New Column Technologies for Better and Faster Separations in UHPLC

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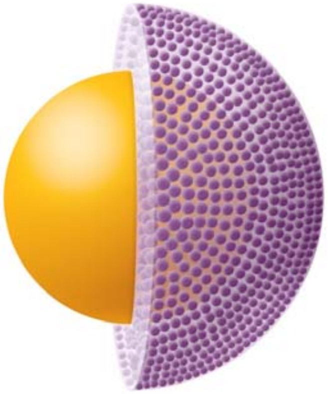
Outline

- Review new column technologies
  - Properties
  - Theoretical considerations
- Applications of superficially porous particles
  - Fast separations
  - “Common” separations
  - High efficiency separations
- Practical considerations
  - Instrument impact
  - Method transfer
- Conclusions

Column technologies for small molecules

Particle	Particle size	Special need	Optimum applications
Traditional fully porous	2.5-5.0 µm	n/a	Widely used in many established methods
Sub-2-µm fully porous	1.5-2.0 µm	UHPLC; low extra-column broadening	Fast separation or high efficiency?
Sub-3-µm superficially porous	2.6-2.7 µm	low extra-column broadening	Fast separation or high efficiency?
Sub-2-µm superficially porous	1.7 µm	UHPLC; low extra-column broadening	Fast separation or high efficiency?
Monolith	n/a	n/a	High efficiency separations

Modern superficially porous particles



- Physical-chemical properties
  - Small particle size distribution: d90/d10 < 1.20
  - Available particle size: 2.7, 2.6, 1.7 μm
  - Small particle porosity

- Chromatographic properties
  - Smaller h_{min} compared to fully porous
    - Smaller A term: better packed bed homogeneity
    - Smaller B term: less porosity thus less longitudinal diffusion
    - Similar C term for small molecules

- What are the optimum applications for superficially porous particles?

Gritti *et al. J Chrom A*, **2010**, *1217*, 3819

Theory to optimize efficiency

- Three-parameter optimization (Knox-Saleem limit)
  – Simultaneously optimize particle size, column length and flow rate
  – Useful to assess the *performance potential* of different particle types

$$d_p^* = \left[\frac{\Phi\eta^{B}/_{C}}{P_{max}}\right]^{1/4} (\lambda t_o)^{1/4} D_m^{\ 1/2} \qquad L^* = \left[\frac{P_{max}{}^{B}/_{C}}{\Phi\eta}\right]^{1/4} (\lambda t_o)^{3/4} D_m^{\ 1/2} \qquad u_e^* = \left[\frac{P_{max}{}^{B}/_{C}}{\Phi\eta}\right]^{1/4} (\lambda t_o)^{-1/4} D_m^{\ 1/2}$$

- Two-parameter optimization (Poppe or kinetic plots)
  – Fix particle size, then optimize column length and flow rate
  – Useful to compare the *real performance* of different particles

$$L^* = \sqrt{\frac{P_{max}\lambda t_o}{\Phi\eta}} d_p = \psi\lambda t_0^{\ 1/2} d_p \qquad u_e^* = \sqrt{\frac{P_{max}}{\Phi\eta\lambda t_o}} d_p = \psi d_p t_0^{\ -1/2}$$

Carr *et al. Anal. Chem.*, **2009**, *81*, 5342

Simple optimization procedure

Goal – To achieve the highest N in desired time

- Step 1
  - Conduct 3-parameter optimization to find optimal particle size, column length and flow rate
- Step 2
  - Choose the closest particle size available (compromise)
  - Conduct 2-parameter optimization to find optimal column length and flow rate
- Step 3
  - Chose the closest column length available (compromise)
  - Operate at the flow rate that gives the desired time

Wang *et al. LC-GC North America*, **2010**, *28*, 932

Applications of superficially porous particles

- Ultrafast separations
- “Common” separations
- High efficiency separations

Ultrafast LC for dissolution test

	UV analysis	Traditional HPLC analysis
Easy to operate	Yes	Yes
Speed per sample	0.5-2 min	5-10 min
Speed per run	0.5 -2 hrs	3-5 hrs
On-line analysis	Readily available	Not readily available
Sample volume	8-10 mL	< 100 μL
Specificity	Moderate	Excellent
Sensitivity	Moderate	Excellent
Linearity range	Moderate	Excellent
Bubble problem	More likely to occur	Less likely to occur
Information	-	Rich info

Wang *et al. LC-GC North America*, **2010**, *28*, 932

Calculation for fully porous particles

- Optimization goal
  - t_0 = 4 sec or t_R ~ 30 sec
  - Use sub-2-µm fully porous particles at 1100 bar and 80 °C

Table V: Stepwise optimization for t_0 = 4 s separation on ZORBAX RRHD SB-C18 at 1100 bar and 80 °C*

Step	t_0 (s)	d_p (µm)	L (mm)	u_e (cm/s)	ΔP (bar)	N	% Loss
1	4	0.9	38	1.35	1100	18,500	—
2	4	1.8	77	2.70	1100	11,800	36
3	4	1.8	75	2.65	1057	11,700	37

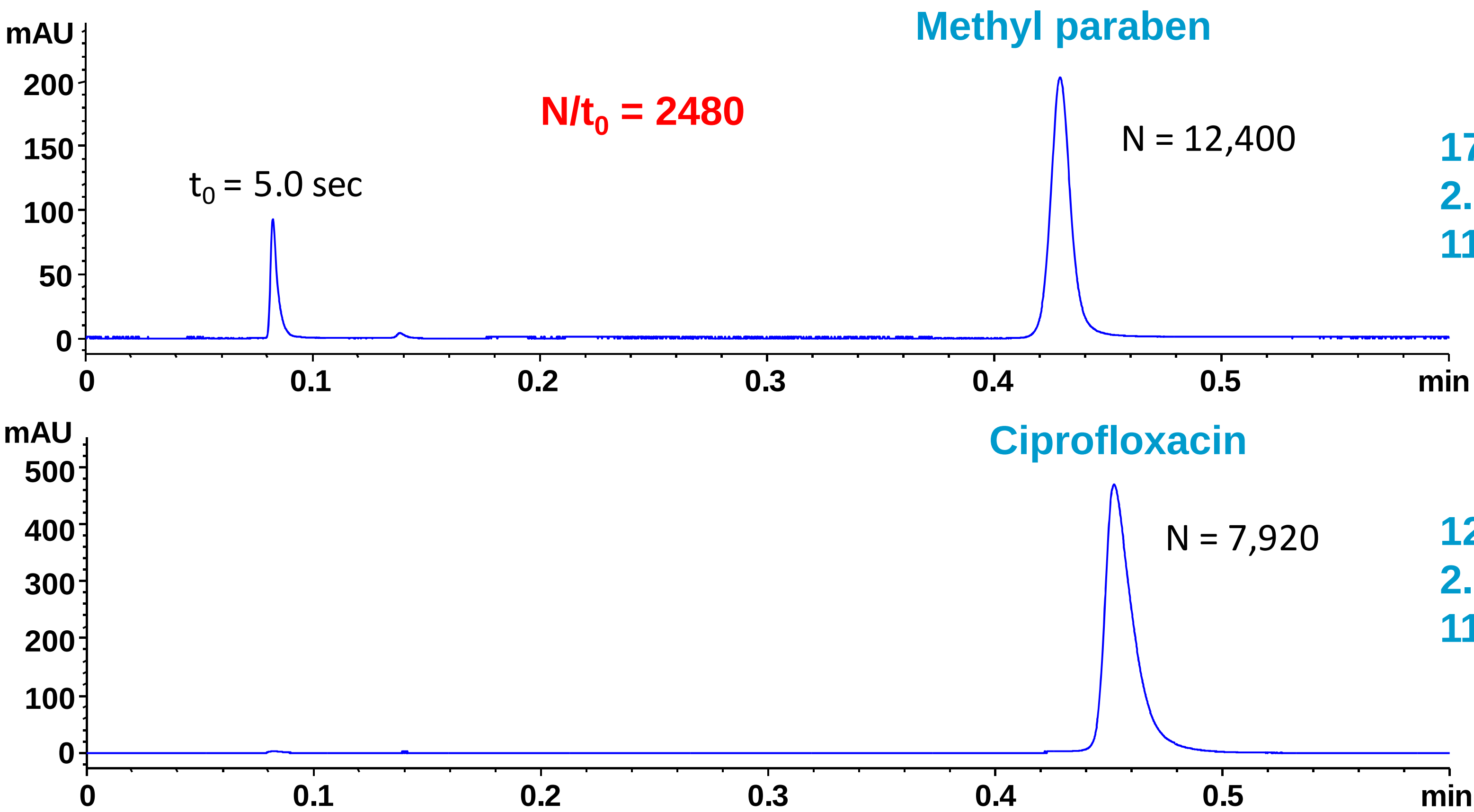
* Conditions: 10% acetonitrile, $D_m = 1 \times 10^{-5}$ cm^2/s for ciprofloxacin at 80 °C.

Wang *et al. LC-GC North America*, **2010**, *28*, 932

Ultrafast separation

Mobile phase A: 0.1% TFA in water, mobile phase B: ACN, **80 °C**, 0.3 μL injection, 278 nm

Zorbax RRHD SB-C18, 1.8 μm, 2.1x75 mm



17% B
2.0 mL/min
1100 bar

12.5% B
2.0 mL/min
1100 bar

Wang *et al. LC-GC North America*, **2010**, *28*, 932

Calculation for superficially porous particles

- Optimization goal
  - t_0 = 4 sec or t_R ~ 30 sec
  - Used sub-3-µm superficially porous particles at 550 bar and 80 °C

Table VI: Stepwise optimization for t_0 = 4 s separation on Poroshell 120 SB-C18 at 550 bar and 80 °C*

Step	t_0 (s)	d_p (µm)	L (mm)	u_e (cm/s)	ΔP (bar)	N	% Loss
1	4	1.2	37	1.33	550	15,500	—
2	4	2.7	82	2.90	550	9200	41
3	4	2.7	75	2.67	466	8900	43

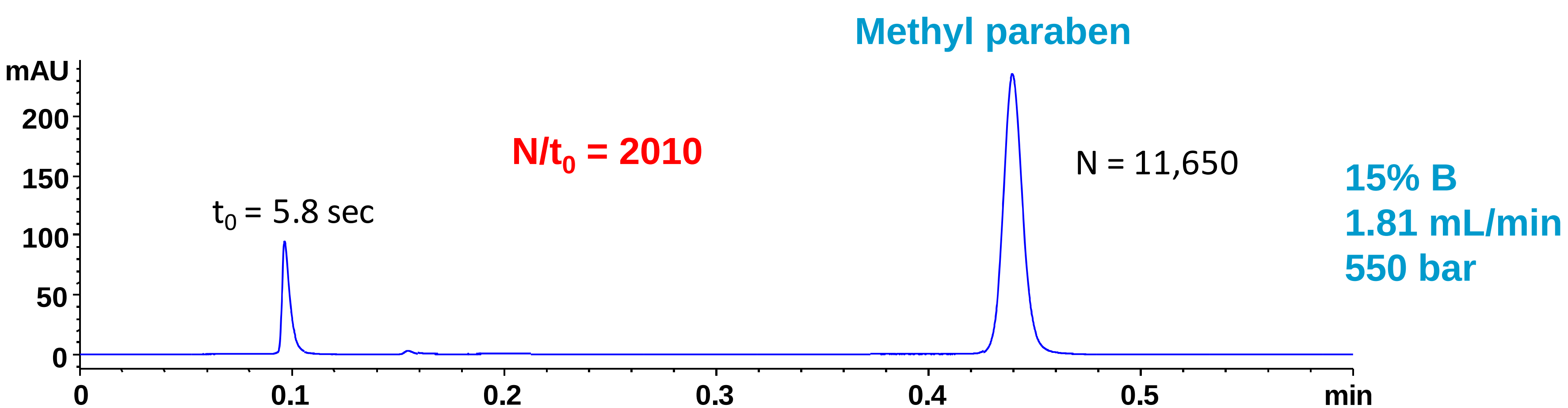
* Conditions: 10% acetonitrile, $D_m = 1 \times 10^{-5}$ cm²/s for ciprofloxacin at 80 °C.

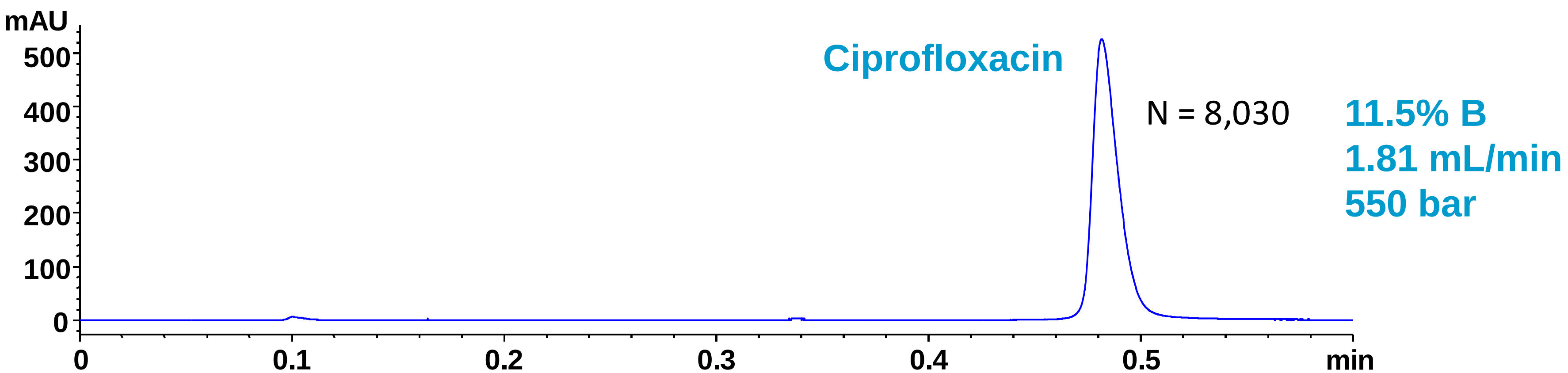
Wang *et al. LC-GC North America*, **2010**, *28*, 932

Ultrafast separation

Mobile phase A: 0.1% TFA in water, mobile phase B: ACN, **80 °C**, 0.3 μL injection, 278 nm

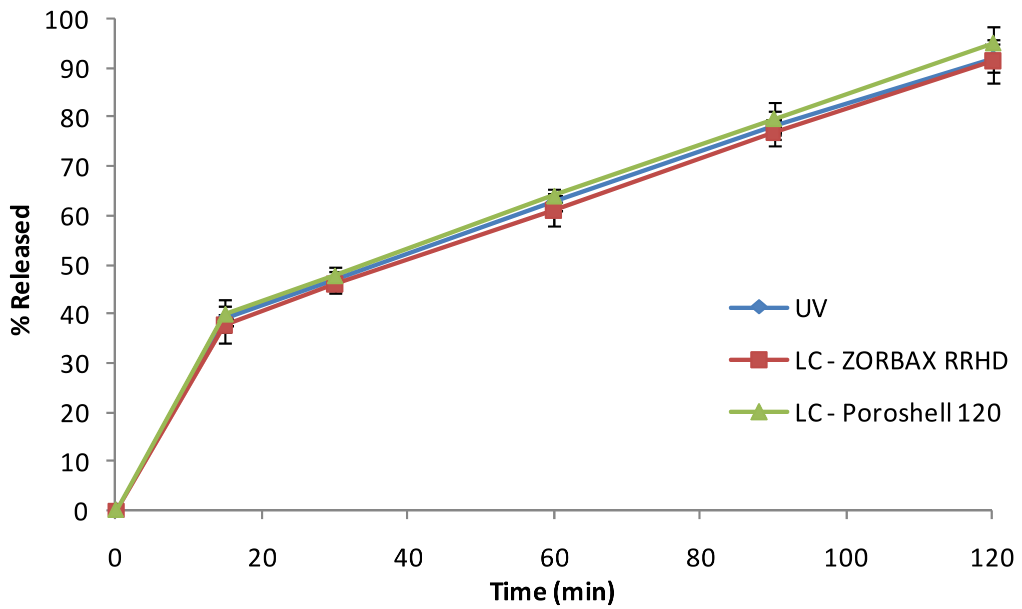
Poroshell 120 SB-C18, 2.7 μm, 2.1x75 mm





Wang *et al. LC-GC North America*, **2010**, *28*, 932

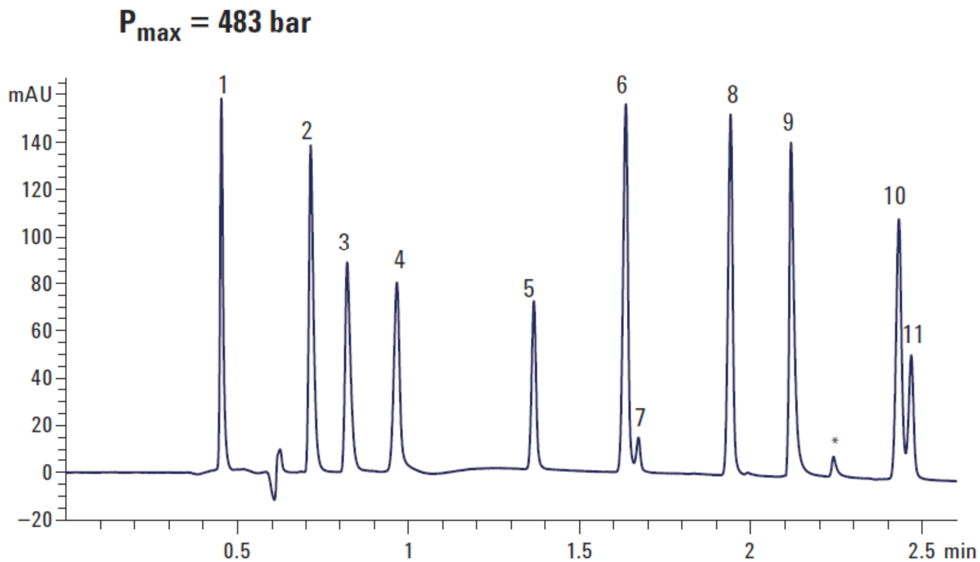
Ultrafast separation for dissolution test



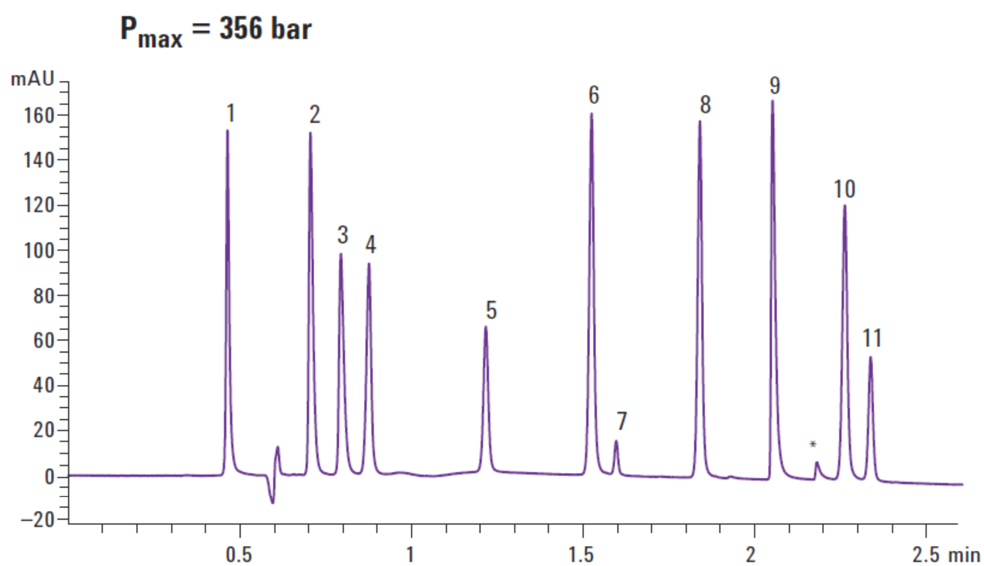
Dissolution test of Ciprofloxacin Extended Release 500 mg tablets

- The ultrafast LC methods provide equivalent dissolution results but is
  - 3-5 times faster than traditional HPLC methods
  - similar to automated UV methods in speed
- Such ultrafast separations can be useful for other quick assay applications, such as content uniformity and cleaning validation

Food and beverage additives



Eclipse Plus C18
1.8 µm, 3.0x100 mm



Poroshell 120 EC-C18
2.7 µm, 3.0x100 mm

Agilent Application Note 5990-6082en

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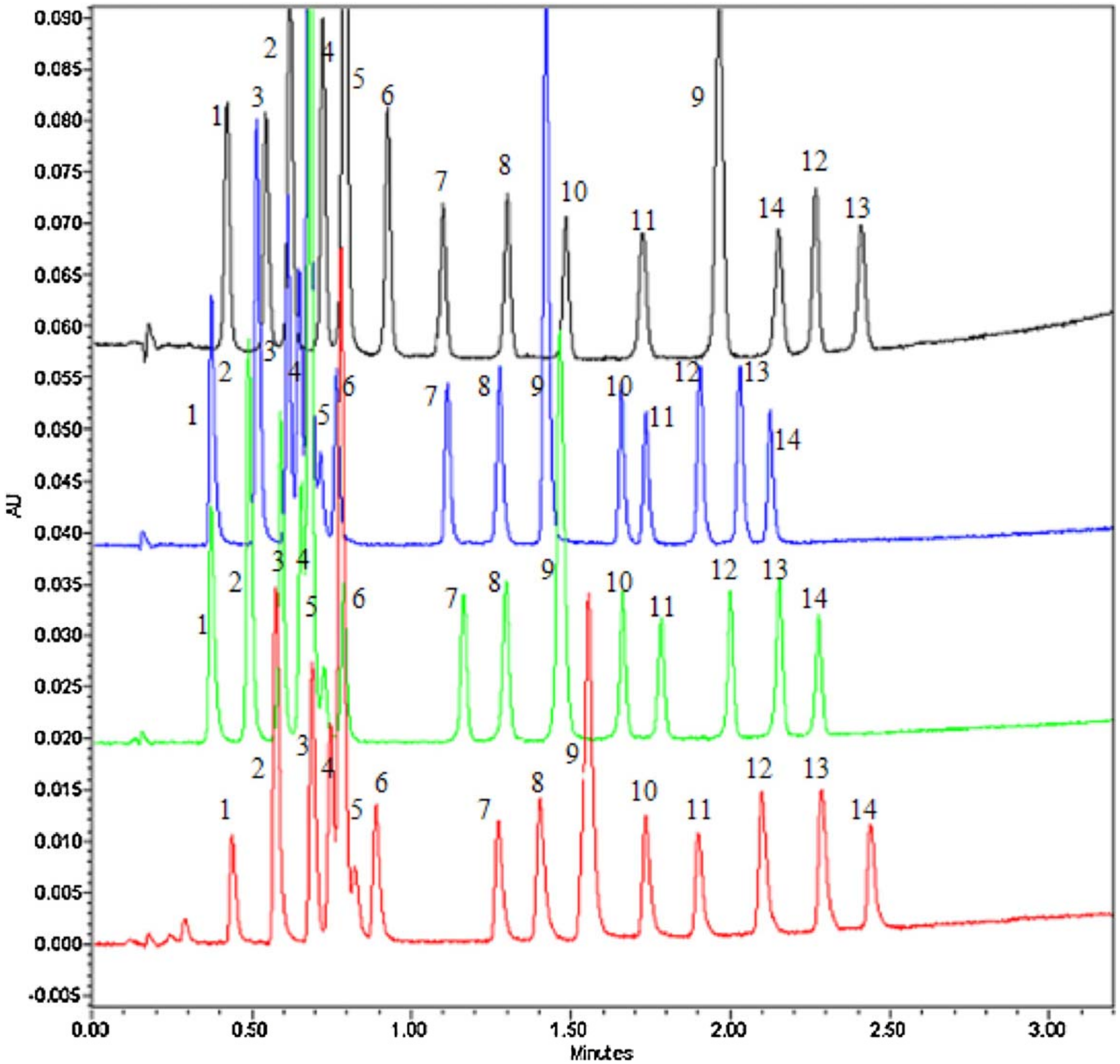
Pharmaceutical screening

14 polar APIs
2.1x50 mm columns
0.8 mL/min, 3 min gradient
from 40% to 90% ACN



Sub-3-μm superficially porous Vendor 1

Sub-3-μm superficially porous Vendor 2

Sub-3-μm superficially porous Vendor 3

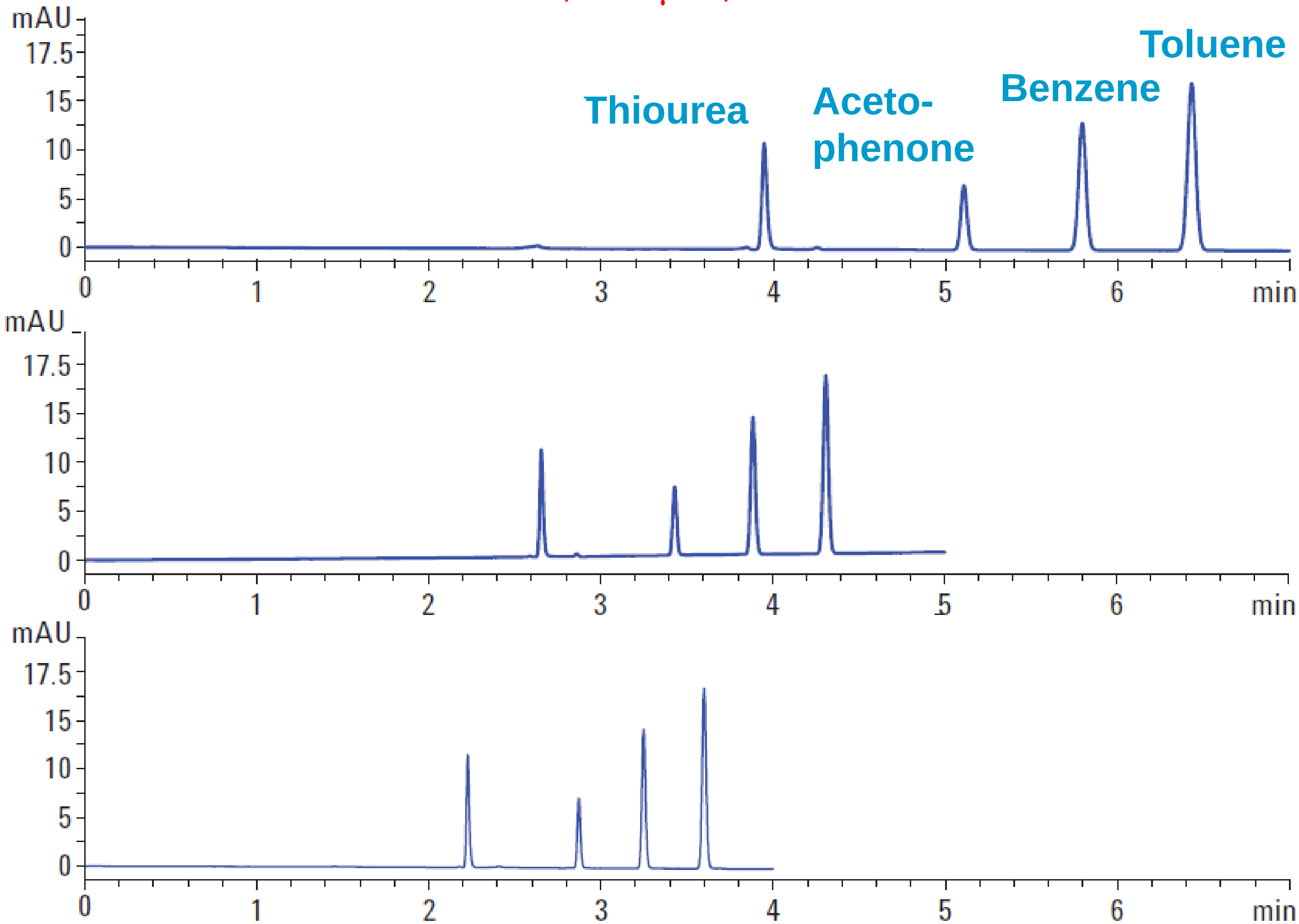
Sub-2-μm fully porous Vendor 4

Fekete *et al. Talanta*, **2011**, *84*, 416

80/20 ACN/H_2O, 60 °C

High efficiency separation

Poroshell 120 SB-C18, 2.7 µm, three 4.6x150 mm columns in series



N = 83,000
1 mL/min
316 bar

N = 103,000
1.5 mL/min
478 bar

N = 115,000
1.8 mL/min
573 bar

Agilent Application Note 5990-5602en

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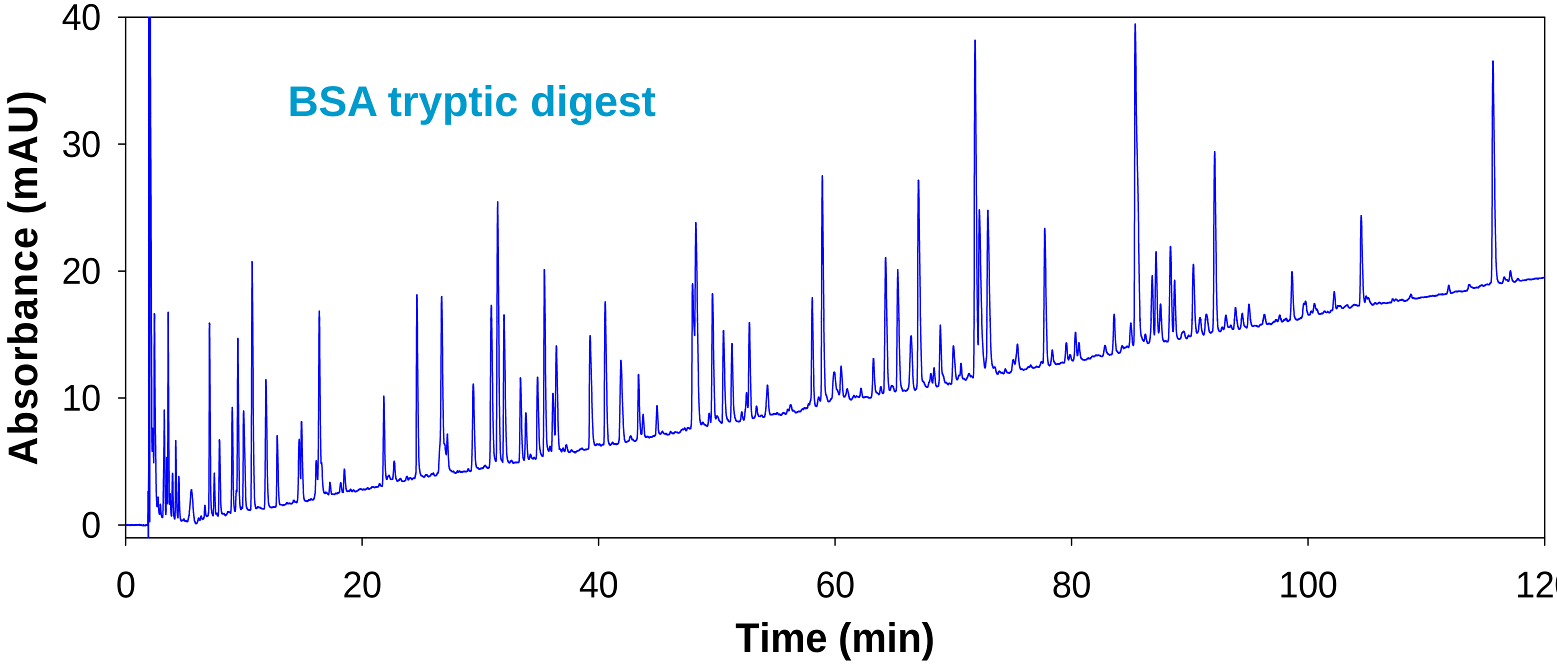
High efficiency separation

Poroshell 300 SB-C18, 5.0 µm, four 2.1x150 mm columns in series



PC = 507
0.50 mL/min
320 bar

Mobile phase A: 0.1% TFA in water with 50 mM $NaClO_4$, mobile phase B: 0.1% TFA in 80/20 ACN/H_2O, gradient from 0% to 42% B in 120 min, 0.50 mL/min, **70 °C**, 214 nm

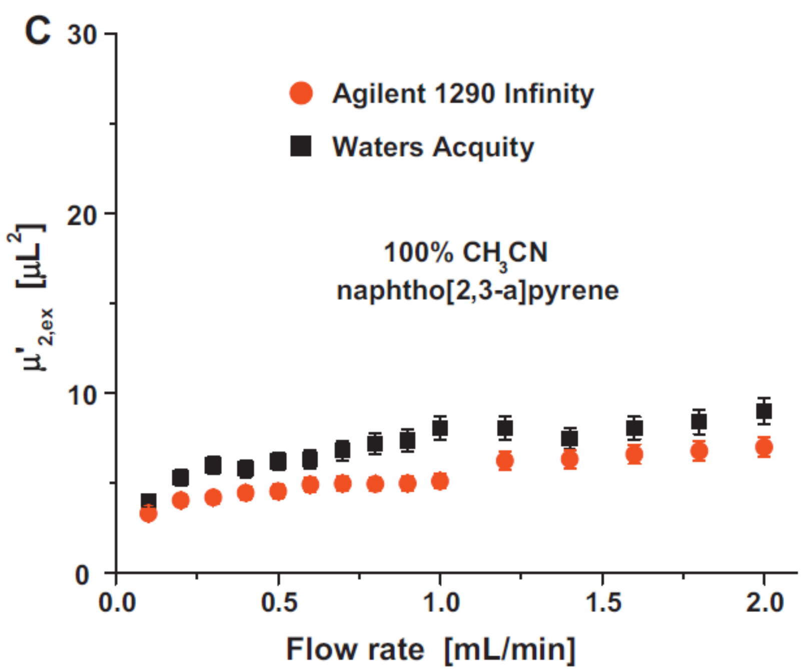
Wang *et al. J Chrom A*, **2006**, *1107*, 139

Practical considerations

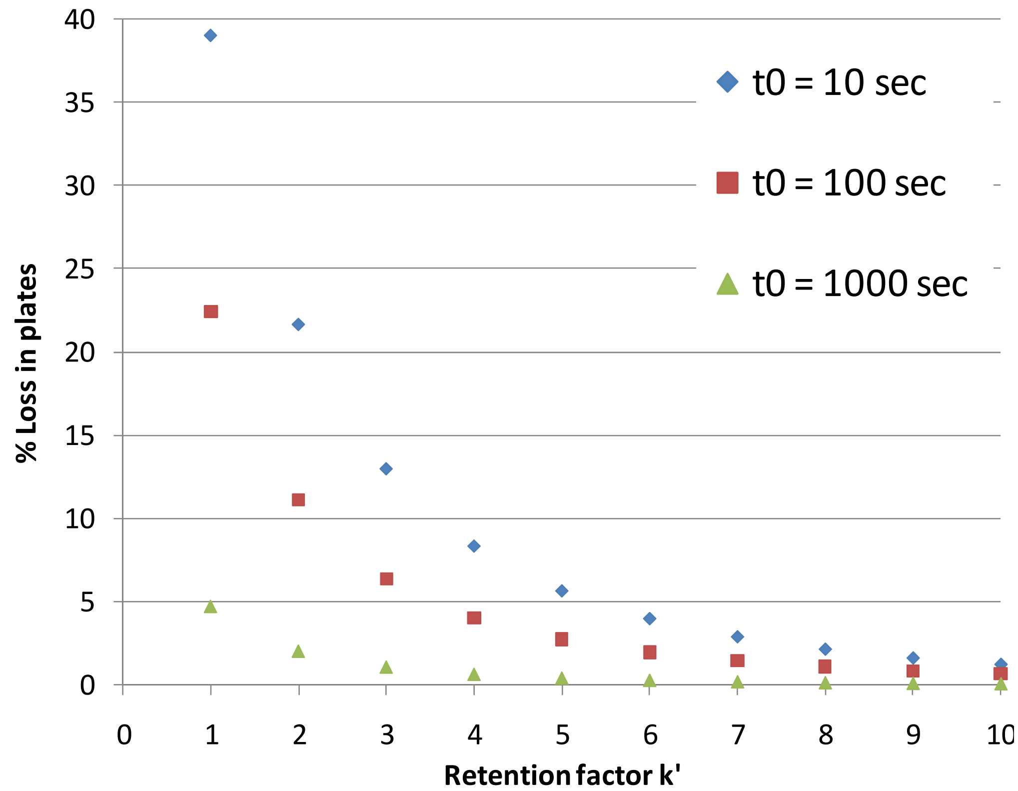
- Instrument effect
- Method transfer from fully porous to superficially porous particles

Instrument broadening

Extra-column broadening on typical UHPLC instruments



Effect of extra-column broadening on 2-parameter optimum of 2.7 μm superficially porous at 600 bar



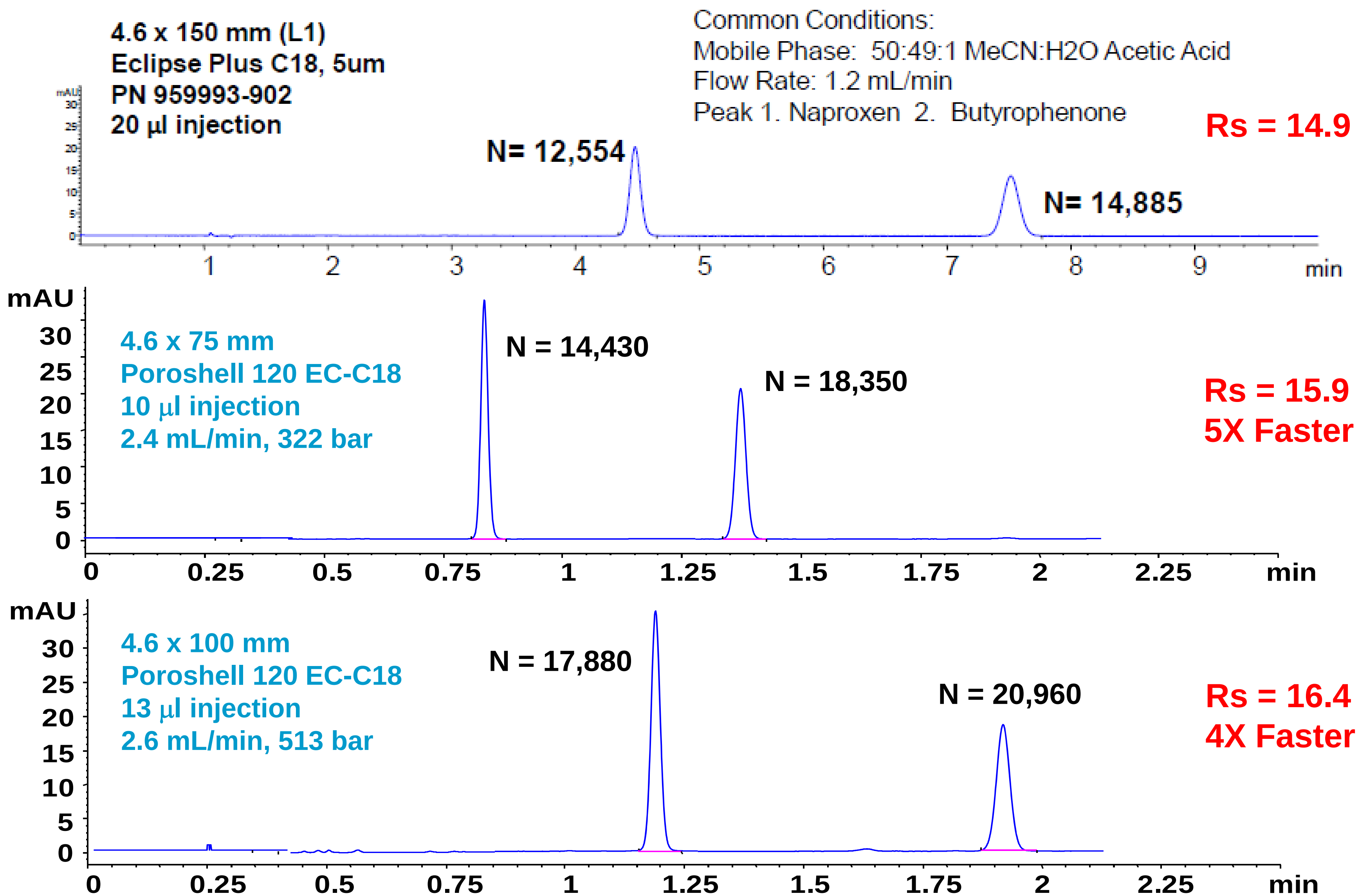
Gritti *et al. J Chrom A*, **2010**, *1217*, 7677

Method transfer

- Consider the case where one wants to transfer an HPLC method to a faster method with 2.7 μm superficially porous particles, but keep the same resolution
- Example – Transfer USP Naproxen method (isocratic)

Method	Original HPLC Method	Quick Transfer #1	Quick Transfer #2
Particles	5 μm fully porous	2.7 μm superficially porous	2.7 μm superficially porous
L (mm)	150	75	100
F (mL/min)	1.2	2.4	2.6
Pressure (bar)	70	300	500

USP Naproxen method transfer



Conclusions

- Superficially porous particles have *great potential* to generate best performance, mainly due to lower h_{min}
- Current superficially porous particles generate best performance in a very wide analysis time range (t_0 > 30 sec or t_R > 3 min)
- Various successful applications confirm the theoretical predictions
- Instrument performance can have strong impact on the performance of superficially porous particles, especially for fast separations

Acknowledgements

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