Solute Attributes and Molecular Interactions Contributing to “U-Shape” Retention on Fluorinated HPLC Stationary Phases

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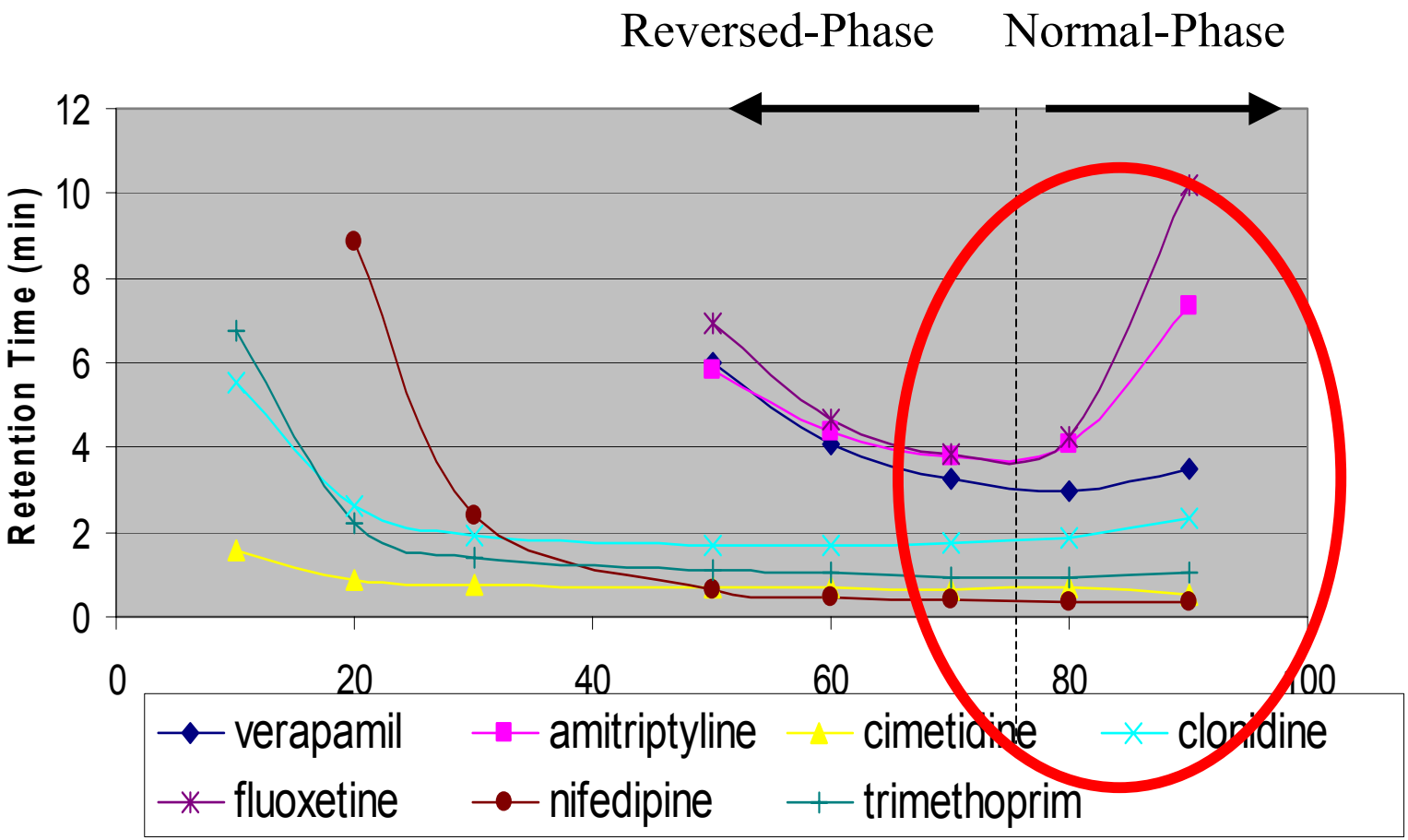
Introduction

- **LC/MS rapidly becoming the cornerstone of analytical chemistry**
  - Pharmaceutical
  - Environmental
  - Agricultural……
- **Many efforts aimed at improving the technique**
  - Improve sensitivity
  - Greater speed
  - More universal
- **Much work centered on interfaces and instrumentation**
- **Less attention paid to LC**
- **Lack of retention/separation leads to**
  - Poor quantification
  - Problematic qualitative analysis

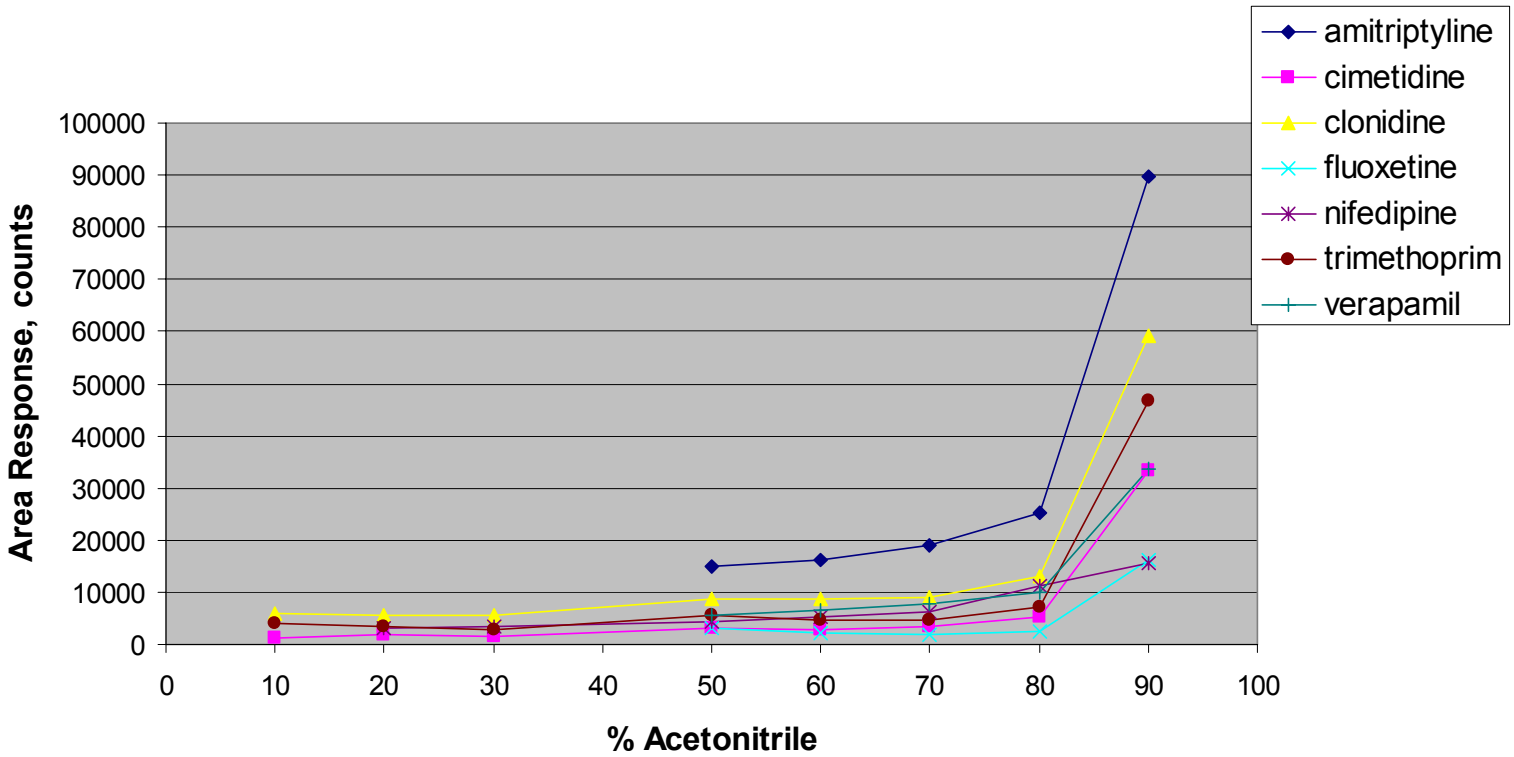
Introduction

"U-Shape Retention"



Introduction

- **Retention at high organic modifier percentages may:**
  - Increase sensitivity in LC/MS experiments through facilitated desolvation.



Introduction

- **Retention at high organic modifier percentages may also:**
  - Speed up analyses.
  - Induce retention otherwise not obtained in RP.
  - Provide alternative mechanisms of interaction.
- **Needham (***J. Chromatogr. A, 869 (2000) 159***)**
  - Examined several stationary phase chemistries
  - Determined that pentafluorophenylpropyl (PFPP)-bonded silica provided greatest retention with good peak shape and reproducibility
- **Certain analytes under certain conditions using certain stationary phases……**

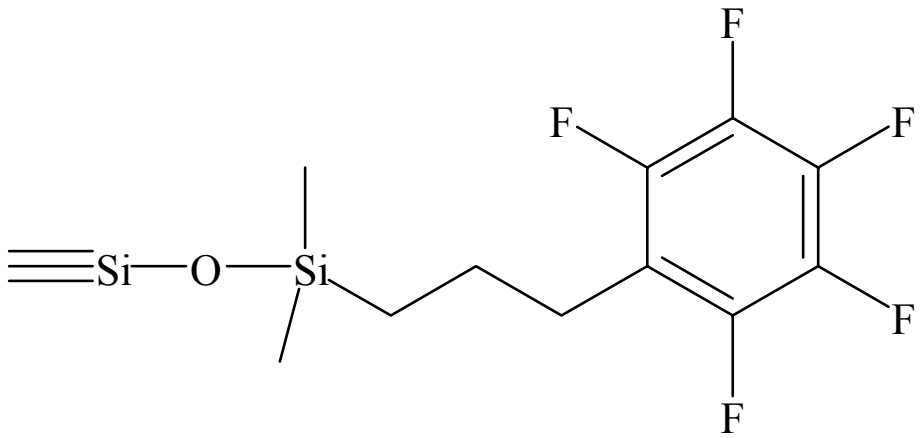
Introduction

- **Paramount to understand**
  - Solute attributes
  - Molecular interactions

- **In this research we sought to better understand "U-Shape" retention by:**
  - Investigating the relationship of retention and percent organic for several pharmaceutical acids, bases and neutrals on PFPP compared to C18.
  - Performing several studies aimed at elucidating the dominant molecular interactions responsible for the observed behavior.

Introduction

- **PFPP offers:**
  - Dipole-dipole interactions
  - Pi-pi interactions
  - Charge-transfer interactions
  - Others?

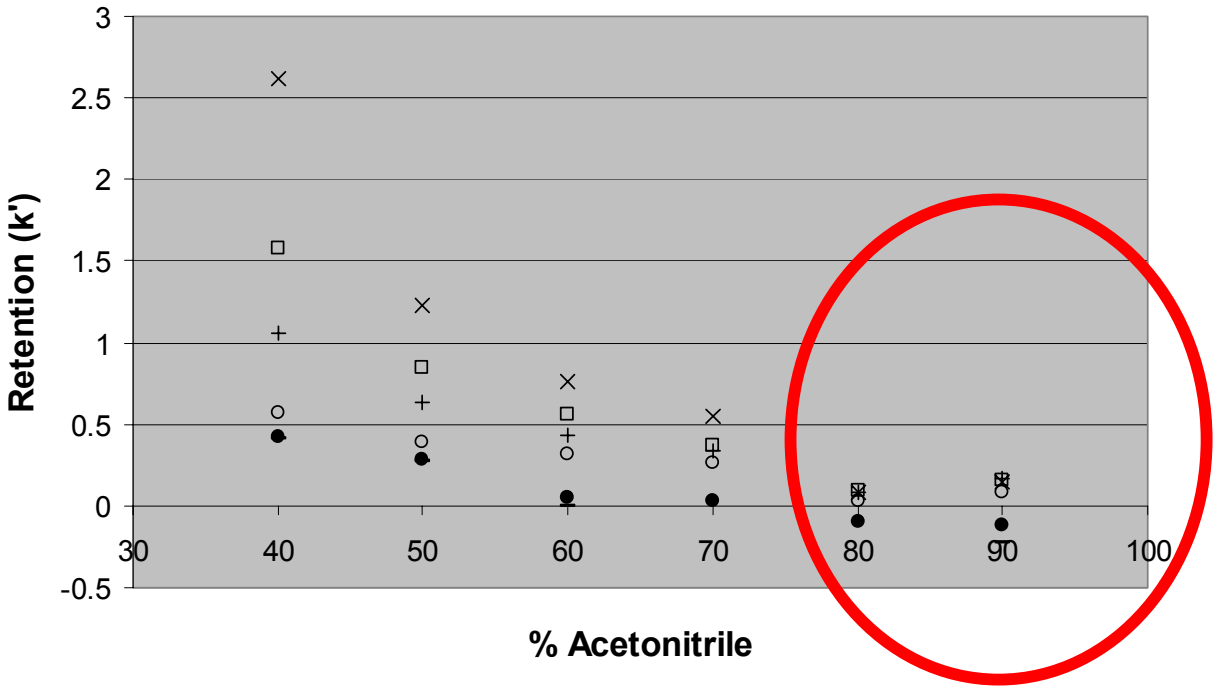


Acidic, Basic and Neutral Retention Profiles

- **Experimental Design:**
  - 6 analytes representing pharmaceutical acids, bases and neutrals
  - Retention monitored from 40 to 90% acetonitrile at two pH levels (4 and 6.7) on Discovery® HS F5 (PFPP) and Discovery HS C18
  - Aqueous component-10 mM ammonium acetate

Acidic Analyte Profiles on PFPP

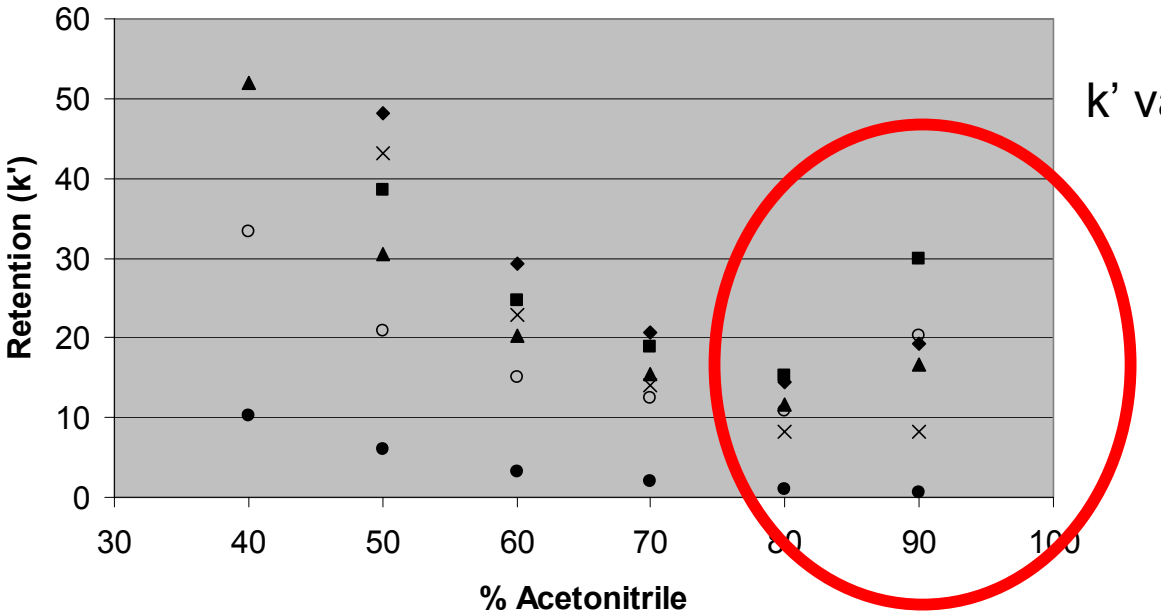
Retention Profiles (k') of Acidic Probes on PFPP at pH 6.7



Retention (k') of acidic probes x ibuprofen, ☎ aspirin, + naproxen, ✉ ketoprofen, ⌨ piroxicam and 🕯 diclofenac using PFPP from 40% to 90% acetonitrile under pH 6.7 conditions.

Basic Analyte Profiles on PFPP

Retention Profiles (k') of Basic Probes on PFPP at pH 6.7

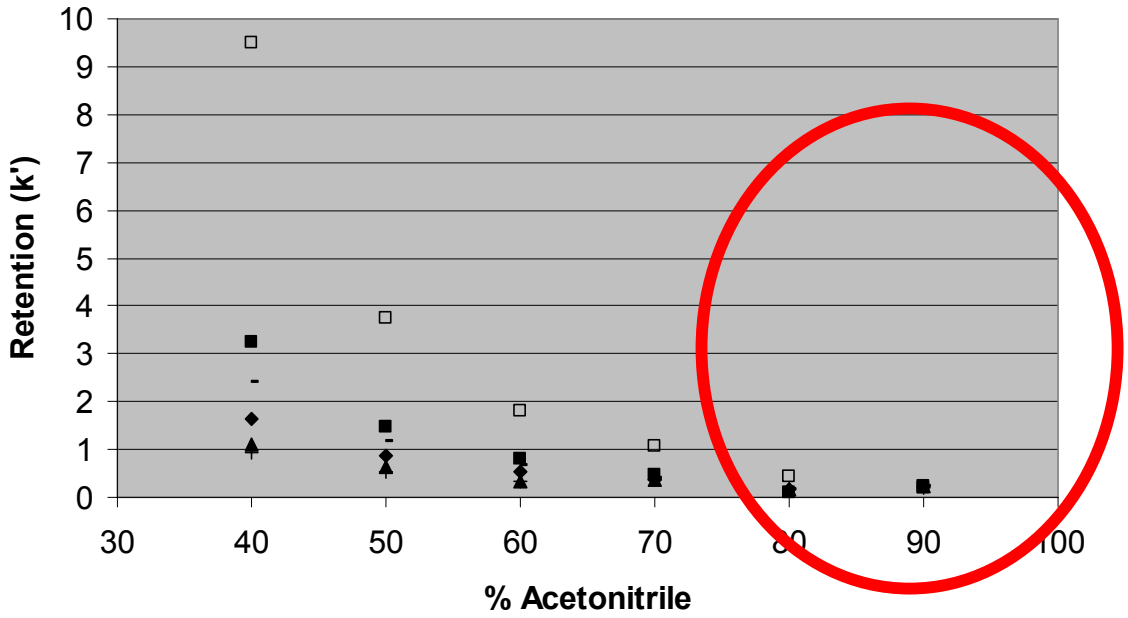


k' values up to 30!

Retention (k') of basic probes ▭ amitriptyline, 🔔 nortriptyline, 🖬 diphenhydramine, x verapamil, 🖂 alprenolol and ☎ lidocaine using PFPP from 40% to 90% acetonitrile under pH 6.7 conditions

Neutral Analyte Profiles on PFPP

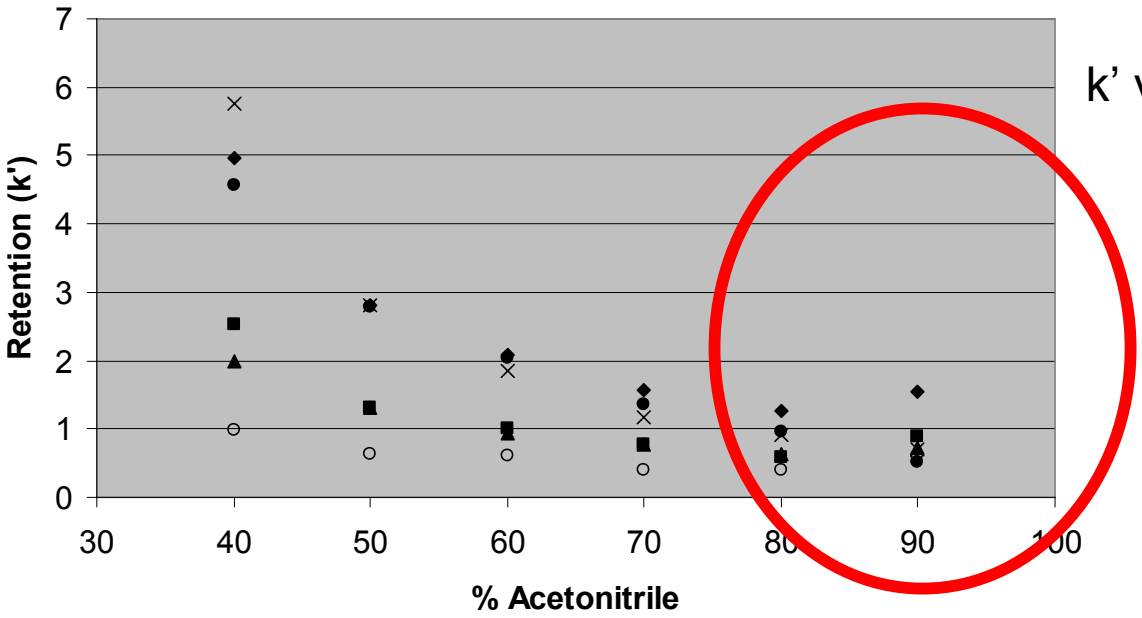
Retention Profiles (k') Neutral Probes on PFPP at pH 6.7



Retention (k') of neutral probes + hydrocortisone, hydrocortisone acetate, progesterone, corticosterone, cortisone acetate and prednisone using PFPP from 40% to 90% acetonitrile under pH 6.7 conditions

Basic Analyte Profiles on C18

Retention Profiles (k') of Basic Probes on C18 at pH 6.7



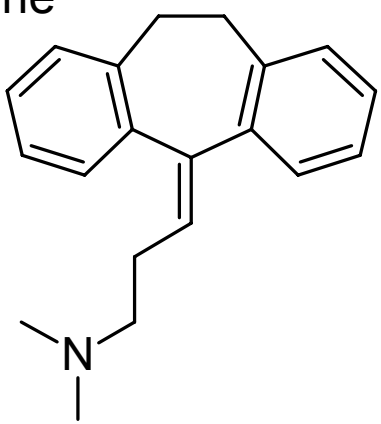
k' values up ~1.5

Retention (k') of basic probes ✉ amitriptyline, 🔔 nortriptyline, 🖬 diphenhydramine, x verapamil, 🖂 alprenolol and ☎ lidocaine using C18 from 40% to 90% acetonitrile under pH 6.7 conditions

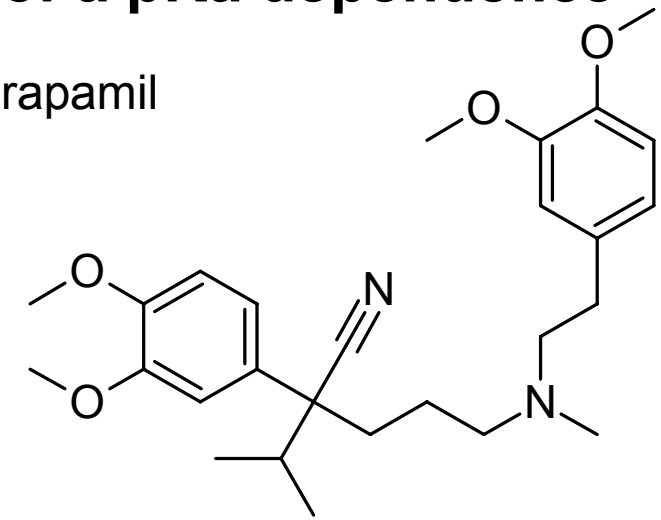
Selectivity Analysis

- Secondary amines show relatively greater increase in the normal-phase region
- Except for verapamil, amines are located on structural “arms”
- Basic moiety in verapamil hindered
- Lidocaine shows evidence of a pKa dependence

Amitriptyline



Verapamil



Retention Profiling Conclusions

- **Only basic analytes exhibit appreciable "U-Shape" retention**
- **Not all bases**
- **Not all to the same extent - selectivity**
- **Also observed to a small degree on C18**
  - Commonality is the silica surface
  - Known to interact with bases via ion-exchange
- **Pointed to ionic interactions with surface silanols**

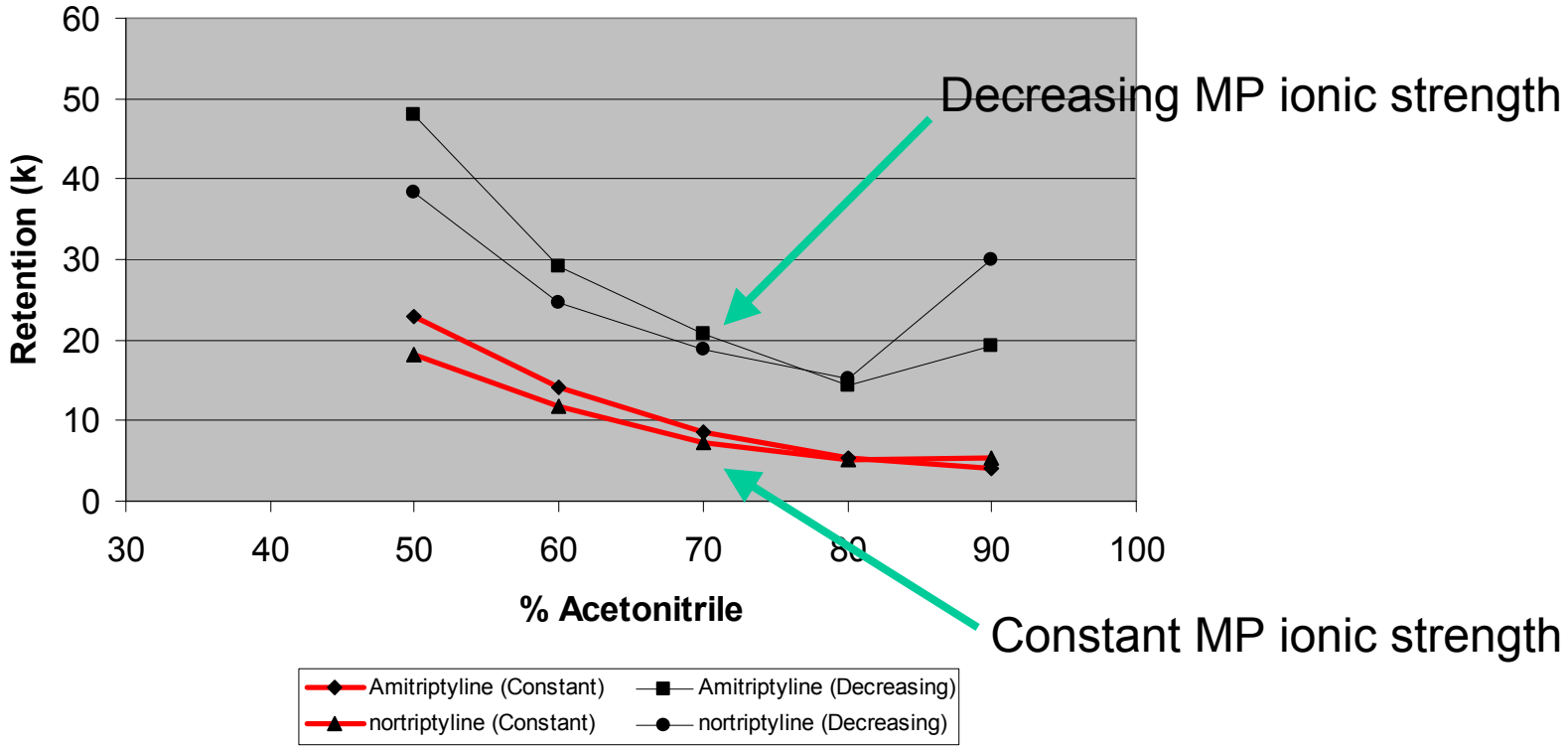
“U-Shape” Retention Profile Dependence on MP Ionic Strength

- **As % organic increased, ionic strength decreased**
- **What if the ionic strength was held constant?**

- **Experimental:**
  - Basic analytes nortriptyline and amitriptyline run from 50 to 90% acetonitrile on PFPP keeping the buffer concentration at 10 mM throughout

“U-Shape” Retention Profile Dependence on MP Ionic Strength

Comparison of Amitriptyline and Nortriptyline Retention on PFPP Phase: Decreasing vs. Constant Buffer Concentration



- Retention at all % acetonitrile levels is attenuated
- Little “U-Shape” character observed at constant ionic strength

Contribution of Bonded Phase to Retention

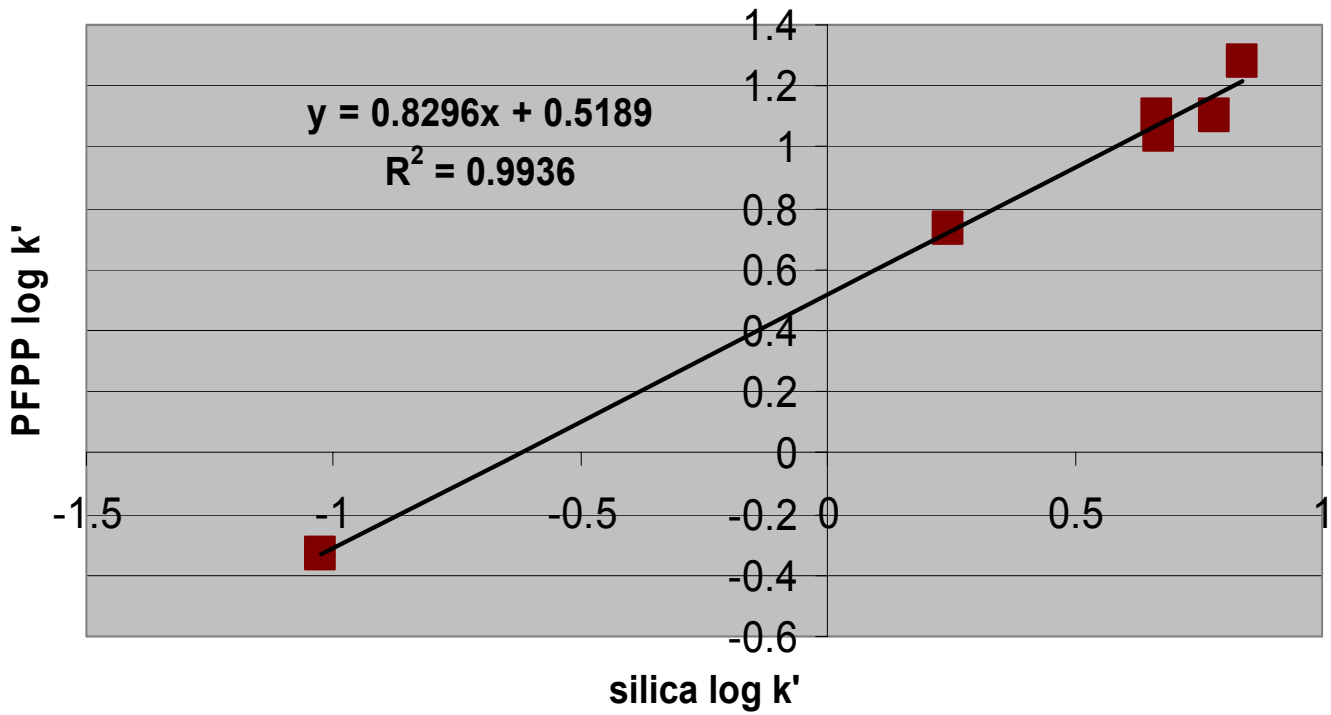
- **Compared basic analyte retention on bare silica to PFPP**
  - 2 mM ammonium acetate (pH 6.7) at 90% acetonitrile

Compound	k' Silica	k' PFPP
amitriptyline	4.63	12.71
nortriptyline	6.92	18.98
diphenhydramine	4.69	10.98
verapamil	1.76	5.40
alprenolol	6.09	12.59
lidocaine	0.09	0.46

Contribution of Bonded Phase to Retention

kappa-kappa plot: Retention of Basic Analytes on PFPP Versus Bare Silica

- good correlation indicative of similar dominant interactions



Dependence of Retention on Ionic Strength at Constant % Acetonitrile

- **Two-site model of retention (***Yang, et. al., J Chromatogr. A 996 (2003) 13***)**

$$\log k' = \log(k'_{RP} + B_{IEX}/[C^+]_m)$$

Reversed-Phase Contribution
(independent of salt concentration)

Ion-Exchange Component
(inversely proportional to salt concentration)

Plot of log k' vs $\log[C^+]_m$

slope = -1, if exclusively ion-exchange

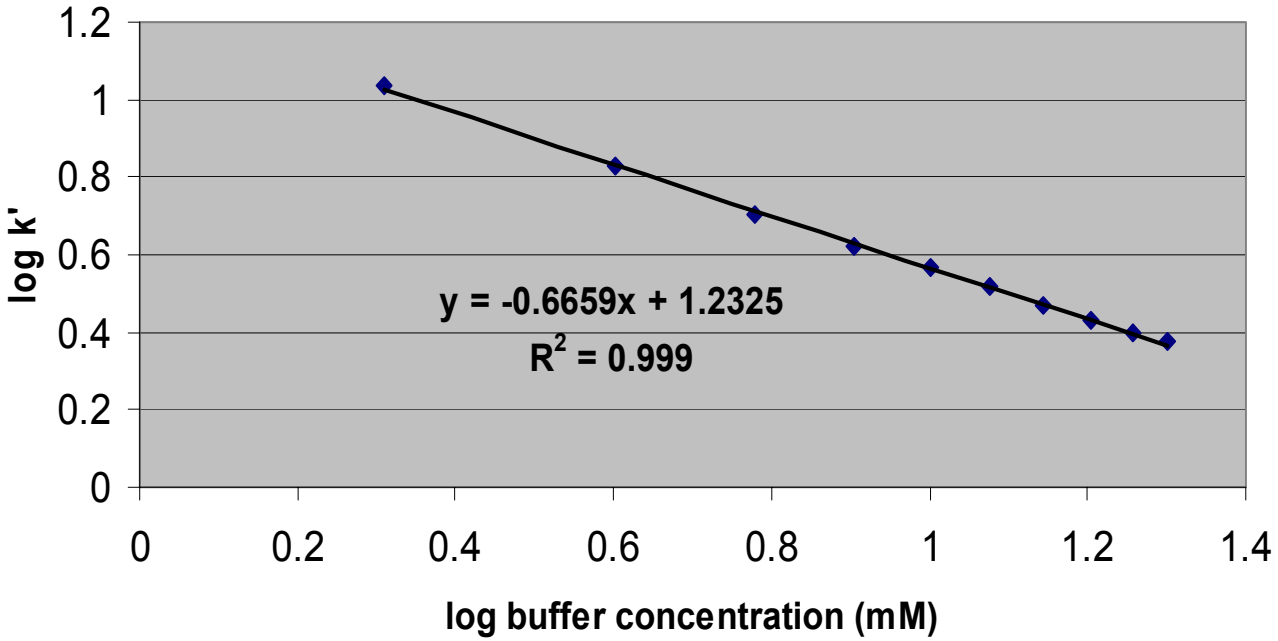
slope = ~0, if RP dominates

Dependence of Retention on Ionic Strength at Constant % Acetonitrile

Amitriptyline Retention (logk') vs. Ammonium Acetate Concentration (log[mM]) on PFPP at 85% Acetonitrile

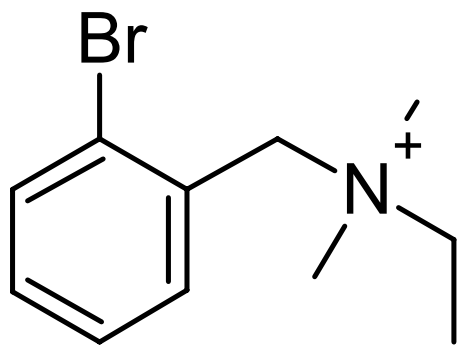
- Slope of –0.6659 indicates more than just ion-exchange

- Note linear dependence of retention on buffer conc.



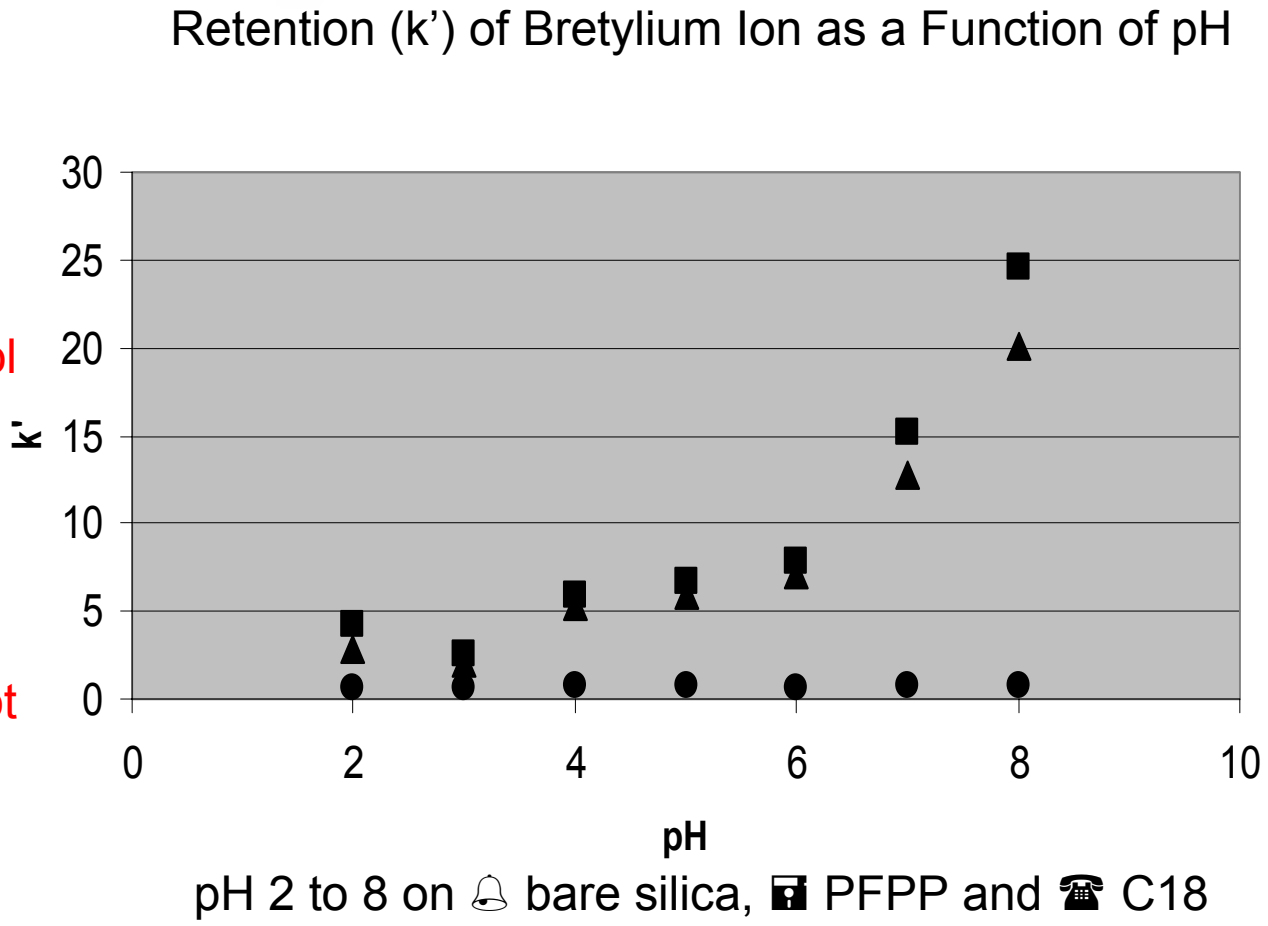
Dependence of Silanol pKa on Bonding Chemistry

- **For IEX silanols must be ionized**
- **Neue (*J. Chromatogr. A 925 (2001) 49*) used quaternary ammonium ion (bretylium) retention as a function of pH to estimate silanol pKa values.**
- **Bare silica, PFPP and C18 were subjected to similar experiment**
  - Ammonium ion concentration held constant at 25 mM
  - pH varied from 2 to 8
  - Bretylium ion retention monitored



Dependence of Silanol pKa on Bonding Chemistry

- Bonded-phase alters silanol acidity
- Bonded phase inhibits interaction
- Explains why PFPP and not C18



Summary

- **Only basic analytes exhibit appreciable**

"U-Shape" retention on PFPP

  - Also observed on C18 to a lesser extent
  - Selectivity and good peak shape are obtained
  - Selectivity appears to be a function of pKa and silanol/base accessibility

- **The "normal-phase" region of the profile was shown to be a function of MP ionic strength**
- **Based on the two-site model of retention, both RP and IEX contribute to retention (hydrophobically-assisted ion-exchange)**
- **Further supported by the preferential retention of bases on PFPP compared to silica**
- **Estimated pKa values for C18 and PFPP also support the existence of IEX mechanisms**

Conclusions

- Ability to retain basic analytes at high organic modifier concentrations offers potential to increase LC/MS sensitivity
- Alternative mechanisms may provide unique options in method development
- Bonded-phase chemistry plays an important role in both RP and IEX

Conclusions

- **PFPP presents new opportunities to manipulate retention and selectivity**
  - Buffer concentration
  - pH (pKa of analytes and surface silanol groups)

- **Knowledge should lead to:**
  - greater utilization
  - more robust methods
  - further advances in stationary phase chemistries
  - expansion to other forms of separation

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