

Advantages of Polar Reversed-Phase Chemistries for LC/MS Analyses

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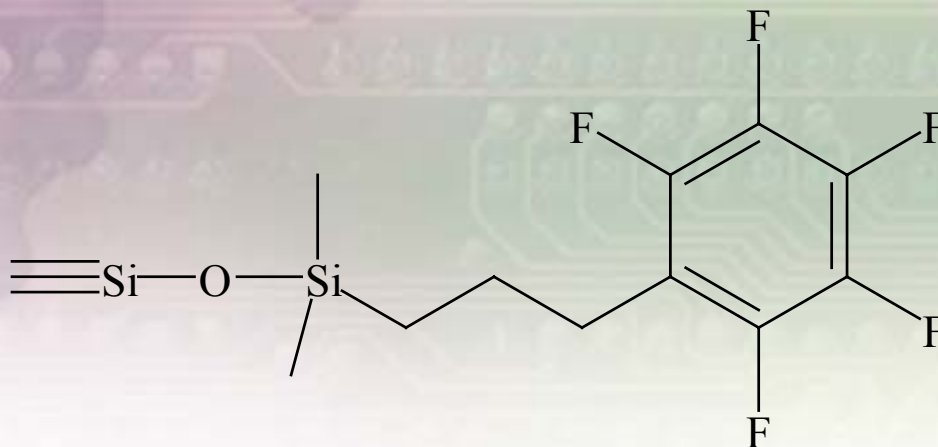
Outline

- General chromatographic and LC/MS-related issues
- How polar-RP phases may combat issues
 - Pentafluorophenylpropyl (HS F5) phase
 - Advantages - examples
 - Interactions
 - Method development
- Summary
- Questions

Chromatographic and LC/MS-Related Problems

- Problems associated with traditional Alkyl phases:
 - Poor retention
 - Poor MS sensitivity
 - General elution problem
 - Too little or too much selectivity
- A variety of functionalized, reversed-phase columns are available

Discovery HS F5



Pentafluorophenylpropyl Phase

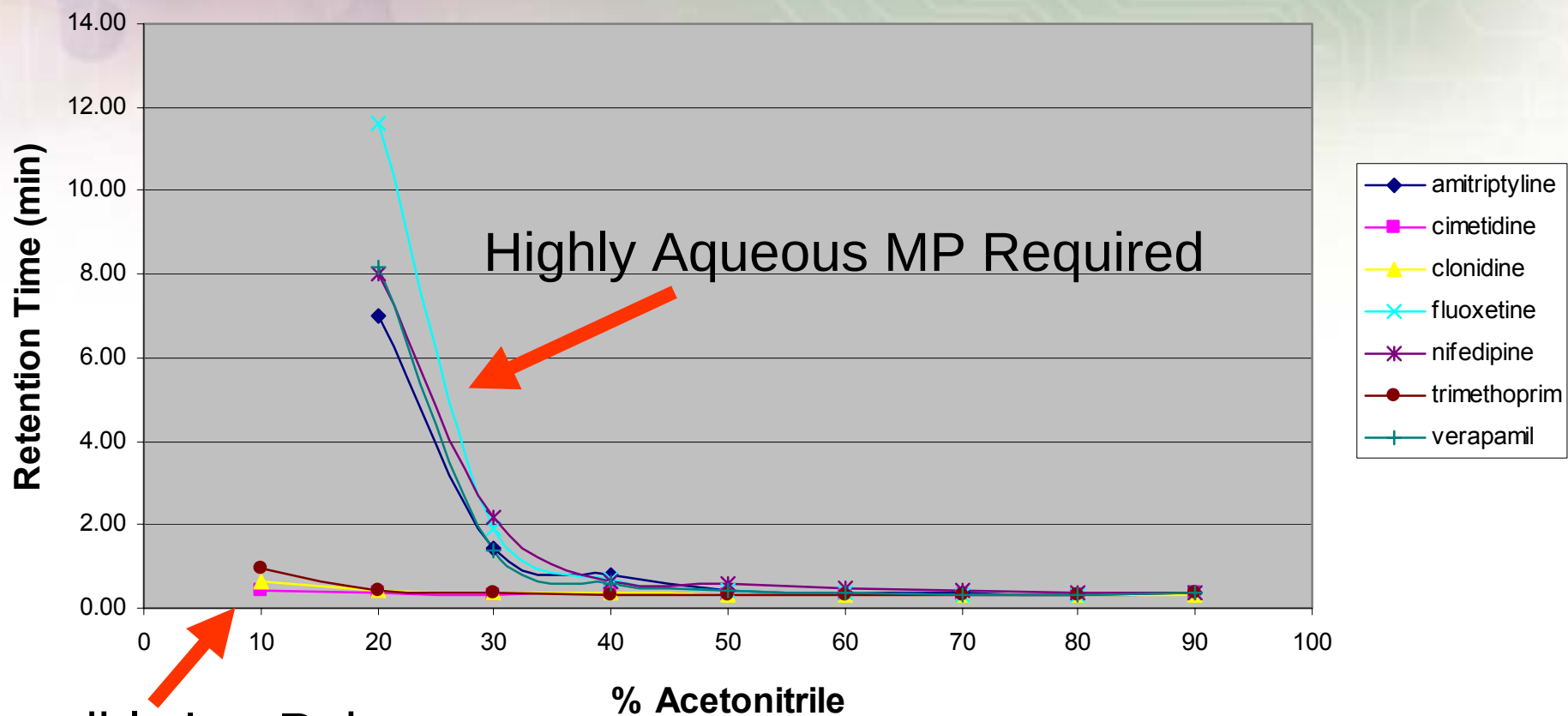
General Characteristics:

- Surface area: 300 m²/g
- Pore Size: 120 Å
- Coverage: 4.0 μmol/m²
- Endcapped: Yes
- %C: 12

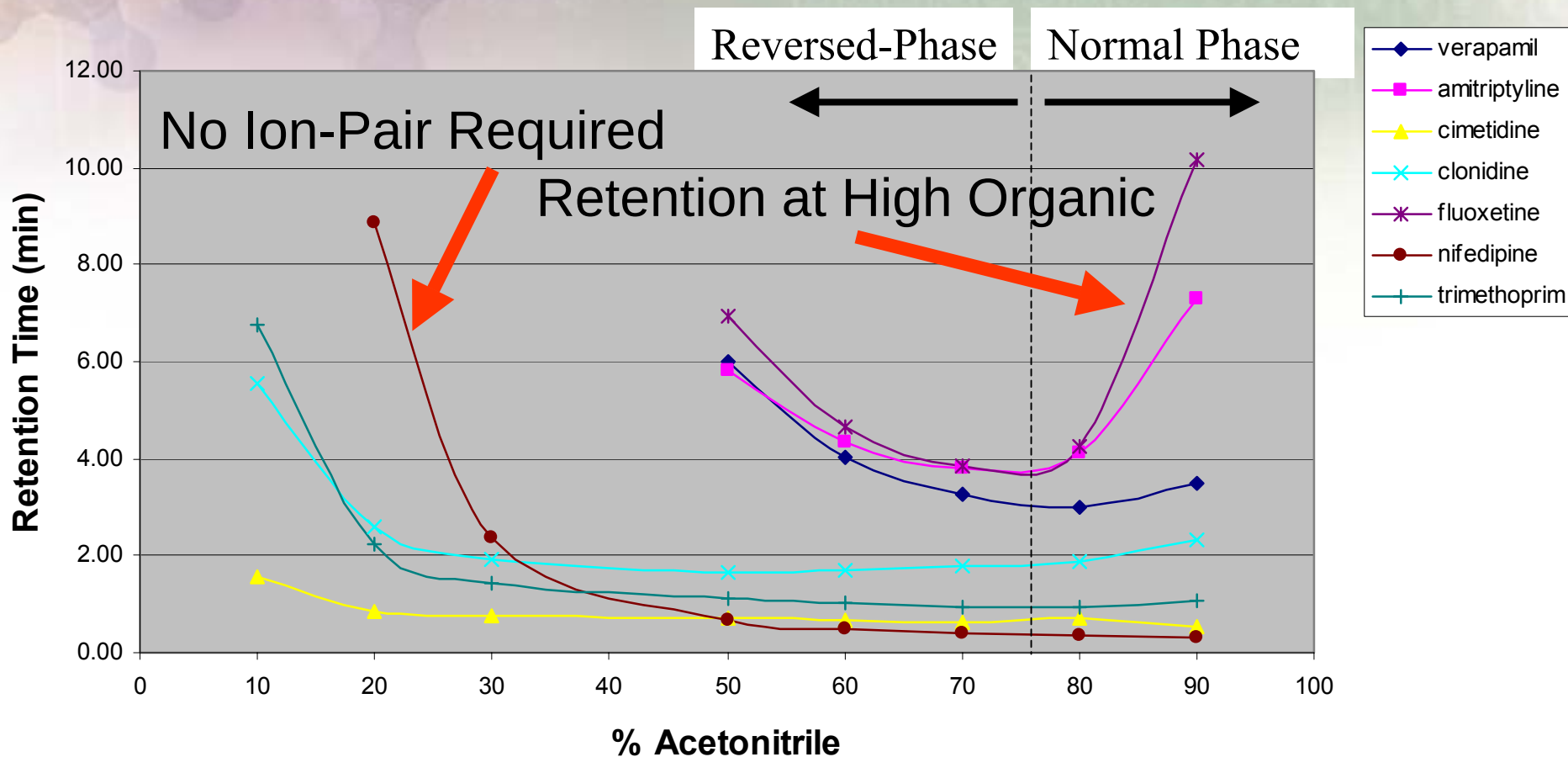
Poor Retention

- Polar analytes, particularly bases, on traditional alkyl phases
 - Often require highly aqueous mobile phases
 - May require ion-pair
 - Both situations can inhibit MS sensitivity

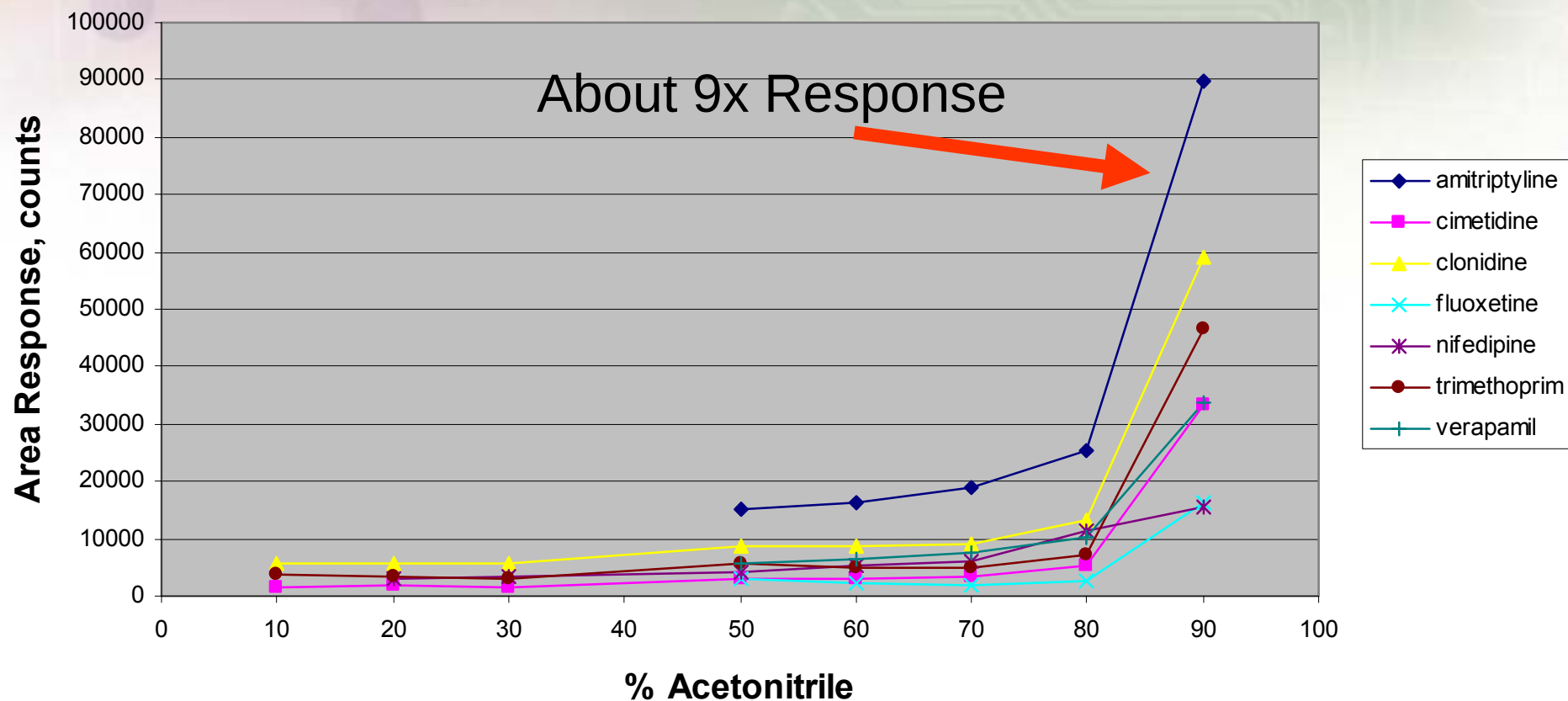
Retention Profile on C18



“U-Shape” Retention on HS F5



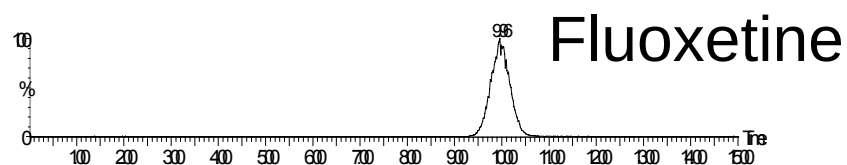
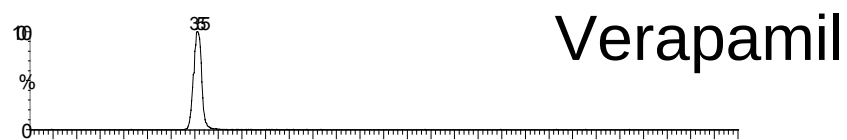
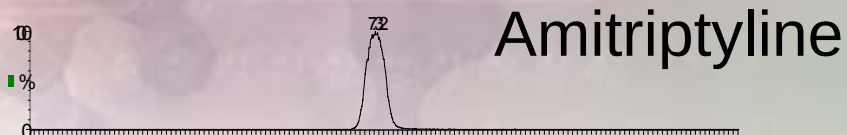
MS (ESI) Response as a Function of % Acetonitrile



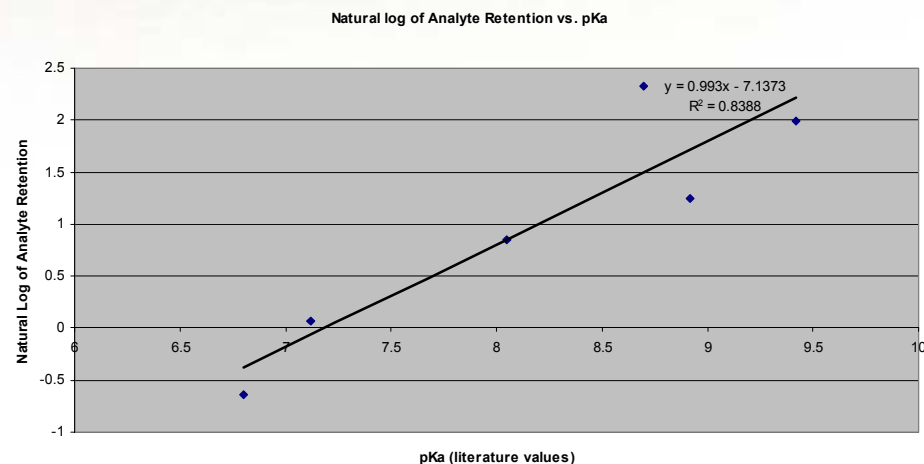
Retention at 90% Acetonitrile

Discovery HS F5

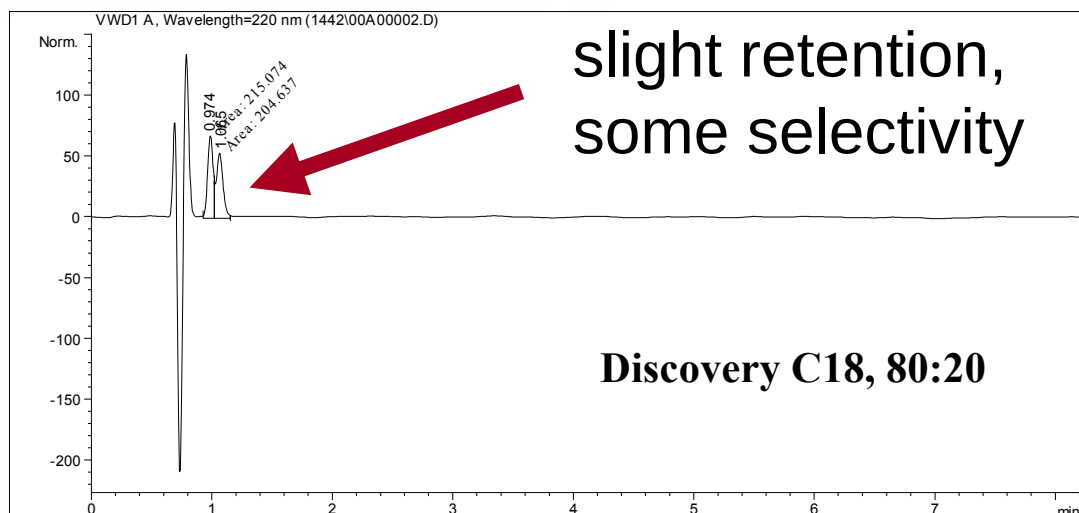
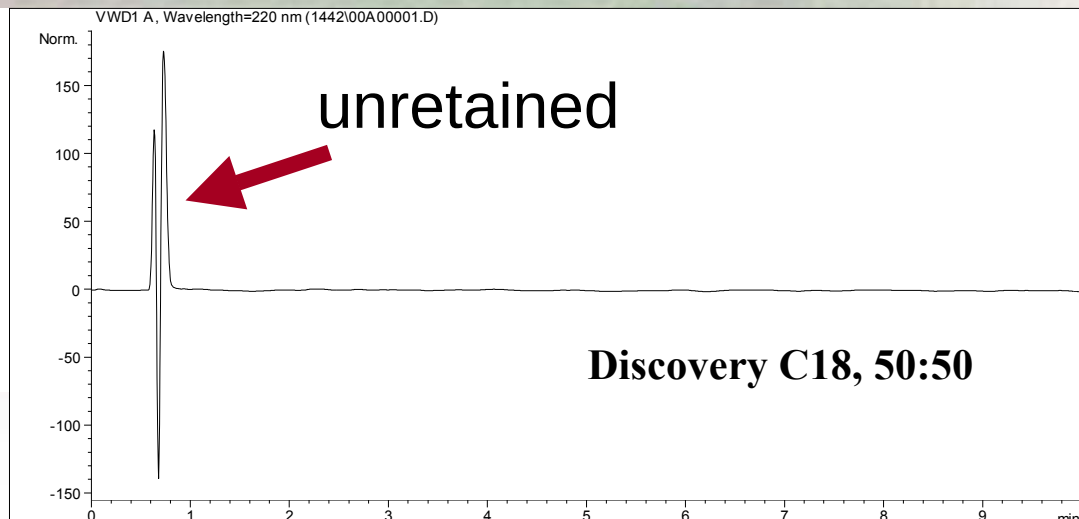
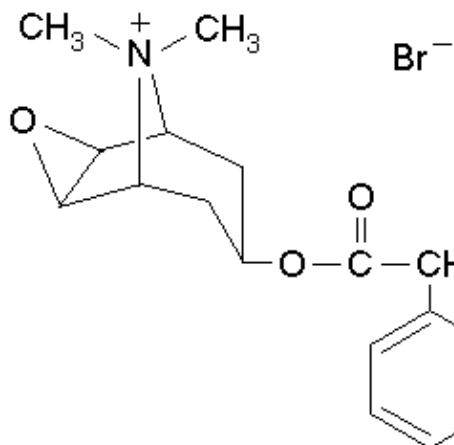
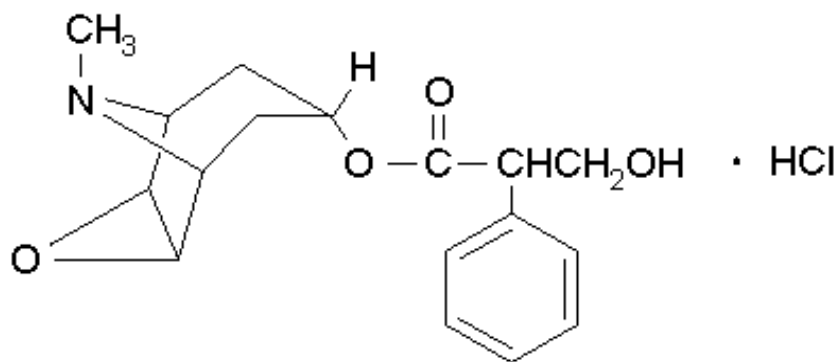
micras05



Relationship between analyte pKa and normal phase retention



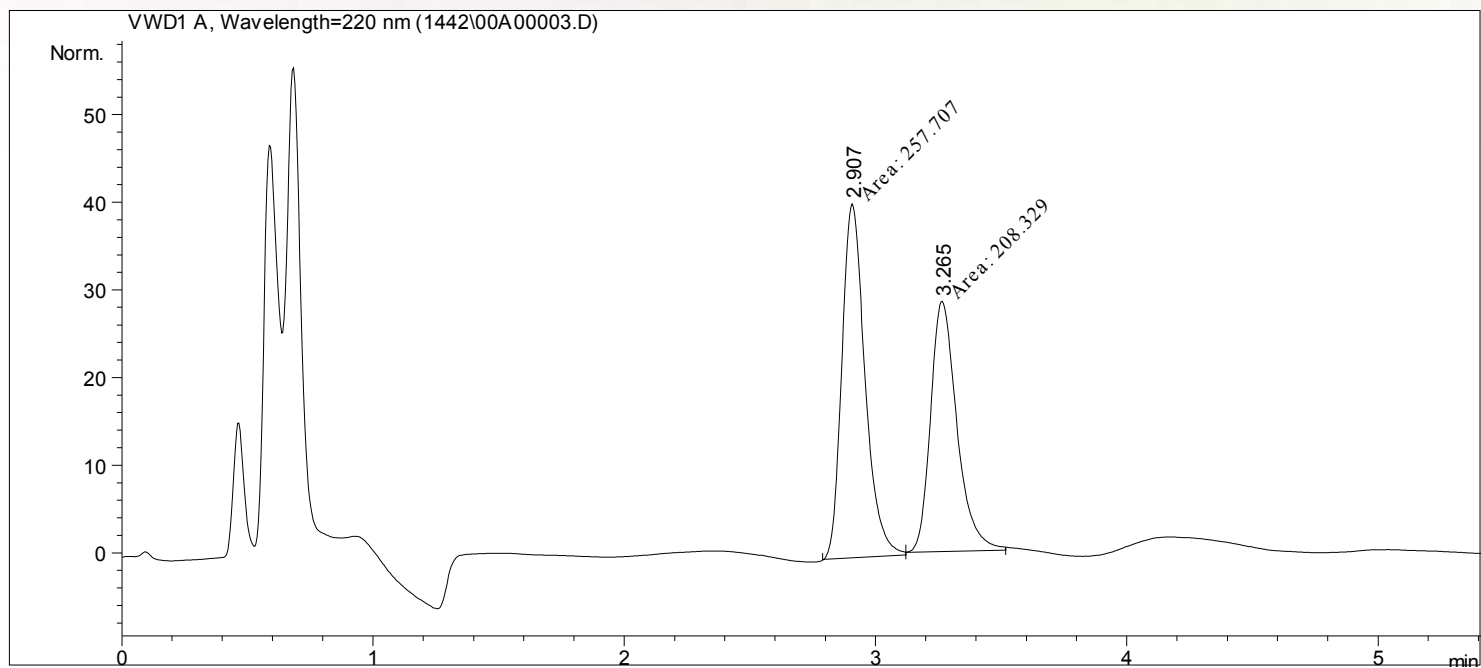
Example: Scopolamines



Example: Scopolamines

Discovery HS F5, 20:80

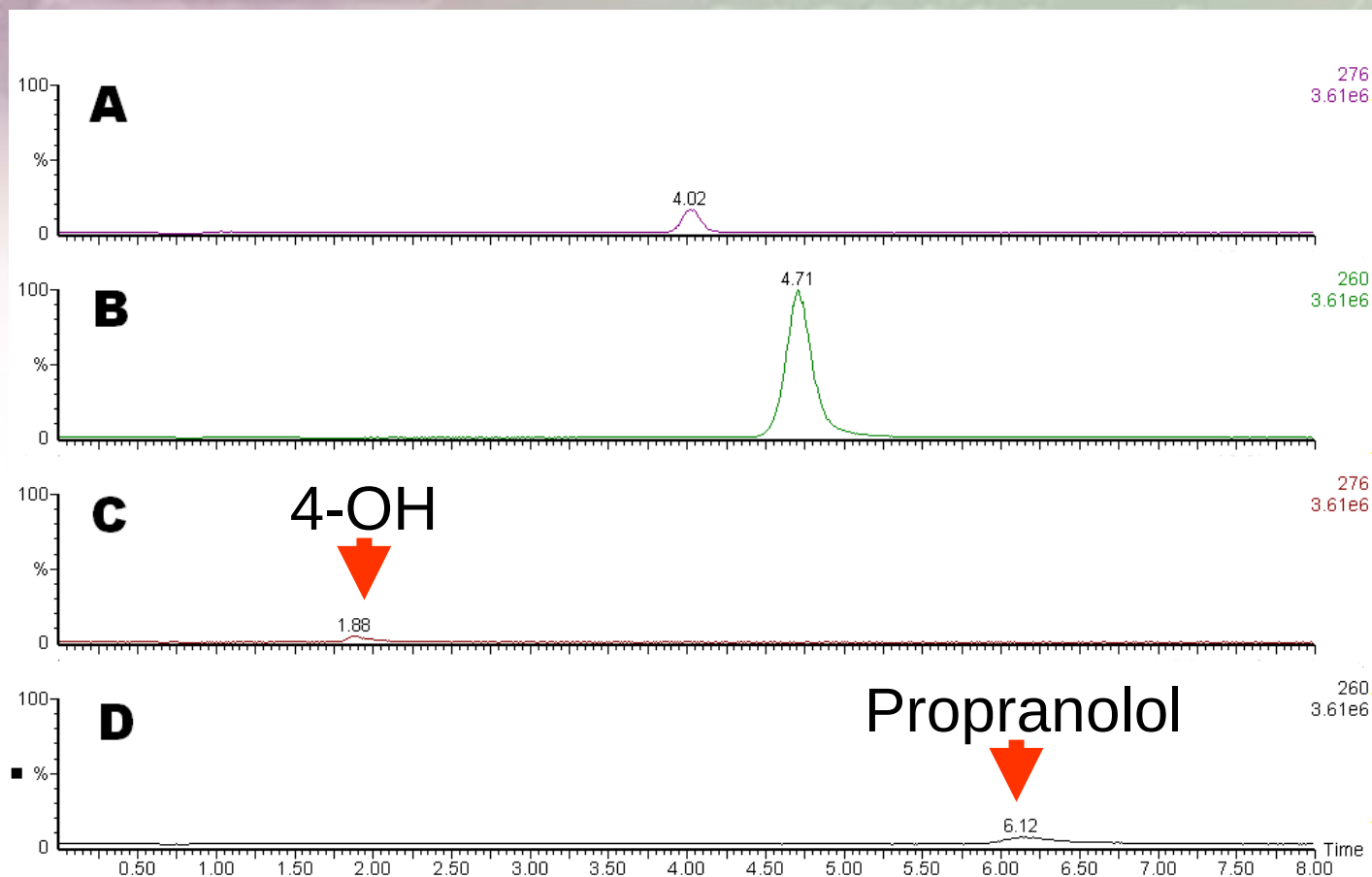
Good retention, good selectivity, “U-shape” retention observed



Example: Propranolol and Metabolite

- Compared use of “normal-phase” region on HS F5 to C18 separation
 - Greater MS sensitivity
 - More efficient separation
 - Faster sample preparation (no evaporation/reconstitution)

Example: Propranolol and Metabolite



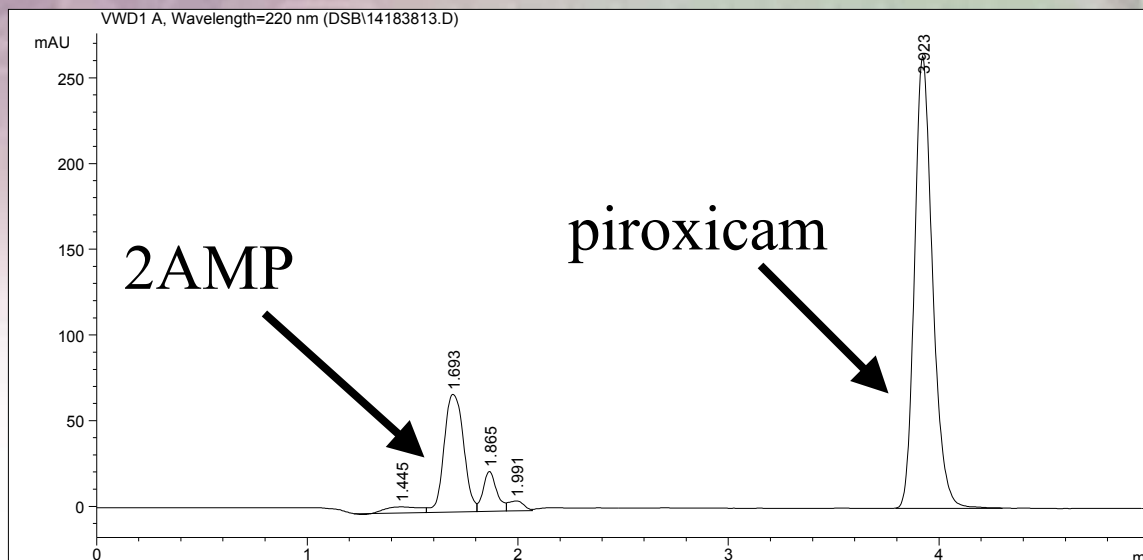
LC/MS Advantages

- The ability to retain at high percent organic:
 - Provides unique selectivity compared to C18
 - Provides retention not possible on C18 without highly aqueous MP or detrimental modifiers such as ion-pair reagents
 - Increases absolute response due to the more efficient desolvation in the ESI source
 - Reduces sample pretreatment requirements, ie. direct injection of highly organic SPE eluents
 - Allows higher flow rates due to the low viscosity of the mobile phase resulting in reduced analysis time
- Each of these aspects can be utilized to generate and/or improve high-throughput LC/MS methods

Retention in “Reversed-Phase” Region

- Only basic analytes have been shown to exhibit “U-Shape” retention
- Often need to retain/separate neutrals and acids
- Polar-RP phases may provide :
 - retention
 - additional selectivity
 - more efficient separations

Example: General Elution Problem



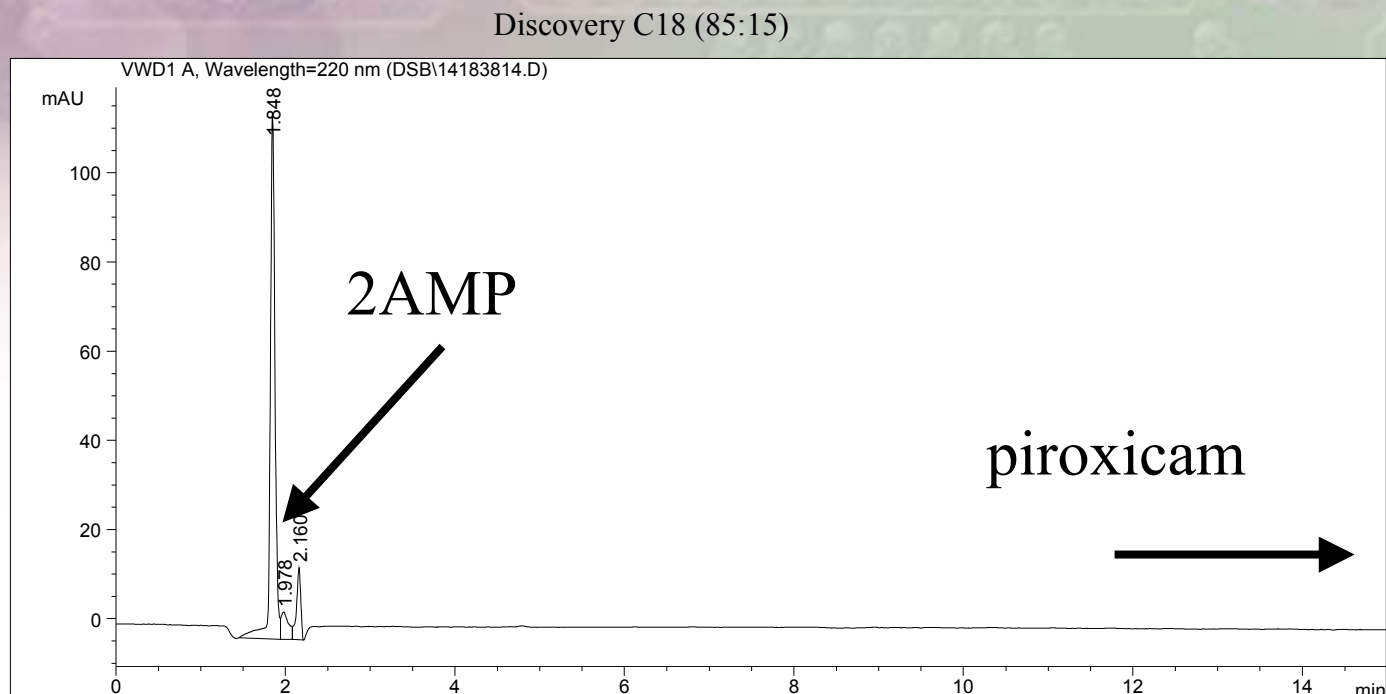
Conditions:

Column: Discovery C18, 150x4.6mm, 5um

Mobile Phase: 10mM KH_2PO_4 , pH 2.5, Acetonitrile (55:45, v/v)

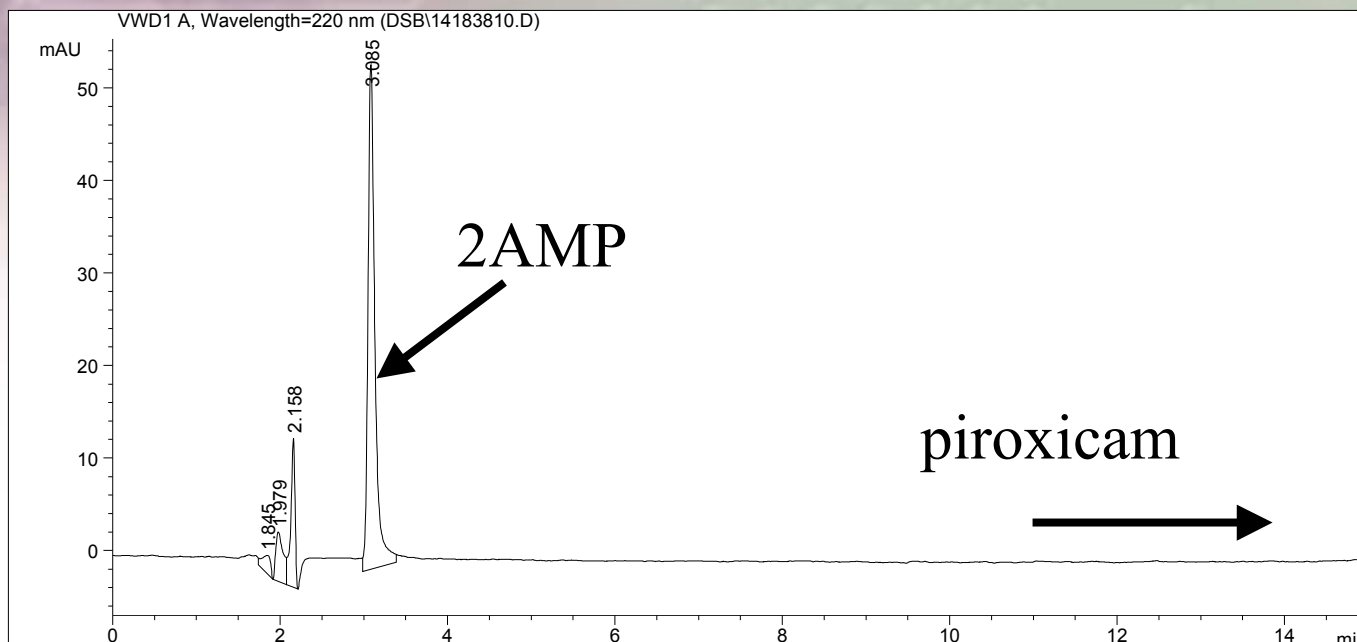
Detection: UV @220nm

Example: General Elution Problem



Decrease solvent strength from 55:45 (buffer:Acetonitrile) to 85:15:
2AMP still unretained, piroxicam too retained

Example: General Elution Problem

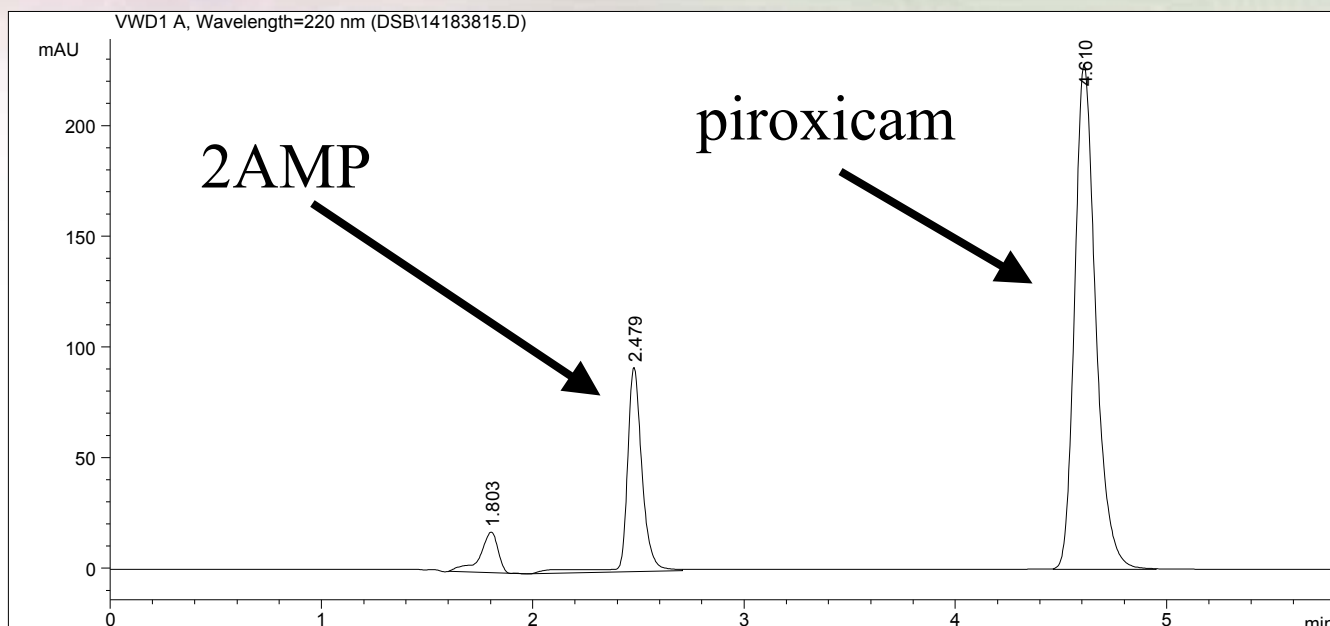


Column: Discovery C18, 150 x 4.6 mm, 5 μ m

Mobile Phase: 10mM K₂HPO₄, pH 6.8:Acetonitrile (85:15, v/v)

Example: General Elution Problem

Discovery HS F5 10mM phosphate, pH 2.5:Acetonitrile (55:45)

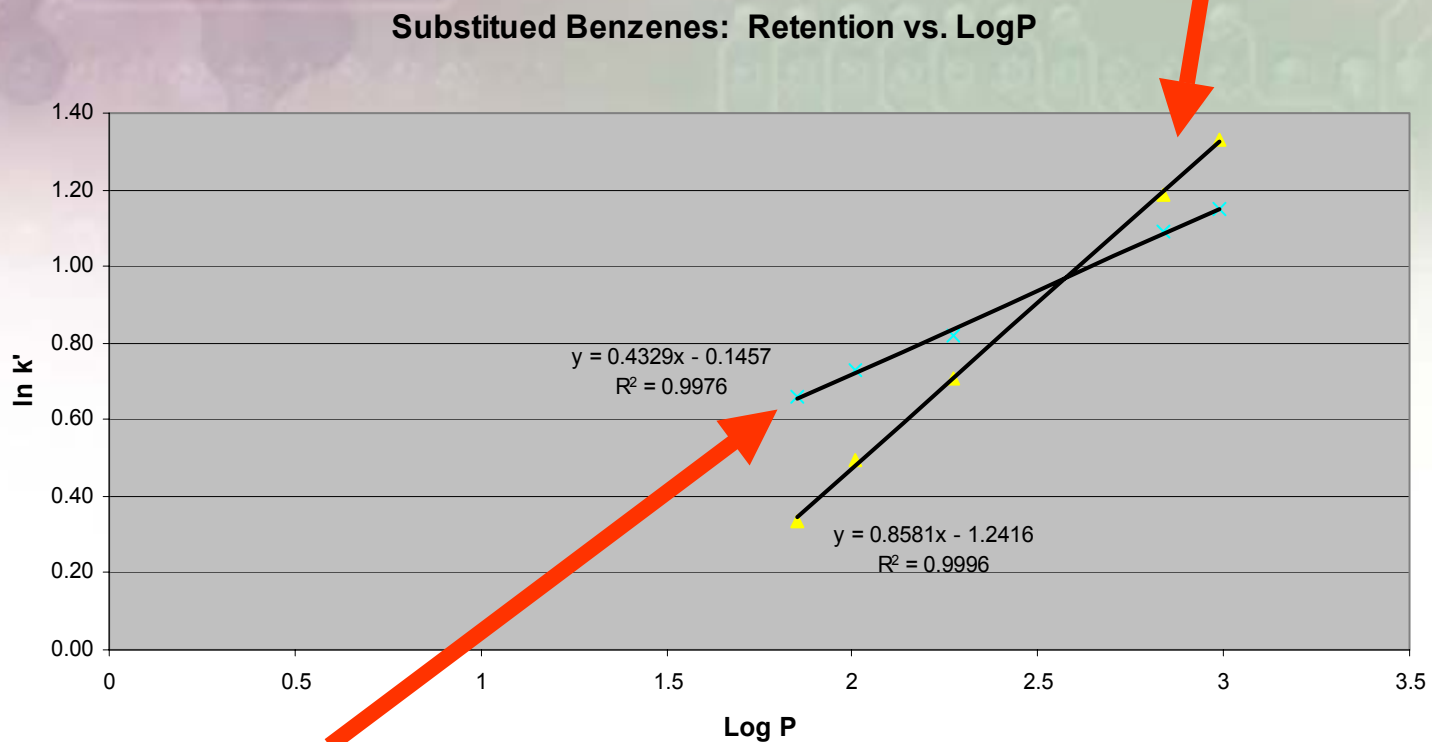


Interactions

- Retention is a result of multiple types of interactions
 - Dispersive: transient electronic interactions
 - Polar: permanent and induced dipole, H-bonding
 - Ionic: permanent charge interactions
- dispersive interactions are dominant in reversed phase systems

Interactions: Dispersive

More hydrophobic = Greater Rt on HS C18

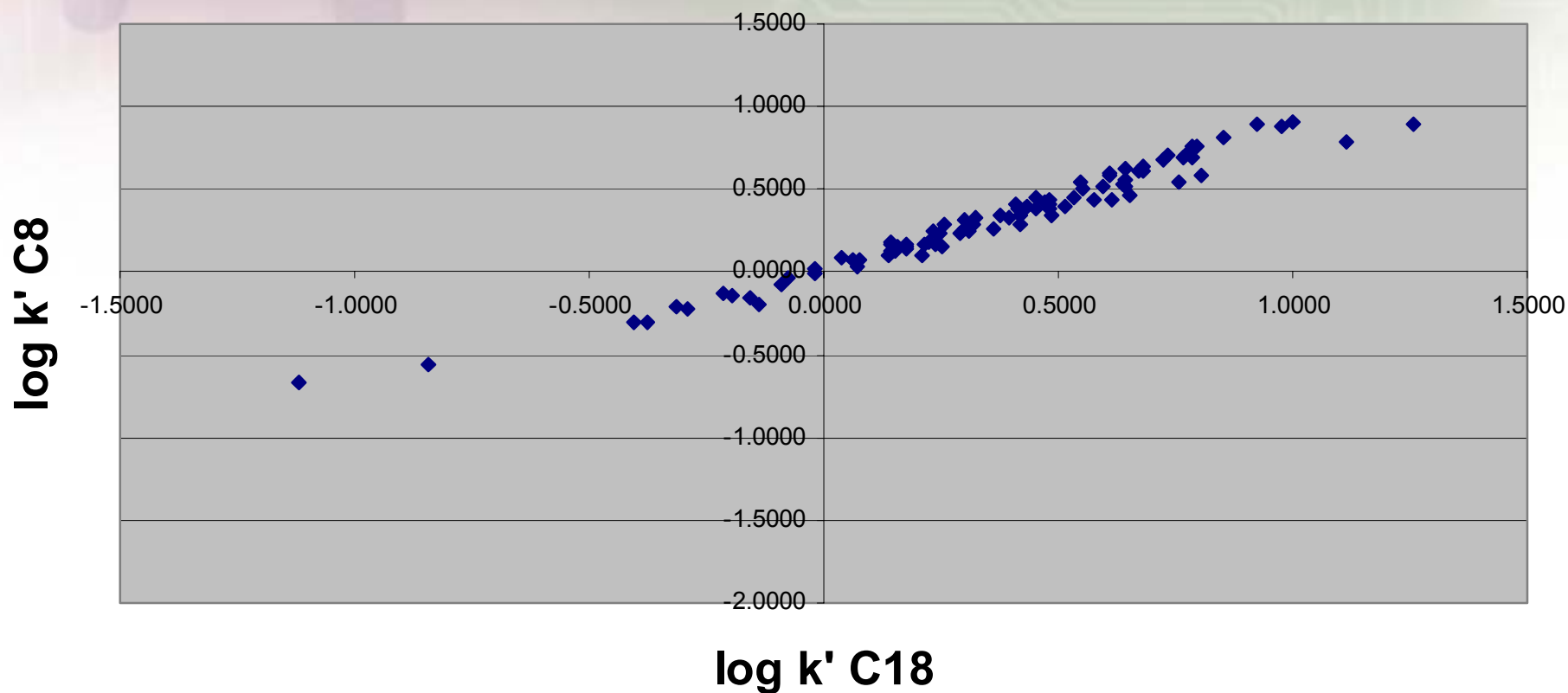


More hydrophilic = Greater Rt on HS F5

“Ideal” RP Analytes

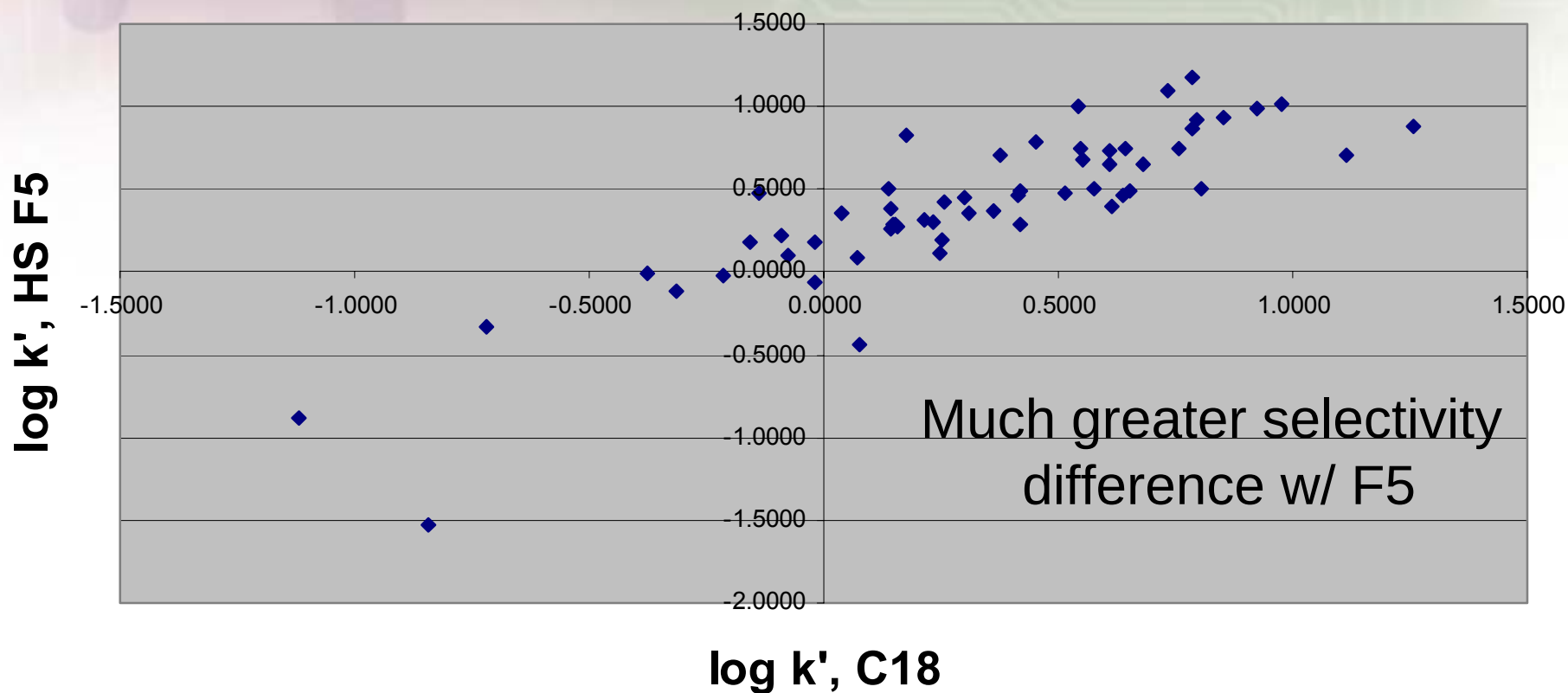
Interactions: Dispersive + Polar

log k' C18 vs Log k' C8



Interactions: Dispersive + Polar

log k' correlation between C18 and HS F5

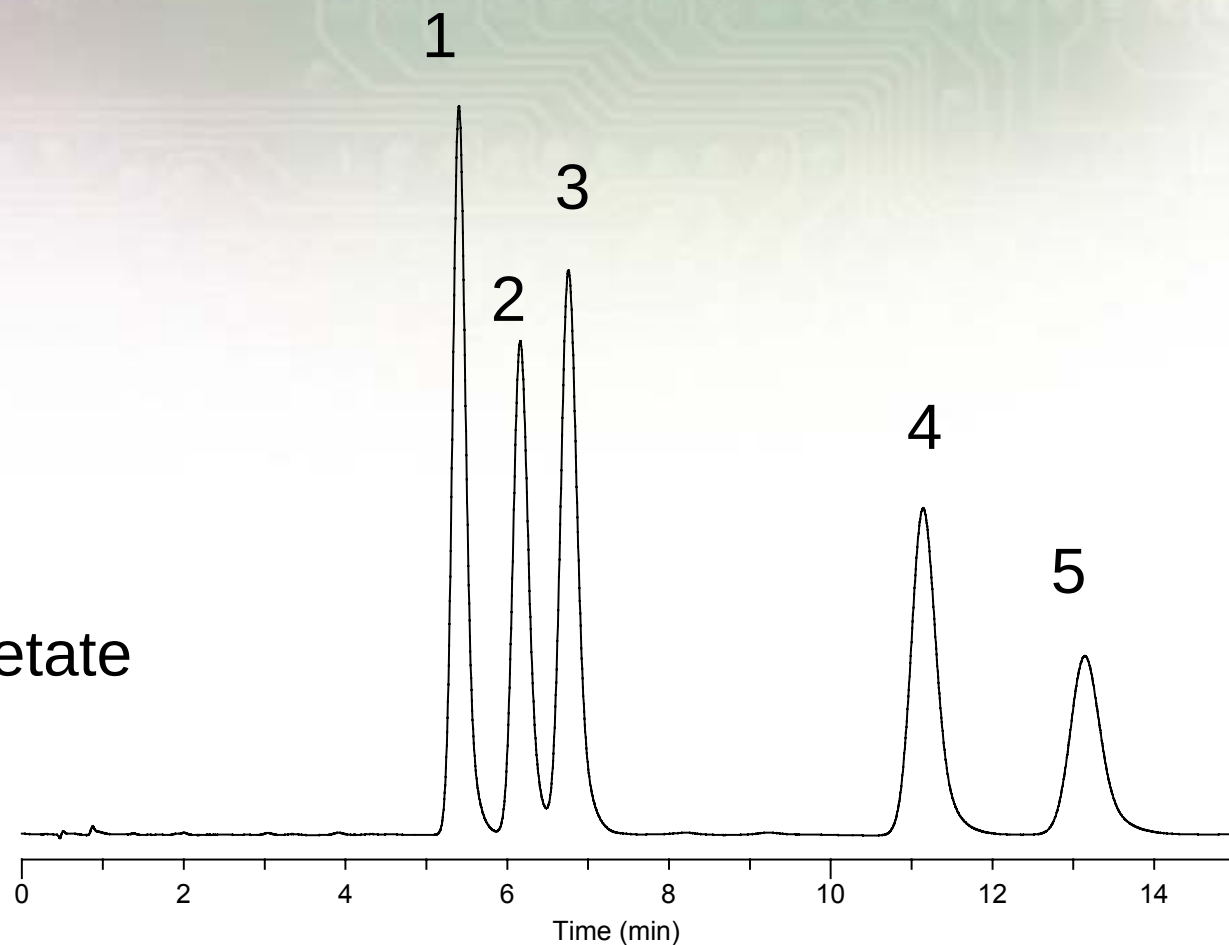


Interactions: Dispersive + Polar

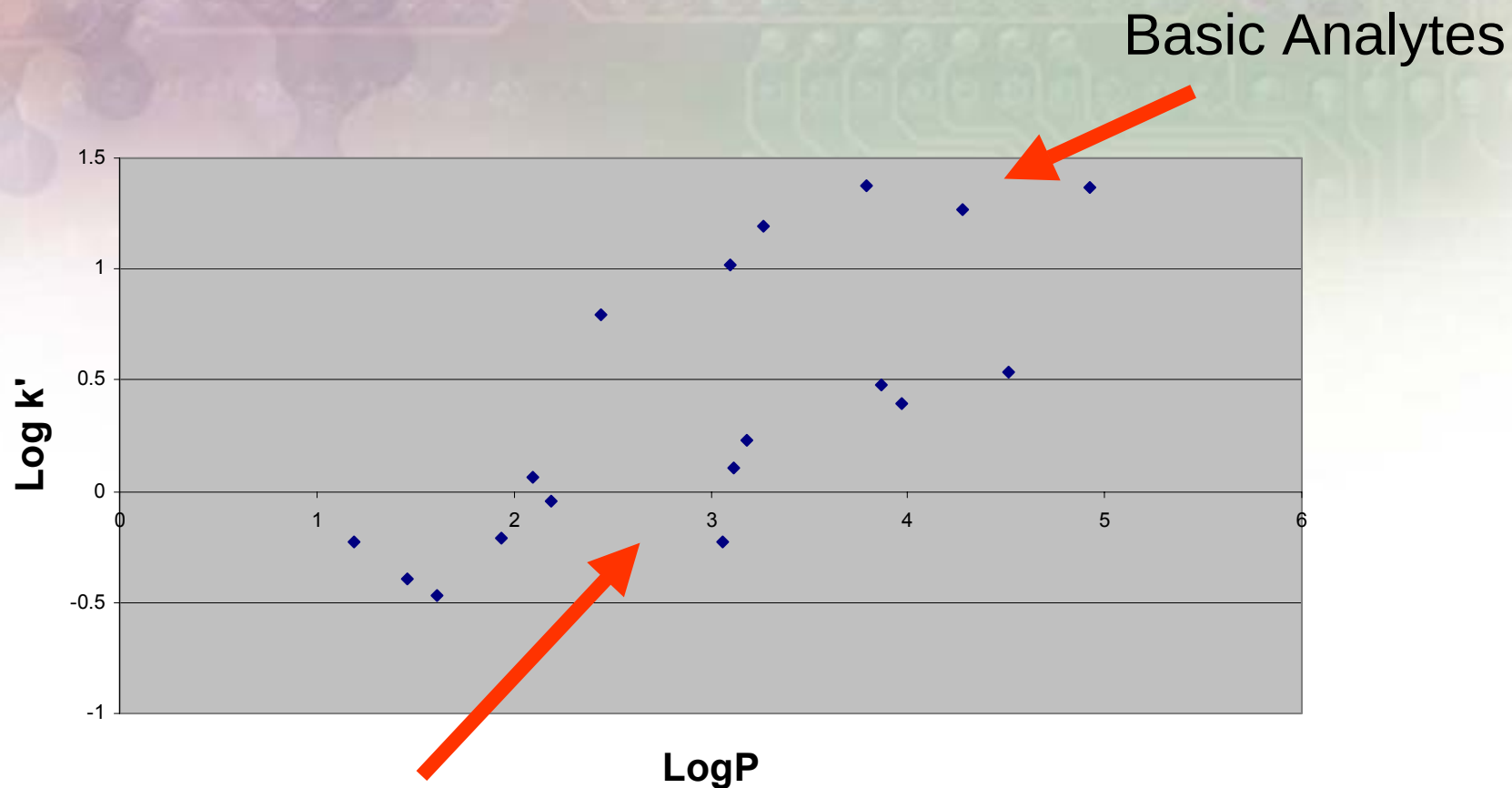
- Alkyl phases do not complement polar interactions
- Polar interactions are of a secondary nature
 - Difficult to control
 - Highly variable
- Polar-RP phases, by design, complement polar interactions, enhancing selectivity

Additional Selectivity – Example: Corticosteroids

- Hydrocortisone
- Prednisolone
- Prednisone
- Corticosterone
- Hydrocortisone Acetate



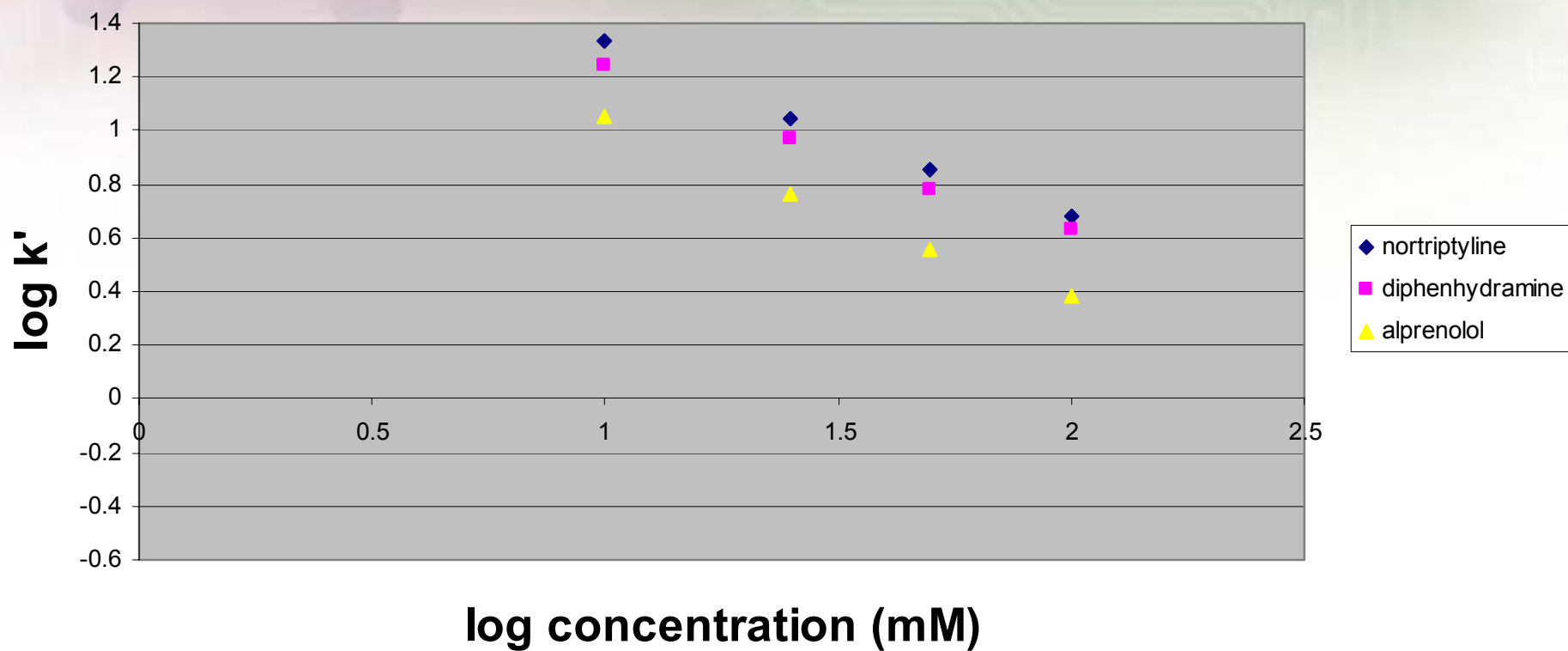
Interactions: Dispersive + Polar + Ionic



Neutral and Acidic Analytes

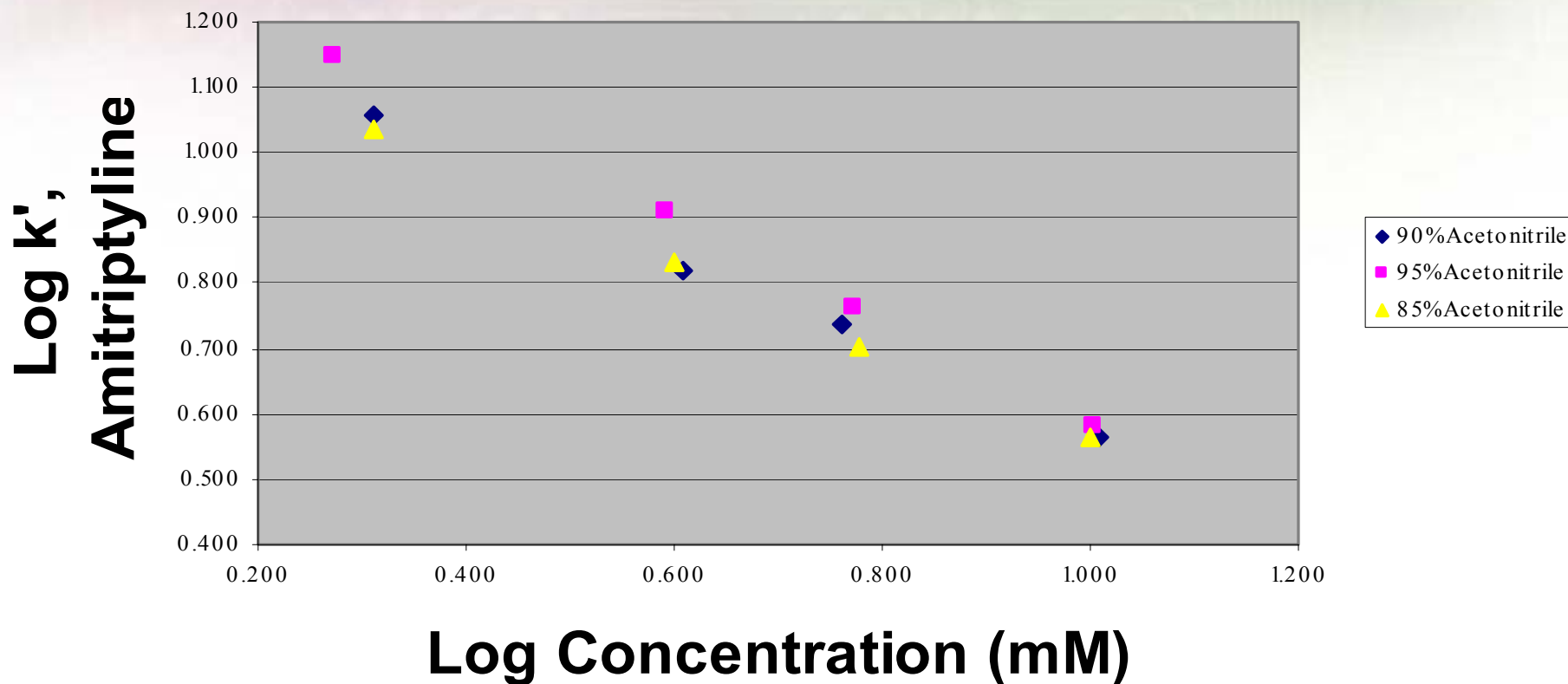
Interactions: Dispersive + Polar + Ionic

Base Retention Response to Ionic Strength in RP Region



Interactions: Dispersive + Polar + Ionic

Comparison of Log-Log plots for 85, 90 and 95% Acetonitrile



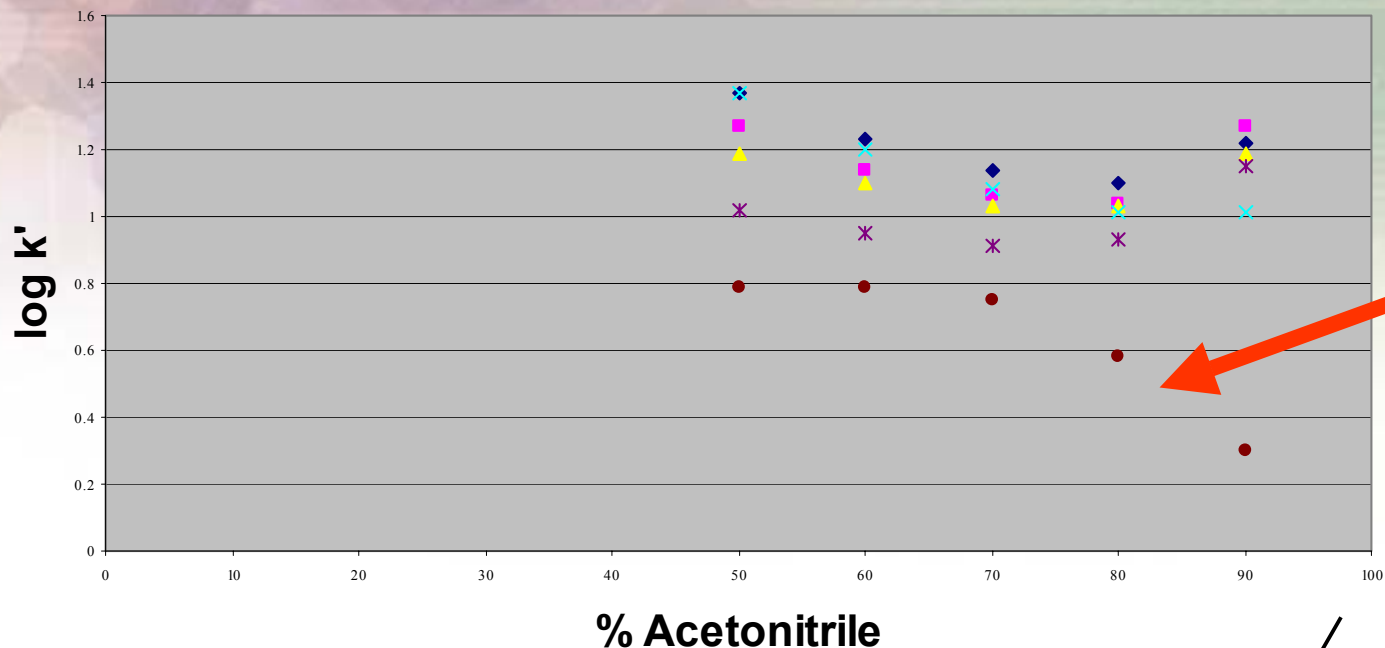
Interactions, Summary

- Retention is a manifestation of various interactions
 - Dispersive, polar, and ionic
- Traditional alkyl phases primarily complement dispersive
 - Polar interactions are secondary
- Polar RP phases are designed to complement polar and ionic interactions
 - Improved control
 - Increased ruggedness
 - Better selectivity

Method Development: Tips for HS F5

- LC/MS analysis of basic compounds
 - “U-shape” retention profile
 - Ammonium formate/acetate
 - Soluble in the high organic systems
 - 2-10mM range (solubility issues at higher levels)
 - Counter ion required
 - Not all bases show “U-shape”

Method Development: Retention Profile of Some Bases

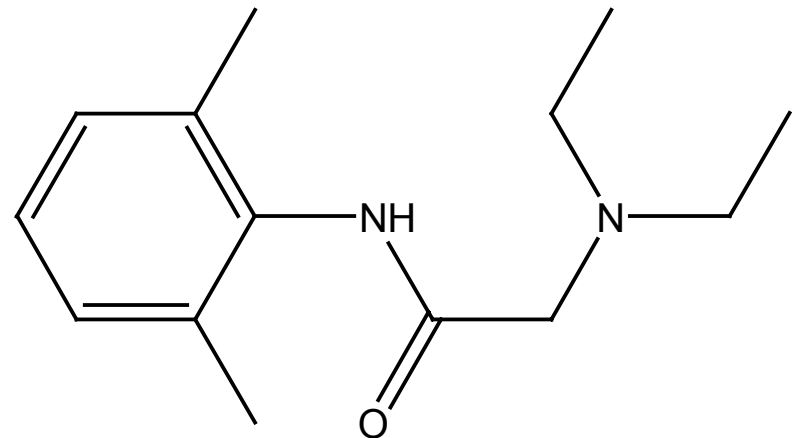


Lidocaine

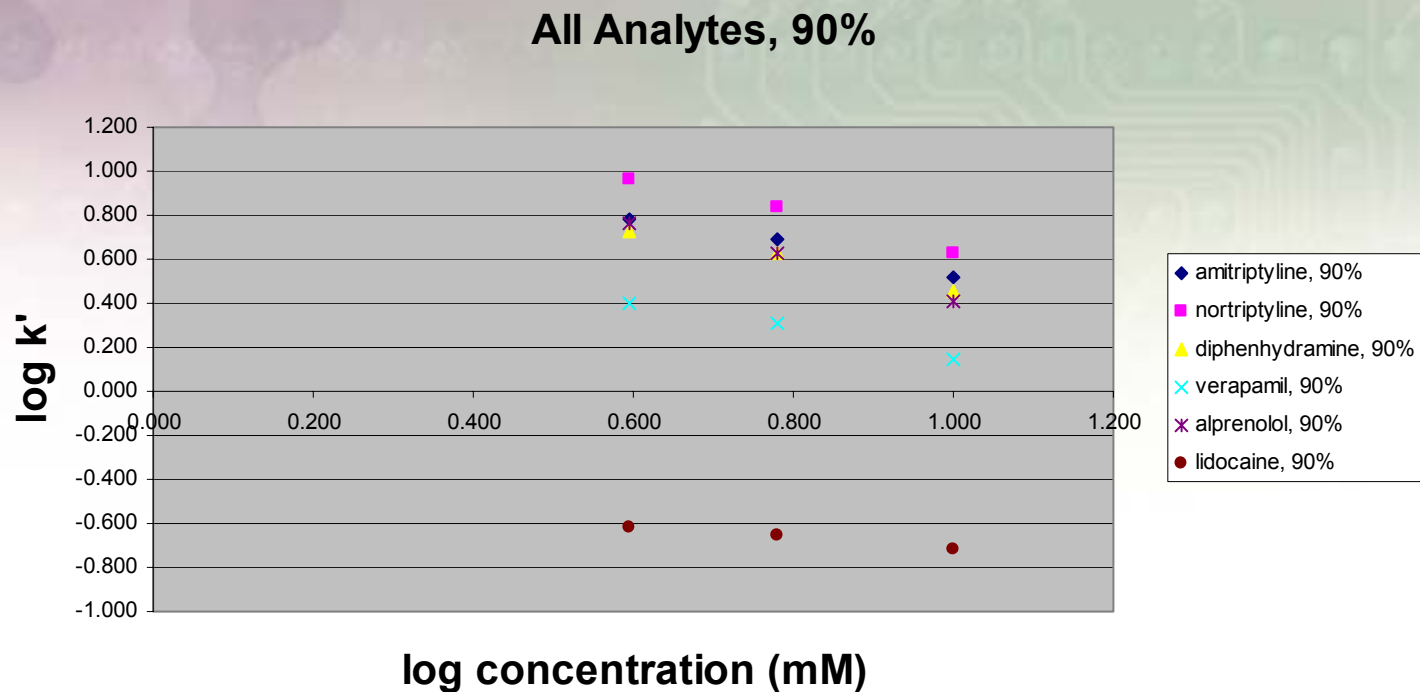
Internal hydrogen bonding

Also Remember:

- pKa changes w/organic
- pH changes w/organic



Method Development: Selectivity Adjustments



Use ionic strength and % organic

Method Development

- Use pH to maximize/minimize ionic interactions
- Use different organic modifiers to adjust polar interactions
- Generally obtain better peak shape at higher pH
- Ionic strength important in RP mode too
- Remember the counter ion

Summary

- General chromatographic and LC/MS-related issues
- How polar-RP phases combat issues
 - Pentafluorophenylpropyl (HS F5) phase
 - Advantages - examples
 - Interactions
 - “polar interactions, by design”
 - Method development