High Speed 2D-HPLC Through the Use of Ultra-Fast High Temperature HPLC as the Second Dimension

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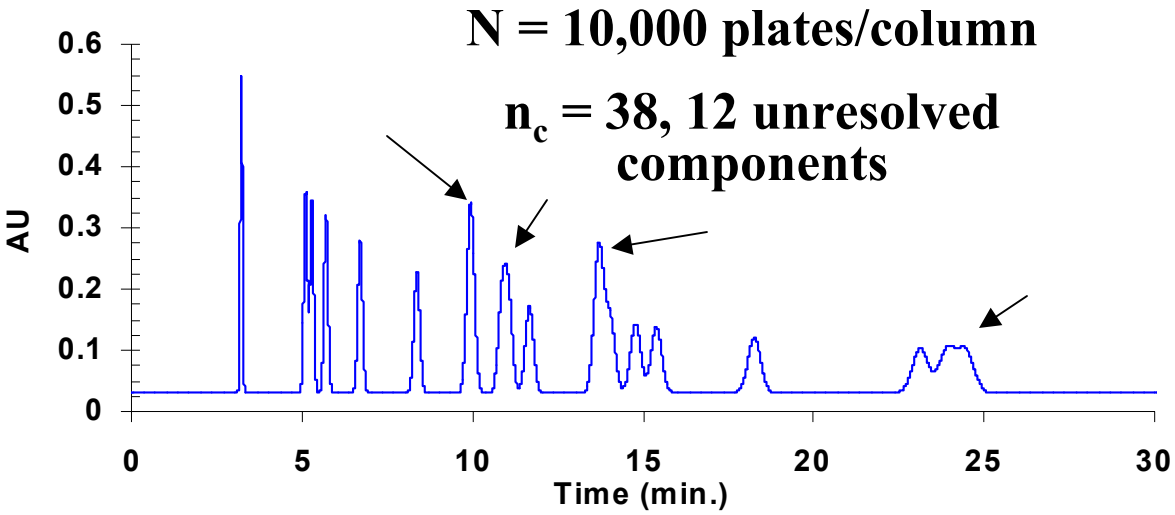
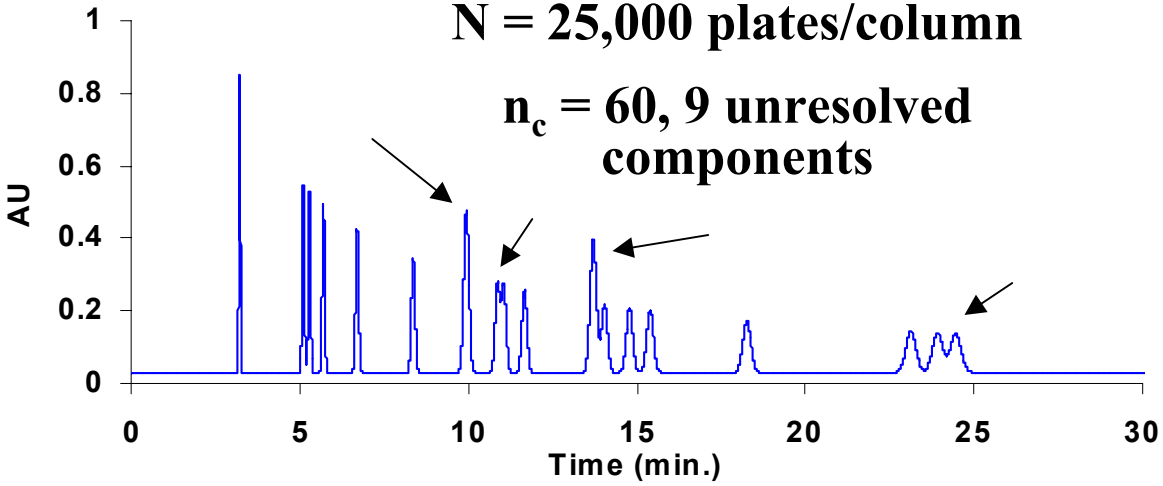
A Common Problem in HPLC

Sample composed of 20 components with random k' values

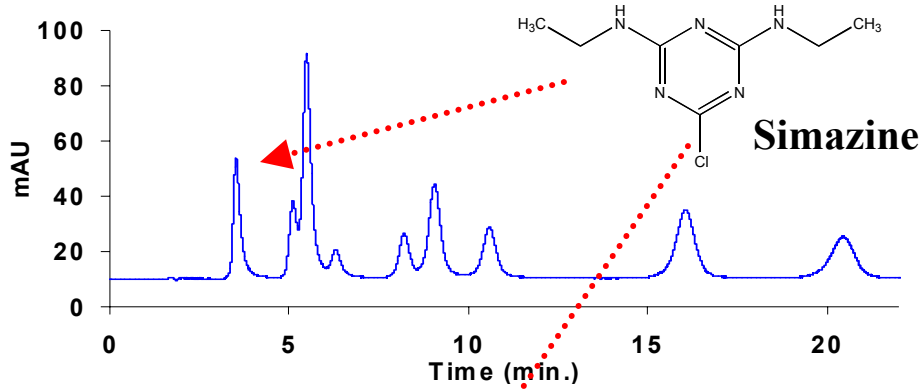
150 mm x 4.6 mm i.d. column

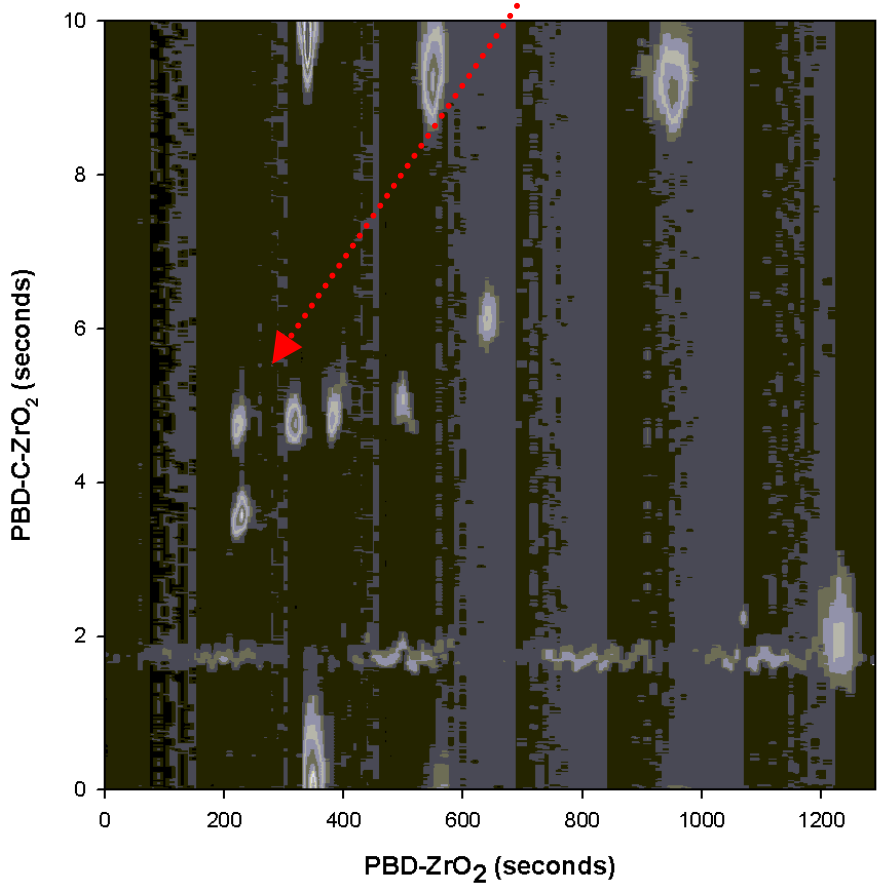


Even with state-of-the-art HPLC, only half of the components in this sample can be resolved !!!



LC × UFHTLC Separation of Ten Triazine Herbicides





1st Dimension Conditions: Column, 50 mm x 2.1 mm i.d. PBD-ZrO_2; Mobile phase, 20/80 ACN/Water; Flow rate, 0.08 ml/min.; Injection volume, 20 µl; Temperature, 40 °C

2nd Dimension Conditions: Column, 50 mm x 2.1 mm i.d. PBD-C-ZrO_2; Mobile phase, 20/80 ACN/Water; Flow rate, 7.0 ml/min.; Injection volume, 15 µl; Temperature, 150 °C; 1st dimension sampling frequency, 0.1 Hz

Total LC × UFHTLC peak capacity = 185

Using a single column, it would take a 2.5 meter long column and 44 hours to generate the same peak capacity

Outline

- **Background**
  - Limitations of one-dimensional HPLC (1DLC)
  - Requirements and advantages in two-dimensional HPLC (2DLC)
  - Improving 2DLC by applying UFHTLC (Ultra Fast High Temperature Liquid Chromatography) to the second dimension separation

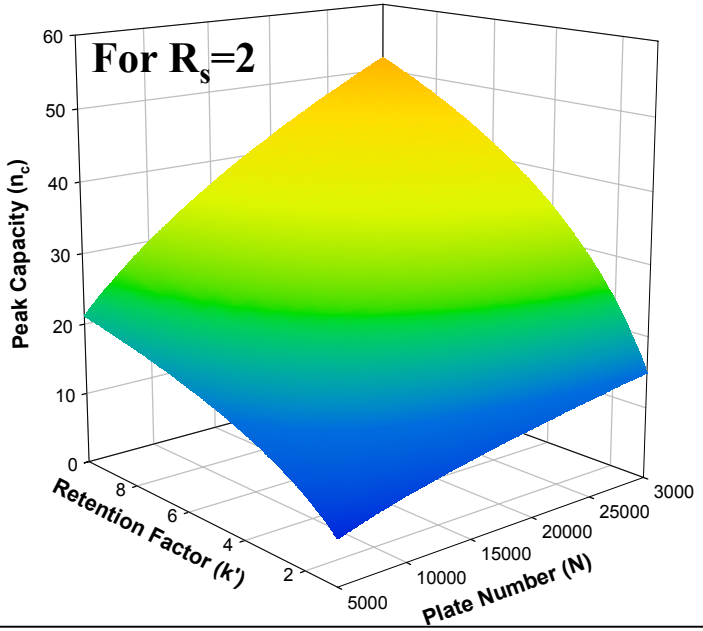
- **Results**
  - Assembly of a preliminary LC × UFHTLC instrument
  - Fast 1DLC separations using narrow-bore columns
  - A preliminary, fast LC × UFHTLC separation

- **Conclusion** – Implementation of UFHTLC in the second dimension separation of 2DLC will ***increase*** both the ***practical limit*** and the ***rate*** of peak capacity production in HPLC.

Limitations of One-Dimensional HPLC

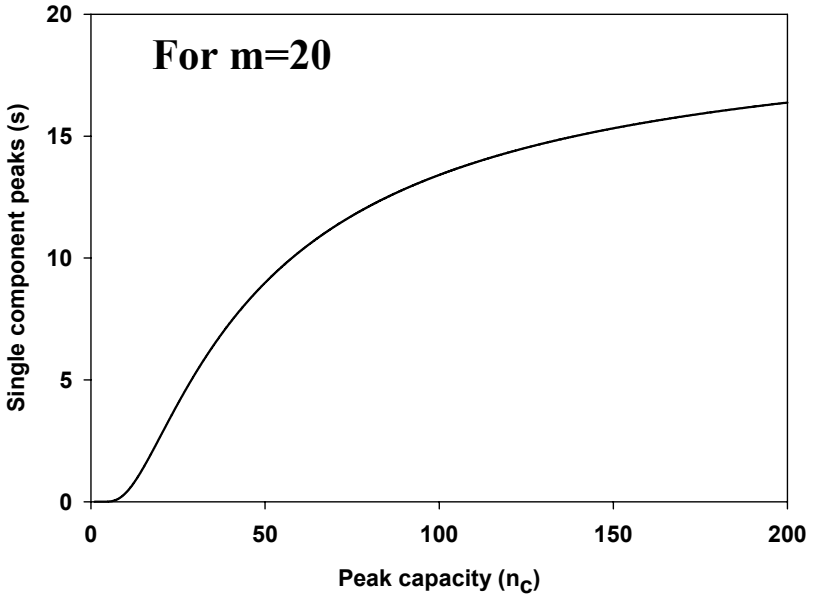
#1a - Low peak capacity

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



#1b – The number of components observable as ***single*** peaks is even lower

$$s = me^{-2m/n_c}$$



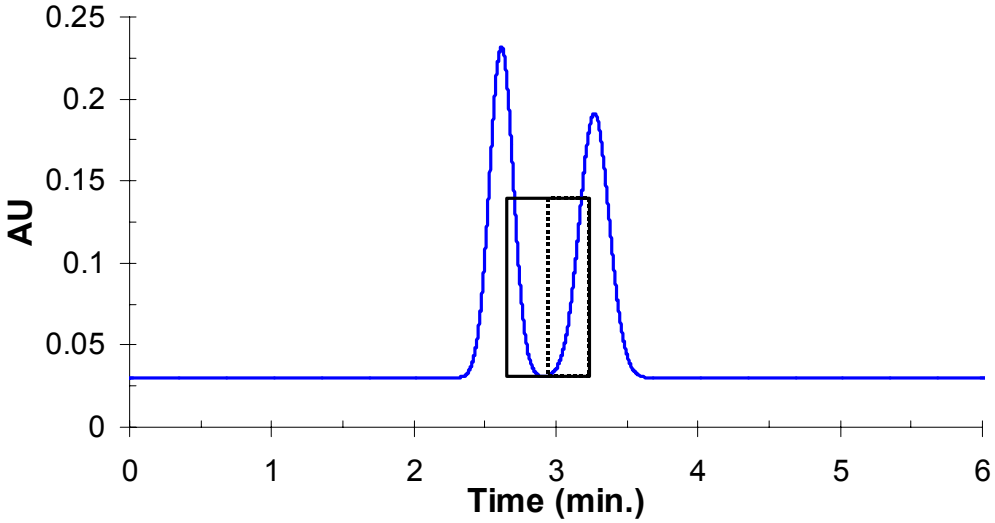
Comprehensive two-dimensional HPLC is the most efficient way to greatly increase the peak capacity of HPLC

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

Requirements and Advantages in Two-Dimensional HPLC

Two conditions must be met for the technique to be considered "two-dimensional"

1. Orthogonality of separation mechanisms – This is a requirement imposed on the stationary phase chemistry
2. Separation gained in one dimension cannot be diminished by separation in the other dimension



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

If these two conditions are satisfied, the maximum total peak capacity of the two-dimensional system is:

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

Murphy, R. E.; M. R. Schure; J. P. Foley *Anal. Chem.*, 1998; Vol. 70, pp 1585-1594

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

Comparison of Peak Capacity Production

Technique	Peak Capacity Limit (n_c)	Analysis Time (hr)	Peak Capacity Production (n_c/hr)
Capillary GC	10^3	10^0-10^1	10^2
GC x GC	10^4-10^5	10^1	10^3-10^4
HPLC	10^2-10^3	10^0-10^1	10^1-10^2
LC x LC	10^3-10^4	**10^1-10^2**	10^2
LC x UFHTLC	10^3-10^4	**10^0-10^1 ??**	**10^3 ??**
2D-Gel Electrophoresis	10^3-10^4	10^2	10^1-10^2

Goal: **To increase the speed of peak capacity production in HPLC such that 10-20-fold increases in peak capacity can be achieved for separations under 60 minutes**

Potential Approaches to Improving the Speed of HPLC

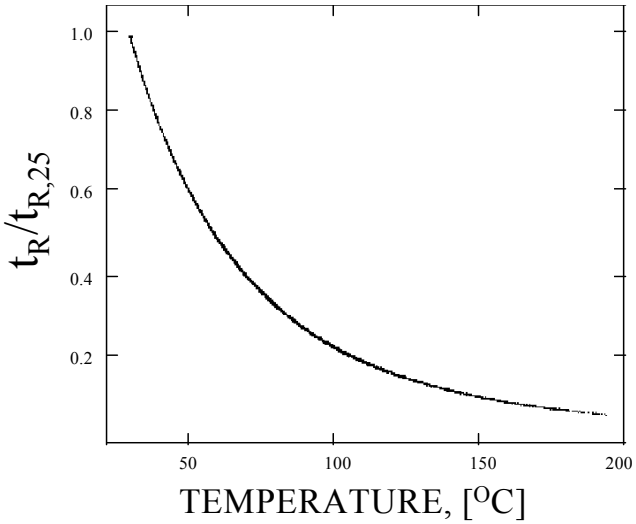
Approach	Advantage	Disadvantage
Shorter Columns	Works with most equipment, stationary phases	Low plate count and resolution
Monolithic Columns	Low backpressure	Narrow-bore columns are not available, high sovent useage
Ultra-High Pressure LC	High plate counts with small particles	Specialized equipment needed, losses in N at high velocity
High Temperature LC	Low backpressure, high efficiency at high velocity	Requires adequate heating, stable phases, stable analytes.

High temperature LC* is the only approach that allows a significant fraction of the column plate count to be retained as the column linear velocity is increased to values that allow *significantly faster HPLC

Improving Two-Dimensional HPLC by Applying UFHTLC to the Second Dimension Separation

#1 – High column temperatures dramatically lower eluent vicosities allowing higher column linear velocities

#2 – High column temperatures dramatically increase analyte diffusivity in the eluent producing more efficient separations at high linear velocities



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

Antia, F. D.; C. Horvath In *J. Chromatogr.*, 1988; Vol. 435, pp 1-15

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Developing Instrumentation: Guidelines for System Parameters in LC × UFHTLC

Target second dimension peak capacity = 20 (within 10 seconds)

Theoretical work by Thompson suggests the 2.1 mm column diameter is most suitable for UFHTLC

	Flow rate required (ml/min.)		
	Column Inner Diameter (mm)		
u_e (cm/s)	1.0	2.1	4.6
0.1	0.02	0.08	0.38
1.0	0.18	0.79	3.8
10.0	1.8	7.9	38

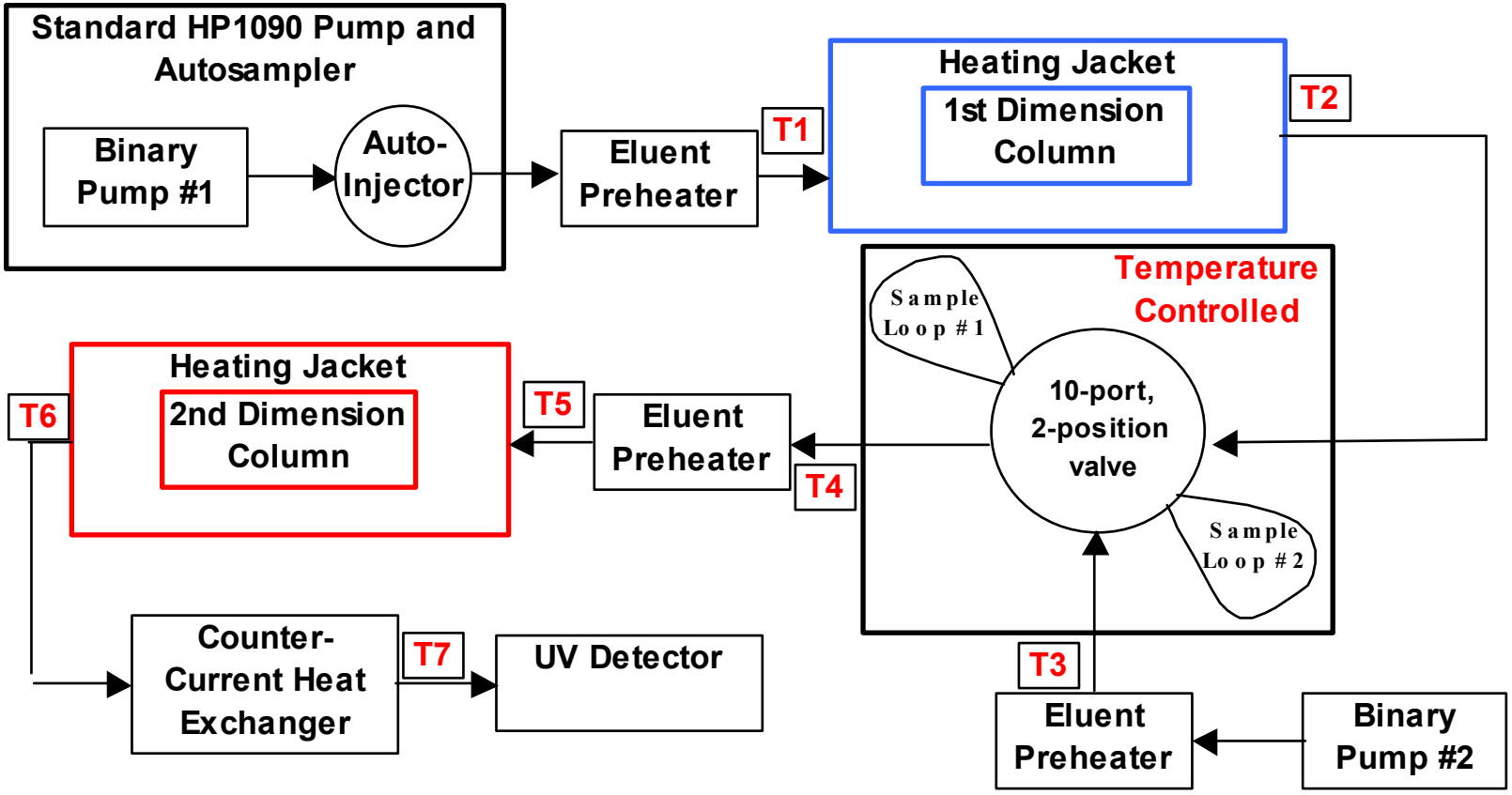
For a maximum of 10% decrease in column efficiency due to extra-column broadening:

Column Temperature (°C)	40[a,b]		150[c,d]	
Retention Factor	1	5	1	5
$V_{injection}$ (μl)	3.8	11.4	5.1	15.4
$V_{detector}$ (μl)	3.8	11.4	5.1	15.4
Detector response time (s)	0.3	1.0	0.02	0.05
$L_{connecting\ tubing}$ (cm)[e]	17.2	155	1.3	11.3

a. hypothetical van Deemter coefficients are A=1.5, B=5, C=.03
b. assumes flow rate of 0.2 ml/min.
c. hypothetical van Deemter coefficients are A=1.5, B=5, C=.0075
d. assumes flow rate of 5.0 ml/min.
e. hypothetical diffusion coefficient = 1.0 x 10^{-5} cm^2/s at 30 °C

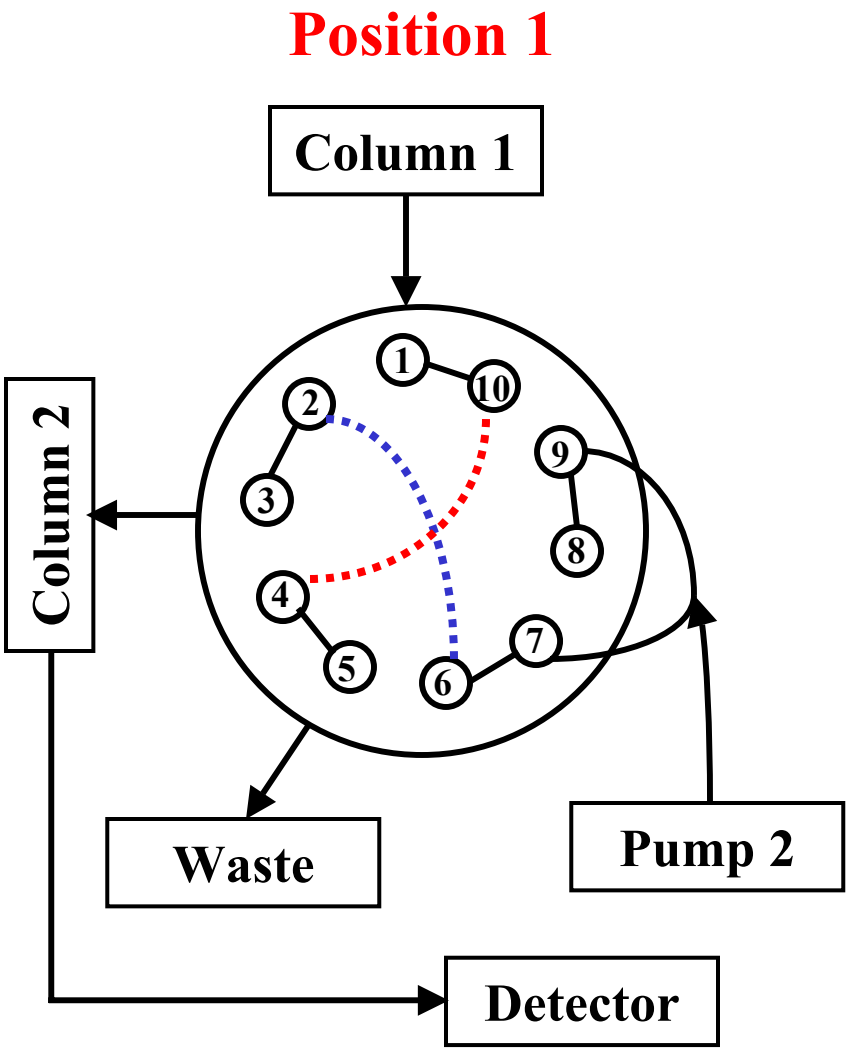
Thompson, J. D.; Carr, P. W. *Analytical Chemistry* **2002**, *74*, 4150-4159

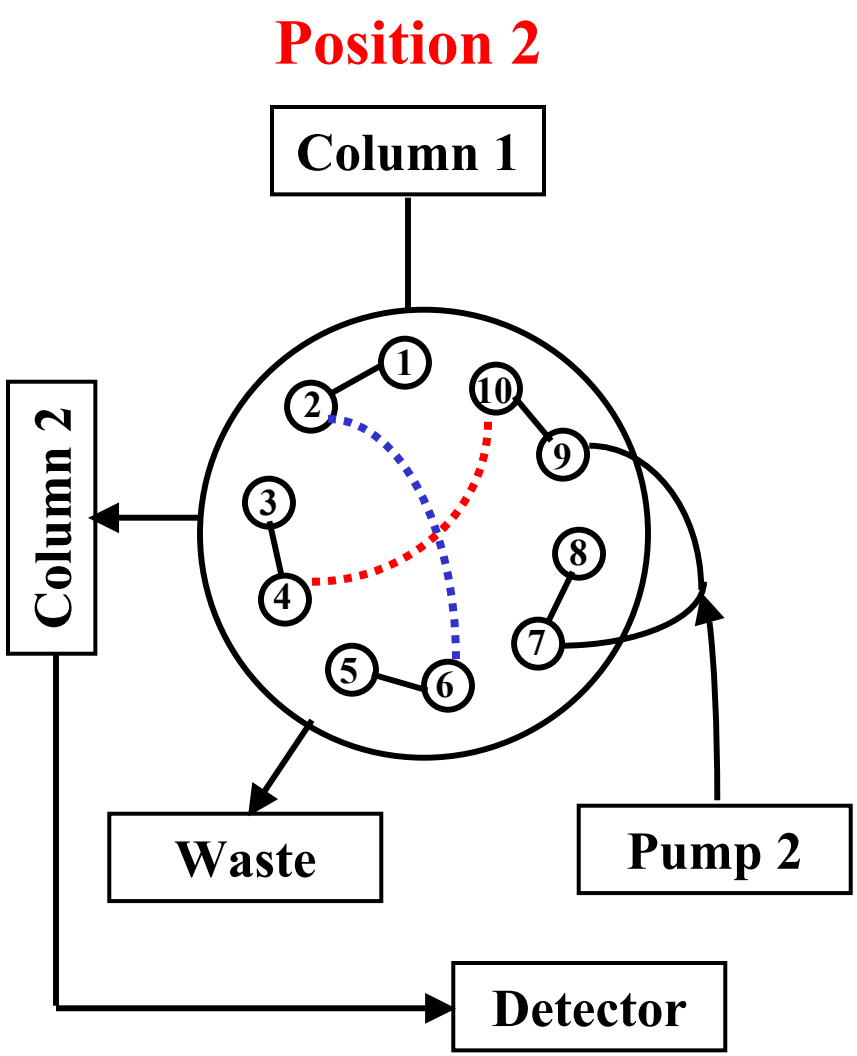
Schematic of a Complete LC × UFHTLC System



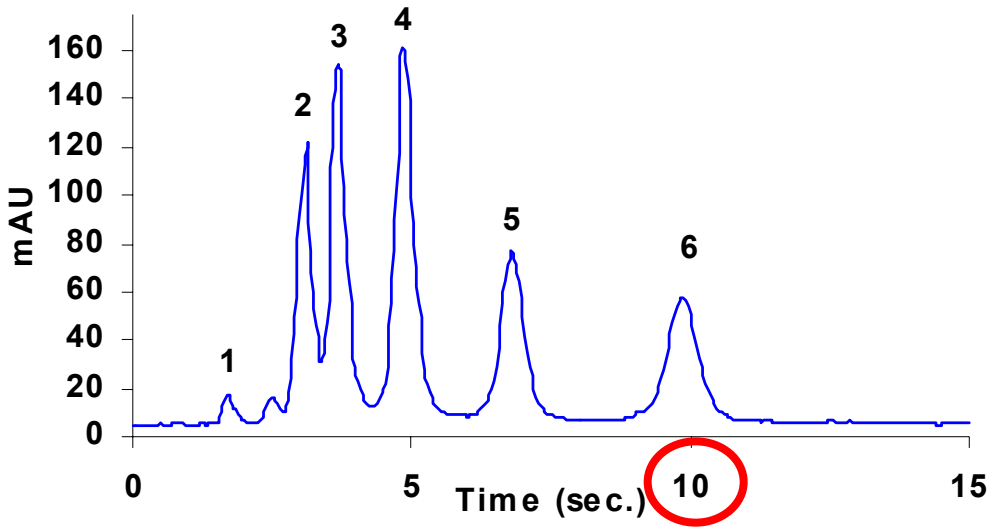
All components are controlled using Labview

Developing Instrumentation - Sample Transfer in 2DLC





A Very Fast One-Dimensional Separation



$u_e = 6.0$ cm/s

$k'_{max} = 4.6$

$N = 1400$

$n_c = 8$

$\Delta P = 300$ 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

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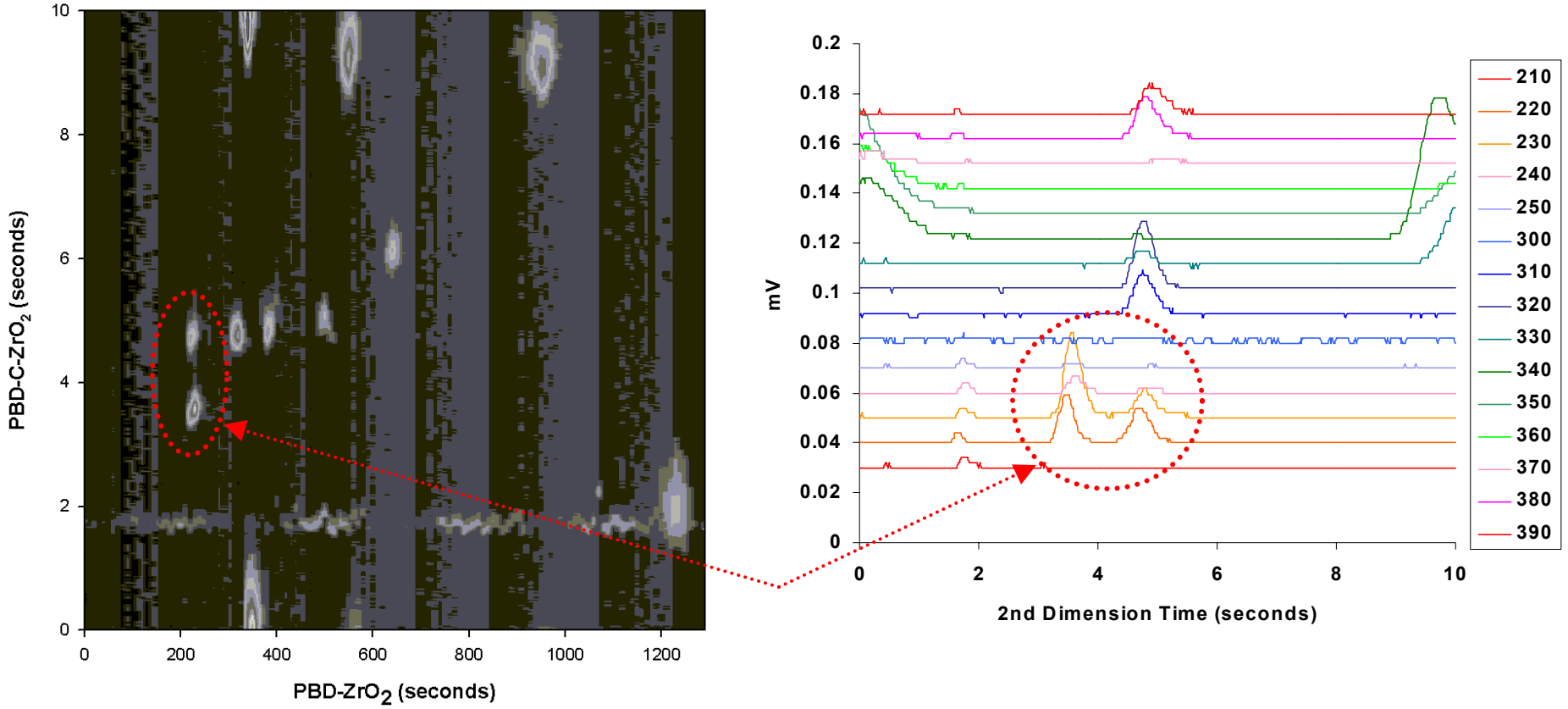

Conclusions

- Implementation of UFHTLC in the second dimension separation of 2DLC will allow comprehensive 2DLC separations on a practical timescale. This will…
  - **Extend** the peak capacity **limit** of practical HPLC
  - **Increase** the **rate** of peak capacity production in HPLC

- The dramatically increased peak capacity will allow faster separations of complex pharmaceutical and biological samples

Acknowledgements

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Systec Inc.