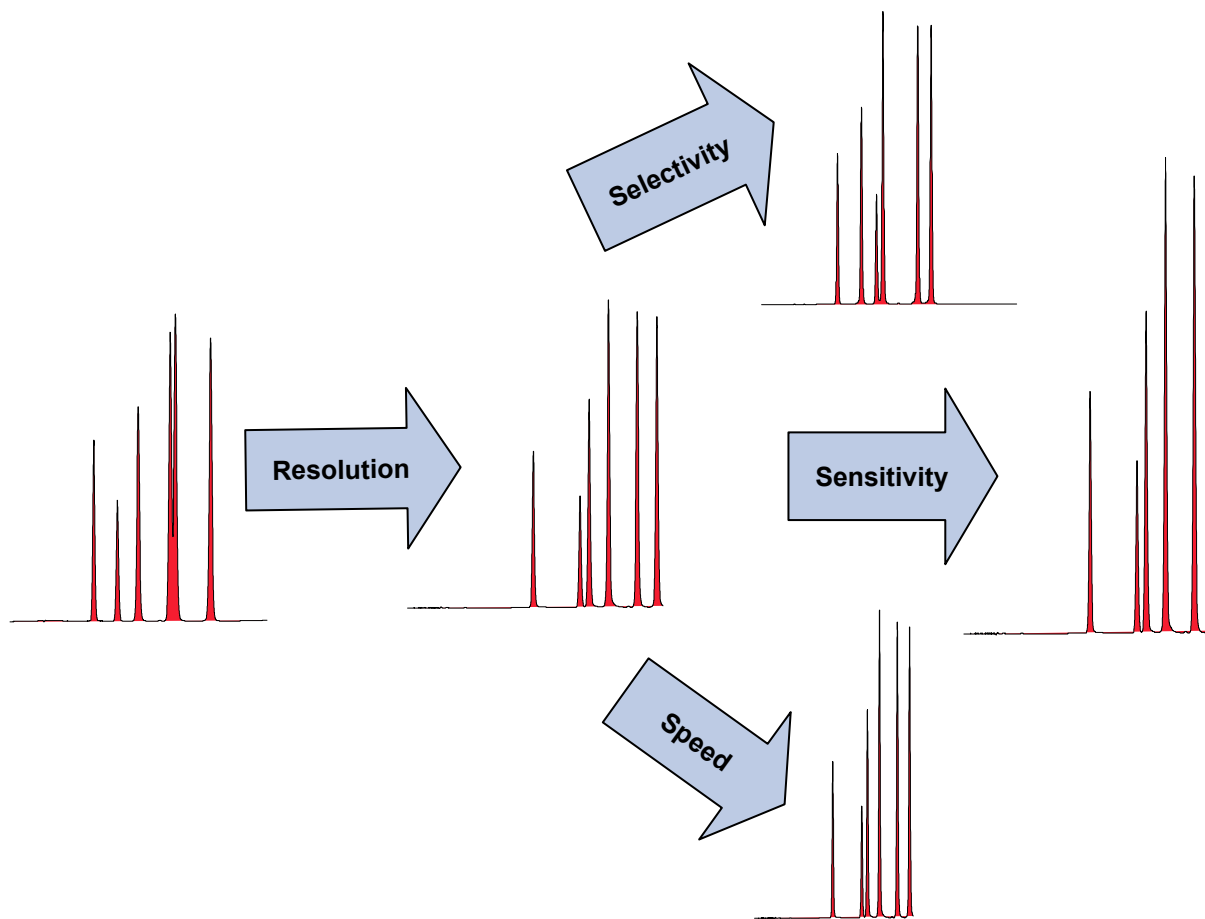


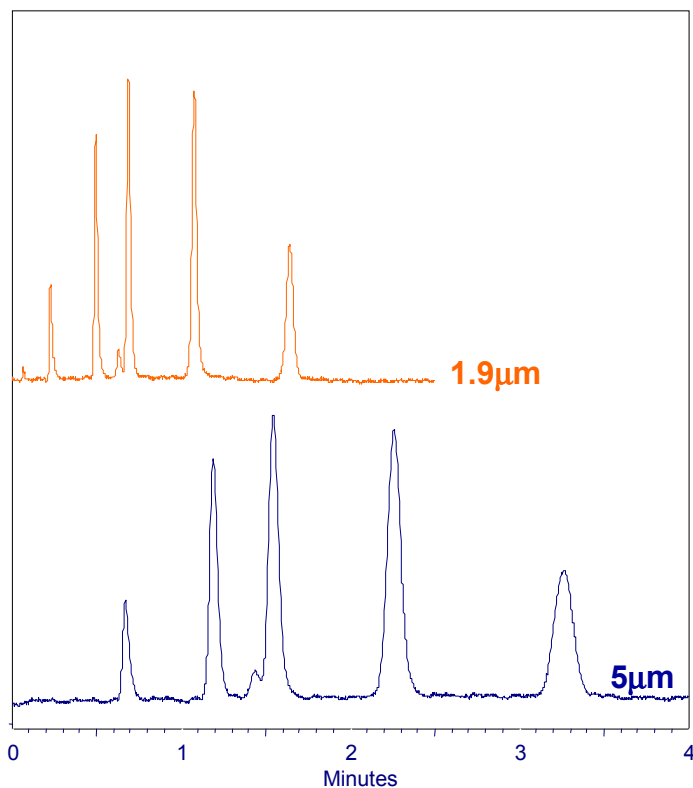
# The use of sub-2 $\mu$ m Particles for Enhanced Speed and Resolution in HPLC and LC/MS Applications

**K. Doolan**  
**KVCV, 12 October 2006**

# The challenge: Making assays more productive



# 1.9 $\mu$ m vs 5 $\mu$ m Hypersil GOLD™



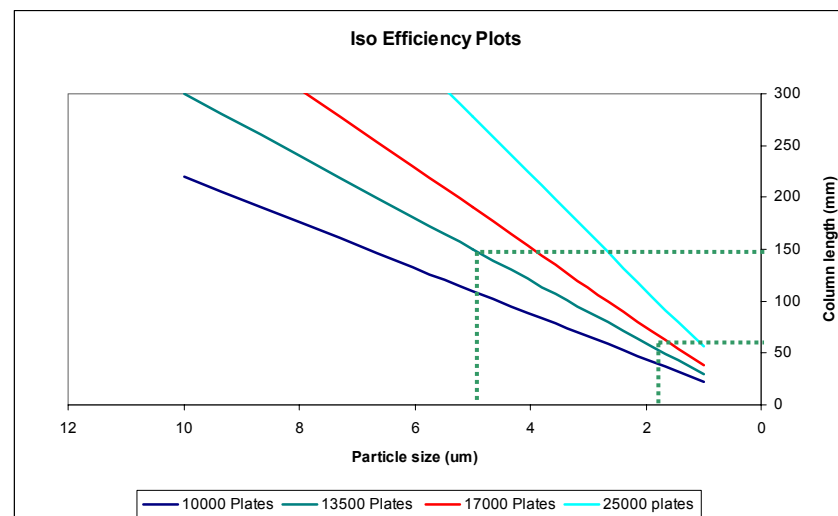
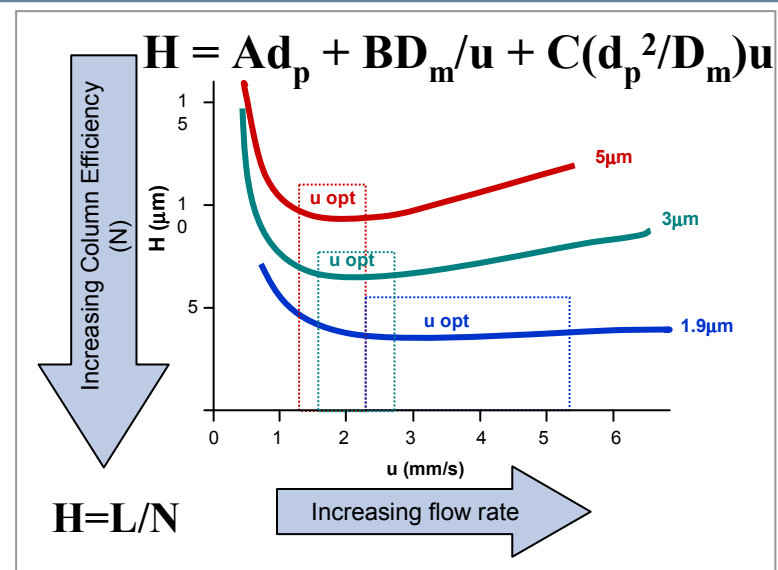
Columns: Hypersil GOLD 50x2.1mm  
Mobile phase: H<sub>2</sub>O / ACN (1:1)  
Peak 5: o-xylene

	5 $\mu$ m	1.9 $\mu$ m
Flow rate (mL/min)	0.2	0.55
$\Delta$ P (psi)	350	3800
Peak 5 Width (s)	13.8	5.4
N/t <sub>0</sub> (plates / s)	93	513
R <sub>s</sub> (4,5)	3.33	5.39

**5.5 x improvement in plate generation rate.**  
**60% reduction in peak width.**  
**62% increase in resolution**  
**2 x faster analysis**

# The advantages of 1.9 $\mu\text{m}$ particles

- 1.9  $\mu\text{m}$  particles give higher efficiency and greater range of optimum linear velocity
- Can operate at higher flow rates without losing performance
- Shorter columns packed with 1.9  $\mu\text{m}$  particles give same efficiency as longer columns with 5  $\mu\text{m}$  particles



# System Optimization

- Minimize Volume Dispersion

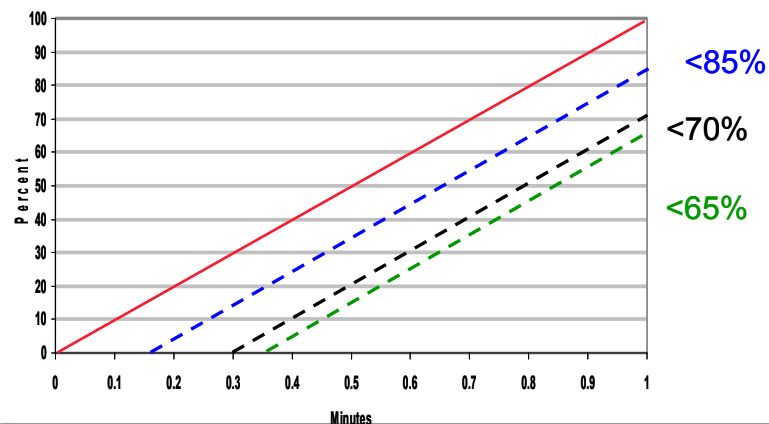
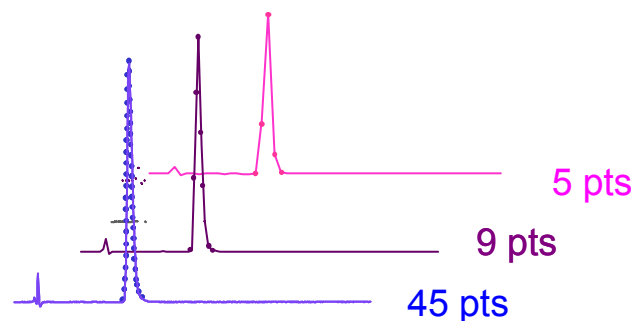
- *Low injection volume*
- *Low volume flow cell*
- *Tubing – short L, narrow ID*

$$\sigma_{ext}^2 = \frac{V_{inj}^2}{K_{inj} \cdot 12} + \frac{V_{cell}^2}{K_{cell} \cdot 12} + \tau^2 F^2 + \frac{r_c^4 \cdot l_c \cdot F}{76 \cdot D_m}$$

- Optimize Detector Sampling Rate

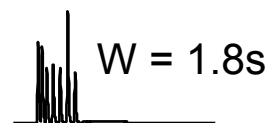
- *Reduce detector time constant*
- *Increase sampling frequency*
- *Fast scanning MS*

- Low Dwell Volume Pump for Fast Gradients

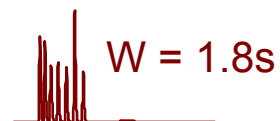


# Effect of column geometry, particle size and operating conditions on run time and peak width at base. 6 mins → 0.5mins

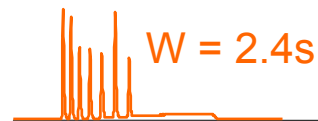
Column: 50 x 2.1 mm, 1.9  $\mu\text{m}$   
 Flow rate: 1000  $\mu\text{L}/\text{min}$   
 $t_g = 0.4$  min; Temp.: 60  $^\circ\text{C}$



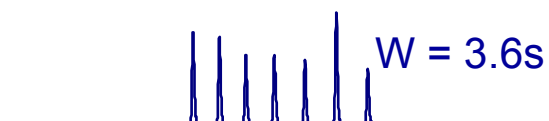
Column: 50 x 2.1 mm, 1.9  $\mu\text{m}$   
 Flow rate: 1000  $\mu\text{L}/\text{min}$   
 $t_g = 0.4$  min



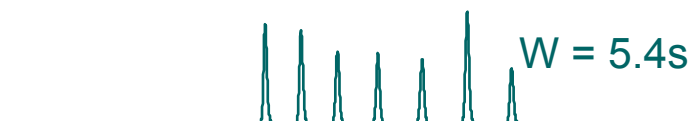
Column: 100 x 2.1 mm, 1.9  $\mu\text{m}$   
 Flow rate: 1000  $\mu\text{L}/\text{min}$   
 $t_g = 0.7$  min



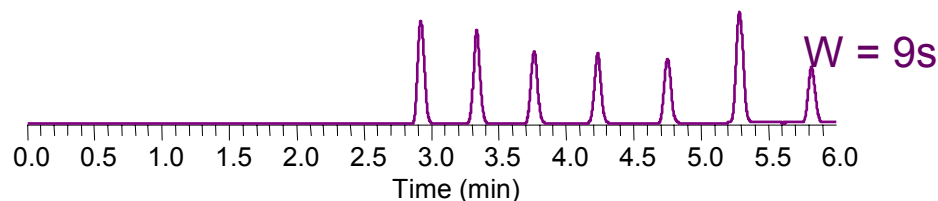
Column: 200 x 2.1 mm, 1.9  $\mu\text{m}$   
 Flow rate: 600  $\mu\text{L}/\text{min}$   
 $t_g = 1.5$  min



Column: 200 x 2.1 mm, 3  $\mu\text{m}$   
 Flow rate: 400  $\mu\text{L}/\text{min}$   
 $t_g = 2.3$  min



Column: 200 x 2.1 mm, 5  $\mu\text{m}$   
 Flow rate: 250  $\mu\text{L}/\text{min}$   
 $t_g = 3.5$  min



Columns: Hypersil GOLD

Mobile phase: A -  $\text{H}_2\text{O}$ ; B - MeCN

Gradient: 65 to 95%B in  $t_g$

Temperature: 40  $^\circ\text{C}$

Detection: 247 nm (0.1s rise time)

Injection volume: 0.2  $\mu\text{L}$

HPLC System: Accela

Analytes:

Acetophenone

Propiophenone

Butyrophenone

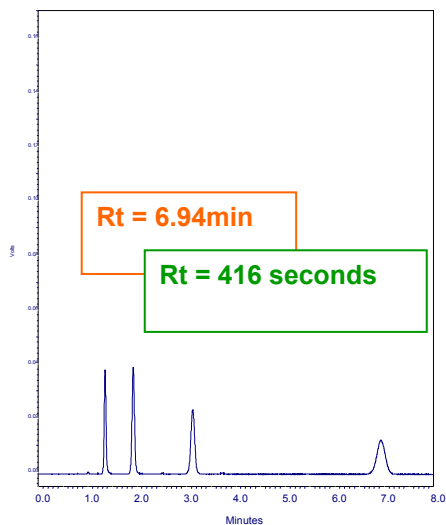
Valerophenone

Hexanophenone

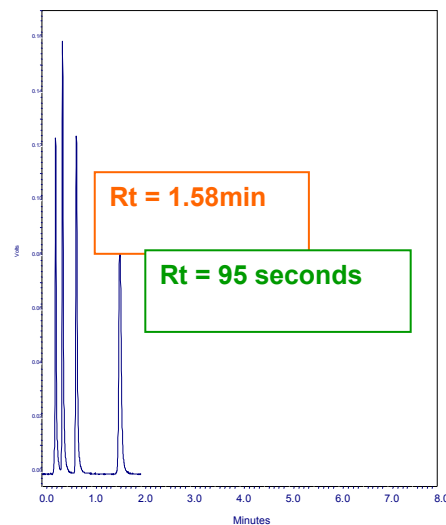
Heptanophenone

Octanophenone

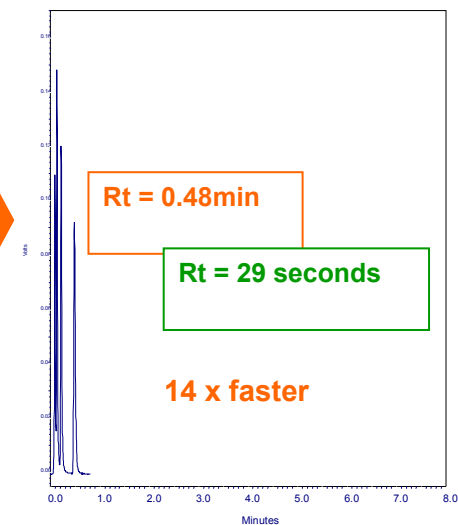
# Optimize for Speed



100x4.6mm, 5 $\mu$ m  
Flow rate: 1 mL/min

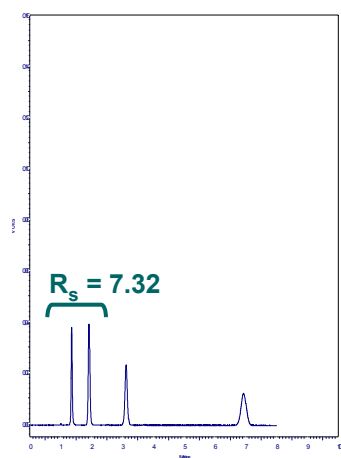


50x2.1mm, 1.9 $\mu$ m  
Flow rate: 0.55 mL/min



20x2.1mm, 1.9 $\mu$ m  
Flow rate: 0.8 mL/min

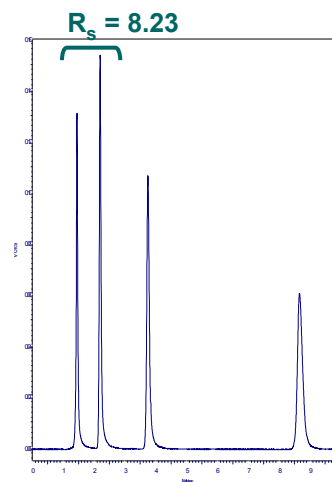
# Increase Resolution with Hypersil GOLD 1.9 $\mu$ m



100x4.6mm, 5 $\mu$ m  
Flow rate: 1 mL/min



13% higher  
Resolution



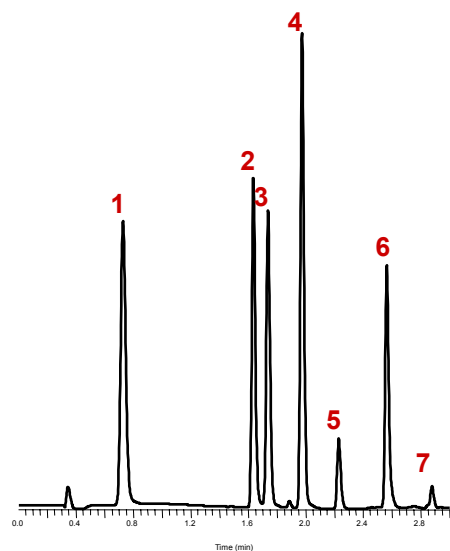
100x2.1mm, 1.9 $\mu$ m  
Flow rate: 0.2 mL/min



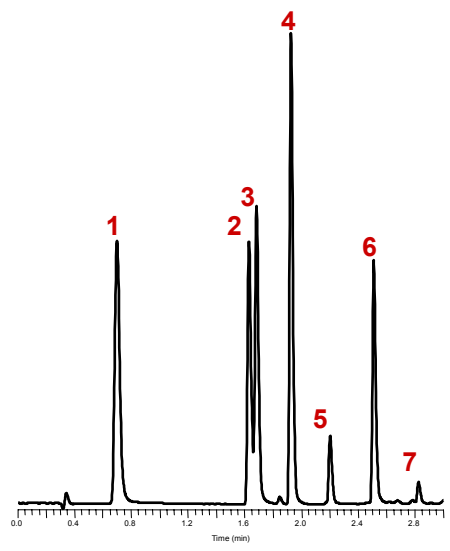
# Hypersil GOLD: Alternative Selectivity

Effect of column chemistry (C18 selectivity, C18 polar endcapped and pentafluorophenyl) on the separation of aromatic amines.

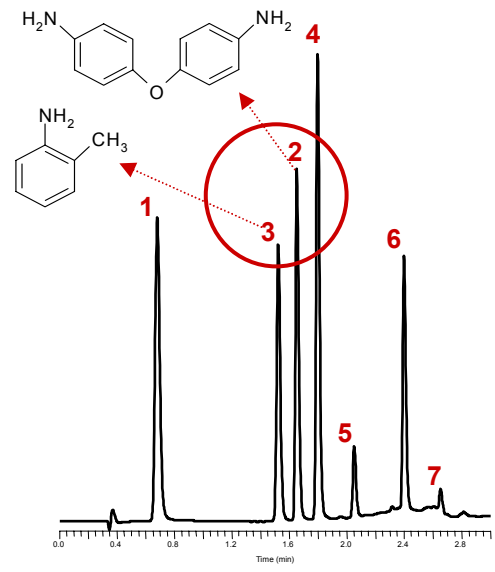
Hypersil GOLD



Hypersil GOLD aQ



Hypersil GOLD PFP



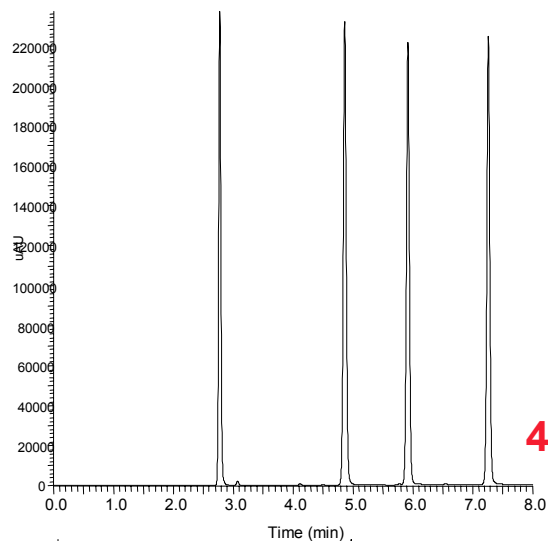
Columns: Hypersil GOLD 1.9  $\mu$ m, 50 x 2.1 mm  
Mobile phase: 25mM NH<sub>4</sub>OAc pH 5 / ACN  
Gradient: 10 – 100% B in 3 mins  
Flow rate: 0.5 mL/min  
Temperature: 40 °C  
Detection: UV@ 254 nm (2  $\mu$ L flow cell)  
Injection Volume: 0.5  $\mu$ L

Analytes:

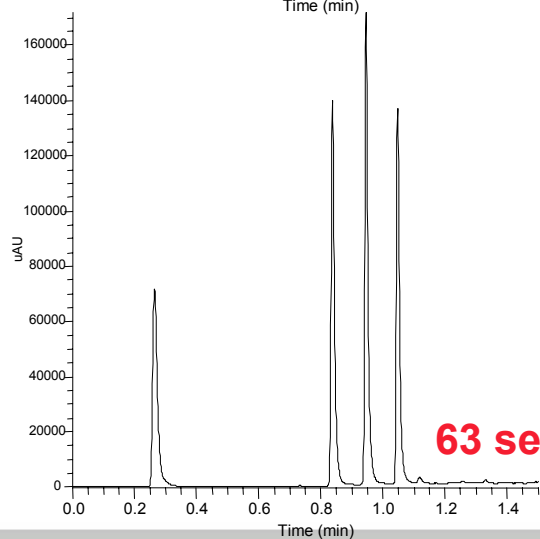
1. 2,4-diaminotoluene
2. 4,4-oxydianiline
3. o-toluidine
4. 2-methoxy-5-methylaniline
5. 2,4,5-trimethylaniline
6. 4,4-methylene-bis(2-chloroaniline)
7. Impurity from analyte No. 6

# Gradient Method Transfer (Sulphonamides)

150x4.6mm, 5 $\mu$ m



433 seconds



63 seconds

30x2.1mm, 1.9 $\mu$ m

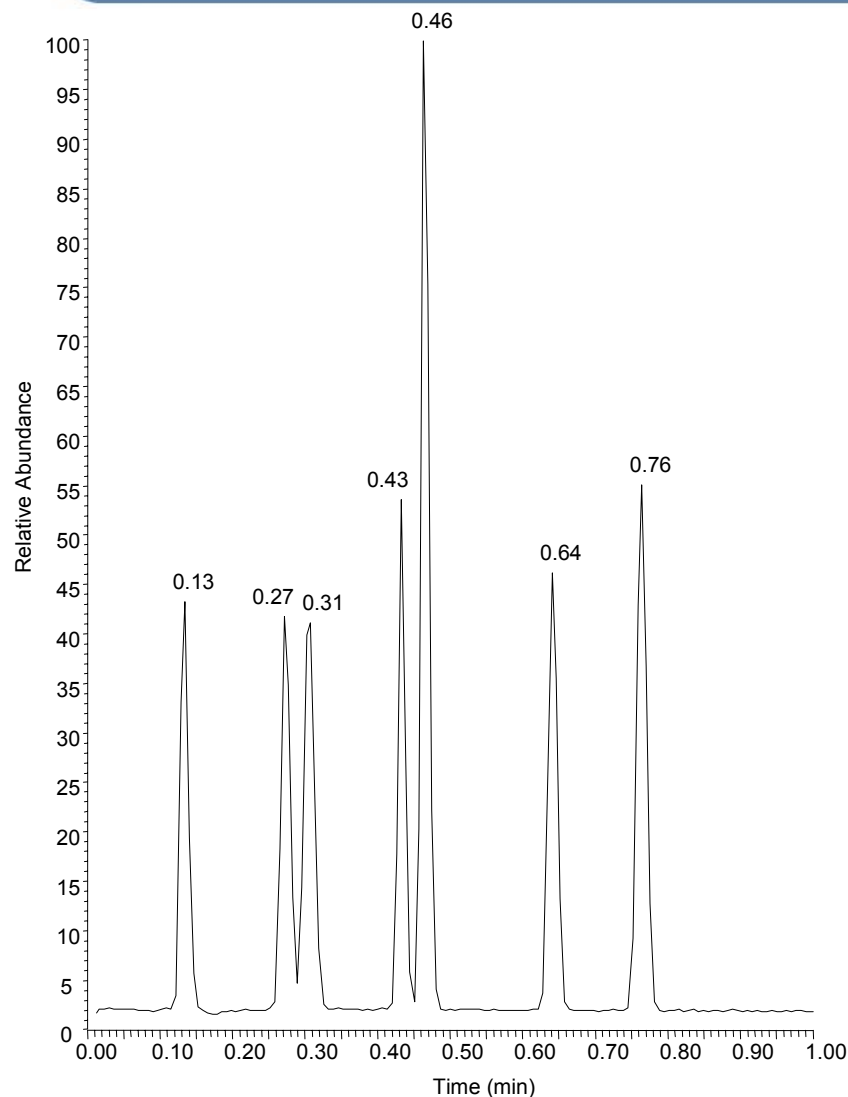
## Summary

	150x4.6mm 5 $\mu$ m	30x2.1mm 1.9 $\mu$ m
Peak width* (s)	9	4.2
	13.2	3.6
	10.2	3
	11.4	2.4
Resolution*	11.2	8.8
	5.4	2.0
	7.4	2.2
Peak height	236331	71764
	231151	139283
	220165	170483
	222985	135603

\* baseline

Cycle time reduced by 10 fold

# LC/MS analysis of $\beta$ -Blockers in under 1 minute



Column:

30 x 2.1mm 1.9  $\mu$ m Hypersil Gold

Mobile phase:

A - H<sub>2</sub>O + 0.1% HCOOH

B - MeCN + 0.1% HCOOH

Gradient: Time	%A	%B
0.00	85	15
0.10	75	25
0.50	70	30
1.00	60	40

Flow rate: 1000  $\mu$ l/min

Pressure: 310 bar

Temperature: 30  $^{\circ}$ C

Injection: 5  $\mu$ L

Detection: LTQ-Orbitrap

Resolution ( $\Delta m / m$ ): 15,000 (FWHM)

Interface: ESI +

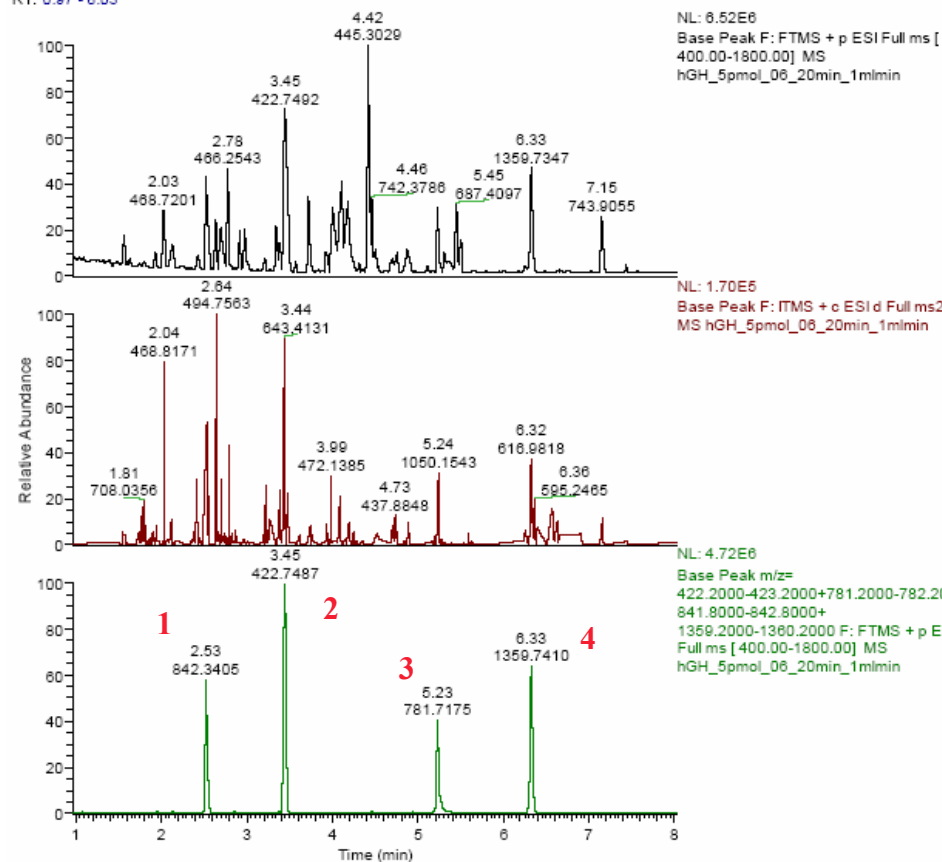
Analytes:

1. Atenolol
2. Nadolol
3. Pindolol
4. Timolol
5. Metoprolol
6. Labetalol
7. Propranolol

# Protein Digest on Accela + LTQ Orbitrap

## Run #1

RT: 0.97 - 8.03



**Peak:**            **1**            **2**            **3**            **4**

**Width:**        **1.32s**      **2.52s**      **0.84s**      **1.80s**

Run	RT (842.3)	RT (422.7)	RT (781.7)	RT (1359.7)
1	3.88	4.72	6.32	7.54
2	3.91	4.76	6.36	7.56
3	3.94	4.78	6.37	7.58
4	3.88	4.74	6.35	7.56
5	3.92	4.78	6.36	7.58
6	3.89	4.74	6.35	7.56
7	3.89	4.75	6.34	7.56
8	3.88	4.73	6.35	7.57
9	3.89	4.73	6.35	7.57
10	3.88	4.72	6.32	7.54

m	3.90	4.75	6.35	7.56
s	0.02	0.02	0.02	0.01
CV (%)	0.53	0.47	0.26	0.18

### Chromatogram:

Top: MS (400-1800)  
Middle: full MS/MS  
Down: 4 ions extracted

# Summary

- Advantages of 1.9  $\mu\text{m}$  particles
  - Higher efficiency with wider range of optimum flow rate
  - Can use shorter column length without losing efficiency
- Optimize system
  - Minimise extra column dispersion: Injector, tubing, detector
  - Increase sampling rate`
  - Minimise pump dwell time
- Optimize analysis
  - Speed: Use shorter column, higher flow rate, fast gradient for fast simple separations
  - Resolution: Use longer column with narrow ID and lower flow for more complex matrices
  - Selectivity: Use Hypersil GOLD, aQ, or PFP

[www.thermo.com/columns](http://www.thermo.com/columns)

