ZirChrom®

Method Development on Next Generation RPLC Supports

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ZirChrom®

1-866-STABLE-1
www.zirchrom.com

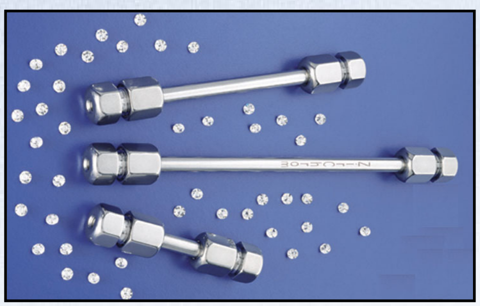
... For Peak Performance

Specialists in High Efficiency, Ultra-Stable Phases for HPLC

ZirChrom Separations, Inc.

ZirChrom Separations, Inc. is a company formed in 1995 located in Anoka, Minnesota, USA. ZirChrom manufactures a full line of zirconia-based high performance chromatographic materials for the analytical analysis of compounds primarily by high performance liquid chromatography (HPLC).

The extreme stability of zirconia and its unique chromatographic selectivity allows for the optimization of separation conditions, which are totally incompatible with other types of supports. ZirChrom columns will give you full access to all chromatography tools that have traditionally been limited when using silica (I.e. pH, high temperature, and buffers).



Zirconia-based supports promise to revolutionize the way that liquid chromatographic analyses are done. These supports will last longer, and allow for faster more selective separations resulting in significant cost savings per analysis

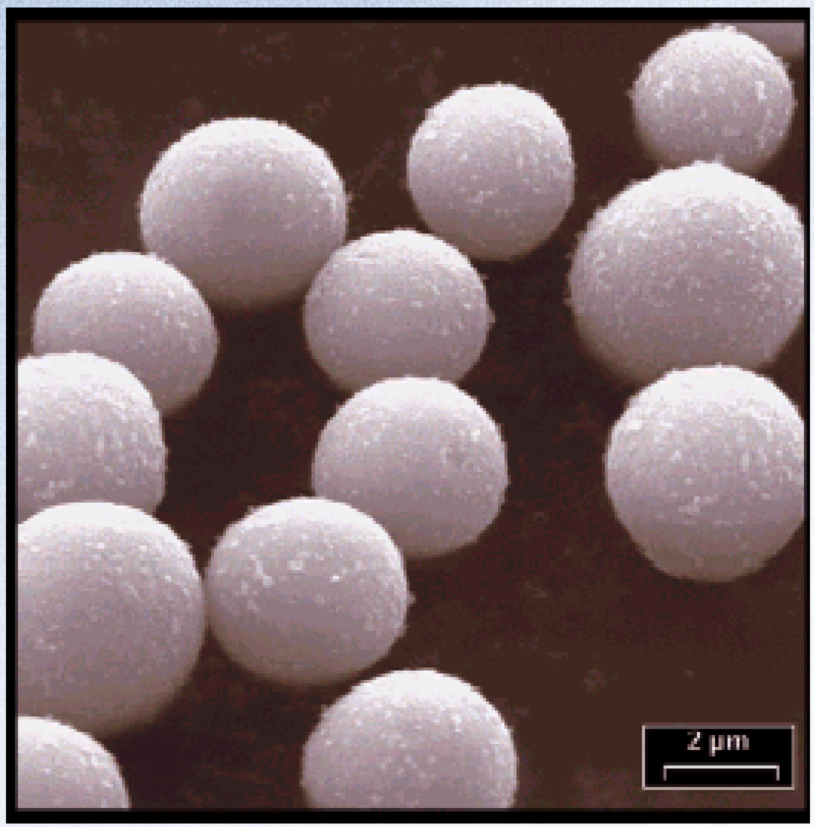
Outline

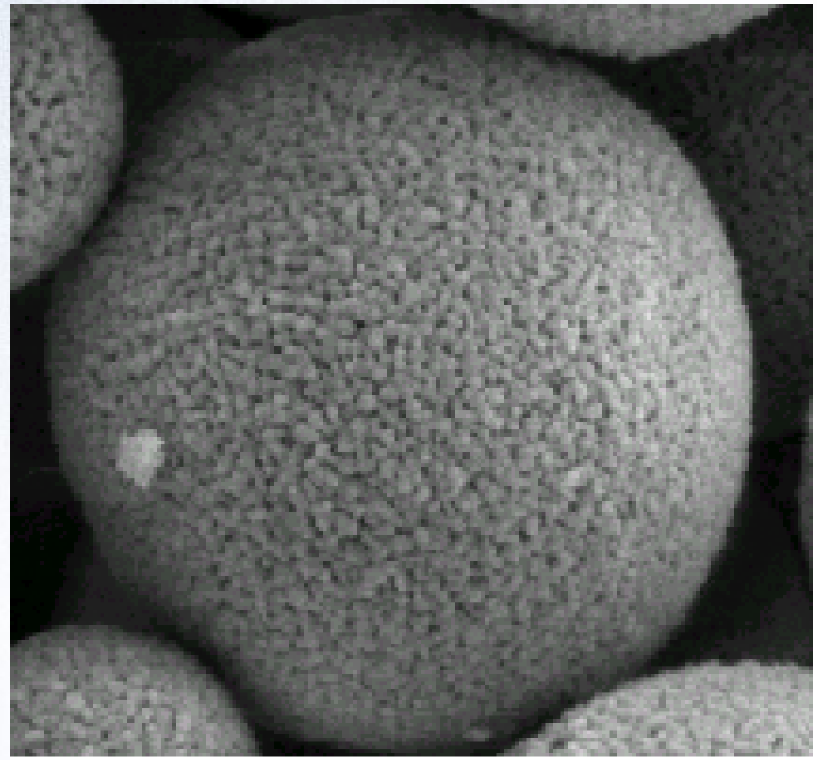
- RPLC Columns Sold By ZirChrom
- Surface Chemistry Considerations
- Method Development on ZirChrom Columns – Where Do I Start
  - Column Choice
  - Mobile Phase
  - pH
  - Column Temperature
- Example Method Development of A Real Pharmaceutical Samples

Why Use ZirChrom Phases?

- Selectivity - similar, different, or very different to ODS-silica
- Stability - run the operating conditions needed to get the separation, don’t worry about the column
- Speed - get 3x - 5x faster analysis by modest increases in temperature.
- Cost - Longer column life, greater productivity

Analytical Diameter Porous Zirconia Particles





1µm 25000X

Properties of Porous Analytical Zirconia

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Characteristic	Property
Surface area (m^2/g)	22
Pore volume (cc/g)	0.13
Pore diameter (Å)	250-300
Porosity	0.45 (silica 0.48)
Density (gm/cc)	2.6 (2.5x silica)
Particle size(μm)	3.0 (130,000 p/m)

Prep-scale particles also available

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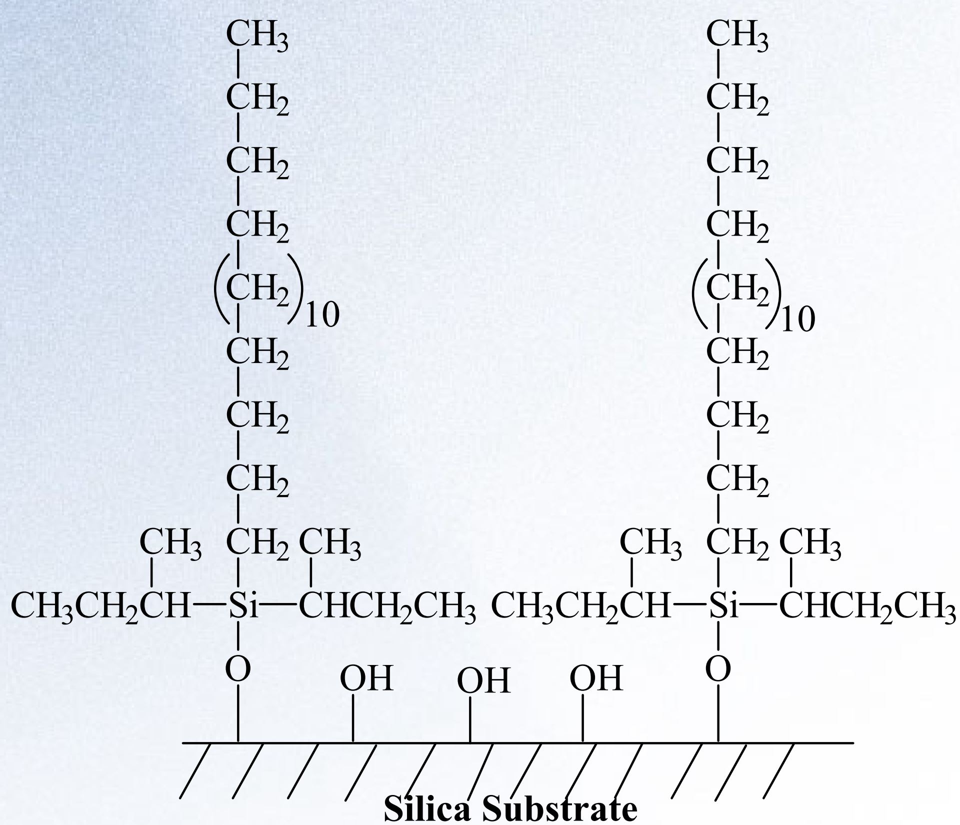
4 RPLC Stationary Phases

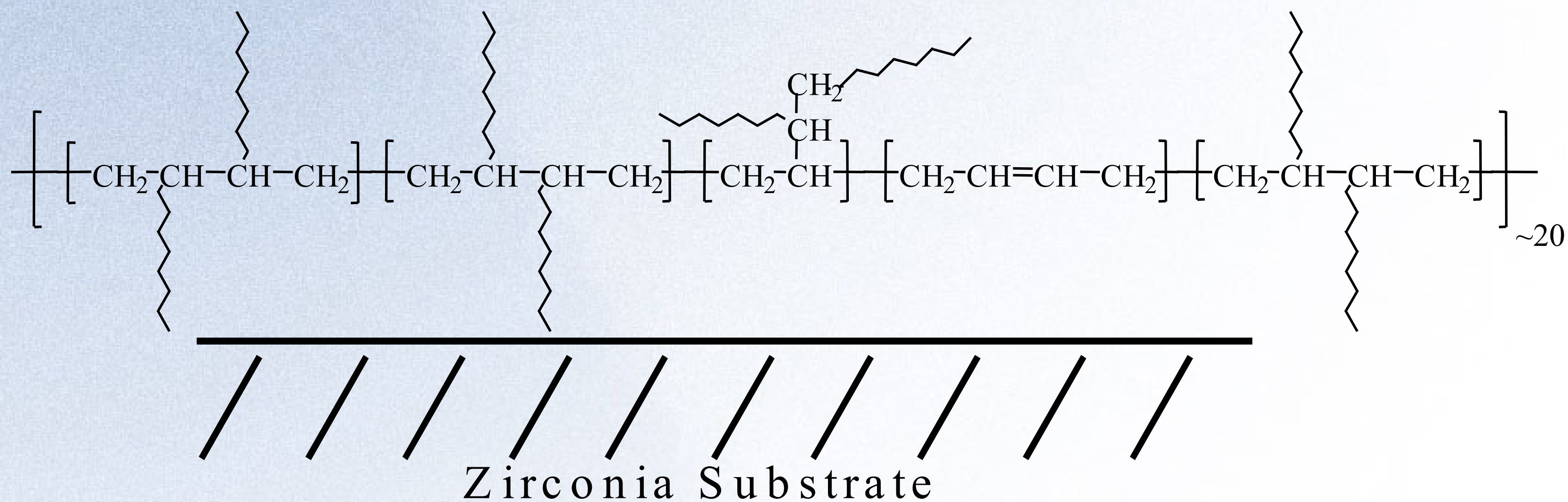
Column Name	Stationary Phase
ZirChrom-PBD	Polybutadiene
ZirChrom-PS	Polystyrene
ZirChrom-CARB	Carbon
DiamondBond-C18	Octadecylphenyl-Carbon

Typical Silica C18

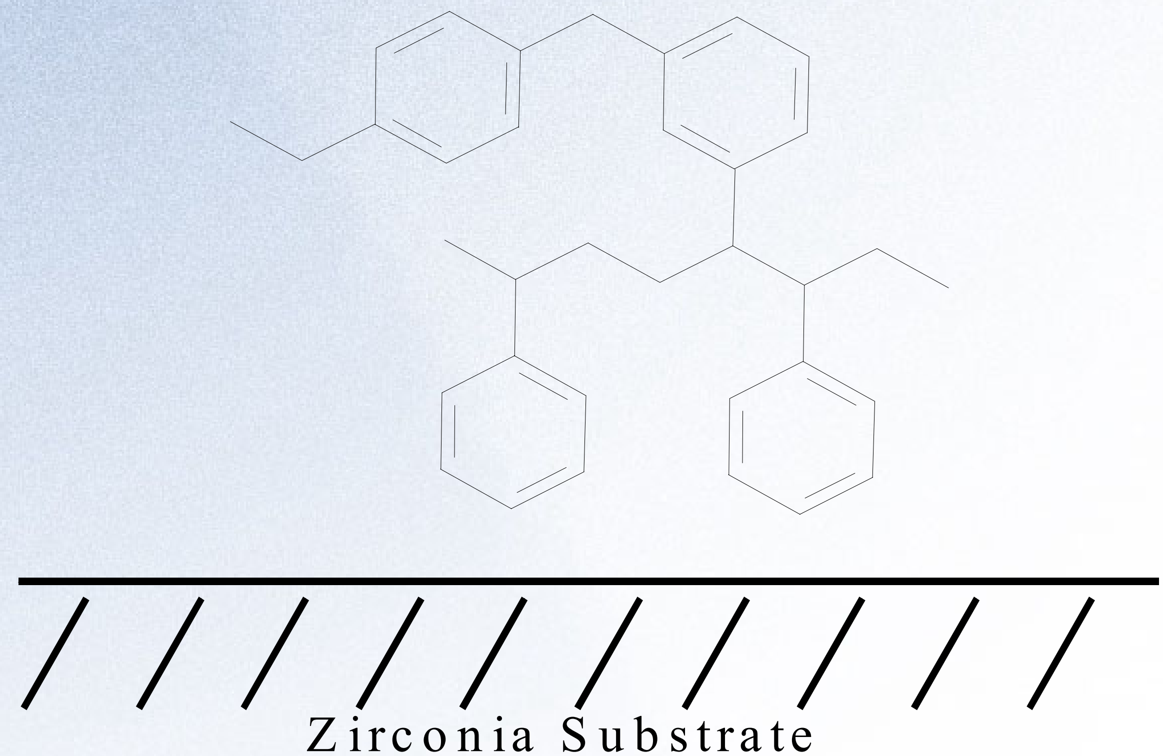
ODS



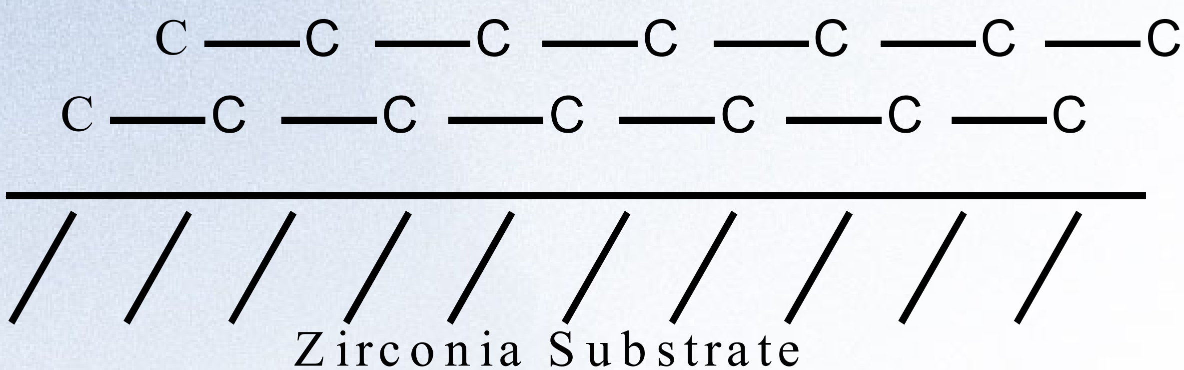
ZirChrom®-PBD



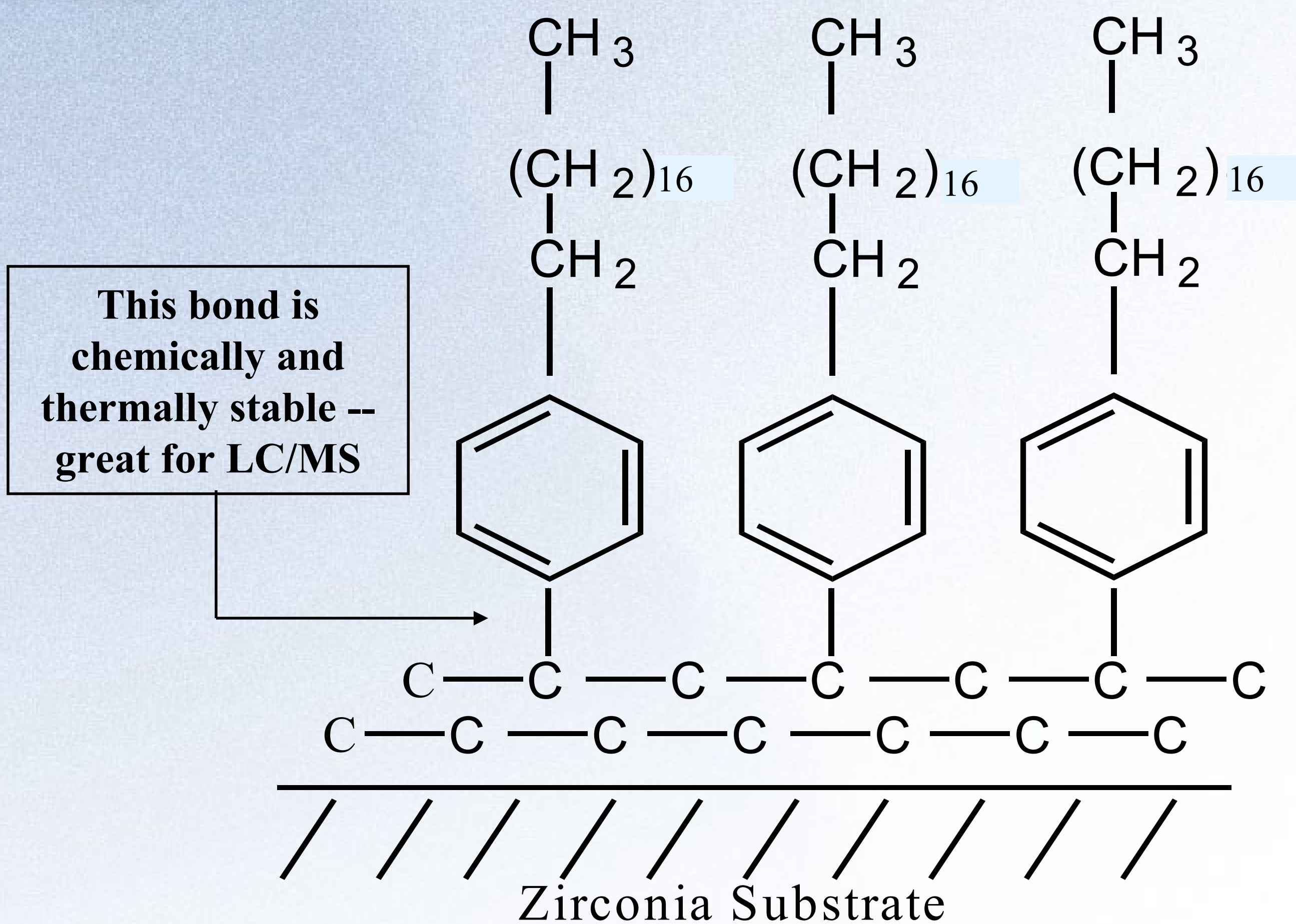
ZirChrom®-PS



ZirChrom®-CARB



DiamondBond®-C18



How do I Start?

- Sample – size, matrix and solubility?
- Column(s) Selection
- Mobile Phase Optimization
  - Organic modifier
  - pH
  - Buffer choice
- Temperature– Can I speed it up?

General Starting Point

Separation Variable	Preferred Initial Choice
Dimension	10 x 0.46 cm
Particle Size	3 μm
Stationary Phase	ZirChrom-PBD or DBC18
Mobile Phase	Water/Acetonitrile
%B	Variable
Buffer	25 mM phosphate, pH 7.0
Additives	Variable
Flow Rate	1-2 mL/min
Temperature	30-80°C
Sample Volume	5 microliters
Sample Mass	100 μg

Which Column Do I Use?

- Are the analytes acidic, basic or neutral?
- Are the analytes very polar?
- Are the analytes closely related? Chiral?
- Are the analytes highly aromatic?
- Are the analytes thermally stable?
- How complex is the sample?

Reversed-Phase Column Selection Guide

CURRENT PROBLEM/CONCERN	COLUMN	SUGGESTED CONDITIONS
Improve Selectivity		
Need improved selectivity for nonelectrolytes, isomers, disastereomers. Currently using carbon, cyano, phenyl or fluoro phases	DiamondBond™ C18, ZirChrom®-CARB	Temp ≥50 °C, with acetonitrile or preferably THF eluent.
Need improved selectivity for bases.	ZirChrom®-PBD	Use with Lewis base buffer (e.g. phosphate, citrate, fluoride) at pH 7.0, 5-50 mM. If not effective, increase pH to 11.0 or higher - See Technical Bulletin #241.
Need improved selectivity for acids.	DiamondBond™ C18	Use with 10 mM phosphate and 2 mM fluoride at pH 7.0. If not effective, drop pH to 2.0 or lower.
Change Retention		
Need more retention for very polar (hydrophilic) nonelectrolytes. Currently using nearly 100% water eluent or polar embeded phase	DiamondBond™ C18, ZirChrom®-CARB	Can use in high water mobile phase. Temperature can be lowered to room temperature if needed to increase retention.
Need more retention for very polar bases. Currently using nearly 100% water eluent or polar embeded phase or *sulfonic acid paired ion reagent*	ZirChrom®-PBD	Use at pH 7.0 with < 5mM phosphate. Vary pH as needed. Can use in high water mobile phase.
Need more retention for very polar acids. Currently using nearly 100% water eluent or polar embeded phase or *quaternary amine paired ion reagent*	DiamondBond™ C18	Use low pH to protonate acid. Choose a pH << pKa, even pH 0.5 is no problem.
Need less retention with any solute type.	ZirChrom®-PS	Least hydrophobic ZirChrom phase. Can easily achieve use of 100% water eluent.
Improve Efficiency / Productivity		
Inadequate stability and selectivity. Having trouble with silica-based phases, changed to alumina or polymer column and problems were *still not* sufficiently resolved	DiamondBond™ C18, ZirChrom®-PBD	pH range: 0.5 - 13, Temp < 200 °C. Any of the listed reversed phases will give higher efficiency and better peak shape than alumina or polymer columns.
Poor column stability. Experiencing retention drift at low or high pH, at above ambient temperature or when using phosphate or carbonate buffer.	DiamondBond™ C18, ZirChrom®-PBD	Our phases are the "Most Durable" phases on the market
Separations taking too long.	DiamondBond™ C18, ZirChrom®-PBD	Increase temperature up to max. operating range for LC &/or analyte. Increase flow rate. Easily improves speed 2-3 fold.
Column overloaded too easily with basic solutes.	ZirChrom®-PBD	Use phosphate buffer.
Improve Detection Sensitivity		
Need to go to shorter wavelength to enhance sensitivity in UV. Solute does not have long wavelength absorption or is very dilute	ZirChrom®-PS	Use a high water or pure water eluent and go deep into UV.
Need to decrease bleed in LC/MS.	DiamondBond™ C18	Recommend THF or acetonitrile as eluent modifier; can also enhance sensitivity.

See www.zirchrom.com literature articles numbers 1, 3, 4, 18, 27, 28, 30, 35, 38, 39, 41, 44, 46, 50, 51, 55, 59 *Technical Bulletin #240*

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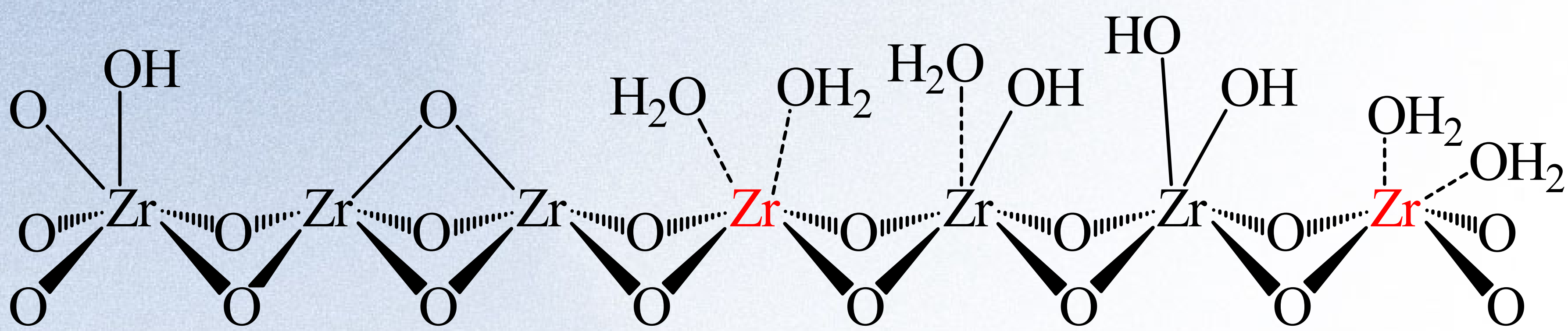
Part II.
Zirconia
the "***Un-Silica***".
The difference is the surface chemistry.

Surface Chemistry of Zirconia



Brönsted Acid: $ZrOH + {}^{-}OH \rightleftharpoons ZrO^{-} + H_2O$

Brönsted Base: $Zr{-}O{-}Zr + H^{+} \rightleftharpoons Zr{-}O(H^{+}){-}Zr$

Lewis Acid: $Zr(OH_2) + {}^{-}OOC{-}R \rightleftharpoons ZrOOC{-}R + H_2O$

Interaction Strength of Lewis Bases with Lewis Acid Sites on Zirconia

Interaction Strength	Lewis Base (A)
Strongest	Phosphate
↓	Fluoride
↓	Citric acid
↓	Sulfate
↓	Acetic acid
↓	Formic acid
↓	Nitrate
Weakest	Chloride

- Lewis bases with higher electron density and lower polarizability interact more strongly with zirconia.

Resolution: The Importance of Selectivity

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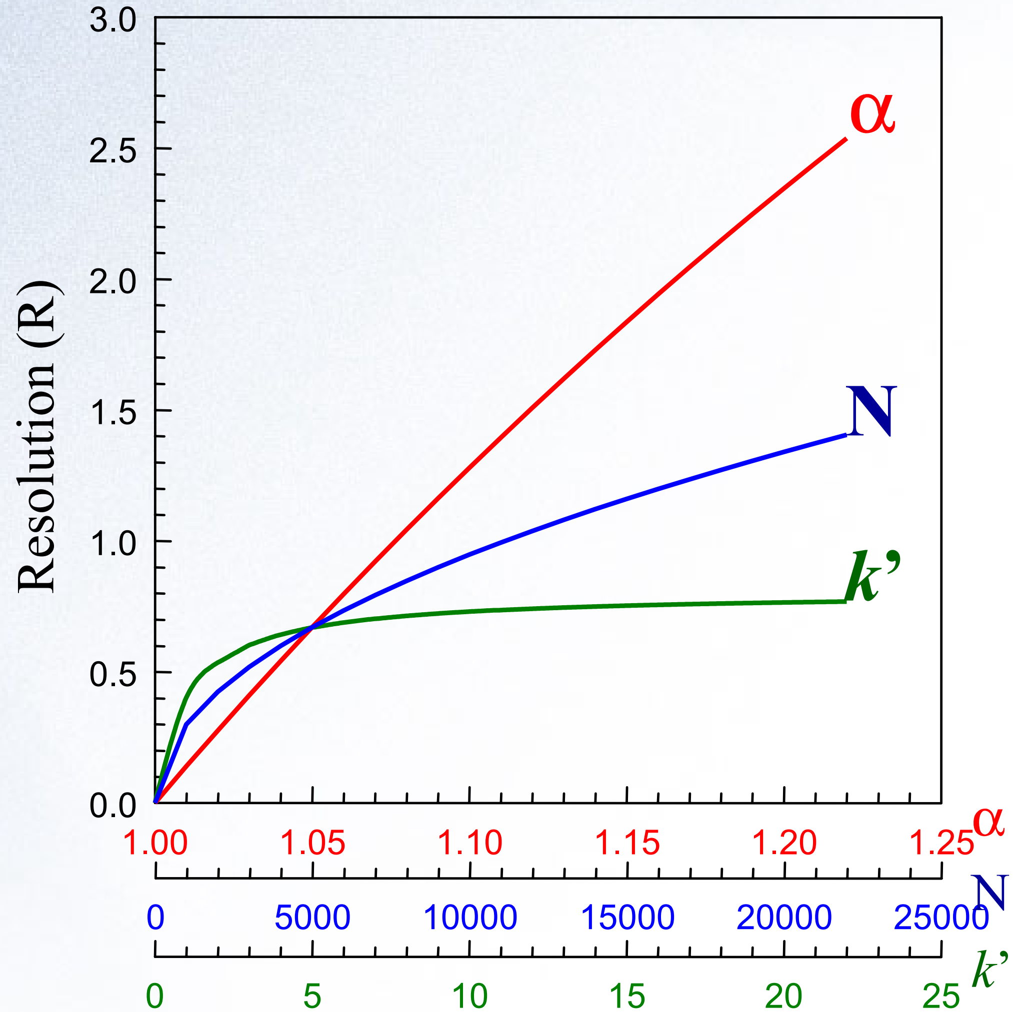
Efficiency → $\frac{\sqrt{N}}{4}$ Retention → $\frac{k'}{k'+1}$ Selectivity → $\frac{\alpha-1}{\alpha}$

$$R = \frac{\sqrt{N}}{4} \cdot \frac{k'}{k'+1} \cdot \frac{\alpha-1}{\alpha}$$

$$\alpha = \frac{k_j'}{k_i'}$$



➢ **Selectivity (α) has the greatest impact on improving resolution.**

Effect of α and N on Resolution

In general it is better to optimize selectivity rather than column efficiency as resolution is directly related to selectivity changes, but only varies with the square root of N.

α	Column	N	Run Time
1.10	4 cm, 3 μm	6,000	2-5 min
1.05	30 cm, 5 μm	25,000	30-60 min
1.02	5 m, 10 μm	160,000	8-15 h

Method Development Knobs on Zirconia RP Phases

Stationary Phase 4 Types	**pH (1-14)**	**Organic Modifier Type (many)**
Temperature up to 200°C	**Percent Organic Modifier**	**Buffer - Type and Concentration**

Method Development Knobs on Silica RP Phases

Stationary Phase Many Types

pH (2-9)

Organic Modifier Type (many)

Temperature up to 50^{o}C

Percent Organic Modifier

Why Choose Zirconia?

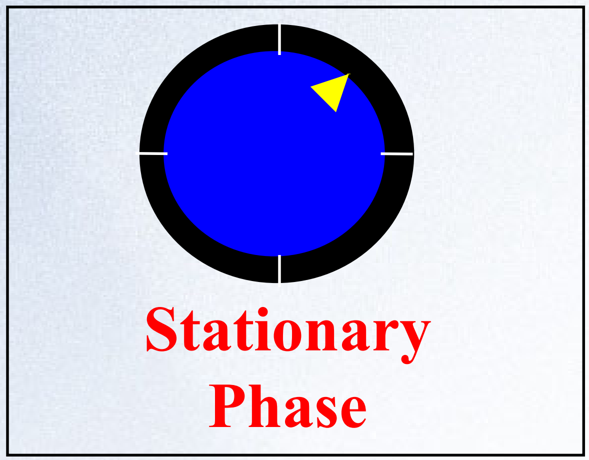
Silica

Zirconia

Which tool set would you rather have to keep your method development vehicle going?

When Do We Use the Stationary Phase Knob?

Before we try anything else . . .



- To change ***selectivity***
- To change ***retention***

22 Non-electrolyte Solutes

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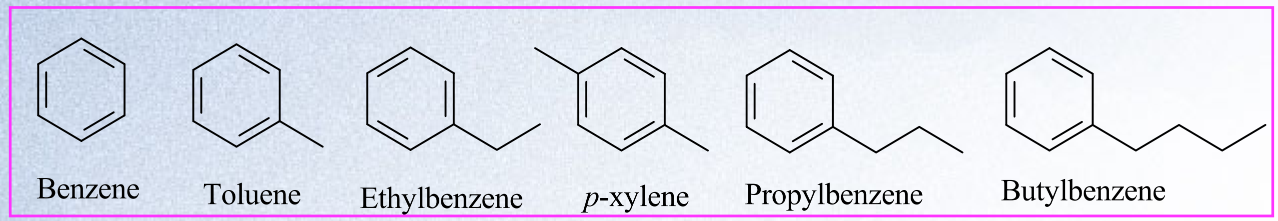
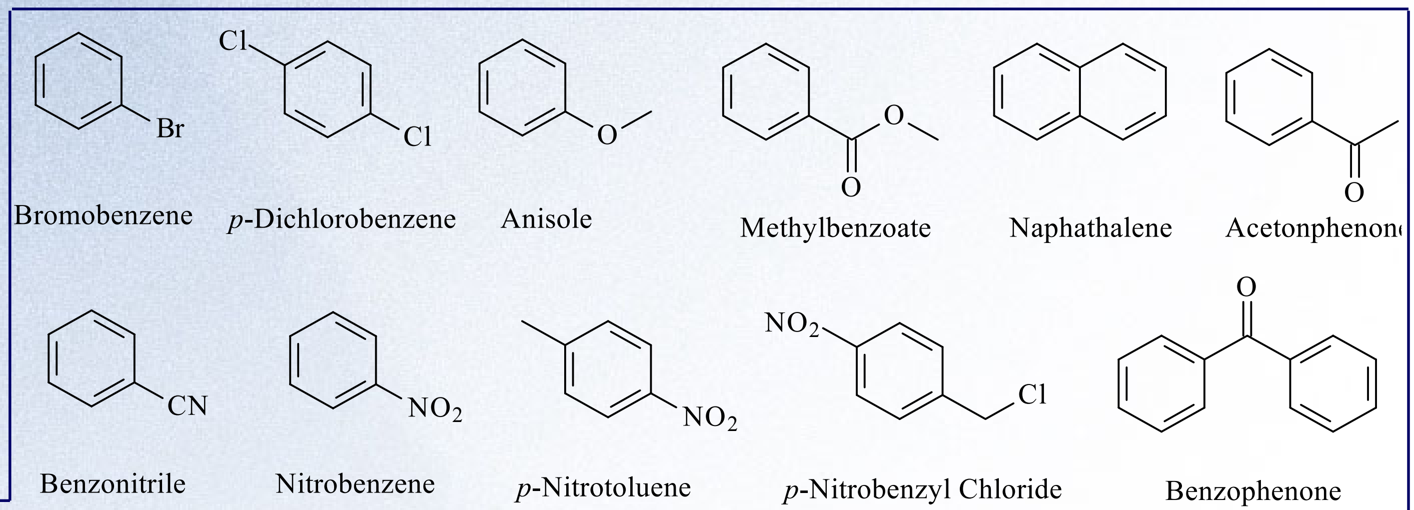
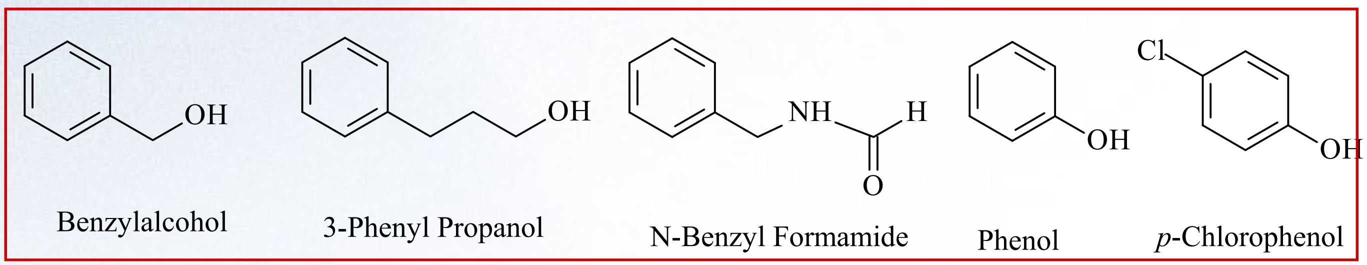
Nonpolar



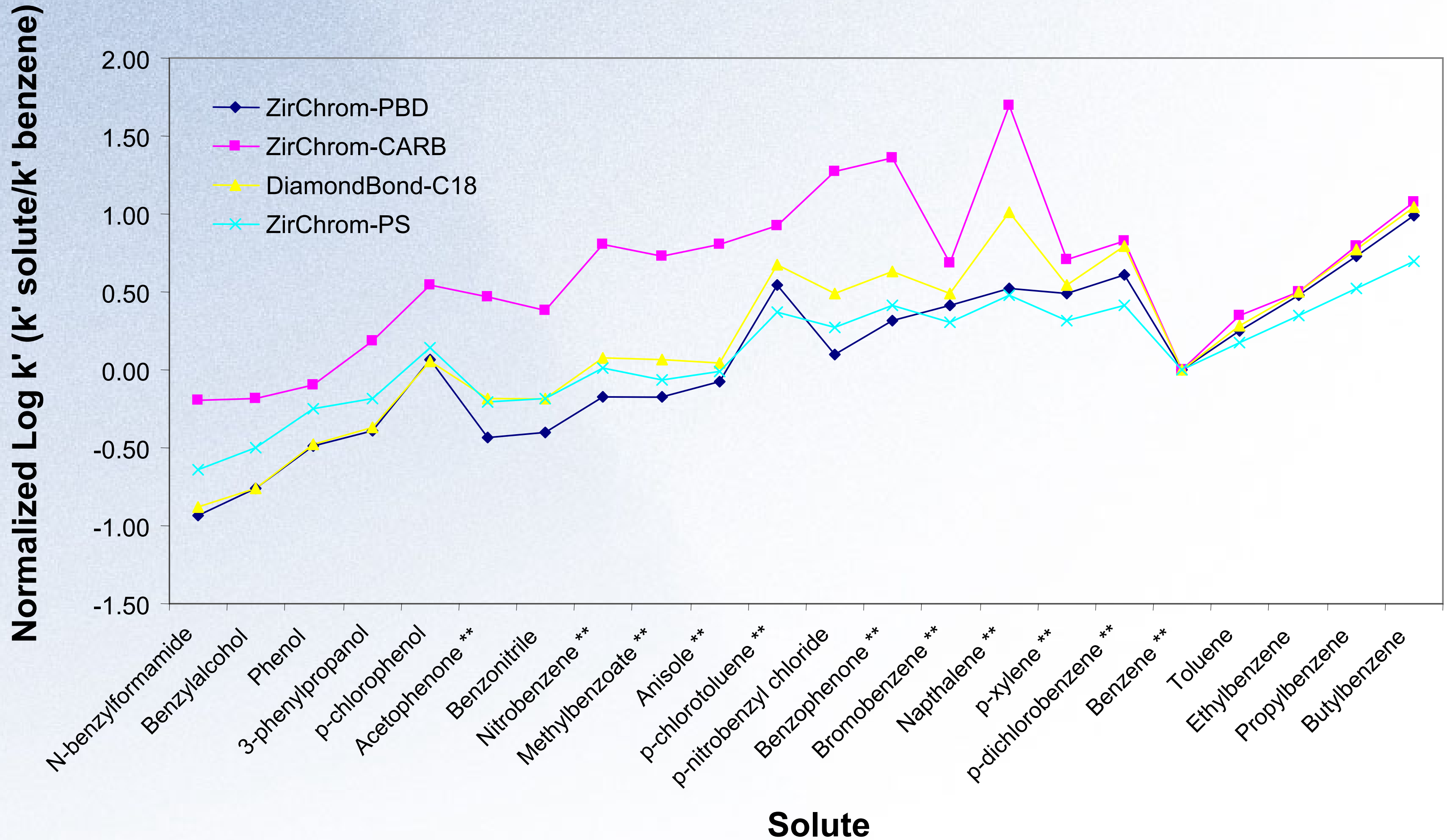
Polar



HB Donor



Selectivity Comparison



Selectivity Matrix*

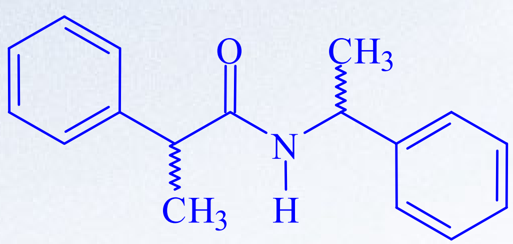
	CARB	DB-C18	PBD	RP18	C18 (2)	PLRP-S	RP-1
ZirChrom-CARB	1						
DiamondBond-C18	0.80	1					
ZirChrom-PBD	0.51	0.90	1				
Xterra RP18	0.53	0.85	0.90	1			
Luna C18 (2)	0.53	0.86	0.93	0.97	1		
PLRP-S	0.60	0.90	0.93	0.92	0.96	1	
Gammabond RP-1	0.52	0.88	0.96	0.97	0.98	0.95	1

* Column names are the trademarks of their respective manufacturers.

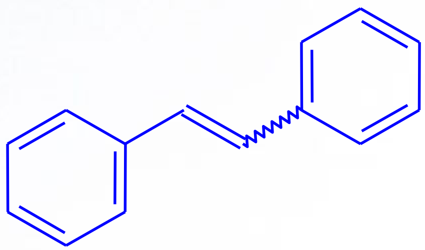
- **ZirChrom-PBD is the most similar to ODS for non-ionic analytes**
- **ZirChrom-CARB is the most different to ODS**
- **DiamondBond-C18 is ODS-like but with some CARB selectivity (generally better peak shapes than CARB)**

Selectivity and Shape: Isomeric Analytes

Compound	ODS	Carbon
di(phenethyl)amide	1.19	1.20
cis-/trans-stilbene	1.02	22



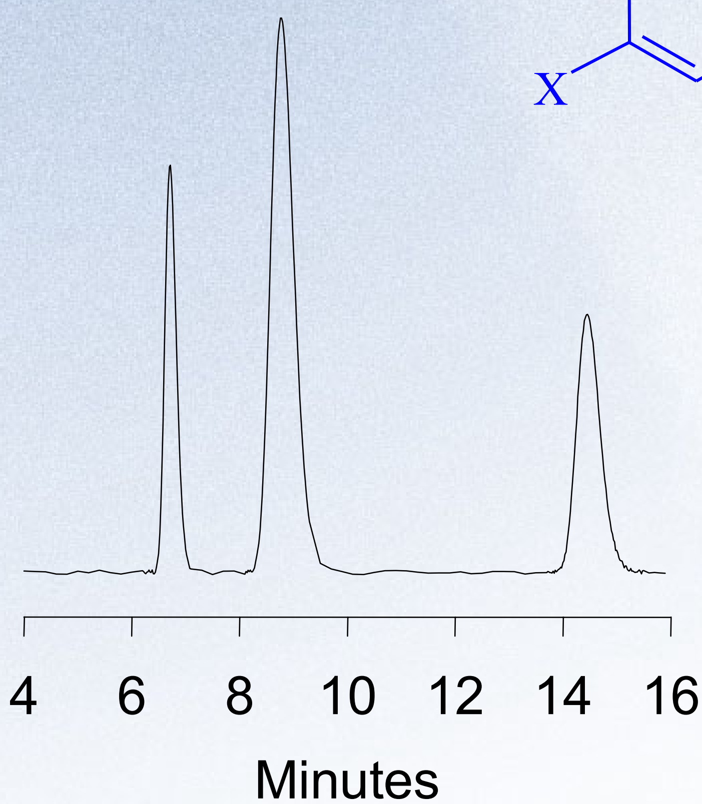
di(phenethyl)amide

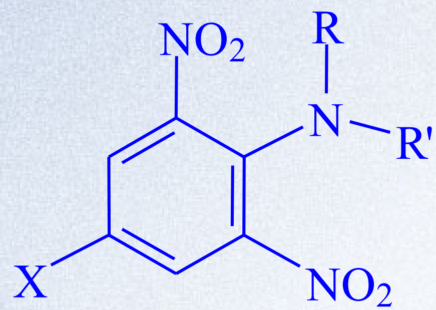


cis-/trans-stilbene

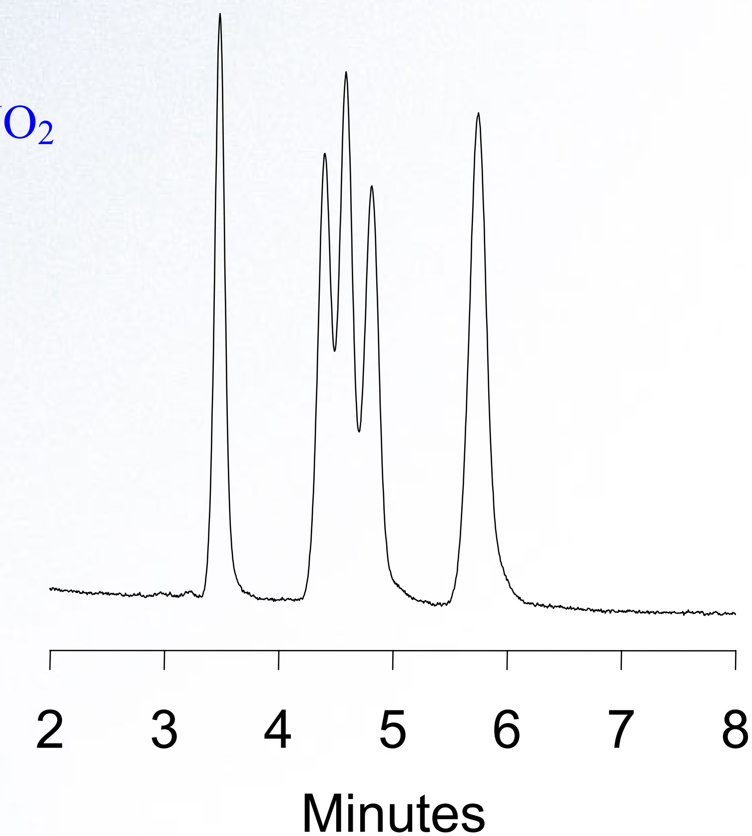
Dinitroaniline Herbicide Separation (EPA Method 627)

ODS

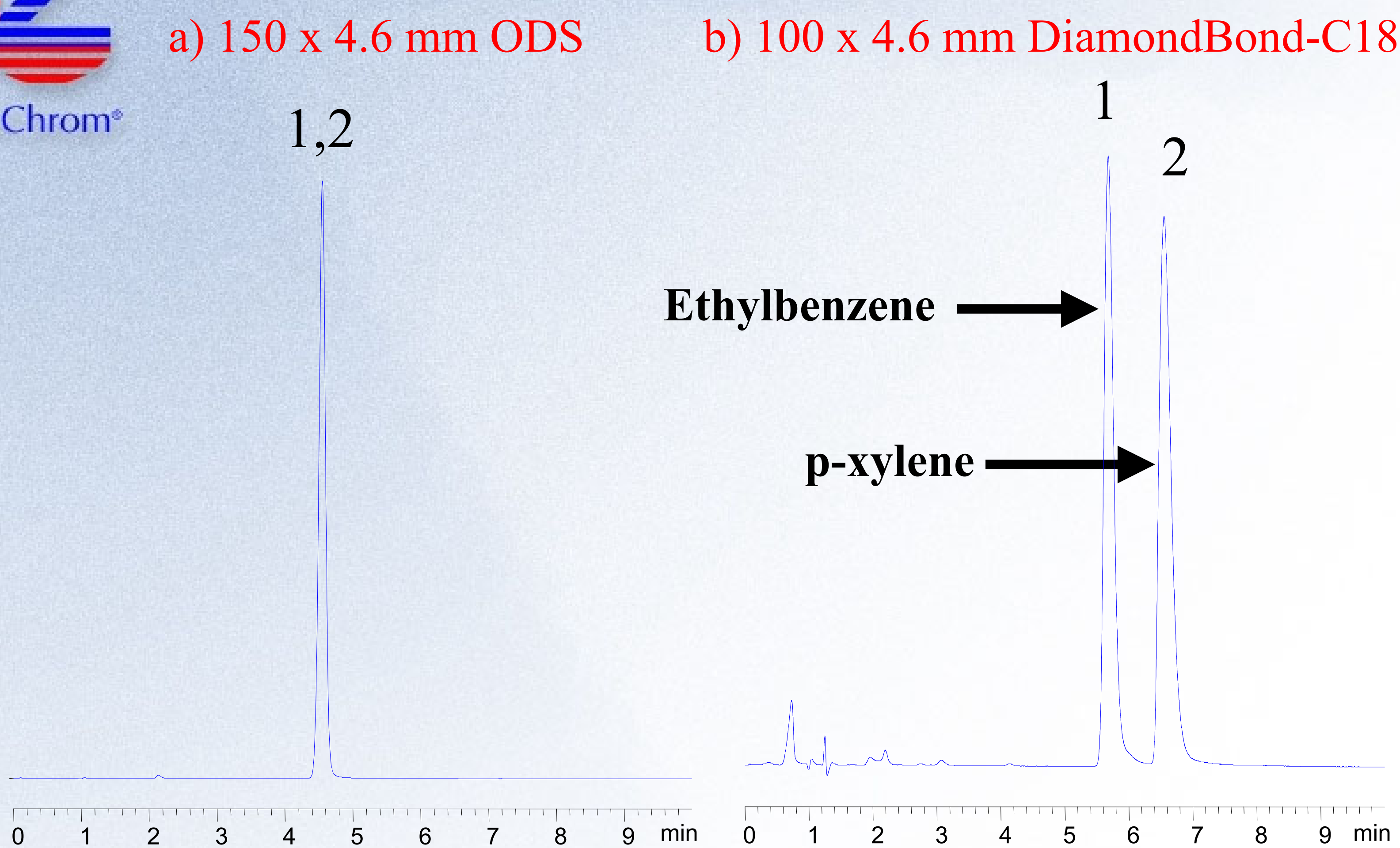




Carbon



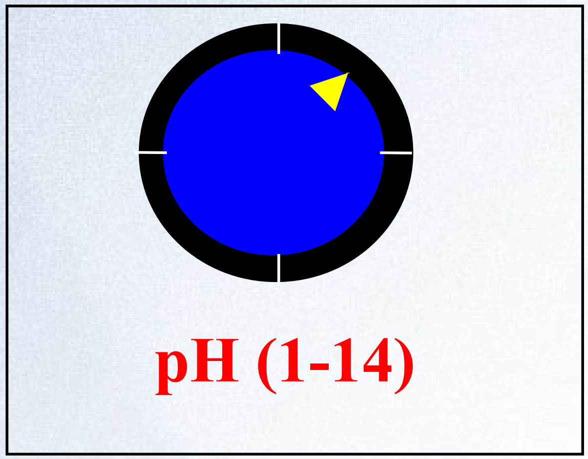
DiamondBond-C18 Selectivity*



LC Conditions: a) Column, 150 x 4.6 Zorbax Eclipse XDB-C8 S/N: USRK010769; Mobile phase, 65/35 ACN/Water; Temperature, 30 ºC; Flow rate, 1.0 ml/min.; Injection volume, 5 ul; Detection at 254 nm; Solutes: 1=Ethylbenzene, 2=p-xylene. b) Column, 100 x 4.6 DiamondBond-C18, OD082401A; Mobile phase, 37.5/5/57.5 ACN/THF/Water; Temperature, 60 ºC; all other conditions the same as a).

* Column names are the trademarks of their respective manufacturers.

When Do We Use the pH Knob?



- For ***ionizable*** compounds
- To improve ***peak shape*** of acidic or basic compounds
- To change ***selectivity*** of acidic or basic compounds
- To change ***retention*** of acidic or basic compounds

Silica after aging with Base*

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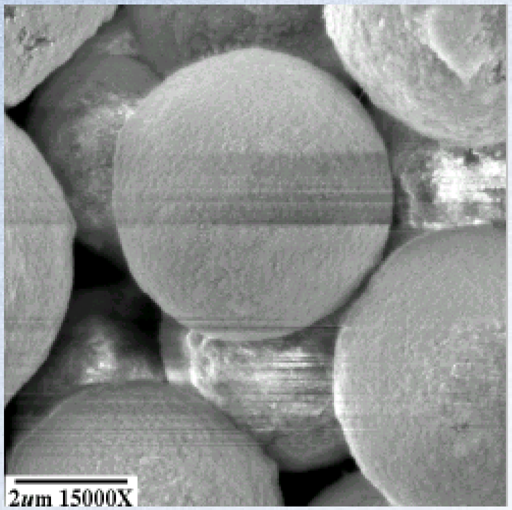
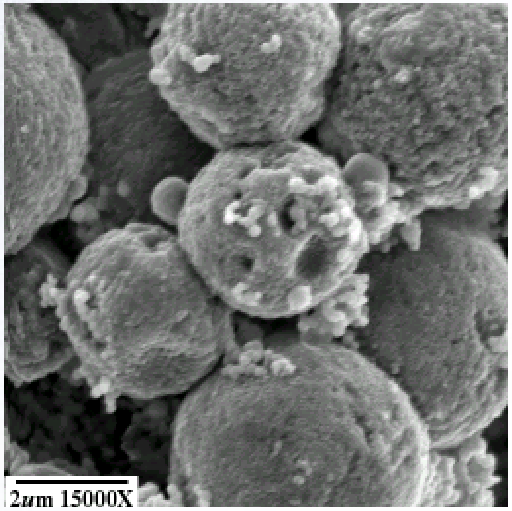


Extend C18 before aging

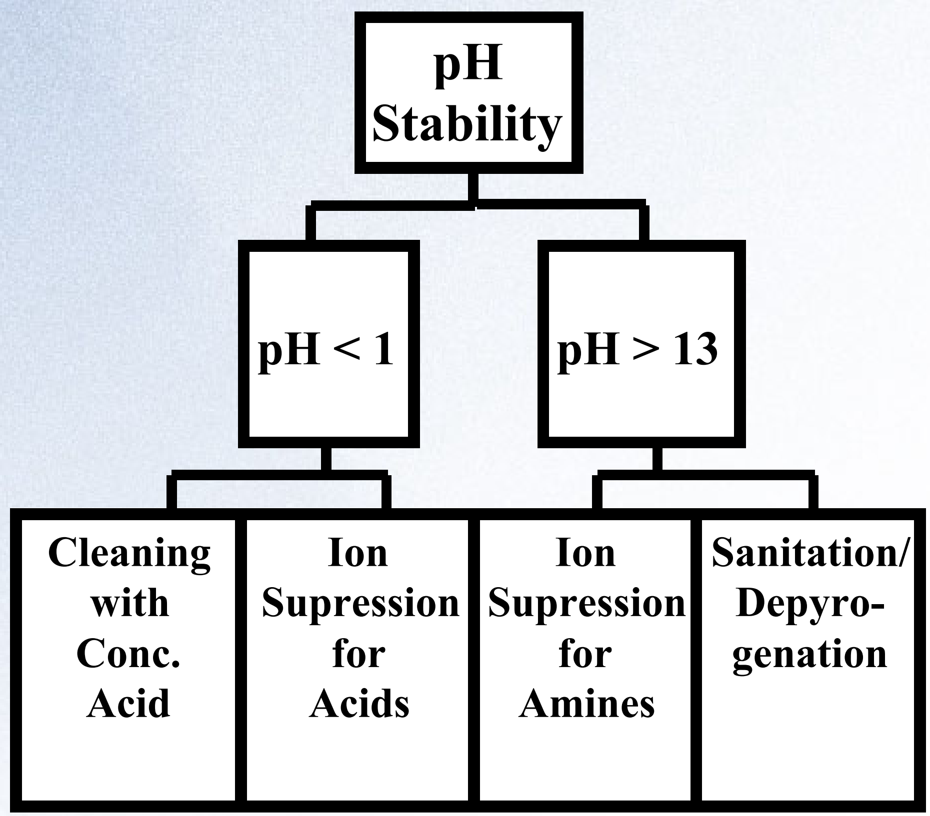


Extend C18 after aging

Aging conditions: 0.1g of Extend C18 was placed in 50mls of 40:60 - Methanol:1M Potassium hydroxide solution. It was aged for 24 hours at room temperature in a shaking bath.

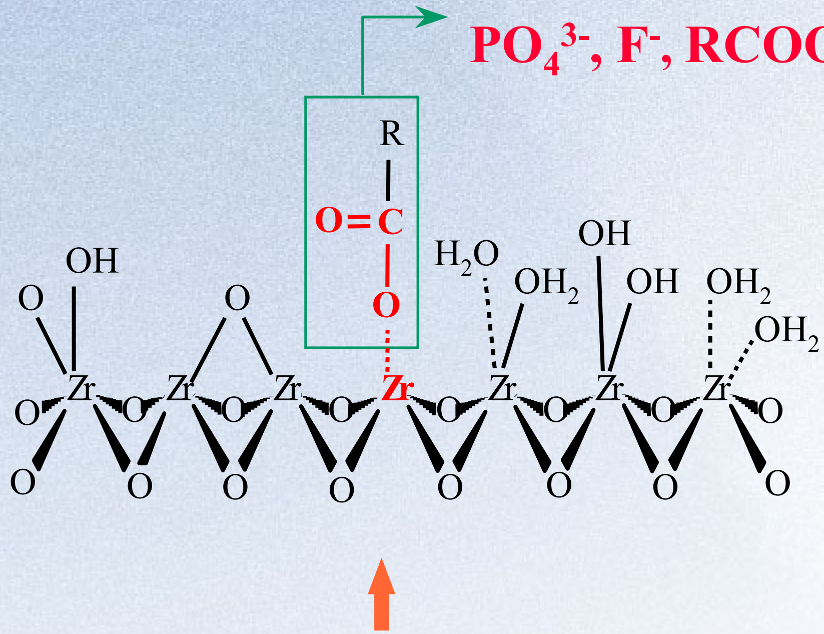
* Column names are the trademarks of their respective manufacturers.

Why Use pH Extremes?

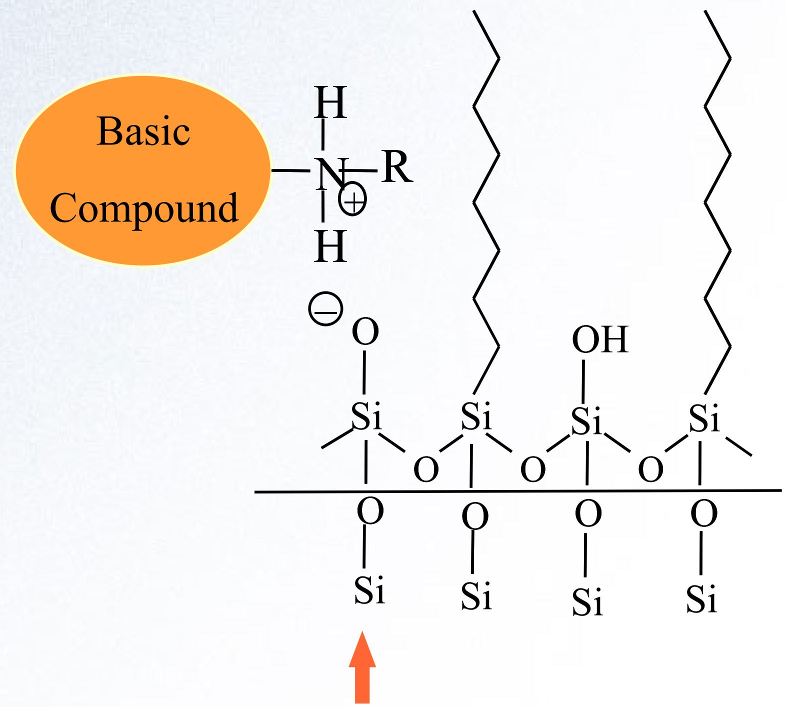


ZrO_2 / SiO_2 Surface Chemistry

Lewis base:
PO_4^{3-}, F^-, $RCOO^-$



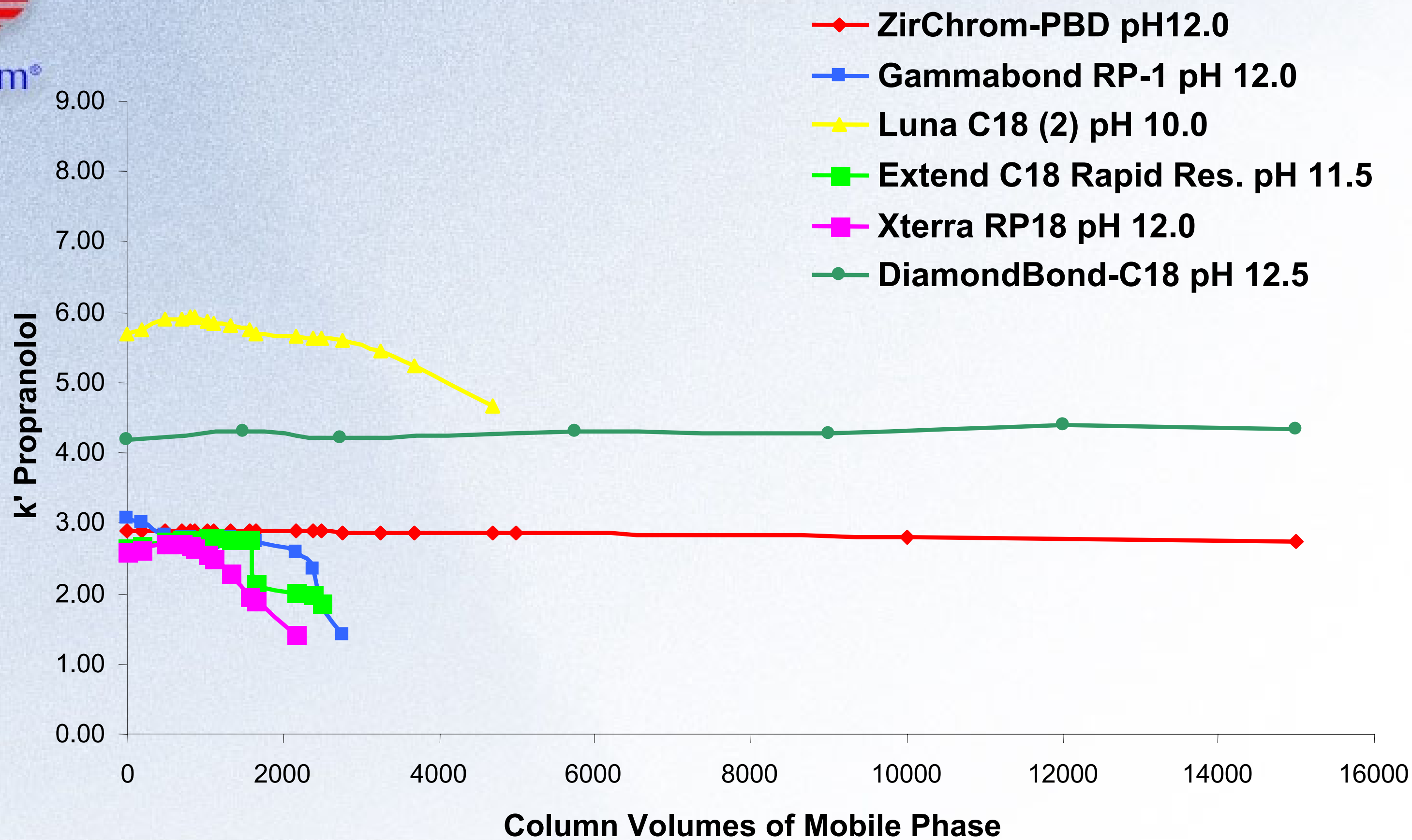
Lewis Acid Site



Active Silanol

Correct buffering can eliminate unwanted residual surface interactions (or enhance desirable residual surface interactions)

High pH Stability Comparison*



<u>Exposure Conditions</u>: Mobile phase, ACN/50mM Potassium phosphate buffer at indicated pH; Temperature, 30 ^{o}C.

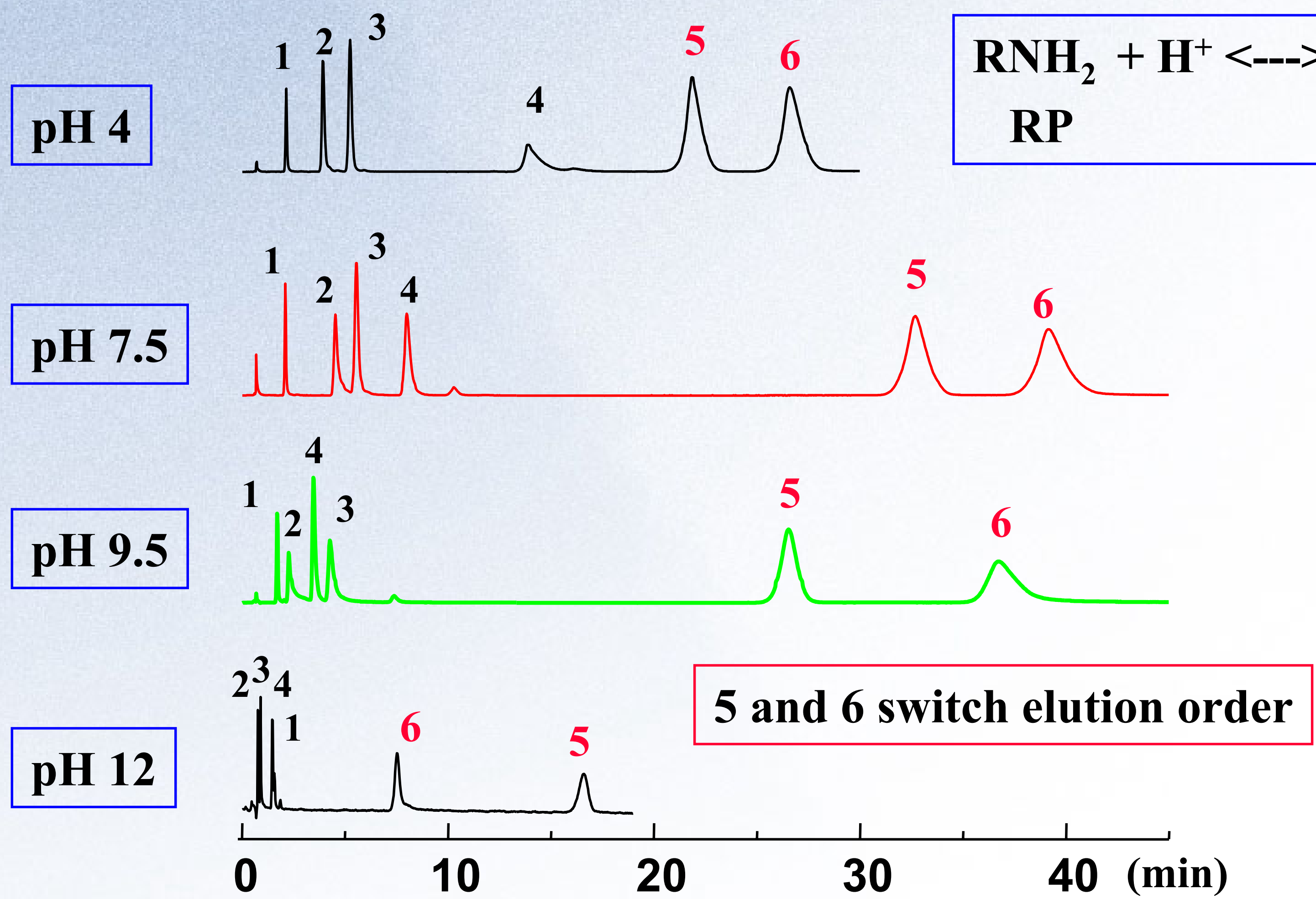
<u>LC Conditions</u>: Mobile phase, ACN (or THF)/50mM Potassium phosphate buffer at indicated pH; Flow Rate, 1.0 mL/min.; Temperature, 30 ^{o}C; Injection Volume, 5 uL; Detection, 254nm.

* Column names are the trademarks of their respective manufacturers.

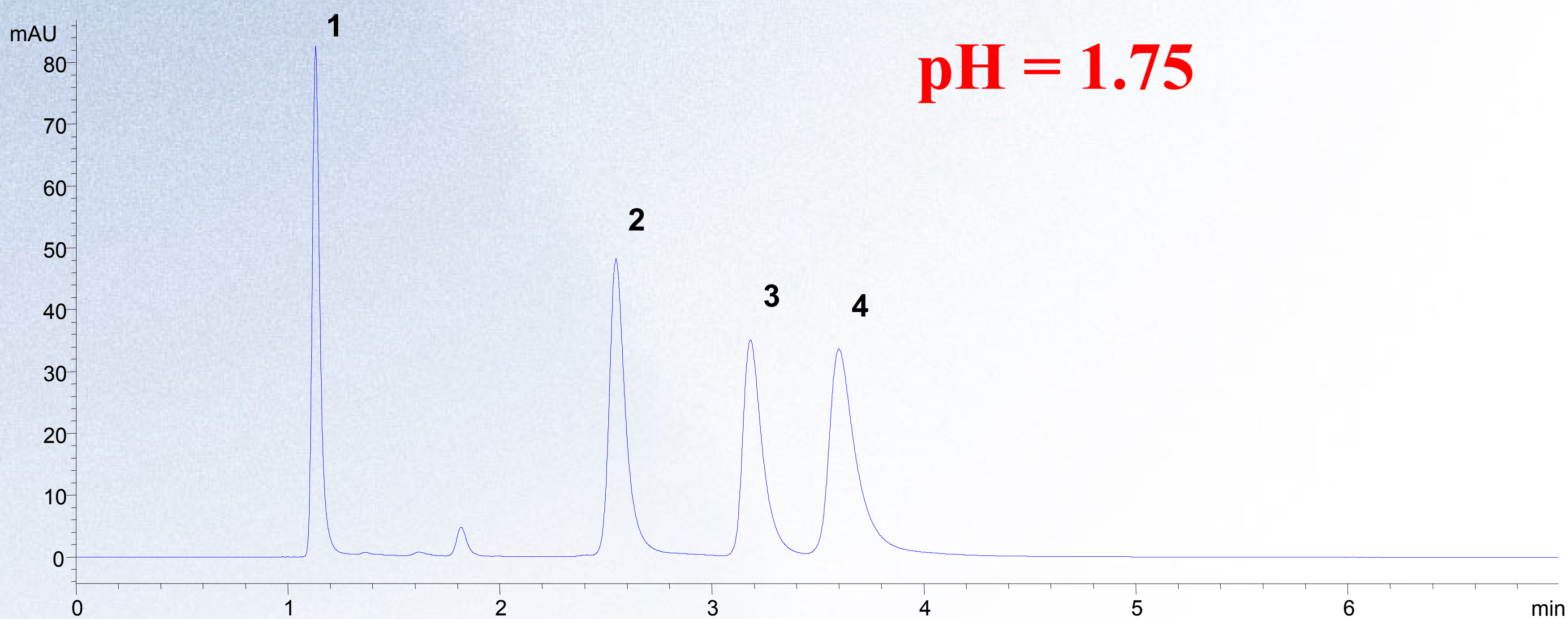
Effect of pH on Bases

$RNH_2 + H^+ \text{<--->} RNH_3^+$

RP | RP + CEX



Fast Separations of NSAIDs on DiamondBond™-C18



LC Conditions: Column, 100 x 4.6 DiamondBond™-C18; LC Conditions: Mobile phase, 50/50 ACN/50mM Phosphoric acid, pH 1.75; Flow rate, 1.0 ml/min.; Temperature, 65 °C; Injection volume, 1.0 ul; Detection at 254nm; Solutes: 1=Acetominaphen, 2=Ketoprofen, 3=Ibuprofen, 4=Naproxen

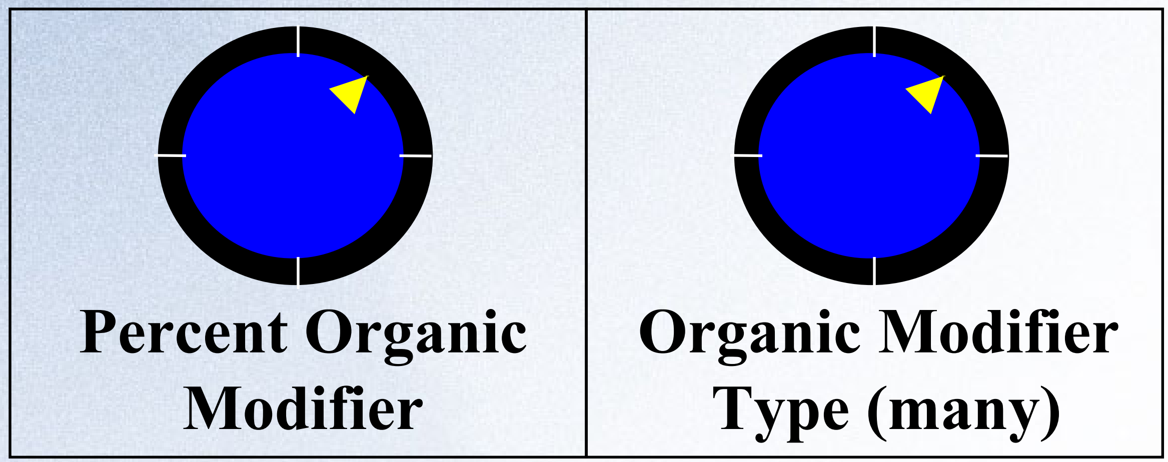
Why Work at High pH?

- ✓ To adjust retention
- ✓ To improve peak shape by deprotonating amines
- ✓ To adjust selectivity

Why tie your hands with columns with limited lifetime, limited buffer types and limited pH range?

ZirChrom RPLC phases are stable: 1< pH < 14

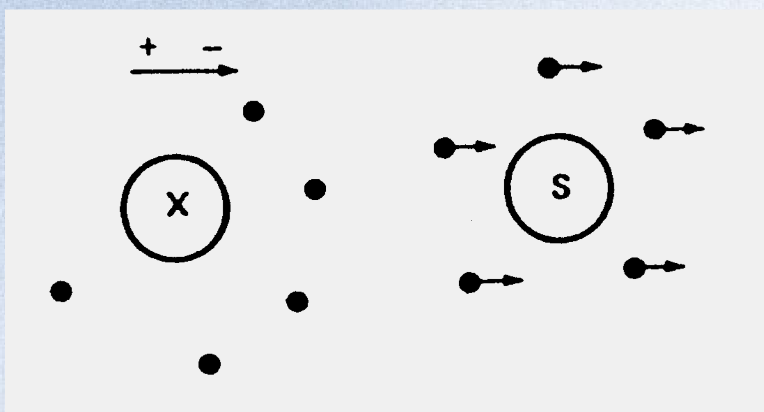
When Do We Use the Mobile Phase Knobs?



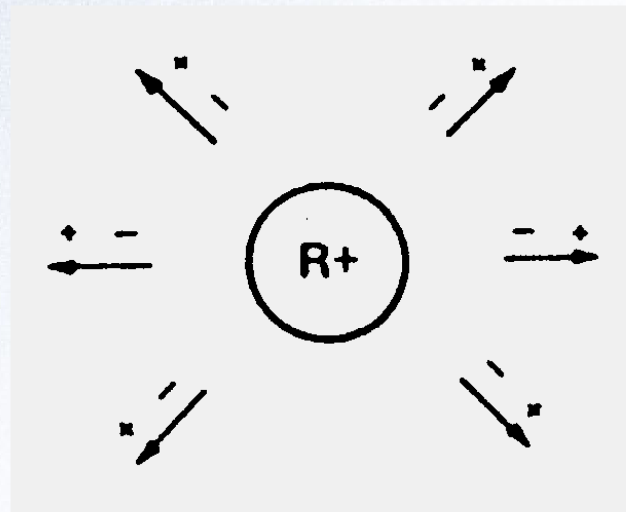
- To change ***selectivity***
- To change ***retention***

Molecular Interactions in LC

Dispersion



Dielectric Interactions



Dipole

$$CH_3-\overset{+}{C}\equiv\overset{-}{N}\longleftrightarrow CH_3-\overset{+}{C}\equiv\overset{-}{N}$$

Hydrogen Bonding

$$Cl_3\,C-H\longleftrightarrow :N-(CH_3)_3$$

Overview of Mobile Phase Optimization

- Define optimum solvent strength so that

$$1 < k' < 20$$

  a. Do stepwise isocratic study in 20% steps starting at 100% organic.

  b. Do gradient determination of % organic.

- Define the best type of modifier; MeOH, ACN or THF - - - TRIANGLE OPTIMIZATION.
- Keep in mind that the relative “strengths of MeOH, ACN and THF are related.

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Separation Optimization

EASY SAMPLES

Initial run with ACN/water → Define k′ range vs % ACN → Best % ACN for k′ range and band spacing

AVERAGE SAMPLES

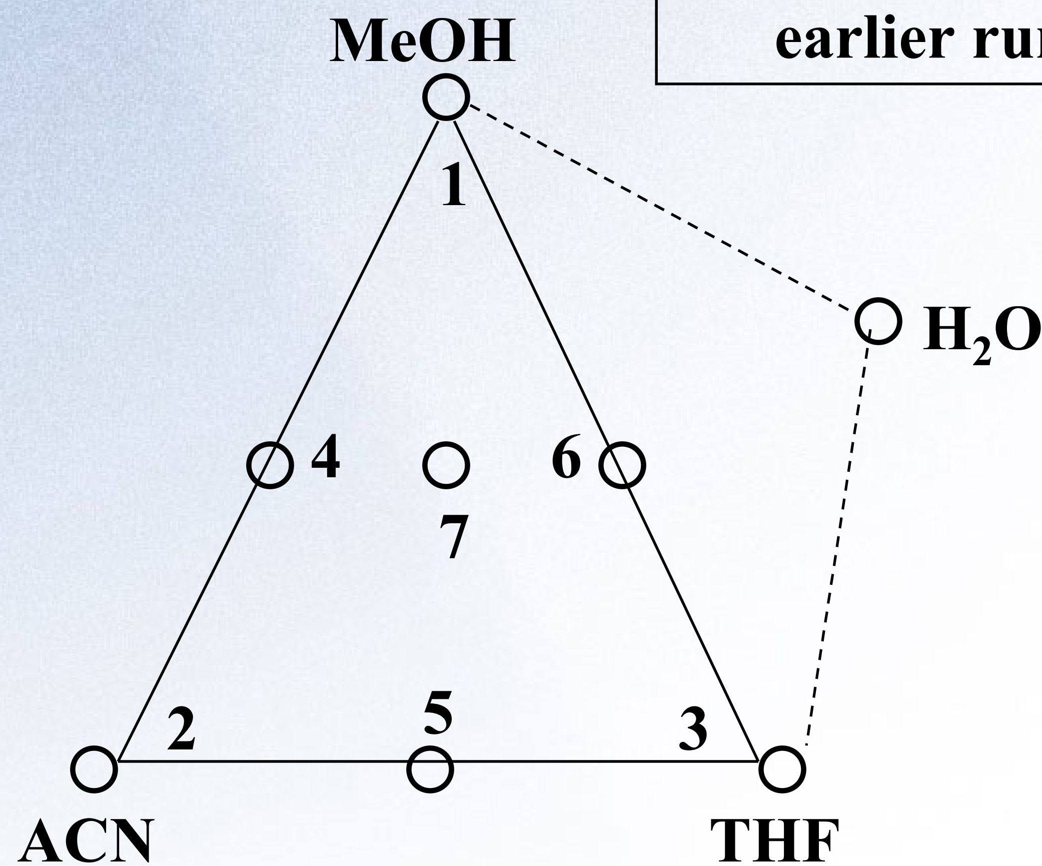
Initial run with MeOH/water → Define k′ range vs % MeOH → Best % MeOH for k′ range and band spacing

Initial run with THF/water → Define k′ range vs % THF → Best % THF for k′ range and band spacing

Triangle Optimization

HARD SAMPLES

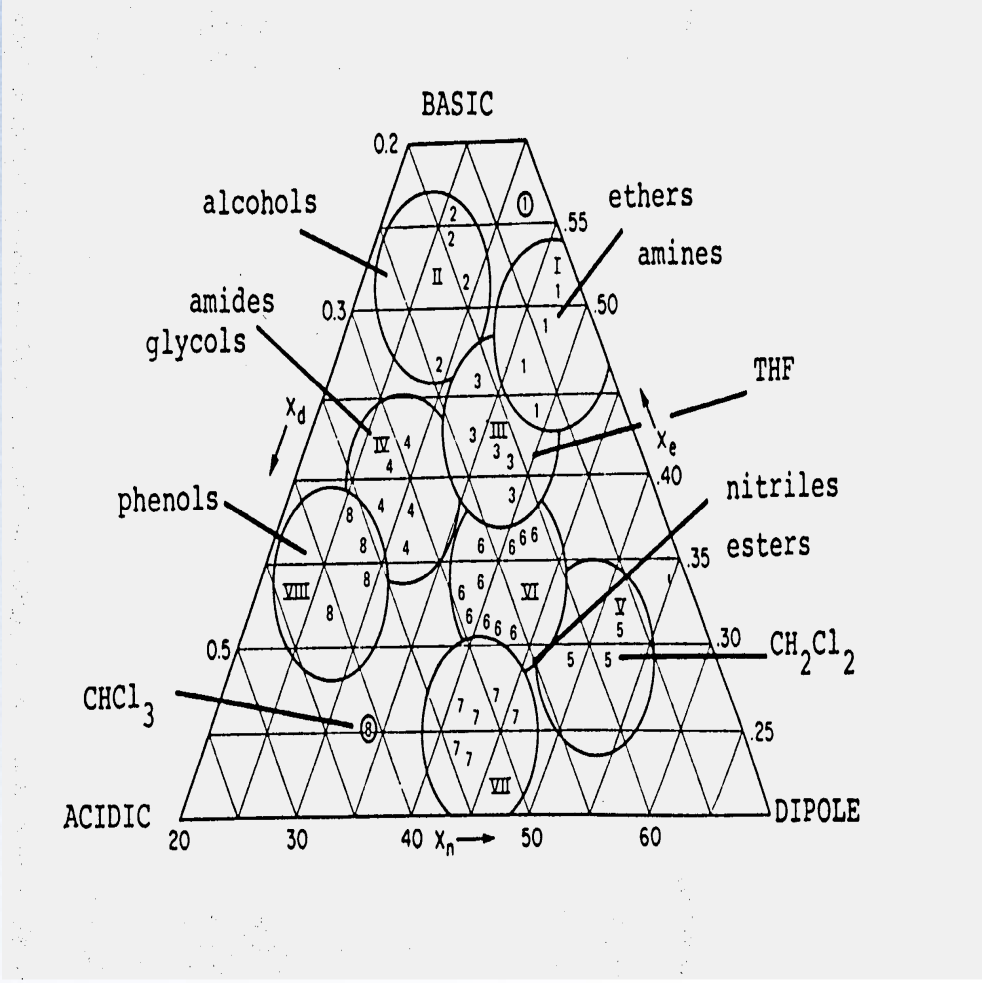
Use k′ range from earlier runs



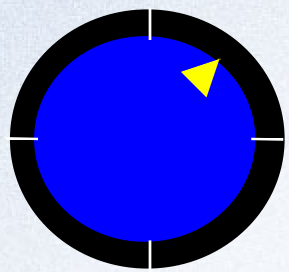
Snyder Scheme for RPLC Optimization

- Define % solvent for 1 < k' < 20 in MeOH(**Solvent 1**).
- If band spacing is not OK then use equieluotropic mixtures for ACN and THF. (**Solvents 2 and 3**)
- If 2 or 3 are not OK then do mid-points as shown. (**Solvents 4,5,6**).
- If a significant improvement is seen then look for intermediate ternary mixture.
- If no significant improvement is seen then do mixture for all three solvents (**Solvent 7**).

Solvent Properties Triangle



When Do We Use the Buffer Knob?



Buffer - Type and Concentration

- To improve ***peak shape*** of acidic or basic compounds
- To change ***selectivity*** of acidic or basic compounds
- To change ***retention*** of acidic or basic compounds
- To modify ***band spacing*** or elution order

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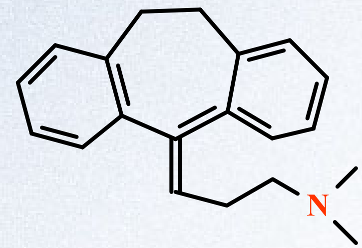
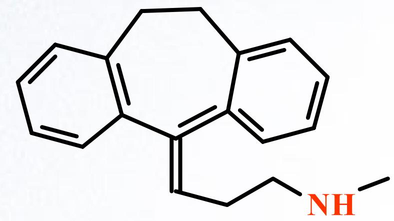
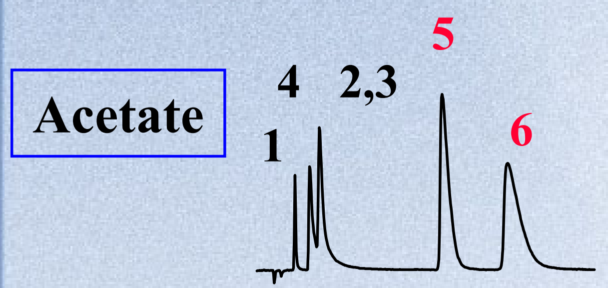
Effect of Different Lewis Base Additives

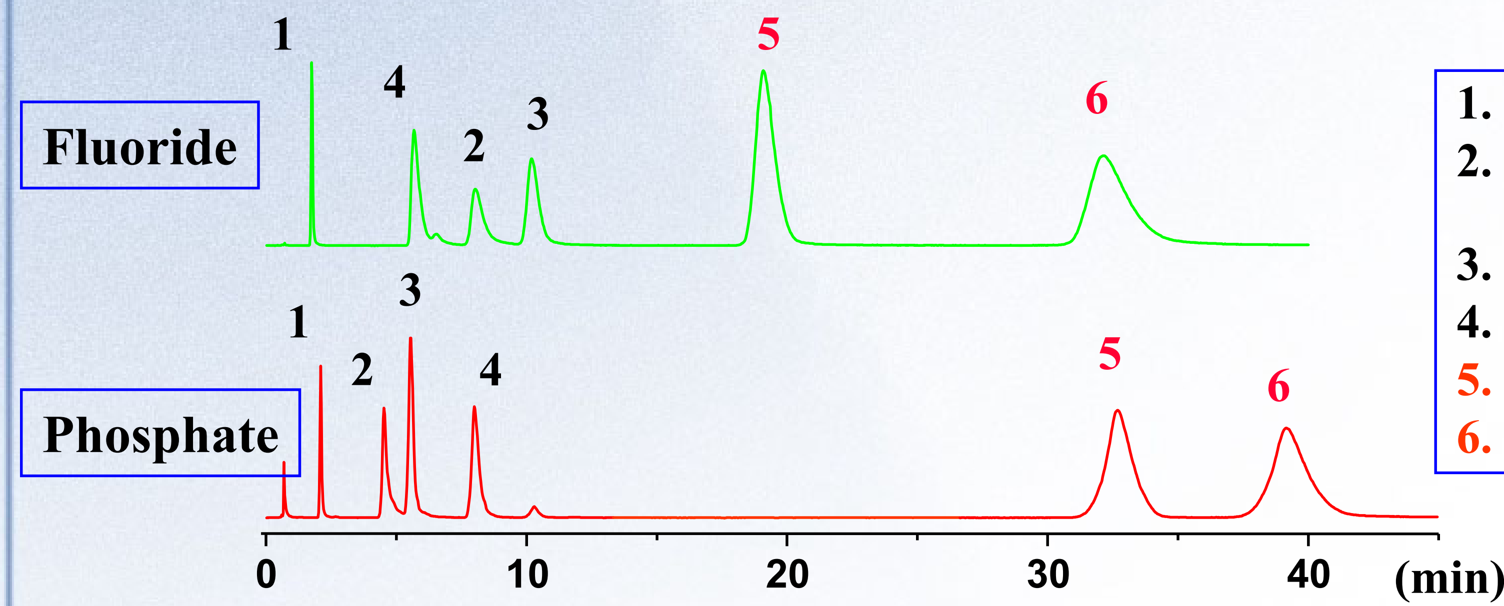


(5) pK_a 9.4



(6) pK_a 9.7





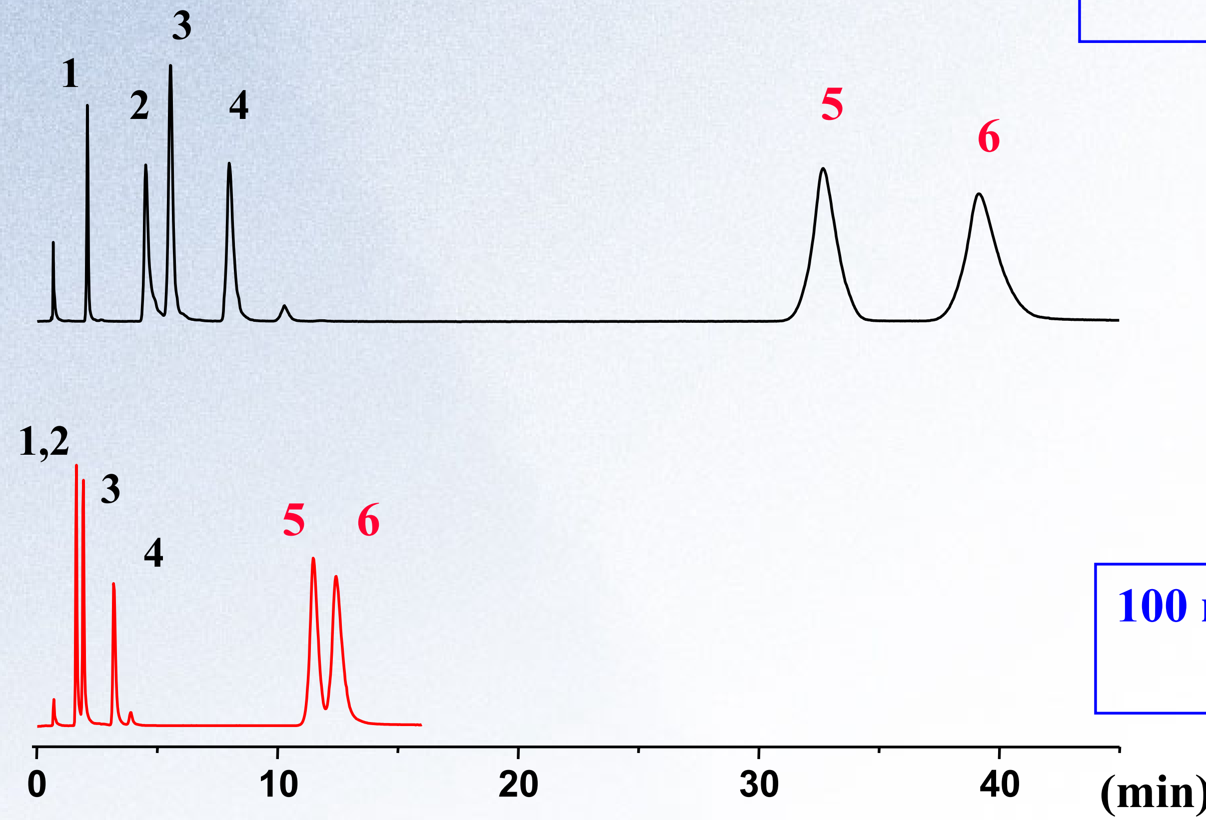
1. Lidocaine
2. Norpseudo ephedrine
3. Tryptamine
4. Quinidine
5. Amitriptyline
6. Nortriptyline

20% ACN, 20 mM Lewis base additive, pH 7.5; 0.8 mL/min; 30 °C

Effect of Additive Concentration on Bases

ZirChrom®

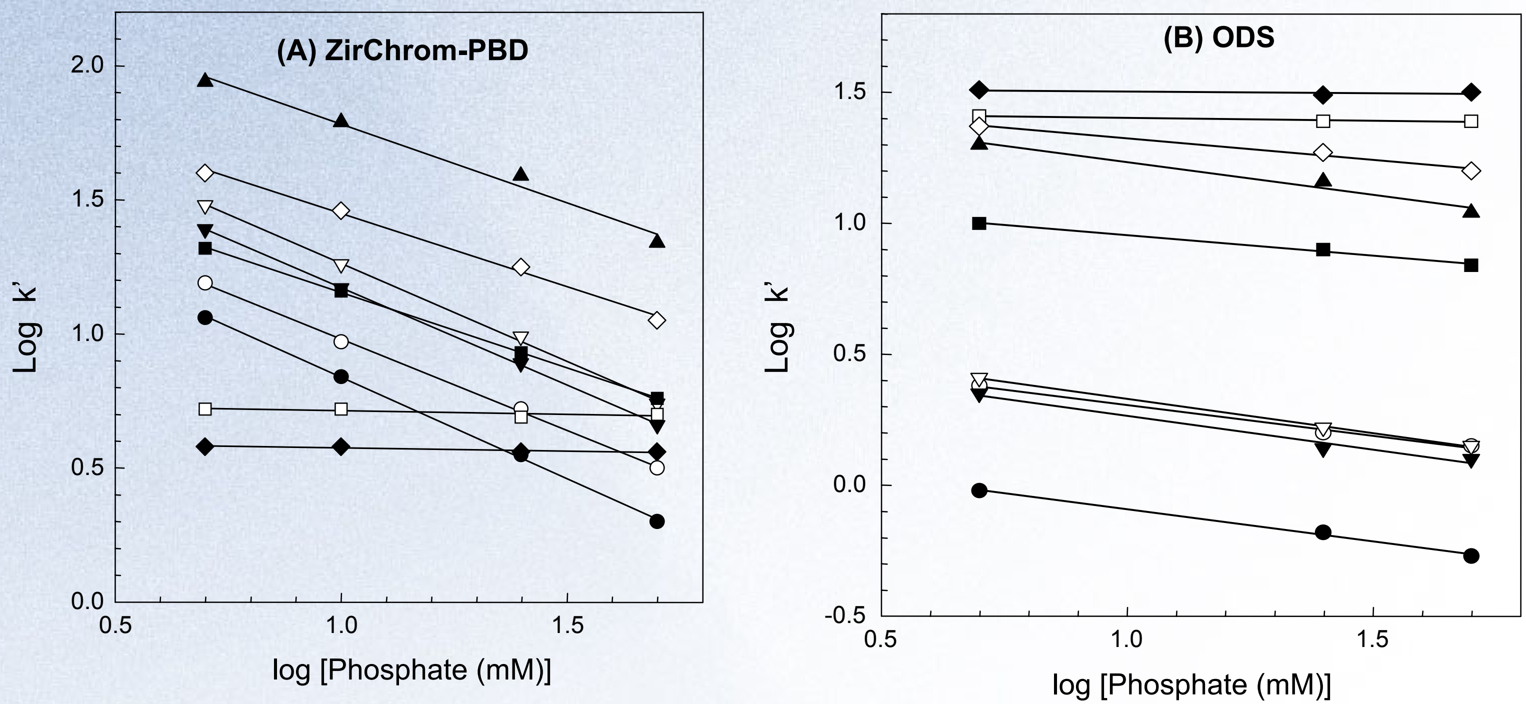
20 mM $NH_4H_2PO_4$
pH 7.5



100 mM $NH_4H_2PO_4$
pH 7.5

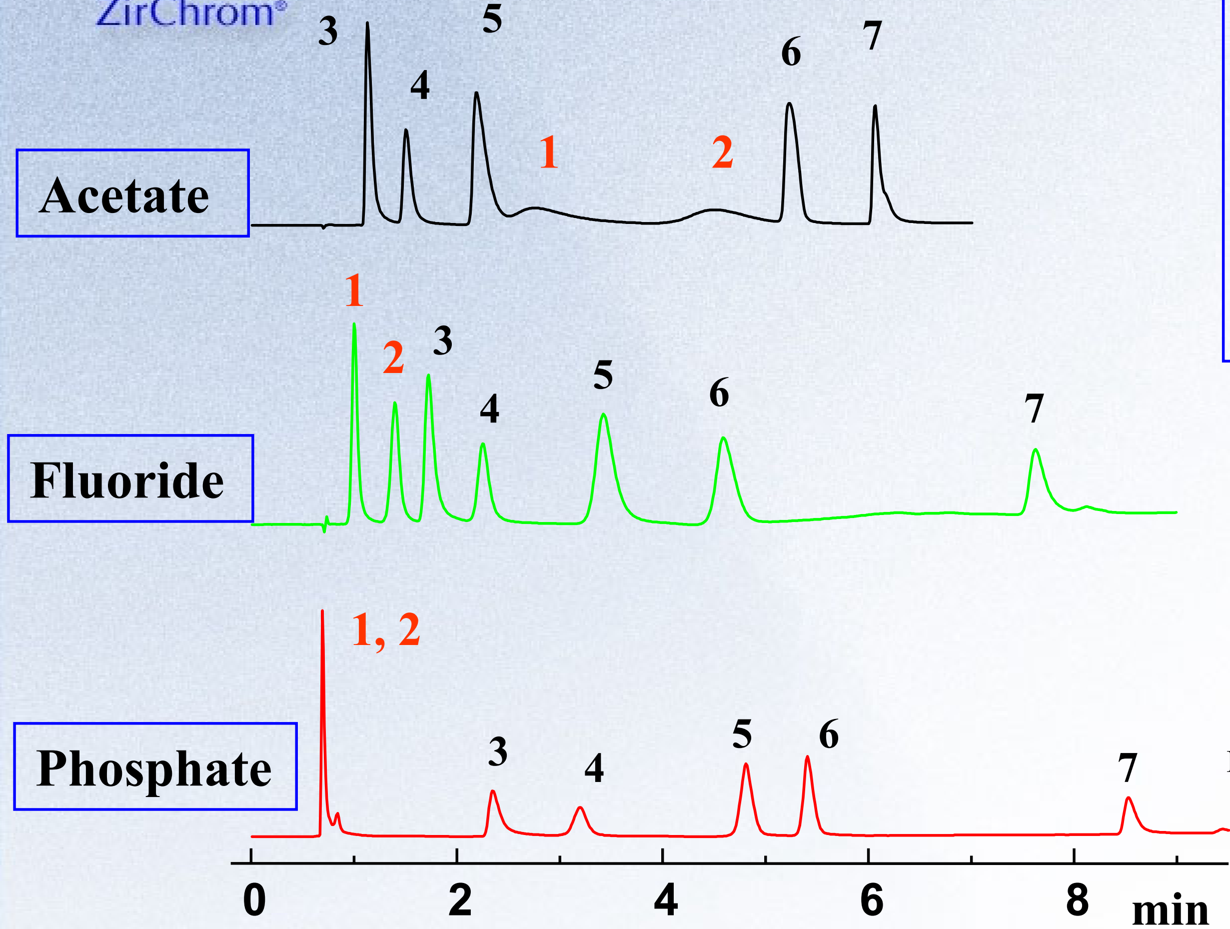
- **CEX is adjustable by ionic strength of mobile phase**

Effect of Buffer Concentration on Retention



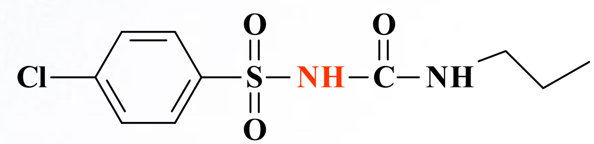
Plot of log *k*' for antihistamines versus logarithm of phosphate buffer concentration in milimole on (A), ZirChrom-PBD column and (B), ODS column. Experimental conditions: mobile phase, 40/60 acetonitrile/potassium phosphate buffer at pH 7.0; temperature, 30°C; solutes: (●), pheniramine; (○), thenyldiamine; (▼), chlorpheniramine; (▽), brompheniramine; (■), cyclizine; (□), thonzylamine; (◆), meclizine; (◇), chlorcyclizine; (▲), pyrrobutamine.

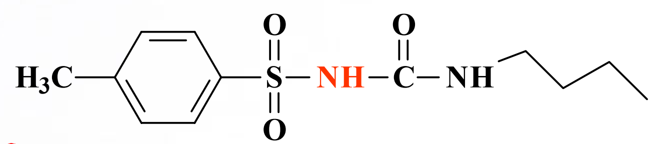
Separation of Antiarrythmic Drugs



1. Chlorpropamide
2. Tolbutamide
3. Procainamide
4. Acetylprocainamide
5. Propionylprocainamide
6. Lidocaine
7. Quinidine

(1) $pK_a = 4.9$





(2) $pK_a = 4.9$

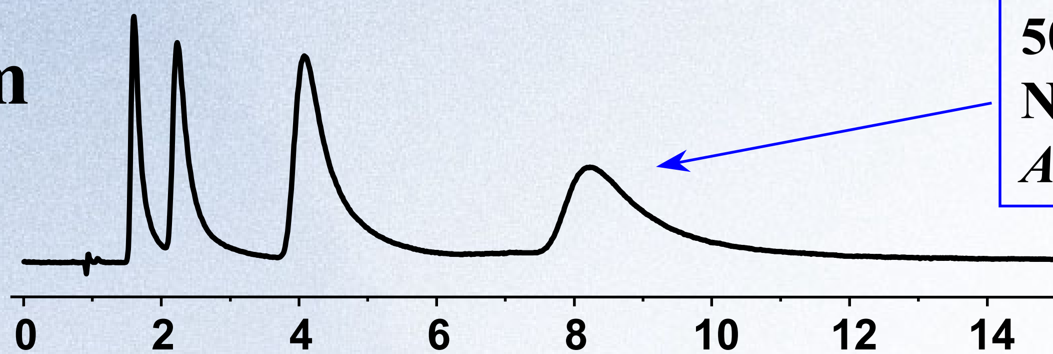
Gradient elution; 30mM Additive, 15 mM TRIZMA , pH 7.5; 0.8 mL/min; 40 °C

Separation of Alkoxybenzoic Acids on ZirChrom®-PBD

ZirChrom®

40 mM Acetate pH 4

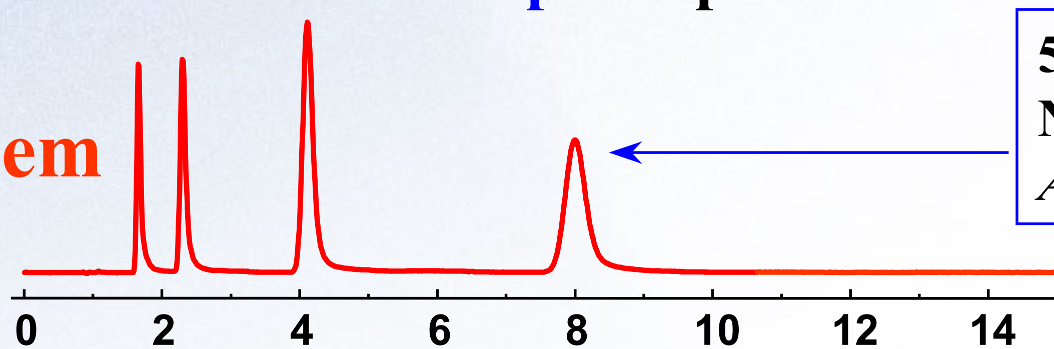
Problem



50 x 4.6 mm
N = 260
A_s = 3.35

40 mM Phosphate pH 2.15

No Problem



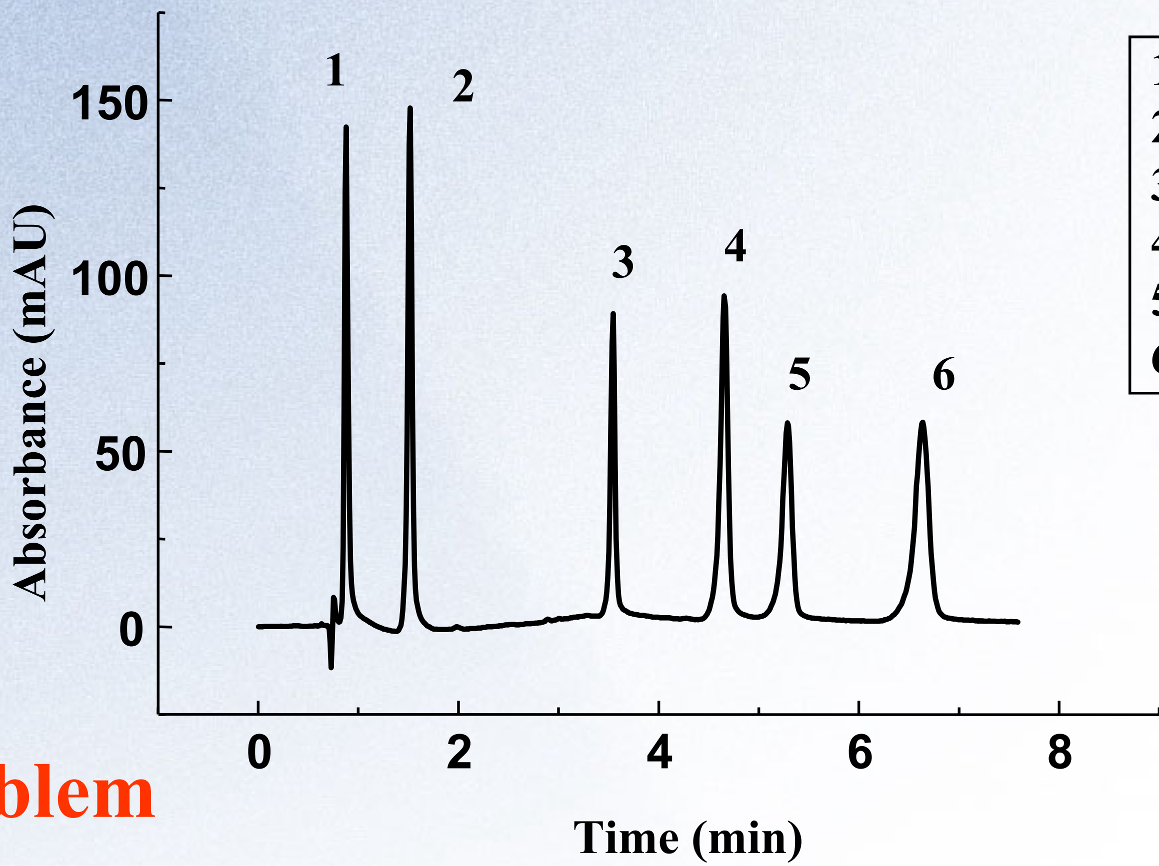
50 x 4.6 mm
N = 3250
A_s = 1.07

25% ACN, 40 mM above additive, 5 mM NH_4F; 0.6 mL/min; 30 °C; 254 nm.

Carboxylate Problem - Solved!

- Phosphate as buffer ($[PO_4] > 20$-30 mM).
- Small amount of fluoride (5mM).
- Low pH (< 3).
- High temperature (40 ^oC).
- ACN as organic modifier.

Separation of Carboxylic Acid NSAIDs on ZirChrom®-PBD



1. Acetaminophen
2. Aspirin
3. Ketoprofen
4. Fenoprofen
5. Ibuprofen
6. Indomethacin

No Problem

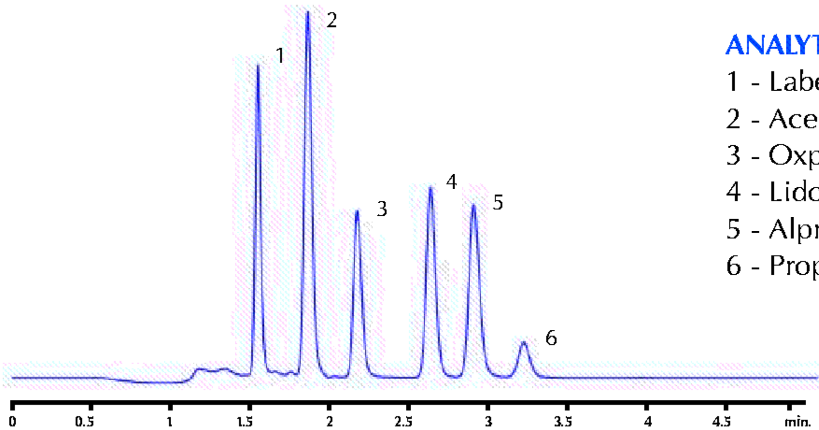
A, 40 mM H_3PO_4, 5 mM NH_4F, pH 2.1; B, 50% ACN + A; 10-60% B, 0-2 min; 60% B, 2-10 min; 0.8 mL/min; 40 °C.

Solve Any Tailing Problem

Use High pH on Zirconia Phases

ZirChrom-PBD Gives Excellent Peak Shapes For Basic Drugs at High pH

...In LC/MS-friendly amine buffer



LC CONDITIONS

Mobile phase: 35/65 ACN/10mM Triethylamine, 50mM Tetramethylammonium hydroxide, pH 12.2

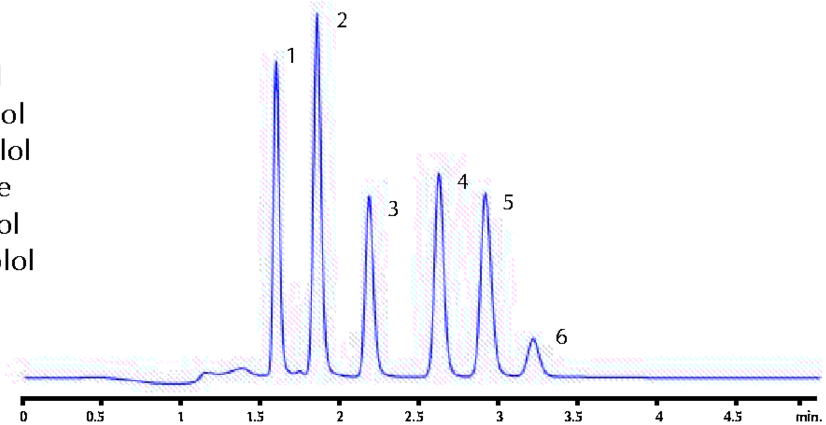
Flow rate: 1.0 ml/min Injection volume: 5 ∝l

Temperature: 30 °C Detection: 254 nm

ANALYTES

1 - Labetalol
2 - Acebutolol
3 - Oxprenolol
4 - Lidocaine
5 - Alprenolol
6 - Propranolol

...Or in phosphate buffer



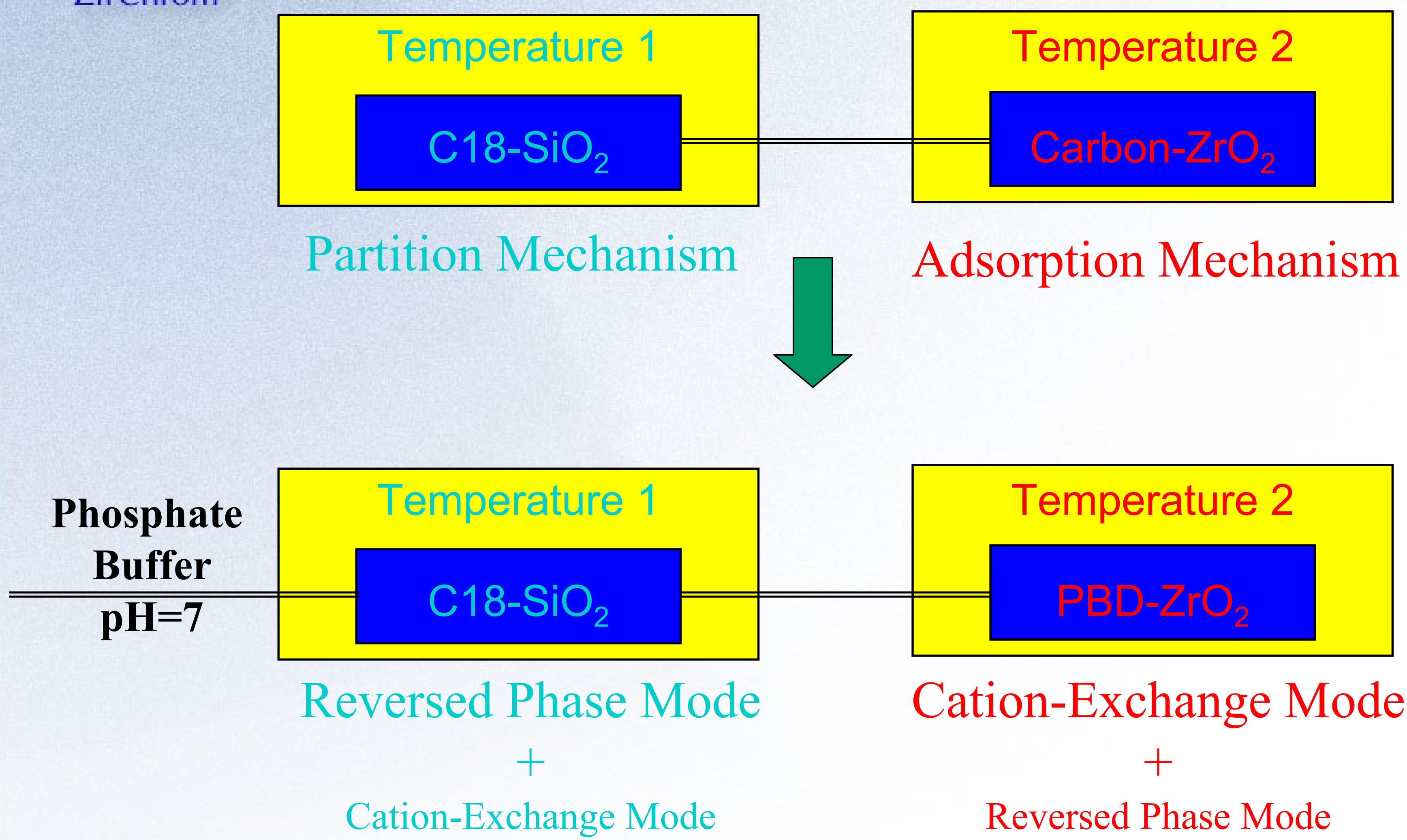
LC CONDITIONS

Mobile phase: 35/65 ACN/20mM Potassium Phosphate, pH 12.2

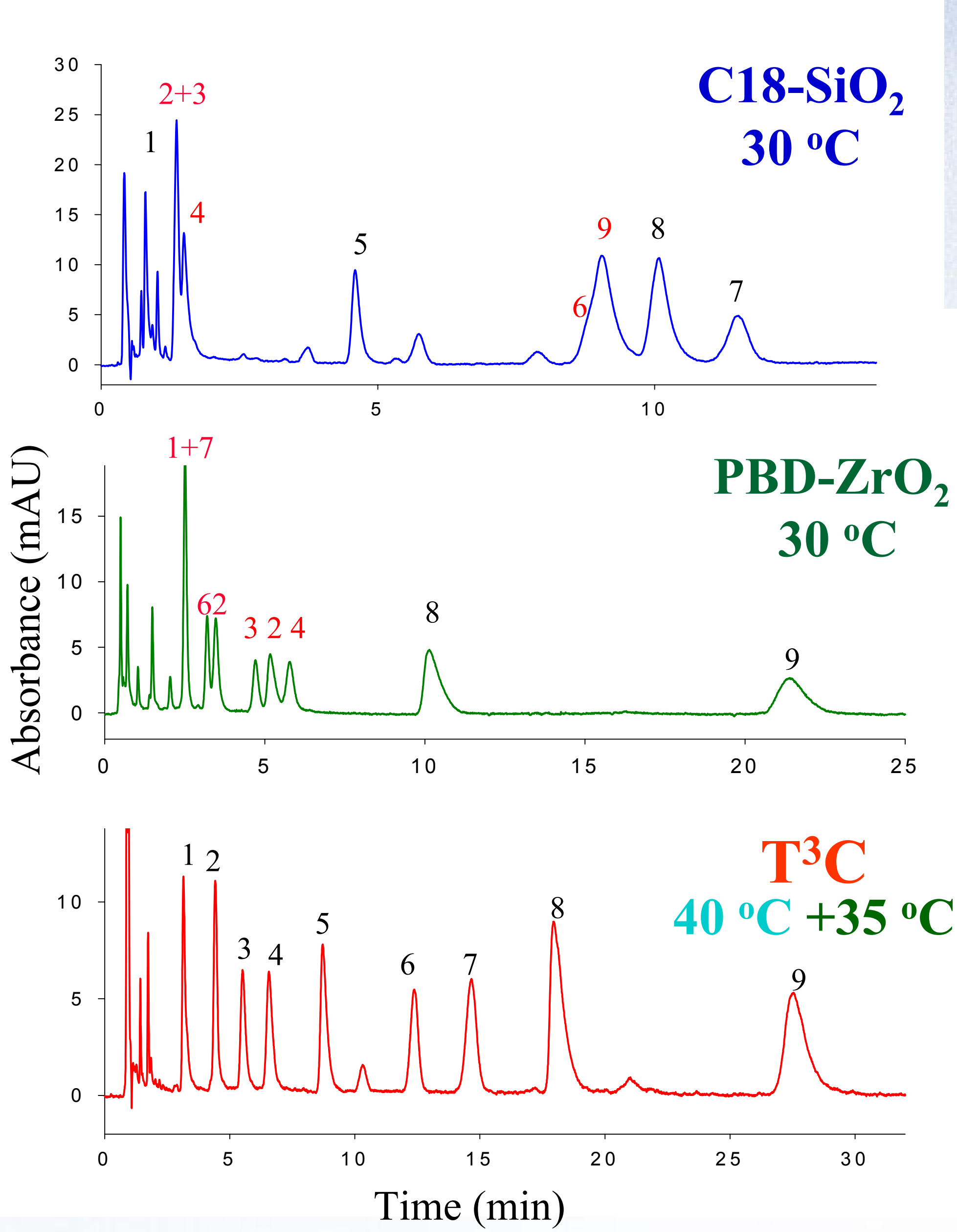
Flow rate: 1.0 ml/min Injection volume: 5 ∝l

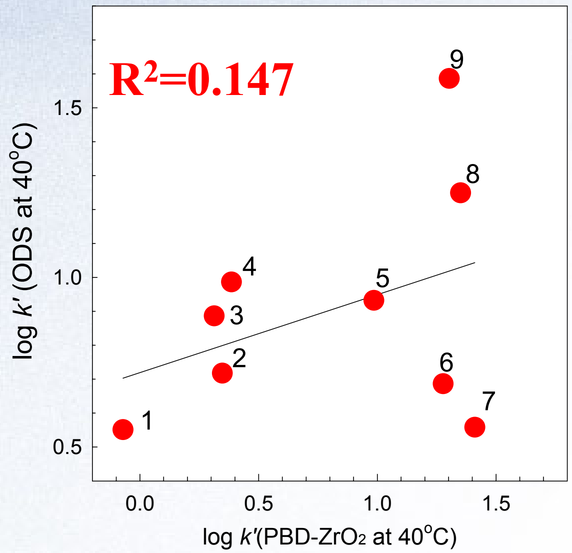
Temperature: 30 °C Detection: 254 nm

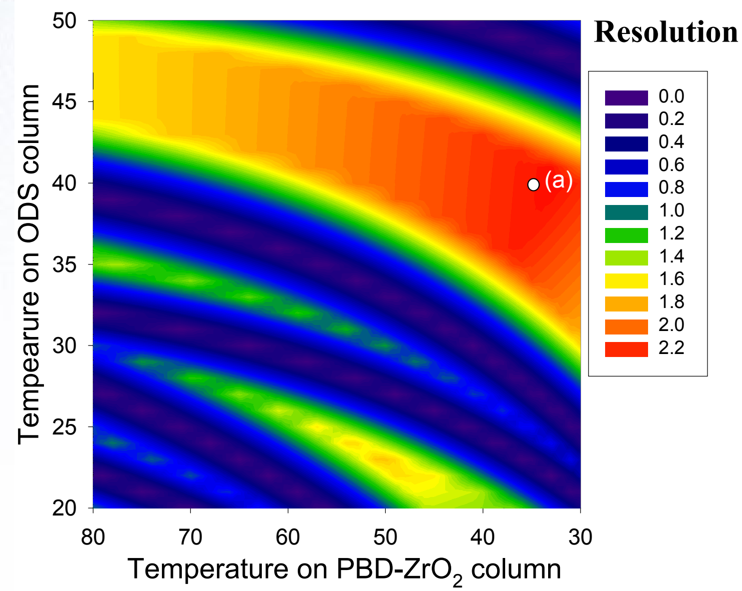
Combination of ODS and PBD-ZrO_2 for the separation of basic drugs



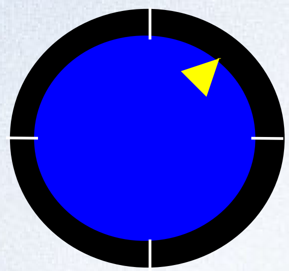
Separation of Anti-Histamine Drugs by T^3C







When Do We Use the Temperature Knob?



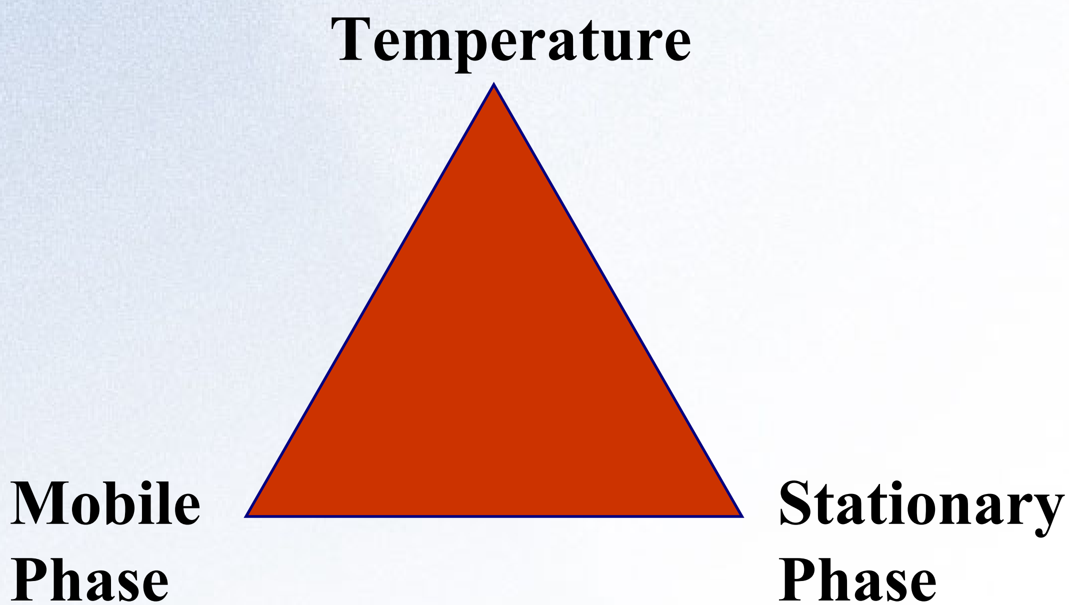
Temperature up to 200^{o}C

- To ***speed up*** analyses
- To modify ***band spacing***

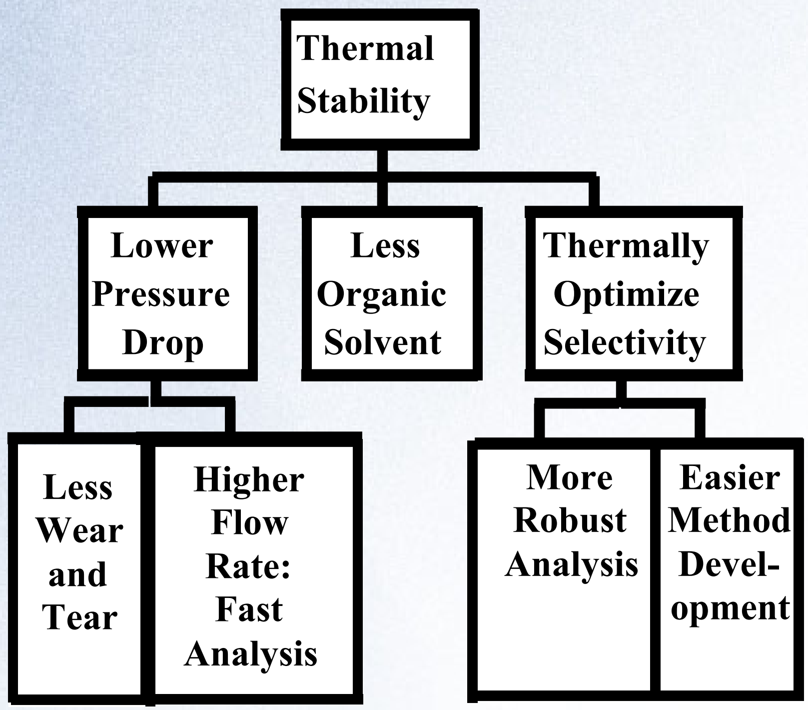
ZirChrom®

Temperature

The Third Dimension in HPLC

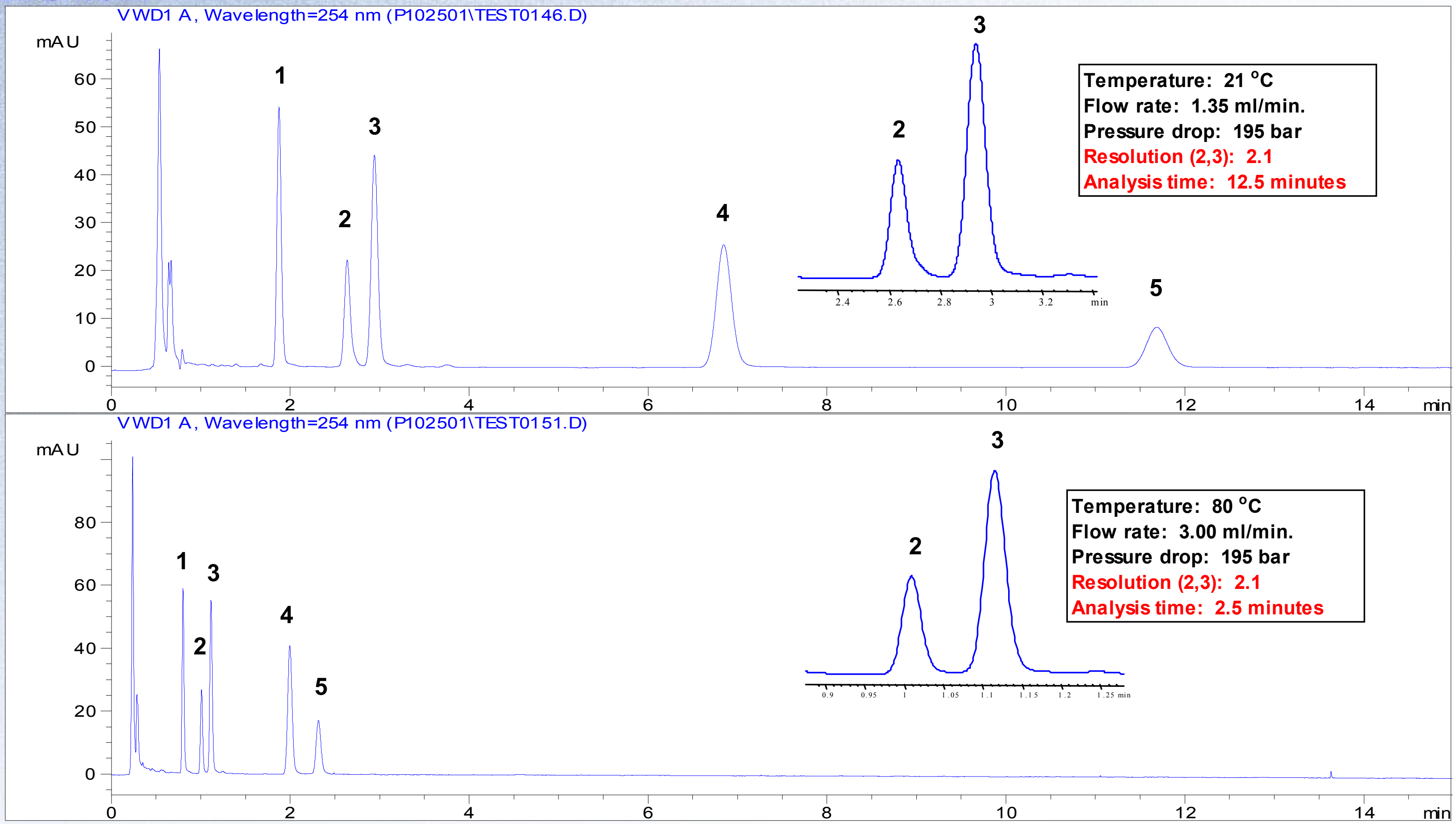


Why Use Temperature?



Antihistamine Example:

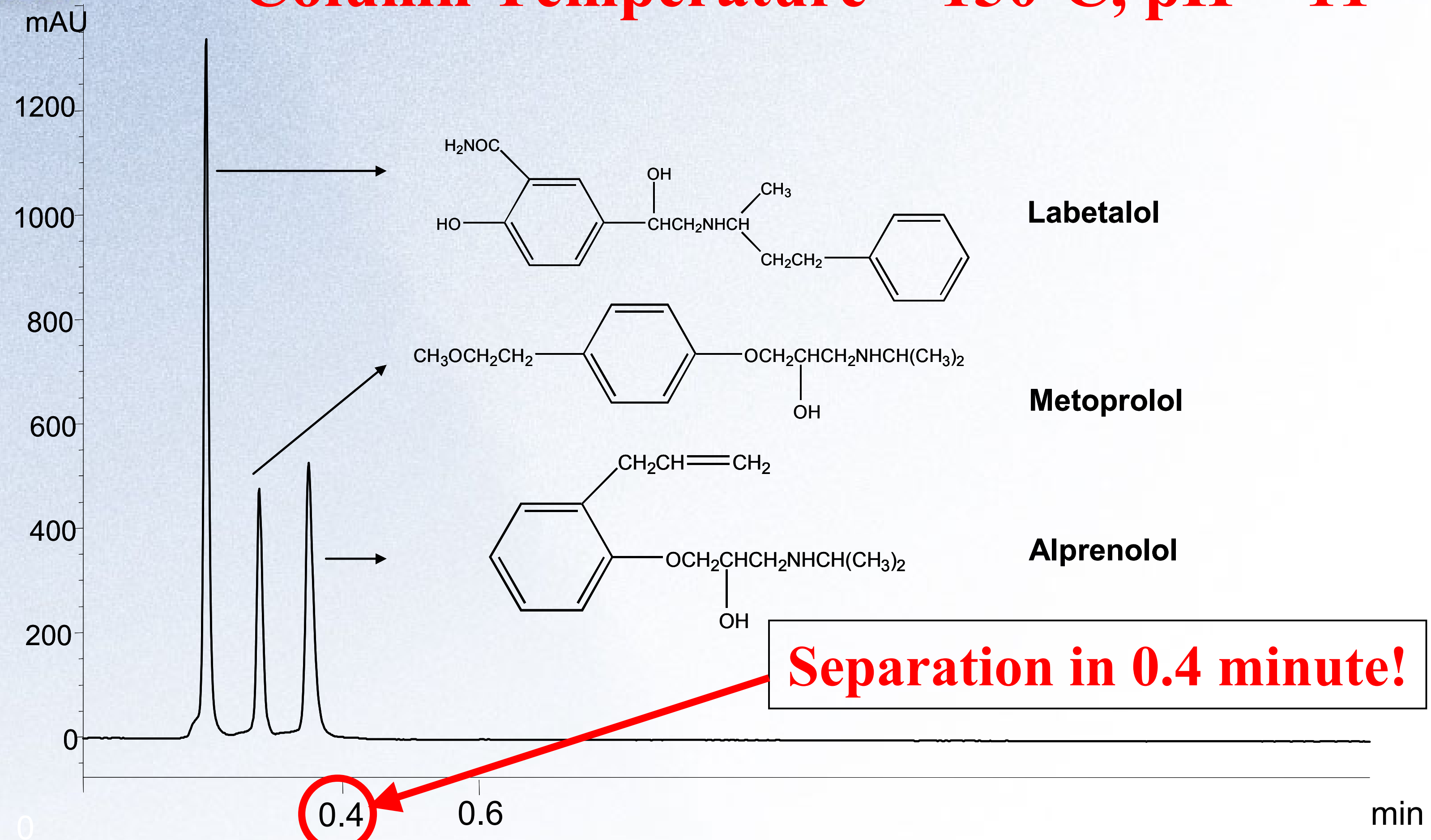
Speed it up!



LC Conditions: (A) Mobile Phase, 29/71 ACN/50mM Tetramethylammonium hydroxide, pH 12.2; Flow Rate, 1.35 mL/min.; Injection volume, 0.5 ul; 254 nm detection; Column Temperature, 21°C; Pressure drop = 195 bar; Solutes: 1=Doxylamine, 2=Methapyrilene, 3=Chlorpheniramine, 4=Triprolidine, 5=Meclizine4=Triprolidine, 5=Meclizine, 100 x 4.6 ZirChrom-PBD (B) same as A, except Mobile Phase, 26.5/73.5 ACN/50mM Tetramethylammonium hydroxide, pH 12.2; Flow Rate, 3.00 mL/min.; Column Temperature, 80°C; Pressure drop = 195 bar.

Fast β-Blockers Separation

Column Temperature = 150°C, pH = 11



LC Conditions: Column, 50 x 4.6 Diamondbond-C18, OD0121601A; Mobile phase, 45/55 ACN/20mM Ammonium Phosphate pH11.0; Flow rate, 3.0 ml/min; Temperature, 150 °C; Injection volume, 1.0 ul; Detection at 210 nm; Solutes, 1=Labetalol, 2=Metoprolol, 3=Alprenolol

Summary

Causes of Bad Peak Shape

ZirChrom®

- "Bad" Column (poorly packed).
- Build up of "GARBAGE" on the column.
- Too much sample per injection.
- Wrong solvent for sample.
- Extra-column effects.
- Chemical or secondary effects (Lewis acid site interactions).
- Poor buffering.

Operational Similarities for SiO_2 and ZrO_2 RP Media

ZirChrom®

- k' increases with molecular hydrophobicity (CH_3, CH_2, phenyl, etc.).
- Elution sequence of non-electrolytes similar.
- k' decreases 2 fold per 10% increase in volume % modifier.
- log k' linear with % organic modifier.
- k' decreases as temperature is increased (3 fold/50 ^{o}C).
- Solvent strength: THF > ACN > MeOH.

Operational Differences in SiO_2 and ZrO_2 RP Media

- Lewis base modifier at mid-range pH creates mixed-mode RPC/IEC possibilities.
- Cations are typically more retained and sometimes much more retained on ZrO_2 in buffered (PO_4) eluents than on SiO_2 phases.
- Elution sequence of anions and cations can be very different on ZrO_2 & SiO_2 at neutral pH.
- Must use Lewis base eluent for carboxylic analytes. Strongly advise use of 5mM or more phosphate (or TMAH) for all electrolytes.

Conclusions

- Silica-based HPLC columns work well between pH 3 and 8; outside these limits, these columns can have short lifetimes
- Zirconia is stable at pH 1-14 and at elevated temperature
- Zirconia can be functionalized by coating / cross-linking polymers, for very stable supports.
- ZirChrom's zirconia columns reproducibly combine silica's efficiency with polymer stability

Thanks *very much* for listening!

ZirChrom®

ZirChrom Separations & Cabot Corporation

Partners in Chromatography

ZirChrom®

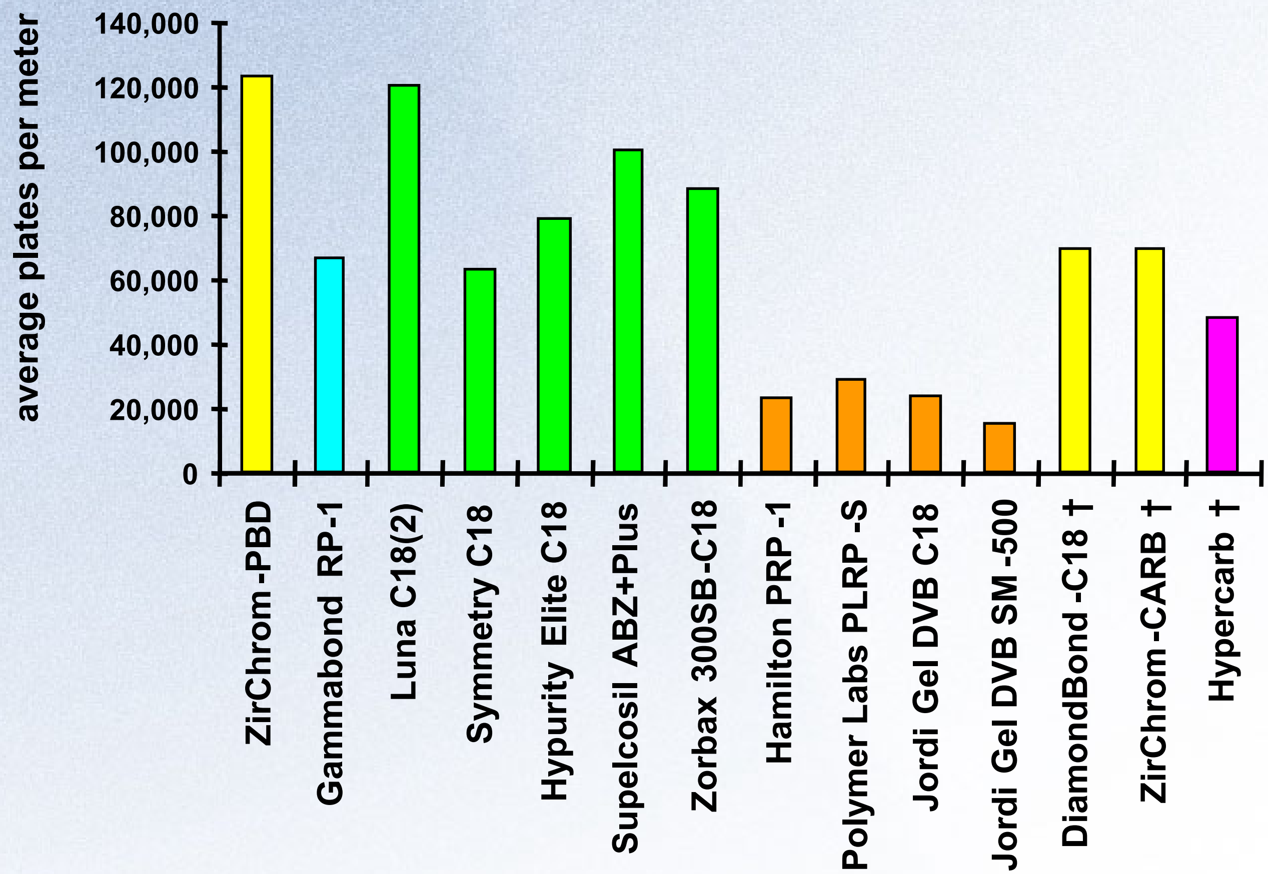
1-866-STABLE-1
www.zirchrom.com

... For Peak Performance

For more information and web access to the free **Buffer Wizard:** www.zirchrom.com

Additional Information

Efficiency Comparison of Leading HPLC Columns*



LC Conditions: Mobile Phase, 65/35 Acetonitrile/50mM Potassium phosphate buffer, pH 3.2; Flow Rate, 1.0 mL/min.; Injection volume, 1 ul; 254 nm detection; Column Temperature, 21°C. Solutes: uracil, phenol, pyridine, 4-butylbenzoic acid, N,N – dimethylaniline, toluene.

† Mobile Phase: 45/5/50 Acetonitrile/THF/50mM Potassium phosphate buffer, pH 7.0; Column Temperature: 30°C; all other conditions are identical.

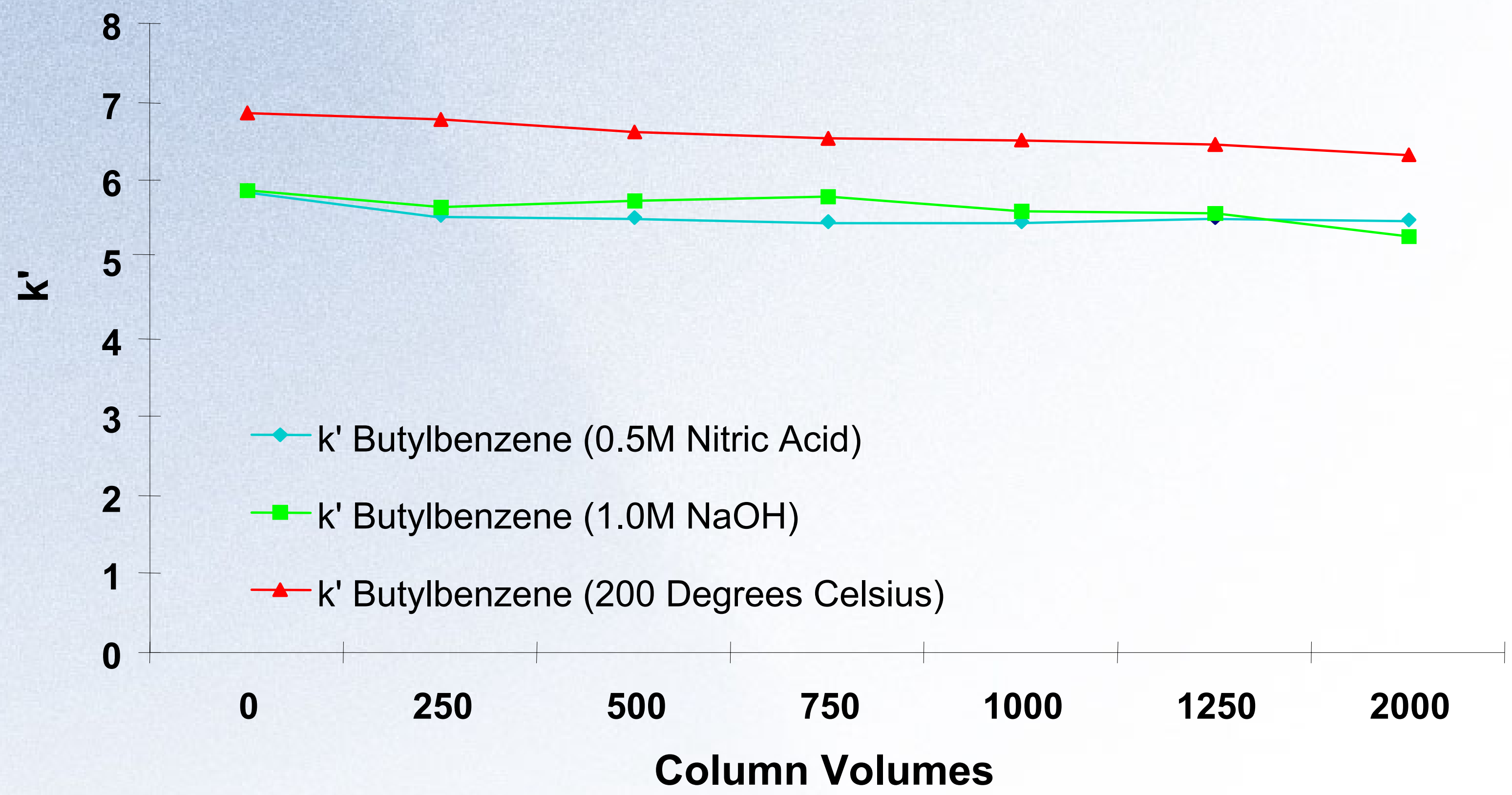
* Column names are the trademarks of their respective manufacturers.

Reproducibility Data for 50 ZirChrom®-PBD Columns

Parameter	Average	%RSD
k' (Toluene)	2.32	2.4%
α (Methylbenzoate/ Benzonitrile)	1.80	1.1%
N (plates/m)	151,000	6.8%
Symmetry	0.93	7.2%

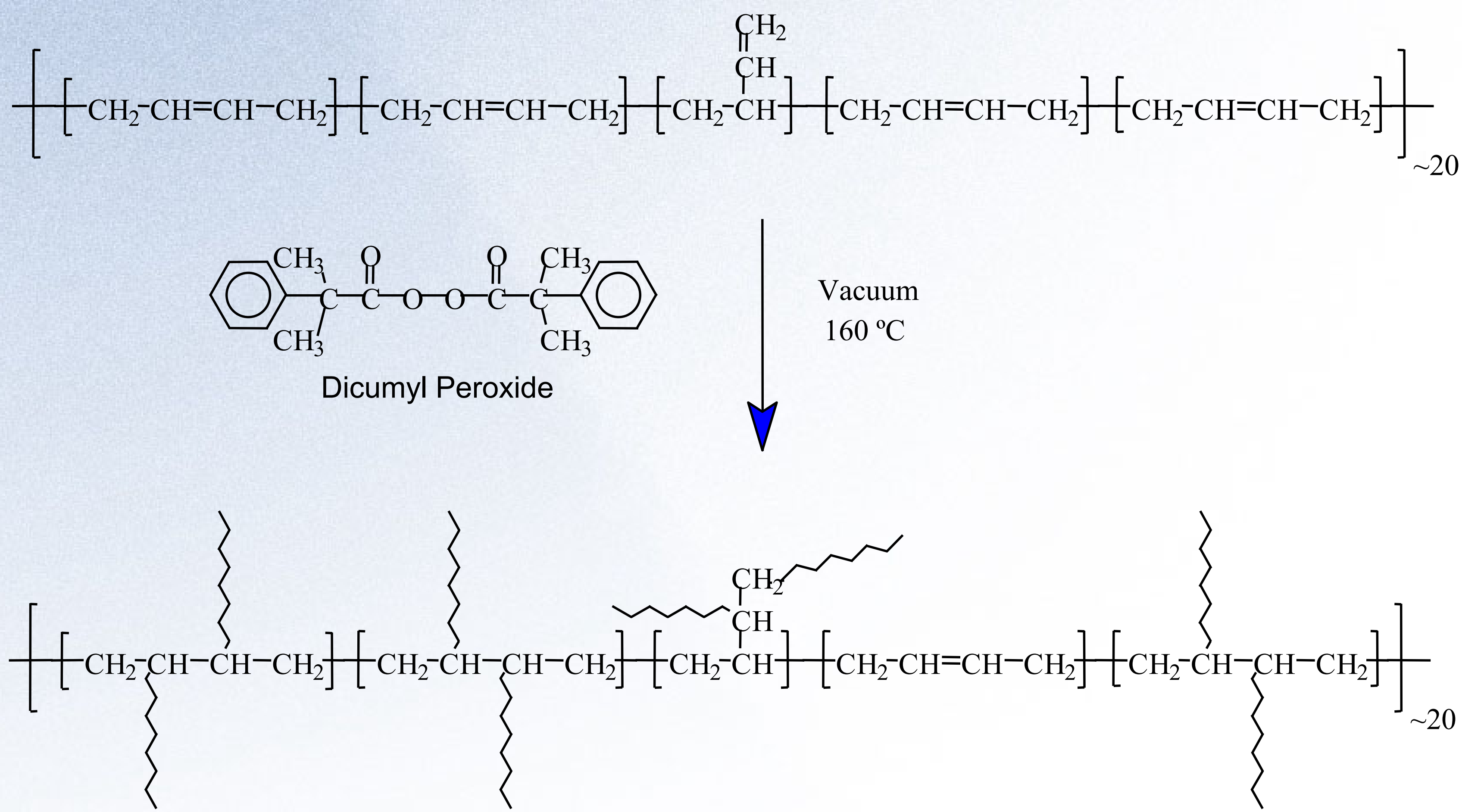
LC Conditions: Mobile phase, 35/65 ACN/Water; Flow rate, 1.0 ml/min.; Temperature, 30 ^{o}C; Injection volume, 5 μl; Detection at 254 nm

Testing of DiamondBond Stability



<u>LC Conditions</u>: Base Stability—DiamondBond™ Phase A, 30 x 4.6 mm id; Mobile phase, 50/50 ACN/Water; Flow rate, 1.0 ml/min.; Temperature, 30 °C; Injection volume, 5ul; Detection at 254nm. Acid Stability—DiamondBond™ Phase A, 50 x 4.6 mm id; Mobile phase, 50/50 ACN/Water; Flow rate, 1.0 ml/min.; Temperature, 30 °C; Injection volume, 5ul; Detection at 254nm. Temperature Stability-- DiamondBond™ Phase B, 50 x 4.6 mm id; Mobile phase, 50/50 ACN/Water; Flow rate, 1.0 ml/min.; Temperature, 30 °C; Injection volume, 5ul; Detection at 254nm.

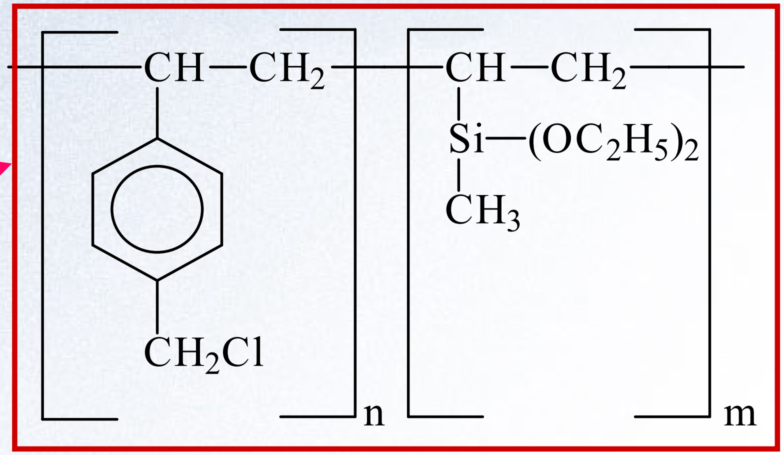
Synthesis of ZirChrom-PBD (Polybutadiene)



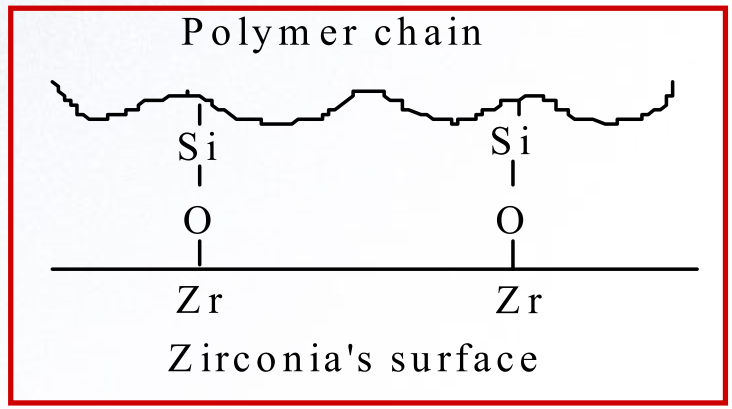
ZirChrom-PS Synthesis

ZirChrom®

Step 1. Synthesis of Copolymer (CMS/VMS)



Step 2. Adsorption of Copolymer



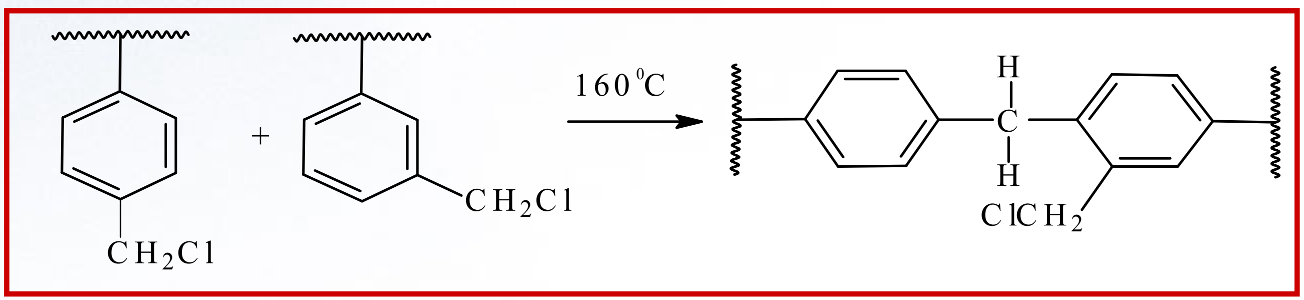
Step 3. Thermal Crosslinking

↓

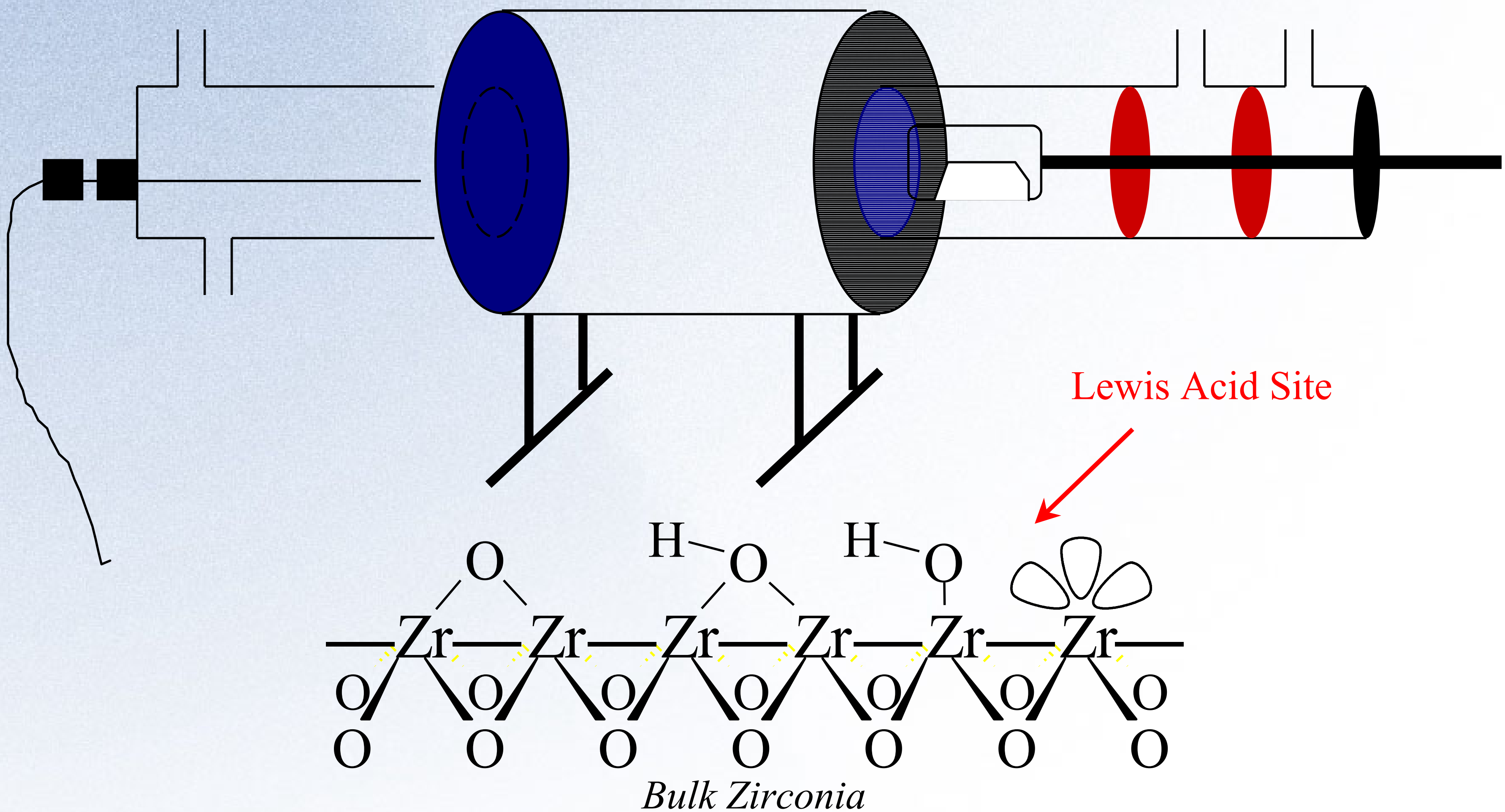
$CMS/VMS\text{-}ZrO_2$

||

$PS\text{-}ZrO_2$

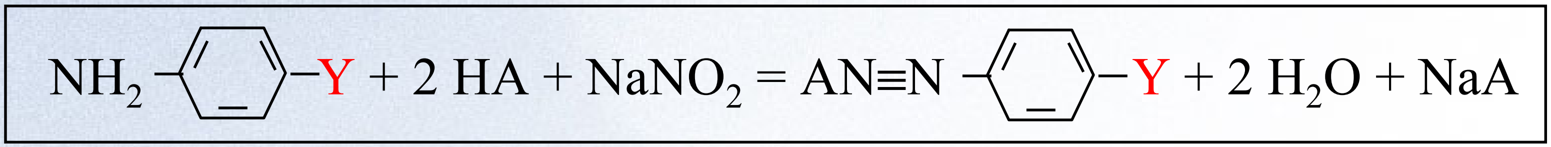


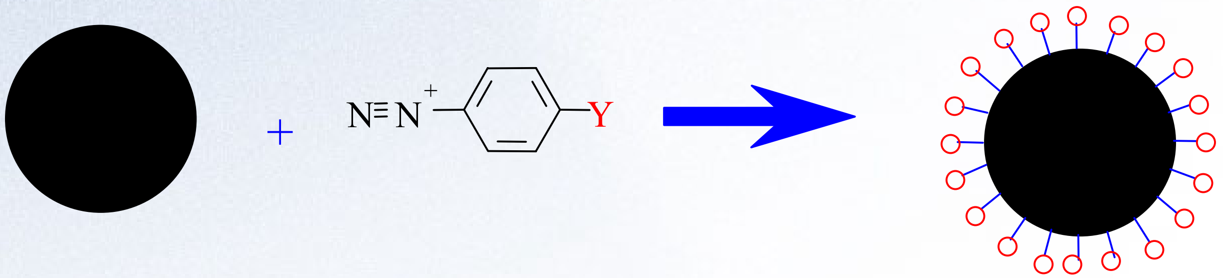
Synthesis of ZirChrom-CARB



Synthesis of DiamondBond-C18

- General approach - Cabot Corporation (Billerica, MA):
  - functionalizing agent X-R-Y
  - X reacts with surface
  - Y = functional group
- X is typically a diazonium salt





Carbon Clad Zirconia Diazonium Salt Modified Carbon Clad Zirconia

ZirChrom-CARB

ZirChrom®

- ZirChrom-CARB has RPLC like properties but has radically different selectivity than ODS or ZirChrom®-PBD -- great for steroids, and for geometric isomers.
- Much more hydrophobic & retentive than ODS -- great for polar analytes

- ZirChrom®-CARB is very stable. Can be used at low and high pH. Can be used at high temperatures.
- Lewis acid-base properties are still important on all zirconia-based phases.
- Peak shapes best with THF (10%) and/or elevated temperature.

Role of Temperature in LC

ZirChrom®

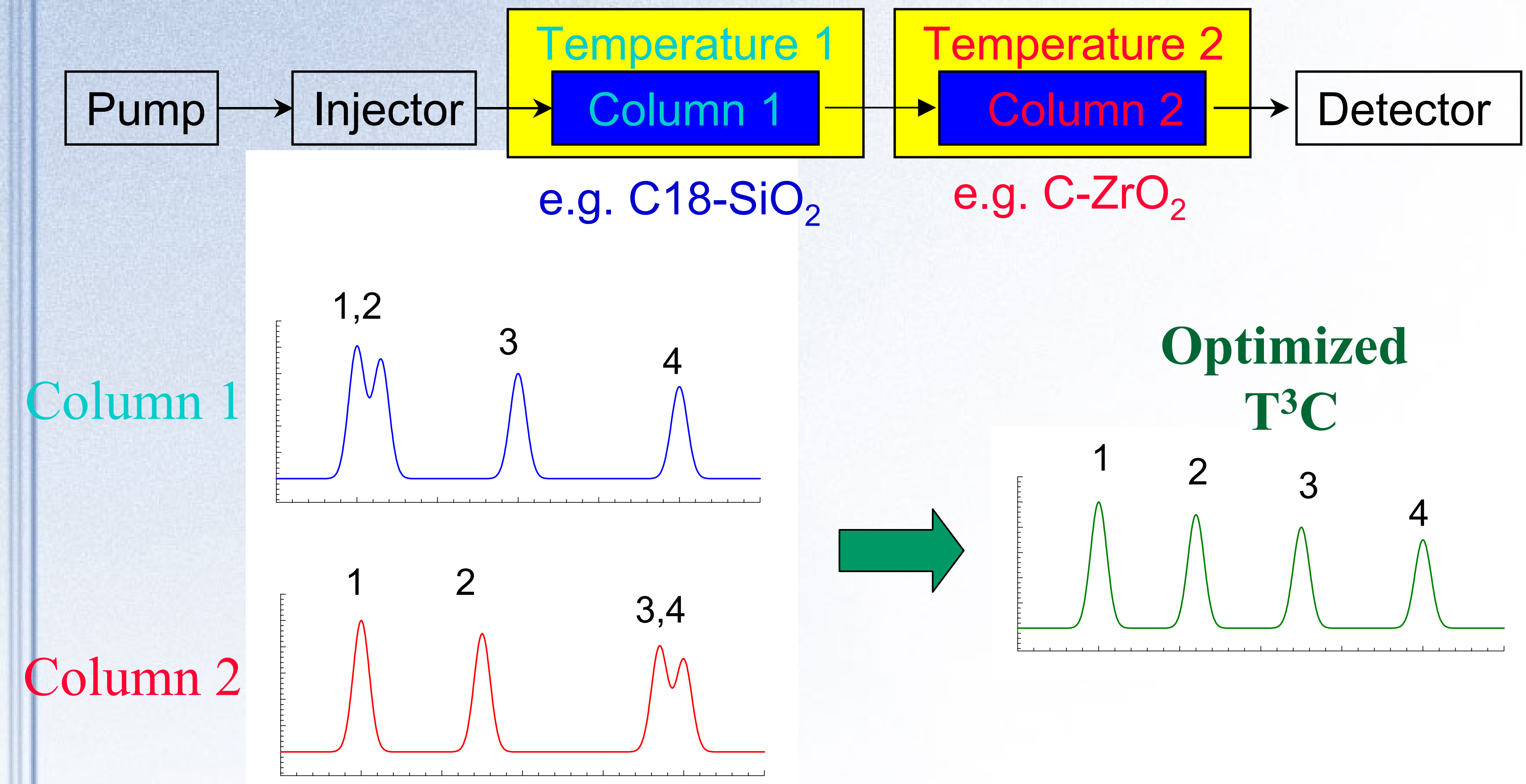
"High-Performance Liquid Chromatography at Elevated Temperatures: Examination of Condition for the Rapid Separation of Large Molecules", R. D. Antia and Cs. Horvath, *J. Chromatogr*., 435, 1-15 (1988).

"Temperature as a Variable in Reversed –Phase High-Performance Liquid Chromatographic Separations of Peptide and Protein Samples", W. S. Hancock, R. C. Chloupek, J. J. Kirkland and L. R. Snyder, *J. Chromatogr. A*, 686, 31-43 (1994)

"Superheated Water: A New Look at a Chromatographic Eluent for Reversed-Phase Liquid Chromatogrpahy",
R. M. Smith and R. J. Burgess, *LC-GC*, 17, 938-945 (1999)

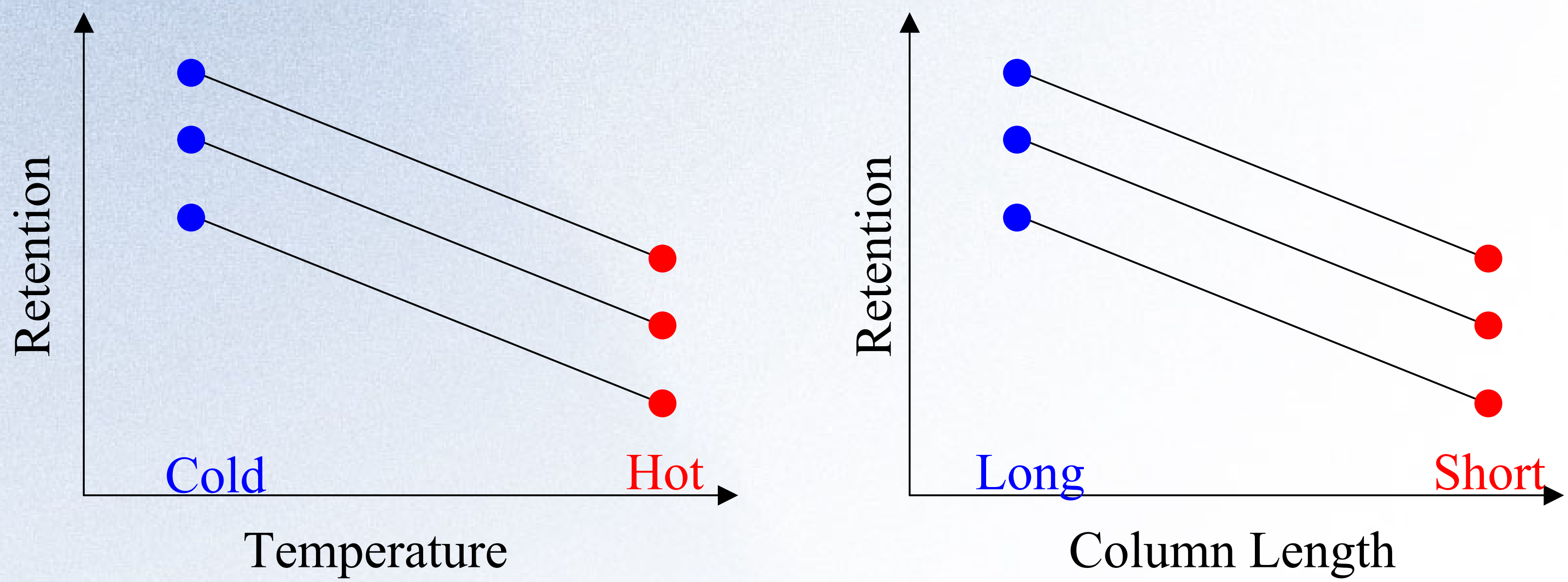
Thermally Tuned Tandem Columns (T^3C)

A Mechanism to ***Continuously*** ***Adjust*** the Stationary Phase



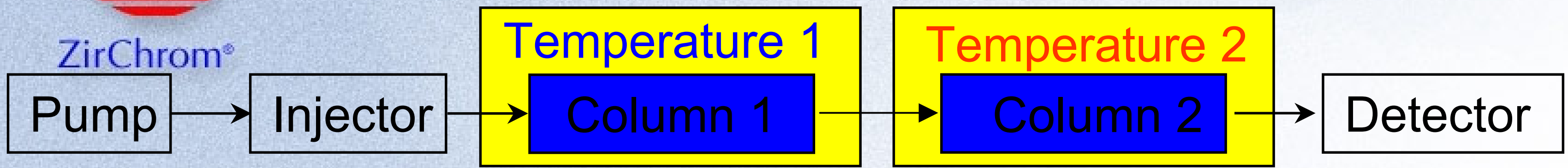
Analogy between Column Temperature and Column Length

T increases 50 °C ⟹ *k*' decreases 3-fold



Increasing temperature and decreasing column length both decrease retention time

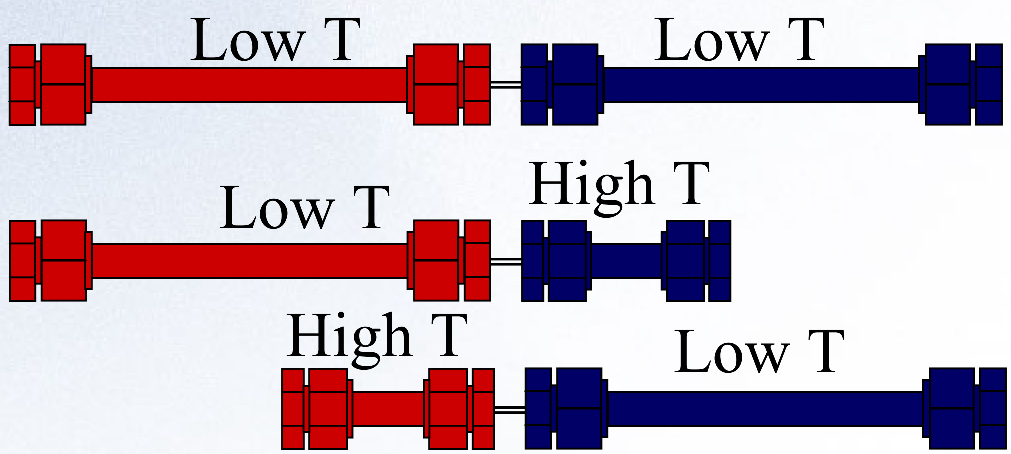
Effect of Temperature on T^3C Selectivity



$$\alpha_{net} = f_1\alpha_1 + f_2\alpha_2$$

$$f_1 = \frac{k_1'}{k_1' + k_2'}$$

$$f_2 = \frac{k_2'}{k_1' + k_2'}$$



$$\alpha_{net} \rightarrow \alpha_1$$

$$\alpha_{net} \rightarrow \alpha_2$$

Temperature *continuously* changes the T^3C selectivity between α_1 and α_2.

Requirements for T^3C

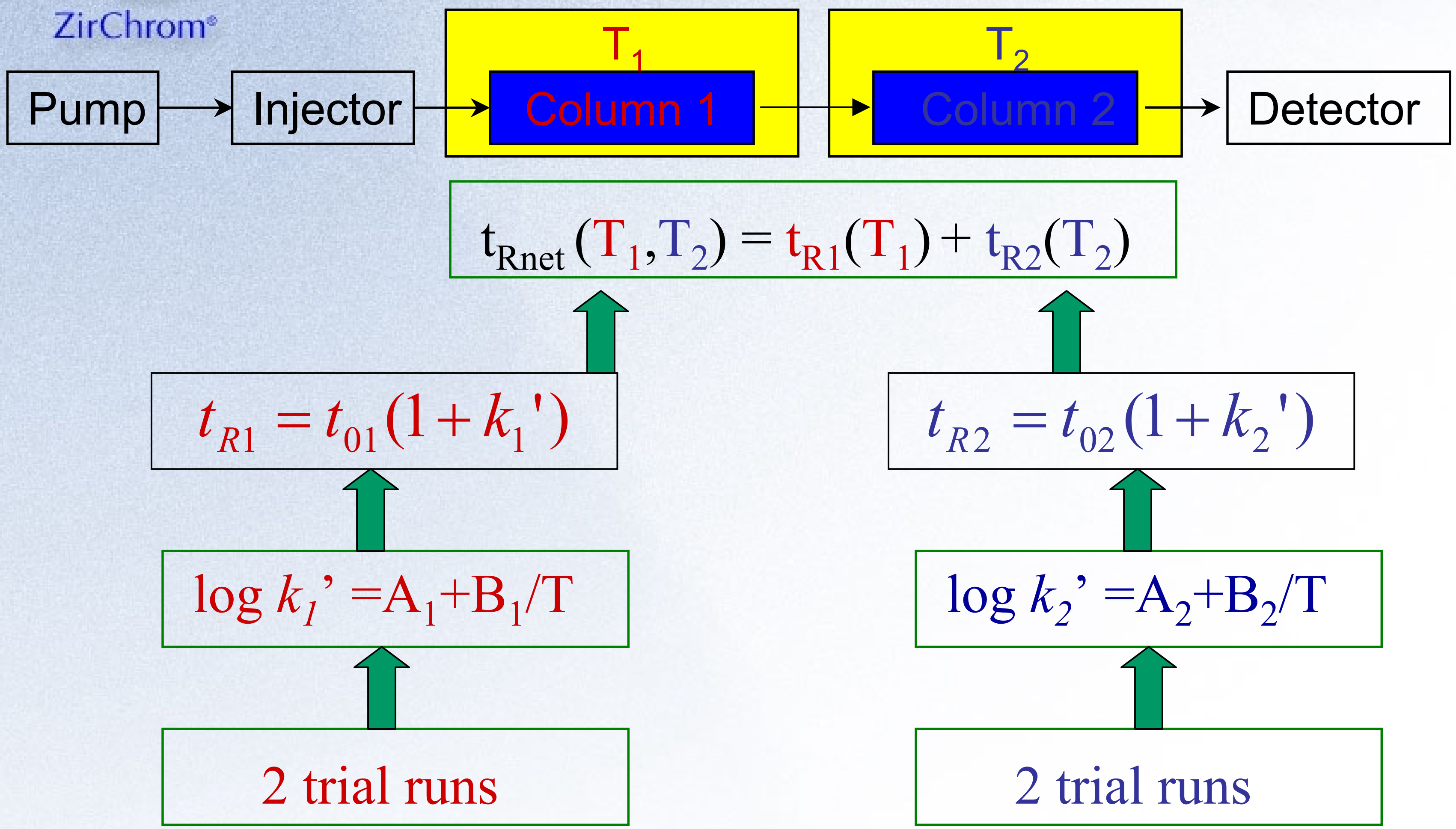
1. Two columns with different (ideally orthogonal) selectivities

2. One column must be thermally stable
   - Zirconia based phase are thermally stable up to 200 ^oC
   - Polybutadiene-coated zirconia (PBD-ZrO_2)
   - Carbon-coated zirconia (C-ZrO_2)

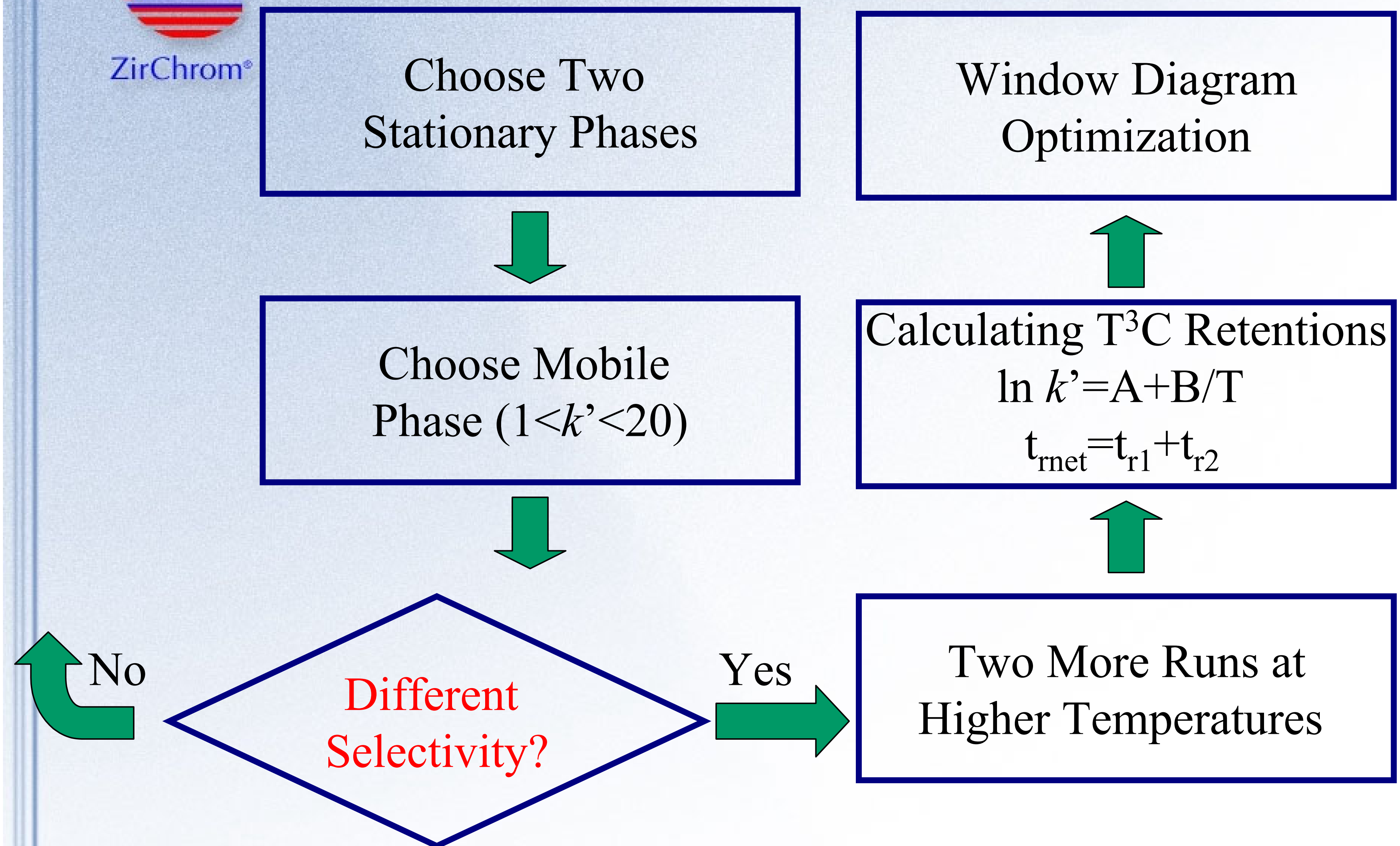
3. Method development must be easy
   - Theory for T^3C
   - Guideline for method development

Method Development for T^3C

ZirChrom®

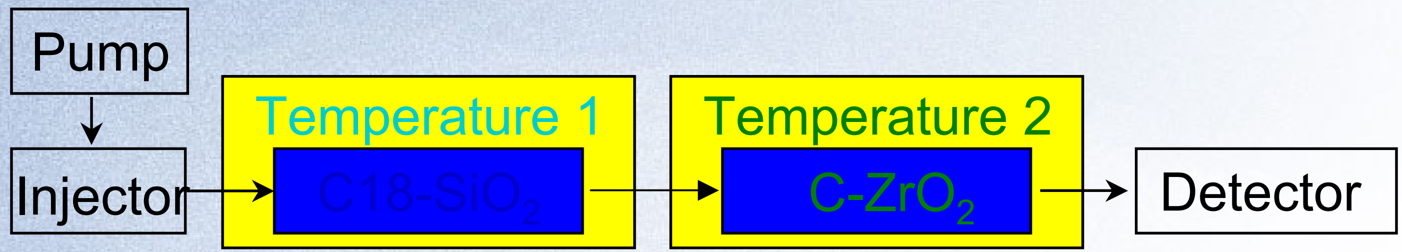


Guidelines for Optimizing T^3C

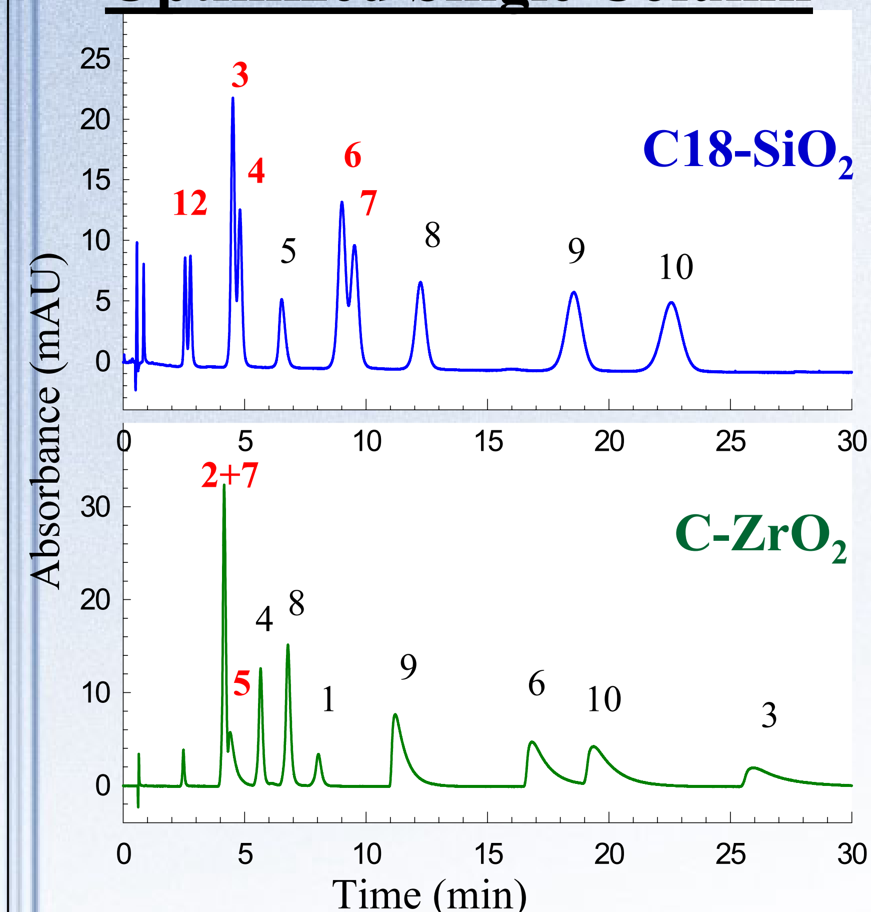


Thermally Tuned Tandem Column (T^3C) Concept

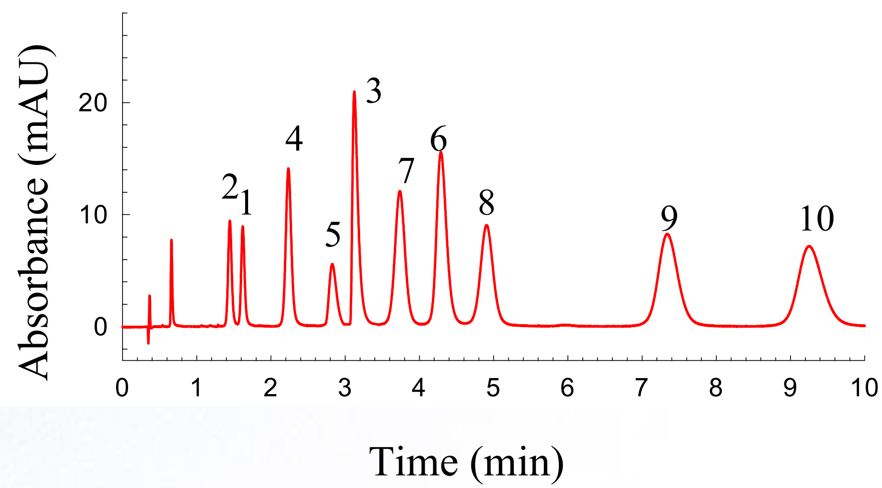
ZirChrom®



Optimized Single Column

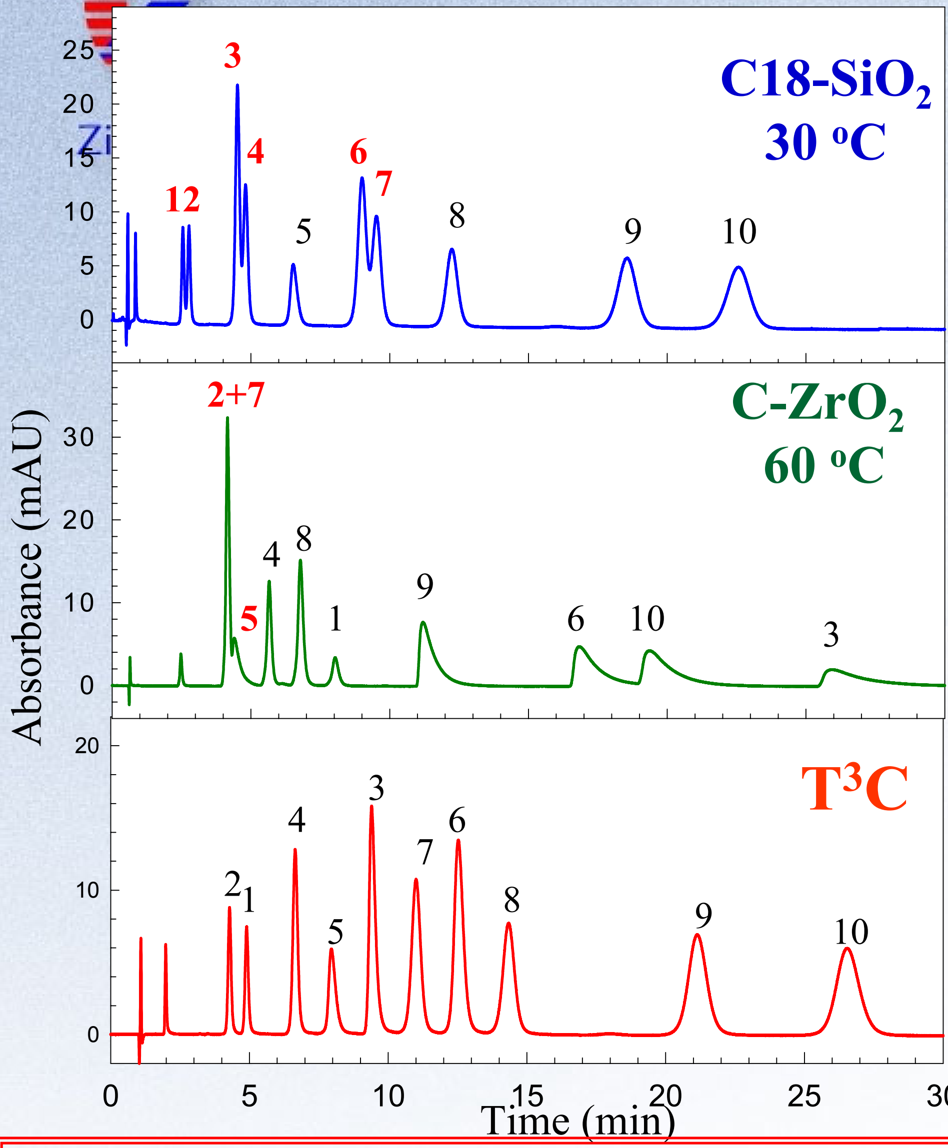


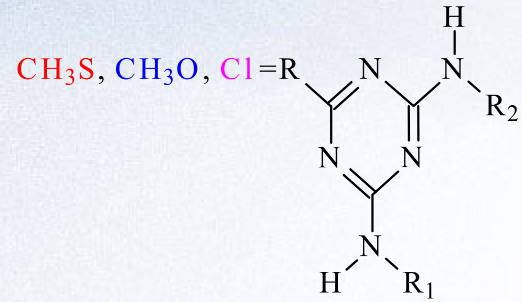
Optimized T^3C



T^3C Works!

Separation of Ten Triazine Herbicides by T^{3}C





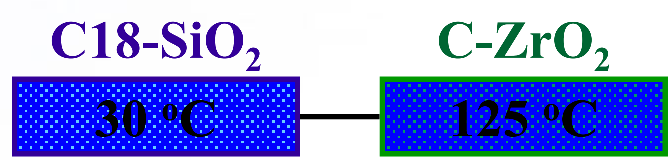
Solutes:

1. Simazine
2. Cyanazine
3. Simetryn
4. Atrazine
5. Prometon
6. Ametryn
7. Propazine
8. Terbutylazine
9. Prometryn
10. Terbutryn

Other conditions:

30/70 ACN/water

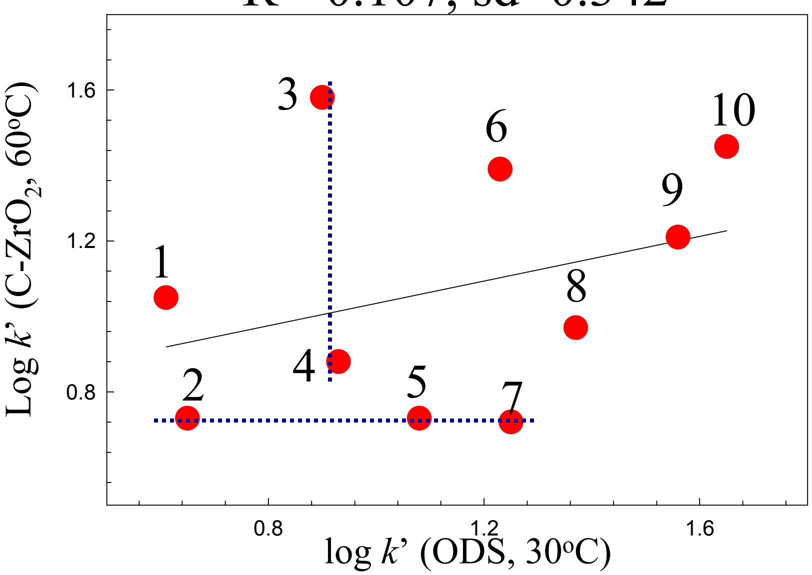
1ml/min; 254 nm detection

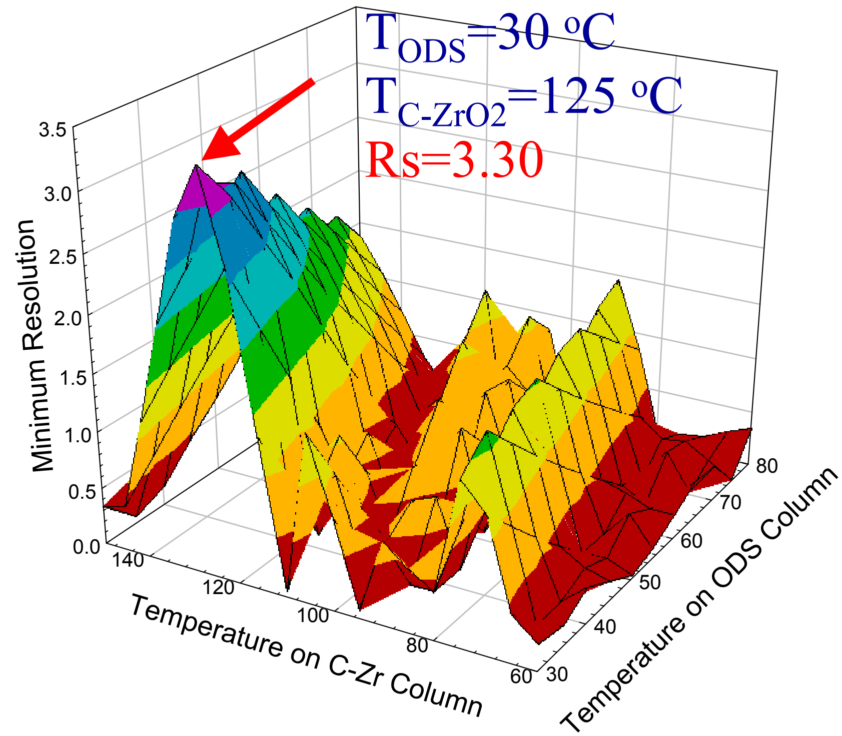


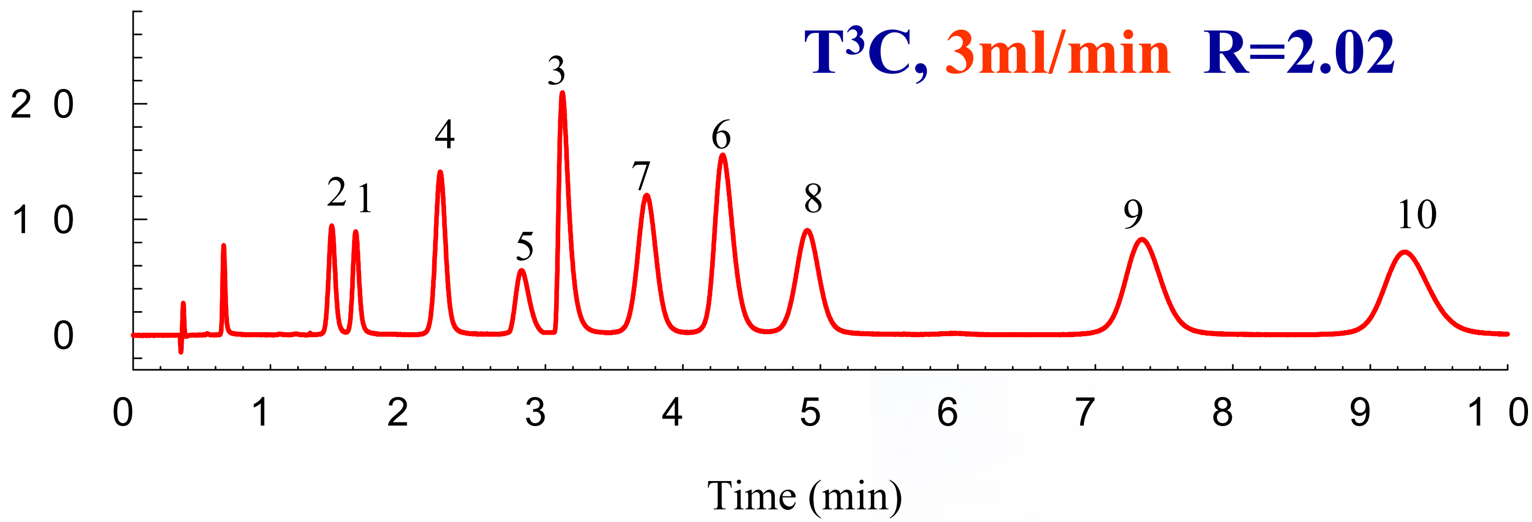
❖ **T^{3}C can improve separation without increasing analysis time.**

Steps in T³C Optimization of Triazine Herbicides

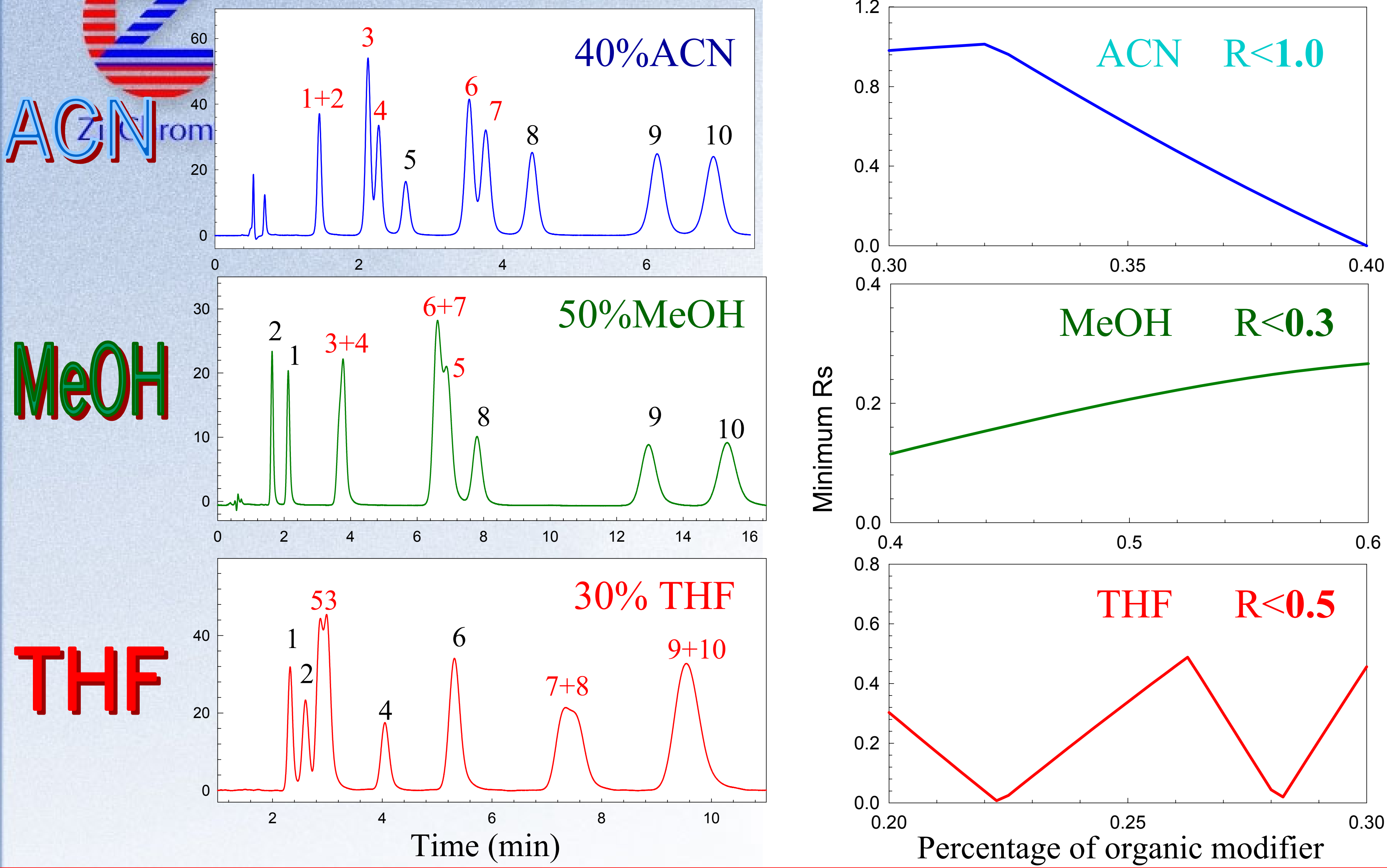
R^2=0.107, sd=0.342







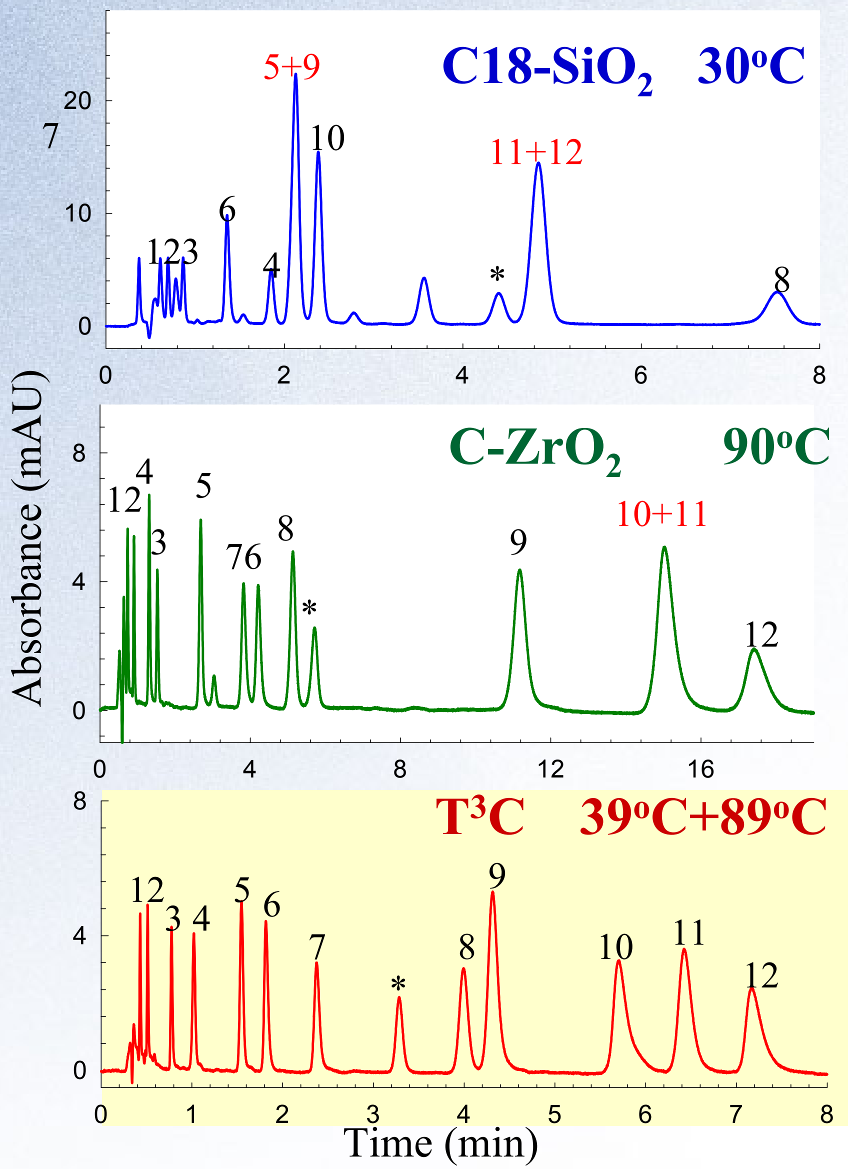
Compare T^{3}C with Mobile Phase Optimization



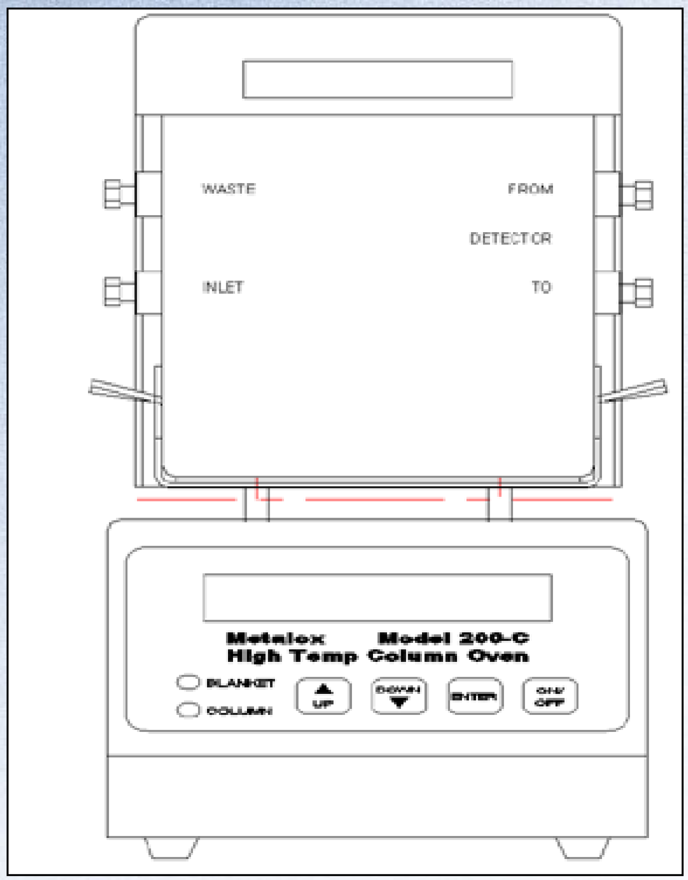
❖ **T^{3}C is more powerful than mobile phase optimization on ODS**

More Applications of T³C Concept

Urea and Carbamate Pesticides



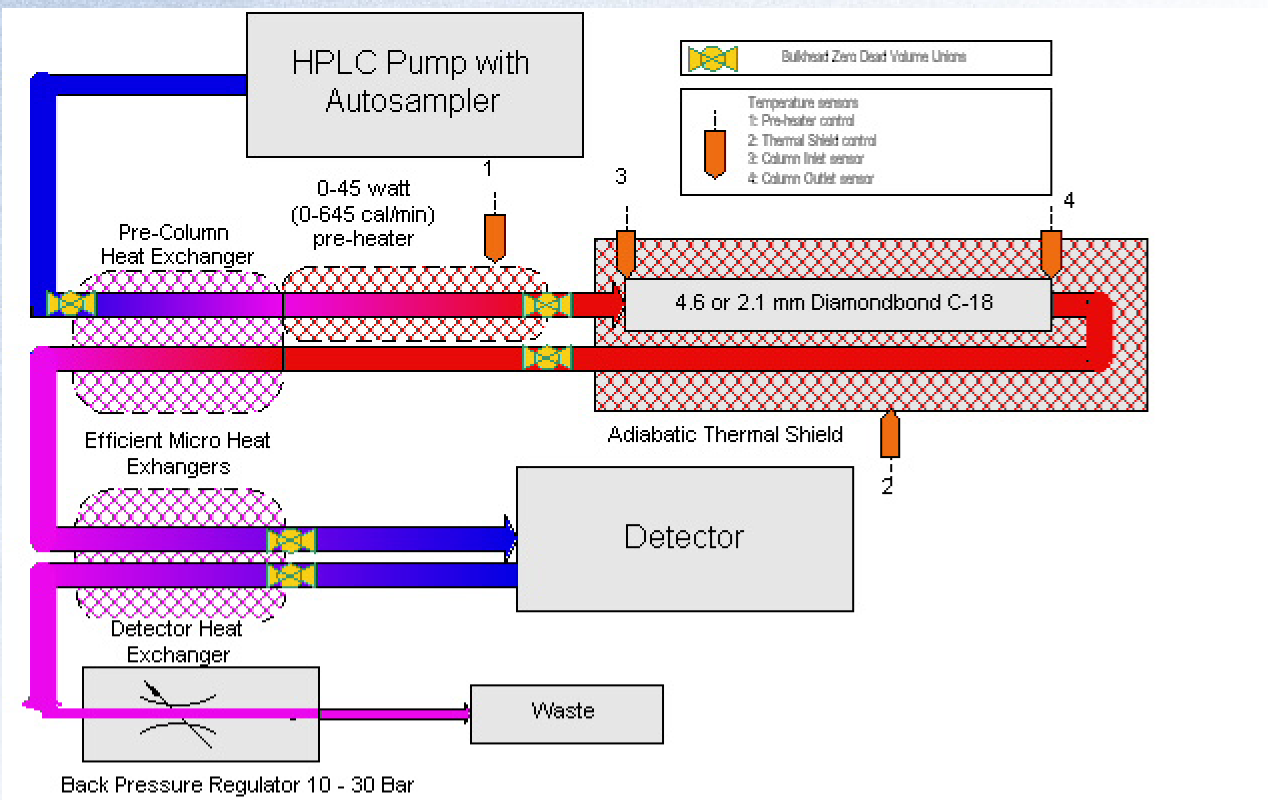
Introducing the Metalox™ 200-C High Temperature Column Heater



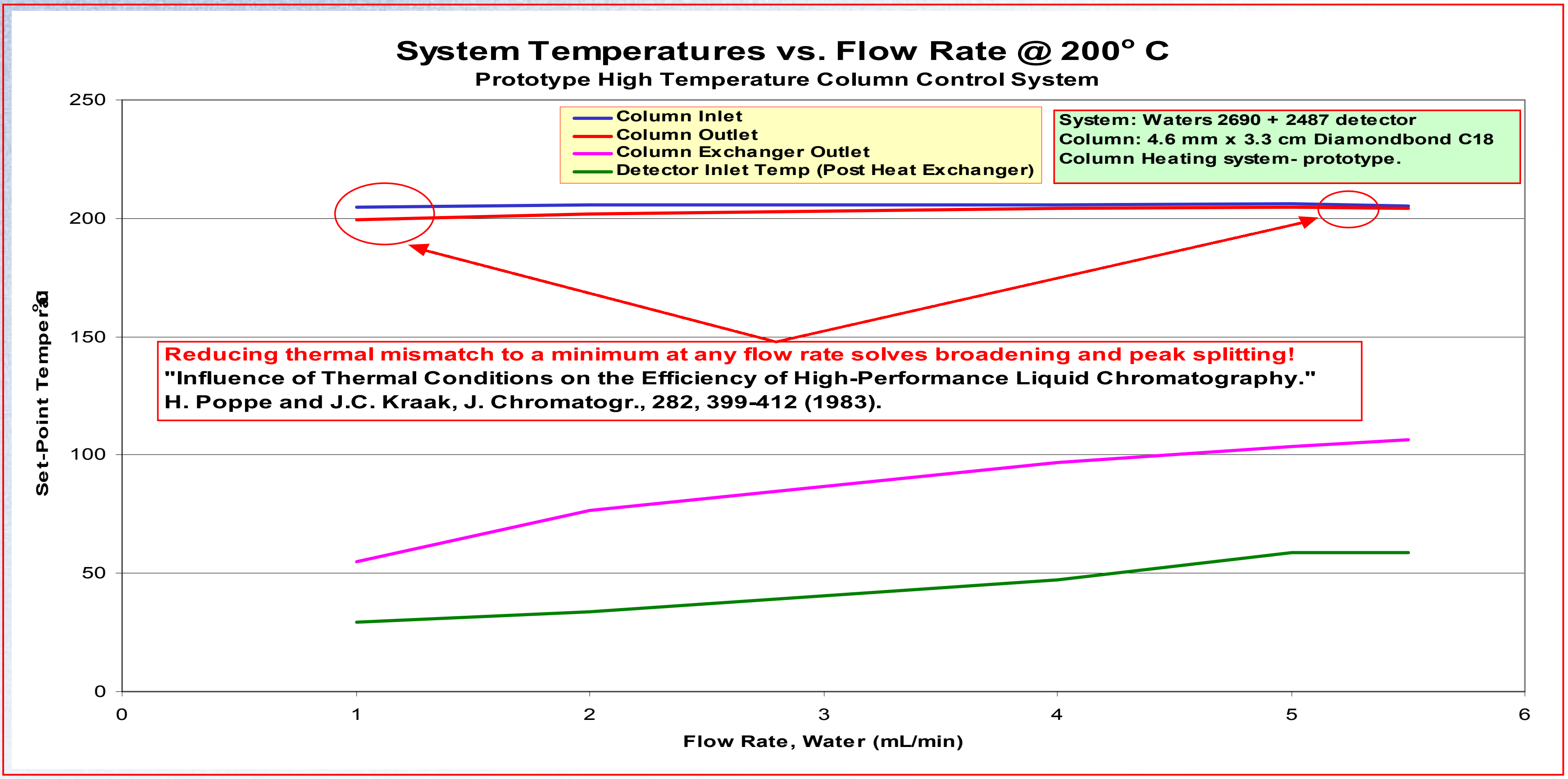
- Designed from the ground up for chromatographers.
- Overcomes issues such as thermal mismatch and inaccurate column temperature reporting.

Metalox™ 200-C Diagram

Patent Pending



Metalox™ 200-C Four Zone Temperature Performance



Conclusion: Regardless of flow rate, the column inlet and outlet temperature are within 3 °C of each other.

Metalox™ 200-C Features Advantages, and Benefits

Feature	Advantage	Benefit
Adiabatic type oven (Adiabatic: Occuring without loss or gain of heat), *Patent Pending*	All critical components (column insulation and mobile phase) are at a stable and defined temperature	Greatly reduces retention time shifts and band broadening commonly found in other column heater designs and thus improves overall data quality
Advanced oven design includes reduced internal dead volume by using laser welded components in the heat exchanger	Reduces band broadening normally associated with adding a "modular column heater"	Allows use of narrow bore columns and/or gradient elution without excessive increase in run time or loss of column efficiency
Close coupled heater element and temperature sensors *Patent Pending*	Provides for more efficient heat transfer to mobile phase over air bath only type	Allows for faster and more responsive heat transfer with smaller (less expensive) instrument design.

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Metalox™ 200-C Features Advantages, and Benefits

Feature	Advantage	Benefit
Heat exchanger at mobile phase inlet	Pre-heats incoming mobile phase closer to desired temperature as well as reducing exiting mobile phase temperature as it enters the detector	Reduces needed heater size which lowers instument size and cost. Lower detector temp. means less thermal noise and drift from detector cell.
Four monitored temperature zones	Monitors temperature at critical points in flow path	Ensures that column is at or near uniform temp., reducing thermal band broadening. Provides built in capabilities for instrument validation. Column is at constant temp. during gradient elution and/or flow programming.
Column resides in defined location (max length 15 cm, max id 4.6 mm)	When column is changed the positioning geometry will be the same even with different operators	Eliminates the problem caused by temp. variability inside large air bath ovens and thus removes another point of instrumental error and lowered chromatographic performance.

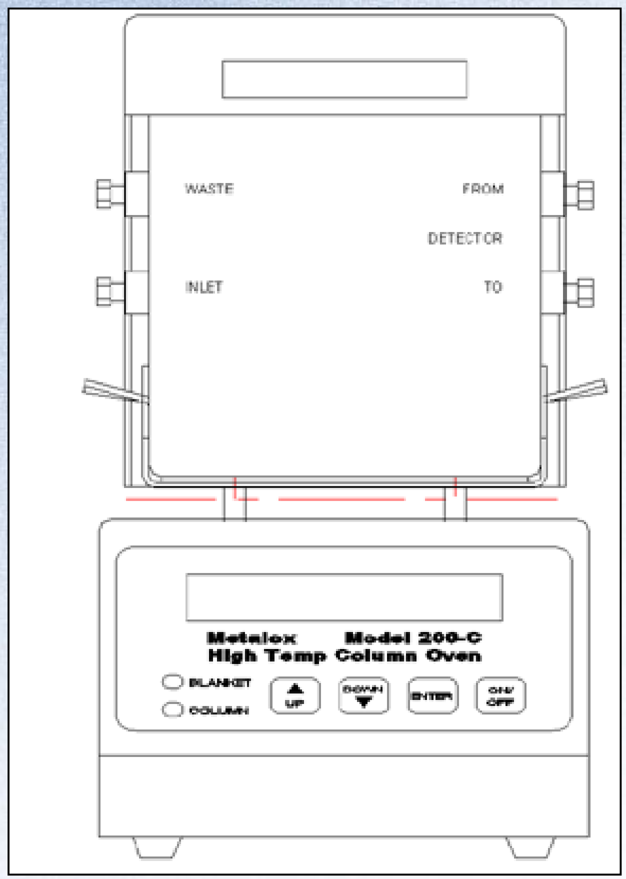
Metalox^{TM} 200-C Features Advantages, and Benefits

Feature	Advantage	Benefit
Small footprint and stand alone operation.	Easy to attach to existing systems and/or move between systems for added flexibility	User get top chromatographic performance in modular format
Front panel displays all four temperature controlled zones as well as column inlet and outlet differential temperature	Operator can easily tell when oven and column are truly ready for use - instead of just a single readout of an air temperature or block sensor	Avoids making reruns because column was not truly at desired operating temperature
RS-232 and analog output of all four temperature zones	Provides two convenient monitoring modes to log data to external devices	Very useful when setting up validation protocols, no need to attach extra sensor and recording devices

Metalox^{TM} 200-C Features Advantages, and Benefits

Feature	Advantage	Benefit
Bonded heaters are powered by isolated 24V DC	No line voltage in close proximity to chromatographic flow path or operator	Provides extra safety to both instrument and personnel in case of shorted heater component
Back pressure regulator down stream of detector	Keep constant back pressure on entire chromatographic system	Prevents mobile phase form boiling in detector and prevents mobile phase boiling in column upon loss of mobile phase supply
Two thermal cutout sensors inside of oven	Shuts off heater power in case of thermal runaway	Provides extra measure of safety for unattended operation

The Metalox™ 200-C High Temperature Column Heater



- Introductory Offer
  - The new Metalox 200-C High Temperature Column Heater
  - Back Pressure Regulator
  - A set of three ZirChrom revolutionary RP HPLC Columns.
    - ZirChrom-PBD
    - ZirChrom-CARB
    - DiamondBond C18

Metalox™ 200-C Preliminary Specifications

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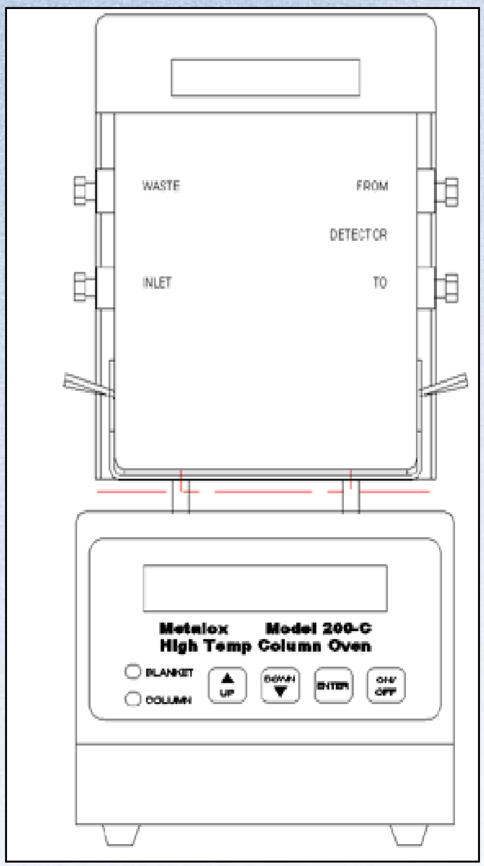

- Operational Capabilities
  - Max. Column Operating Temp: 200° C (6 ml/min with water)
  - Min. Temperature: 7° C above ambient
  - Max. Flow Rate: 6ml/min (200° C, water mobile phase)
  - Max. Cal./s: 17.9
  - Temperature Reproducibility: ± 0.5° C
  - Accuracy of Temp. Reading: ± 1%
  - Display Resolution: 1° C
- Physical Specifications
  - Weight: 15 lbs
  - Footprint: 6”x10”x14”
  - Power Requirements: 115-230V 47-440 Hz
  - Internal Transfer Volumes:
    - Pre-Column: 10.5 uL
    - Post-Column 4.0 uL

Note: Metalox 200-C design and specifications are pending patent

Quantitative Value Assessment
Steroids Separation

Customer: Contract Lab XYZ **Date: 1/31/02**

	Leading ODS	ZirChrom-PBD
Column Cost	$395.00	$595.00
Analysis Time (min)	10.0	5.0
Average Time per Successful Cycle (min)	12.3	7.2
Possible Cycles per Instrument per Year	**42,705**	**73,474**
Cost Components		
Column Cost per Successful Cycle	*$1.01*	*$1.30*
Solvent Cost per Successful Cycle	*$0.02*	*$0.04*
Waste Disposal Cost per Successful Cycle	*$0.01*	*$0.01*
Total Fixed Cost per Successful Cycle	*$2.37*	*$1.38*
Total Operator Cost per Successful Cycle	*$0.26*	*$0.15*
Total Cost per Analysis*	**$3.67**	**$2.88**