UPLC in Pharmaceutical Process Development - Comparison with Theoretical Promise

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Outline

- Fast LC for pharmaceutical development
- Approaches to fast LC
- Comparison between theoretical and practical performances in UPLC
  - Efficiency
  - Retention and selectivity factors
  - Pressure resistance
- Applications of fast LC to API process development

Fast HPLC Applications in the Pharmaceutical Industry

Discovery → Development → Manufacturing

Discovery

- **Drug screening**
- Drug impurity profiling
- PK/PD study
- Safety study

Development

- **In-process control**
- **Impurity profiling**
- Stability study
- Safety study
- PK/PD
- Delivery testing
- Raw material testing

Manufacturing

- **In-process control**
- **Product release testing**
- Raw material testing

HPLC is one of the most widely used analytical tools in the pharmaceutical industry

Why Fast LC/UPLC?

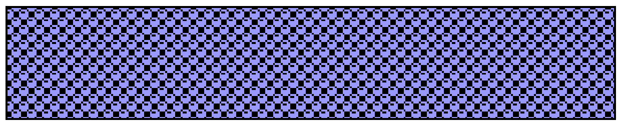
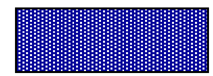
- Fast LC significantly shortens turnaround times
  - Method run time as much as 5 - 10 x faster than traditional
  - Faster method screening/development
  - For turn around time for ‘bottleneck’ in-process analyses
  - High-throughput analysis for a large number of samples
- Fast LC uses significantly less solvent
  - Estimated 10 L/instrument solvent savings
  - Estimated 25000 L solvents reduction for 2500 LC in a big pharmaceutical company.
- Fast LC is cost effective
  - The price of some UPLCs is comparable to that of a conventional HPLC
  - One UPLC can replace two HPLC’s
  - Estimated half million savings in acetonitrile and waste disposal costs for 2500 LCs

Consider cumulative savings if implemented at commercial supply sites

Approaches to Fast HPLC

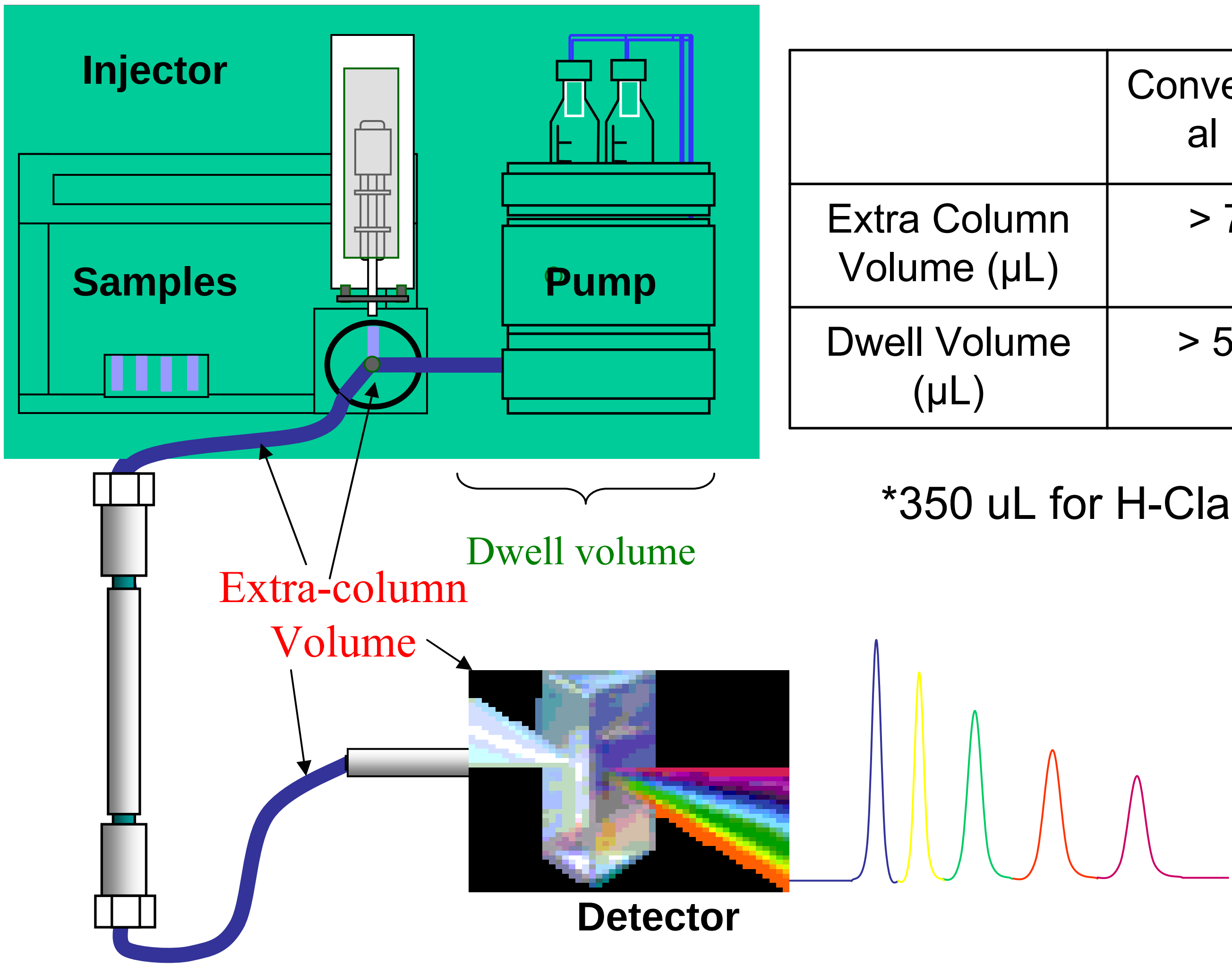
- New column technologies
  - Monolithic columns
  - Sub-2 µm packing materials
  - Fused-core particles
- New instrumentation (UPLC/UHPLC)
  - High pressure capability (>= 15,000 psi)
  - Minimized system volumes
  - Fast data acquisition speed
- Fast screening systems (short columns in parallel)

Column Dimension vs. Extra-column Volume

	Conventional LC (6000 psi)	**UPLC/UHPLC (>15,000 psi)**
Column Dimensions	150 - 250 × **4.6** mm, 5 μm 	50 – 100 × **2.1** mm, 1.7 μm 
Column Volume	2.5 - 4.1 mL	0.17 – 0. 34 mL
Extra-column volume	70 μL	< 20 μL

Are extra-column effects significant in UPLC?

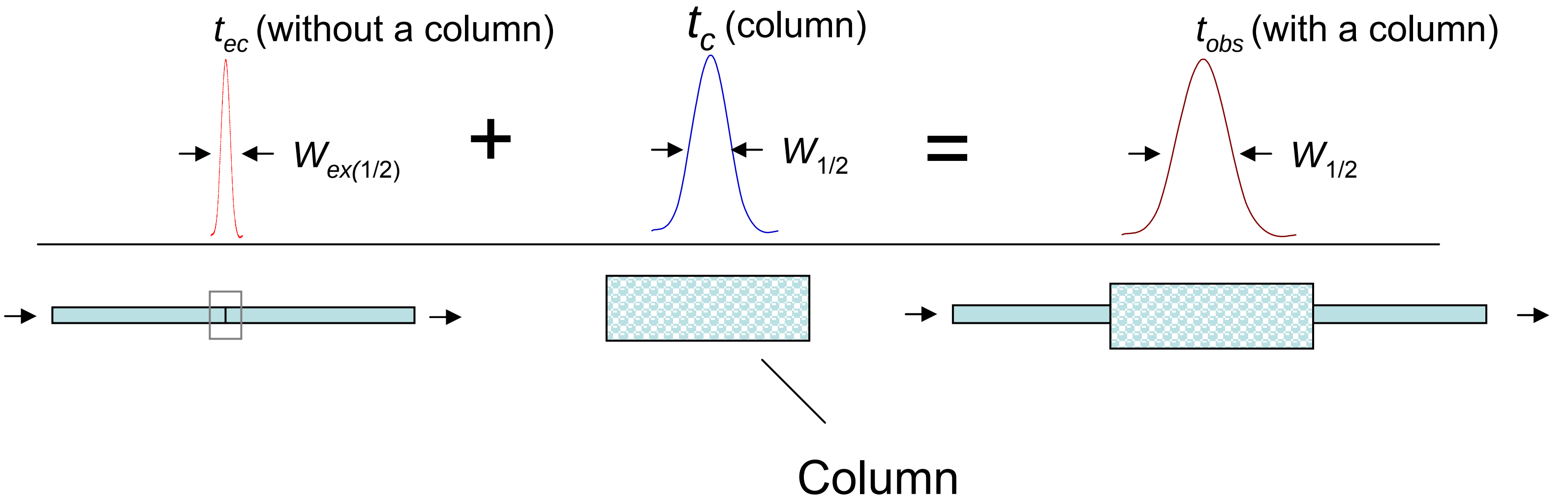
Extra Column Volumes



	Conventional LC	UPLC /UHPLC
Extra Column Volume (µL)	> 70	< 20
Dwell Volume (µL)	> 500	< 200*

*350 uL for H-Class

Effect of Extra-column Volume on Performance



Impact on performance:

- Apparent efficiency

$$\sigma^2_{obs} = \sigma^2_c + \sigma^2_{ec}$$

- Apparent retention

$$k_{obs} = \frac{(t_R)_{obs} - (t_0)_{obs}}{(t_0)_{obs}} \quad , t_{obs} = t_c + t_{ec}$$

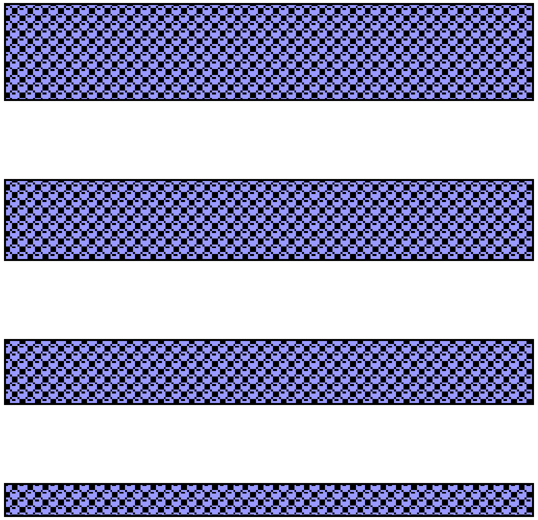
- Apparent column resistance

$$\Delta P_c = \frac{\phi \eta L}{d_p^2} u = Ku \qquad \Delta Pc\,(obs) = \Delta P_c + \Delta P_{ec}$$

Performance vs. Column Internal Diameter

Theoretical Promise

N ~12,000 plates



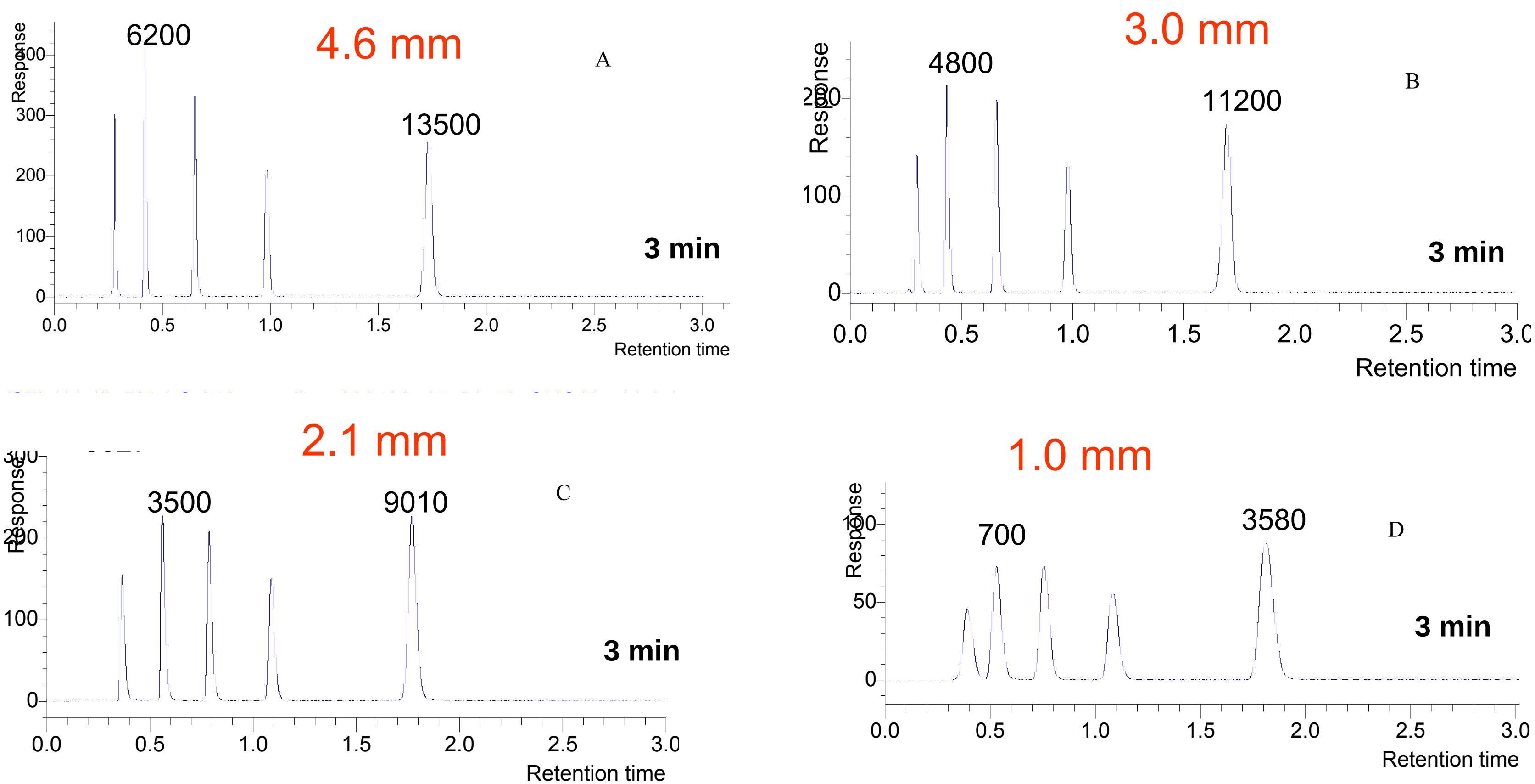
50 mm	Flow rate for 3 mm/s	Separation Time
4.6 mm	1.4 mL/min	2 min
3.0 mm	0.60 mL/min	2 min
2.1 mm	0.29 mL/min	2 min
1.0 mm	0.067 mL/min	2 min

$$F_2 = F_1 \left(\frac{r_2}{r_1} \right)^2$$

$$t = \frac{L}{u}(1 + k')$$

Performance vs. Column Internal Diameter

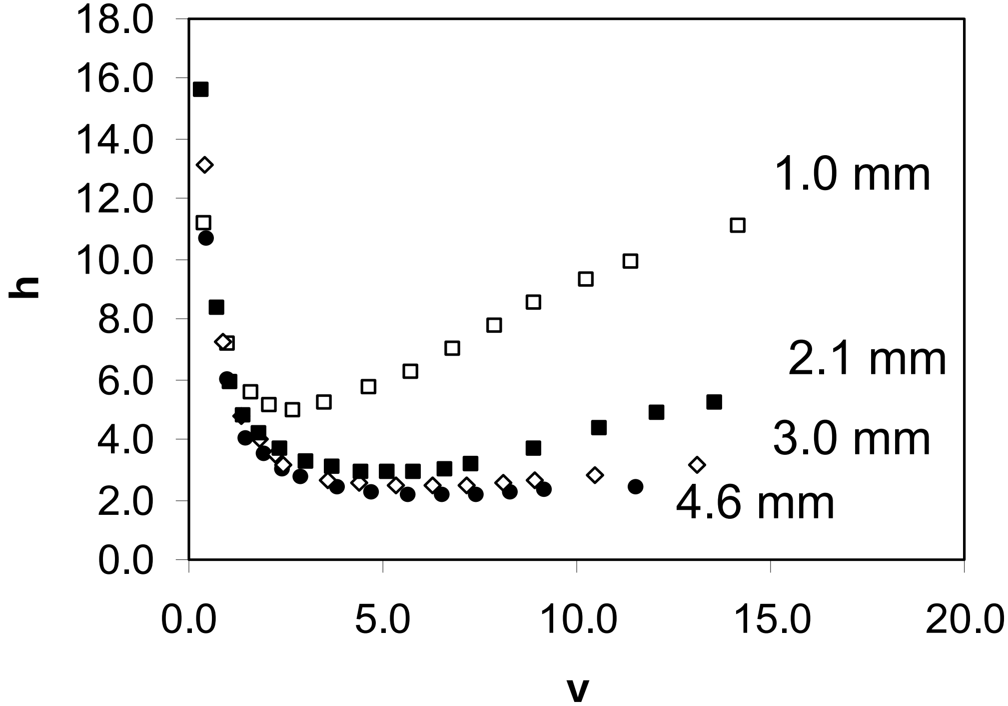
Observation 1: Efficiency decreased as the column internal diameter decreased in UPLC.



Conditions:
50/50 MeCN/H2O, 25C, 210 nm, 1.8 um, Zorbax Extend C18
50x4.6 mm, 4.8 uL; 50x3.0 mm, 2.0 uL; 50x2.1 mm, 1.0 uL;
50x1.0 mm, 0.23 uL; Phenone mix as solutes

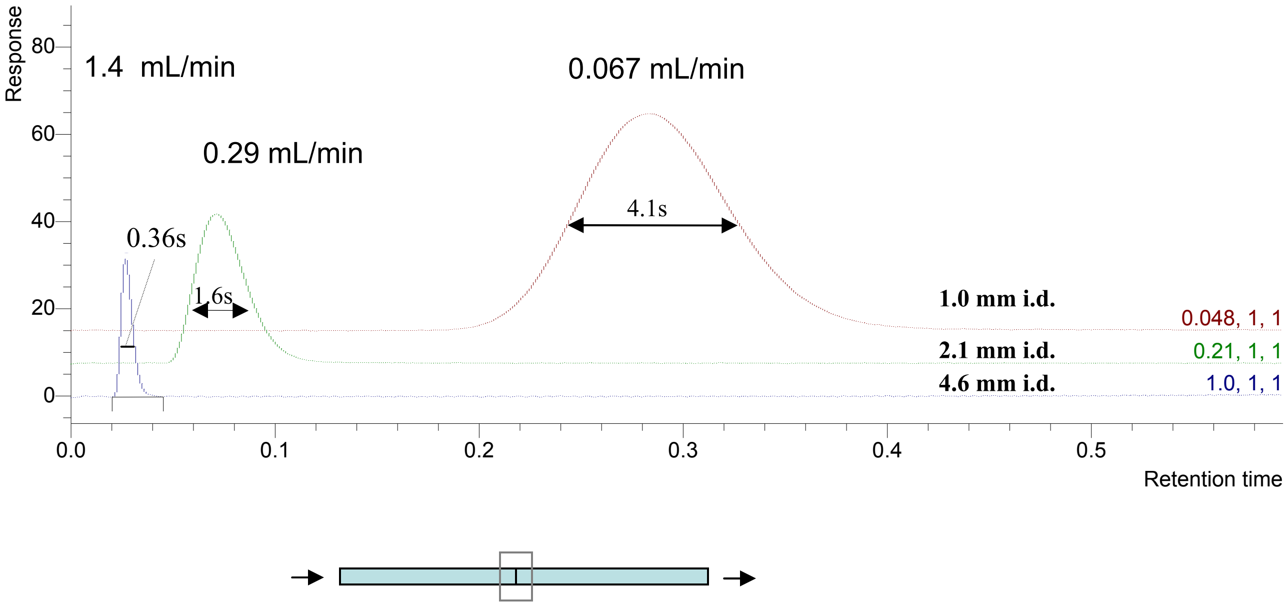
Performance vs. Column Internal Diameter

Observation 1: Efficiency decreased as the column internal diameter decreased.



Conditions: premixed 50/50 (v/v) water to acetonitrile as the mobile phase; 0.05 mg/mL uracil as unretained marker.

Migration Times and Band-broadening from Extra-column Volume

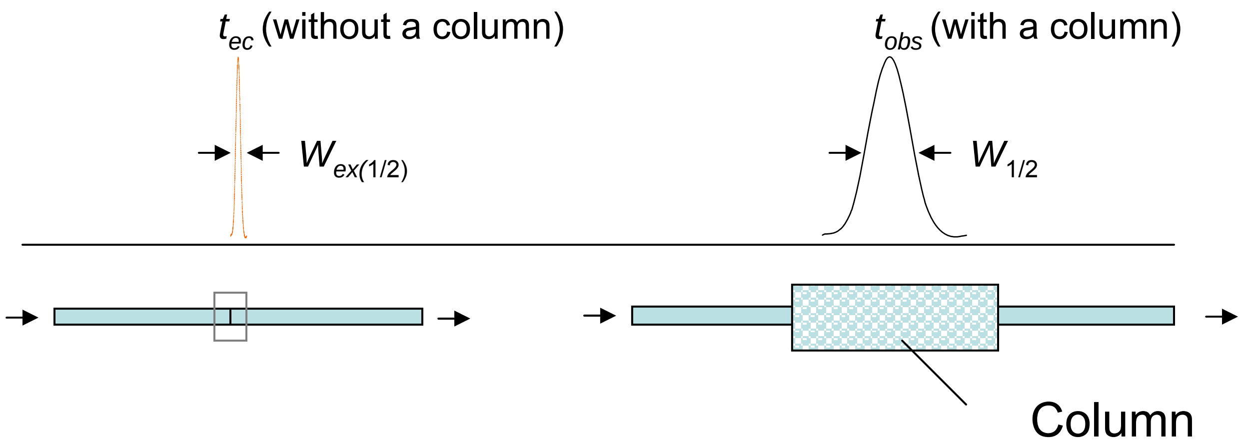


Conditions: premixed 50/50 (v/v) water to acetonitrile as the mobile phase; 0.05 mg/mL uracil as unretained marker.

Efficiency and Linear Velocity Corrected with Extra-column Volume

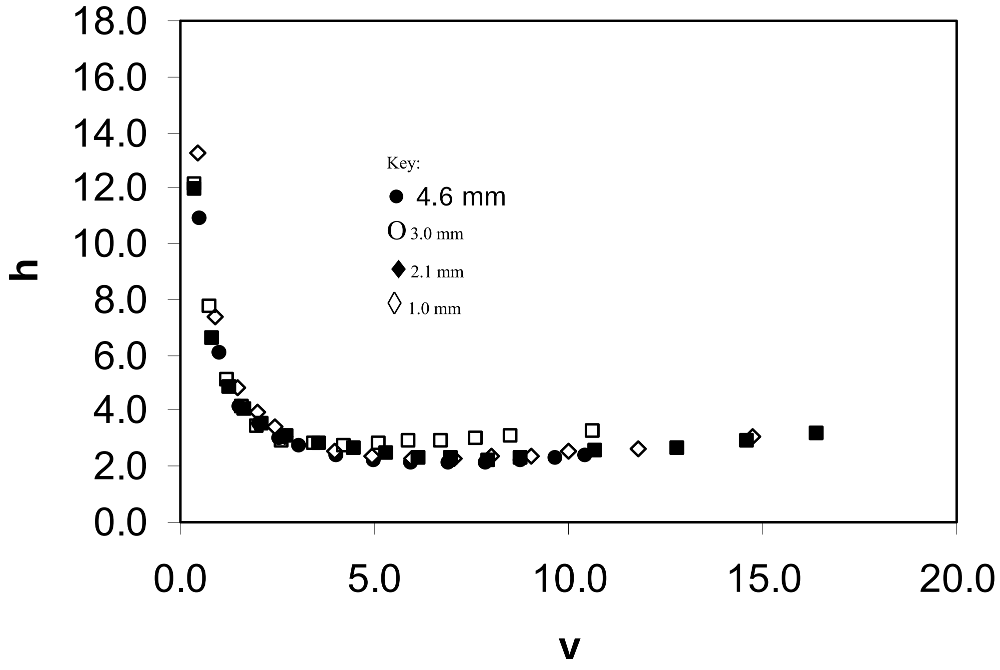
$$N_c = 5.54 \frac{\left[(t_R)_{obs} - (t_R)_{ec}\right]^2}{(w_{1/2}^2)_{obs} - (w_{1/2}^2)_{ec}}$$

$$u_{corr} = \frac{L}{(t_0)_{obs} - (t_0)_{ec}}$$



N: theoretical plate number
t_R: retention time
t_{ex}: extra-column contribution to the retention time
$w_{1/2}$: peak width at half peak height
$W_{ex(1/2)}$: extra-column contribution to peak width at half peak height

Column Efficiency Corrected with Extra-Column Band-Broadening.



Conditions: premixed 50/50 (v/v) water to acetonitrile as the mobile phase; 0.05 mg/mL uracil as unretained marker.

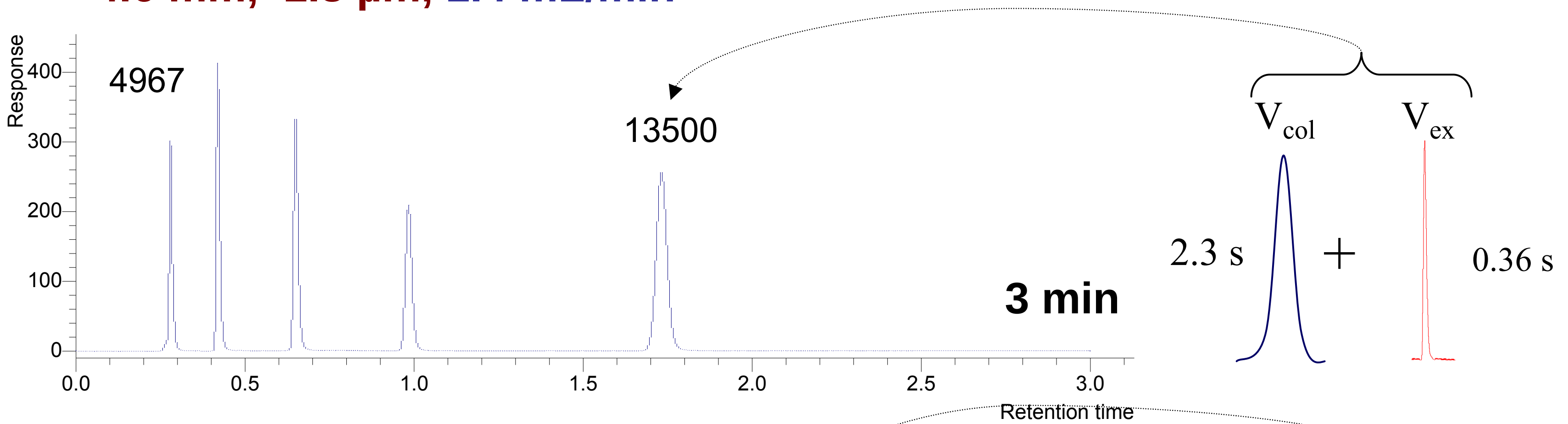
Column Efficiency Corrected with Extra-Column Band-Broadening

Column i.d. (mm)	4.6		3.0		2.1		1.0	
	Obs.	Corr.	Obs.	Corr.	Obs.	Corr.	Obs.	Corr.
k = 2.73	12982	13235	11040	12220	9242	12563	3971	10163
Relative Difference (%)	1.9		9.7		26.4		60.9	
k = 5.50	13500	13583	11278	12103	10927	12542	5570	10431
Relative Difference (%)	0.6		6.8		12.9		46.6	

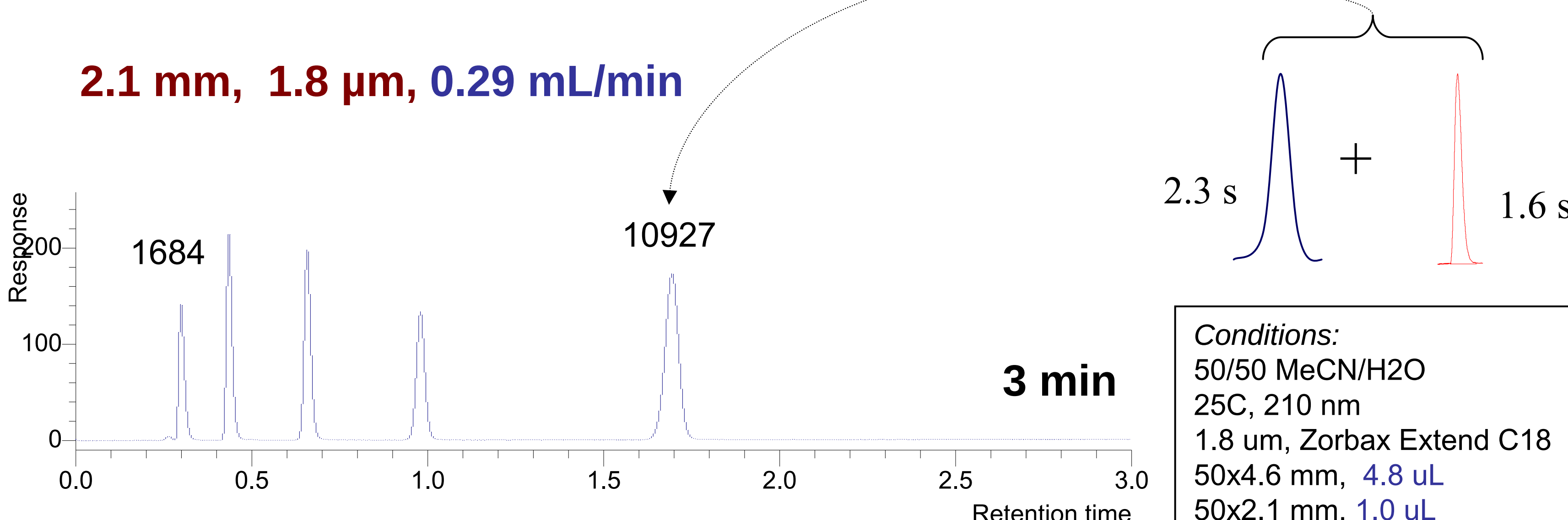
Conditions: premixed 50/50 (v/v) water to acetonitrile as the mobile phase; 0.05 mg/mL uracil as unretained marker.

4.6 vs. 2.1 mm i.d. Column on UPLC

4.6 mm, 1.8 µm, 1.4 mL/min



2.1 mm, 1.8 µm, 0.29 mL/min



Conditions:
50/50 MeCN/H2O
25C, 210 nm
1.8 um, Zorbax Extend C18
50x4.6 mm, 4.8 uL
50x2.1 mm, 1.0 uL
Phenone mix as solutes

4.6 mm i.d. Columns are less sensitive to extra column volume
2.1 mm columns are greener

Performance vs. Column Length

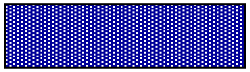
Theoretical Prediction

$$L \propto N \propto d_p$$

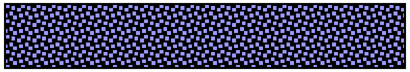
$$t_R = \frac{(1+k)Nh}{D_m v} d_p^2$$

N ~12,000 plates

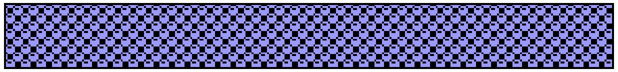
50 mm, 1.7 µm, **3 mm/s**

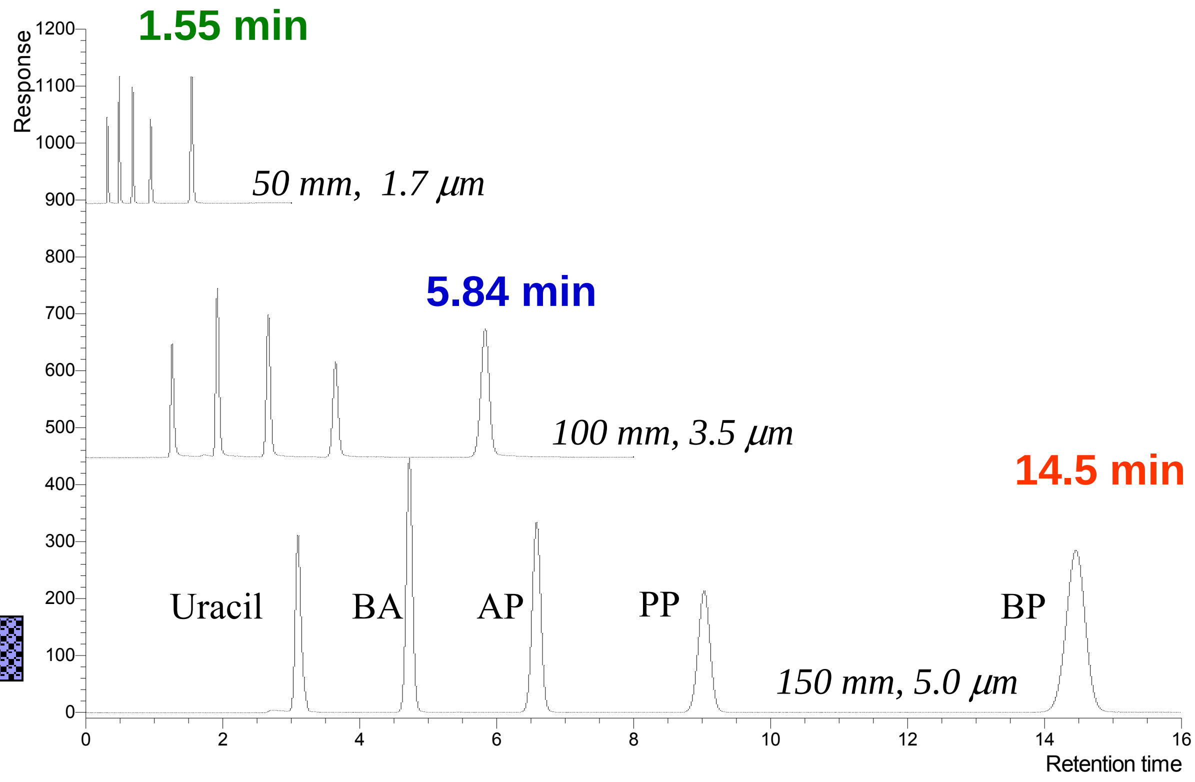


100 mm, 3.5 µm, **2 mm/s**



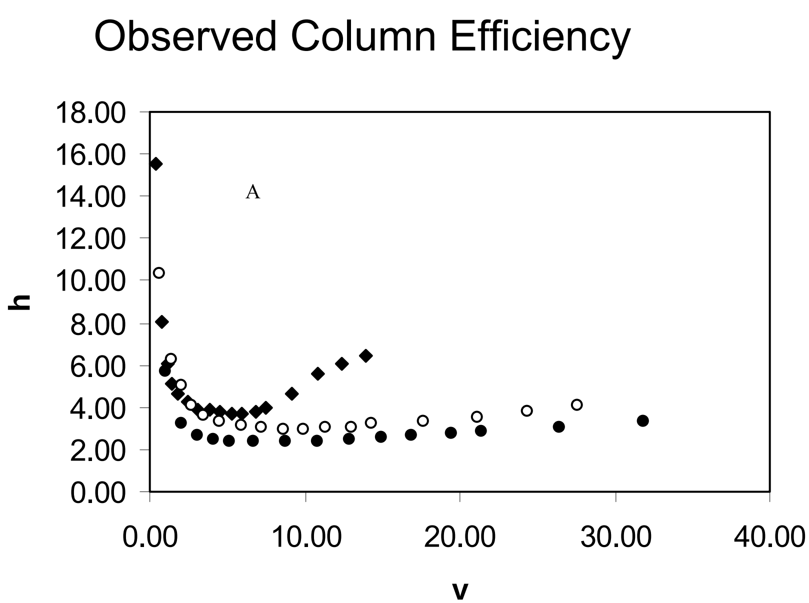
150 mm, 5 µm, **1 mm/s**

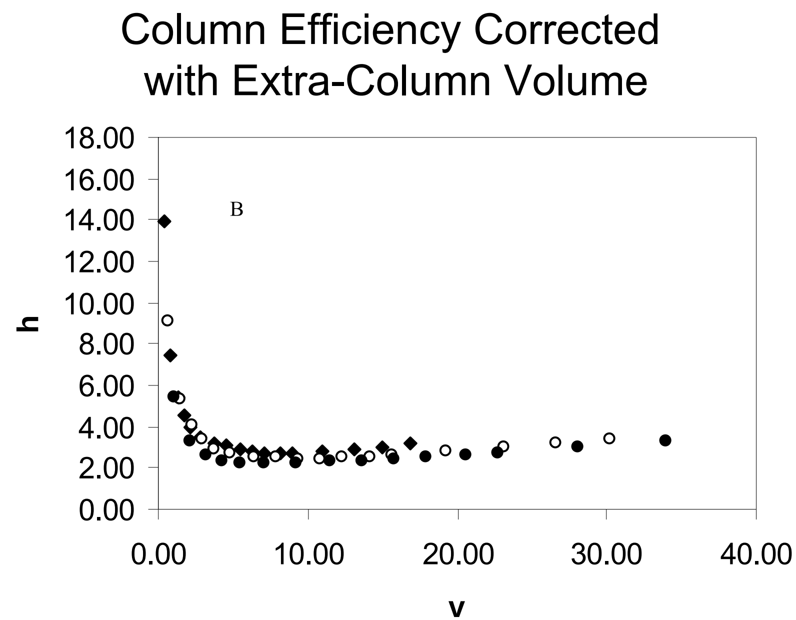




Performance vs. Column Length

Observation 2: Observed efficiency decreased as the column length decreased from 150 to 50 mm.



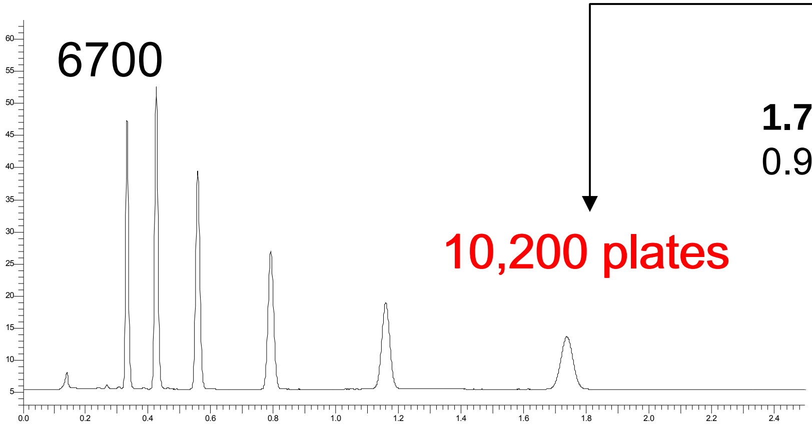


L (mm) x i.d	150 x 2.1		100 x 2.1		50 x 2.1	
u (mm/s)	1.05		2.1		2.94	
	App.	Corr	App.	Corr	App.	Corr
N	12631	12847	10948	11588	9448	11432
Rel. Error	1.7%		4.0%		21%	

Conditions: BEH Shield RP C-18 columns; premixed 50/50 (v/v) water to acetonitrile as the mobile phase; 0.2 mg/mL benzophenone as an analyte and uracil as unretained marker. The flow rate range and injection volumes for various columns are shown in Table 2.
Key: (♦) 50 mm; (○) 100 mm; (●) 150 mm.

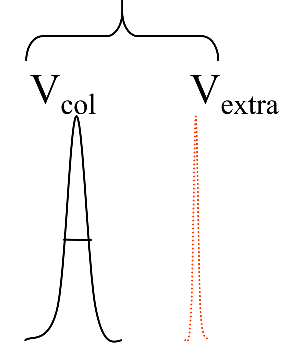
Fast Separation Is More Sensitive to Extra Column Volume

N_{theor} = 12,000 plates



1.7 µm, 50 x 2.1mm,
0.9 mL/min

10,200 plates

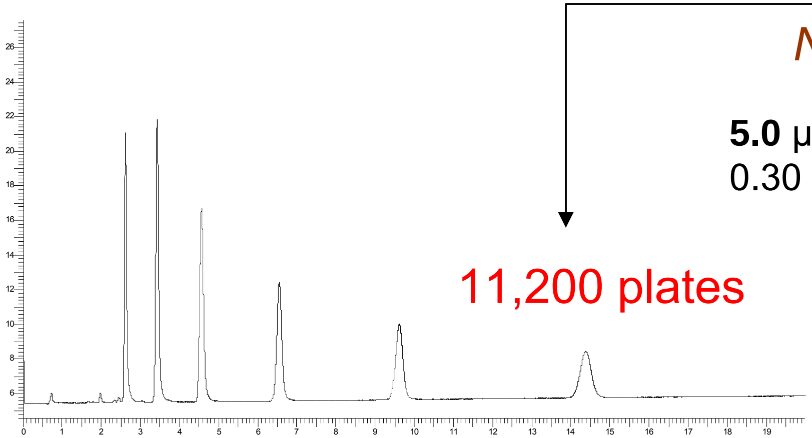


21%

2.4 s + 0.41s

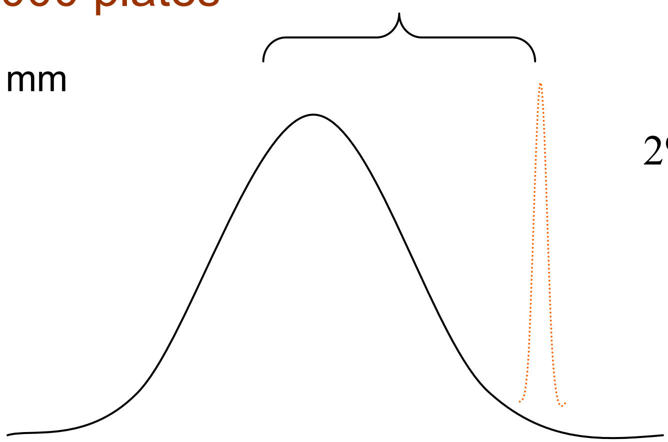
1.7 min

N_{theor} = 12,000 plates



5.0 µm, 150 x 2.1 mm
0.30 mL/min

11,200 plates



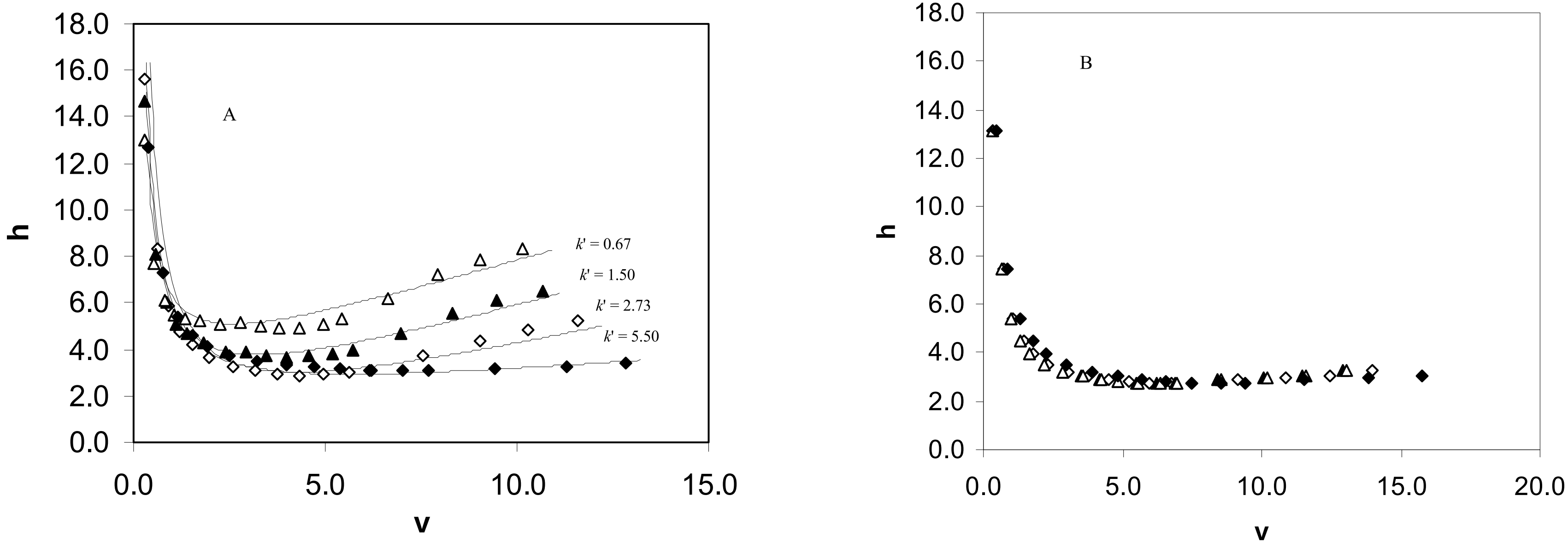
2%

15 min

18s + 1.2s

Early Eluted Peaks Are More Sensitive to Extra Column Volume

Effect of Retention Factor on Apparent Efficiency



Suggested Column Dimensions for Sub-2 um Packed Columns

$$\sigma^2_{ec(acc)} \le 0.10\sigma^2_c \le 0.10\frac{\pi^2 L^2 r^4 \varepsilon^2 (1+k)^2}{N_c}$$

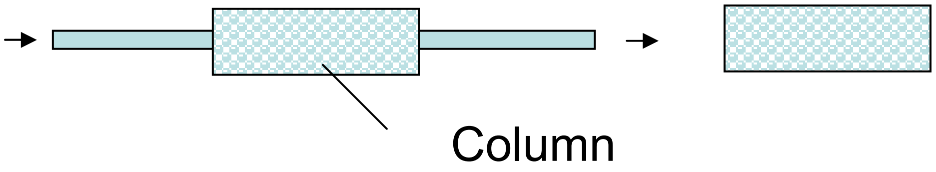
LC System	Conventional HPLC	UPLC/UHPLC	Capillary LC
Column I.D. (mm)	3.0 – 4.6	2.0 – 4.6 2.0 - 3.0 preferred	< 1 mm
Column Length (mm)	100	50 - 150	>100

Performance vs. Column Internal Diameter

Observation 3: The observed retention factor decreased from 4.6 to 3.0 to 2.1 to 1.0 mm although the retention time increased slightly

Benzene, 50/50 MeCN/H2O

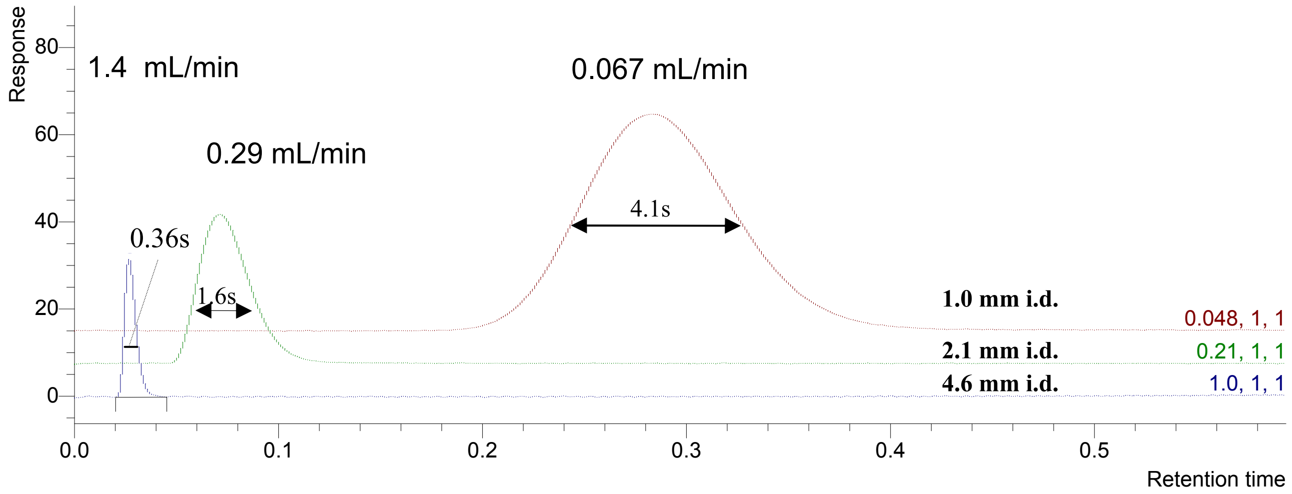
N ~12,000 plates	50 mm	Flow Rate for 3 mm/s	Retention Time (min)	t0 (min)	k	k (corr)
	4.6 mm	1.4 mL/min	1.18	0.27	3.37	3.34
	3.0 mm	0.60 mL/min	1.20	0.29	3.14	3.41
	2.1 mm	0.29 mL/min	1.26	0.34	2.71	3.31
	1.0 mm	0.067 mL/min	1.29	0.38	2.39	3.24



Performance vs. Column Internal Diameter

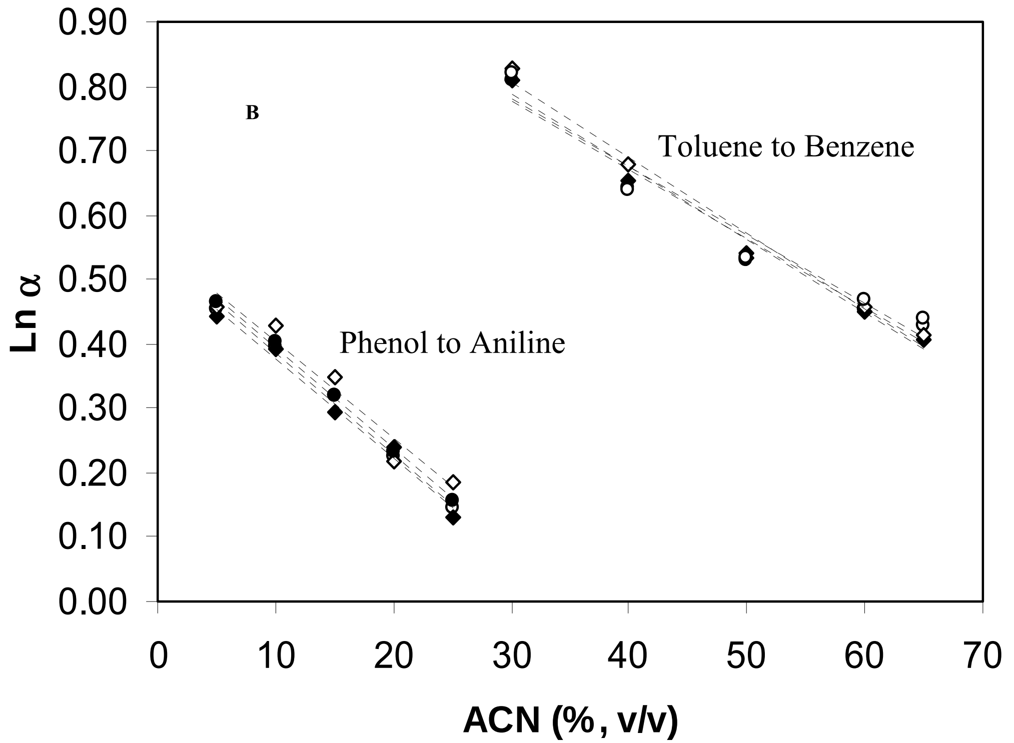
Observation 3: The observed retention factor decreased from 4.6 to 3.0 to 2.1 to 1.0 mm

$$k_{obs} = \frac{(t_R)_{obs} - (t_0)_{obs}}{(t_0)_{obs}} \approx \frac{(t_R)_{column} - (t_0)_{column}}{(t_0)_{obs}}$$



Performance vs. Column Internal Diameter

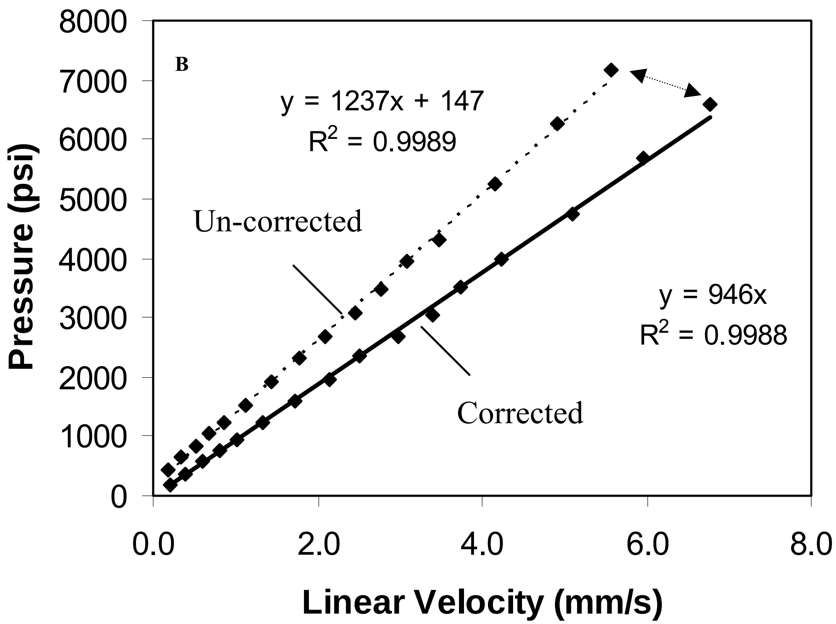
Observation 4: The observed selectivity factor remained unchanged from 4.6 to 3.0 to 2.1 to 1.0 mm



$$\alpha_{obs} = \frac{k_{2(obs)}}{k_{1(obs)}} = \frac{\dfrac{(t_R)_{2(obs)} - (t_0)_{2(obs)}}{(t_0)_{2(obs)}}}{\dfrac{(t_R)_{1(obs)} - (t_0)_{1(obs)}}{(t_0)_{1(obs)}}} = \frac{k_{2(corr)}}{k_{1(corr)}} = \alpha_{corr}$$

Performance vs. Internal Diameter

Observation 5: Observed pressures are higher than the corrected column pressures; the observed column resistance is 30% higher than actual column resistance



Column i.d. (mm)	Relative Column Resistance*		Relative Error (%)
	Apparent	Corrected	
4.6	1083	829	31
3.0	1174	862	36
2.1	1237	946	31
1.0	1291	1004	29

$$\Delta P_c\,(\mathrm{corr}) = \Delta P_{obs} - \Delta P_{ec}$$

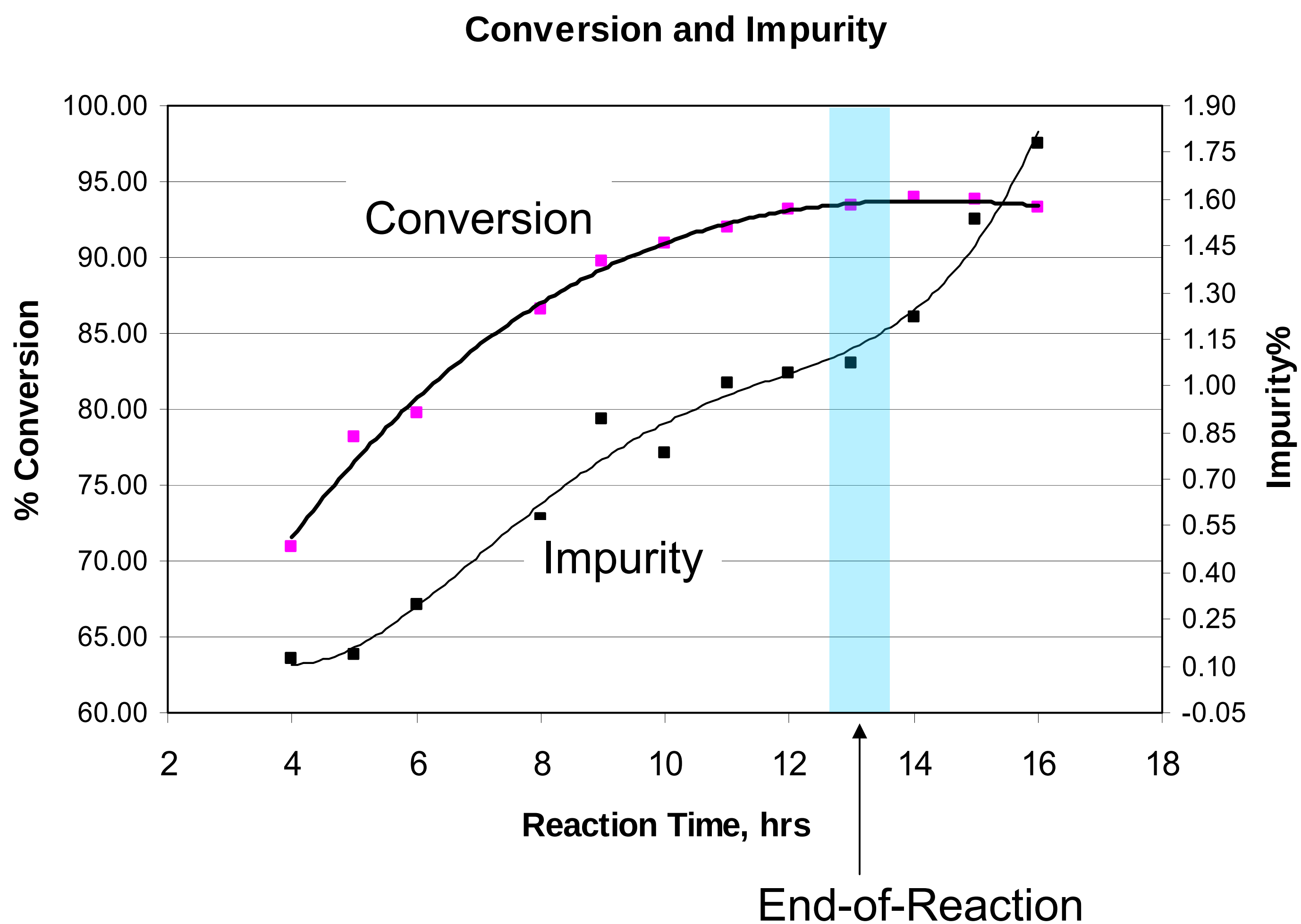
$$\Delta P_c = \frac{\phi \eta L}{d_p^2} u$$

$$u_{corr} = \frac{L}{(t_0)_{obs} - (t_0)_{ec}}$$

Column resistance

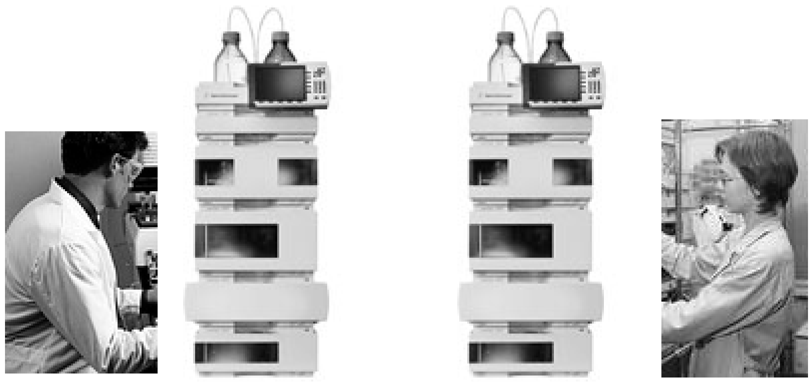
Applications – Real-time Reaction Monitoring in API Process Development

One sample per hour at least!



Real-time Reaction Monitoring
– 50 min method for hourly samples

2 LCs, 2 Analysts



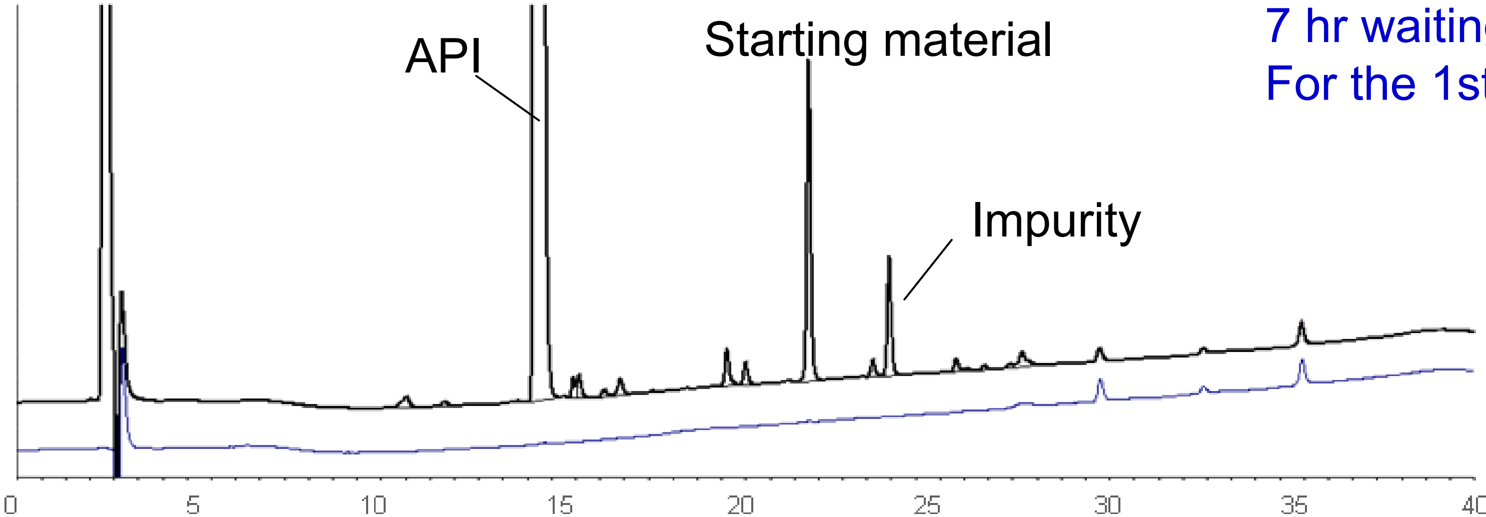
1, 3, 5, …19 hr

2, 4, 6, …20 hr

40 min run
+ 10 min post run
= 50 min

2 blanks
5 system suitabilities
1 standard

7 hr waiting time
For the 1st sample

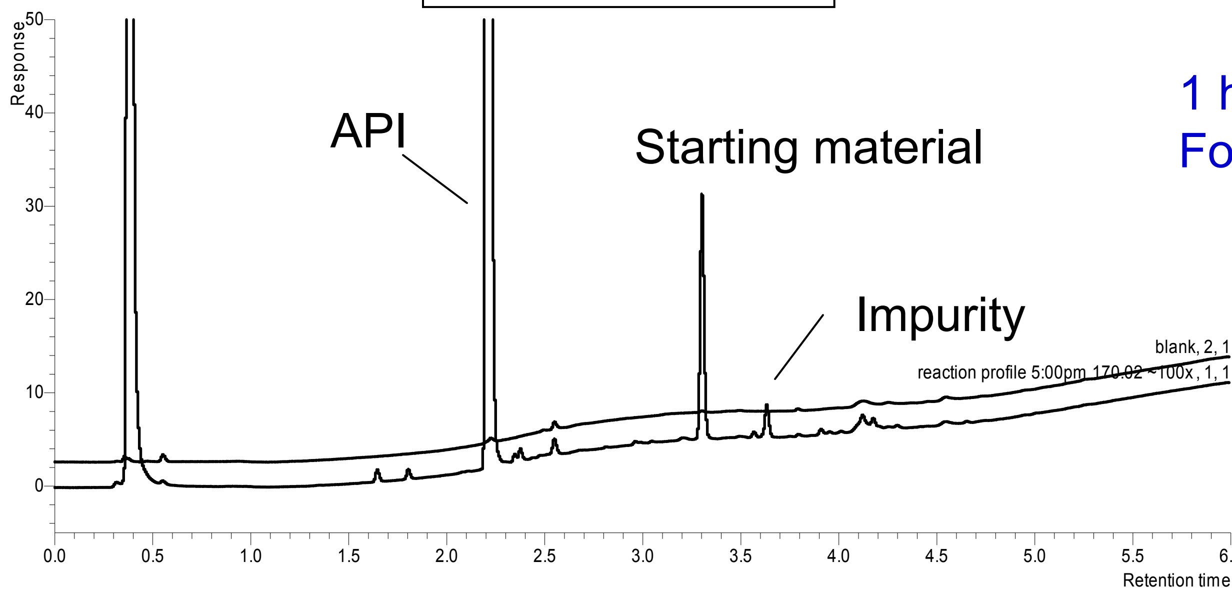


40 min

Real-time Reaction Monitoring
– 8 min method for hourly samples

1 LC, 1 Analyst

1, 2, 3, 4,….20 hr



6 min

6 min run
+ 2 min post run
= 8 min

2 blanks
5 system suitabilities
1 standard

1 hr waiting time
For the 1st sample

Applications- Fast LC for Release Testing

One Merck product: 360 lots need to be released per year

	50 min	**8 min**
Cycle time (hr)	6300 (or 263 days)	1008 (42 days)
Solvent consumption (Liter)	567	91
Waste (Liter)	1134	182

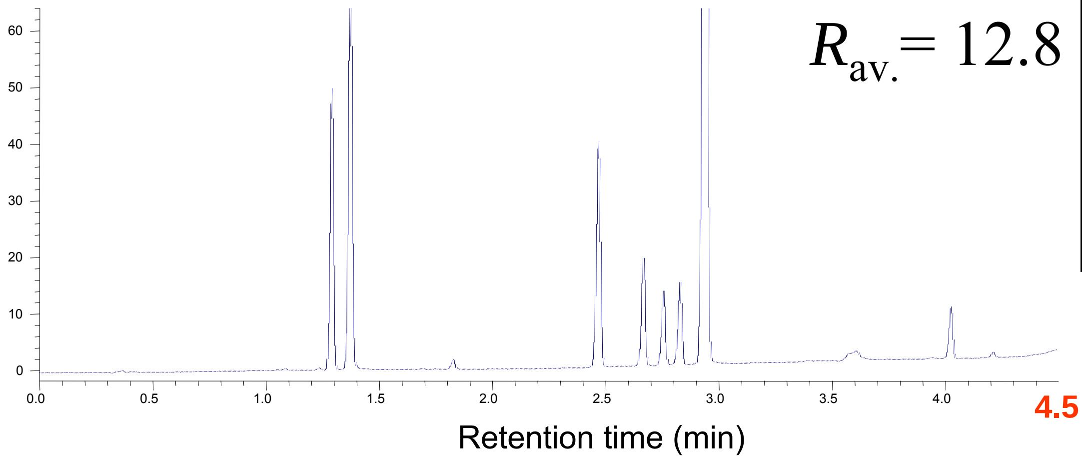
Total 21 runs for one release:
2 blanks
2 System suitability (sensitivity)
5 Injections for precision
2 Standards x 2
2 Sample preparations x 2
2 Standards x 2 (Bracketing)

Applications - Using UPLC

Impurity profiling for an Intermediate

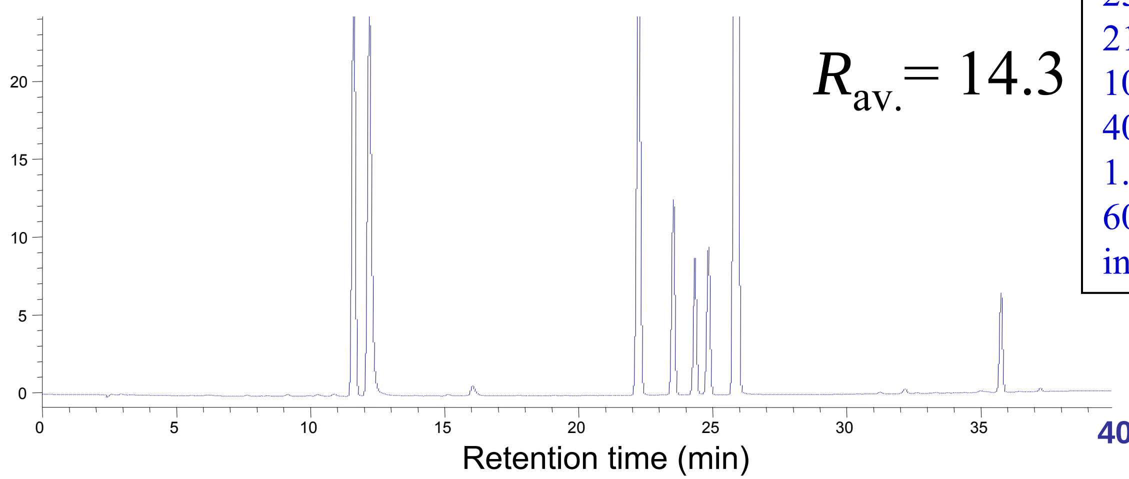
9 x faster

$R_{av.} = 12.8$



100 x2.1mm, 1.7 µm Acquity BEH C_{18}
210 nm detection @ 40 Hz
2 µL injection
40 ºC, Water Acquity System
0.60 mL/min, 13,000 psi
60/40 to 5/95 0.1%H_3PO_4/MeCN
in 4.5 min, 2 min post run

$R_{av.} = 14.3$



250 x 4.6mm, 5 µm Symmetry C_{18}
210 nm detection @ 5 Hz
10 µL injection
40 ºC, Agilent1100
1.0 mL/min, 1450 psi
60/40 to 5/95 0.1%H_3PO_4/MeCN
in 40 min, 10 min post run

Conclusions

- The effect of extra-column volume on performance can be significant in UPLC although the system was optimized for fast separation.
- Columns with small internal diameters and / or short column lengths are more susceptible to extra-column band-broadening for high-speed separation in UPLC.
- Apparent column efficiency decreased by ~20% with a decreasing column internal diameter or a decreasing column length. The speed gain usually overweighed the loss in column efficiency.
- Apparent retention factors also decreased with a decreasing column internal diameter, while observed selectivity is practically the same for different internal diameter columns.
- The observed column pressure resistance is 30% higher than the actual column resistance.
- Fast LC proved to be useful for pharmaceutical in-process monitoring, releasing testing, and intermediate profiling with a significant reduction in cycle time and in solvent consumption.

Selected Recent Publications on Fast LC

- Wu, N.; Li, Z. "*Applications of Monolithic Chromatography in Support of Pharmaceutical Development of Drug Substances and Formulations*," in "***Monolithic Chromatography and Its Modern Applications***", Ed. by Perry Wang, ILM Publications, Chapter 15, p.427-449, 2010.
- Ahmed Abrahim , Mohammad Al-Sayah, Peter Skrdla, Yuri Bereznitski, Yadan Chen, Naijun Wu*, *"Practical Comparison of 2.7 μm Fused-Core Silica Particles and Porous Sub-2 μm Particles for Fast Separations in Pharmaceutical Process Development",* ***J. Pharm. Biomed. Anal. 2010,*** 51*, 131-137.*
- Wu, N*; Clausen, A.; Wright, L.; Vogel, K.; Bernardoni, F. "*Fast UHPLC Using Sub-2 μm Particles and Elevated Pressures for Pharmaceutical Process Development".* ***American Pharmaceutical Review*** **2008**, *11*, 24-33.
- Al-Sayah, A.A.; Rizos, P.; Antonucci, V.; Wu, N. "*High throughput screening of active pharmaceutical ingredients by UPLC,"* ***J. Sep. Sci.*** **2008**, *31 (12)*, 2167-2173.
- Wu, N; Clausen, A. "*Fundamental and practical aspects of ultrahigh pressure liquid chromatography for fast separations".* ***J. Sep. Sci.*** **2007**, *30*, 1167-1182.
- Wu, N. *"Increasing the Speed of Enantiomeric Separation Using Supercritical Fluid Chromatography*," in ***Advances in Chromatography***, Schmidt Periodicals GmbH, Germany, **2007**, Vol. 46, Chapter 4.
- Wu, N.; Liu, Y.; Lee, M.L. "*Sub-2mum porous and nonporous particles for fast separation in reversed-phase high performance liquid chromatography."* ***J. Chromatogr. A*** **2006,** *1131,* 142-150
- Wu, N.; Thompson, R. *"Fast and Efficient Separations Using Reversed Phase Liquid Chromatography,"* ***J. Liquid Chromatography***, **2006**, *29*, 949-988.
- Wu, N. ; Dempsey, J.; Yehl, P.M.; Dovletoglou, A.; Ellison, D.K.; Wyvratt, J.M. "*Practical Aspects of Fast HPLC Separations for Pharmaceutical Process Development Using Monolithic Columns* " ***Analytica Chimica Acta***, **2004**, *523*, 149–156.

Acknowledgements

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