Ultra-Stable Stationary Phases for HPLC: Assembly, Advantages, and Applications

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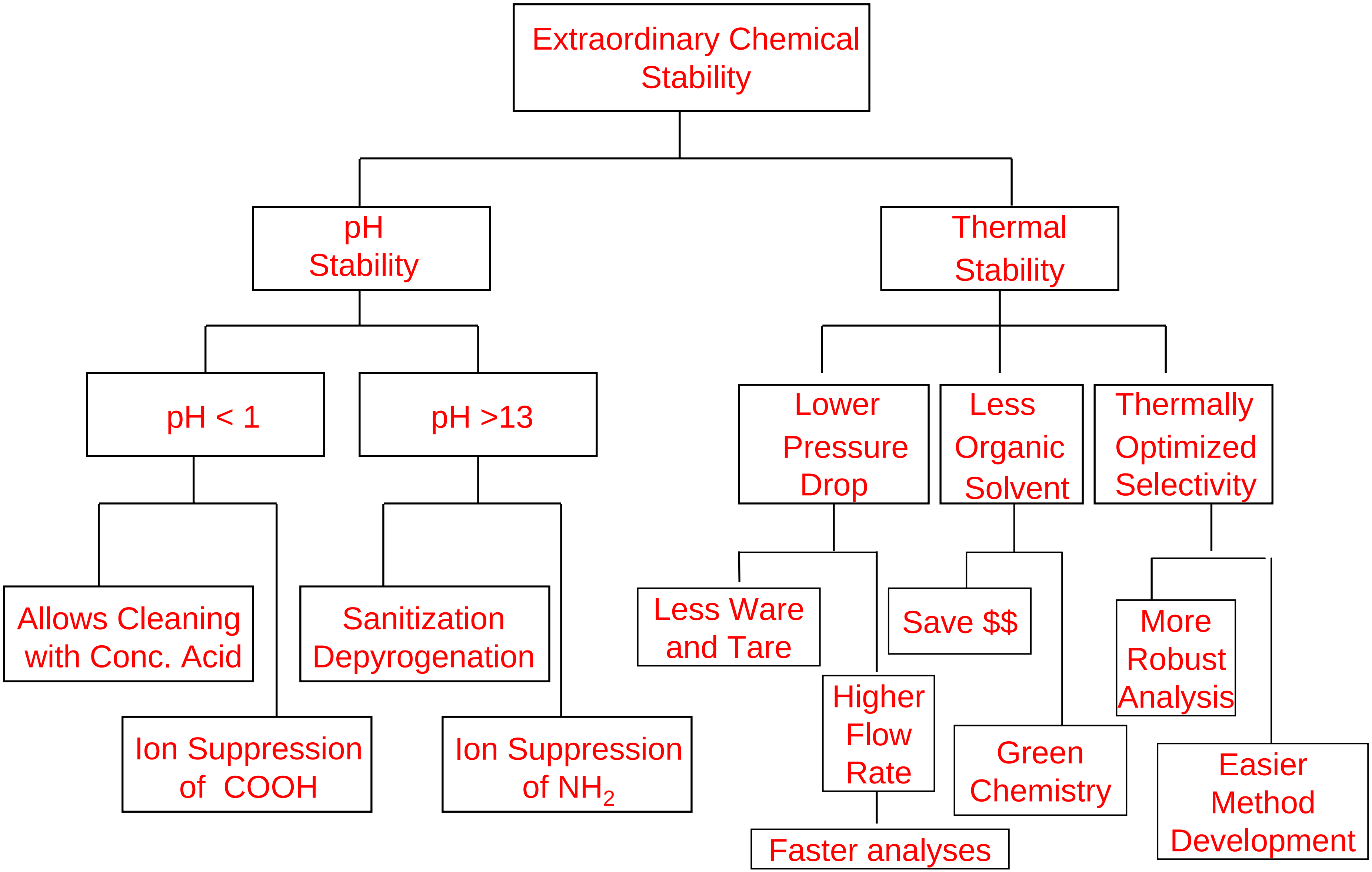
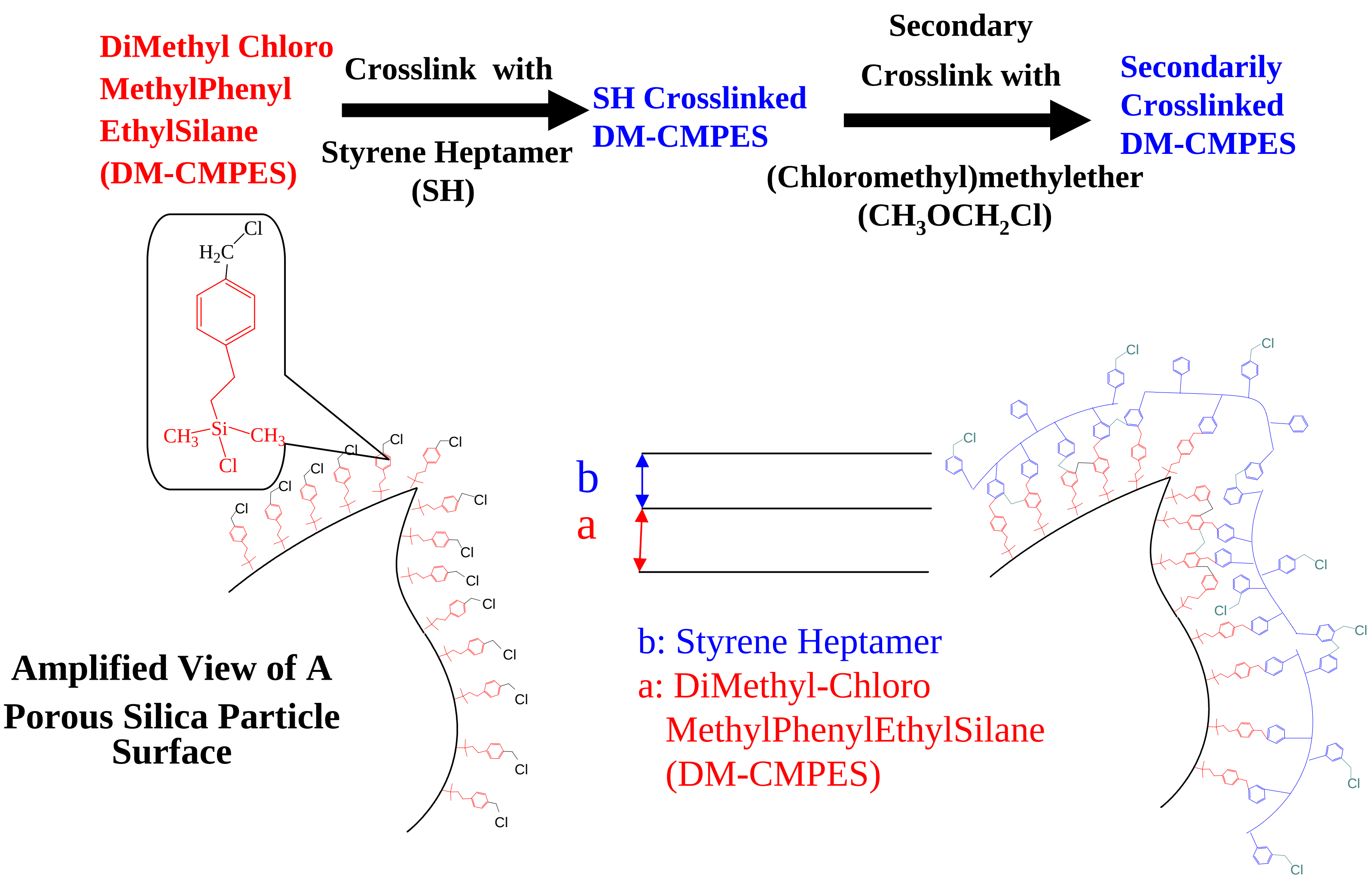
Group Goals

- Demonstrate advantages
  - ✓Chemical and thermal stability
- Synthesize highly stable stationary phases for RPLC
  - ✓Silica-based hypercrosslinked reversed and ion exchange phases
- Apply
  - ✓Ultra-Fast High Temperature Liquid Chromatography (UFHTLC)
  - ✓Two-Dimensional HPLC (2DLC)
  - ✓Fast gradient elution chromatography of forensic samples.
  - ✓Optimization of gradient elution peak capacity for proteomics studies.

What are the Advantages of Highly Stable Stationary Phases?

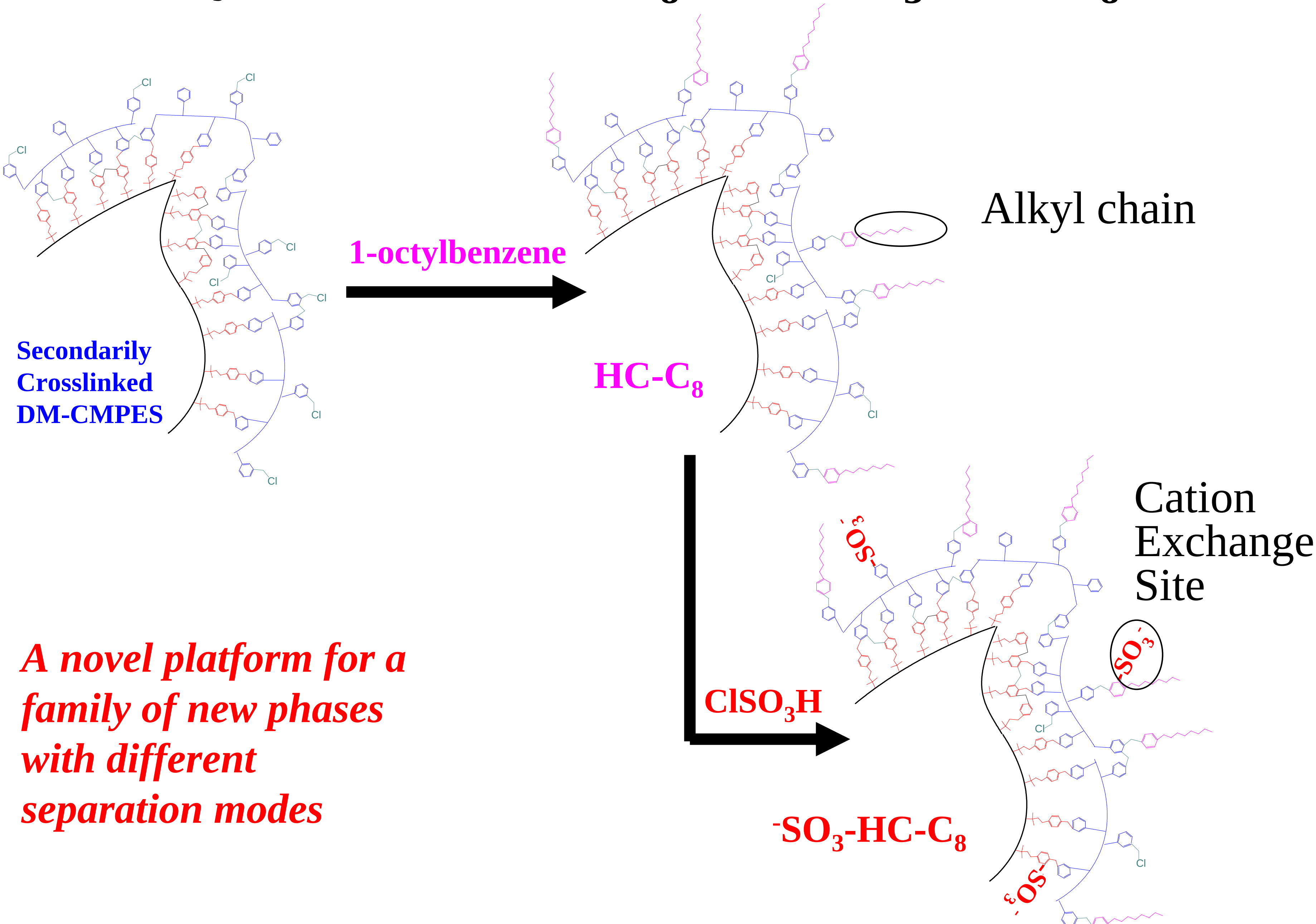


Hyper-Crosslinked (HC) Platform Prepared by Orthogonal Friedel-Crafts Chemistry



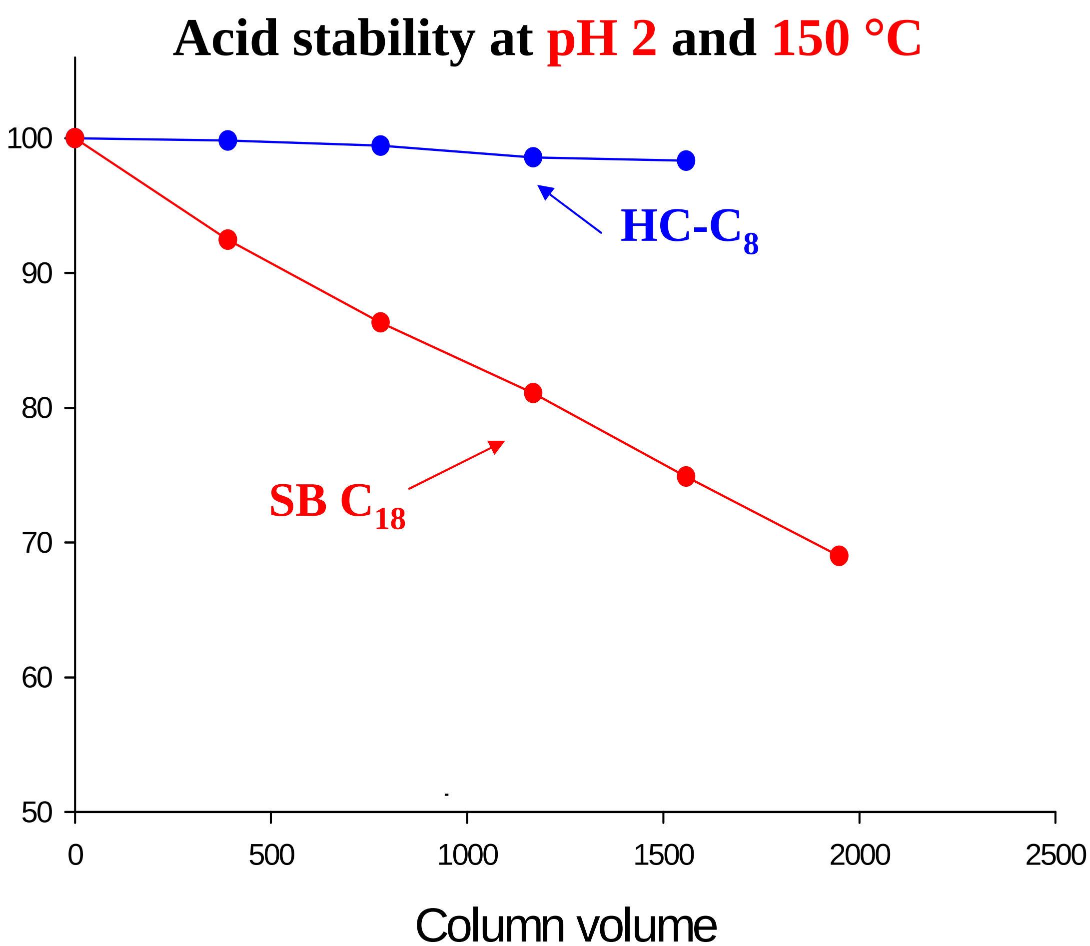
Friedel-Crafts reactions catalyst: $SnCl_4$

Synthesis of HC-C_8 and $^{-}SO_3$-HC-C_8

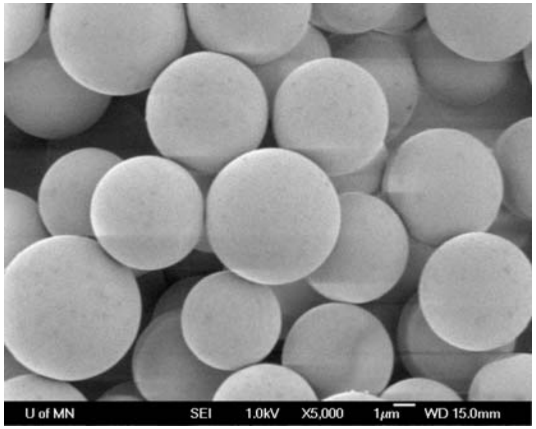


A novel platform for a family of new phases with different separation modes

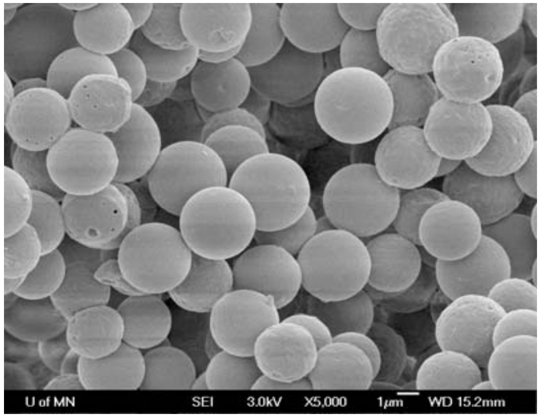
Ultra High Acid Stability of HC-C_8



Column: 3.3×0.21cm
49.95/49.95/0.1 ACN/Water/TFA**,** 150 °C, 0.5 mL/min
Solute: hexadecanophenone



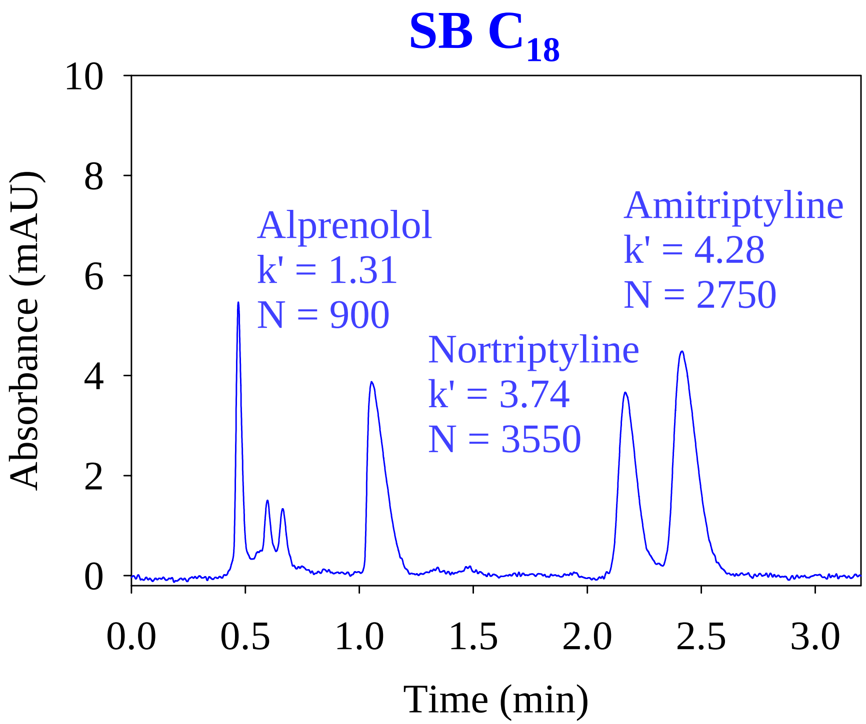
HC-C_8 before HF digestion

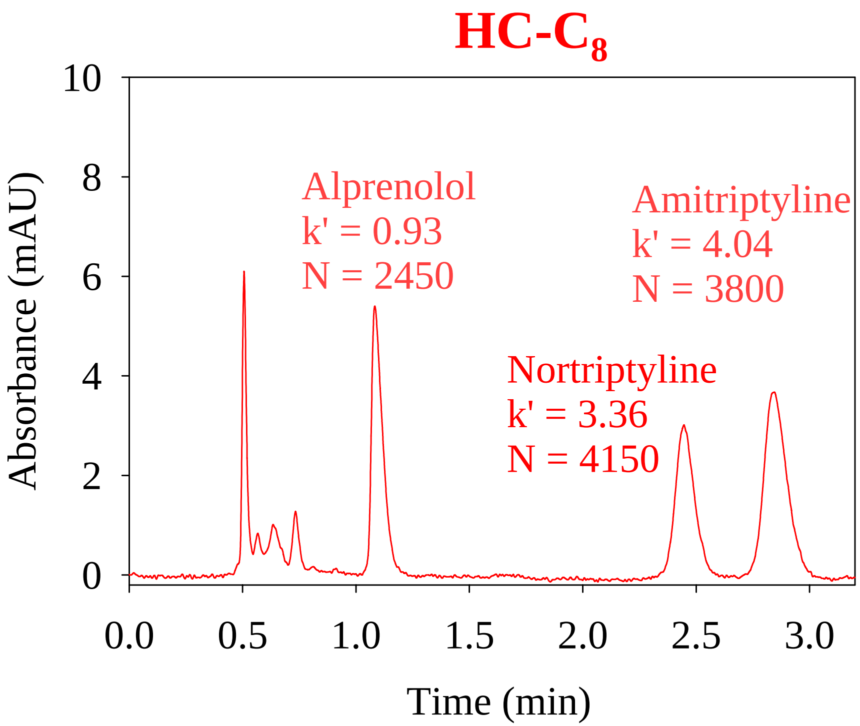


HC-C_8 After HF digestion all silica was removed

Very good acid stability is achieved by the formation of hyper-crosslinked polymer networks

Basic Drugs Performance Comparison of SB C_{18} and HC-C_8 in Formic Acid



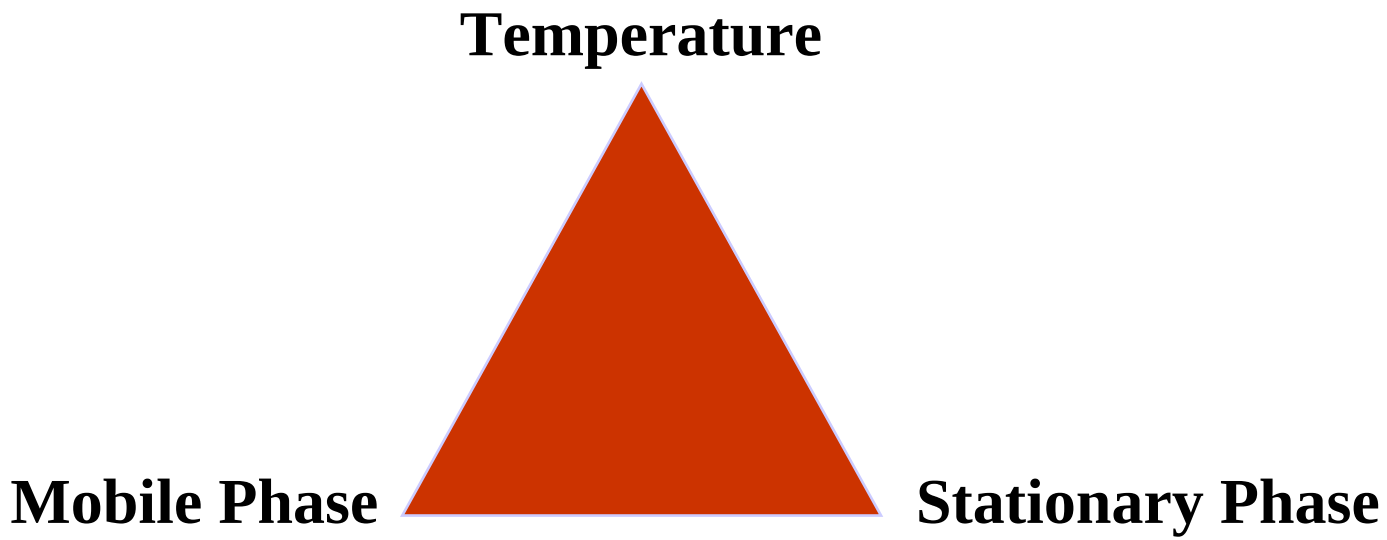


HC-C_8: 34/66 ACN/water, SB C_{18} 38/62 ACN/water. For both columns: **0.1% formic acid**, 40ºC, 1mL/min

5 × 0.46 cm column

HC-C_8 provides excellent efficiency for basic drugs even in weak ion pairing reagent formic acid

Temperature
The Third Dimension in HPLC



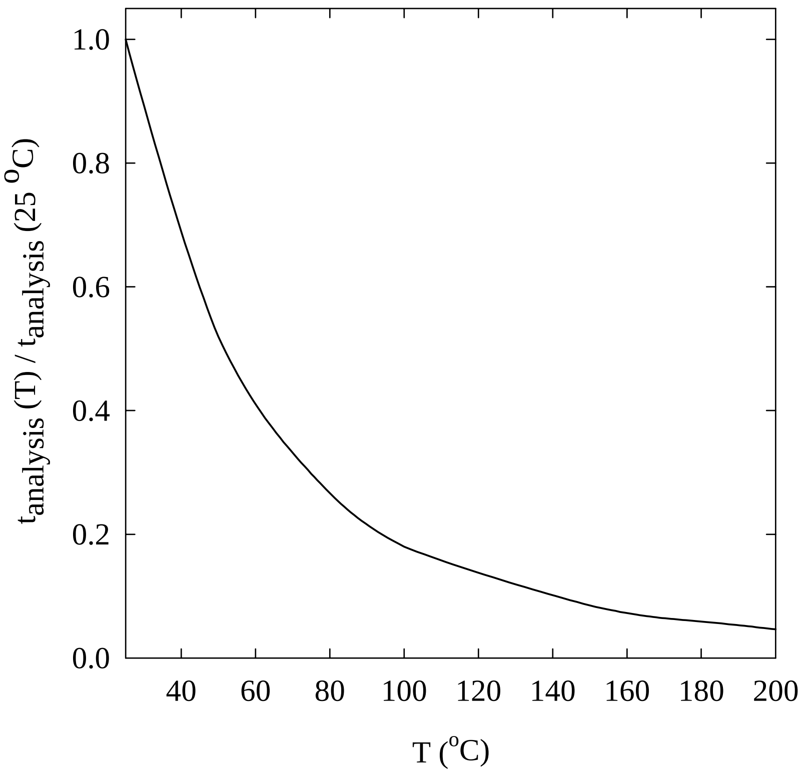
Applications:

- Affects speed - UFHTLC
- Affects selectivity – T^3C

Limitations:

- Stationary phase stability
- Analyte stability
- Thermal mismatch broadening

Ultra-Fast High Temperature Liquid Chromatography (UFHTLC) - Effect of Temperature on *Analysis Time*



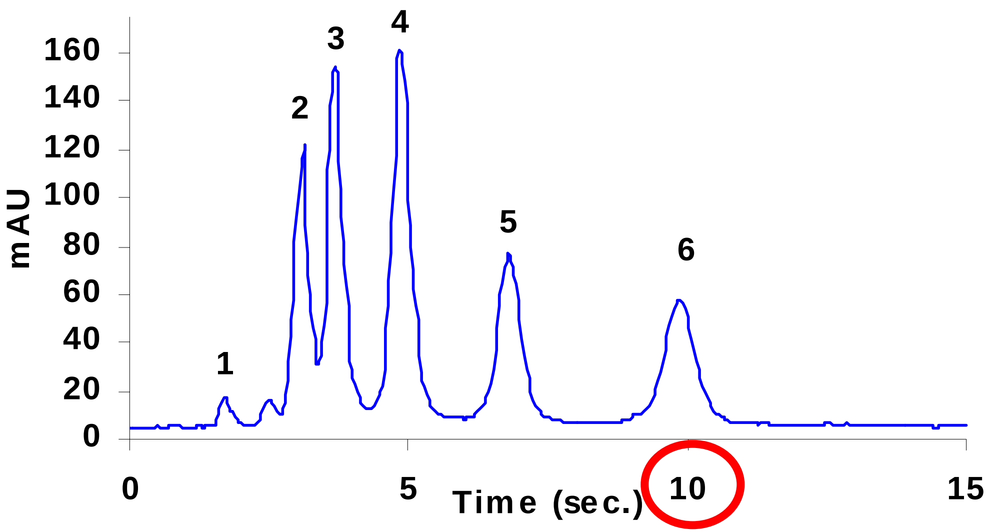
"High-Performance Liquid Chromatography at Elevated Temperatures: Examination of Condition for the Rapid Separation of Large Molecules", R. D. Antia and Cs. Horvath, *J. Chromatogr*., 435, 1-15 (1988).

$$\left.\frac{t}{N}\right|_{\frac{h}{\nu}\to A\nu^{-2/3}} \propto (1+k')\,\frac{L^{2/3}}{\Delta P_{\max}^{2/3}}\,\frac{\eta}{T^{1/3}}$$

Applications of UFHTLC

- Dramatically increases throughput for routine analyses, decreasing total analysis cost
- Increase screening rate in combinatorial chemistry (speed up LC side of LC-MS)
- Make 2D-HPLC practical and thus greatly enhance resolving power of HPLC

Ultra-Fast Separation of Alkylphenones



$u = 3.3$ cm/s ($\nu = 150$)

$k'_{max} = 4.6$

$N = 1400$

$n_c = 8$

$\Delta P = 300$ bar

Column: 50 mm x 2.1 mm i.d. PBD-C-ZrO_2

Temperature: 150 °C

Flow rate: 4.75 ml/min.

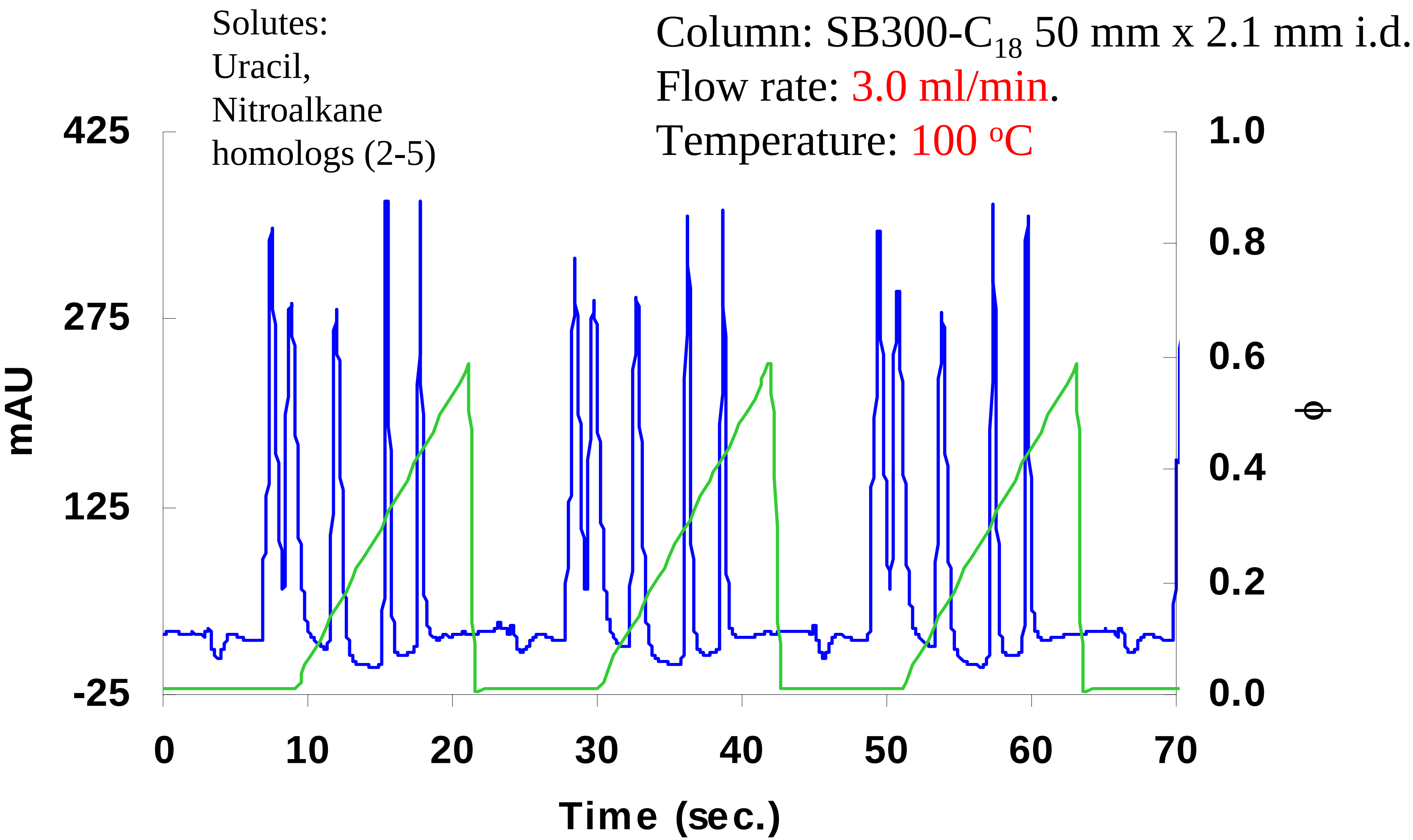
Injection volume: 1 μl

Detection at 254 nm with 6 μl flow cell and 50 ms detector response time

Solutes: Acetone, propiophenone, butyrophenone, valerophenone, hexanophenone, and heptanophenone

World's Fastest Gradient Elution RPLC

Gradient from 0-100% B in 21 seconds



Gradient Conditions

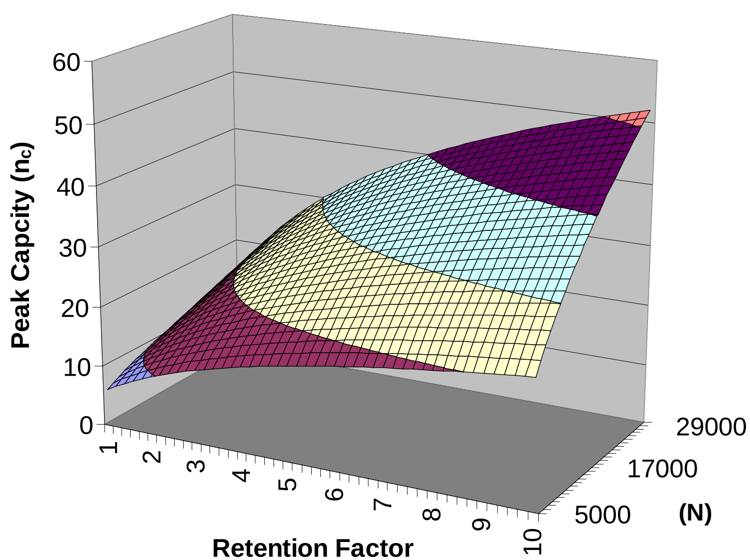
A: 0.1% Trifluroacetic acid in water; B: 0.1% Trifluroacetic acid in ACN

Gradient from 0-100% B in 21 seconds

Fast, Comprehensive Two-Dimensional HPLC – An Approach to Dramatically *Increasing the Resolving Power* of HPLC

One-dimensional separations in HPLC are limited by low peak capacity

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



Comprehensive two-dimensional HPLC can dramatically increase total peak capacity

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

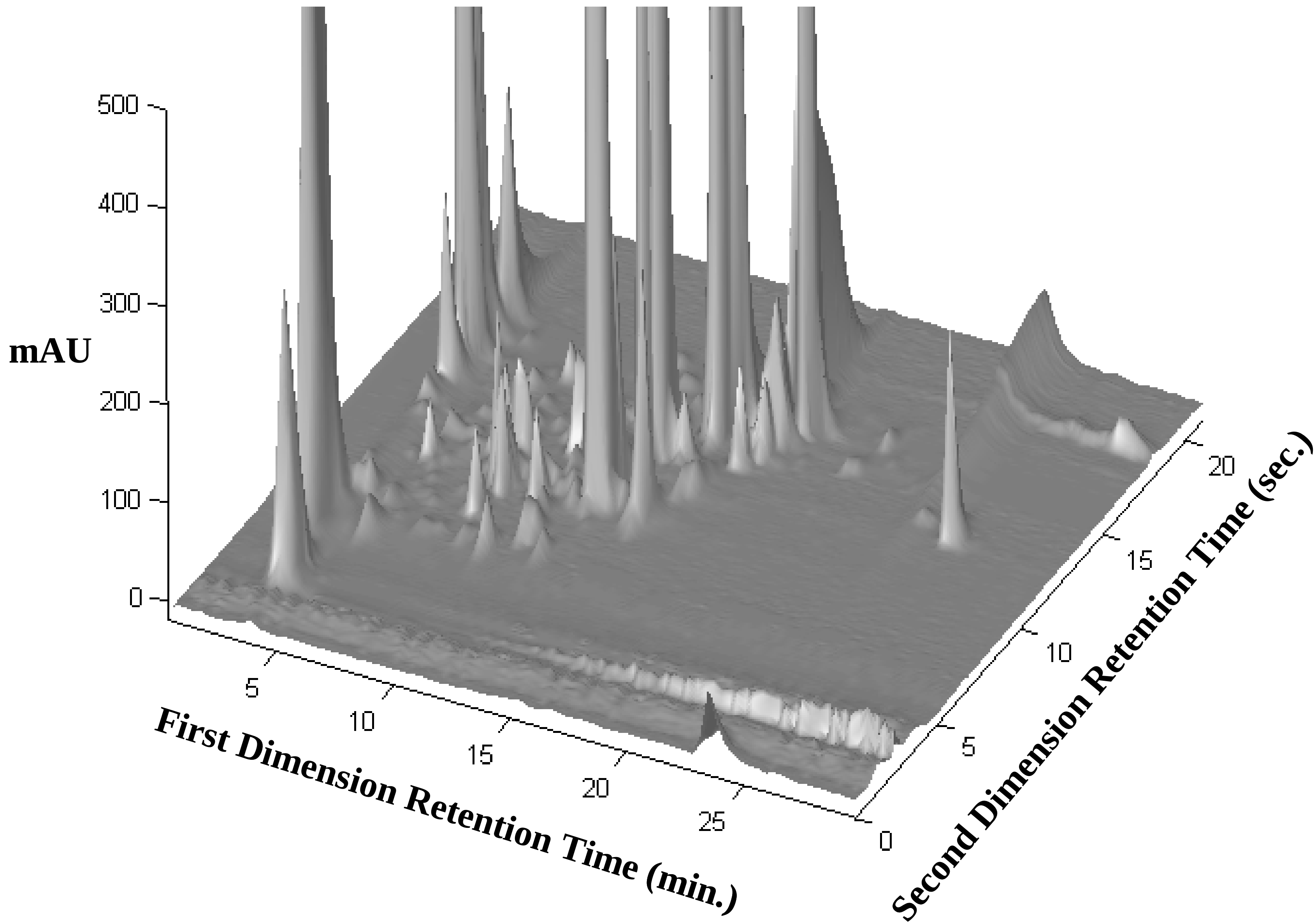
A major limitation, however, is the slow speed, which is related to the second dimension linear velocity, u_2

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

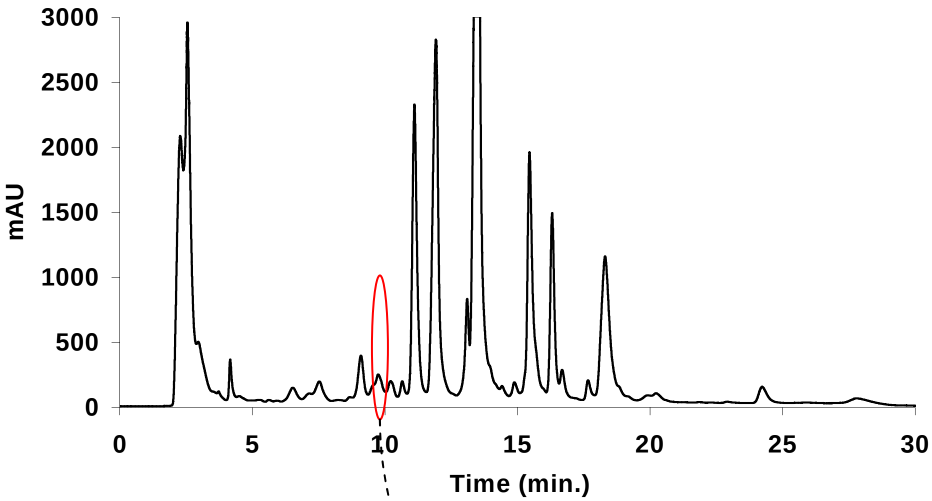
Giddings, J. C. *Multidimensional Chromatography: Techniques and Applications*; Marcel Dekker: New York, 1990

2DLC Separation of Corn Seedling Extract

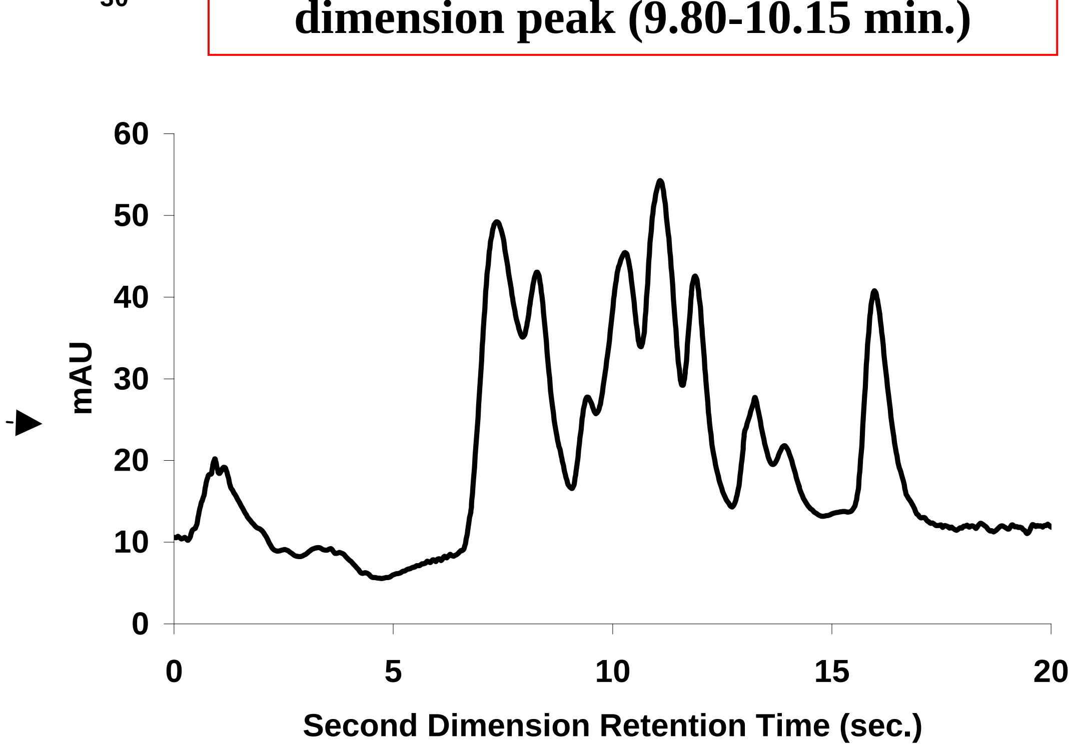
> 200 Peaks in 30min



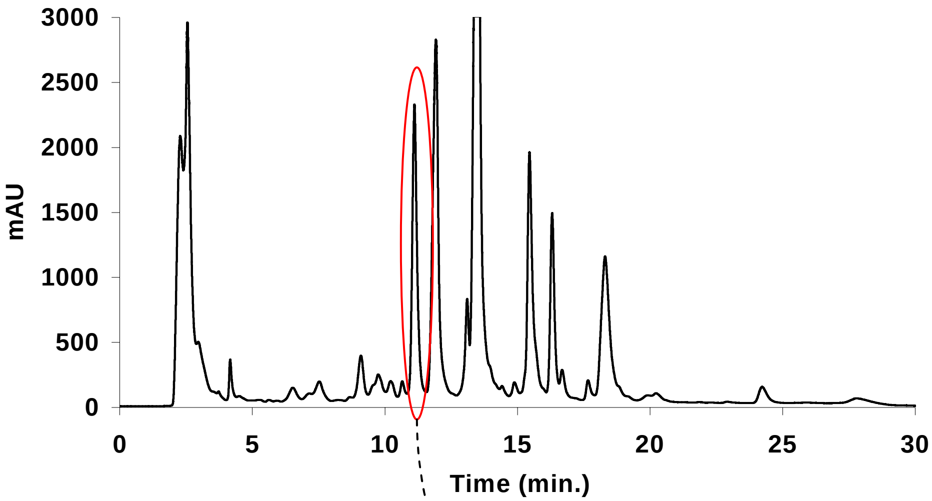
Two Dimensional Separations are Rich with Information



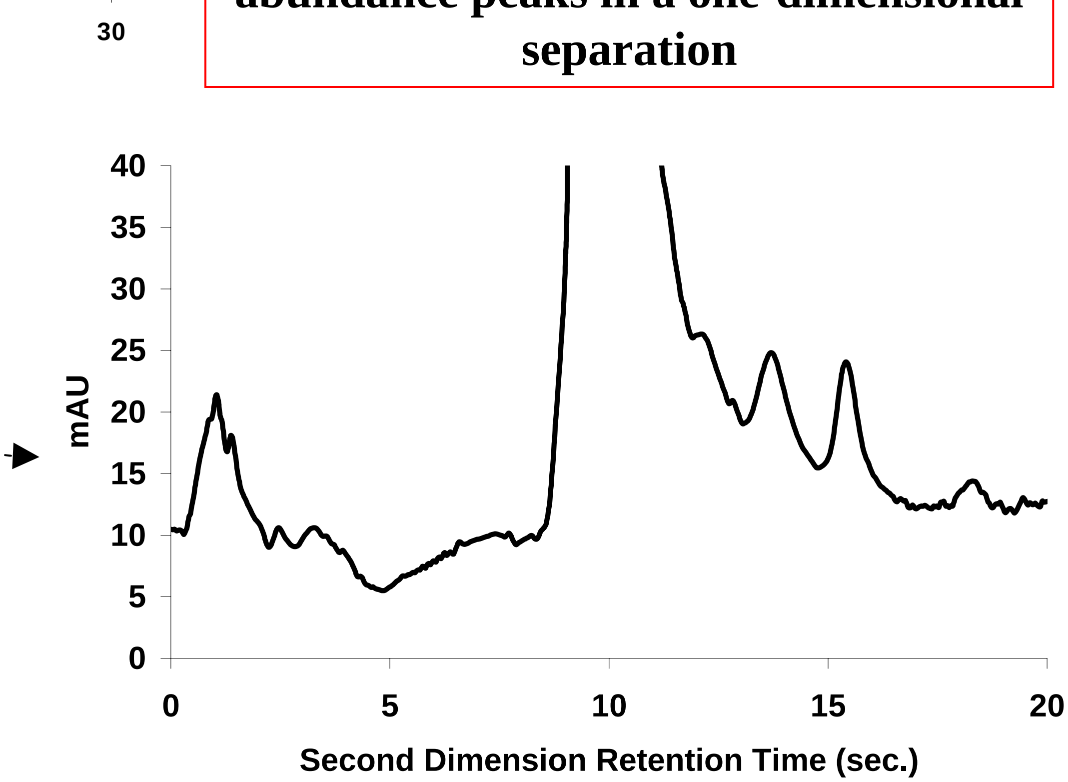
At least nine peaks are observed in the second dimension from single first dimension peak (9.80-10.15 min.)



Increased Peak Capacity Begins to Mitigate the Dynamic Range Problem that Plagues Bioanalytical Separations

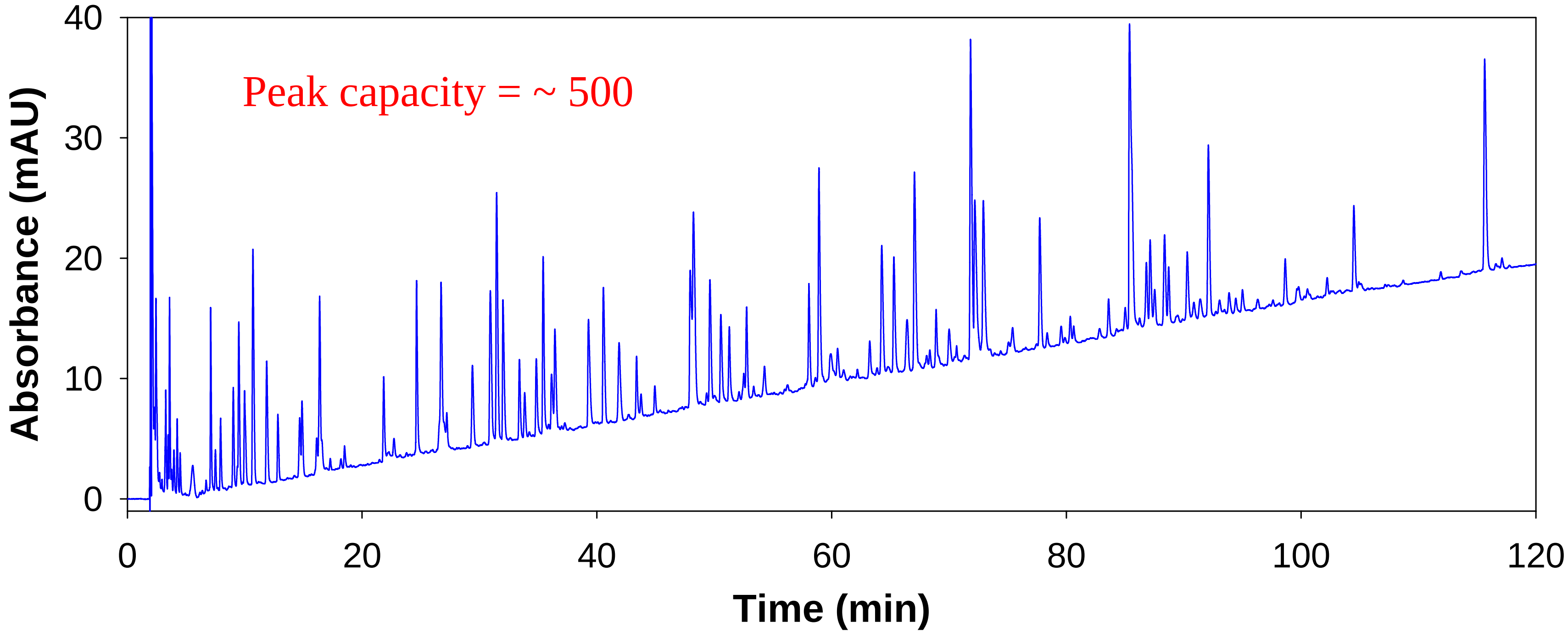


Several low abundance species are detected in the 2DLC separation that would otherwise be obscured by high abundance peaks in a one-dimensional separation



Optimized Peak Capacity Production in Gradient Elution Separation of Peptides Separation

Retention window = 180.8 min, Average peak width = 0.231 min



Chromatographic Conditions:

Five Poroshell 300SB-C18 columns connected in series, 2.1mm i.d., 5µm, L = 60 cm

Solvent A: 0.1% TFA in H_2O, Solvent B: 0.1% TFA in 80:20 ACN:H_2O

Gradient: 0 – 40 – 100 – 0 %B at 0 – 120 – 160 – 200 min, Pressure = 315 bar

0.50 mL/min, 70 ^{o}C, 5 µL injection, 13 µL flow cell, 214 nm, HP 1100

Conclusions

- Highly crosslinked silica phases are VASTLY more stable in acid media than sterically protected ODS phase
- UFHTLC dramatically increases throughput for routine analyses, decreasing total analysis time and cost
- Fast gradient elution allows **rapid identification**
- LC × UFHTLC is a very useful approach to enhance the peak capacity of HPLC