

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 pK_a 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.

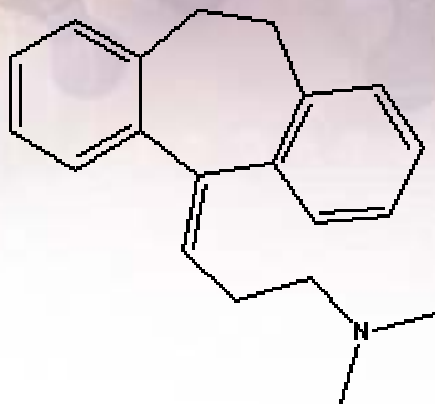
Methods

- 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

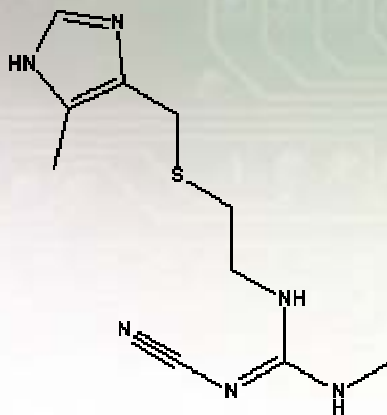
Amitriptyline

Mass 277.2, pKa = 9.42



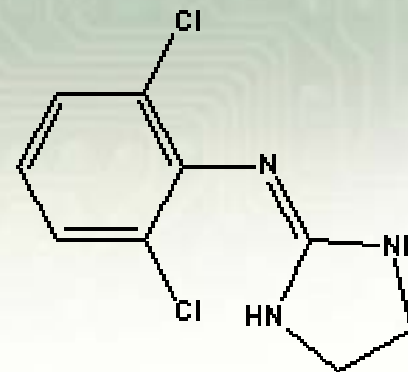
Cimetidine

Mass 252.1, pKa = 6.8



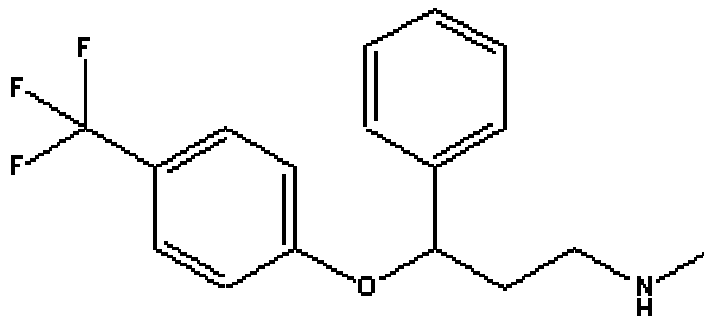
Clonidine

Mass 229.0, pKa = 8.05



Fluoxetine

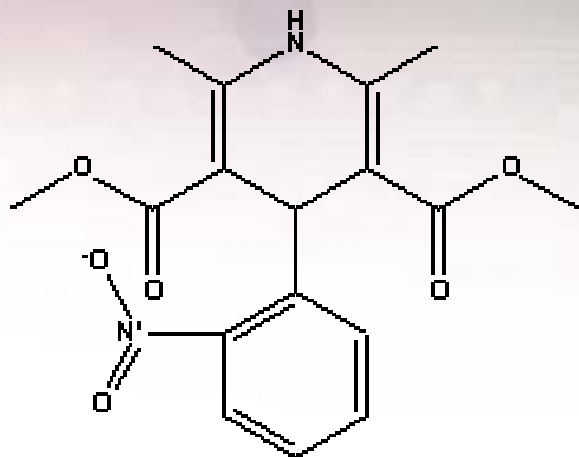
Mass 309.1, pKa unknown



Methods, Cont

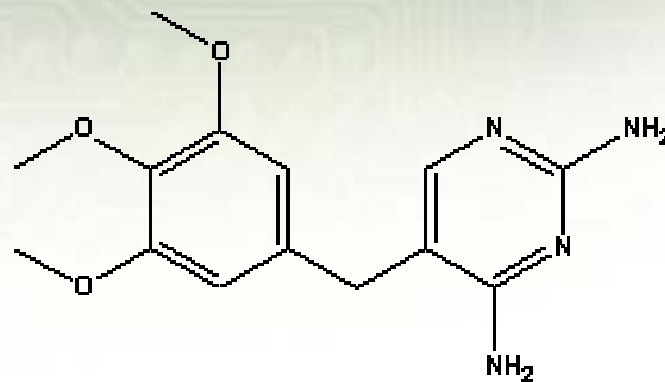
Nifedepine

Mass 346.1, pKa unknown



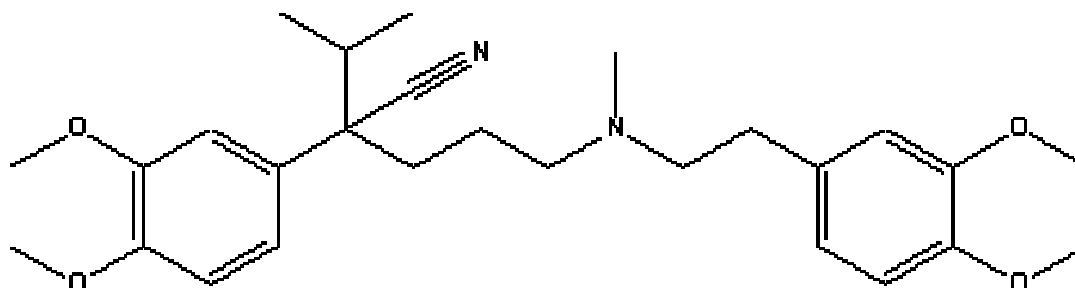
Trimethoprim

Mass 290.1, pKa = 7.12



Verapamil

Mass 454.3, pKa 8.92



Methods, Cont.

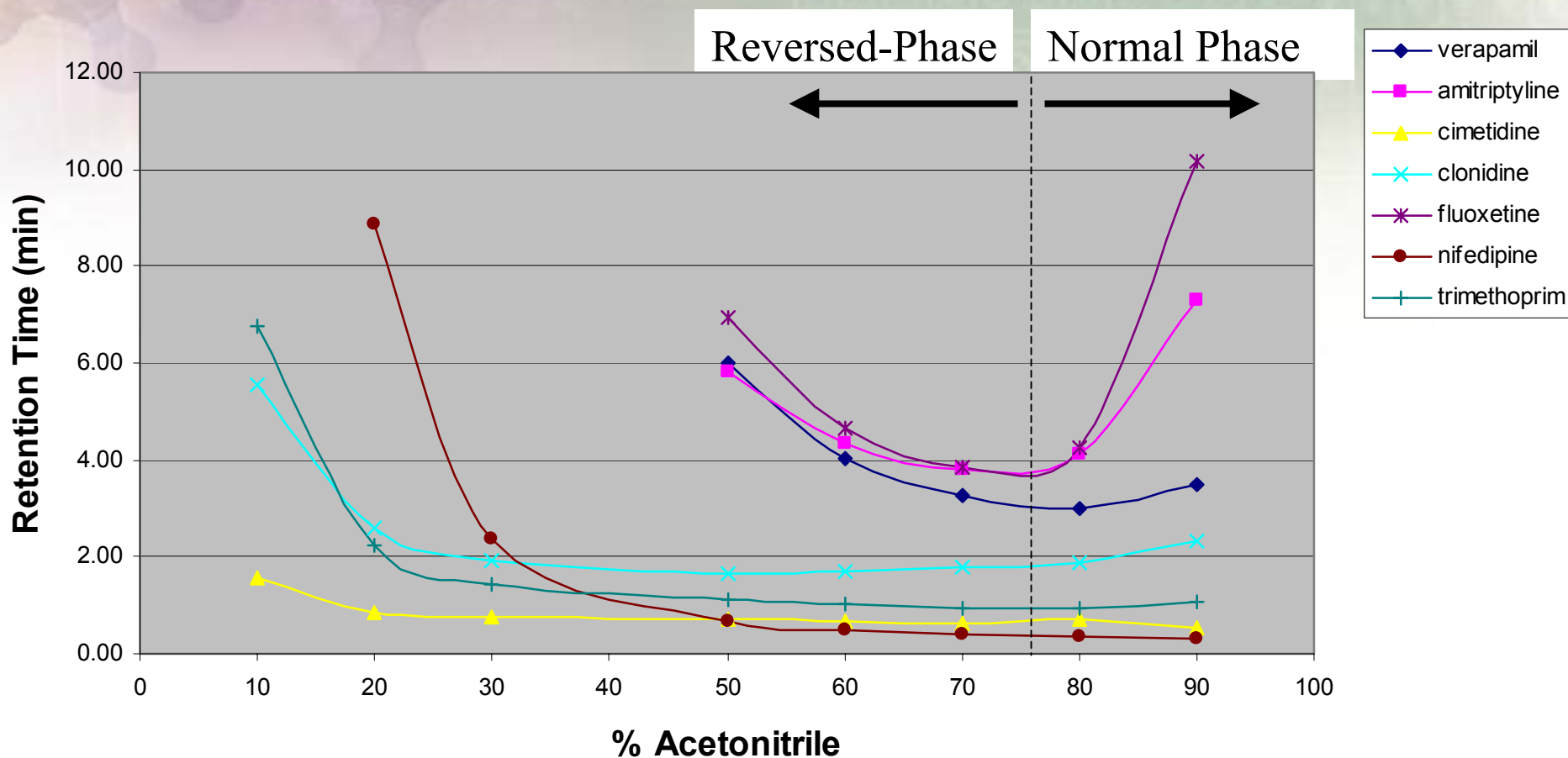
- LC/MS Conditions:
 - System: Waters Alliance HT equipped with a Micromass ZQ single-quadrupole mass analyzer via ESI interface operating in positive ion mode
 - LC Parameters:
 - Mobile Phase: 5mM Ammonium Acetate, pH 4 w/ acetic acid: Acetonitrile (varying percentages)
 - Flow Rate: 0.5mL/min, split to ~0.2mL/min
 - Temperature: 60°C
 - 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: 140°C
 - Desolvation Temperature: 350°C
 - Scan Range: m/z 100-m/z 500,
w/0.5 sec. scan time

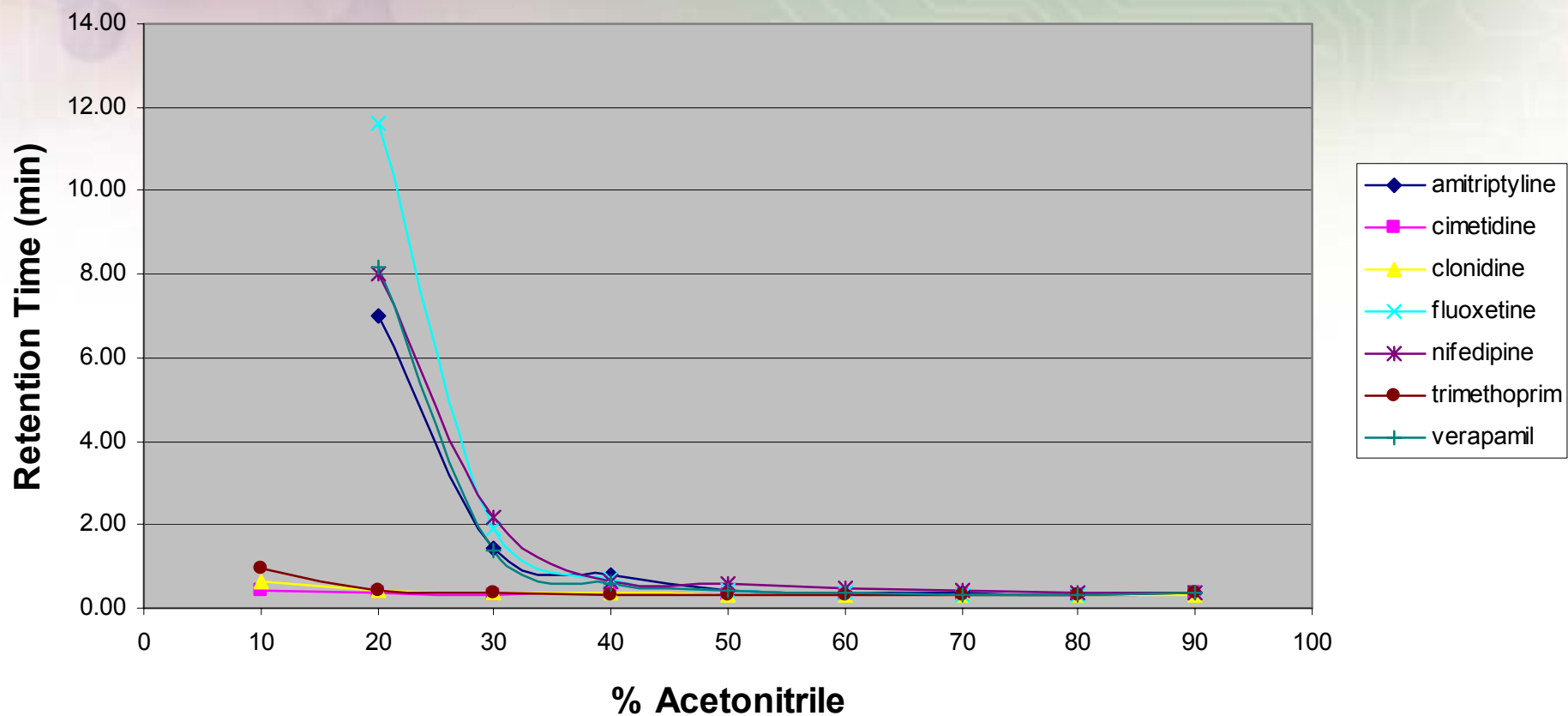
Results

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



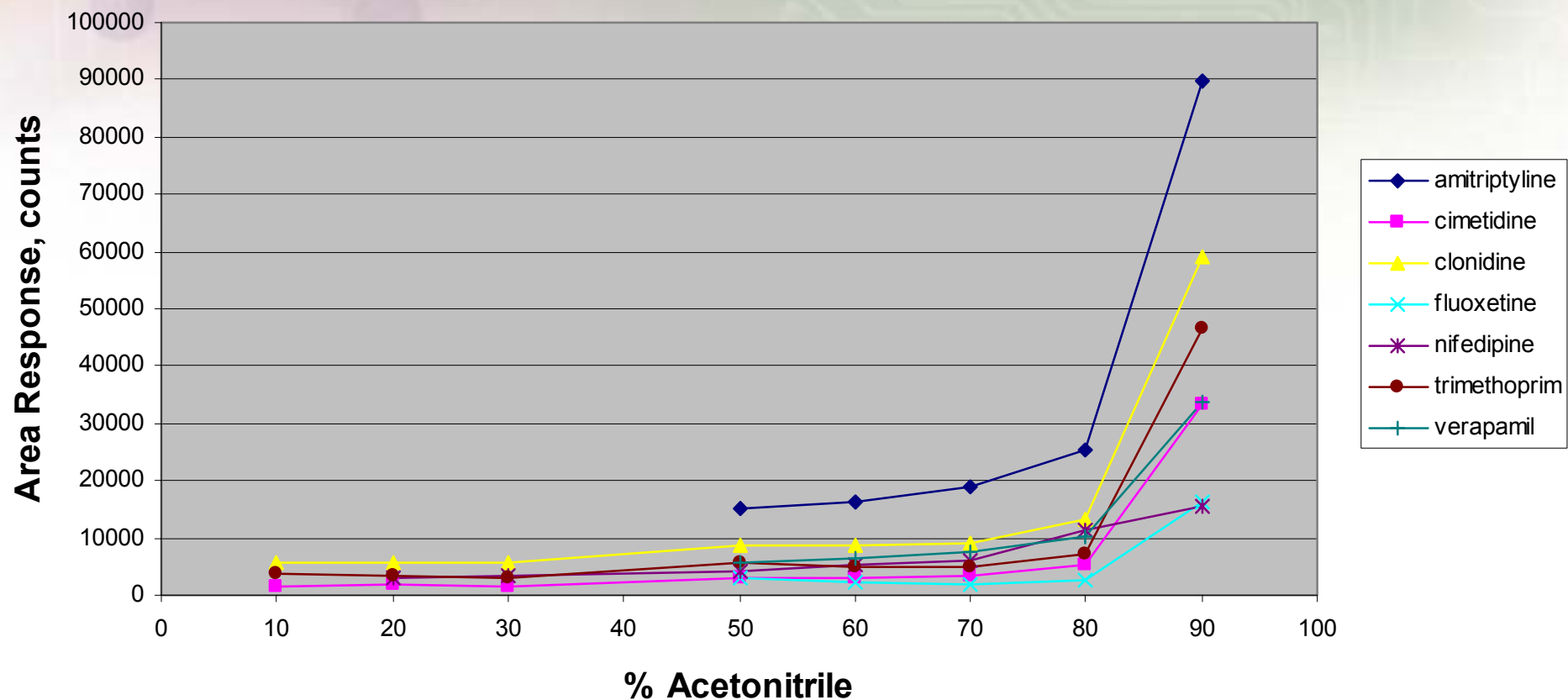
Results, Cont

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



Results, Cont

% Acetonitrile vs. Area Response on Discovery HS F5

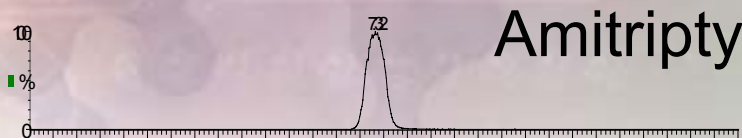


Results, Cont

Retention at 90% Acetonitrile

Discovery HS F5

minutes



Amitriptyline



Cimetidine



Nifedipine



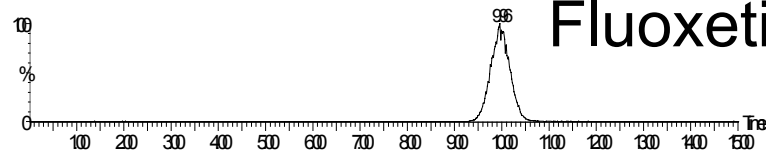
Clonidine



Verapamil

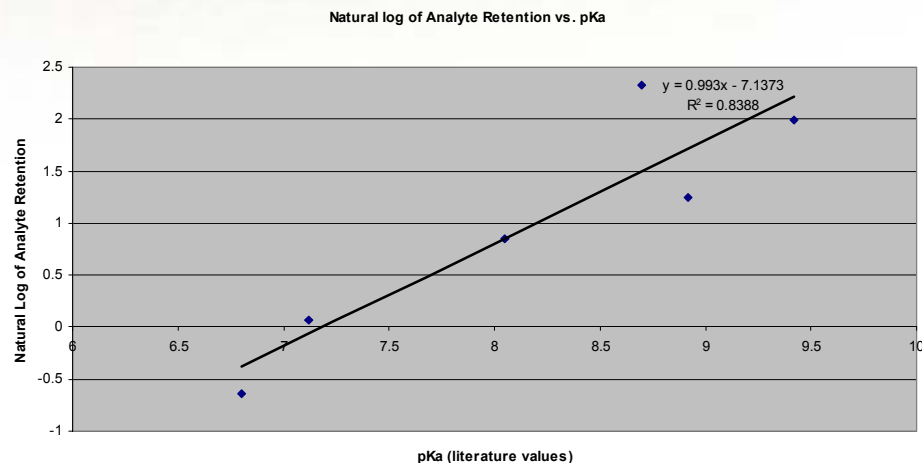


Trimethoprim



Fluoxetine

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