Method development with Zirconia-based Stationary Phases

EAS 2014

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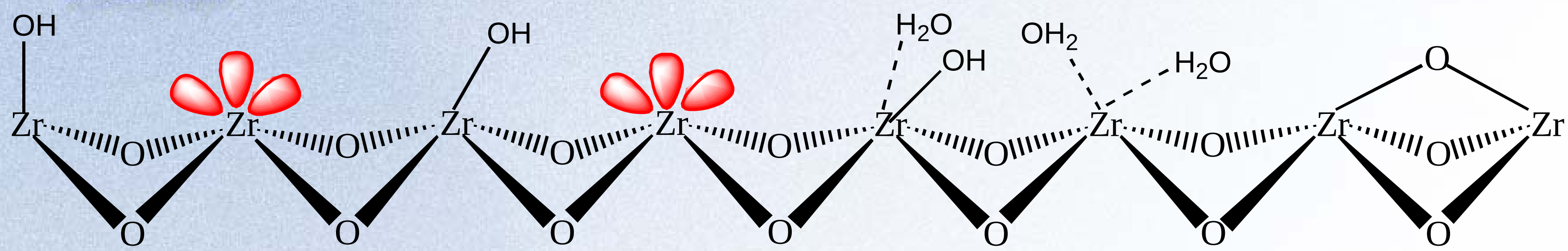
Method Development Tools

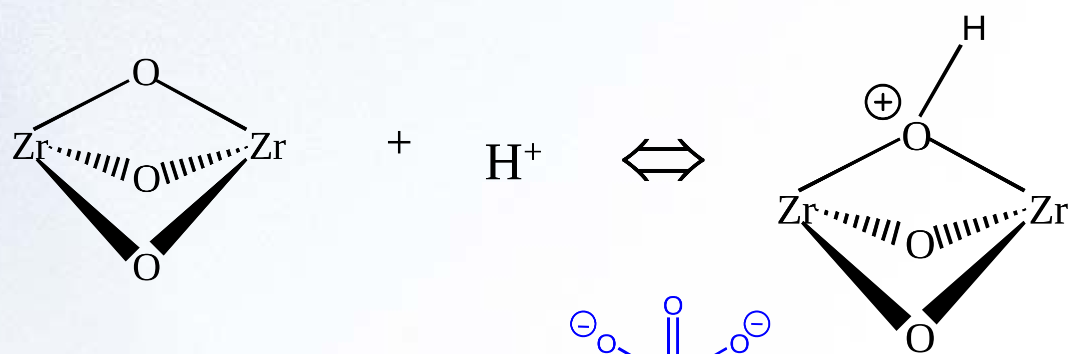
- Stationary Phase
- Organic Modifier (Type)
- Organic Modifier (%)
- Buffer Type and Concentration
- pH (1-14)
- Temperature (up to 200^{o} C)

Zirconia Surface Chemistry

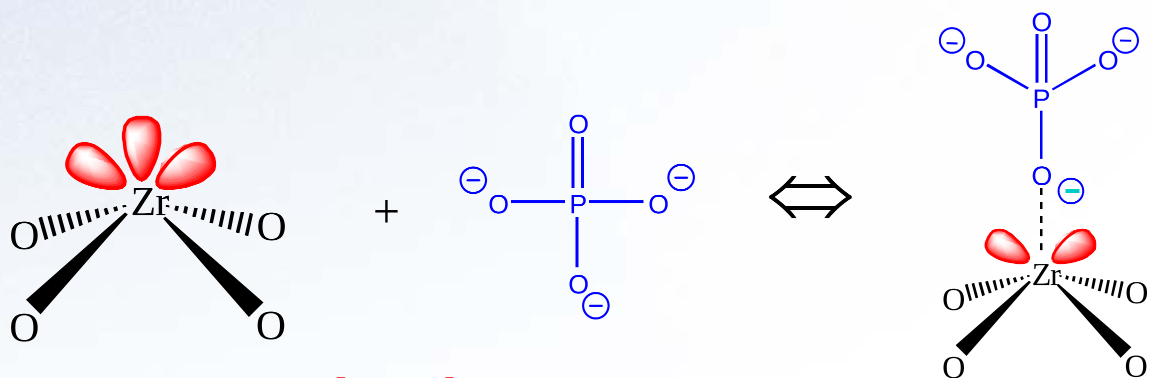


Weak Brönsted Acid: $ZrOH + OH^- \Leftrightarrow ZrO^- + H_2O$

Weak Brönsted Base:



Strong Lewis Acid:



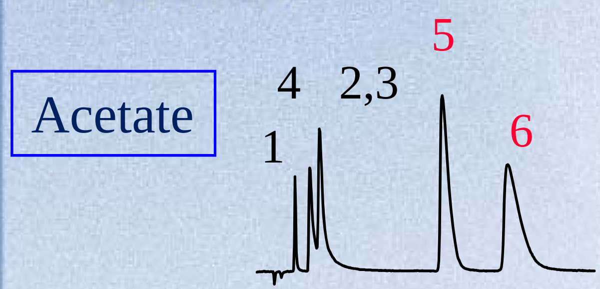
Ligand Exchange Interaction

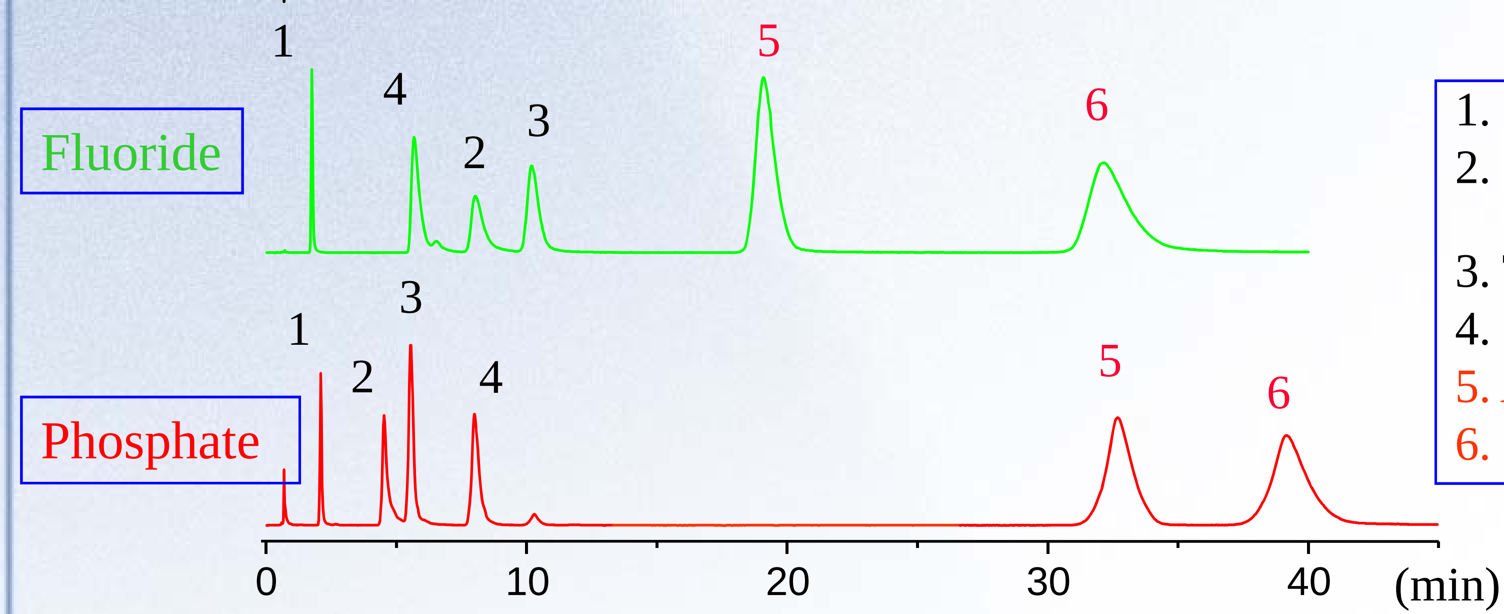
Choosing Buffer Type

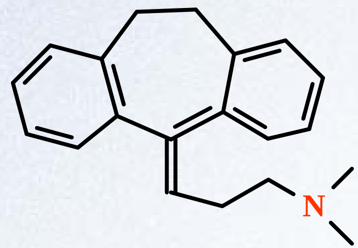
Interaction Strength	Lewis Base (A)
Strongest	Hydroxide
	Phosphate
	Fluoride
	Citrate
	Sulfate
	Acetate
	Formate
Weakest	Chloride

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

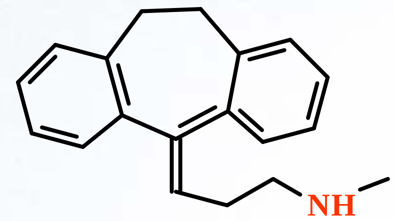
Effect of Buffer Type & Concentration







(5) pK_a 9.4



(6) pK_a 9.7

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

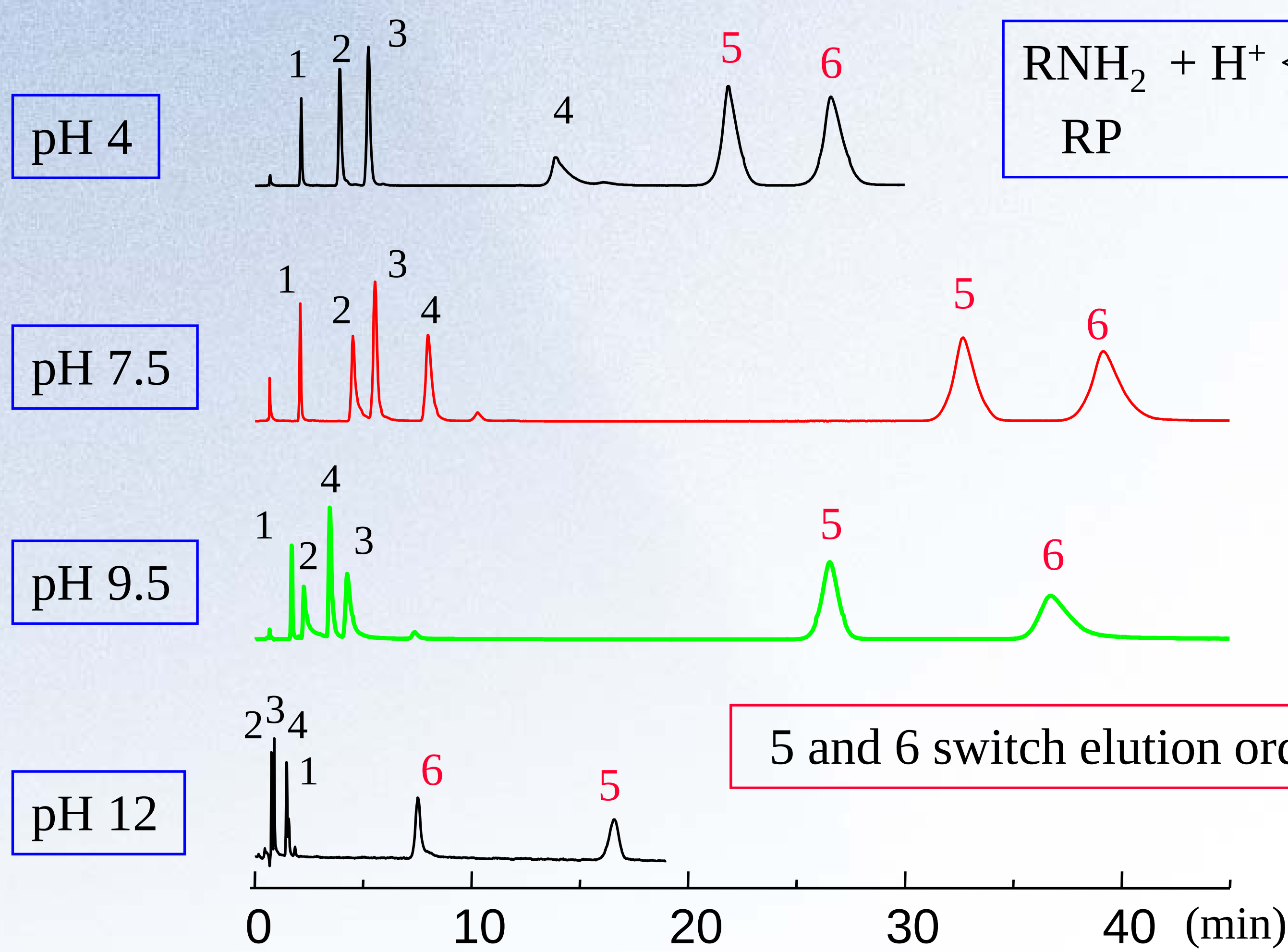
20% ACN, 20 mM Lewis base additive, pH 7.5; 0.8 mL/min; 30 °C

Effect of pH on Selectivity

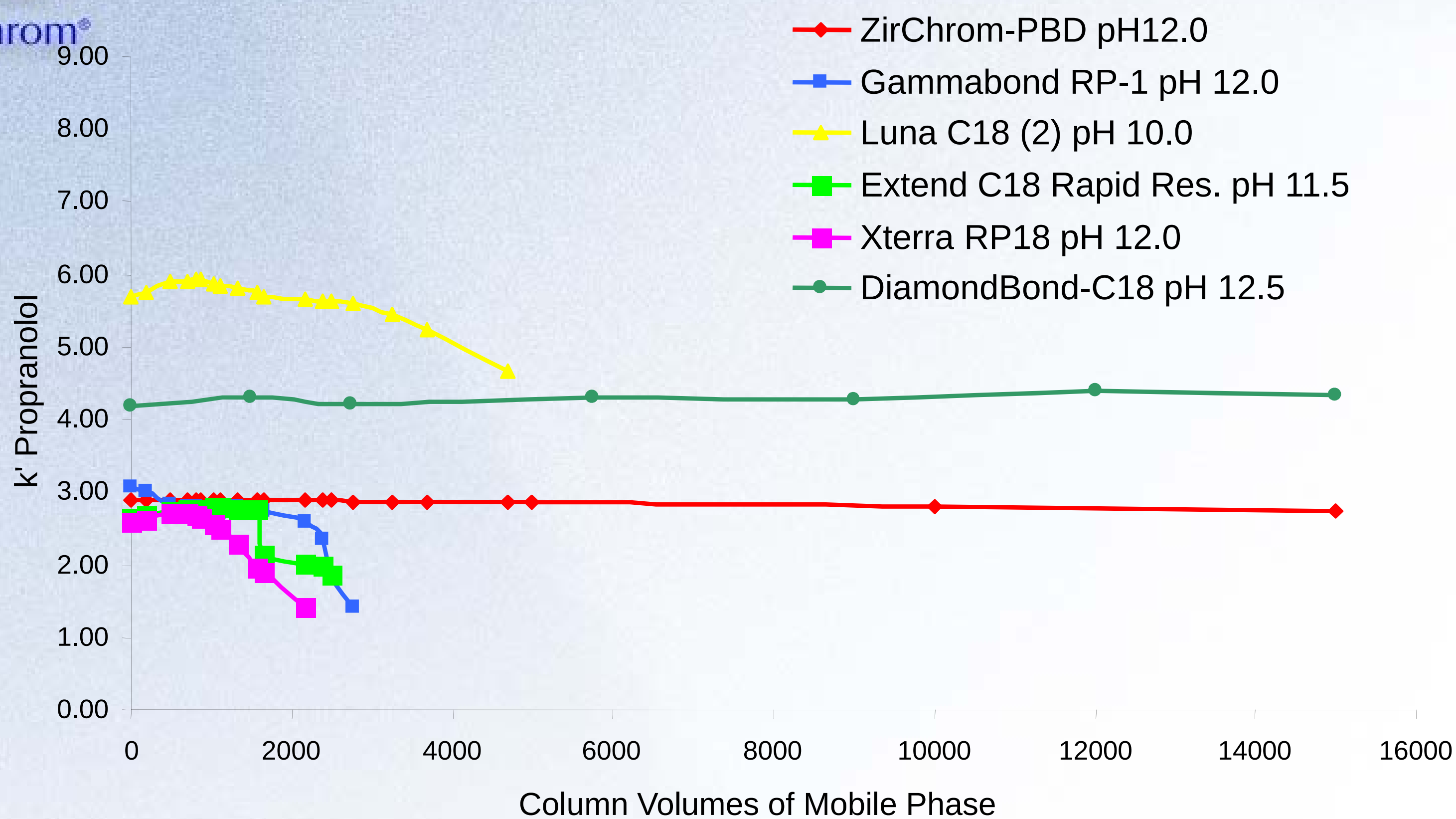
ZirChrom®

$RNH_2 + H^+$ <---> RNH_3^+

RP   RP + CEX



High pH Stability Comparison*



Exposure Conditions: Mobile phase, ACN/50mM Potassium phosphate buffer at indicated pH; Temperature, 30 ºC.

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

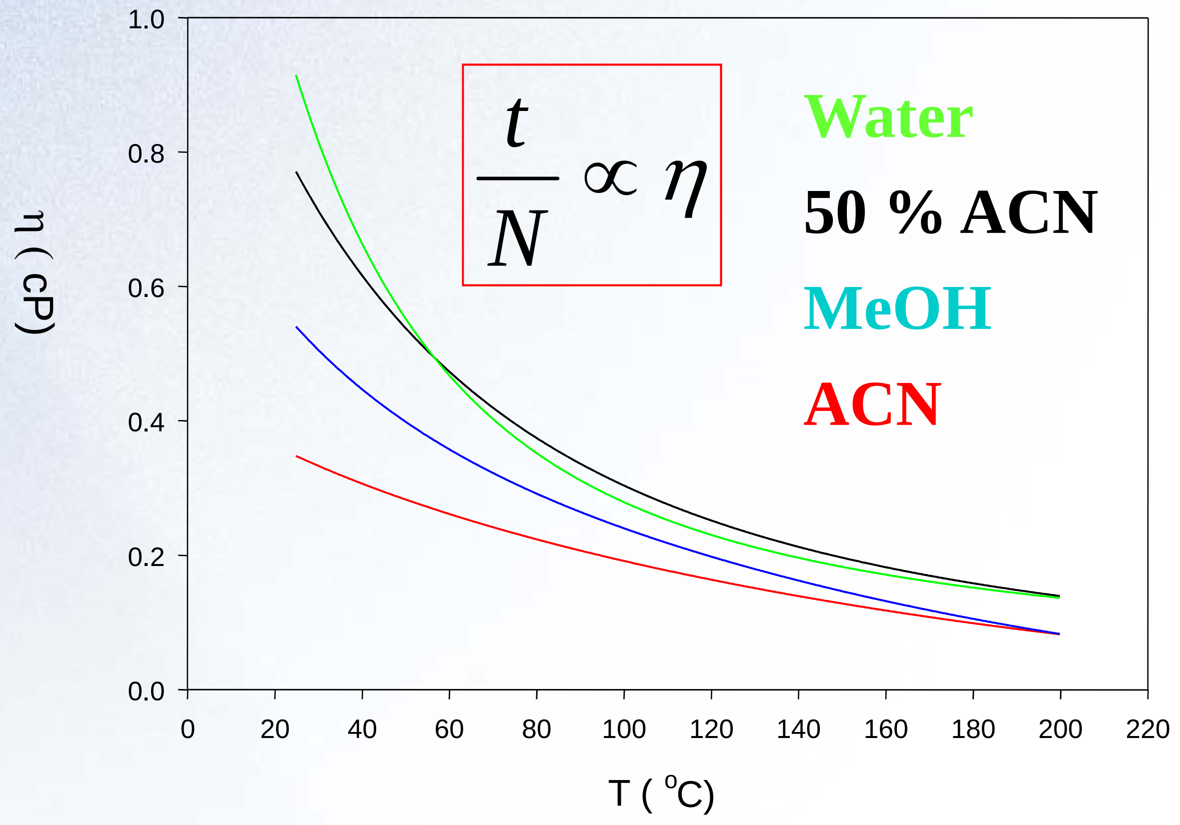
* Column names are the trademarks of their respective manufacturers.

Why Use Elevated Column Temperature?

- Reduce viscosity/improve mass transfer
  - Enables higher flow rates
    - Compress the analysis time in isocratic separations
    - Fast Gradients
- Reduces organic content
  - Lowers cost
  - “Greener” Separations

Effect of Temperature on Mobile Phase Viscosity

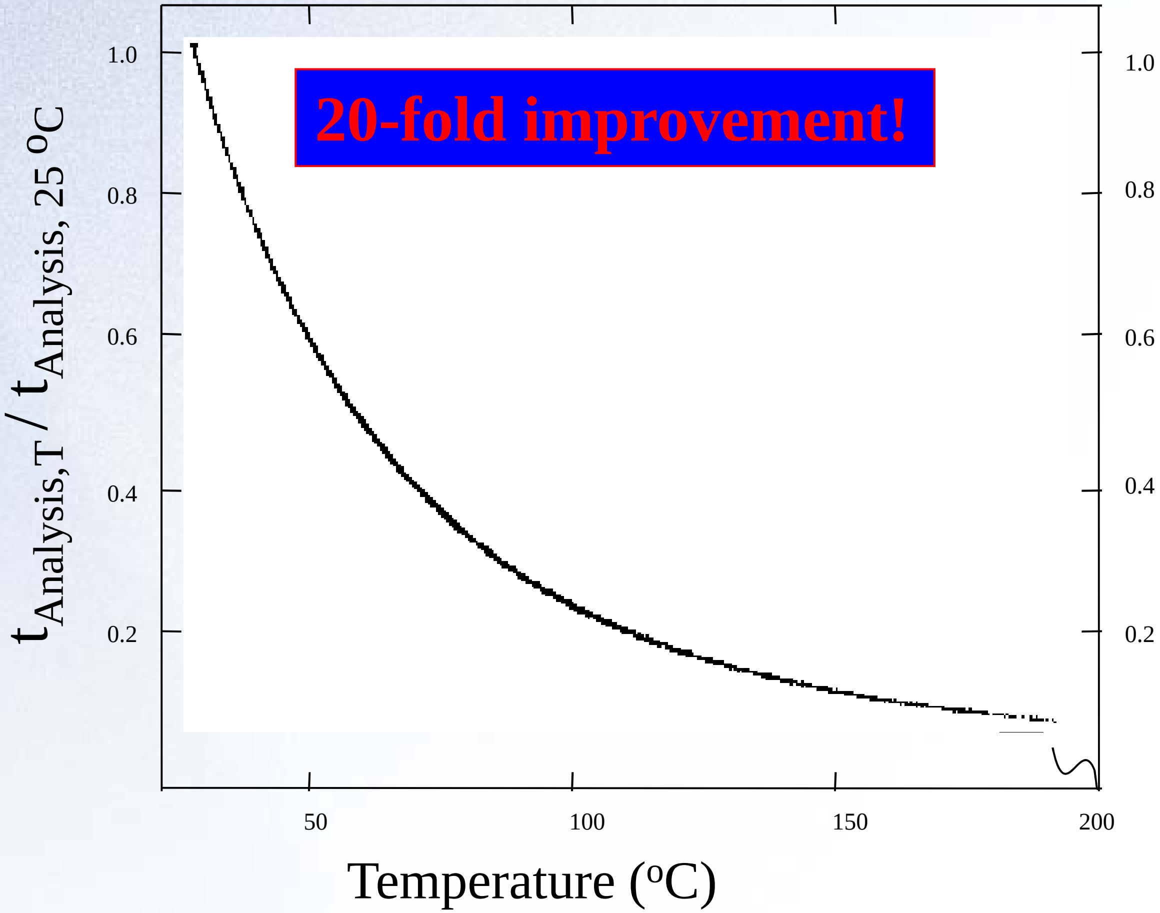
Estimated Effect of Temperature on Viscosity*



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

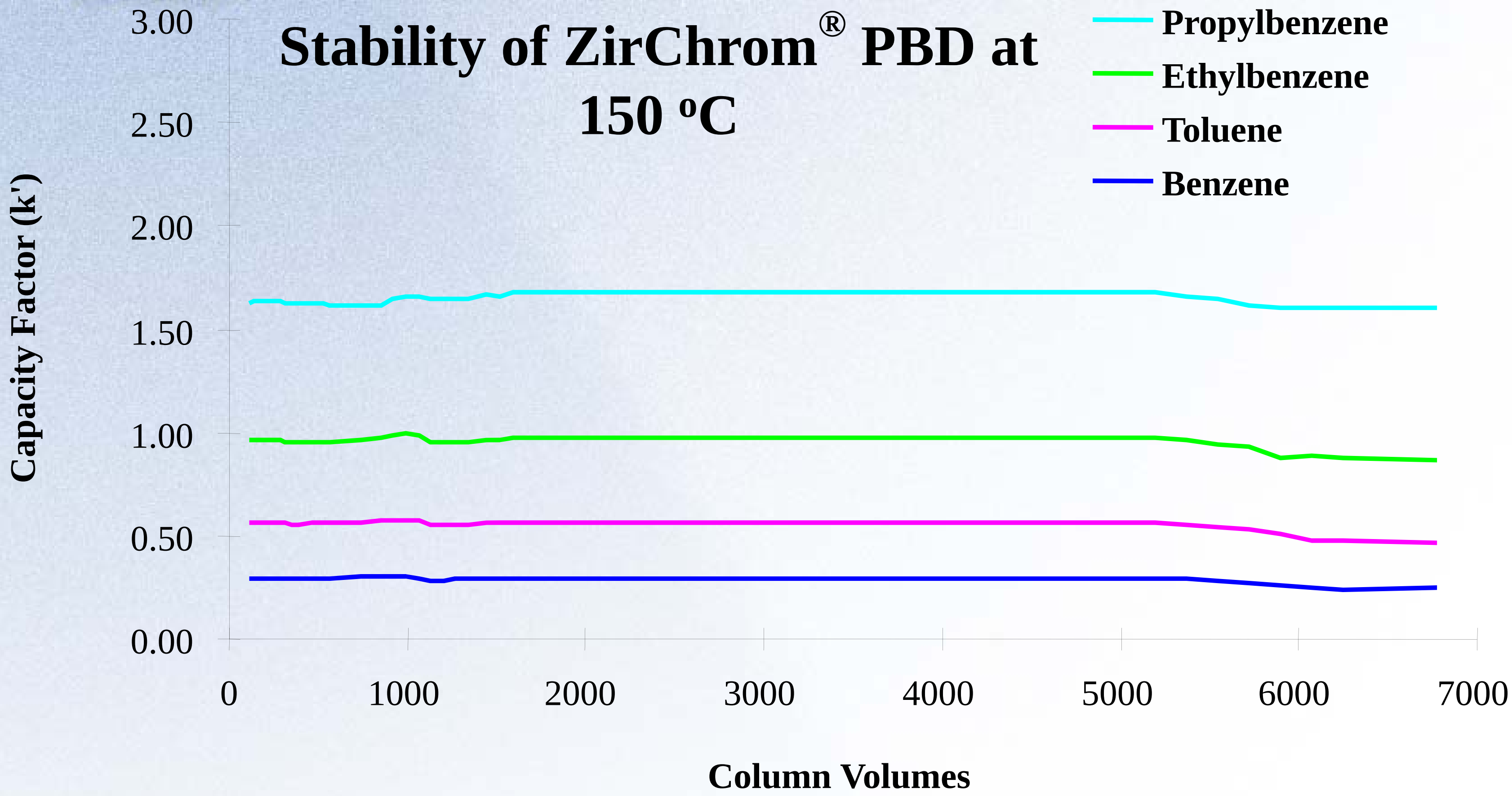
Why Temperature?

Effect of Temperature on Analysis Time at Constant Pressure, Plate Count and Retention Factor*



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

Thermal Stability



Column, ZirChrom®-PBD, 150 x 4.6 mm i.d.; Flow rate, 1.0 ml/min.; Mobile phase: 15/85 ACN/H_2O; Solutes, benzene, toluene, ethylbenzene, propylbenzene; Column Temperature = 150ºC.

The Effect of Incomplete Thermal Equilibration

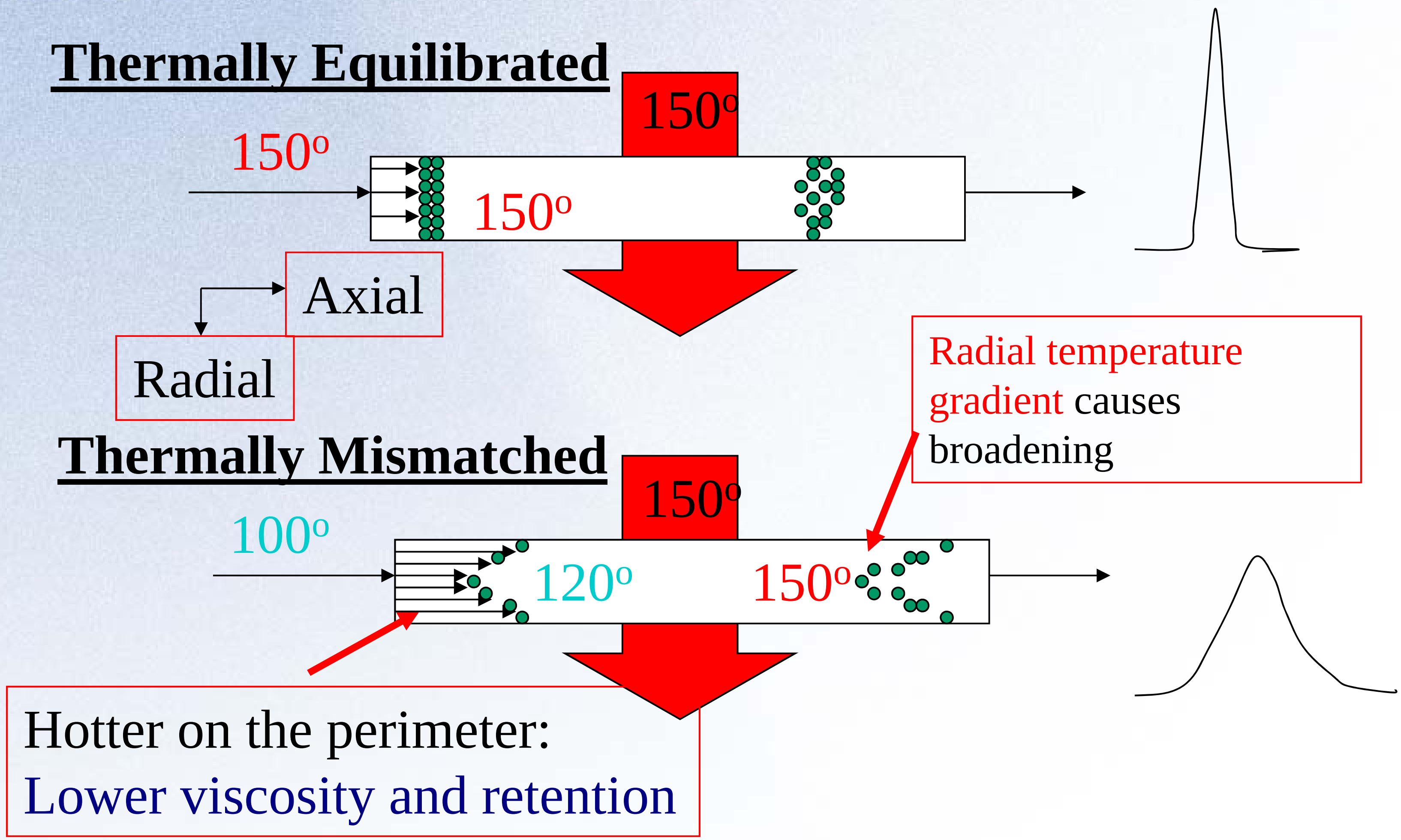
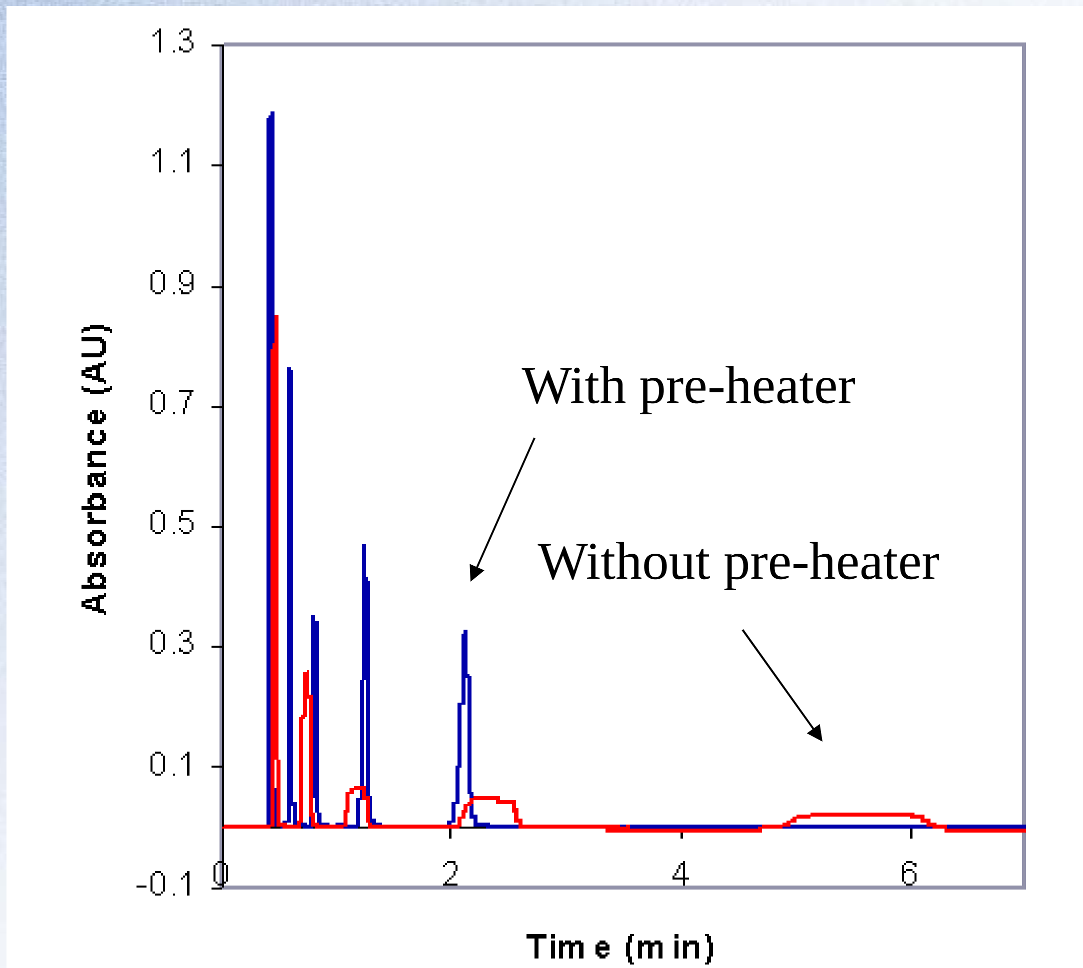


Diagram Courtesy Dr. Jon Thompson, Systec

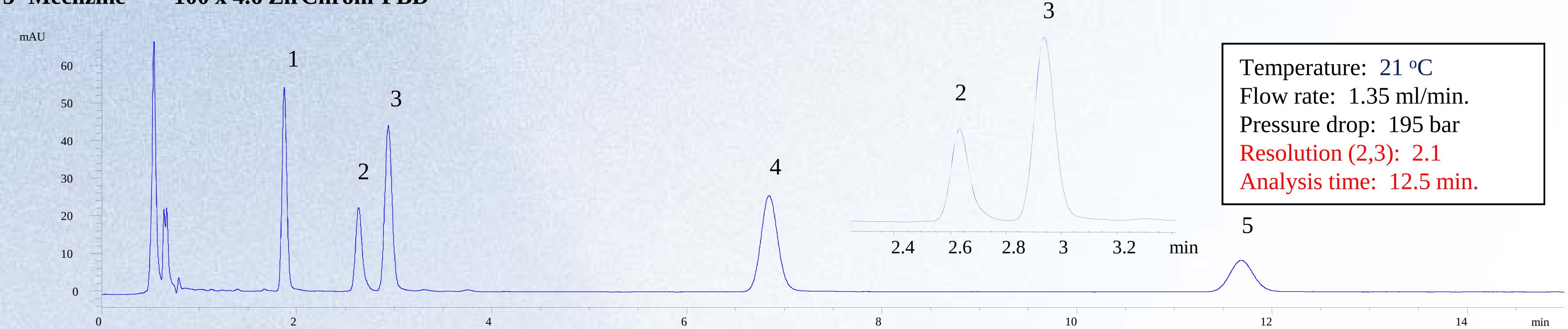
Thermal Mismatch Broadens Peaks



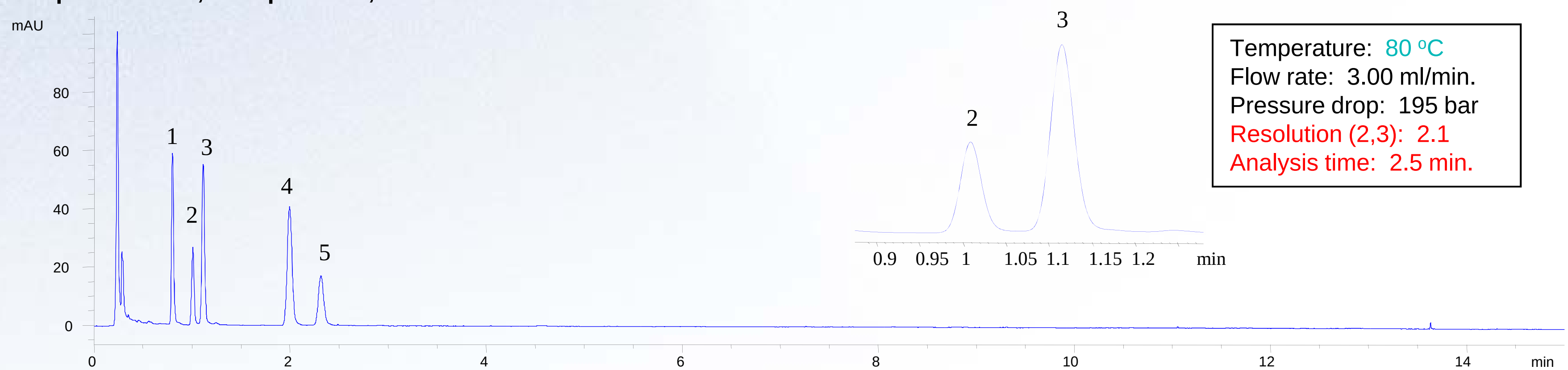
Conditions:_ Mobile phase, 50/50 ACN/water; Setpoint temperature, 165 °C, Flow rate, 3 l/min.; Heater, F with 1.68 m preheater tubing; 100 mm x 4.6 mm i.d. ZirChrom®-PBD; Solutes, alkylphenones.

Analysis Time May Be Reduced Without Loss of Resolution Through *Column Heating*

LC Conditions: 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=Meclizine 100 x 4.6 ZirChrom-PBD



LC Conditions: Mobile Phase, 26.5/73.5 ACN/50mM Tetramethylammonium hydroxide, pH 12.2; Flow Rate, 3.00 mL/min.; Injection volume, 0.2 ul; 254 nm detection; Column Temperature, 80°C; Pressure drop = 195 bar; Solutes: 1=Doxylamine, 2=Methapyrilene, 3=Chlorpheniramine, 4=Triprolidine, 5=Meclizine 100 x 4.6 ZirChrom-PBD

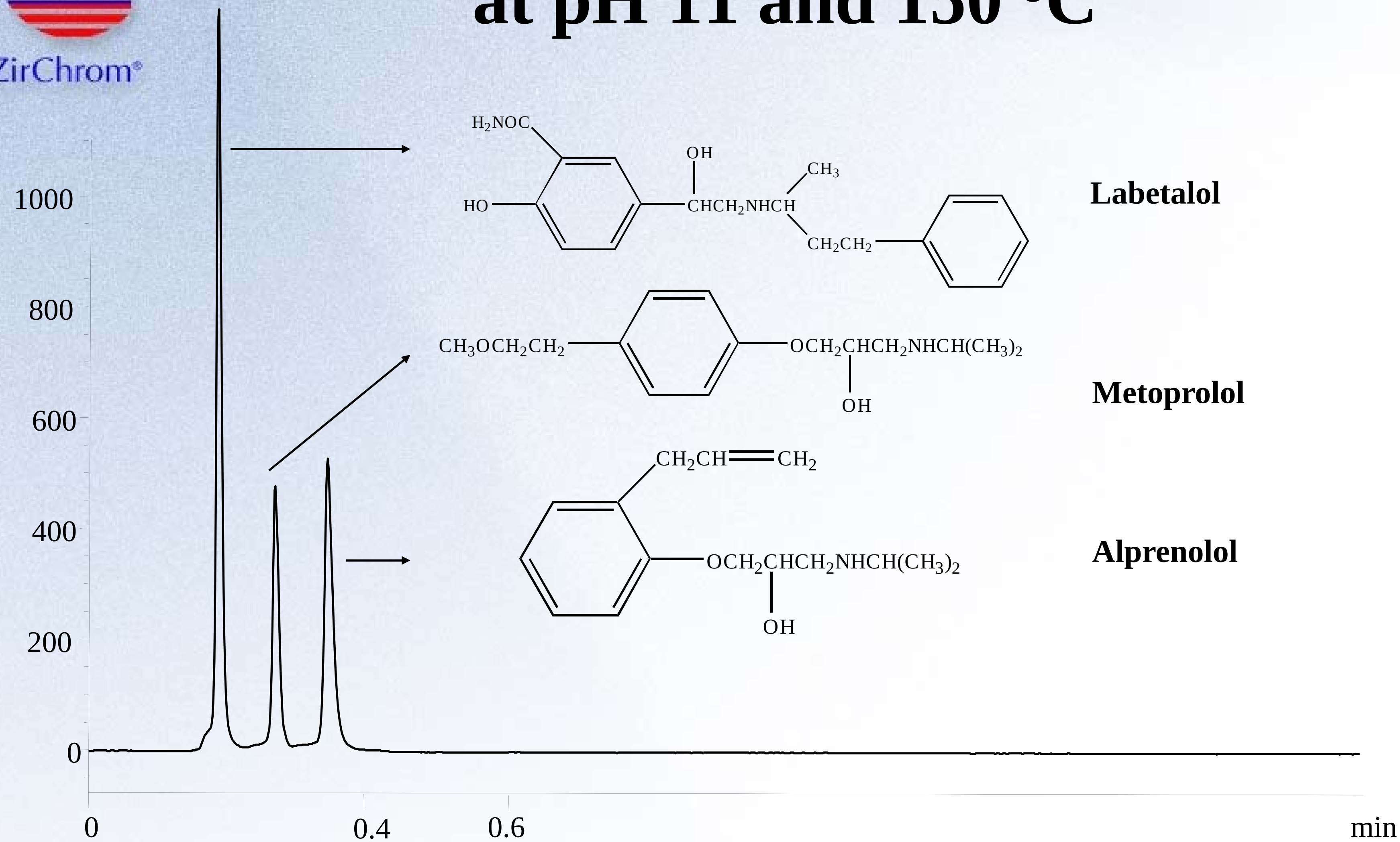


The Importance of Analysis Time Reduction Through *Column Heating*

Temperature (degrees C)	Cost per Analysis*	Throughput per Instrument*
21	$2.66	1 X
50	$1.87	2.1 X
80	$1.50	3.3 X
140	$1.32	5.2 X

* Based on Quantitative Value Assessment Tool - http://www.zirchrom.com/documents/value.xls

Fast Pharmaceutical Separation at pH 11 and 150 °C



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

- Zirconia-based stationary phases have very different selectivity from silica-based phases.
- Zirconia phases have selectivity that can be tuned by the addition of different buffers.
- Zirconia phases offer the ultimate in stability
  - Faster separations at high temperature
  - Allows the use of the full pH range