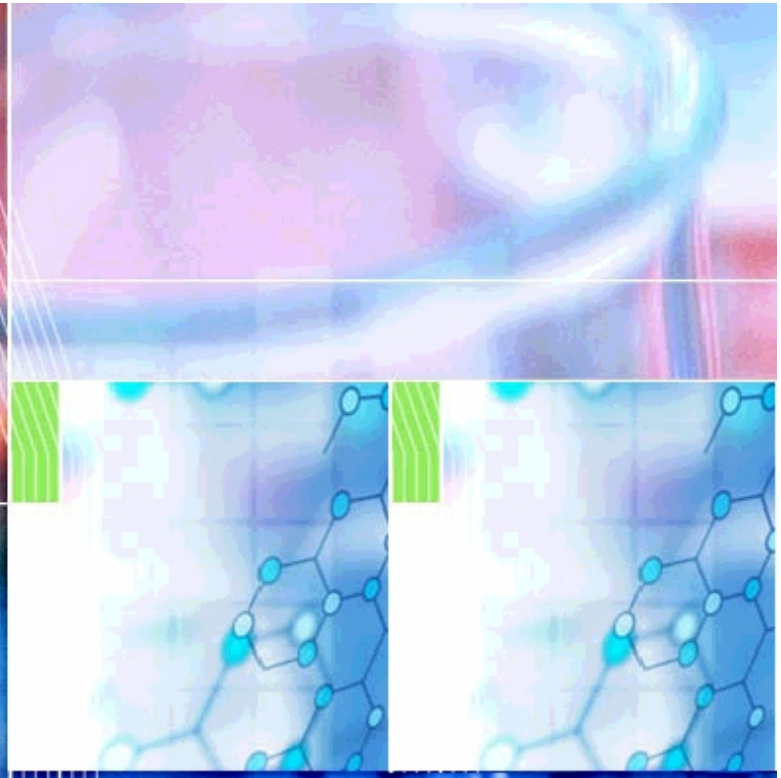
New Developments in Hybrid Particle Technology: Stability, Selectivity Separation and SPEED



Diane M. Wagrowski-Diehl, KimVan Tran, Eric Grumbach, Jon Belanger, Bob Brennick, Jeffrey R. Mazzeo, Uwe D. Neue

AAPS 2002 Toronto

Abstract

Pharmaceutical companies have been driven to bring products to market faster by increasing sample throughput. To meet these goals, this has required new chromatographic approaches which expand the limits currently imposed by silica gel based reversed-phase separations. The development of hybrid particles for HPLC columns has set new limits for speed of analysis, operating temperatures and a wider useable pH range (1-12).

Because of the mandate to run faster separations, researchers have tried to move away from hour-long separations on 15 or 25 cm columns. Many have turned to the monolith technology due to claims of reductions in separation time and increased sample throughput. However, running at such high flow rates (up to 10 mL/min) increases solvent consumption dramatically. Smaller i.d. monoliths have been promised, but are not yet available. Additionally, monolith technology is not yet available in preparative dimensions which creates a barrier to direct scale-up possibilities.

New column hardware in 4.6 x 20 mm dimensions has been developed and packed with 2.5 µm and 3.5 µm XTerra® MS C_{18} particles. We have measured the peak capacities of these columns and found them to be comparable to results obtained with the monolith columns. We have developed several applications with total run times of 5 minutes or less, using reasonable flow rates. We will show how an application run on a long column can be transferred to these shorter columns resulting in optimum chromatographic performance in a much shorter run time. We also show 1000 injections of a protein precipitation sample on a 4.6 x 20 mm column packed with 2.5 µm particles.

Organic vs. Inorganic Packings

	Advantages	Disadvantages
Inorganic (C_{18} -Silica)	**Mechanically strong** **High efficiency**	**Tailing peaks for bases** **Limited pH range**
Organic (Poly-styrene)	**Wide pH range** **No tailing for bases**	**Low efficiency** **Mechanically weak**

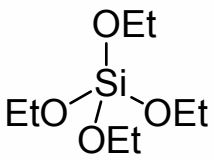
Hybrid Organic/Inorganic Technology:

Combining the Best of Inorganic and Organic Particles

Hybrid Packing Material

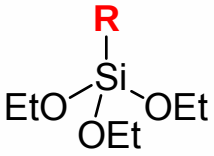
- Hybrid packing materials bring new capabilities to HPLC
- Classical surface chemistry with high-pH and higher temperature stability than silica-gel based columns
  - Provides new selectivity options in method development
  - Provides options for faster separations
  - Basic compounds in non-ionic form give improved peak shape
  - Basic compounds in non-ionic form give high retention
  - Basic compounds in non-ionic form high preparative loadability
- Lower silanol content than classical silica-based packings
  - Less tailing for basic compounds
  - Simpler retention mechanism

Synthesis of Hybrid Particles

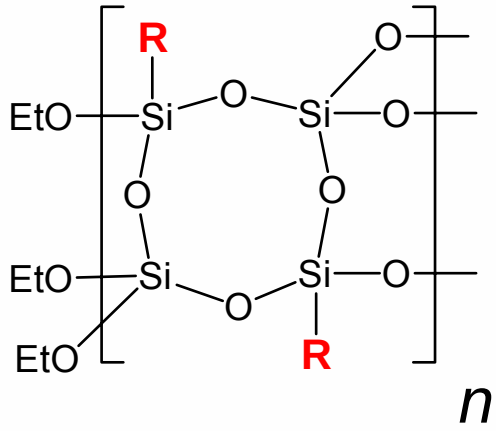


Tetraethoxysilane

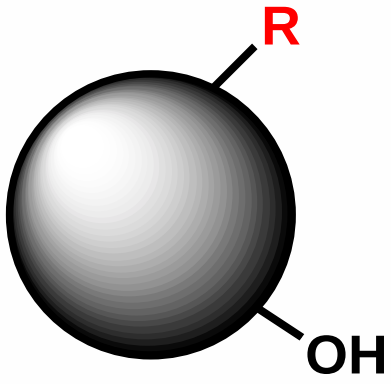
+



Organofunctional triethoxysilane



Polyethoxyoligosiloxane Polymer



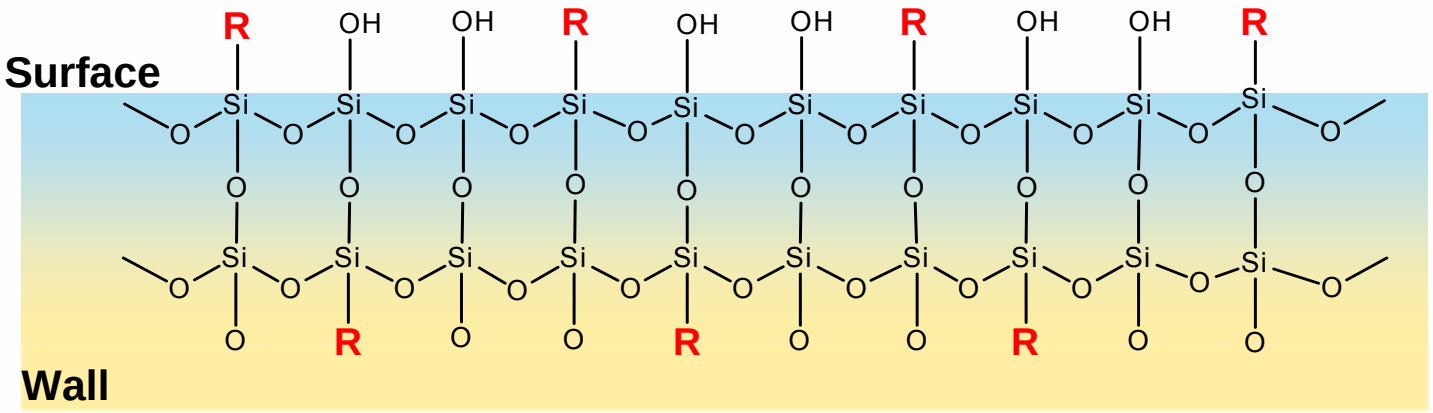
Porous Hybrid Particles

SA	=	140 – 330 m^2/g
TPV	=	0.4 – 1.0 cc/g
MPD	=	90 – 300 Å

- First generation Hybrid, **R** = CH_3
- Characterized by %C, SEM, TGA, BET, NMR

Patent Pending

First Generation Hybrid: R = CH_3



Methyl Hybrid Attribute	RP-HPLC Consequence
Surface CH_3 groups reduce surface silanol concentration and the pKa of surface silanols	Reduced USP peak tailing factors
Internal CH_3 groups provide hydrophobicity	Increased base stability of column

Bonded methyl hybrid packings show significantly increased base stability vs. bonded silica packings!

We can use these particles to achieve FAST separations…

Achieving Faster Separations

- To help decrease sample run times, we know that we can move to shorter length columns run at higher flow rates
  - Many chemists have already moved from 25 or 30 cm columns to 10 and 15 cm columns to decrease run times
- In recent years, fast separations (under 5 min) have been developed on the monolith column technology, but at extremely high (i.e. 10 mL/min) flow rates
  - Many researches seem to believe that fast separations can only be performed on silica-rod technology

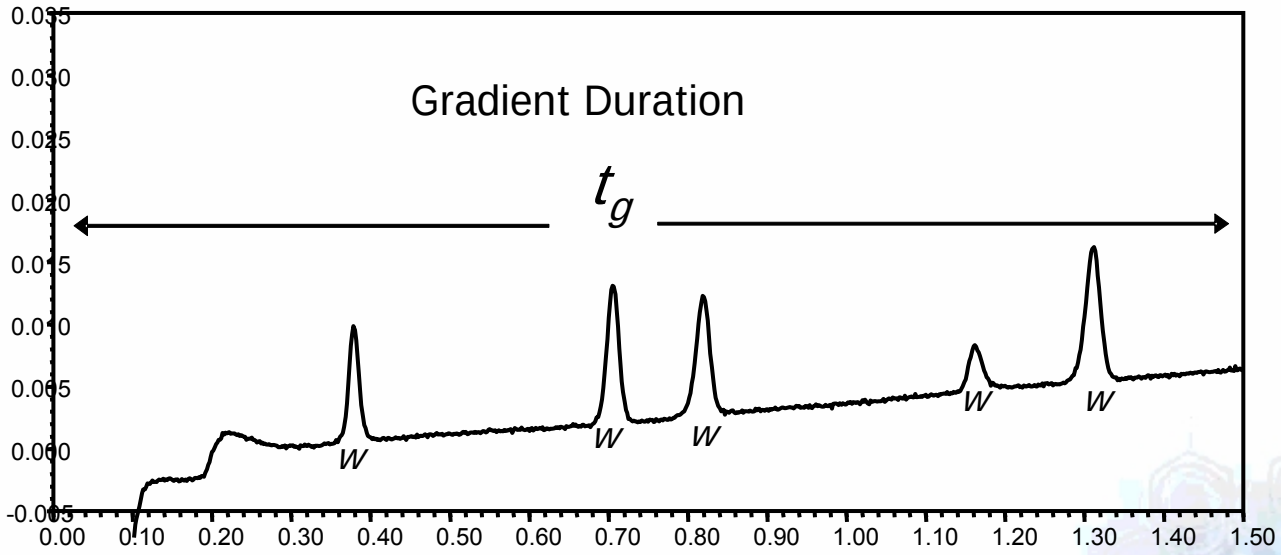
This is a misconception!

- The development of 4.6 x 20 mm **column** hardware can also give us shorter run times on columns packed with spherical particles.

Peak Capacity (*P*) in Gradients

Number of peaks (*P*) with a peak width (*w*)
that can be separated in a given Gradient Time (t_g)

$$P = 1 + \frac{t_g}{w}$$



We can use Peak Capacity to compare column performance.

Chromatographic Conditions for Measuring Peak Capacities

Conditions

Columns: XTerra® MS C_{18}, 4.6 x 20 mm, 2.5 µm
XTerra® MS C_{18}, 4.6 x 20 mm, 3.5 µm
Monolith Column, 4.6 x 50 mm
Mobile Phase A: H_2O
Mobile Phase B: ACN
Mobile Phase C: 100 mM $NH_4^+CH_3COO^-$, pH 5.0
Flow Rates: As Indicated
Gradient:

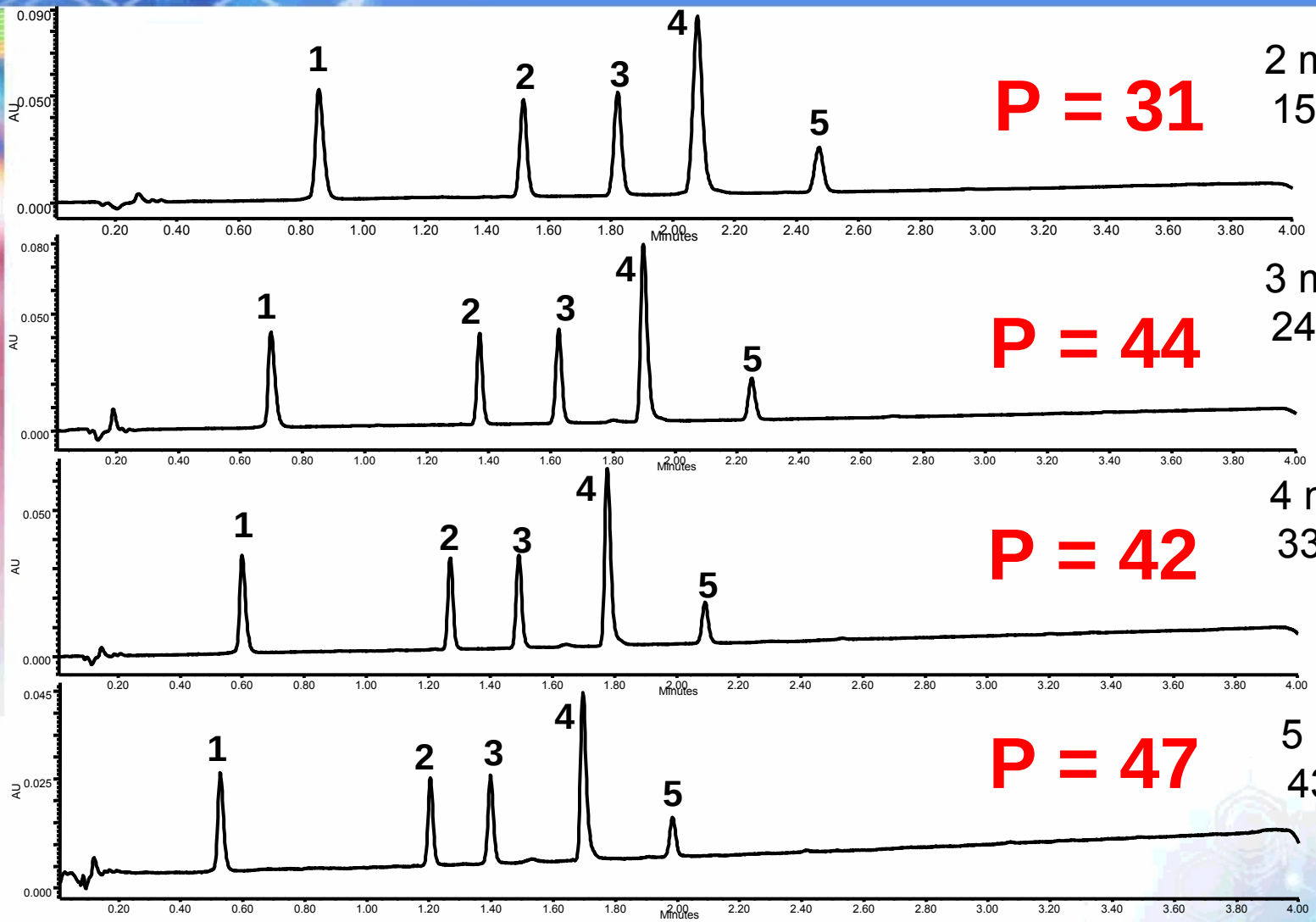
Time (min)	%A	%B	%C
0	85	5	10
4	20	70	10

Injection Volume: 20.0 µL
Temperature: Ambient
Detection: UV @ 238 nm
Instrument: Alliance™ 2695 with 2996 PDA

Analytes

1. Lidocaine (40 µg/mL)
2. Prednisolone (10 µg/mL)
3. Naproxen (3 µg/mL)
4. Amitriptyline (10 µg/mL)
5. Ibuprofen (40 µg/mL)

Peak Capacities for XTerra® MS C_{18} 4.6 x 20 mm, 2.5 µm



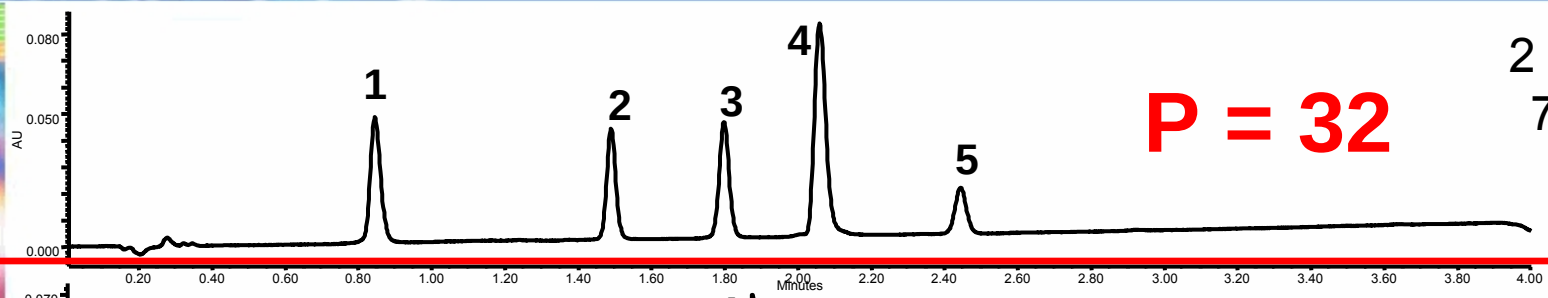
P = 31 — 2 mL/min, 1550 psi

P = 44 — 3 mL/min, 2400 psi

P = 42 — 4 mL/min, 3350 psi

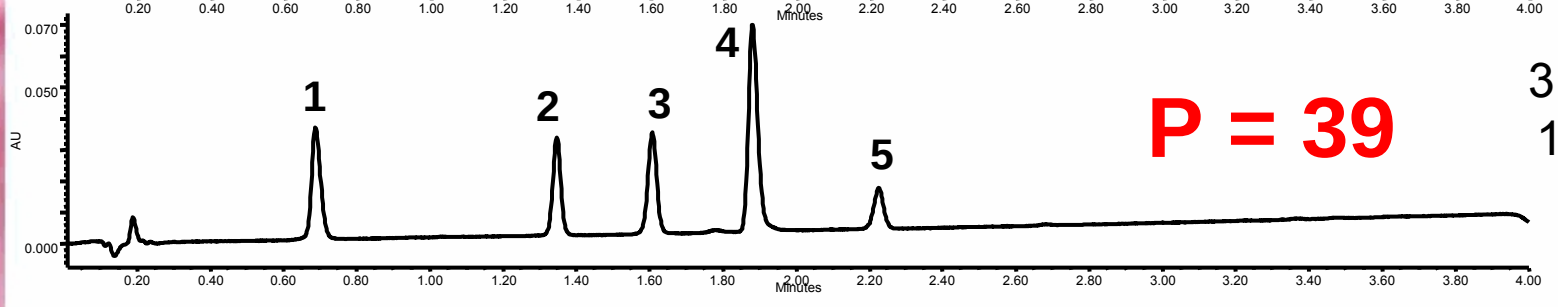
P = 47 — 5 mL/min, 4330 psi

Peak Capacities for XTerra® MS C_{18} 4.6 x 20 mm, 3.5 µm



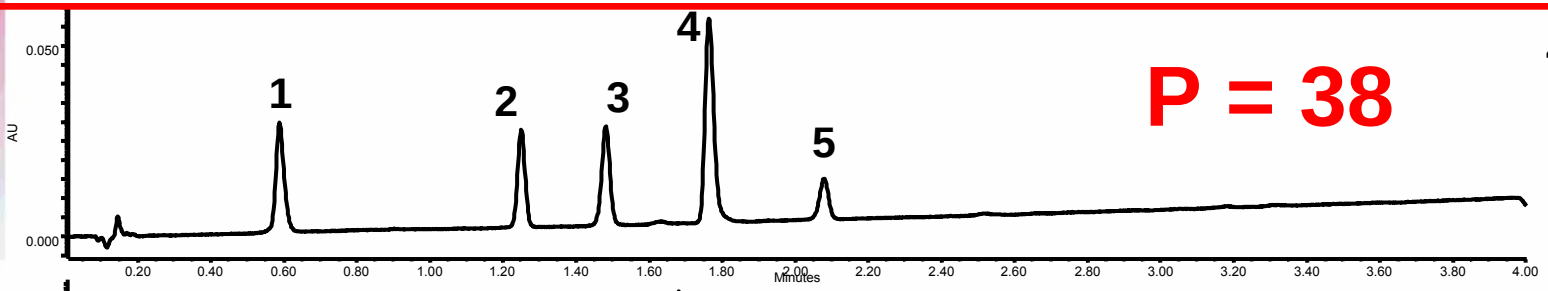
P = 32

2 mL/min
790 psi



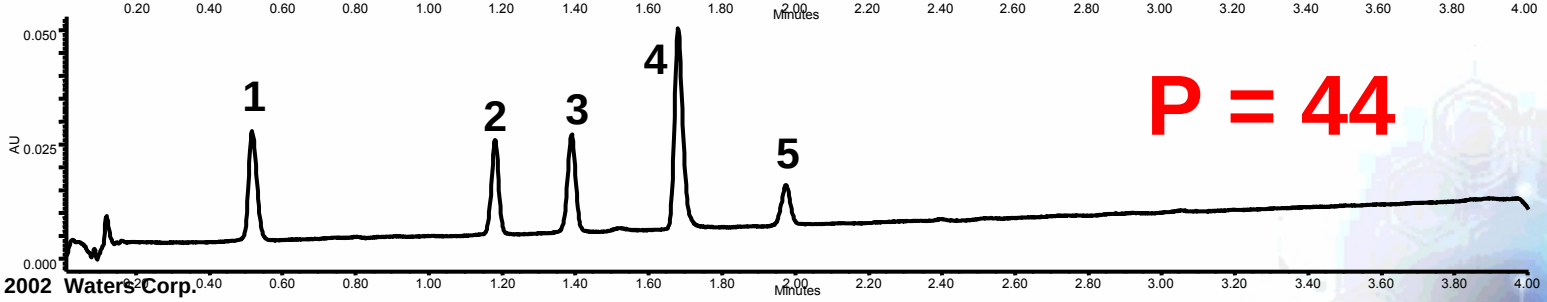
P = 39

3 mL/min
1200 psi



P = 38

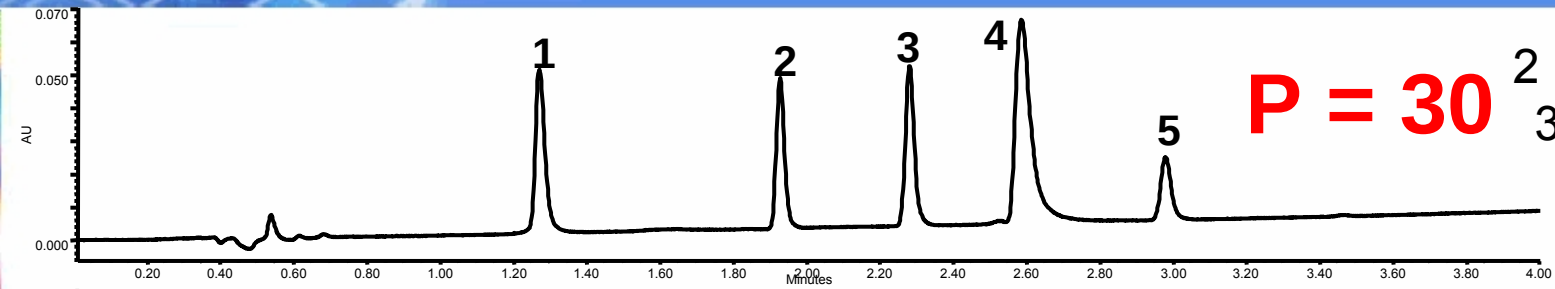
4 mL/min
1650 psi



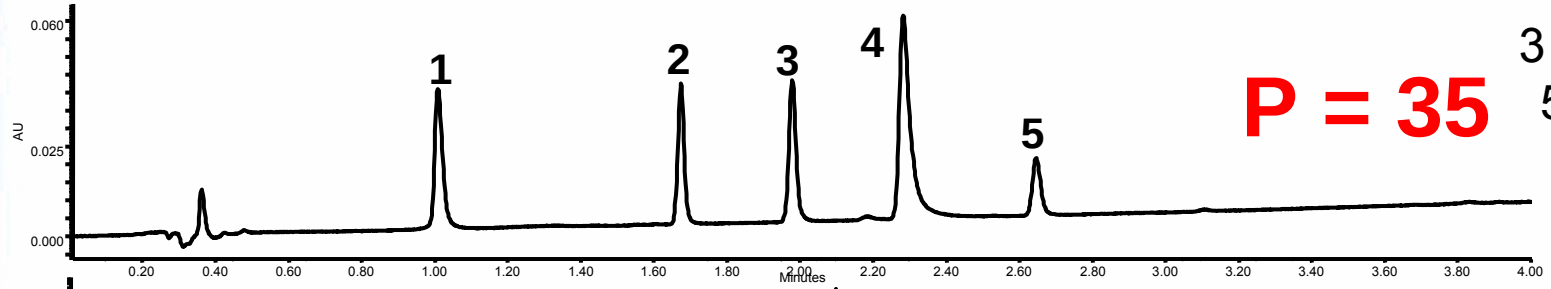
P = 44

5 mL/min
2100 psi

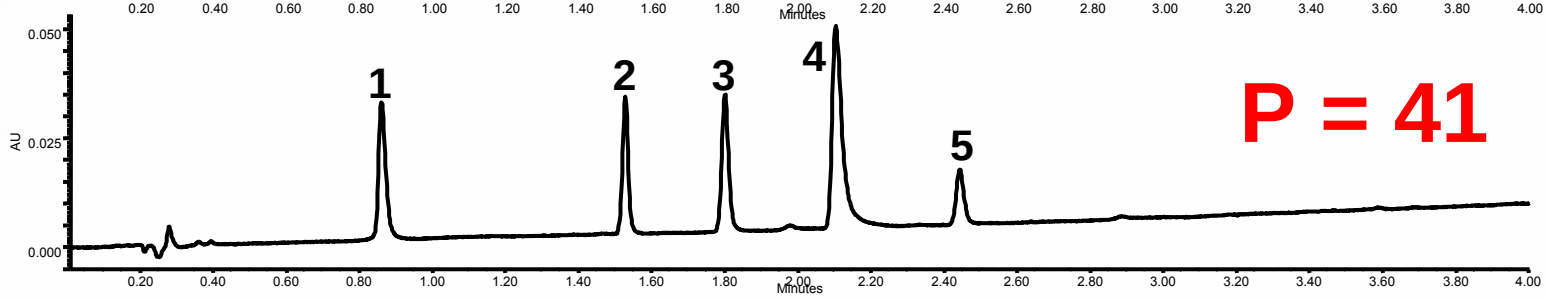
Peak Capacities for Monolith Technology



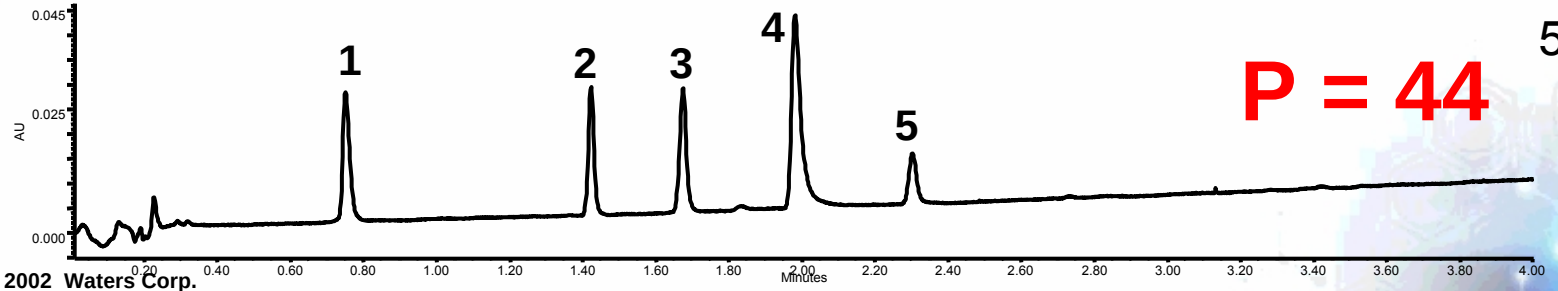
P = 30 2 mL/min 345 psi



P = 35 3 mL/min 520 psi



P = 41 4 mL/min 700 psi



P = 44 5 mL/min 865 psi

Typical Reversed-Phase Separation on a 4.6 x 150 mm Column

Conditions
Column: **XTerra® MS $C_{18,}$ 4.6 x 150 mm, 5.0 µm**
Mobile Phase A: Water
Mobile Phase B: Acetonitrile
Mobile Phase C: 100 mM $NH_4HCO_{3,}$ pH 10
Flow Rate: 1.4 mL /min

Gradient:

Time (min)	Profile %A	%B	%C
0	90	0	10
50	60	30	10

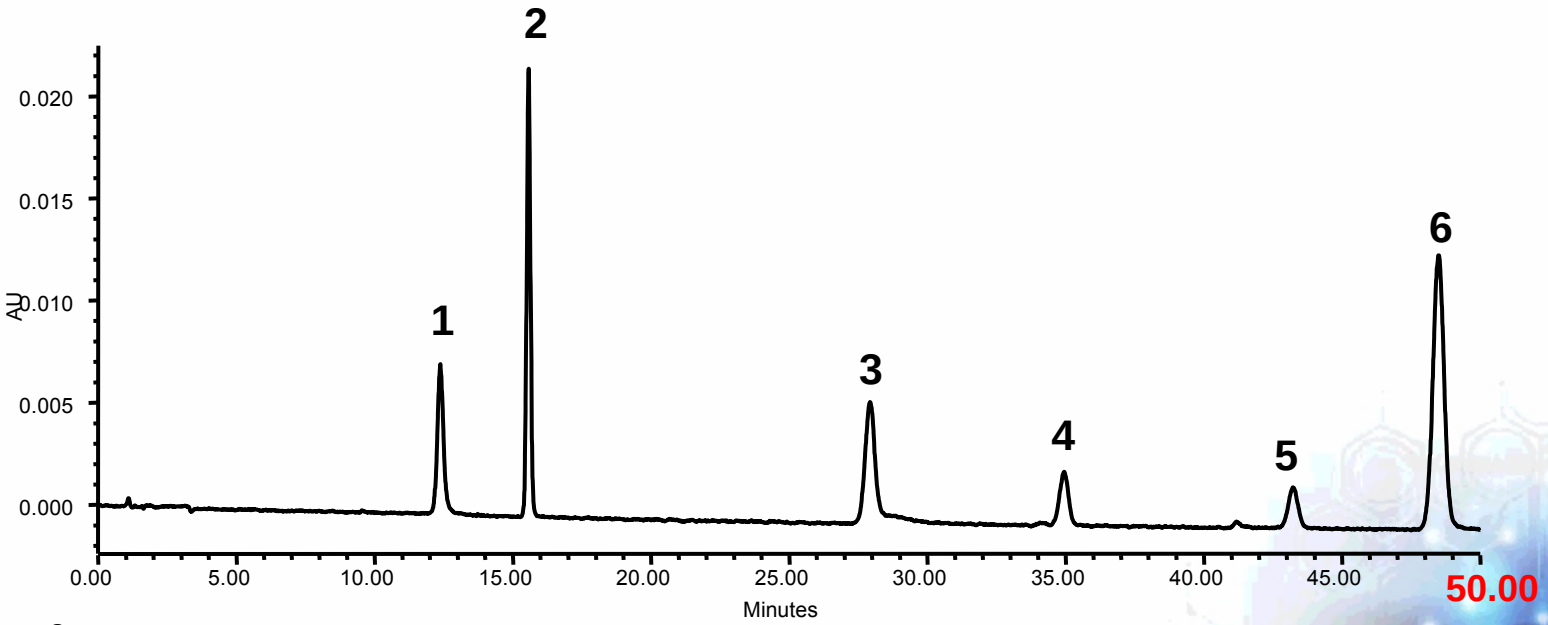
Injection Volume: 10.0 µL
Sample concentration: 20 µg/mL
Temperature: 30 °C
Detection: UV @ 254 nm
Instrument: Alliance™ 2795 w/996 PDA

1. Caffeine
2. Aniline
3. N-Methylaniline
4. 2-Ethylaniline
5. 4-Nitroanisole
6. N-N-Dimethylaniline

P = 52
PSI = 1575

Six peaks fully resolved in almost ONE HOUR.

How can we improve our sample throughput?



Step One: First Pass at a Gradient Run on a 4.6 x 20 mm Column

Conditions
Column: **XTerra® MS $C_{18,}$ 4.6 x 20 mm, 3.5 µm**
Mobile Phase A: Water
Mobile Phase B: Acetonitrile
Mobile Phase C: 100 mM $NH_4HCO_{3,}$ pH 10
Flow Rate: **3.0 mL/min**

Gradient:	Time (min)	Profile %A	%B	%C
	0	90	0	10
	7	60	30	10

Injection Volume: 10.0 µL
Sample concentration: 20 µg/mL
Temperature: 30 °C
Detection: UV @ 254 nm

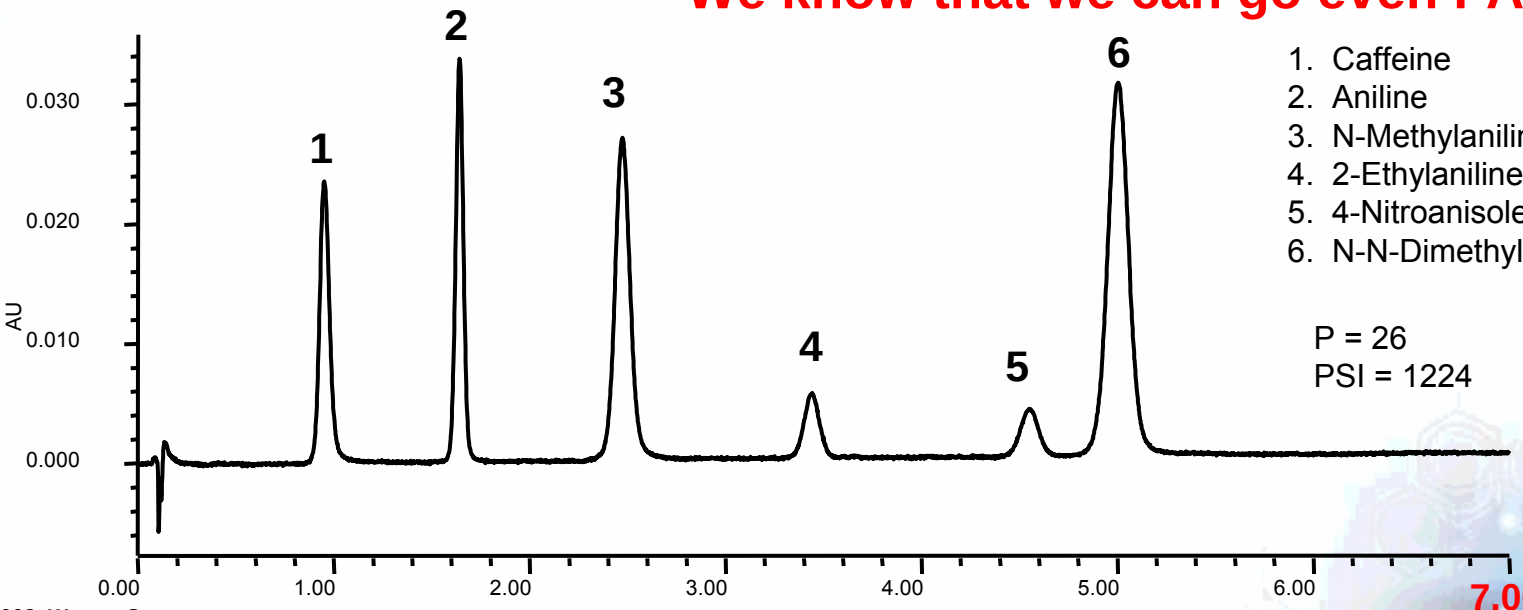
Ratio of column length to gradient time:

150 mm/ 50 min = 3

Try: 20 mm/ 7 min = 2.8

Results: Total run time of 7 min – much better than the 50 minutes!

We know that we can go even FASTER.



1. Caffeine
2. Aniline
3. N-Methylaniline
4. 2-Ethylaniline
5. 4-Nitroanisole
6. N-N-Dimethylaniline

P = 26
PSI = 1224

Step Two: Reduce Gradient Time to 4 Minutes

Conditions

Column: **XTerra® MS $C_{18,}$ 3.5 µm 4.6 x 20 mm, 3.5 µm**

Mobile Phase A: Water

Mobile Phase B: Acetonitrile

Mobile Phase C: 100 mM NH_4HCO_3 pH 10

Flow Rate: 3.0 mL/min

Gradient:

Time (min)	Profile %A	%B	%C
0	90	0	10
4	60	30	10

Injection Volume: 10.0 µL

Sample concentration: 20 µg/mL

Temperature: 30 °C

Detection: UV @ 254 nm

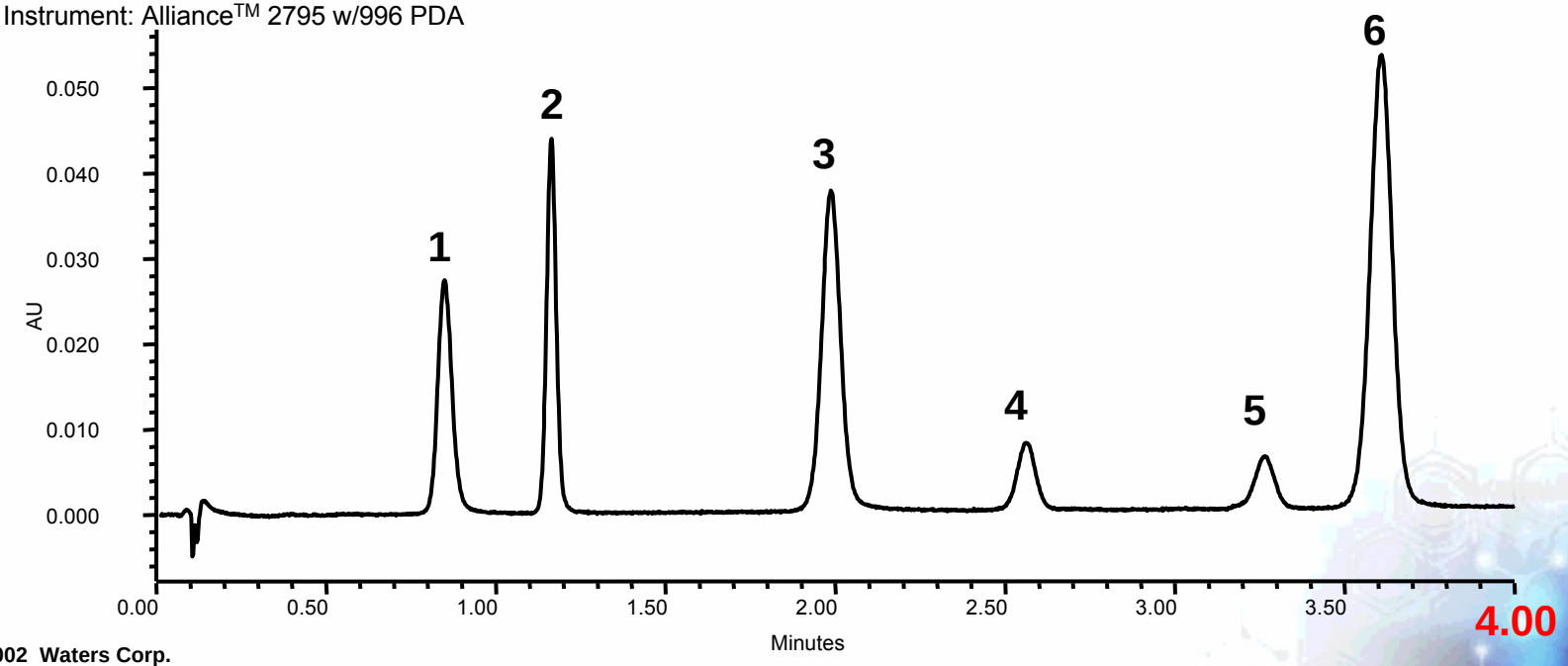
Instrument: Alliance™ 2795 w/996 PDA

Analytes

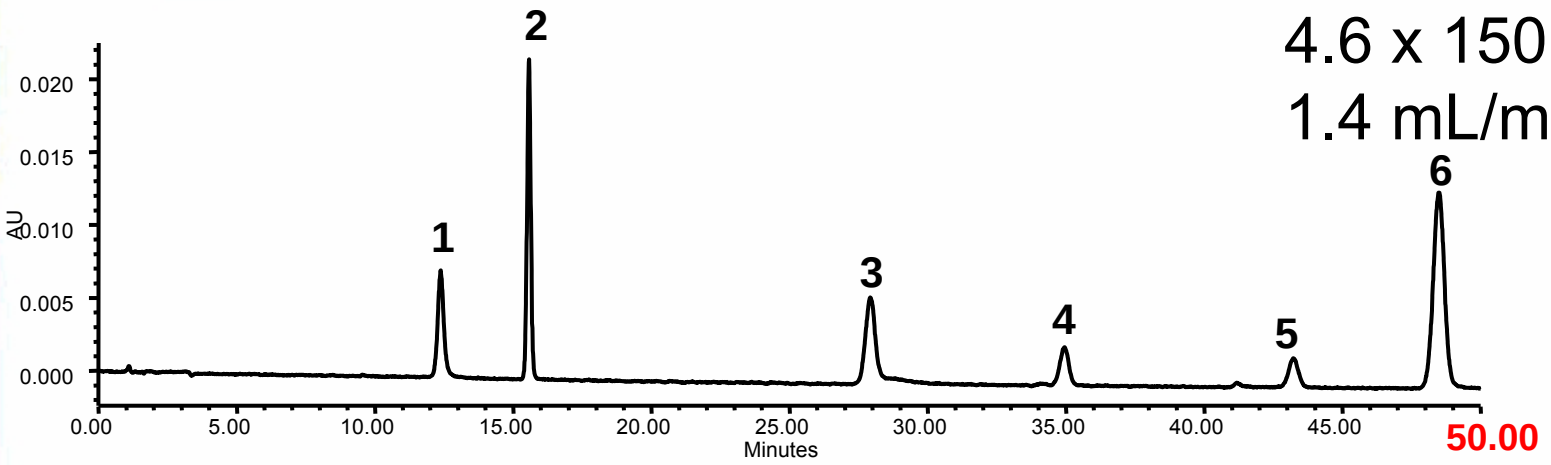
1. Caffeine
2. Aniline
3. N-Methylaniline
4. 2-Ethylaniline
5. 4-Nitroanisole
6. N-N-Dimethylaniline

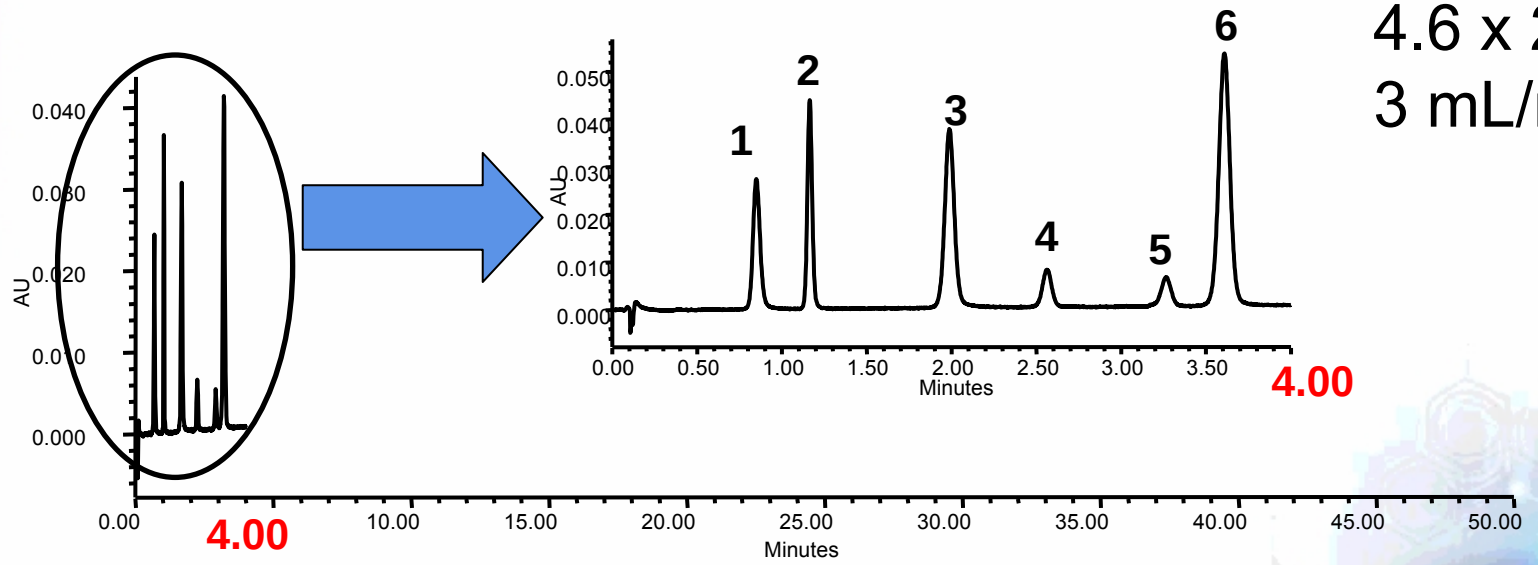
P = 23

PSI = 1235



Reduction in Total Run Time by a Factor of 12!





Data Comparison: Monolith vs. XTerra® MS C_{18} 3.5 µm

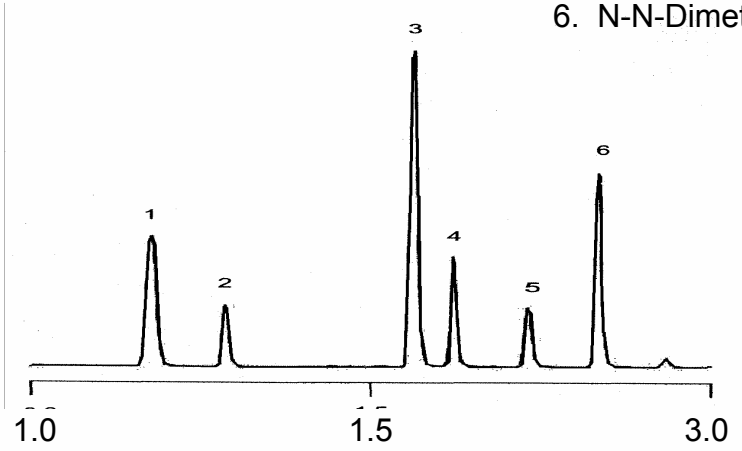
XTerra® at pH 10 makes it possible to separate 6 analytes in 4 minutes and operate at a lower flow rate.

Conditions
Column: Monolith, 4.6 x 100 mm
Mobile Phase A: 20 mM Phosphate buffer pH 7.7
Mobile Phase B: Acetonitrile
Flow Rate: **7.0 mL/min**

Gradient:	Time (min)	Profile %A	%B
	0	90	10
	2.5	40	60
	3.0	40	60

Injection Volume: 10.0 µL
Temperature: Ambient
Detection: UV @ 254 nm

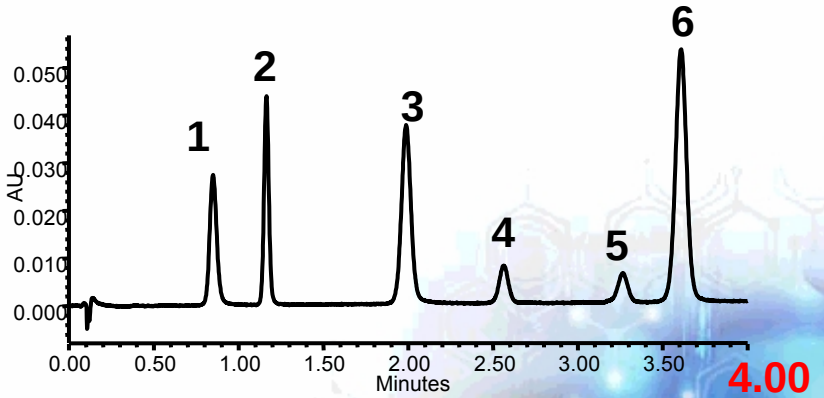
1. Caffeine
2. Aniline
3. N-Methylaniline
4. 2-Ethylaniline
5. 4-Nitroanisole
6. N-N-Dimethylaniline



Conditions
Column: **XTerra® MS $C_{18,}$ 3.5 µm 4.6 x 20 mm, 3.5 µm**
Mobile Phase A: Water
Mobile Phase B: Acetonitrile
Mobile Phase C: 100 mM NH_4HCO_3 pH 10
Flow Rate: **3.0 mL/min**

Gradient:	Time (min)	Profile %A	%B	%C
	0	90	0	10
	4	60	30	10

Injection Volume: 10.0 µL
Sample concentration: 20 µg/mL
Temperature: 30 °C
Detection: UV @ 254 nm



http://www.merck.de/english/services/chromatographie/hplc/chromolith/pdf/appl_05.pdf

Application: Separation of 9 Analytes in 4 Minutes on a 4.6 x 20 mm Column

Conditions

Column: XTerra® MS $C_{18,}$ 4.6 x 20 mm, 3.5 µm
Mobile Phase A: 0.1% Formic Acid in Water
Mobile Phase B: 0.1% Formic Acid in ACN
Flow Rate: 3.0 mL/min
Gradient:

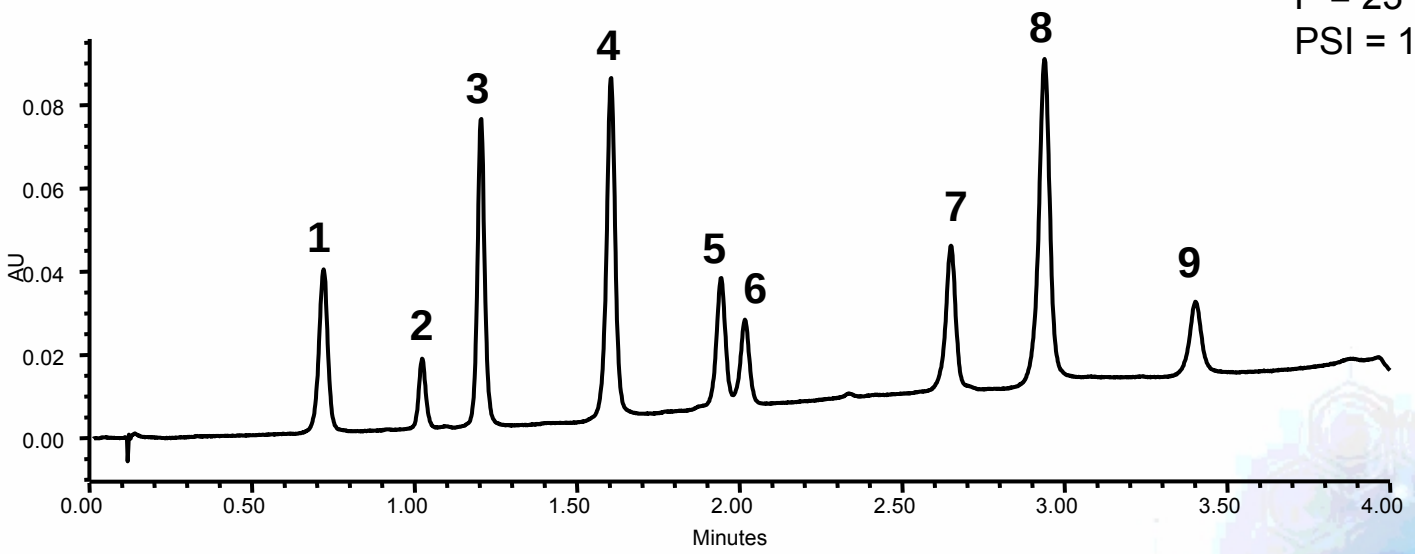
Time (min)	Profile %A	%B
0	100	0
4	0	100

Injection Volume: 10.0 µL
Sample concentration: 10 µg/mL
Temperature: 30 °C
Detection: UV @ 254 nm
Instrument: Alliance™ 2795 w/996 PDA

Compounds

1. Acetanilide
2. Triamcinolone
3. Hydrocortisone
4. 2-Amino-7-chloro-5-oxo-5H-[1]benzopyrano[2,3-b]pyridine-3-carbonitrile
5. 6α-Methyl-17α-hydroxyprogesterone
6. 3-Aminofluoranthene
7. 2-Bromofluorene
8. Perylene
9. Naphtho(2,3-a)pyrene

P = 23
PSI = 1440



Application: Sulfonamides

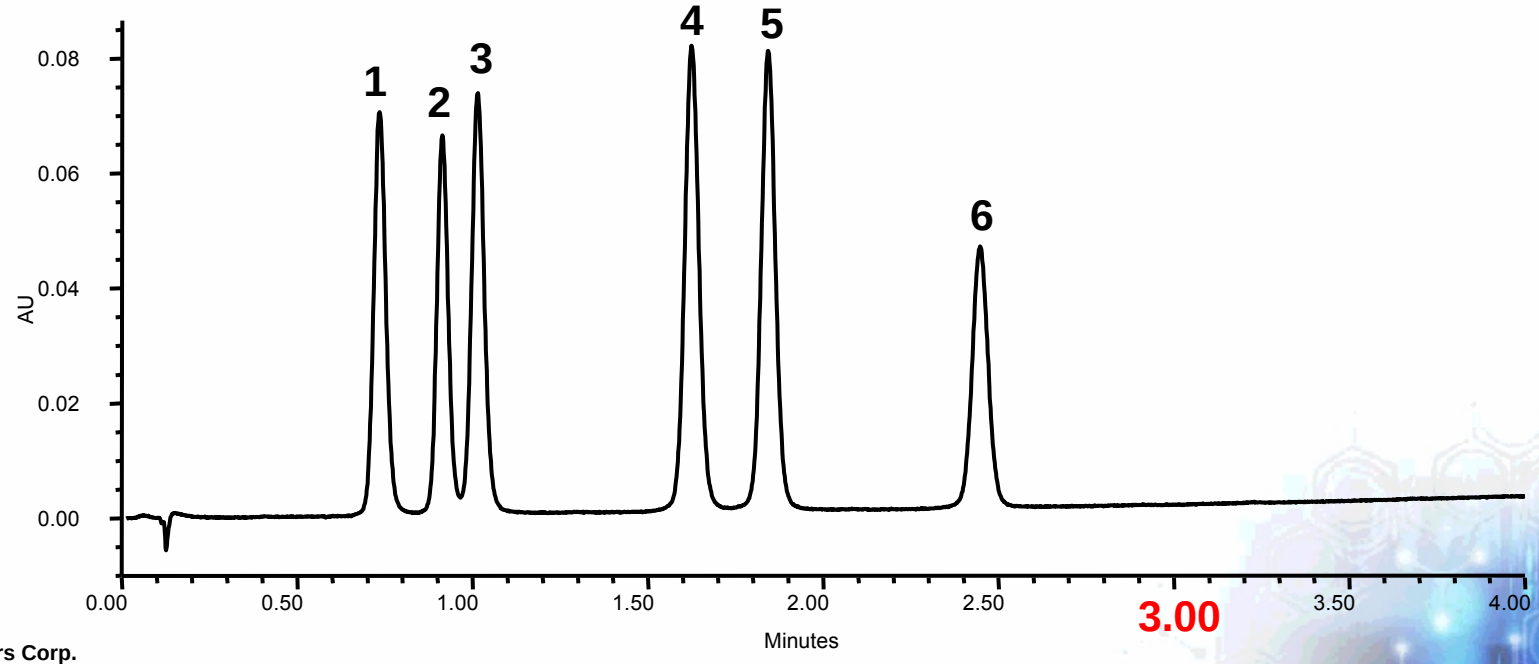
Conditions
Column: XTerra™ MS $C_{18,}$ 4.6 x 20 mm, 3.5 µm
Mobile Phase A: 0.1.% FA in Water
Mobile Phase B: 0.1 % FA in MeOH
Flow Rate: 3.0 mL/min
Gradient:

Time (min)	Profile %A	%B
0	100	0
4	50	50

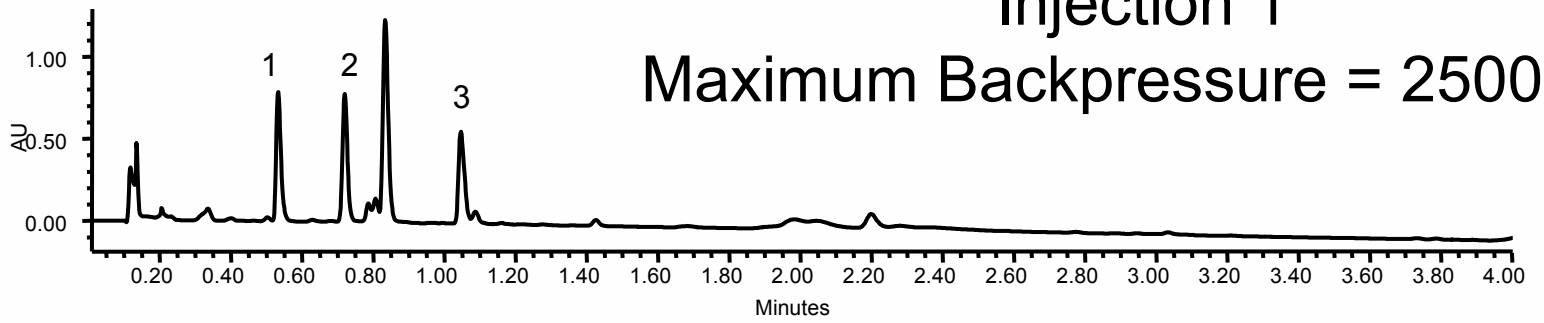
Injection Volume: 10.0 µL
Sample concentration: 20 µg/mL
Temperature: 30 °C
Detection: UV @ 254 nm
Instrument: Alliance™ 2795 w/996 PDA

Analytes
1. Sulfadiazine
2. Sulfathiazole
3. Sulfamerazine
4. Sulfamethoxazole
5. Sulfisoxazole
6. Sulfadimethoxine

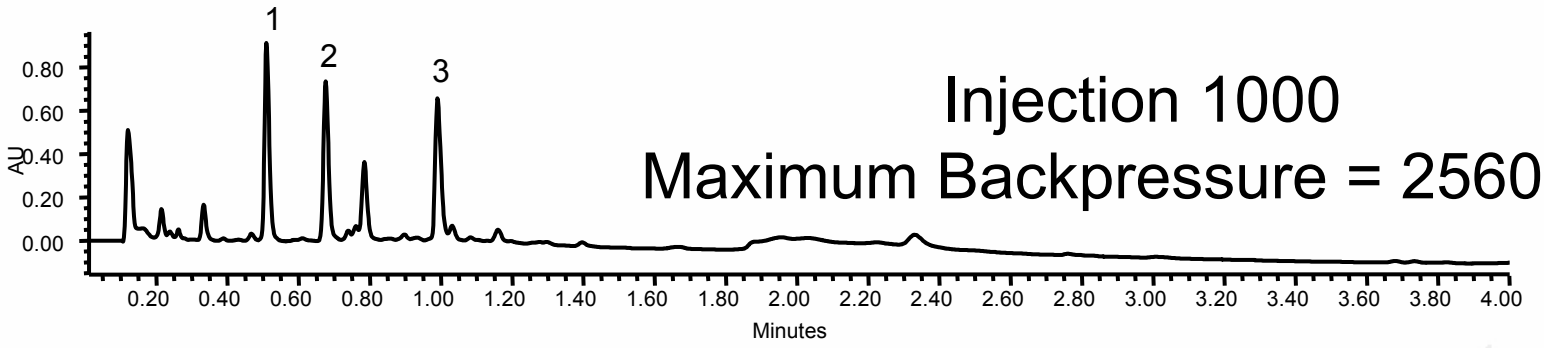
P = 23
PSI = 2045



Column Lifetime: Protein Precipitation Samples



Injection 1
Maximum Backpressure = 2500 psi



Injection 1000
Maximum Backpressure = 2560 psi

1000 injections on a 2.5 µm particle column with only a slight increase in backpressure!

Protein Precipitation Sample Preparation and HPLC Conditions

Protein Precipitation Procedure:

Analytes*: atenolol (10 mg/mL in MeOH)
pindolol (5 mg/mL in MeOH/H_2O)
metoprolol (10 mg/mL in MeOH)

*prepared in strong conc. to spike into plasma

Spiked Plasma Sample:

50 µL of atenolol
50 µL of metoprolol
50 µL of pindolol
4750 µL of Rat Plasma
100 µL of H_3PO_4
5000 µL total sample volume

Protein Precipitation:

-Multiple samples prepared from 5000 µL spiked plasma
350 µL of Spiked Plasma sample
1000 µL of acetonitrile
1350 µL total sample volume
-Samples were centrifuged at 3000 RPM for 30 minutes.
-Supernatent transferred to culture tube and evaporated.
-Sample reconstituted in 350 µL water and injected.

Conditions

Column: XTerra® MS C_{18}, 4.6 x 20 mm, 2.5 µm
Mobile Phase A: 0.1% TFA in H_2O
Mobile Phase B: ACN
Flow Rate: 3.0 mL/min
Gradient:

Time (min)	Profile %A	%B
0	100	0
4	20	80

Injection Volume: 20.0 µL
Sample Concentrations: 0.1 mg/mL of atenolol,
0.05 mg/mL of pindolol
0.1 mg/mL of metoprolol
Temperature: Ambient
Detection: UV @ 220 nm
Instrument: Alliance™ 2695 w/2996 PDA

Peaks

1. Atenolol
2. Pindolol
3. Metoprolol

Conclusions

- Many different hybrid organic/inorganic packing materials can be designed for HPLC
- C_{18}-bonded hybrid packing materials show:
  - Low silanol activity = improved peak shape compared to the best silica-based packings
  - Improvements in high-pH stability compared to silica-based materials
- These new 4.6 x 20 mm columns packed with XTerra® offer a better advantage over the monolith technologies by providing:
  - Better peak shapes for basic analytes
  - Ability to run at high pH and high temperature
  - Available in MS C18, MS C8, RP18 and RP8 chemistries and
  - Availability of Preparative dimensions for direct scale-up
  - Lower flow rates allow for less solvent waste and easier split into a mass spectrometer without a loss in sensitivity
- Chemists do not need the monolith technology
  - To achieve faster separations
  - To run 100's of protein precipitation samples
    - This can all be accomplished on the new 4.6 x 20 mm columns