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# Utility of U-Shaped Retention Profiles Under Common Reversed Phase HPLC Conditions

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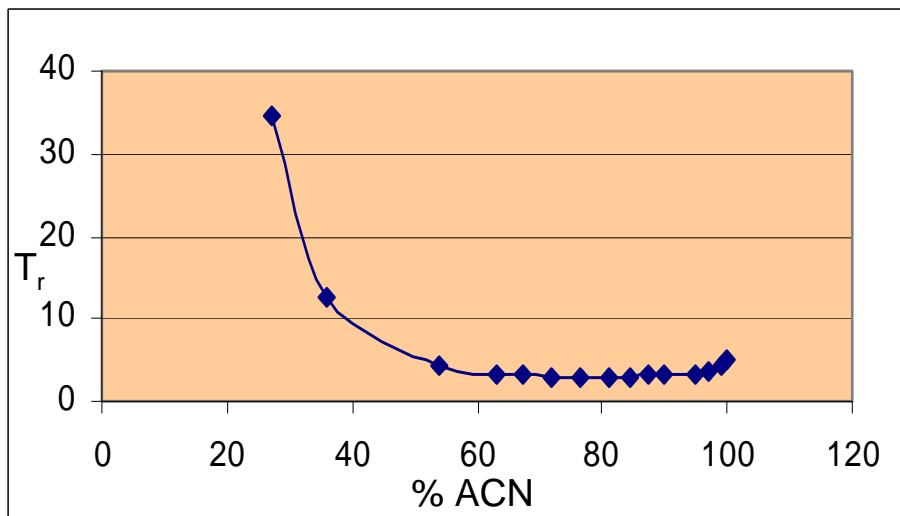


# Introduction

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- **RP & NP retention profiles**
- **HILIC phases & U-shaped retention**
- **RP packings - prediction of silanol activity level**
- **Phases showing U-shaped retention**
- **Advantages**

# Reversed-Phase Chromatography



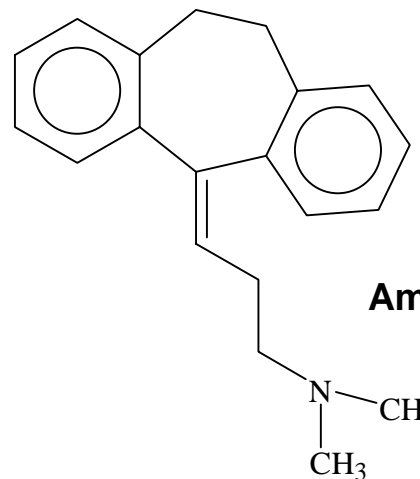
Discovery® C18

Flow = 1mL/min

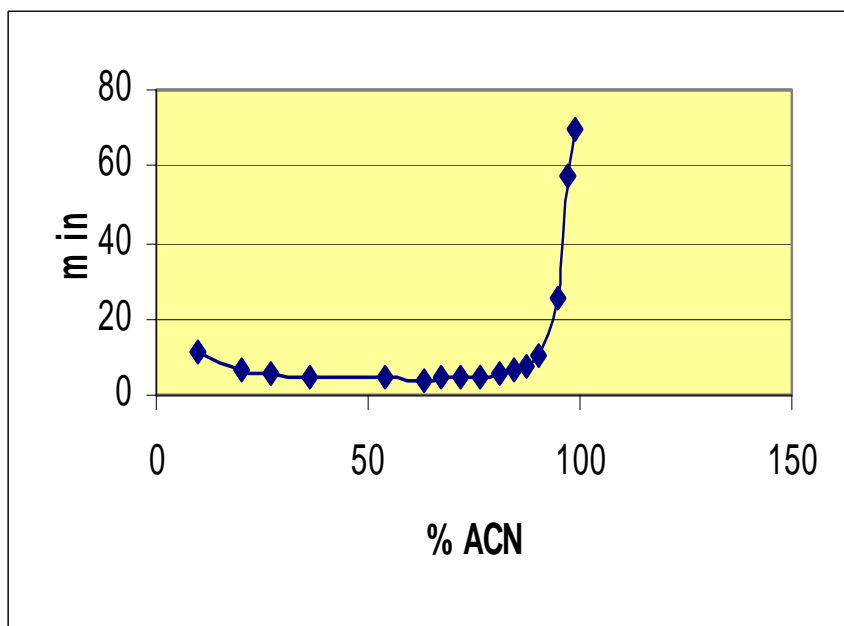
MP: A = ACN w/ 5mM Ammonium acetate

B = Water w/ 5mM Ammonium acetate (pH 6.8)

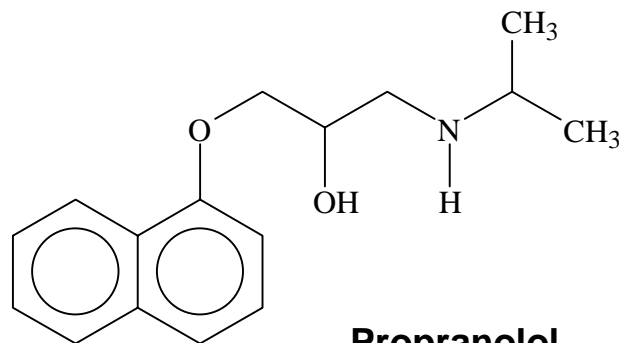
- Retention decreases with increasing organic
- Strong solvents: MeOH, ACN, etc.



# Normal Phase Chromatography



- Retention increases with increasing organic
- Strong solvent: Aqueous



Propranolol

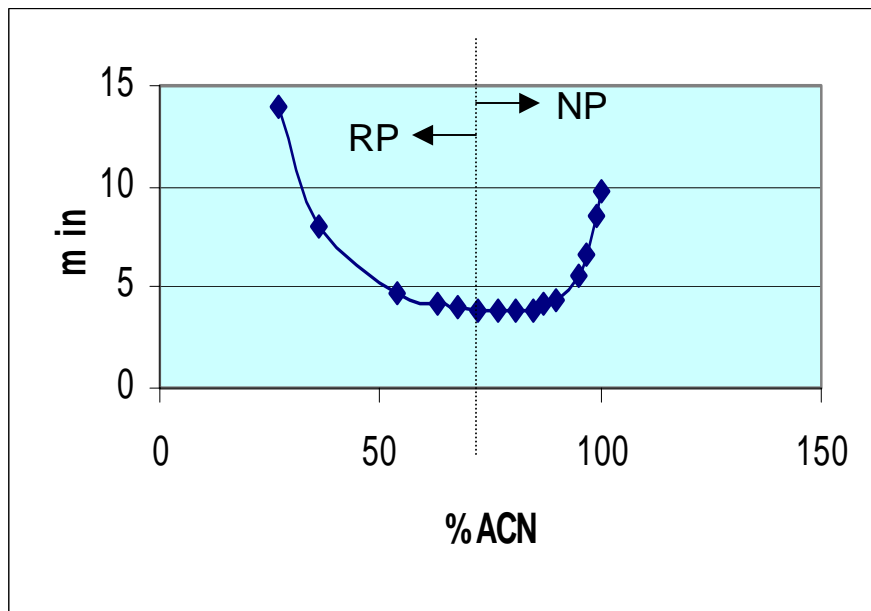
SUPELCOSIL® Si (Silica)

Flow = 1mL/min

MP: A = ACN w/ 5mM Ammonium acetate

B = Water w/ 5mM Ammonium acetate (pH 6.8)

# U-Shaped Retention



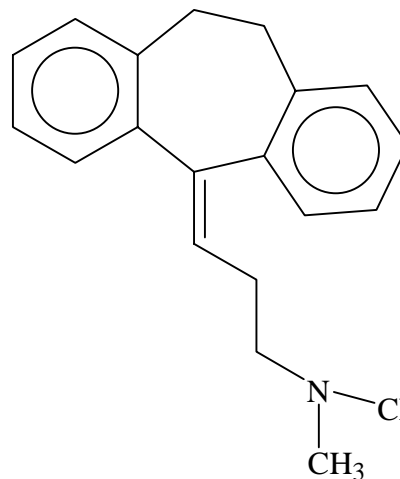
- Retention increases at high organic and high aqueous MP
- Strong solvent: Mixture of Aqueous:Organic

Discovery Cyano

Flow = 1mL/min

MP: A = ACN w/ 5mM Ammonium acetate

B = Water w/ 5mM Ammonium acetate (pH 6.8)



Amitriptyline

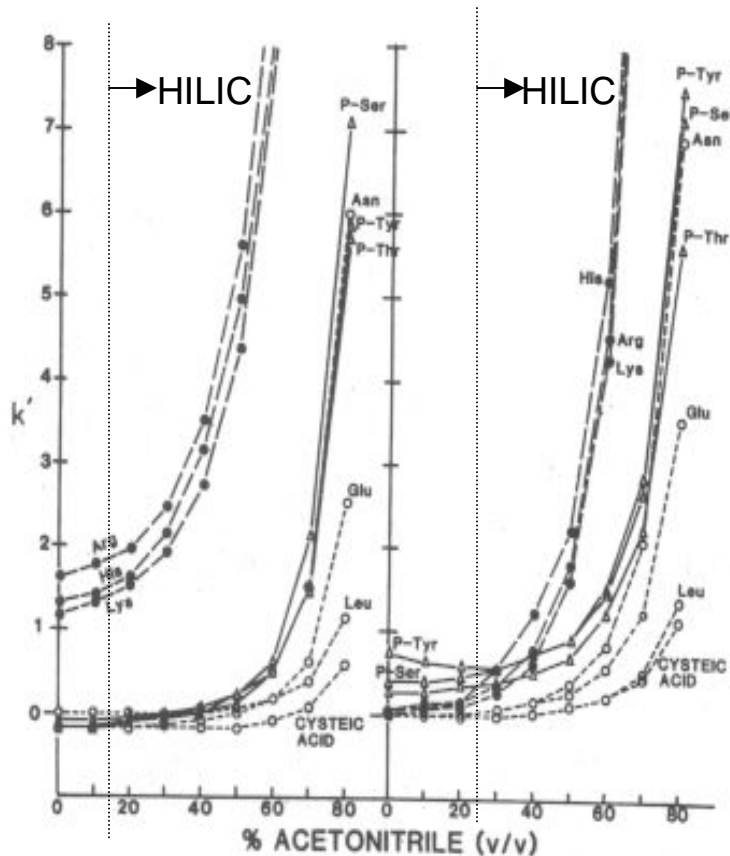


# HILIC Phases

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- **“HILIC” first proposed by Andrew Alpert (1990)**
- **Polar phases can show U-shaped retention profiles**
- **Normal phase region explained by polar interactions**

# HILIC Chromatography



- Polar stationary phase - polar analyte interactions
- Example - Amino acids on PolySulfoethyl Aspartamide (left) and PolyHydroxyethyl Aspartamide (right) phases

Ref: A. Albert, J. Chromatogr., 499 (1990) 177.





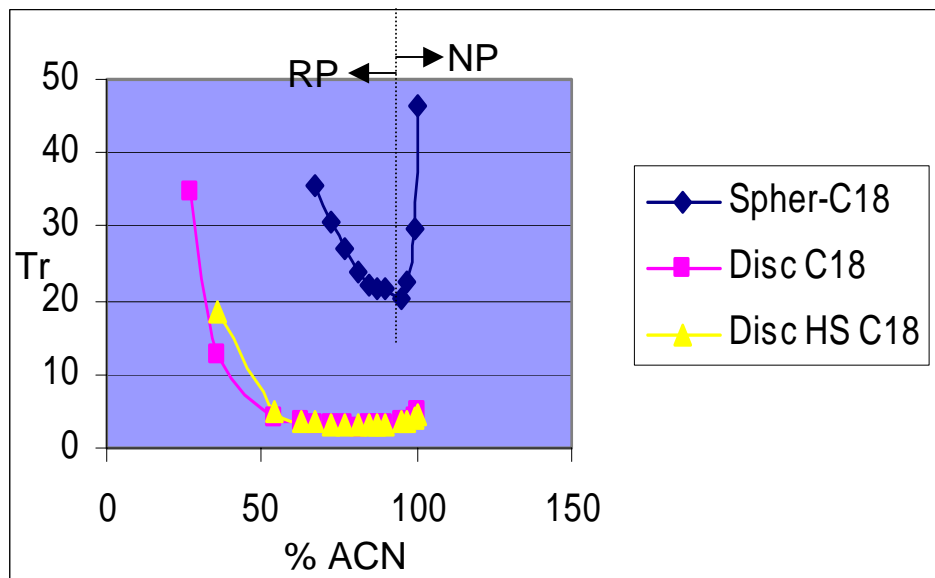
# HILIC On Reversed-Phase Columns

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- **Bij, et. al., found U-shaped curves on C8 & C18 phases for dibenzo-crown ethers, some peptides, and free amino acids (Ref.: J. Chromatogr., 203, (1981) 65).**
- **Bare silica columns also showed U-shaped retention.**
- **Hypothesis: The level of base deactivation can be approximated by the NP (HILIC) region of RP packings.**



# U- Shaped Retention Profile of C18 Phases



Flow = 1mL/min

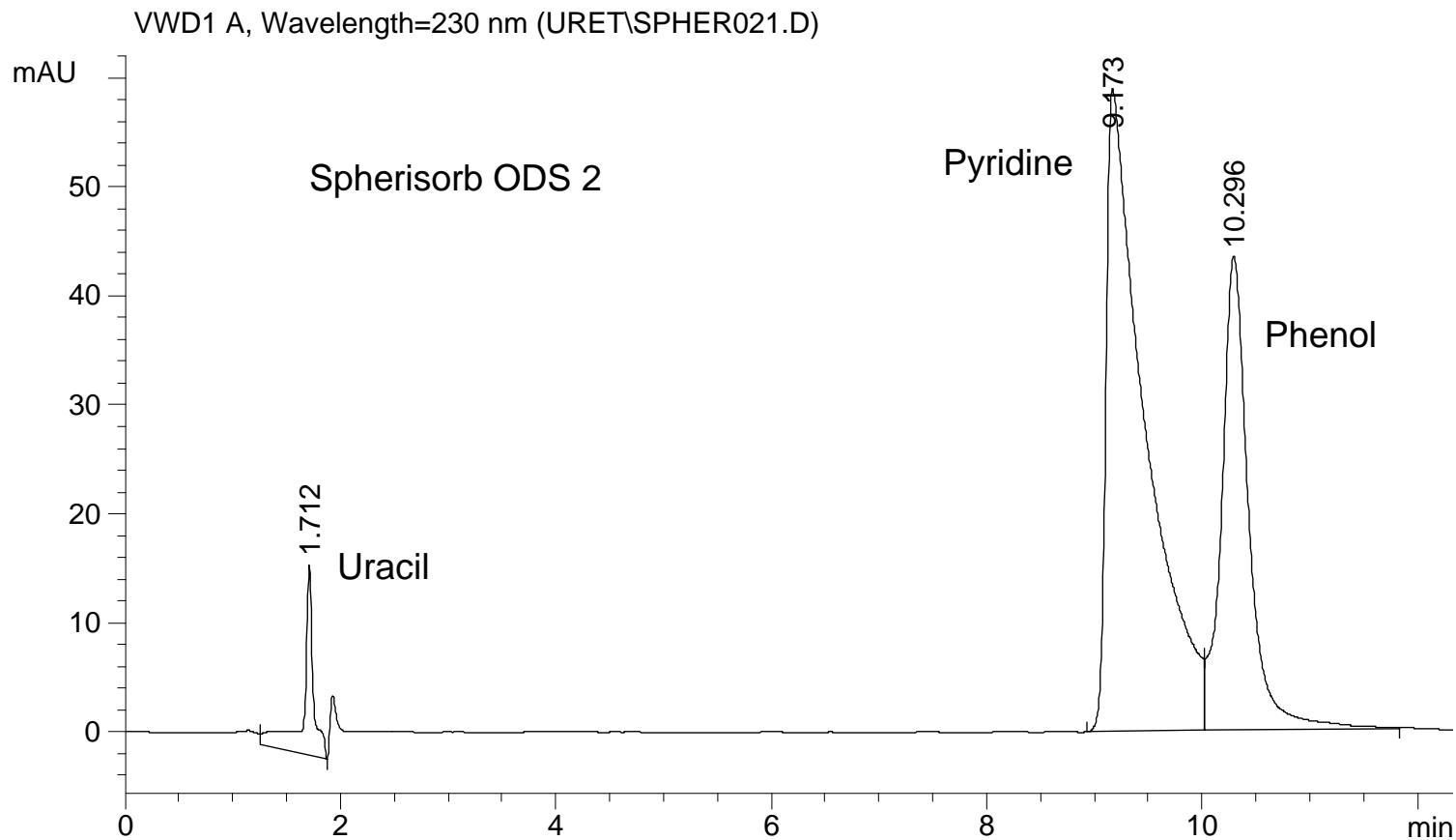
Propranolol, 0.33mg/mL

MP: A = ACN w/ 5mM Ammonium acetate

B = Water w/ 5mM Ammonium acetate (pH 6.8)

- NP (HILIC) region attributed to polar silanol interactions with polar analyte
- More active, Type A C18 phases (e.g., Spherisorb ODS 2) show more NP character
- Base deactivated, Type B C18 phases (e.g., Discovery C18, Discovery HS C18) show little NP character

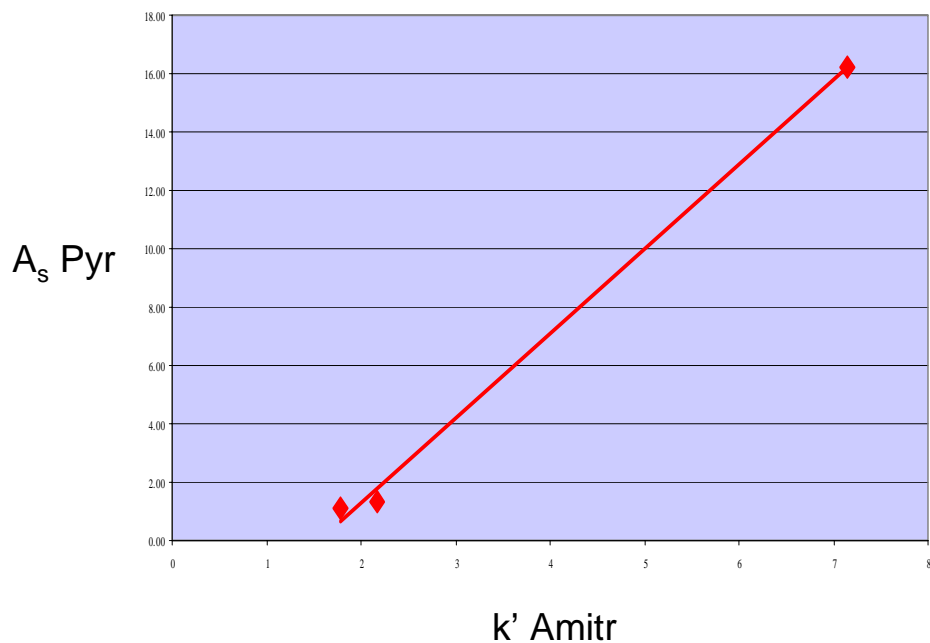
# Pyridine/Phenol Test



MP = 90:10 - H<sub>2</sub>O:ACN, each w/ 5mM Ammonium Acetate; 35oC; Flow = 1mL/min; UV @ 230nm

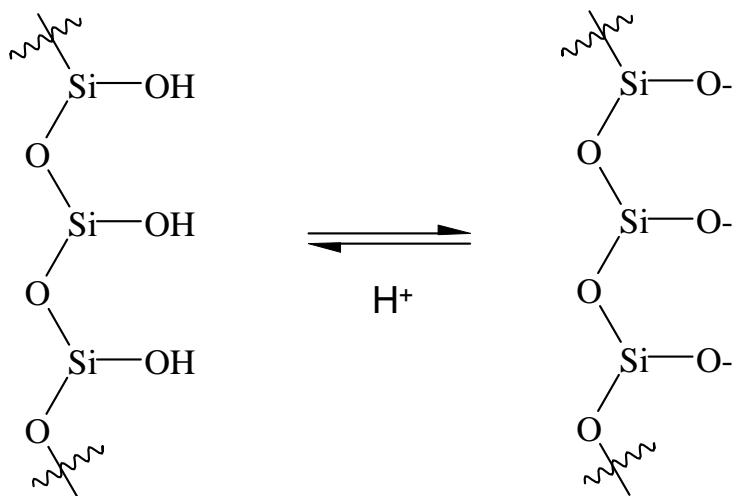
# Prediction of Level of Base Deactivation In RP Packings

Amitriptylene  $k'$  (99% ACN) VS. Pyridine Asymmetry (10% ACN)



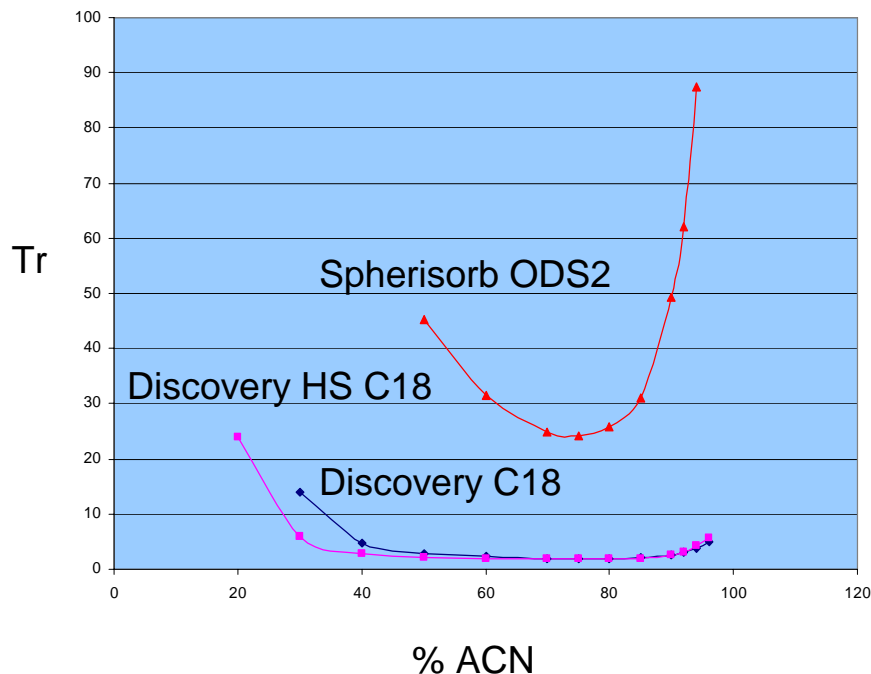
- **HILIC region relates well to traditional methods of measuring level of base deactivation for RP packings (e.g., Pyridine/Phenol Test)**
- **Pyridine asymmetry may be used to predict amount of tailing of basic compounds in RP region**

# Surface Silanol Equilibrium



- Surface silanol equilibrium
- Range of silica pK<sub>a</sub> ~4-5 (Ref.: Snyder & Kirkland, Intro. To Modern Liq. Chromatogr., 2nd Ed., John Wiley & Sons (1990) 273)

# HILIC - pH 4



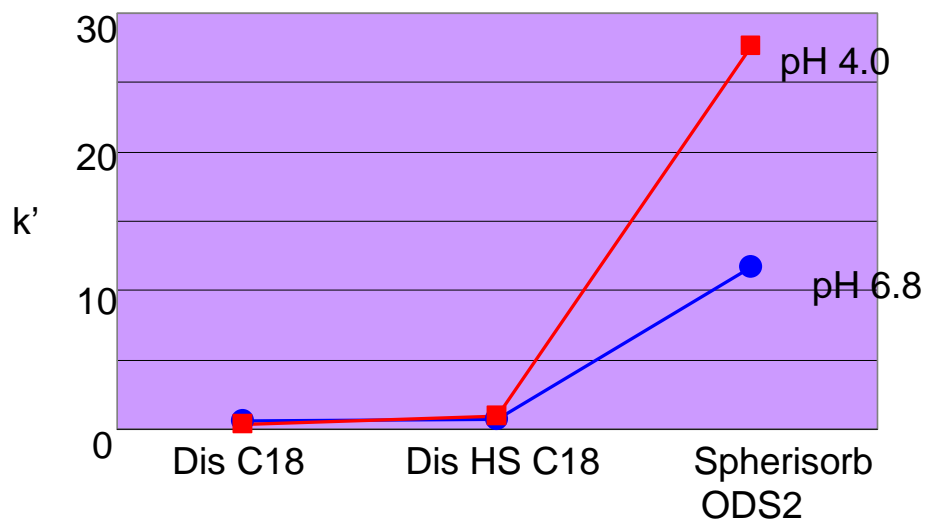
- HILIC increases when pH is lowered below pKa of basic compounds
- HILIC interaction can still occur even when surface silanols are protonated

Flow = 1mL/min

MP: A = ACN w/ 5mM Ammonium acetate

B = Water w/ 5mM Ammonium acetate (pH 4.0)

# C18 Packing vs. Capacity Factor



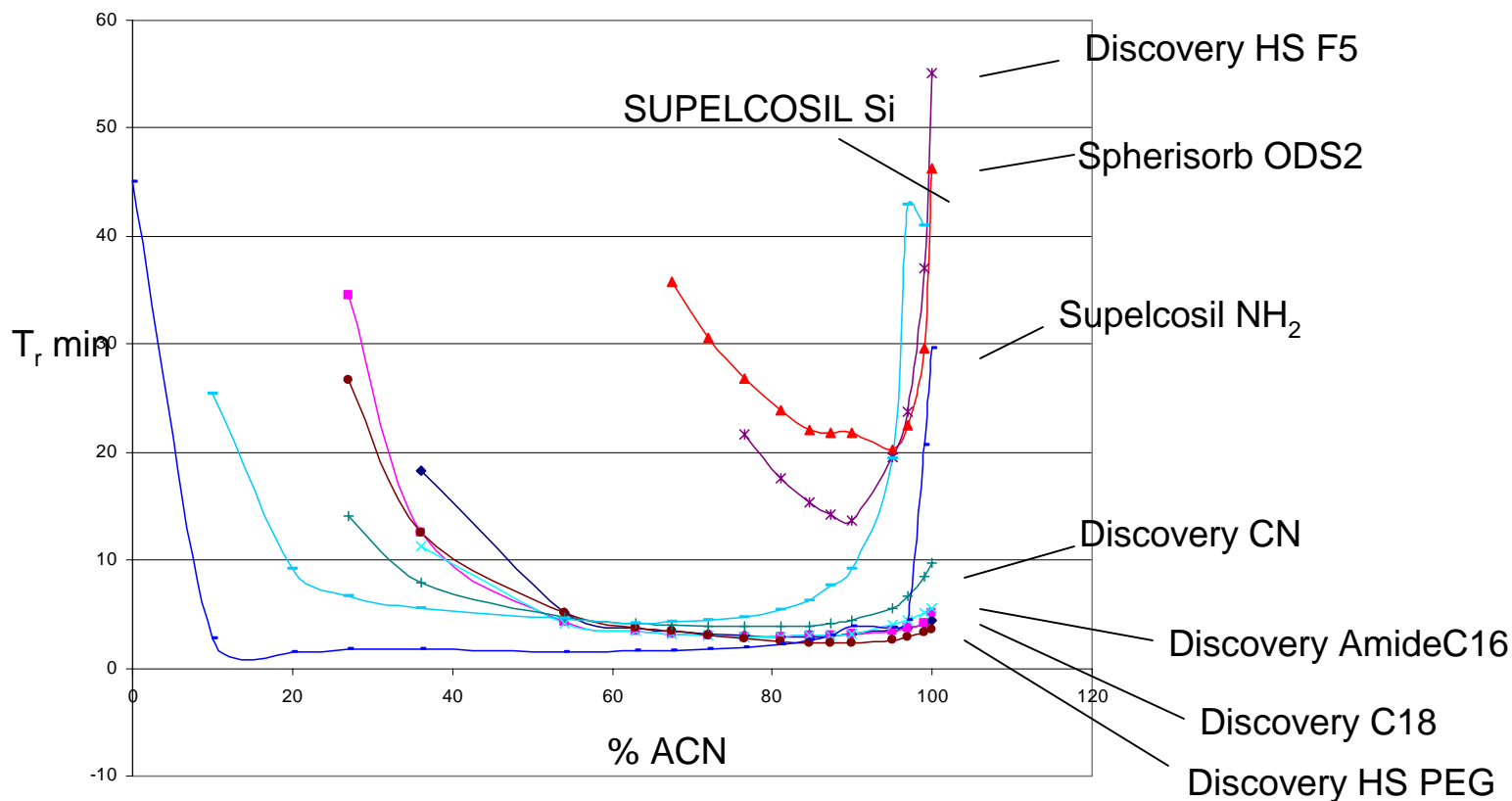
- More HILIC retention with lower pH
- Dominant mechanism not electrostatic

Amitriptyline

MP = 99:1 - ACN/ H<sub>2</sub>O both w/ 5mM NH<sub>4</sub>OAc

B = ACN w/ 5mM NH<sub>4</sub>OAc

# Other Phases Exhibiting HILIC Character



Amitriptyline; flow = 1 mL/min; M.P. = 5mM NH<sub>4</sub>OAc, pH 6.8; 35°C; 230nm



# Advantages of Using Phases Exhibiting U-Shaped Retention

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- Provide unique selectivity vs. C18 phases
- Presents twice the opportunity for retention/resolution (high and low % organic)
- Can use simple water:organic MP's
- Often faster analysis
- Retain aqueous-soluble analytes
- Retain organic-soluble analytes
- Increase LC/MS sensitivity at high organic MP's
- Reduce sample pre-treatment requirements
- Do not collapse



# Summary

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- **HPLC phases can contain both RP and NP modes of retention**
- **For basic analytes on C18 phases, the mechanism in the HILIC region is not readily explained by siloxide anion electrostatic interactions as the major contributing factor**
- **Level of base deactivation of C18 phases can be approximated by their amount of HILIC character**
- **Several advantages exist for developing methods on phases that give U-shaped retention curves**
- **A wide variety of phases exist that do show U-shaped curves, including fluorinated, C18, silica, NH<sub>2</sub>, and CN**



# Acknowledgment

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- **The authors would like to thank Mr. Yasuyuki Kurosu for helpful ideas on measuring base deactivation levels.**