

# The Role of Selectivity in Chromatographic Separations (GC and LC)

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Turning Visions into Reality

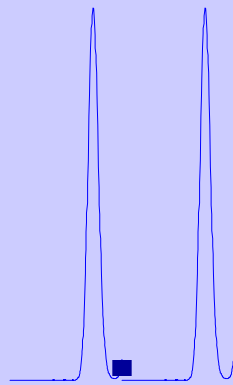
[www.restek.com](http://www.restek.com)

# How do we get Separation ?



Chemistry  
(Selectivity)  
**Separation/Resolution**

Separation in time



Physical  
(Introduction and transport)  
**Helpful for Throughput**

Separation in width

# HPLC Column Market

- Early days:
  - Many stationary phases
  - Phase chemistry was addressed first

$$R = 1/4 \sqrt{N} \times (k' / k'+1) \times (\alpha-1 / \alpha)$$

- Spherical silica refinements improved efficiency
- Current situation
  - C18 dominates market (~85%)
  - 5-10% normal phase separations
  - ~5-10% of separations are on “other” phases
    - Is this reasonable?
- UHPLC (sub-2 $\mu$ m) columns further increase efficiency

# HPLC Column Market Continued Developments

- Particle size !
- Particle types and media
- Pressures, flow rates, diameters, etc...
- Instrumental refinements

**“We should put our efforts on gaining selectivity  
rather than hunting for plates”**

**-Professor Klaus Unger HPLC 2008**

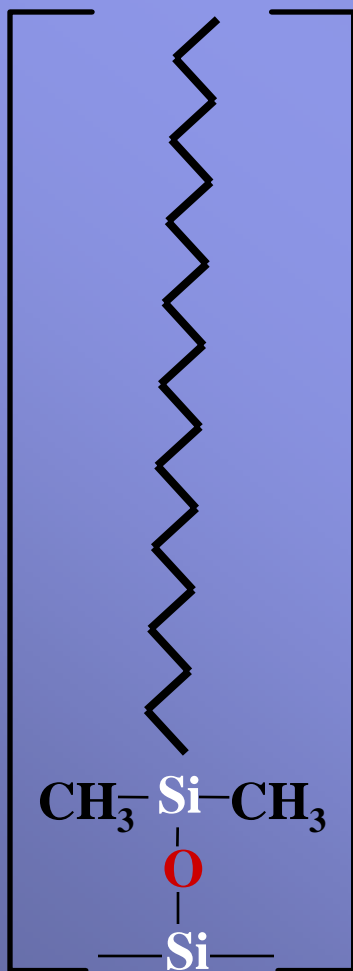
# What do we separate?

- Functionalized molecules:
  - Unsaturated molecules
  - Heteroatomic-containing molecules
  - Aromatics
  - Others
- Need to address the differential selectivity, not bulk solvation of the target analytes
  - What makes the hard-to-separate molecules different
  - Increase/maximize the liquid-liquid partition term

# Comparing Alkyl and Aromatic Phases

## The C18

C18



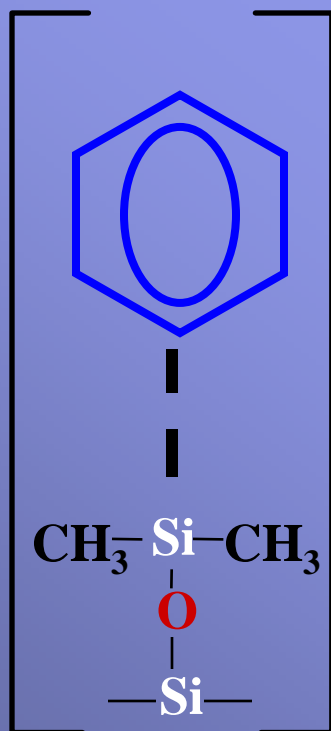
### Characteristics

- Strong hydrophobic attraction/retention
- Best for Neutral Compounds
- Ionizable groups can lead to decreased retention capacity
- High Ratio of Carbon: Heteroatom Preferred
- Predictable Retention Capacities
- Separates best when major distinction between analytes is hydrophobicity

# Comparing Alkyl and Phenyl Phases

## The Phenyl Phase Family

### Conventional Phenyl



### Characteristics

- Moderate hydrophobic attraction/retention
- Best for Aromatic or Unsaturated Compounds
- Alternate Selectivity to a C18 possible (pi-pi)
- Separates best when major distinction between analytes is unsaturation
- Retention Capacities not as predictable
- Methanol can be used to enhance retention and selectivity to some degree

# Project Scope

Scope: *Investigate New “Phenyl” column chemistry to accomplish the following:*

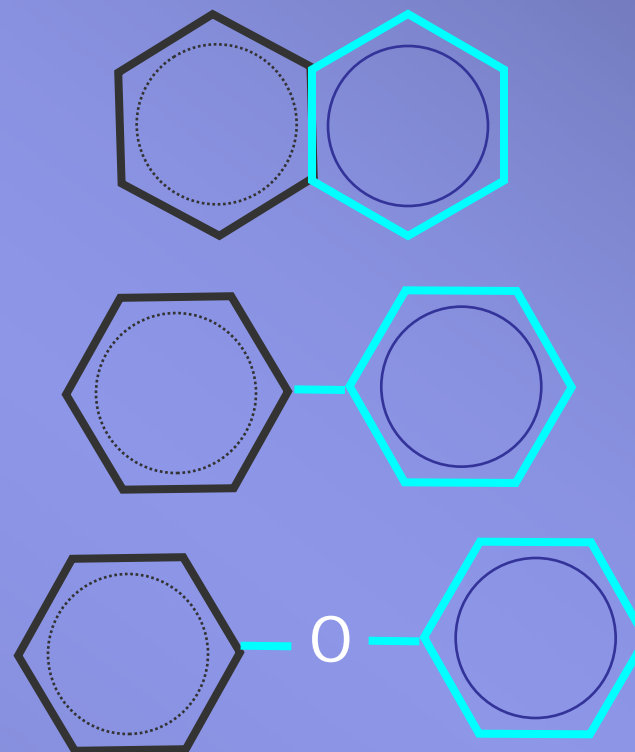
1. Increase overall hydrophobicity of phenyl phase
  - comparable non-polar retention to C18
2. Reduce the Retention Loss for Ionizable groups
  - improve MS sensitivity
3. Improve Aromatic Selectivity
  - separation based upon unsaturation or electron withdrawing groups
4. Allow More Predictable Retention
  - Target specific functional groups
5. Tunable Selectivity
  - Acetonitrile vs Methanol
6. True Orthogonal Separations to C18

# Choosing Stationary Phase Ligands

Increased Alkyl Spacer or  
Multiple Rings?

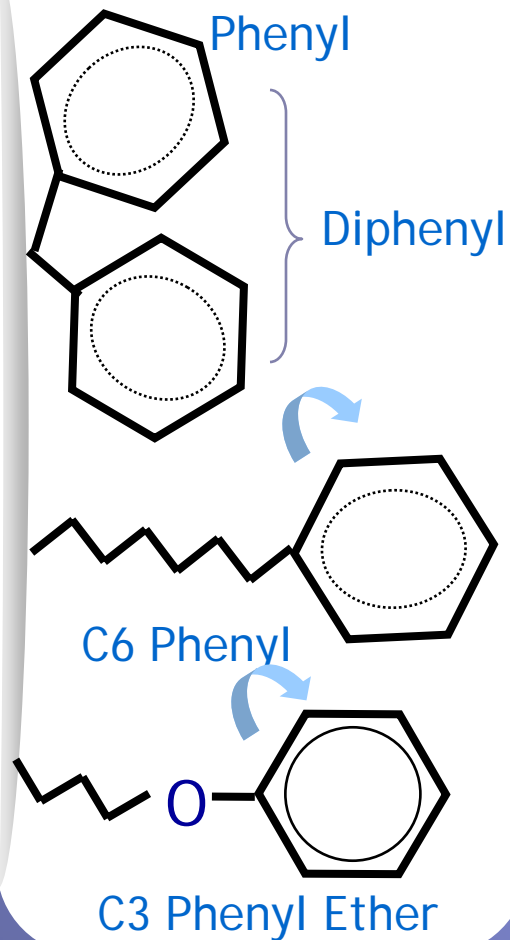


Fused, Conjugated Rings?  
Addition of Polarity?

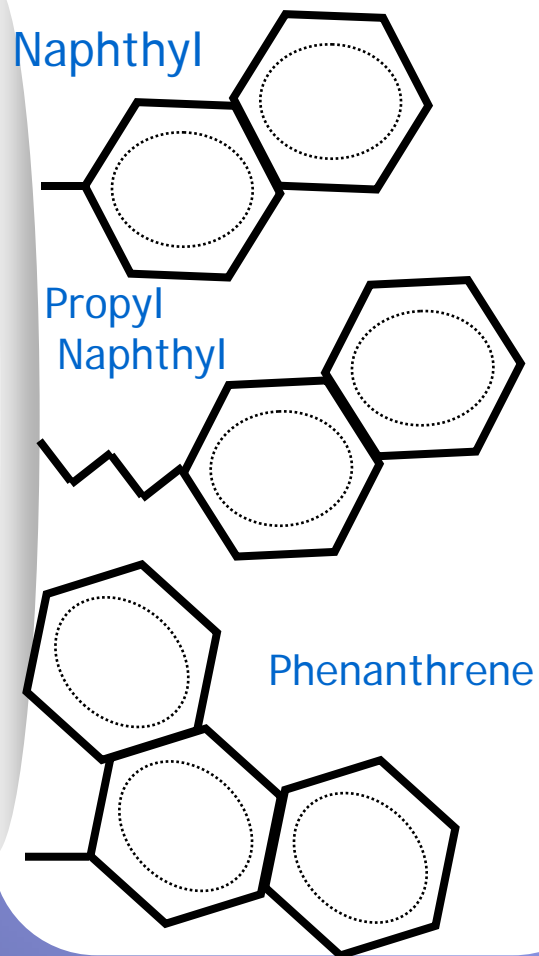


# Stationary Phase Ligands Investigated

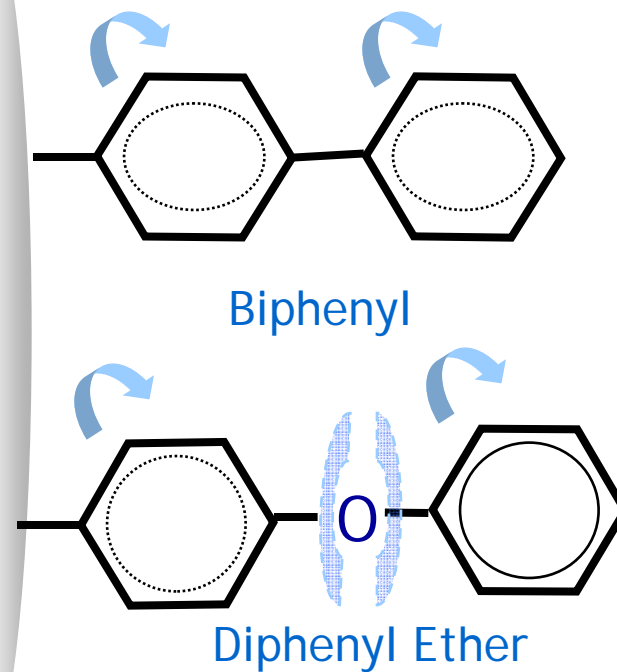
## Alkyl Phenyls



## Fused Ring Systems



## Non-Fused Ring Systems



# Testing Protocol

Determine:

- Increase in Non-Polar Retention
- Pi-Pi Interactions
- Shape Selectivity
- Effect of analyte Polarity on Retention
- Effect of an alkyl spacer
- Advantageous Use of Methanol versus Acetonitrile

# Experimental and Test Phases

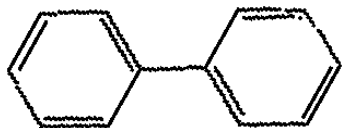
Phase	Carbon Load (%)
Monomeric C18	11
Polar Embedded Alkyl	7
Phenyl	5.3
Biphenyl	8.3
Diphenylether	8.4
Naphthalene	7.3
Propylnaphthalene	8.9
Phenanthrene	8.4

All Experimental phases were bonded to identical 5- $\mu\text{m}$  140 $\text{\AA}$  base-deactivated silica.

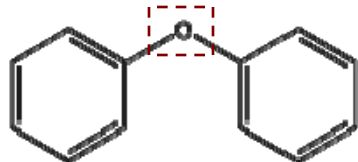
Phase	Pore Size ( $\text{\AA}$ )	Carbon Load (%)	Surface Area( $\text{m}^2/\text{g}$ )
Diphenyl	---	---	---
Phenyl Hexyl	100	17.5	400
Propyl Phenyl Ether	80	11	475
Allure <sup>TM</sup> Biphenyl	60	20	525

# Determining Hydrophobic Retention Biphenyl Test Probe

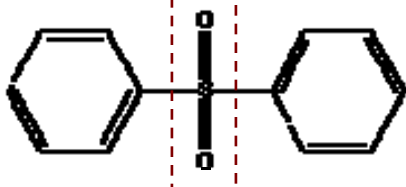
Biphenyl



Diphenyl Ether



Phenyl Sulfone



## Analysis of test probes on a biphenyl phase

**Pinnacle DB Biphenyl**

150 x 4.6mm 5um

30:70 0.1% FA in Water:

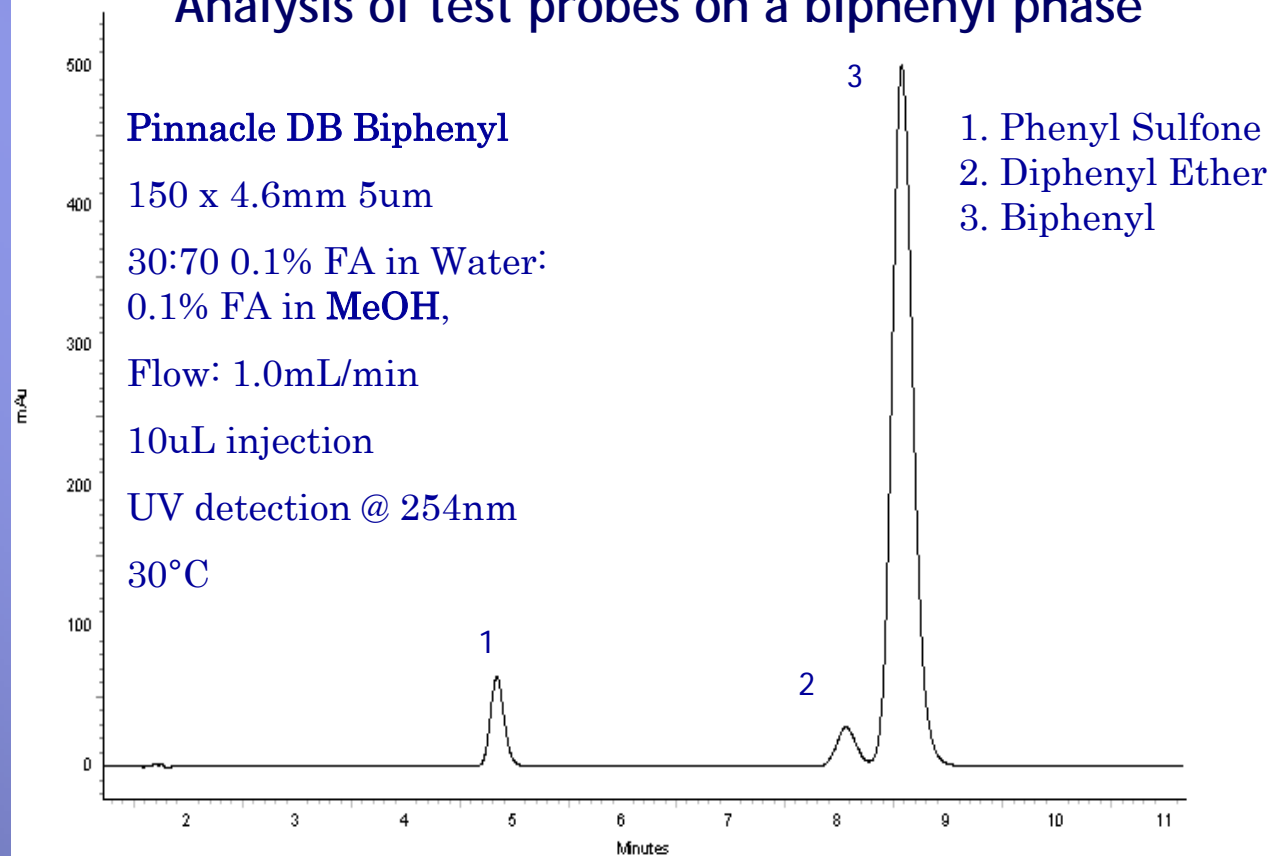
0.1% FA in MeOH,

Flow: 1.0mL/min

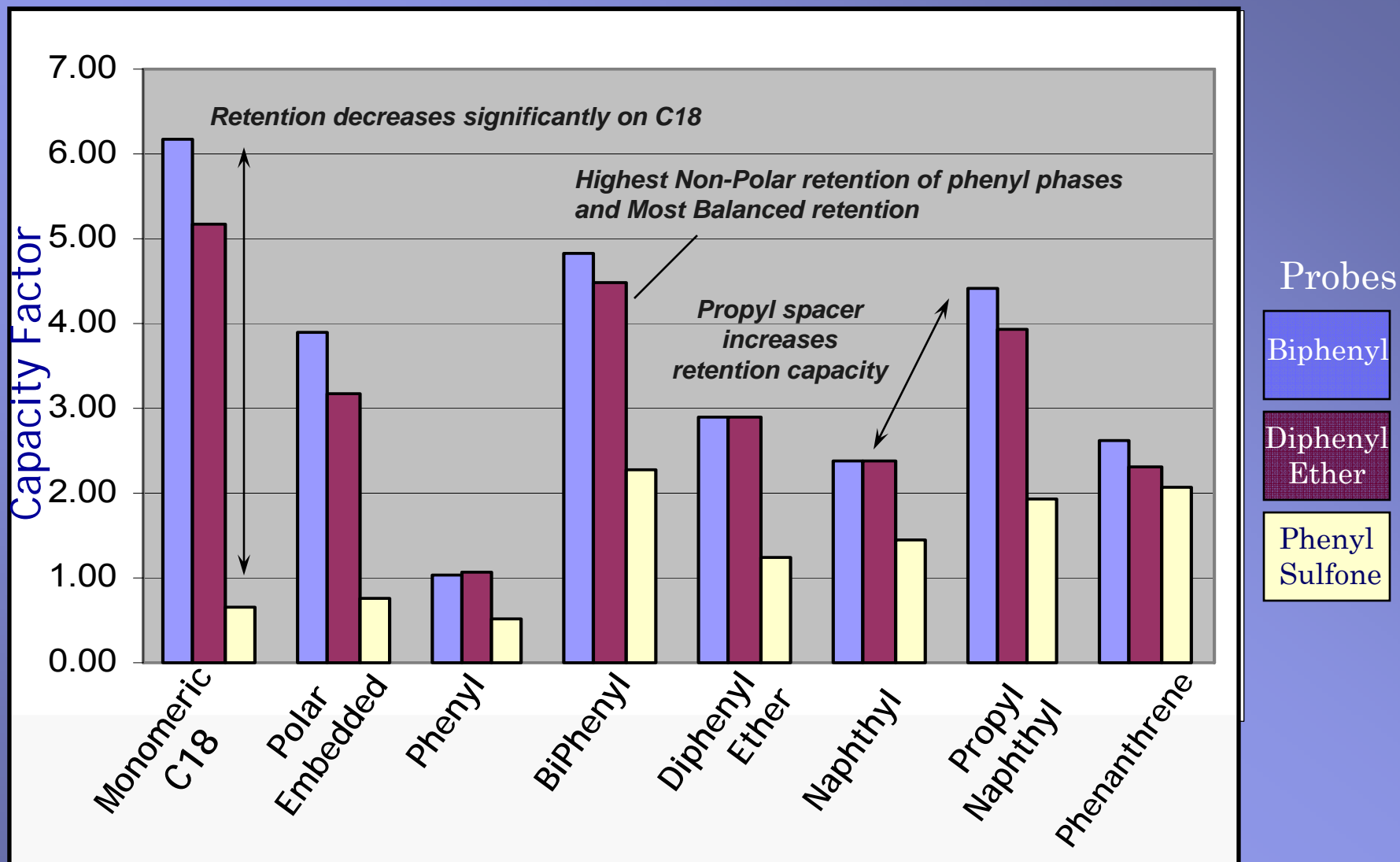
10uL injection

UV detection @ 254nm

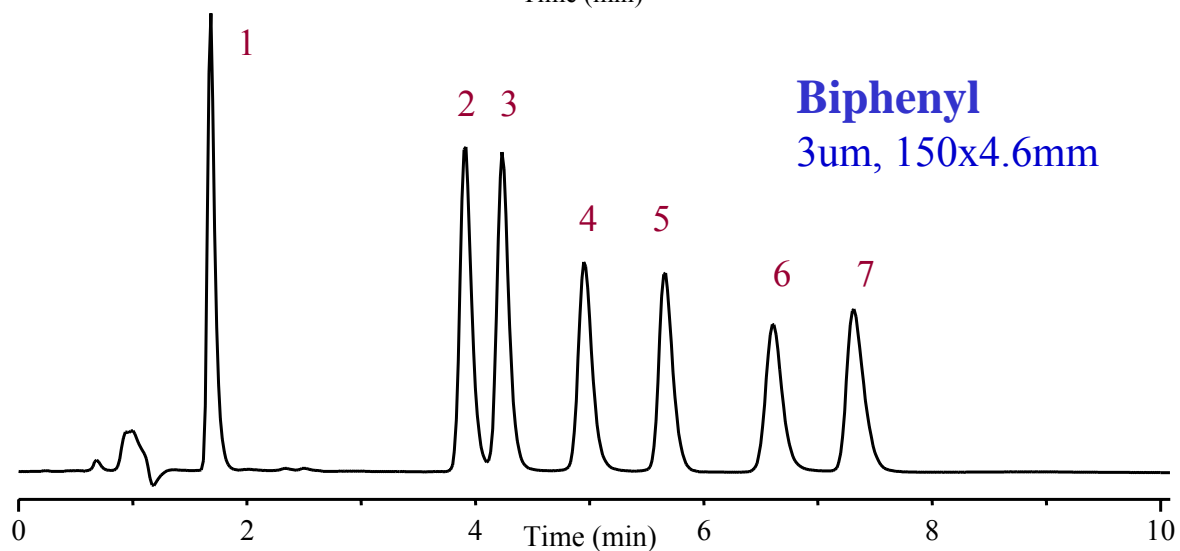
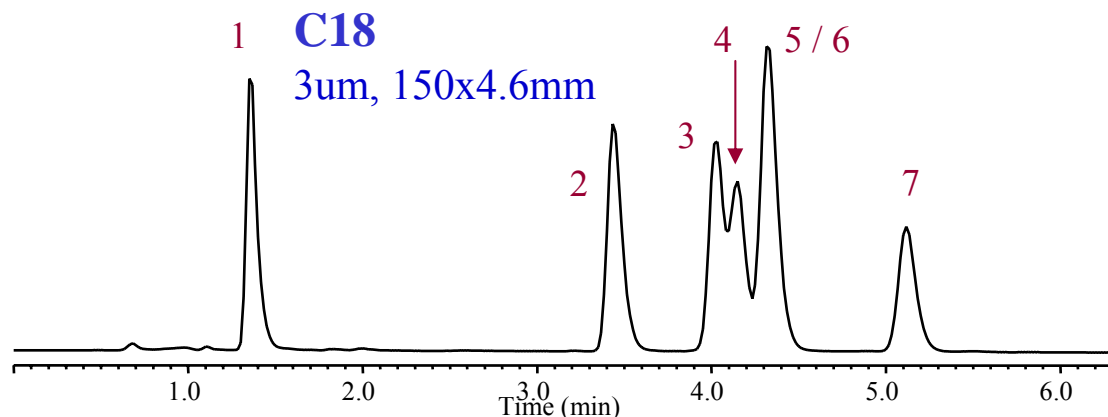
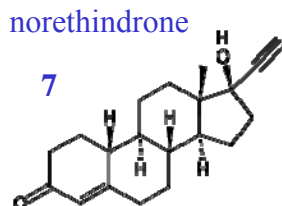
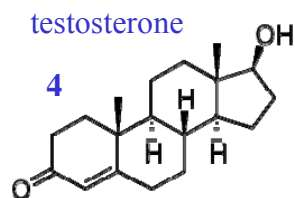
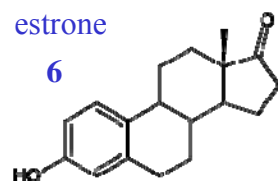
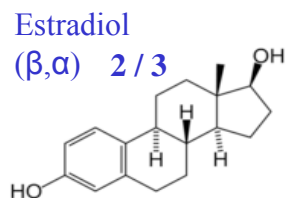
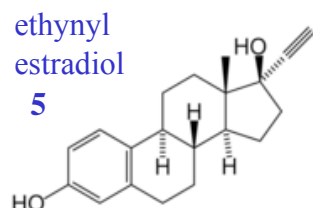
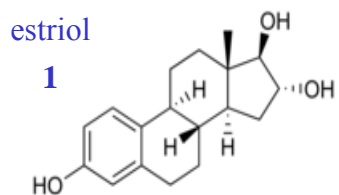
30°C



# k' Values for Aromatic Test Probes



# Biphenyl Retention and Selectivity Analysis of Steroids



50:50 Water:ACN,  
1.5 ml/min, Isocratic,  
UV @ 254 nm

# Biphenyl Selectivity : UHPLC Analysis of Steroids

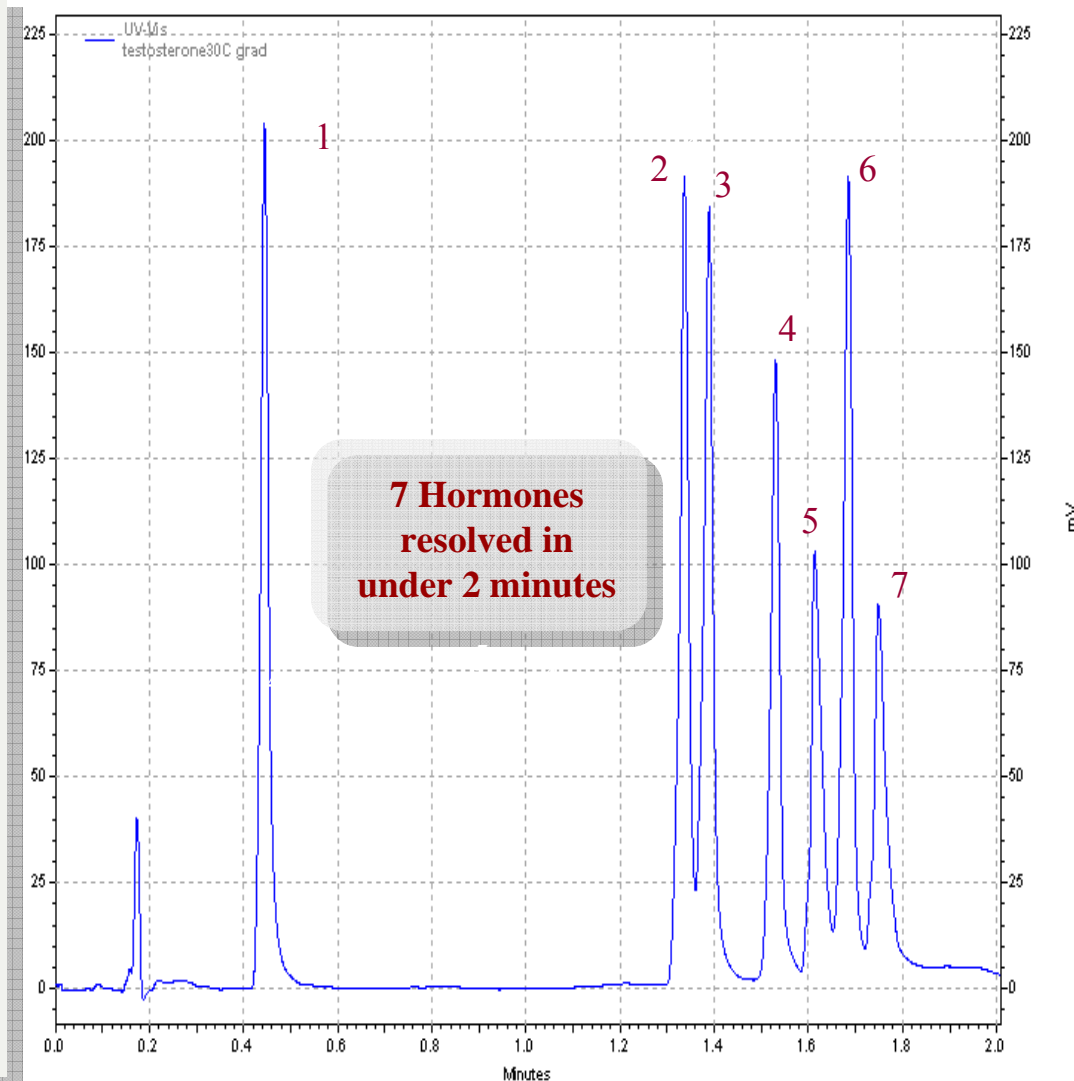
**Pinnacle DB Biphenyl 1.9 $\mu$ m,  
50x2.1mm**

A: Water  
B: Acetonitrile

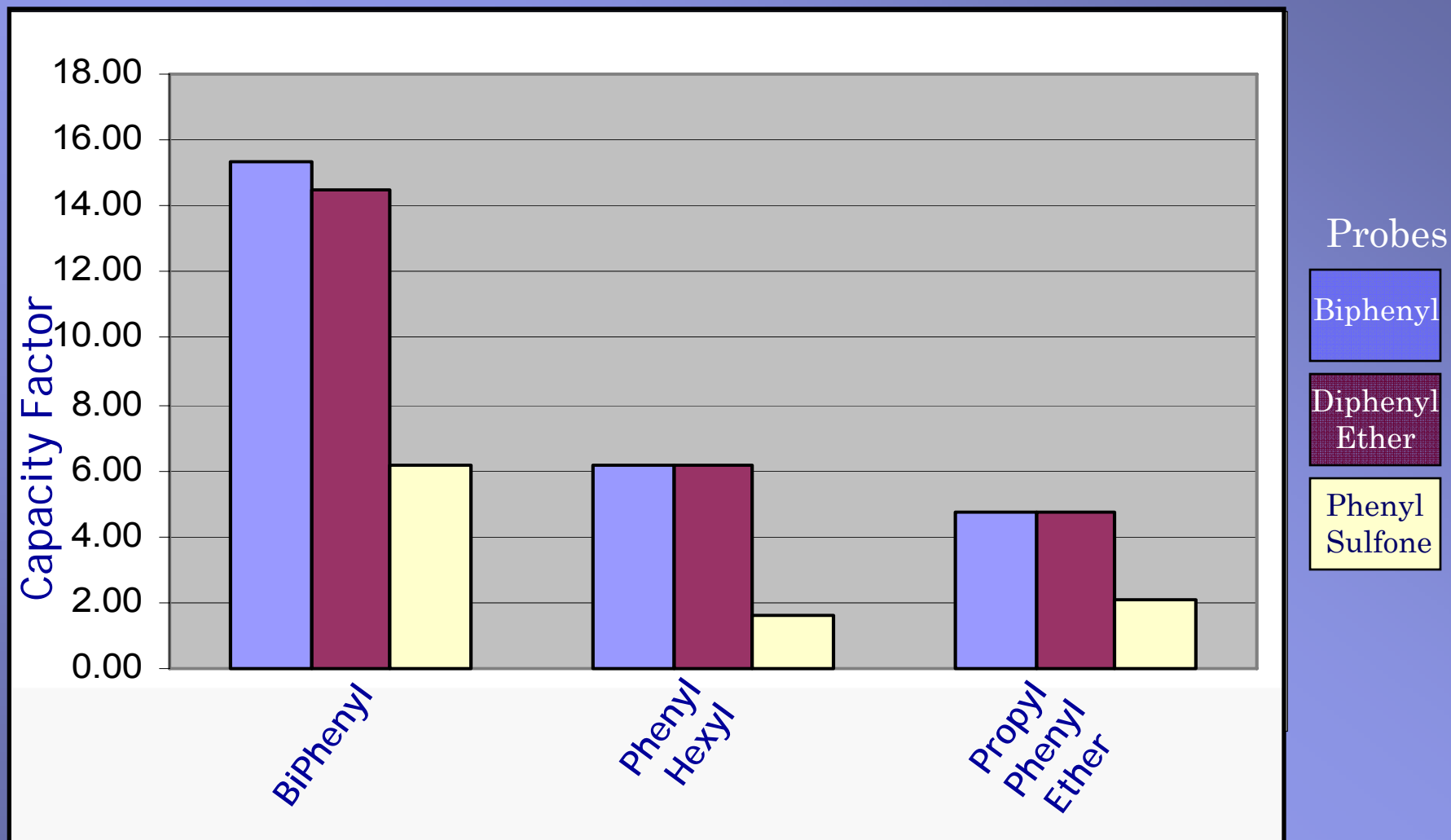
Time (min.)	%A	%B
0	70	30
1	70	30
3	30	70

Flow: 0.8 mL/min  
Temp.: 30°C  
Det.: UV @ 220 nm  
1  $\mu$ L injection, ~100 $\mu$ g/mL each

1. estriol
2. 17 $\beta$ -estradiol
3. 17 $\alpha$ -estradiol
4. ethynyl estradiol
5. testosterone
6. estrone
7. norethindrone



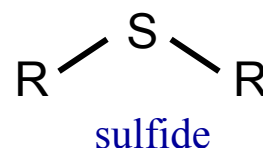
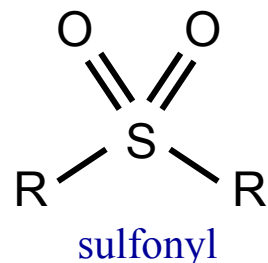
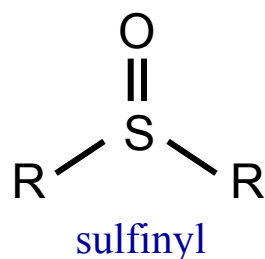
# k' Values for Aromatic Test Probes different "tether" chemistry



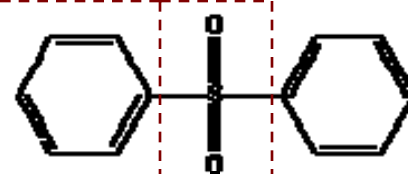
# Selectivity Towards Specific Functional Groups

## Sulfur Groups – Retention of Sulfonyl

### Common Sulfur Moieties

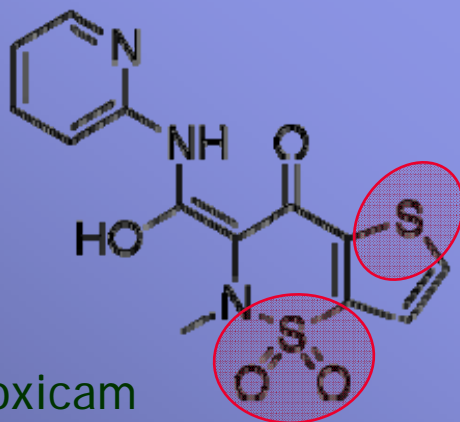


### Test Probe

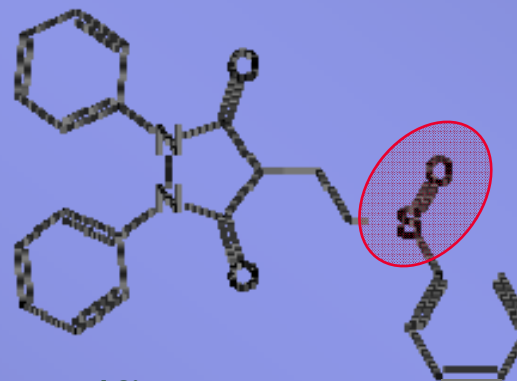


Phenyl Sulfone

Application Examples: NSAIDs

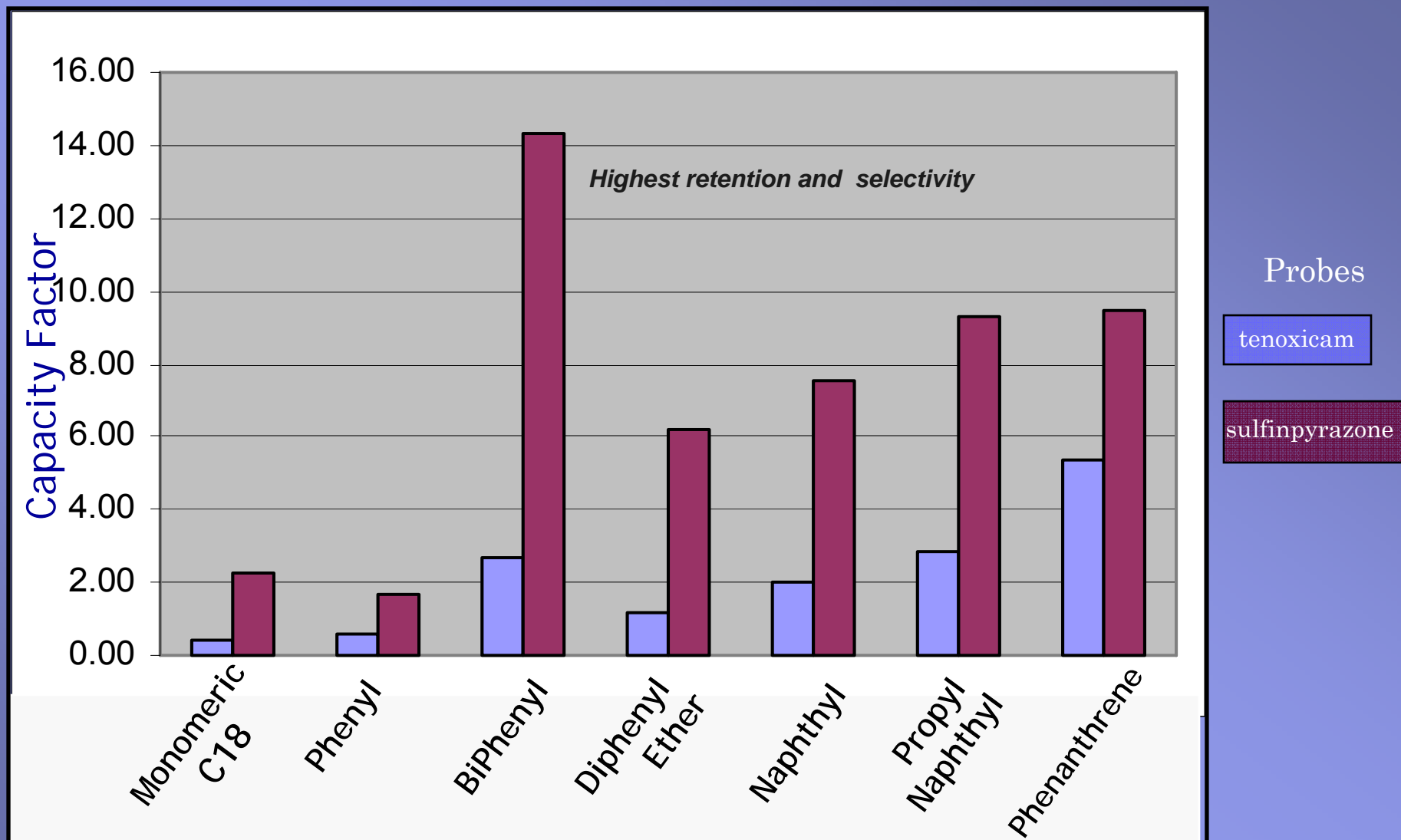


tenoxicam



sulfinpyrazone

# k' Values for Tenoxicam and Sulfinpyrazine



# Biphenyl Selectivity : Acetonitrile

Mobile Phase – 50 : 50 0.5% Formic acid in water (pH 2.25): **ACN** 0.1% formic acid

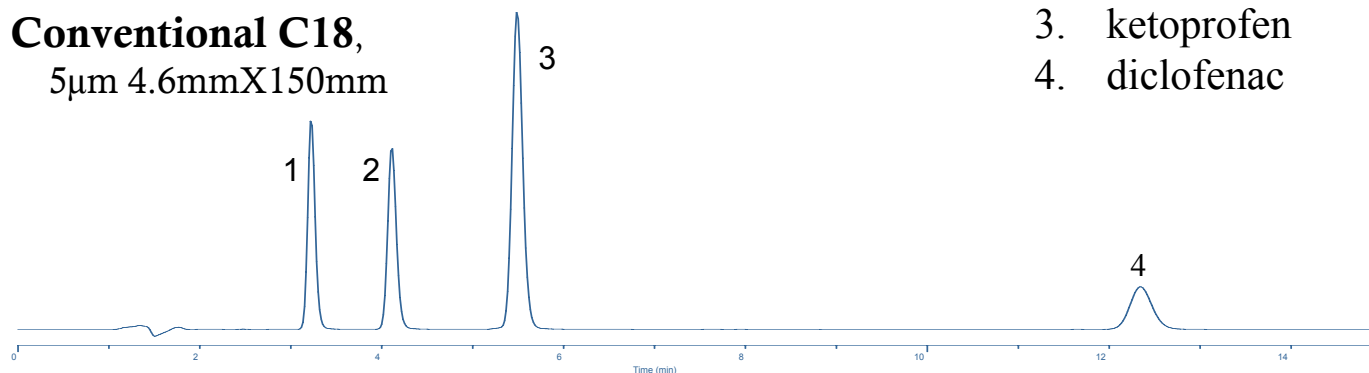
Flow rate: 1.0 ml/min, Isocratic

UV detection @ 254 nm

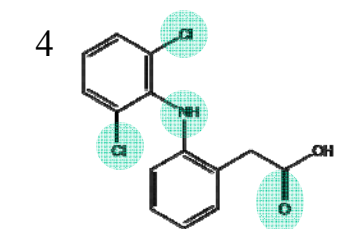
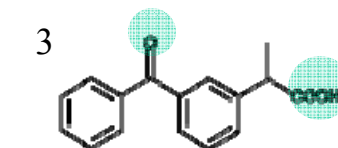
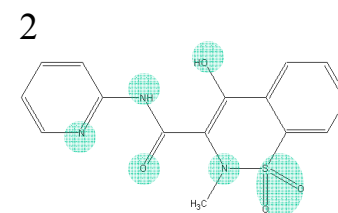
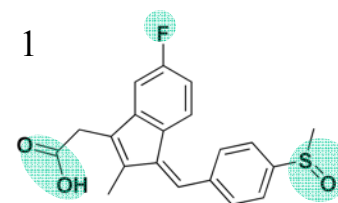
Temperature: Ambient

Sample Volume: 5µl of 100µg/ml

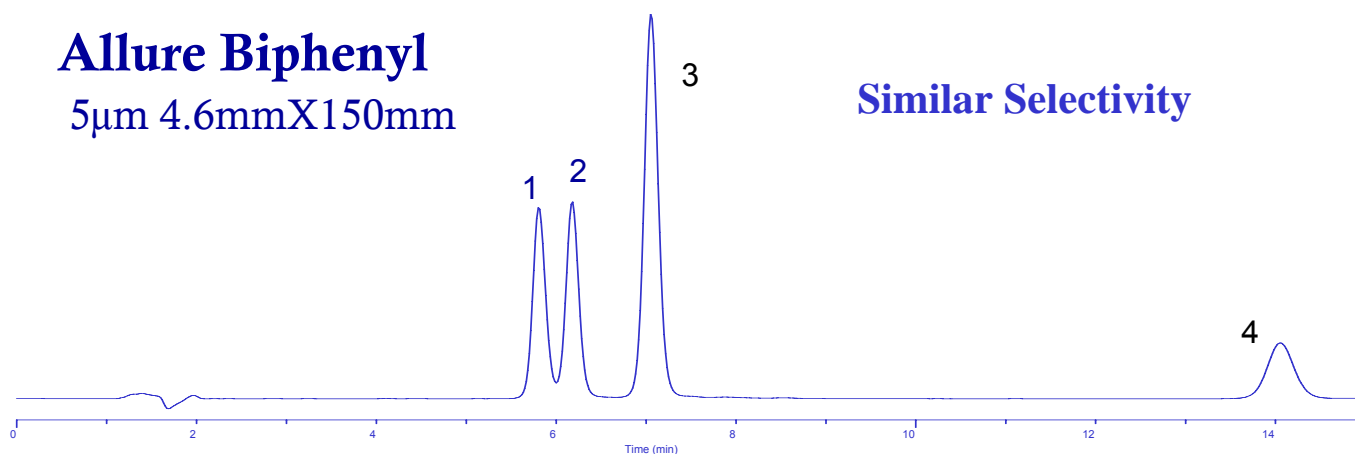
**Conventional C18,**  
5µm 4.6mmX150mm



1. sulindac
2. piroxicam
3. ketoprofen
4. diclofenac



**Allure Biphenyl**  
5µm 4.6mmX150mm



**Similar Selectivity**

# Biphenyl Selectivity : Methanol

Mobile Phase – 0.5% Formic acid in water (pH 2.25): **MeOH** 0.1% formic acid

Flow rate: 1.0 ml/min, Isocratic

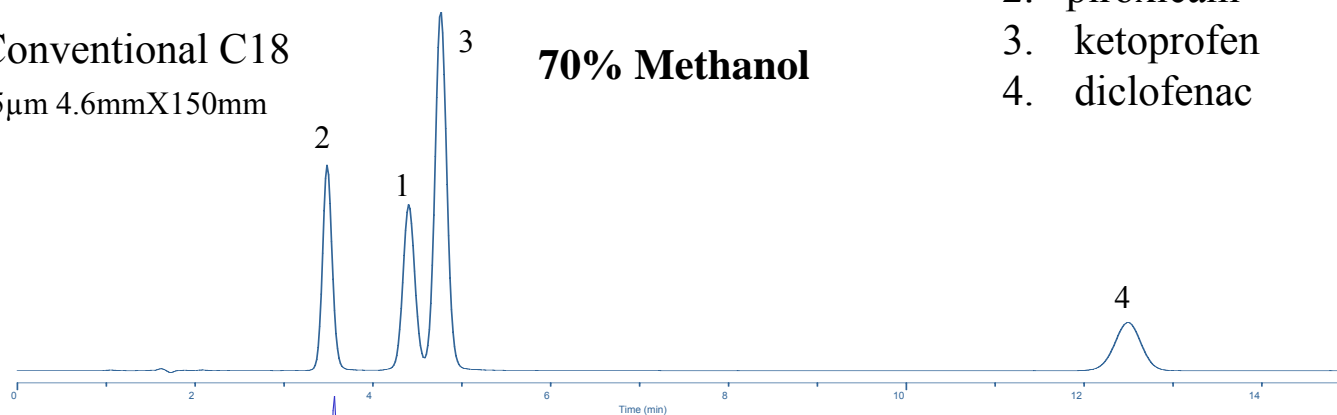
UV detection @ 254 nm

Temperature: Ambient

Sample Volume: 5µl of 100µg/ml

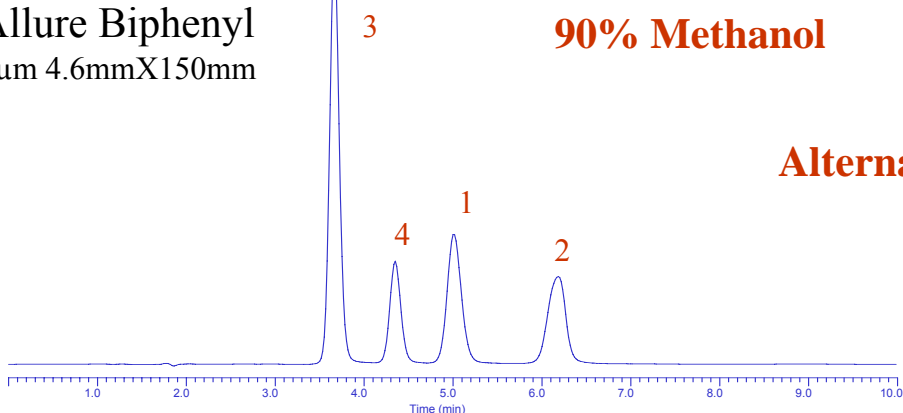
Conventional C18

5µm 4.6mmX150mm

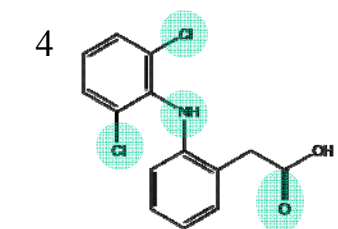
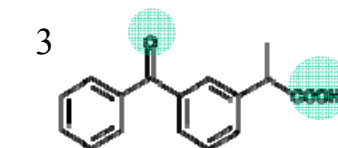
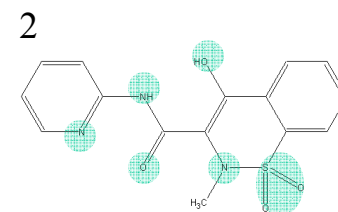
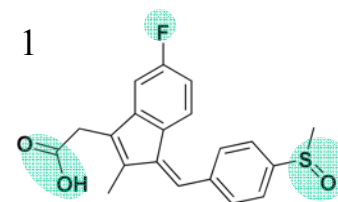


Allure Biphenyl

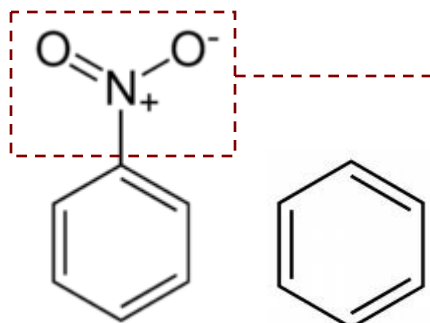
5µm 4.6mmX150mm



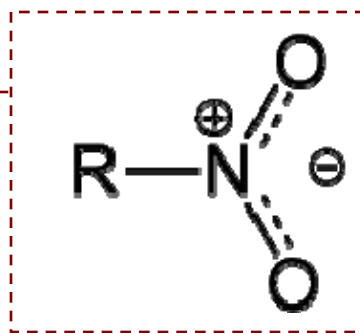
1. sulindac
2. piroxicam
3. ketoprofen
4. diclofenac



# Selectivity Towards Electron Withdrawing Groups



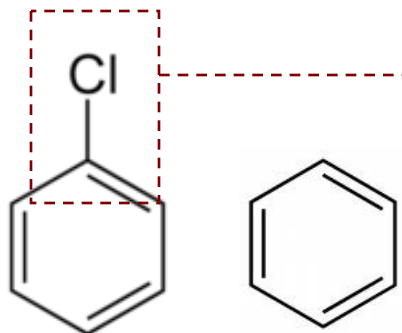
nitrobenzene    benzene



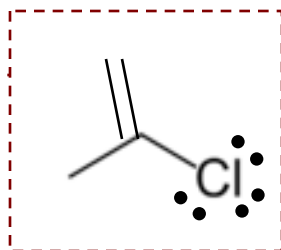
*Strongly  
electron withdrawing*

$\alpha$

Benzene/Nitrobenzene



Chlorobenzene    benzene



*Weakly  
electron withdrawing*

$\alpha$

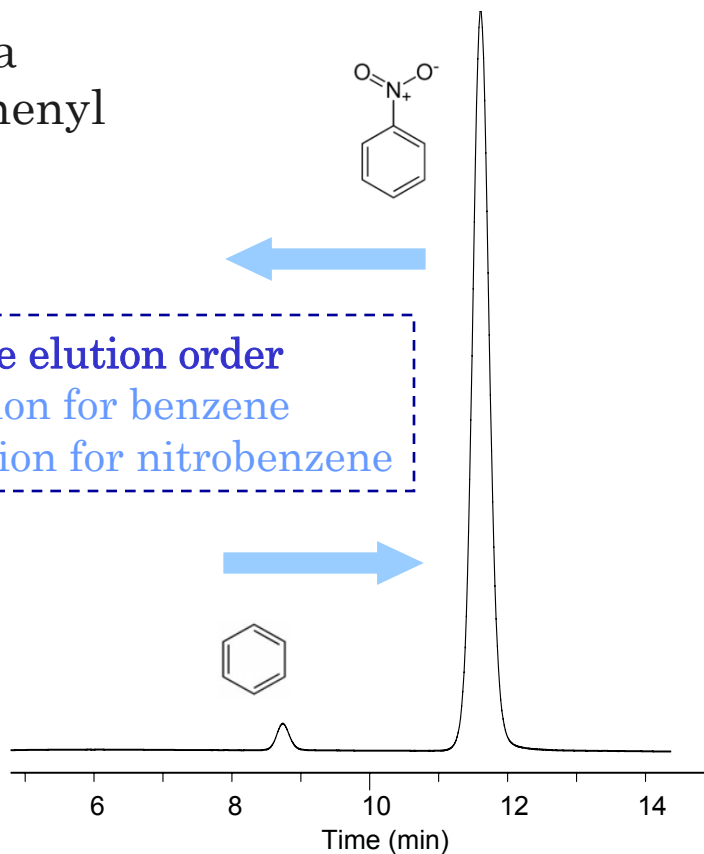
Benzene/Chlorobenzene

# Selectivity Towards Electron Withdrawing Groups Test Probe Analysis

Elution order on a  
Pinnacle DB Biphenyl

**C18 shows opposite elution order**

- increased retention for benzene
- decreased retention for nitrobenzene

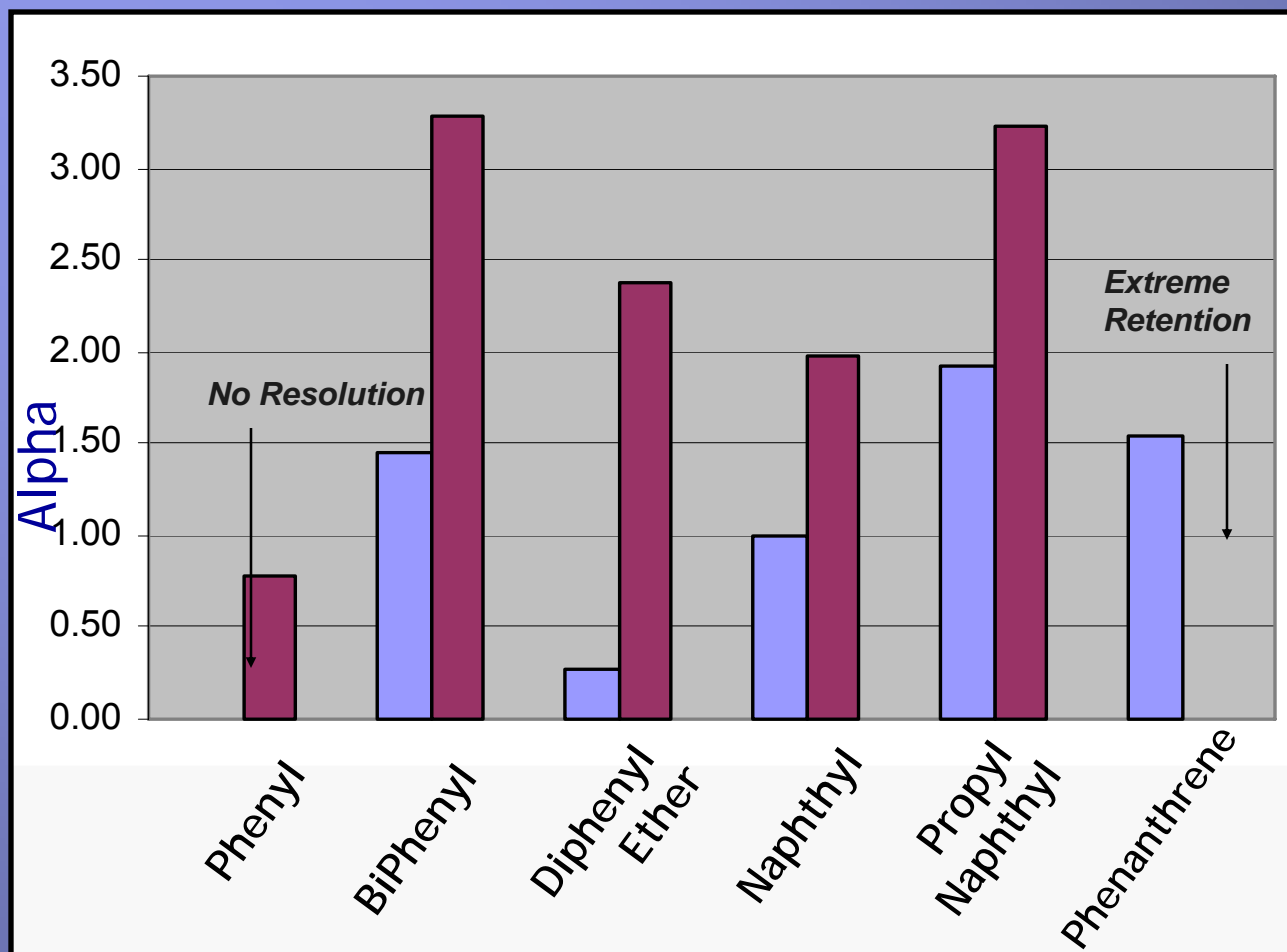


Pinnacle DB Biphenyl  
150 x 4.6mm 5 $\mu$ m  
40:60 Water: MeOH,  
Flow: 1.0mL/min  
10 $\mu$ L injection  
UV detection @ 254nm  
30°C

# Selectivity Towards Electron Withdrawing Groups

$\alpha$  Benzene/Nitrobenzene and

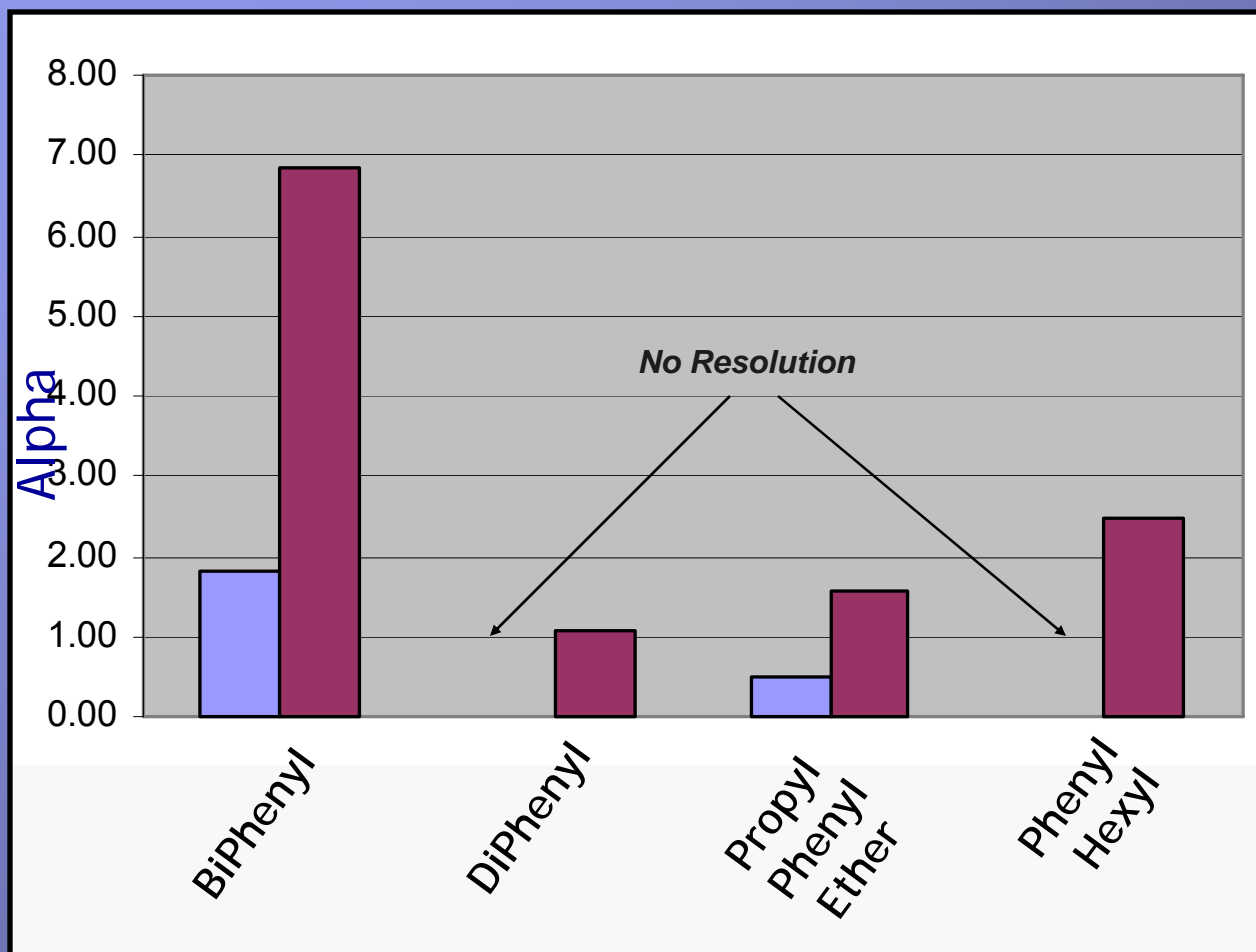
$\alpha$  Benzene/Chlorobenzene



# Selectivity Towards Electron Withdrawing Groups

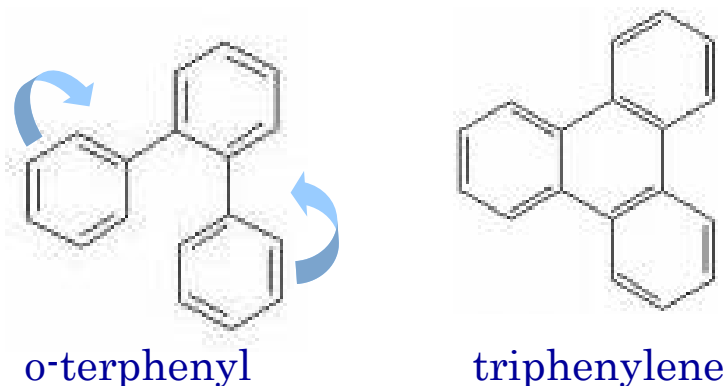
$\alpha$  Benzene/Nitrobenzene and

$\alpha$  Benzene/Chlorobenzene



# Selectivity Towards Shape

## $\alpha$ o-terphenyl/triphenylene (Tanaka)



Column Dimensions: 150 x 4.6mm 5 $\mu$ m

Pore Size 140Å

MPA = Water , MPB = Methanol,

80%B Isocratic

Flow: 1.0mL/min, 5 $\mu$ L injection,

254nm,

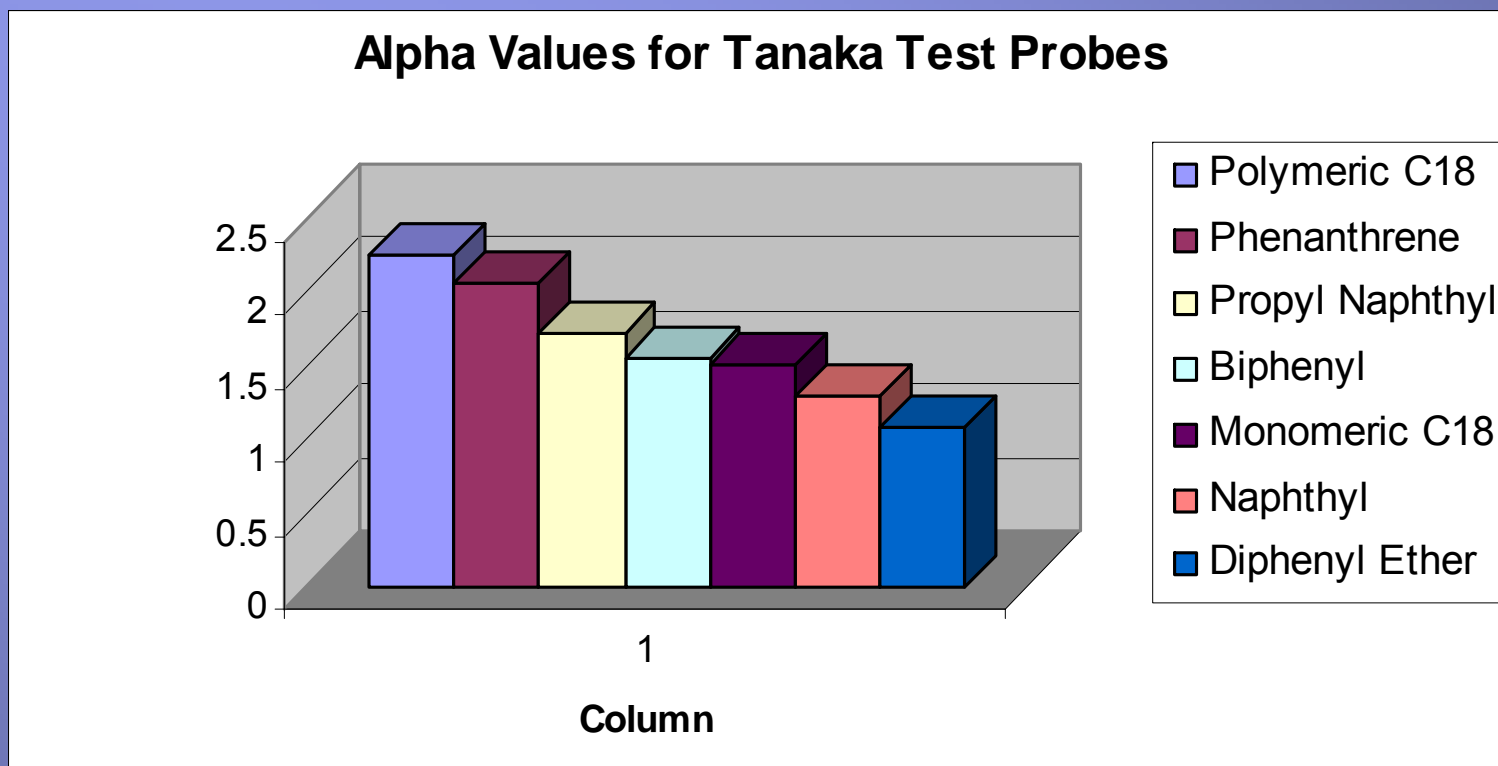
40°C

Phase	$\alpha_{o/t}$
Polymeric C18	2.25
Phenanthrene	2.05
Propyl Naphthyl	1.71
Biphenyl	1.55
Monomeric C18	1.51
Naphthyl	1.3
Diphenyl Ether	1.08

Reduced  $k'$  of 50%  
observed with 10°C  
increase from 30°C to  
40°C

# Selectivity Towards Shape

## $\alpha$ o-terphenyl/triphenylene (Tanaka)



# Column Test Probe Rankings

	k'	k'	k'	k'	k'	$\alpha$	$\alpha$	$\alpha$	$\alpha$	$\alpha$
	Biphenyl	Diphenyl Ether	Phenyl Sulfone	Nitro benzene	Chloro-benzene	Tanaka	Nitro	Chloro	Sulfone	DPE
Phenyl 	6	6	6	6	6	NA	6	6	6	NR
Biphenyl 	1	1	1	1	2	3	3	2	1	2
Diphenyl Ether 	3	3	5	5	4	5	5	4	3	NR
Naphthyl 	5	4	4	3	5	4	4	5	4	NR
Propyl Naphthyl 	4	5	2	2	3	2	1	3	5	3
Phenanthrene 	2	2	3	4	1	1	2	1	2	1
Biphenyl 						1	1	1	1	
Diphenyl 						NR	4	NA	NA	
Phenyl Hexyl 						NR	3	2	NR	
Propyl Phenyl Ether 						NR	2	3	NR	

NR = No Resolution  
NA = Not assayed

# HPLC Conclusions

- Fused ring systems did not yield as good of general purpose properties, though they did give a measure of shape selectivity
- Use of aliphatic spacer in fused ring systems improved shape selectivity and phase load (~1.5% increase)
- Biphenyl ligand gives best retention and selectivity as “general purpose” RPLC column for moieties studied
- Biphenyl allows for additional mechanisms of solvation, relative to C18 for functionalized molecules
- Biphenyl allowed for alternative selectivity to be expressed by varying the mobile phase composition

# Reality of the GC Chromatography Market

- Knowledge level of end users decreasing
  - Most users have not had a single course in separations
- Column offerings generally limited
  - Manufacturers produce what sells
- 85% of separations are performed on 1's, 5's or wax columns
- Benefits of MS have often lead to compromised chromatography

# What Does the Industry Need?

- Low Bleed?
- “MS” Phases?
- Inertness !
  - Professor Walt Jennings, ISCC Conference 2004
  - Jaap has just addressed.
- New Selectivity Phases !
  - Targeted analyses
  - GCxGC applications

# Chlorinated Pesticides Target Analysis

2,4,5,6-tetrachloro-m-xylene

alpha HCH

gamma HCH

beta HCH

delta HCH

heptachlor

aldrin

heptachlor epoxide

gamma chlordane

alpha chlordane

4,4'-DDE

endosulfan I

dieldrin

endrin

4,4'-DDD

endosulfan II

4,4'-DDT

endrin aldehyde

methoxychlor

endosulfan sulfate

endrin ketone

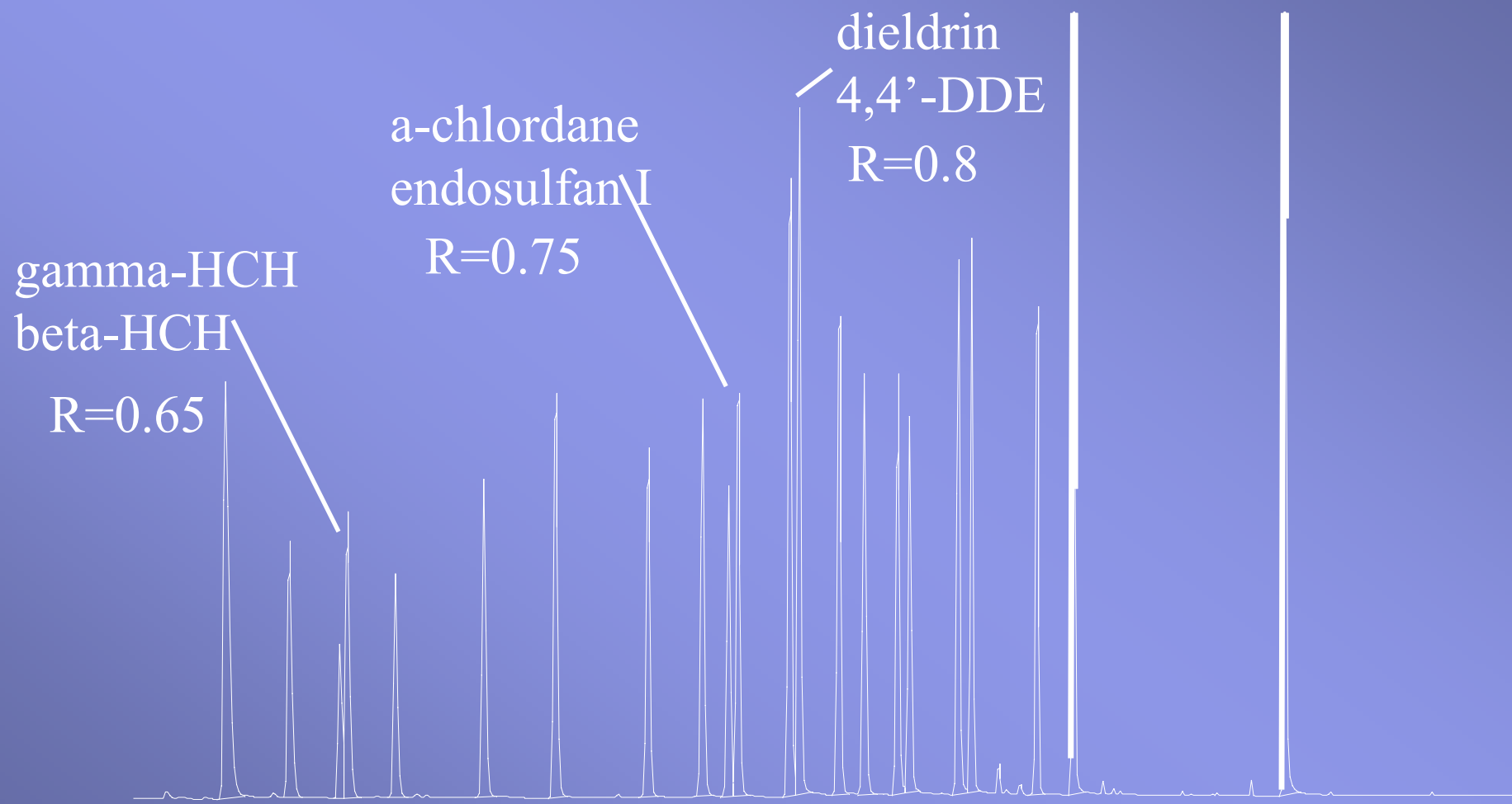
decachlorobiphenyl

## Column Selection?

-5, -35, -1701, many others...

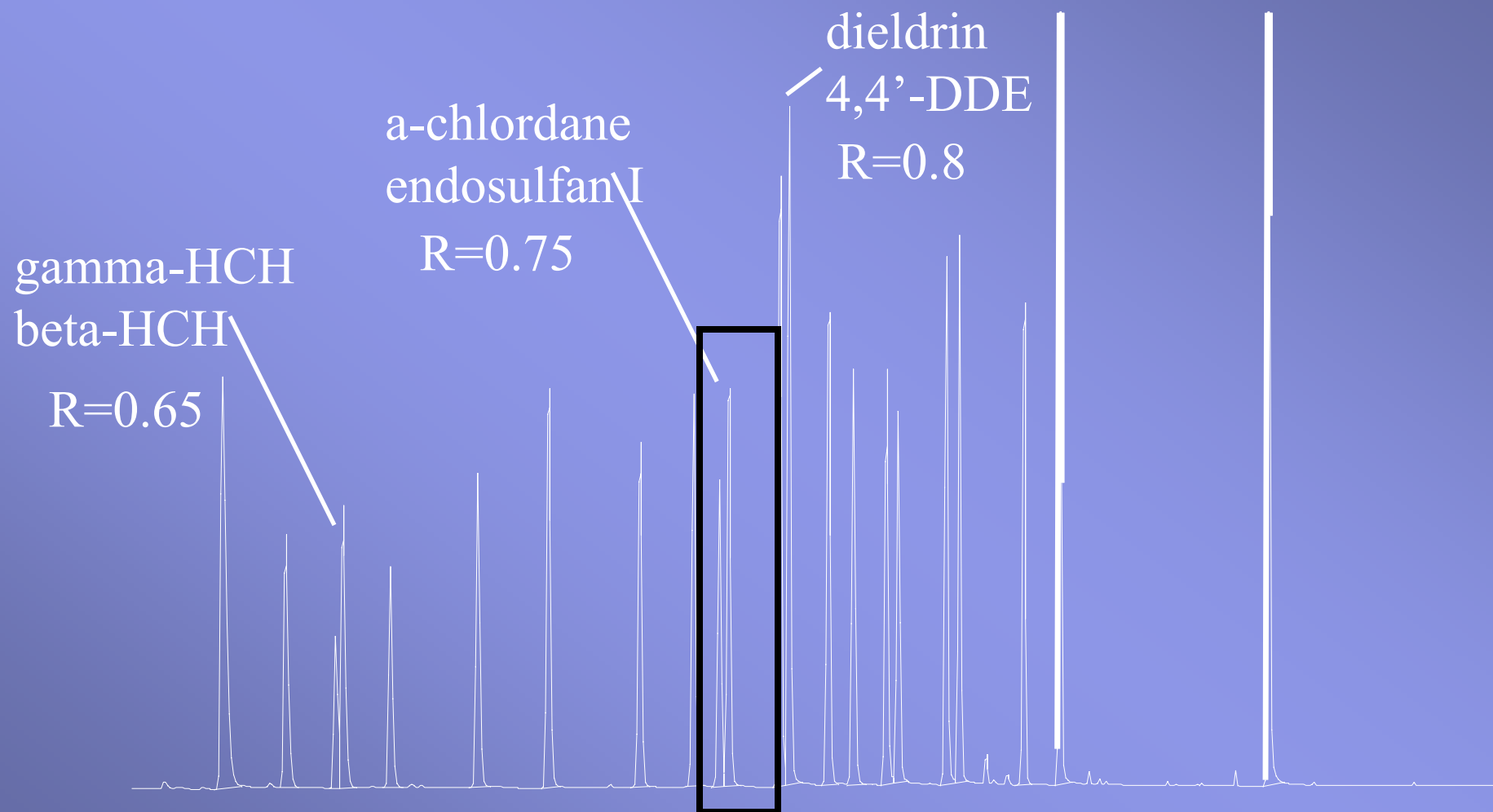
# Chlorinated Pesticide Analysis

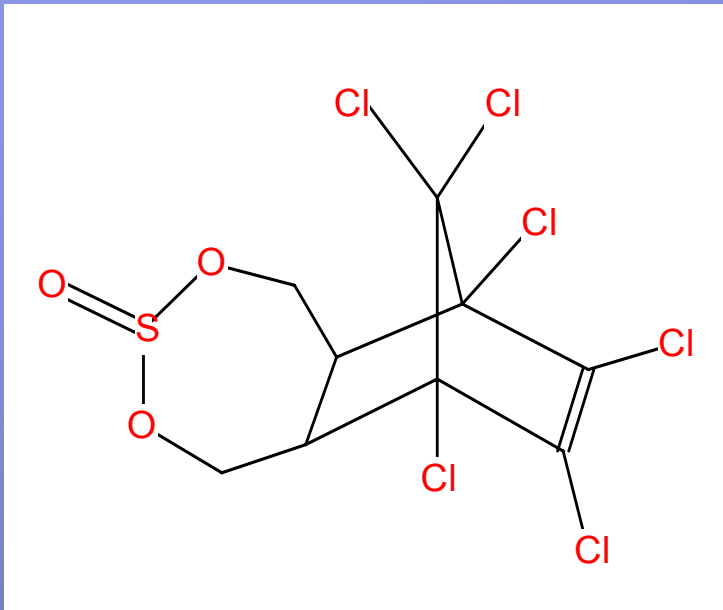
## Rtx<sup>®</sup>-5 Column



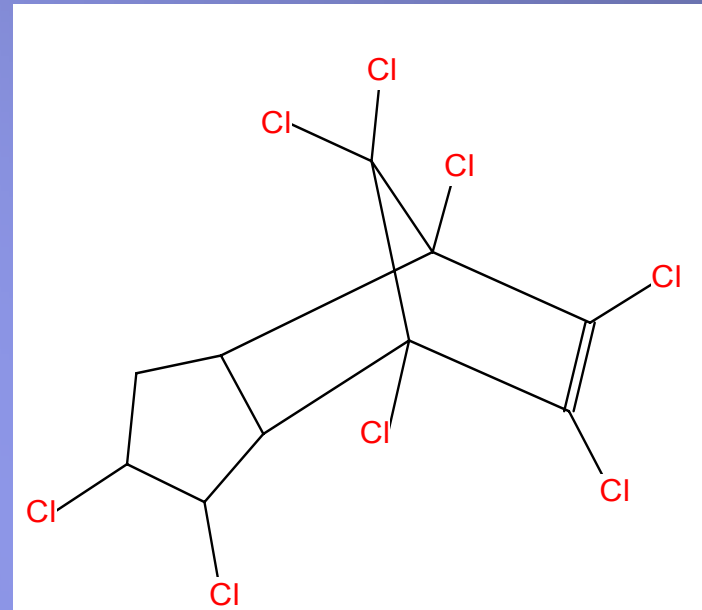
# Chlorinated Pesticide Analysis

## Rtx<sup>®</sup>-5 Column



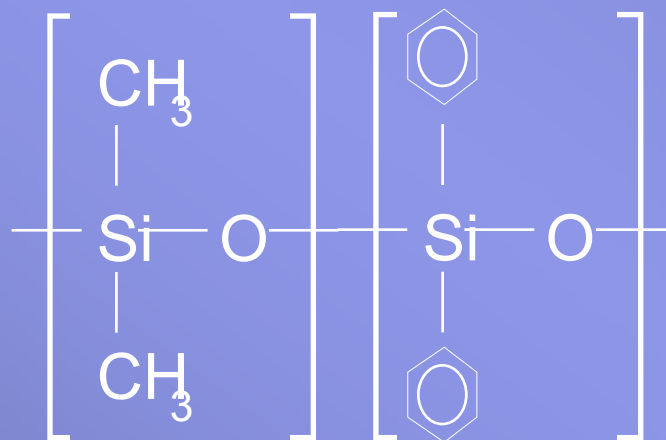


Endosulfan



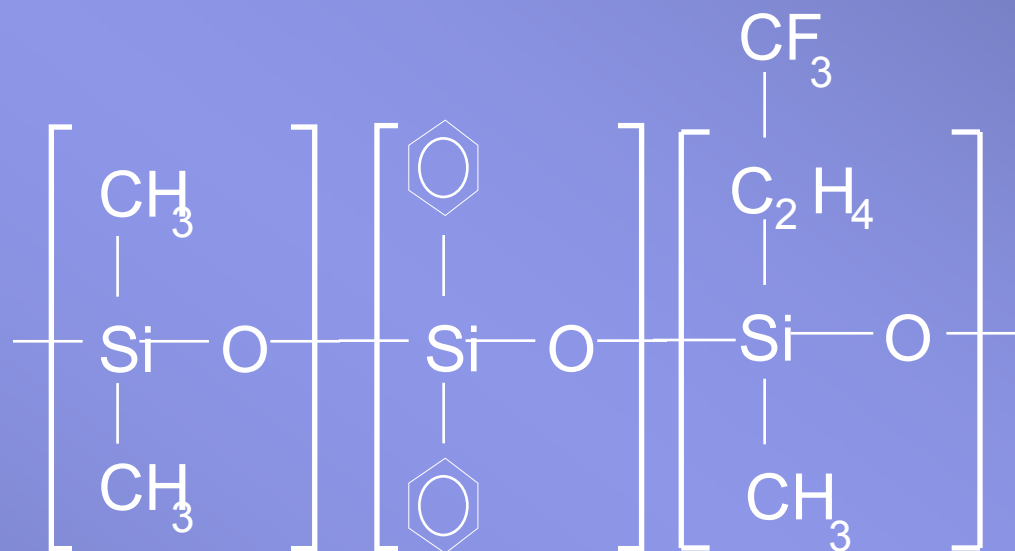
Chlordane

# Which should be more selective?



-5, -35, etc

-OR-



(*Anal. Chem.* **74**(9), 2133-2138, 2002.)

# Alpha chlordane and Endosulfan I

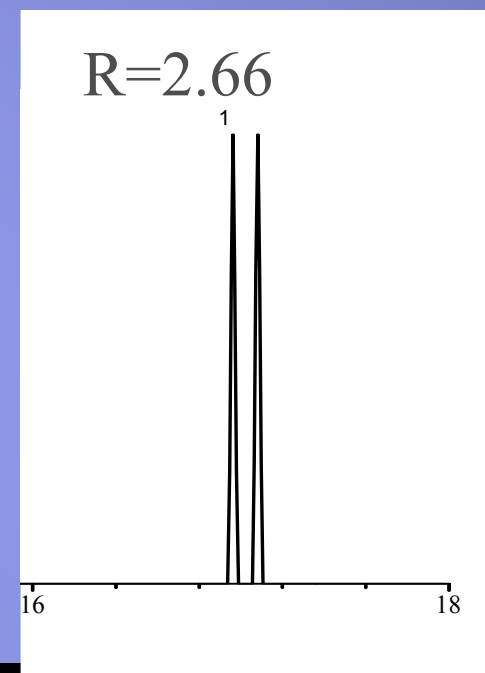
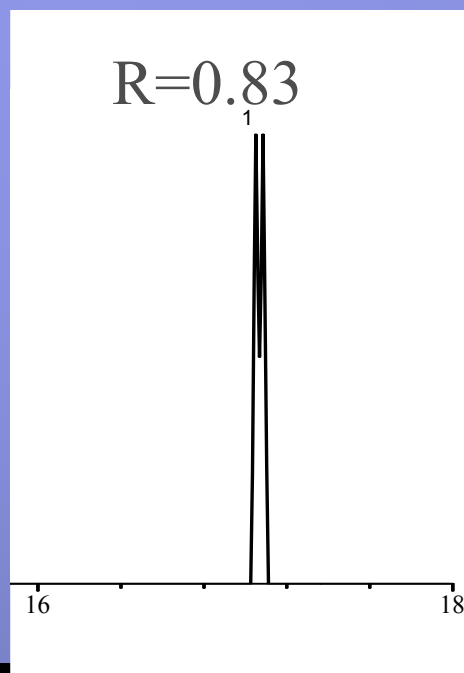
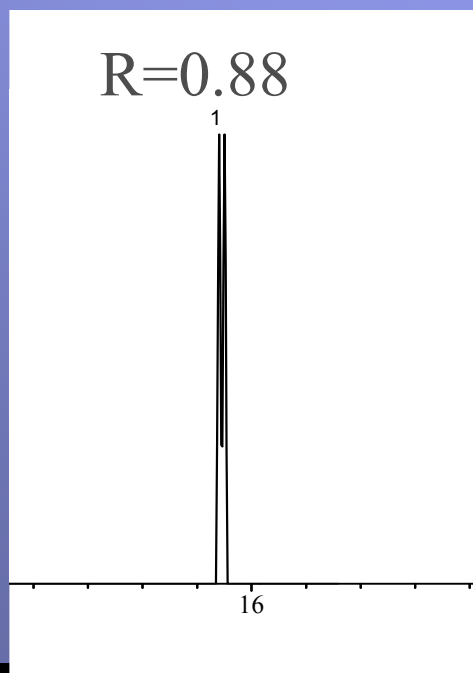
30 M X 0.25 mm i.d. X 0.25 um d.f.

1.0 mL/min, 100 C (1), 10 C/min to 300C

Rtx-5

Rtx-35

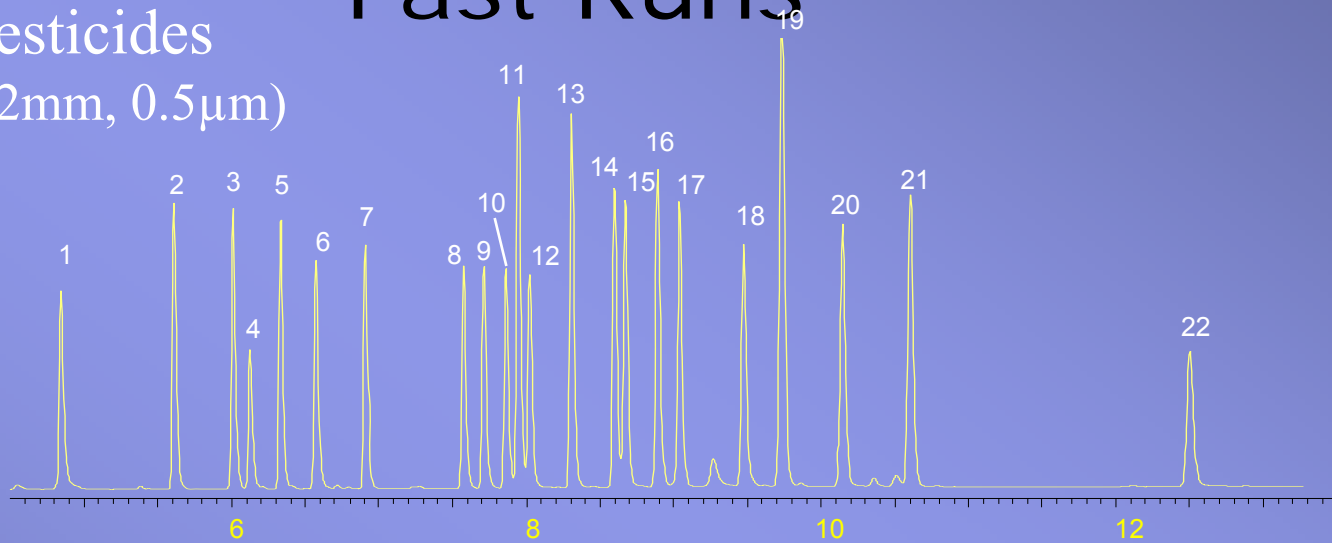
Rtx-CLP2



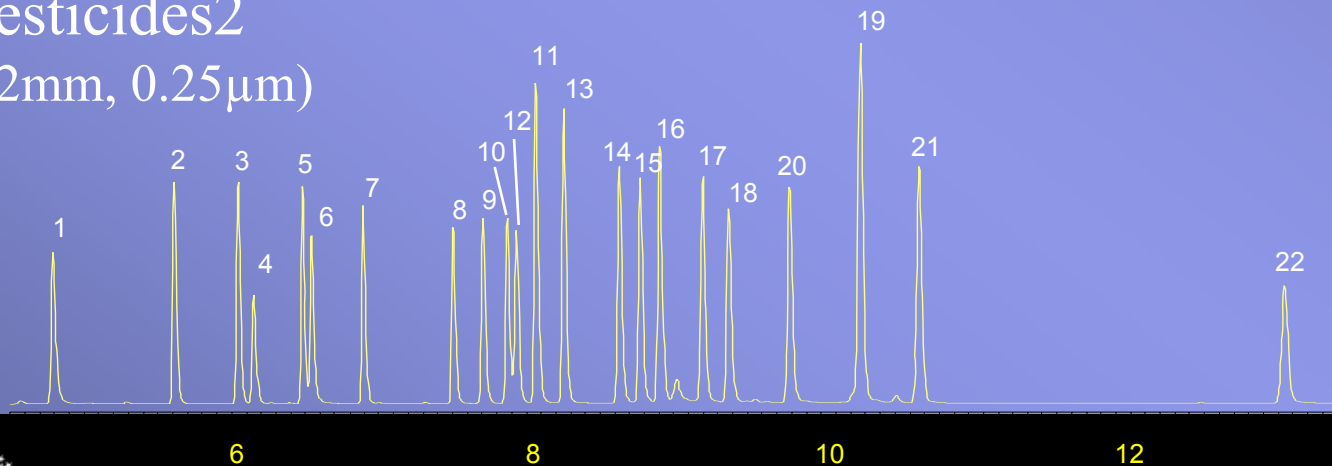
# Chlorinated Pesticides

## Fast Runs

Rtx-CLPesticides  
(30m x 0.32mm, 0.5 $\mu$ m)



Rtx-CLPesticides2  
(30m x 0.32mm, 0.25 $\mu$ m)



# Organochlorine Pesticides Summary

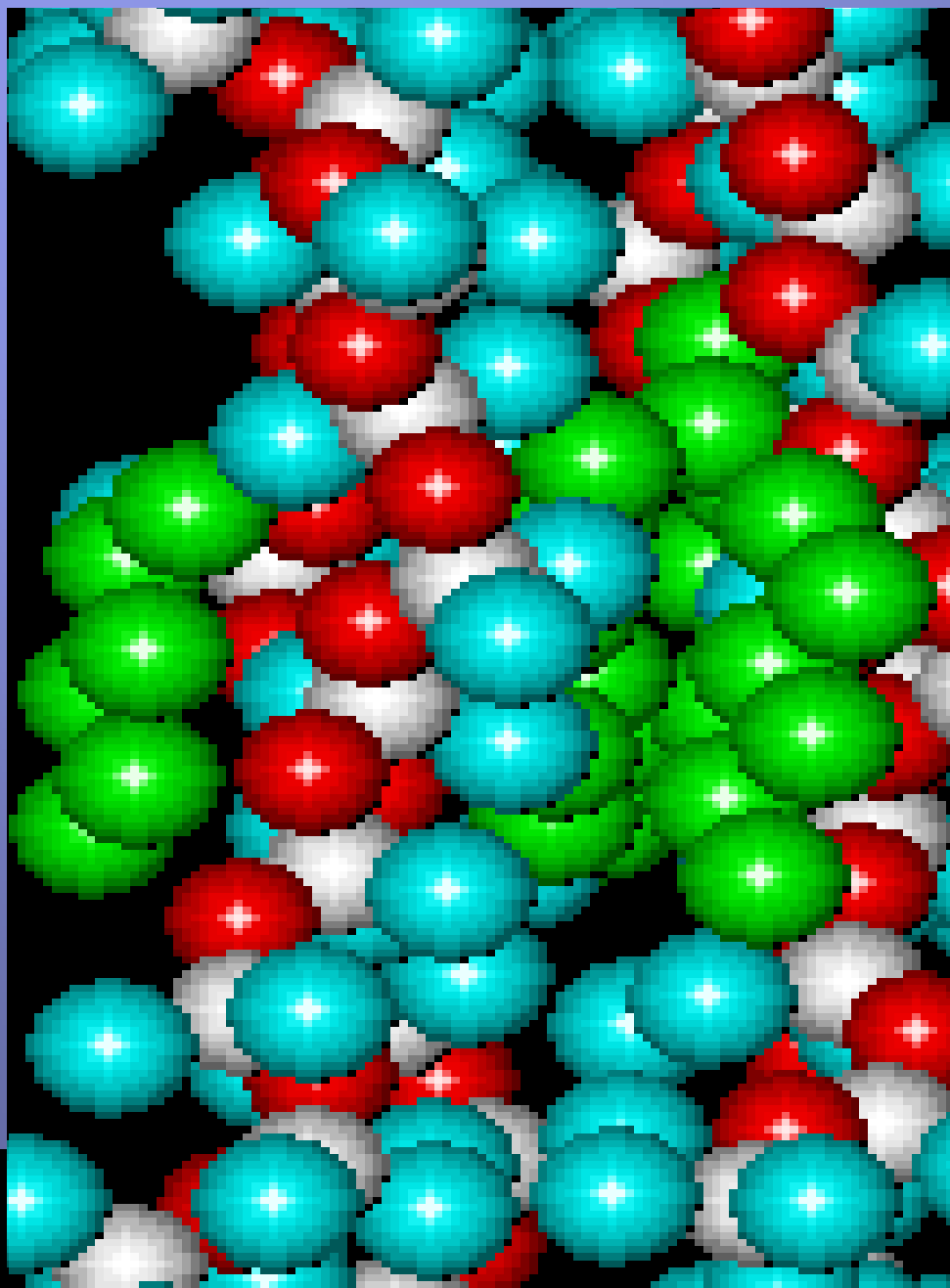
- Stationary phase selectivity dramatically improves separation of targets
  - Not really a surprise, but most users don't consider stationary phase structure
- Works well in many different column dimensions
  - Does not rely largely on peak width to achieve resolution of target compounds
    - Resolution driven from alpha, not capacity factor or plates

# What is in the Future?

- Predictive modeling to develop new phases in the absence of laboratory data
  - Using MD simulations, we can “calculate”  $\Delta G$
  - We can use this  $\Delta G$  to then predict retention times of compounds on a phase under various chromatographic conditions.

# Simplified Statistical Approach

- Partition coefficient determined (A.Z. Panagiotopoulos)
  - Define unit volume
  - Add analyte molecule
  - Add polymer
  - Determine presence of analyte in sub-unit volume when polymer is and is not present
  - Difference is  $K_D$
  - Perform at two temperatures to obtain retention indices ( $\Delta H$  and  $\Delta S$ )

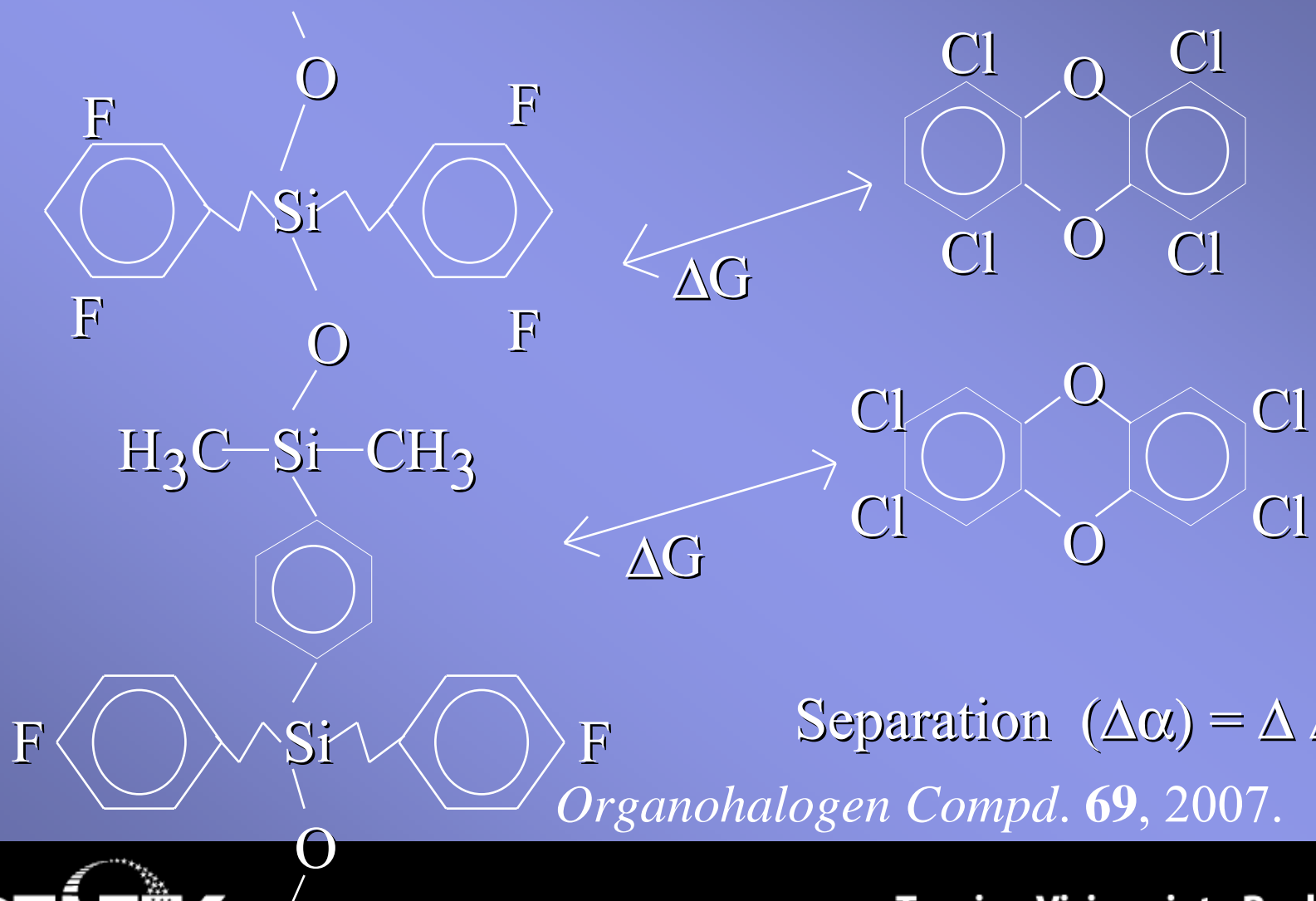


Example Result:  
Demeton-O on  
PDMS:

Observed  $\Delta G =$   
 $-1.14E4$  J/mol

Calculated  $\Delta G =$   
 $-1.13E4$  J/mol

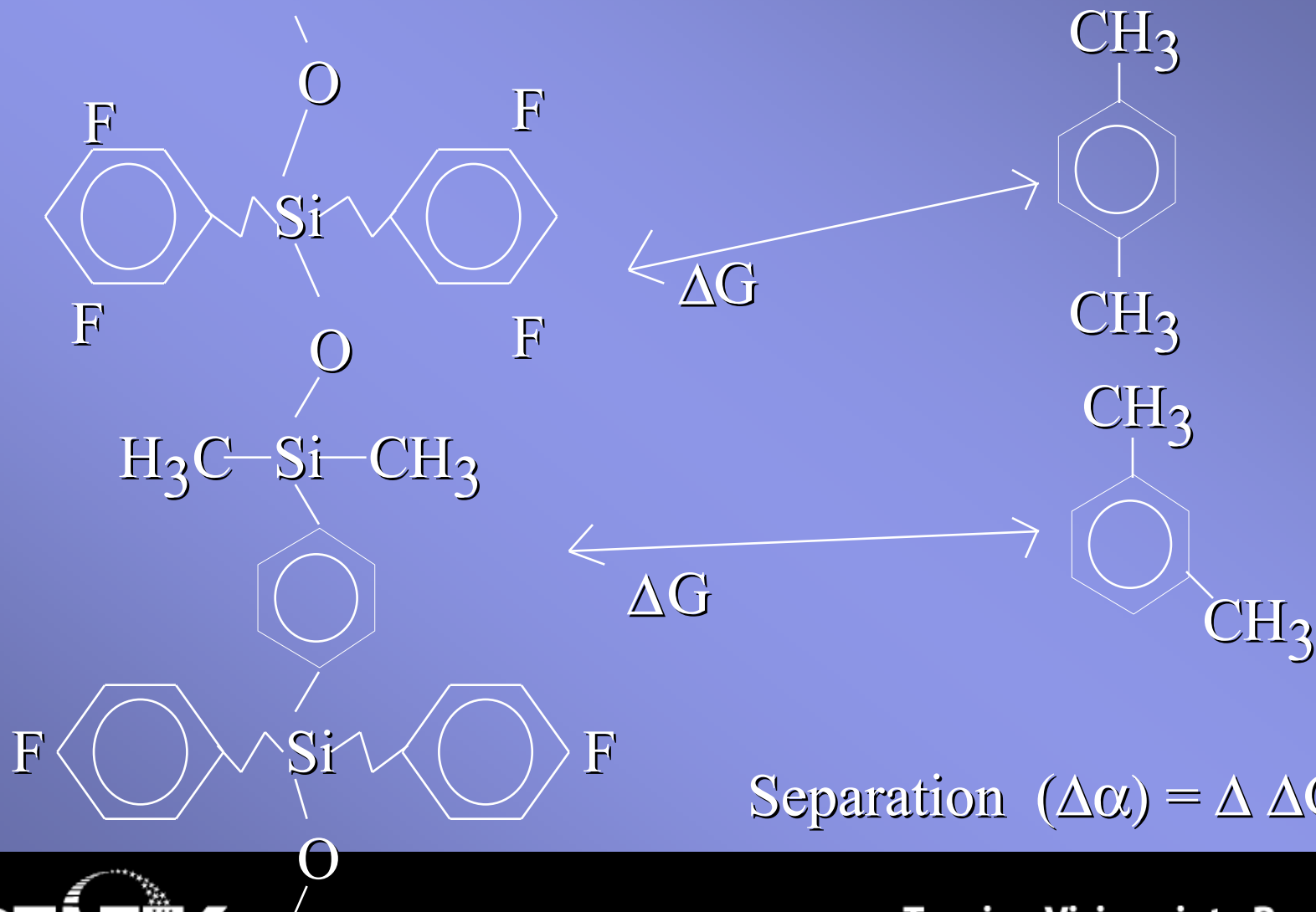
# Modeling - Energies of Interaction length to breadth separations?



Separation ( $\Delta\alpha$ ) =  $\Delta \Delta G$

*Organohalogen Compd.* **69**, 2007.

# Modeling - Energies of Interaction length to breadth separations?



Separation ( $\Delta\alpha$ ) =  $\Delta \Delta G$

## MD Simulation Run Conditions: Xylene isomers

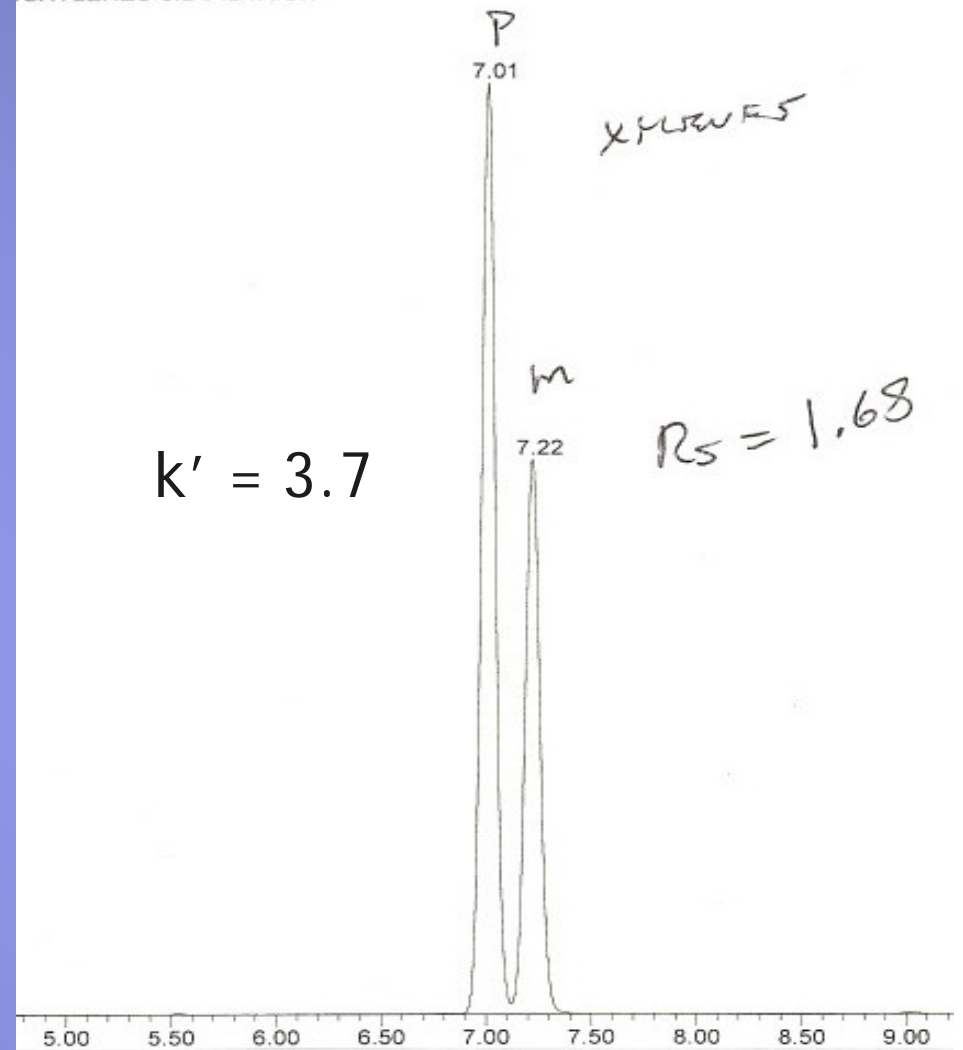
- Don't use moieties with poor thermal stability or reactivity
- Liquid crystals are too restrictive
- Can this be done with a polysiloxane with 5-type thermal characteristics?
  
- Polymer predicted to give  $R > 1.5$  for m- and p-xylene on 30-M X 0.25 mm i.d. X 0.25  $\mu\text{m}$  d.f.

Meta- and para- xylene  
Separated on an achiral  
polysiloxane column with  
320C-plus thermal stability.

Polymer under development  
As a potential phase for  
Length/breadth selectivity

Stay tuned.....

IBXYLENES-3.D\FID1A.CH



# GC Columns

- Bleed is very good
- Inertness has been addressed
- Selectivity must be our focus
  - Everything shouldn't be run on a "5"
  - Targeted separations will benefit from specific selectivity
  - Need to address common needs (general purpose) phases with other available mechanisms of solvation
  - Shape selectivity with rugged columns?

# Overall Summary

- GC and HPLC are governed by the same physics and chemistry
- They have followed similar evolutionary tracks
- Both techniques need to drive selectivity for best resolution
- GC has higher efficiencies (for now), but efficiency is a benefit that can only go so far.
- We must consider the role of alpha, and what we can do to provide the best selection of selectivity
- Restek is focused on delivering on this last challenge as well!