HPLC Column Technology: Smaller and Faster

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HPLC 2008

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Outline of Talk*

- Drivers for HPLC Column Improvements
- Approaches to increase throughput in HPLC
  - Small porous particles (10- → 5- → 3- → 2-3- → < 2-µm)
  - Superficially porous** and non-porous particles (40 →1.5- →5- → 2.7-µm)
  - Monoliths
    - Silica-based
    - Polymer-based
  - Brief Comparisons of above
- Parallel LC

* Focus on commercial products, not research products
** Also called pellicular, porous layer beads, fused core

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Drivers for Improvements in HPLC Column Technologies

1. **Productivity Gains (faster separations, rapid method development, automation, time-to-market, QC department—quicker results)**
2. Improvement in Quality of Analysis [reproducible columns, improved recovery (bio-compounds), lower activity (less tailing, especially for basic compounds)]
3. **Cost of Analysis (column lifetime & stability, guard columns, solvent reduction, lower cost solvents, narrow bore and smaller, high throughput LC)**
4. Smaller, more complex samples, trace analysis (proteomics) (nanocolumns, selectivity improvements-specialty columns, 2-D & comprehensive chrom./column switching)
5. **Widespread use of LC-MS & LC/MS-MS (cap/nano columns, short columns, smaller particles, packings with wider range of solvent compatibility, low bleed)**
6. Increasing Importance of Biologically-Derived Molecules (wide pore packings, rugged packings, biocompatible surfaces, inert column materials)
7. **Environmental Reasons; solvent reduction (smaller diameter columns, shorter columns, less toxic solvents, disposal costs)**

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Some Ways to Increase Sample Throughput
(and Resolution)

1) **Shorter column lengths (to reduce analysis time) packed with small porous particles (to maintain resolution). (Reasonable # of plates and reasonable pressure, fast separation)**
2) **Longer column lengths (to increase efficiency) packed with even smaller porous and non-porous particles (to maintain resolution), with the ultimate being the so-called "Ultra-High Pressure LC". (Many plates, fast separation, high pressure)**
3) **Columns packed with various small superficially porous particles (pellicular) particle sizes, pore sizes and phase thickness to allow the rapid resolution of biomolecules such as proteins as well as small molecules. (Large and small molecules, fast separations, lower pressure)**
4) **Columns designed with silica- and polymer-based monolith stationary phase formats (fast separation, low pressure, in-series columns)**
5) **Parallel LC (multiple capillary columns/channels, increased samples/hour)**

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History of Commercial HPLC Particle Development

Year(s) of Acceptance	Particle Size	Most Popular Nominal Size	Plates / 15cm (Approximate)
1950's		100µm	200
1967		50 µm (pellicular)	1,000
1972		10 µm	6,000
1985		5 µm	12,000
1992		3-3.5 µm	22,000
1998*		1.5 µm* (non-porous)	30,000
1999		5.0 µm (pellicular)	8,000**
2000		2.5 µm	25,000
2003		1.8 µm	32,500
2007/2008		2.7 µm (pellicular)	32,000***

* non-porous silica or resins
** 300 A pore for protein MW 5,700

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*** 90-120 A pore

How to go Faster With Minimal Loss of Resolution?

Shorter Columns with Smaller Particles

Plates Selectivity Retention

$$R_s = \frac{\sqrt{N}}{4} \cdot \frac{\alpha-1}{\alpha} \cdot \frac{k'}{k'+1}$$

$$N \propto \frac{L}{d_p}$$

To Maintain Rs:

e.g.: L/2 ⇨ $d_p/2$

- ↓ Column Length = ↓ N
- ↓ Particle Size = ↑ N
- ↓ Particle Size = ↑ P

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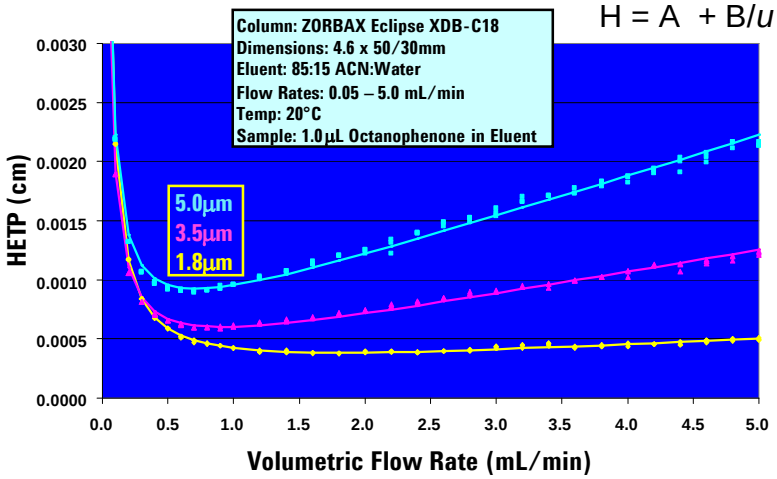
Van Deemter Curve: HETP vs. Volumetric Flow Rate

$H = A + B/u + Cu$



➢Smaller particle sizes have flatter curves, minima shift out slightly

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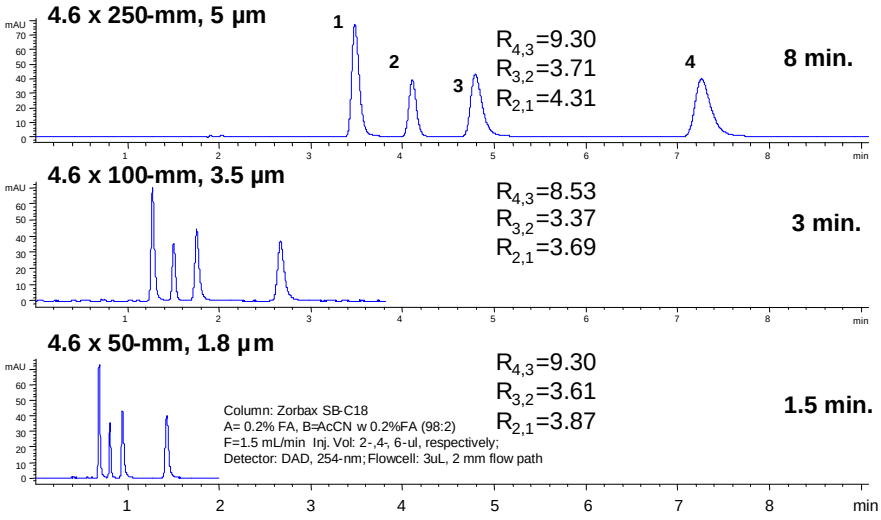
Column Scalability: Change in Column Configuration to Increase Speed While Maintaining Resolution



Solutes:
1-methylxanthine; 2) 1,3-dimethyluric acid; 3) 3,7-dimethylxanthine; 4) 1,7-dimethylxanthine

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But What About Pressure? Pressure Increases Dramatically with Decreasing Particle Size

Equation For Pressure Drop Across An HPLC Column

$$\Delta P = \frac{\eta \cdot L \cdot v}{\theta \cdot d_p^2}$$

- ΔP = Pressure Drop
- η = Fluid Viscosity
- L = Column Length
- v = Flow Velocity
- d_p = Particle Diameter
- θ = Dimensionless Structural Constant of Order 600 For Packed Beds in LC

- ❖ Many parameters influence column pressure
- ❖ But the particle size and column length are most critical
- ❖ Long length and smaller particle size mean more resolution and pressure
- ❖ HPLC systems are now available that can handle these higher pressures

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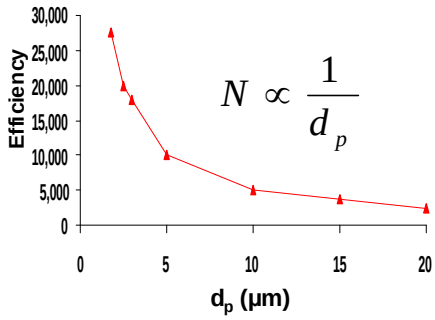
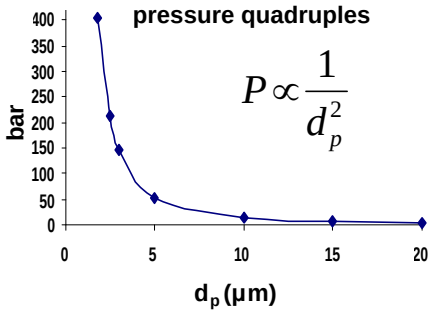
Pressure and Efficiency Vary Inversely with d_P

…but the effect of d_P on pressure is greater

We need to try something more than continue to reduce particle size; monoliths and nonporous particles may play a role.



As efficiency doubles, pressure quadruples



Dr. Richard A. Henry, Supelco, Minn. Chromatography Symp. May, 2008

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Pressures for Various Particle Sizes

$$N \propto \frac{1}{d_p}$$

$$P \propto \frac{1}{d_p^2}$$

$$P_{N_{opt}} \propto \frac{1}{d_p^3}$$

Particle Size	Pressure, psi	Bar	N
1.8	5889	406	27,500
2.5	3089	213	20,000
3	2118	146	16,500
5	769	53	10,000
10	189	13	5,000
15	87	6	3,750
20	44	3	2,500

Dr. Richard A. Henry, Supelco, Minn. Chromatography Symp. May, 2008

100x4.6mm column, 1.0 mL/min, 50/50 Water/ACN, 30

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Commercial Two and Sub-Two Micron Totally Porous HPLC Columns (Pittcon '08)*

Manufacturer	Product Name	Aver. d_p, µm
Agilent	Zorbax Rapid Resolution HT	1.8
Alltech	VisionHT	1.5
Bischoff	ProntoPEARL TPP Ace-EPS	1.8
Macherey-Nagel	Nucleodur	1.8
MicroSolv Technology	Cogent Diamond & Silica-C	1.8
Orachem Technologies	Emerald	1.7
Phenomenex	Luna	2.0
Restek	Pinnacle DB	1.9
Sepax	GP-8 and GP-18	1.7
Shant Laboratories	Pathfinder	1.5
Thermo	Hypersil Gold	1.9
Tosoh Haas	TSKgel SuperODS	2.0
Waters	Acquity BEH	1.7
YMC	Ultra-Fast	2.0

* Non-porous & Superficially Porous Particles Not Included

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Commercial Two to Three Micron Totally Porous HPLC Columns (Pittcon '08)*

Manufacturer	Product Name	Aver. d_P, µm
Fortis Technologies	Fortis	2.1
Phenomenex	Luna HST and Synergi	2.5
Sepax	GP-8 and GP-18	2.2
Shimadzu	XR	2.2
Tosoh Bioscience	TSK-Gel ODS HTP	2.3
Varian	Pursuit UPS and XRS	2.4, 2.8
Waters	XBridge, SunFire	2.5

* Non-porous & Superficially Porous Particles Not Included

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Some Ways to Increase Sample Throughput
(and Resolution)

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Calculating Peak Capacity - Equations

Peak Capacity (Zp) - Isocratic Elution

$$P_c = 1 + \frac{\sqrt{N}}{4R_S} \ln[1+k_{last}]$$

k_{last} :retention factor last peak determines:
N : efficiency
R_s : required resolution (base line separation: Rs→ 1.5)
P_c : peak capacity

Peak Capacity (Zp) - Gradient Elution Equations

Equation 1 used in next examples

1. $$P_C = 1 + \frac{t_g}{\frac{1}{n}\sum_1^n w} = 1 + \frac{t_g}{w_{av}}$$

Pc = peak capacity
tg = gradient time (min)
w = peak width (min)

$t_{R,n}$ = last peak
$t_{R,m}$ = first possible peak (unretained)
$t_{R,1}$ = first actual peak

2. $$P_c = 1 + \frac{t_{R,n} - t_{R,m}}{W_{av}}$$

3. $$P_c = 1 + \frac{t_{R,n} - t_{R,1}}{W_{av}}$$

W impacted by N, which includes column length and particle size

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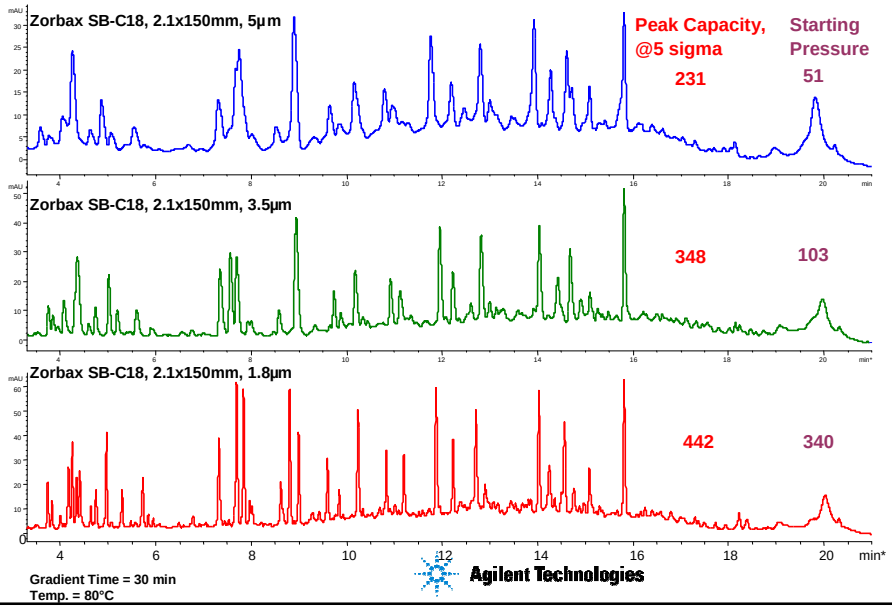
Peptide Map of BSA for Three Different Particle Size Columns



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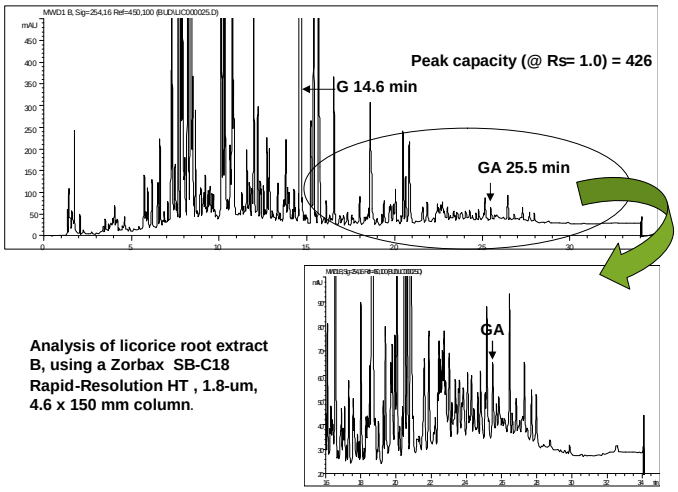
Gradient Reversed-Phase Separation of Licorice Root Extract B on 15 cm, 1.8-µm Column



Analysis of licorice root extract B, using a Zorbax SB-C18 Rapid-Resolution HT , 1.8-um, 4.6 x 150 mm column.

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Optimum Chromatographic Efficiency

What is the optimum flow rate for a small vs. a large molecule?

Assumptions: 4.6mm i.d. Column
300 Angstrom Pore Diameter
5µm Porous Packing, $\varepsilon_e \approx 0.4$
$k \approx 10$, $\nu_{opt} \approx 13$

Small Molecule
MW ≈ 100 g/mol
Dm ≈ 1×10^{-5} cm²/sec
u_e = 0.26 cm/sec
F_{opt} = 1.04 mL/min

$$F_{opt} = \frac{\nu_{opt} D_m \pi r^2 \varepsilon_e}{d_p}$$

Large Molecule
MW ≈ 10,000 g/mol
Dm ≈ 1×10^{-6} cm²/sec
u_e = 0.026 cm/sec
F_{opt} = 0.104 mL/min

Large (e.g., proteins) should be chromatographed at low flow rates (slow analyses) on conventional, totally porous packings for maximum resolution.

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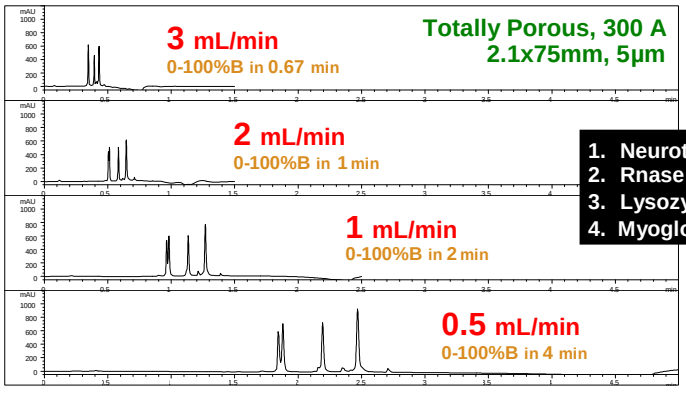
Effect of Increasing Flow Rate in Macromolecule (Protein) Analysis with Use of Totally Porous Silica



AutoBypass; Piston Stroke: 20µL, Temp.: 70° C, Det.: 215 nm
Mobile Phase: A= 95% H_2O, 5% AcN with 0.1%TFA; B= 5% H2O, 95% AcN, with 0.07%TFA

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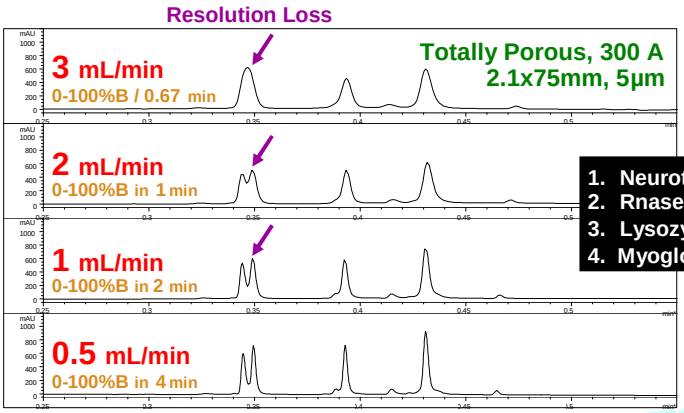
Effect of Increasing Flow Rate in Macromolecule (Protein) Analysis with Use of Totally Porous Silica



Agilent 1100 WPS with AutoBypass; Piston Stroke: 20µL, Temp.: 70°C, Det.: 215 nm
Mobile Phase: A= 95% H_2O, 5% AcN with 0.1%TFA; B= 5% H2O, 95% AcN, with 0.07%TFA

Aligned

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Slower Diffusion of Large Molecules Broadens Peaks at High Flow Rates

Decrease the diffusion time for macromolecules!

How?

- Increase the Diffusion Rate
  - Elevate operating temperature
  - Decrease solvent viscosity
- Decrease the Diffusion Distance
  - Develop very small particles
  - Limit diffusion distance into a particle ← Poroshell Approach

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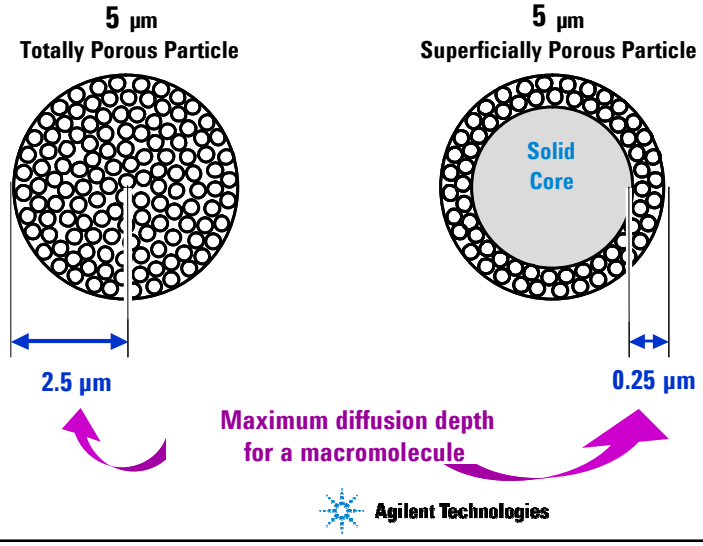
Comparison of Diffusion Distances

Totally porous silica versus superficially porous silica



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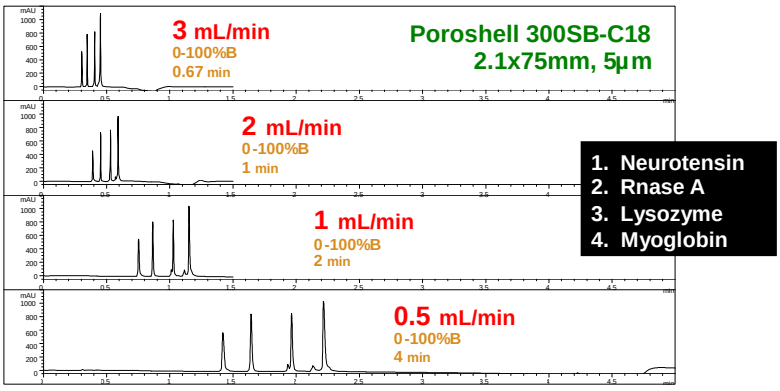
Effect of Increasing Flow Rate in Macromolecule (Protein) Analysis with Use of Superficially Porous Silica



1100 WPS with AutoBypass; Piston Stroke: 20µL, Temp.: 70°C, Det.: 215 nm
Mobile Phase: A= 95% H_2O, 5% AcN with 0.1%TFA; B= 5% H2O, 95% AcN, with 0.07%TFA

5 min

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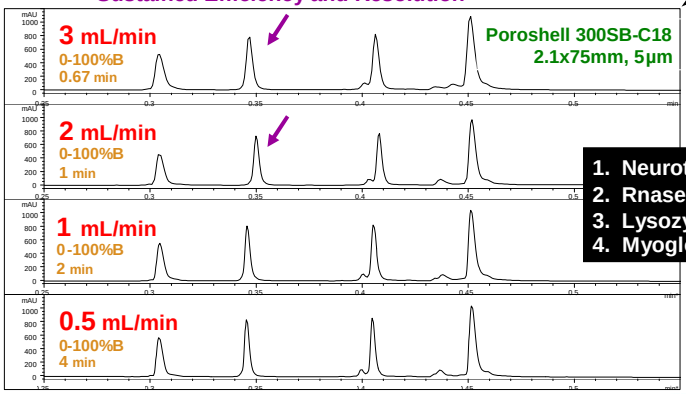
Effect of Increasing Flow Rate in Macromolecule (Protein) Analysis with Use of Superficially Porous Silica

VERY High Linear Velocity!

Flow = 3.0 mL/min
(5 - 100 %B / 0.67 min)

Sustained Efficiency and Resolution



1. Neurotensin
2. Rnase A
3. Lysozyme
4. Myoglobin

Aligned

Agilent 1100 WPS with AutoBypass; Piston Stroke: 20µL, Temp.: 70°C, Det.: 215 nm
Mobile Phase: A= 95% H_2O, 5% AcN with 0.1%TFA; B= 5% H2O, 95% AcN, with 0.07%TFA

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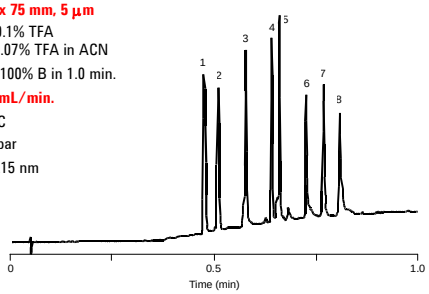
High Flow Rates with 2.1 mm ID Poroshell 300 for High Resolution and Fast Separations

Columns: **Poroshell 300SB-C18**
2.1 x 75 mm, 5 µm
Mobile Phase: A: 0.1% TFA
B: 0.07% TFA in ACN
Gradient: 5 – 100% B in 1.0 min.
Flow Rate: **3.0 mL/min.**
Temperature: 70°C
Pressure: 250 bar
Detection: UV 215 nm



Sample:
1. Angiotensin II
2. Neurotensin
3. Rnase
4. Insulin
5. Lysozyme
6. Myoglobin
7. Carbonic Anhydrase
8. Ovalbumin

- High efficiency at higher flow rates for extremely rapid separations of proteins and peptides.
- This is due to more rapid mass transfer of the superficially porous particle

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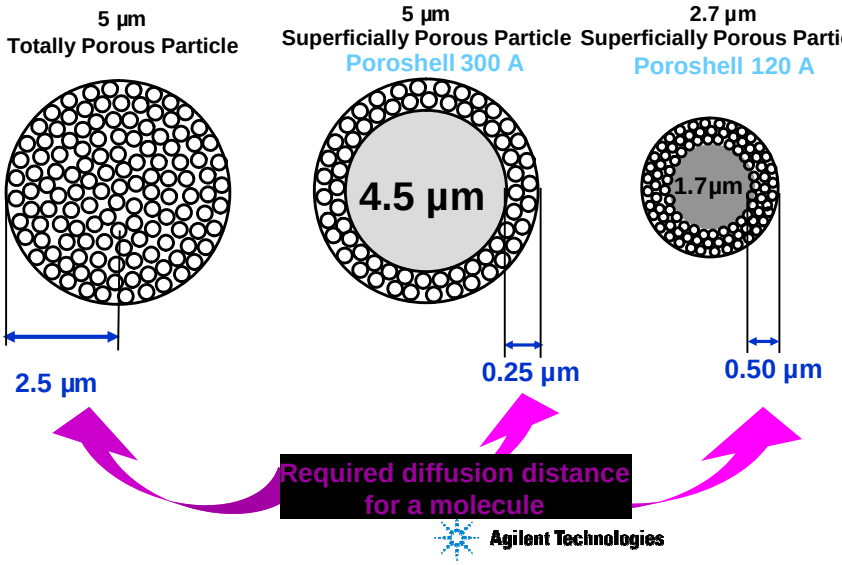
Comparison of Diffusion Distances

Totally porous silica versus superficially porous silicas



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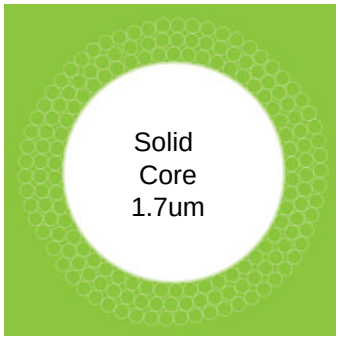
What is Poroshell 120?

A new HPLC column with:

1. **Sub-2 µm like high efficiency**
2. **At ~40-50% lower pressure**
3. **A 2.7 µm particle size with 1.0 µm porous shell**
4. **120Å pores for small molecule separations**
5. **A "standard" frit to reduce potential clogging**



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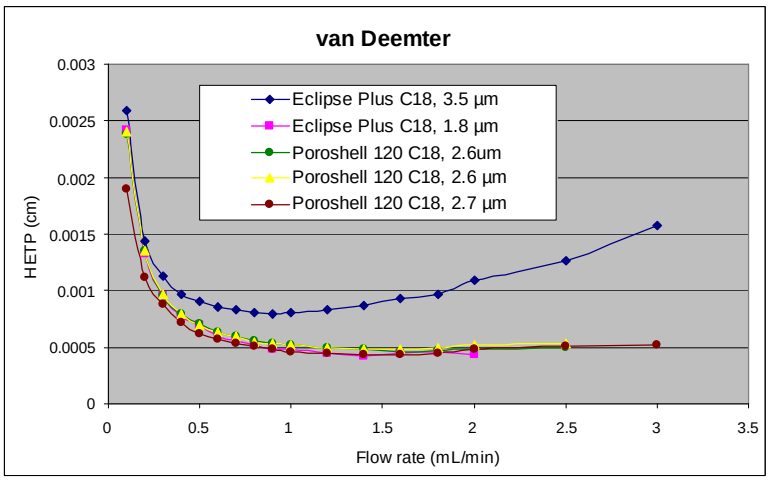
Superficially Porous Column for Small Molecules can Achieve Equal Performance to 1.8 µm



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Comparing Efficiency and Pressure with Different Types of Columns

Particle Size/Type	Pressure	Efficiency	LC Compatibility
3.5um – Totally Porous	123 bar	7,800	All 400 bar instruments
2.7um – Poroshell 120	180 bar	11,000	All LC's
1.8um – Totally Porous	285 bar	11,000	All LC's but pressure limits may be reached early

Columns: 4.6 x 50mm, Mobile Phase: 60% ACN:40% Water Flow Rate: 2 mL/min

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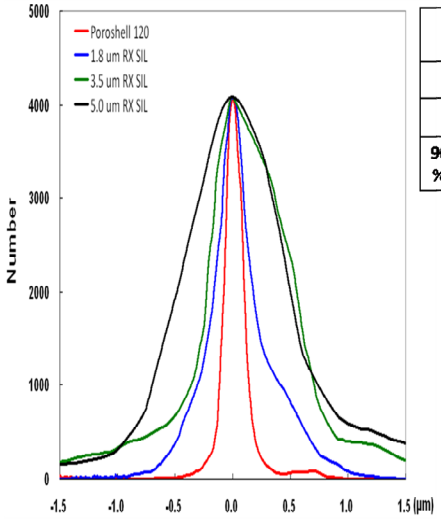
A Term - Comparison of Particle Size Distributions Between Totally Porous and Poroshell 120 Particles



	Poroshell 120	1.8 µm	3.5 µm	5.0 µm
10%	2.48 µm	1.67 µm	3.07 µm	4.59 µm
90%	2.75 µm	2.45 µm	4.44 µm	6.21 µm
90%/10 % ratio	1.11	1.47	1.45	1.35

- The 1.8, 3.5 and 5µm particles all have a normal particle size distribution.
- A number below 1.5 would be expected for the totally porous particles
- **The Poroshell 120 particle is 25% narrower.**
- This narrower particle size distribution improves column efficiency over a totally porous particle.

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Monoliths as Separation Media

Monoliths: chromatography sorbents **cast** as homogeneous phases into chromatographic columns

Typical Commercial Types:

1. **Agglomerates of polyacrylamide particles (UNO-BioRad Laboratories)**
2. **Polymethacrylate copolymerisates (CIM-BIA Separations, Ljubljana, Slovenia; Dionex LC Packings)**
3. **PS-DVB Copolymerisates [Dionex LC Packings]**
4. **Silica rod columns (Chromolith-Merck, Onyx-Phenomenex)**

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Characteristics of Silica-Based Monolithic Phases Relative to Packed Microparticulate Columns

1. **Flow-through pores with macroporosity (1-2-µm in width)**
2. **Mesopore- diffusive pores, average pore diameter can be controlled.**
3. **Higher porosity than packed microparticulate columns (i.e. reduced pressure drop)**
4. **Efficiency about the same as a 3-5-µm silica gel**
5. **Flatter h vs. v curves than "efficiency-equivalent" microparticulate packed column.**

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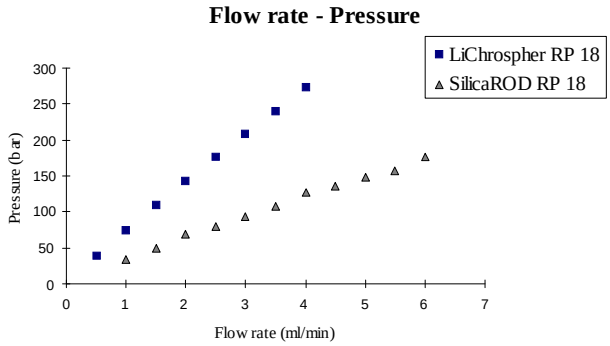
Chromolith™ & Microparticulate HPLC Column
Comparison of Pressure vs. Flow Rate



Column 1. Chromolith™ RP 18 (100-4.6mm)
2. LiChroCART®(125-4mm) LiChrospher® RP 18, 5mm

Mobile Phase: Acetonitrile/ water (35/ 65; v/v)

(courtesy of Merck,Darmstadt)

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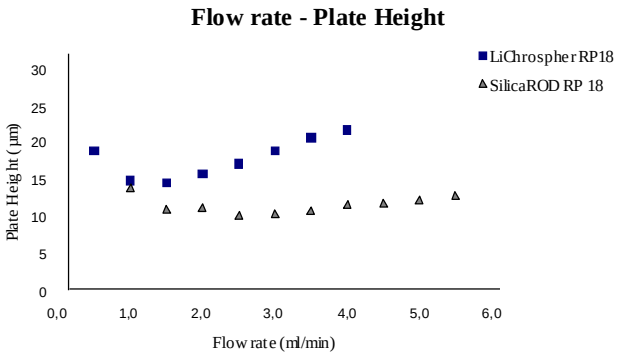
Chromolith™ & Microparticulate HPLC Column
Comparison of Plate Height & Flow Rate



column: 1. Chromolith™ RP 18 (100-4.6mm)
2. LiChroCART® (125-4mm) LiChrospher® RP 18, 5um

eluent: Acetonitrile/ water (35/ 65; v/v)

sample: Benzene

(courtesy of Merck,Darmstadt)

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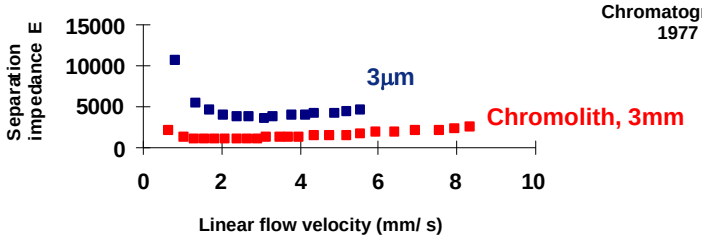
Separation Impedence: E vs. u as a Measure of Monolith Column Performance

Chromolith® 100 x 3mm, i.d.

$$E^* = \frac{\Delta p \cdot t_0}{N^2 \cdot \eta}$$

*J.H. Knox
Chromatographia
1977



Chromolith® Performance RP-18e, 3mm i.d.
Purospher™ STAR RP 18e, 3µm

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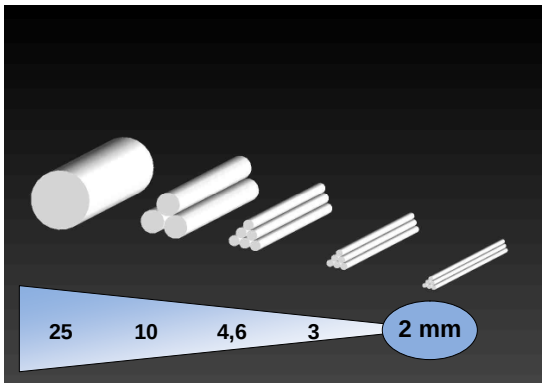
(Courtesy of Merck KGaA)

Monolithic Silica Columns
Chromolith FastGradient – 2mm i.d.



Silica-based monolithic columns of different diameters

[K. Cabrera, LCGC No. Amer. 26 (S4) 32 (2008)]

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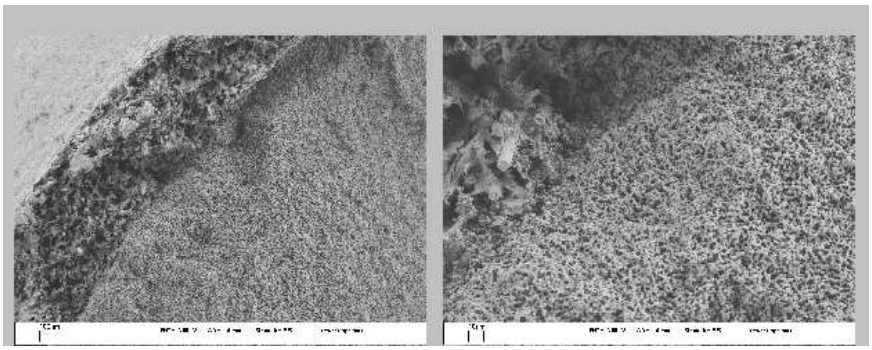
Interface of Silica Monolith and PEEK Encapsulation SEM Picture



[K. Cabrera, LCGC No. Amer. 26 (S4) 32 (2008)]

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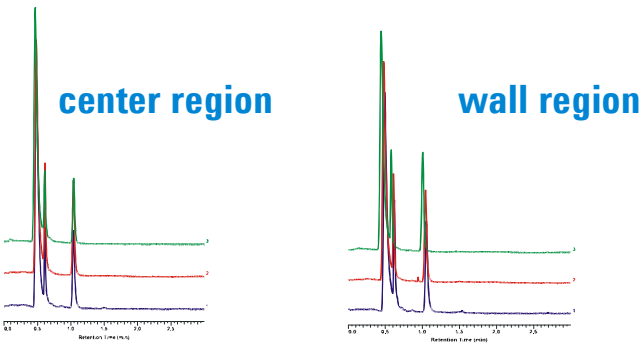
With Chromolith PEEK Cladding-Minimized Wall Effects



Overlap of three chromatograms after manual injections of 0.1mL substance mixture (toluene, nitrobenzene, 2-nitroanisole) on top of a silica-based monolith with a syringe in the center or at the wall region. After replacement of the end fitting, connection to an HPLC-system and operation with n-heptane/ dioxane (95/5; v/v) and a flow rate of 0,38mL/ min.

[K. Cabrera, LCGC No. Amer. 26 (S4) 32 (2008)]

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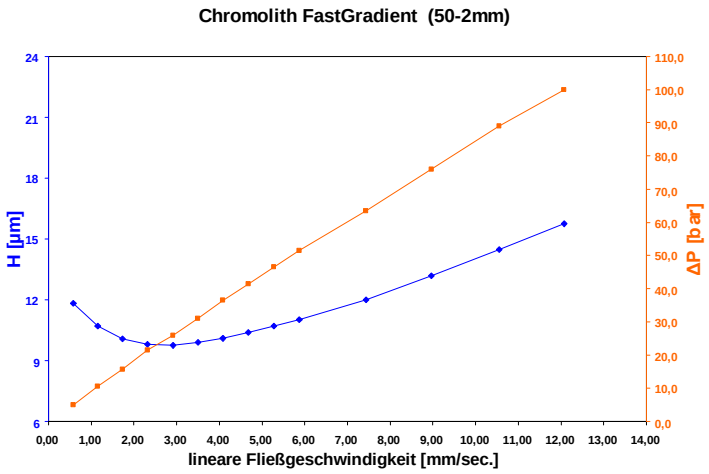
Chromolith FastGradient H/u and ΔP/u Curves



[K. Cabrera, LCGC No. Amer. 26 (S4) 32 (2008)]

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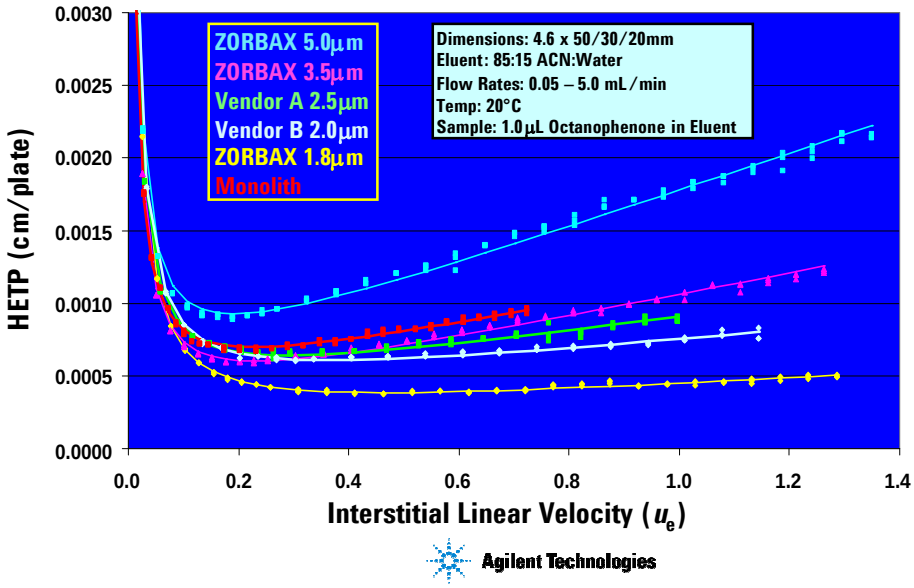
Van Deemter Plot A: HETP vs. Linear Velocity

Small Particle Columns including Monolith



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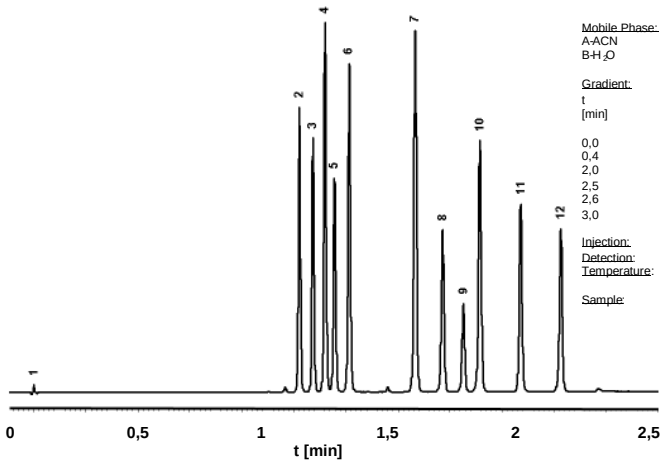
Chromolith® FastGradient (50-2mm)
Fast Analysis of Steroids (Doping Substances)



Mobile Phase:
A-ACN
B-H_2O

Gradient:

t [min]	A [%]	B [%]	flow [ml/min]
0,0	15	85	1,50
0,4	15	85	1,50
2,0	100	0	1,00
2,5	100	0	1,00
2,6	15	85	1,00
3,0	15	85	1,00

Injection: 0,5µl
Detection: 240 nm UV
Temperature: 40°C

Sample:
1.) Fluoxymesterone
2.) Boldenone
3.) Methandrostenolone
4.) Testosterone
5.) Methyltestosterone
6.) Boldenone acetate
7.) Testosterone acetate
8.) Nandrolone propionate
9.) Testosterone propionate
10.) Nandrolone phenylpropionate
11.) Testosterone isocaproate

(Courtesy of Merck KGaA)

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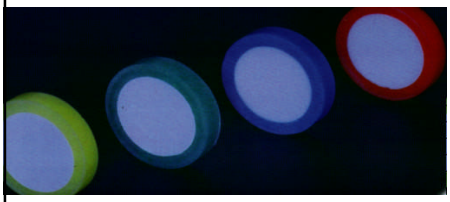
Example of Monolithic Polymer for Biochromatography

CIM = **C**onvective **I**nteractive **M**edia (BIA Separations)

- Crosslinked, porous monolithic polymer (GMA-EDMA)
- Available in disks and tubes
- IEX, HIC, RPC, affinity phases available
- Can be stacked and used for multi-modal separations
- Purification, process control, SPE, trace enrichment



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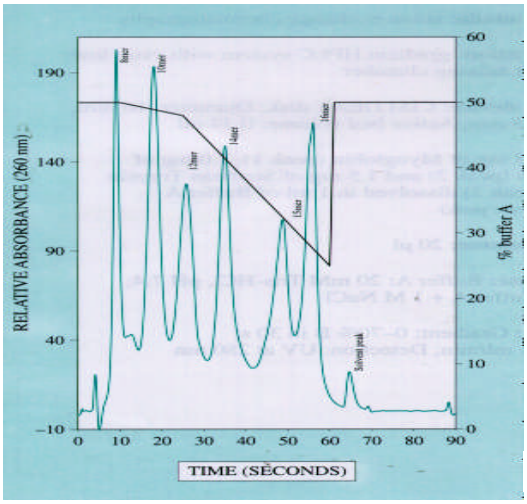
Separation of Oligodeoxynucleotides on CIM DEAE Disk



Separation Device: CIM DEAE Disk,
diameter: 16 mm, 3 mm thick,
active bed volumne: 0.34 mL
Instrument:: HPLC system with low dead volume mixing chamber
Sample: 10-ug of each in 1 mL buffer A
Injection Volume: 20-uL
Buffer A: 20 mM Tris-HCl, pH 7.4
Buffer B: Buffer A + NaCl
Gradient: Shown on chromatogram
Flow Rate: 6 mL/min
Detection: UV at 260-nm
Sample:

No.of Bases	5'-3' Sequence	Short Name
8	CCA TGT CT	8mer
10	GTC CAT GTC T	10mer
12	AGG TCC ATG TCT	12mer
14	CGA GGT CCA TGT CT	14mer
15	CCGAGGTCC ATG TCT	15mer
16	GCCG AGG TCC ATG TCT	16mer

(Courtesy of BIA Separations)

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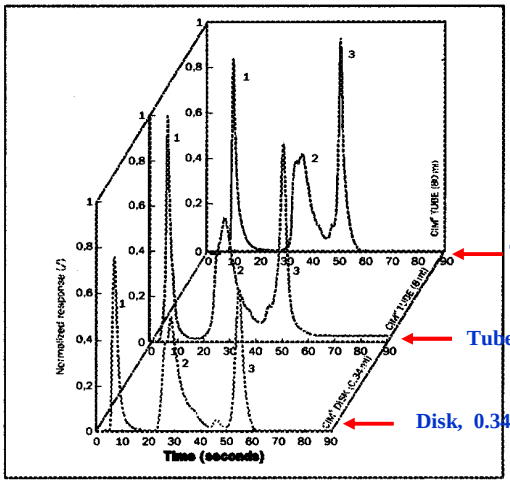
Preparative Scaleup with CIM Column Modules



Tube, 80-mL

Tube, 8-mL

Disk, 0.34-mL

Latest Additions:
- 800-mL
- 8-L (200g protein, 2-L/min flow)

(Courtesy of BIA Separations)

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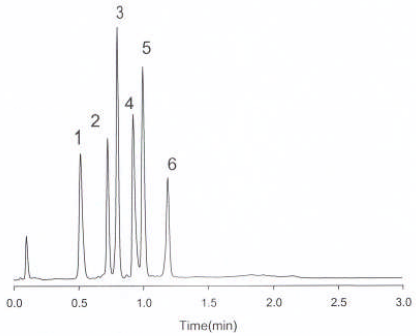
Protein Separation using Dionex ProSwift Monolithic Column

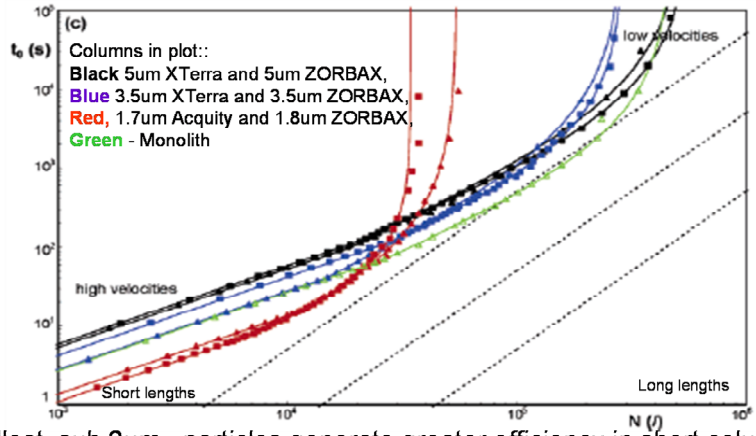


Column: **Dionex ProSwift Monolithic RP-all 4.6 x 50 mm**
Buffer A: Water + 0.15% TFA
Buffer B: Acetonitrile + 0.15% TFA
Gradient: 20-65% B in 1.4 min
Flow Rate: 10 mL/min
Absorbance: 280 nm
Sample: 1. Ribonuclease A 2. Cytochrome C 3. Lysozyme
4. Carbonic anhydrase 5. BSA 6. Ovalbumin
Temperature: 25ºC

(courtesy of Dionex)

Kinetic Plot for Comparison of Different Particle Sizes, based on u_{max}

Desmet et. al. Anal. Chem. **2006**, 78, 2150-2162



Smallest, sub 2um, particles generate greater efficiency in short columns at high velocities than other particle sizes, larger particle sizes in longer columns can generate more efficiency

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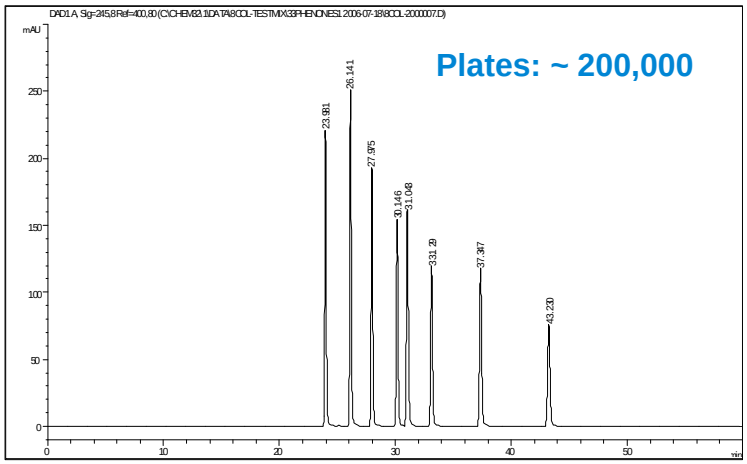
Analysis of Alkylphenone Standard on a Set of 8 Columns of 25 cm x 2.1 mm i.d. Packed with 5 µm Particles of Zorbax 300SB-C18 material.



Isocratic (70% acetonitrile – 30% water), 0.2 mL/min, 60 °C
Compounds: acetanilide (23.98 min), acetophenone (26.14 min), propiophenone (27.97 min), butyrophenone (30.15 min), benzophenone (31.04 min), valerophenone (33.13 min), hexanophenone (37.35 min), heptanophenone (43.23 min).

(courtesy of Pat Sandra, RIC)

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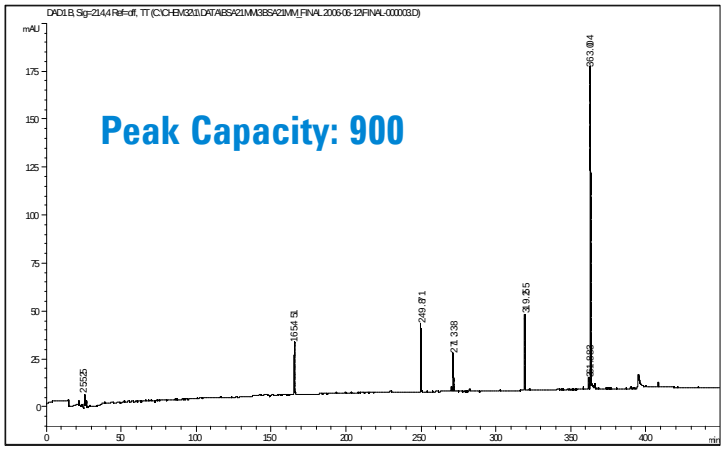
Analysis of Peptide Standard Mixture on a Set of 8 columns of 25 cm x 2.1 mm i.d. x 5 µm Zorbax 300SB-C18 material.



Standard mixture: Proteomix (containing 5 peptides and a-cyano-4-hydroxy-cinnamic acid, LaserBio Labs, Sophia-Antipolis, France). **Gradient:** 2% to 70% acetonitrile (+0.1% TFA) in water (+0.1 % TFA) in 500 min. Flow rate: 0.2 mL/min. Column temperature: 60°C.

(courtesy of Pat Sandra, RIC)

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Some Ways to Increase Sample Throughput
(and Resolution)

1) **Shorter column lengths (to reduce analysis time) packed with small porous particles (to maintain resolution). (Reasonable # of plates and reasonable pressure, fast separation)**

2) **Longer column lengths (to increase efficiency) packed with even smaller porous and non-porous particles (to maintain resolution), with the ultimate being the so-called "Ultra-High Pressure LC". (Many plates, fast separation, high pressure)**

3) **Columns packed with various small superficially porous particles (pellicular) particle sizes, pore sizes and phase thickness to allow the rapid resolution of biomolecules such as proteins as well as small molecules. (Large and small molecules, fast separations, lower pressure)**

4) **Columns designed with silica- and polymer-based monolith stationary phase formats (fast separation, low pressure, in-series columns)**

5) **Parallel LC (multiple capillary columns/channels, increased samples/hour)**

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What is Parallel LC?

Conventional Single-Channel HPLC

One Lab

8-Channel HPLC

Eksigent®

Sepiatec®

30" Benchtop

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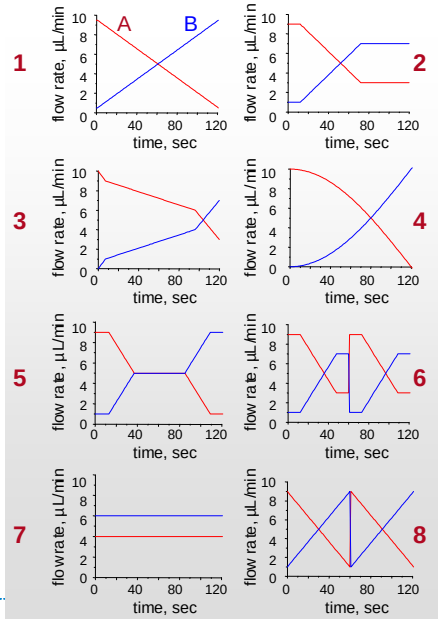
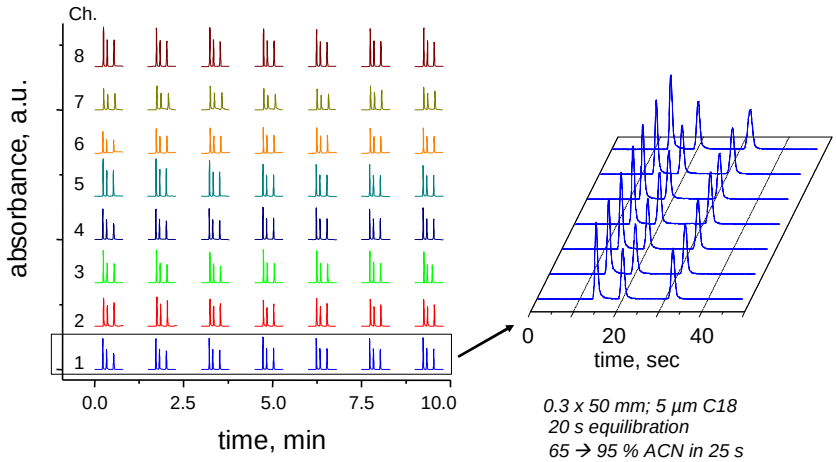
Eksigent ExpressLC-800 System Design

- **Eight Independent Channels**
  - **Allows 8 independent methods to run simultaneously**
  - **Optimized for 300 µm ID columns**
- **Each Channel Incorporates**
  - **High pressure binary mixing**
  - **Independent injector**
  - **Independent UV detector arrays**
- **Dual Headed Autosampler**
  - **4 injections / minute**

(courtesy of Eksigent)



High Throughput Performance of Parallel LC



0.3 x 50 mm; 5 µm C18
20 s equilibration
65 → 95 % ACN in 25 s
20 s hold;
12 µL/min

(courtesy of Eksigent)

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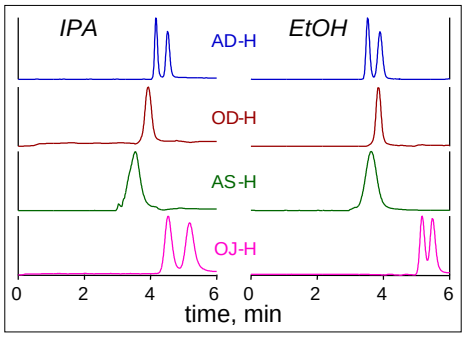
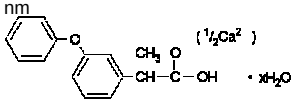
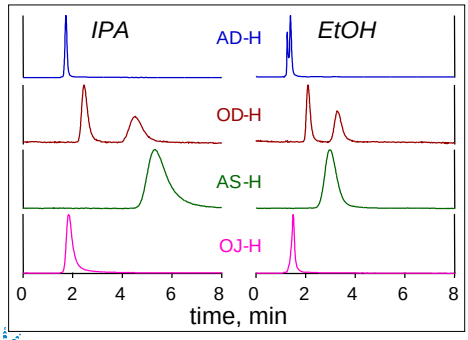
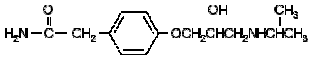
Chiral Application
8 Channel Method Development – Isocratic



Fenoprofen
A: ETOH and IPA as marked
B: 0.1% TEA, 0.1% TFA, 5% IPA/hexanes
- isocratic 10% A; 4 µL/min
- detection at 220 nm







Atenolol
A: ETOH and IPA as marked
B: 0.1% TEA, 0.1% TFA, 5% IPA/hexanes
- isocratic 10% A; 7 µL/min
- detection at 230 nm

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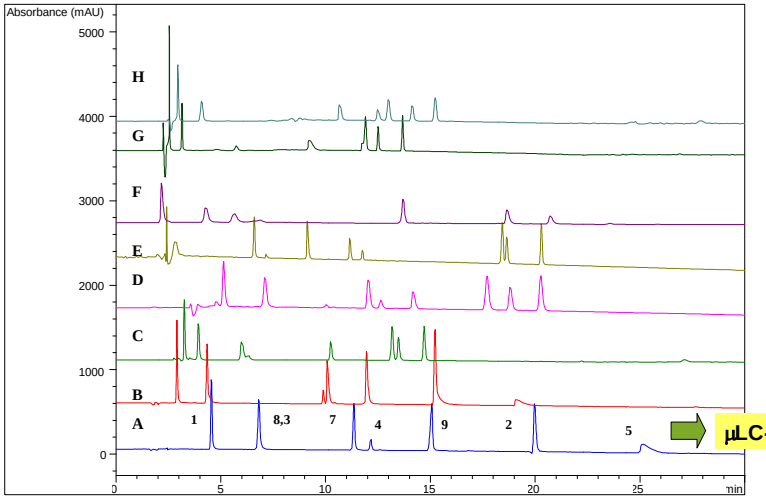
Parallel Screening Using 8 Different Conditions



Find one condition which gets most peaks resolved, then use µLC-MS to obtain the peak identity if needed

Data courtesy of Yining Zhao (Team leader)
Gang Xue, Nate Lacher of Pfizer, Groton,CT

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Conclusions

- High-speed and/or high resolution HPLC separations can be performed by a variety of approaches depending on time, sample complexity and sensitivity requirements
- Short columns, small particles (3.5- and sub-2-µm particles) (reasonable # of plates, fast separations, reasonable pressure)
- Long columns, smaller particles (down to 1.5-µm) (complex samples, reasonable separation times, high- or ultra-high pressure)
- Superficially porous particles for fast separations of both biomolecules and small molecules
- Polymeric or silica-based monoliths (low pressure drop, fast separations, macromolecular or small molecule separations)
- An alternative approach is to use parallel LC with capillary columns

- Instrumental configurations may have to be modified to make these very fast separations possible and practical with available hardware. These changes involve decreasing extra column effects, increasing data rate, decreasing dwell volume, smaller flow cells and so on.

Acknowledgements

- Colleagues at Agilent-Cliff Woodward (deceased), Bill Long, Maureen Joseph, John Henderson, Bud Permar and Bill Barber
- Manufacturers who supplied data on their products (Merck, Eksigent, Supelco, BIA Separations)
- Yining Zhang at Pfizer for parallel LC data

AND LAST BUT NOT LEAST

- Thanks to you the audience for listening to my marathon presentation!