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

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


Practical Limit Temperature Dependence

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


*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!

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

Separation in 0.4 minute!

mAU

min

0

0.4

0.6

Labetalol

Metoprolol

Alprenolol

CHCH2NHCH

CH2CH2

H2NOC

OH

HO

CH3

CH2CH2

OCH2CHCH2NHCH(CH3)2CH3OCH2CH2

OH

OCH2CHCH2NHCH(CH3)2

OH
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

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 (T³C)

A Mechanism to Continuously Adjust the Stationary Phase

- Column 1: e.g. C18-SiO₂
- Column 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

C³-ZrO²

T³C can improve separation without increasing analysis time.
Schematic of a Complete LC × UFHTLC System

- Standard HP1090 Pump and Autosampler
- Binary Pump #1
- Auto-Injector
- Eluent Preheater
- 1st Dimension Column
- Heating Jacket
- T1
- T2
- T3
- T4
- T5
- T6
- T7
- 2.1 mm column
- Counter-Current Heat Exchanger
- UV Detector
- High flow pump
- Temperature Controlled
- Sample Loop #1
- Sample Loop #2
- 10-port, 2-position valve
- Eluent Preheater
- Binary Pump #2
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 Na₃PO₄ 20 mM NaClO₄ 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 HClO₄ 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

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 (*T³C 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 ultrafast analyses.

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