Ultra-Fast 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
- **Using Temperature to Control Selectivity**
  - Importance of Selectivity in HPLC Optimization
- **Using Temperature in 2D Separations**
  - Basic Design Elements of 2D Separations
  - Example of 2D-UFHTLC Capability

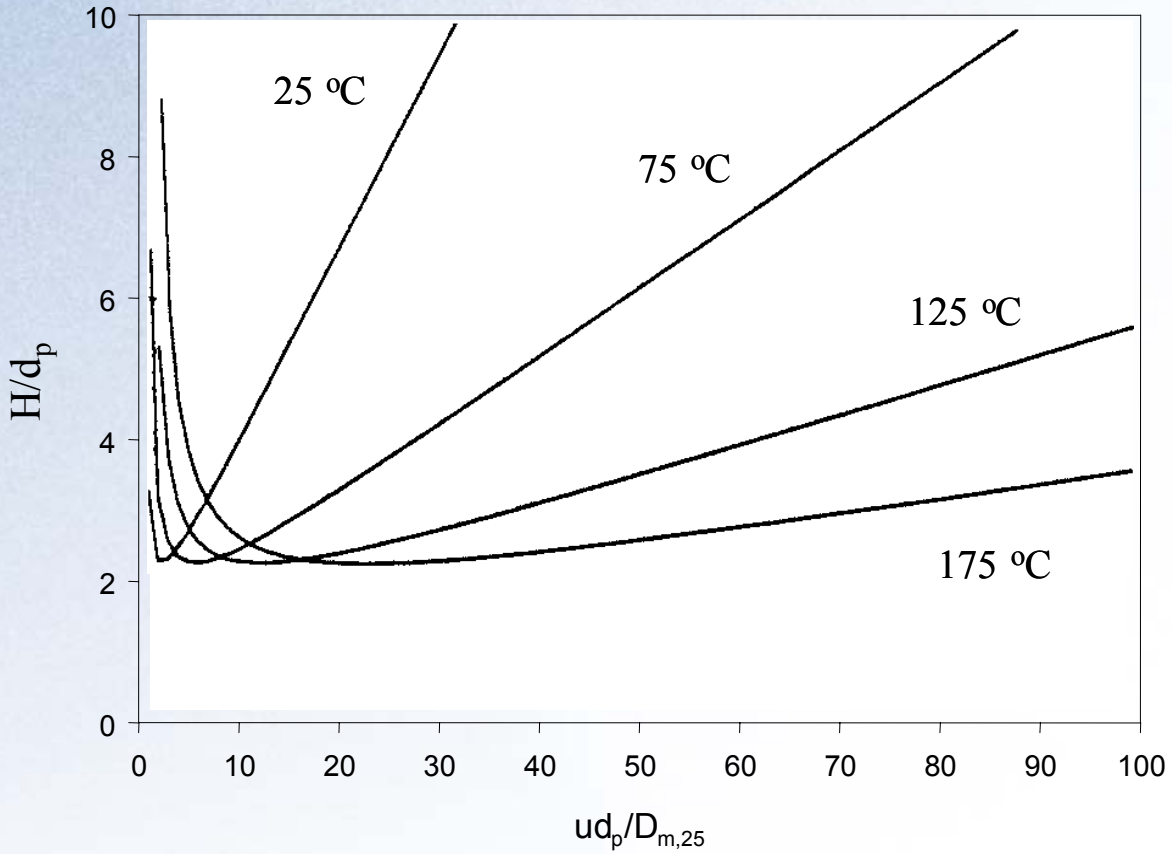
Theoretical Advantages to High Temperature LC

van Deemter Plot F. D. Antia and Cs. Horvath, *J. Chromatogr.*, 435, 1-15 (1988).	$h = A + \frac{B}{\nu} + C\nu + D\nu^{2/3} + \frac{3D_m}{8k_d d_p^2}\nu$
Practical Limit Temperature Dependence Guiochon, Georges, *Anal. Chem.*, 52, 2002-2008 (1980).	$\frac{t}{N} \propto (1+k')\frac{L^{2/3}}{\Delta P_{max}^{2/3}}\frac{\eta}{T^{1/3}}$

Three ways that temperature increases efficiency and speed

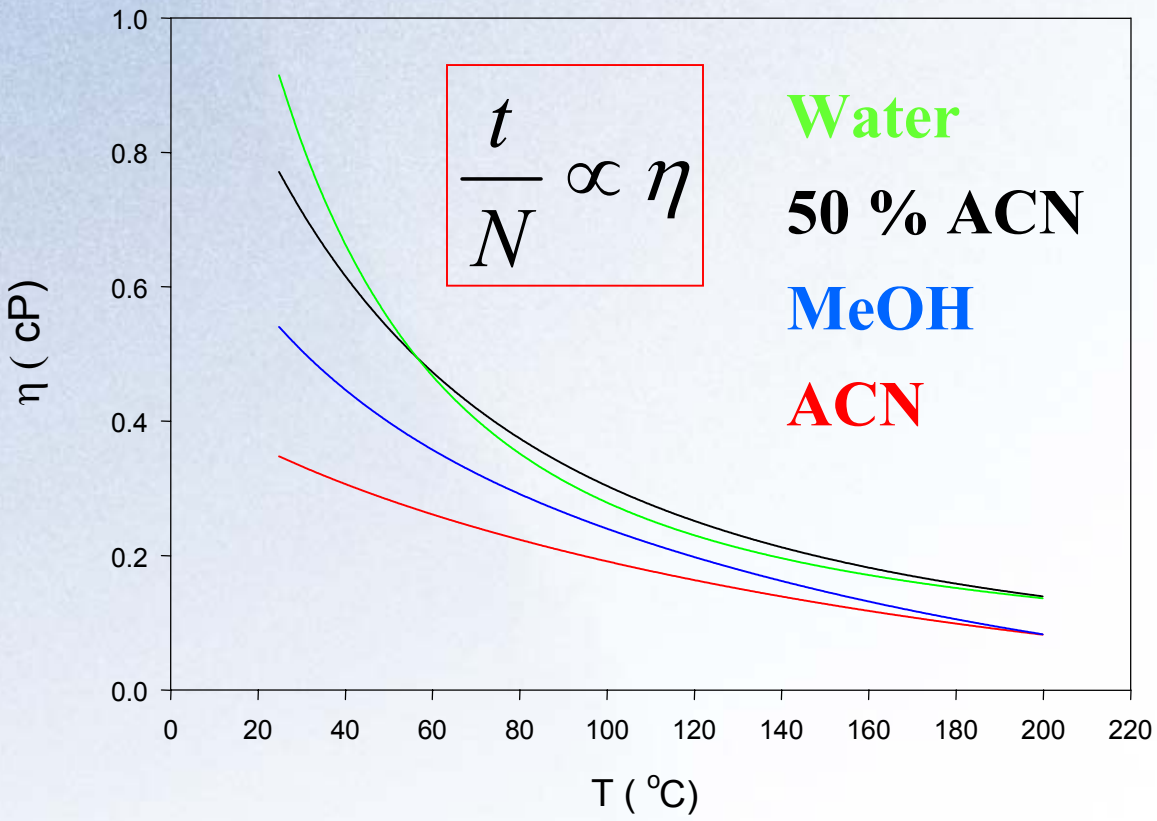
- Increased temperature increases diffusivity, thus decreasing the reduced velocity
- Increased temperature accelerates sorption kinetics
- Increased temperature decreases mobile phase viscosity

Theoretical Effect of Temperature on Column Efficiency



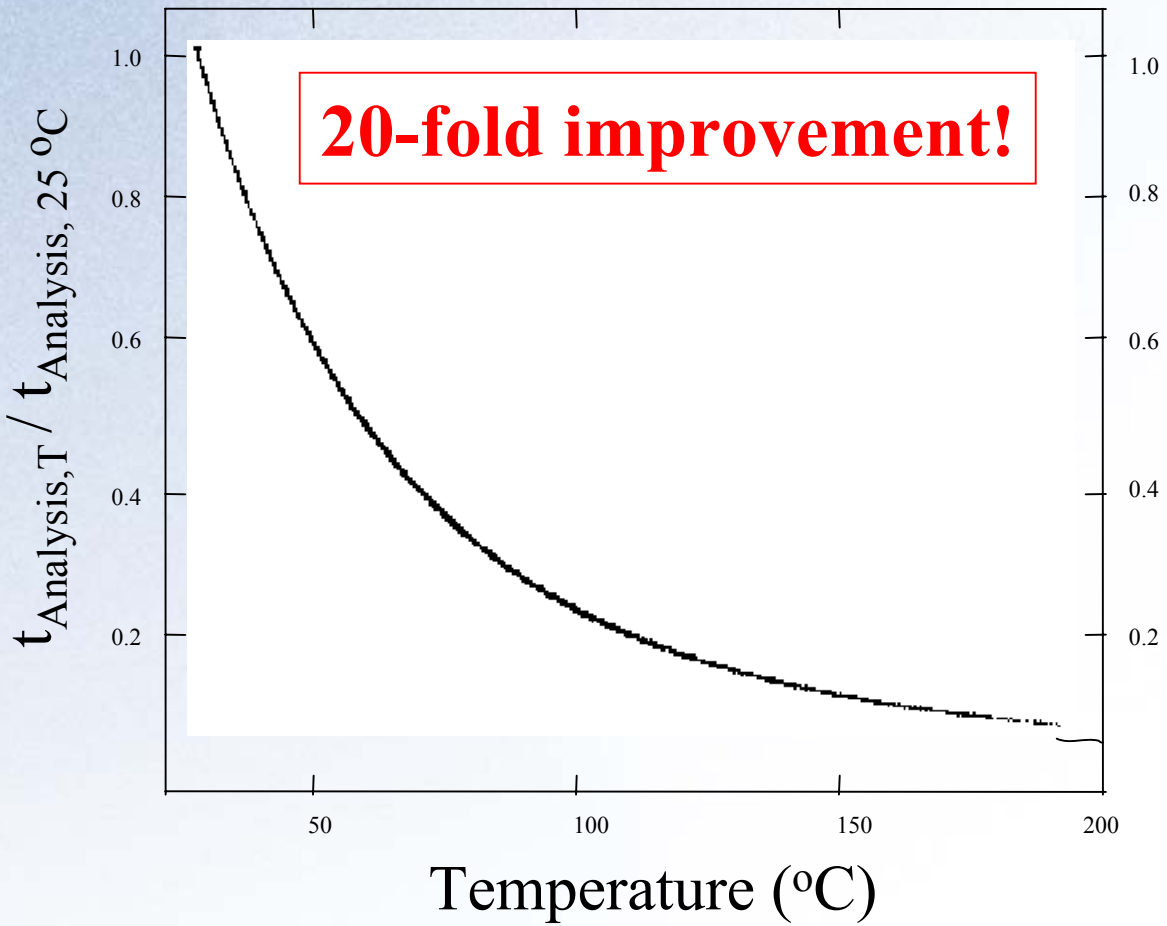
F. D. Antia and Cs. Horvath, *J. Chromatogr.*, 435, 1-15 (1988).

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*



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

Comparison of Variables Affecting Selectivity

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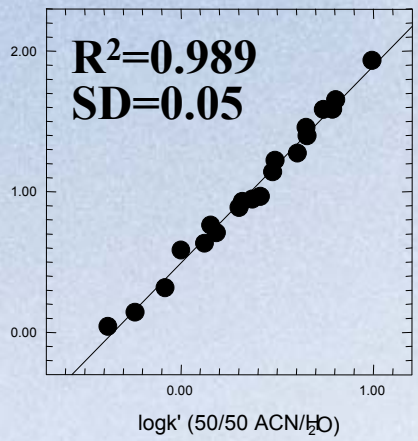
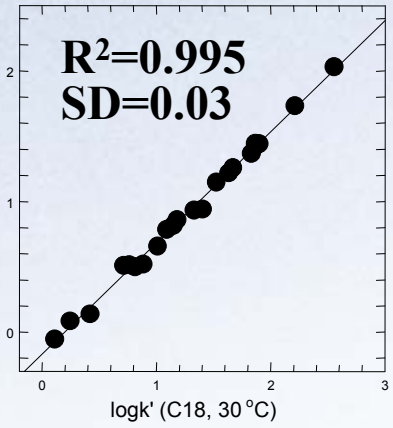
30% ACN vs. 50% ACN

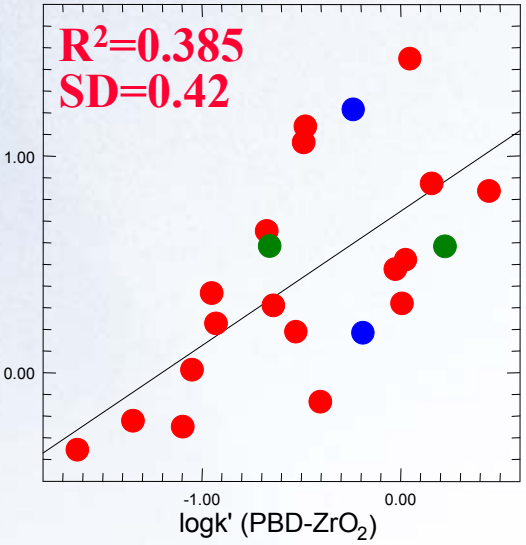


80°C vs. 30°C

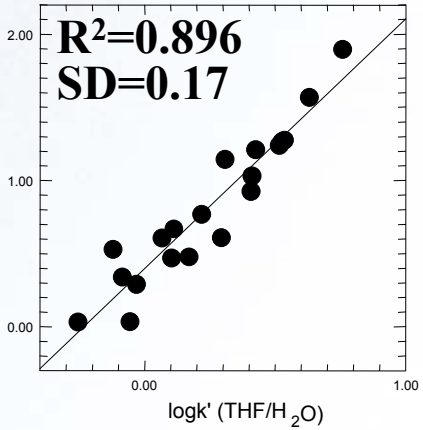


Stationary Phase Type

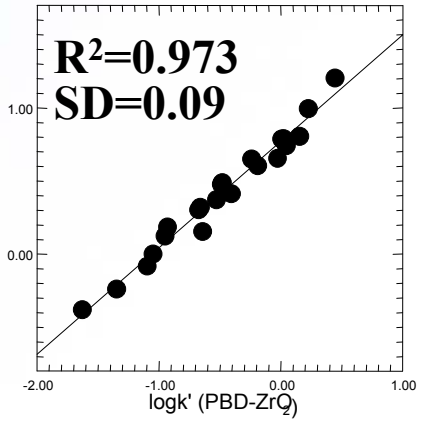
Carbon-ZrO_2 vs. PBD-ZrO_2



MeOH vs. THF

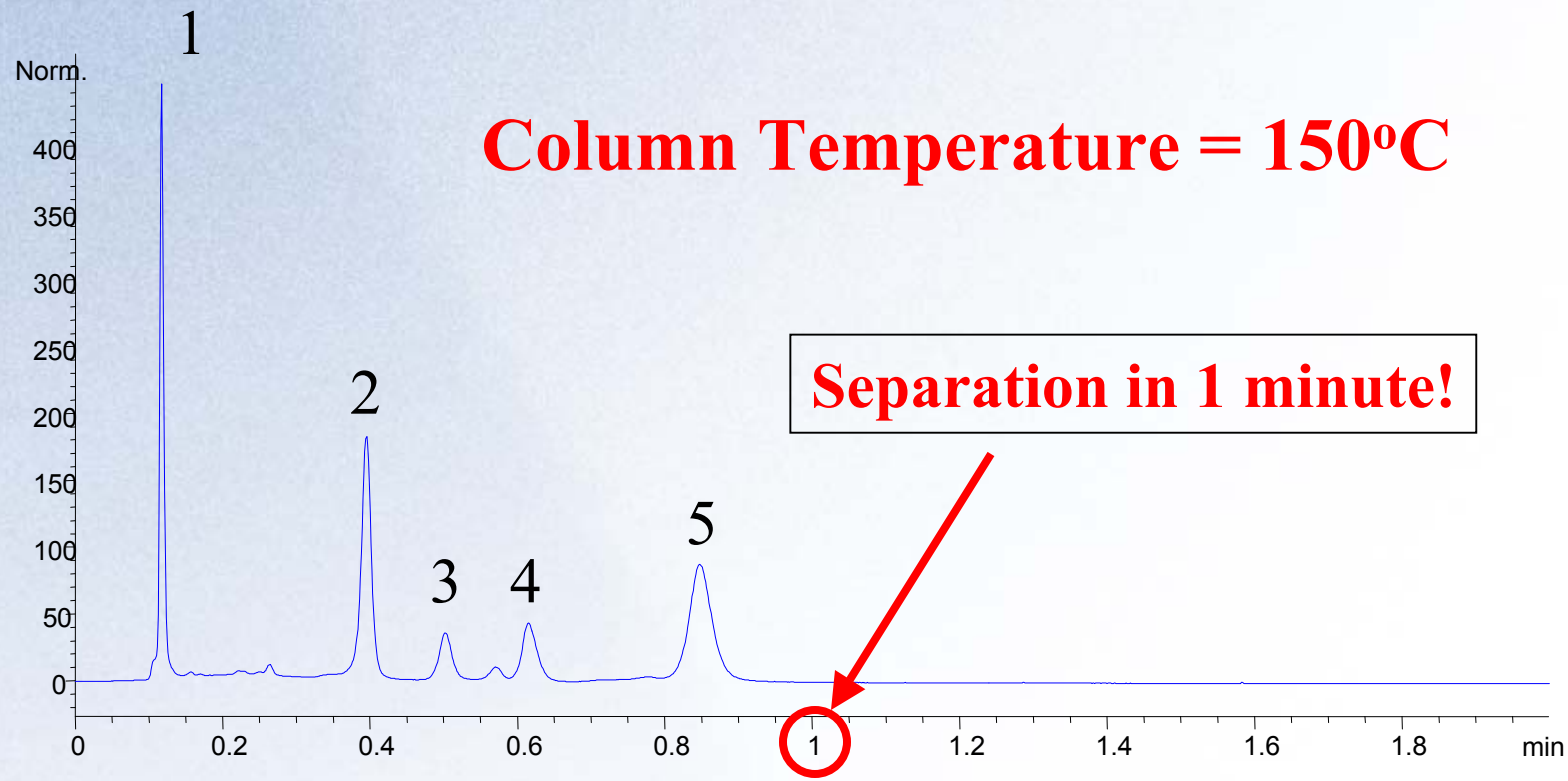


C18-SiO_2 vs. PBD-ZrO_2



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

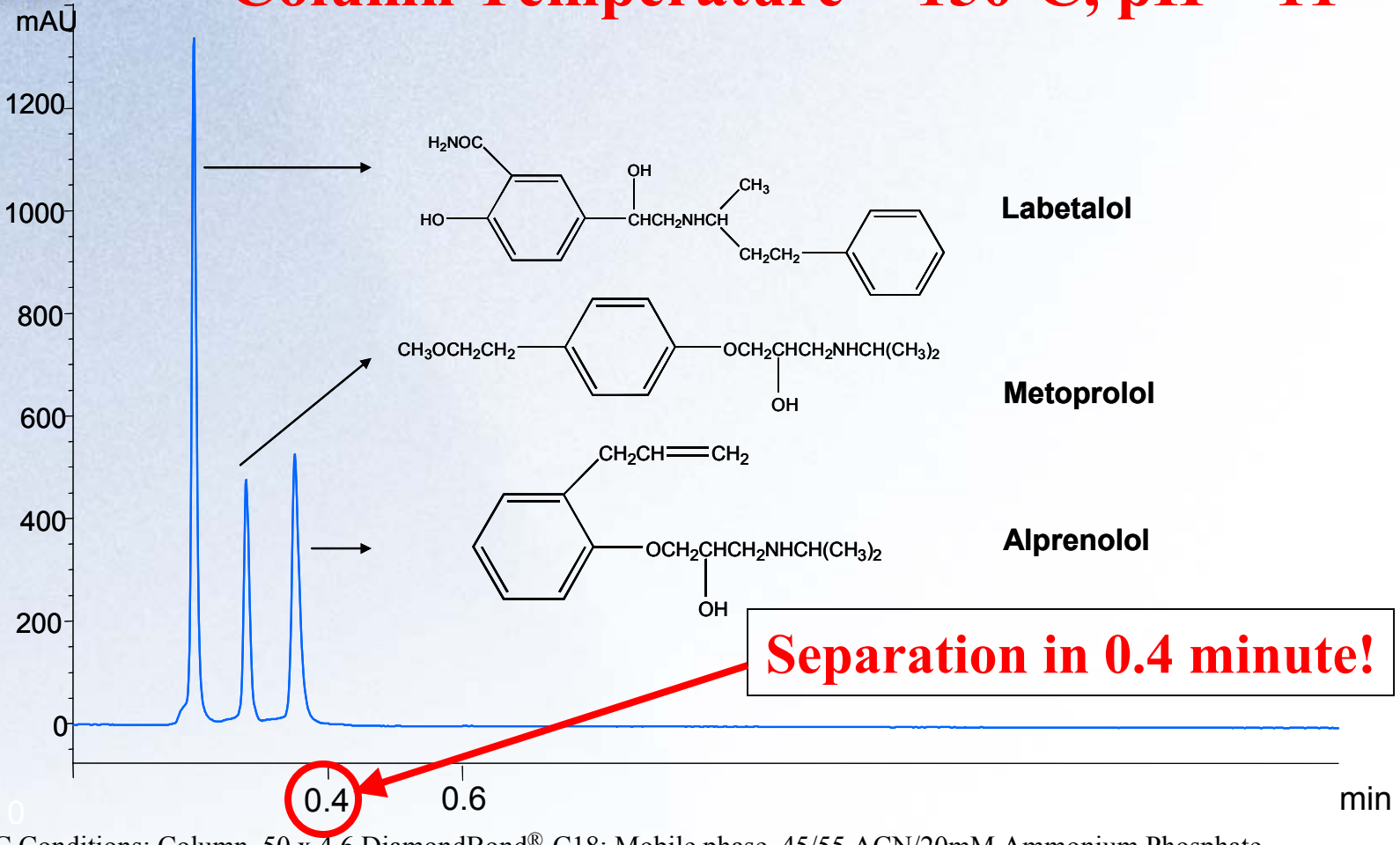
Fast Separations Non-Steroidal Anti-Inflammatories



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, 1µL; Detection at 254nm; Solute concentration, 0.15 mg/mL.; Solutes, 1= Acetaminophen, 2=Ketoprofen, 3=Naproxen, 4=Ibuprofen, 5=Oxaprofen.

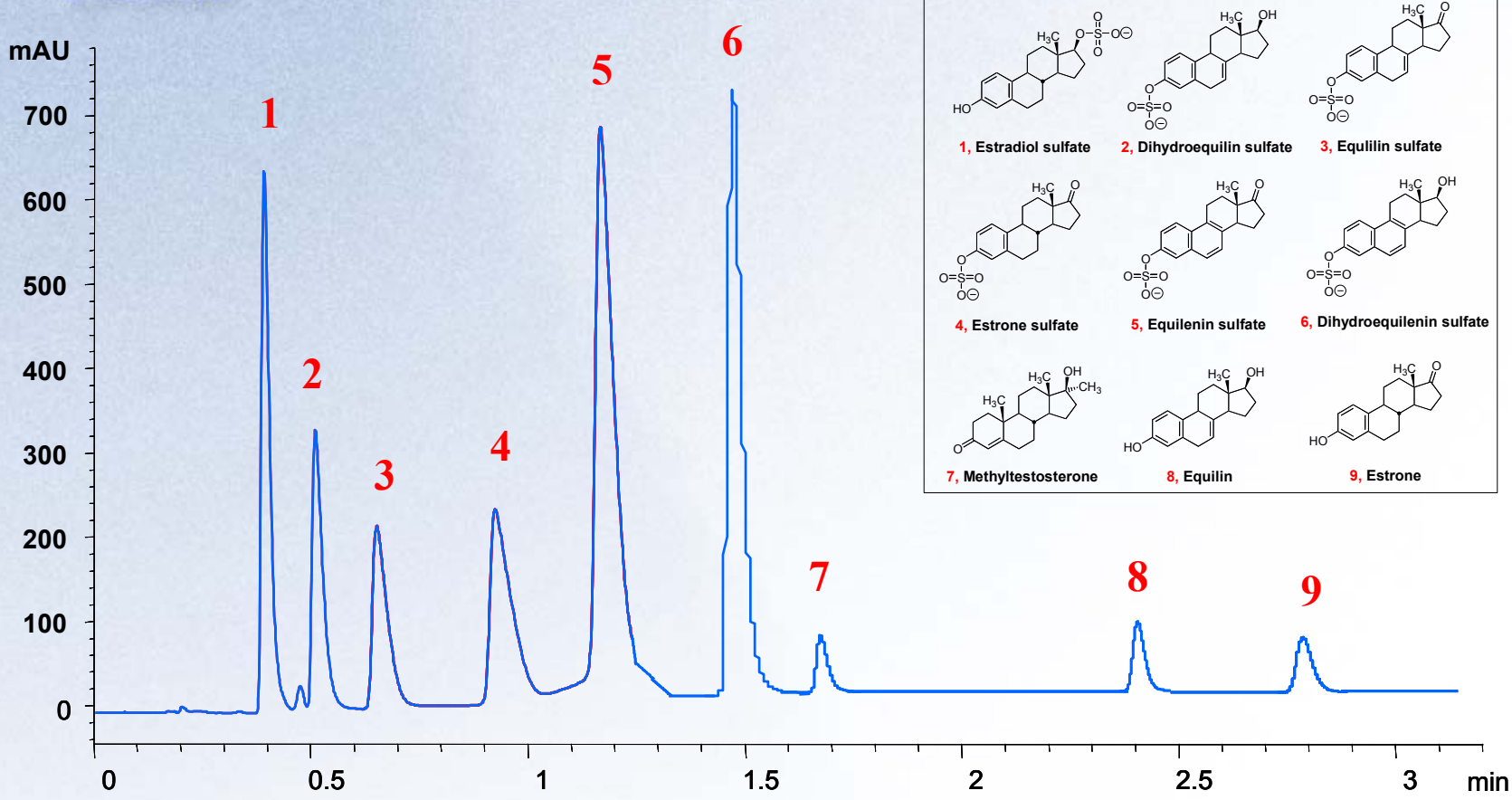
Fast β-Blockers Separation

Column Temperature = 150°C, pH = 11



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

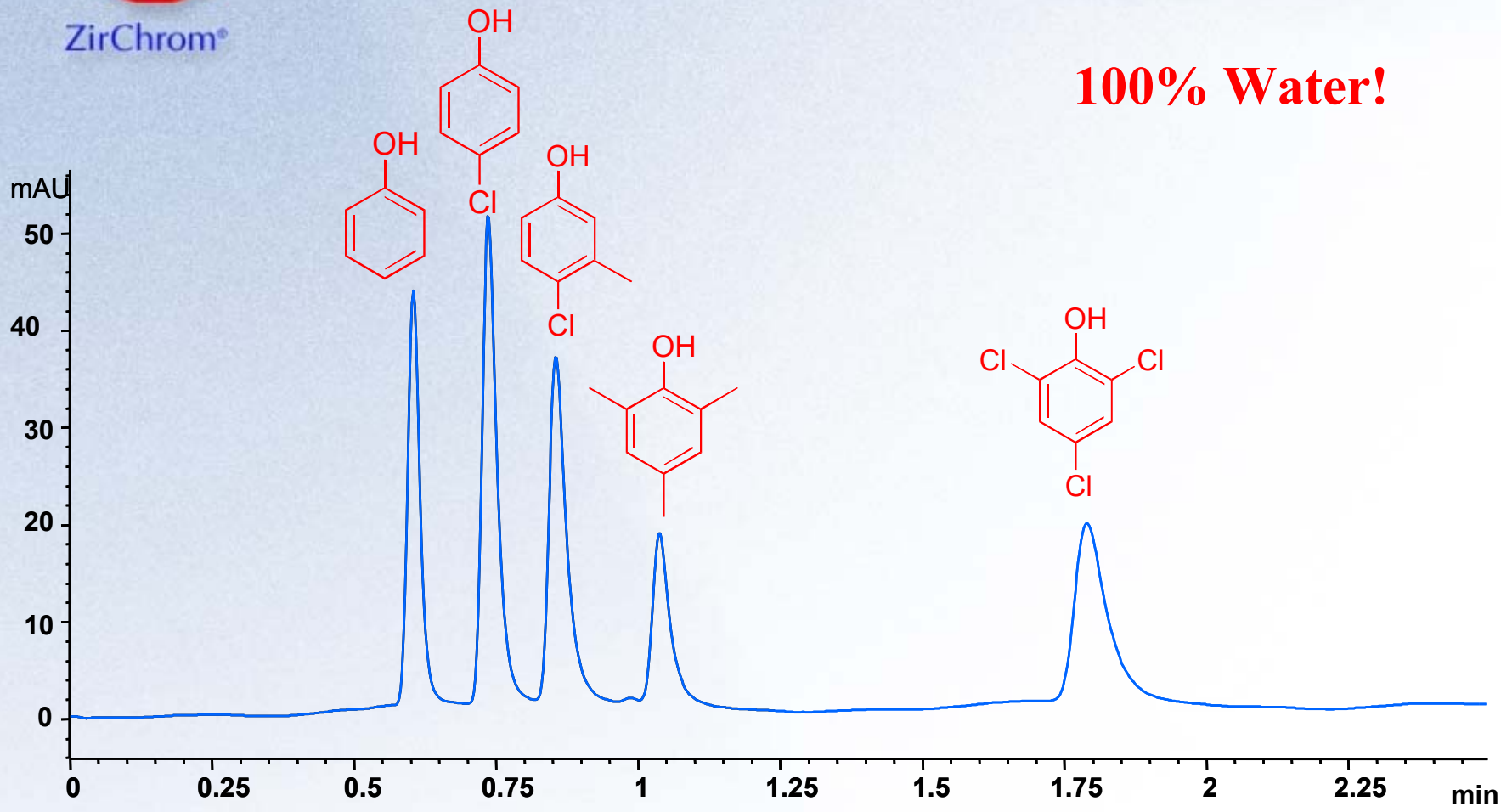
Fast Steroid Separation at 125°C Using a ZirChrom®-CARB Column



LC Conditions: Column, 100 mm x 3.0 mm i.d. ZirChrom®-CARB, gradient elution 2-90% B from 0.3-3.9 minutes, A = 40/60 ACN/25 mM ammonium fluoride, pH 5.6, B = 40/60 ACN/THF, Flow rate: 2.5 mL/min., Temperature, 125 °C (using Metalox 200C), Injection volume, 2 ml, UV Detection at 215 nm.

Two Minute "Green" Separation of Chlorophenols at 200 °C

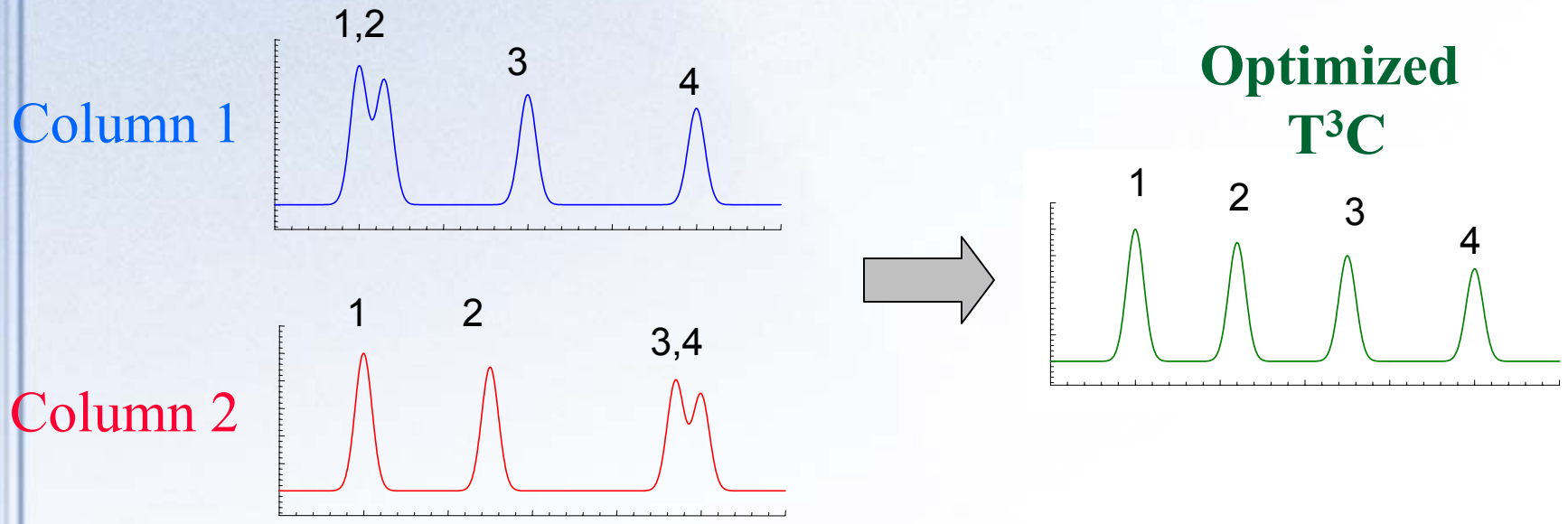
100% Water!



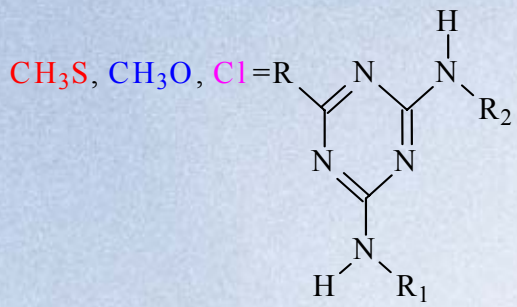
Chromatographic conditions: Column, ZirChrom®-PBD, 150 x 4.6 mm i.d., Mobile Phase, 100% Water, Flow Rate, 3.0 mL/min., UV detection at 280 nm, Column Temperature, 200 °C using a Metalox® 200C column heater (ZirChrom Separations, Anoka, MN).

Thermally Tuned Tandem Columns (T^3C)

A Mechanism to *Continuously Adjust* the Stationary Phase



Separation of Ten Triazine Herbicides by T³C



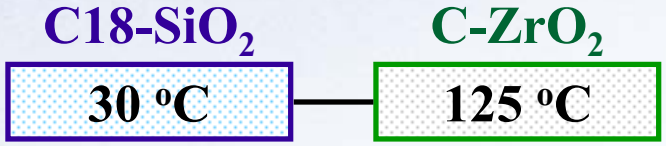
Solutes:

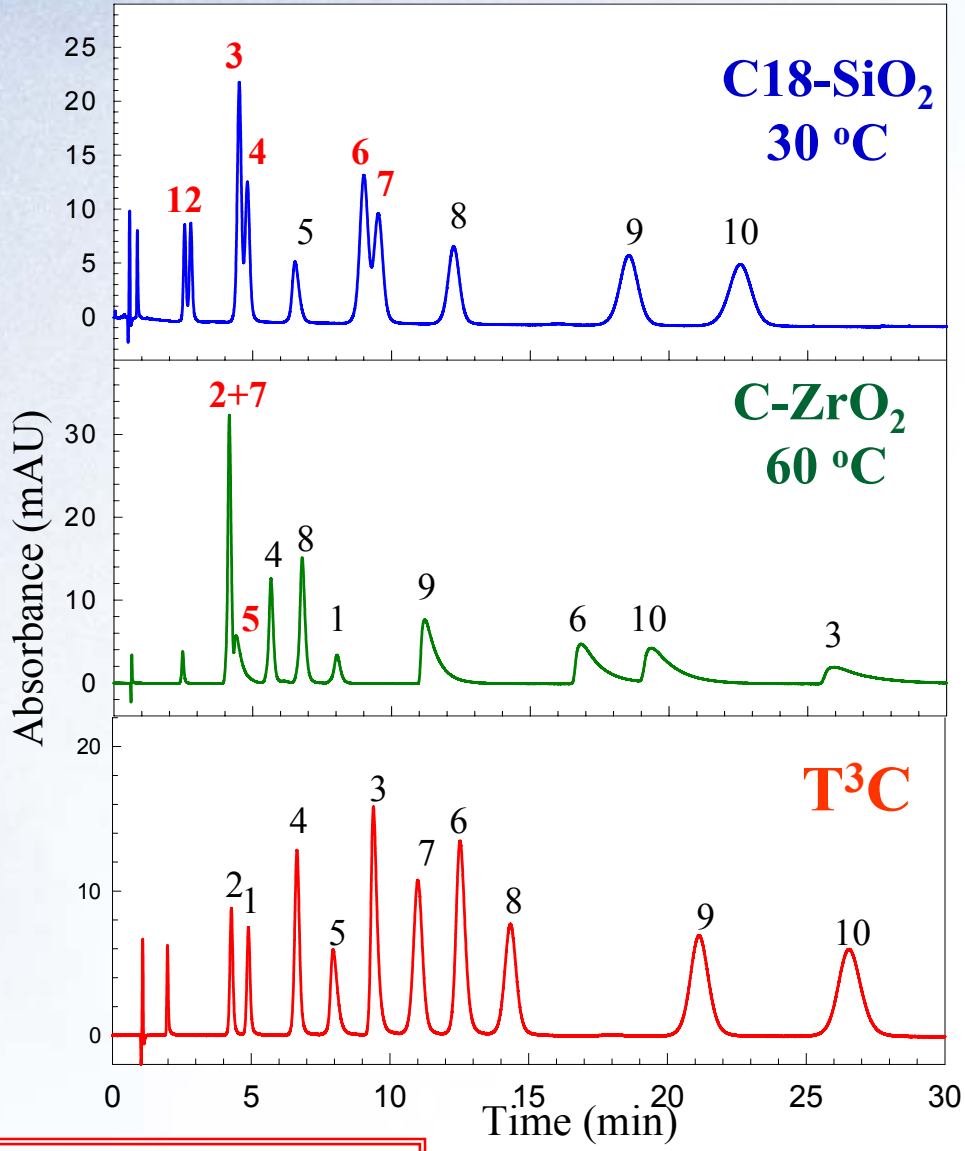
1. Simazine
2. Cyanazine
3. Simetryn
4. Atrazine
5. Prometon
6. Ametryn
7. Propazine
8. Terbutylazine
9. Prometryn
10. Terbutryn

Other conditions:

30/70 ACN/water

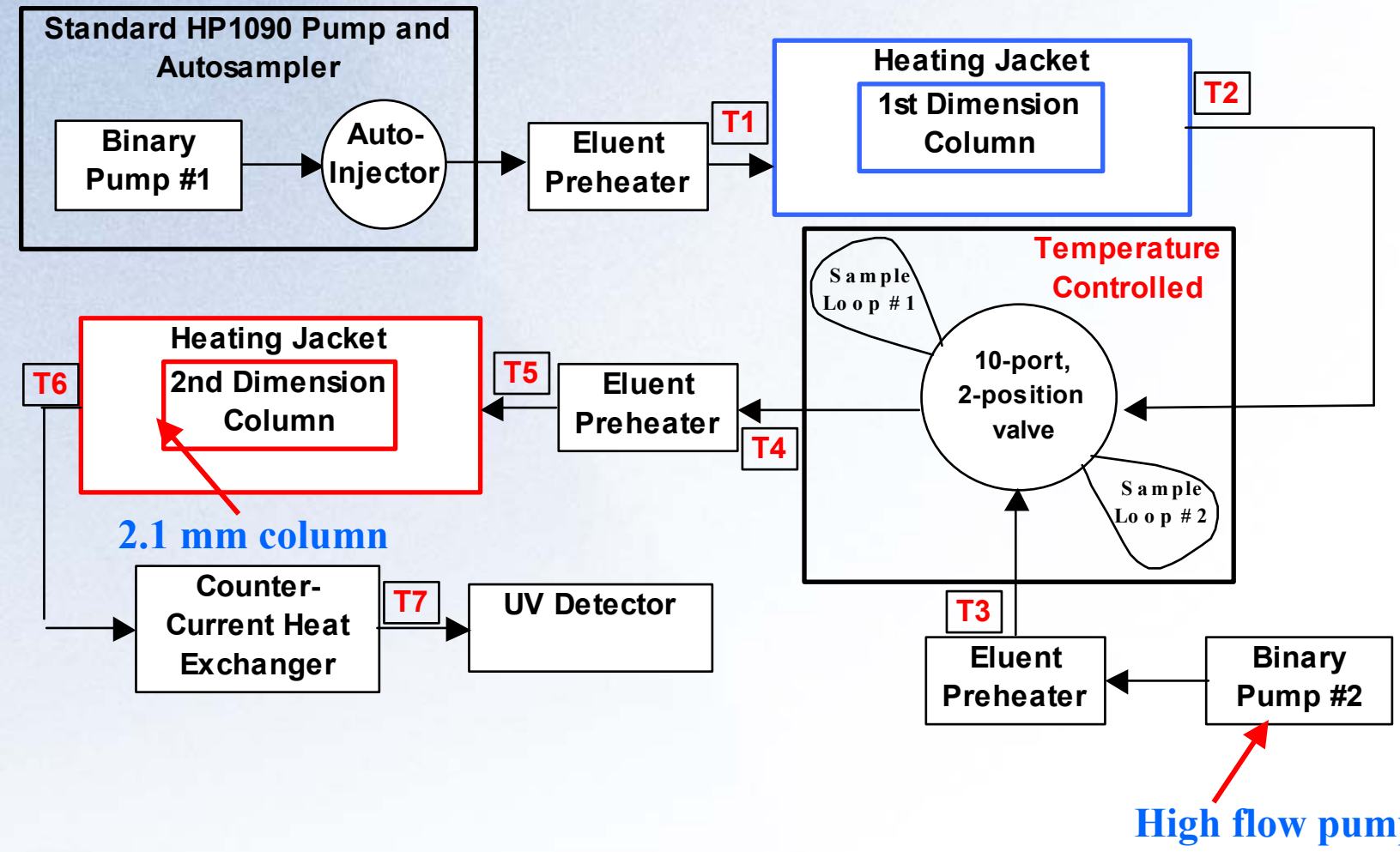
1mL/min; 254 nm detection



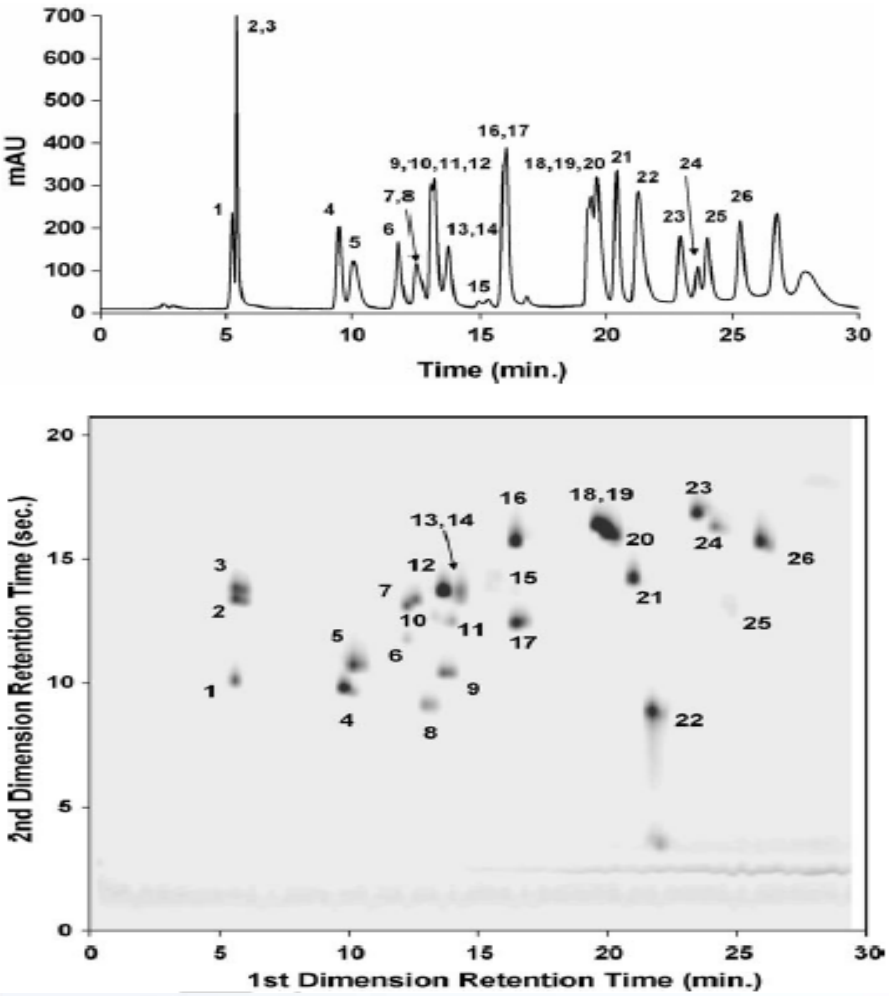


➢ T³C can improve separation without increasing analysis time.

Schematic of a Complete LC × UFHTLC System



2DLC Separation Of A 26 Component Indolic Metabolite Mixture



1st Dimension Conditions: Column, 50 mm x 2.1 mm i.d. Discovery® HS-F5; Mobile phase, A: 20 mM Na_3PO_4 20 mM $NaClO_4$ pH=5.7 B: ACN; Gradient: 95/5 A/B to 60/40 20 min, hold 2 min, 60/40 to 30/70 1 min, 30/70 to 95/5 0.01 min, hold 7 min. Flow rate, 0.10 mL/min.; Injection volume, 10 µL; Temperature, 40 °C; UV 220 nm

2nd Dimension Conditions: Column, 50 mm x 2.1 mm i.d. ZirChrom®-CARB; Mobile phase, A: 20 mM $HClO_4$ B: ACN, Gradient: 0-70% B in 17.4 s ; Flow rate, 3.0 mL/min.; Injection volume, 13.4 µL; Temperature, 110 °C; UV 220 nm

Stoll, D. R.; Cohen, J. D.; Carr, P. W. *Journal of Chromatography A*, **2006**, *1122*, 123-137.

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°C***.

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

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

(4) 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.

(5) Fast ***2D-LC*** separations have been successfully achieved allowing for the ***rapid analysis*** of complex mixtures.