



Practical Aspects of High Performance Column Selection in HPLC/UHPLC: Superficially Porous or Sub-Two Micron Porous

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Outline

Newer particles/columns

HPLC particle development history

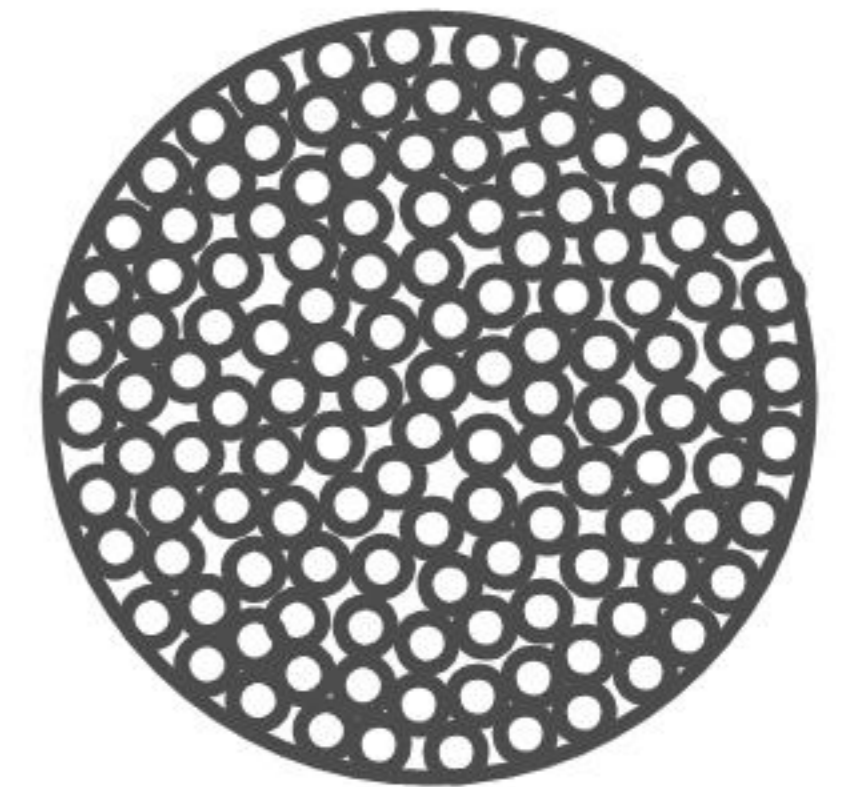
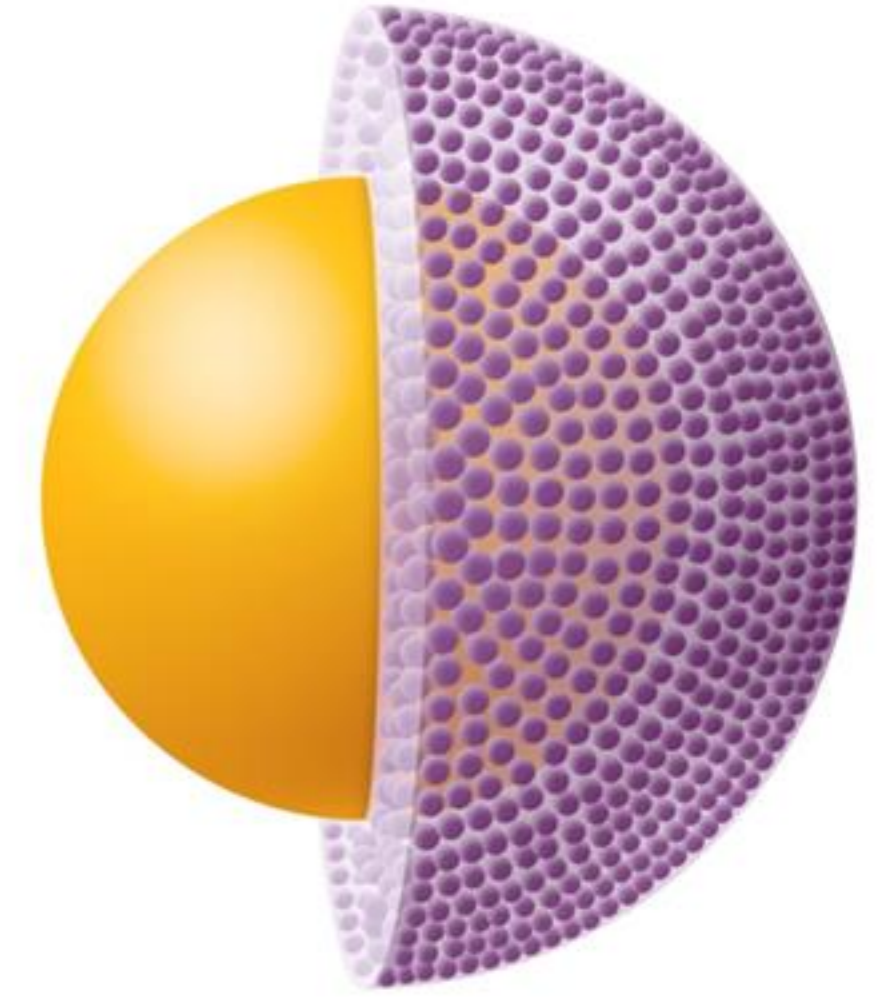
Particles used in HPLC/UHPLC

Intro to Superficially Porous Particles

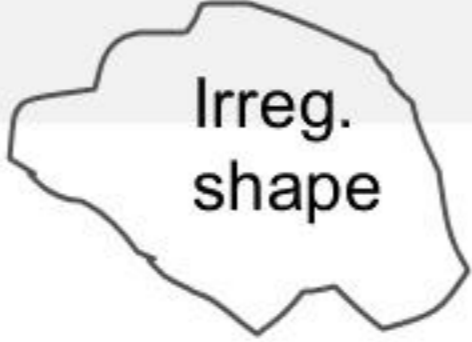










Compare: Totally Porous <2- μm Particles (TPP) and Superficially Porous Particles (SPP)

- Efficiency
- Pressure
- Peak capacity
- Sample capacity
- Practical aspects; plugging, dimensions, method transfer, available phases, pressure, instrument requirements

Best for your application



History of Commercial HPLC Particle Development

Year of Acceptance	Particle Size	Nominal Size	~Plates/15 cm
1950s	 Irreg. shape	100 μm	200
1967	 Glass Bead	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)	33,000
1999		5.0 μm (pellicular)	8,000**
2000		2.5 μm	25,000
2003		1.8 μm	32,500
2007/2008		2.7 μm	32,000***
2013		1.6 μm (pellicular)	

* non-porous silica or resins, ** 300 A pore for proteins, *** 90-120 A pore

Superficially Porous Particles

Past

- Pellicular
- Porous Layer(ed) Beads
- Superficially Porous Particles

Current

- Superficially Porous Particles (SPP)
- Fused Core Particles
- (Core) Shell Particles
- Poroshell

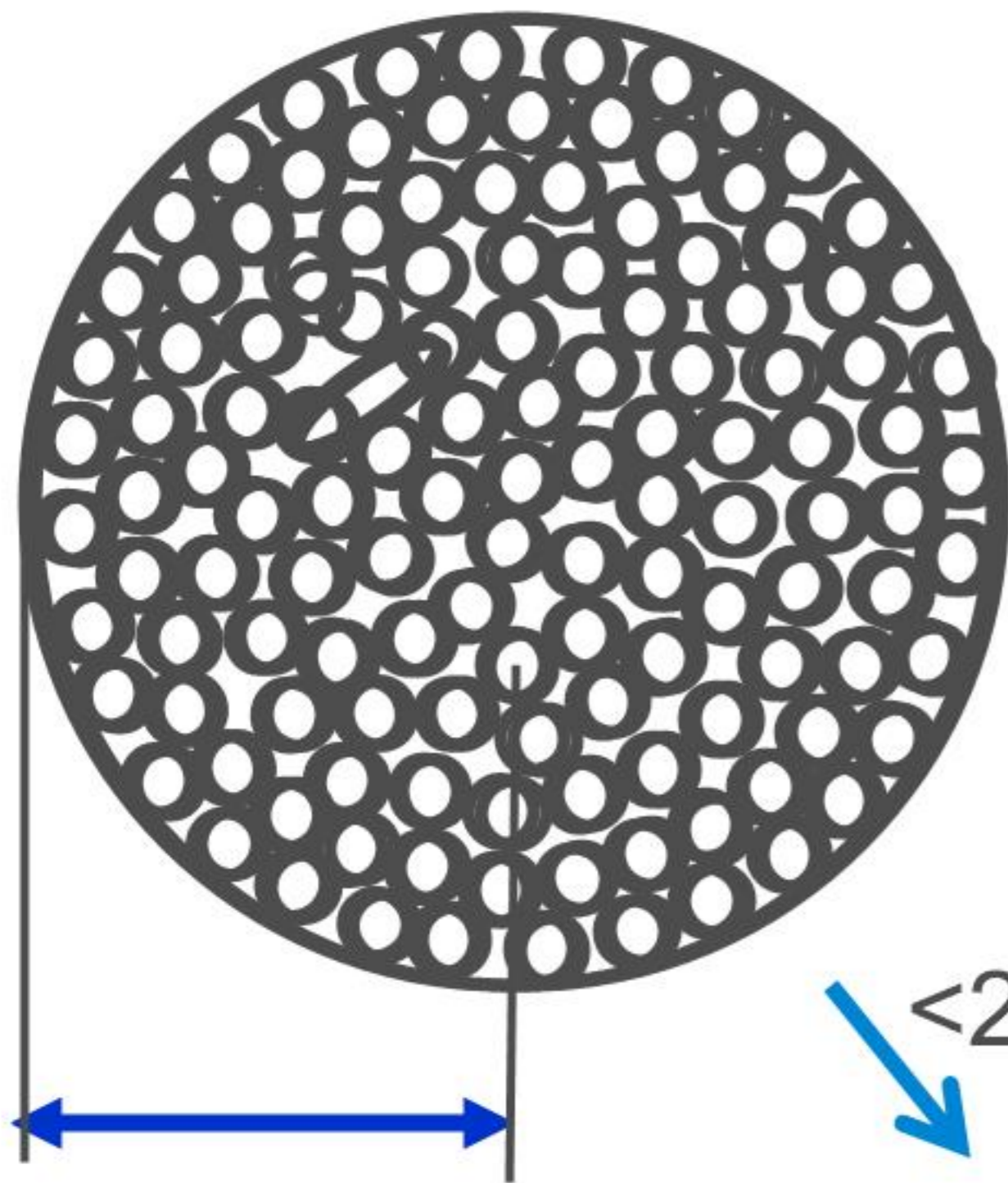
Comparison of Different Particles

Diffusion Distances

Totally porous silica versus superficially porous silicas

5 μm

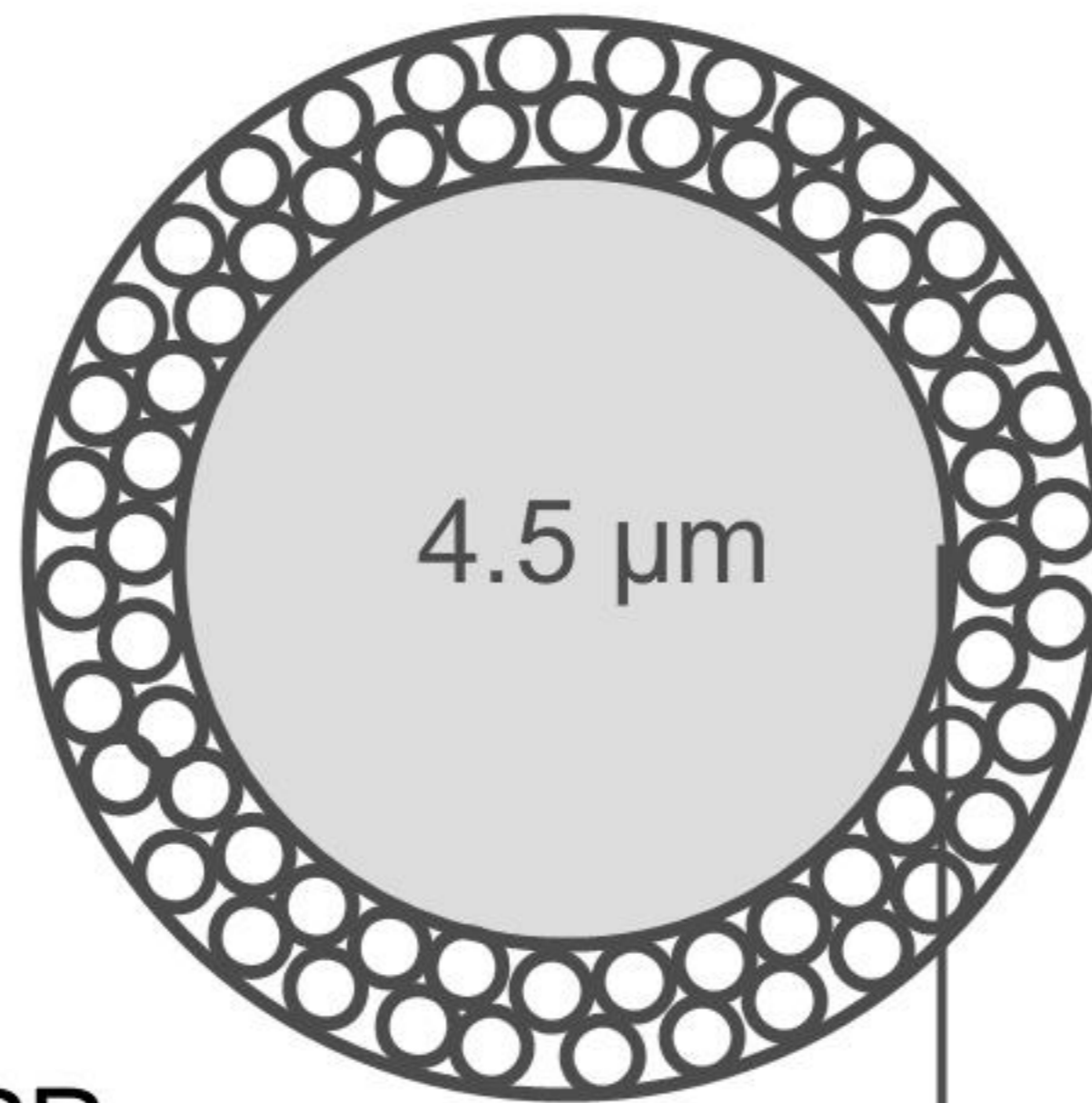
Totally Porous Particle (TPP)



2.5 μm

5 μm

Superficially Porous Particle



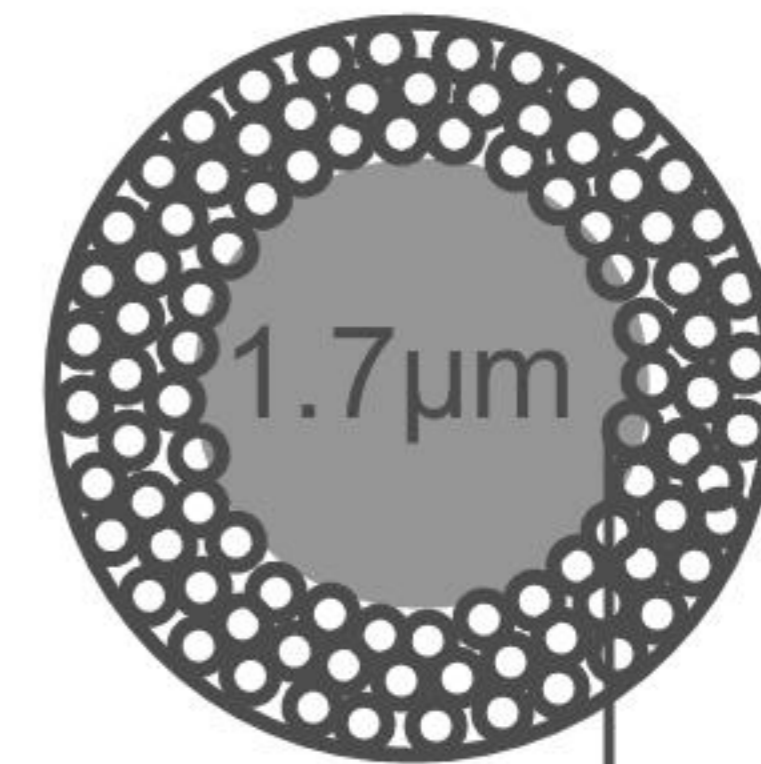
4.5 μm

2000

0.25 μm

2.7 μm

Superficially Porous Particle (SPP)

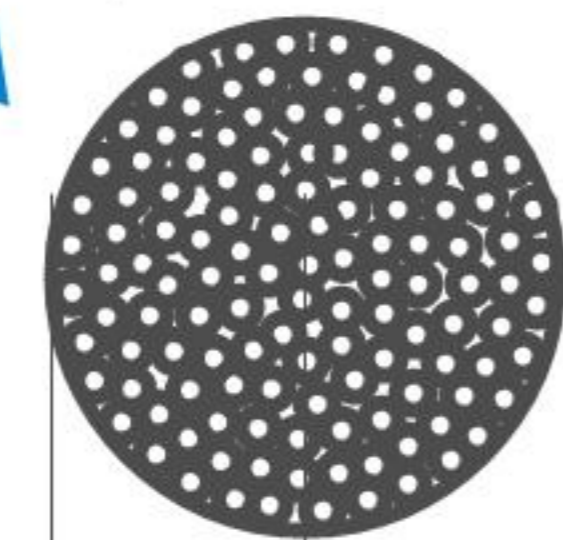


1.7 μm

2008/2010

0.50 μm

<2 μm TPP



0.90 μm

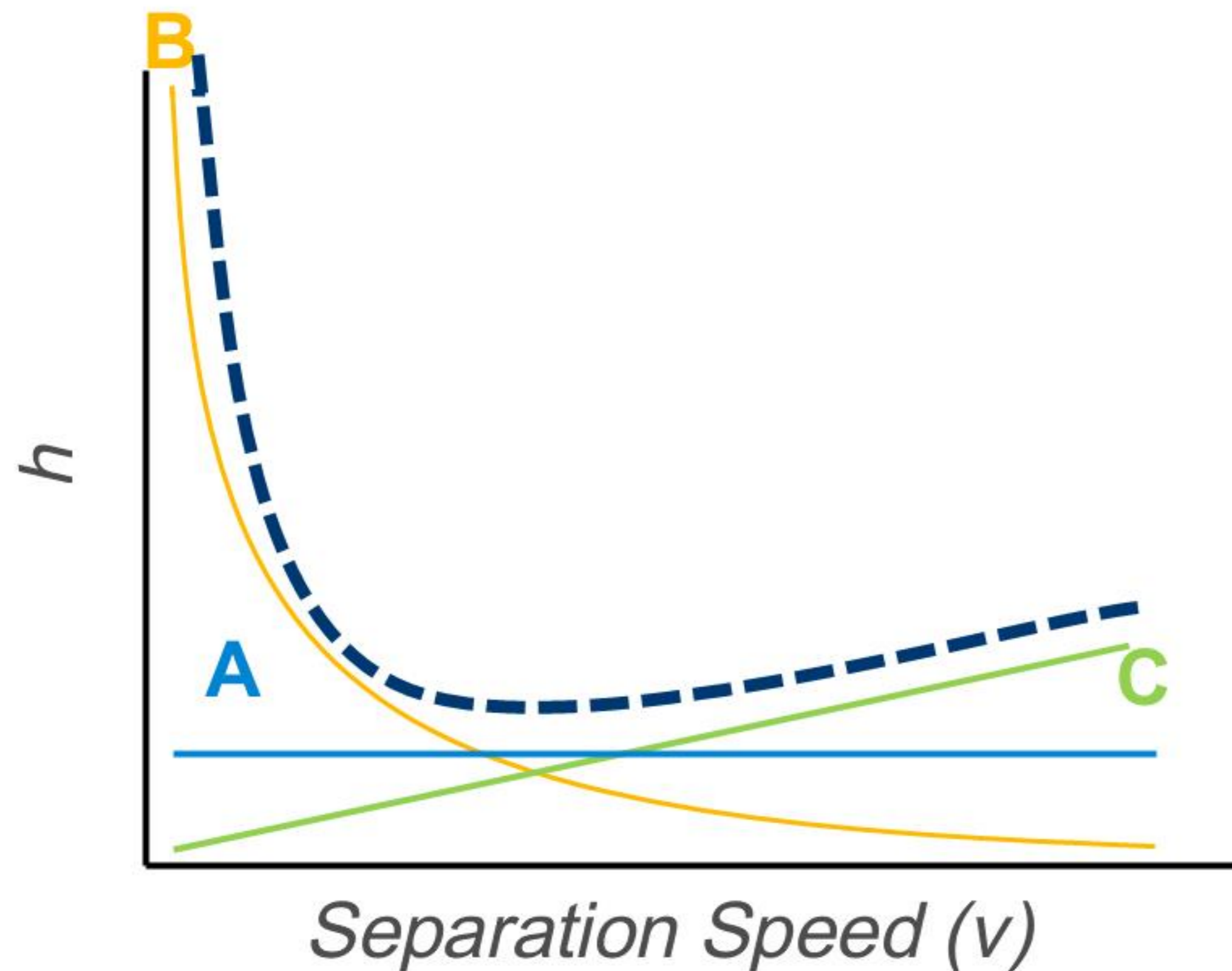
Required diffusion distance for a molecule



Efficiency Improvement with Superficially Porous Particles

van Deemter equation:

$$h = A + B/v + C \cdot v$$

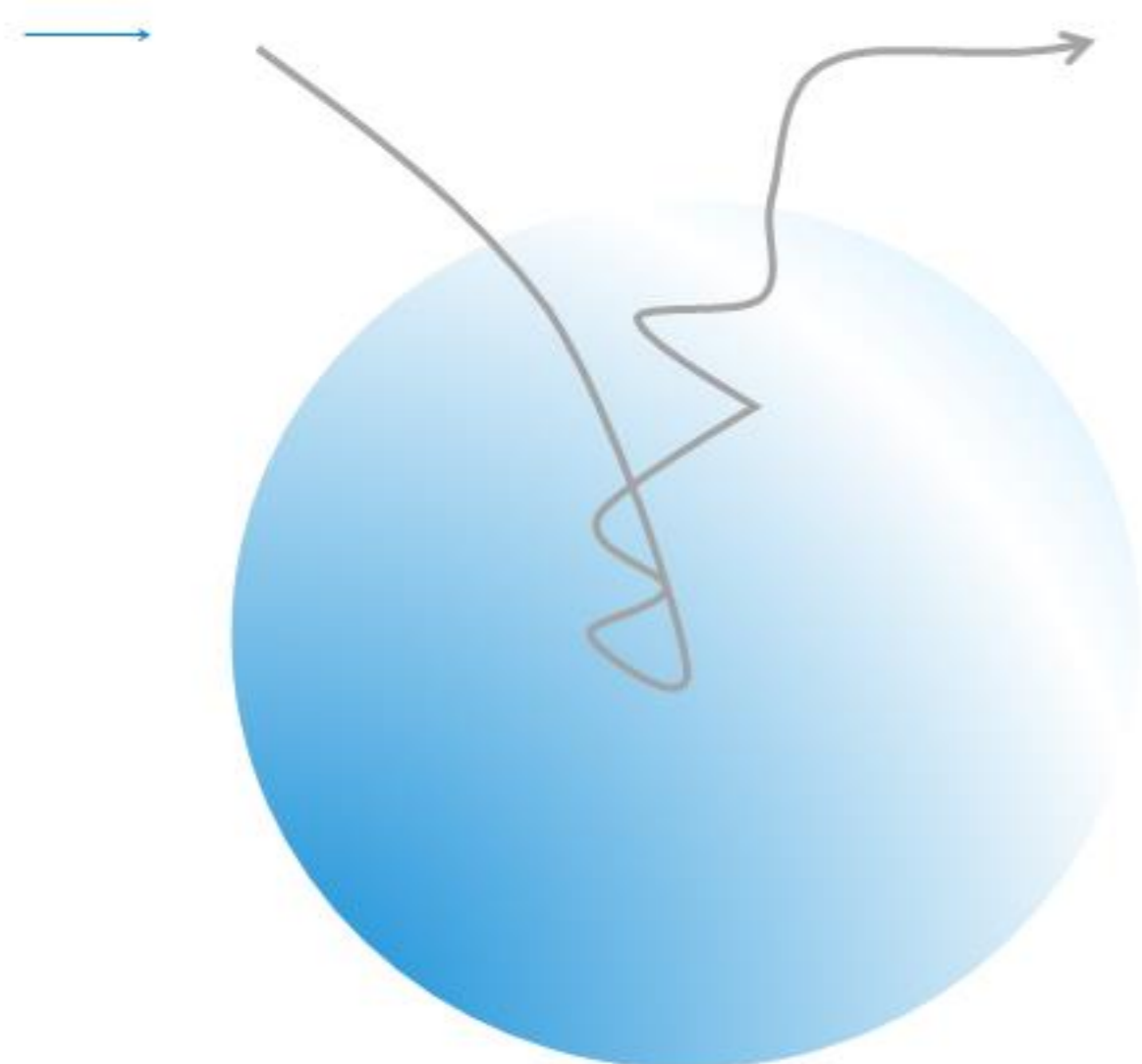


Lower h = higher efficiency!

- **A term** – eddy diffusion and flow distribution
 - particle size & packing quality important
 - narrow particle size distribution
- **B term** – longitudinal diffusion
 - Minimal impact to longitudinal diffusion
- **C term** – mass transfer
 - shorter diffusion paths
 - better with superficially porous particles
 - more effect on large molecules

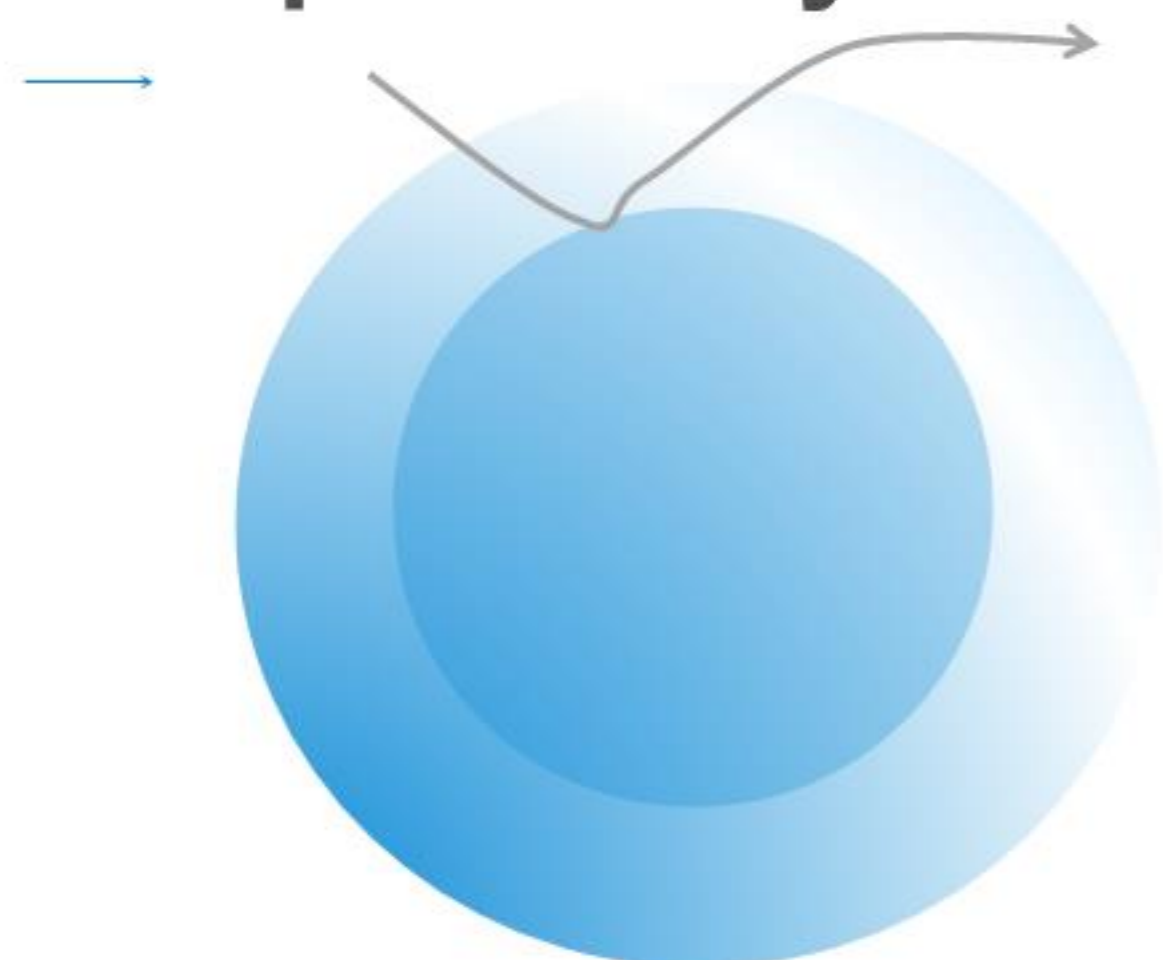
Analyte Mass Transfer Improvements through Lower Diffusion

Totally Porous



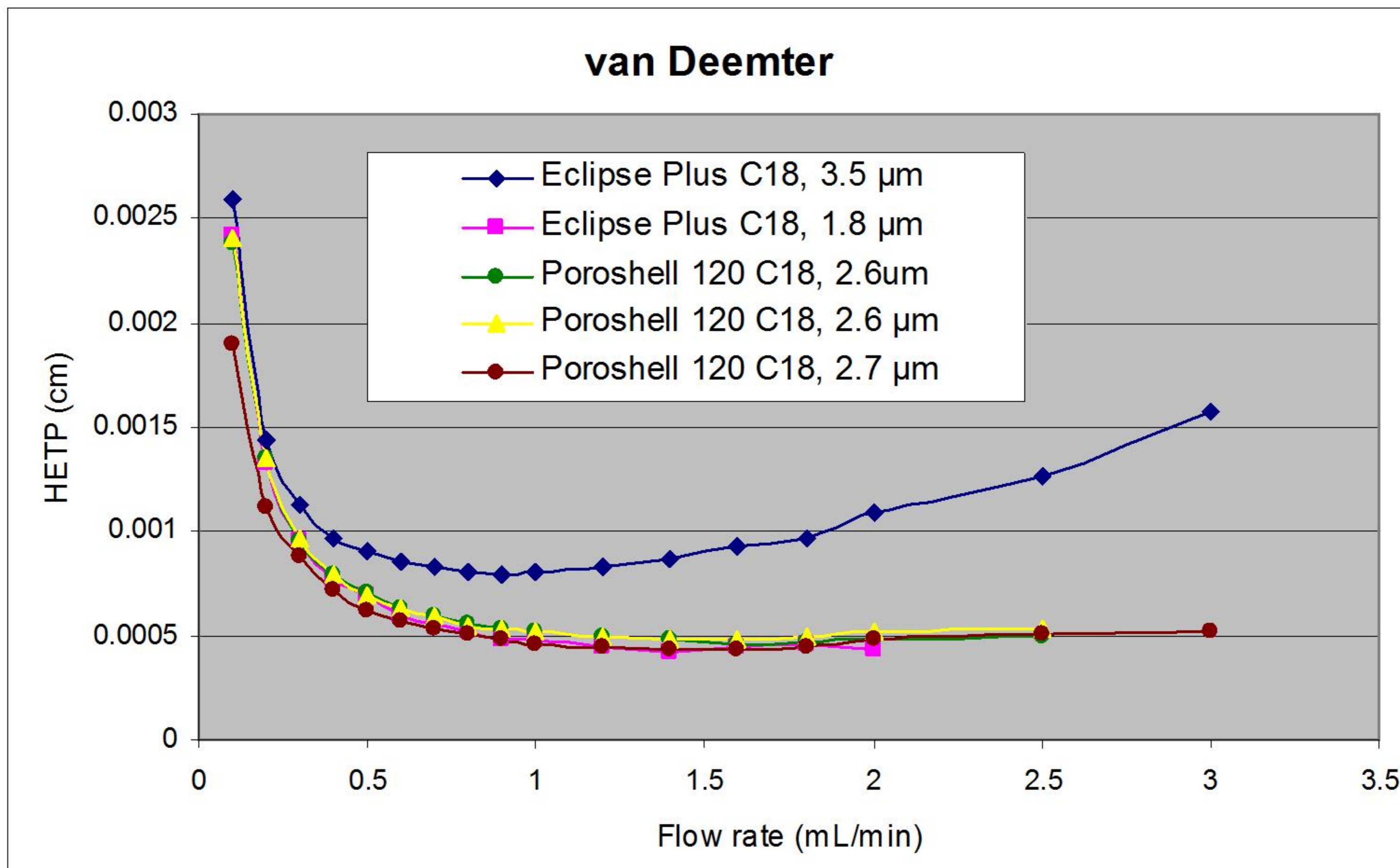
- **Totally porous particles**
 - **diffusion throughout particle**
- **Poroshell 120**
 - **diffusion limited to outer shell**

Superficially Porous



- **Results:**
 - **Lower C term**
 - **Higher efficiency**
- **And**
 - **Higher flow rate with**
 - **Minimal impact on efficiency**

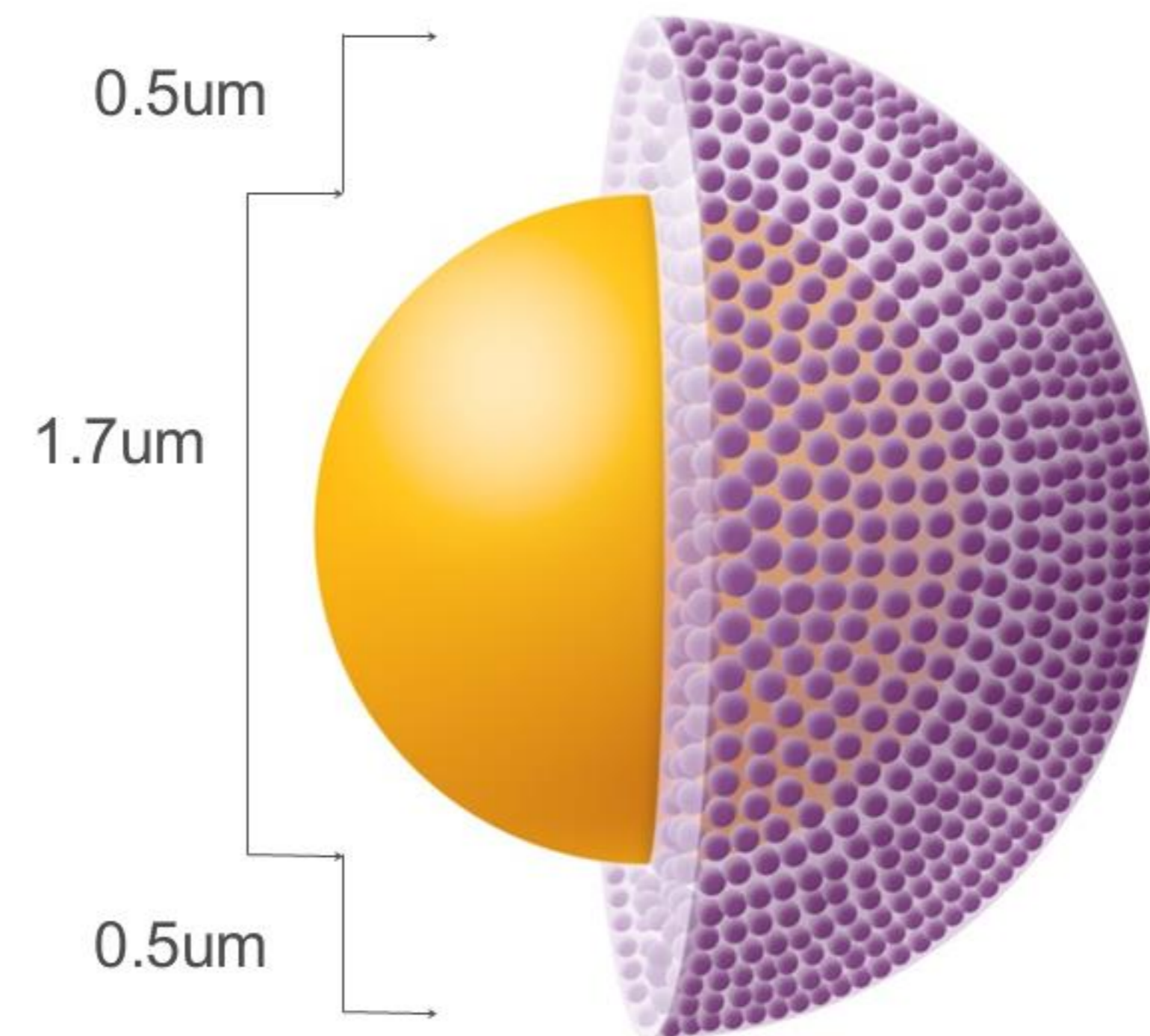
Superficially Porous (SPP) Column for Small Molecules Can Achieve Equal Performance to 1.8 μm (TPP)



Poroshell 120 SPP Columns for HPLC and UHPLC:

Poroshell 120 columns:

- 90-100% efficiency of sub 2 μm
- 2X efficiency of 3.5 μm (totally porous)
- A ~40-50% lower pressure
- A 2 μm frit to reduce clogging
- 600 bar pressure limit for HPLC or UHPLC use
- The particle has 2.7 μm outer diameter with a solid core (1.7 μm) and porous outer layer with a 0.5 μm diffusion path. The average pore diameter is 120 \AA .



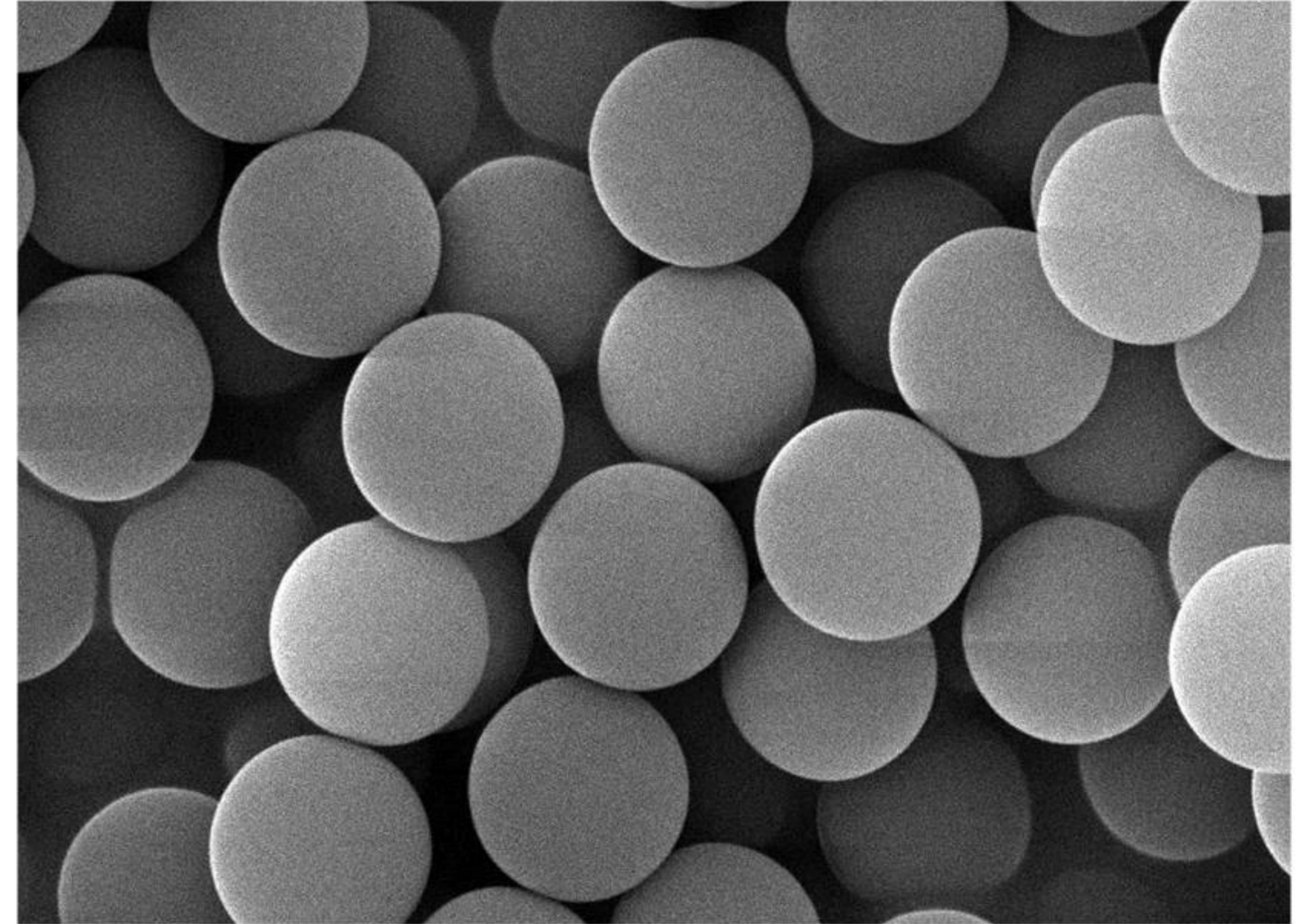
How is a Poroshell 120 Particle Made

Step 1 – Make the Solid Core

Cores uniform 1.7 μ m size with a smooth surface - monodisperse

RSD of 7 batches of cores = 0.04%

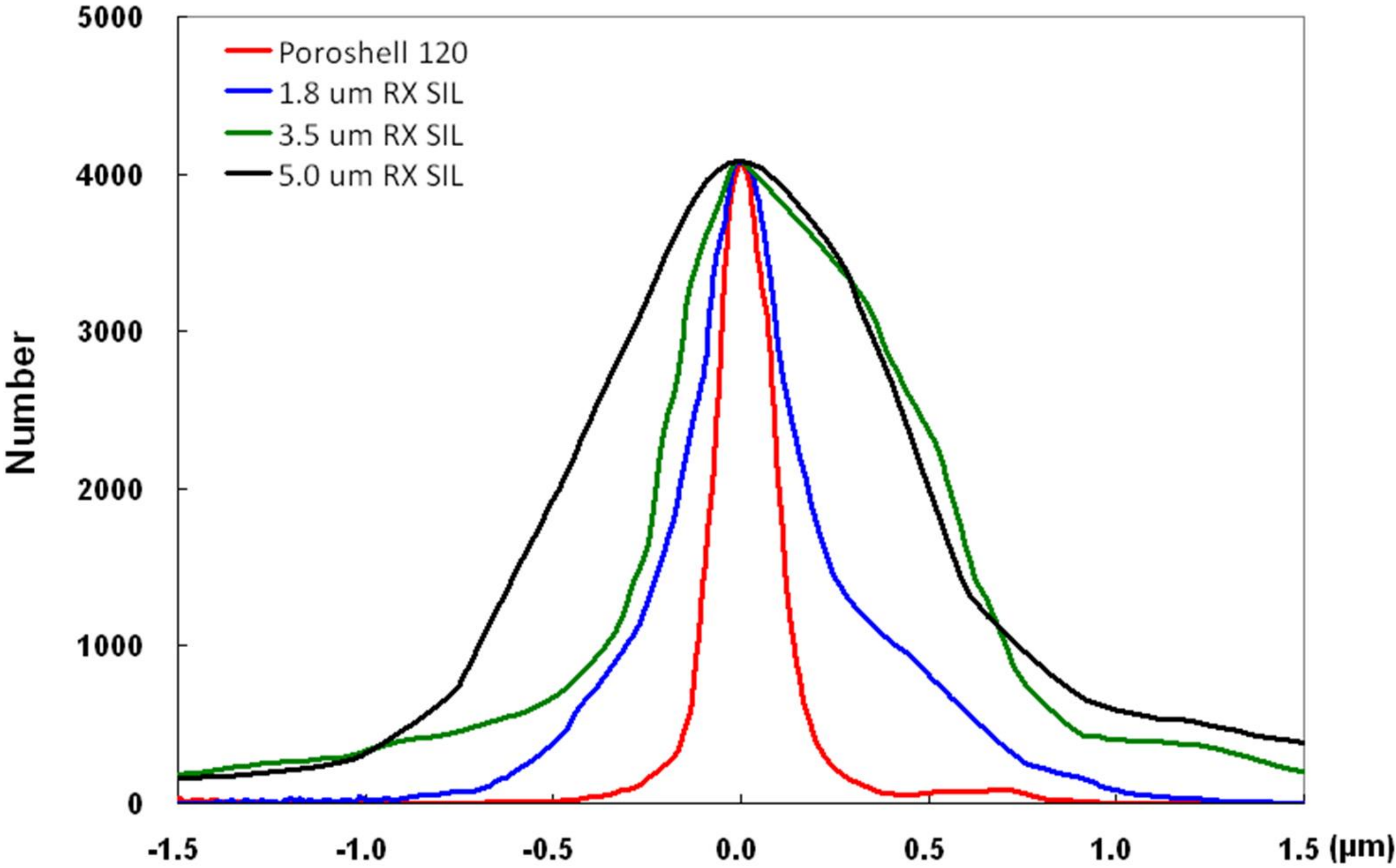
Tight final particle size distribution
(tighter than with totally porous particles)



SEM of Cores

Particle Size Distributions

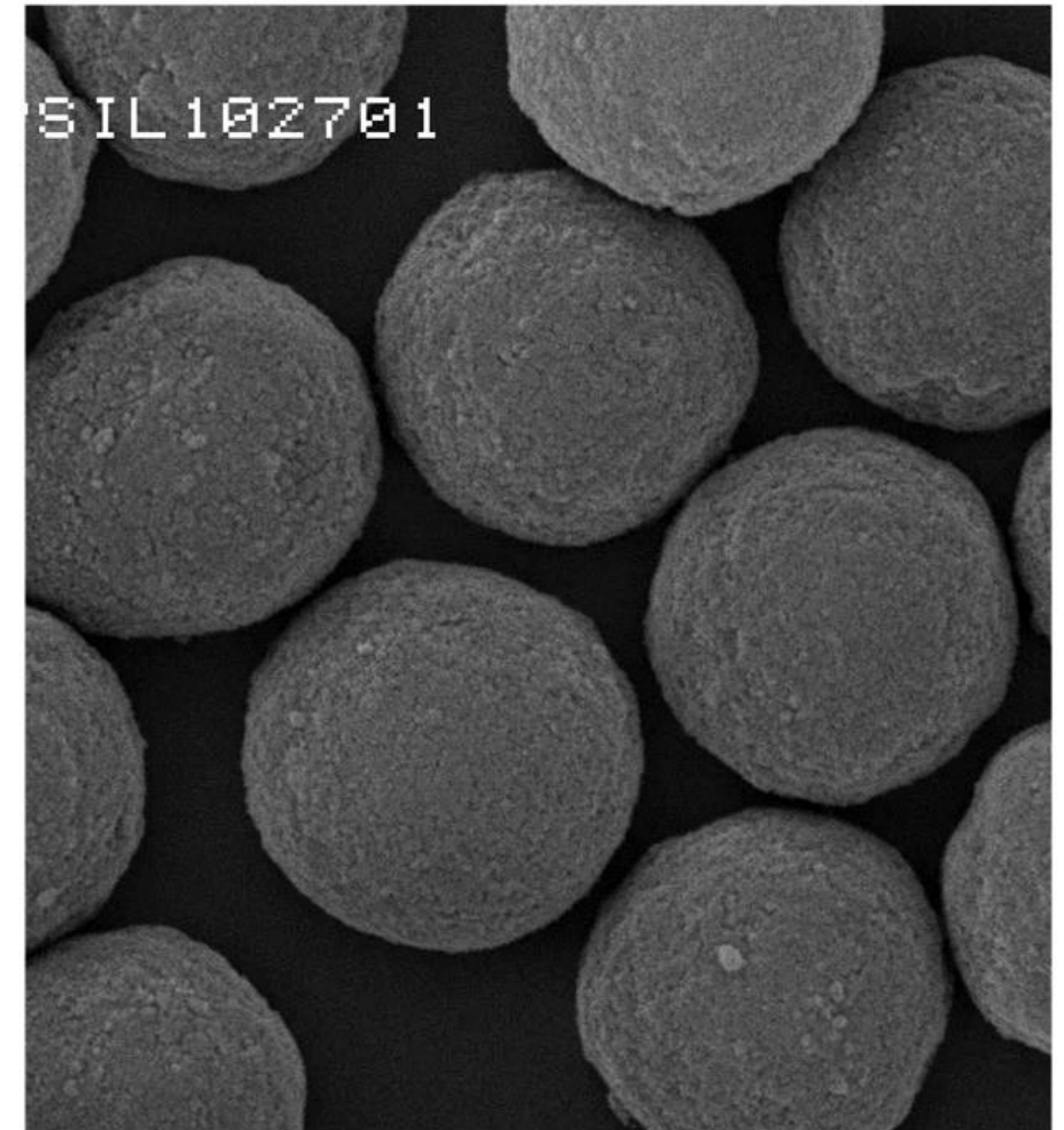
Totally Porous and Poroshell 120 Particles



Step 2 – The Porous Outer Layer

Poroshell 120 differs from other processes for superficially porous particles

- Single step coacervation process used versus multi-layering
 - Coacervation - The process by which small sol particles come together to form the porous particle
- 1 step reduces variability = more reproducible



Step 3 – Attach the Bonded Phase

- Poroshell 120 EC-C18 & EC-C8
 - Endcapped for best peak shape
 - C18 is also used for peptide mapping
- Poroshell 120 Stablebond SB-C18 & SB-C8
 - Great alternate selectivity – non-endcapped
 - Best performance at low pH, i.e. longest lifetime
- Additional selectivities for method optimization
 - EC-CN, Phenyl-Hexyl, SB-Aq, Bonus-RP, HILIC
- *New Phases*
 - PFP
 - HPH-C18 & C8
 - Poroshell 120 based
 - 110 Å pore size

Comparison

Efficiency

- Totally Porous Particles (TPP)
- Superficially Porous Particles (SPP)

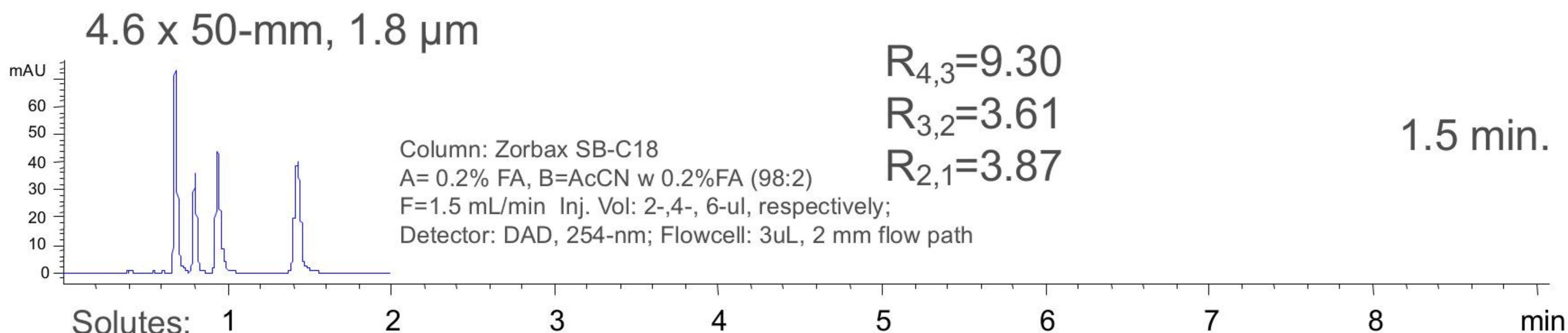
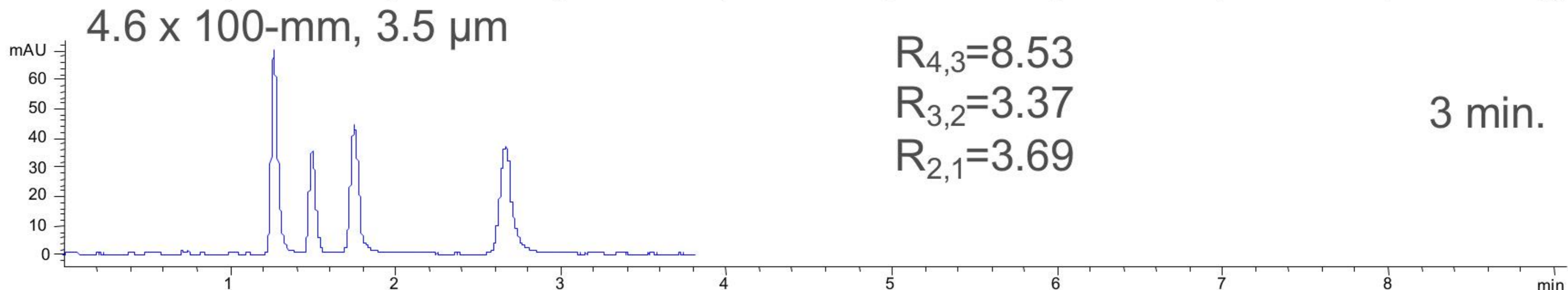
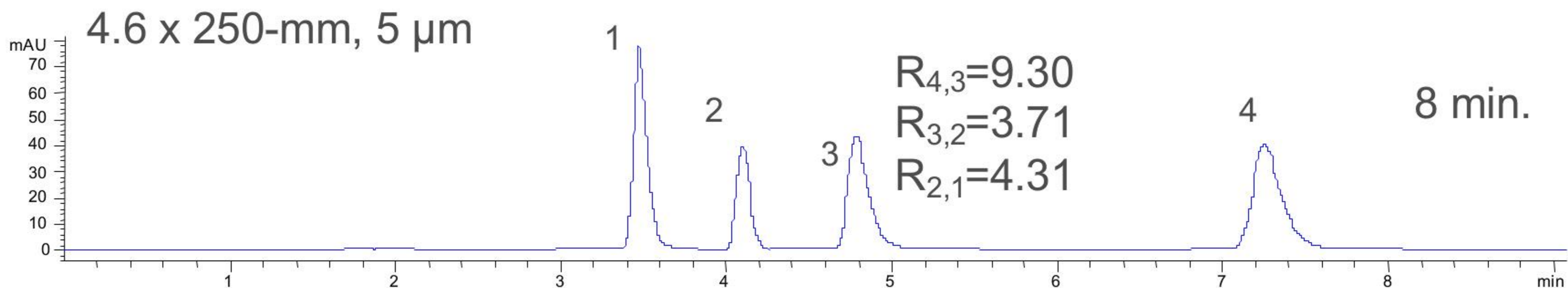
Sub 2- μm Short Totally Porous Particle (TPP) Columns Provide Efficiency of Longer Columns with Larger Particles

Column Length (mm)	Efficiency N(5 μm)	Efficiency N(3.5 μm)	Efficiency N(1.8 μm)	Typical Pressure Bar (1.8 μm)		Analysis Time*
150	12,500	21,000	32,500	580		
100	8,500	14,000	24,000	420		Analysis Time -33%
75	6000	10,500	17,000	320		Peak Volume -50%
50	4,200	7,000	12,500	210		Solvent Usage -67%
30	N.A.	4,200	6,500	126		
15	N.A.	2,100	2,500	55		

* Reduction in analysis time compared to 150 mm column
 • pressure determined with 60:40 MeOH/water, 1mL/min, 4.6mm ID

TPP Column Scalability

Change Dimensions & d_p to Increase Speed; Maintain Resolution



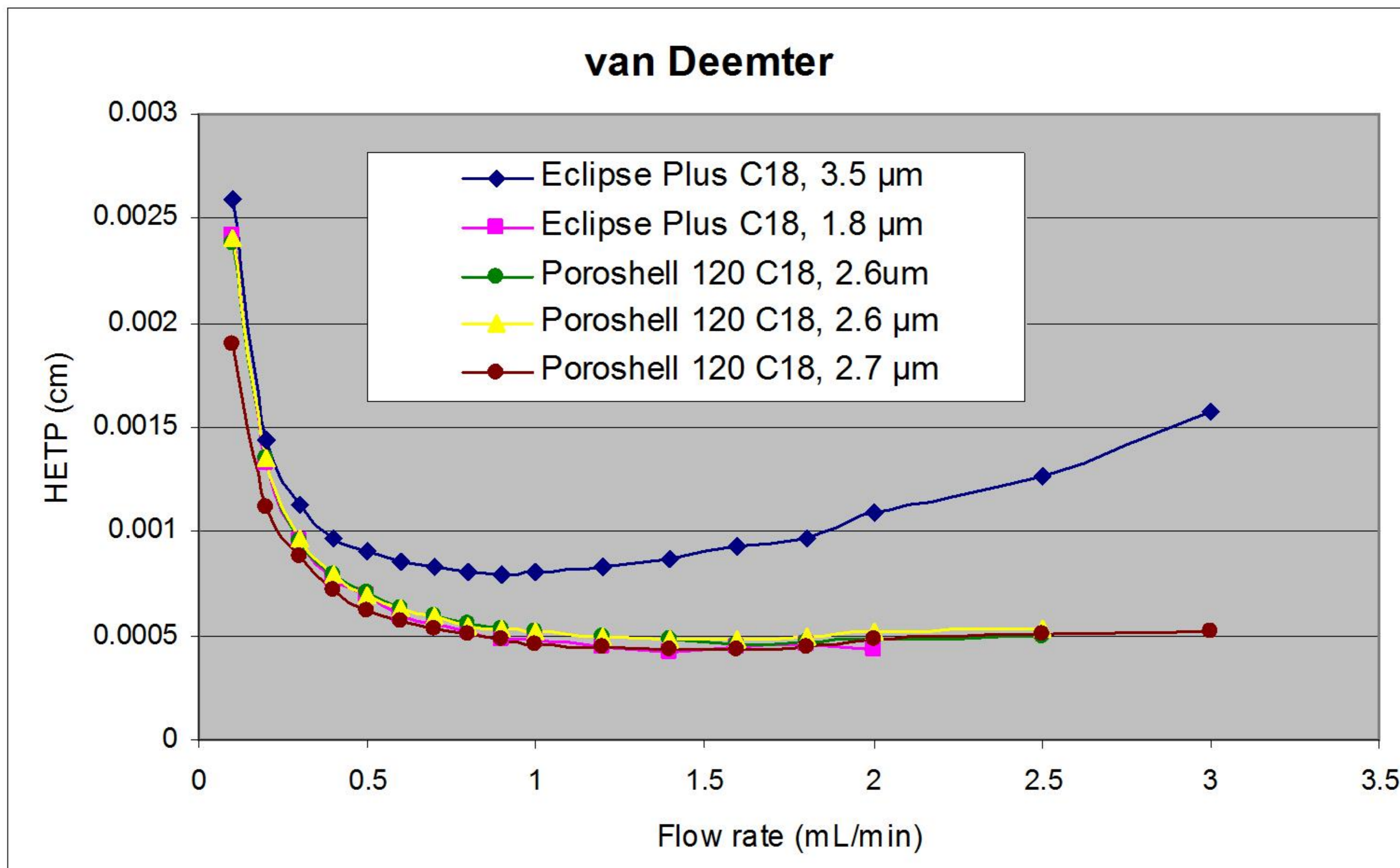
Column: Zorbax SB-C18
A= 0.2% FA, B=AcCN w 0.2%FA (98:2)
F=1.5 mL/min Inj. Vol: 2-,4-, 6-ul, respectively;
Detector: DAD, 254-nm; Flowcell: 3uL, 2 mm flow path

Solutes: 1 2 3 4 5 6 7 8 min

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

Small Molecules

SPP Similar Performance to 1.8 μm TPP

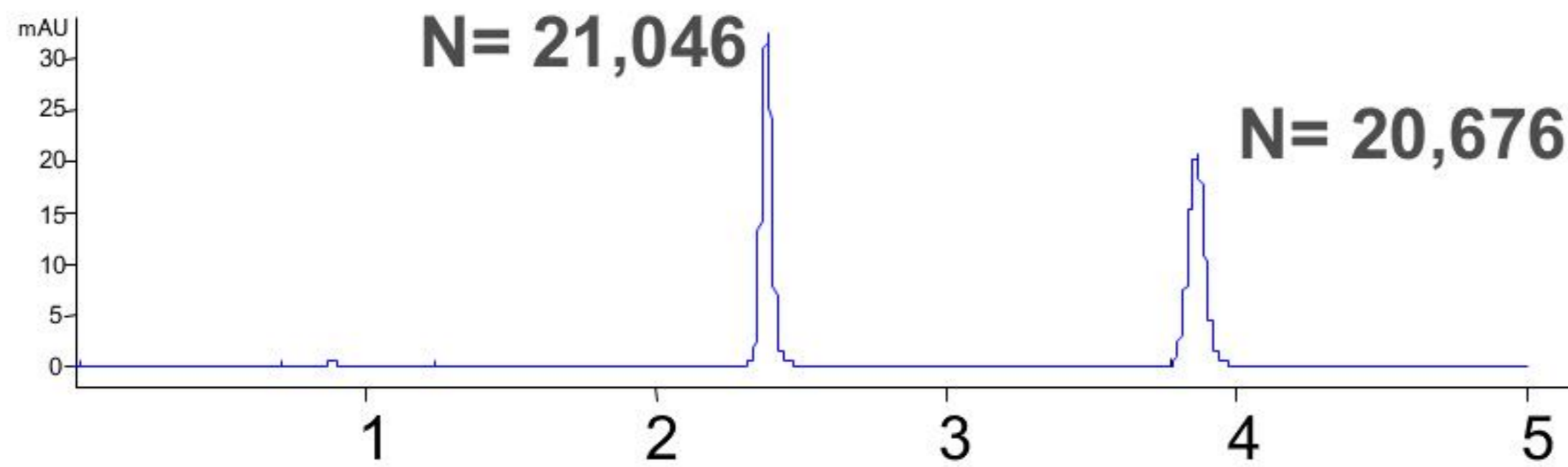
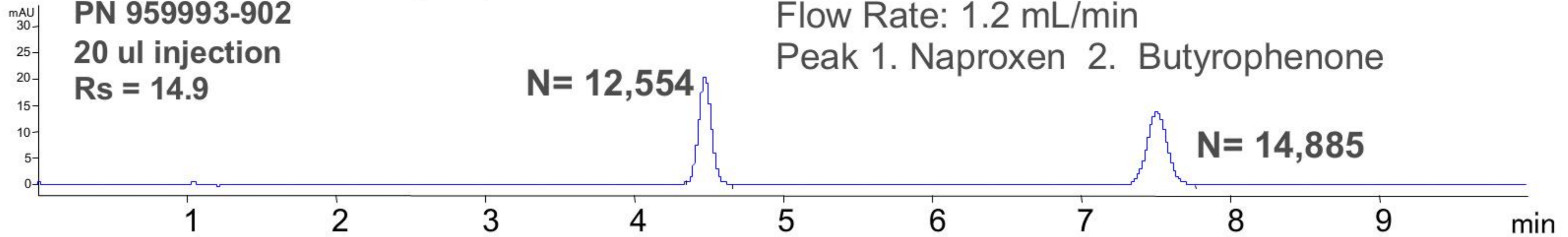


Naproxen Tablets - USP Method Faster Analysis on Poroshell 120

Method Requirement $N > 4000$, R_s better than 11.5

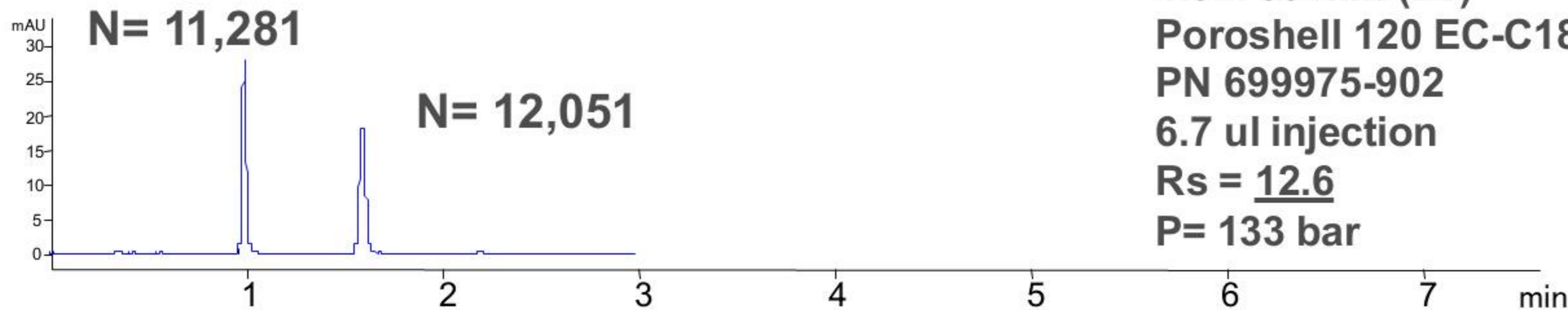
4.6 x 150 mm (L1)
Eclipse Plus C18, 5um (TPP)
PN 959993-902
20 ul injection
 $R_s = 14.9$

Common Conditions:
Mobile Phase: 50:49:1 MeCN:H2O Acetic Acid
Flow Rate: 1.2 mL/min
Peak 1. Naproxen 2. Butyrophenone



4.6 x 100 mm (L1)
Poroshell 120 EC-C18, 2.7 um
PN 695975-902
13.67 ul injection
 $R_s = 17.0$
 $P = 238$ bar

2X Faster



4.6 x 50 mm (L1)
Poroshell 120 EC-C18, 2.7 um
PN 699975-902
6.7 ul injection
 $R_s = 12.6$
 $P = 133$ bar

4.5X Faster

Comparison

Pressure

But What About Pressure?

Pressure Increases Dramatically with Decreasing Particle Size

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

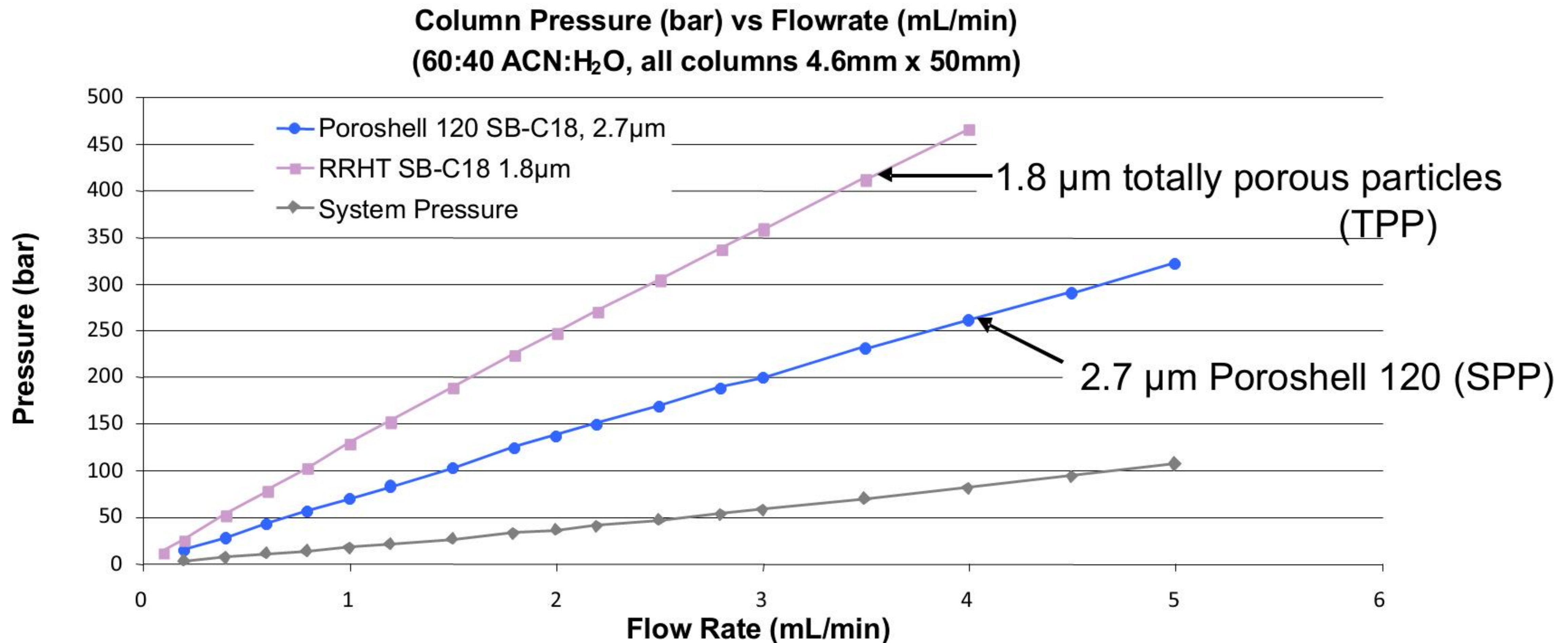
v = Flow Velocity

d_p = Particle Diameter

θ = Dimensionless structural constant

- ✓ Many parameters influence column pressure
 - Particle size and column length are most critical
- ✓ Long length and smaller particle size = more resolution and pressure

Backpressure: Comparison of 2.7 μm Poroshell 120 with 1.8 μm Totally Porous Particles



- Superficially porous particles have ~~50%~~ 45% lower backpressure than 1.8 μm totally porous particles when used under the same conditions.

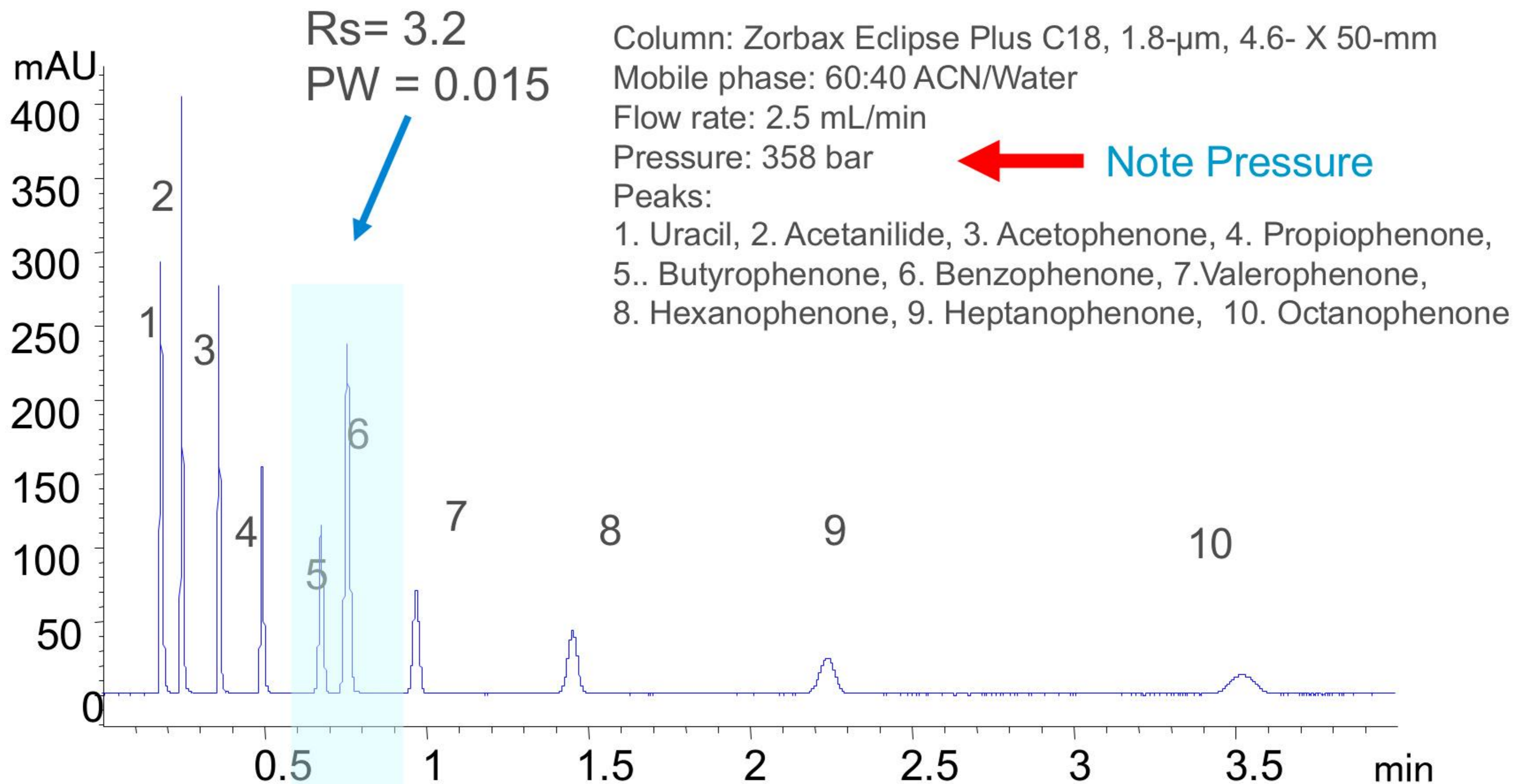
Efficiency and Pressure Tradeoffs

Totally Porous Sub-2- μm Columns (TPP) and 2.7- μm Superficially Porous Particle (SPP) Columns

- **High Throughput (Speed)**
- **High Efficiency (Plate Number)**

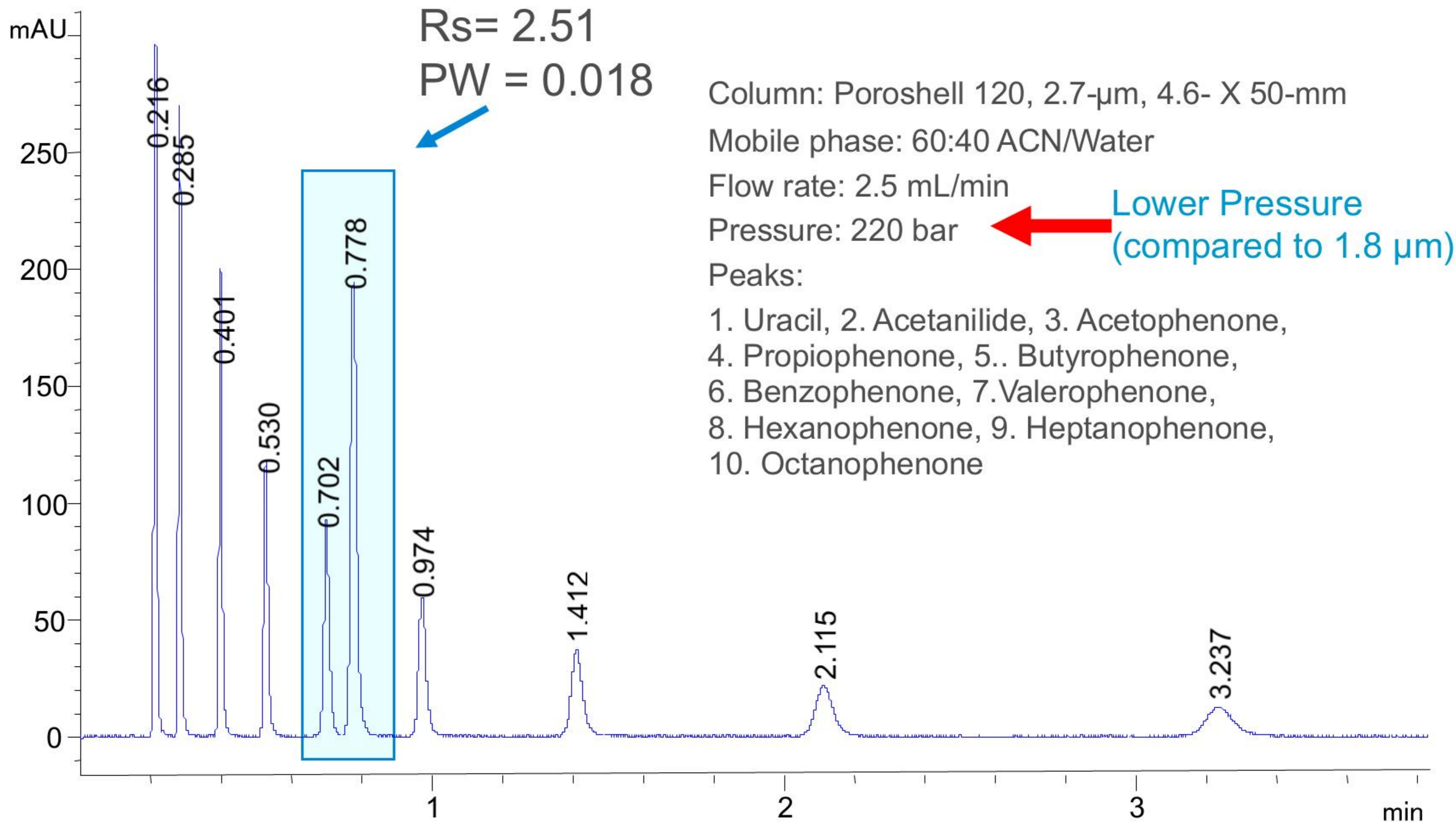
Eclipse Plus C18 Totally Porous 1.8- μ m

Isocratic Separation of Alkylphenones



Poroshell 120 Superficially Porous Particle (2.7- μm) (SPP)

Isocratic Separation of Alkylphenones



Comparing Efficiency and Pressure Different Types of Columns

Particle Size/Type	Pressure	Efficiency	LC Compatibility
3.5 μ m – Totally Porous	123 bar	7,800	All 400 bar instruments
2.7 μ m – Poroshell 120* (Superficially Porous)	180 bar	12,000	All LCs/UHPLCs (up to 600 bar)
1.8 μ m – Totally Porous	285 bar	12,500	All LCs/UHPLCs (up to 1200 bar)

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

12 Phenols on SPP Under 400 bar

Column: Poroshell 120 EC-C18, 4.6 x 50mm, 2.7 μ m

Mobile Phase A: Water w/0.1% Formic Acid

B: Acetonitrile

Gradient	Time	%B
	0.8	5%
	6.8	60%

Instrument: 1200 SL; 2 mm flow cell;

Temp: 25 C

Column: Poroshell 120 EC-C18, 4.6 x 100mm, 2.7 μ m

Mobile Phase A: Water w/0.1% Formic Acid

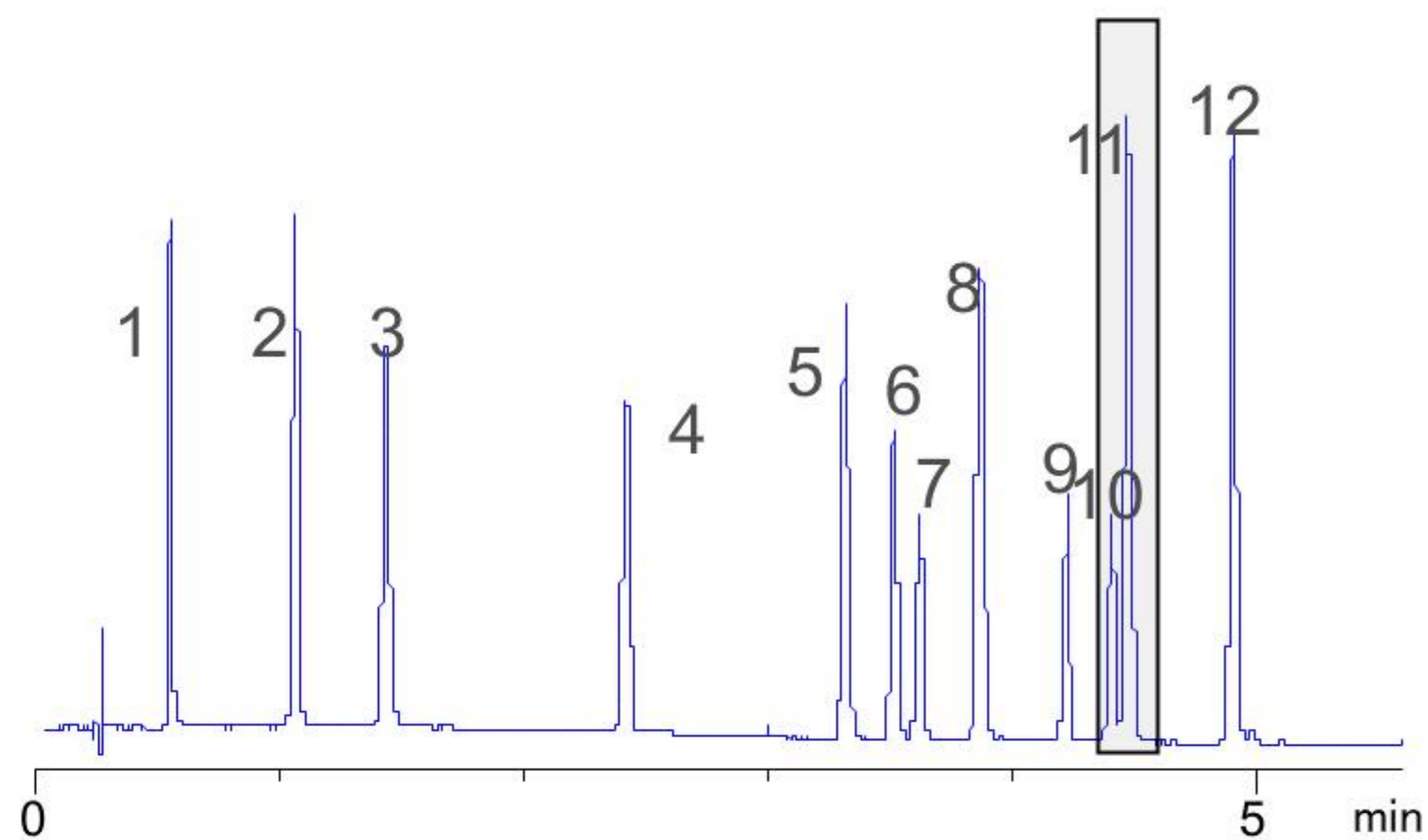
B: Acetonitrile

Gradient	Time	%B
	2.0	5%
	17	60%

Instrument: 1200 SL; 2 mm flow cell;

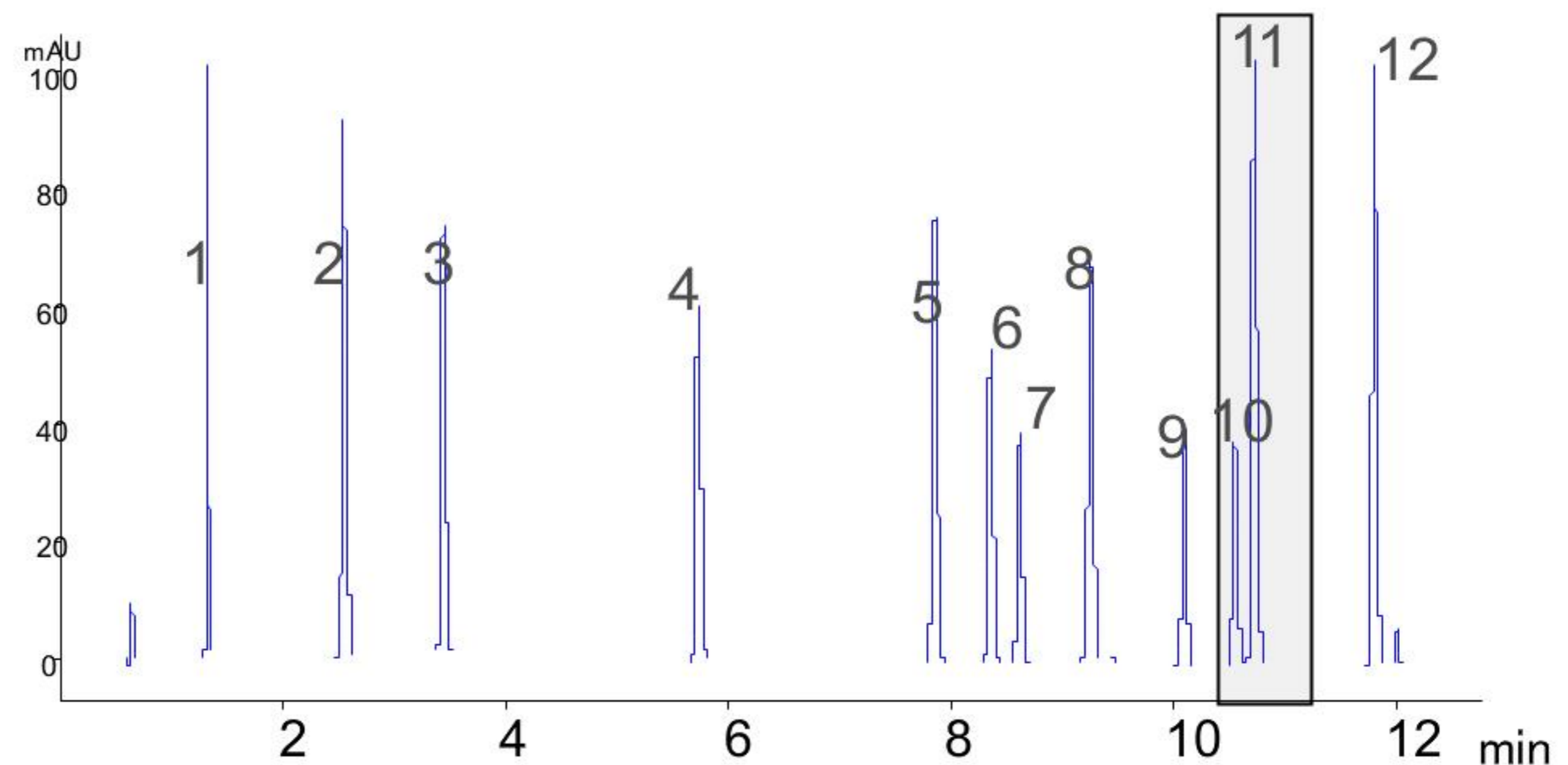
Temp: 25 C

274 bar 2.5 ml/min



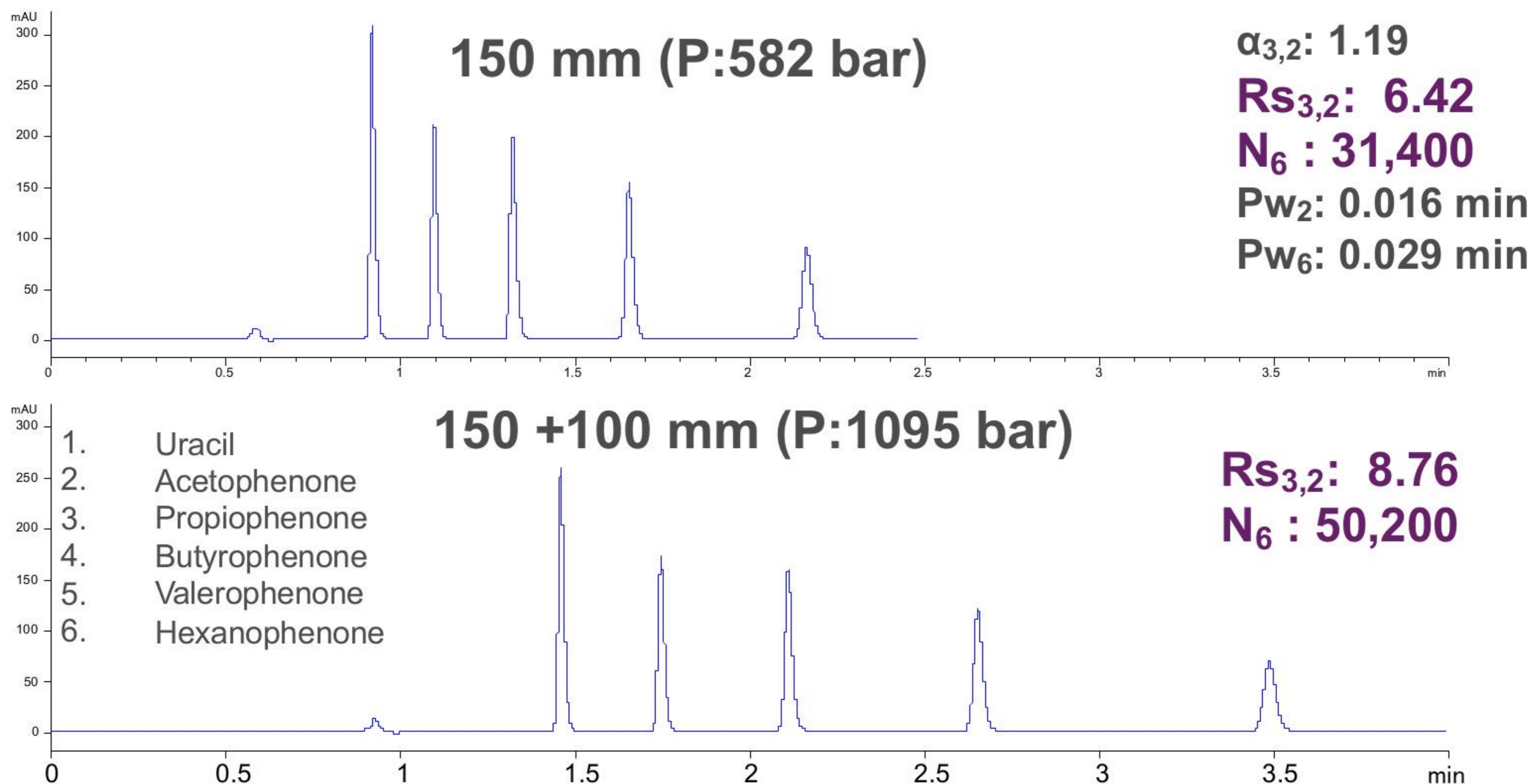
Sample: 1. Hydroquinone, 2. Resorcinol, 3. Catechol, 4. Phenol, 5. 4-Nitrophenol, 6. p-cresol, 7. o-cresol, 8. 2-Nitrophenol, 9. 3,4 di methyl phenol, 10. 2,3 di methyl phenol, 11. 2,5 di methyl phenol, 12. 1-naphthol

**Improve Resolution with 100mm
2.0 ml/min, 394 bar**



Gain Efficiency and Resolution 1.8 μ m TPP

Mobile Phase 25:75 water:acetonitrile, Flow Rate: 0.5 mL/min, ambient temperature, ZORBAX RRHD SB-C18, 1.8 μ m columns

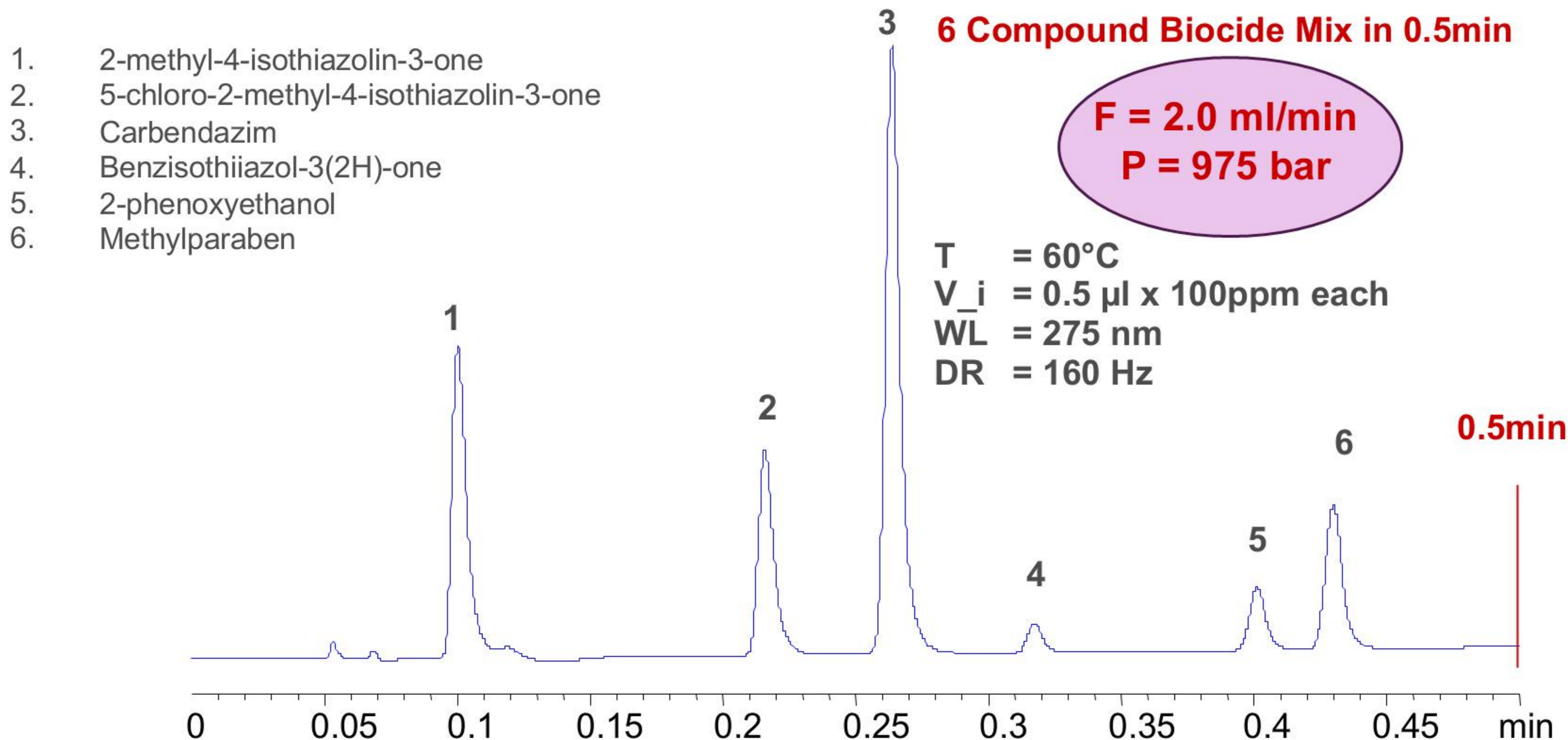


High R_s in 3.5 minutes, 250mm, 1.8 μ m at **1100 bar**
Resolution is fine on 150mm column, but longer column has even more

High Throughput: Sub 30 Second Separations with Short 1.8- μm (TPP) Columns

Column: ZORBAX RRHD SB-C18 2.1 x 50mm 1.8 μm

Gradient: H₂O (0.05% TFA) / 10-40 % ACN; 1min

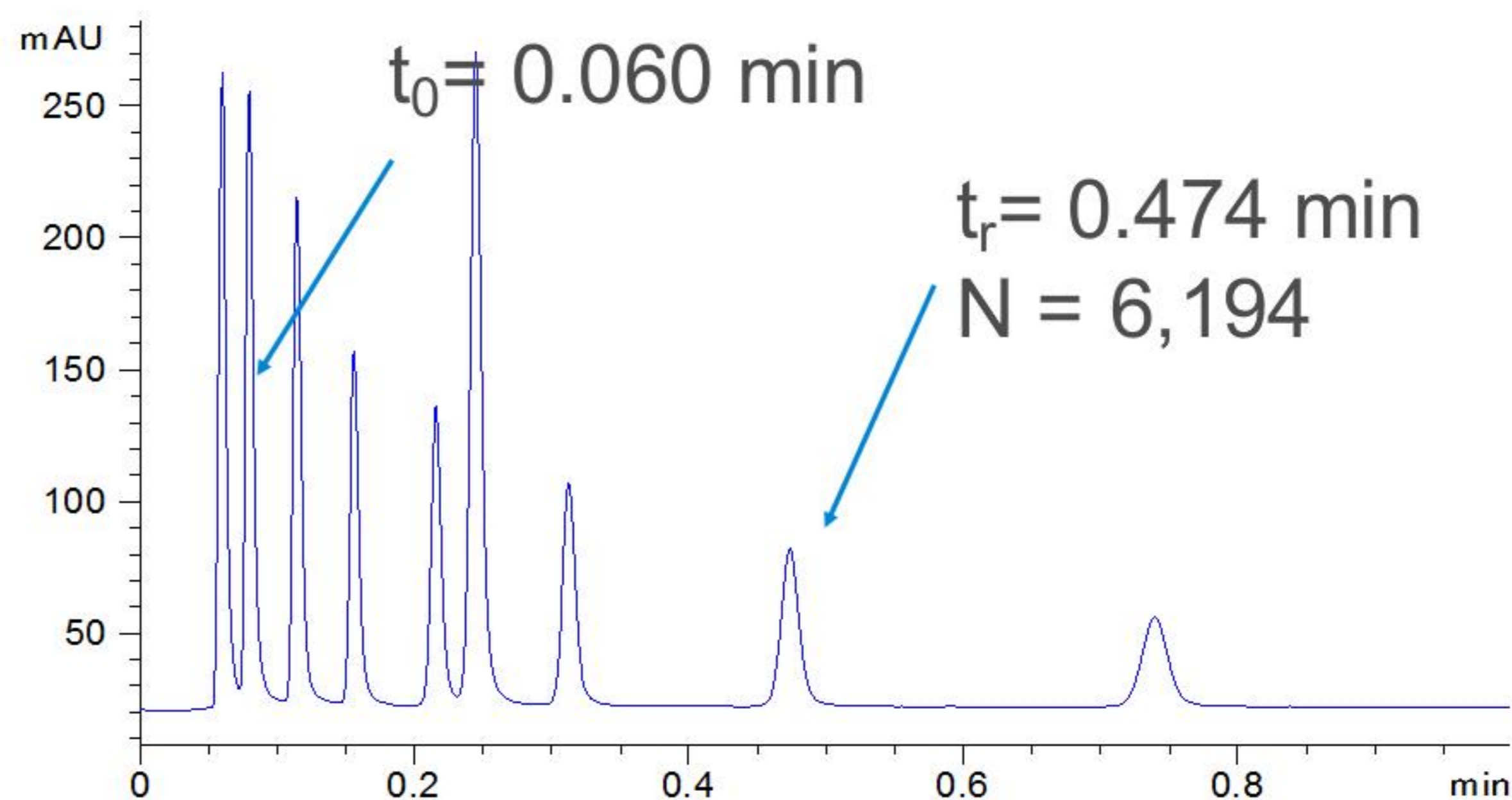
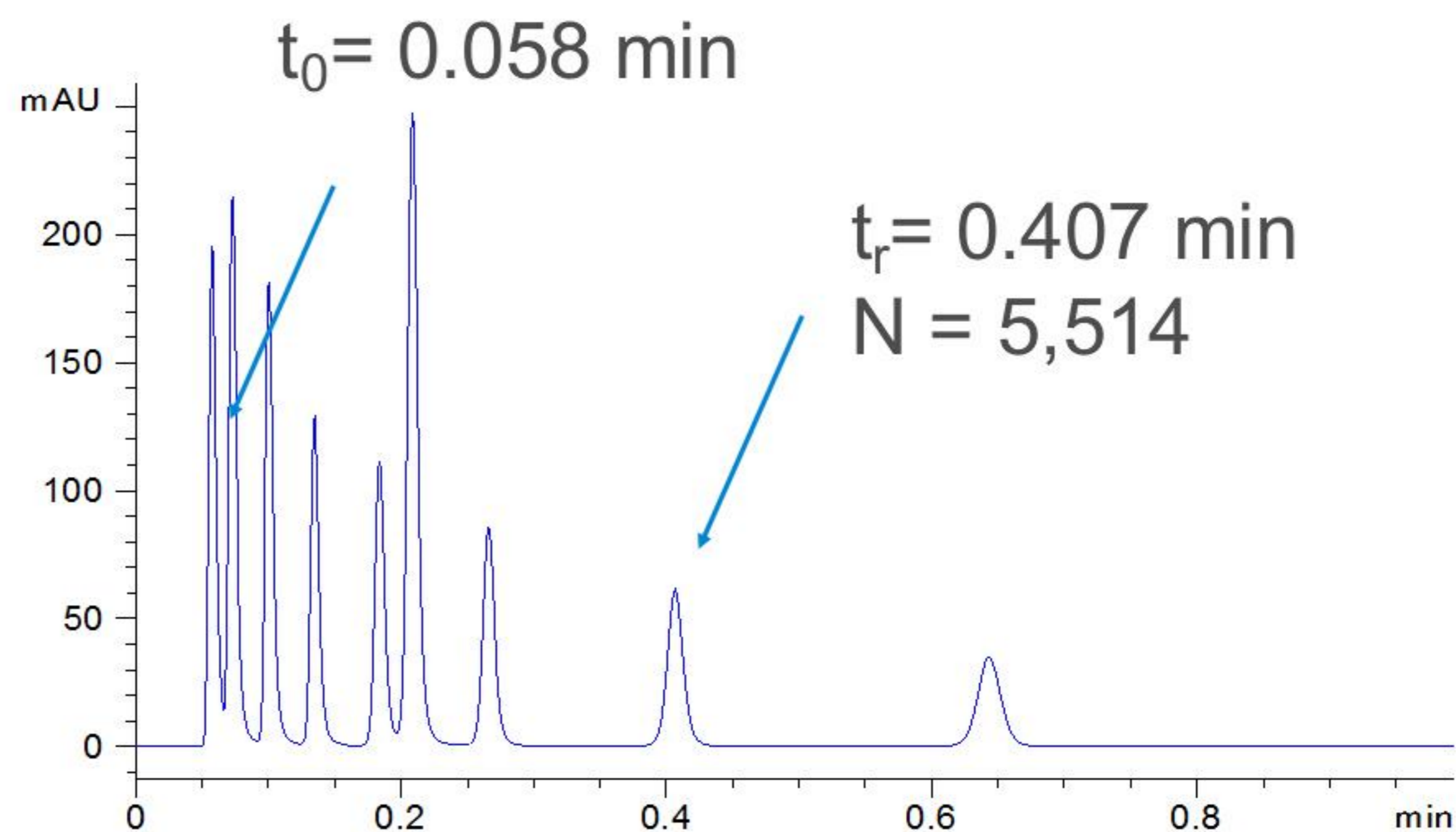


Achieving this speed takes advantage of the higher pressure limits of the UHPLC Systems (like Agilent 1290 Infinity)

TPP v SPP for Ultra-Fast Analysis on 50 mm Columns

2.7 μ m Poroshell 120
EC-C18
2.1 x 50 mm
F = 1.94 mL/min, 40°C
56% ACN, 44% Water
549 bar

1.8 μ m ZORBAX RRHD
Eclipse Plus C18
2.1 x 50 mm
F = 1.82 mL/min, 40°C
59% ACN, 41% Water
1003 bar

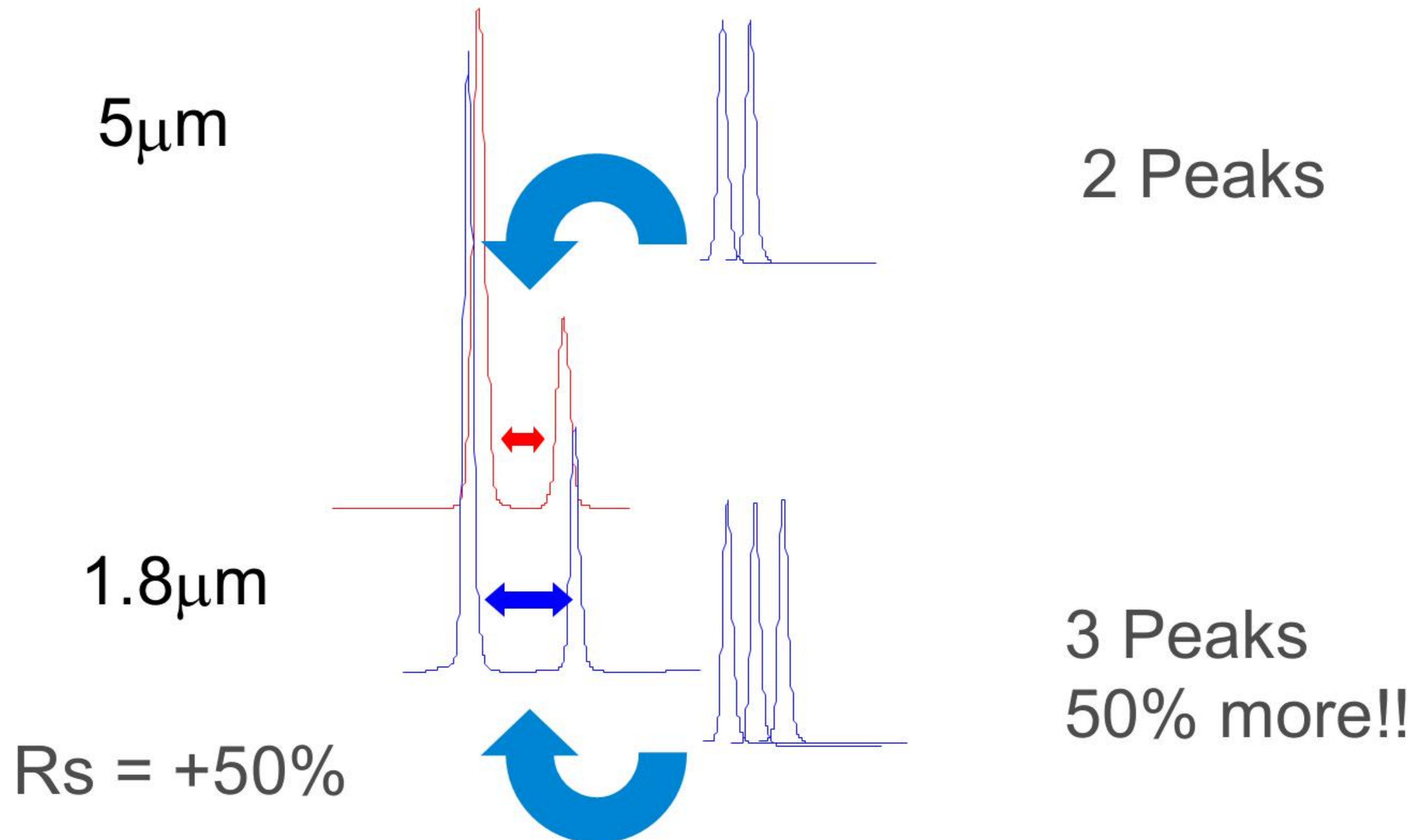


Comparison

Peak Capacity

Defining Peak Capacity

- Peak capacity is the number of peaks which can be separated with defined resolution (example: $R=1$) in a certain period of time for a given system (column length and particle size)
- Peak capacity is another measure of separation efficiency (esp. for gradients) and is especially useful for complex chromatograms.



Calculating Peak Capacity - Equations

Peak Capacity (P_c) - Gradient Elution Equations

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

P_c = peak capacity
 t_g = 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

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

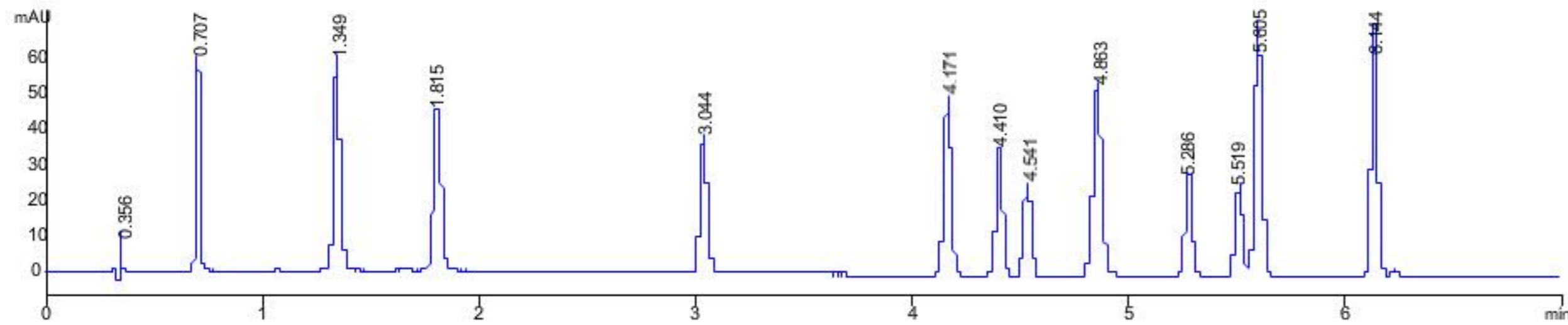
$$3. P_c = 1 + \frac{t_{R,n} - t_{R,1}}{W_{av}}$$

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

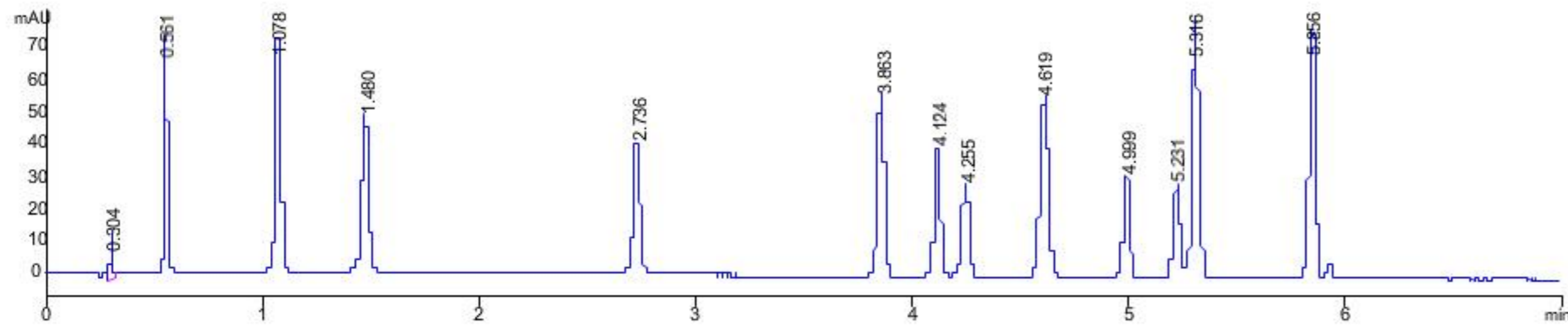
Peak Capacity

(P_c) of TPP (1.8 μm) Slightly Higher Than SPP

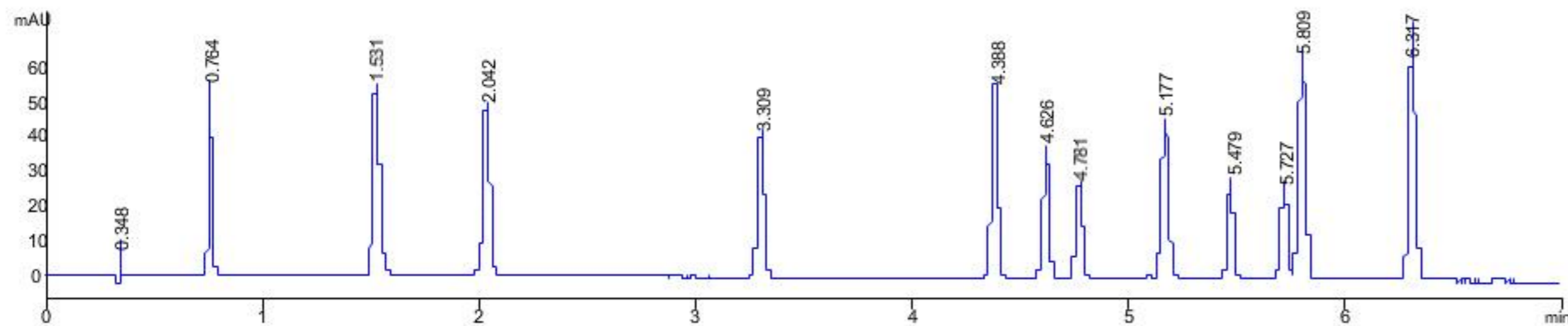
Columns: 4.6 x 50mm Flow Rate: 2 mL/min



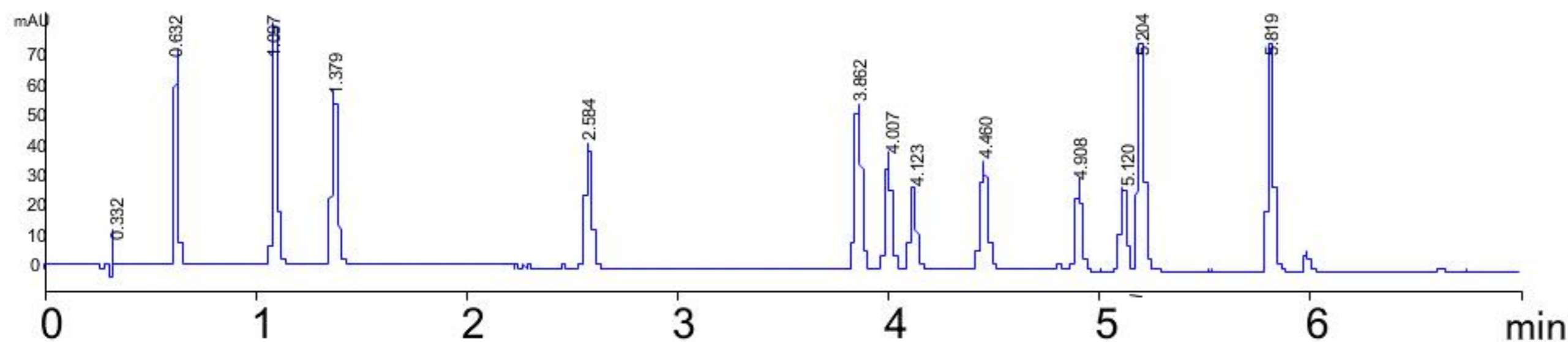
Poroshell 120 EC C18
2 ml/min 220 Bar (SPP)
 P_c Ave=54



Other C18
2 ml/min 210 Bar (SPP)
 P_c Ave=55



Eclipse Plus C18 RRHT
2 ml/min 364 Bar
 P_c Ave=61 (TPP)



Other C18 (2.6um)
2 ml/min 232 Bar (SPP)
 P_c Ave=50

All compounds prepared at 1 mg/ml (prepared at 0.1 mg/ml) in water. Compounds were combined in equal volumes to yield approximately 0.1 mg/ml
10ul injection volume , 4.6 x 50 mm
A: 0.1 % Formic Acid in Water
B: Acetonitrile
Flow rate: 2 mL/min

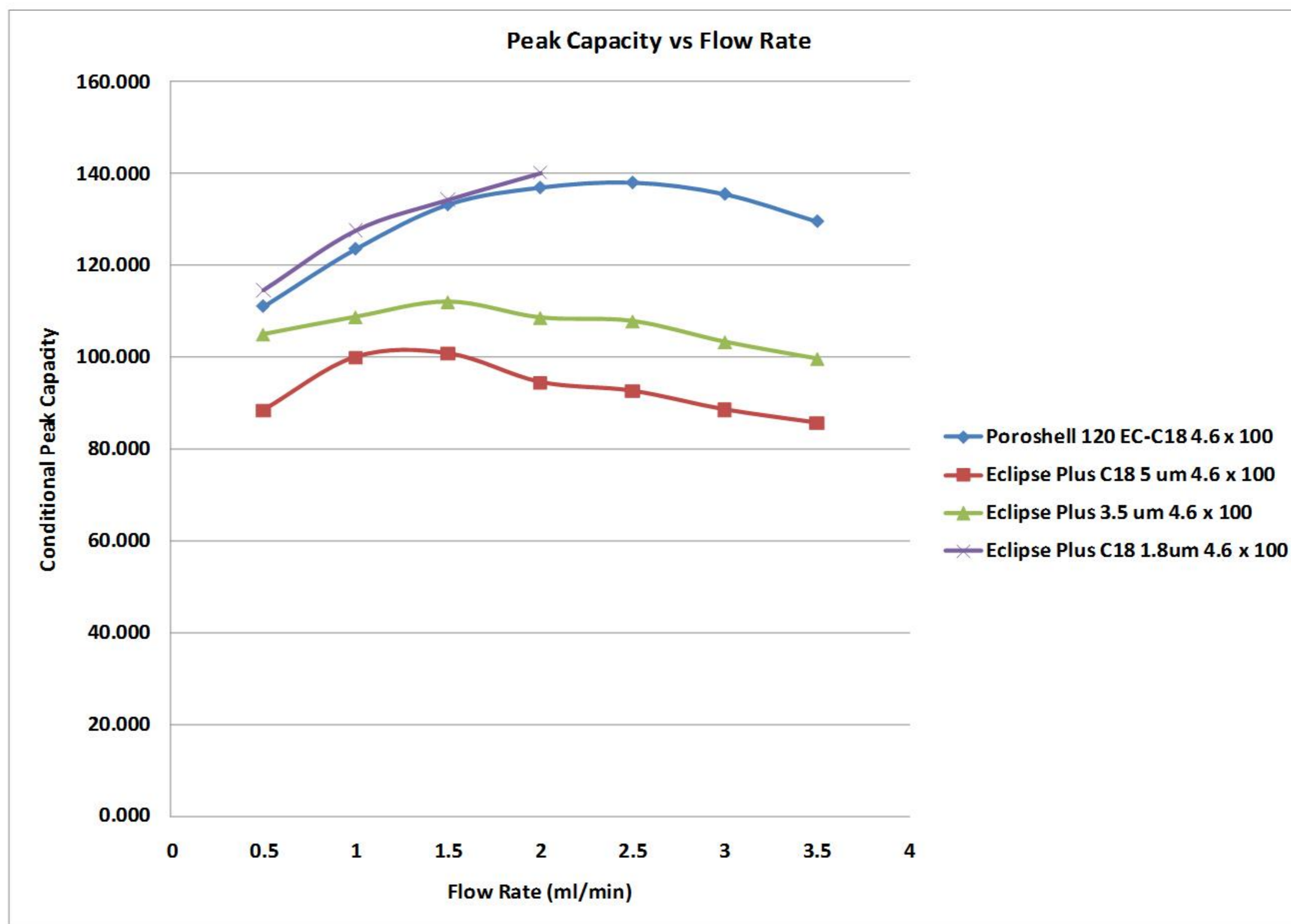
270 nm, no reference

1. Hydroquinone
2. Resorcinol
3. Catechol
4. Phenol
5. 4-Nitrophenol
6. p-cresol
7. o-cresol
8. 2-Nitrophenol
9. 3,4 di methyl phenol
10. 2,3 di methyl phenol
11. 2,5 di methyl phenol
12. 1-naphthol

Gradient	Profile	Time	% B
1	1	1	5
2	8.5	60	
3	10	60	
4	11	5	

Comparison of Peak Capacity

Poroshell 120 (SPP) vs. Totally Porous 1.8 μ m, 3.5 μ m and 5 μ m Columns



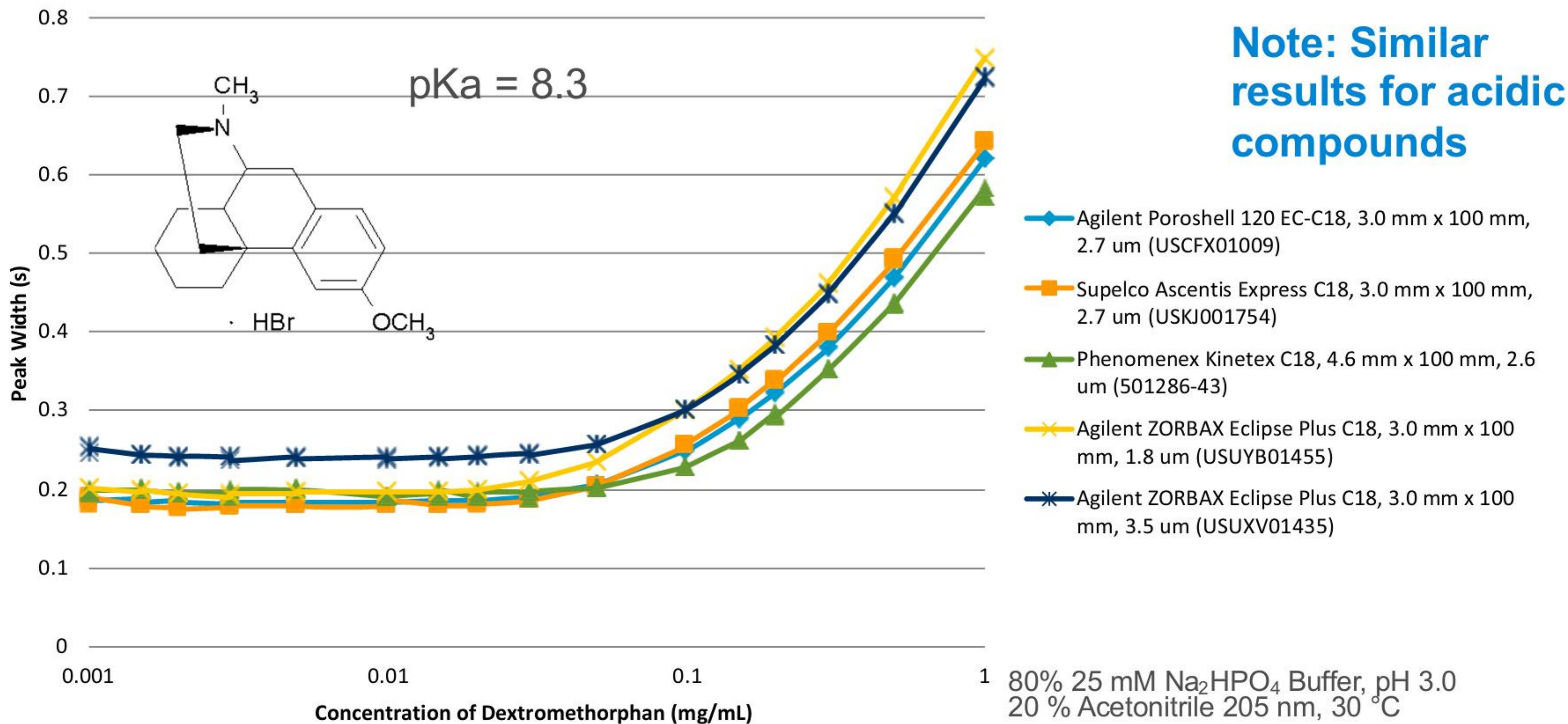
Peak capacity is similar on Poroshell 120 2.7 μ m to 1.8 μ m column of same size and higher than 3.5- or 5- μ m columns of similar size.

Comparison

Sample (Loading) Capacity

Sample Loading of Basic Compounds on Poroshell 120 (SPP) Comparable to sub-2 micron Columns

Base Loading with Dextromethorphan



The increase in peak width for the Poroshell 120 is at the same loading as the 1.8 μm.

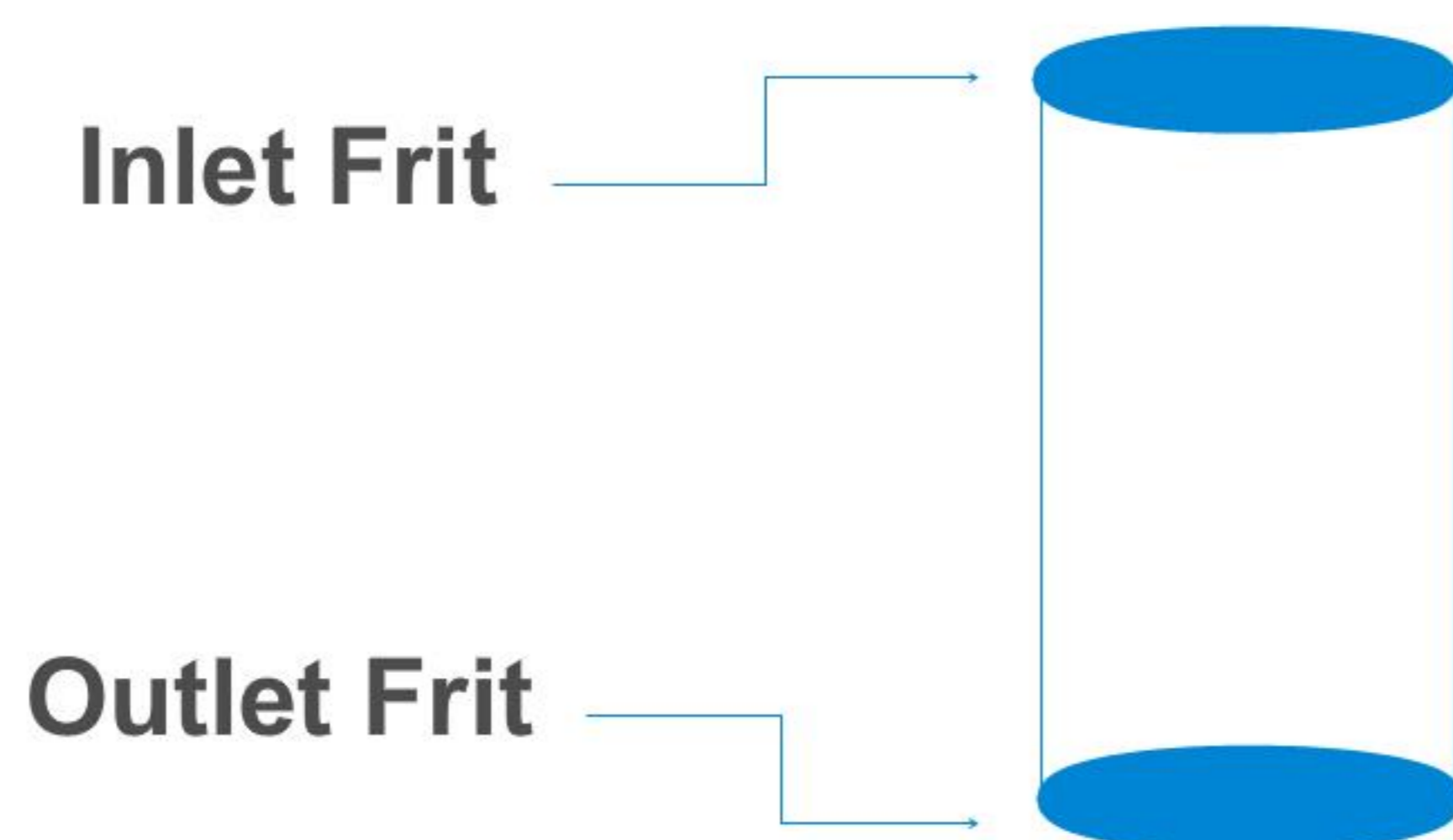
Compare

Practical Aspects

- Tolerance for Dirty Samples (Plugging)
- Available Phases
- Method transfer
 - ▶ See Appendix
- Instrument

Most Column Pressure Problems in HPLC/UHPLC... Caused by Sample/Mobile Phase Matter Plugging Inlet Frit

- Filter sample and buffers
- Use in-line filter to keep particulates out of inlet frit
- Outlet frit must be smaller than smallest particles in column packing



Quick Summary of Families and Bonded Phases

Poroshell 120

Particle size: 2.7um

EC-18, EC-C8, SB-C18, SB-C8, Phe-Hex, SB-Aq, PFP, EC-CN, Bonus-RP, HPH-C18, HPH-C8, HILIC

Eclipse Plus

Particle sizes: 5, 3.5, 1.8um

Available phases: C18, C8, Phenyl-Hexyl, PAH, HILIC

StableBond

Particle sizes: 7, 5, 3.5, 1.8um

Pore sizes 80Å & 300Å

Available phases: C18*, C8*, Phenyl, CN, C3, Aq*

*Also available in Poroshell 120

Eclipse XDB

Particle sizes: 7, 5, 3.5, 1.8um

Available phases: C18, C8, Phenyl, CN

Also available in Poroshell 120

Bonus-RP

Particle sizes: 5, 3.5, 1.8um

Also available in Poroshell 120

Additional Phases

Extend-C18

Particle sizes: 5, 3.5, 1.8um

Pore sizes 80Å & 300Å

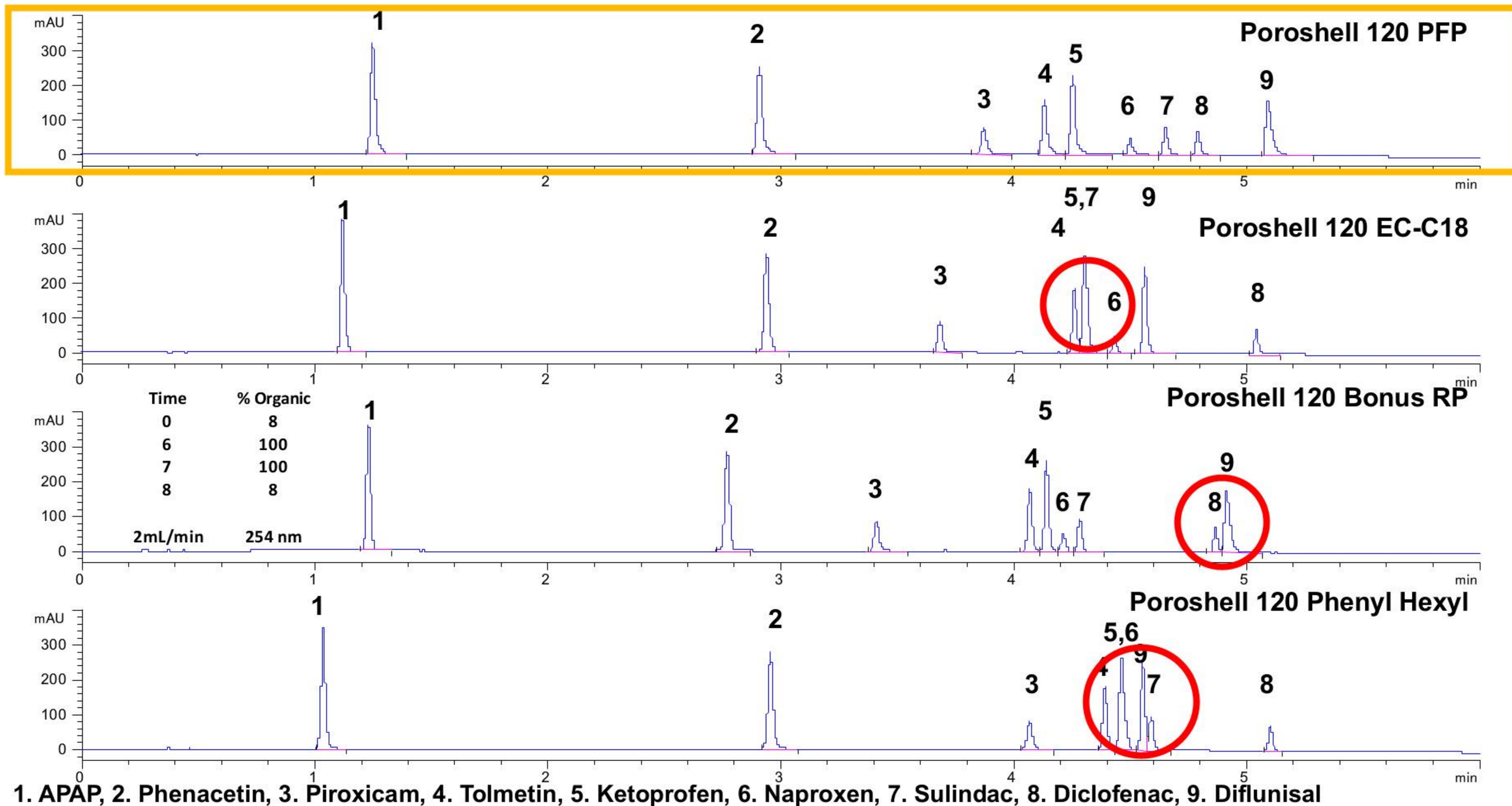
300-Diphenyl

Particle size: 1.8um



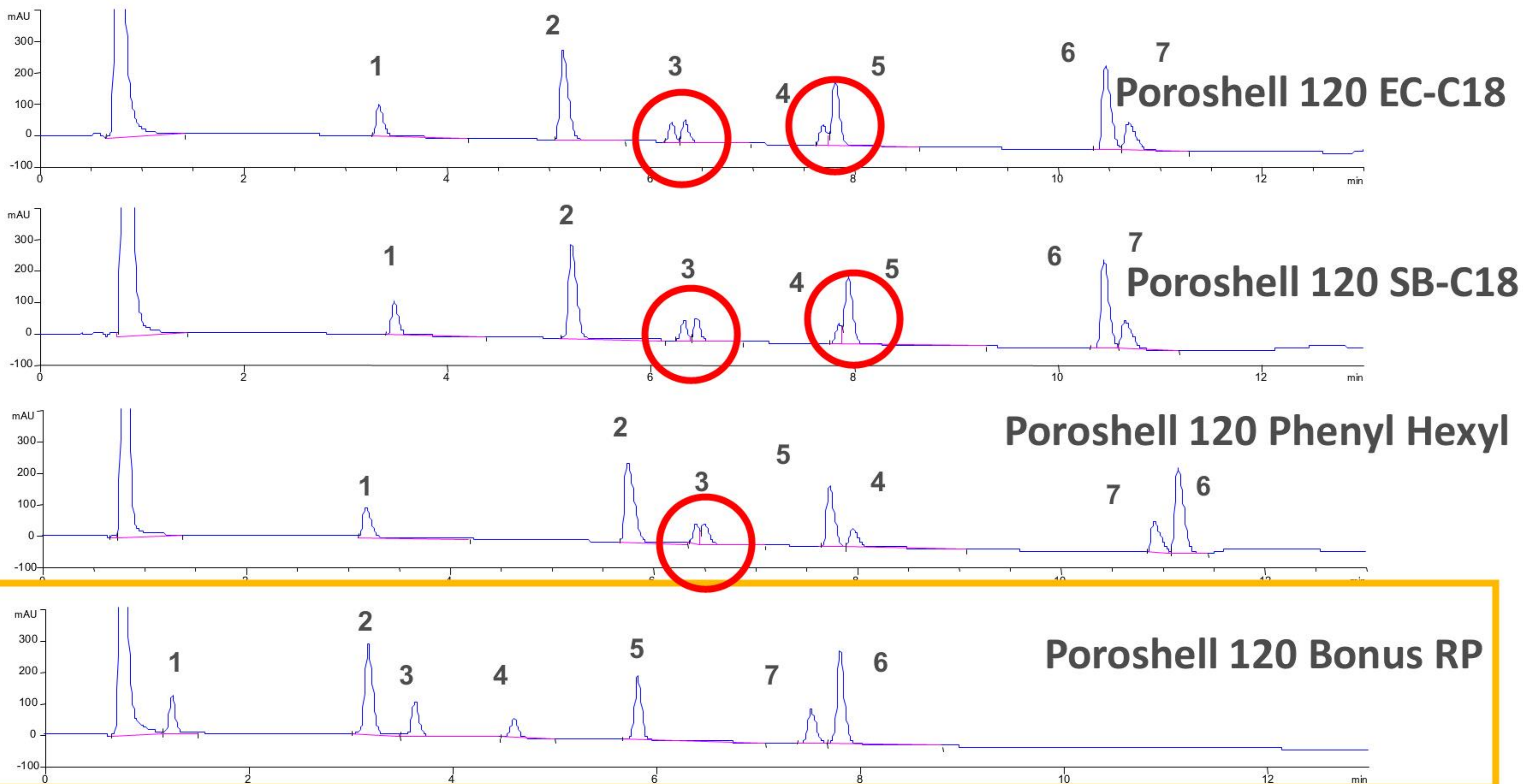
NSAID Separation Poroshell 120 MeOH Gradient

Best Resolution of all analytes with Poroshell 120 PFP



Comparison of Bonded Phases - Beta Blockers

Best Resolution of all analytes with Poroshell Bonus-RP

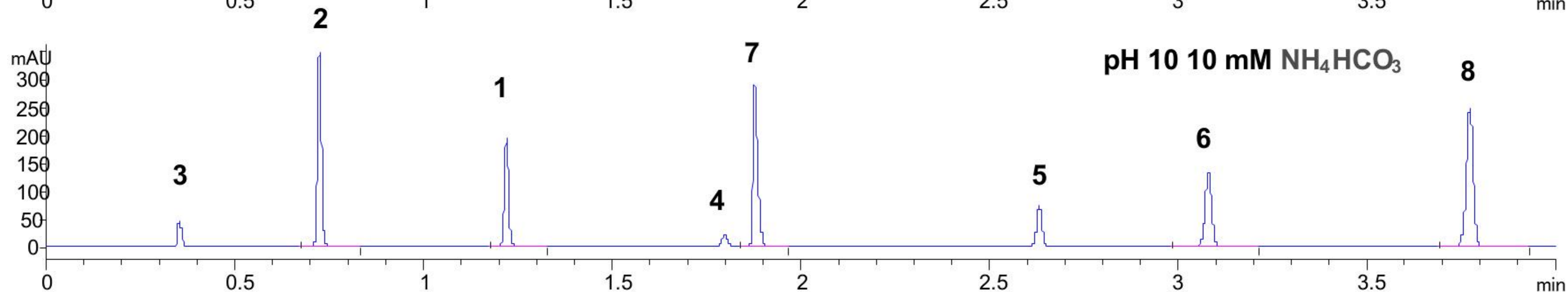
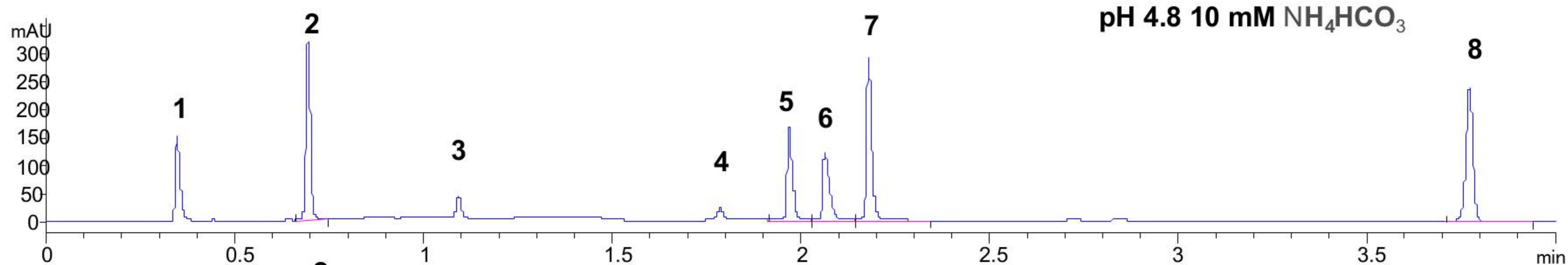
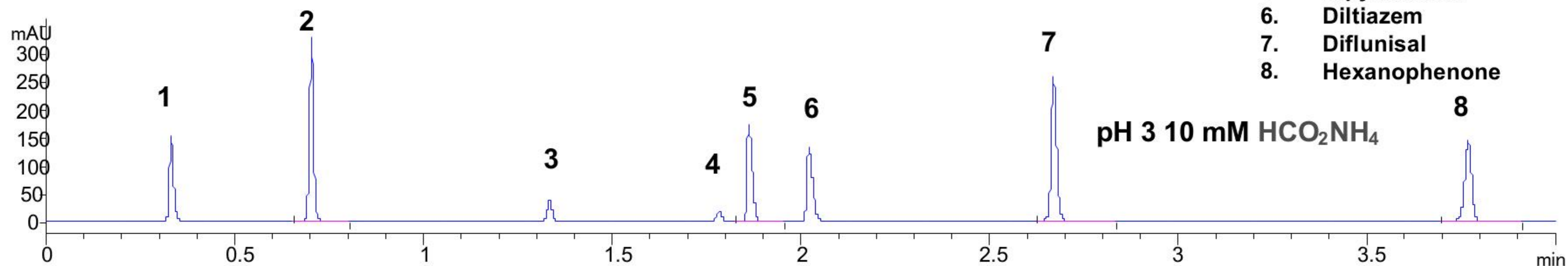


1. Atenolol, 2. Pindolol, 3. Naldolol, 4. Metoprolol, 5. Acebutolol, 6. Propranolol, 7. Alprenolol

Poroshell HPH Low, Mid, & High pH with Acetonitrile

Why use multiple pH's?

1. Procainamide
2. Caffeine
3. Acetyl Salicylic Acid
4. Hexanophenone Deg.
5. Dipyrimadole
6. Diltiazem
7. Diflunisal
8. Hexanophenone



Column/Instrument Compatibility

Particle Size/Type	Pressure	Efficiency	LC Compatibility
3.5µm – Totally Porous	123 bar	7,800	All 400 bar instruments
2.7µm – Poroshell 120* (Superficially Porous)	180 bar	12,000	All LCs/UHPLCs (up to 600 bar)
1.8µm – Totally Porous	285 bar	12,500	All LCs/UHPLCs (↑1200 bar)

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

Comparison

TPP sub-2 μm and SPP, 2.7 μm

Parameter	TPP (sub-2- μm)	SPP (2.7- μm)	Conclusion
Efficiency	12,500 pI/50 mm	12,000 pI/50 mm	Similar
Pressure	~ 2X SPP	~ 1/2 TPP	SPP lower
Selectivity (bonded phase)	Same particle size to particle size	Only slightly different for same phase for TPP	Transfer of method from TPP \rightarrow SPP
Particle size distribution	1.47 (90%/10% ratio)	1.20 (90%/10% ratio)	SPP 25% narrower; helps packing efficiency; "A" term
Peak capacity	About the same as SPP	About the same as TPP	May be compound dependent
Plugging	0.5 micron porosity frit	2 micron porosity frit	SPP less prone
Sample capacity	Same	Same	SPP 75% of surface volume; particles pack more densely so a "wash"
Scale-up for prep (particle sizes)	1.8, 3.5, 5.0 (7.0)	Only 2.7- μm	TPP better
Pressure limit	Up to 1200 bar	Up to 600 bar	TPP goes higher; hardware different
Column diameters	2.1, 3.0 (1200 bar) 2.1, 3.0, 4.6 (600 bar)	2.1, 3.0, 4.6	Highest pressure (1200 bar) columns have one less id
Number of phases	Largest (17)	Large (12)	TPP wins out here since SPP newer

Agilent Technical Support

LC or GC Column Support

800-227-9770 (US & Canada)

Select opt. 3, opt. 3, then option 1 for GC or option 2 for LC.

Email: lc-column-support@agilent.com,
gc-column-support@agilent.com

Website: www.agilent.com/chem



Questions?



Appendix

New Phases on Poroshell 120

Poroshell 120 PFP

- A USP L43 bonded chemistry consisting of a perfluorophenyl ring attached with a propyl linker
- Excellent choice for polar analytes and analytes with pi-character due to its ability to have unique pi-pi interactions
- Position as an alternative phase chemistry for unique selectivity that is orthogonal to C18 chemistries
- **Recommended operating range**
 - **pH 2-8**
 - **Maximum temp: 60°C**

Poroshell HPH-C18 (June 1) and HPH-C8 (July 1)

- A chemically modified Poroshell 120 particle using proprietary technology designed to give high pH stability
- Utilizes the same bonding chemistry as EC-C18 and EC-C8
- **Recommended operating range**
 - **pH 3-11**
 - **Maximum temp: 60°C**

Columns are available in 2.1, 3.0, and 4.6mm ID's, and in lengths of 50, 100, and 150mm; Other dimensions available through the custom columns process.

Poroshell 120 Bonded Phases

EC-C18
EC-C8
EC-CN

- Endcapped for best peak shape
- **EC-C18 – 1st choice for most separations, use C8 for less retention**
- EC-CN similar to Zorbax Eclipse XDB -CN; alternate selectivity for normal and reversed-phase

SB-C18
SB-C8

- Non-endcapped
- **Best performance and longest lifetime at low pH (1 -2)**
- Poroshell 120 SB-C8 enables method transfer from existing Zorbax SSB-C8 columns in many established USP methods

NEW
HPH-C18
HPH-C8

- Chemically modified Poroshell 120 particle using proprietary technology to give high pH stability
- **Recommended operating range: pH 3-11**
- .Alternate selectivity to other C18 and C8 phases

Bonus-RP

- Embedded polar group provides unique selectivity for polar compounds
- **Often changes elution order of acidic and basic compounds**
- Small polar acids can be separated on Bonus -RP with 0.1% TFA mobile phases



Poroshell 120 Bonded Phase, cont'd

Phenyl-Hexyl

- Utilizes the same bonding process utilized in ZORBAX Eclipse Plus Phenyl -Hexyl
- **Excellent choice for resolution of analytes such as aromatic compounds**, due to unique pi-pi interactions, or when other phenyl type columns are recommended
- Alternative selectivity to EC-C18 or SB-C18

PFP

- USP L43
- **Can give extra retention and selectivity for positional isomers of halogenated compounds**
- Use for the separation of aromatic compounds and polar molecules containing hydroxyl, carboxyl, nitro and other polar groups

SB-Aq

- Proprietary bonded phase is an excellent choice for polar analytes that don't retain well on C18, and when LC-UV is used
- StableBond chemistry enhances low-pH stability, for the most acidic mobile phases
- Good choice for small, polar compounds where 100% aqueous mobile phase is needed.

HILIC

- Bare silica HILIC (ships in HILIC solvents) for separations of polar compounds
- Ideal for retention and analysis of polar compounds by LC/MS in high organic for maximum sensitivity



How To “Match” a Column to ZORBAX RRHT/RRHD

General Phase Type	Starting ZORBAX Choice
Typical “endcapped” C18 or C8 bonded phases, newer columns	Eclipse Plus C18 or C8
Endcapped C18 or C8 columns, older generation	Eclipse XDB-C18 or C8
Non-endcapped columns	StableBond C18
Older types of columns	StableBond C18, C8 etc.
Aqueous “type” columns	SB-Aq
CN or Phenyl	Eclipse Plus Phe-Hex, SB-Phenyl, SB-CN

Phenyl-Hexyl and Bonus-RP are for general method development use for alternate selectivity
 Use SB-AQ for LC-UV and HILIC for LC/MS of polar, difficult to retain analytes

Method Translation Software

Adobe Flash Player 10

Agilent Technologies Method Translator and Cost Savings Calculator

Basic Mode **Advanced Mode** Viscosity Table Cost Savings Calculator Agilent 1260 Infinity LC

Original Method

System Info

Max System Pressure (bar) 400

Allowed pressure (%) 90

Max. System Flow (mL/min) 5

System Dispersion (µL) 50

Column Info

Column ID (mm) 4.6

Column length (mm) 150

Particle Size (µm) 5

Flow Resistance Factor 900

Porosity 0.6

Red. Plate Height 2.3

Calculated values

Pressure (bar) 41

Linear Velocity (mm/s) 1.67

Estimated k* (grad. k') 2.50

Column Volume (mL) 1.495

Plate Numbers 13,043

Effective Plate Numbers 8,786

Plate Number Yield (%) 67

Peak Volume (mL) 0.279

Peak Width (min) 0.279

Peak Width (s) 16.734

Peak Capacity 51

Solvent consumption (mL) 25.000

Method Info

Max. Solvent Viscosity (cP) 0.75

Flow Rate (mL/min) 1

Injection Volume (µL) 20

	Time	%B	Flow
Initial:	0.00	25.0	1.00
Initial Hold:	1.00	25.0	1.00
Gradient:	15.00	100.0	1.00
Hold to:	20.00	100.0	1.00
Return by:	20.10	25.0	1.00
End of Run:	25.00	25.0	1.00

Alerts!

New Method

System Info

Max System Pressure (bar) 1200

Allowed pressure (%) 90

Max. System Flow (mL/min) 5

System Dispersion (µL) 10

Column Info

Column ID (mm) 2.1

Column length (mm) 50

Particle Size (µm) 1.8

Flow Resistance Factor 900

Porosity 0.6

Red. Plate Height 2.3

Calculated values

Pressure (bar) 104

Linear Velocity (mm/s) 1.67

Estimated k* (grad. k') 2.50

Column Volume (mL) 0.104

Plate Numbers 12,077

Effective Plate Numbers 4,688

Plate Number Yield (%) 39

Peak Volume (mL) 0.027

Peak Width (min) 0.127

Peak Width (s) 7.636

Peak Capacity 38

Solvent consumption (mL) 1.737

Method Info

Max. Solvent Viscosity (cP) 0.75

Flow Rate (mL/min) 0.21

Injection Volume (µL) 1.39

	Time	%B	Flow
Initial:	0.00	25.0	0.21
Initial Hold:	0.33	25.0	0.21
Gradient:	5.00	100.0	0.21
Hold to:	6.67	100.0	0.21
Return by:	6.70	25.0	0.21
End of Run:	8.33	25.0	0.21

Time Saving Factor

3.0

Solvent Saving

93%

Simple Conversion
 Speed Optimized
 Resolution Optimized

Print Reset Profiles Help Disclaimer Version 2.4

http://www.chem.agilent.com/en-US/Products-Services/Instruments-Systems/Liquid-Chromatography/pages/1200infinity_cost_calculator.aspx

New Method Development Kits

Choose from a variety of kits to suit your needs

- **Poroshell 120 L1, L7, and L10 USP Kits** make it easier to improve speed and sample throughput – without sacrificing resolution – by transferring your 5µm USP methods to Poroshell 120 columns
- **Poroshell 120 Selectivity Kits** provide a variety of column chemistries to help quickly adjust your analyte retention & selectivity
- **ZORBAX RRHD and Poroshell 120 Aqueous Method Development Kits** are ideal for polar compounds and 100% aqueous conditions, so you can achieve greater analyte retention without the phase collapse that can occur with C18 chemistries

Method Development Kits	Description (One of each)	Dimension	Part No.
Poroshell 120 Selectivity	EC-C18, Phenyl-Hexyl, Bonus-RP	2.1 x 50 mm	5190-6155
Poroshell 120 Selectivity	EC-C18, Phenyl-Hexyl, Bonus-RP	4.6 x 50 mm	5190-6156
Poroshell 120 Aqueous	SB-Aq, Phenyl-Hexyl, Bonus-RP	2.1 x 50 mm	5190-6157
Poroshell 120 Aqueous	SB-Aq, Phenyl-Hexyl, Bonus RP	4.6 x 50 mm	5190-6158
Poroshell 120 L1, L7, and L10 USP	EC-C18, EC-C8, EC-CN	4.6 x 100 mm	5190-6159
Poroshell 120 L1, L7, and L10 USP	EC-C18, EC-C8, EC-CN	3.0 x 100 mm	5190-6160
ZORBAX RRHD pH	SB-C18, Eclipse Plus C18, and Extend-C18	2.1 x 50 mm	5190-6152
ZORBAX RRHD Eclipse Plus	Eclipse Plus C18, Eclipse Plus C8, Eclipse Plus Phenyl-Hexyl	2.1 x 50 mm	5190-6153
ZORBAX RRHD Aqueous	SB-Aq, Bonus RP, Eclipse Plus Phenyl-Hexyl	2.1 x 50 mm	5190-6154

Validation Kits Available

Same column (dimensions, bonded phase, particle size); different mfg. lots

Manufacturing lots:

Agilent ZORBAX Rapid Resolution High Definition (RRHD) Method Validation Kits											
Size (mm)	Particle Size (µm)	Eclipse Plus C18	Eclipse Plus C8	Eclipse XDB-C18	Extend-C18	Eclipse Plus Phenyl-Hexyl	Bonus-RP	SB-C18	SB-C8	SB-Phenyl	SB-Aq
3.0 x 150	1.8	959759-302K	959759-306K	981759-302K				859700-302K	859700-306K		
3.0 x 100	1.8	959758-302K	959758-306K	981758-302K	758700-302K	959758-312K		858700-302K	858700-306K	858700-312K	858700-314K
3.0 x 50	1.8	959757-302K	959757-306K	981757-302K	757700-302K	959757-312K		857700-302K	857700-306K	857700-312K	857700-314K
2.1 x 150	1.8	959759-902K	959759-906K	981759-902K	759700-902K	959759-912K	859768-901K	859700-902K	859700-906K	859700-912K	859700-914K
2.1 x 100	1.8	959758-902K	959758-906K	981758-902K	758700-902K	959758-912K	858768-901K	858700-902K	858700-906K	858700-912K	858700-914K
2.1 x 50	1.8	959757-902K	959757-906K	981757-902K	757700-902K	959757-912K	857768-901K	857700-902K	857700-906K	857700-912K	857700-914K

Agilent ZORBAX Method Validation Kits												
Size (mm)	Particle Size (µm)	Eclipse Plus C18	Eclipse Plus C8	Eclipse XDB-C18	Eclipse XDB-C8	Extend-C18	Eclipse Plus Phenyl-Hexyl	Bonus-RP	SB-Aq	SB-C18	SB-C8	SB-Phenyl
4.6 x 250	5	959990-902K	959990-906K	990967-902K	990967-906K	770450-902K	959990-912K	880668-901K	880975-914K	880975-902K	880975-906K	880975-912K
4.6 x 150	5	959993-902K	959993-906K	993967-902K	993967-906K	773450-902K		883668-901K	883975-914K	883975-902K	883975-906K	883975-912K
3.0 x 150	5	959993-302K										
4.6 x 250	3.5									884950-567K		
4.6 x 150	3.5	959963-902K	959963-906K	963967-902K	963967-906K	763953-902K	959963-912K	863668-901K	863953-914K	863953-902K	863953-906K	863953-912K
4.6 x 100	3.5	959961-902K	959961-906K	961967-902K	961967-906K	764953-902K	959961-912K	864668-901K	861953-914K	861953-902K	861953-906K	861953-912K
4.6 x 50	3.5	959943-902K	959943-906K	935967-902K	935967-906K	735953-902K	959943-912K	835668-901K	835975-914K	835975-902K	835975-906K	835975-912K
4.6 x 150	1.8	959994-902K							829975-914K	829975-902K	829975-906K	829975-912K
4.6 x 100	1.8	959964-902K	959964-906K	928975-902K	928975-906K	728975-902K	959964-912K	828668-901K	828975-914K	828975-902K	828975-906K	828975-912K
4.6 x 50	1.8	959941-902K	959941-906K	927975-902K	927975-906K	727975-902K	959941-912K	827668-901K	827975-914K	827975-902K	827975-906K	827975-912K
3.0 x 100	1.8				928975-306K			828668-301K				
3.0 x 50	1.8				927975-306K			827668-301K				
2.1 x 100	1.8				928700-906K							
2.1 x 50	1.8				927700-906K							