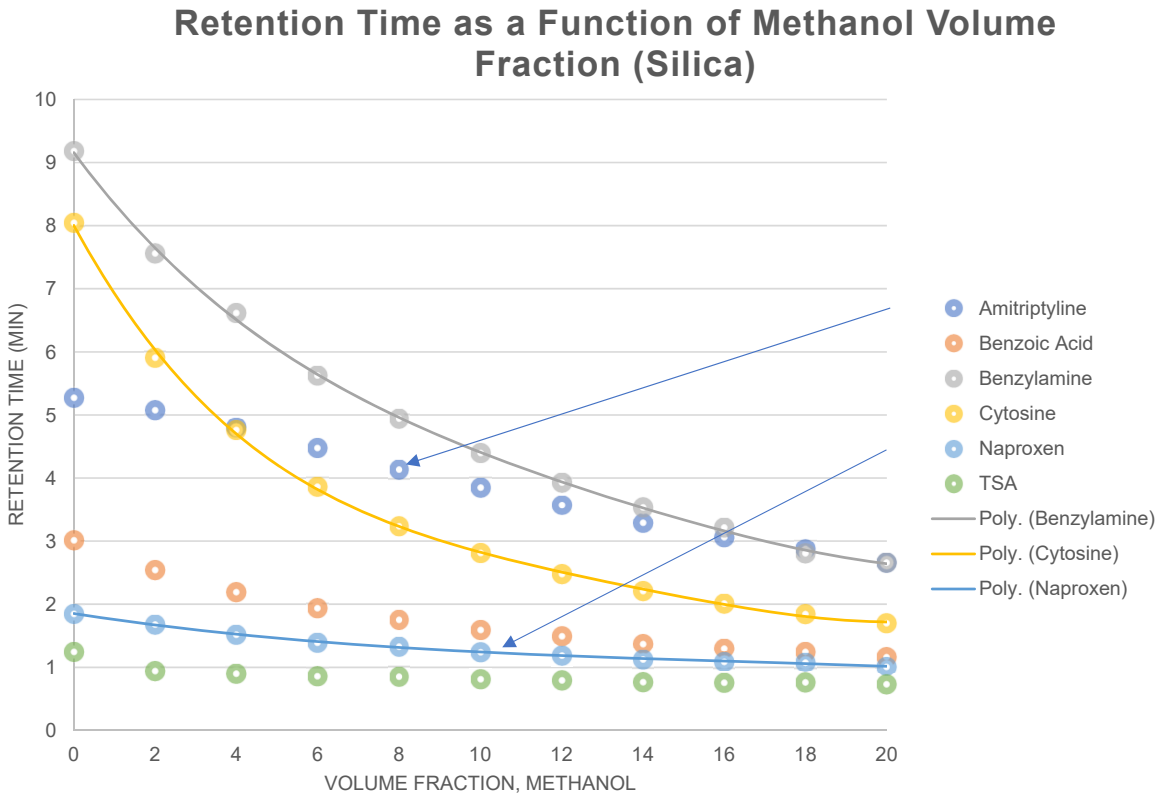
Naproxen
LogP 1.794, pKa - 5



Toluene sulfonic acid
LogP -0.032, pKa -1.34

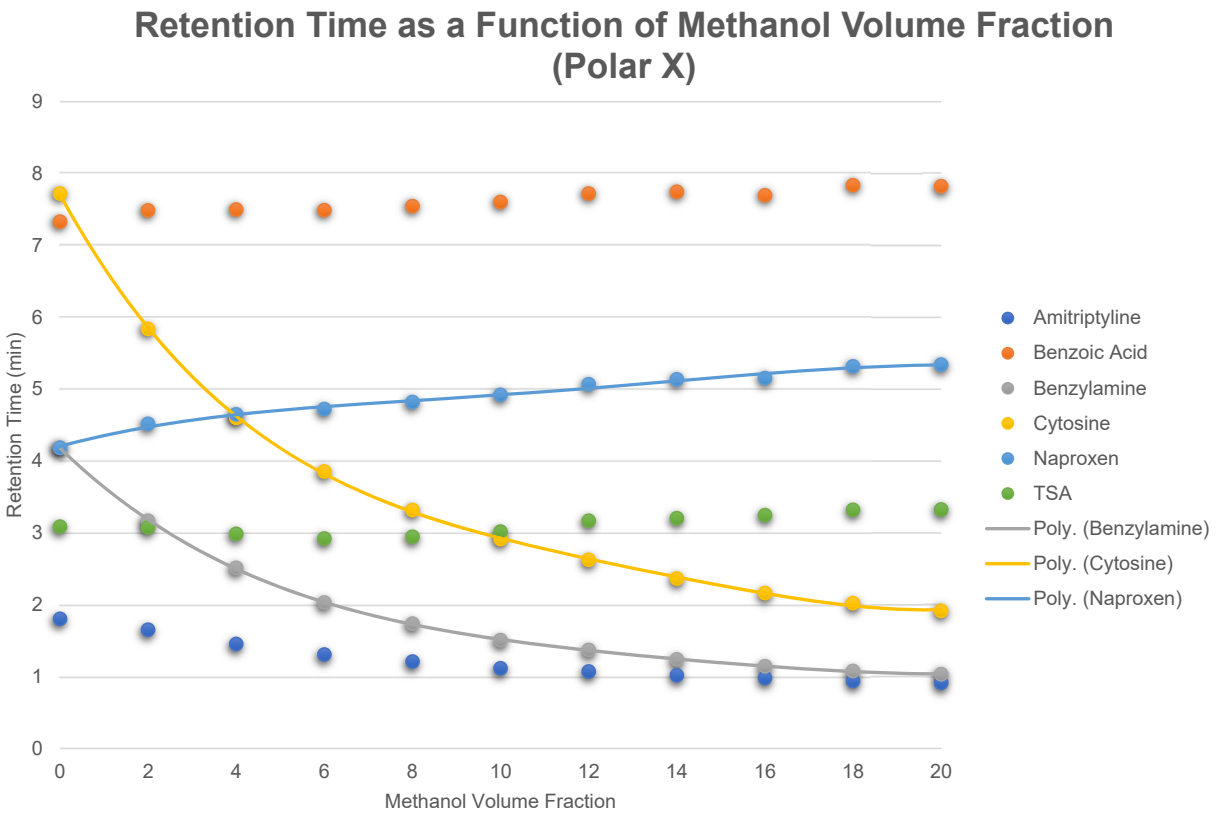
Results – Silica Phase

- Cytosine (neutral polar) and benzylamine (hydrophilic base) (again) most impacted –
- Retention also decreased for amitriptyline at a slower rate (nonpolar base)
- Each of the acids slightly decreased in retention



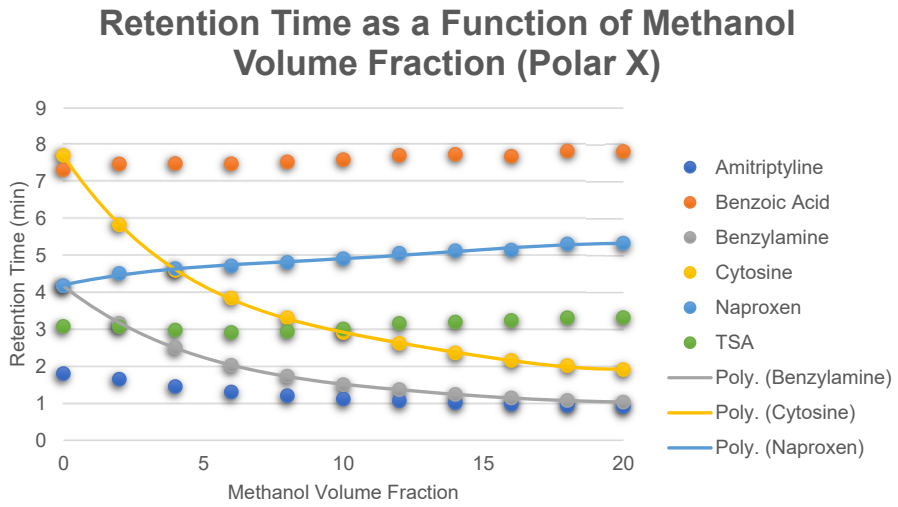
Results – Positively Charged Surface

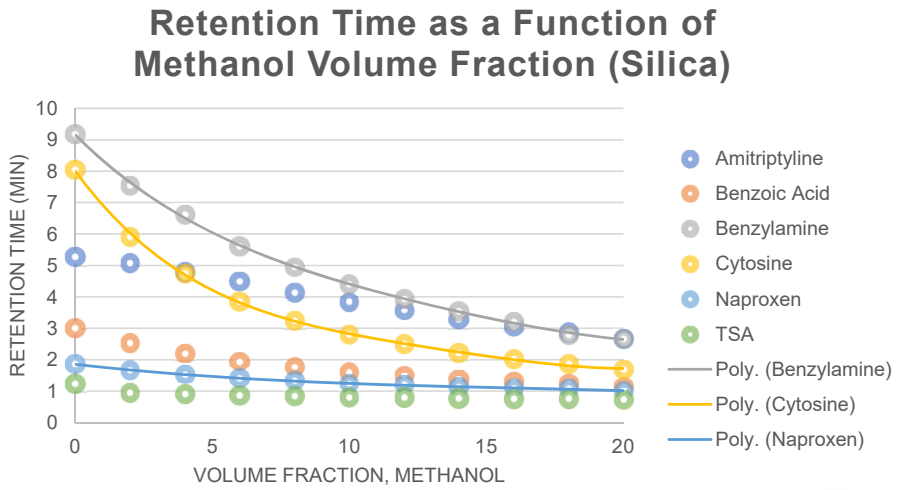
- Cytosine (neutral polar) and benzylamine (hydrophilic base) most impacted –
- Retention also decreased slightly for amitriptyline (nonpolar base)
- Each of the acids increased in retention, slightly



Results

- Partitioning impact consistent between the phases
- Where ion-exchange dominates, (positively or negatively) methanol introduction has less impact
- Similar compounds impacted similarly
- Compounds from different classes/different retention mechanisms = change in selectivity





Interpretation

Melnikov, *Anal. Chem*. 2013, **85**, 8850

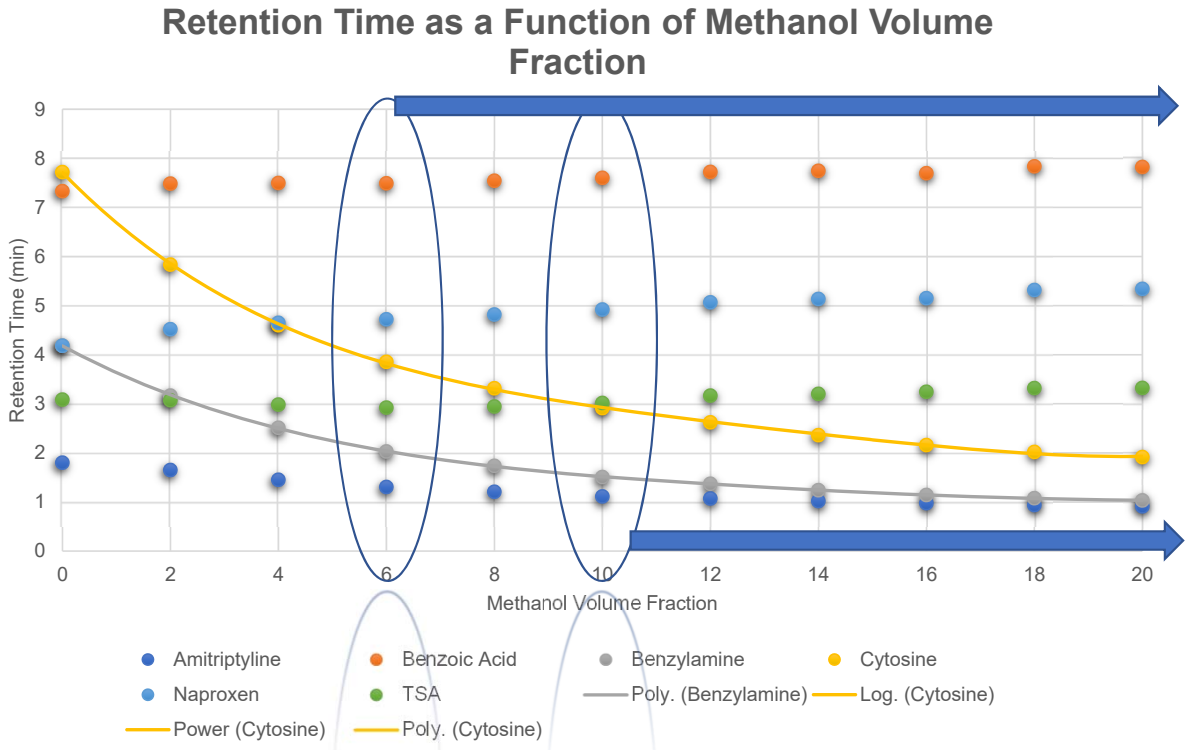
Simulations substituting alcohol for water in 10:90 water:acetonitrile to 5:90:5 water:acetonitrile:alcohol

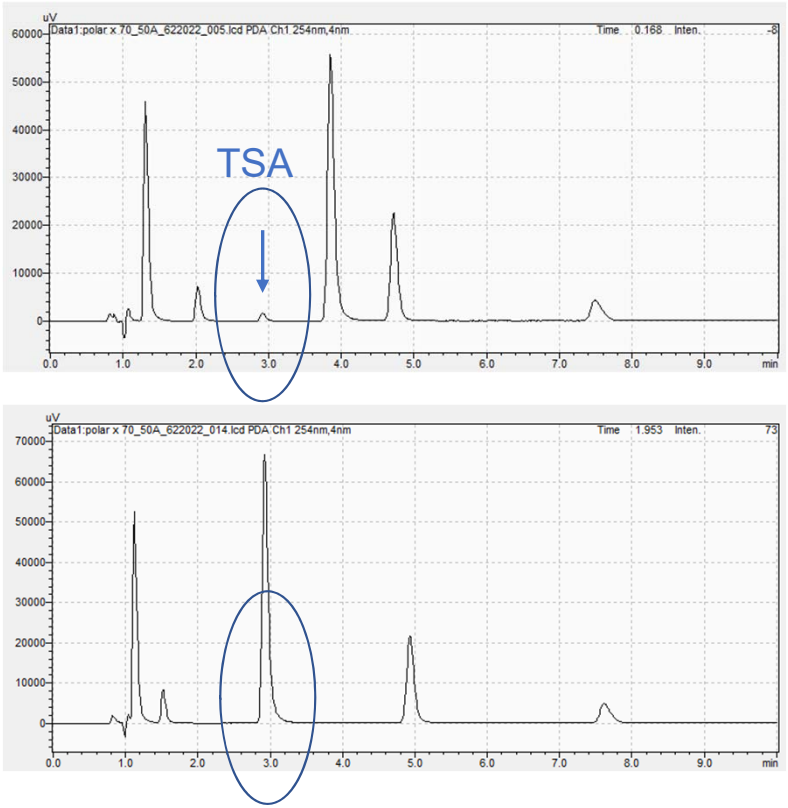
- Greatest impact on diffuse layer
- Low impact on water layer close to surface – strengthened?
- **Methanol for Water?**

Highly Stable

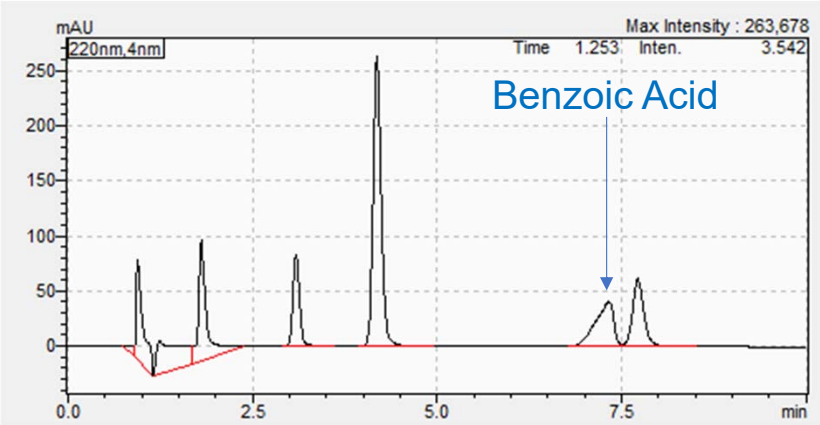
Active partitioning zone

Results – Selectivity Manipulation with Methanol – Polar X

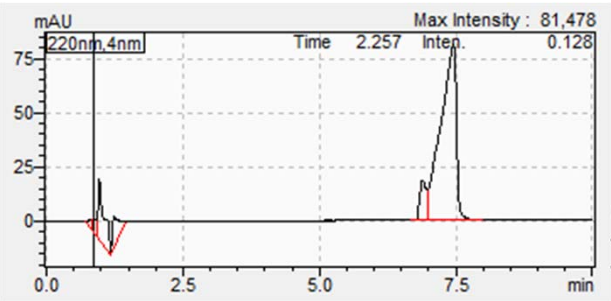




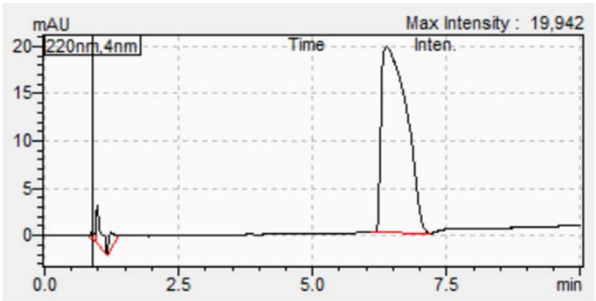
Sidebar - Peak Shape Issues and ‘Disturbances’ –



5 mM ammonium formate (pH unadjusted) in 5:95 water:acetonitrile, Polar X phase



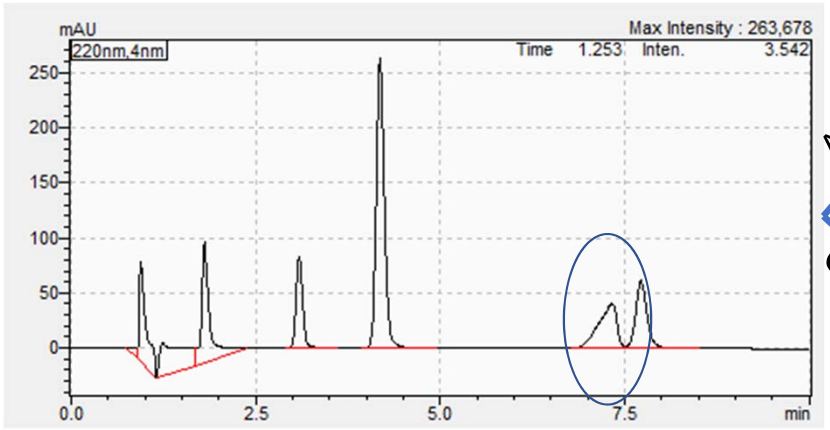
Benzoic Acid



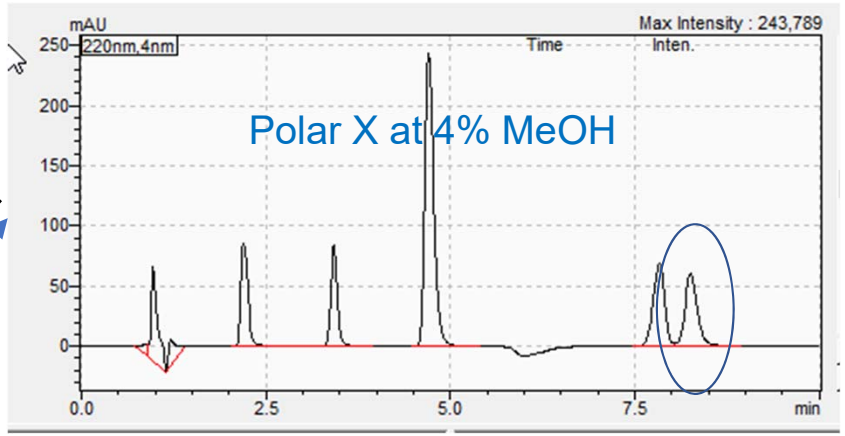
Formic Acid

Gritti, *J. Chromatogr. A*, 1489 (2017) 95-106

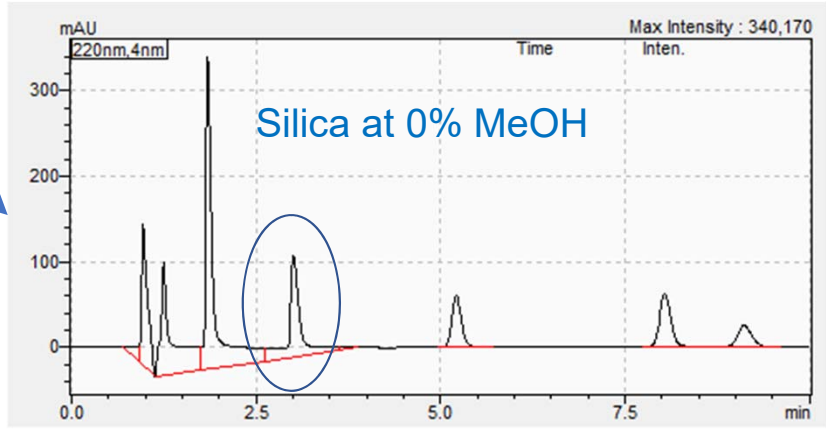
Peak Shape Issues – common HILIC complaint



Add some methanol



Change phase



Conclusions

- **Replacement of small portions of acetonitrile with methanol primarily impacts partition mechanisms in HILIC**
  - Likely due to a co-solvent effect
  - Consistent with simulation studies
  - Points to diffuse layer as the active partition zone
- **Analyte retention dominated by IEX mechanisms (and possibly adsorption) much less impacted by methanol presence**
  - Methanol does not appear to be interacting directly with the surface appreciably
- **Selectivity between classes of analytes impacted significantly – useful tool**
- **Selectivity changes of like analytes less apparent (within study)**

Conclusions

- **Replacement of water with methanol – future need**

- **Mobile phase components retain well in HILIC (sometimes very well)**
  - Disruptions due to sample injection can lead to baseline disturbances and peak shape issues
  - Alterations of stationary phase and mobile phase (methanol incorporation) can help

- **Refined model of HILIC dynamics**
- **Practical implications/uses**
- **More questions!**

Acknowledgements

Organizers! Great to be back!

Restek colleagues