Selerity Technologies Inc.

Convert Your Existing HPLC for High Throughput Separations

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Abstract

There is great demand for increased productivity in analytical laboratories, particularly in the pharmaceutical industry. Faster methods are desired so that an increased number of compounds can be characterized and quantitated using existing resources. High throughput analysis in drug discovery requires the analysis of thousands of compounds. The key to achieving quality high-speed separations is to maintain resolution as the flow rate is increased. When developing high-speed separation methods, if resolution is not maintained as retention time is reduced, no improvement in productivity is realized. Productivity is proportional to resolution per unit time. Maximizing productivity while simultaneously maintaining resolution and minimizing retention time requires that peak width also be minimized. This reduction in peak width is achieved by optimizing several parameters at higher linear velocities, as well as making simple modifications to the HPLC system. The parameters that need to be optimized are: stationary phase particle size and column dimensions, stationary phase and mobile phase choice, system dwell volume, flow rate, and temperature. This poster outlines an easy to follow "recipe" for performing high-throughput HPLC separations with existing, conventional HPLC equipment, without a loss of peak resolution.

Introduction

High Throughput Separation Parameters

As the demands of the separation scientist's time increase, fast, quality analyses become intrinsically more important. Optimized parameters enable the chemist to perform existing work faster, can find information otherwise missed, and impacts the productivity of the laboratory and shortens the turnaround delay on time sensitive samples.

It is not enough just to increase the mobile phase flow rate. There are several other parameters involved in boosting resolution of the separation without compromising speed. If approached in a systematic way, the process is easy to follow, allowing ready solutions to existing and upcoming analytical challenges using your existing HPLC system.

Although optimizing the separation parameters are the most important aspect in achieving the high throughput separation goal, the HPLC system hardware must first be optimized to recognize the improvements. Therefore, optimized plumbing diagrams and mobile phase preheating are presented.

Theory

The resolution (R_S) of any two peaks in a chromatogram can be changed by varying the conditions of the separation. The parameters that are directly related to the separation conditions are: selectivity, width and retention. The peak retention time t_r must be reduced to shorten the analysis but not at the expense of resolution or no improvement in productivity is realized (productivity is proportional to resolution per unit time).

Maximizing productivity (R_S/t_r), while minimizing t_r, requires that peak width (w) also be minimized.

$R_S = 2(t_{r2}-t_{r1}) / (w_1 + w_2)$

This is done by focusing on:

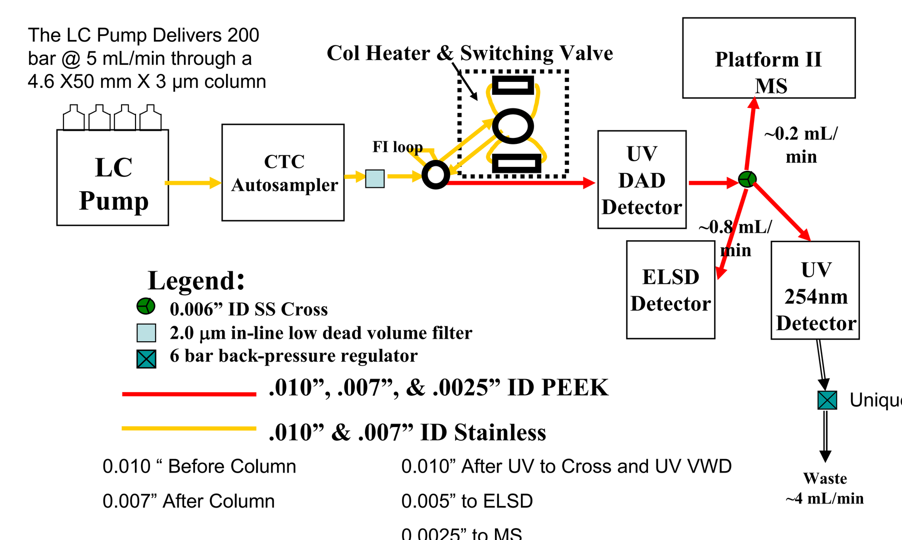
- •Reducing system volume
- •Optimizing temperature
- •Reducing particle size[1]
- •Increasing flow rate

It becomes obvious to the chromatographer that pressure will become the limiting factor when changing these parameters. However, these limitations can be reduced by slight increases in temperature if applied efficiently, and augmented by choice of an optimized column format yielding lower pressure losses.

Steps to Optimization

Step 1: Optimize System Plumbing

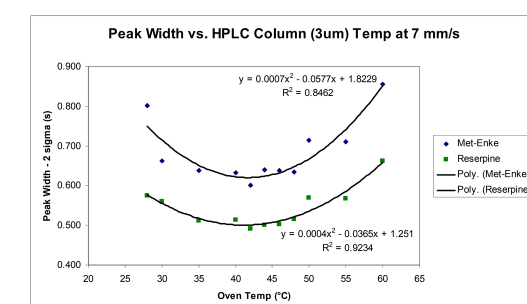
(The System Used Here Is More Than 10 Years Old)



Step2: Use Active Mobile Phase Preheating

- Stand Alone Mobile Phase Preater, capable of efficiently preheating the mobile phase on any HPLC system in only 2.25" length

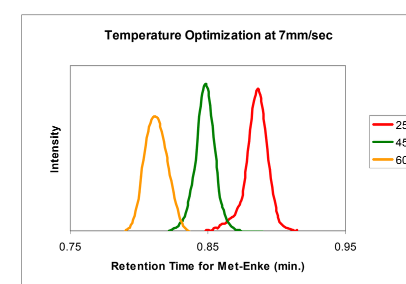
Selerity Calorametherm Pre-Heater: non-invasive and easy to use



Temperature optimization curves at 5 mL/min (7mm/s) through 4.6 mm ID column with 3 µm particles.

Temperature Optimization

- Temperature optimization at 7 mm/s ≈ 45°C.
- 7 mm/s = 5 mL/min. through a 4.6 X 50 mm column.
- At 45-50°C, peaks are symmetric with little impact on width.
  - 25°C peak fronting occurs.
  - 60°C peak width increases.
- With an ordinary HPLC system use a flow of 5 mL/min through a 4.6 X 50 mm column with 3-3.5 µm particles.

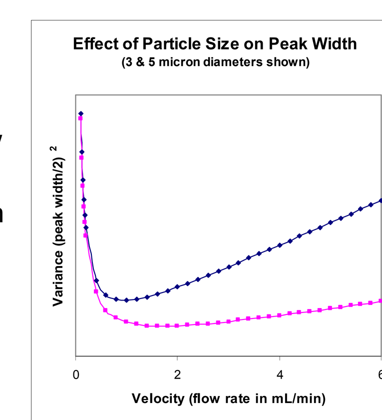


Step 3: Column Dimensions

Smaller particles:

- Narrow peaks under all conditions
- Less broadening of peaks as flow (velocity) is increased
- Same separation is achieved with shorter columns
- Allows shorter columns to reduce retention times (50 mm best compromise)

In practice, particles less than 3 µm do not produce enough reduction in peak width to offset large pressure increases (even when using UHPLC) pressure proportional to $1/d^2$ 3-3.5 µm appears optimal, with 4.6 X 50 mm dimensions



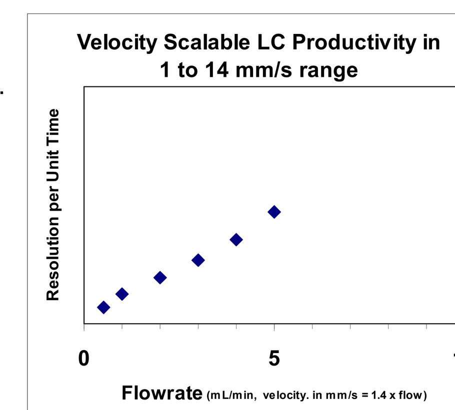
Step 4: Column / Mobile Phase Choice

Column / Mobile Phase Choice

- **None of the other efforts will matter without high quality here.**
- Peak width differences between best/worst columns are at least 5 fold.
- Head to head comparisons are the only way to optimize this parameter. (test new columns frequently and don't believe only one or two tests, seek low pressures).
- 50 mm lengths with 3µm particles balance speed vs. resolution (resolution is similar to 150 mm x 5µm).
- Pick mobile phase for pH control, MS ionization, viscosity, & eluting strength (not to correct poor column performance).
- Good choices:
  - ACN (residue/UV grade → purity) – low viscosity & good eluting strength. B&J solvents were used in this study.
  - High purity CH_3COOH (acid pH) and NH_4COOH (neutral pH) for buffers in ultra high quality H_2O (not bottled) - pH control & MS ionization (not recommended if one must use low λ UV).
  - 3rd + generation high purity silica "well packed & bonded" in 50 mm – 3-3.5 µm columns **(packing consistency & pressure matters).** Inertsil columns were used in the work shown here.
- For standard HPLC: use 4.0 – 4.6 mm ID

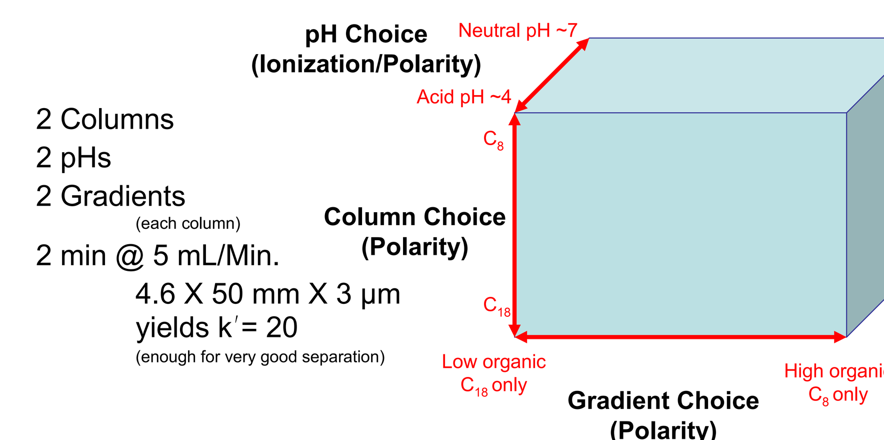
Step 5: Use High Flow

- •HPLC systems can span 0.5 to 5 mL/min without exceeding the 200 to 240 bar (3000 –3600 psi) maximum base pressure.
- •Ideally, the slope equals 1.
- •In reality the slope equals 0.75, using the plumbing described.
- •Can be extended further with higher pressure limits although, high pressure usually comes at the expense of reduced flow rate.
- •5 mL/min. can be operated with the same reliability as 1 mL/min. (1-4 k inj./col.) (>99% uptime on an open access LC/MS)

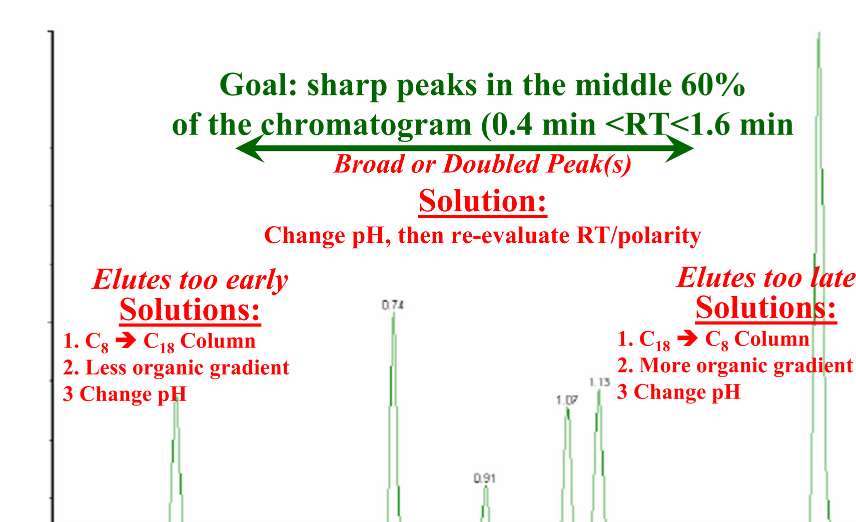


Note: Optimized temperature is required at each flow rate.

Generic Gradient Method Set for Addressing a Wide Range of Compounds

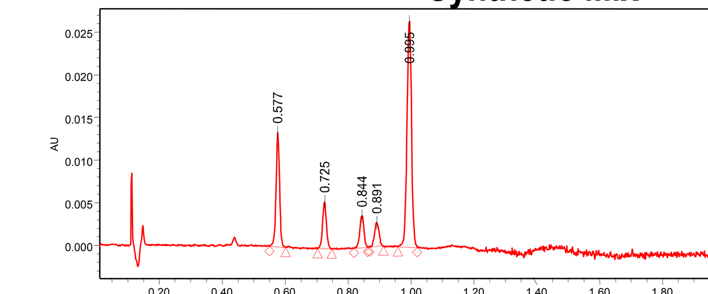


What to do if the First Run Does Not Provide Sufficient Performance



Examples

Synthetic Mix



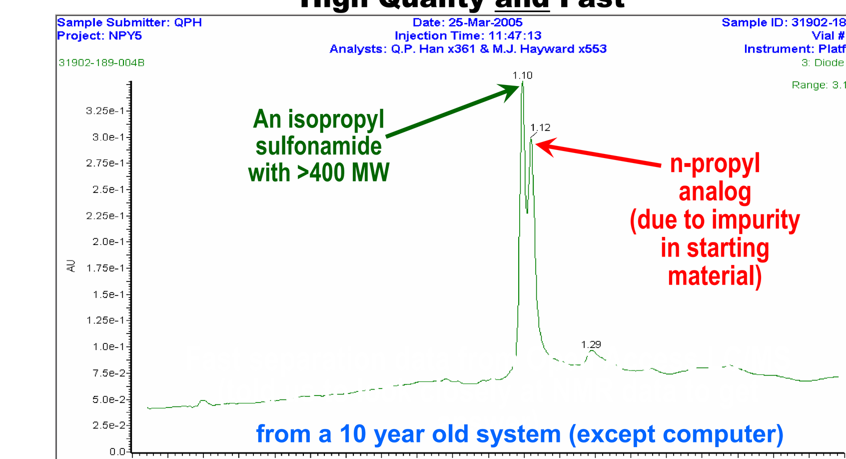
Analysis Conditions:

Fast Gradient
Detector: PDA 254 nm
Velocity: 7mm/sec
Injection Volume: 3 µL

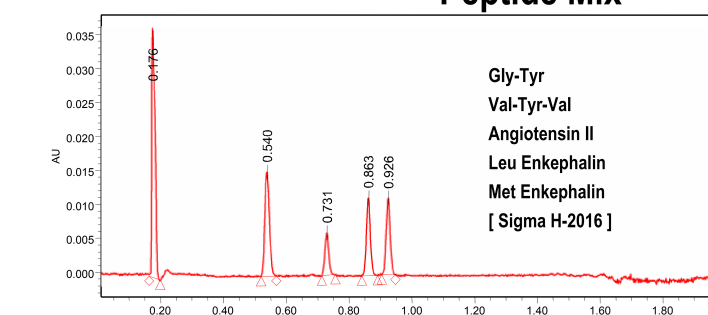
Peak Results

	Name	RT	Area	Height	USP Plate Count	USP Tailing	Width @ 60.7%	Width @ 50%
1		0.577	10942	13522	1.260802e+004	1.000425e+000	1.036469e-002	1.221940e-002
2		0.725	4877	5510	1.656218e+004	9.9558500e-001	1.136114e-002	1.334410e-002
3		0.844	3581	3785	1.837554e+004	9.844788e-001	1.255780e-002	1. 463615e-002
4		0.891	2772	2818	1.885785e+004	9.759476e-001	1.295368e-002	1.522987e-002
5		0.995	26516	26631	2.411252e+004	9.962392e-001	1.281727e-002	1.509342e-002

Example: Compound Purity
High Quality and Fast



Peptide Mix



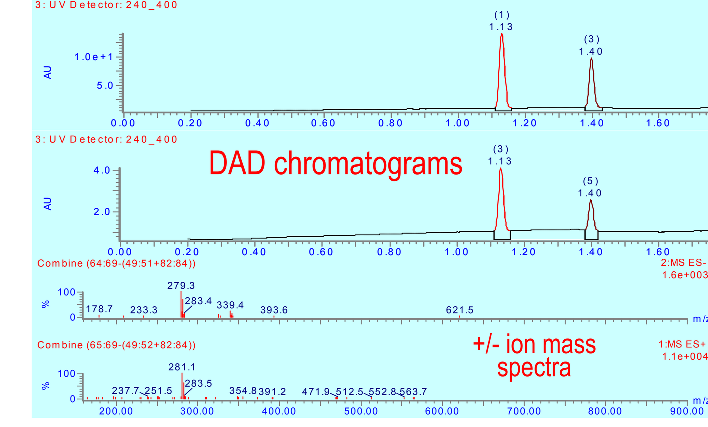
Analysis Conditions:

Fast Gradient
Detector: PDA 276 nm
Velocity: 7mm/sec
Injection Volume: 2 µL

Peak Results

	Name	RT	Area	Height	USP Plate Count	USP Tailing	Width @ 60.7%	Width @ 50%
1		0.176	26527	38203	1.284183e+003	1.835363e+000	1.171566e-002	1.302404e-002
2		0.540	15965	15358	6.121671e+003	1.185411e+000	1.390078e-002	1.638247e-002
3		0.731	5453	6336	1.673794e+004	1.010993e+000	1.125222e-002	1.331217e-002
4		0.863	10234	11491	2.248976e+004	1.074621e+000	1.141224e-002	1.346443e-002
5		0.926	10231	11310	2.377559e+004	1.058166e+000	1.161939e-002	1.384282e-002

Example: Open Access - LC/MS
from a 10 year old system (except computer)



A random synthetic chemistry sample

Same sample 2 weeks later (re-diluted)

Similar results on most any HPLC system.

Conclusions

- UPLC-like performance can be obtained on any LC where 7 mm/s velocity and k's of 10/min are readily achievable.
- 3-3.5 µm particles give the best balance of resolution and pressure drop. Same performance levels as sub 2 µm without the pressure.
- High pressure is not required with 3-3.5 µm particles and thus ordinary LC components can be employed.
- Generic gradient analyses reaching k′ = 20 can be performed in 2 min. with a 2.5 min cycle time using ordinary HPLC instruments.
- Such systems have been proven with many hundreds of thousands of analyses for qualitative and quantitative drug discovery applications.

Reference

[1] Kinetic Conditions for Optimum Speed and Resolution in Column Chromatography, J H Knox and M Saleem, J. Chromatogr. Sci., 7 (1969) 614-622.

Contact Information

We are investigating making optimization kits available to allow conversion of standard HPLCs for high throughput separations. For more information and additional data, please leave your business card or visit www.selerity.com.