High Temperature Liquid Chromatography

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OUTLINE

- **Advantages of High Temperature HPLC**
  - Theoretical Effects of High Temperature HPLC
  - Practical Analytical Advantages of Using High Temperature HPLC
- **Development of New Stationary Phases**
  - Selectivity Comparison of Zirconia Based Stationary Phases with C18 Silica and Other Columns
  - High Temperature Separations
- **Using Temperature to Control Selectivity**
  - Importance of Selectivity in HPLC Optimization

Theoretical Advantages to High Temperature LC

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).

Practical Limit
Temperature Dependence

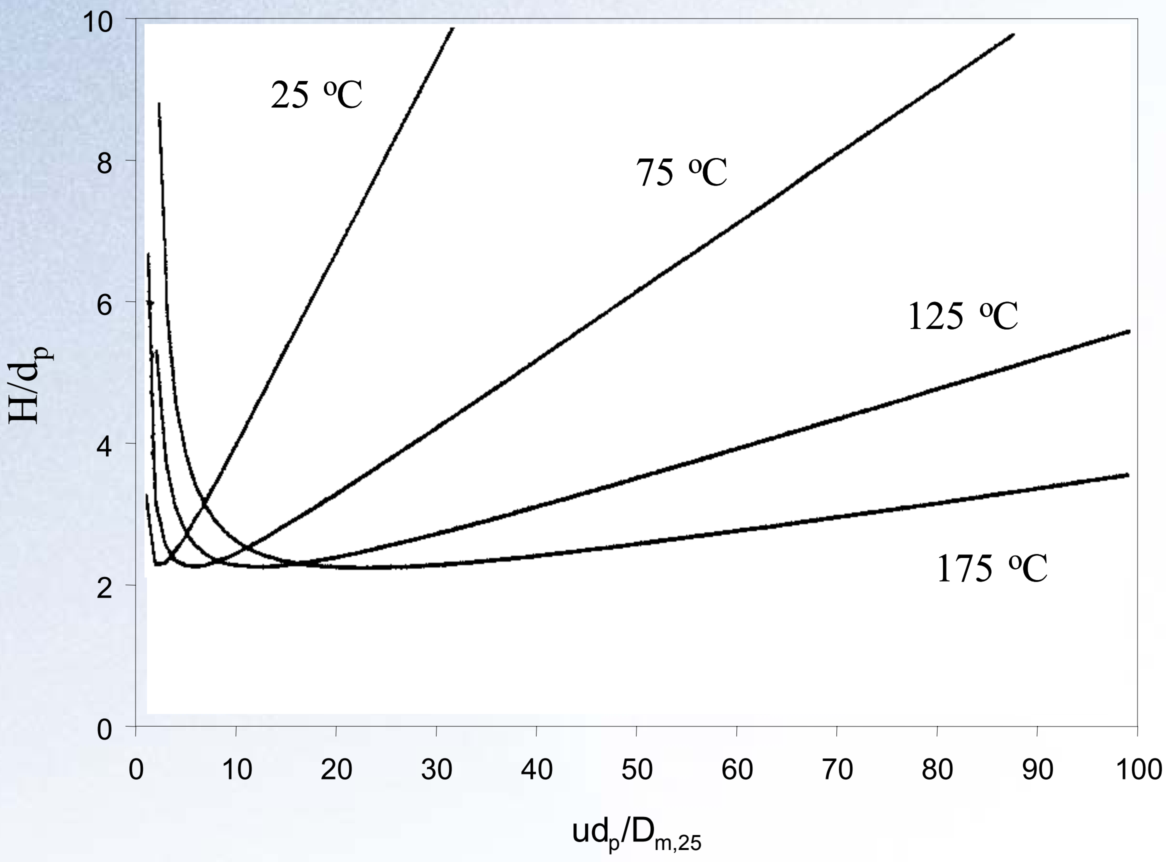
$$\frac{t}{N} \propto (1+k')\frac{L^{2/3}}{\Delta P_{max}^{2/3}}\frac{\eta}{T^{1/3}}$$

Guiochon, Georges, *Anal. Chem.*, 52, 2002-2008 (1980).

Three ways that temperature increases efficiency and speed

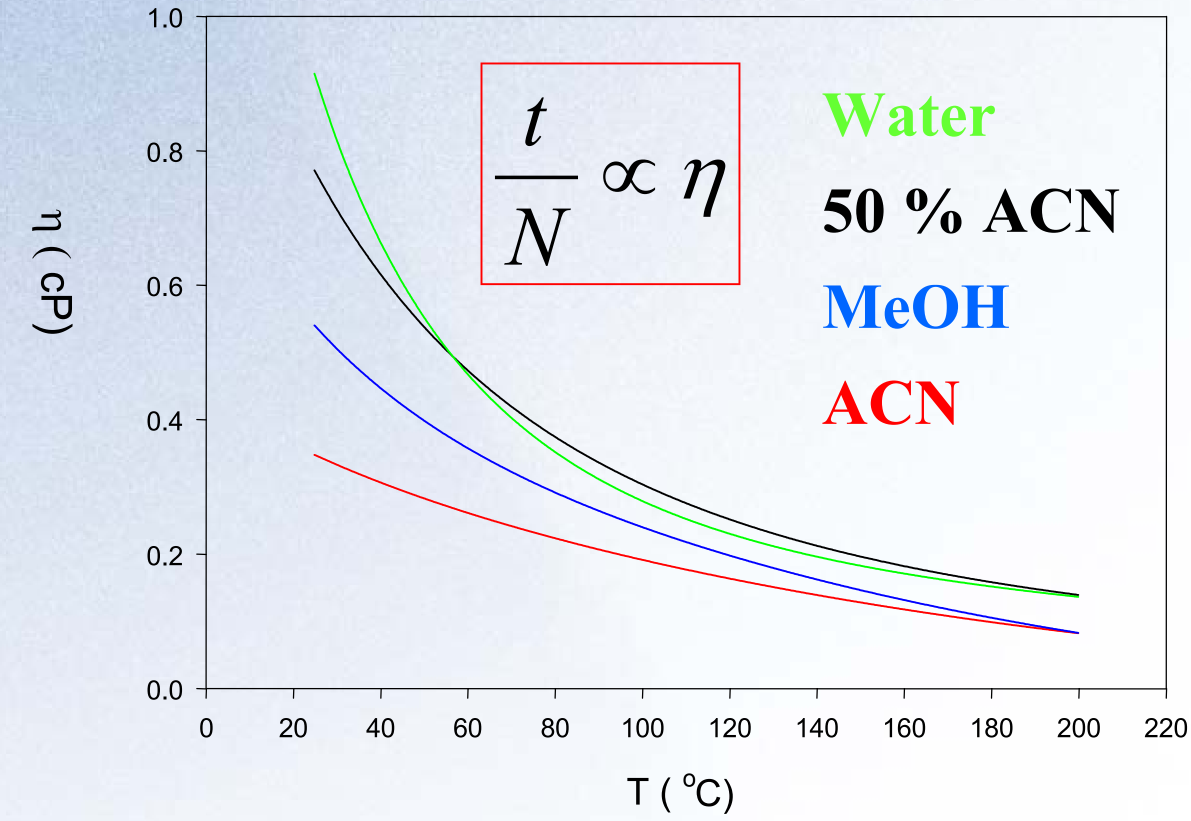
- Increased temperature increases diffusivity, thus decreasing the reduced velocity
- Increased temperature accelerates sorption kinetics
- Increased temperature decreases mobile phase viscosity

Theoretical Effect of Temperature on Column Efficiency



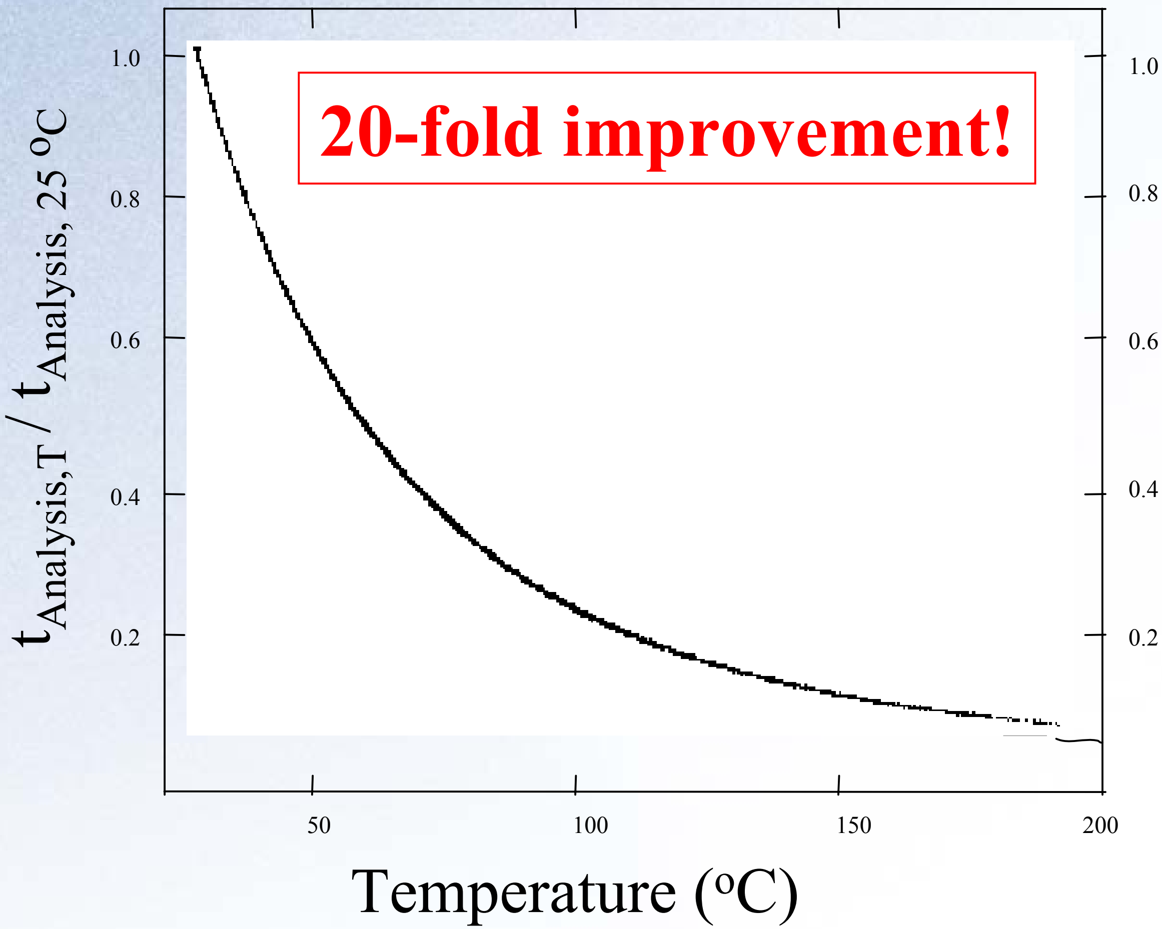
R. D. Antia and Cs. Horvath, *J. Chromatogr.*, 435, 1-15 (1988).

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).

Effect of Temperature on Theoretical Analysis Time at Constant Pressure and Plate Count*



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

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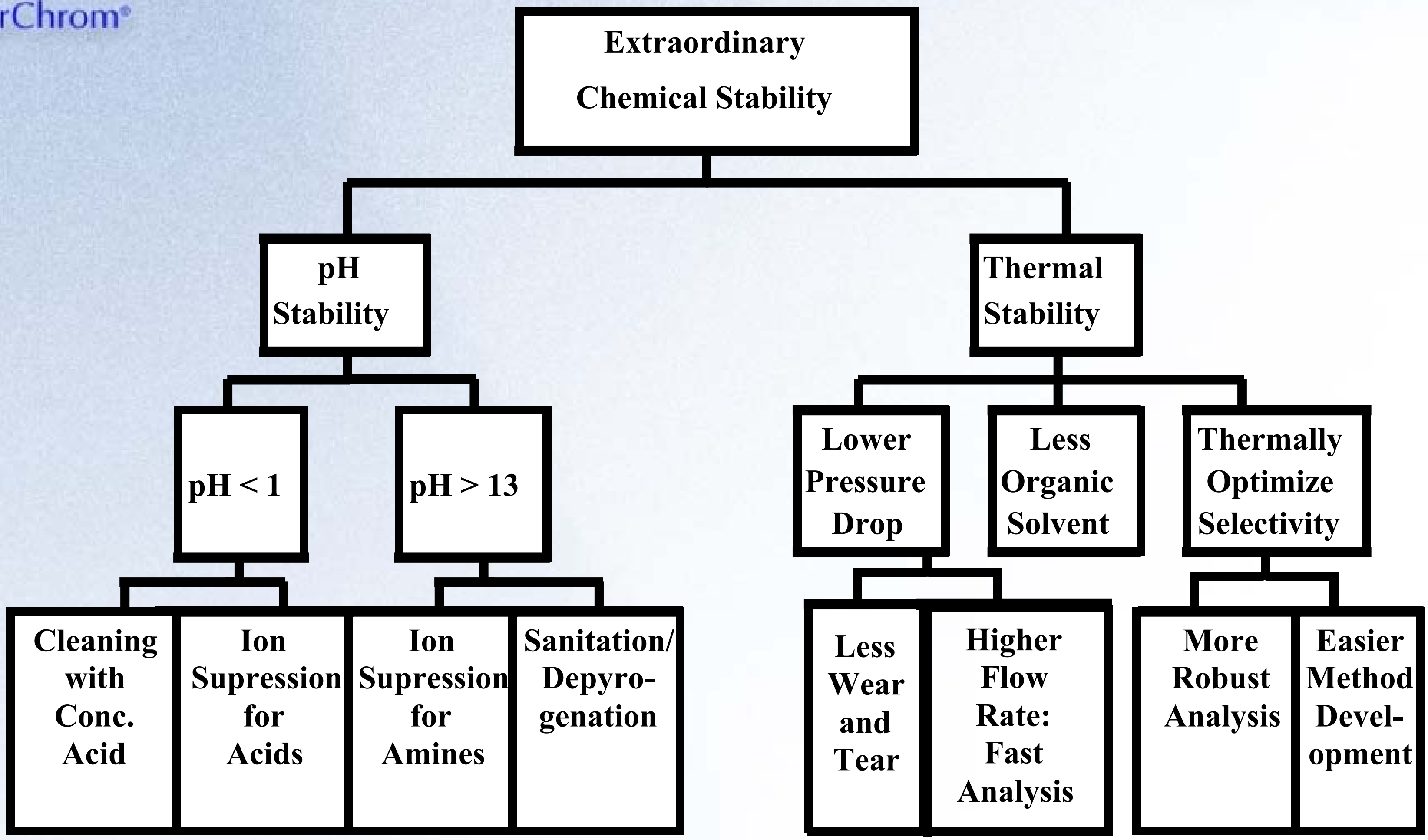
Practical Advantages of Column Stability

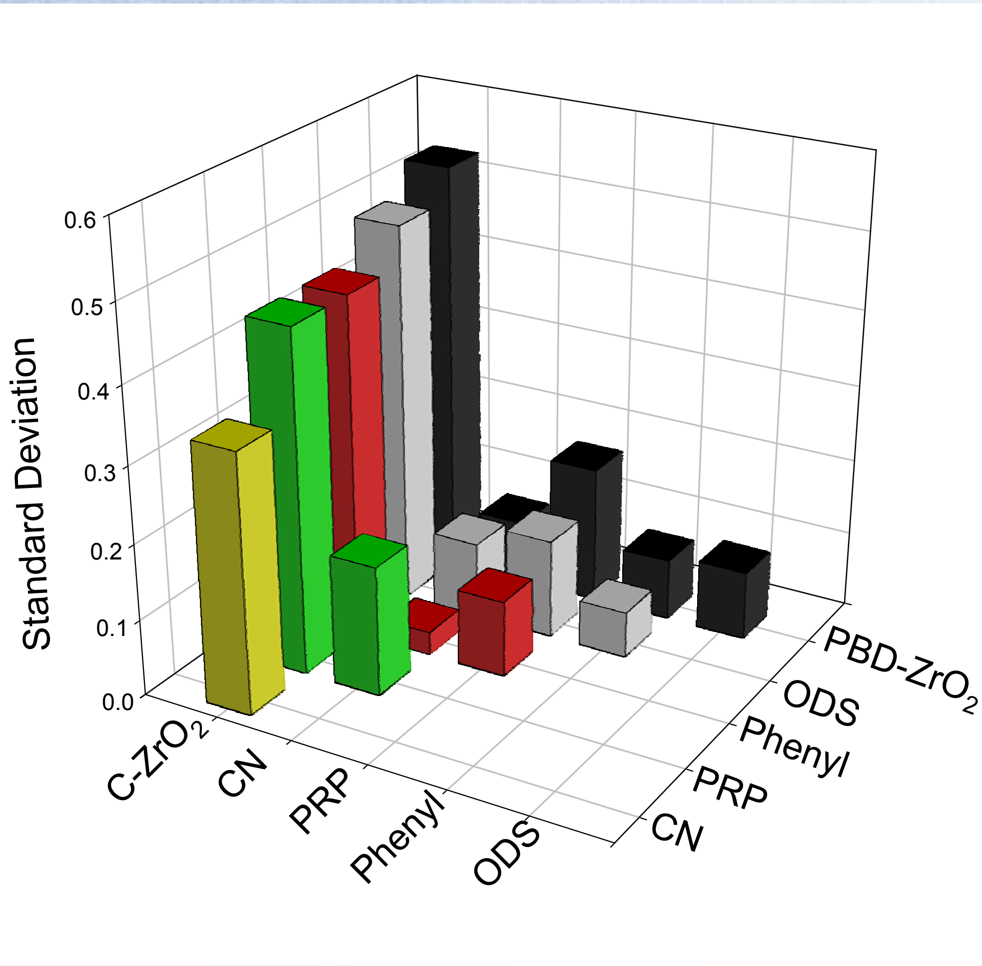


List of HTLC compatible reversed-phase columns

Manufacturer	Column Name	Stationary Phase Type	Temperature Limit (°C)	Selectivity versus C18
Polymer Laboratories	PLRP	Polymer	200	Different
Selerity	Blaze	Silica	200	Different
Supelco	DiscoveryZR-Carbon	Carbon Clad Zirconia	200	Different
Supelco	DiscoveryZR-CarbonC18	Modified Carbon on Zirconia	200	Different
Thermo-Electron	Hypercarb	Carbon	200	Similar
ZirChrom Separations, Inc.	ZirChrom-CARB	Carbon Clad Zirconia	200	Different
ZirChrom Separations, Inc.	Diamondbond-C18	Modified Carbon on Zirconia	200	Different
Jordi	Jordi DVB	Polymer	150	Different
Sachtleben	Sachtopore-RP	Polymer coated Titania	150	Different
Supelco	DiscoveryZR-PBD	Polymer Coated Zirconia	150	Different
Supelco	DiscoveryZR-PS	Polymer Coated Zirconia	150	Different
ZirChrom Separations, Inc.	ZirChrom-PS	Polymer Coated Zirconia	150	Different
ZirChrom Separations, Inc.	ZirChrom-PBD	Polymer Coated Zirconia	150	Different
Agilent	SB Extend-C18	Silica	90	Similar
Waters	X-Bridge	Silica	80	Similar

Stationary Phase Comparison

Average Scatter of κ–κ Plots for Two Kinds of Stationary Phases Using 22 Solutes



- Carbon-ZrO_2
- PBD-ZrO_2
- C18-SiO_2 (ODS)
- Phenyl-SiO_2
- CN-SiO_2
- PRP

➢ **For *non-electrolytes*, C-ZrO_2 and aliphatic phases have the most different selectivities.**

Fast Separations Non-Steroidal Anti-Inflammatories

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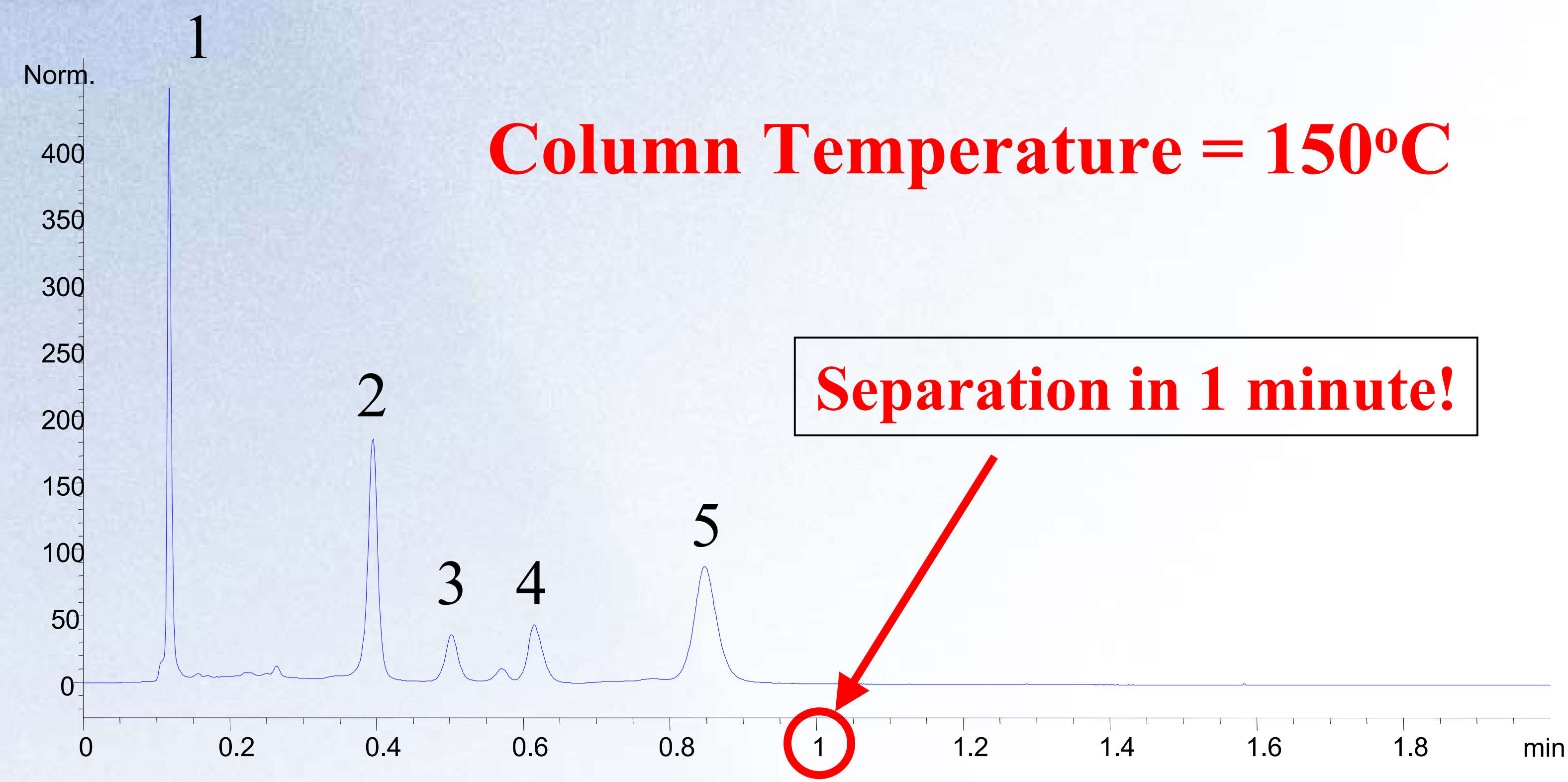
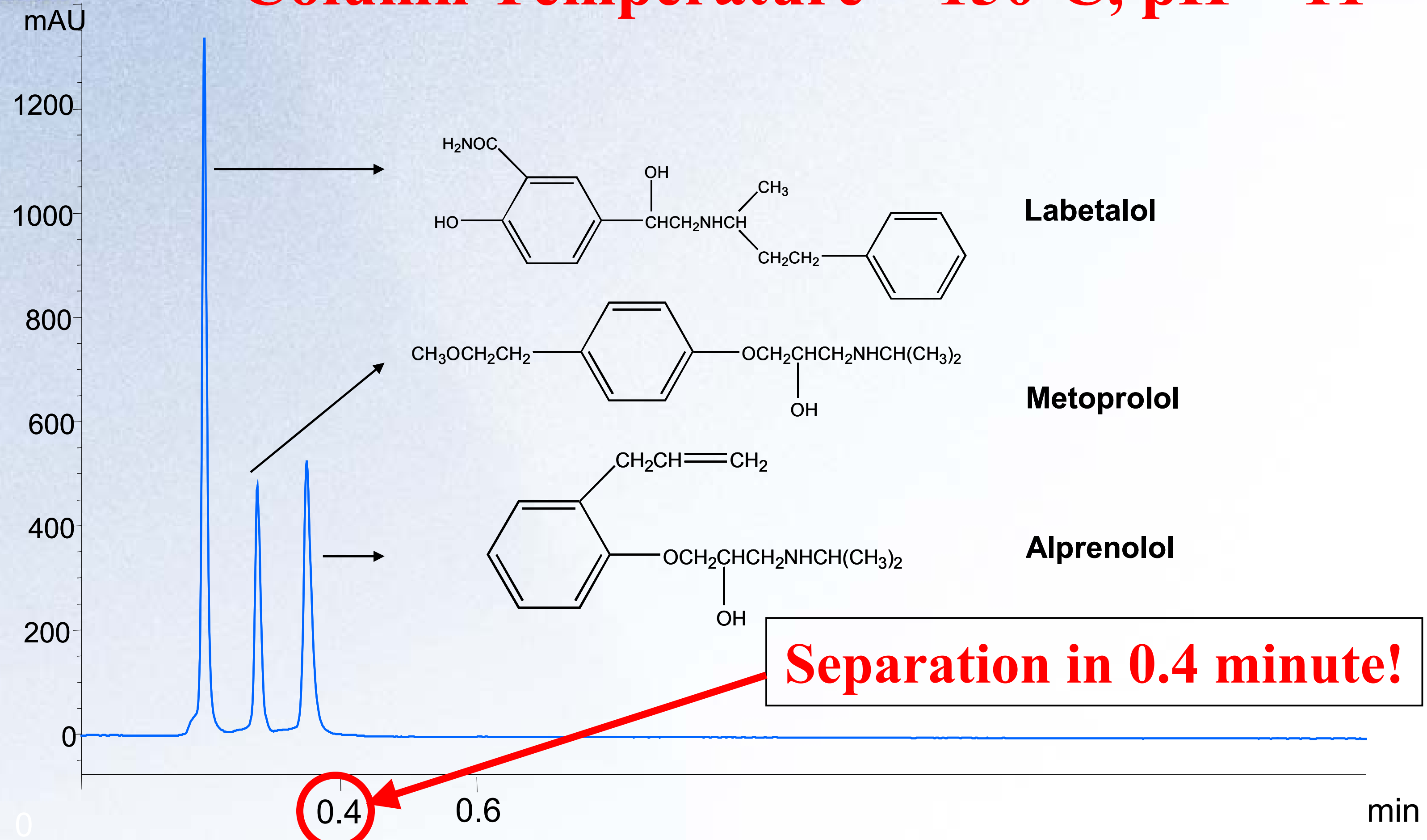


LC Conditions: Column, 50 x 4.6 DiamondBond™-C18; Mobile phase, 25/75 ACN/40mM phosphoric acid, pH 2.3; Flow rate, 5.5 ml/min.; Temperature, 150 °C; Injection volume, 1ul; Detection at 254nm; Solute concentration, 0.15 mg/ml.; Solutes, 1= Acetaminophen, 2=Ketoprofen, 3=Naproxen, 4=Ibuprofen, 5=Oxaprofen.

Fast β-Blockers Separation

Column Temperature = 150°C, pH = 11



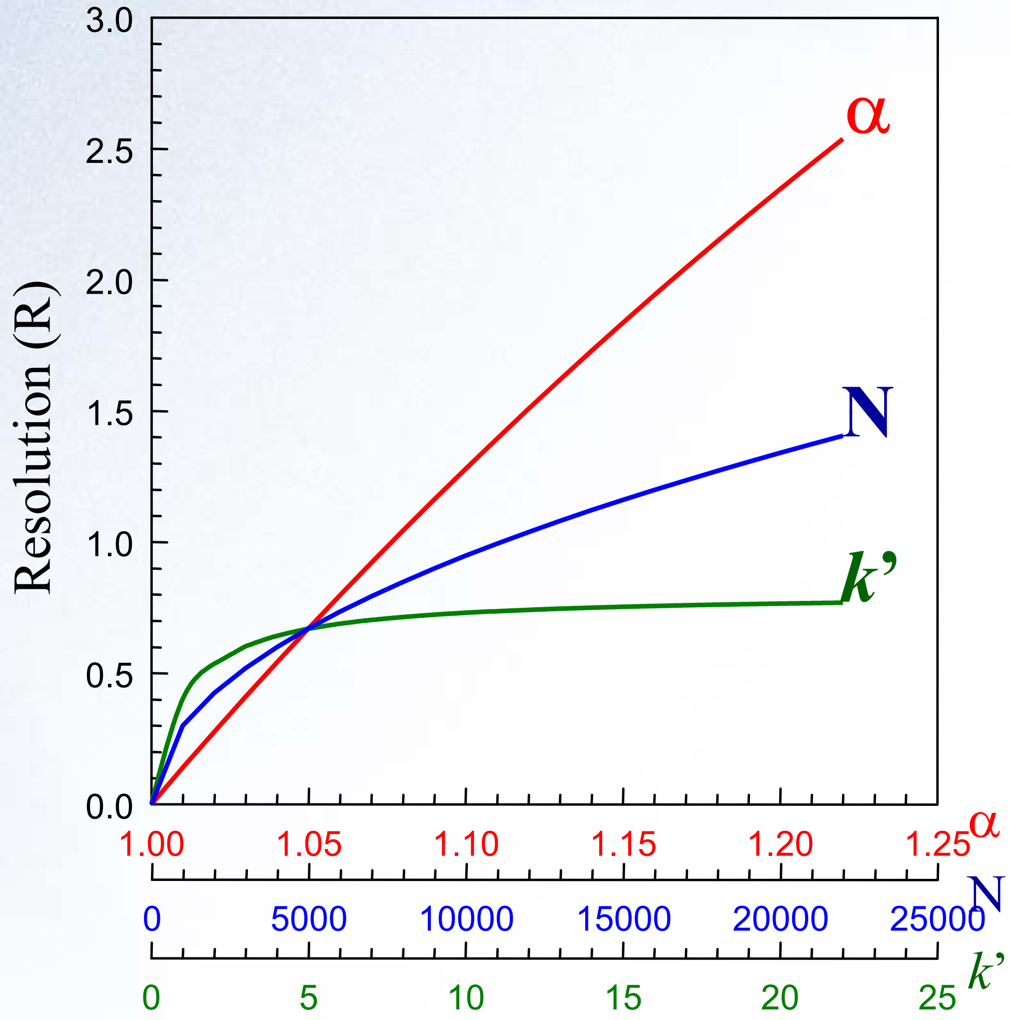
LC Conditions: Column, 50 x 4.6 Diamondbond-C18, OD0121601A; Mobile phase, 45/55 ACN/20mM Ammonium Phosphate pH11.0; Flow rate, 3.0 ml/min; Temperature, 150 °C; Injection volume, 1.0 ul; Detection at 210 nm; Solutes, 1=Labetalol, 2=Metoprolol, 3=Alprenolol

Resolution: The Importance of Selectivity

Efficiency Retention Selectivity

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

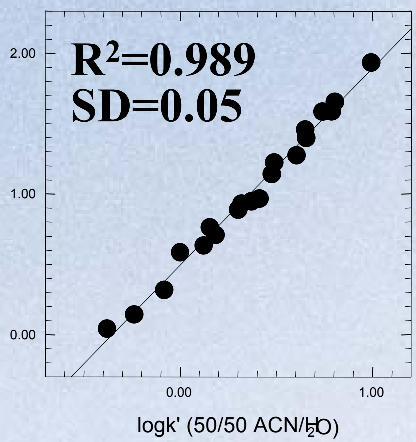
$$\alpha = \frac{k_j'}{k_i'}$$



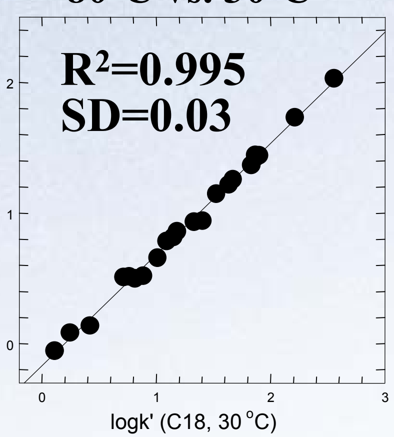
- **Selectivity (α) has the greatest impact on improving resolution.**

Comparison of Variables Affecting Selectivity

30% ACN vs. 50% ACN

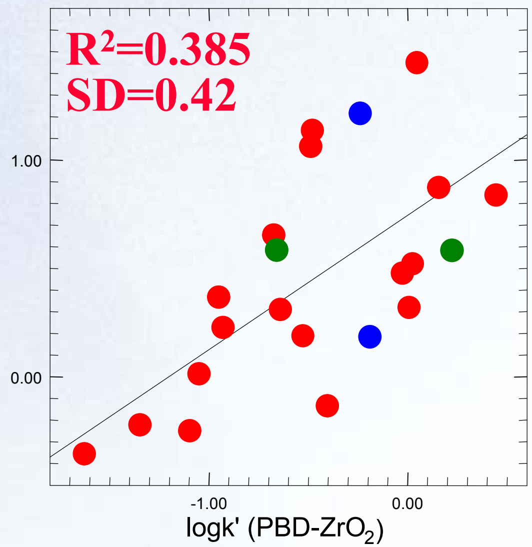


80°C vs. 30°C

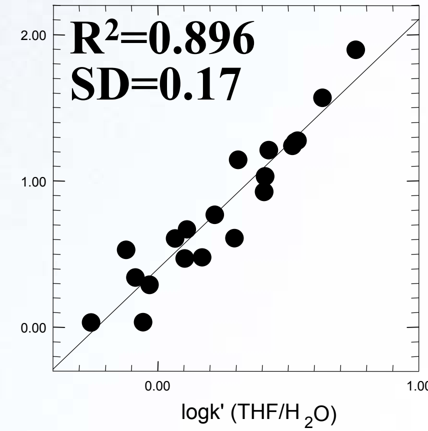


Stationary Phase Type

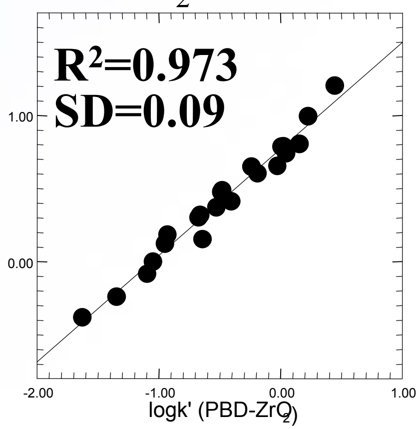
Carbon-ZrO_2 vs. PBD-ZrO_2



MeOH vs. THF



C18-SiO_2 vs. PBD-ZrO_2



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

Thermally Tuned Tandem Columns (T^3C)

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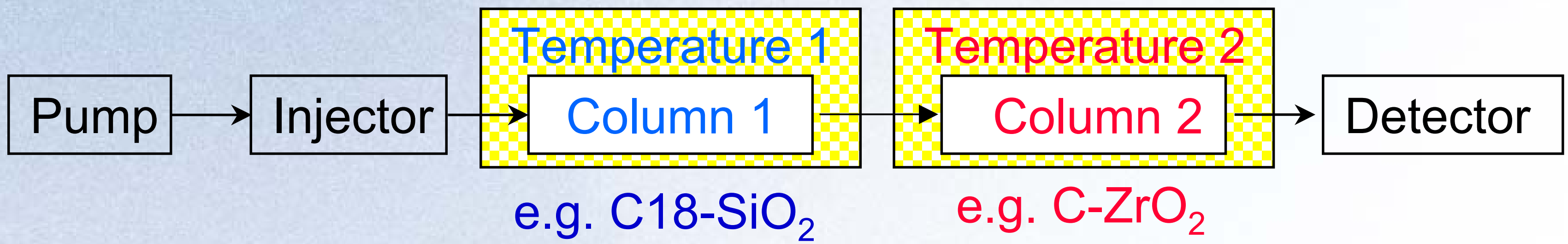
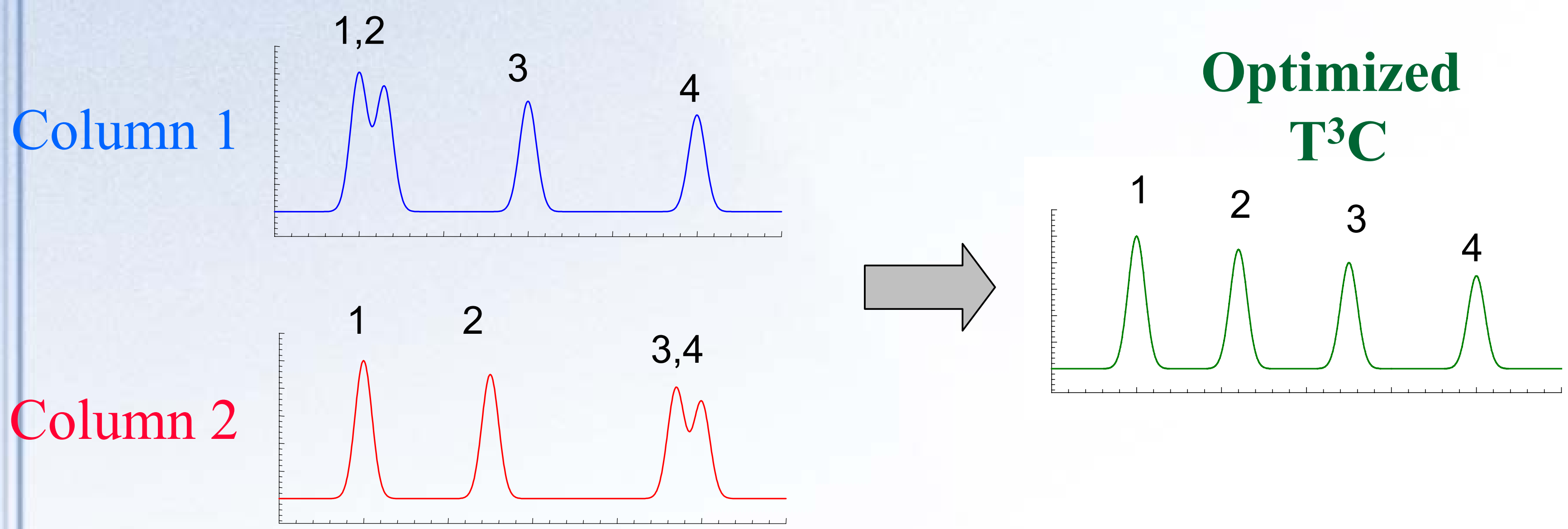
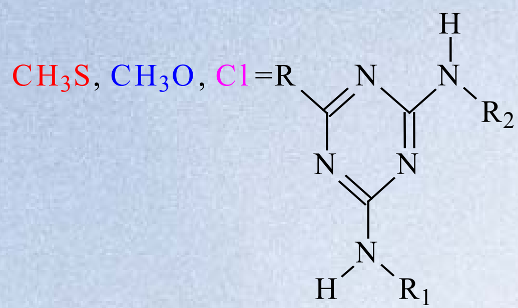
A Mechanism to ***Continuously*** ***Adjust*** the Stationary Phase





Separation of Ten Triazine Herbicides by T^3C



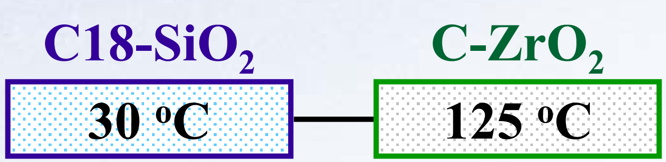
Solutes:

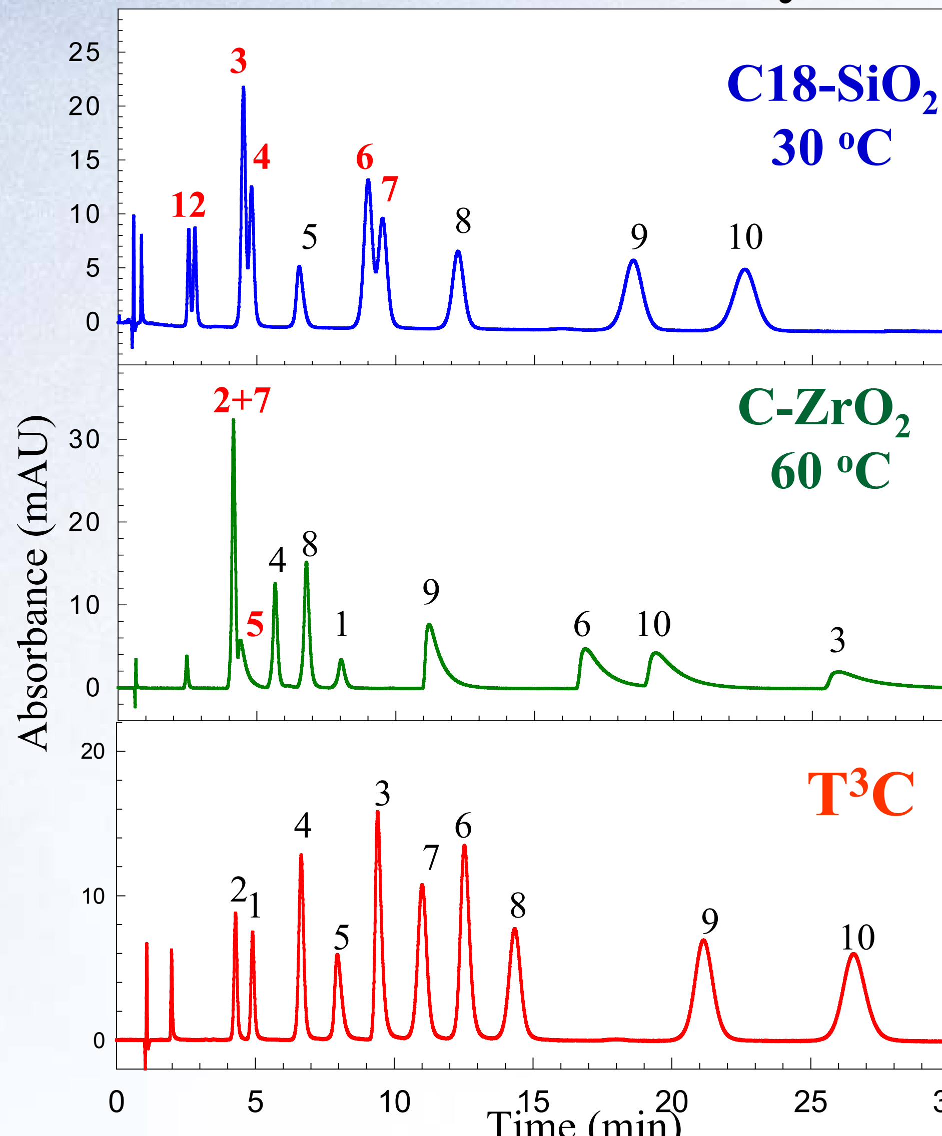
1. Simazine
2. Cyanazine
3. Simetryn
4. Atrazine
5. Prometon
6. Ametryn
7. Propazine
8. Terbutylazine
9. Prometryn
10. Terbutryn

Other conditions:

30/70 ACN/water

1ml/min; 254 nm detection





➤ **T^3C can improve separation without increasing analysis time.**

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Conclusions

(1) Zirconia Based Stationary Phases are ***ultra-durable*** and ***efficient , stable*** at the ***extremes of pH*** and at column temperatures as high as ***200^{o}C***.

(2) ***ZirChrom®-CARB*** has the ***most different selectivity*** relative to conventional ODS phases for the 22 selected non-ionizable compounds.

(3) High Temperature Liquid Chromatography (HTLC) is a ***powerful technique*** that can be used as a ***routine analytical tool*** in the development of separation methods.

(4) HTLC is a ***unique tool*** in altering chromatographic selectivity (***T^{3}C method***), increasing analysis speed.

(5) HTLC capability will become an ***important*** part of HPLC ***system design*** in order to fully utilize the benefits of columns prepared with ultra-small particles and utrafast analyses.