High Temperature Liquid Chromatography

C.V. McNeff¹*, B. Yan¹, D. R. Stoll², R.A. Henry³

¹ ZirChrom Separations, Inc., 617 Pierce Street, Anoka, MN 55303
² Department of Chemistry, University of Minnesota, 207 Pleasant St.
   Minneapolis, MN 55455.
³ Independent Consultant, 983 Greenbriar Drive, State College, PA 16801

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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

\[ h = A + \frac{B}{\nu} + C\nu + D\nu^{2/3} + \frac{3D_m}{8k_d d_p^2} \nu \]


Practical Limit Temperature Dependence

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


*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

Estimated Effect of Temperature on Viscosity*

\[ \frac{t}{N} \propto \eta \]

Water
50 % ACN
MeOH
ACN

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

*20-fold improvement!

Practical Advantages of Column Stability

Extraordinary Chemical Stability

pH Stability

pH < 1
- Cleaning with Conc. Acid

pH > 13
- Ion Supression for Acids
- Ion Supression for Amines
- Sanitation/Depyroegenation

Thermal Stability

Lower Pressure Drop
- Less Wear and Tear

Less Organic Solvent
- Higher Flow Rate: Fast Analysis

Thermally Optimize Selectivity
- More Robust Analysis
- Easier Method Development

pH > 13
- pH < 1

List of HTLC compatible reversed-phase columns

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Column Name</th>
<th>Stationary Phase Type</th>
<th>Temperature Limit (°C)</th>
<th>Selectivity versus C18</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polymer Laboratories</td>
<td>PLRP</td>
<td>Polymer</td>
<td>200</td>
<td>Different</td>
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<tr>
<td>Selerity</td>
<td>Blaze</td>
<td>Silica</td>
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<td>Supelco</td>
<td>DiscoveryZR-Carbon</td>
<td>Carbon Clad Zirconia</td>
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<td>DiscoveryZR-CarbonC18</td>
<td>Modified Carbon on Zirconia</td>
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<tr>
<td>Thermo-Electron</td>
<td>Hypercarb</td>
<td>Carbon</td>
<td>200</td>
<td>Similar</td>
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<td>ZirChrom-CARB</td>
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<tr>
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<td>Diamondbond-C18</td>
<td>Modified Carbon on Zirconia</td>
<td>200</td>
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<tr>
<td>Jordi</td>
<td>Jordi DVB</td>
<td>Polymer</td>
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<tr>
<td>Sachtleben</td>
<td>Sachtopore-RP</td>
<td>Polymer coated Titania</td>
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<tr>
<td>Agilent</td>
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<td>Silica</td>
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<tr>
<td>Waters</td>
<td>X-Bridge</td>
<td>Silica</td>
<td>80</td>
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</table>
Stationary Phase Comparison
Average Scatter of κ–κ Plots for Two Kinds of Stationary Phases Using 22 Solutes

- Carbon-ZrO₂
- PBD-ZrO₂
- C18-SiO₂ (ODS)
- Phenyl-SiO₂
- CN-SiO₂
- PRP

➢ For non-electrolytes, C-ZrO₂ and aliphatic phases have the most different selectivities.
Fast Separations Non-Steroidal Anti-Inflammatories

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.
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

\[ R = \frac{\sqrt{N}}{4} \cdot \frac{k'}{k'+1} \cdot \frac{\alpha-1}{\alpha} \]

\[ \alpha = \frac{k_j'}{k_i'} \]

➢ Selectivity (\(\alpha\)) has the greatest impact on improving resolution.
Comparison of Variables Affecting Selectivity

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

Stationary Phase Type
Carbon-ZrO₂ vs. PBD-ZrO₂

MeOH vs. THF

- R²=0.989
  - SD=0.05

- R²=0.896
  - SD=0.17

30% ACN vs. 50% ACN

- R²=0.995
  - SD=0.03

80°C vs. 30°C

- R²=0.973
  - SD=0.09

- R²=0.385
  - SD=0.42
Thermally Tuned Tandem Columns (T³C)

A Mechanism to Continuously Adjust the Stationary Phase

Pump → Injector → Column 1 → Column 2 → Detector

Temperature 1: e.g. C18-SiO₂
Temperature 2: e.g. C-ZrO₂

Optimized T³C
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.
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) 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 (**T3C 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 ultrafast analyses.