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ZirChrom®

Applications of Ultra-Stable Phases for HPLC: High Temperature Ultra-Fast Liquid Chromatography and Thermally Tuned Tandem Column (T^3C) Liquid Chromatography

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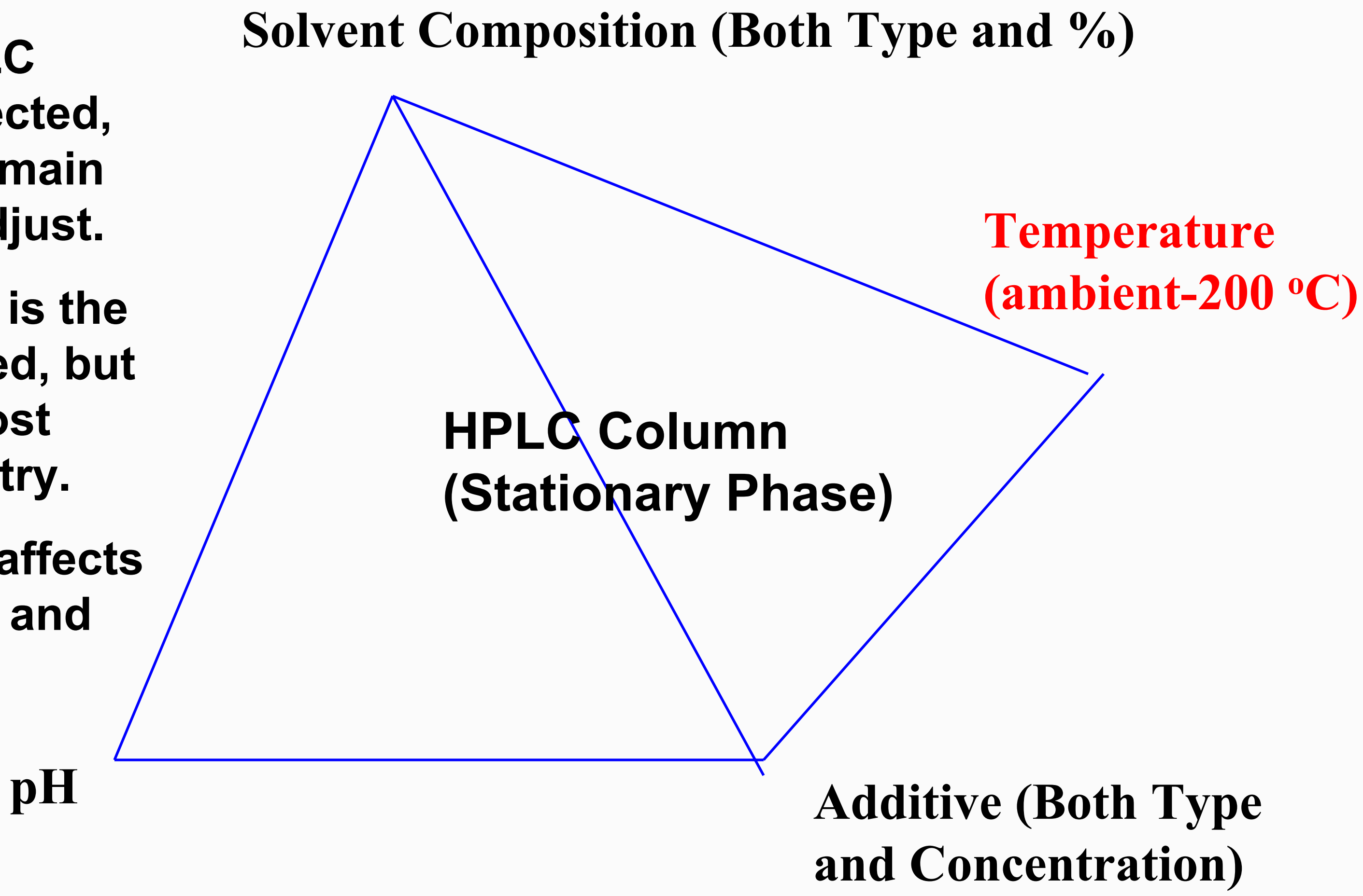
DIAMONDBOND™
HPLC Columns

Outline

- Development of Ultra-Stable Stationary Phases
  - Theoretical and Practical Benefits of High Temperature HPLC
  - Stability of Zirconia-based HPLC Columns (Z-phases)
  - Examples of Ultra-Fast High Temperature Separations
- Using Temperature to Control Selectivity
  - Importance of Selectivity in HPLC Optimization
  - Selectivity of Zirconia-based HPLC Columns (Z-phases)
  - Thermally Tuned Tandem Columns (T^3C) Concept
  - Examples of T^3C Applications
- Conclusions

Retention and Selectivity Variables

- Once an HPLC column is selected, there are four main variables to adjust.
- Temperature is the least often used, but may be the most convenient to try.
- Temperature affects both retention and selectivity.



Solvent Composition (Both Type and %)

Temperature (ambient-200 ^{o}C)

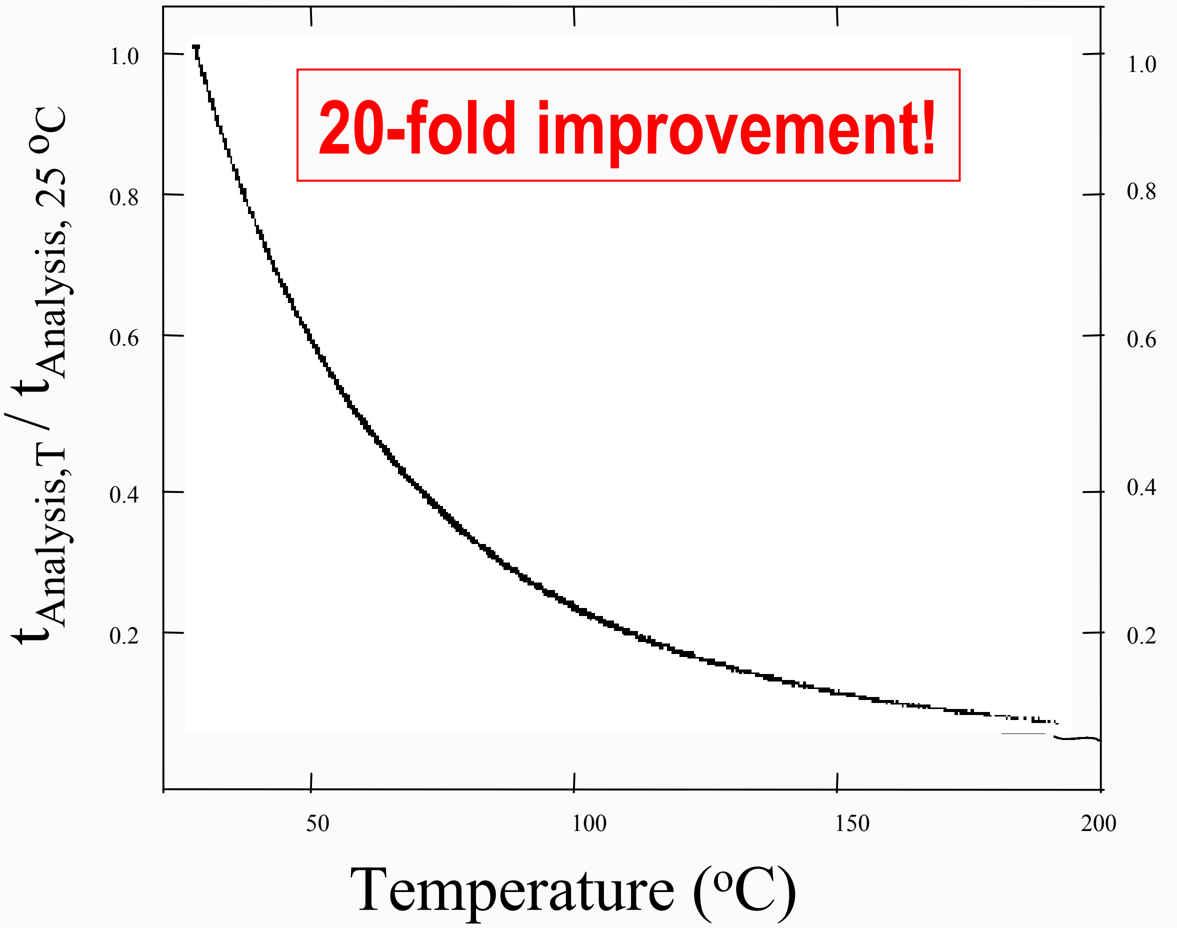
HPLC Column (Stationary Phase)

pH

Additive (Both Type and Concentration)

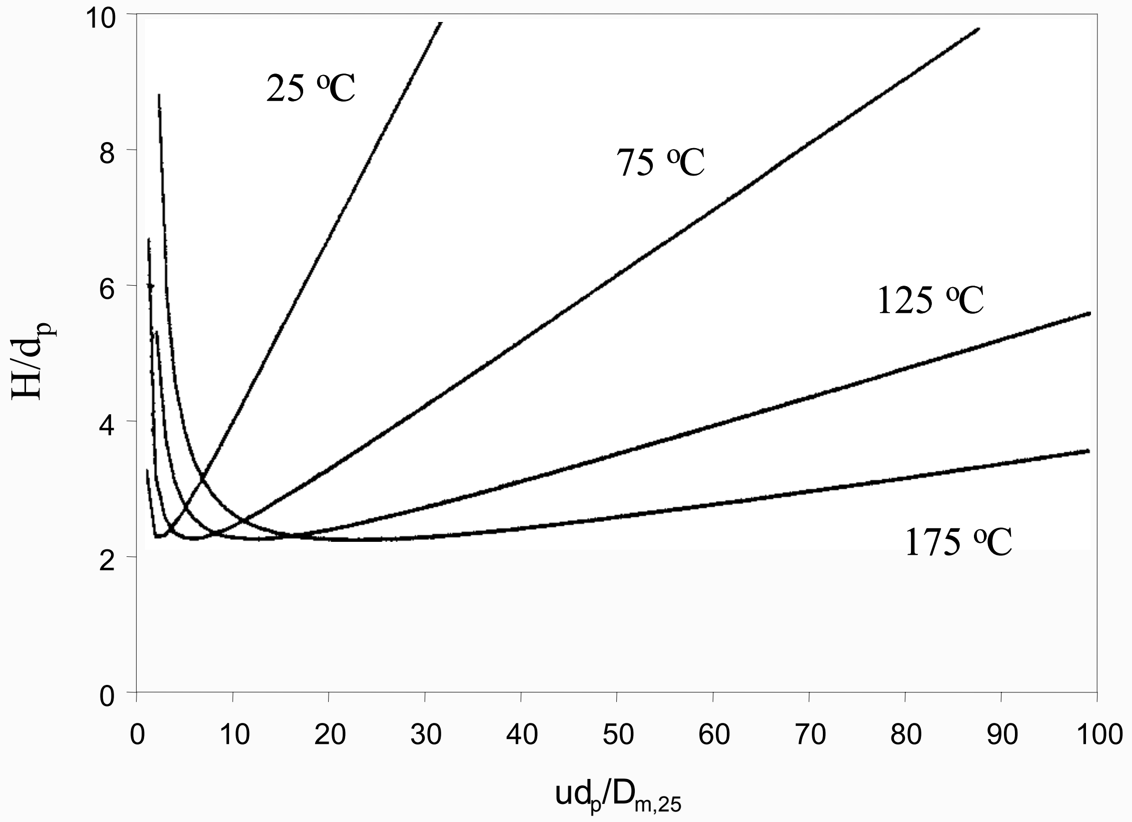
Effect of Temperature on Retention Time*

*R. D. Antia and Cs. Horvath, *J. Chromatogr.*, **435**, 1-15 (1988).



Keeping Pressure and Plate Count Constant

Effect of Temperature on Column Efficiency



- ***Ways that temperature increases efficiency and speed:***
  - Increased temperature increases diffusivity, thus decreasing the reduced velocity
  - Increased temperature accelerates sorption kinetics
  - The result is speed without loss of resolution and sensitivity, even at high flow rates

van Deemter Plot $$h = A + \frac{B}{\nu} + C\nu + D\nu^{2/3} + \frac{3D_m}{8k_d d_p^2}\nu$$

R. D. Antia and Cs. Horvath, *J. Chromatogr.*, 435, 1-15 (1988).

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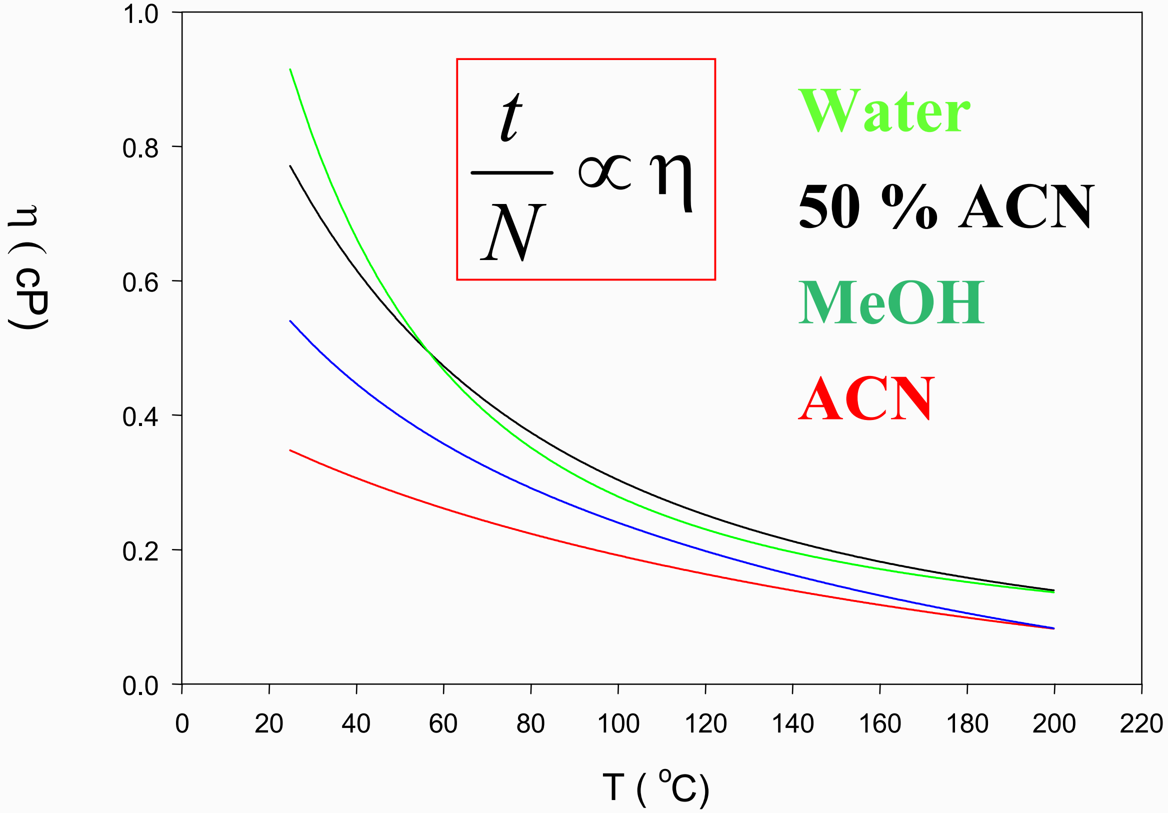
Estimated Effect of Temperature on Viscosity*

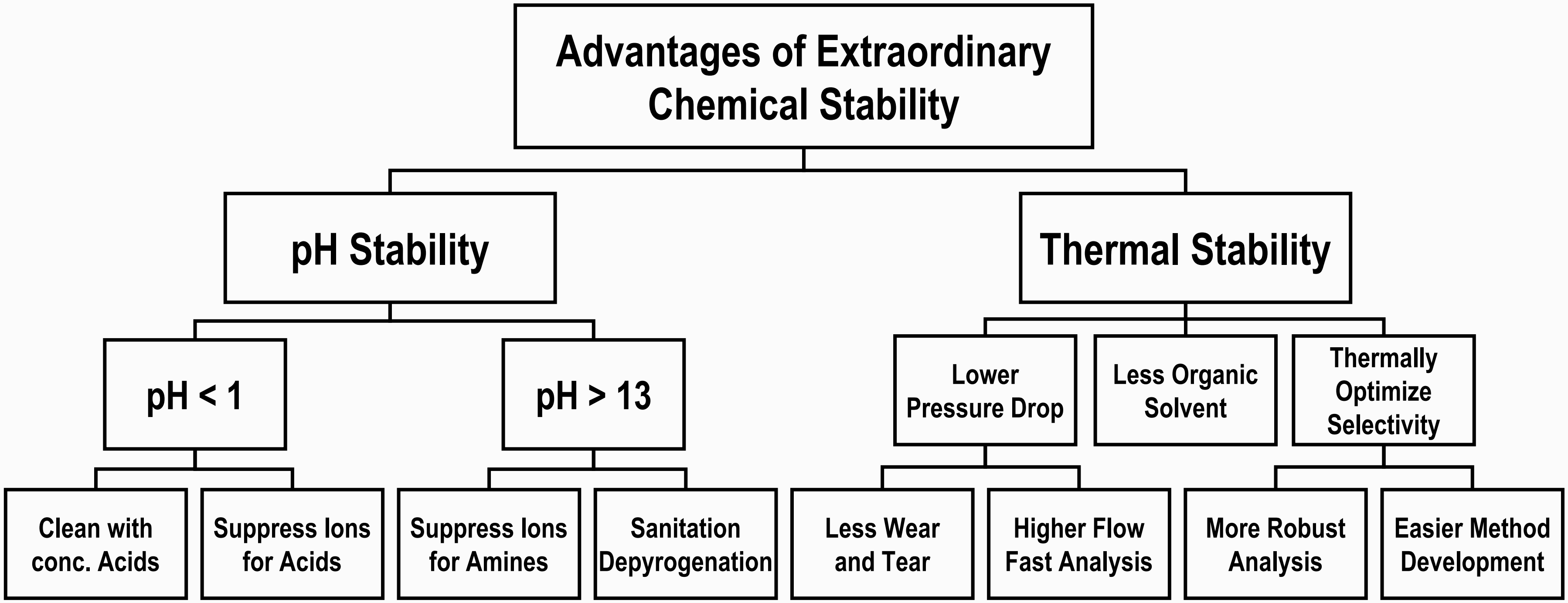
* H. Chen and Cs. Horvath, "Rapid Separation of Proteins by RP-HPLC at Elevated Temperatures," *Anal. Methods Instrum.*, **1**, 213-222 (1993).



Ways that increased temperature increases efficiency and speed:

1. **Increased temperature increases diffusivity, thus decreasing the reduced velocity**
2. **Increased temperature accelerates sorption kinetics**
3. **Increased temperature reduces k values**
4. **Increased temperature decreases mobile phase viscosity which enables higher flow rates**

Why Stable Phases?



- Advantages of Extraordinary Chemical Stability
  - pH Stability
    - pH < 1
      - Clean with conc. Acids
      - Suppress Ions for Acids
    - pH > 13
      - Suppress Ions for Amines
      - Sanitation Depyrogenation
  - Thermal Stability
    - Lower Pressure Drop
      - Less Wear and Tear
      - Higher Flow Fast Analysis
    - Less Organic Solvent
    - Thermally Optimize Selectivity
      - More Robust Analysis
      - Easier Method Development

Stable stationary phases have advantages in terms of *analysis time, selectivity and column lifetime*. If LC/MS is employed, stable phases exhibit *low bleed background*.

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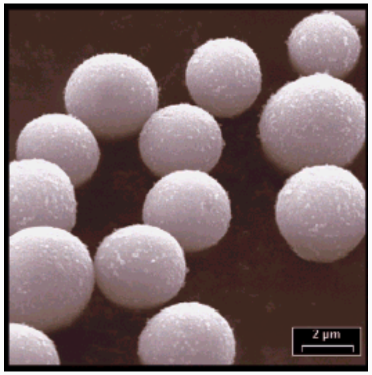
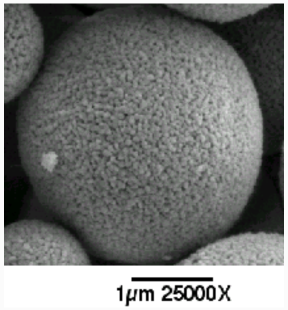
ZirChrom® Particle Properties





Characteristic	Property
Surface Area (m^2/g)	22
Pore Volume (cc/g)	0.13
Pore Diameter (Å)	250-300
Porosity	0.45
Density (g/cc)	5.8 (2.5x silica)
Particle Diameters (µ)	3.0, 5.0, 10.0

ZirChrom®-Carb and DiamondBond particles are prepared by coating base particles with a thin layer of carbon using a chemical vapor deposition process

ZirChrom®-PBD and -PS particles are prepared by coating with a layer of highly crosslinked polymer

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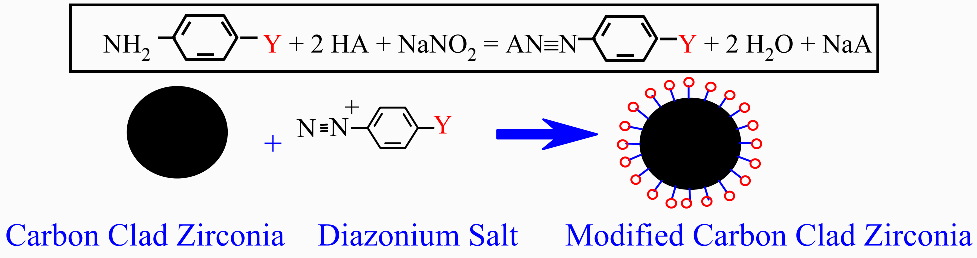
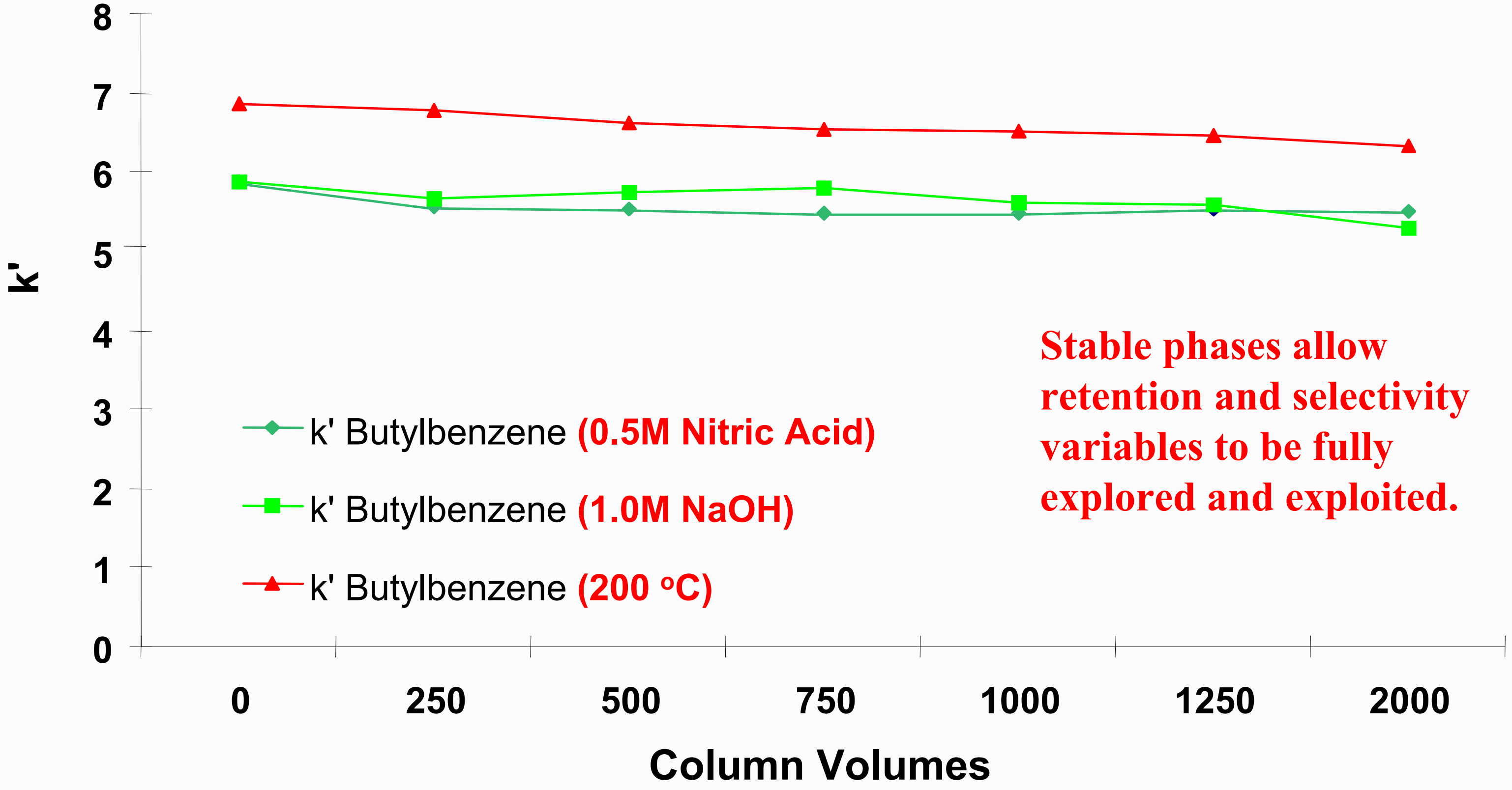
DIAMONDBOND™ - C18

Bonding Reaction on Carbon Clad Zirconia



DiamondBond-C18 Stability



Stable phases allow retention and selectivity variables to be fully explored and exploited.

<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.

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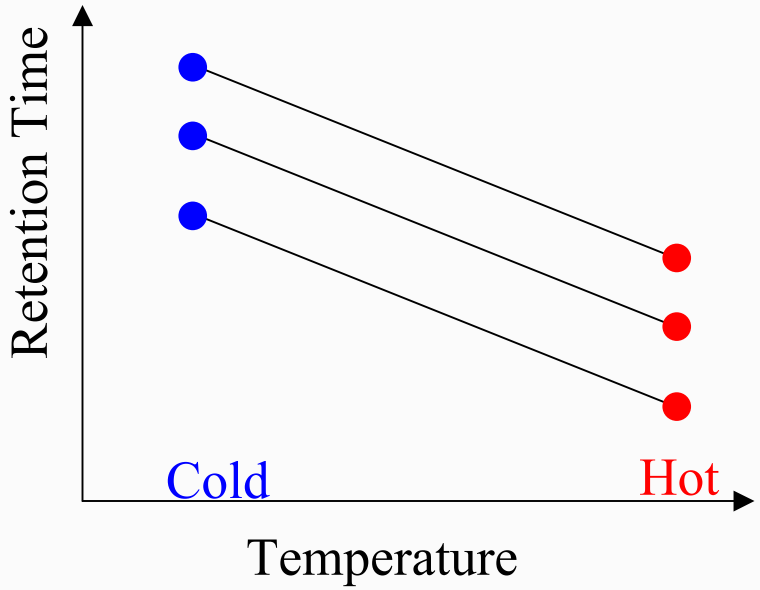
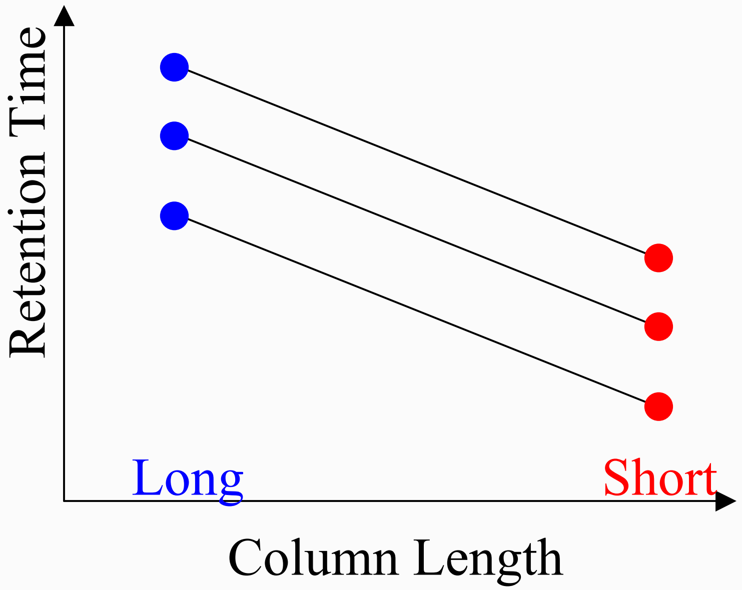
Increasing Separation Speed

Column Temperature vs. Column Length vs. Flow Rate

T increases 50 ^{o}C ⟹ *k*' decreases 3-fold





Increasing only flow rate to reduce retention time is the least desirable option because pressure increases and performance drops. Increasing temperature and decreasing column length are better ways to decrease retention time and speed up analysis; however, decreasing length reduces efficiency and resolution, while increasing temperature doesn't.

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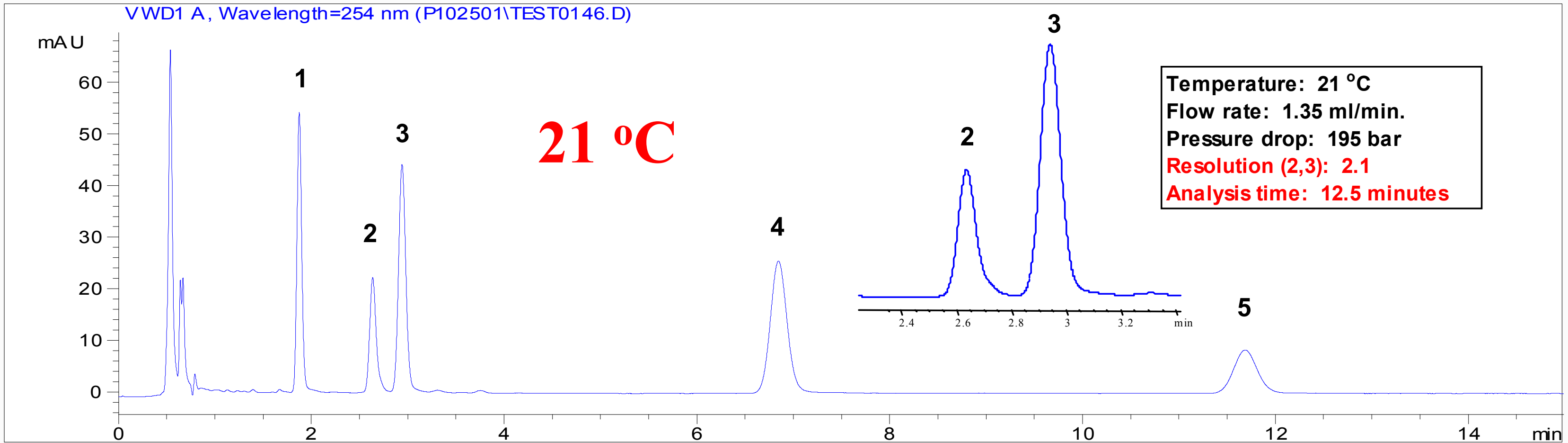
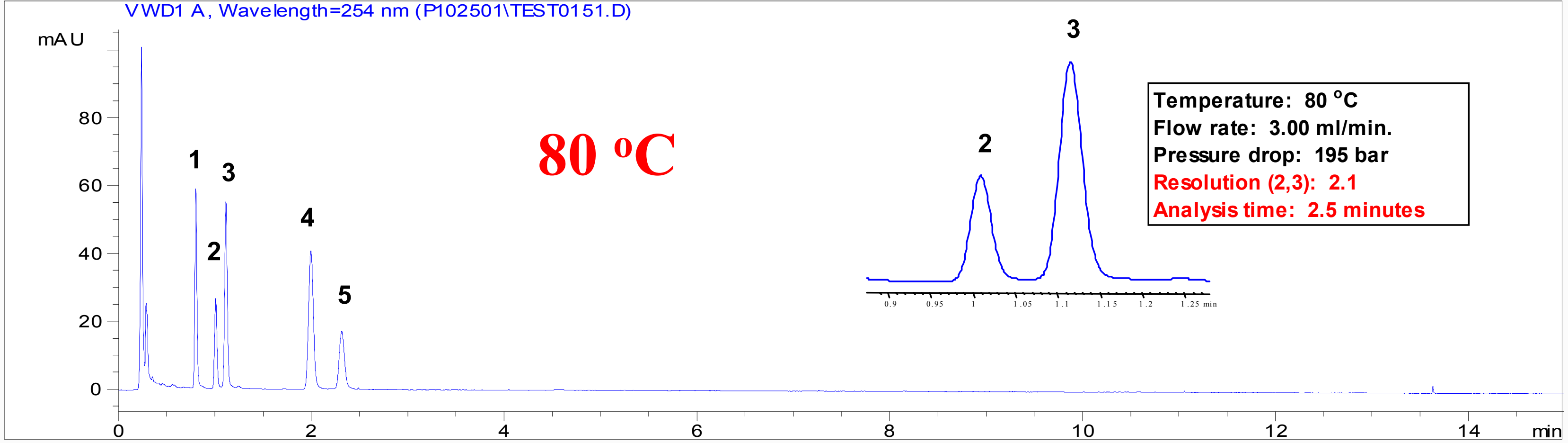
Antihistamines- Fast Isocratic



Temperature: 21 °C
Flow rate: 1.35 ml/min.
Pressure drop: 195 bar
Resolution (2,3): 2.1
Analysis time: 12.5 minutes



Temperature: 80 °C
Flow rate: 3.00 ml/min.
Pressure drop: 195 bar
Resolution (2,3): 2.1
Analysis time: 2.5 minutes

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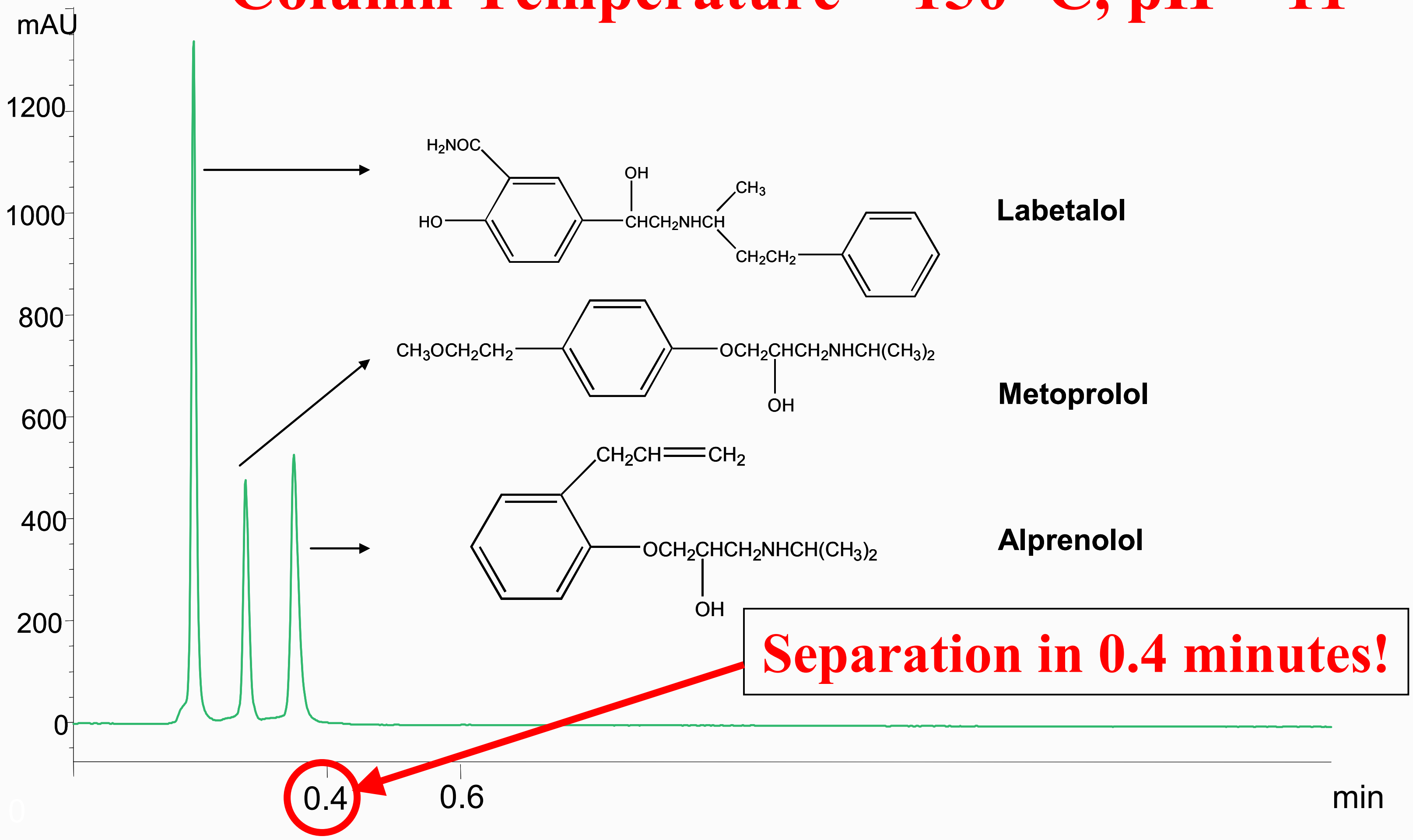
LC Conditions: (A) Mobile Phase, 29/71 ACN/50mM Tetramethylammonium hydroxide, pH 12.2; Flow Rate, 1.35 mL/min.; Injectionl, 0.5 ul; 254 nm detection; Column Temperature, 21°C; Pressure drop = 195 bar; Solutes: 1=Doxylamine, 2=Methapyrilene, 3=Chlorpheniramine, 4=Triprolidine, 5=Meclizine4=Triprolidine, 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.

Beta-Blockers- Fast Isocratic

Column Temperature = 150 °C, pH = 11



Separation in 0.4 minutes!

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;

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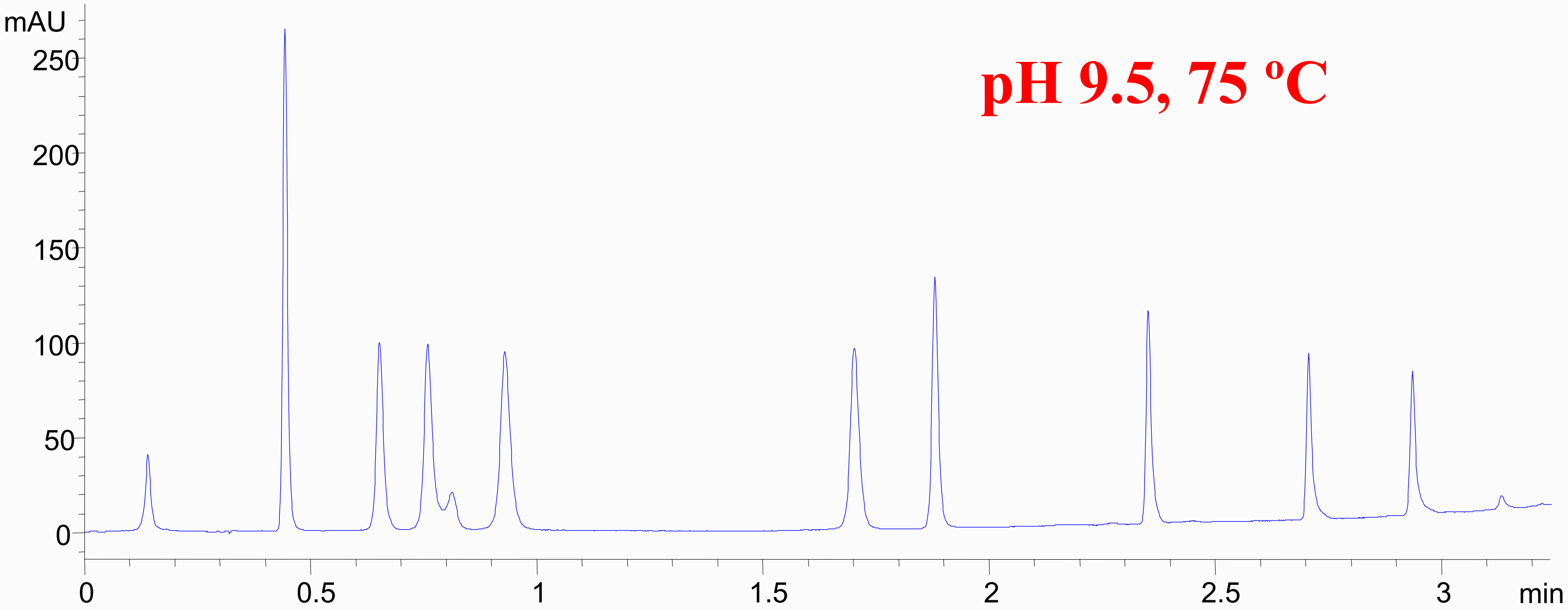
Nitrosamines- Fast Gradient



Column: DIAMOND BOND™-C18, 100 × 4.6mm	
Mobile Phase: 2.5-90%B from 1-3 minutes	
A: 10mM Ammonium hydroxide, pH 9.5	
B: 100% Acetonitrile	

Flow rate:	4.0 mL/min.
Temperature:	75 ºC
Injection volume:	1.0 µL
Detection:	230 nm
Back Pressure:	200 bar

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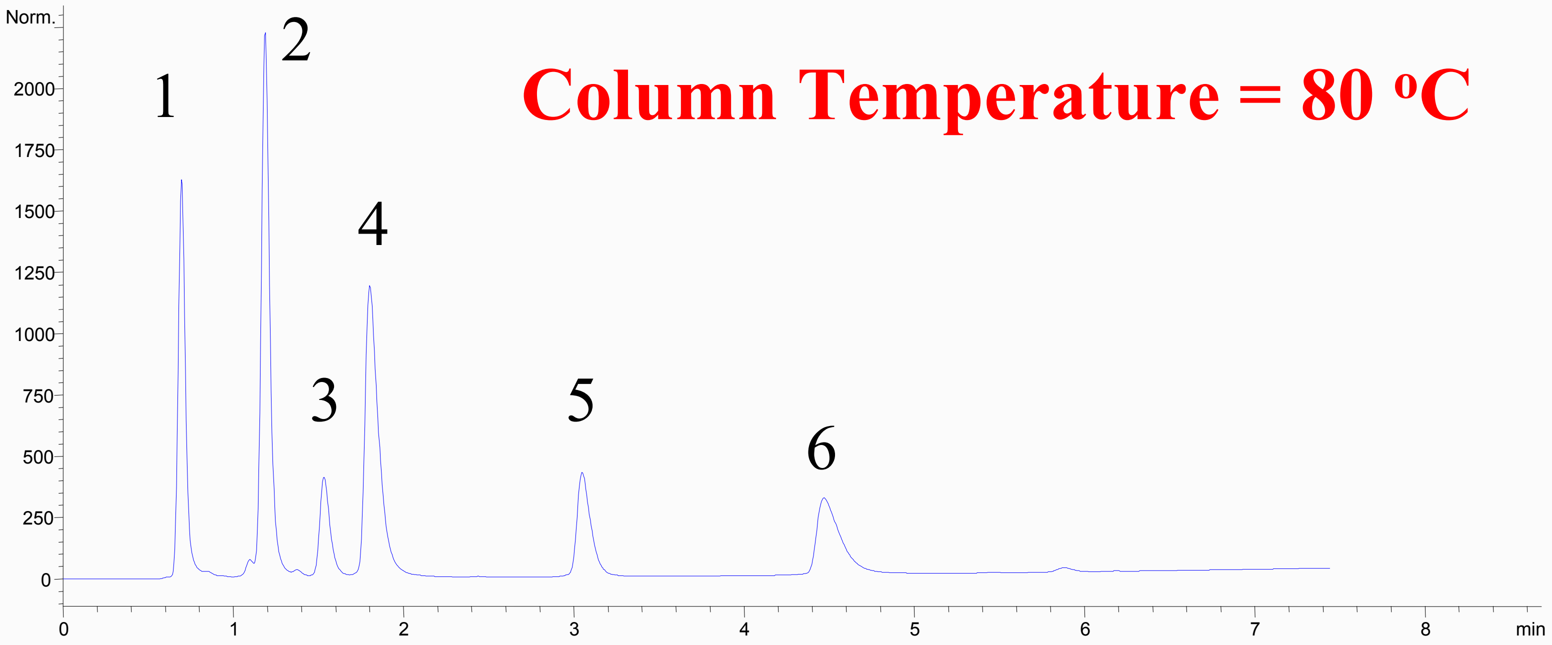
Non-Steroidal Anti-inflammatories



LC Conditions: Column, 50 x 4.6 DiamondBond^{TM}-C18; Mobile phase, 50-80 A over 10 minutes, A=ACN, B=40mM ammonium phosphate, 5mM ammonium fluoride, pH 2.0; **Flow rate, 1.0 ml/min**.; Temperature, 80 ^{o}C; Injection volume, 5ul; Detection at 254nm; Solute concentration, 0.15 mg/ml.;
Solutes, 1=Acetaminophen, 2=Ketoprofen, 3=Ibuprofen, 4=Naproxen, 5=Oxaprofen, 6=Indemethacin.

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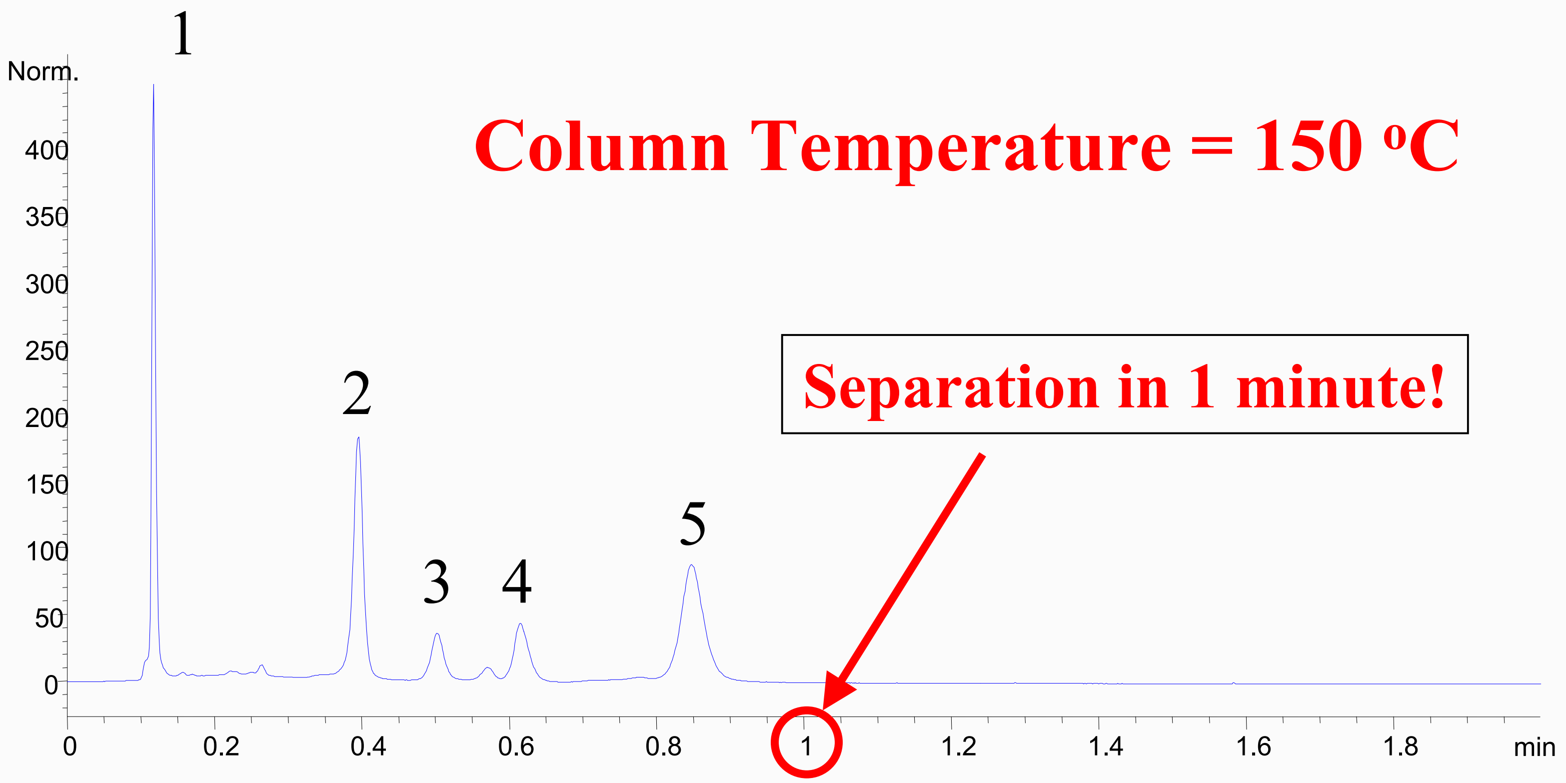
Non-Steroidal Anti-inflammatories (FAST)



Column Temperature = 150 °C

Separation in 1 minute!

LC Conditions: Column, 50 x 4.6 DiamondBond™-C18; Mobile phase, 25/75 ACN/40mM phosphoric acid, pH 2.3; **Flow rate, 5.5 ml/min.**; **Temperature, 150 °C**; Injection volume, 1ul; Detection at 254nm; Solute concentration, 0.15 mg/ml.; Solutes, 1= Acetaminophen, 2=Ketoprofen, 3=Naproxen, 4=Ibuprofen, 5=Oxaprofen.

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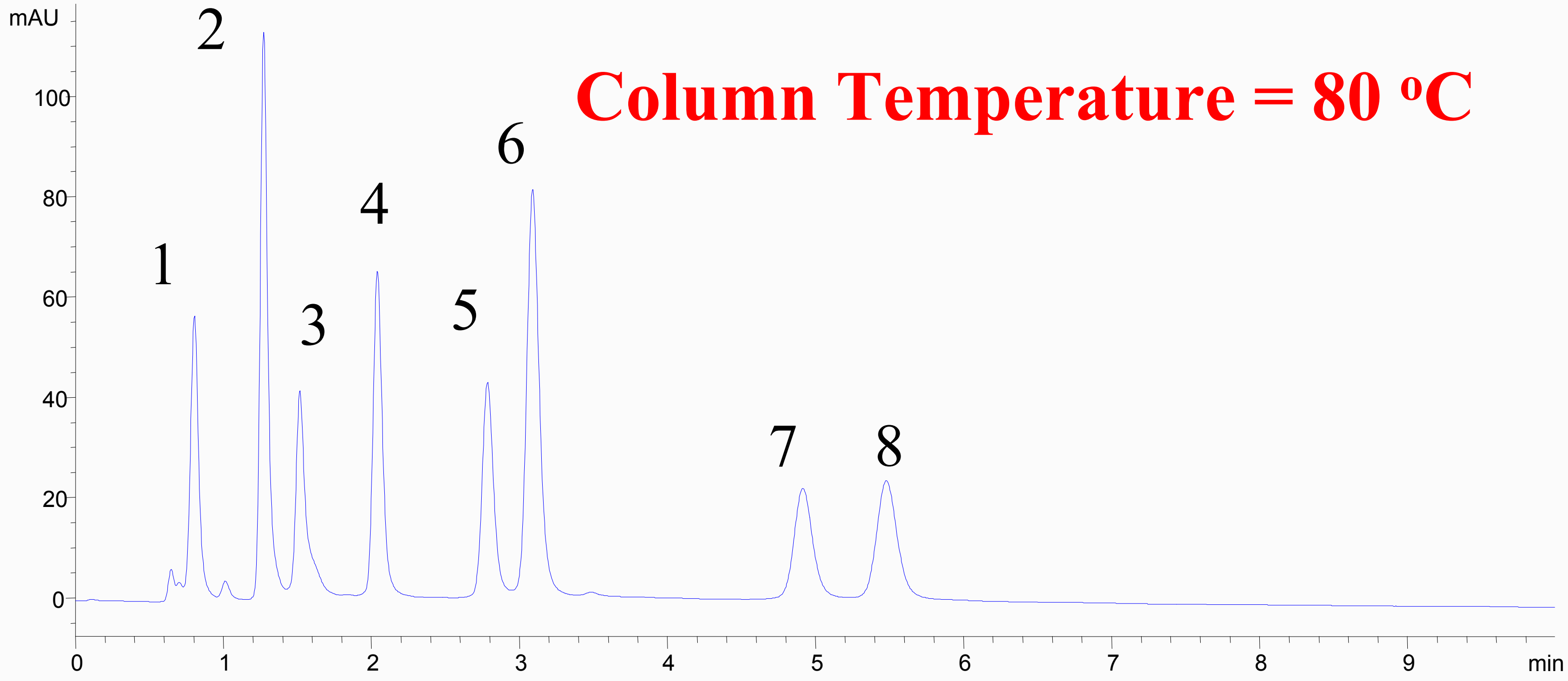
PTH-Amino Acids



Column Temperature = 80 ^{o}C

LC Conditions: Column, 50 x 4.6 DiamondBond^{TM}-C18; Mobile phase, 20/80 ACN/0.1% TFA, pH 2.1; Flow rate, 1.0 ml/min.; Temperature, 80 ^{o}C; Injection volume, 1ul; Detection at 254nm; Solute concentration, 0.15 mg/ml.; Solutes, 1=PTH-Arginine, 2=PTH-Serine, 3=PTH-Glycine, 4=PTH-Alanine, 5=PTH-Isoaminobutyric acid, 6=PTH-Aminobutyric acid, 7=PTH-Valine, 8=PTH-Norvaline.

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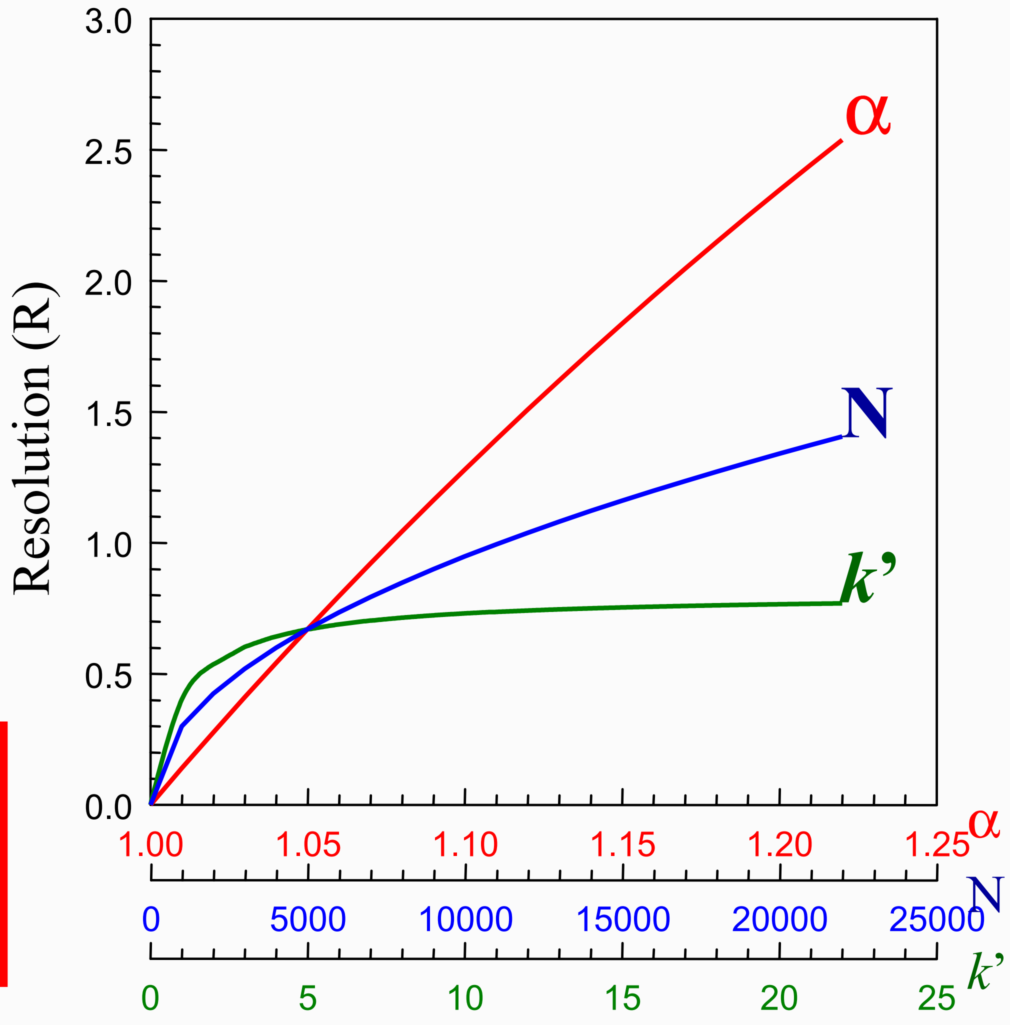
The Impact of Selectivity on Resolution

Efficiency ↘ Retention ↓ Selectivity ↙

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

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

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



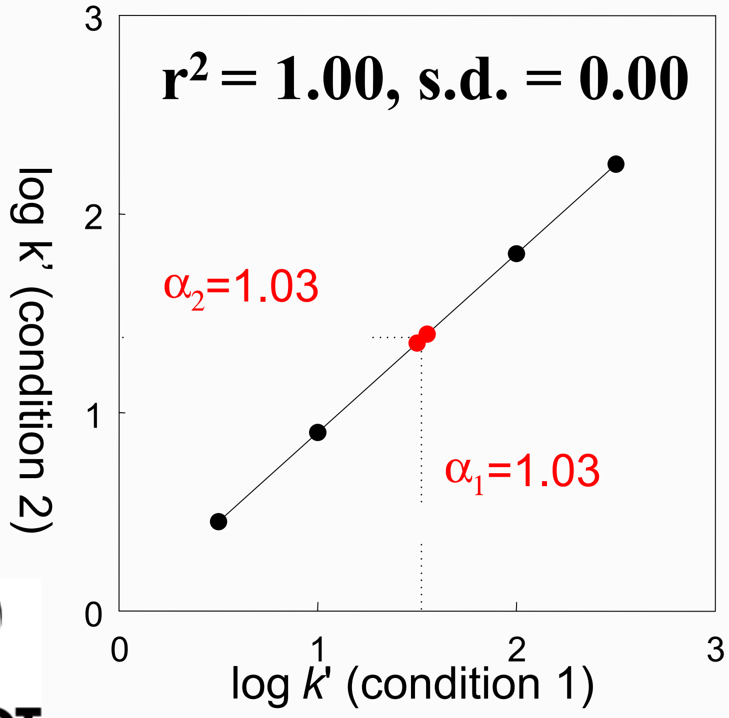
Comparing and Adjusting Selectivity in HPLC

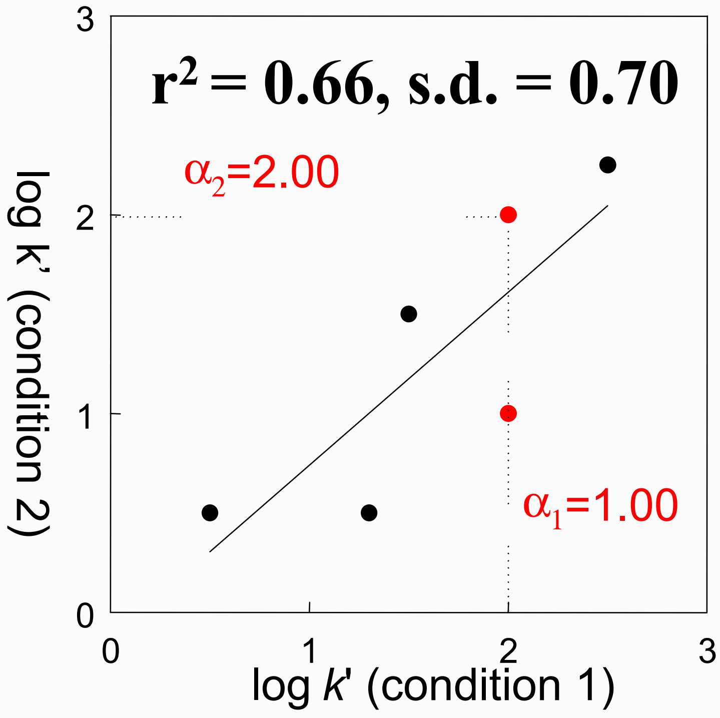
- Mobile Phase Composition (B%)**
- Mobile Phase Type (ACN, MeOH, THF)
- Stationary Phase Type (C18-SiO_2, C-ZrO_2, PBD-ZrO_2)
- Temperature

** Only works in mixed-mode

➢ **Poor correlations in the κ–κ plot indicate changes in selectivity.**

κ–κ plots[1]





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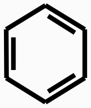
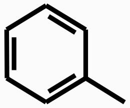
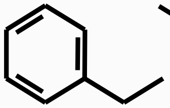
1. Horvath, Snyder and Carr

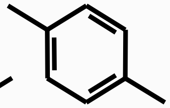
22 Non-Electrolyte Solutes

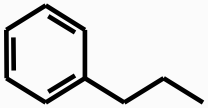
Nonpolar

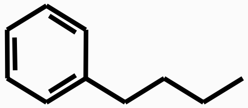

Benzene


Toluene

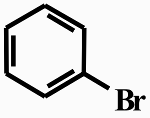

Ethylbenzene


p-xylene

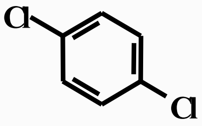

Propylbenzene


Butylbenzene

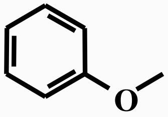
Polar



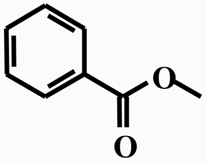
Bromobenzene



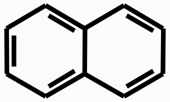
p-Dichlorobenzene

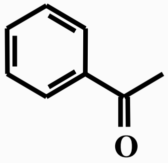


Anisole

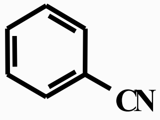


Methylbenzoate

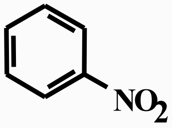

Napthalene



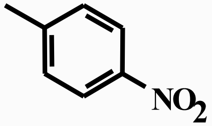
Acetophenone



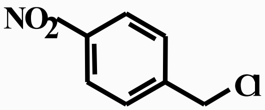
Benzonitrile



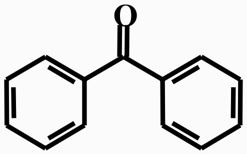
Nitrobenzene



p-Nitrotoluene

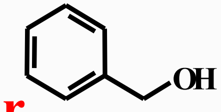


p-Nitrobenzyl Chloride

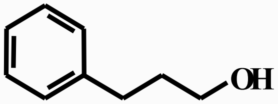


Benzophenone

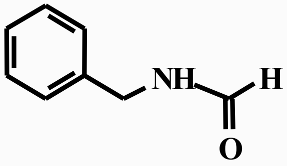
HB Donor



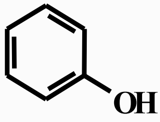
Benzylalcohol



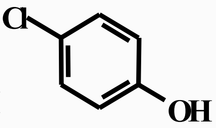
3-Phenyl Propanol



N-Benzyl Formamide



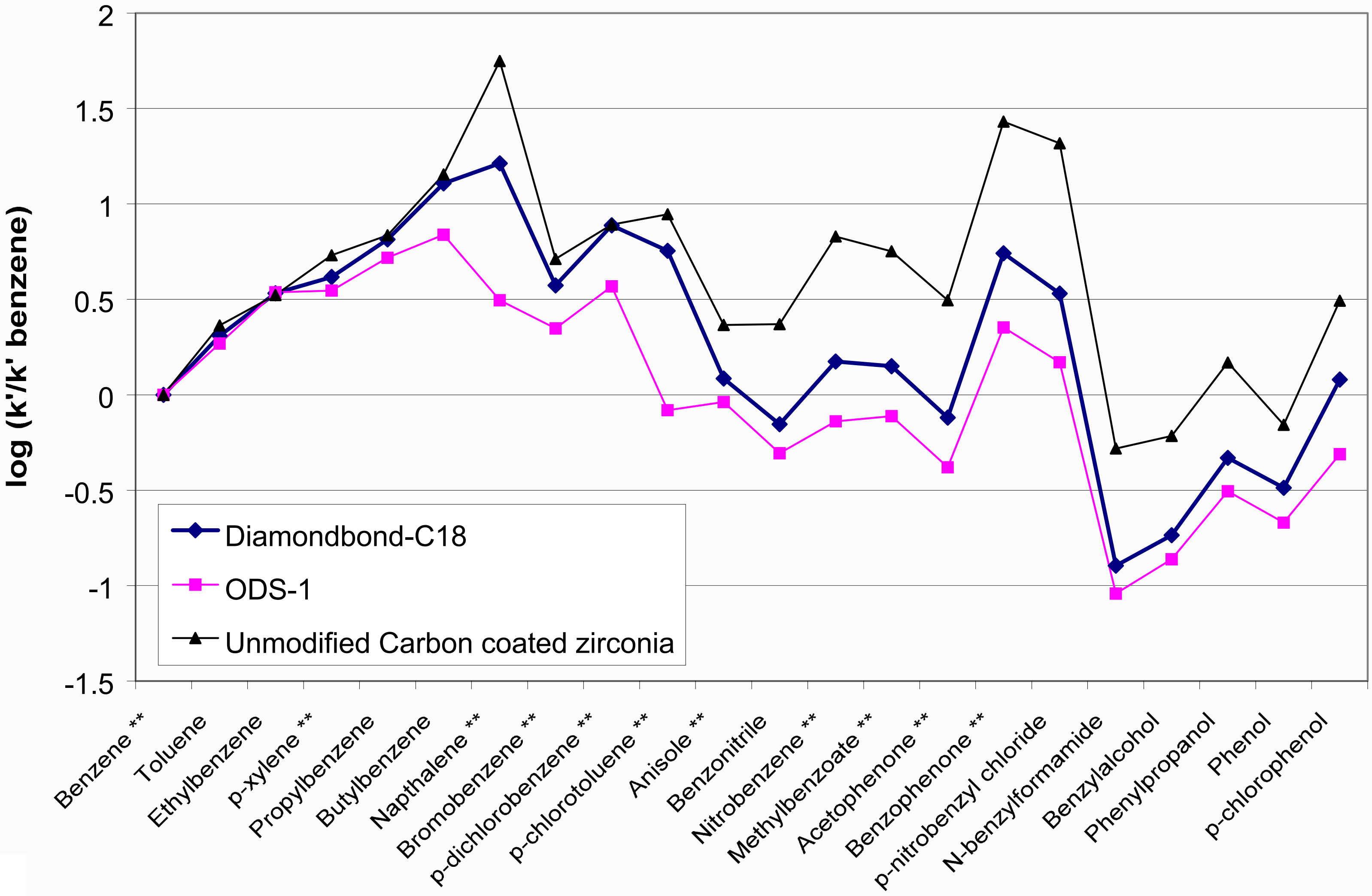
Phenol



p-Chlorophenol

The selectivity of the stationary phase becomes paramount for non-ionic solutes. Temperature and mobile phase variables become less important.

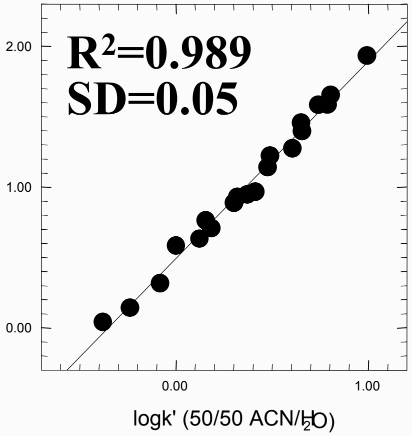
Selectivity Comparison



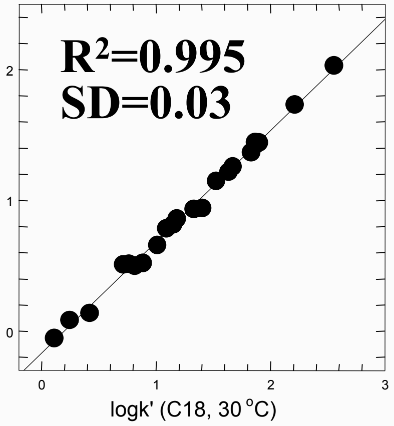
Mobile phase, 40/60 Acetonitrile/Water; Flow rate, 1.0 ml/min.; Temperature, 30 ^{o}C; Detection at 254nm; 5ml Injection volume.

Comparison of Variables Affecting Selectivity

30% ACN vs. 50% ACN

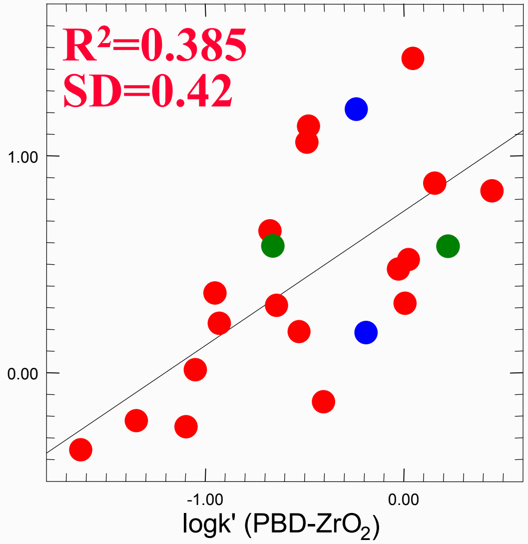


80°C vs. 30°C

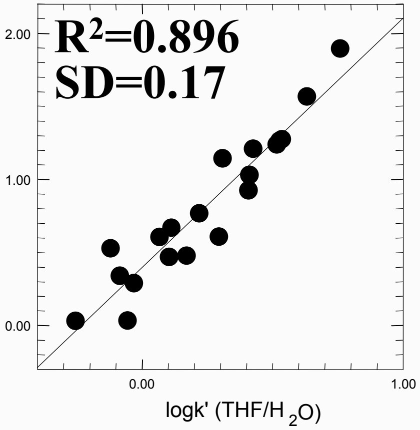


Stationary Phase Type

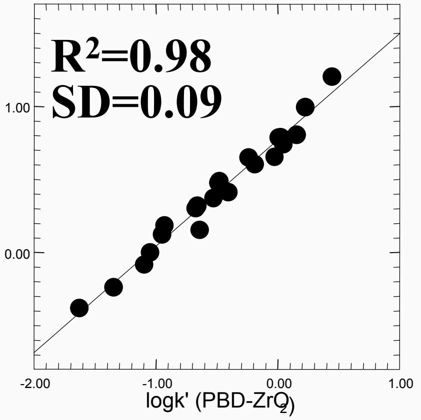
Carbon-ZrO_2 vs. PBD-ZrO_2



MeOH vs. THF



C18-SiO_2 vs. PBD-ZrO_2



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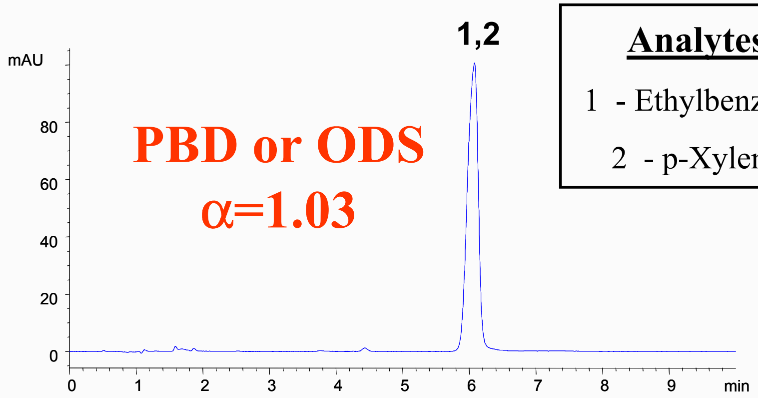
- ❖ **Stationary phase type has a large effect on selectivity.**

Carbon Surface Has Unique Selectivity

LC Conditions

Column: ZirChrom®-PBD, 100 x 4.6 mm

Mobile phase:		Flow rate:	1.0 mL/min.
	35/65 A/B	Temperature:	30 °C
A:	ACN	Injection volume:	5 µL
B:	Water	Detection:	254 nm



Analytes

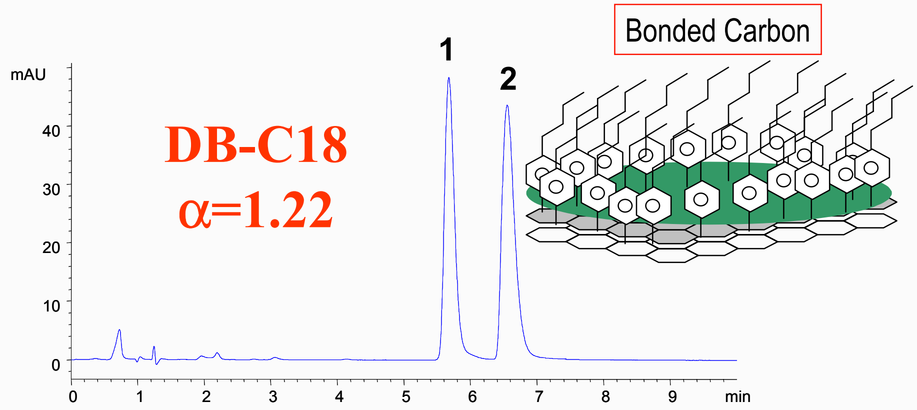
1 - Ethylbenzene

2 - p-Xylene

LC Conditions

Column: DiamondBond-C18, 100 x 4.6 mm

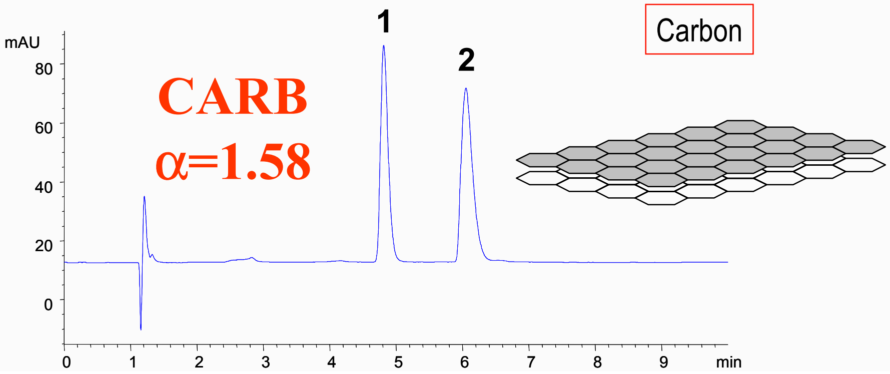
Mobile phase:		Flow rate:	1.0 mL/min.
	37.5/5/57.5 A/B/C	Temperature:	60 °C
A:	ACN	Injection volume:	5 µL
B:	THF	Detection:	254 nm
C:	Water		



LC Conditions

Column: ZirChrom®-CARB, 100 x 4.6 mm

Mobile phase:		Flow rate:	1.0 mL/min.
	32.5/67.5 A/B	Temperature:	60 °C
A:	ACN	Injection volume:	5 µL
B:	Water	Detection:	254 nm



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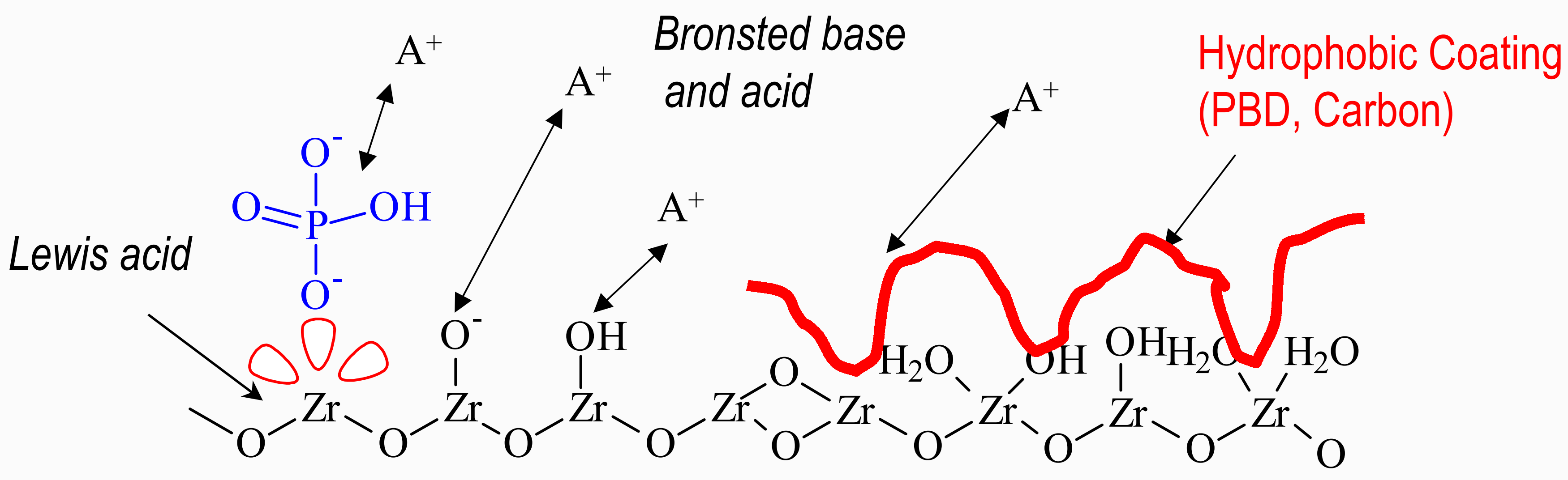
Selectivity Difference vs ODS (Orthogonality)

Column	R^2	Selectivity Difference*
ZirChrom®-PBD	0.985	12
DiamondBond®-C18	0.889	33
ZirChrom®-Carb	0.549	67

*$S=100(1-R^2)^{0.5}$, as described by U. Neue at FACSS 2002

- **For non-ionizable solutes:**
  - **ZirChrom-Carb and Diamondbond-C18 columns have very different selectivity from traditional C18-silica columns, exceeding that of embedded polar phases.**
  - **ZirChrom-PBD has selectivity similar to C18-silica.**
- **For ionizable solutes:**
  - **The selectivity difference is even greater on zirconia phases due to mixed mode possibilities.**

Zirconia Has Unique Surface Chemistry



- Zirconia by itself has very rich surface chemistry
- Coated zirconia phases (Carbon and PBD) have mixed surface properties
- The retention of various basic and acidic analytes can be fine tuned by changing pH, buffer and salt concentration, in addition to mobile phase modifier concentration and type

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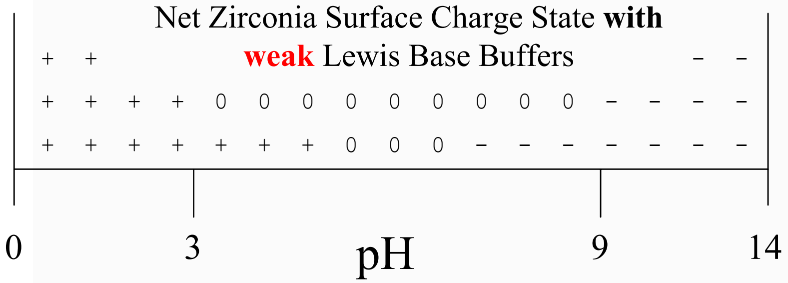
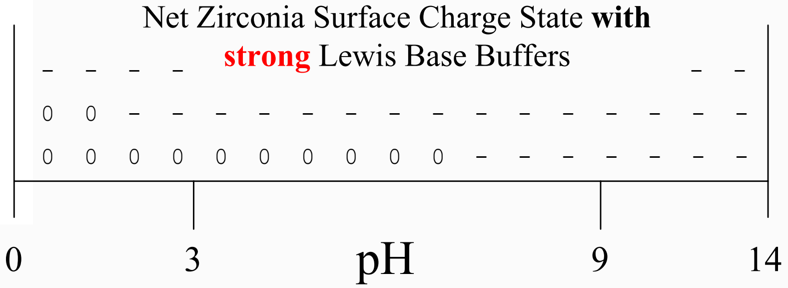
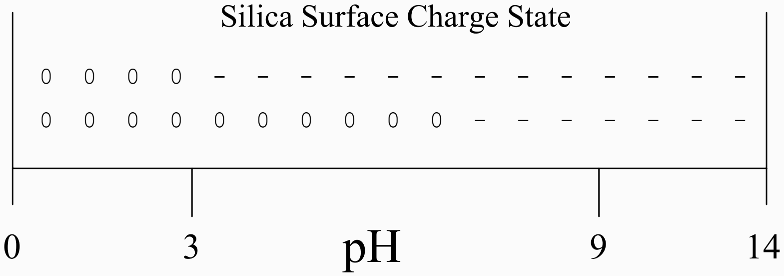
Zirconia Features Tunable Surface Properties

Interaction Strength	Lewis Base
Strongest	Hydroxide
	Phosphate
	Fluoride
	Citrate
	Sulfate
	Acetate
	Formate
Weakest	Nitrate
	Chloride


Net Zirconia Surface Charge State **with** **weak** Lewis Base Buffers

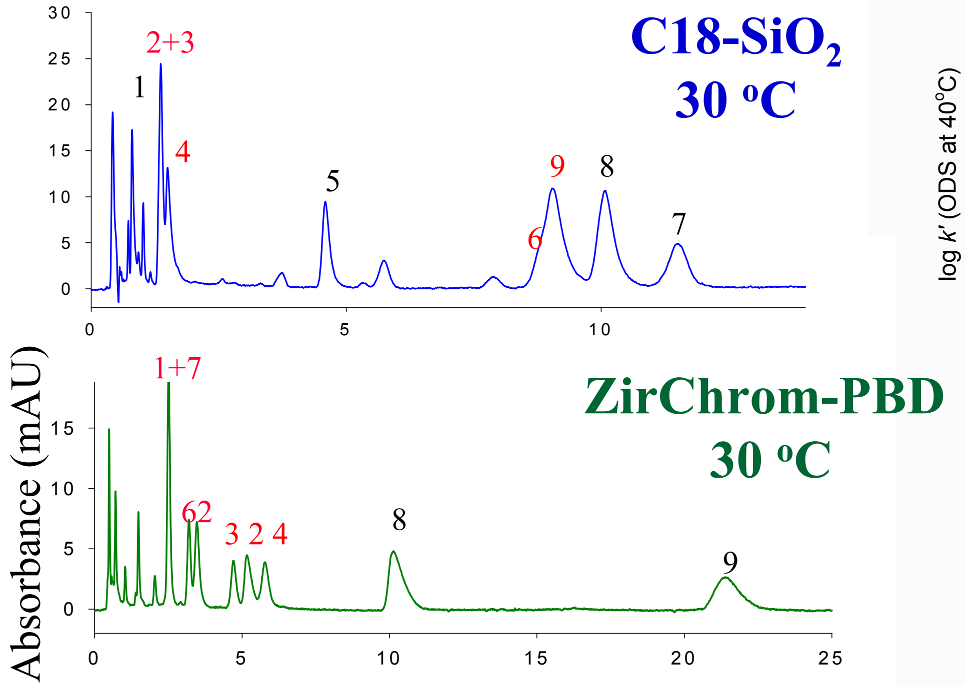

Net Zirconia Surface Charge State **with** **strong** Lewis Base Buffers

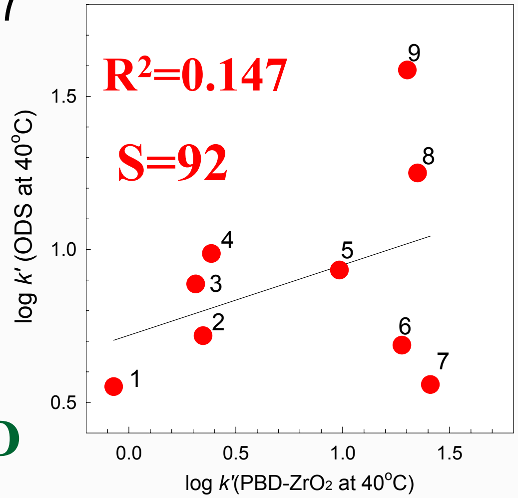
- The choice of buffer and pH on zirconia columns affects the surface charge and the elution properties of ionizable analytes. Mixed-mode (RP plus CEX) selectivity can be created.


Silica Surface Charge State

Antihistamine Drug Selectivity Comparison

Mobile Phase: 40/60 Acetonitrile/25 mM Phosphate, pH=7



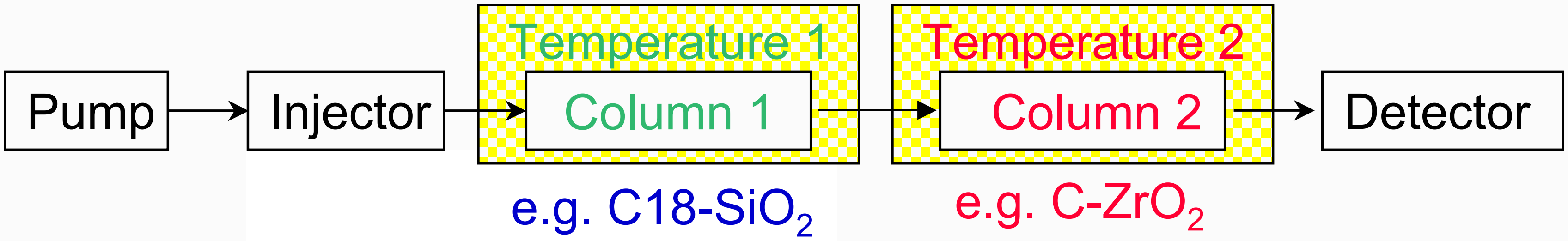


- The selectivity of zirconia based columns towards ionizable compounds becomes very different from that of traditional silica columns. Buffer type and pH have an effect on mixed-mode retention (RP and CEX) by Z-phases.

Thermally Tuned Tandem Columns

- *What if you could continuously adjust the selectivity of your HPLC column?*
- *Think of temperature as a replacement for solvent strength in adjusting retention time.*

Thermally Tuned Tandem Columns (T^3C)



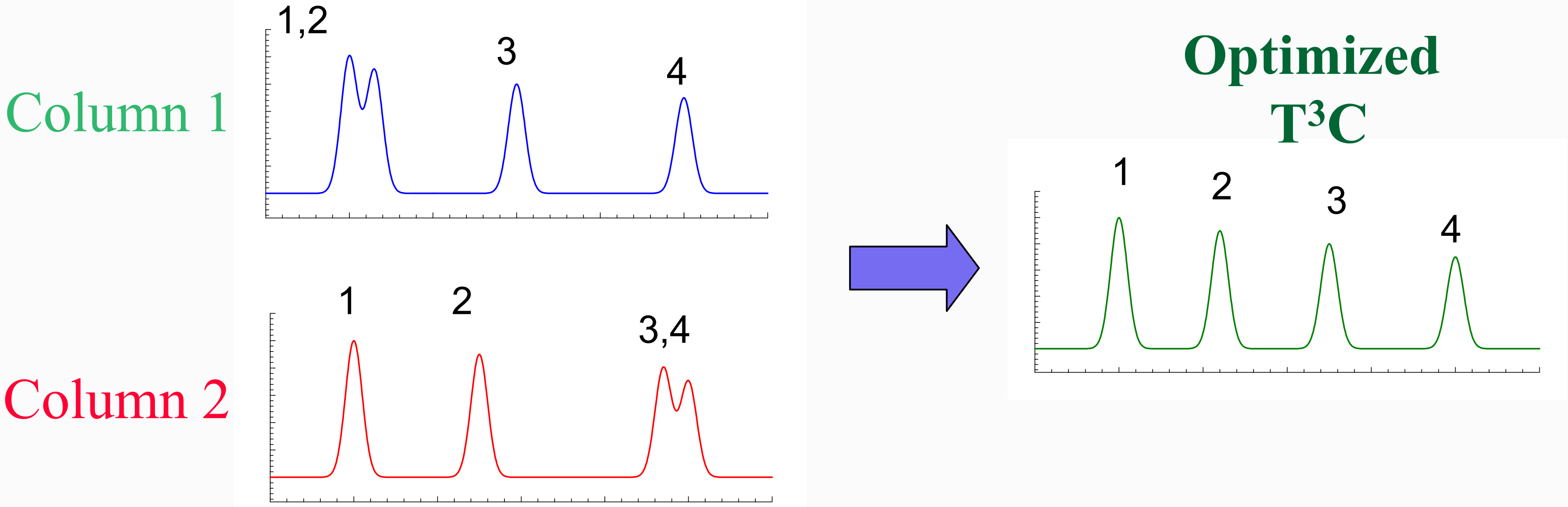
Pump → Injector → Column 1 → Column 2 → Detector

Temperature 1: Column 1

Temperature 2: Column 2

e.g. C18-SiO_2

e.g. C-ZrO_2



- T^3C is a method to continuously adjust HPLC selectivity using tandem, orthogonal columns and dual, independent temperature control.

Requirements for T^{3}C

- Two columns with different (ideally orthogonal) selectivity
- At least one thermally stable column (e.g. Zirconia-based)
- Thermally stable compounds
- Temperature control for at least one column (both preferred)
- Easy method development
  - Theory and practice of T^{3}C (references 1-3)
  - Guidelines for method development

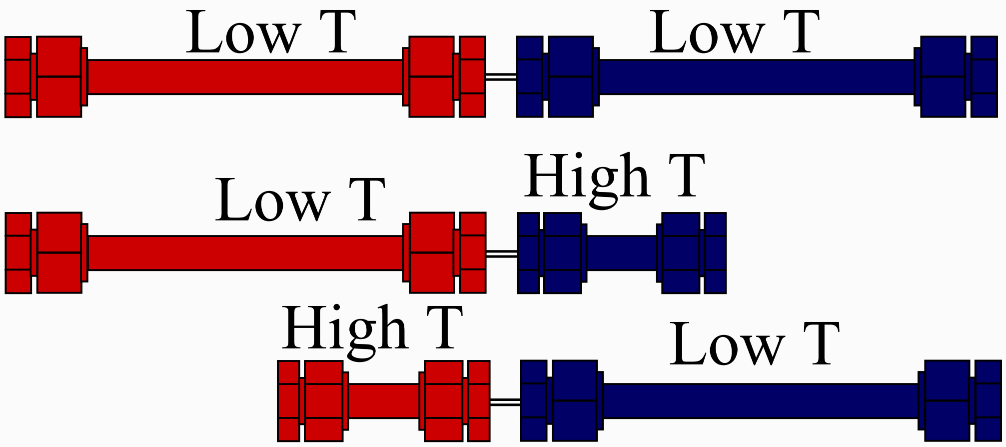
Effect of Temperature on T^{3}C Selectivity

Pump → Injector → Column 1 (Temperature 1) → Column 2 (Temperature 2) → Detector

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

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

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

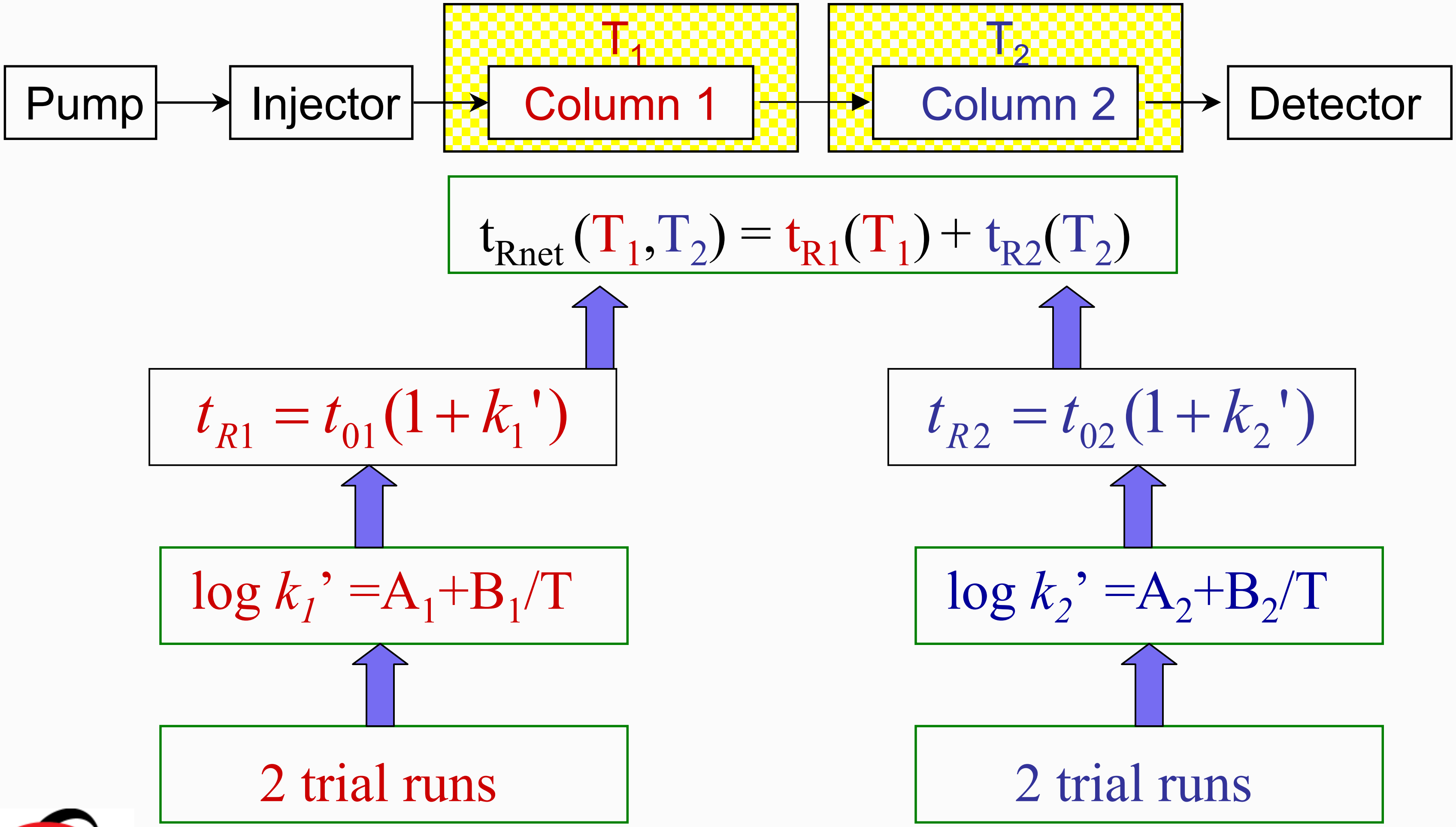


$$\alpha_{net} \longrightarrow \alpha_1$$

$$\alpha_{net} \longrightarrow \alpha_2$$

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

Method Development for T^{3}C



Pump → Injector → Column 1 → Column 2 → Detector

T_1

T_2

$$t_{Rnet}(T_1,T_2) = t_{R1}(T_1) + t_{R2}(T_2)$$

$$t_{R1} = t_{01}(1+k_1')$$

$$\log k_1' = A_1 + B_1/T$$

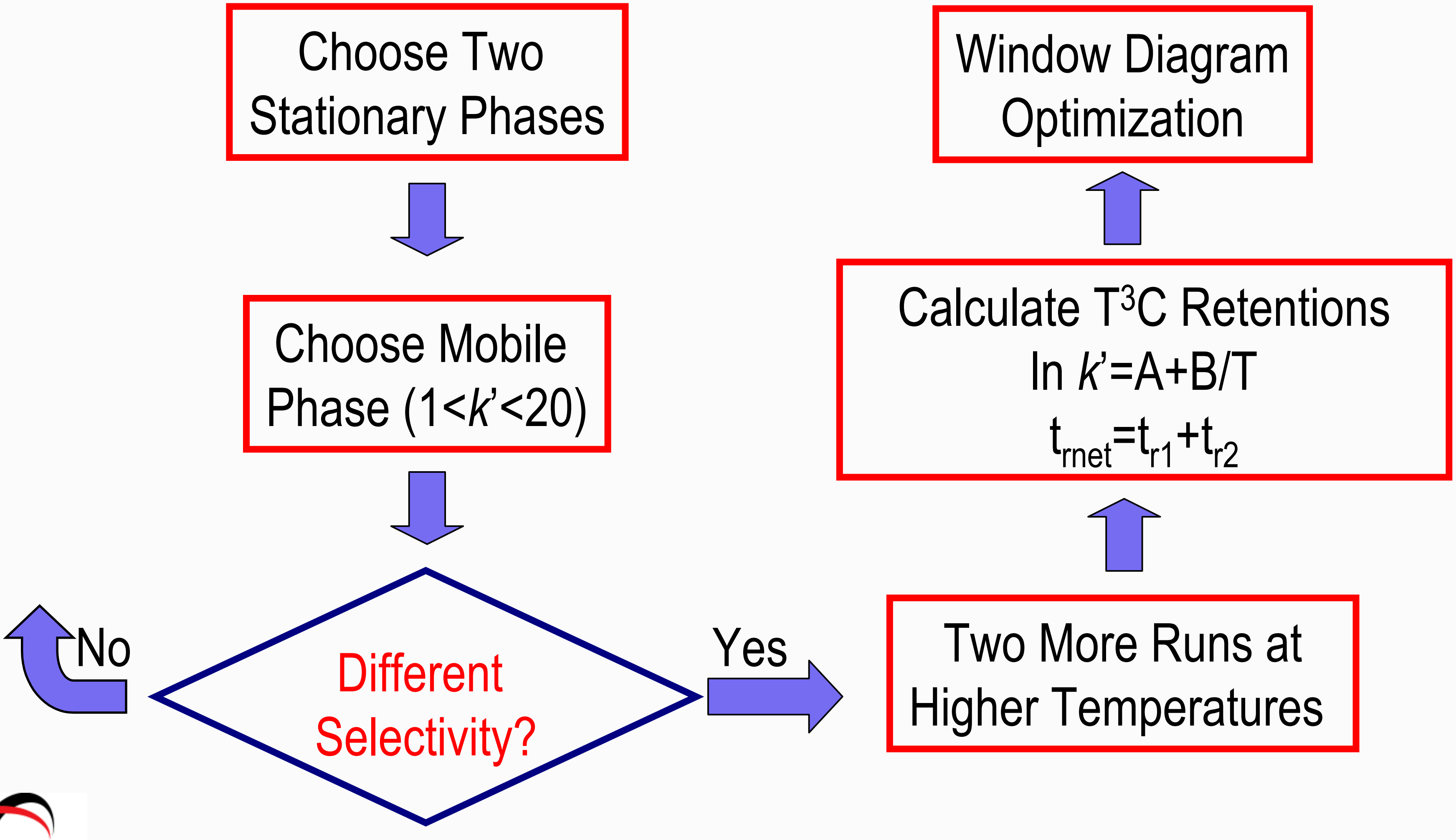
2 trial runs

$$t_{R2} = t_{02}(1+k_2')$$

$$\log k_2' = A_2 + B_2/T$$

2 trial runs

Guidelines for Optimizing T^3C



Choose Two Stationary Phases

Choose Mobile Phase $(1<k'<20)$

Different Selectivity?

No

Yes

Two More Runs at Higher Temperatures

Calculate T^3C Retentions

$\ln k' = A + B/T$

$t_{rnet} = t_{r1} + t_{r2}$

Window Diagram Optimization

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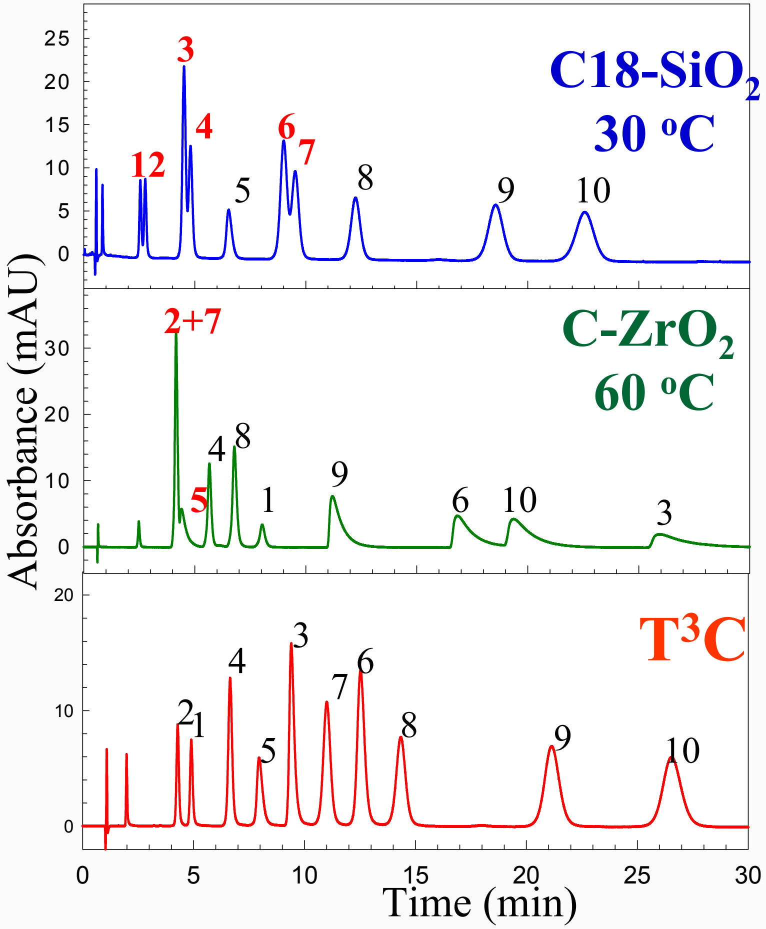
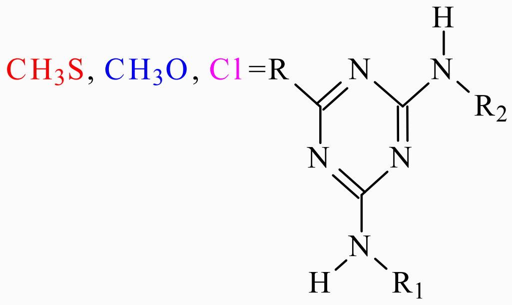
Separation of Ten Triazine Herbicides by T^3C





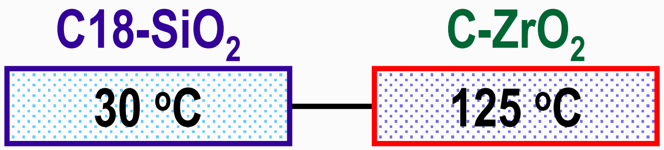
Solutes:

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^3C can improve separation without increasing analysis time. Extra length is offset by shorter k values.

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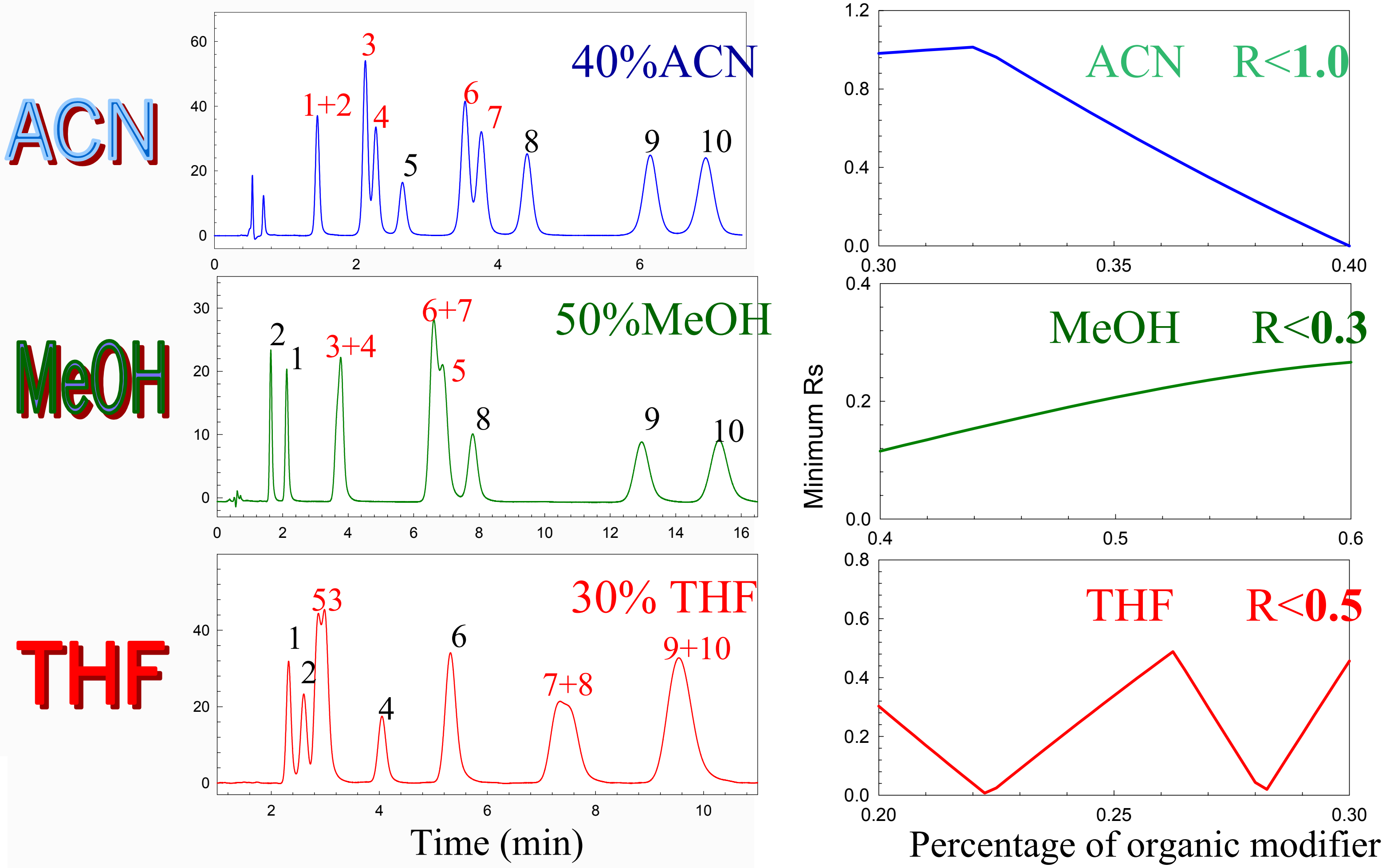
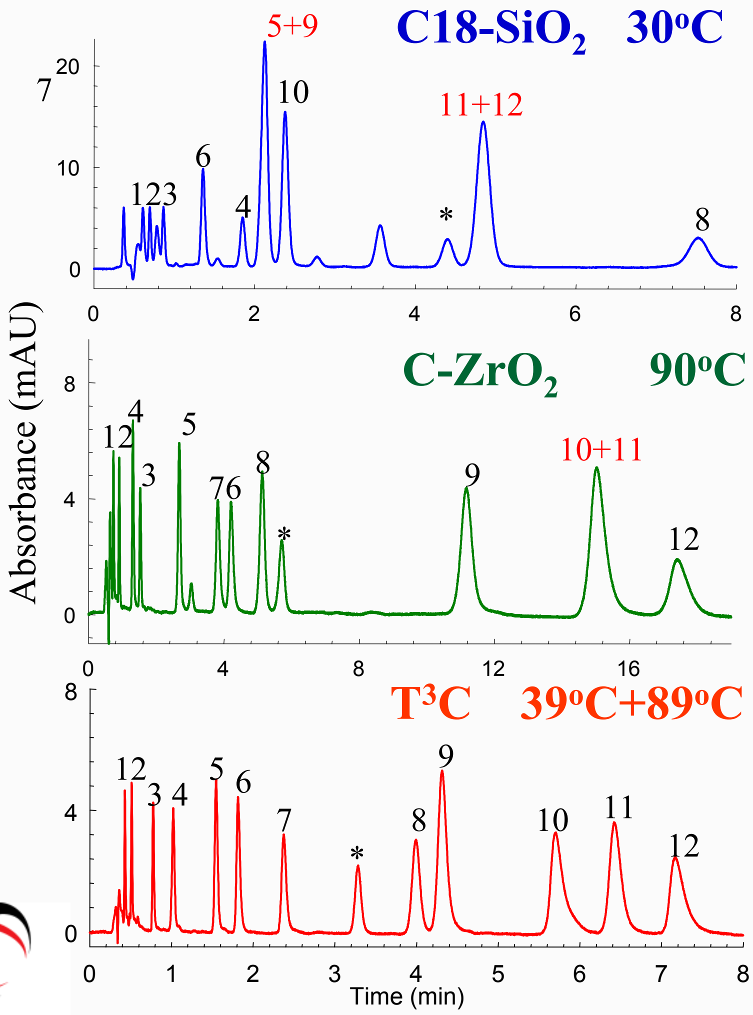
Comparison of T^{3}C with Solvent Optimization

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

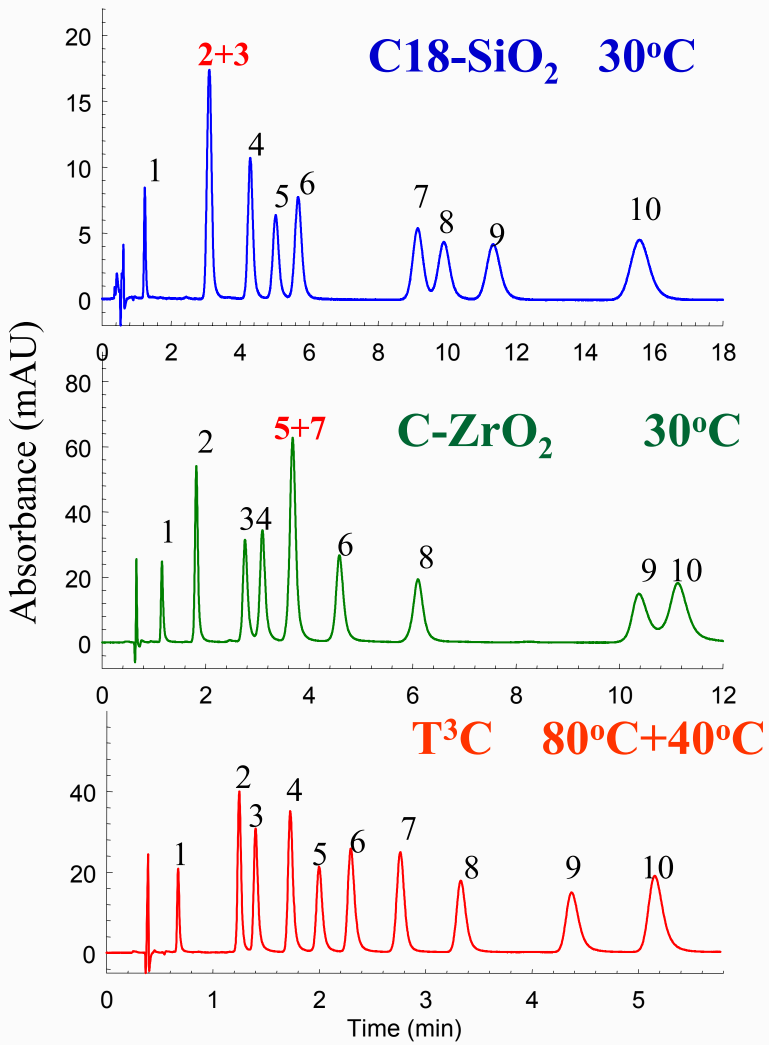


More T³C Separations

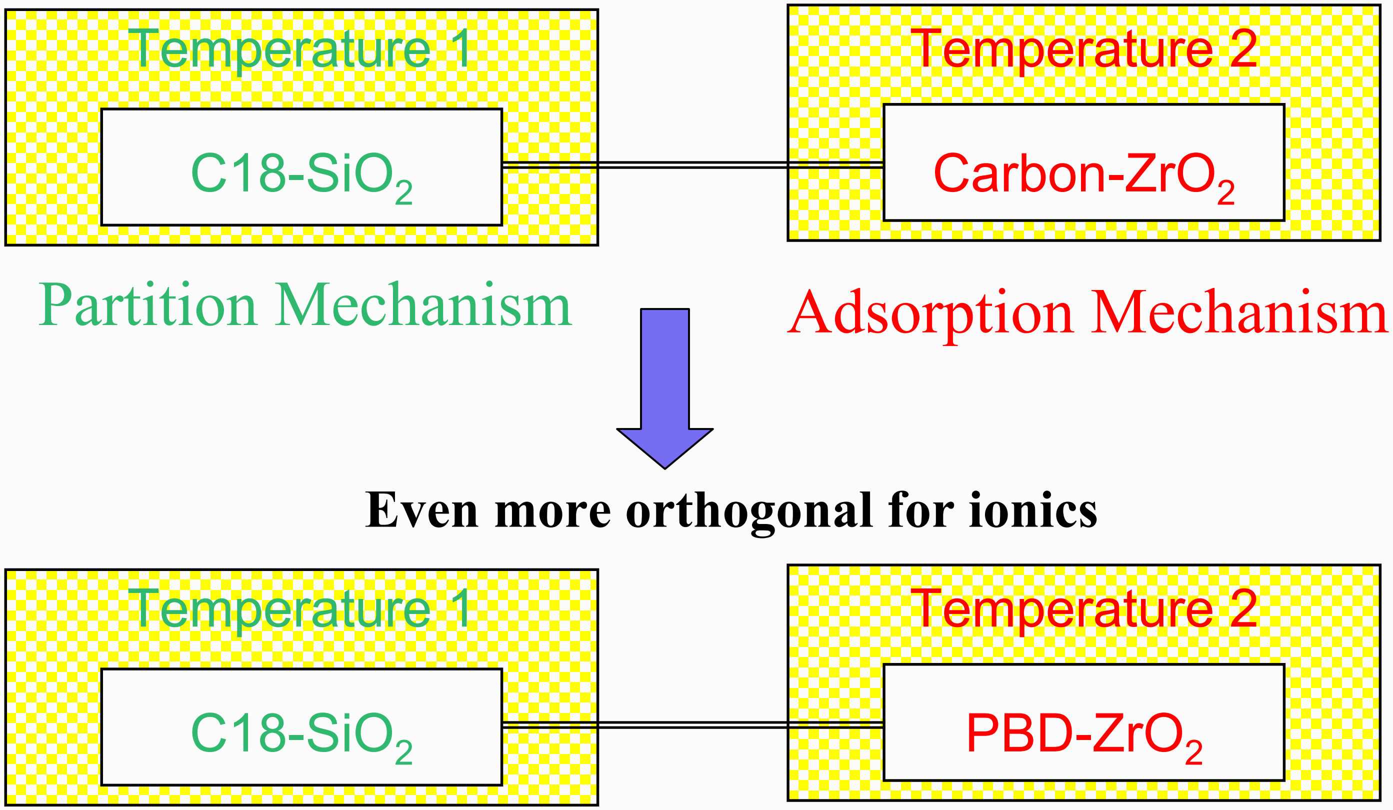
Urea and Carbamate Pesticides



Barbiturates



Basic Drugs: C18-Silica and PBD-Zirconia



orthogonal for nonionics

Temperature 1

C18-SiO$_2$

Temperature 2

Carbon-ZrO$_2$

Partition Mechanism

Adsorption Mechanism

Even more orthogonal for ionics

Phosphate Buffer pH=7

Temperature 1

C18-SiO$_2$

Temperature 2

PBD-ZrO$_2$

Reversed Phase Mode
+
Cation-Exchange Mode

Cation-Exchange Mode
+
Reversed Phase Mode

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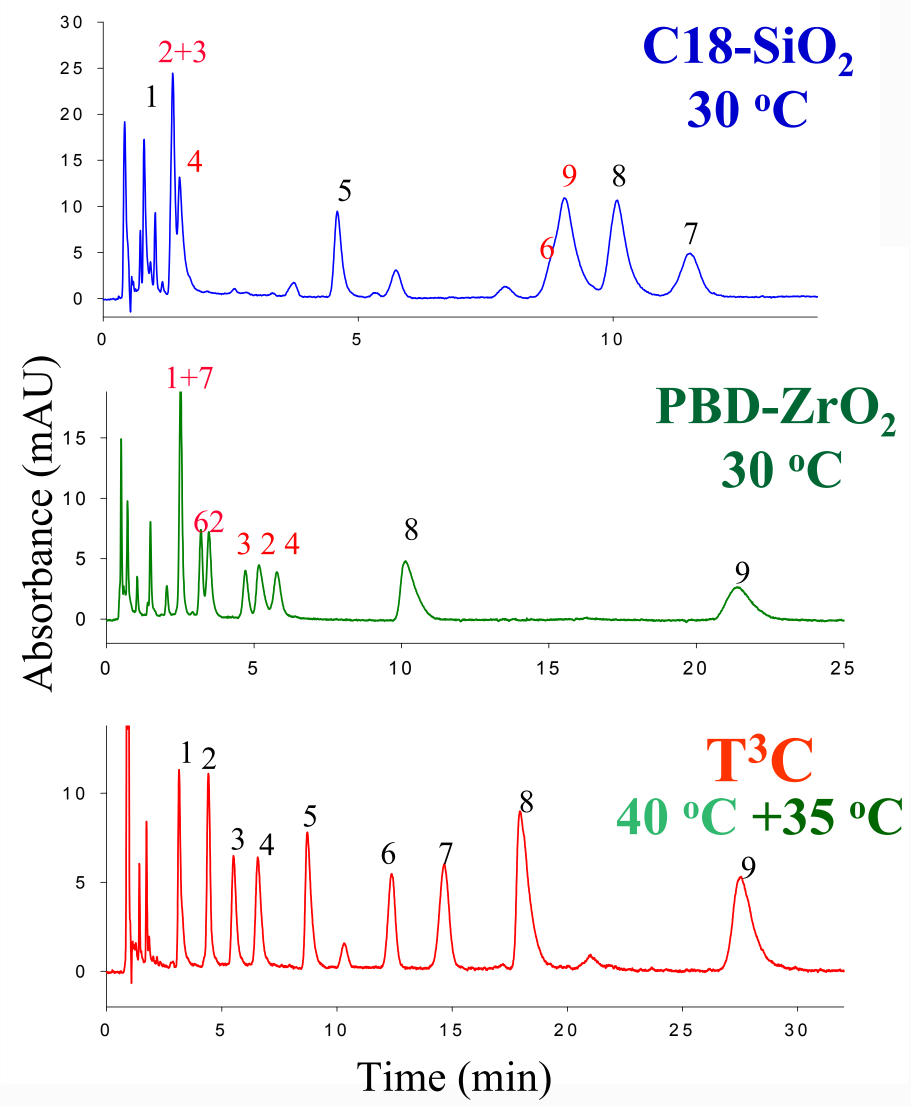
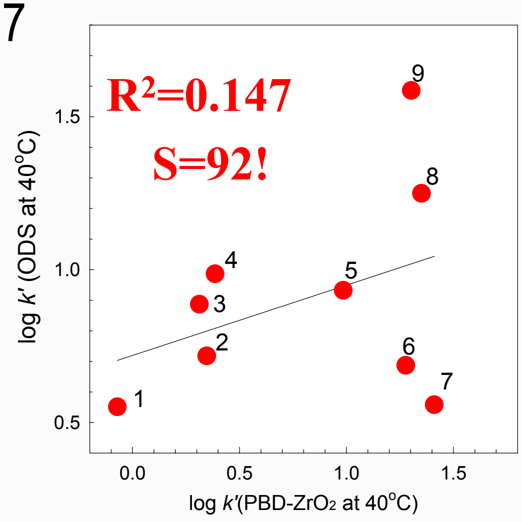
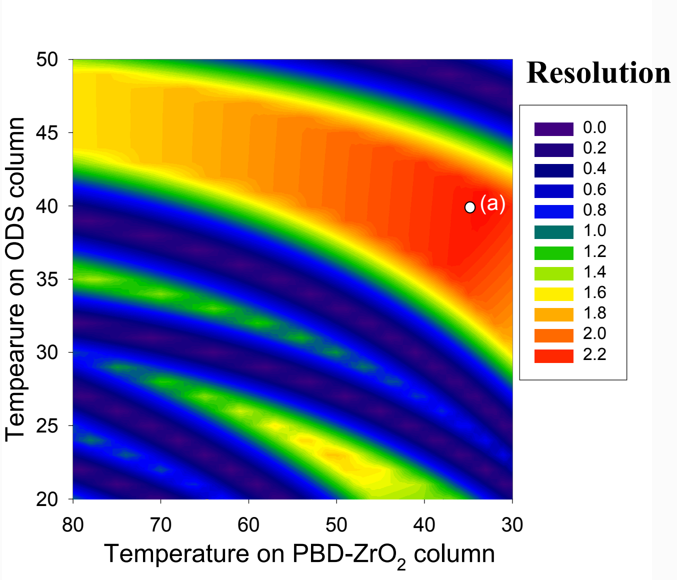
Separation of Antihistamine Drugs by T^3C

Mobile Phase: 40/60 Acetonitrile/25 mM Phosphate, pH=7







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Advantages and Disadvantages of T^3C

- **Advantages**:
  - Provides much better separation
  - Can improve selectivity without big change in analysis time
  - Relatively easy method development
  - Good selection of orthogonal stationary phases available

- **Disadvantages**:
  - Stationary phases and sample must be thermally stable
  - Potential for higher pressure drop and longer run time
  - Need to carefully select stationary and mobile phase
  - Need well-designed column oven; two ovens or dual-zone oven provide more selectivity

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Conclusions

- Zirconia-based phases are ultra-stable to extreme conditions in both temperature and pH
- The durability of Zirconia-based phases allows for ultra-fast separations at elevated temperatures
- ZirChrom® zirconia-based phases offer selectivity that can be very different from traditional silica-based stationary phases
- The combination of unique (orthogonal) selectivity and exceptional thermal stability allows the development of novel separations using the Thermally Tuned Tandem Column (T^3C) approach
- For a given analysis, T^3C requires two stationary phases with orthogonal selectivity and different critical pairs.

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References

1. Jun Mao and Peter W. Carr, Anal Chem. 2000, 72, 110-118.
2. Jun Mao and Peter W. Carr, Anal. Chem. 2001, 73, 4478-4485.
3. Jonathan D. Thompson and Peter W. Carr, Anal. Chem. 2002, 74, 1017-1023.

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