Investigation of retention mechanisms in HILIC chromatography: Important considerations for robust method development

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Abstract

Investigation of Retention Mechanisms in HILIC Chromatography: Important Considerations for Robust Method Development

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Hydrophilic interaction liquid chromatography (HILIC), especially in conjunction with mass spectrometry (MS), has become a powerful tool for the analysis of a wide variety of challenging analytes. Applications of the technique have increased dramatically over the past decade, especially for the analysis of polar analytes where reversed-phase chromatography suffers. HILIC conditions employ a high percentage of acetonitrile which enables facilitated solvent evaporation in LC/MS sources and thus often an increase in analyte response when compared to more aqueous based systems. The increased retention of polar analytes afforded by HILIC provides improved selectivity and decreases the impact of endogenous species, often leading to improved qualitative and quantitative analyses [1].

Although HILIC has proven useful, it has also been thwarted with complications including difficulties in method development and method robustness.

In this presentation, studies investigating the underlying retention mechanisms dominant in HILIC chromatography are presented and discussed. Along with reversed-partitioning HILIC is well known to exhibit, ion-exchange and the interplay of the dominant mechanisms are unveiled and used to develop a model of overall retention and selectivity. Interactions that operate using different stationary phase chemistries and conditions are presented. The impact of analyte polarity and charge as well as the variations caused by high percentages of organic on these physiochemical parameters are highlighted. Throughout the discussion, examples of use and misuse of HILIC are employed to illustrate these important concepts to build a solid fundamental foundation for efficient and effective use of this powerful technique.

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Agenda

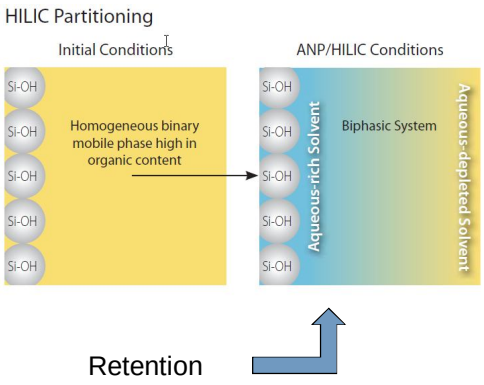
Introduction

Factors affecting the HILIC Separation

Modelling Ionic interactions on polar stationary phases in HILIC

Introduction

HILIC Hydrophilic Interaction LIquid Chromatography



5-40 % Water

Factors effecting the HILIC systems

Column

Mobile Phase

- pH
- Buffer Concentration

Analytes

- pKa
- Log P_{OW} or Log D_{OW}

(Temperature)

Sample

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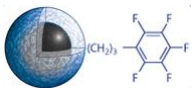
Columns

Ascentis® Express
Extreme Performance on Any LC System

Ascentis Express Fused Core Particle Columns

HILIC (Si)

OH5 (Pentalol) Branched hydroxylated alkane New!!

F5 (PFP) 

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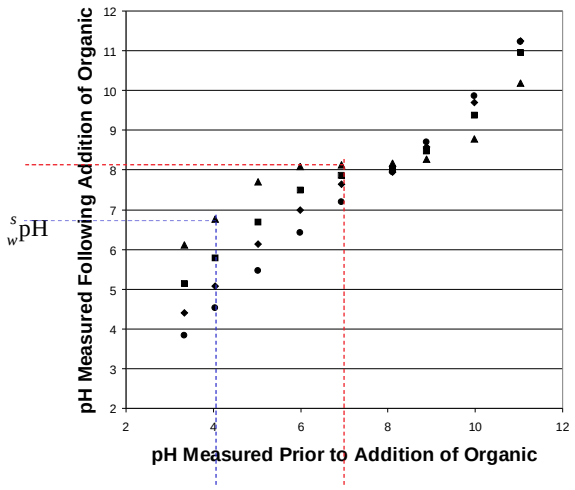
pH Effect of Acetonitrile on pH of Ammonium Acetate [4]



Measurements were taken at 25ºC.

Triangle: 90.0% ACN,
Square: 75% ACN,
Diamond: 50% ACN,
Circle: 32.5% ACN

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pKa

Analyte pK_a values have also been shown to be impacted by the presence of organic modifiers

Figure 2 shows the results of an NMR experiment conducted that explored the chemical shift of a proton near the ionizable group for amitriptyline in 90% acetonitrile. From data such as this, effective pK_a values can be established for a variety of compounds.

Table 1 shows the results for several basic pharmaceutical compounds. The data indicates that the effective pK_a value for a basic analyte in 90% acetonitrile is approximately 1 pK_a unit less than the aqueous-based value

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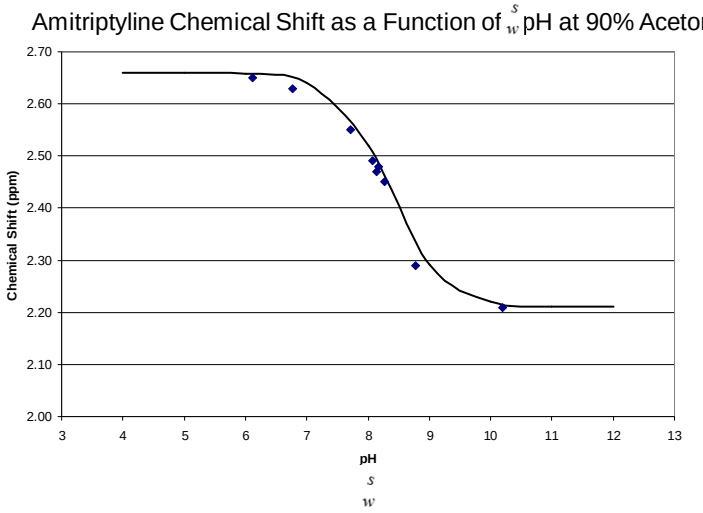
Determination of pK_a Values using ^1H NMR [4]



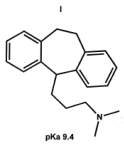


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Table 1: Determination of pK_a Values using NMR [4]

Amitriptyline

% Acetonitrile	pK_a	Correlation (R^2)
25	9.32	0.9997
50	9.02	0.9996
75	8.88	0.9956
90	8.34	0.9923

- pK_a values for bases decrease with increasing acetonitrile
- At 90% each analyte exhibited a pK_a value about 1 full pH unit less than the literature pK_a value

Analyte	Literature pK_a	$^{s}_{w}pK_a$	Correlation (R^2)
Amitriptyline	9.4	8.34	0.9923
Nortriptyline	9.7	8.92	0.9920
Diphenhydramine	9.0	8.33	0.9978
Verapamil	8.9	7.98	0.9976
Alprenolol	9.7	8.73	0.9855

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Investigation of Retention Mechanisms on Different HILIC Phases

interaction differences for three different HILIC stationary phases: PFPP (F5), bare silica (HILIC) and a new pentalol phase (OH5)

Using ephedrine as a probe molecule, retention as a function of buffer concentration was collected and interpreted.

Related compounds and dominant interactions prevalent using each phase is studied

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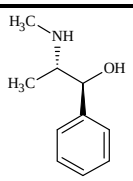
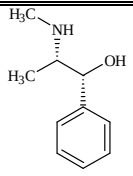
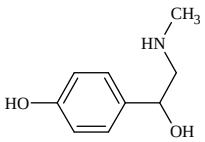
Selected Probes

ACD/Labs, PhysChemProp, v. 12

Structure	pKa(MB)	LogD(8.0)	LogP	MW	name
	9.38	-0.37	1.08	165.23	pseudoephedrine
	9.38	-0.37	1.08	165.23	ephedrine
	9.37	-1.35	0.13	167.21	synephrine

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Experimental

Conditions:

Instrument: #9, Waters 2690/Micromass ZQ single quadrupole interfaced via ESI operating in pos. ion mode

Columns:

- Ascentis Express Pentalol (OH5), 10 cm x 3.0 mm
- Ascentis Express HILIC, 10 cm c 3.0 mm,
- Ascentis Express F5, 10 cm x 3.0 mm,

Mobile Phase A: **10 mM ammonium acetate** (pH unadjusted) in 10:90 water:acetonitrile

Mobile Phase B: 10:90 water:acetonitrile

Mixtures of A and B were run at 0%B, 20%B, 40%B, 60%B and 80%B corresponding to 10 mM, 8 mM, 2 mM, 4 mM and 2 mM buffer concentrations, respectively

Flow rate: 0.4 mL/min

Temperature: ambient

Detection: MS, ESI, pos ion mode, scan m/z 125 – 300

Injection volume: 2 uL

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Calculation for ion exchange impact in HILIC

Samples were injected in triplicate at each buffer concentration using each of the phases. A sample of ephedrine only was also injected under each condition to discriminate from pseudoephedrine in the mix.

$\text{Log } k = -\log[C^+]_m + \log\beta_{IEX}$

$[C^+]_m$ concentration of the competing ion in the mobile phase and
β_{IEXC} constant for a given system

- phase ratio,
- ion-exchange capacity of the stationary phase ion-exchange equilibrium constant.

Log k Retention

A plot of log k vs log $[C^+]_m$ will thus yield a slope of -1 when ion-exchange is solely responsible for retention,

The plot would yield a slope of 0 where ion-exchange is not present.

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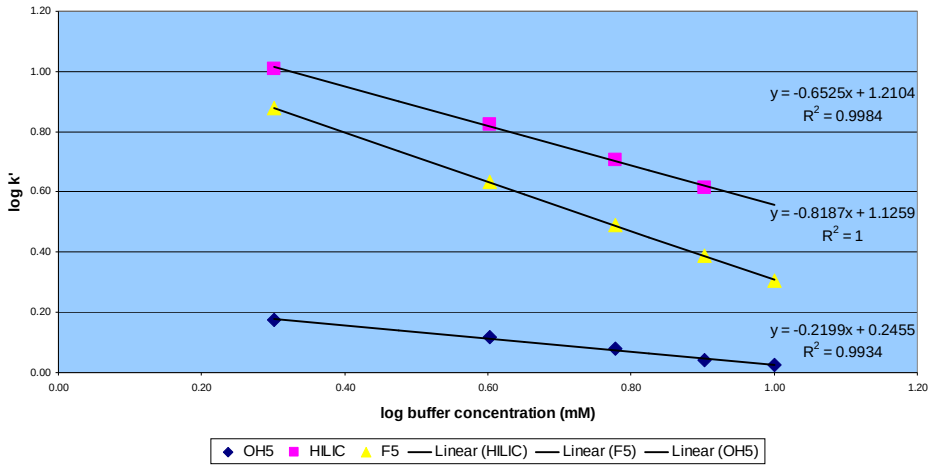
Response of Ephedrine Retention on Buffer Concentration on Three HILIC Phases



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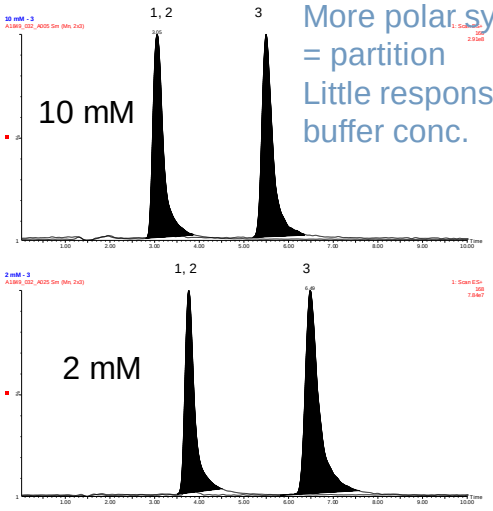
HILIC OH5 column at 10 and 2 mM ammonium acetate



More polar synephrine elutes last = partition

Little response of retention on buffer conc.

1. Ephedrine
2. Pseudoephedrine
3. Synephrine

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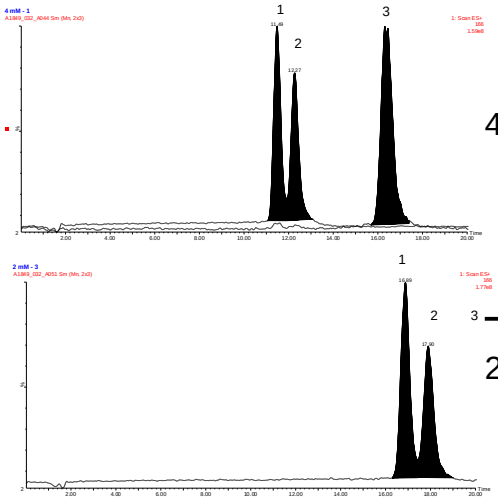
HILIC (bare silica) column at 4 and 2 mM ammonium acetate



Synephrine still last
More Rt change
Both partition and IEX

1. Ephedrine
2. Pseudoephedrine
3. Synephrine

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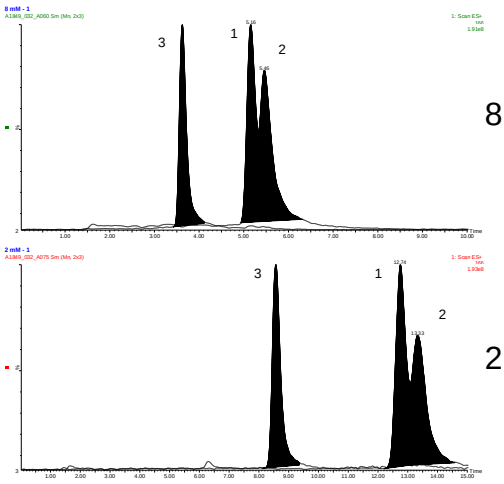
F5 column at 8 and 2 mM ammonium acetate



Synephrine early!
Rt change with Buffer
Little or no partition,
IEX present

1. Ephedrine
2. Pseudoephedrine
3. Synephrine

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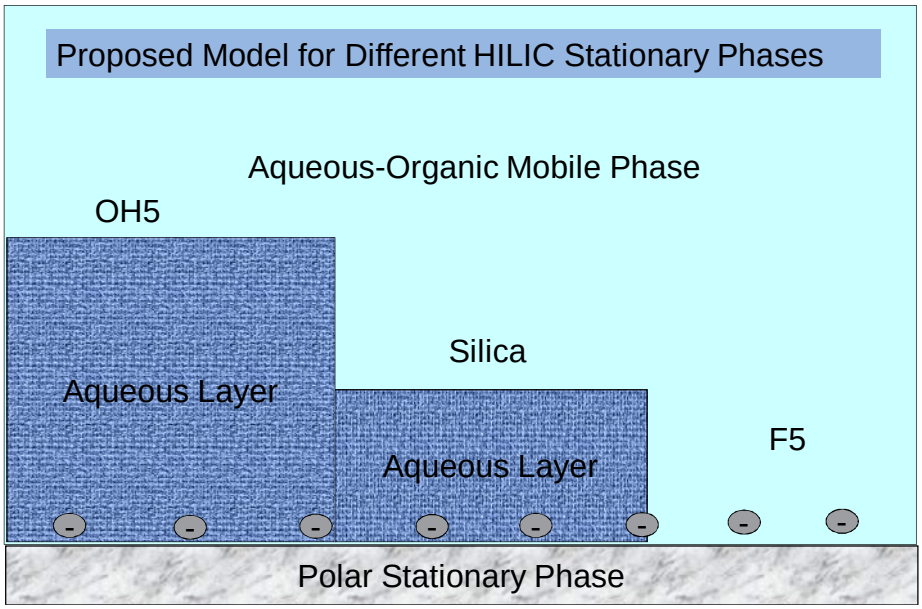

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Conclusions

Columns described in this presentation

- Pentalol (OH5)– dominated by partition
- Bare silica (HILIC)– both partition and IEX
- Pentafluorophenylpropyl – mainly IEX

In order to develop robust and reliable methods using HILIC chromatography thefollowing factors should be considered

- pH changes in high % organics
- pKa of analytes in high % organics
- Mobile phase modifiers concentrations

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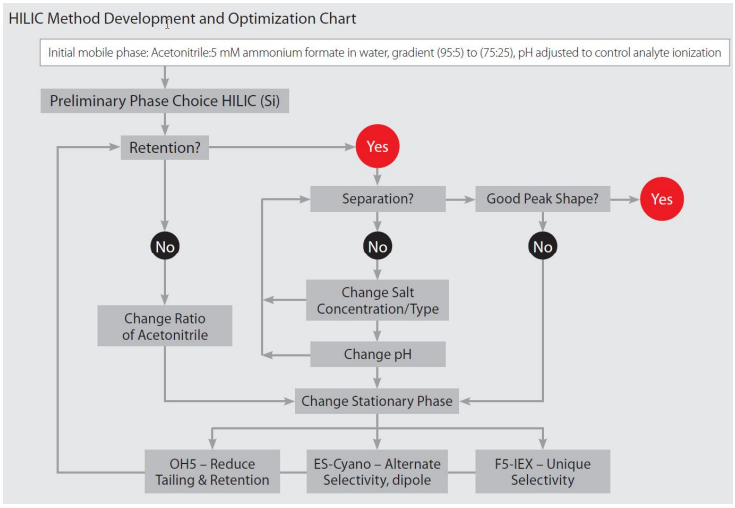
Method Development and Optimization Chart



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