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

David S. Bell
Supelco, Bellefonte, PA
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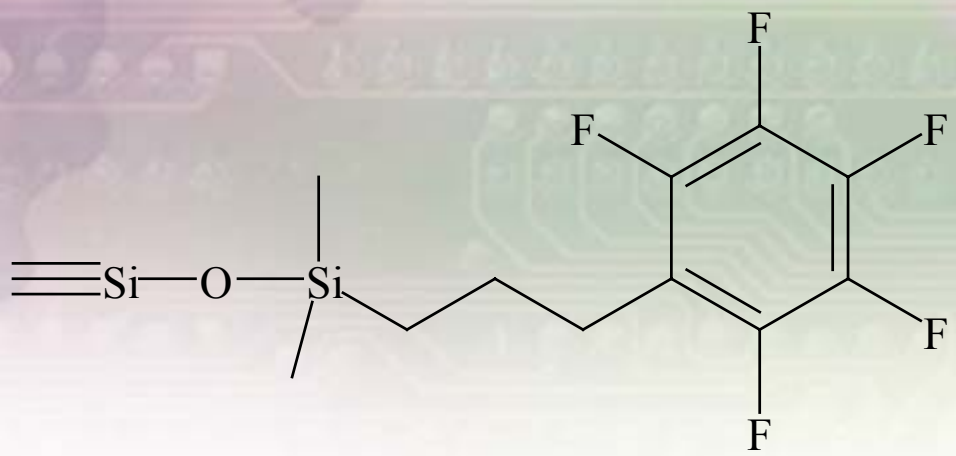
Outline

- 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

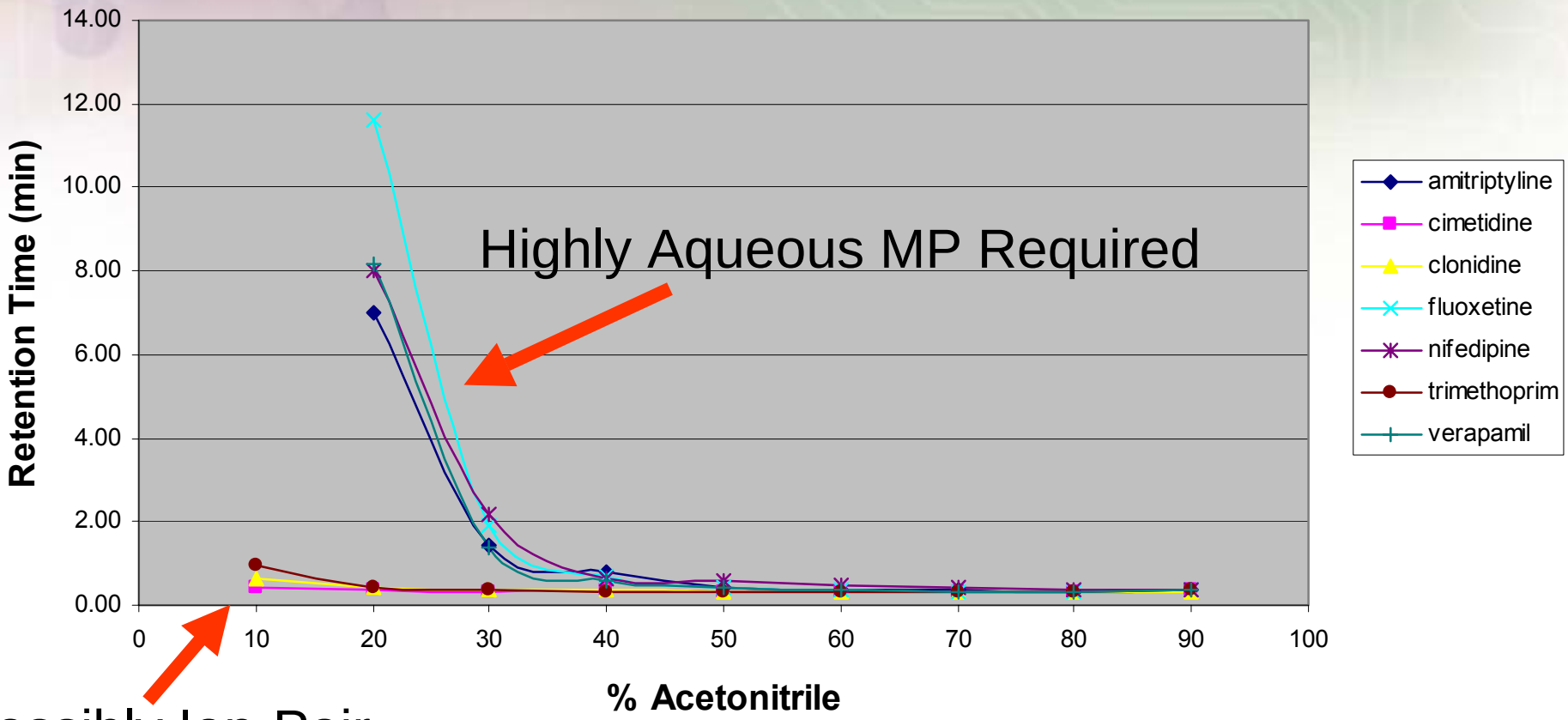
General Characteristics:

- Surface area: 300 m^2/g
- Pore Size: 120 Å
- Coverage: 4.0 $\mu mol/m^2$
- Endcapped: Yes
- %C: 12

Poor Retention

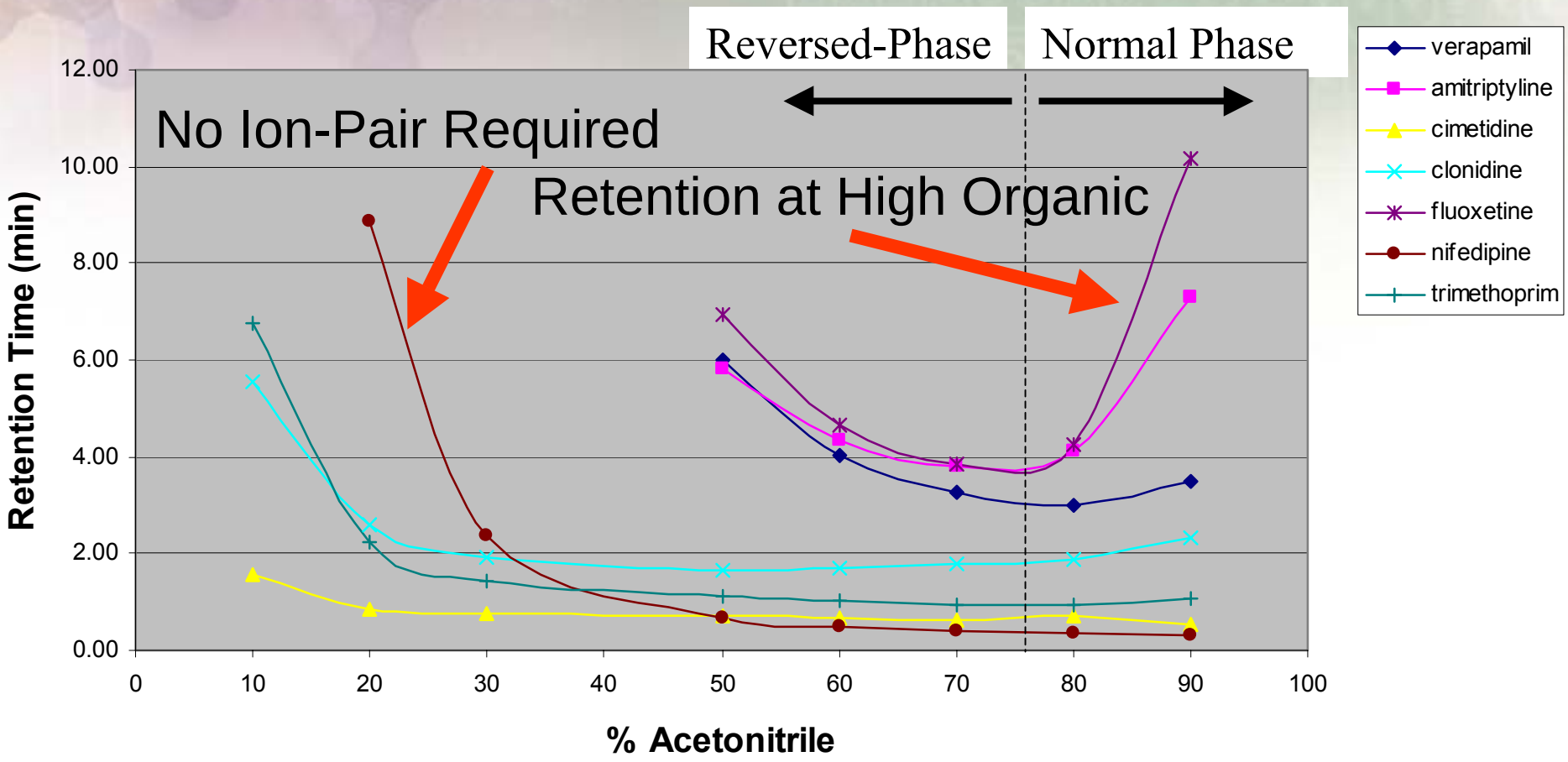
- Polar analytes, particularly bases, on traditional alkyl phases
  - Often require highly aqueous mobile phases
  - May require ion-pair
  - Both situations can inhibit MS sensitivity

Retention Profile on C18

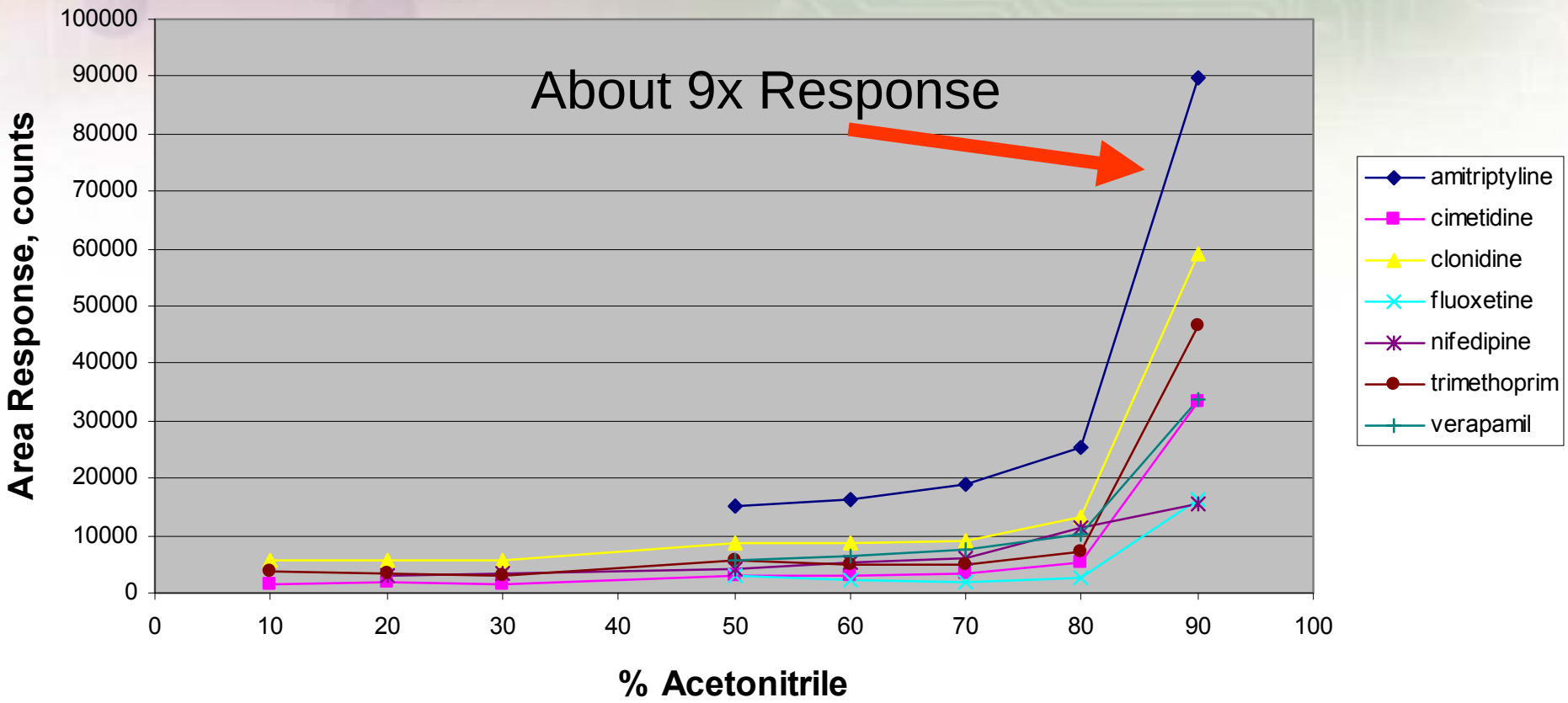


Possibly Ion-Pair

“U-Shape” Retention on HS F5

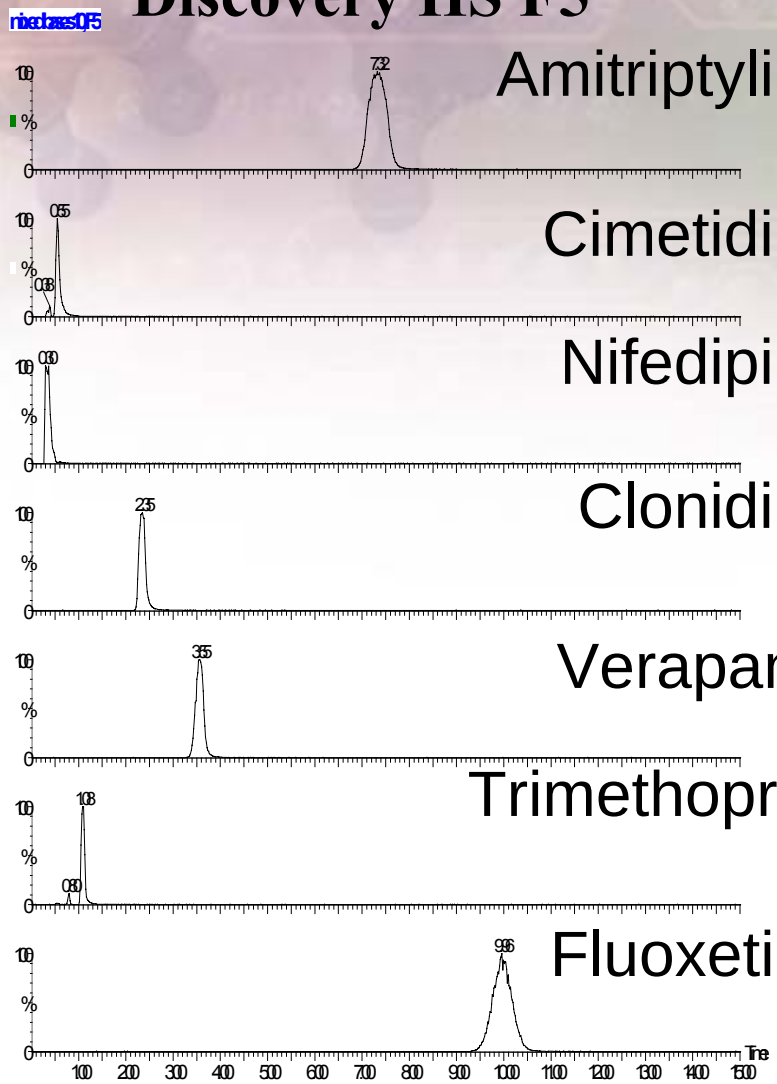


MS (ESI) Response as a Function of % Acetonitrile

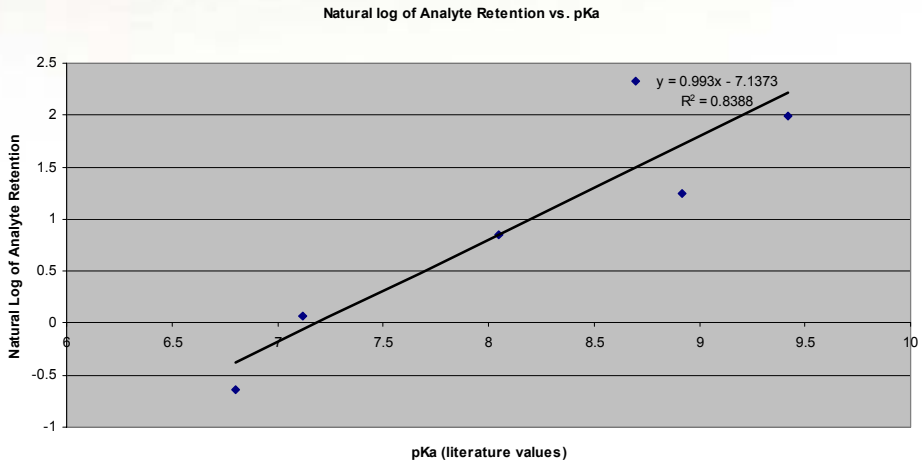


Retention at 90% Acetonitrile

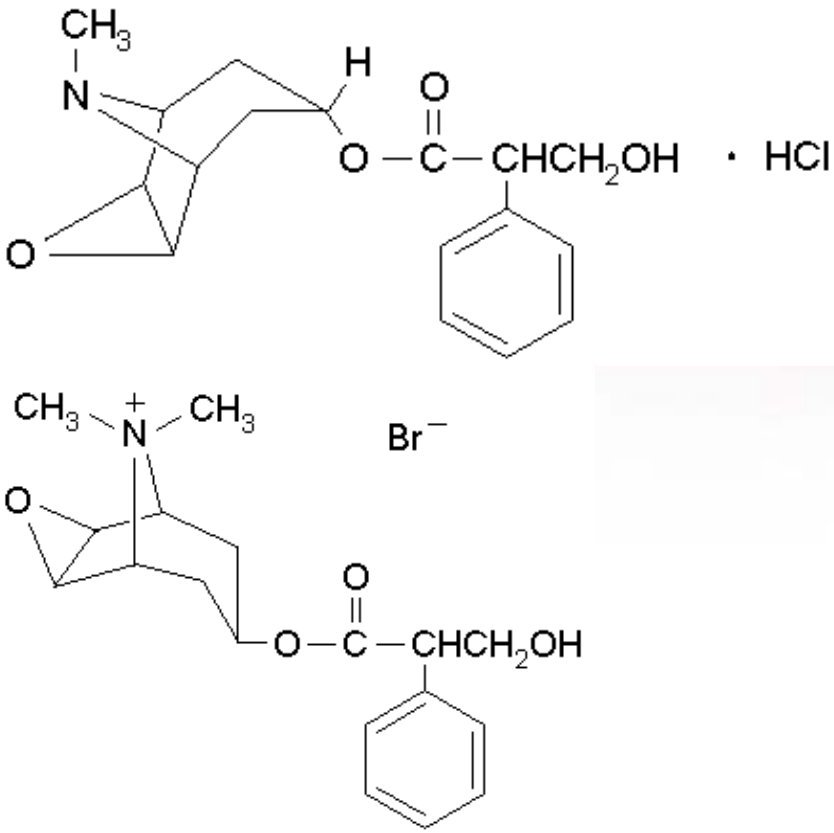
Discovery HS F5

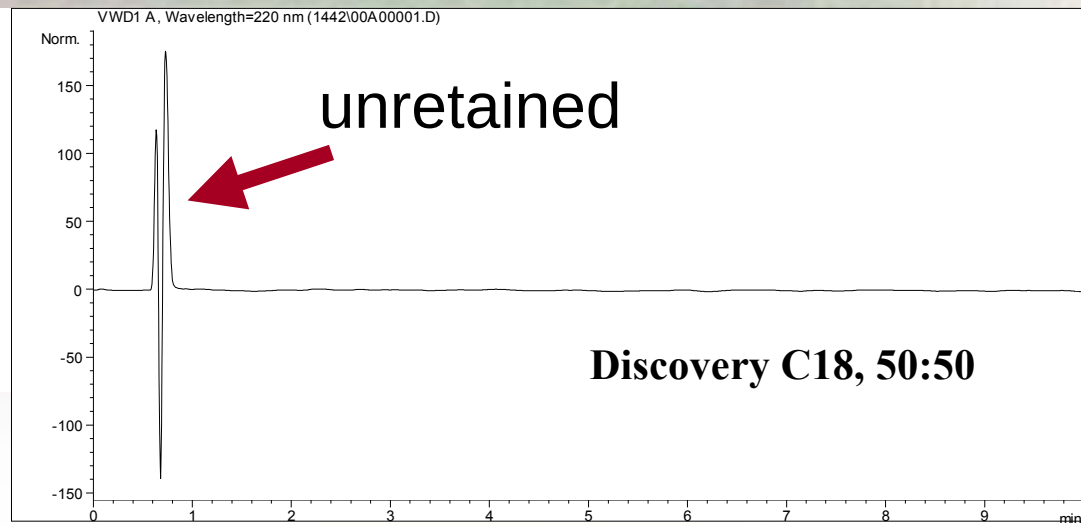


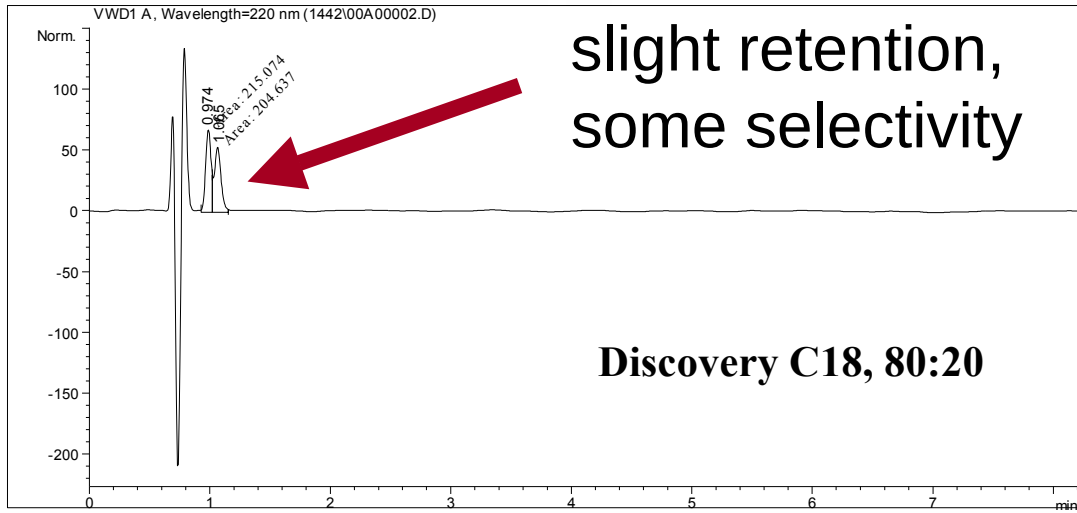
Relationship between analyte pKa and normal phase retention



Example: Scopolamines



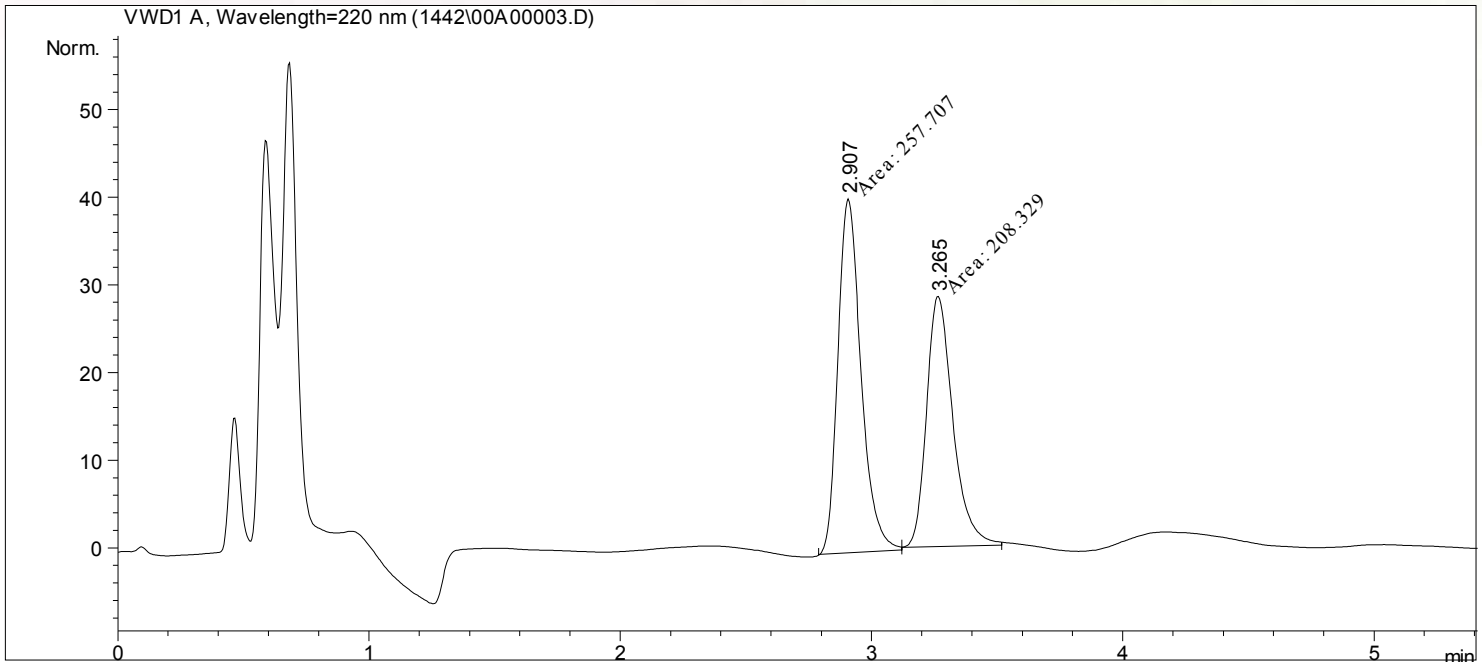




Example: Scopolamines

Discovery HS F5, 20:80

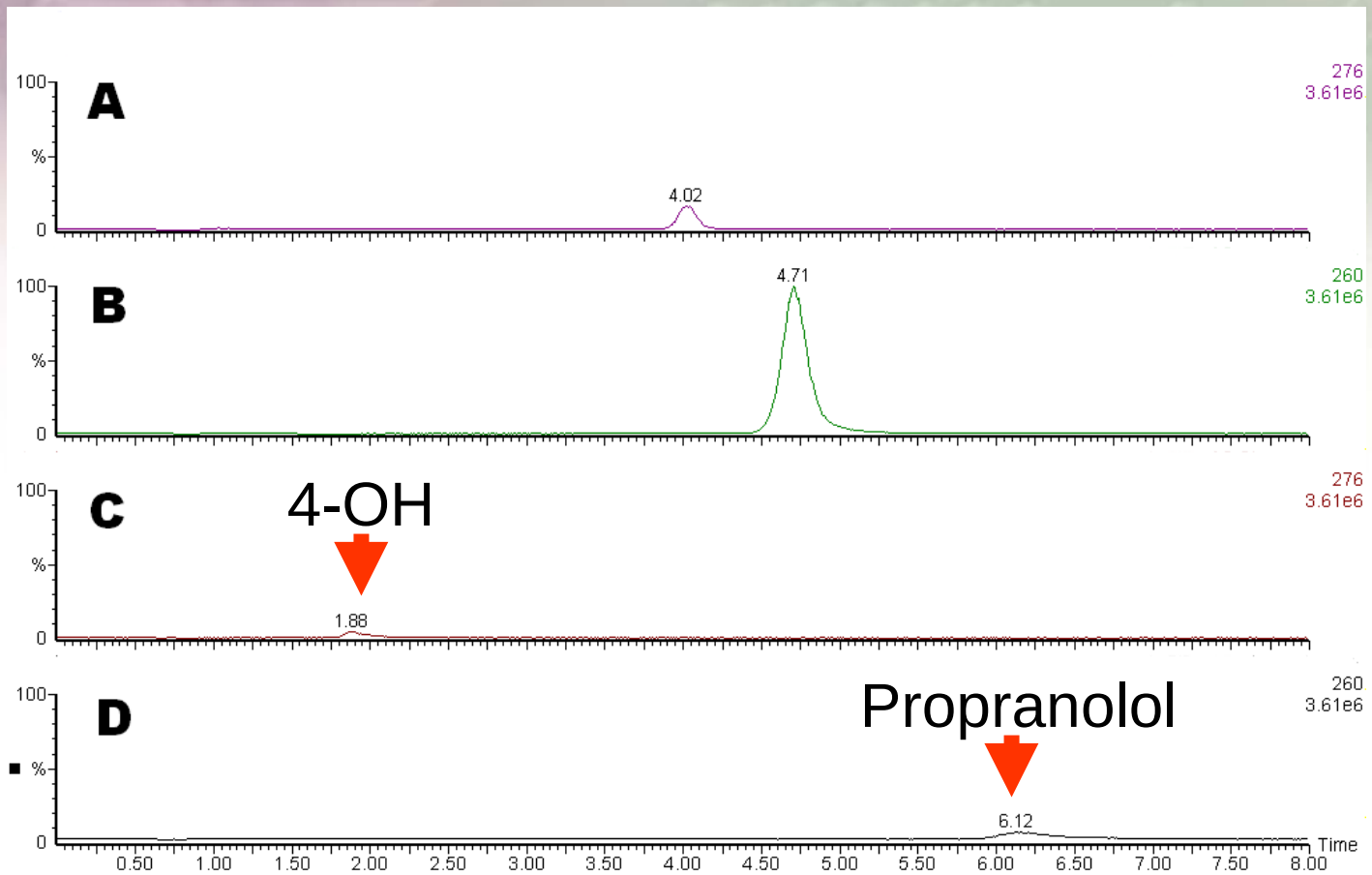
Good retention, good selectivity, “U-shape” retention observed



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



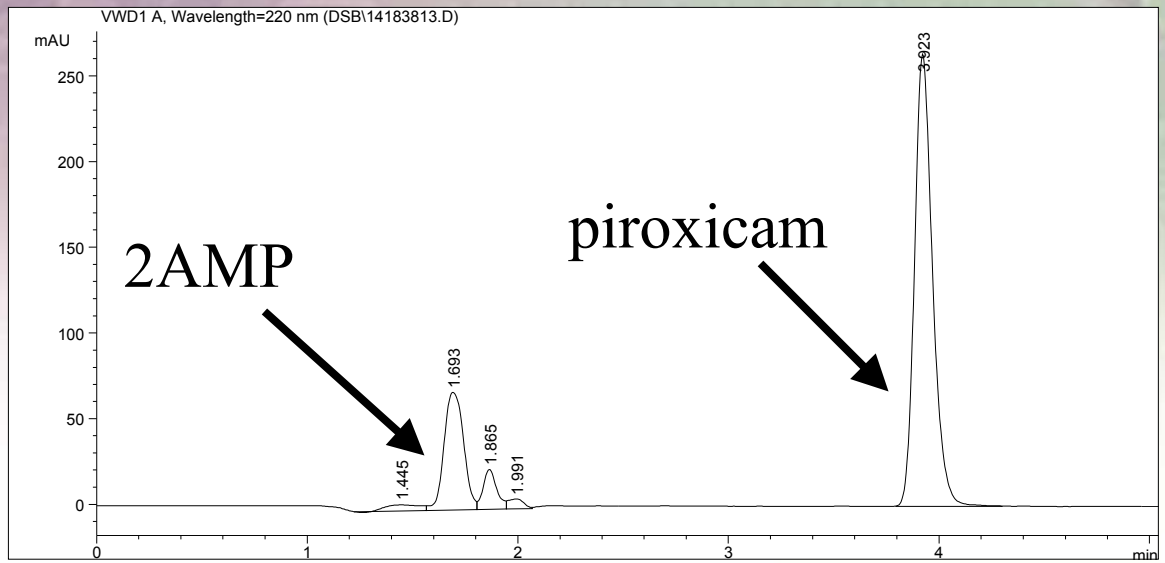
LC/MS Advantages

- 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

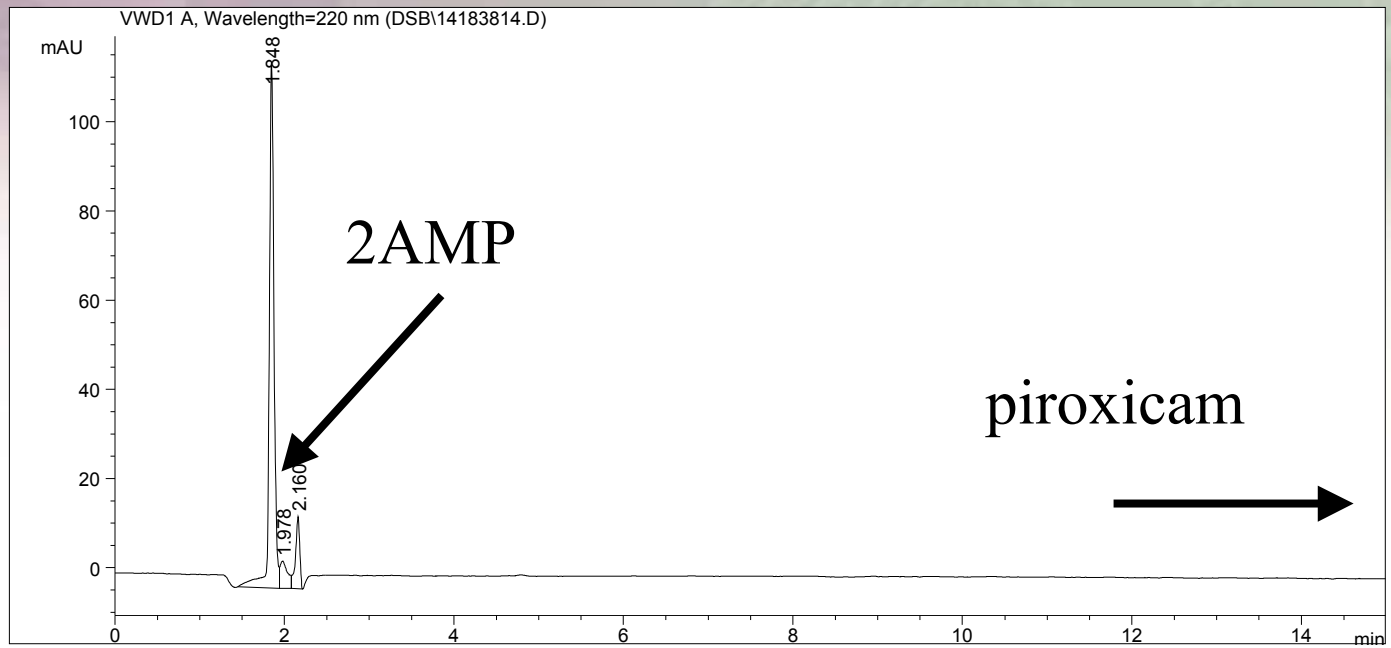
Example: General Elution Problem



Conditions:
Column: Discovery C18, 150x4.6mm, 5um
Mobile Phase: 10mM KH_2PO_4, pH 2.5, Acetonitrile (55:45, v/v)
Detection: UV @220nm

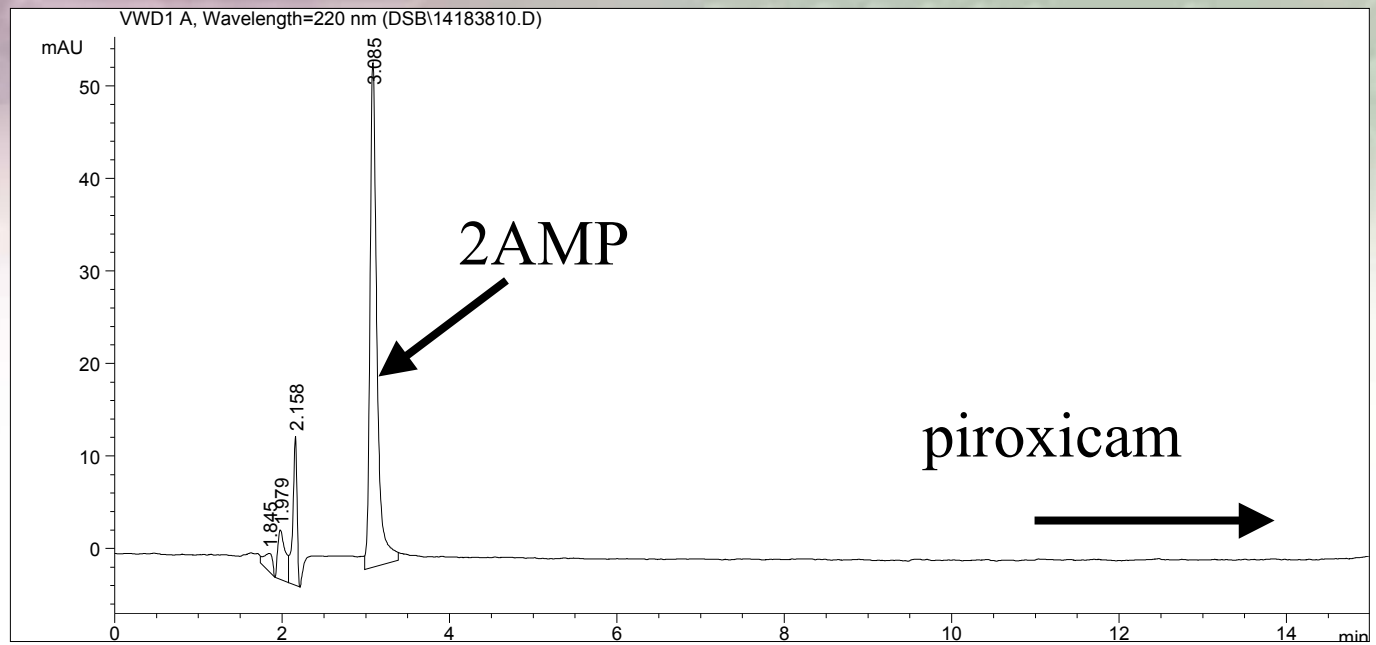
Example: General Elution Problem

Discovery C18 (85:15)



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

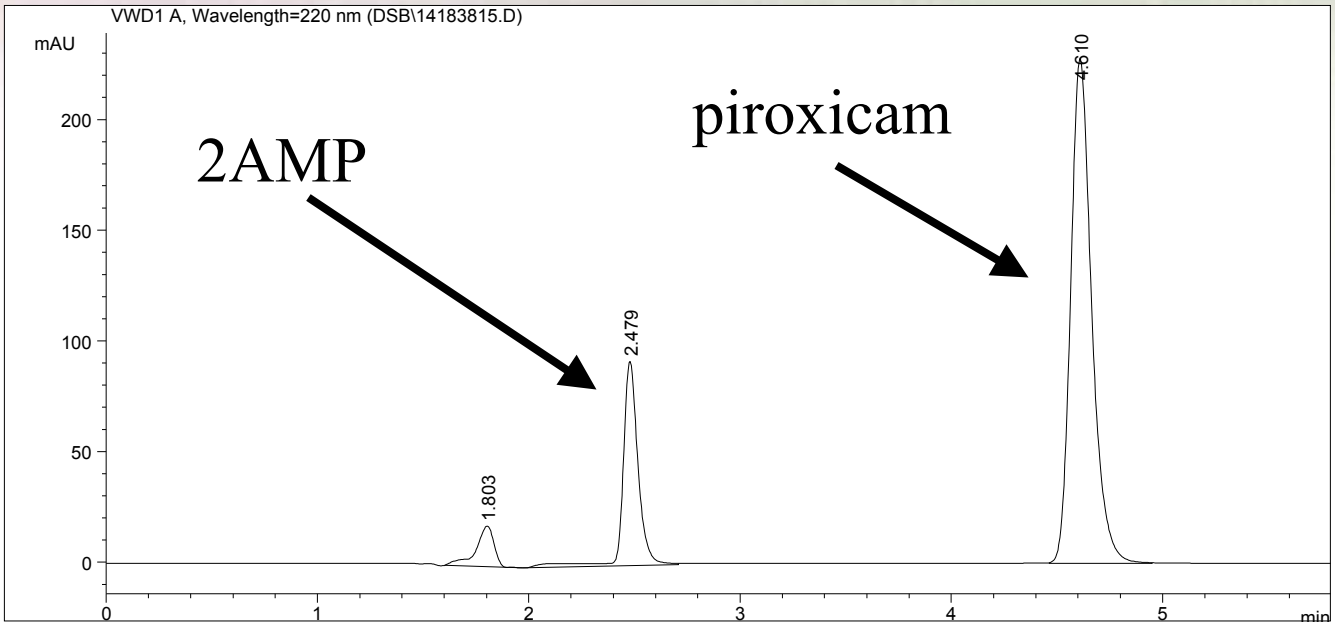
Example: General Elution Problem



Column: Discovery C18, 150 x 4.6 mm, 5um
Mobile Phase: 10mM KxHyPO4, pH 6.8:Acetonitrile (85:15, v/v)

Example: General Elution Problem

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

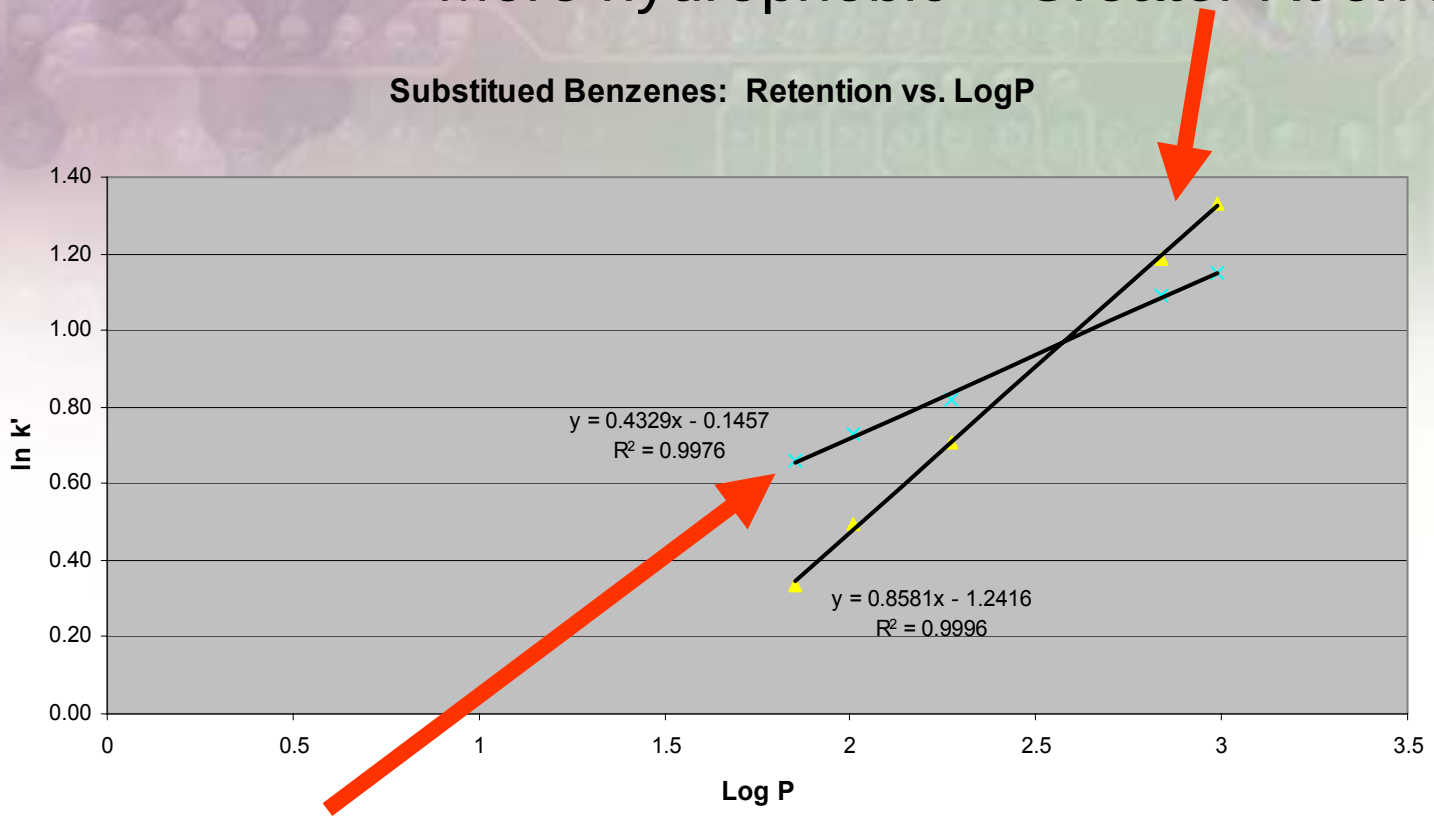


Interactions

- 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

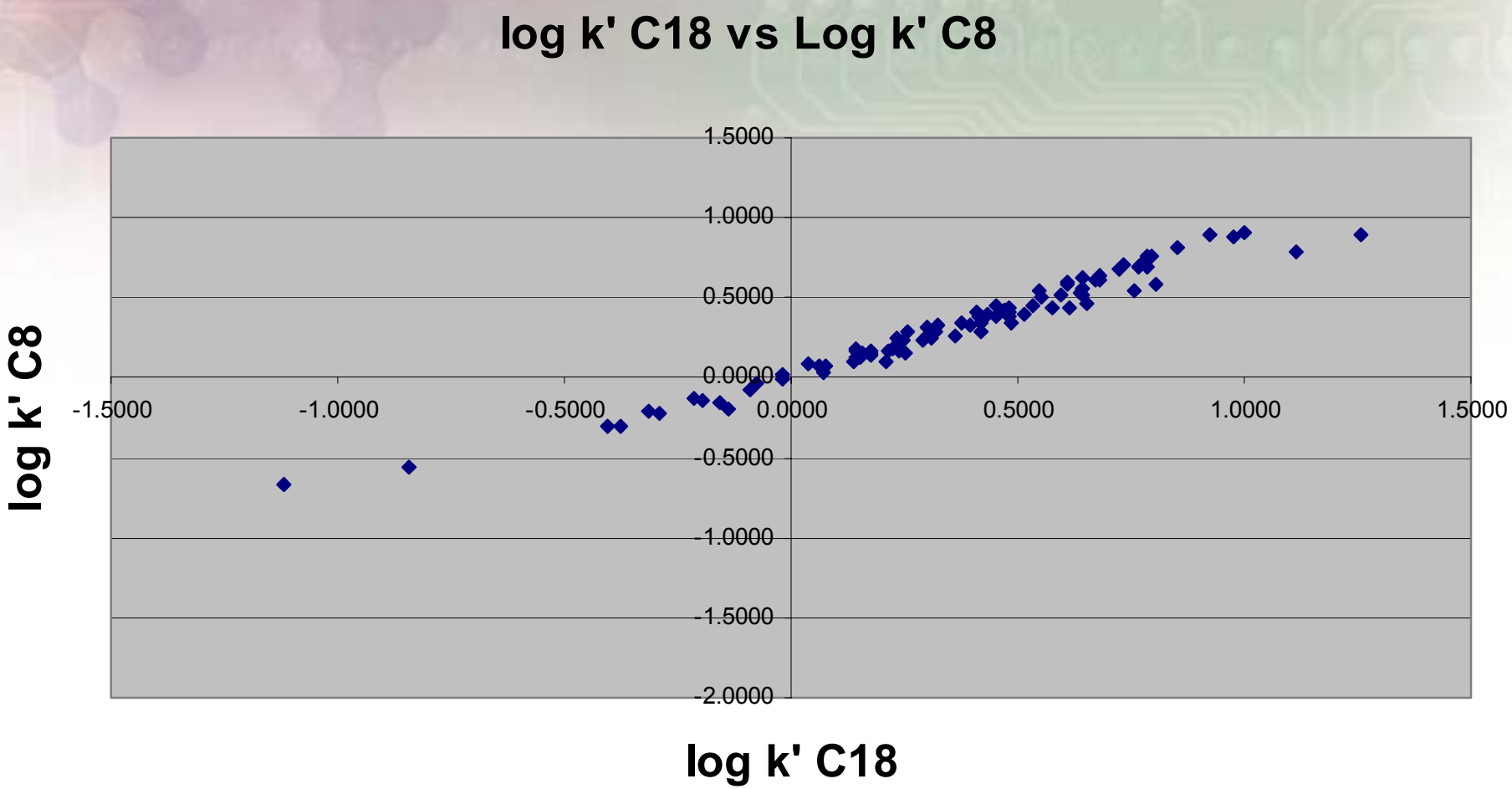
More hydrophobic = Greater Rt on HS C18



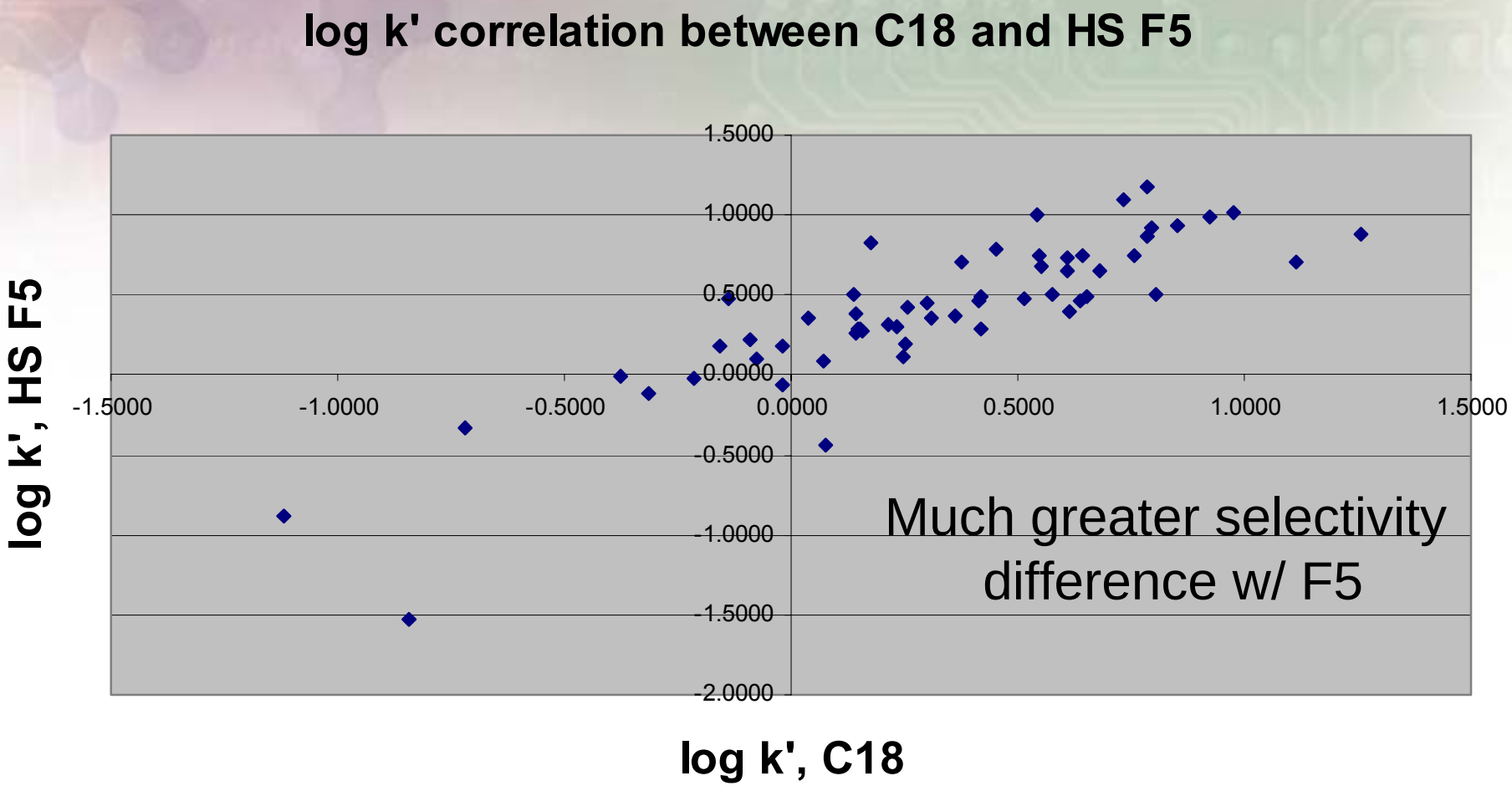
More hydrophilic = Greater Rt on HS F5

"Ideal" RP Analytes

Interactions: Dispersive + Polar



Interactions: Dispersive + Polar

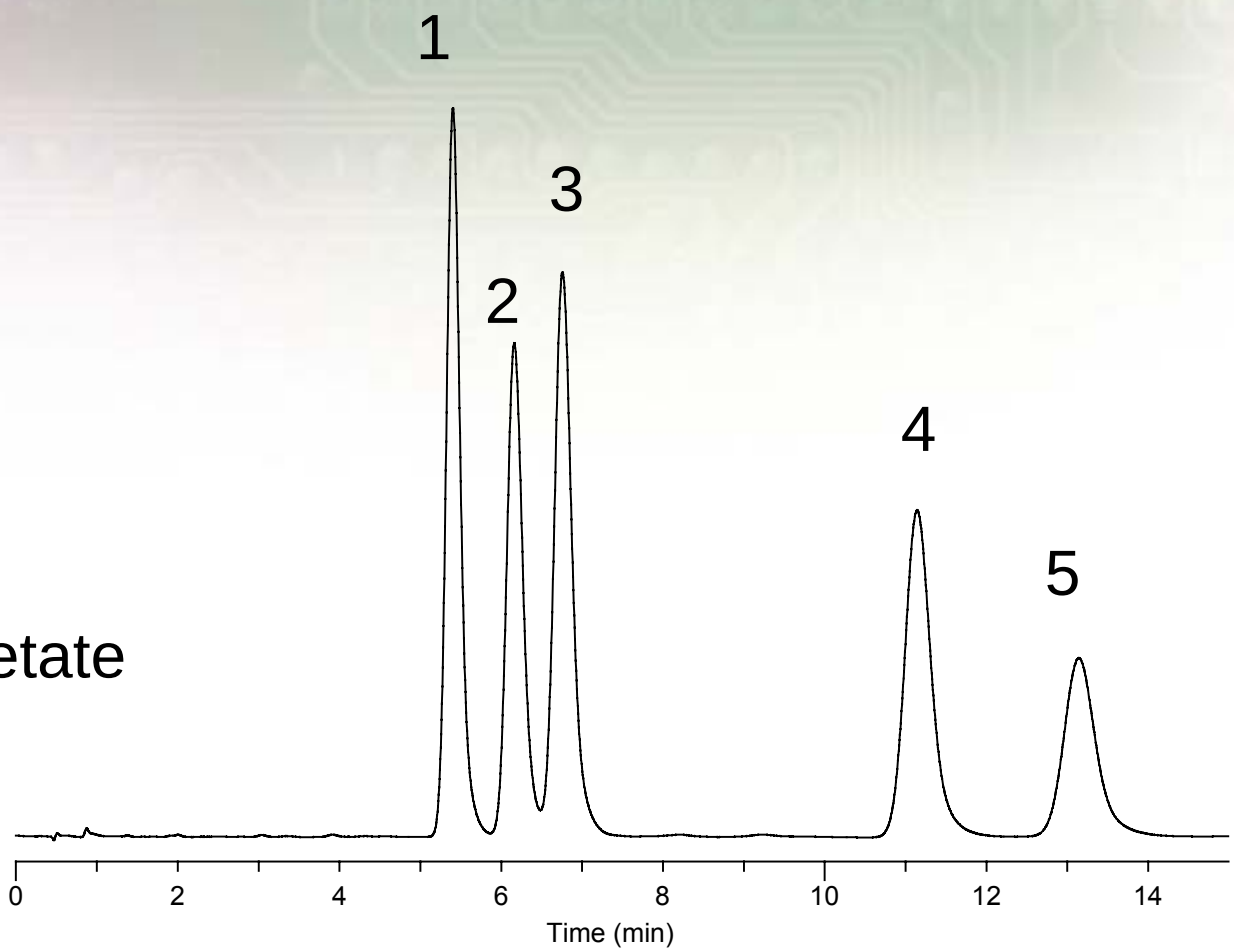


Interactions: Dispersive + Polar

- 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

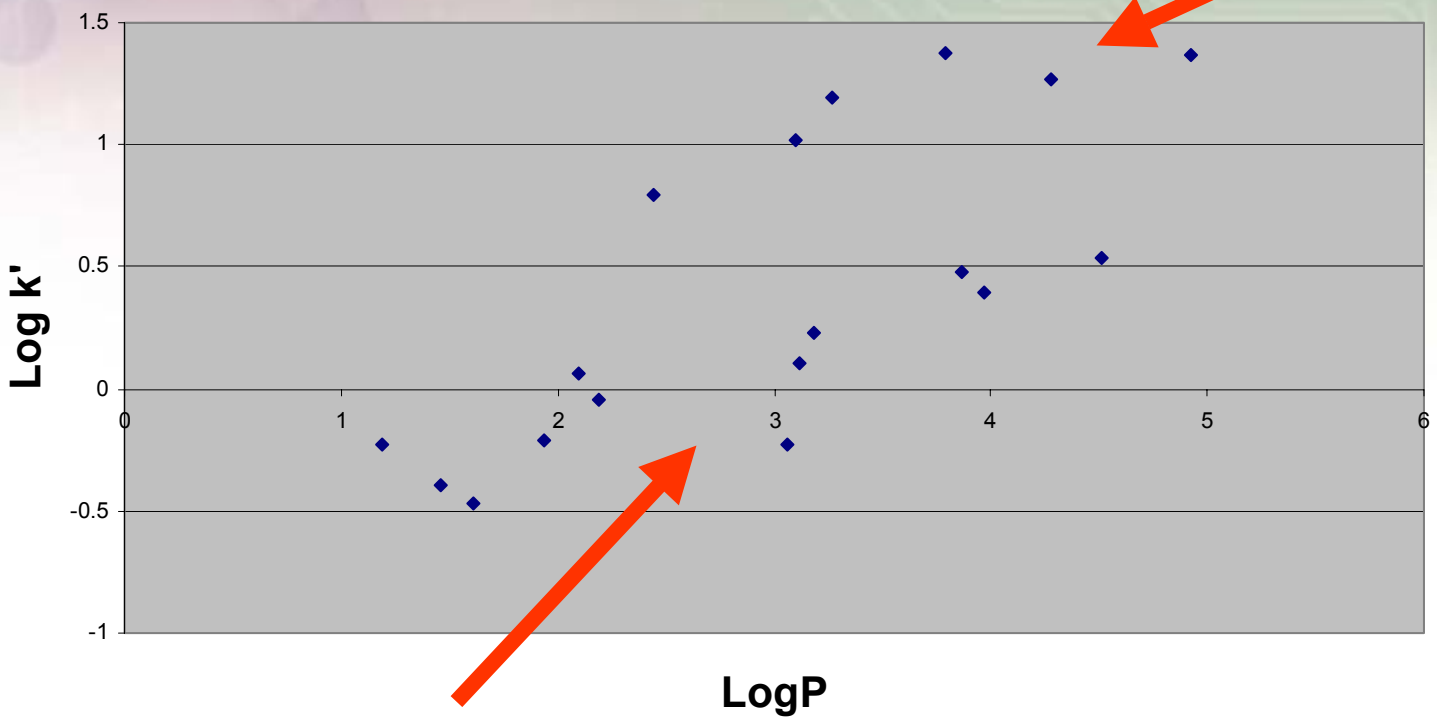
Additional Selectivity – Example: Corticosteroids

- Hydrocortisone
- Prednisolone
- Prednisone
- Corticosterone
- Hydrocortisone Acetate



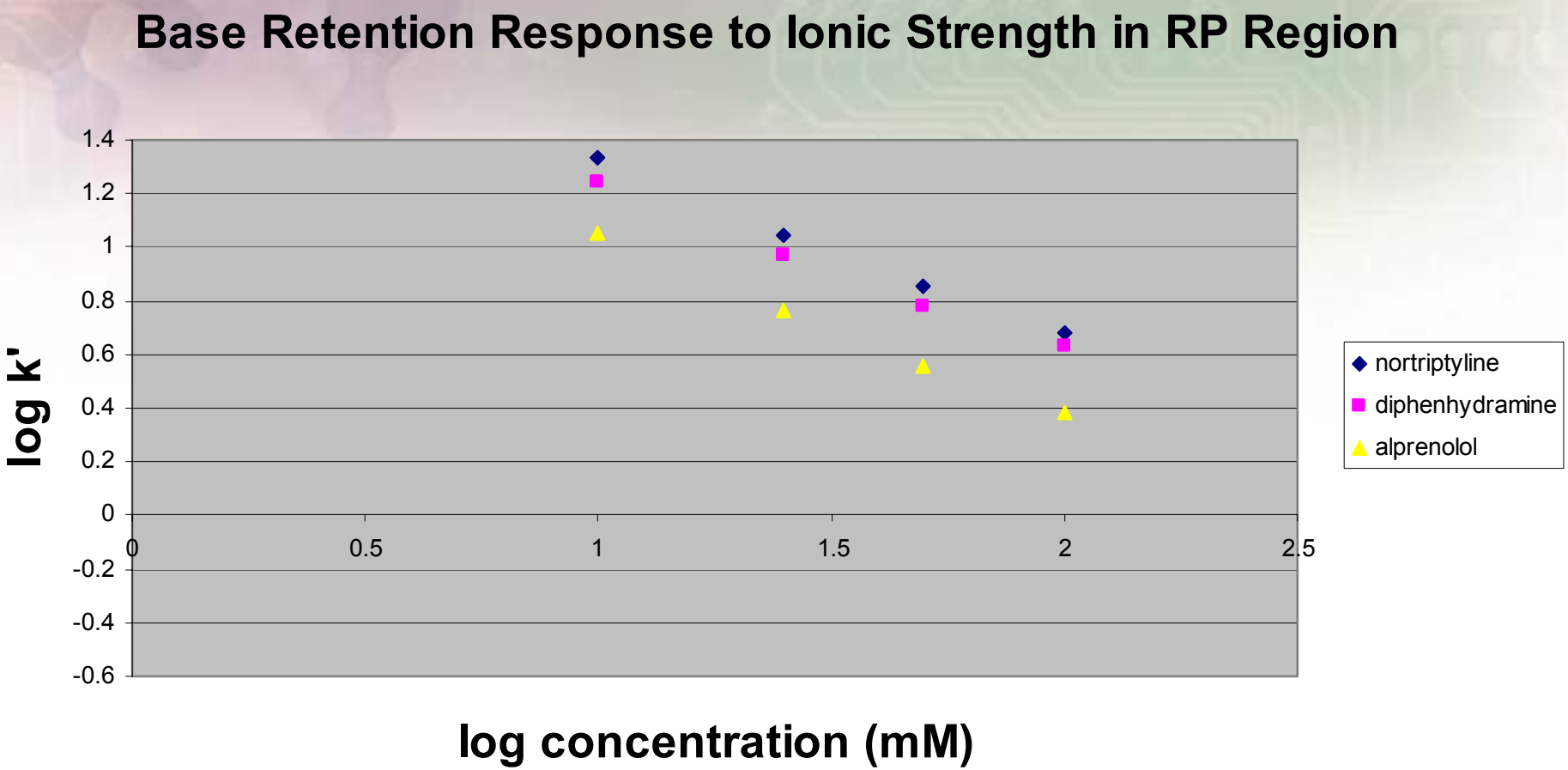
Interactions: Dispersive + Polar + Ionic

Basic Analytes



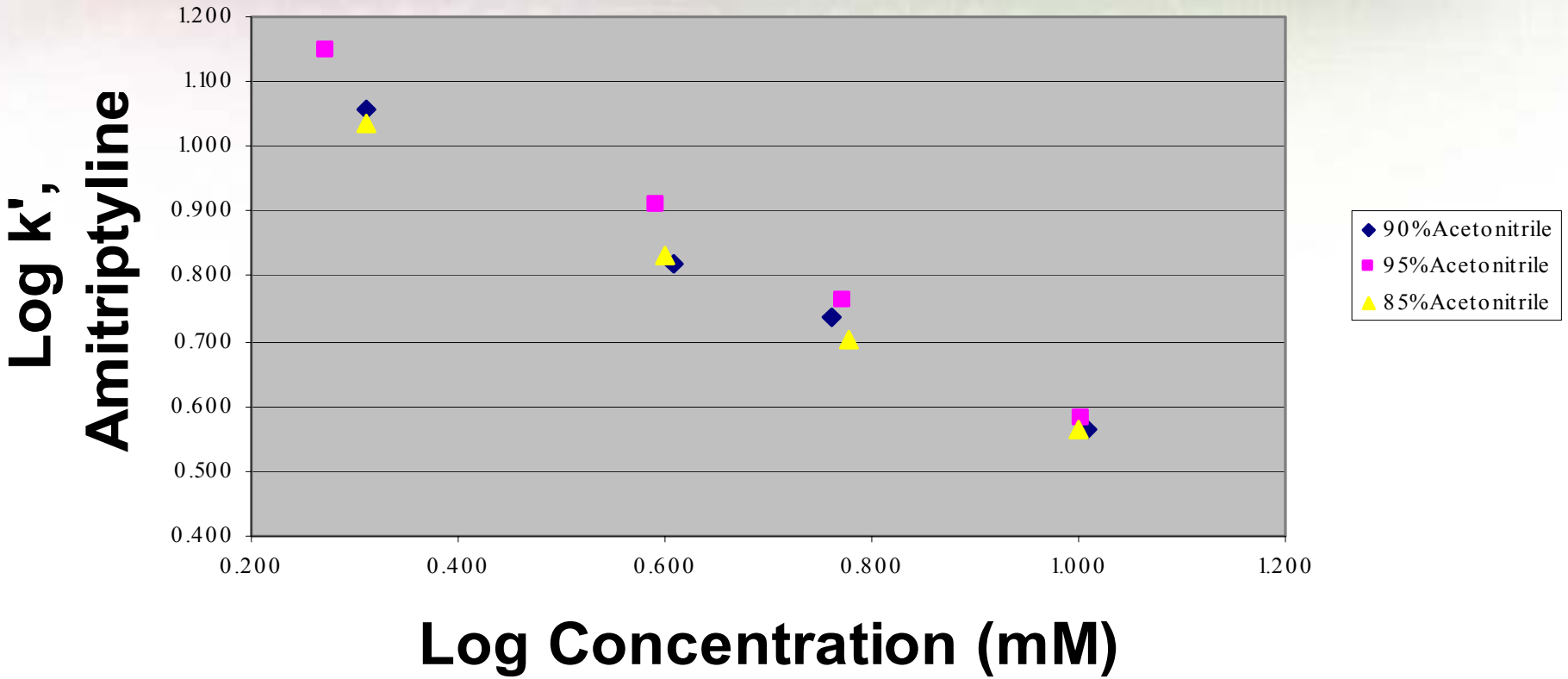
Neutral and Acidic Analytes

Interactions: Dispersive + Polar + Ionic



Interactions: Dispersive + Polar + Ionic

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



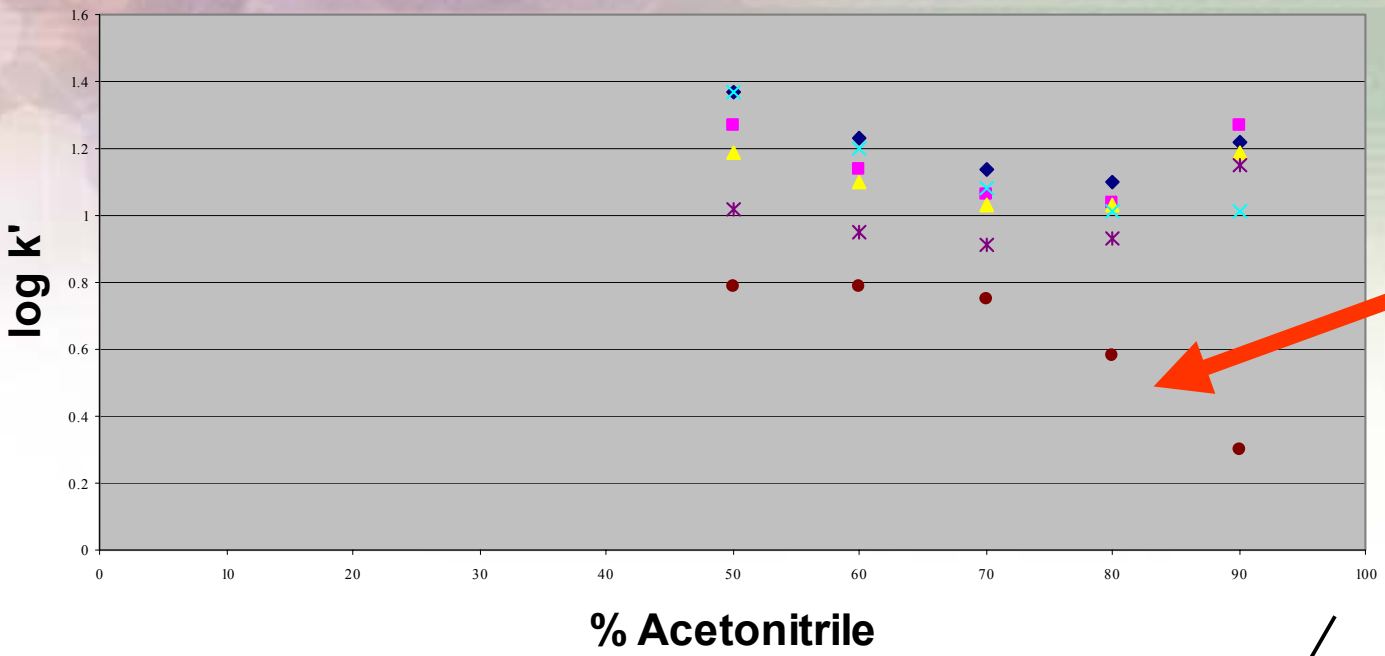
Interactions, Summary

- 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"

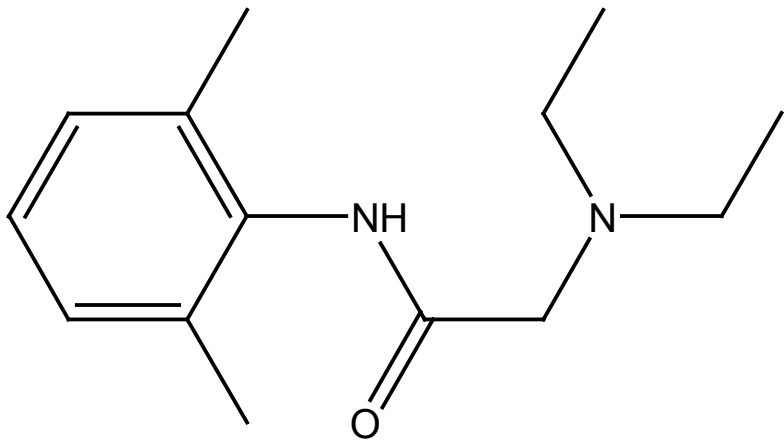
Method Development: Retention Profile of Some Bases



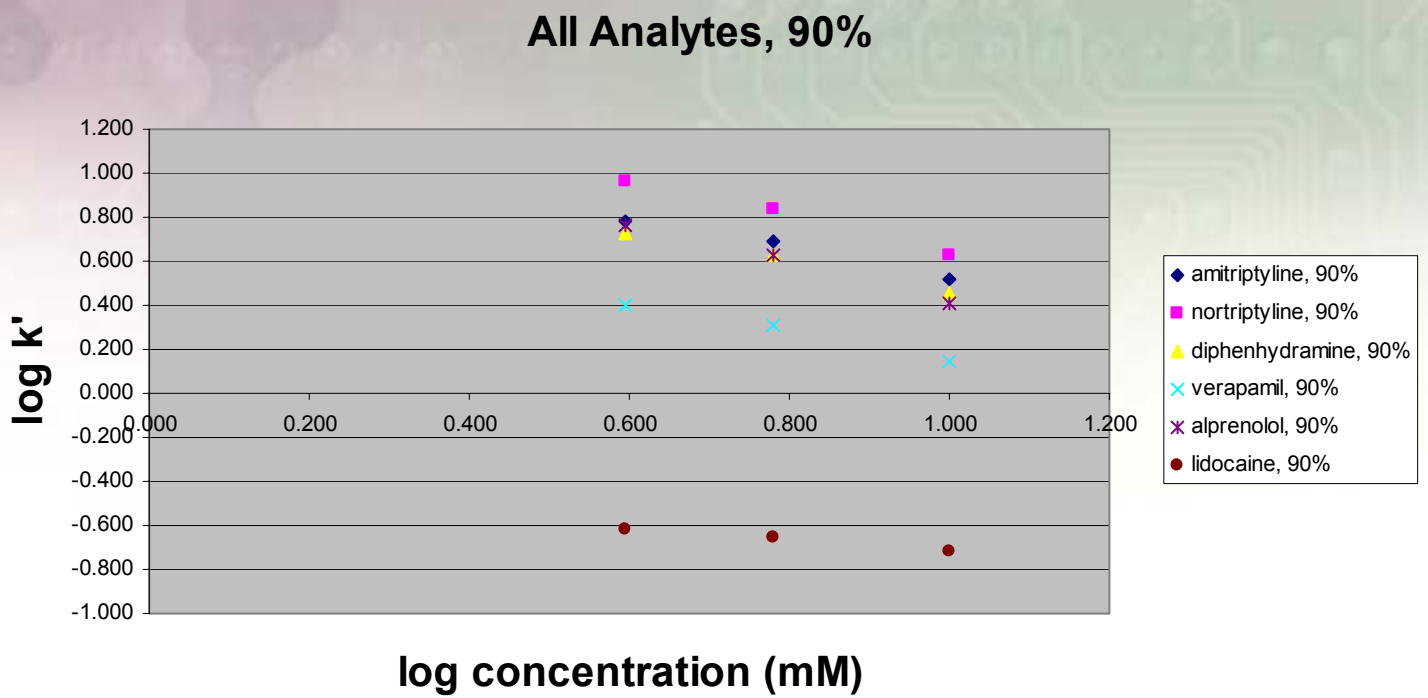
Lidocaine

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