# *Unique Retention and Selectivity of Pentafluorophenylpropyl Phases for High-Throughput LC/MS Analysis*

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# Overview

- The LC/MS retention and sensitivity for several classes of pharmaceutical analytes were studied on a pentafluorophenylpropyl stationary phase and compared to the chromatography obtained on a traditional C18
- Unique retention and selectivity are demonstrated on the pentafluorophenylpropyl phase. The results show a “U-Shape” retention profile as a function of percent organic modifier. This profile is a result of both reversed-phase and normal phase retention character
- The extent of normal phase character appears to be correlated with the pKa values of the analytes

# Overview, Cont.

- The ability to retain at high organic percentages:
  - Provides unique selectivity compared to traditional reversed-phase systems
  - Increases absolute response due to the more efficient desolvation in the ESI source
  - Reduces sample pretreatment requirements by allowing the direct injection of highly organic samples without peak distortion
  - Allows for higher flow rates due to the lower mobile phase viscosity
- Each of these attributes can be utilized to generate and/or improve high-throughput LC/MS methods

# Introduction

- Pentafluorophenylpropyl-modified silica has previously been shown to provide retention of tricyclic antidepressants and calcium channel blockers using mobile phases with high percentages of organic modifier[1]
- This study is intended to extend the work to several classes of pharmaceutical compounds and to discuss the advantages of retention using mobile phases with high organic content to high-throughput analysis


- Needham, S.R., et al., *Optimized stationary phases for the high-performance liquid chromatography-electrospray ionization mass spectrometric analysis of basic pharmaceuticals.* Journal of Chromatography A, 2000. **869**(1-2): p. 159-170.

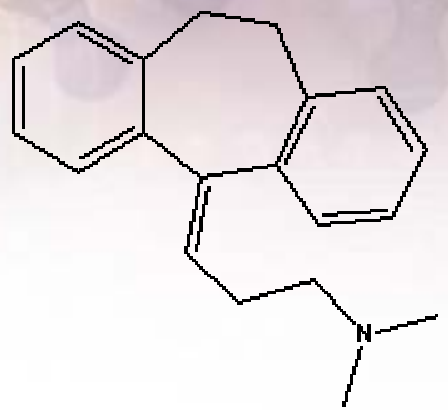
# Methods

- The retention of the following compounds were monitored with varying percentages of organic modifier:
  - Amitriptyline (tricyclic antidepressant)
  - Cimetidine (anti-ulcerative)
  - Clondine (antihypertensive)
  - Fluoxetine (antidepressant)
  - Nifedipine (anti-anginal)
  - Trimethoprim (antibacterial)
  - Verapamil (anti-anginal)

# Methods, Cont

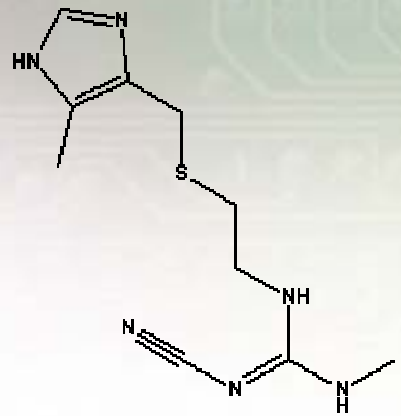
## Amitriptyline

Mass 277.2, pKa = 9.42



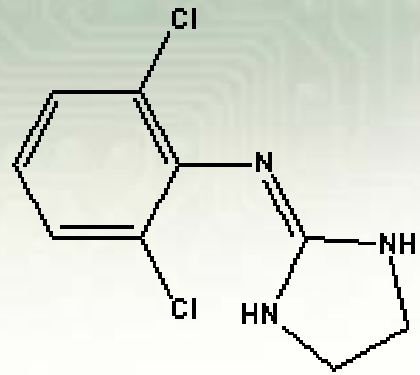

## Cimetidine

Mass 252.1, pKa = 6.8



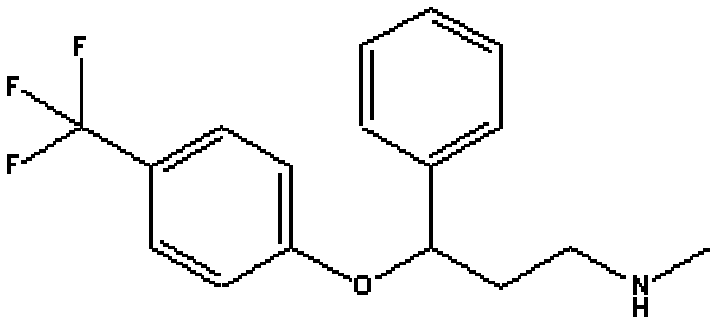

## Clonidine

Mass 229.0, pKa = 8.05




## Fluoxetine

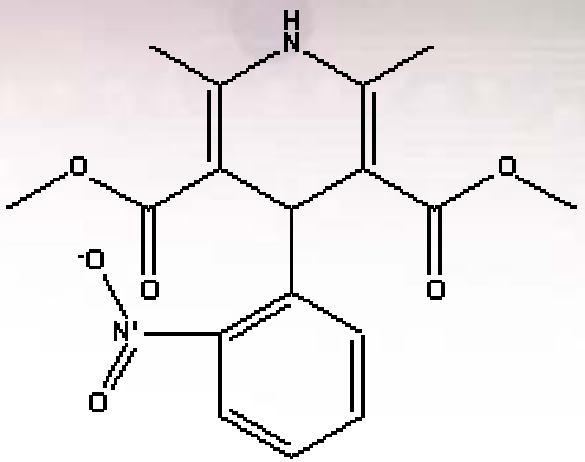
Mass 309.1, pKa unknown



# Methods, Cont

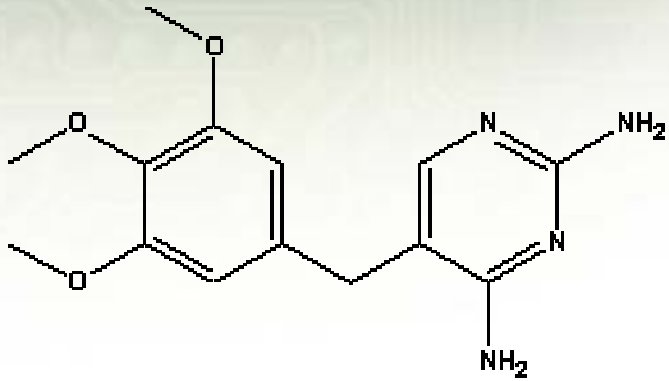
## Nifedepine

Mass 346.1, pKa unknown



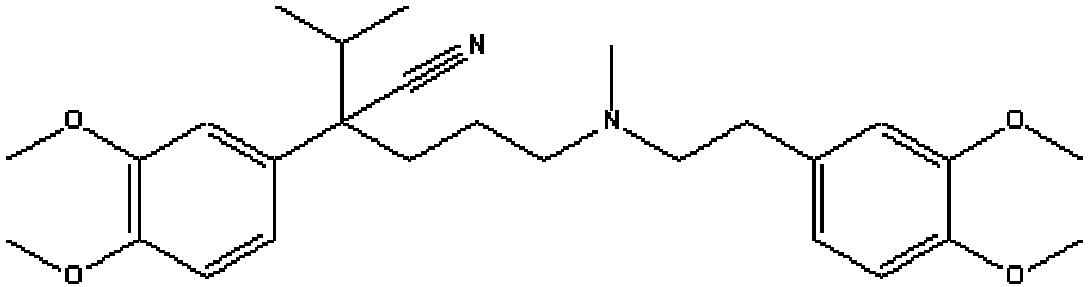
## Trimethoprim

Mass 290.1, pKa = 7.12



## Verapamil

Mass 454.3, pKa 8.92



# Methods, Cont.

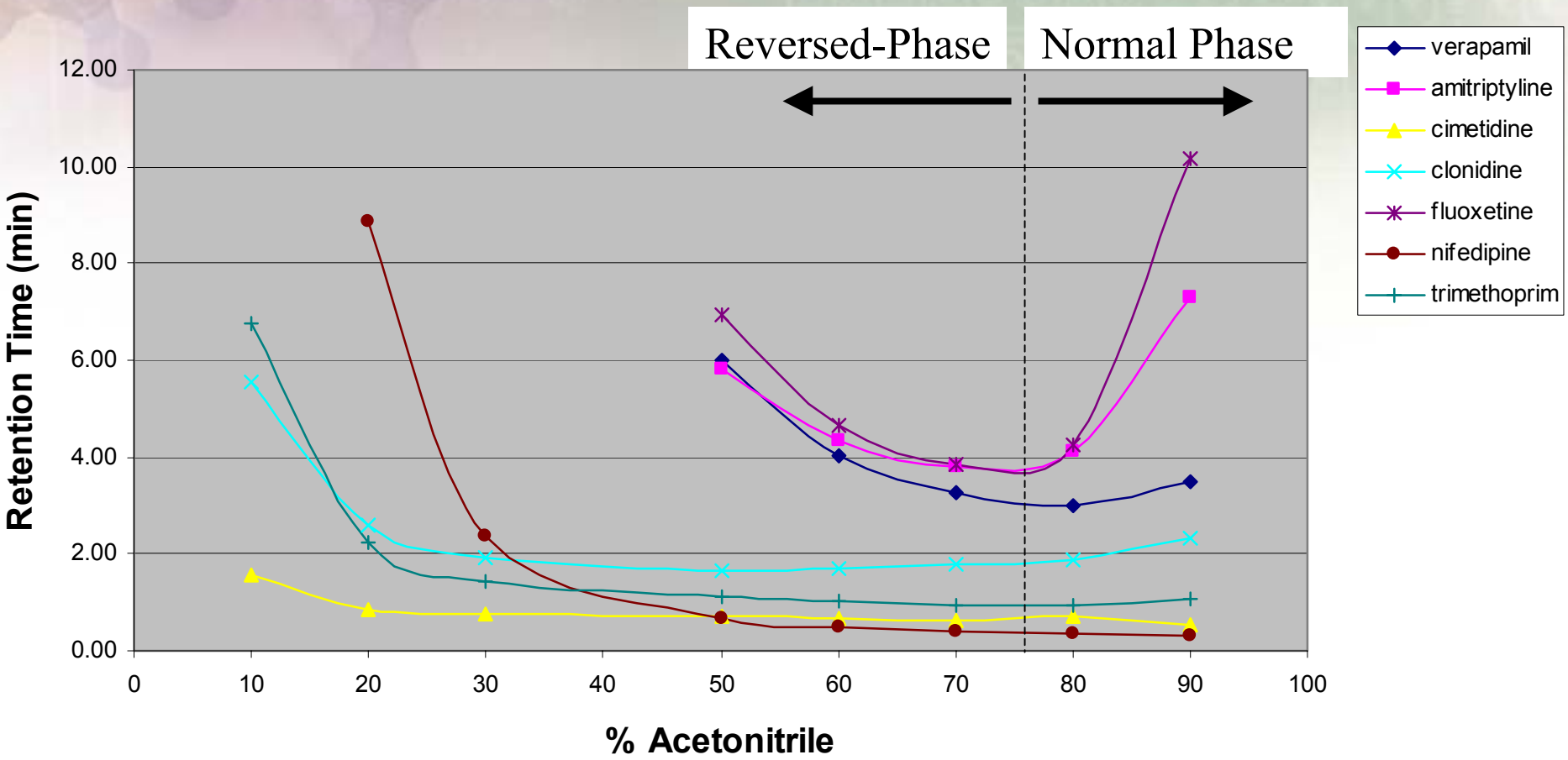
- LC/MS Conditions:
  - System: Waters Alliance HT equipped with a Micromass ZQ single-quadrupole mass analyzer via ESI interface operating in positive ion mode
  - LC Parameters:
    - Mobile Phase: 5mM Ammonium Acetate, pH 4 w/ acetic acid: Acetonitrile (varying percentages)
    - Flow Rate: 0.5mL/min, split to ~0.2mL/min
    - Temperature: 60ºC
    - Injection volume: 10uL
    - Column 1: Discovery HS F5 (pentafluorophenylpropyl), 50 x 2.1mm, 5um
    - Column 2: Discovery C18, 50 x 2.1mm, 5um

# Methods, Cont.

- MS Parameters
  - Capillary:  3.5kV
  - Cone:  25V
  - Extractor:  5V
  - Source Temperature:  140ºC
  - Desolvation Temperature:  350ºC
  - Scan Range:  m/z 100-m/z 500, w/0.5 sec. scan time

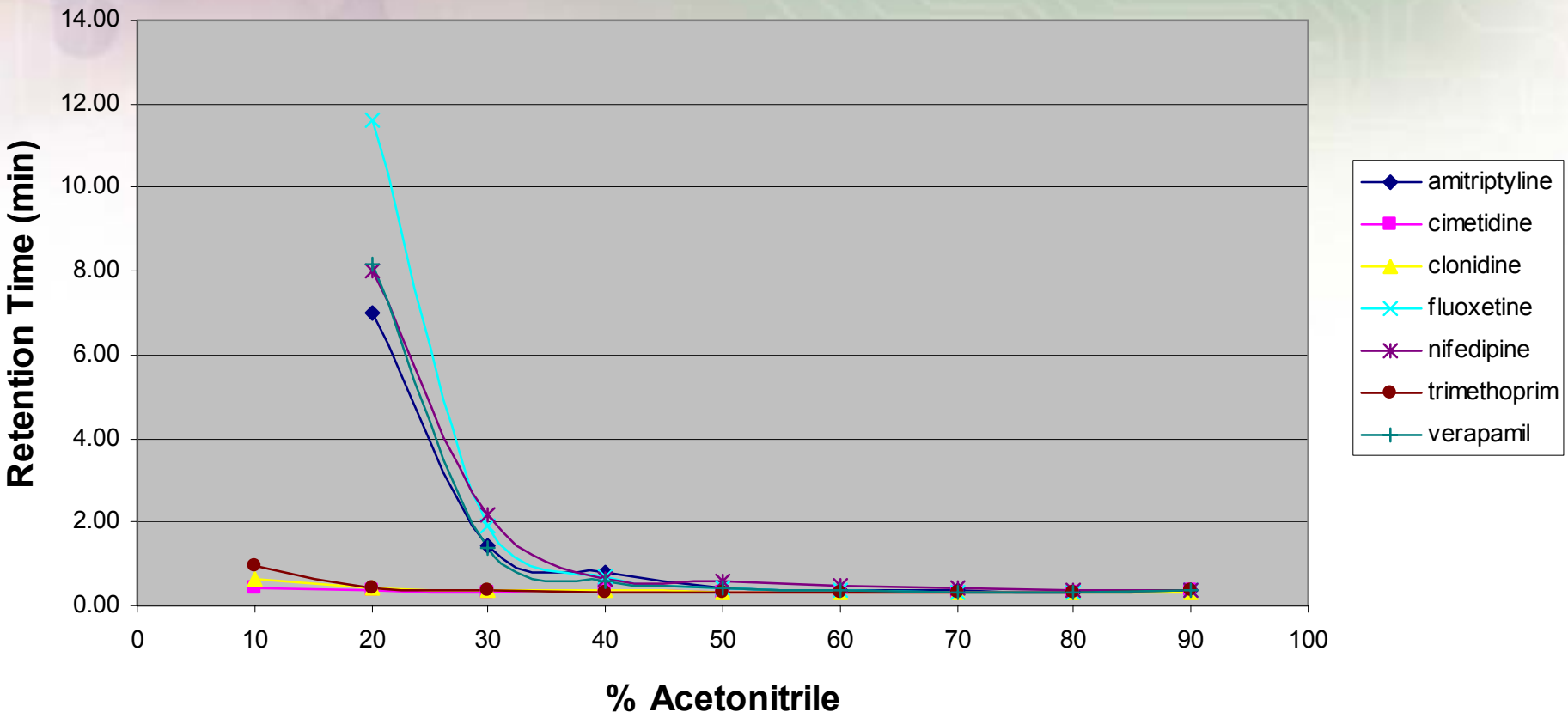
# Results

## % Acetonitrile vs. Retention Time, (min) on Discovery HS F5



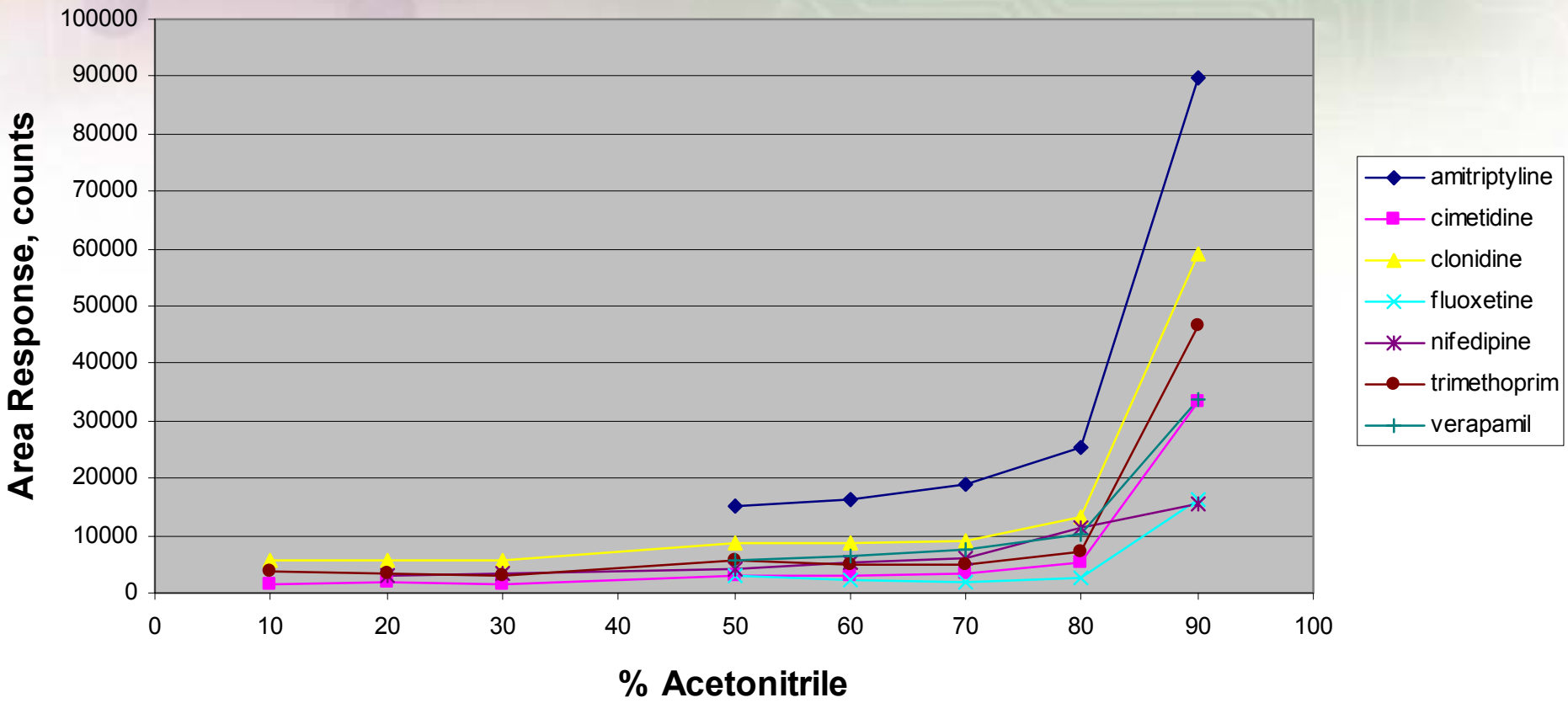
# Results, Cont

## % Acetonitrile vs. Retention Time (min) on Discovery C18



# Results, Cont

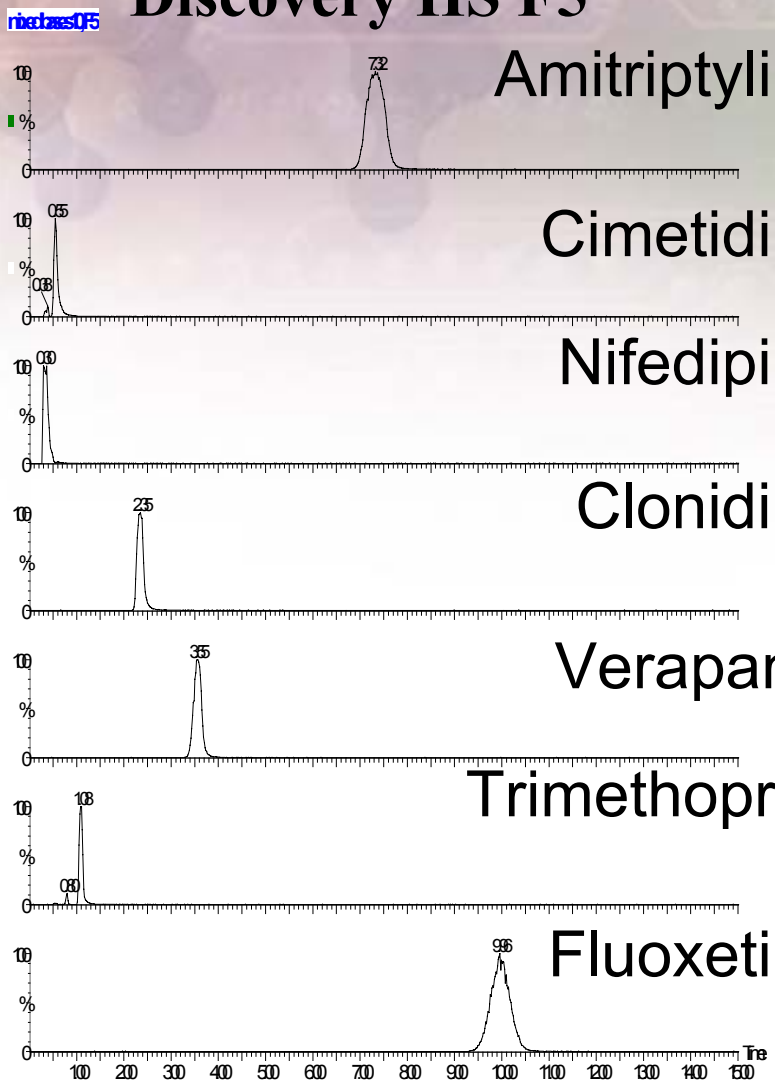
## % Acetonitrile vs. Area Response on Discovery HS F5



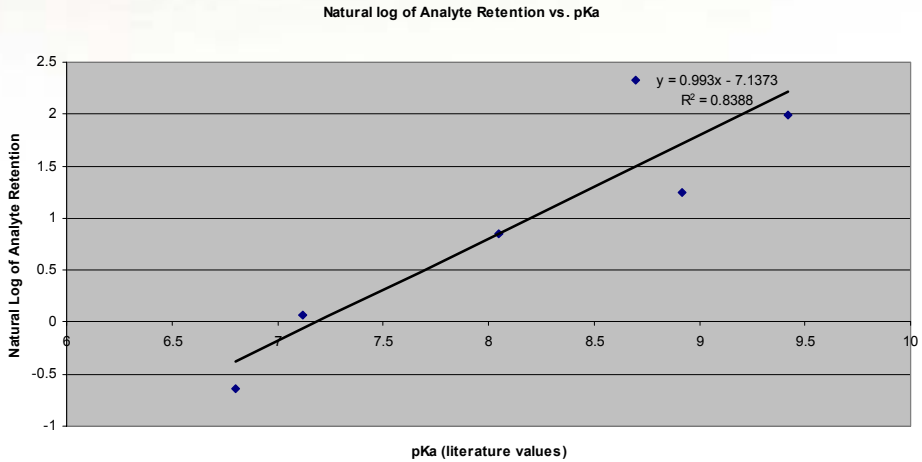
# Results, Cont

Retention at 90% Acetonitrile

**Discovery HS F5**




Relationship between analyte pKa and normal phase retention



# Conclusions

- Pentafluorophenylpropyl phase is shown to exhibit unique retention and selectivity as compared to traditional C18 separations with excellent peak shape for several classes of pharmaceutical analytes
- A plot of retention as a function of percent organic modifier for several of the analytes results in a “U-shape” profile indicating both reversed-phase and normal phase regions of retention
- The normal phase character of the phase results in the ability to retain many analytes at high percentages of organic modifier

# Conclusions, Cont.

- The degree of retention at high percent organic seems to correlate with the pKa of the tested analytes
  - Nifedipine and Cimetidine showed typical reversed-phase behavior
  - The more basic analytes Clonidine, Trimethoprim and Verapamil exhibited a slight increase in retention around 80% Acetonitrile
  - The most basic compounds, Amitriptyline and Fluoxetine showed the greatest increase in retention at high organic
- To better understand the retention mechanisms, parameters such as pH and organic modifier will be studied in terms of their effects on the retention profile

# Conclusions, Cont.

- The ability to retain at high percent organic:
  - Provides unique selectivity compared to C18 and often produces retention not possible on C18 without 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 protein-precipitated samples and/or 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