An Overview of the the Development of Stationary Phases for Reversed-Phase Liquid Chromatography

Analytical Potential of Stable Phases for Reversed-Phase Liquid Chromatography

by

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Key Papers in History of Stable Reversed-Phases:

1. A. Wehrli, J.C. Hildenbrand, H.P. Keller, R. Stampfli, R.W. Frei, *"Influence of organic bases on the **stability** and separation properties of reversed-phase chemically bonded silica gels",* J. Chromatogr. **149 (1978)**, 199-210.

2 . J.J. Kirkland, J.L. Glajch, R.D. Farlee, *"Synthesis and characterization of **highly stable bonded phases** for high-performance liquid chromatography column packings*", Anal. Chem. **61** (**1989**), 2-11.

Outline

Part I. Overview of analytical potential of high phase stability.

- **Chemical stability.**
- **Thermal stability.**

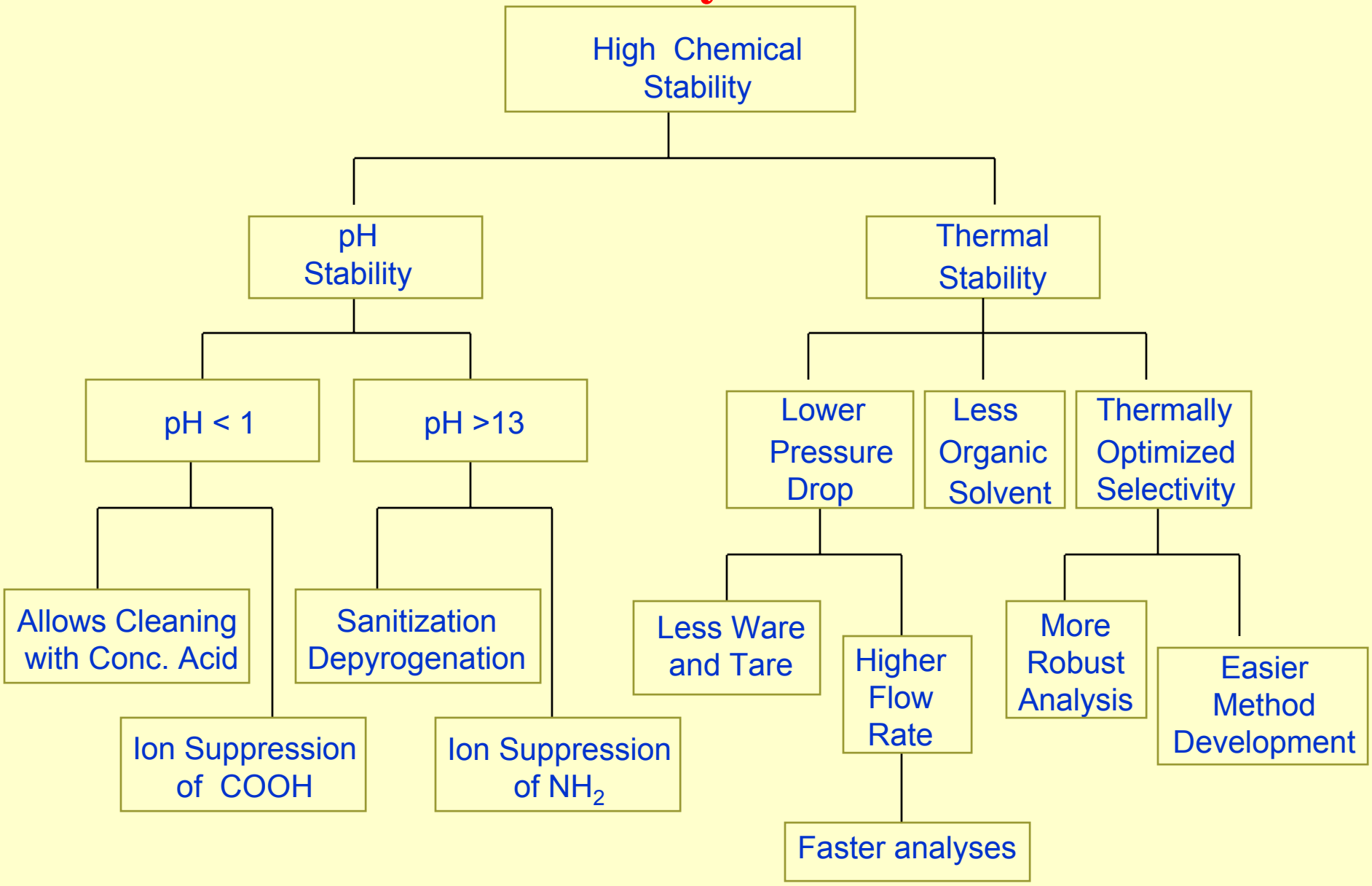
Part II. Using stability to achieve selectivity.

- **Thermally tuned tandem columns in HPLC.**

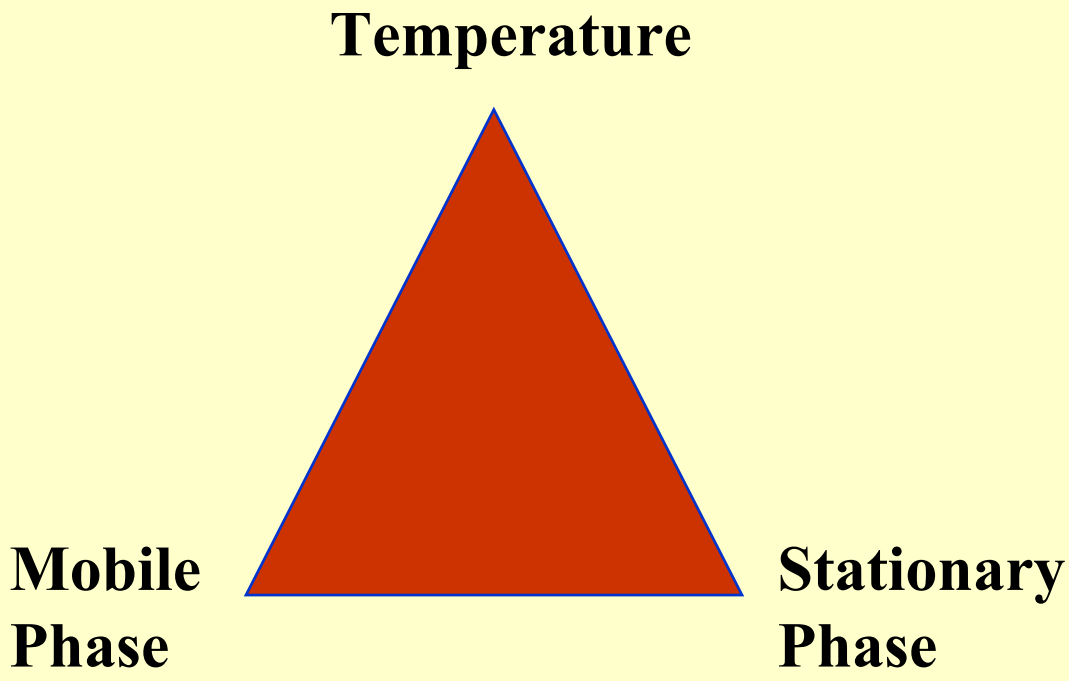
Part III. Using stability to speed up HPLC.

- **High temperature ultrafast liquid chromatography.**
- **High temperature fast two dimensional liquid chromatography.**

Advantages of Highly Stable Stationary Phases



Temperature
The Third Dimension in HPLC



Role of Temperature in LC

"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 Chromatography", R. M. Smith and R. J. Burgess, *LC-GC*, 17, 938-945 (1999)

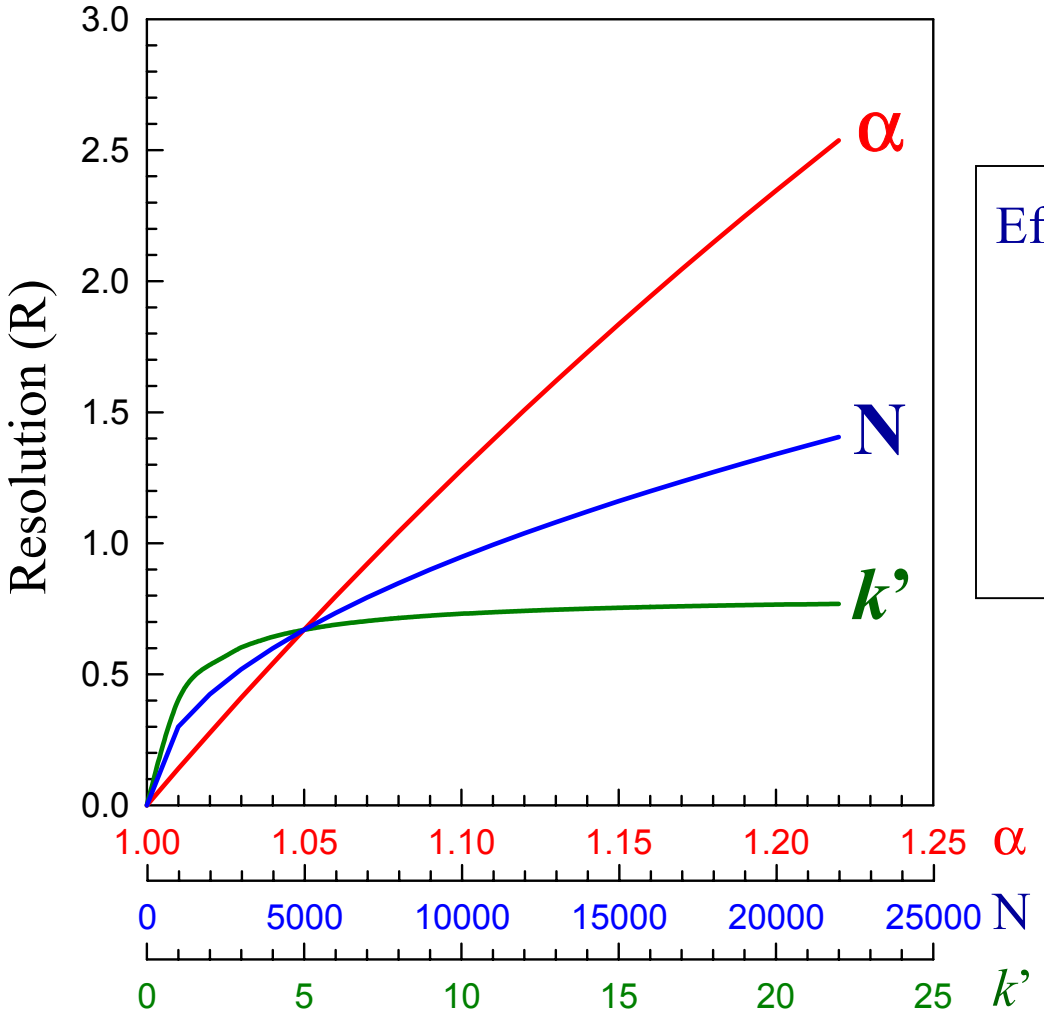
Part II.

The Thermally Tuned Tandem Column (T^3C) Concept

Outline

- Importance of Selectivity in HPLC Optimization
- Thermally Tuned Tandem Column (T^3C) Concept
  - ✓ Theory
  - ✓ Optimization
- An Example – Ten Triazine Herbicides
- Applications
  - ✓Urea and Carbamate Pesticides
  - ✓ Barbiturates
  - ✓ Antihistamine Drugs
- Conclusions
  - ✓ T^3C Works
  - ✓ It Can Save Time or Do Difficult Separations
  - ✓ Only Four or Five Initial Runs Are Needed

The Ultimate Goal of Separation: Resolution (R)



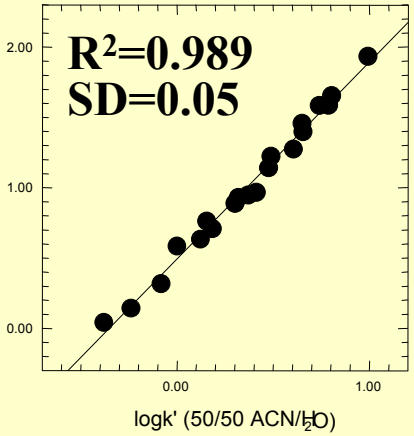
Efficiency Selectivity Retention

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

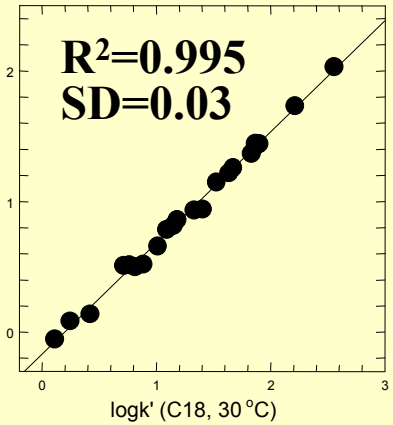
❖ **Selectivity (α) has the greatest impact on improving resolution**

Comparison of Variables Affecting Selectivity

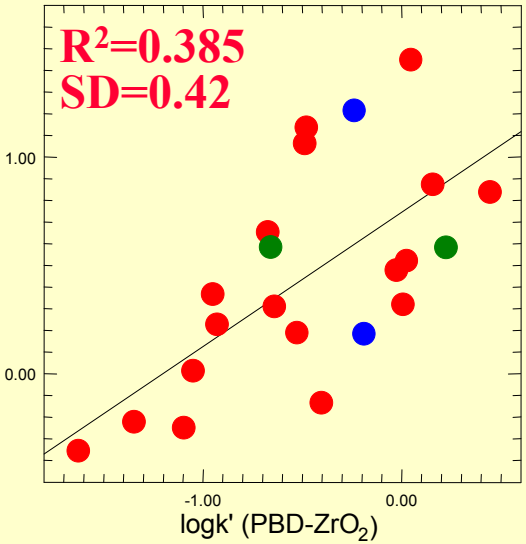
30% ACN vs. 50% ACN



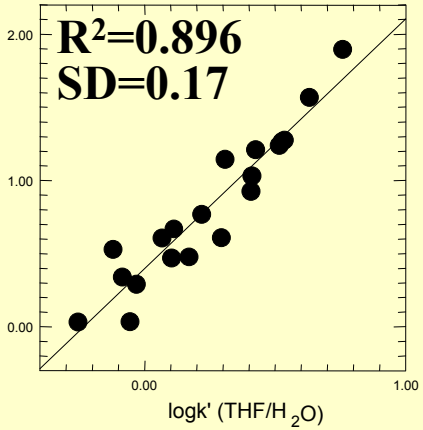
80°C vs. 30°C



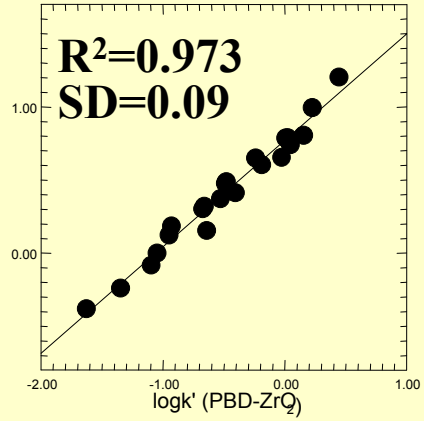
Carbon-ZrO_2 vs. PBD-ZrO_2



MeOH vs. THF



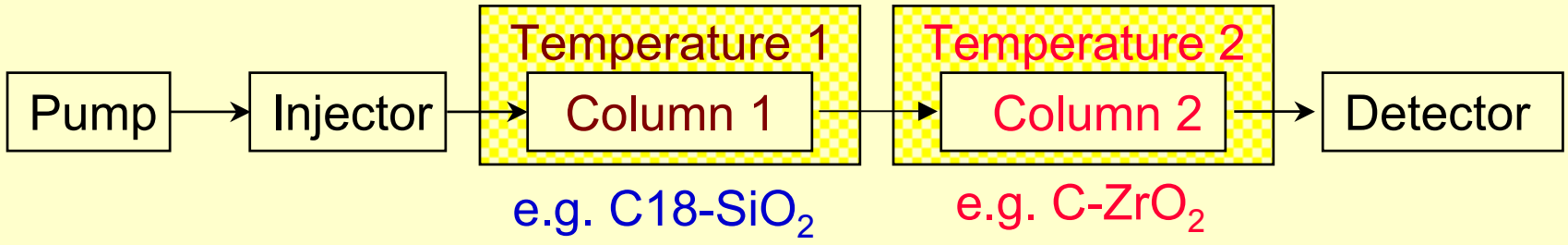
C18-SiO_2 vs. PBD-ZrO_2

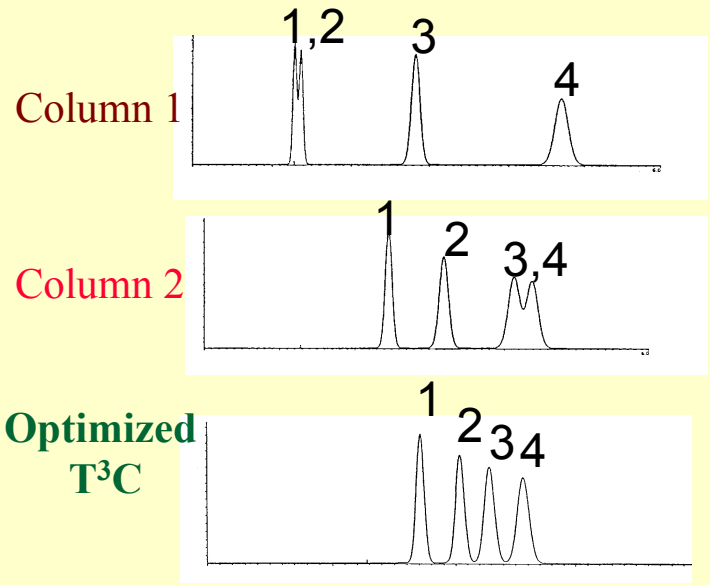


❖ **Stationary phase type can have a very large effect on selectivity.**

The Concept: Thermally Tuned Tandem Columns (T^3C)

A Mechanism to Continuously Adjust the Stationary Phase

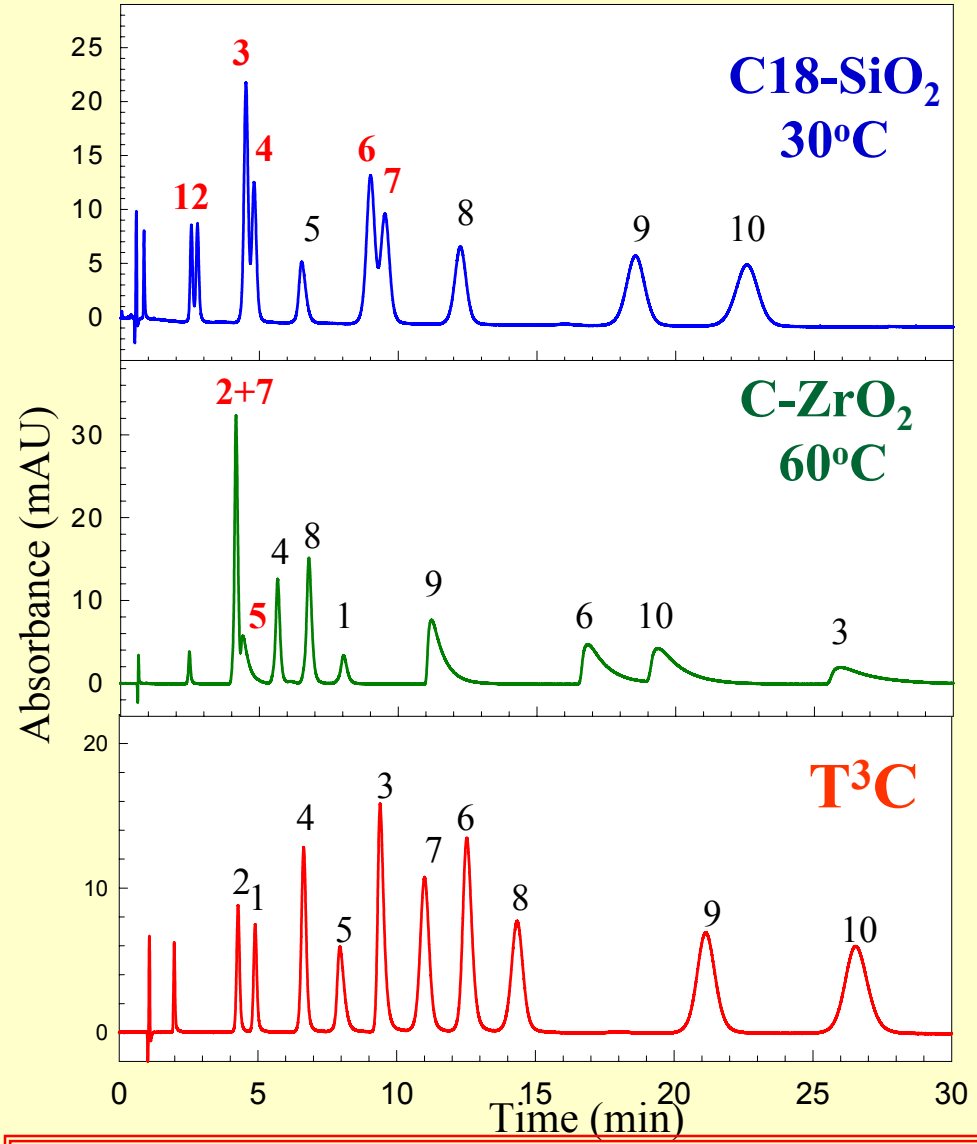


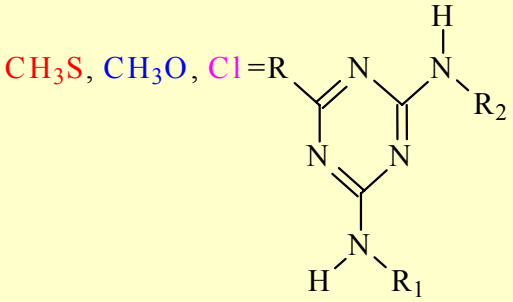


Requirements for T^3C:

- Two columns with different (ideally orthogonal) selectivity
- One very thermally stable column
- Method development must be easy

Separation of Ten Triazine Herbicides by T³C





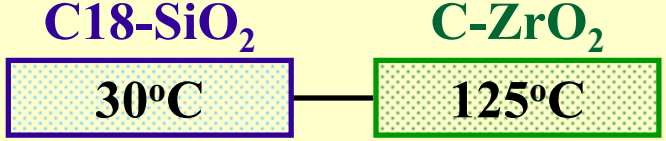
Solutes:

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

Other conditions:

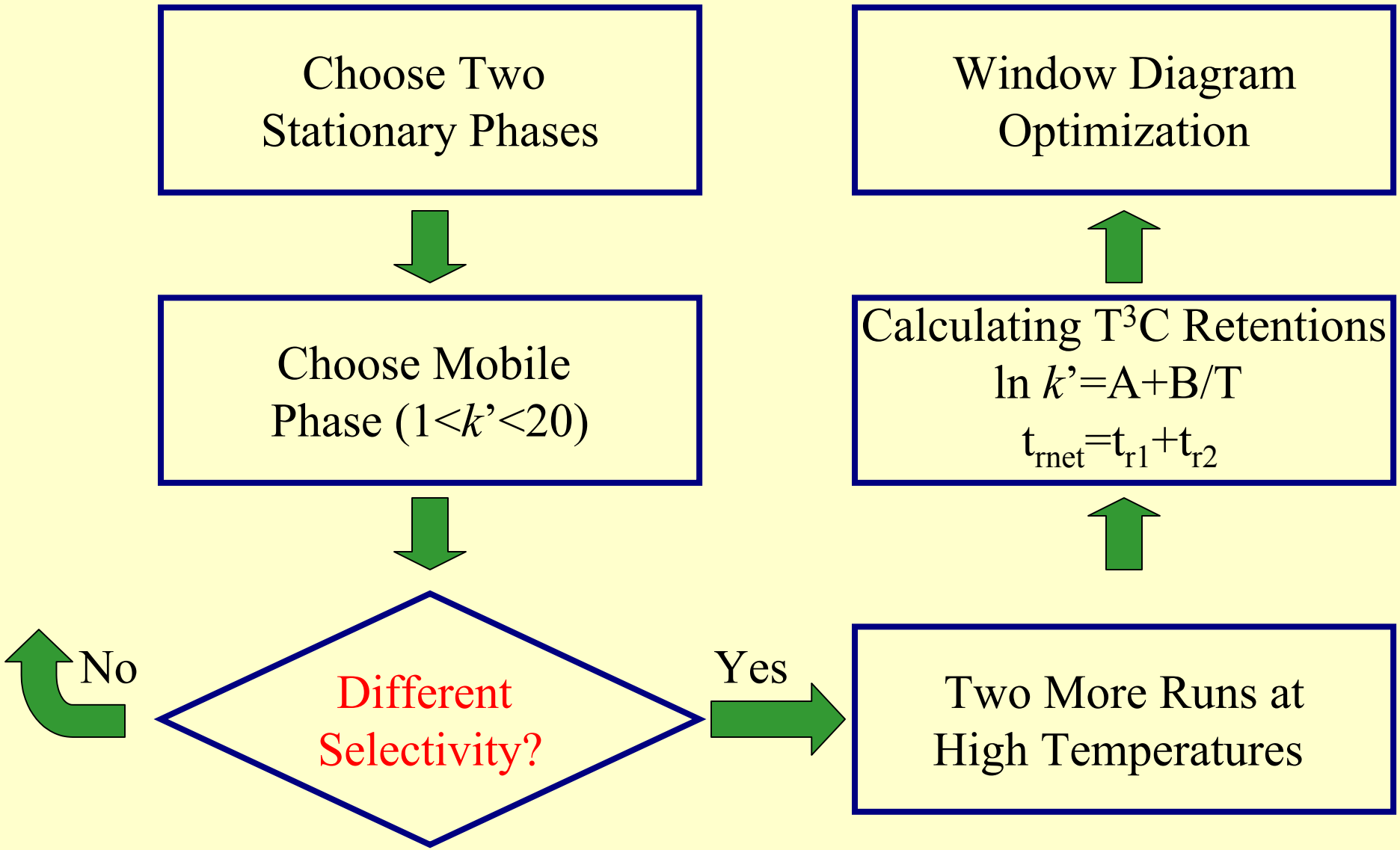
30/70 ACN/water

1ml/min; 254 nm detection

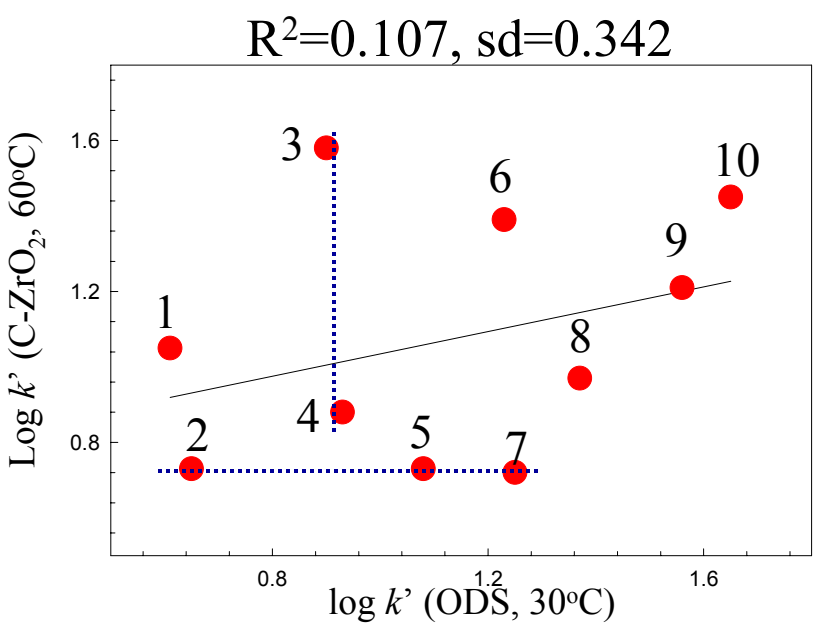


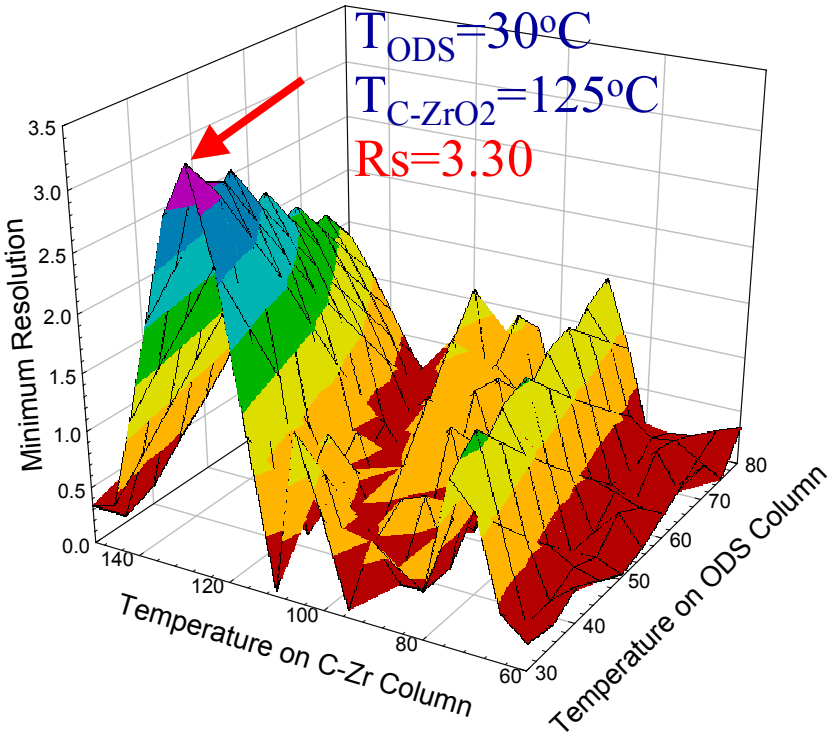
❖ **T³C can improve separation without increasing analysis time**

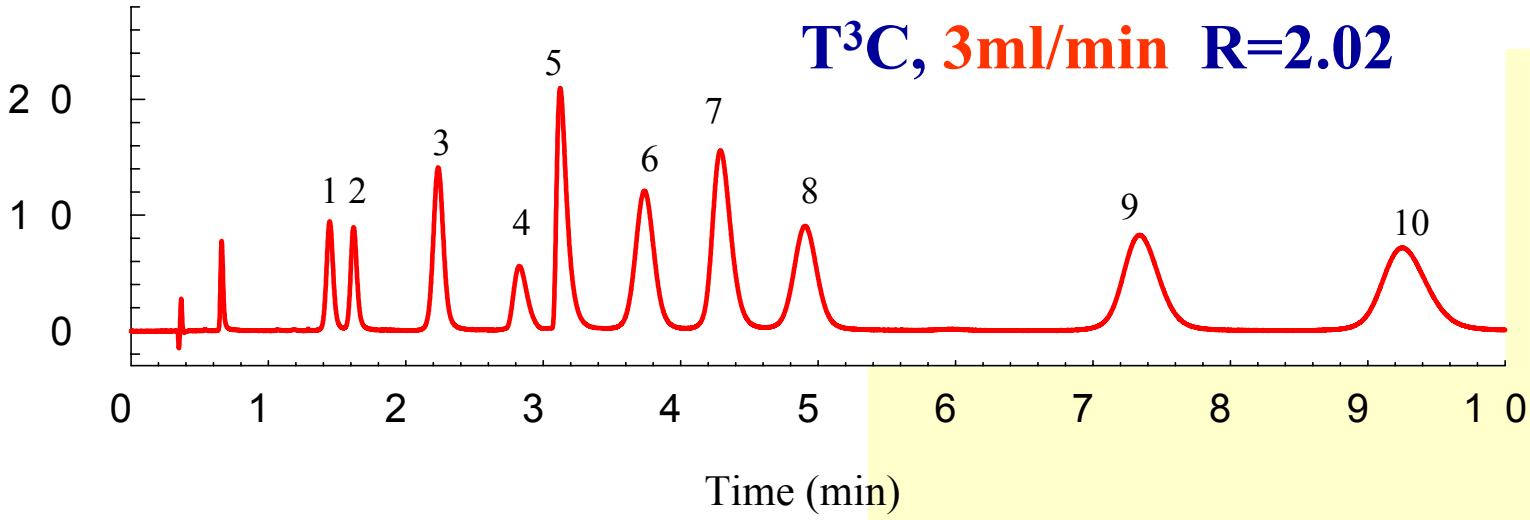
Guidelines for Optimizing T^3C



Steps in T³C Optimization of Triazine Herbicides

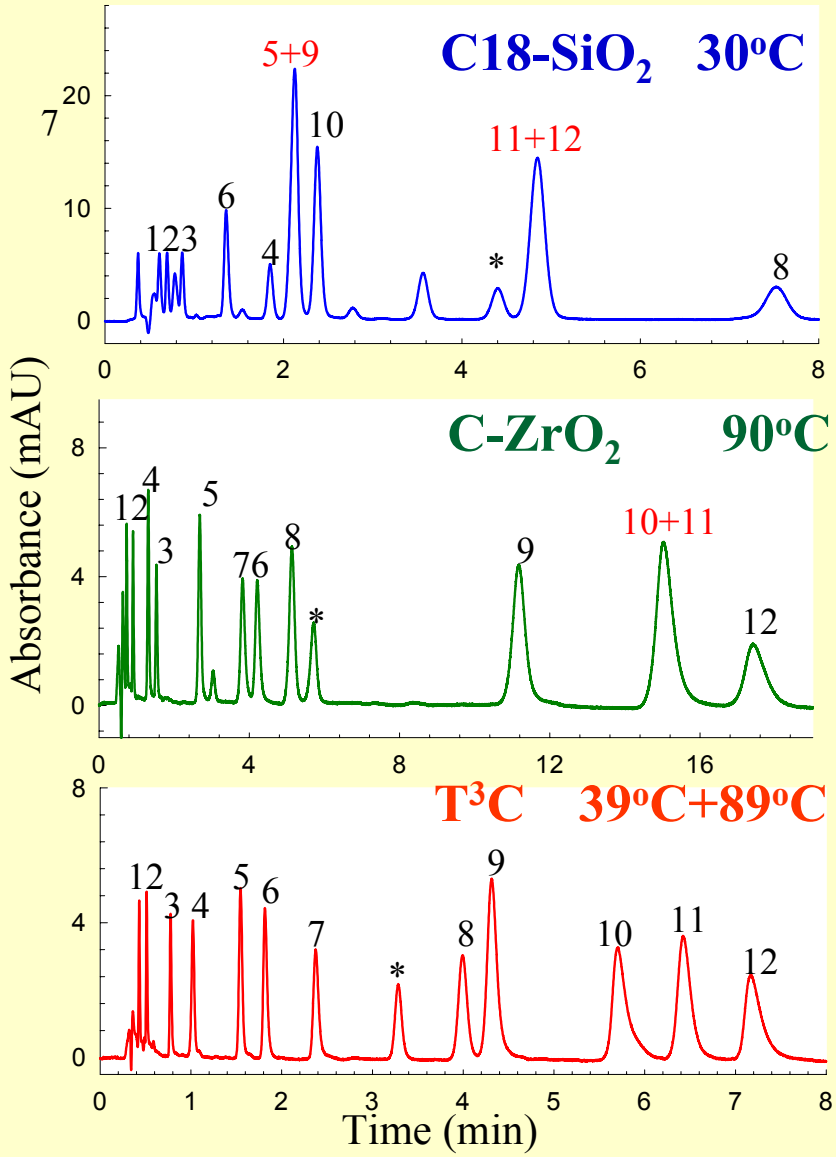




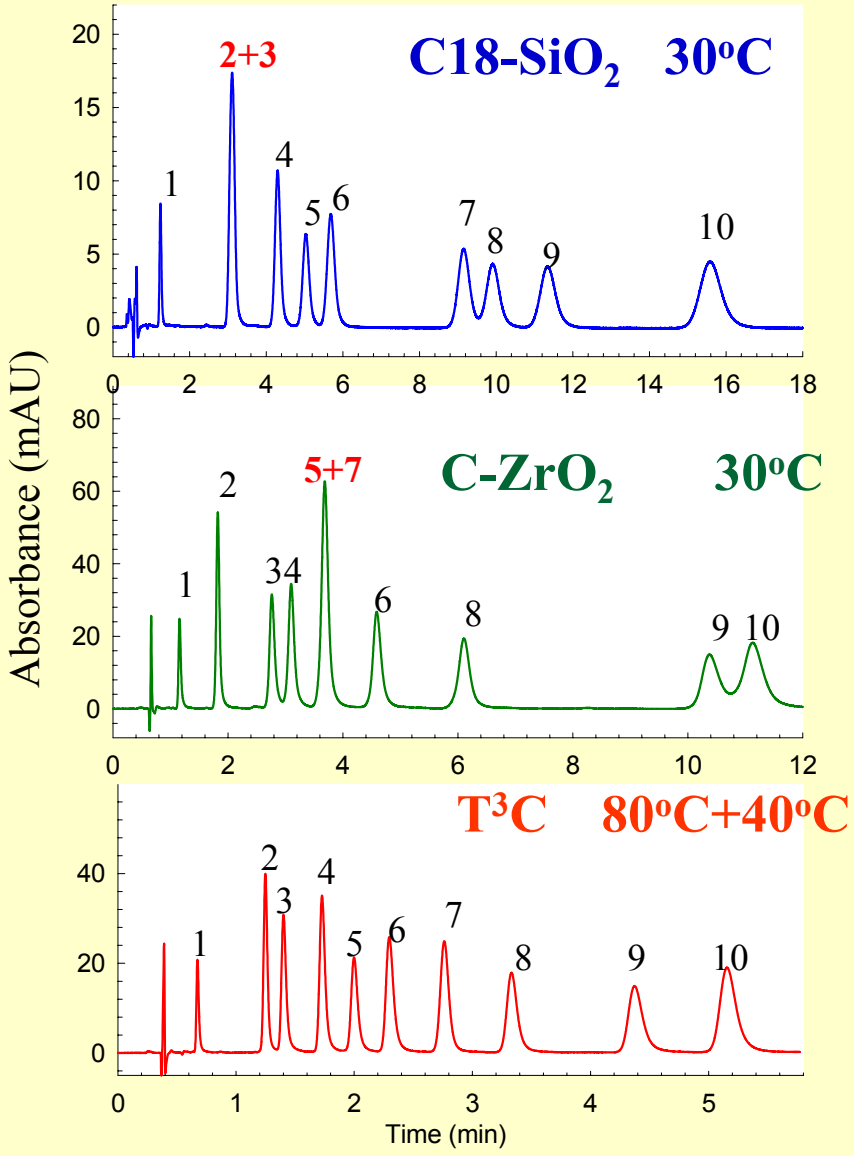


Applications of T³C Method

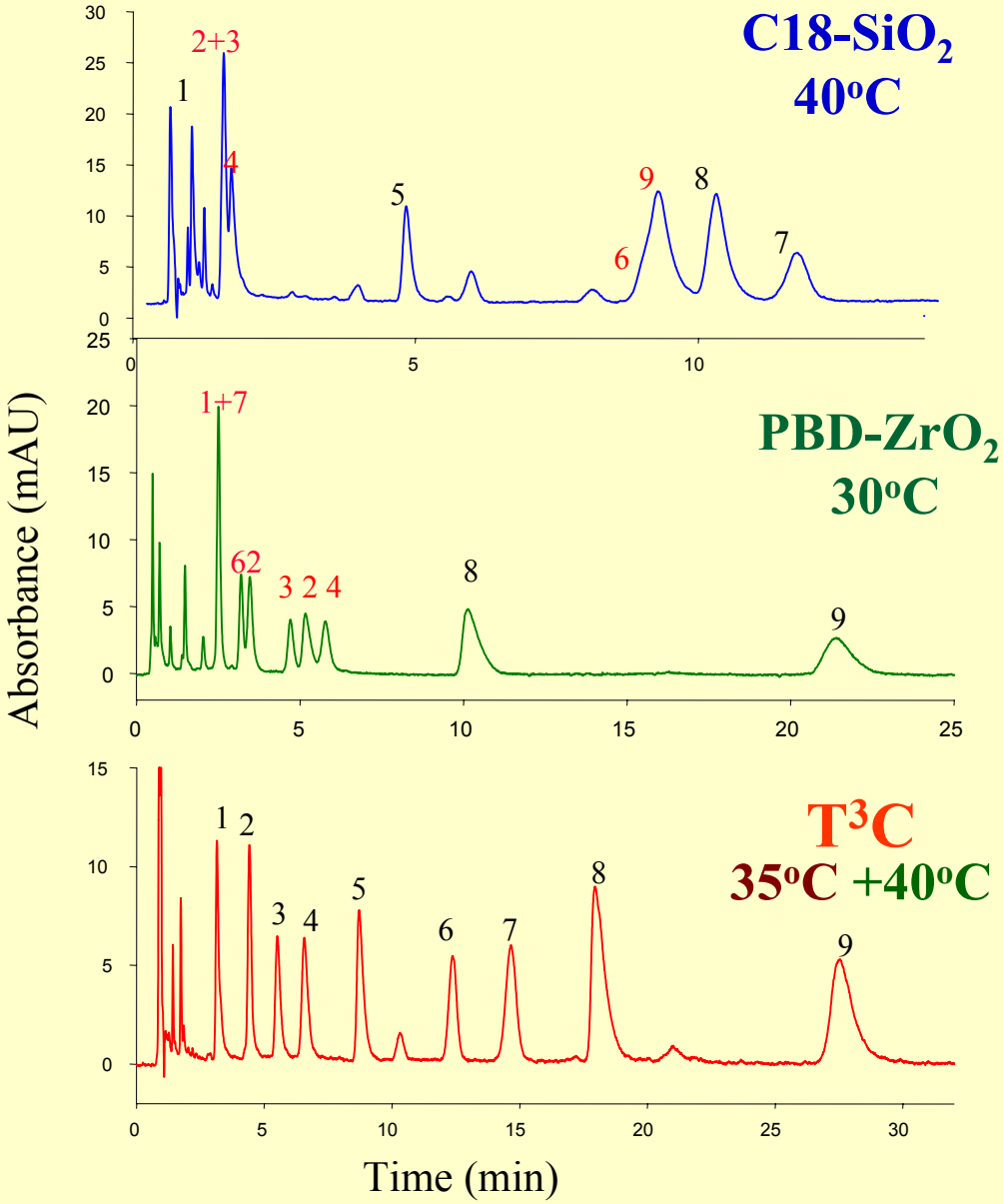
Urea and Carbamate Pesticides



Barbiturates



Separation of Anti-Histamines by T^3C



Conclusions

- T^3C offers unique selectivity for the separation of complex mixtures.
- T^3C requires that on the two phases the critical pairs must be different.
  - Carbon Phase + Aliphatic Phase → Neutral Compounds
  - Reversed Phase + PBD-ZrO_2 Phase Phosphate Buffer → Basic Compounds
- Optimization needs only 4 or 5 trial runs.
- In many cases, T^3C:
  - ✓ is superior to mobile phase optimization.
  - ✓ provides better resolution than a single phase.
  - ✓ improves analysis speed.

Part III. High Temperature Ultra-Fast Liquid Chromatography

Why Fast HPLC?

- Monitor reaction rates with half-lives on order of minutes not hours.
- Monitor prep scale chromatography.
- Increase sample through-put thus lower cost.
- Increase screening rate in combinatorial chemistry (speed up LC side of LC-MS).
- Make 2D-HPLC practical and thus greatly enhance peak capacity of HPLC.

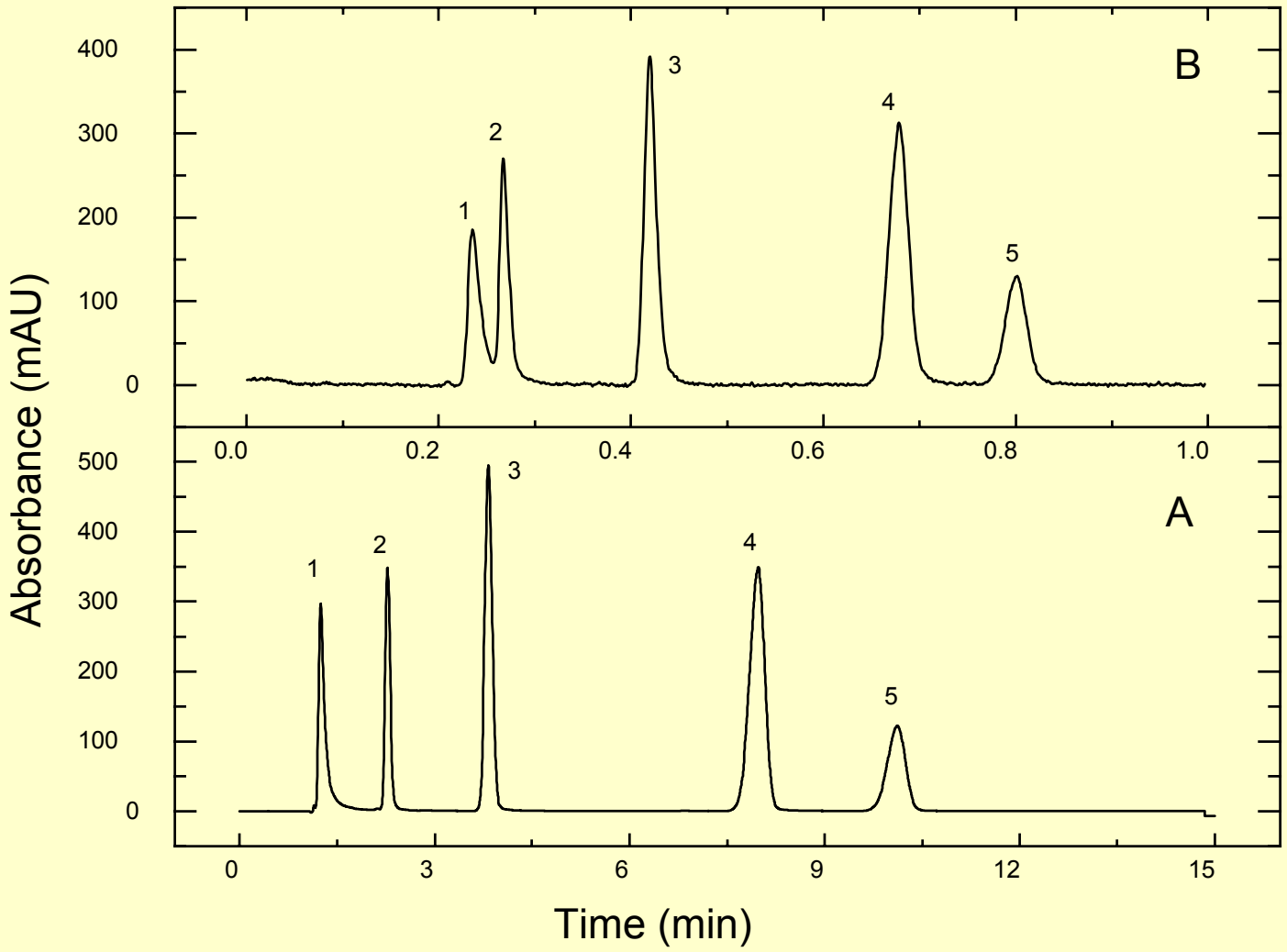
HPLC is Slow Compared to Other Methods*

Technique	d_p (μm)	N_{eff}/t (plates/s)
TLC	150	0.01
Open Column LC	150	0.02
Early HPLC	20-50	2
Current HPLC	2-5	15
Packed GC	10	40
Open Capillary GC	d_c= 0.03 mm	100
CE**	d_c = 0.1-.05 mm	100

*L.R. Snyder; J.J. Kirkland, ***Introduction to Modern Liquid Chromatography*****;** Wiley: New York, 1979**.**

R. Kennedy et al., *Chem. Rev.*, **99, 3081-3140 (1999).

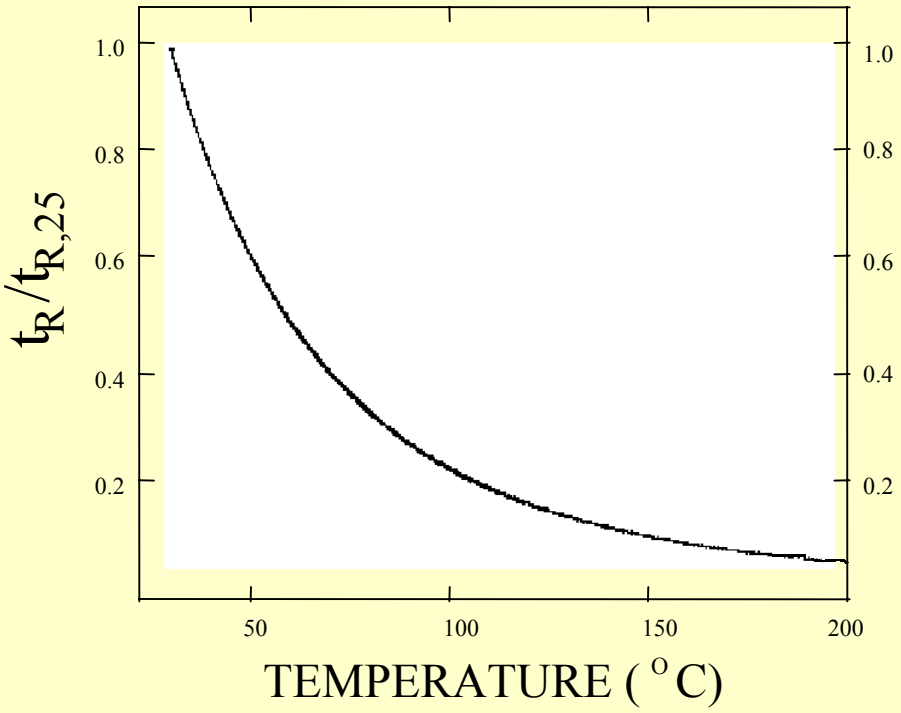
Fast HPLC at High Temperature



100 °C
5 ml/min

30 °C
1 ml/min

Effect of Temperature on ***Analysis Time*** at Constant N and P



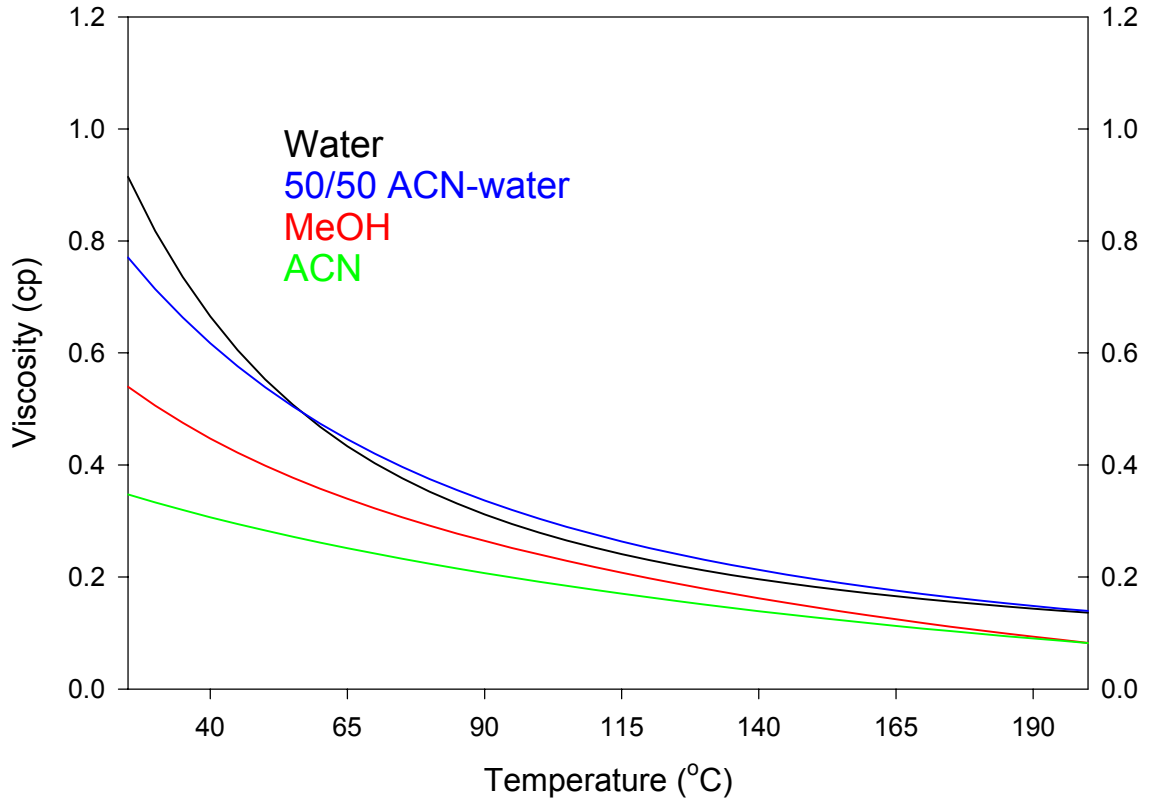
"High-Performance Liquid Chromatography at Elevated Temperatures: Examination of Condition for the Rapid Separation of Large Molecules", R. D. Antia and Cs. Horvath, *J. Chromatogr.*, 435, 1-15 (1988).

Theoretical and Practical Limits of Speed in HPLC

Fixed Pressure*	$\frac{t}{N} = \frac{(1+k')}{D_m}\frac{h}{\nu}d_p^2$
Theoretical Limit*	$\frac{t}{N}\Big\|_{\nu\to\infty} \cong \frac{C(1+k')}{D_m}d_p^2$
Reduced Velocity Limit	$\nu_{\max} = \frac{k_o d_p^3}{D_m \eta}\Delta P_{\max}$
Practical Limit	$\frac{t}{N} \cong \frac{A(1+k')}{D_m^{1/3}}\eta^{1/3}\frac{L^{2/3}}{\Delta P_{\max}^{2/3}}$
Practical Limit Temperature Dependence	$\frac{t}{N} \propto (1+k')A\frac{L^{2/3}}{\Delta P_{\max}^{2/3}}\frac{\eta}{T^{1/3}}$

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

Solvent Viscosity vs. Temperature



Data from Horvath and Chen.

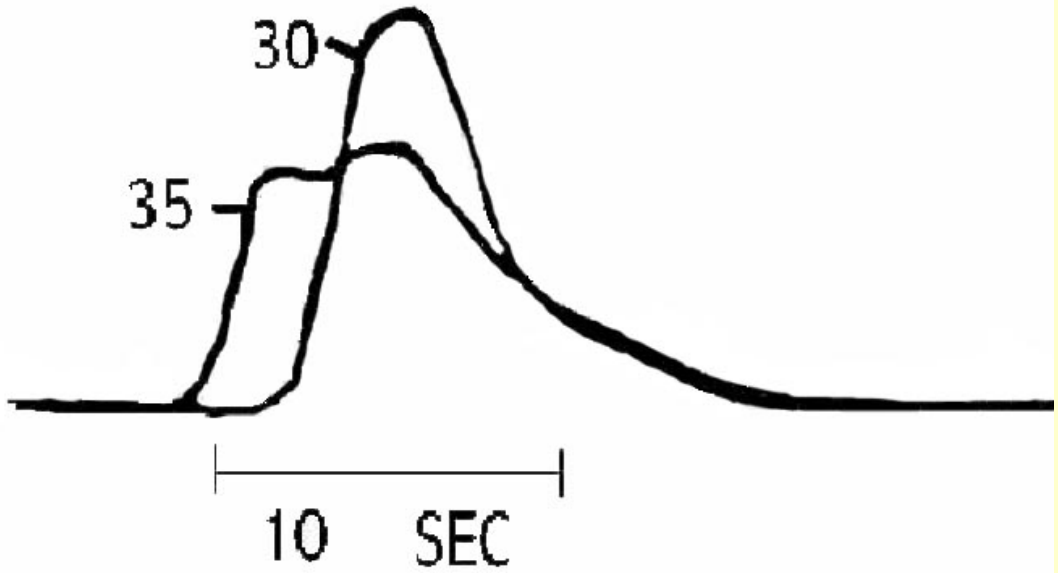
Thermal Mismatch Broadening

"Influence of Thermal Conditions on the Efficiency of High-Performance Liquid Chromatography."

H. Poppe and J. C. Kraak, *J. Chromatogr.*, 282, 399-412 (1983).

Peak Shapes Observed for Various Mobile-Phase Feed Temperatures*

$$\sigma^2_{obs} = \sigma^2_{column} + \sigma^2_{extra-columnn} + \sigma^2_{thermal-mismatch}$$

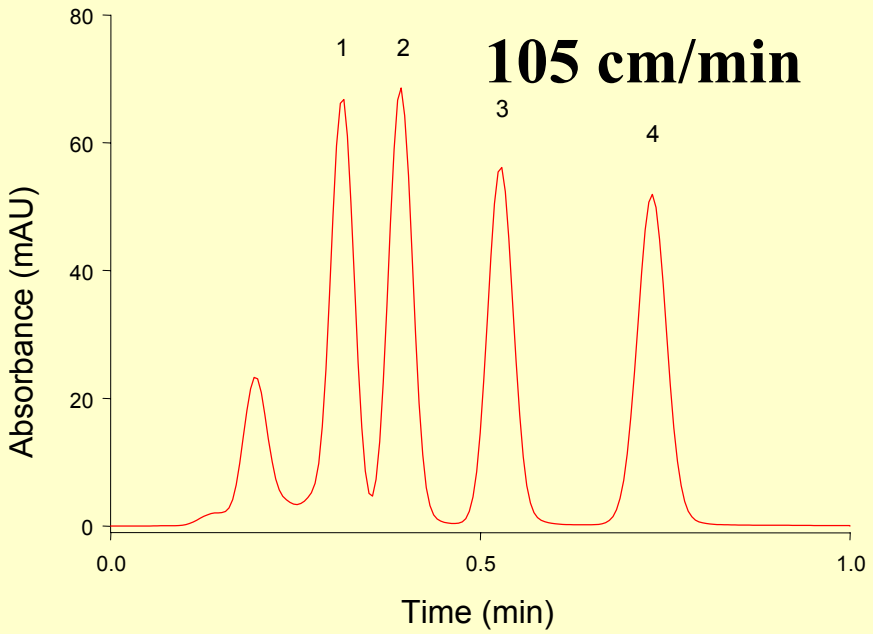


LC conditions: Column water jacket, 30 °C; 6.2 mm IDx8cm;
3µ Zorbax ODS; at 5 mL/min; 50/50 (v/v) ACN,H_2O;
nitrobenzene

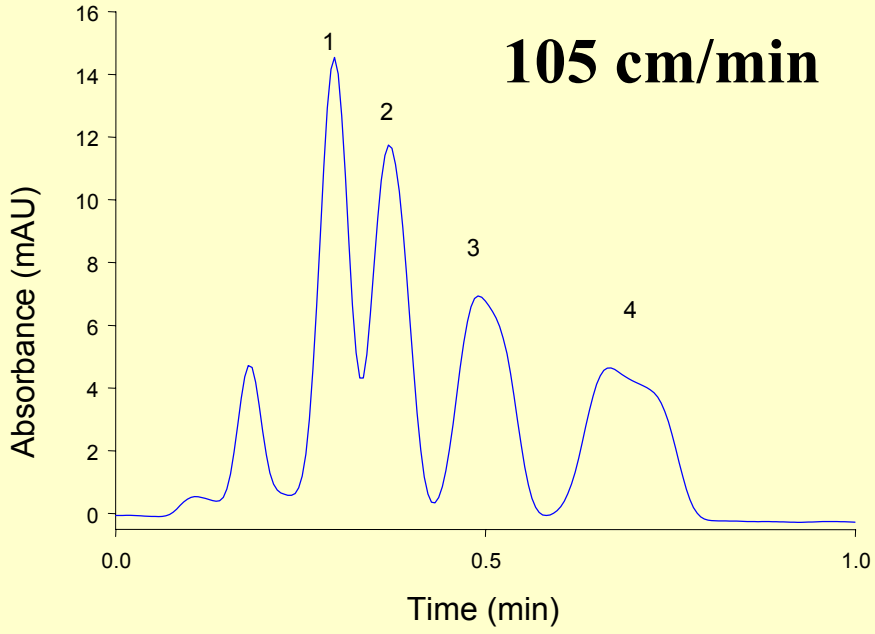
*H. Poppe and J.C. Kraak

Comparison of the Effect of Incomplete Thermal Equilibration on Column Performance

2.1 mm ID



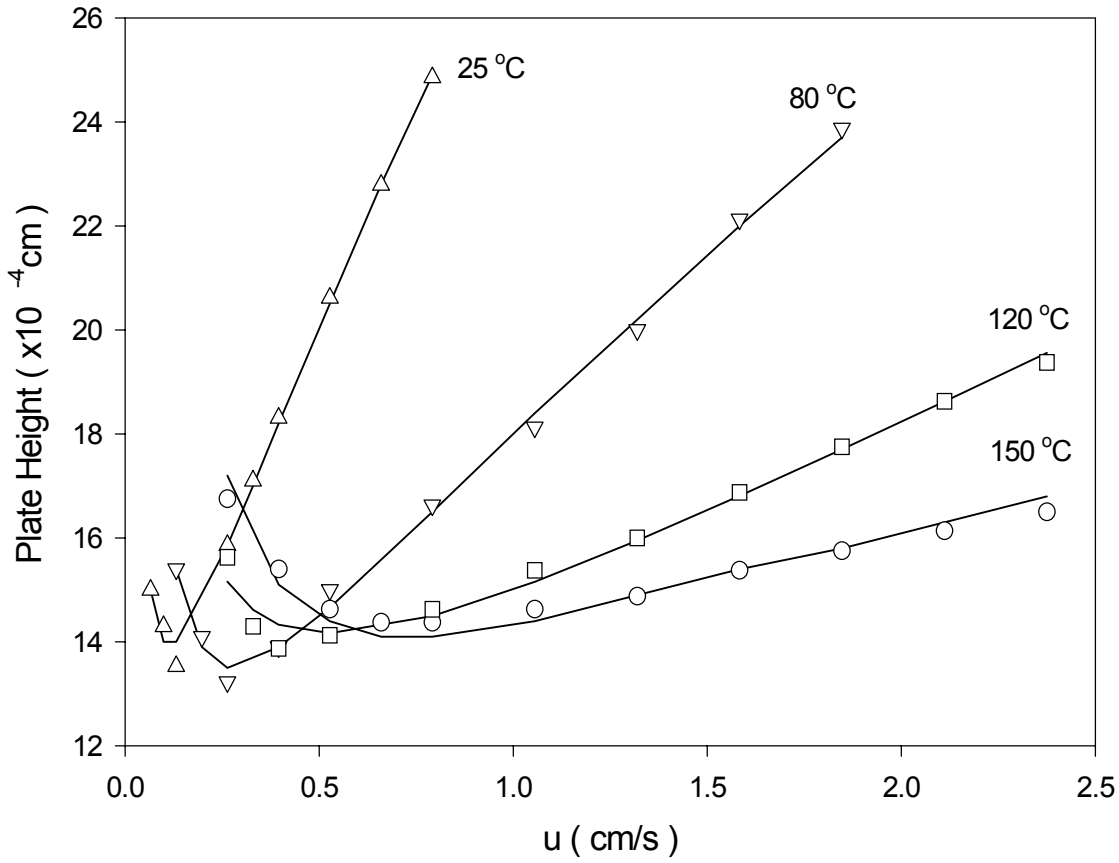
4.6 mm ID



LC conditions: 2.1 x 5 cm, C-18 INERT, 55 % ACN, 5 cm preheater, 60 °C
4.6 x 5 cm, C-18 INERT, 60% ACN, 5 cm preheater, 60 °C.

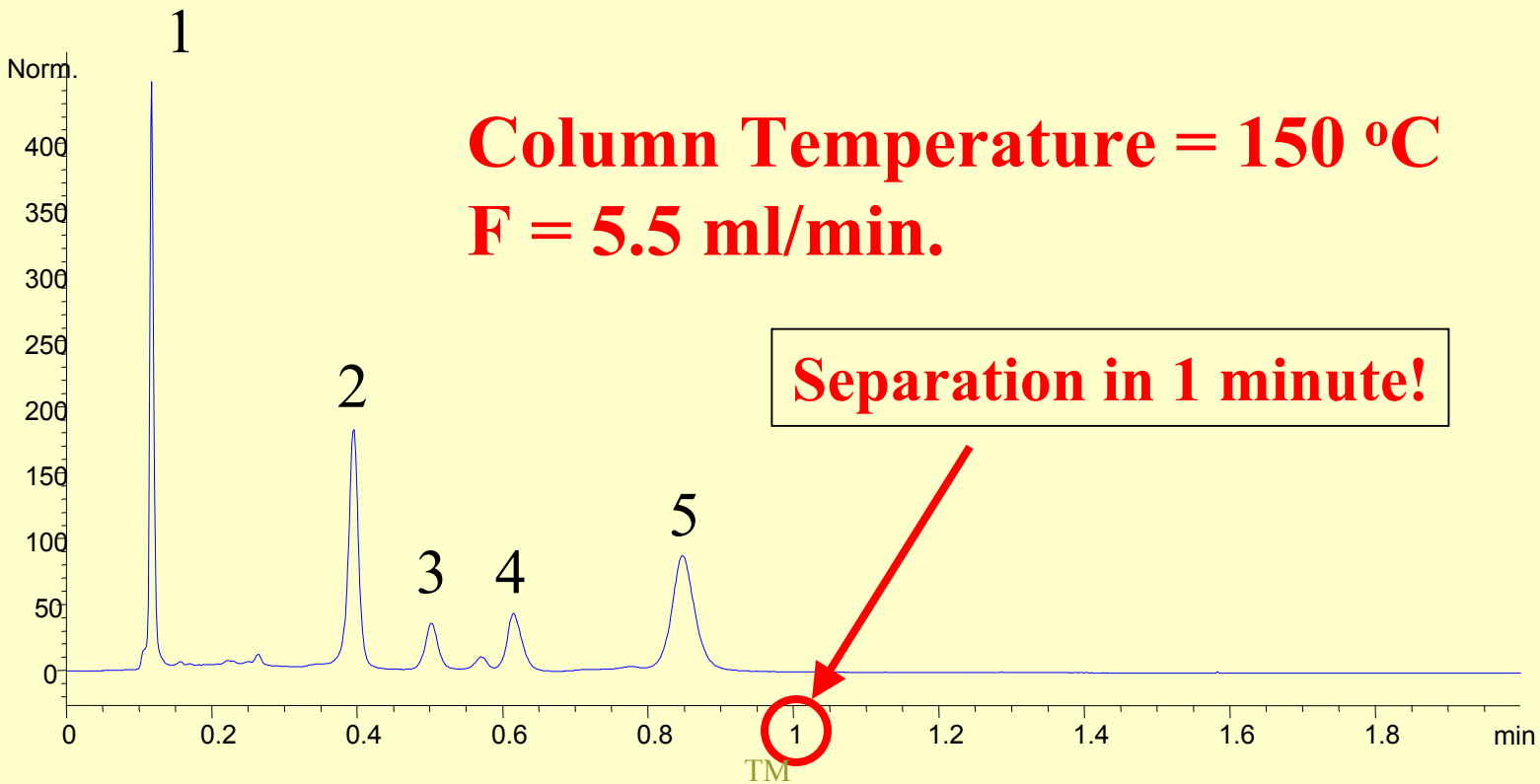
Peaks: 1. toluene, 2. ethylbenzene, 3. propylbenzene,4. butylbenzene

Effect of Temperature on Column Efficiency in HTUFLC



Conclusion: Resistance to mass transfer is greatly reduced as the column temperature is increased. Δ , 25 °C (decanophenone, k'=12.23), ∇, 80 °C (dodecanophenone, k'=7.39), □ , 120 °C (tetradecanophenone, k'=12.32).

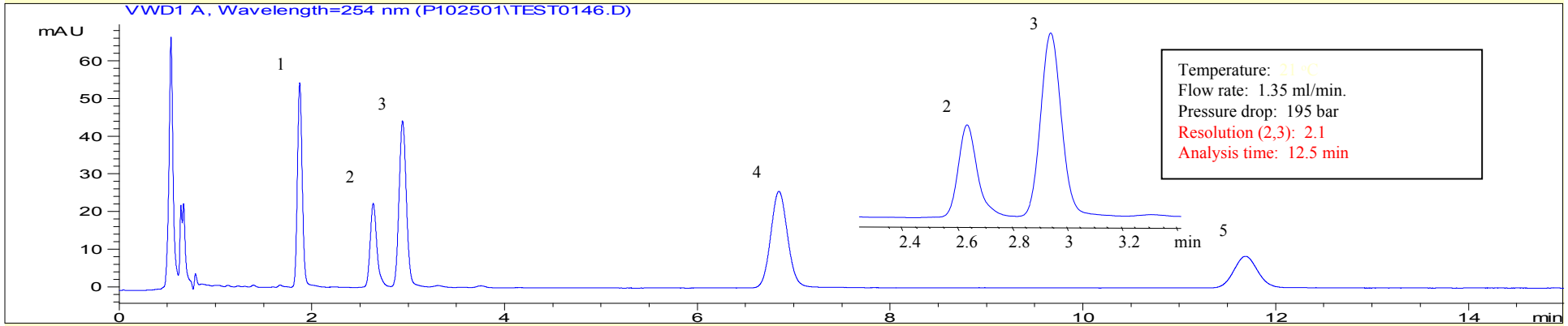
Fast Separations NSAIDs at High Temperature



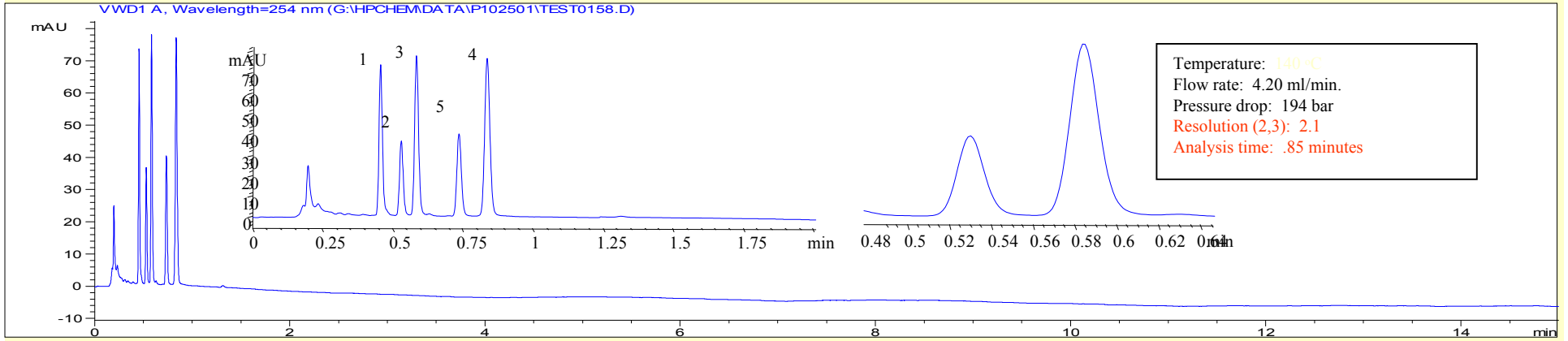
LC Conditions: Column, 50 x 4.6 DiamondBond -C18; Mobile phase, 25/75 ACN/40mM phosphoric acid, pH 2.3; Flow rate, 5.5 ml/min.; Temperature, 150 °C; Injection volume, 1ul; Detection at 254nm; Solute concentration, 0.15 mg/ml.; Solutes, 1= Acetaminophen, 2=Ketoprofen, 3=Naproxen, 4=Ibuprofen, 5=Oxaprofen.

High Speed HPLC

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, 20.5/79.5 ACN/50mM Tetramethylammonium hydroxide, pH 12.2; Flow Rate, 4.20 mL/min.; Injection volume, 0.5 ul; 254 nm detection; Column Temperature, 140°C; Pressure drop = 194 bar; Solutes: 1=Doxylamine, 2=Methapyrilene, 3=Chlorpheniramine, 4=Triprolidine, 5=Meclizine **100 x 4.6 ZirChrom-PBD**

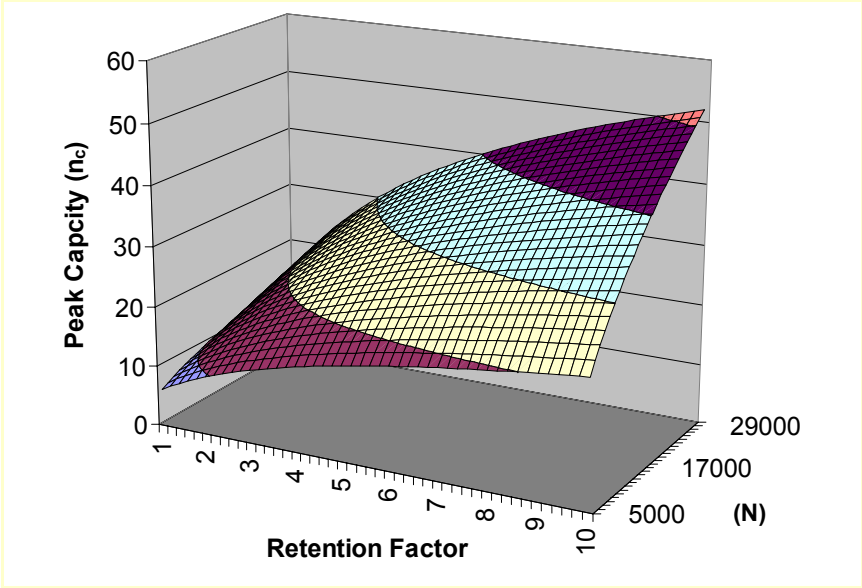


Courtesy ZirChrom

Fast, Comprehensive Two-Dimensional HPLC

One-dimensional HPLC has low peak capacity

$$n_c = 1 + \frac{\sqrt{N}}{4R_s}\ln\left(k'_n + 1\right)$$



Comprehensive two-dimensional HPLC has high peak capacity

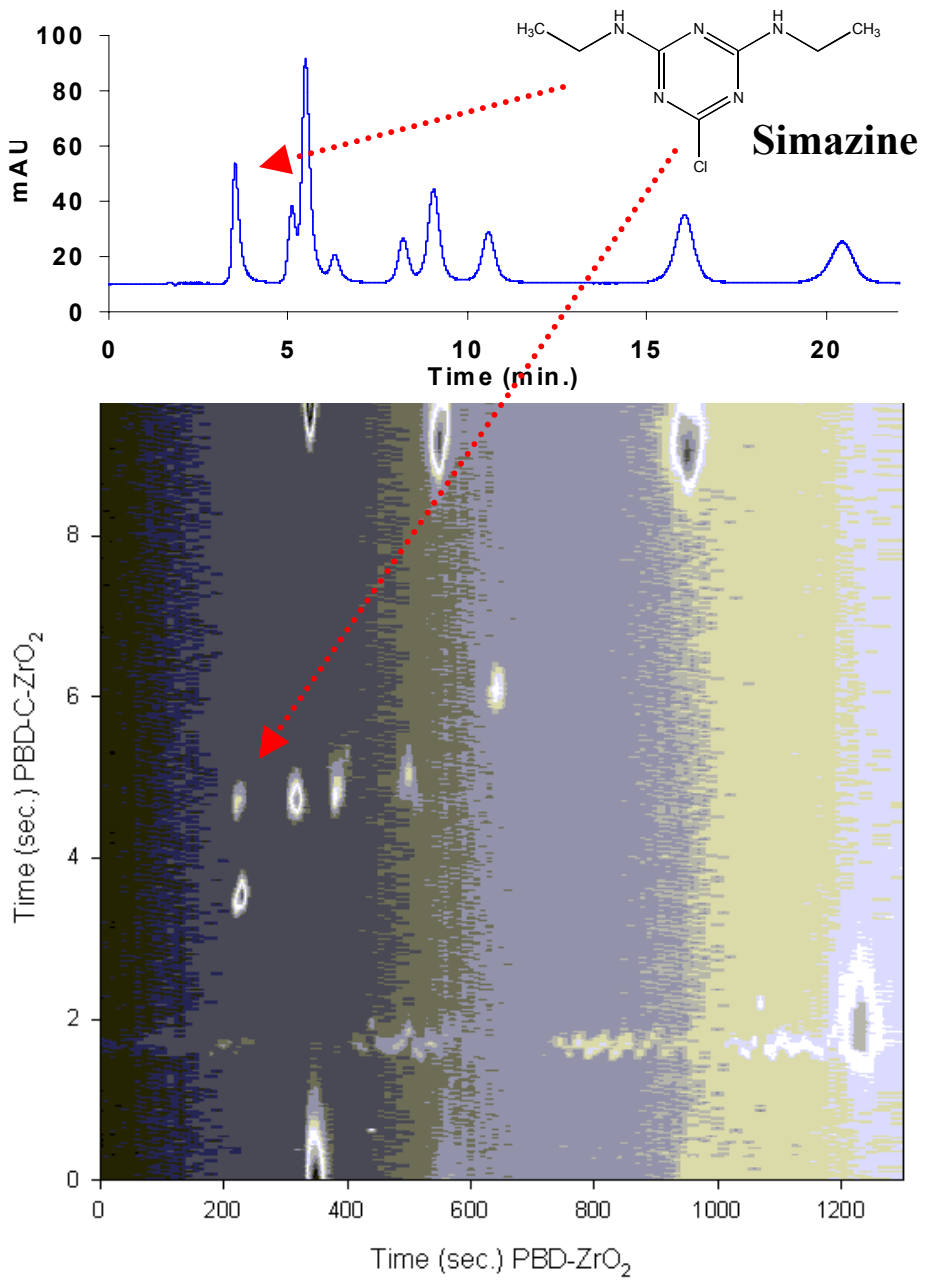
$$n_{cTotal} = n_{c1} \times n_{c2}$$

A major limitation is low speed related to the second dimension linear velocity, u_2

$$t_{rtotal} = \frac{\left(k'_{\max 1} + 1\right)\left(\sqrt{N_1}\left[L_{c2}\left(k'_{\max 2} + 1\right)\right]\right)}{\upsilon_2}$$

Giddings, J. C. *Multidimensional Chromatography: Techniques and Applications*; Marcel Dekker: New York, 1990

LC × UFHTLC Separation of Ten Triazine Herbicides



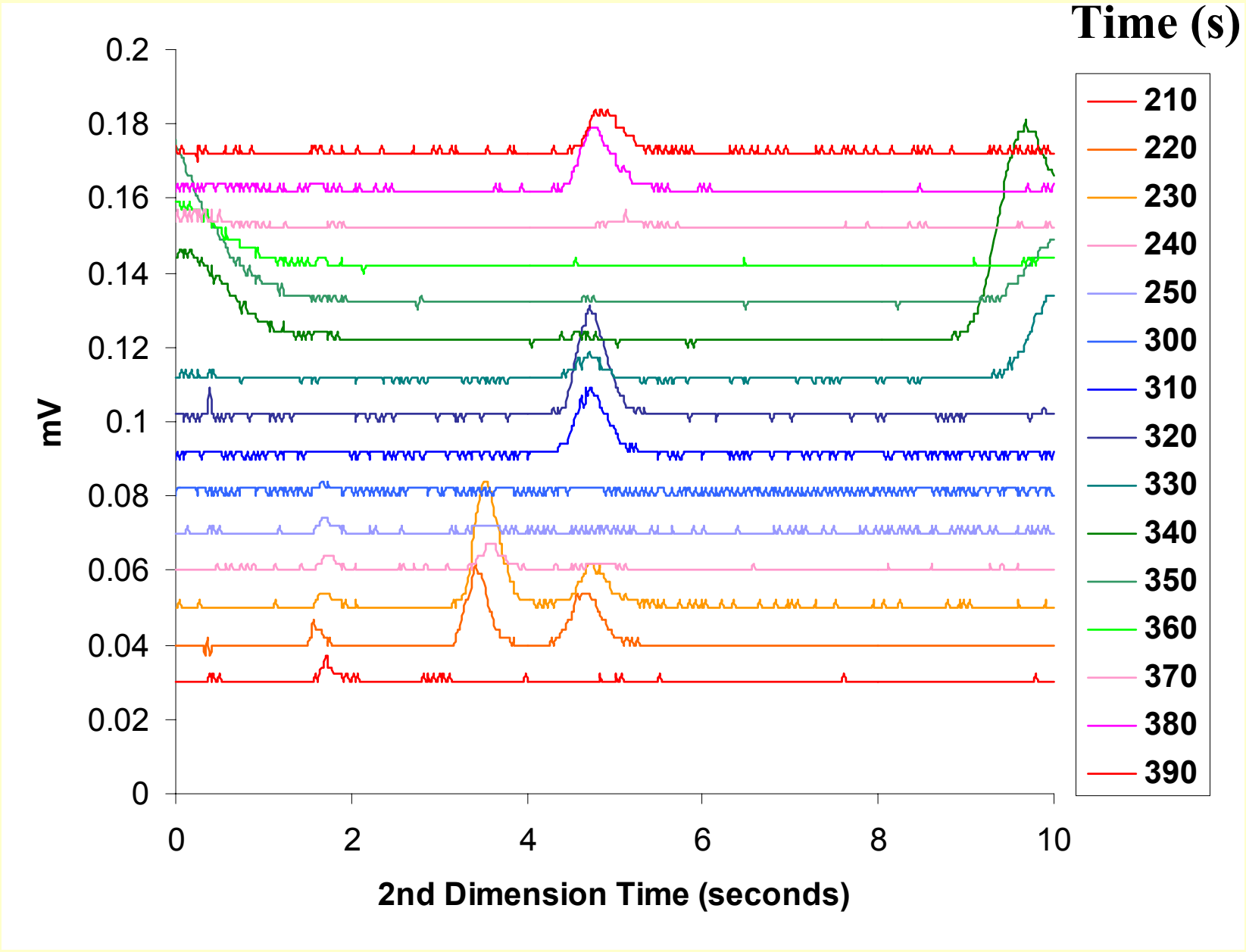
1st Dimension Conditions: Column, 50 mm x 2.1 mm I.d. PBD-ZrO_2;
Flow rate, 0.08 ml/min.;
Temperature, 40 °C

2nd Dimension Conditions: Column, 50 mm x **2.1 mm I.d. PBD-C-ZrO_2**;
Flow rate, 7.0 ml/min.;
Temperature, 150 °C; 1st dimension sampling frequency, 0.1 Hz

Total LC × UFHTLC peak capacity = **185**

A single column would be **2.5 meter** and take **44 hours** to generate same peak capacity

Fast Chromatography on the 2nd Column



Conclusions:

(1) Heat transfer, pressure drop and extra-column broadening considerations are key to design HTUFLC.

(2) Tubing pressure drop is important.

(3) HTUFLC can be as much as 50 times faster than room temperature HPLC.

(4) HTUFLC can be done with 100% water as the eluent.

(5) Fast (< 0.5 hr.) 2D-LC can be done.

Acknowledgments

National Institutes of Health