High Speed vs. High Resolution Analyses in HPLC: A Critical Performance Comparison of Column Options Using Poppe and Kinetic Plots

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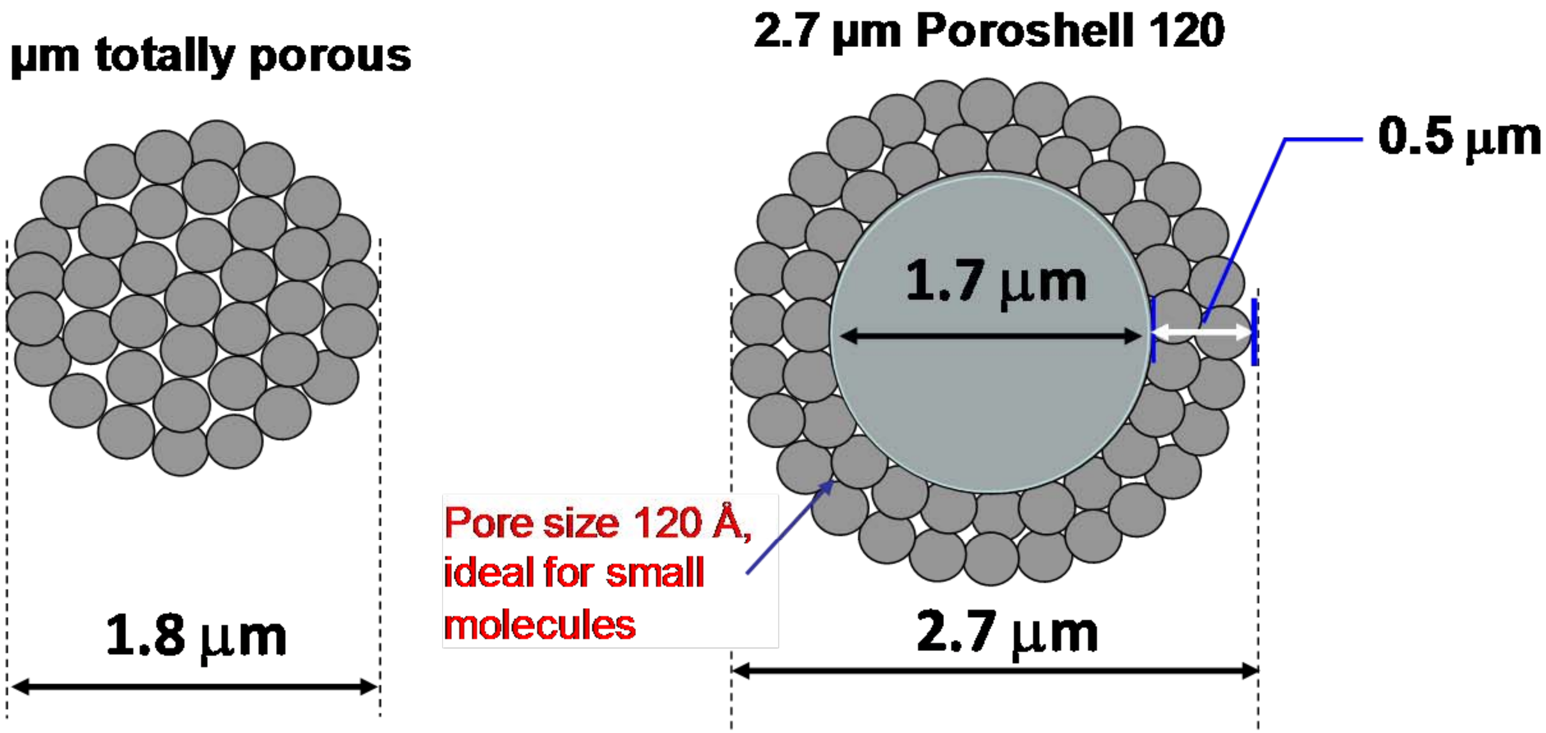
Improvements to HPLC

Approach	Specialized Instrument Required	Key Advantages	Major Limitations
Sub-2 μm Particle	**Yes**	**Very high plate counts in short analysis times**	**Extra-column broadening, frictional heating**
Superficially Porous Particle	**No**	**High plate counts at relatively lower pressure**	**Limited commercial phases**
High Temperature	Yes (above 100°C)	High efficiency maintained at high mobile phase velocity	Solutes degradation, Limited number of stable stationary phases
Monolith	No	High column permeability	Batch to batch reproducibility, Limited column dimensions

Poroshell 120 for Small Molecules



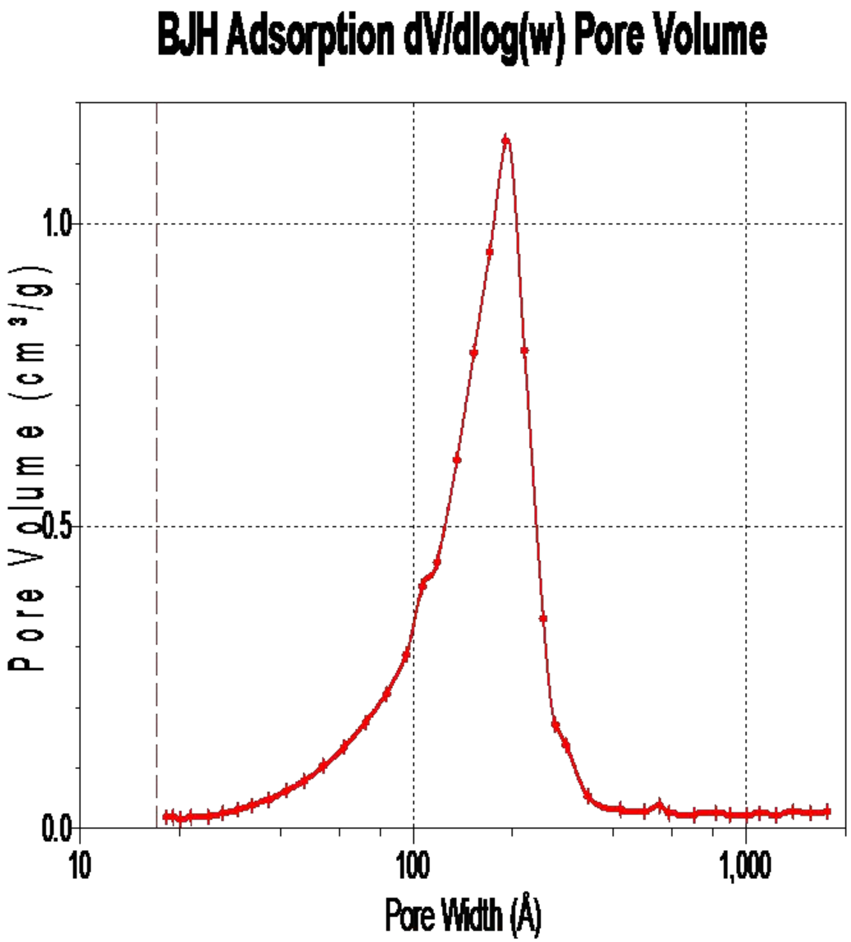
- High efficiency of Poroshell 120 particles similar to sub-2um totally porous particles
- Gains due to shorter mass transfer and narrower particle size distribution
- Low back pressure (40-60% of sub-2um totally porous particles)

Efficiency improvement with superficially porous particles

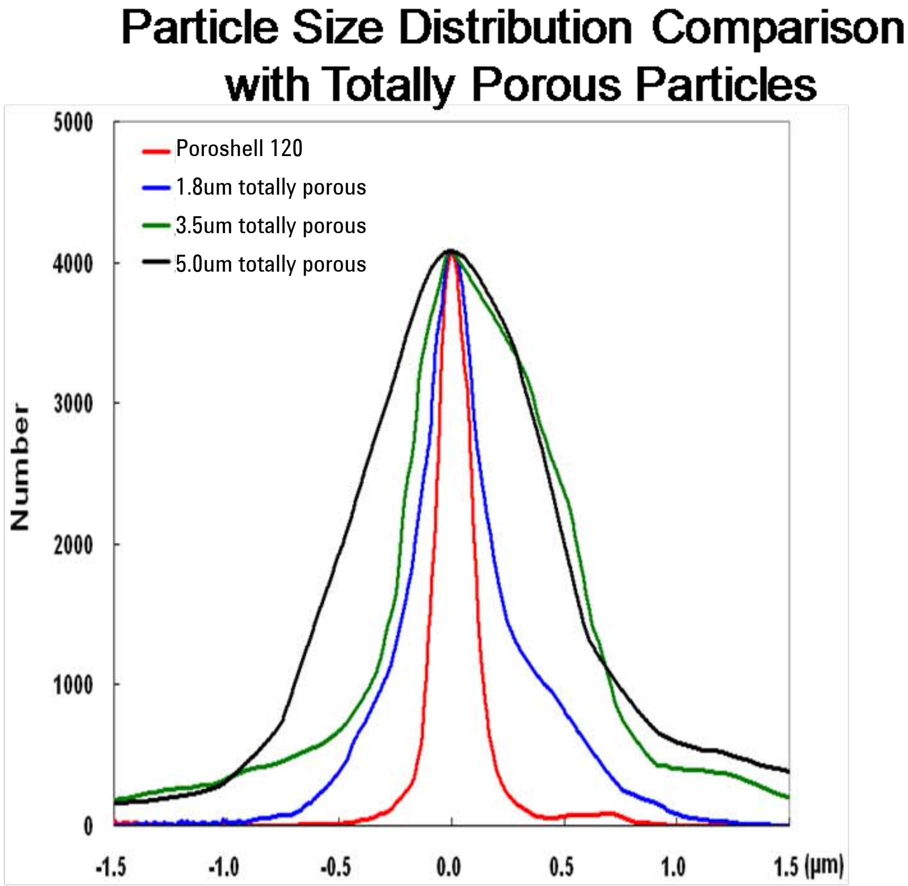
(van Deemter equation) $h = A + B / \nu + C \cdot \nu$

- A term – eddy diffusion and flow distribution
  - Column particle size and column packing quality impact this
  - **Tight particle size distribution improves the A term**
- B term – longitudinal diffusion
  - Impact in superficially porous particle not yet determined
- C term – mass transfer component
  - **Mass transfer is improved by using shorter diffusion paths, improving the C term**
  - This is improved with a superficially porous particle
  - The C term has more effect on large molecules than on small molecules

Poroshell 120 pore and particles size distribution

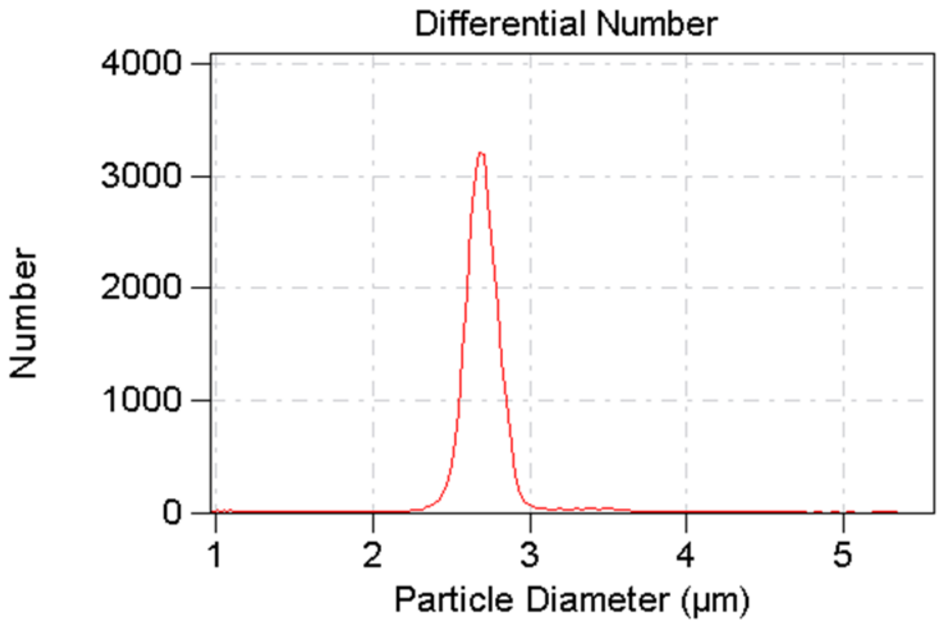


Poroshell 120 particles have an average pore size of 120 Å.

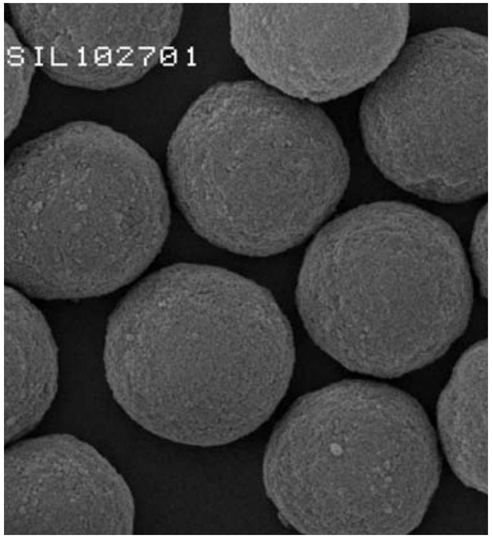


Normalized Particle Size Distribution

Poroshell 120 particles size distribution



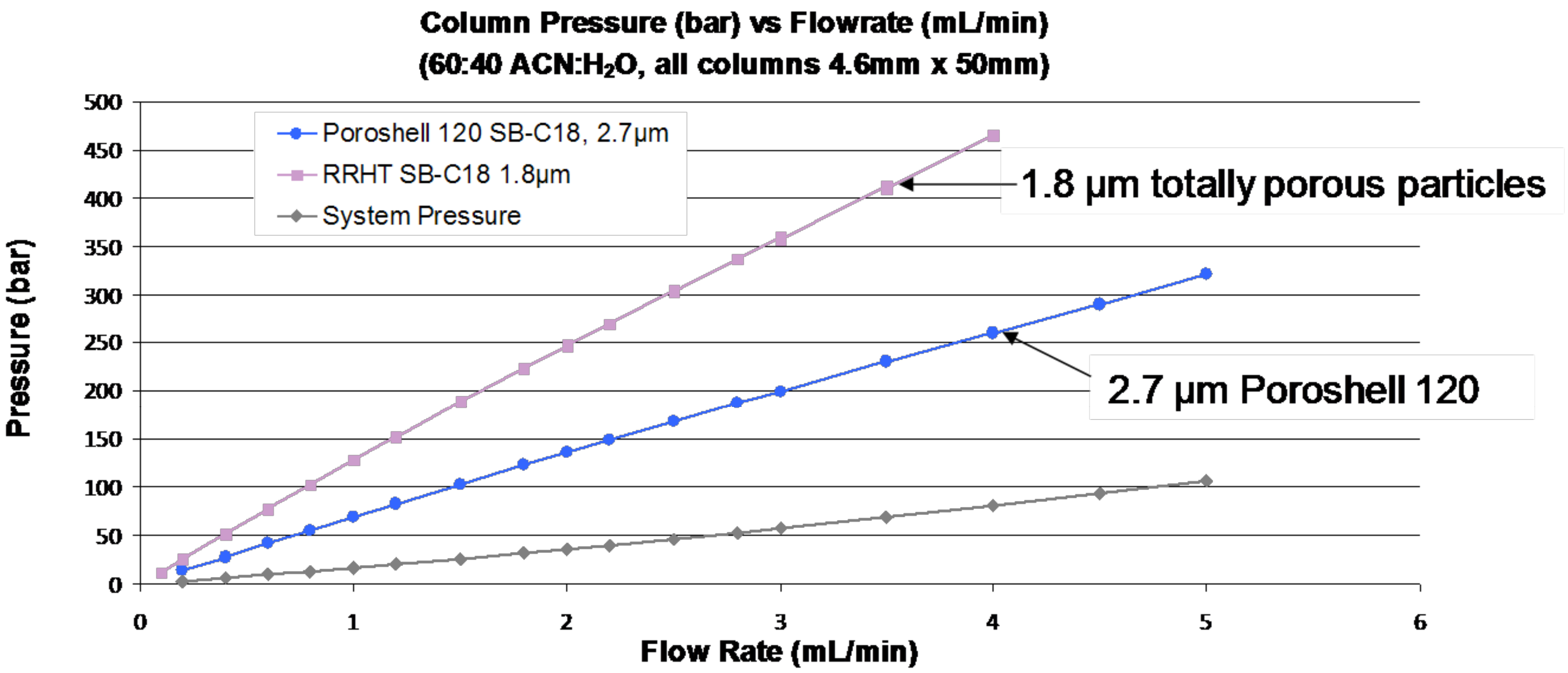
Particle Size Distribution of Poroshell 120



SEM Picture of Poroshell 120

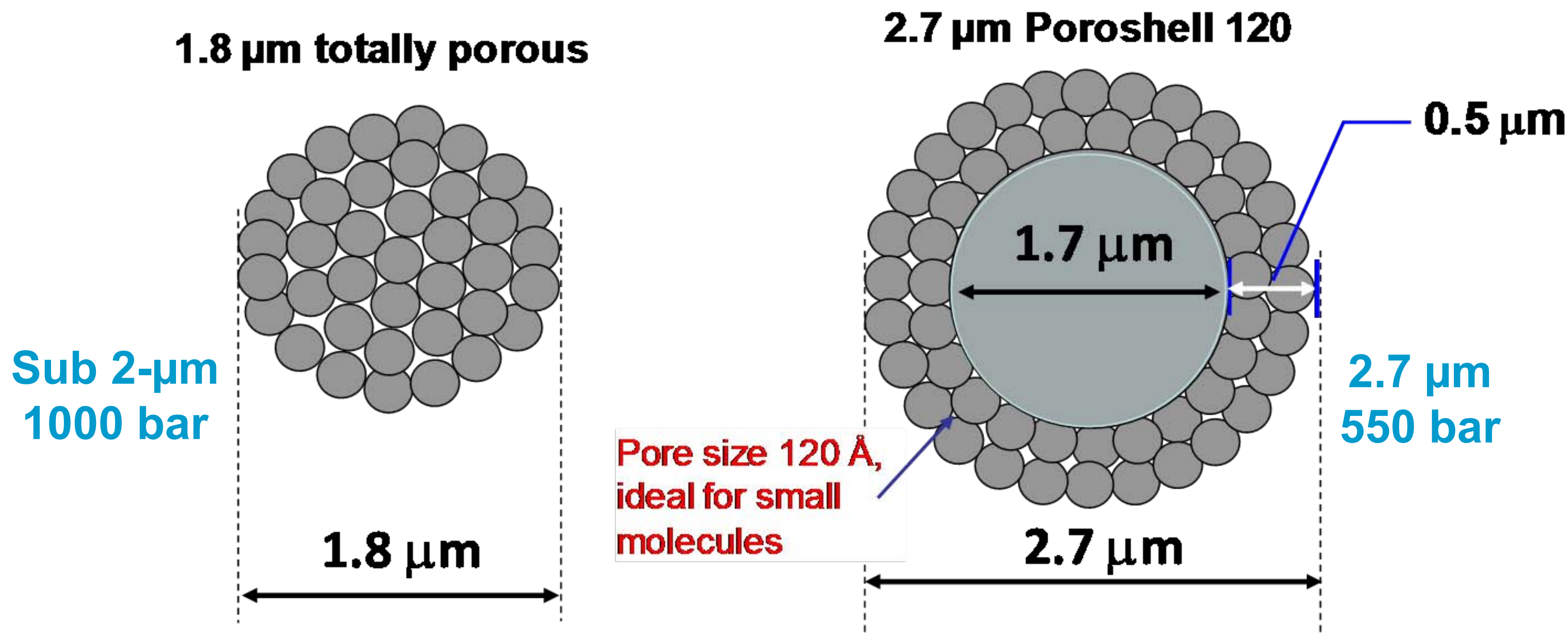
Poroshell 120 particles also show spherical and smooth surface.

Backpressure comparison of 2.7µm Poroshell 120 with 1.8µm totally porous particles



- Superficially porous particles have 40%-50% back pressure of 1.8 µm totally porous particles.

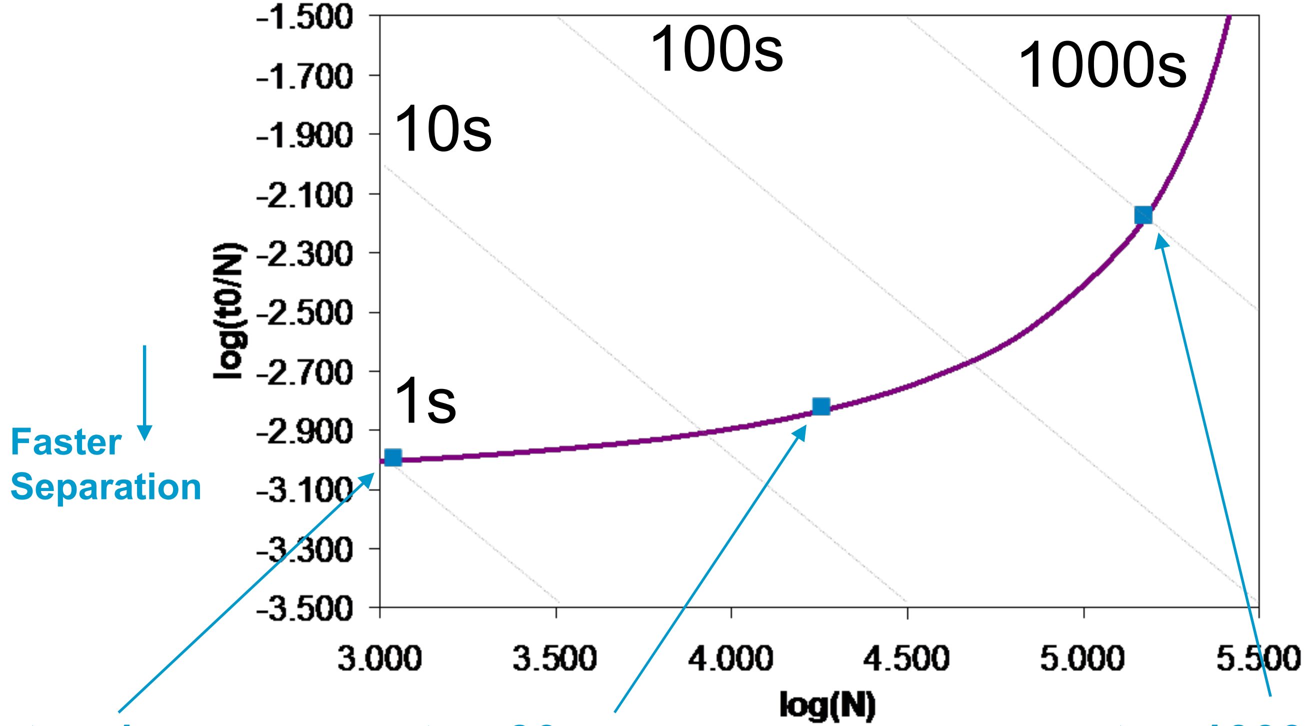
Which Yields Better Performance



- Which one performs better under *optimized* conditions
- Relative performance as a function of separation goal
  - Fast separation (low efficiency with short column)
  - Slow separation (high efficiency with long column)

Theoretical Poppe plot

- Efficiency (N) – Higher N means higher resolving power
- Separation speed (t_0/N) – Lower t_0/N equates to faster separation



Pmax = **400 bar**, T = 40 °C
van Deemter equation

A = 1.0, B = 5.0, C = 0.05
D_m = 1 × 10-5 cm²/sec
η = 6.6 × 10-4 Pa/sec

φ= 500, ε_e = 0.38, ε_i = 0.30

t_0 = 1 sec
L = 3.41 cm
F = 4.36 mL/min
N = 1,000

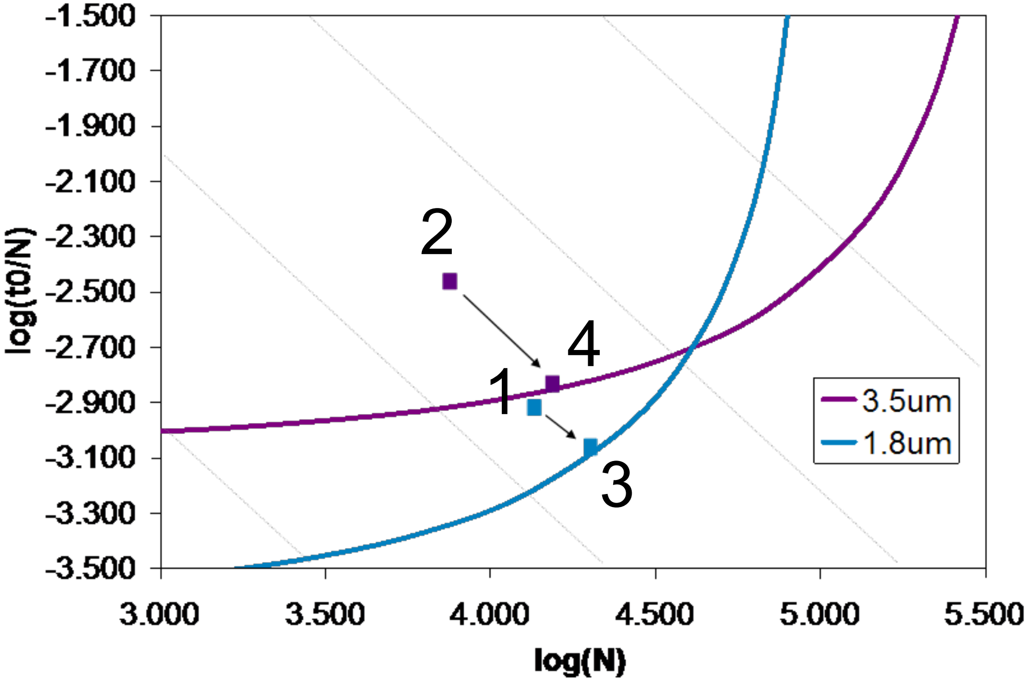
t_0 = 30 sec
L = 18.7 cm
F = 0.796 mL/min
N = 19248

t_0 = 1000 sec
L = 108 cm
F = 0.138 mL/min
N = 141960

http://homepages.gac.edu/~dstoll/calculators/optimize.html

Why use Poppe plots?

Poppe optimum vs H optimum



	van Deemter optimum		Poppe optimum	
	1.8 µm	3.5 µm	1.8 µm	3.5 µm
Point	1	2	3	4
L (cm)	5	5	5.96	16.2
n	10	10	11.9	32.2
F (ml/min)	0.44	0.23	0.52	0.73
ΔP (bar)	282	38	400	400
N	13900	7100	16,400	16,700
t_0 (sec)	13.4	26.1	13.4	26.1
N/t_0	1037	272	1224	640
% N		51%		102%
% N/t_0		26%		52%

Pmax = **400 bar**, T = 40 ^{o}C van Deemter equation

A = 1.0, B = 5.0, C = 0.05 D_m = 1 × 10-5 cm^2/sec

η = 6.6 × 10-4 Pa/sec φ= 500, ε_e = 0.38, ε_i = 0.30

Poppe plot analysis allows for a fair comparison under optimized conditions

Experimental Design

Sub-2 µm fully porous particle

ZORBAX Rapid Resolution High Definition (RRHD) Eclipse Plus C18

vs

2.7 µm superficially porous particle

Poroshell 120 EC-C18

1. Perform flow study on each column type with alkylphenones

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

2. Transform van Deemter analysis into Poppe Plot
3. Test accuracy of method experimentally with columns connected in series to mimic a variety of column lengths

van Deemter metrics:

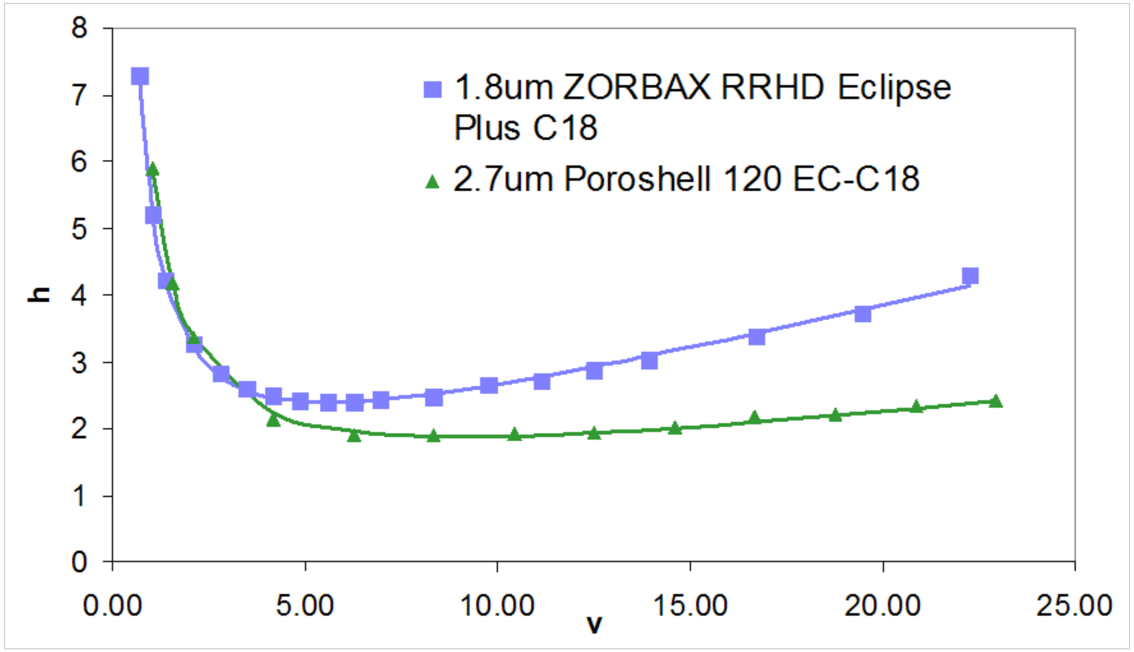
- Plate height (h)
- Mobile phase velocity (ν)

→

Practically meaningful metrics:

- Plate count (N)
- Plate count per unit time (N/t_0)

Reduced van Deemter analysis



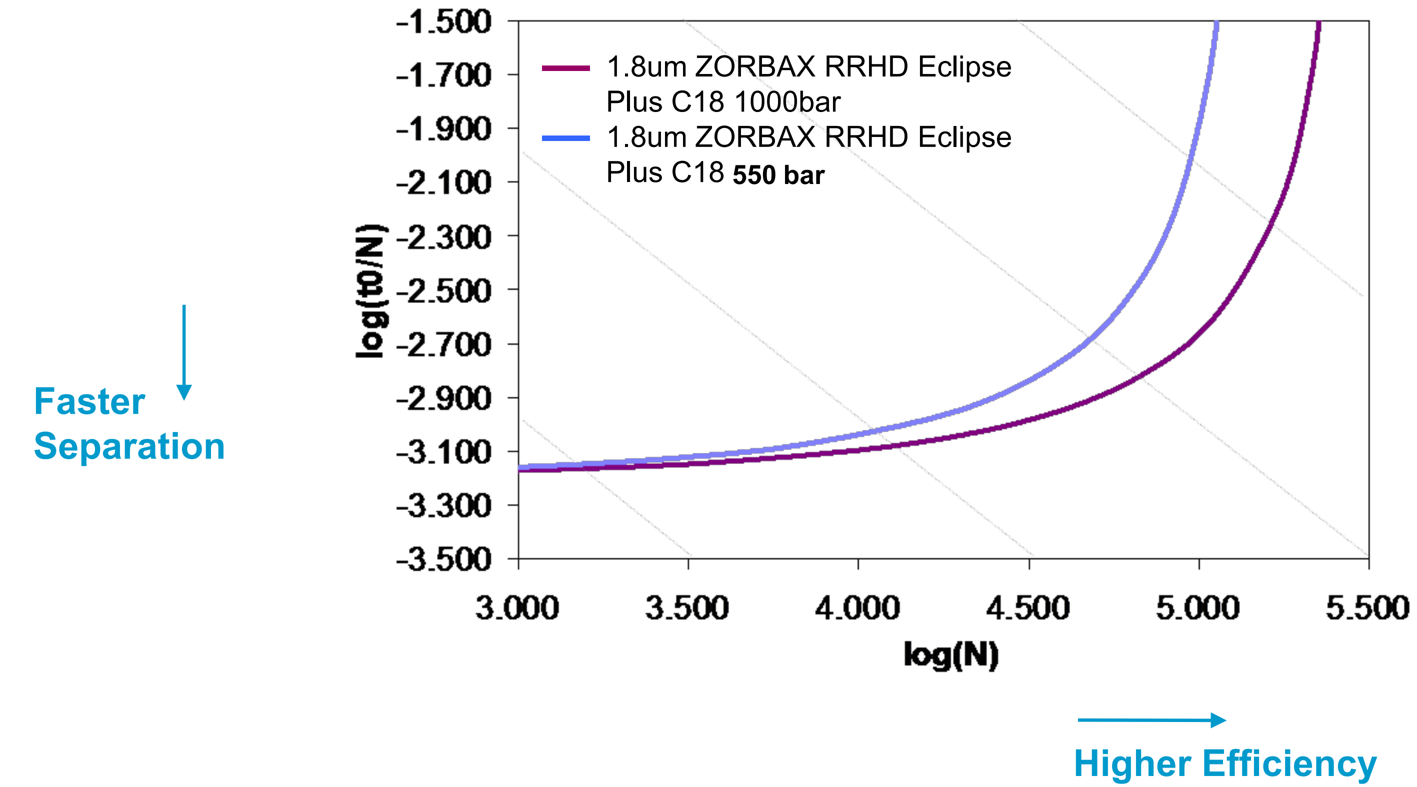
	Poroshell	RRHD
A	**.70**	0.81
B	5.6	**4.7**
C	**0.06**	0.13
h_{min}	**1.90**	2.38

Agilent 1290 Infinity LC System
2.1mm x 50mm columns
Analyte: Hexanophenone
Temp: 40 °C
%ACN adjusted to achieve k'=6 for each column

In the reduced van Deemter plot:

- **Lower C term on the Poroshell 120 column (better mass transfer)**
- **Lower h_{min} on the Poroshell 120 Column**

Theoretical Poppe plot

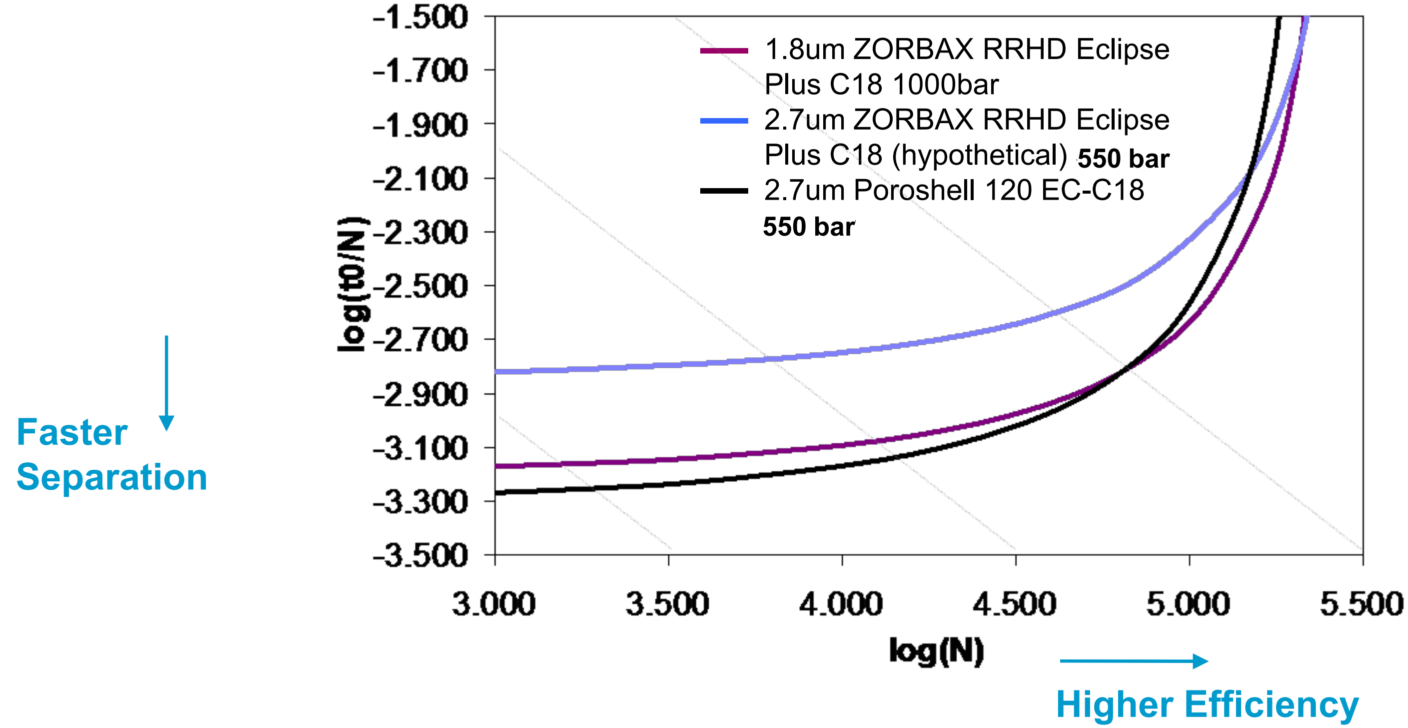


		A	B	C
	1.8µm ZORBAX RRHD C18 @ 1000 bar	0.81	4.66	0.13
	1.8µm ZORBAX RRHD C18 @ 550 bar	0.81	4.66	0.13

Higher pressures result in more efficient separations in longer analyses

http://homepages.gac.edu/~dstoll/calculators/optimize.html

Theoretical Poppe plot



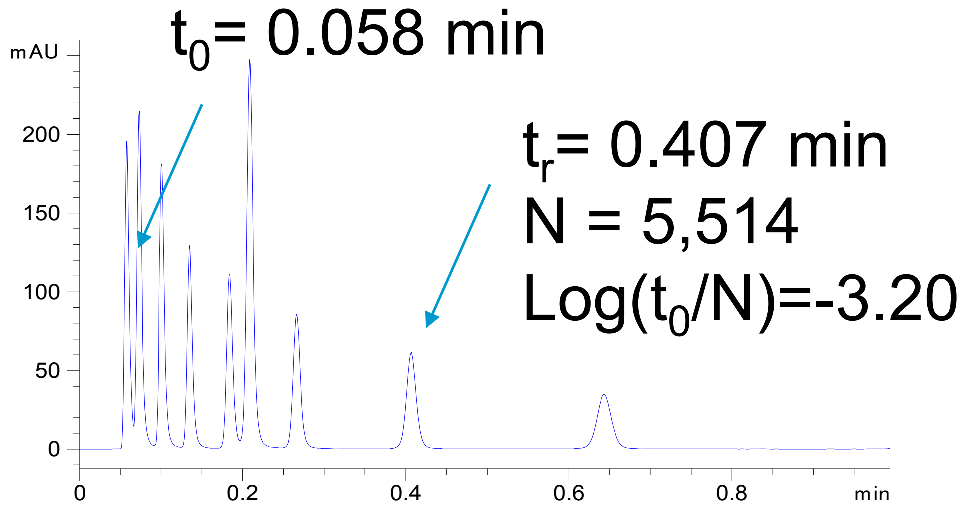
		A	B	C
	1.8µm ZORBAX RRHD C18 @ 1000 bar	0.81	4.66	0.13
	2.7µm ZORBAX C18 @ 550 bar (hypothetical)	0.81	4.66	0.13
	2.7µm Poroshell 120 @ 550 bar	0.71	5.6	0.06

- Poroshell 120 gives similar performance to ZORBAX RRHD ***at 50% backpressure***

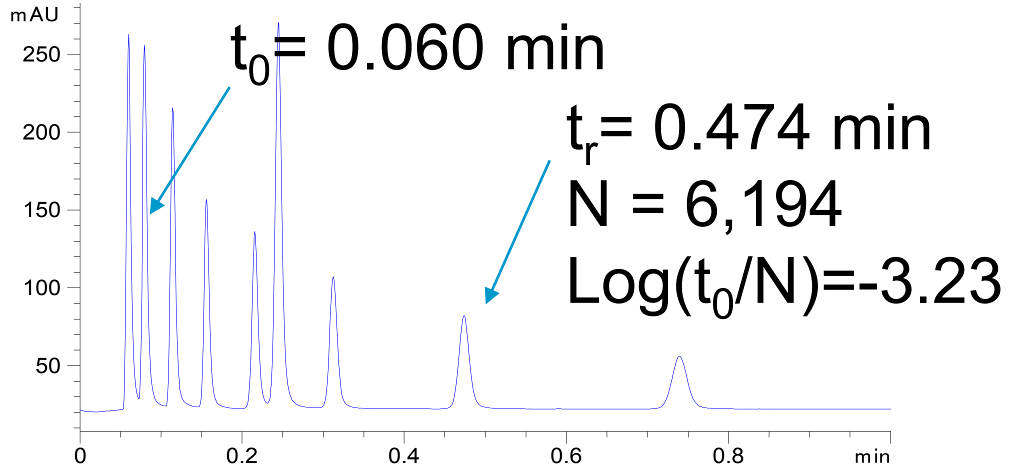
http://homepages.gac.edu/~dstoll/calculators/optimize.html

Fast Analysis (5cm)

2.7um Poroshell 120
EC-C18
2.1x5 cm
F = 1.94 mL/min, 40°C
56% ACN, 44% Water
549 bar

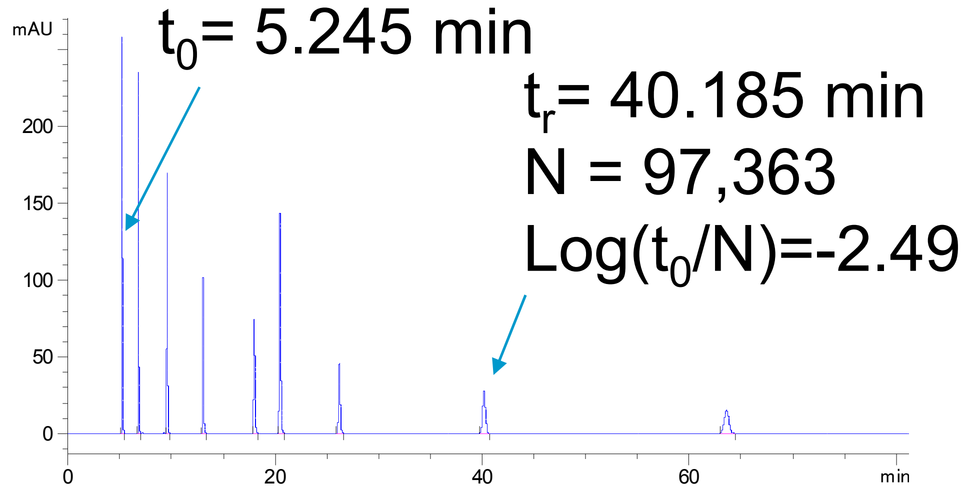


1.8um ZORBAX RRHD
Eclipse Plus C18
2.1x5cm
F = 1.82 mL/min, 40°C
59% ACN, 41% Water
1003 bar

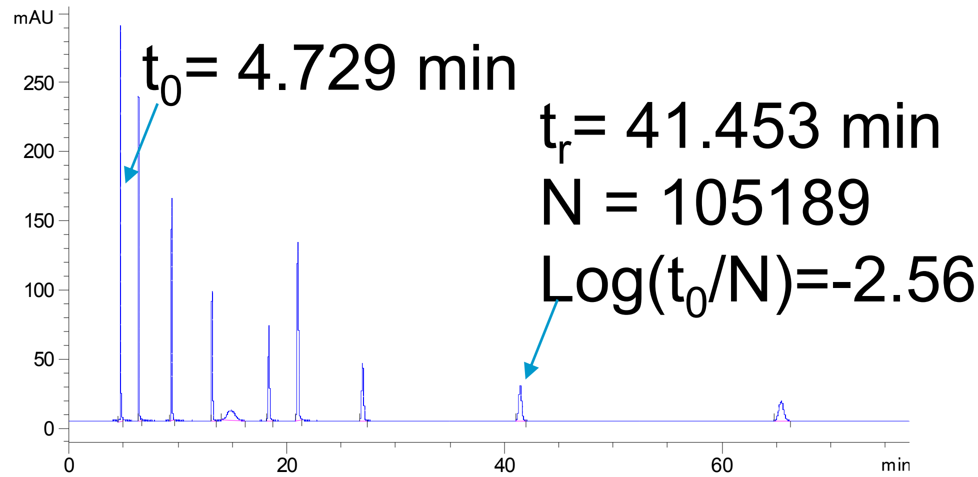


Long Analysis (55cm)

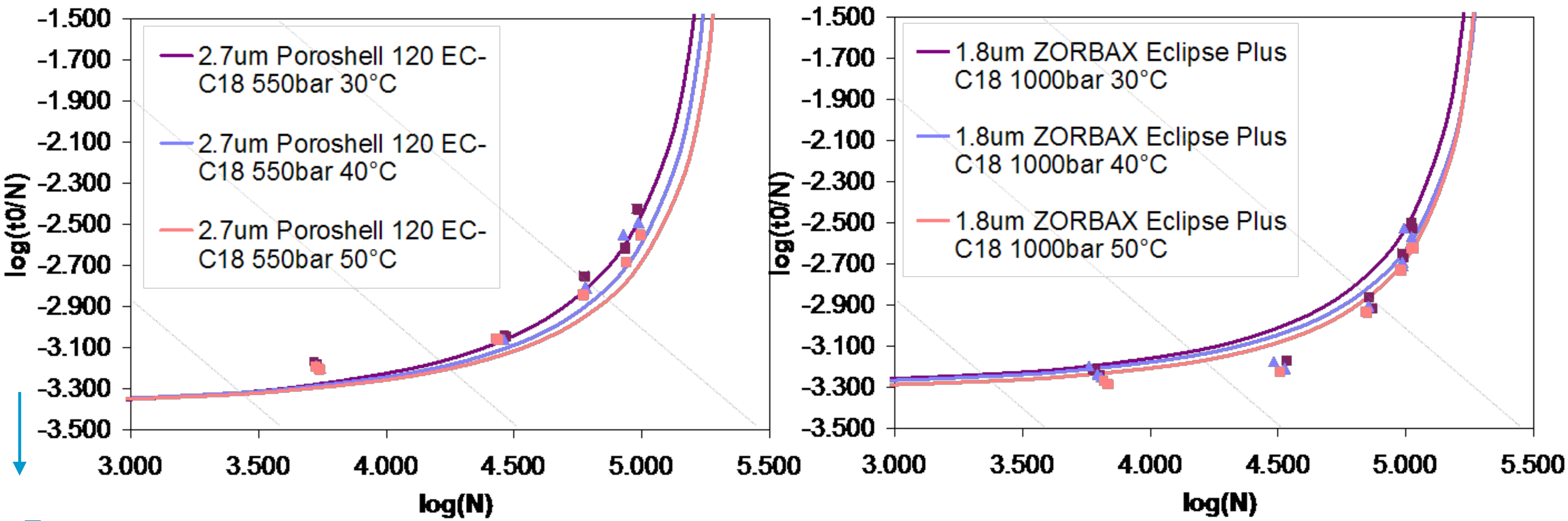
2.7um Poroshell 120
EC-C18
2.1x55cm (3x15cm, 10cm)
F = 0.202 mL/min, 40°C
56% ACN, 44% Water
547 bar



1.8um ZORBAX RRHD
Eclipse Plus C18
2.1x55cm (3x15cm, 10cm)
F = 0.23 mL/min, 40°C
59% ACN, 41% Water
1001 bar



Theoretical Poppe plot



Faster Separation

Higher Efficiency

	RRHD (40°C)			Poroshell 120 (40°C)		
L (mm)	N (Calc)	N (Exp)	% Err	N (Calc)	N (Exp)	% Err
50	4805	6119	27.35%	4333	5390	24.39%
300	69695	71643	2.80%	61220	60264	-1.56%
550	114361	102189	10.64%	101312	97170	-4.09%

Conclusions

Sub-2 µm fully porous particle

ZORBAX RRHD Eclipse Plus C18

vs

2.7 µm superficially porous particle

Poroshell 120 EC-C18

Comparison under optimized conditions

1. Similar performance between Poroshell 120 at 550 bar and 1.8um ZORBAX RRHD at 1000 bar pressures
2. Increasing temperature tends to shift analysis toward slightly higher efficiencies, with a greater impact observed with Poroshell 120
3. More experimentation with other solvents, such as methanol, need to be evaluated to verify performance in a more viscous mobile phase environment

Conclusions

Which column option should be chosen?

Sub 2µm totally porous or 2.7µm Poroshell 120

For an HPLC (<600 bar), consider Poroshell 120

- Unless the chromatography requires scale-up in particle sizes

For a UHPLC, both choices are possible

- Consider the analysis goals: scalability, selectivity, loadability
- Sub 2µm will be an excellent choice for the highest pressure UHPLC systems

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