

Understanding Selectivity in Reversed Phase Chromatography – A Simplified Look at Column Selection

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Pennsylvania State University

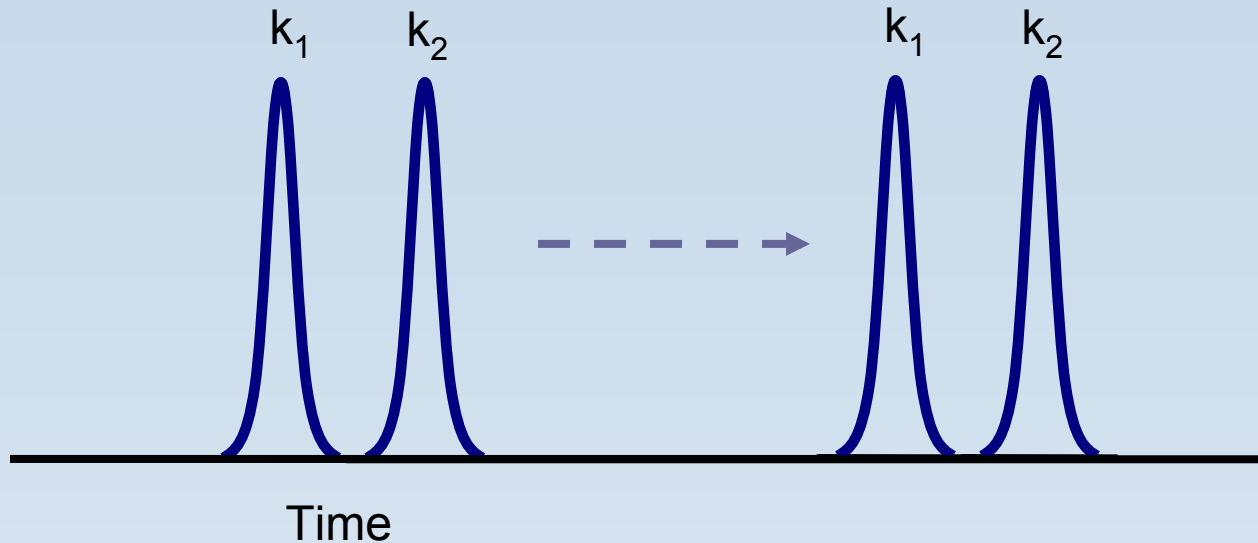
Ty Kahler, Randy Romesberg, Vernon Bartlett, Mike Wittrig,
Bruce Albright, Rick Lake
Restek Corporation



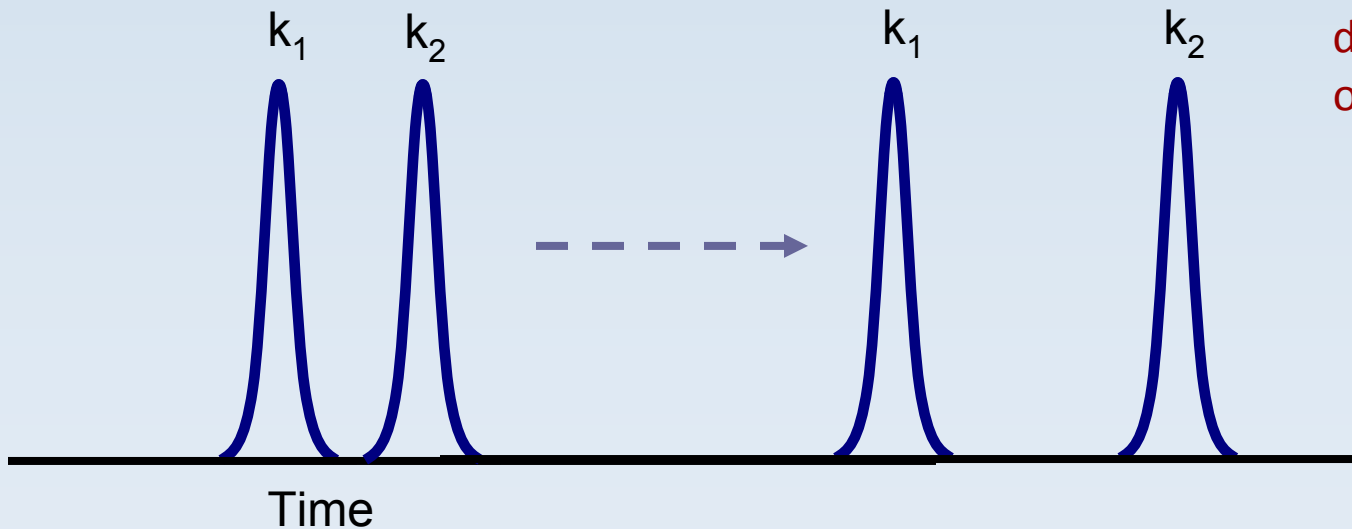
Outline

- Defining Selectivity
- Defining Reversed Phase Separation Mechanisms
- Characterizing Retention Profiles
- Expanding Selectivity Range in RPC to Simplify Column Selection

How we do define selectivity?



Retention is the molecular band elution relative to time



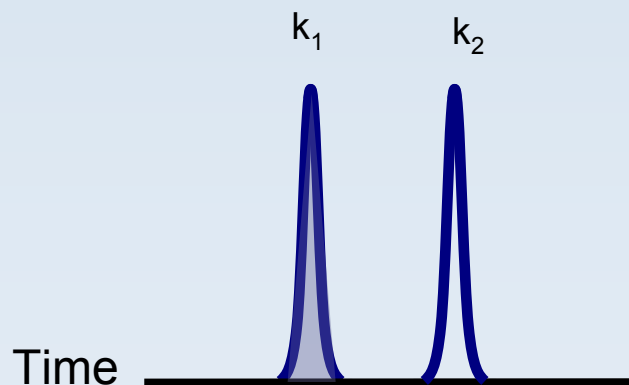
Selectivity is the difference in retention of one molecular band relative to another

$$\alpha = \frac{k_2}{k_1}$$

Determining Retention Profiles

For a “Crude” Simplification...

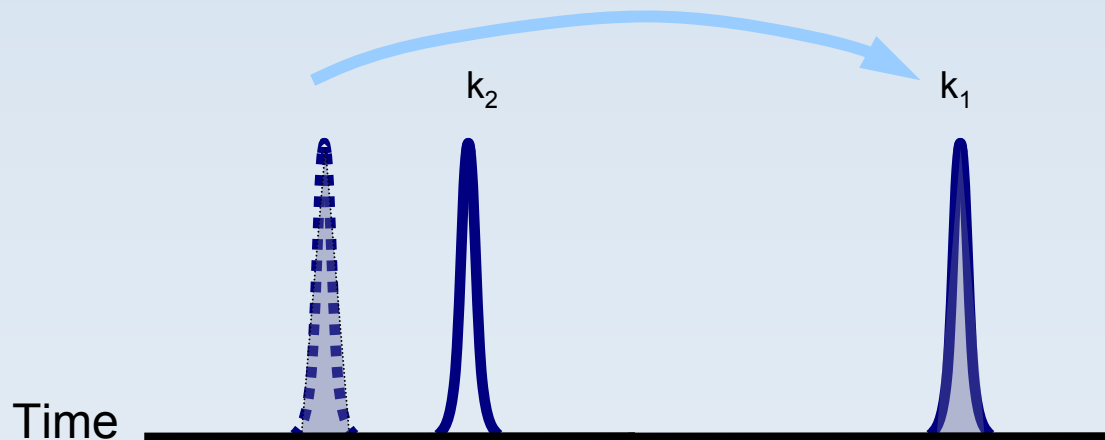
We can define selectivity as the difference between retention (solubility) profiles of solutes relative to the chemical environment (stationary and mobile phase compositions)



Determining Retention Profiles

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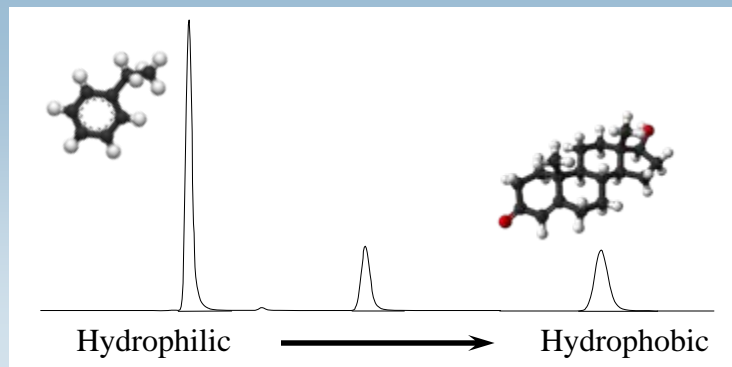
We can define selectivity as the difference between retention (solubility) profiles of solutes relative to the chemical environment (stationary and mobile phase compositions)



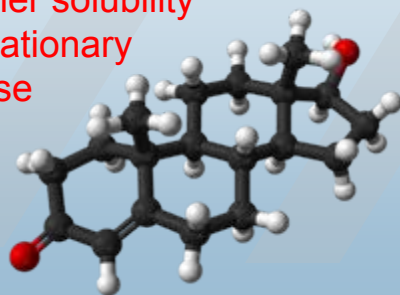
Reversed Phase – Bonded Phase Chromatography

More Polar Mobile Phase

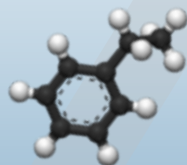
A separation based mainly on differences between the **solubility** of the components in the mobile and stationary phases - molecules partition at different rates



Higher solubility
in stationary
phase



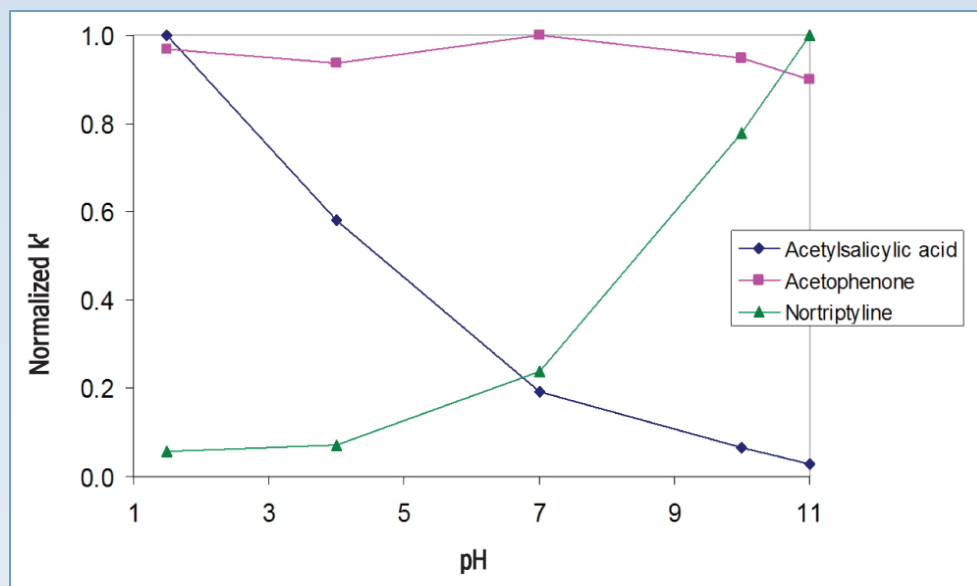
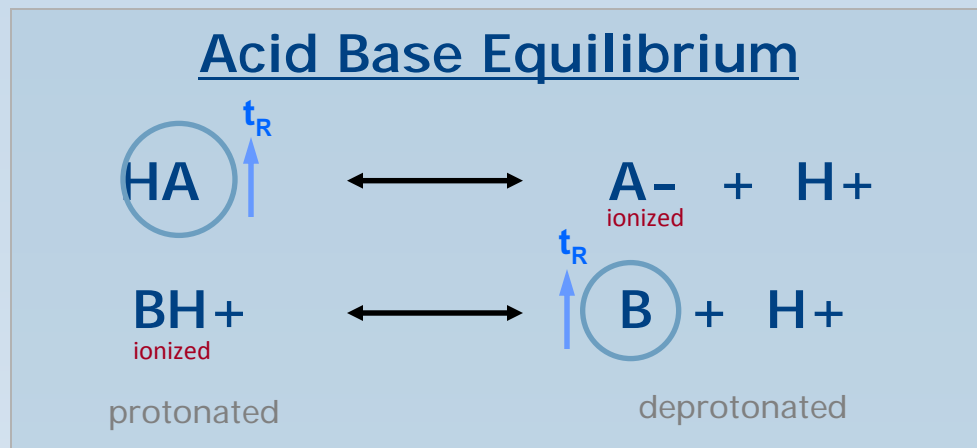
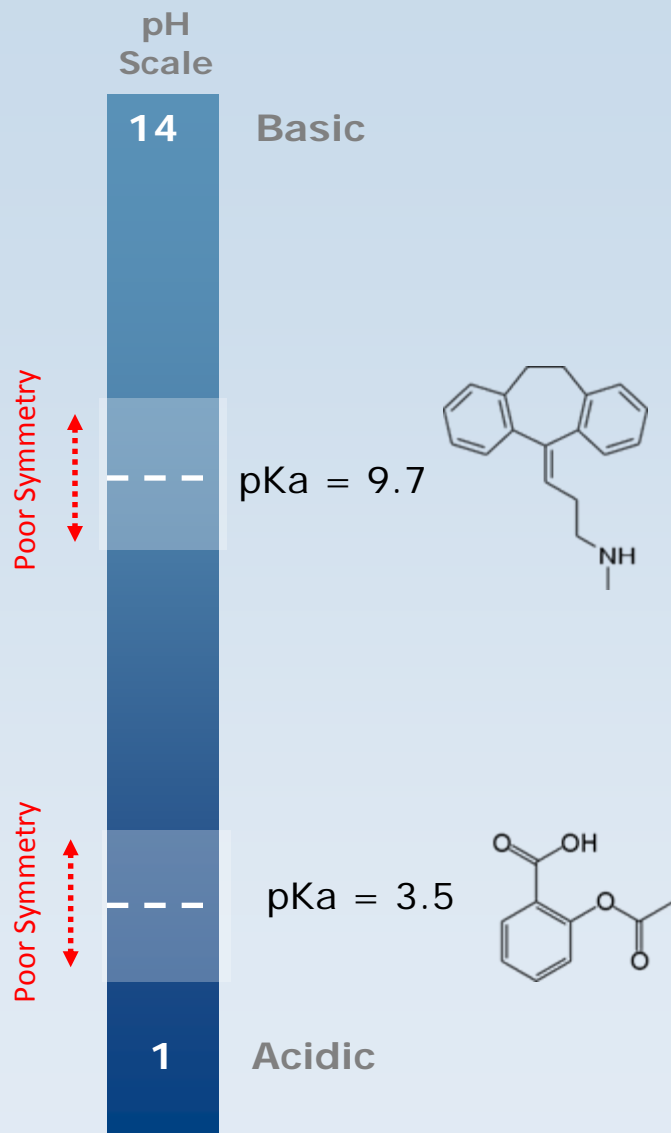
Higher solubility
in mobile phase



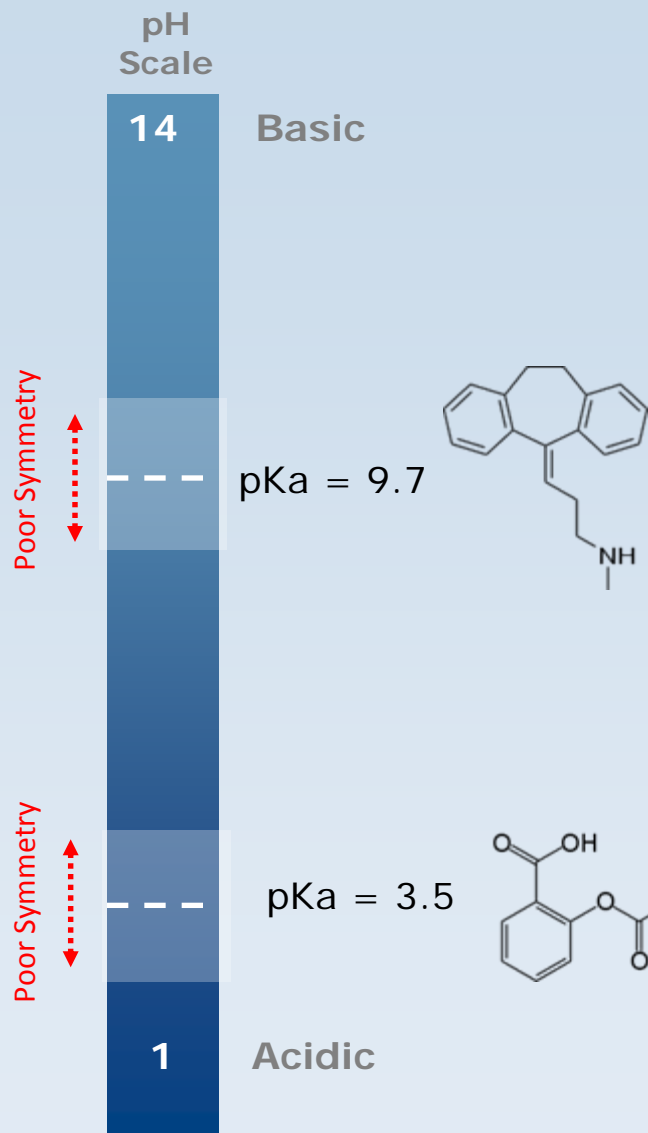
Less Polar Stationary Phase

Silica

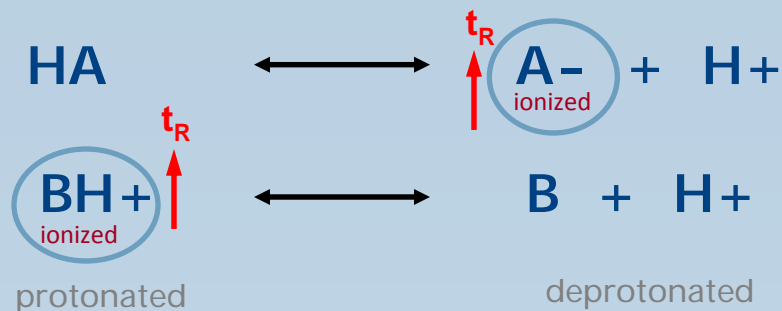
Ion Suppression for RPC on Alkyl Based Phases



Mobile Phase Selection for C18

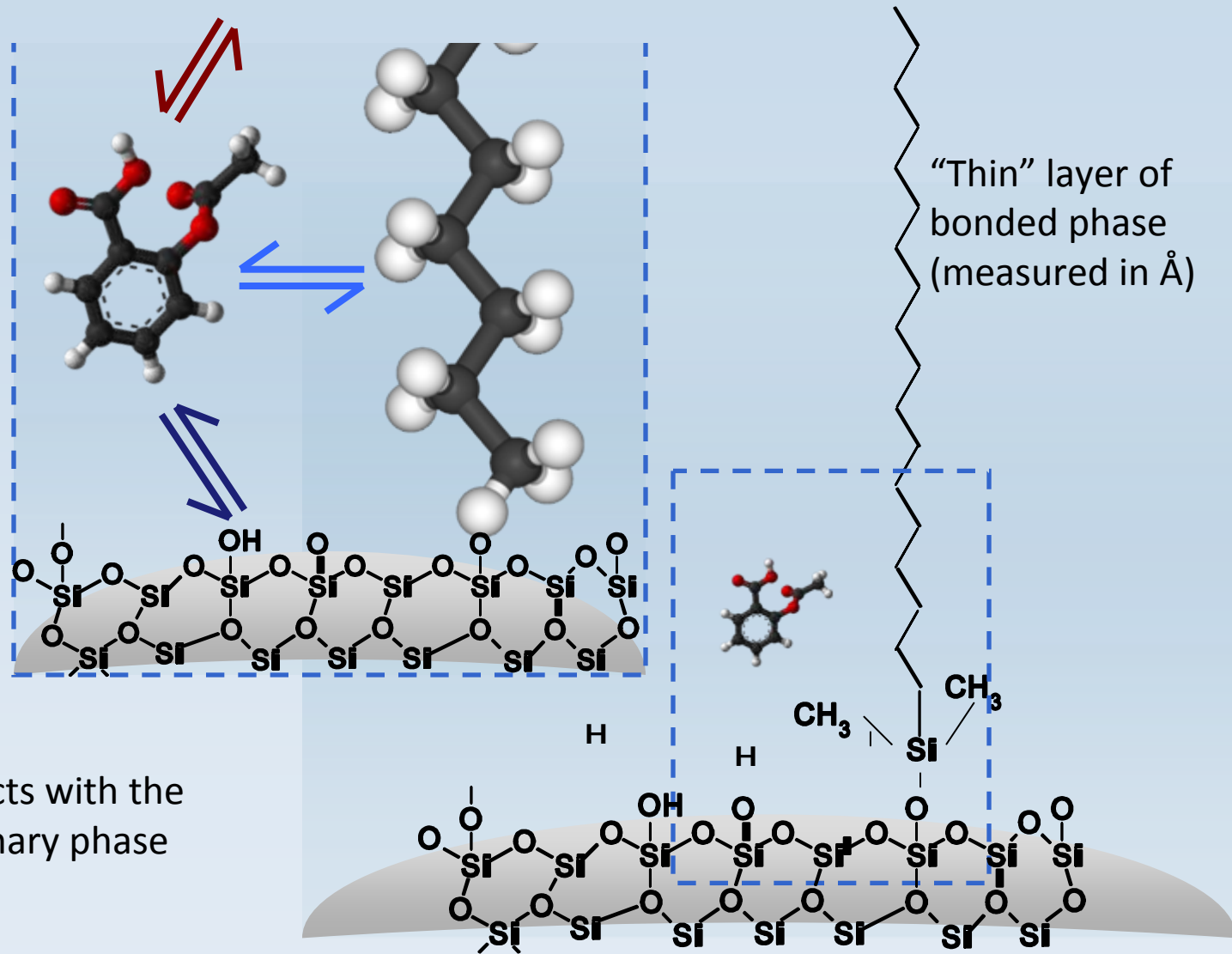


Acid Base Equilibrium



Ion suppression (RP-IS) retention profiles as listed above only hold true for alkyl phases, when applying modified techniques, such as ion-pairing, or alternative stationary phases, often this is not true.

Factors in a Liquid Chromatography Separation



Three Equilibria:

In LC, a solute interacts with the mobile phase, stationary phase and support

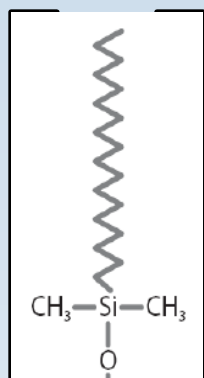
Common RPC Interactions

Molecular Interaction	Retention Profile	Significance to Reversed Phase Chromatography
Dispersion	Hydrophobic	<ul style="list-style-type: none">• Significant driver for RPC and exists to some extent in all organic molecules• Major retention mechanism for alkyl phases (ie, C18).• Retention is proportionate to the hydrophobicity of the molecule,
Charge Transfer (π-π Interaction)	Aromatic	<ul style="list-style-type: none">• Commonly seen in aromatic, unsaturated or aromatics substituted with electron withdrawing groups• Greatly enhanced with methanolic mobile phase
Hydrogen Bonding	Acidic	<ul style="list-style-type: none">• Solutes act as a proton donor with a proton accepting functionality embedded in the stationary phase• Commonly observed as an increase in acidic solute retention
Dipole- Dipole Interaction	Basic	<ul style="list-style-type: none">• Often referred to as electrostatic interactions• Commonly observed as an increase in acidic solute retention

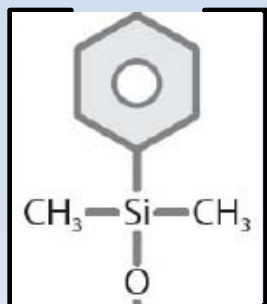
Common Reversed Phase Stationary Phase Ligands

Non Polar

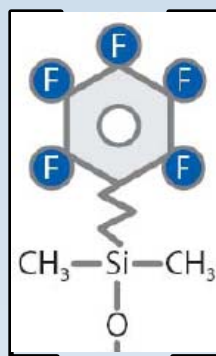
Polar



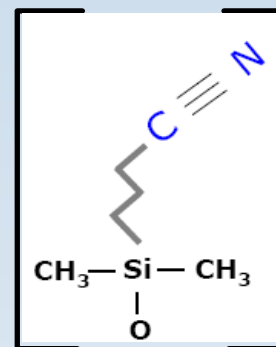
Alkyl



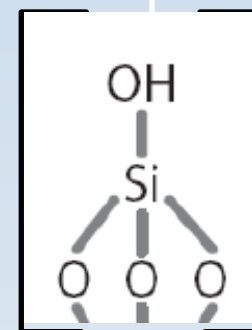
Phenyl



Fluoro

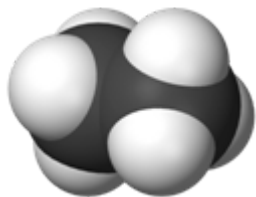


Cyano

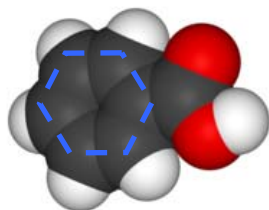


Silica

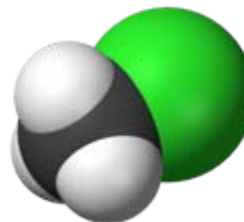
Dispersive



Pi-Pi Interactions



Electrostatic / Dipole



H Bonding



Characterizing Selectivity in RPC

The Hydrophobic Subtraction Model

$$\log \alpha \equiv \log \left(\frac{k}{k_{EB}} \right) = (\eta' H) - (\sigma' S^*) + (\beta' A) + (\alpha' B) + (\kappa' C)$$

Hydrophobicity (H) - *measure of hydrophobic retention*

Steric Resistance (S) - *resistance to penetration of molecules into stationary phase (not shape selectivity)*

Column hydrogen-bond acidity (A) - *measure of free ionized silanols (Proton donating)*

Column hydrogen-bond basicity (B) - *measure of free silanol/siloxanes (Proton accepting)*

Column cation-exchange activity (C) - *ionized silanols - charge measure (pH dependant)*

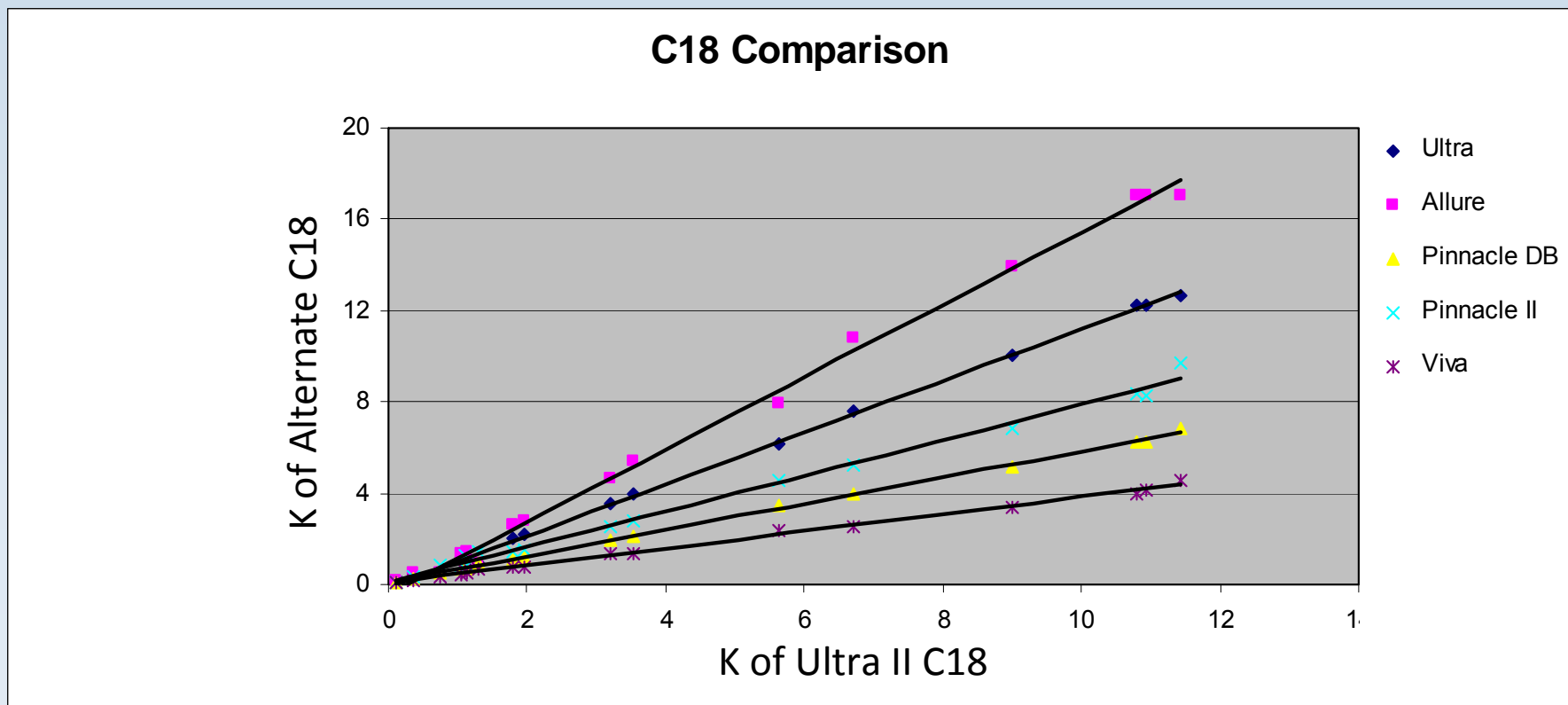
Journal of Chromatography.A 1000 (2003) 757-778, J.Gilroy, J. Dolan, L Snyder



HSM Probes Comparing Ultra II to other Restek C18 Phases

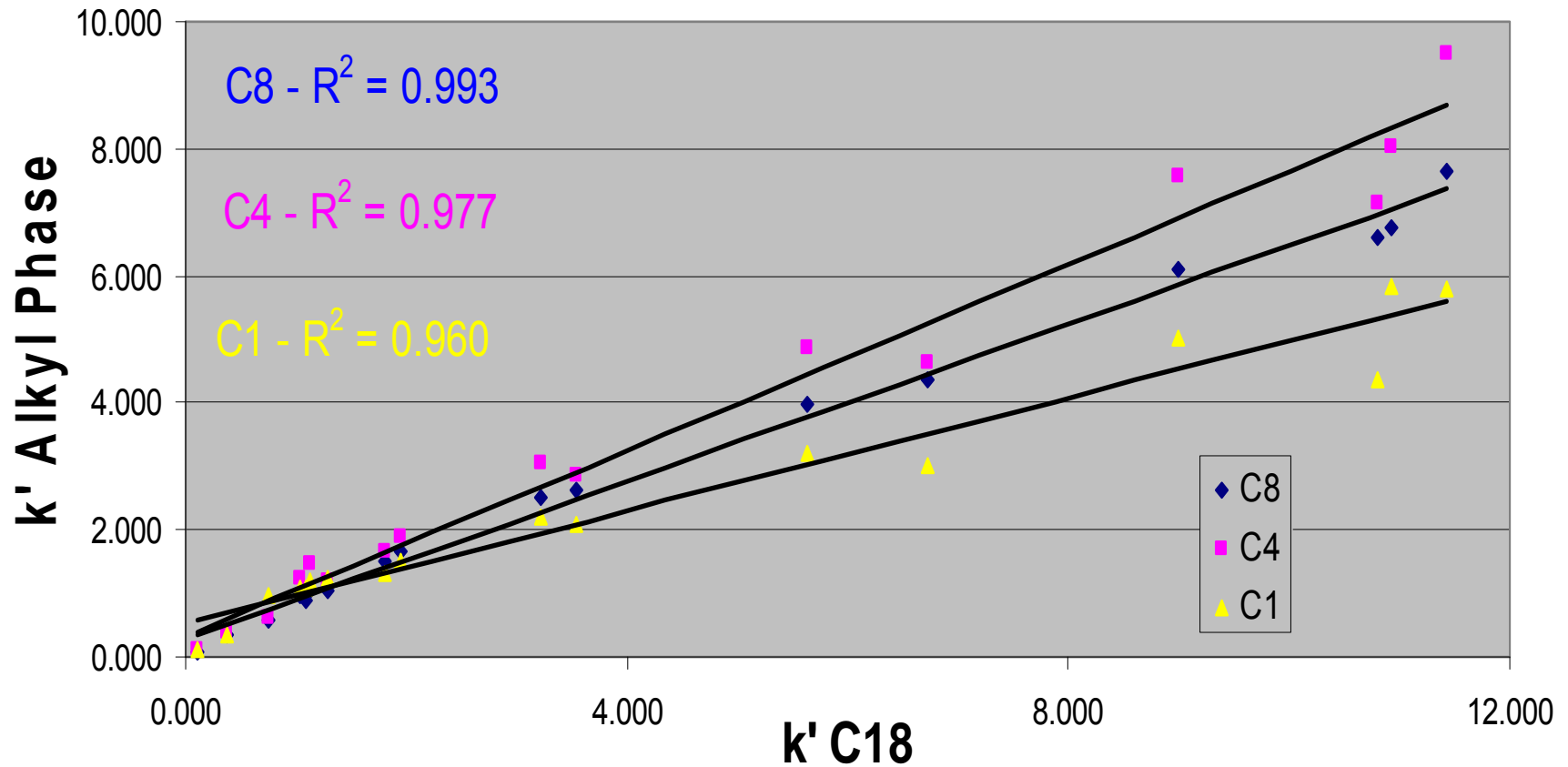
Monomeric C18 columns of differing surface area and silanol activity

H	S*	A	B	C	C-7	F(s)	F(s) - C
1.041	0.021	-0.037	-0.015	0.264	0.181	0.0	0.0
1.051	0.033	-0.032	-0.023	0.057	-0.003	17.2	1.6
1.014	0.025	-0.033	-0.005	0.364	0.280	8.4	1.6
1.038	0.019	-0.030	0.003	0.445	0.349	15.2	2.6
0.980	0.016	-0.076	0.013	0.359	0.305	9.0	4.4
1.131	0.052	0.046	-0.049	-0.037	0.020	25.8	6.3

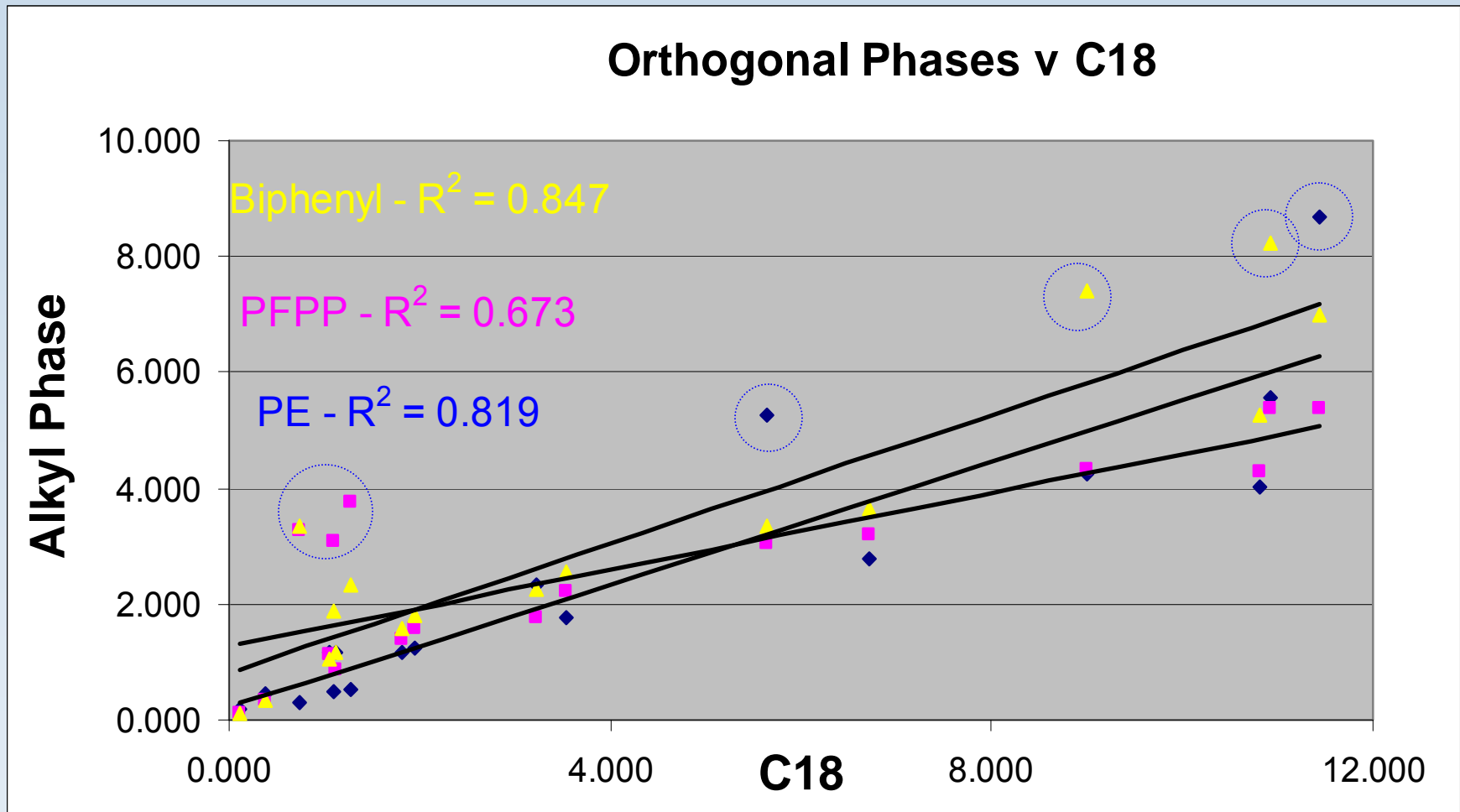


Defining Orthogonal Stationary Phases

Ultra II - Alkyl Phases v C18



Defining Orthogonal Stationary Phases



Finding Column Similarity (“Equivalency”)

Selectivity Function

- Has been previously used to define column similarity
- Can also be used as a guideline for column dissimilarity
- Dissimilar columns can create alternate selectivity

$$F_s = \left\{ \begin{aligned} & [12.5(H_2 - H_1)]^2 + [100(S^*_2 - S^*_1)]^2 \\ & + [30(A_2 - A_1)]^2 + [143(B_2 - B_1)]^2 + [83(C_2 - C_1)]^2 \end{aligned} \right\}^{1/2}$$

[1] L.R. Snyder, J.W. Dolan, P.W. Carr, J. Chromatogr. A 1060 (2004) 77.

Finding Dissimilar Stationary Phases

	Terms Calculated from the Hydrophobic Subtraction Model						
Stationary Phase Type	Hydrophobicity	Steric Hinderance	Hydrogen Bond Acidity	Hydrogen Bond Basicity	Cation Exchange Activity	Selectivity Function	Rank of Dissimilarity
	H	S*	A	B	C	Fs	
C18 (control)	1.041	0.021	-0.037	-0.015	0.264	0	0
C1	0.659	-0.089	-0.396	0.056	0.162	20.9	7
C4	0.806	-0.031	-0.323	0.066	0.093	21.1	6
C8	0.855	0.003	-0.221	0.011	0.177	12.6	8
Aqueous C18	0.784	-0.154	0.321	0.015	0.468	27.2	5
Cyano	0.457	-0.072	-0.828	0.021	0.181	27.9	4
Biphenyl	0.652	-0.198	-0.275	0.047	0.434	28.9	3
PFP Propyl	0.674	-0.084	-0.303	-0.006	0.769	44.2	2
IBD	0.674	-0.043	-0.022	0.225	-0.257	55.8	1

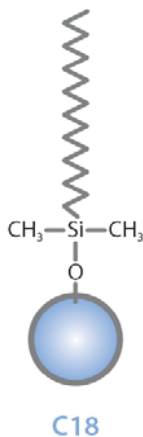
Defines Orthogonal

Characterizing Retention Profiles

Restek Phase:
C18

Stationary Phase
Category:
C18 (L1)

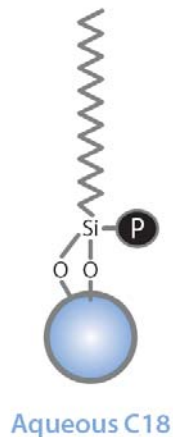
Ligand Type:
Densely bonded and
fully endcapped
octyldecyl silane



Restek Phase:
Aqueous C18

Stationary Phase
Category:
Modified C18 (L1)

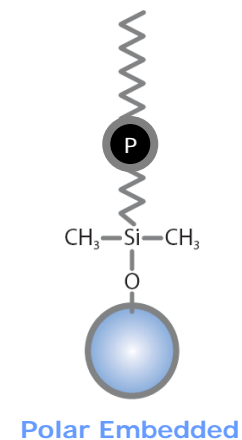
Ligand Type:
Proprietary polar
modified and
functionally bonded
C18



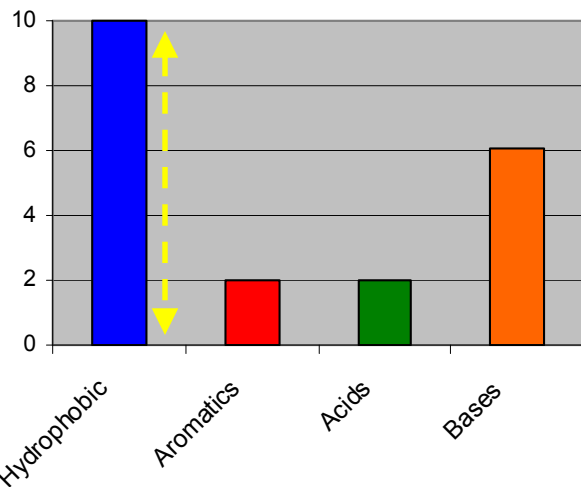
Restek Phase:
IDB

Stationary Phase
Category:
Polar Embedded
Alkyl (L68)

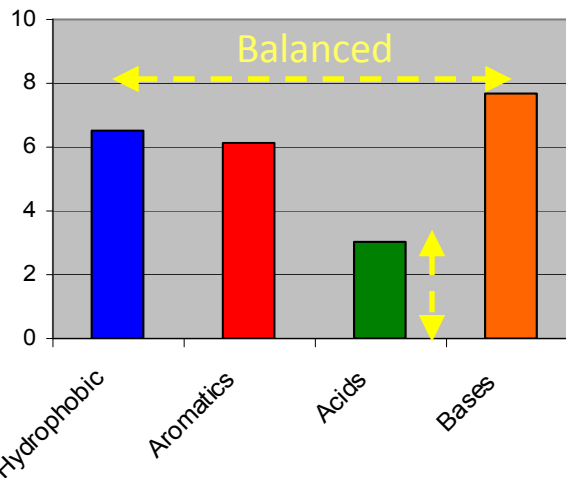
Ligand Type:
Proprietary polar
functional
embedded alkyl



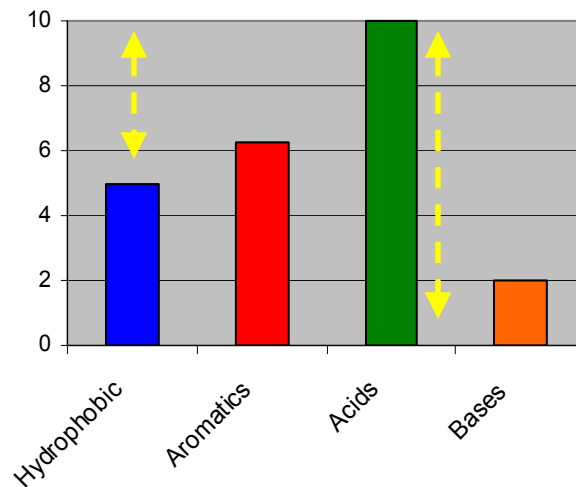
Ultra II C18



Ultra II Aqueous C18



Ultra II IDB

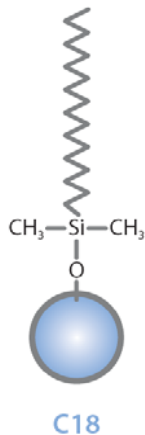


Characterizing Retention Profiles

Restek Phase:
C18

Stationary Phase
Category:
C18 (L1)

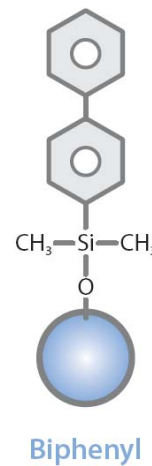
Ligand Type:
Densely bonded and
fully endcapped
octyldecyl silane



Restek Phase:
Biphenyl

Stationary Phase
Category:
Phenyl (L11)

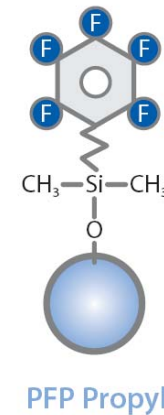
Ligand Type:
Unique Biphenyl



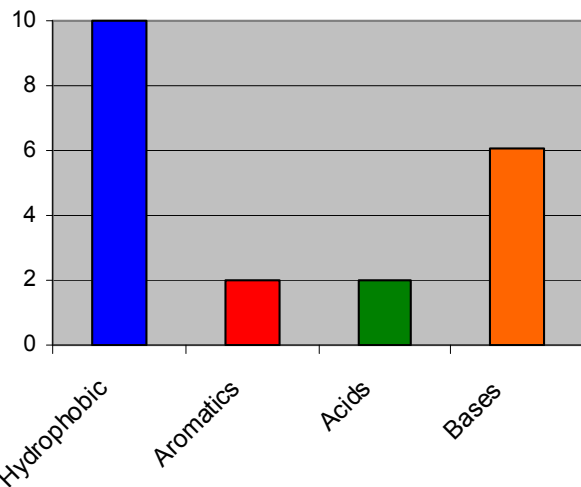
Restek Phase:
PFP Propyl

Stationary Phase
Category:
Proprietary end-
capped
pentafluorophenyl
propyl (L43)

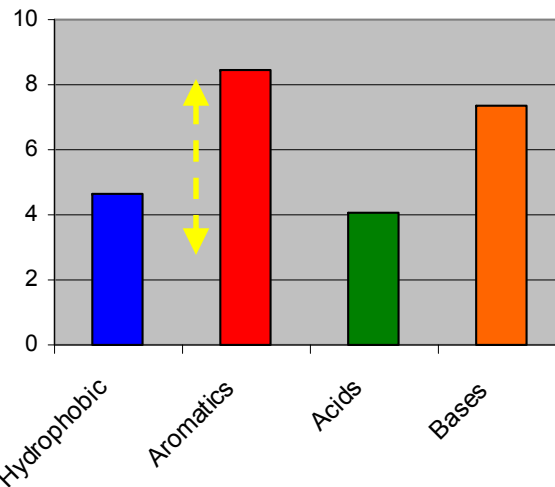
Ligand Type:
Fluorophenyl



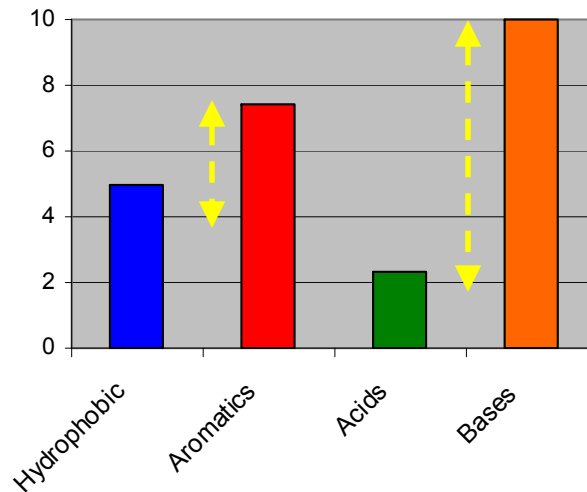
Ultra II C18



Ultra II Biphenyl

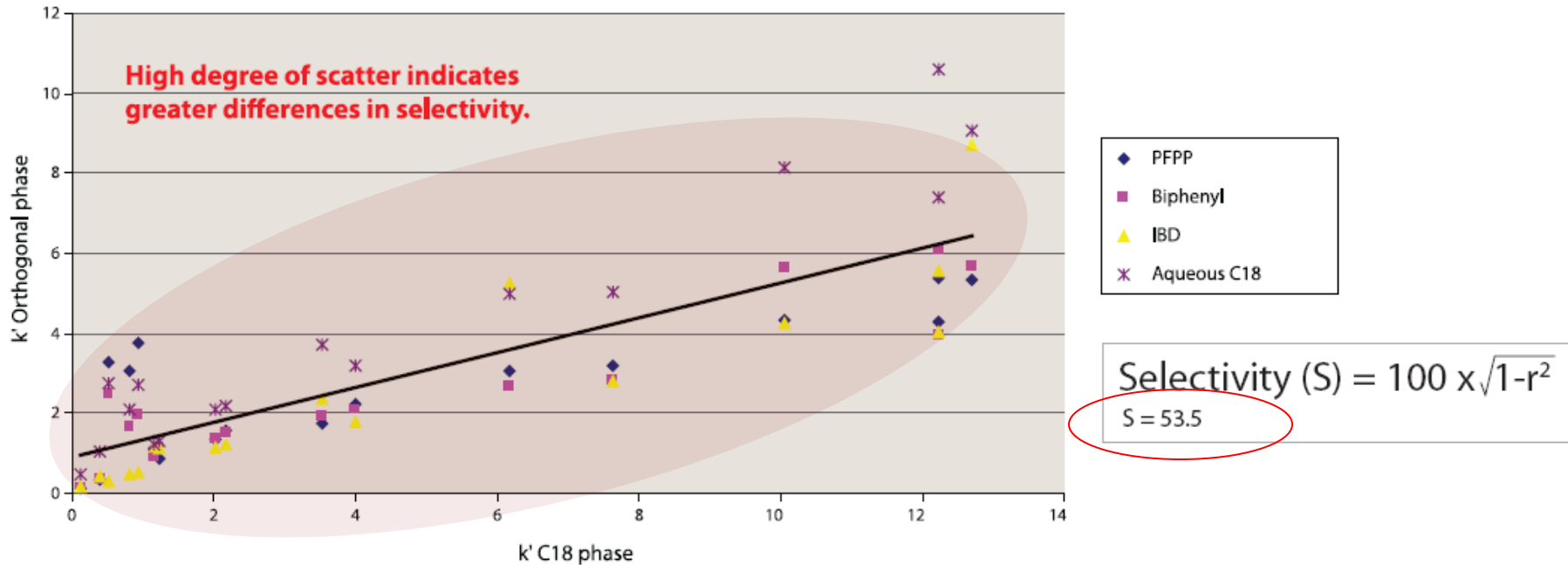


Ultra II PFP Propyl



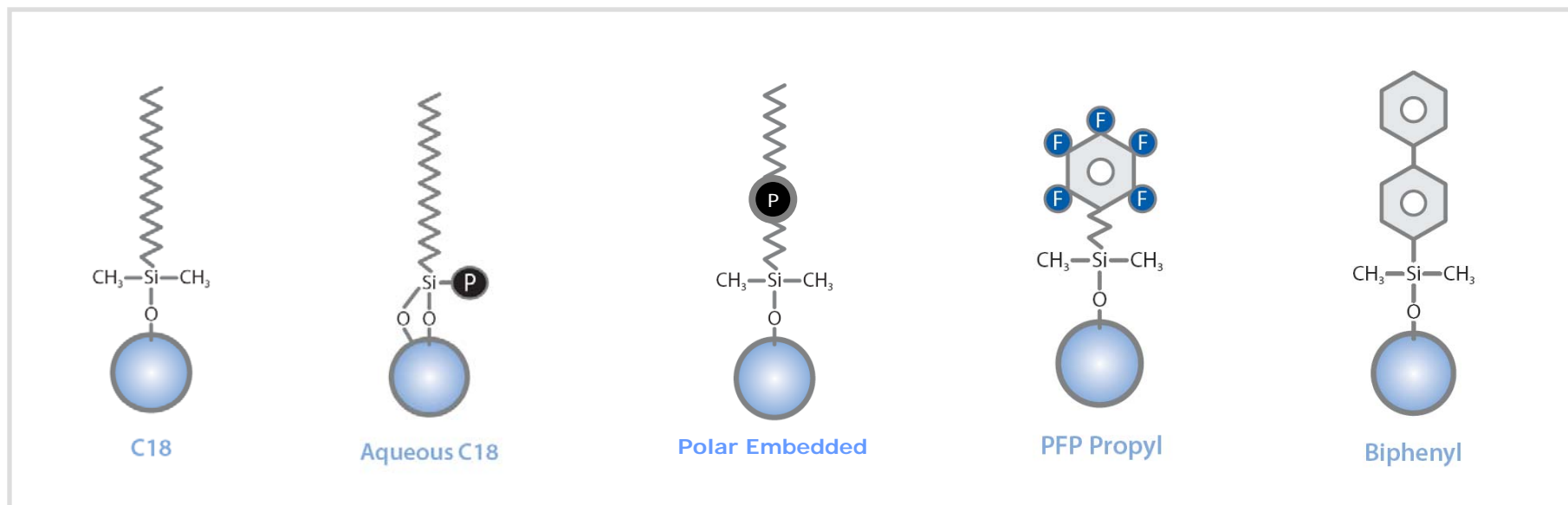
The Value of Selectivity

Correlation of Orthogonal Phase Selectivity Relative to C18



[2] U.D. Neue, J.E. O'Gara, A. Mendez, J. Chromatogr. A 1127 (2006) 161.

Common Reversed Phase Column Categories for Extending Selectivity Range



- General Purpose
- Balanced retention
- Slight Shape Selectivity
- Increased retention for acids
- Symmetry for Bases
- Increased retention for protonated bases
- Increased retention hydrophilic aromatics
- Selectivity for conjugated compounds

Summary

- Selectivity can be seen as differential retention or solubility
- Hydrophobic Subtraction Model is a great treatment for defining a selectivity model
- Expanding the model can match molecular interaction to phase type to produce a definitive guide for stationary phase selection

Acknowledgements

- Dr. Lloyd Snyder
LC Resources