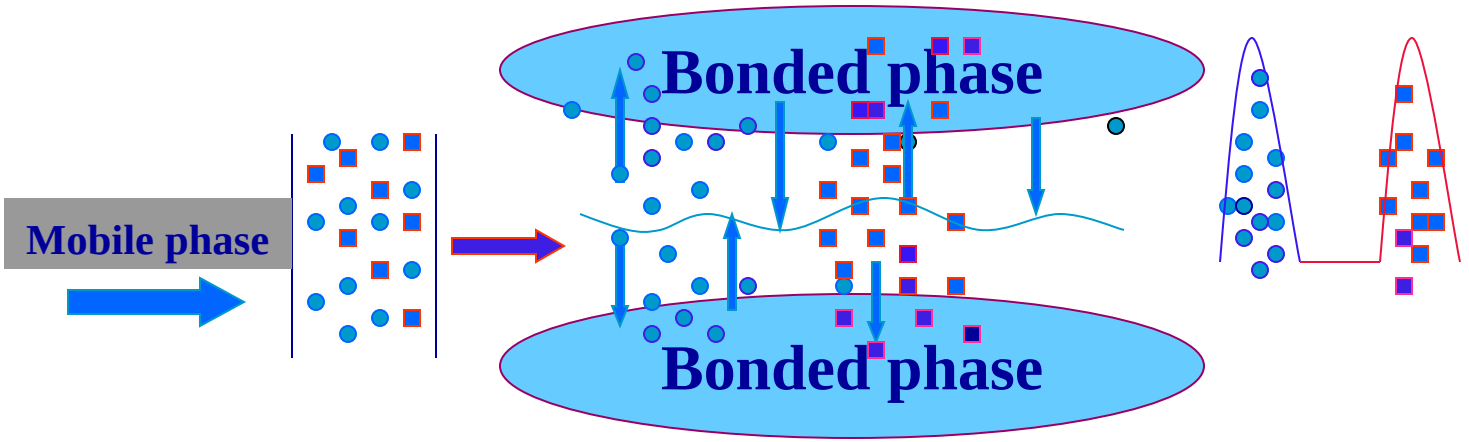
Improving HPLC Speed and Resolution Utilizing Small Particles and Enhanced Bonded Phase Technology

Agilent Technologies, Inc.

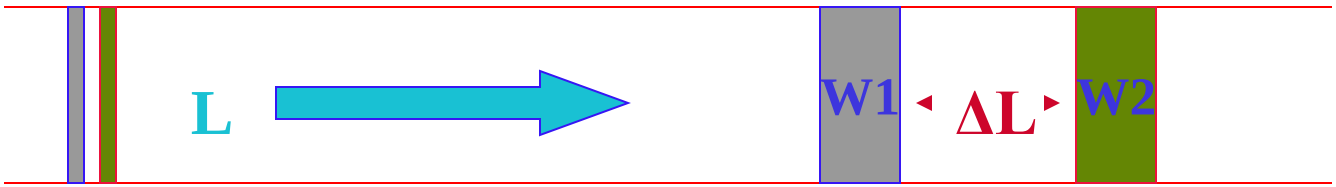
The Separation Process

- Differential partitioning of the components into the stationary and mobile phases.
- Separation controlled by chemical interaction of mobile phase/sample/bonded phase

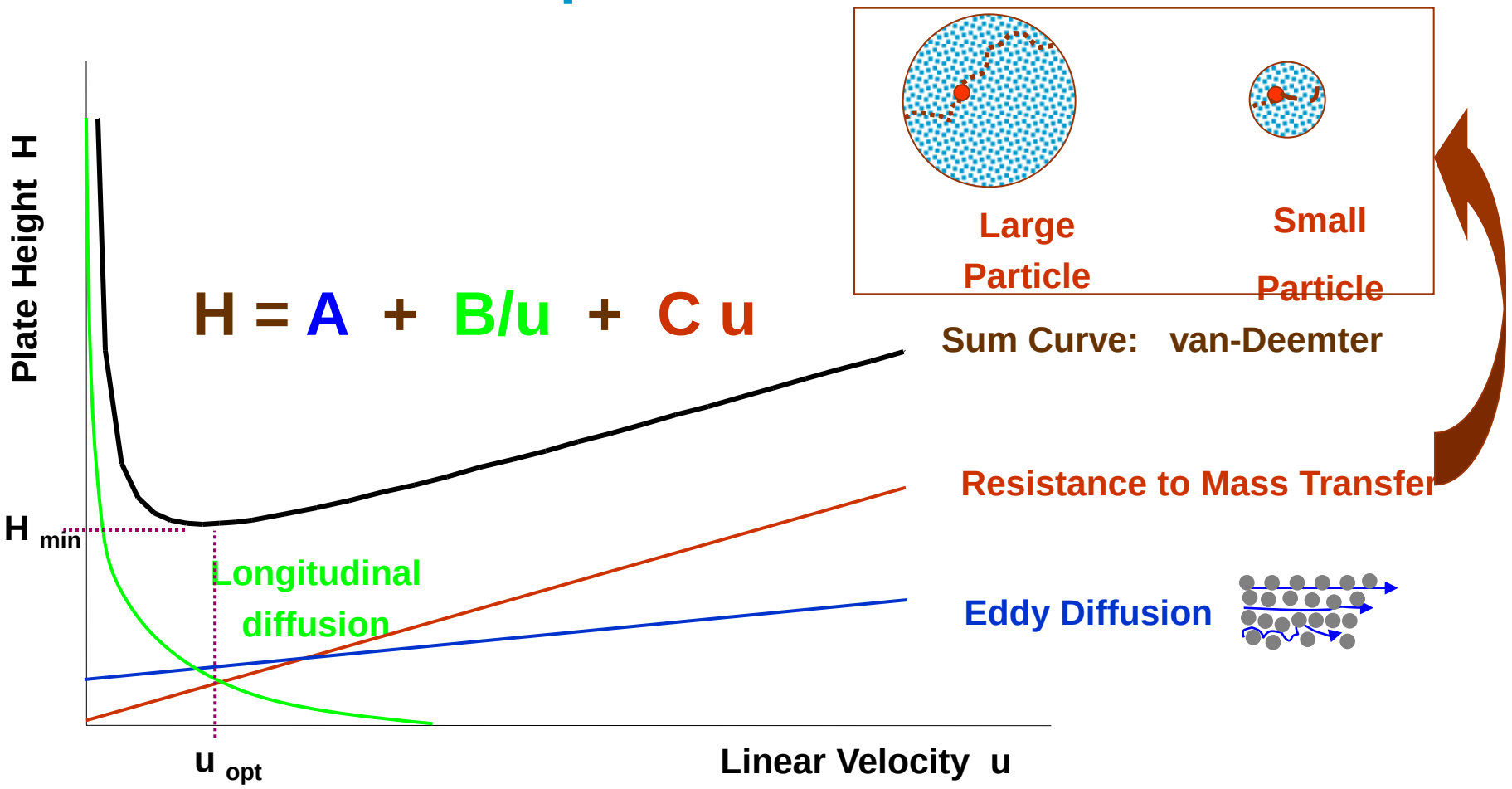


Separation Mechanism

- As Bands Travel the Length of the Column the Respective Distance (ΔL) Increases
- As Bands Move Through the Column Dispersion (while in the Bonded Phase and Mobile Phase) Causes the Band Width to Increase
- Choosing a High Efficiency Short Column Minimizes On-Column Dispersion and Improves Resolution

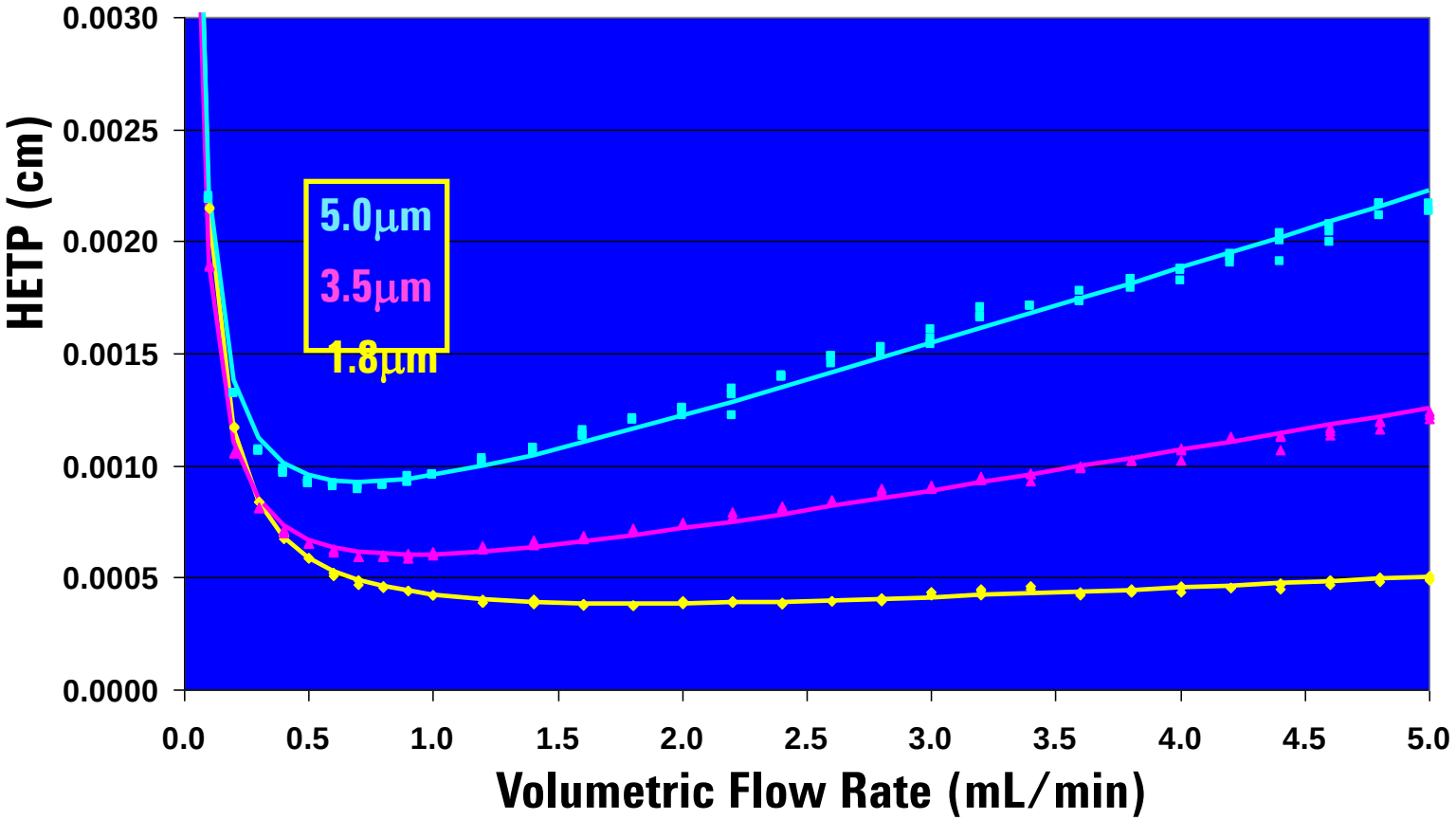


Smaller Particles Improve HETP by Minimizing Dispersion Effects



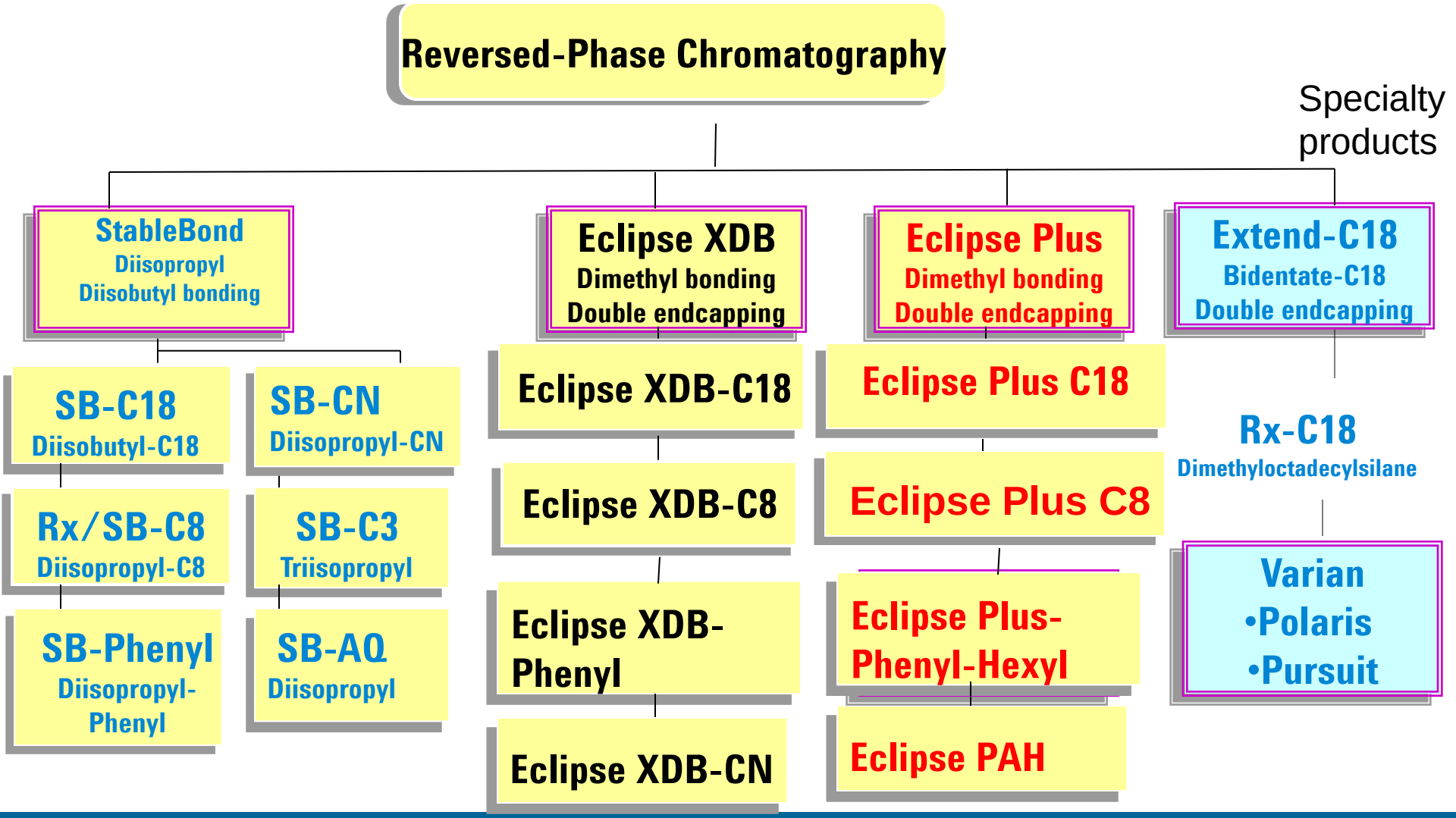
The smaller the plate height, the higher the plate number and the greater the chromatographic resolution

Smaller Particles Maintain Efficiency Over Wider Flow Rate Ranges



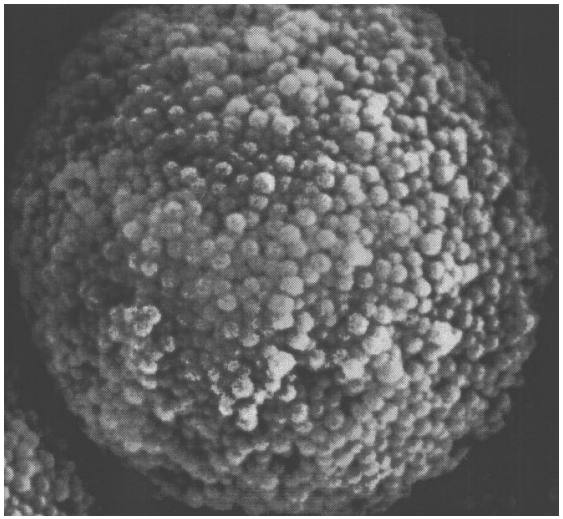
Smaller particle sizes yield flatter curves, minima shift to higher flow rates

Agilent Portfolio of Column Types for RP Chromatography – Eclipse Plus is Newest Family

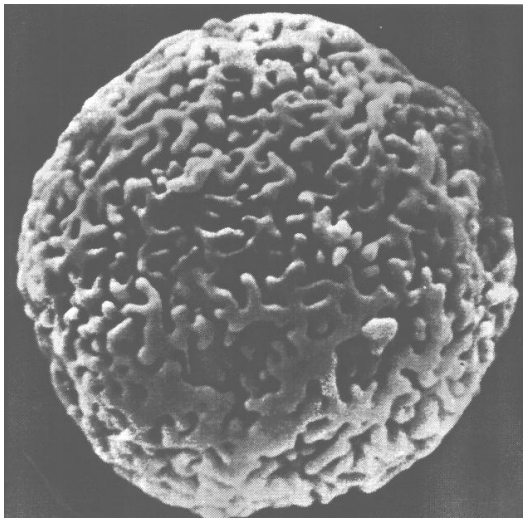


Silica Particles Can Be Made by Different Methods

Silica Sol Aggregation

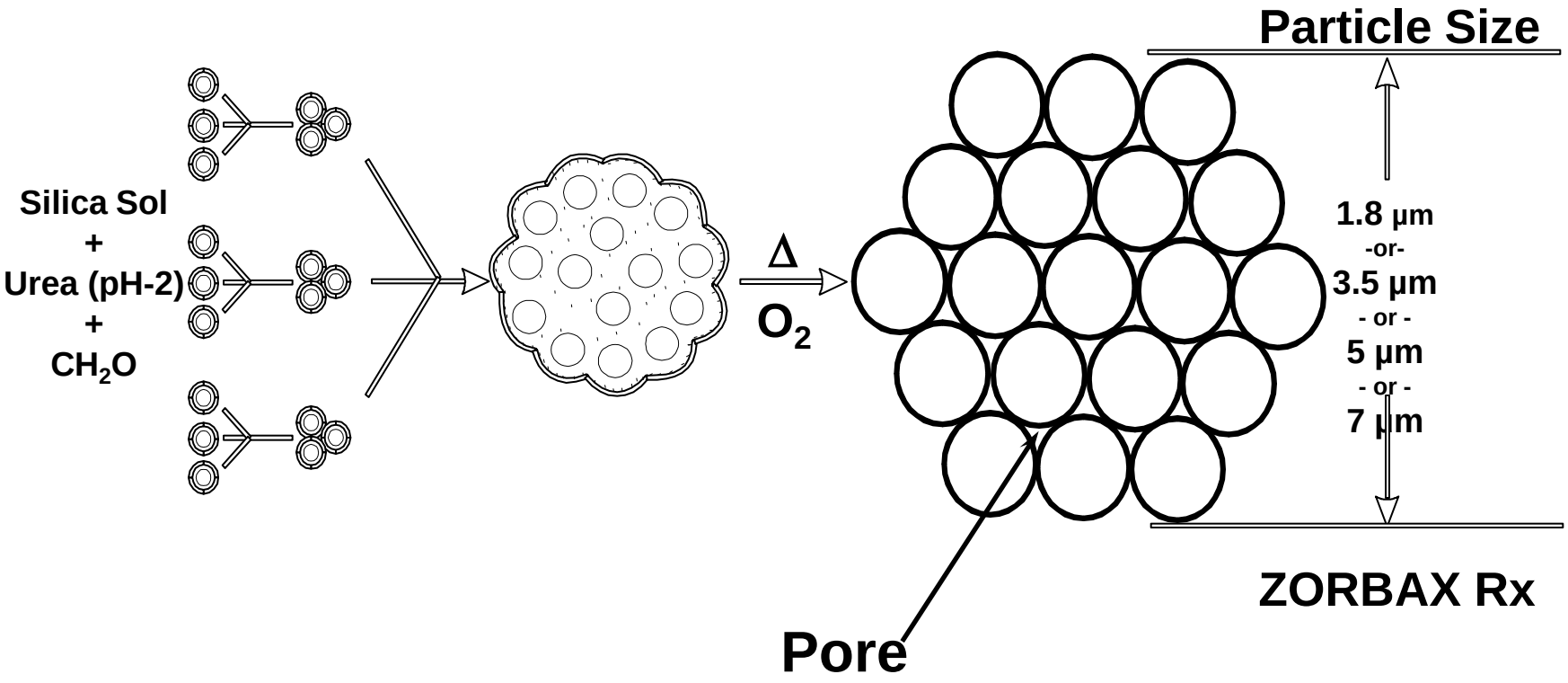


Xerogel



- **The small sol particles that come together to form a larger spherical particle are clearly visible in silica-sol particle.**
- **The silica sol particle will have thicker walls for a more rugged particle.**
- **The more "spongy" structure of particles made by the xerogel process that essentially involves precipitation from a silica solution orsuspension**

ZORBAX® Porous Silica Microsphere (PSM) Manufacturing Process- *Sol Aggregation*



Agilent owns the patent for sol-gel HPLC packings

Uniform Silica Surface Key to Uniform Bonding

- **Silica Particle Production Usually Yields Heterogeneous Surface Chemistry**
  - Underlying -Si-O-Si- Chemical Structure
  - Free Silanols
  - Geminal Silanols
  - Not ideal for Uniform Chemical Bonding

- **Re-Hydrolysis Increases Silanol Surface Population**

- **Theoretical Silanol Surface of ZORBAX PSM Particles**
  - 400µmole/M^2 for 80Angstrom Pore Particle
  - ~180M^2/gram (~1.5gram in 4.6x150mm Column dimension)

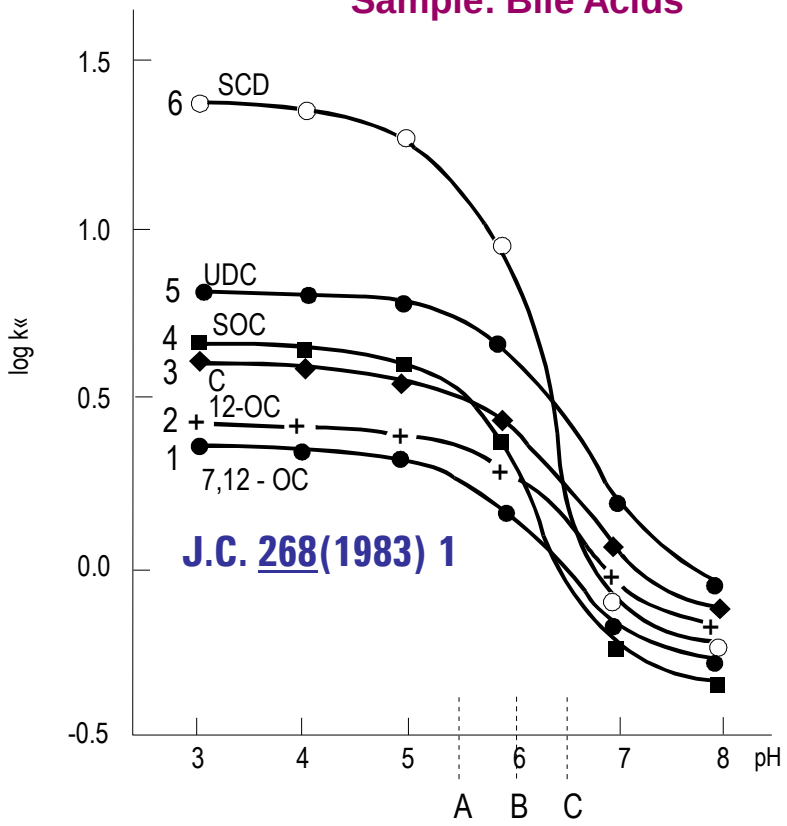
- **Thorough Re-Hydrolysis Produces Uniform Silanol Surface**
  - Maximum Silanol coverage
  - Associated Silanols with lower acidity

pH vs. Selectivity for Acids and Bases

Column: Nucleosil-C18

Mobile Phase: 45% ACN/55% phosphate buffer

Sample: Bile Acids

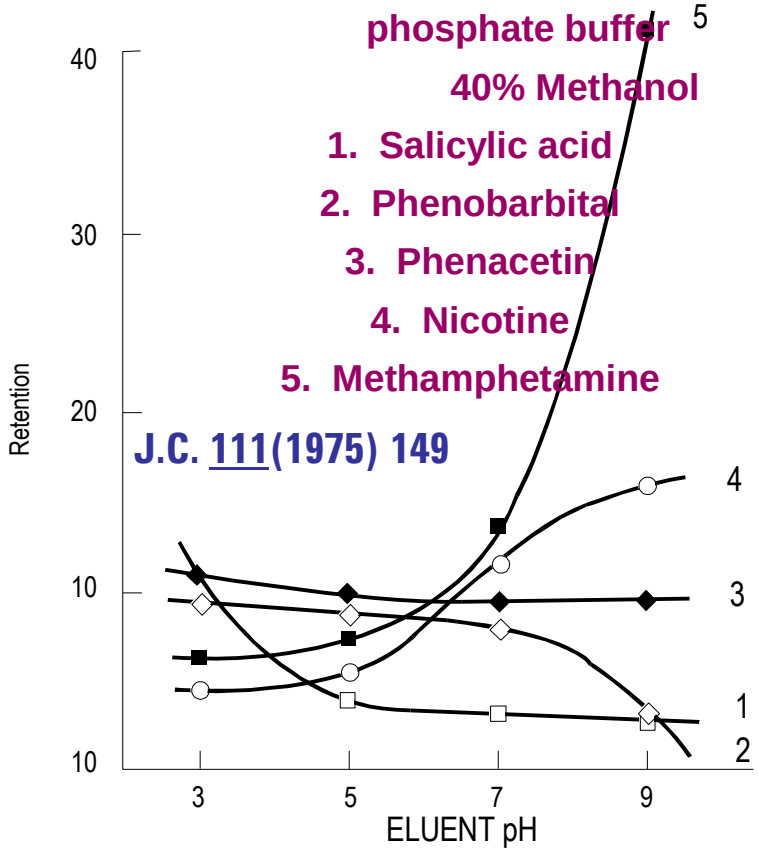


Column: μBondapak-C18

Mobile Phase: 60% 25 mM phosphate buffer

40% Methanol

1. **Salicylic acid**
2. **Phenobarbital**
3. **Phenacetin**
4. **Nicotine**
5. **Methamphetamine**

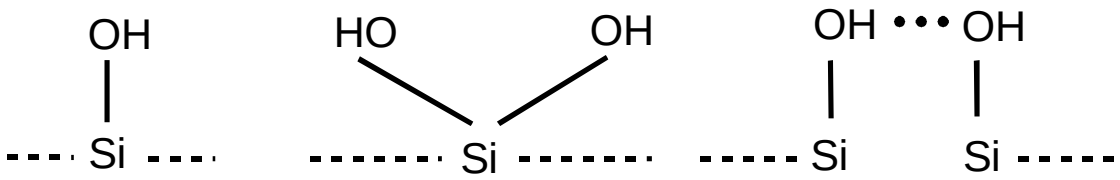


- **Retention and selectivity can change dramatically when pH is changed.**

Silica Particle Surface Chemistry

Non-Ideal Surface Re-Hydrolysis

Ideal Surface State

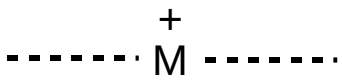


Free Silanols

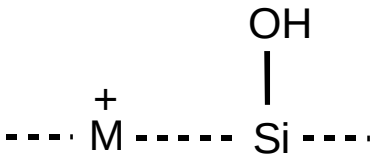
Geminol Silanols

Associated Silanols

decreasing acidity



Surface Metal



Internal Metal (activated silanol) (most acidic)

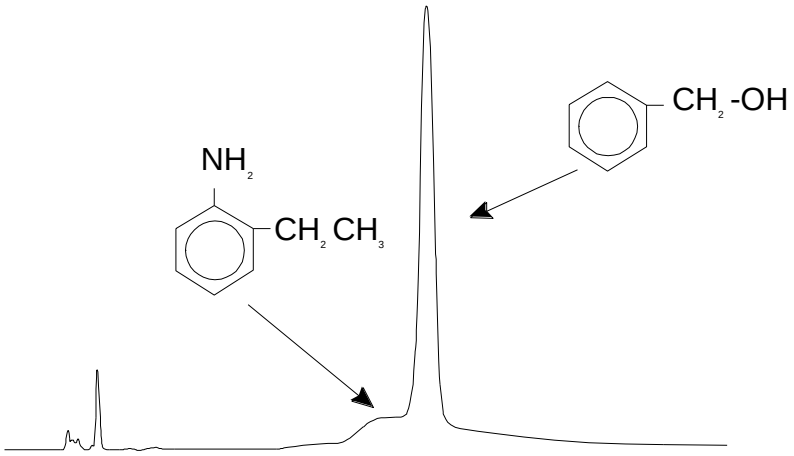
Caused by Using Impure Raw Material

Chromatographic Benefits of Optimized Silica Surface

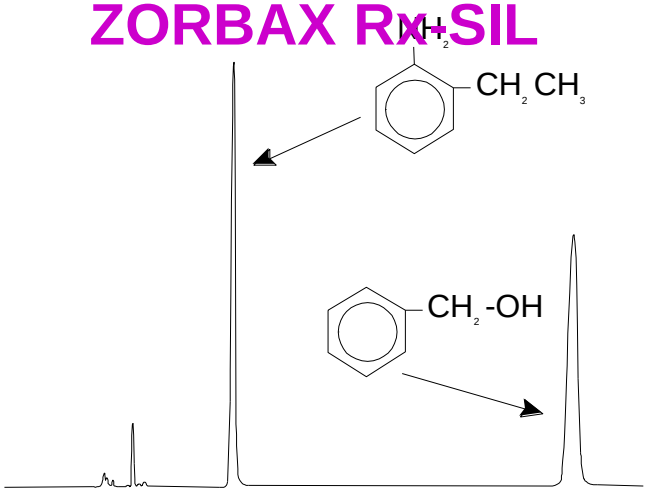
Mobile Phase: 5% 2-Propanol in Heptane

Flow Rate: 2.0 mL/min. Temperature: 35°C

Standard Silica

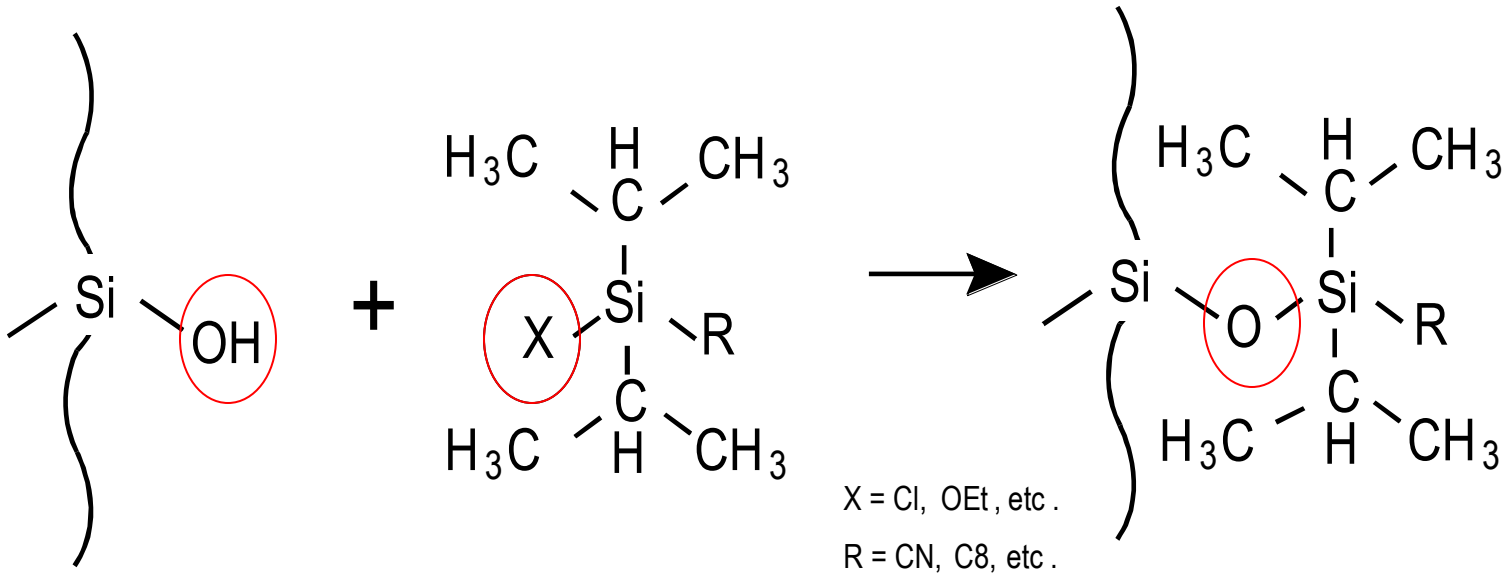


High Purity, Low Acidity ZORBAX Rx-SIL



Improve peak shape for basic compounds with high purity, fully hydroxylated silica such as ZORBAX Rx-SIL

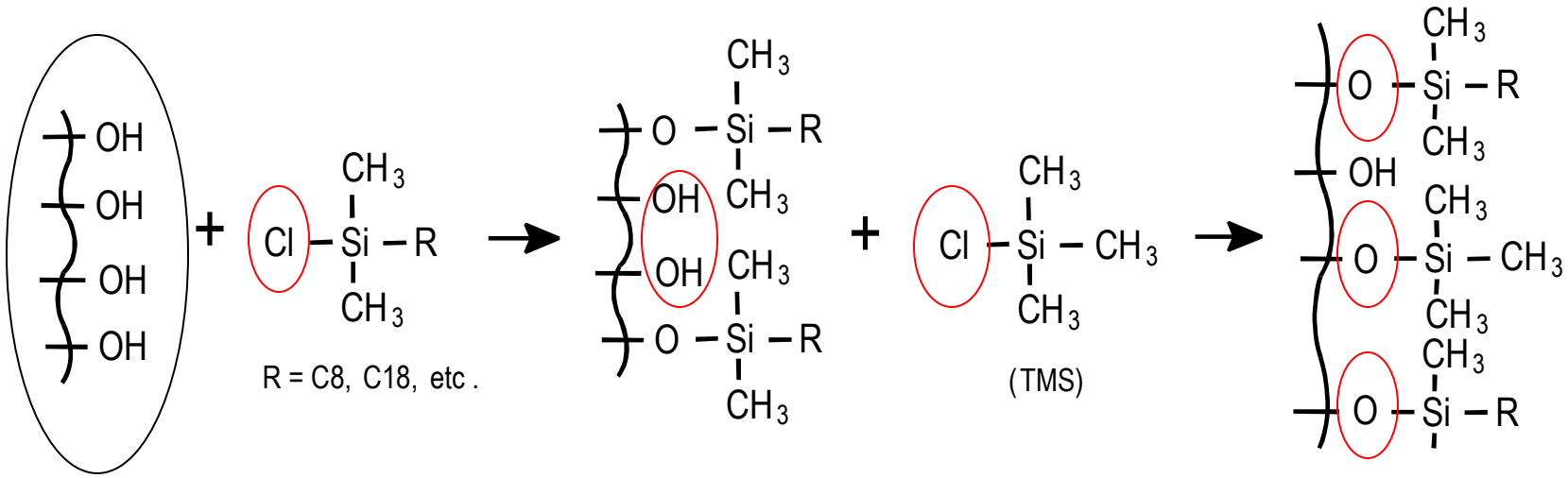
StableBond Silane Bonding Reaction
Sterically Protected Surface, No End Capping



- **Diisopropyl silanes (C8, CN, C3, Phenyl, AQ)**
- **Diisobutyl silanes (C18)**

Traditional Stationary Phase Silane Reaction

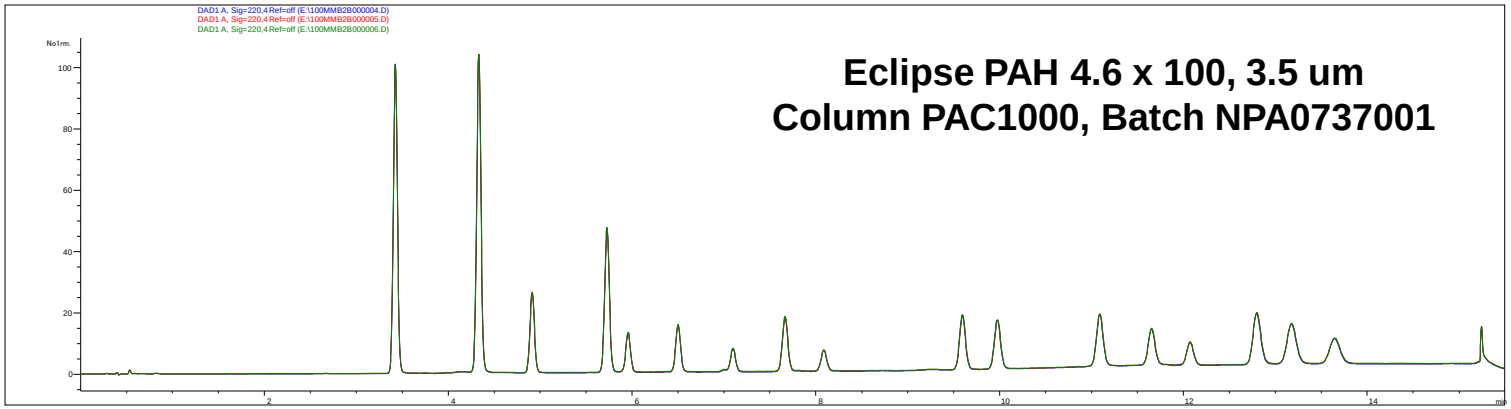
Dense Bonded Phase with Endcapping Reaction

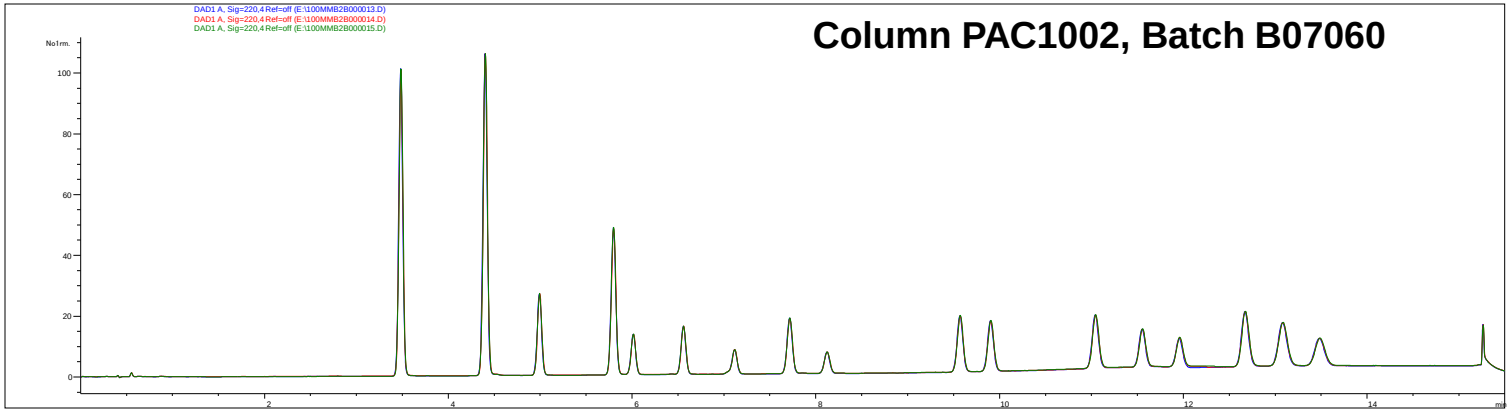


- **Dimethyl silanes**
- **Endcapped with TMS**

If The Process is Done Well, The Result is Very Good Lot-to-Lot Reproducibility

Repeat injections overlaid (3x)

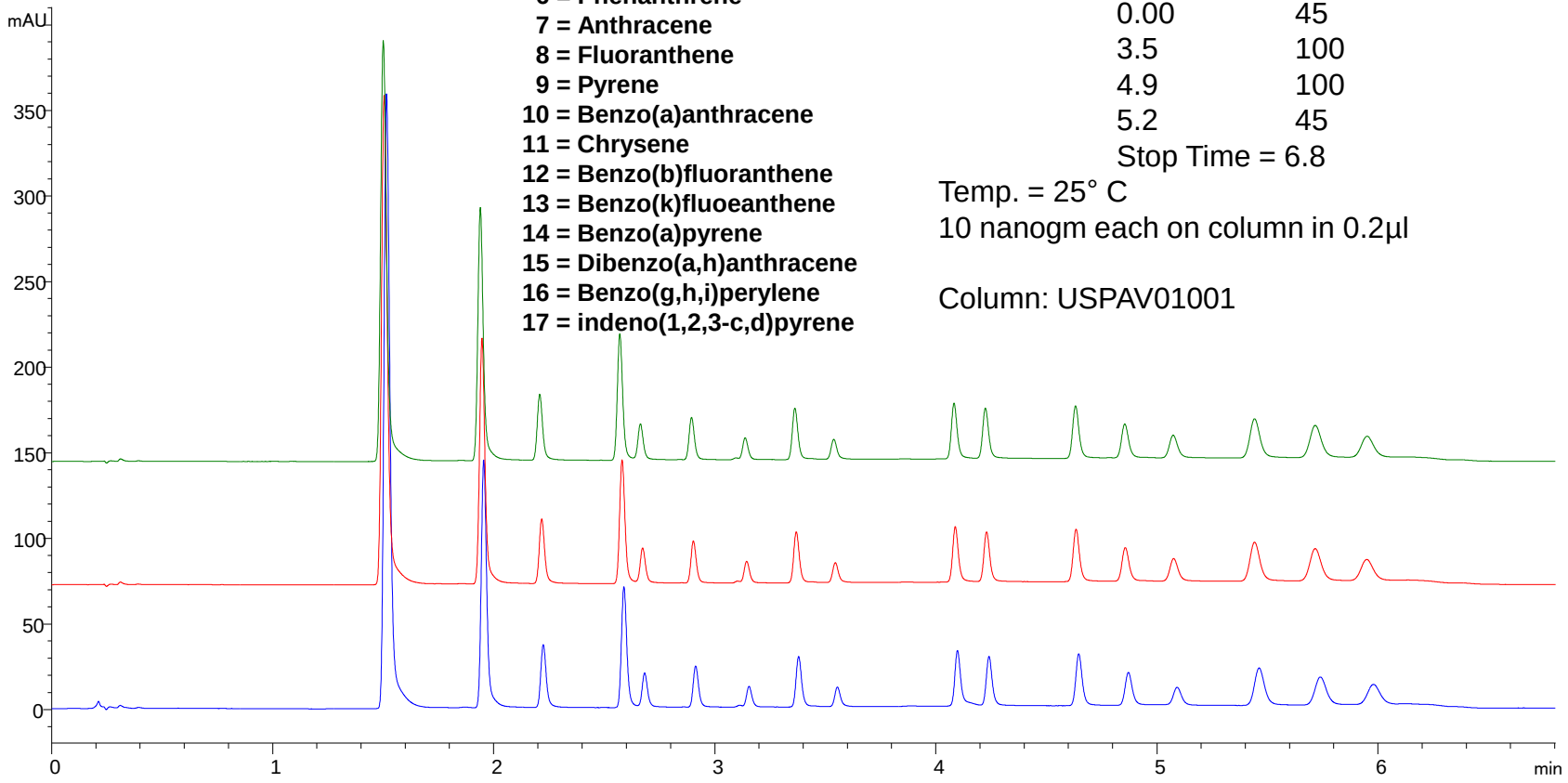




Agilent Technologies

Better Column Surfaces = Better Column Lifetime

Overlay of Runs # 2, 500, 900 injections (Which is Which?)



1 = Toluene
2 = Naphthalene
3 = Acenaphthylene
4 = Acenaphthene
5 = Fluorene
6 = Phenanthrene
7 = Anthracene
8 = Fluoranthene
9 = Pyrene
10 = Benzo(a)anthracene
11 = Chrysene
12 = Benzo(b)fluoranthene
13 = Benzo(k)fluoeanthene
14 = Benzo(a)pyrene
15 = Dibenzo(a,h)anthracene
16 = Benzo(g,h,i)perylene
17 = indeno(1,2,3-c,d)pyrene

Conditions:
Det. 220,4nm No Ref.; Data rate 0.2s, micro flowcell
Flow 0.417 ml/min
Mobile Phase A = Water; B = Acetonitrile
Gradient: Time (Min) % B

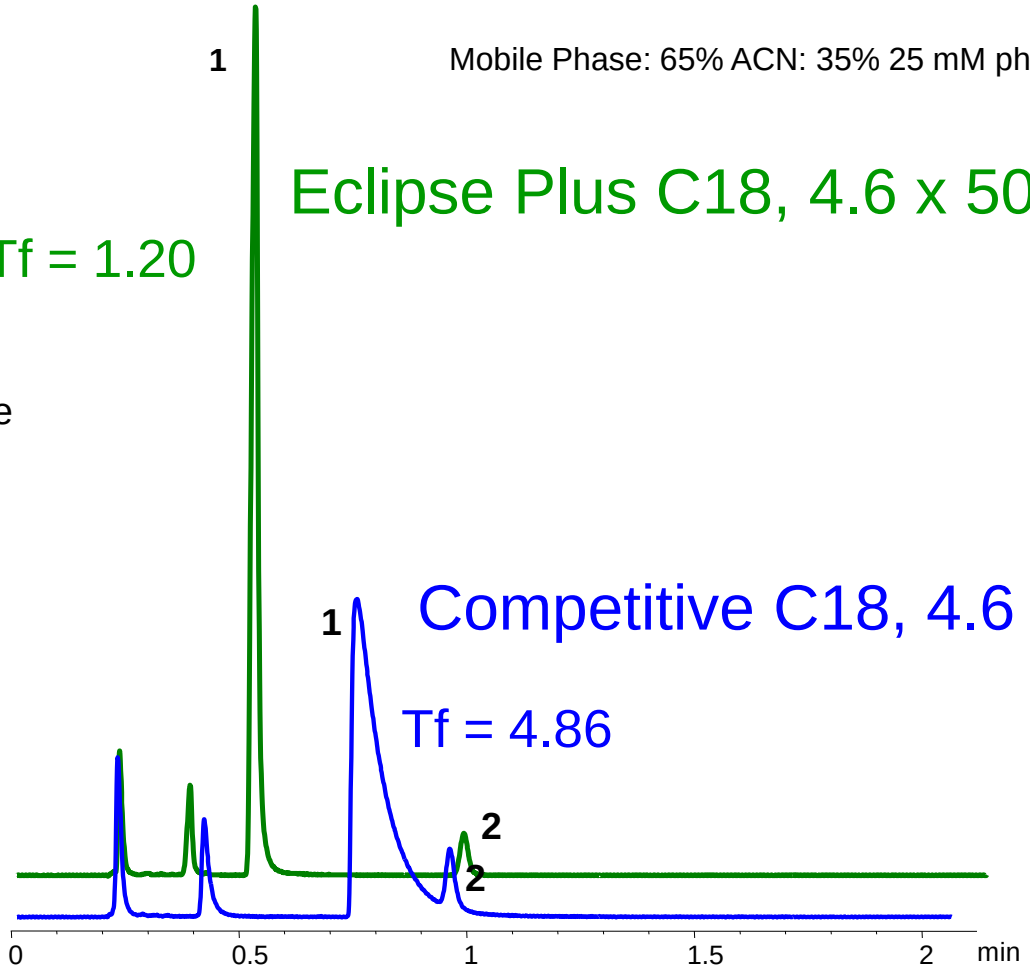
0.00	45
3.5	100
4.9	100
5.2	45

Stop Time = 6.8
Temp. = 25° C
10 nanogm each on column in 0.2µl

Column: USPAV01001

Lifetime is good – this is an example up to 1000 injections RRHT Columns are packed well

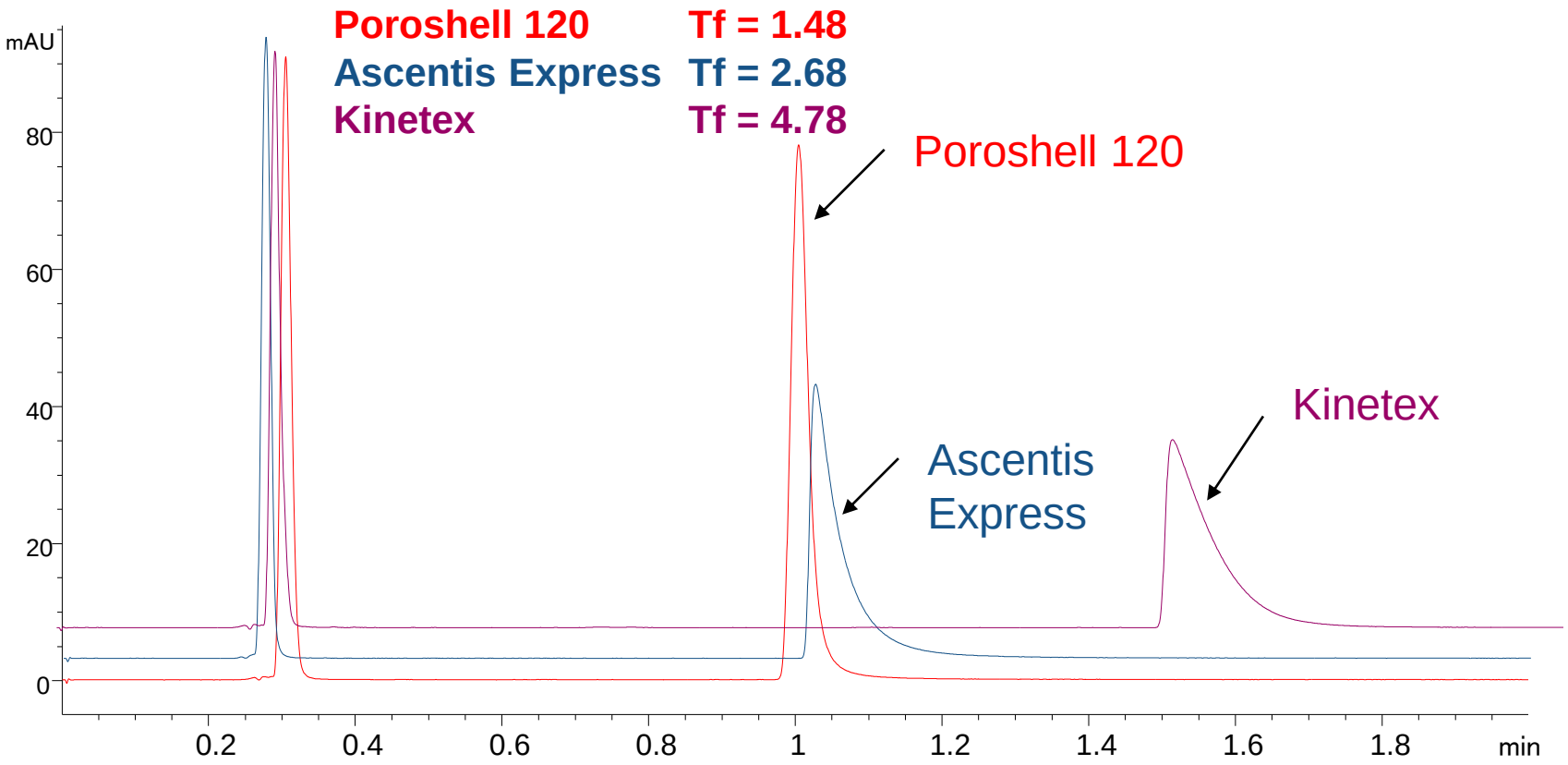
Improved Silica and Bonded Phases Improve Peak Shape



Superior peak shape and better selectivity with Eclipse Plus means more resolution, easier quantitation and better results in your separations.

Comparison of Peak Shape on Superficially Porous Columns

Columns: 4.6 x 50mm, Mobile Phase: 20 mM 40% Na2HPO4, pH 7.00 60% Acetonitrile Flow Rate: 1.5 mL/min
Sample: 2 uL injection of 250 ug/mL amitriptyline, 50 ug/mL uracil in H2O/CH3CN (9:1) Temp: 24°C
Detector 254nm, 2-uL flow cell



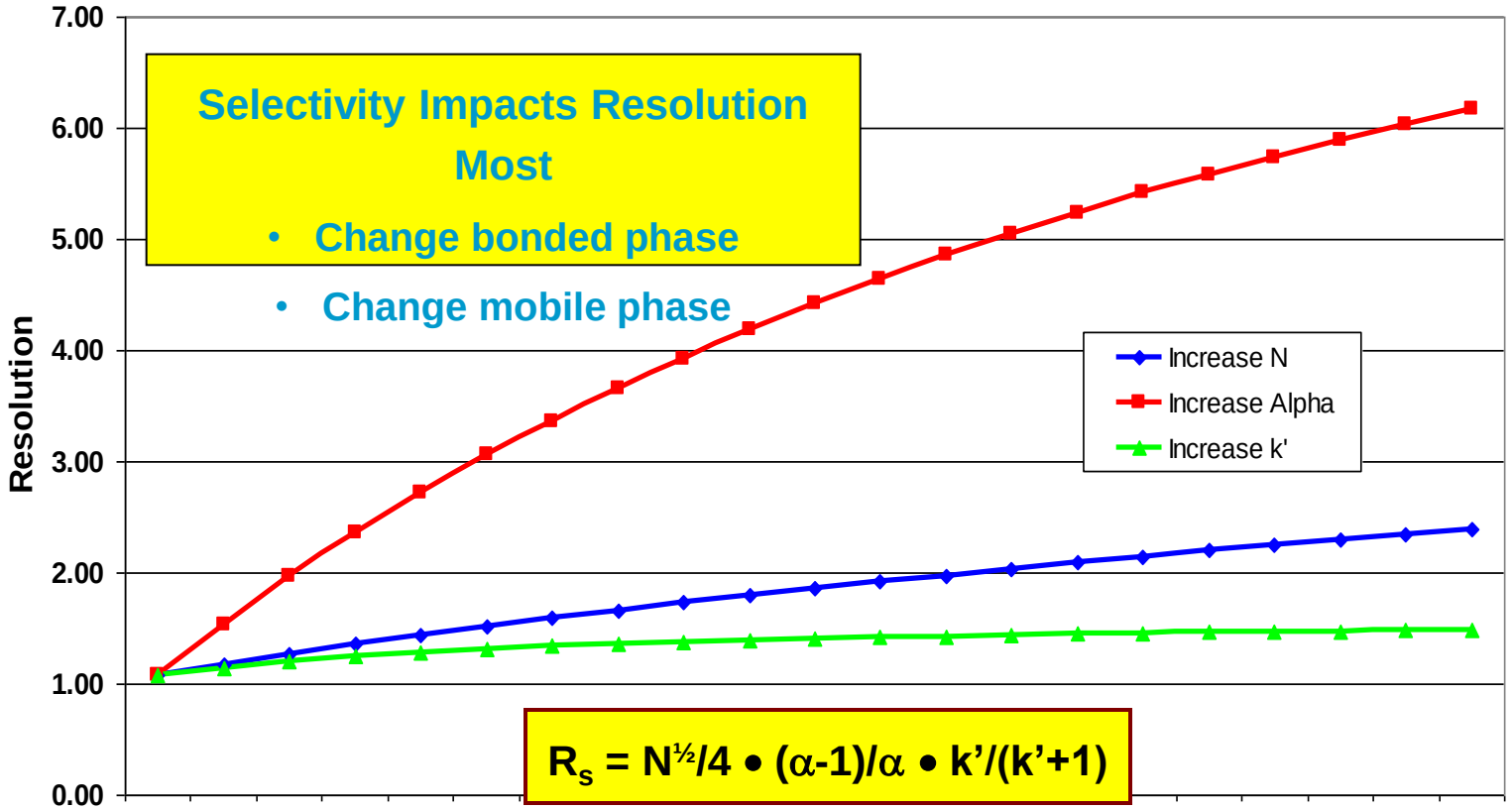
Controlling Factors in Resolution

Each Controlling Factor Can Be Combined to Define and Calculate Resolution

$$R_s = \frac{\sqrt{N}}{4} \cdot \frac{(\alpha-1)}{\alpha} \cdot \frac{k'}{k'+1}$$

Theoretical Plates ***Selectivity*** ***Retention***

Resolution as a Function of Selectivity, Column Efficiency, or Retention



Plates:	5000	10000	15000	20000	25000
Alpha:	1.10	1.35	1.60	1.85	2.1
k':	2.0	4.5	7.0	9.5	12.0

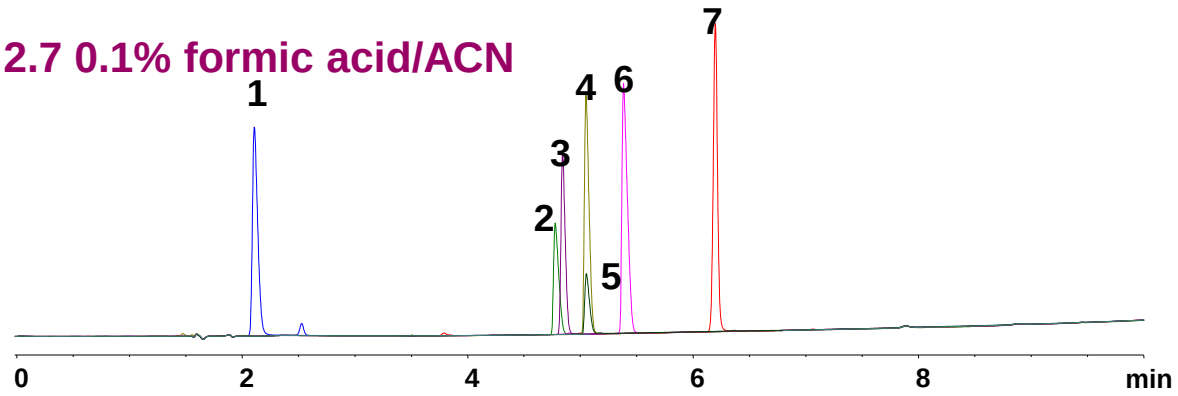
Chemical Parameters That Influence HPLC Separations

- **Sample Chemistry**
- **pH, Buffer Choice and Concentration**
- **Organic Modifier and Concentration**
- **Column Chemistry**

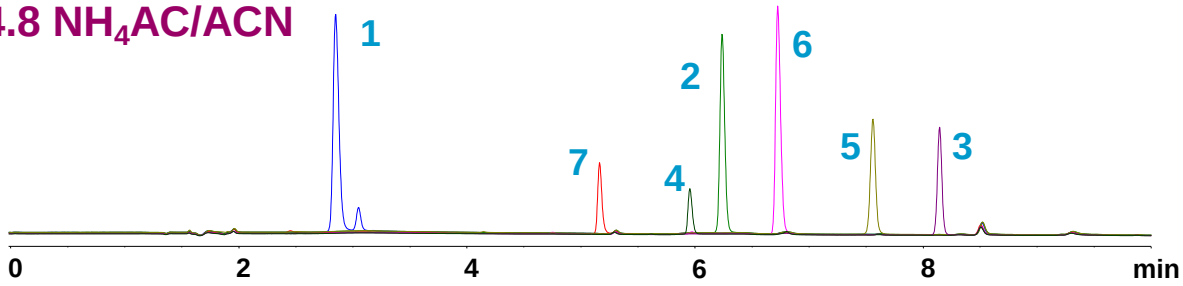
Finding the Correct Interaction of These Parameters Maximizes the Incredible Separating Power of HPLC

pH of Mobile Phase Optimizes Selectivity and Resolution
Changes in Selectivity with pH and Buffer Change

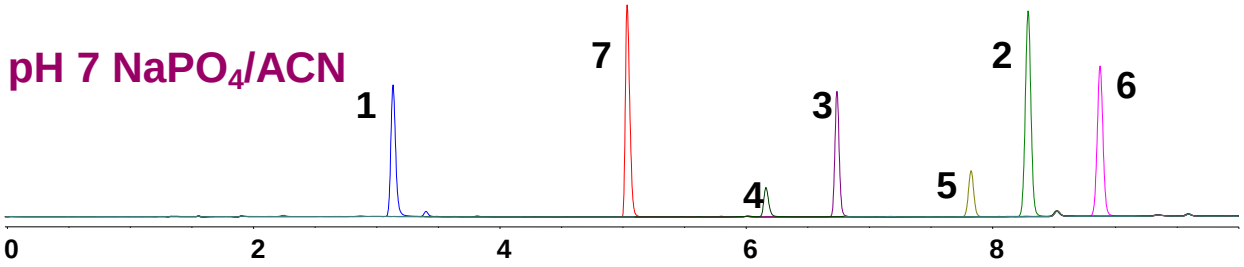
pH 2.7 0.1% formic acid/ACN



pH 4.8 NH_4AC/ACN



pH 7 $NaPO_4$/ACN



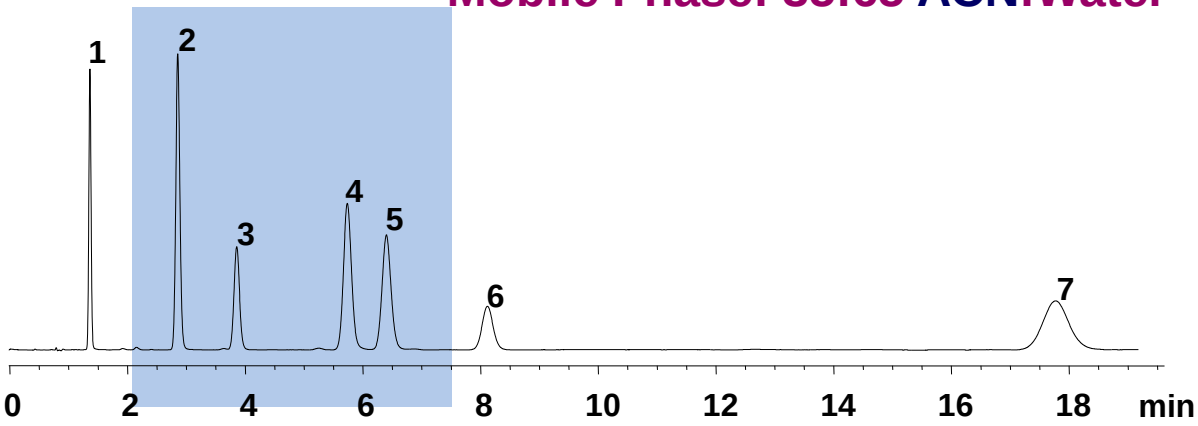
1. procainamide
2. buspirone
3. pioglitazone
4. eletriptan
5. dipyridamole
6. diltiazem,
7. furosemide

Conditions: Column: Eclipse Plus C18 4.6 x 100mm, 5um Gradient: 10 – 90% in 10 minutes Detection: UV 254 nm

Change In Mobile Phase Organic Alters Selectivity Acetonitrile vs. Methanol

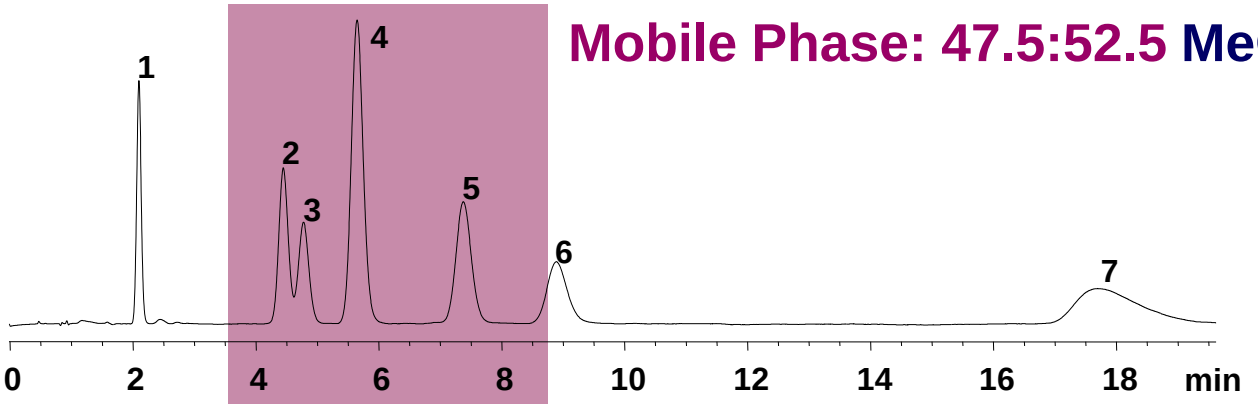
Column: ZORBAX Eclipse XDB-CN Column Dimensions: 4.6 x 150 mm, 5 µm Mobile Phase: As shown
Flow Rate: 2.0 ml/min Injection Volume: 2.00 µl Column Temperature: 25 °C Detector: UV, 210 nm

Mobile Phase: 35:65 ACN:Water

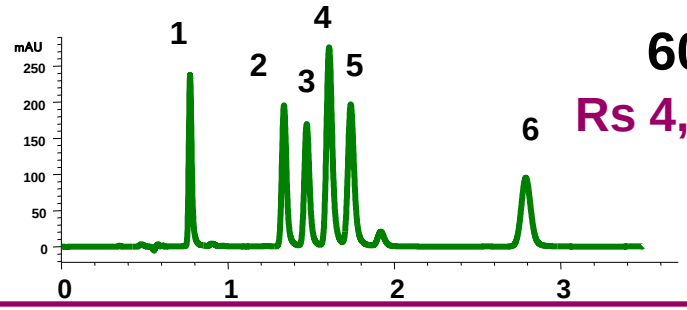


Sample:
1. Estriol (0.00130 µg/µl),
2. b-Estradiol (0.00130 µg/µl),
3. Ethinyl Estradiol (0.00147 µg/µl),
4. Dienestrol (0.00123 µg/µl),
5. Diethylstilbestrol (0.00128 µg/µl)
6. Ethynylestradiol 3-methyl ether (0.00103 µg/µl)
7. Ethynodiol Diacetate (0.00139 µg/µl)

Mobile Phase: 47.5:52.5 MeOH:Water

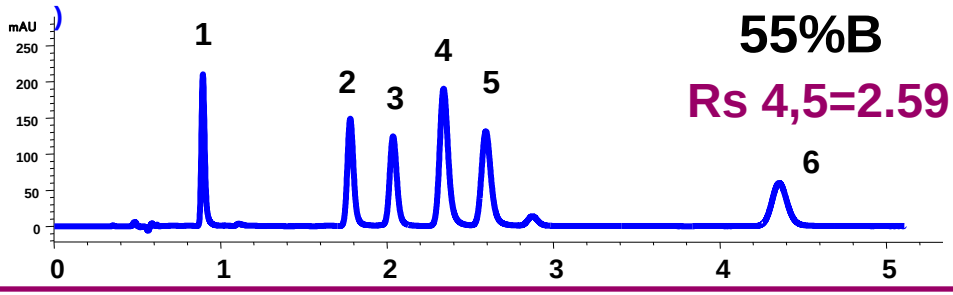


Mobile Phase Optimization on Short RRHT 4.6 x 50 mm, SB-CN Column



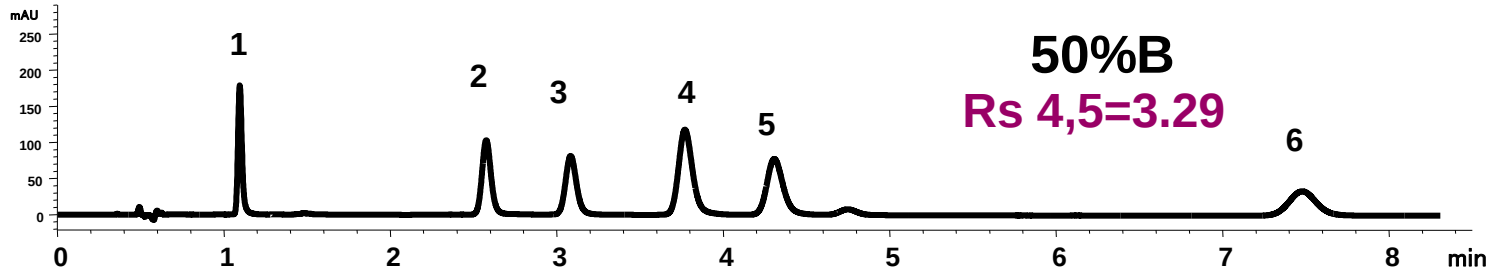
60%B

Rs 4,5=1.96



55%B

Rs 4,5=2.59



50%B

Rs 4,5=3.29

1. Estril
2. Estradiol
3. Ethynyl Estradiol
4. Dienestrol
5. Diethylstilbestrol
6. Ethynl estradiol methyl ester

A: Water

B: 30 % MeOH/70 %MeCN

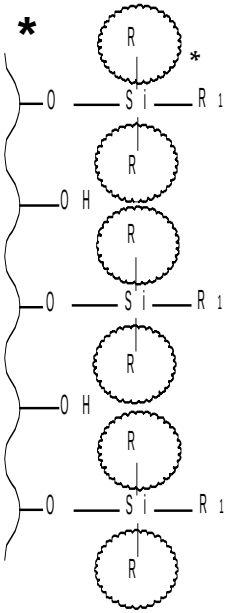
1 µl injection, 210 nm

➢ **RRHT columns allow for very fast optimization of % organic in mobile phase.**

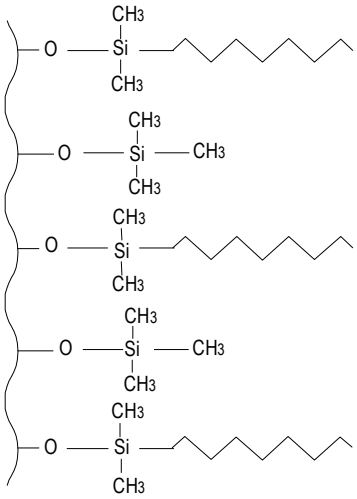
Comparison of ZORBAX Bonded Phase Structures

Surface Structure Alters Interaction of Samples with Phase

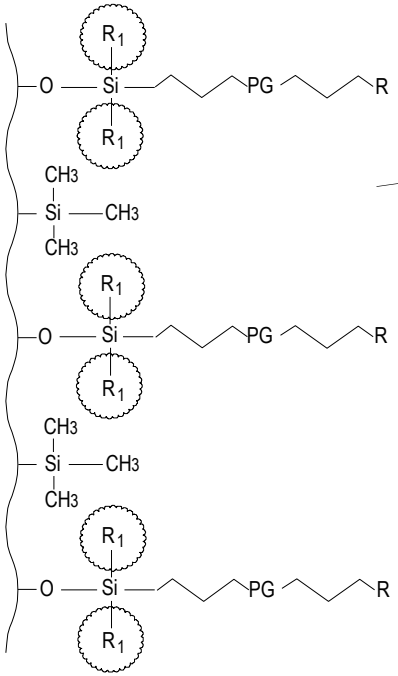
StableBond, pH 1-6
1. Uses bulky silanes
2. Non-endcapped



Eclipse Plus and XDB, pH 2-9
1. eXtra Densely Bonded dimethylalkylsilanes
2. proprietary double-endcapping



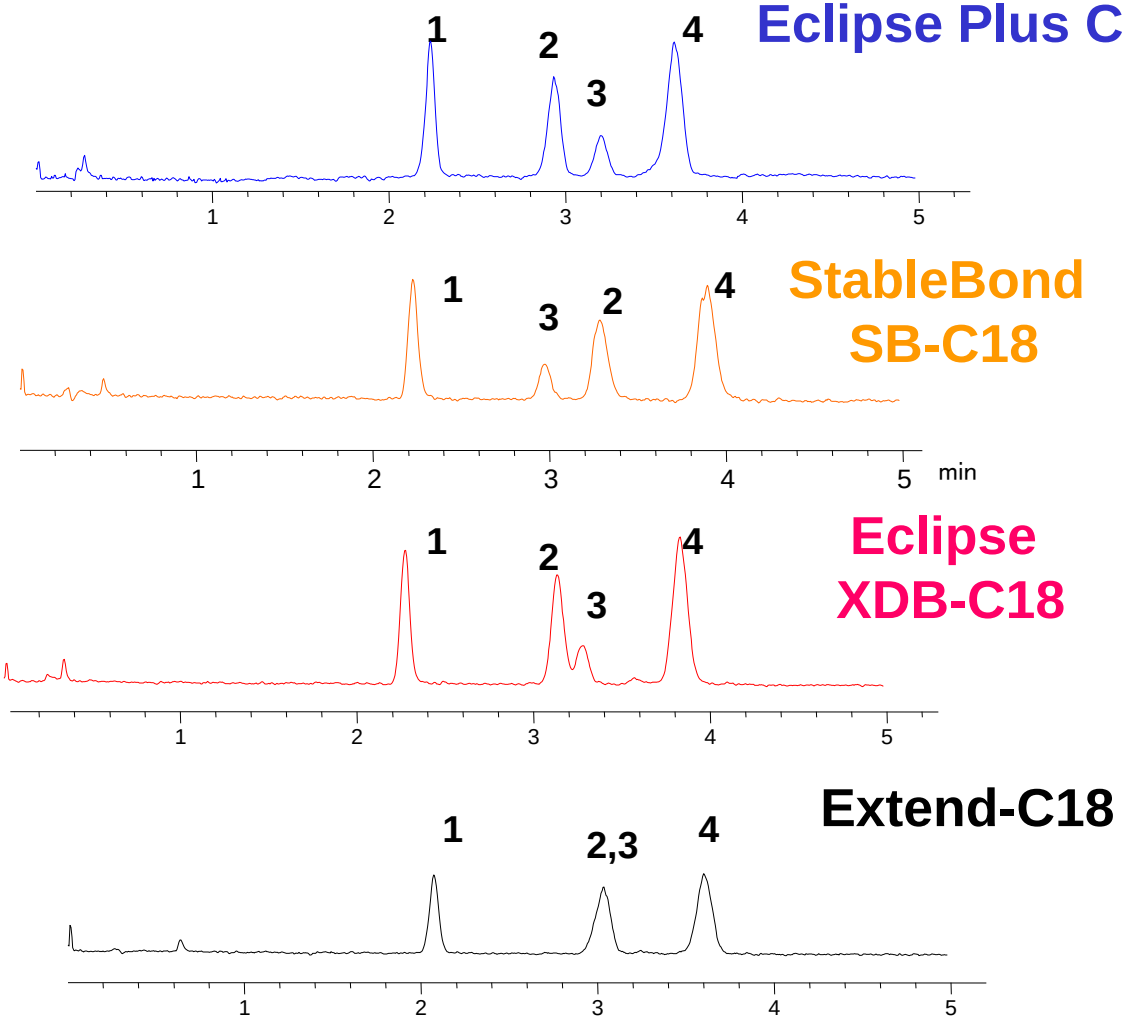
Bonus-RP, pH 2-8
1. polar alkyl phase
2. triple endcapped
3. uses bulky silanes



Extend-C18, pH 2-11.5
1. unique bidentate structure
2. double endcapped

All C18 Phases Do Not Yield the Same Selectivity

Different Surface Chemistry/Structure-Different Selectivity!



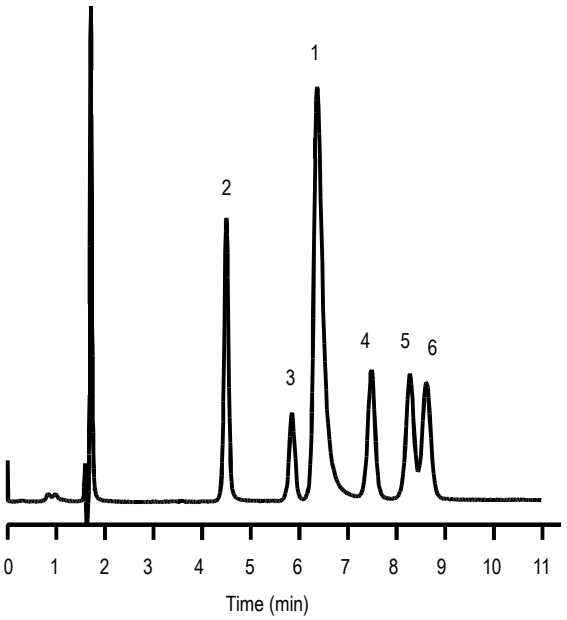
Mobile phase: (69:31) ACN: water
Flow 1.5 mL/min.
Temp: 30 °C
Detector: Single Quad ESI positive mode scan
Columns: RRHT 4.6 x 50 mm 1.8 um

Sample:
1. anandamide (AEA)
2. Palmitoylethanolamide (PEA)
3. 2-arachinoylglycerol (2-AG)
4. Oleoylethanolamide (OEA)

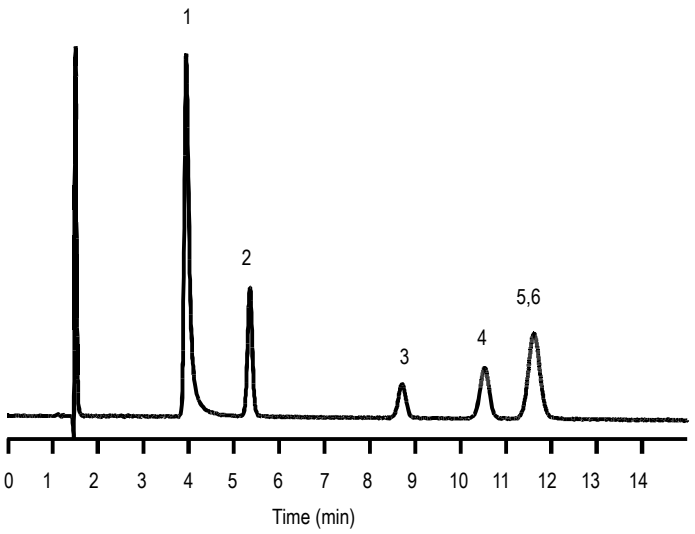
Bonded Phase Surface Structure Alters Selectivity

Identical Silica, Different Phase Structure)

ZORBAX SB-C8



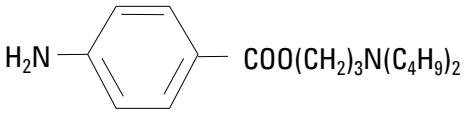
ZORBAX Eclipse XDB-C8



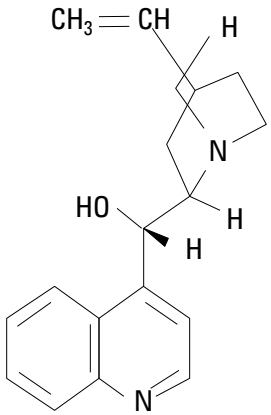
Column: 4.6 x 150 mm, 5 µm, Mobile Phase: 35% Methanol: 65% water Flow Rate: 1. 0 mL/min Temperature: RT
Sample: Modified Engelhardt Mixture 1. Pyridine 2. Aniline 3. Phenol 4. o-Toluidine 5. m-Toluidine 6. p-Toluidine

Basic Anesthetics

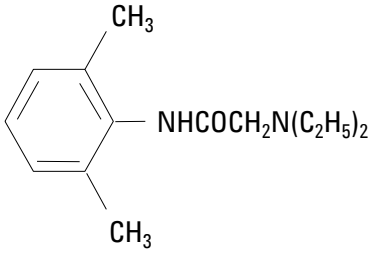
Butacaine



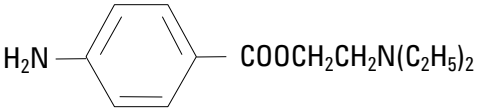
Cinchonine



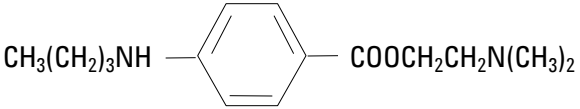
Lidocaine



Procaine



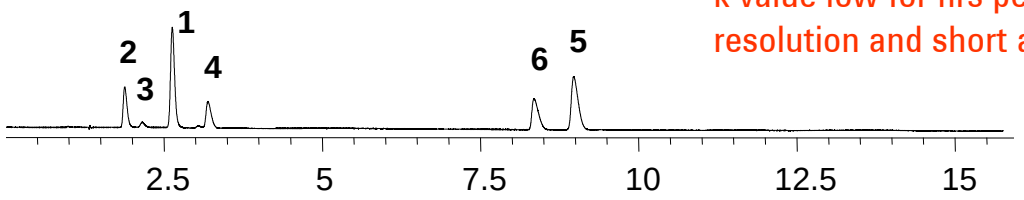
Tetracaine



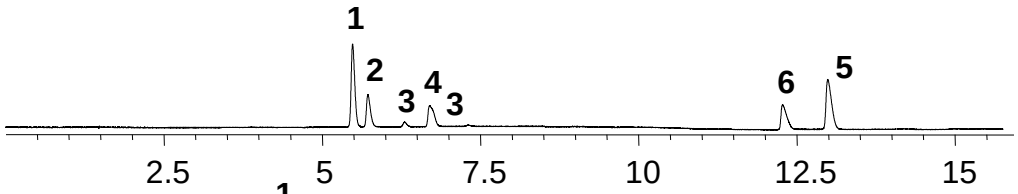
Selectivity Is Different, But No Gain in Speed or Overall Resolution

k value low for firs peak, but excellent resolution and short analysis time

Eclipse XDB-CN

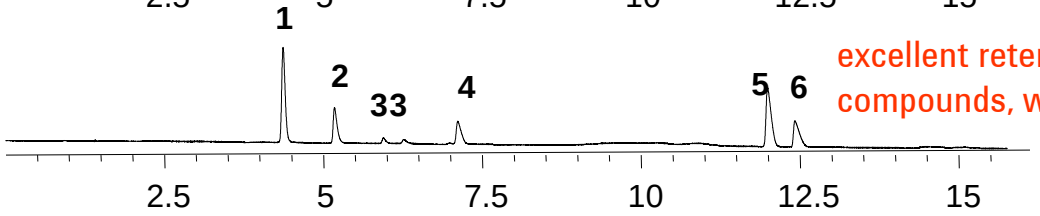


Eclipse XDB-Phenyl

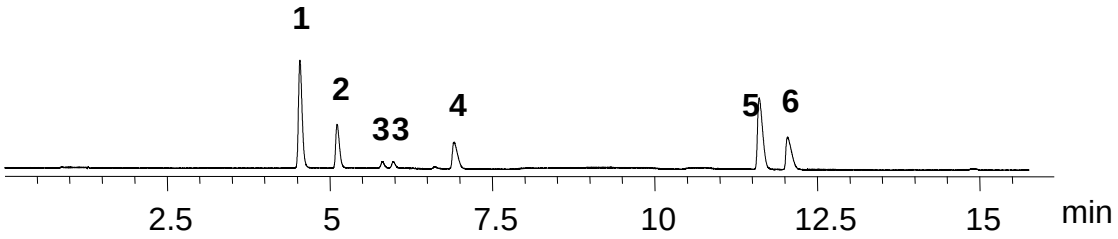


Eclipse XDB-C8

excellent retention and resolution of all compounds, with good analysis time



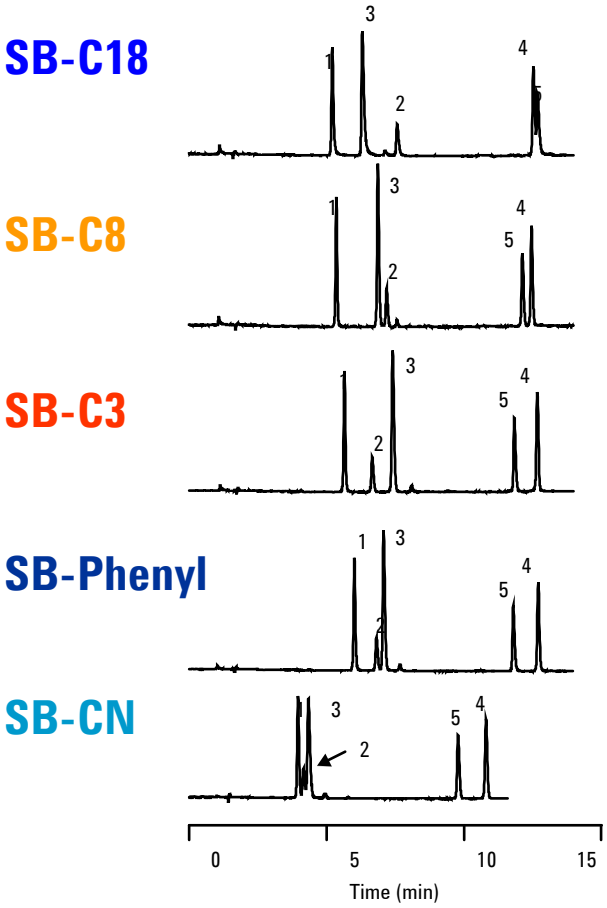
Eclipse XDB-C18



Dimensions: 4.6x150mm, 5µm Mobile Phase: 100% B in 18.8 min; A: 95:5 50mM NaH_2PO_4, pH 2.5:ACN; B: 47:53 50mM NaH_2PO_4, pH 2.5:ACN Flow Rate: 1.5 mL/min Injection: 5µL Temperature: 25°C Detector: UV, 254 nm

Sample: 1. Procaine (0.210µg/µl), 2. Cinchonine (0.224µg/µl), 3. Cinchonine impurity, 4. Lidocaine (0.232µg/µl), 5. Butacaine (0.214µg/µl), 6. Tetracaine (0.232µg/µl), 7. Mobile phase impurity

Selectivity is Different, Resolution is Better, No Increase in Speed



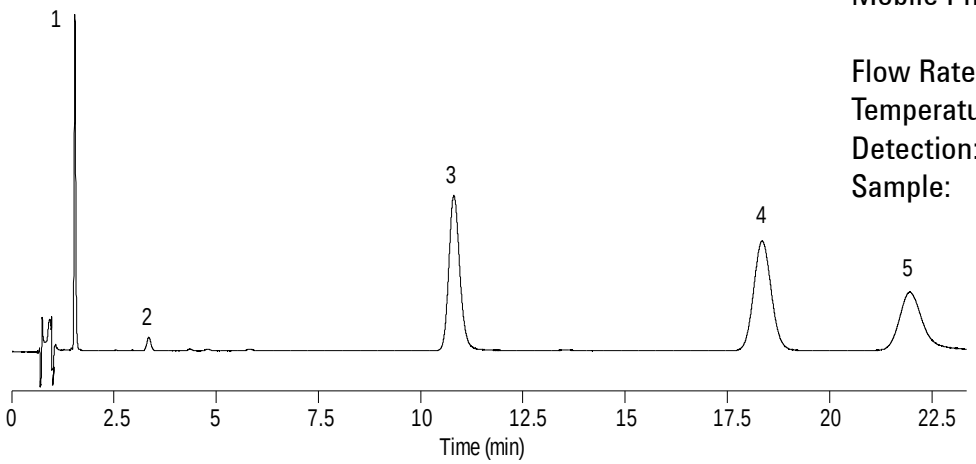
Columns: 4.6 x 250 mm
Mobile Phase:
0 - 100% B in 18.8 min
A: 50 mM NaH_2PO_4, pH 2.5 in 95% H_2O/5% ACN
B: 50 mM NaH_2PO_4, pH 2.5 in 47% H_2O/53% ACN
Flow Rate: 1.5 mL/min
Temperature: 26°C
UV Detection: 254 nm
Sample: 10 µL
1. Procaine
2. Lidocaine
3. d-Cinchonine
4. Butacaine
5. Tetracaine

- **Closely related structures show slight changes in elution order as the polarity of the bonded phase changes.**

Good Separation on C18

Separation of Plant Extract:

Flavones, Flavanones, and Phenolic Esters



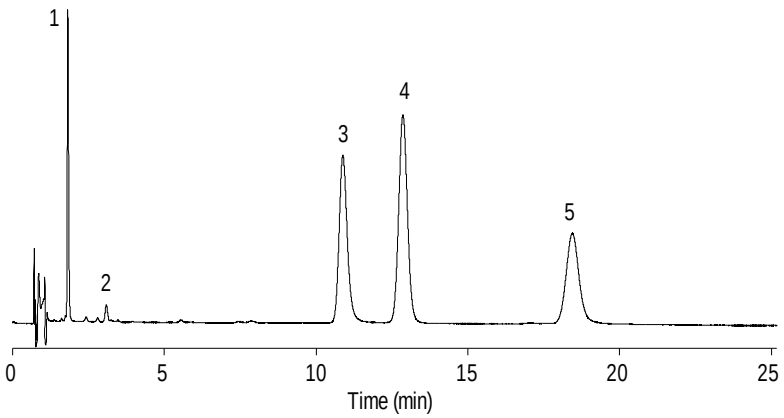
Column: **ZORBAX Rapid Resolution SB-C18**
4.6 x 75 mm, 3.5 µm
Mobile Phase: 22% ACN
78% NaH_2PO_4, pH 2.5
Flow Rate: 1.0 mL/min
Temperature: RT
Detection: UV 254 nm
Sample:
1. Caffeic acid
2. Impurity
3. Luteolin
4. Naringenin
5. Apigenin

Faster with Good Resolution on CN Column!

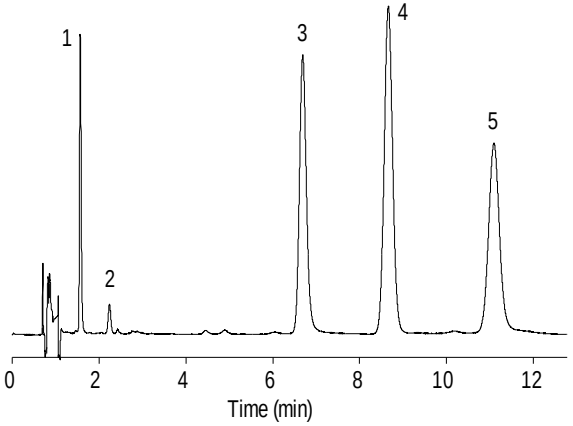
Plant Extract on Cyano Bonded Phase:
Flavones, Flavanones, Phenolic Esters

Column: ZORBAX Rapid Resolution SB-CN, 4.6 x 75 mm, 3.5 µm Mobile Phase: ACN: NaH_2PO_4, pH 2.5 Flow Rate: 1.0 mL/min
Temperature: RT Detection: UV 254 nm Sample: 1. Caffeic acid 2. Impurity 3. Luteolin 4. Naringenin 5. Apigenin

22% ACN: 78% Buffer

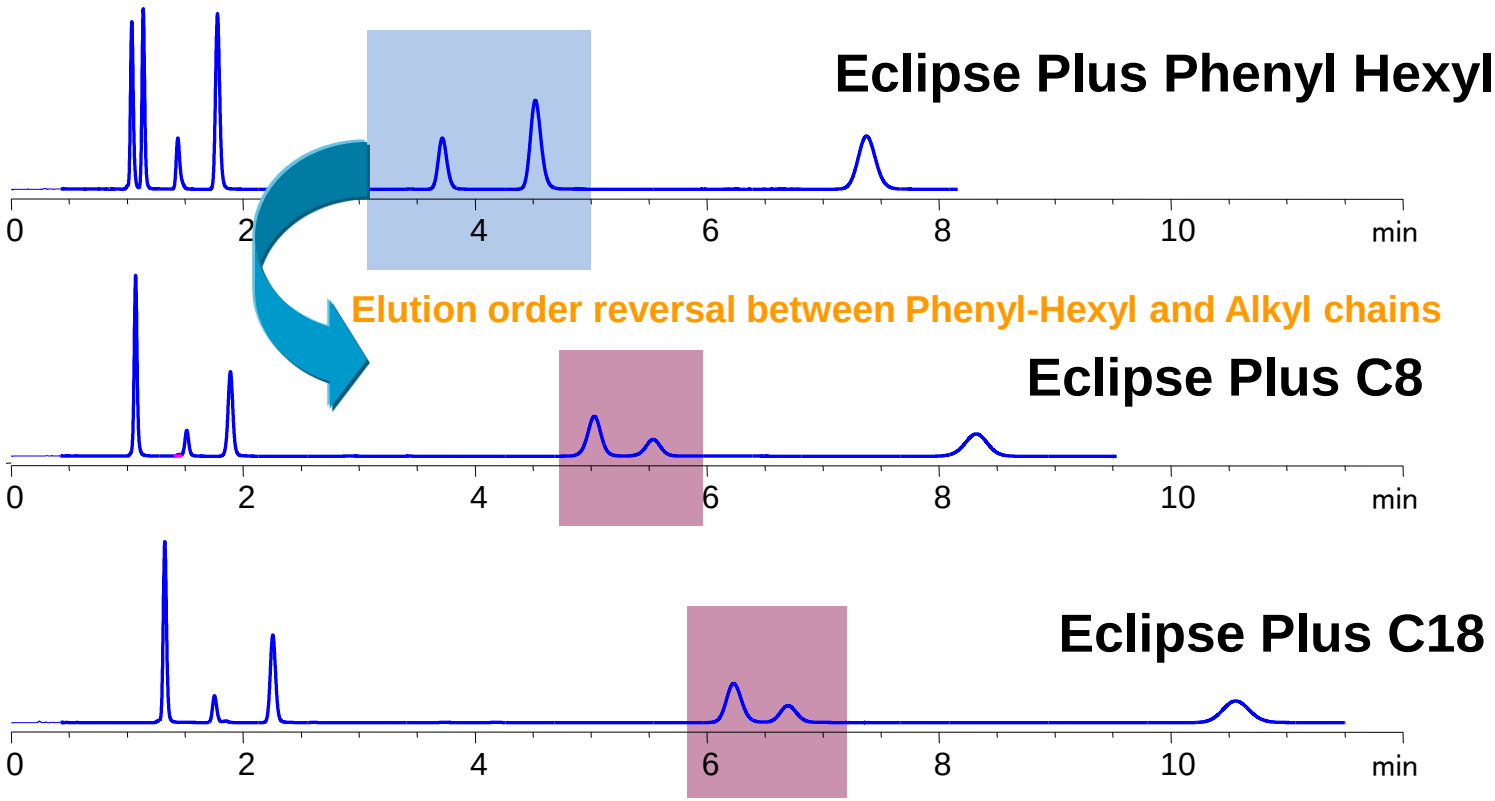


25% ACN: 75% Buffer



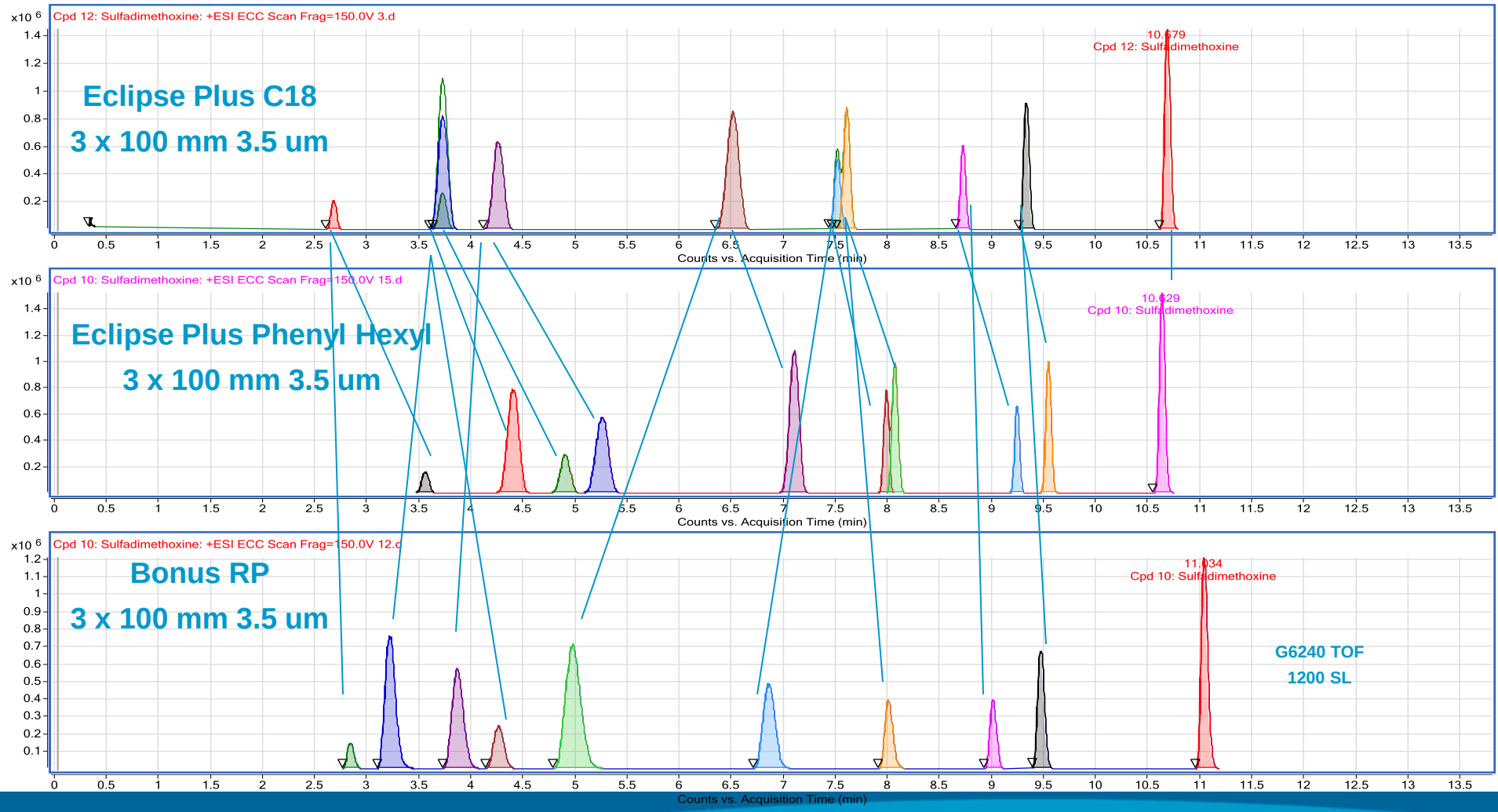
CN bonded phase with stronger mobile phase reduces analysis time by 50% and maintains retention of k=1 on 1st peak.

Improved Speed and Selectivity of Phenyl Hexyl in Relation to C8 or C18 Phase

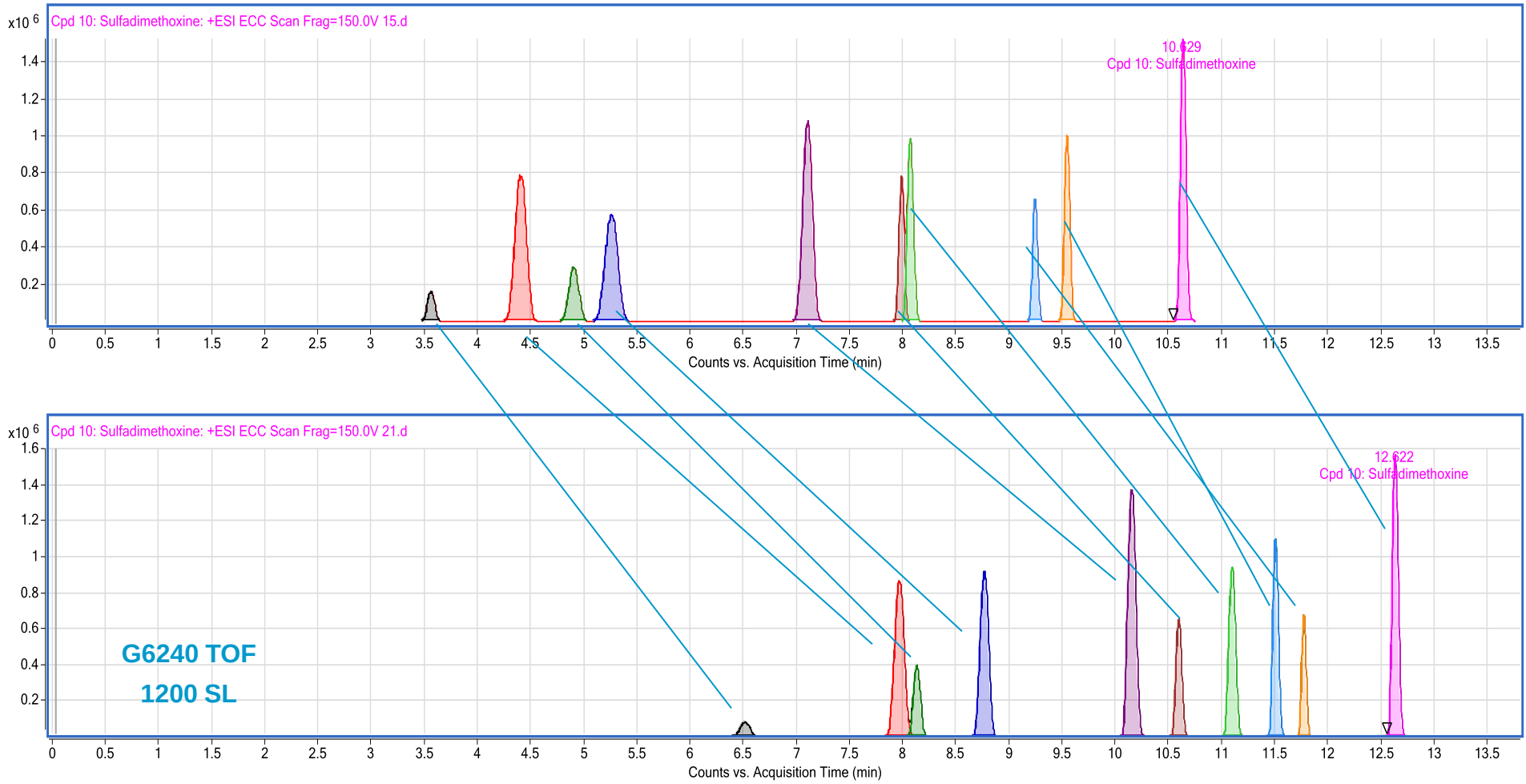


Mobile Phase 40 % ACN 60 % 25 mM Sodium Phosphate Buffer pH 2.4 Flow Rate: 1.5 ml/min 4.6 x 50mm UV 210 nm 2µl Elution order for Eclipse Plus Phenyl Hexyl: (1) Piroxicam, (2) Sulindac,(3) Tolmetin, (4) Naproxen, (5) Ibuprofen, (6) Diclofenac, (7) Celebrex (equal portions of approximately 1 mg/ml solutions

Change Selectivity with very Different Bonded Phases with 0.1 % Formic Acid:Acetonitrile

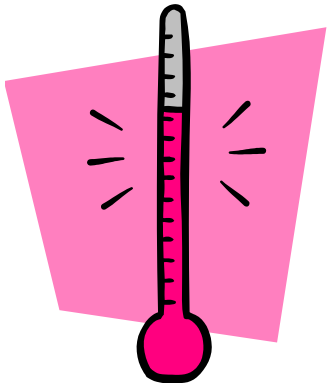


Comparison of Eclipse Plus Phenyl Hexyl in Acetonitrile and Methanol with 0.1 % Formic Acid



Temperature - Higher Temperature as an Aid to Method Development and Faster Operation

Higher Temperature:



Temperature should always be considered as a parameter during method development

Provides more rapid mass transfer:

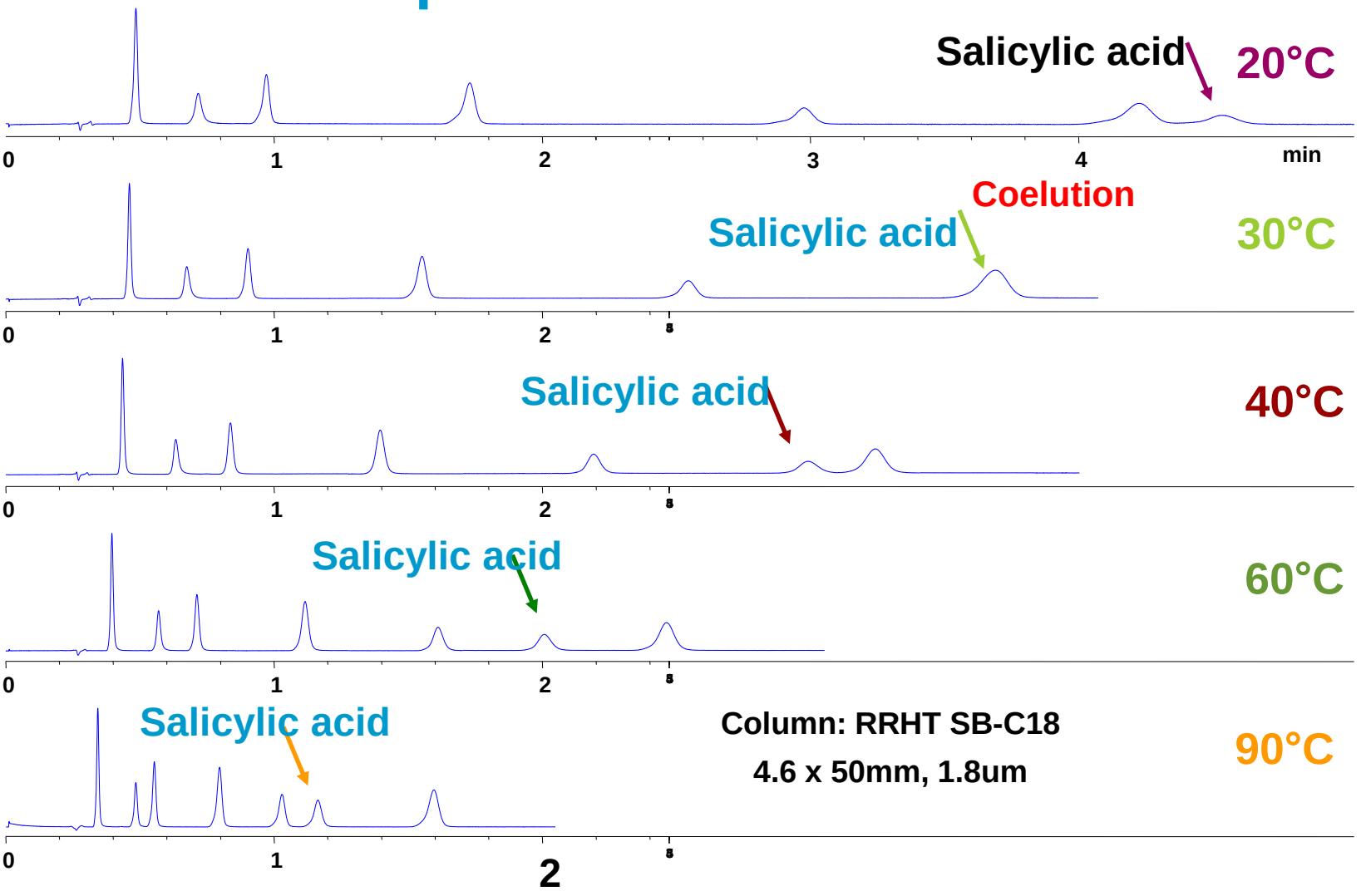
- Improves Efficiency – enhances resolution
- Decreases analysis time – faster separations with no loss in resolution

Decreases Mobile Phase Viscosity

- Lowers backpressure – allows for higher flow rates, faster separations, greater efficiency and use of sub 2-micron columns

Can change selectivity – optimize resolution

Changing Temperature for Selectivity, Resolution Matches the Expected Results

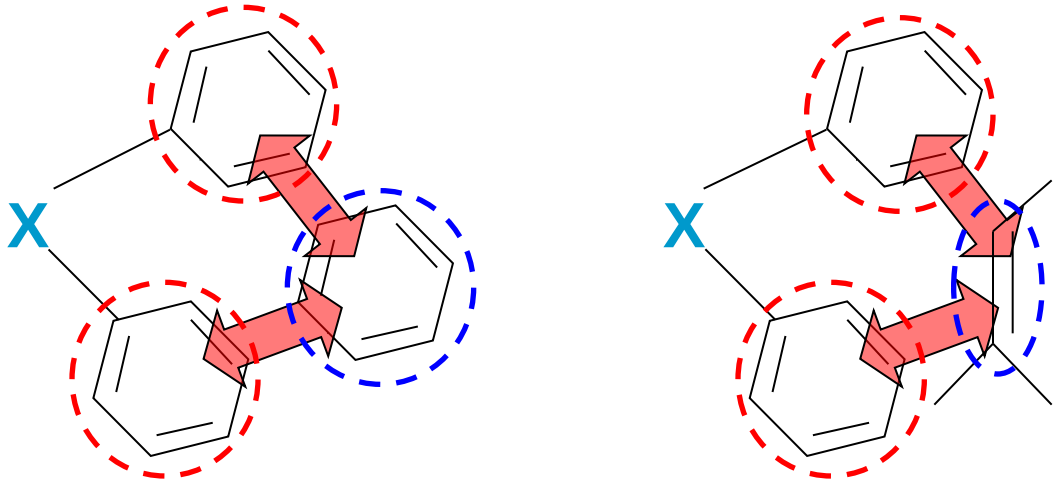


Special Purpose Phases

- Better Resolution for Difficult Samples
- Non-Traditional Mechanisms
- Faster Methods
- “Normal” Dimension Columns and Particles

Pursuit Diphenyl (DP)

- 2 phenyl rings attached to silica via a short aliphatic linker to be used with standard RP mobile phases
- pi-pi election interaction between sorbent & analyte
- Minimal contribution of aliphatic chain to hydrophobic interaction

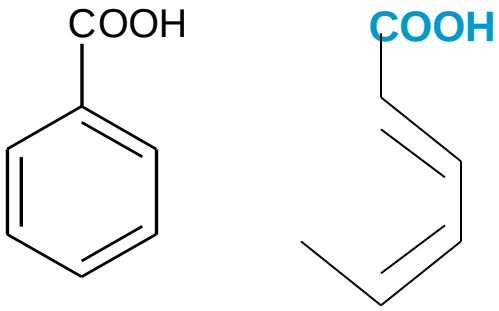


Pursuit DP – Unique Selectivity

Antifungals on Pursuit XRs DP and C18

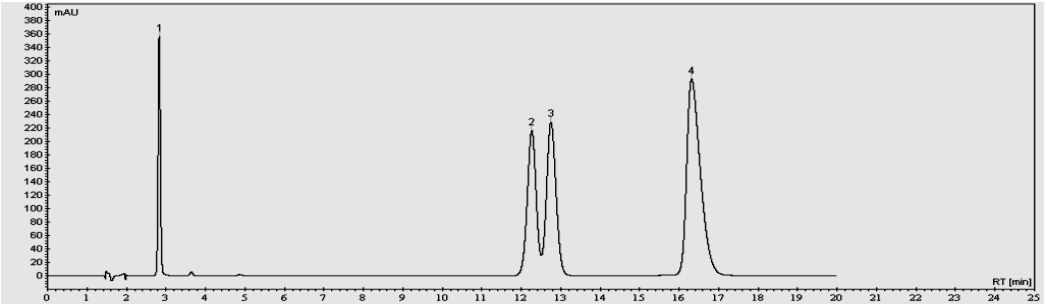
Columns:	Listed on chromatograms
Dimensions:	150 x 4.6 mm, 5µ (all columns)
Mobile Phase:	A: H_2O + 0.1% HCOOH, B: CH_3CN + 0.1% HCOOH A:B - 80:20
Flow Rate:	1.0 ml/min
Temperature:	Ambient
Detection:	254 nm
Sample:	1. 4-Aminobenzoic acid 2. Sorbic acid 3. Benzoic acid 4. Salicylic acid

Critical Pair

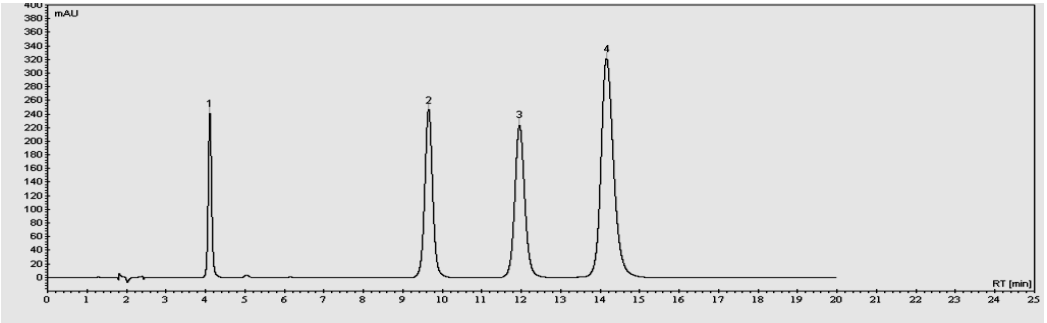


Benzoic Acid **Sorbic Acid**

Pursuit XRs C18



Pursuit XRs DP

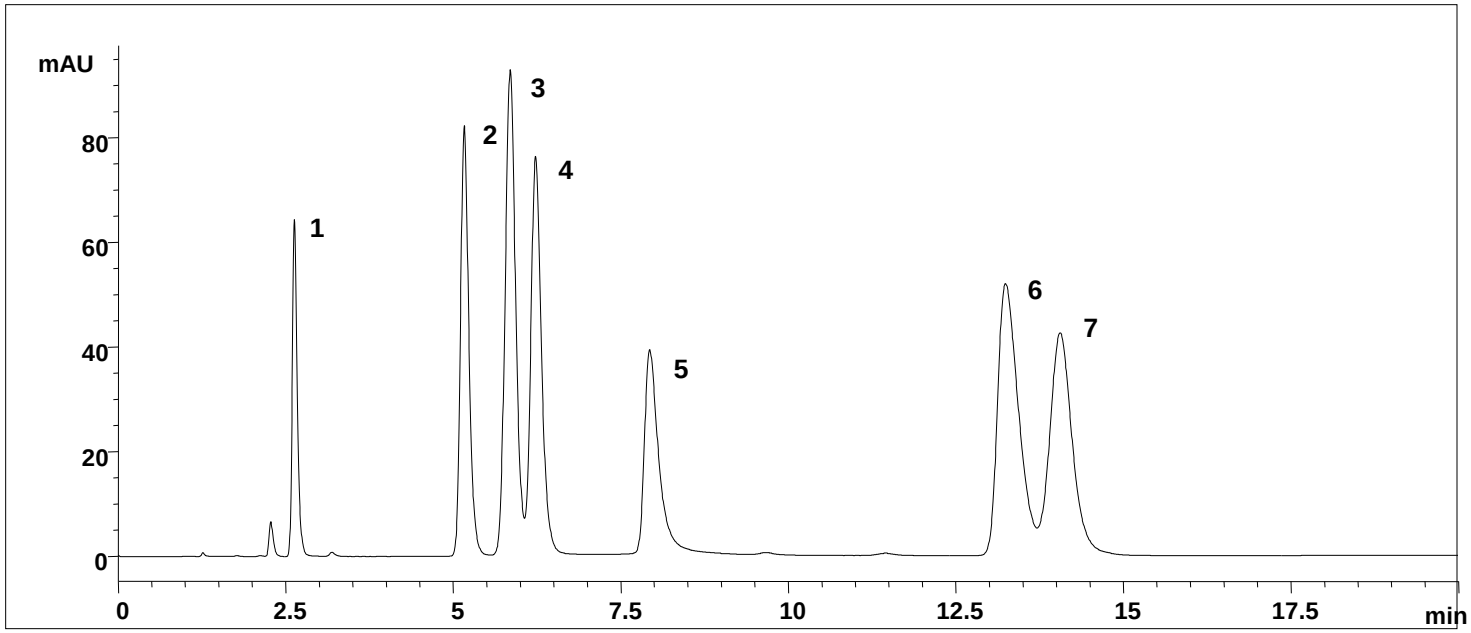


Agilent Technologies

Pursuit PFP

- **Novel selectivity – alternative to traditional C8/C18 as well as π–π /hydrophobic mechanism of DP**
- **Selectivity based on π–π, charge transfer, dipole, hydrogen bonding *and* electrostatic interactions**
- **Pursuit PFP (pentafluorophenyl) applications:**
  - **Aromatics, nitroaromatic compounds, and conjugated systems which allow pi-pi interactions**
  - **Polar analytes which typically require pure aqueous or high aqueous eluents for enhanced polar retention**
  - **Regioselective (positional) isomers**
  - **Halogenated compounds**

Pursuit PFP - Polar Analytes



Separation of Nucleotides and Nucleosides on Pursuit PFP under high aqueous conditions

Column:	Pursuit PFP
Dimensions:	150 x 4.6 mm, 5µ
Mobile Phase:	H_2O + 0.1% HCOOH
Flow Rate:	1.0 ml/min
Temperature:	Ambient
Detection:	254 nm
Sample:	1. 5'-CMP
	2. Uridine
	3. Adenosine
	4. 5'-AMP
	5. 5'-CDP
	6. 5'-UMP
	7. 2-Deoxyguanosine

Positional Isomers

Phenolic Positional Isomers

Column : Pursuit PFP, 150 x 4.6 mm, 5µm; Part Nr. A3050150X046

Mobile Phase : A: Water +0.1% Formic Acid

B: Acetonitrile

Gradient:

Time	%B
0	20
15	60

Flow Rate : 1.5 mL/min

Temperature : Ambient

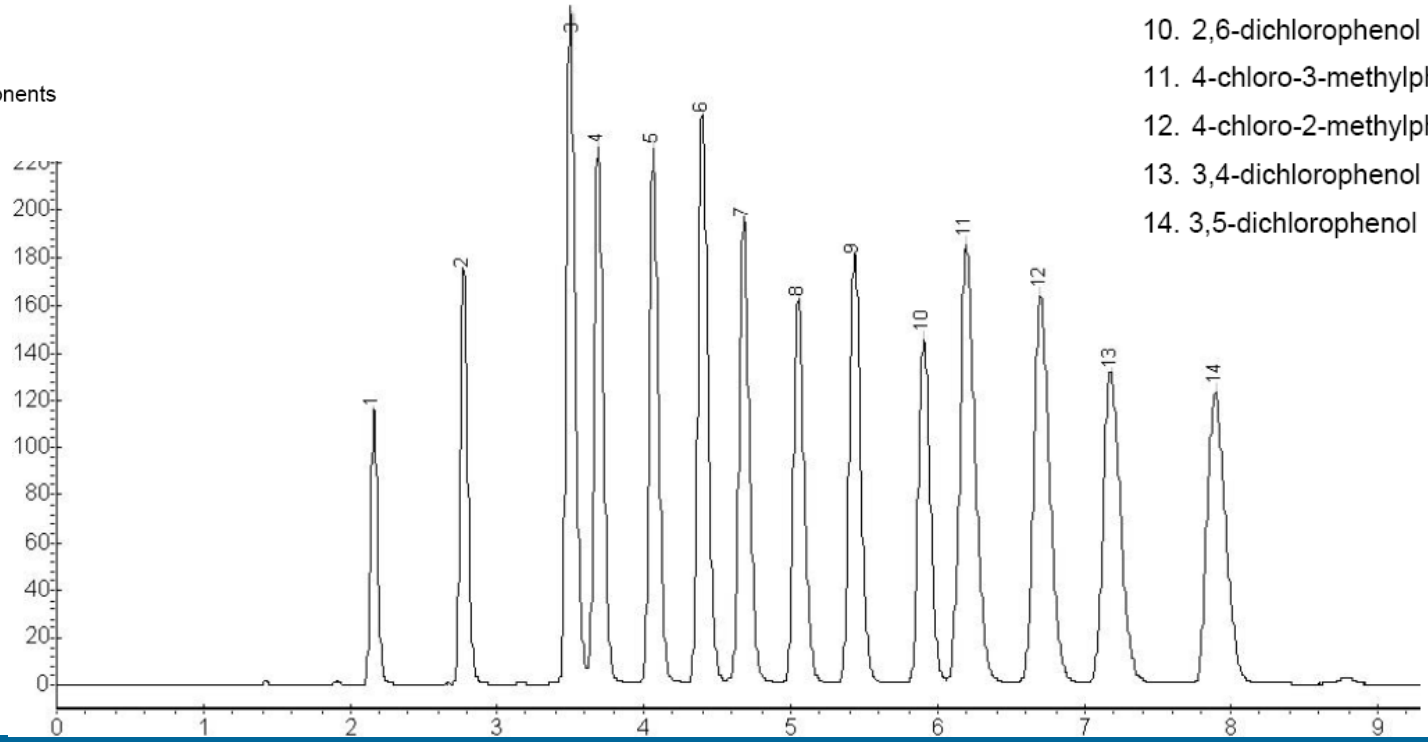
Detection : 254 nm

Injection Vol. : 5 µL

Sample conc. : 1 mg/mL all components

Solvent sample : Water

1. 3,4-dimethoxyphenol
2. 2,6-dimethoxyphenol
3. 3,5- dimethoxyphenol
4. 2,6-difluorophenol
5. 2,4-difluorophenol
6. 2,3-difluorophenol
7. 3,4 difluorophenol
8. 3,5-dimethylphenol
9. 2,6-dimethylphenol
10. 2,6-dichlorophenol
11. 4-chloro-3-methylphenol
12. 4-chloro-2-methylphenol
13. 3,4-dichlorophenol
14. 3,5-dichlorophenol

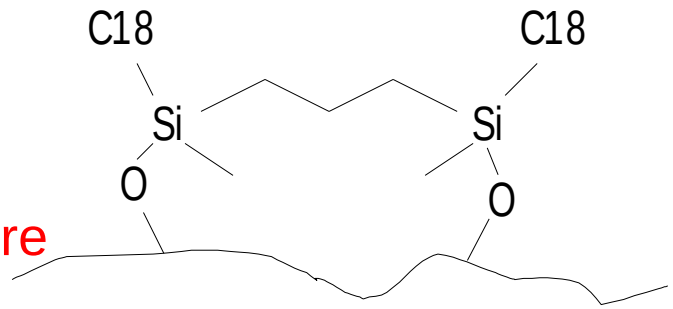


Agilent Technologies

Extend-C18 Originally Designed for High pH Stability

- Patented bidentate C18-C18 bonding for superior high pH stability – up to pH 11.5
- Improved performance over polymeric columns
- Excellent peak shape with double endcapping
- Unique Bonded Surface Improves Interaction with Polar Compounds

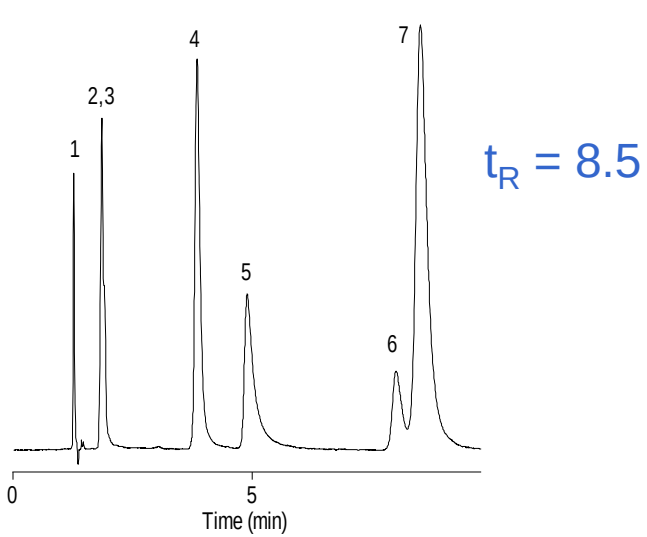
Extend-C18
Bidentate Structure



High pH Increases Retention of Antihistamines

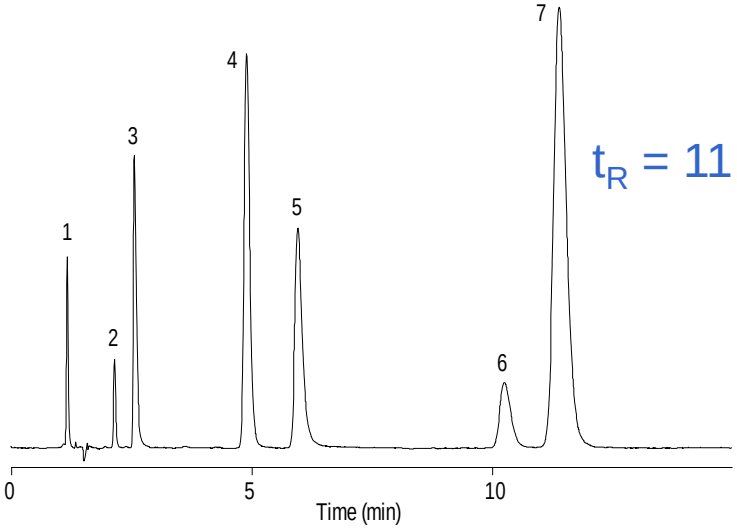
1. Maleate 2. Scopolamine 3. Pseudoephedrine 4. Doxylamine 5. Chlorpheniramine 6. Triprolidine 7. Diphenhydramine

pH 7
30% 20 mM Na_2HPO_4
70% MeOH



t_R = 8.5

pH 11
30% 20 mM TEA
70% MeOH



t_R = 11.4

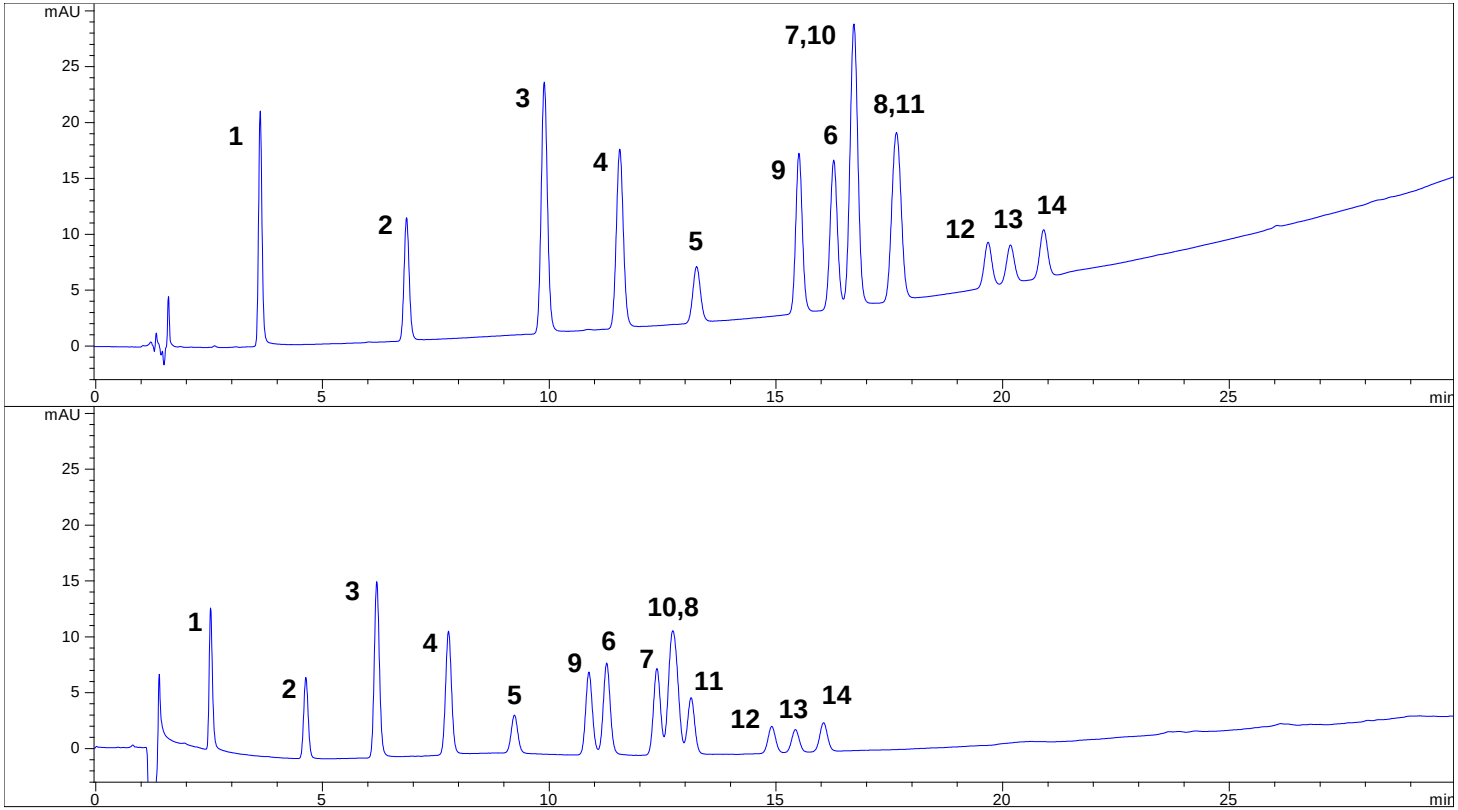
The retention of this sample of basic compounds increases at high pH.

Extend is Useful for More than High pH Separation

- Phase structure is very different from Standard Monomeric and Polymeric C18 phases
- Bi-dentate Bonding adds structure to the phase and causes wider spacing of C18 molecules on surface
- Polar Molecules interact differently with Extend phase structure and give different selectivity

Typical C18 Separations of EPA 8330 Explosives

Densely Bonded Phases – Incomplete Resolution



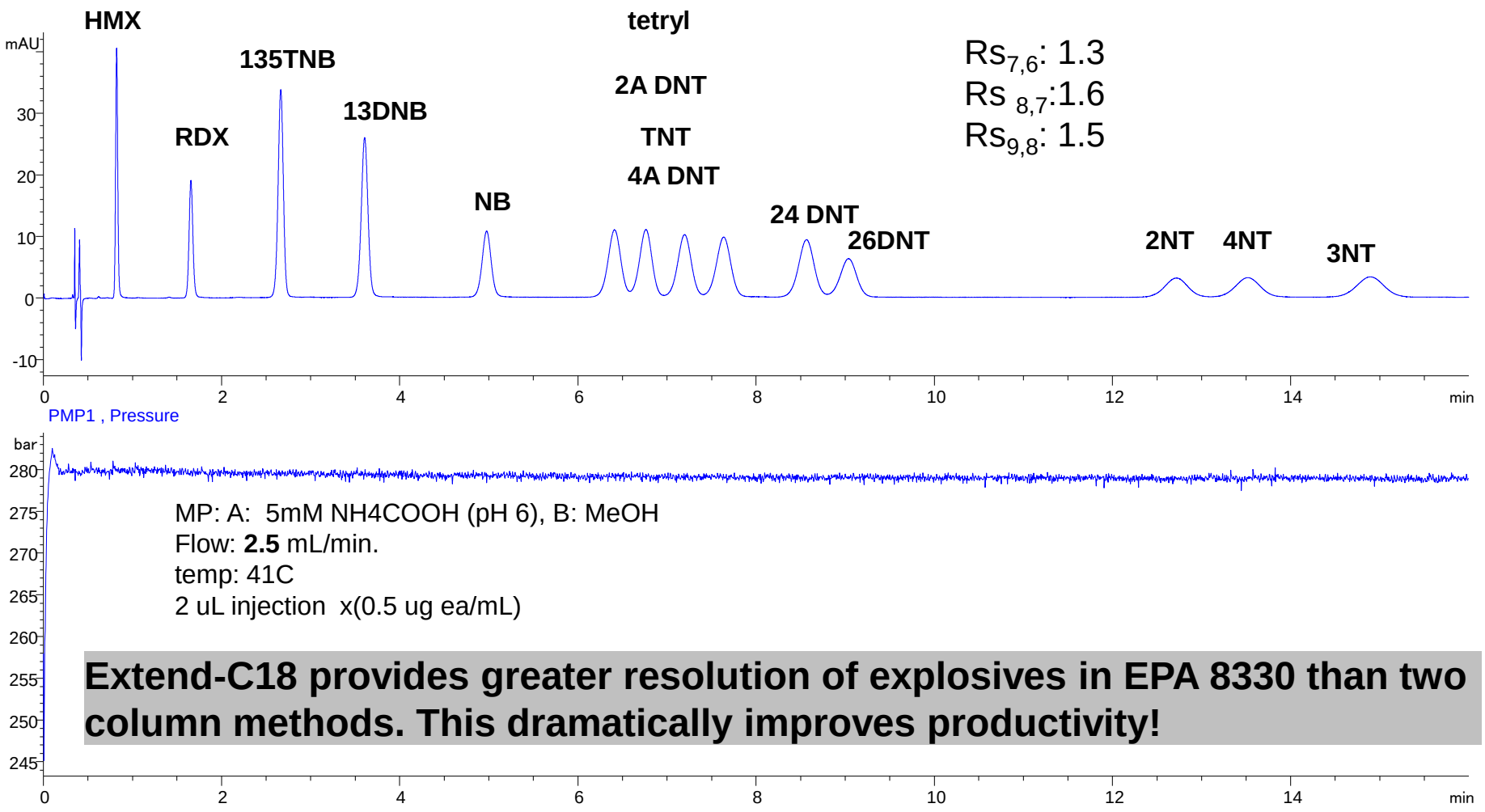
EXPLOSIVES
(5ng/µL in ACN)

1. HMX
2. RDX
3. 1,3,5-Trinitrobenzene
4. 1,3-Dinitrobenzene
5. Nitrobenzene
6. 2,4,6-Trinitrotoluene
7. 2-Amino -4,6-dinitrotoluene
8. 2,4-Dinitrotoluene
9. Tetryl
10. 4-Amino -2,6-dinitrotoluene
11. 2,6-Dinitrotoluene
12. 2-Nitrotoluene
13. 4-Nitrotoluene
14. 3-Nitrotoluene

Sample: EPA8330 Explosives (5ng/µL each); **Injection:** (4µL);
Columns: Zorbax Eclipse XDB-C18, 4.6 x 100mm, 3.5µm, (P/N:961967-902); Hypersil BDS-C18 (4.0 x 150mm, 3µm)
Mobile Phase: A=H2O, B=Methanol; **Gradient:** 26–40%B in 10min, 40–55%B in 10min, 55–70%B in 10min, 70–26%B in 1min; Total=31min.
Flow rate: 0.72 mL/min; **Temperature:** 38°C; **Detection:** UV (Sig=235,40nm, Ref=360,100nm)

Extend C18-Simpler Isocratic Method with Better Resolution in Less Time

Run on any LC – pressure only 280 bar!!



$Rs_{7,6}$: 1.3
$Rs_{8,7}$:1.6
$Rs_{9,8}$: 1.5

MP: A: 5mM NH4COOH (pH 6), B: MeOH
Flow: **2.5** mL/min.
temp: 41C
2 uL injection x(0.5 ug ea/mL)

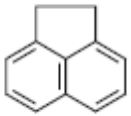
Extend-C18 provides greater resolution of explosives in EPA 8330 than two column methods. This dramatically improves productivity!

PAH Column

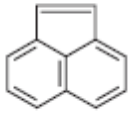
- **Engineered (Proprietary) Bonded Phase for Improved Resolution**
  - Improved Separation by Utilizing Molecular Shape Selectivity
- **Variety of Particle Sizes for Use in Traditional and Higher Pressure Instruments**
  - The 4.6 mm ID will use the most solvent.
  - The 3.0 mm ID will use 50% less solvent for the same analysis time.
- **Shorter columns with smaller particle sizes – 3.5um or 1.8um – allow for faster analyses for higher throughput and increased productivity.**

Example PAH Compounds – PAH's include Isomers that are Separated by Shape

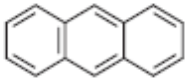
Acenaphthene



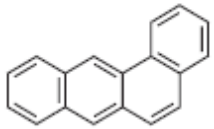
Acenaphthylene



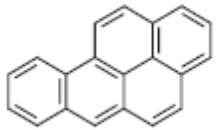
Anthracene



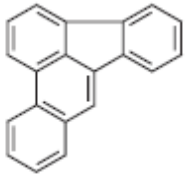
Benz(a)anthracene



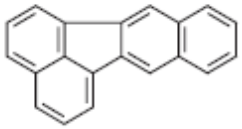
Benzo(a)pyrene



Benzo(b)flouranthene

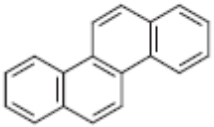


Benzo(k)flouranthene

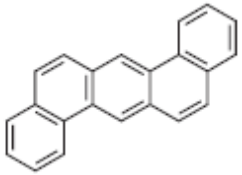


Benzo(g,h,i)perylene

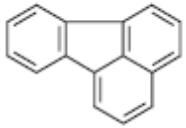
Chrysene



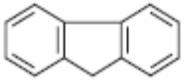
Dibenz(a,h)anthracene



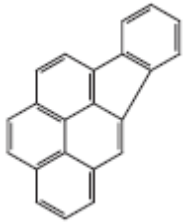
Fluoranthene



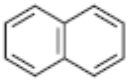
Fluorene



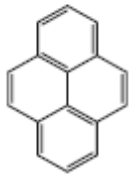
Indeno(1,2,3-c,d)pyrene



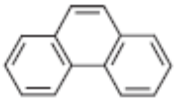
Naphthalene



Pyrene

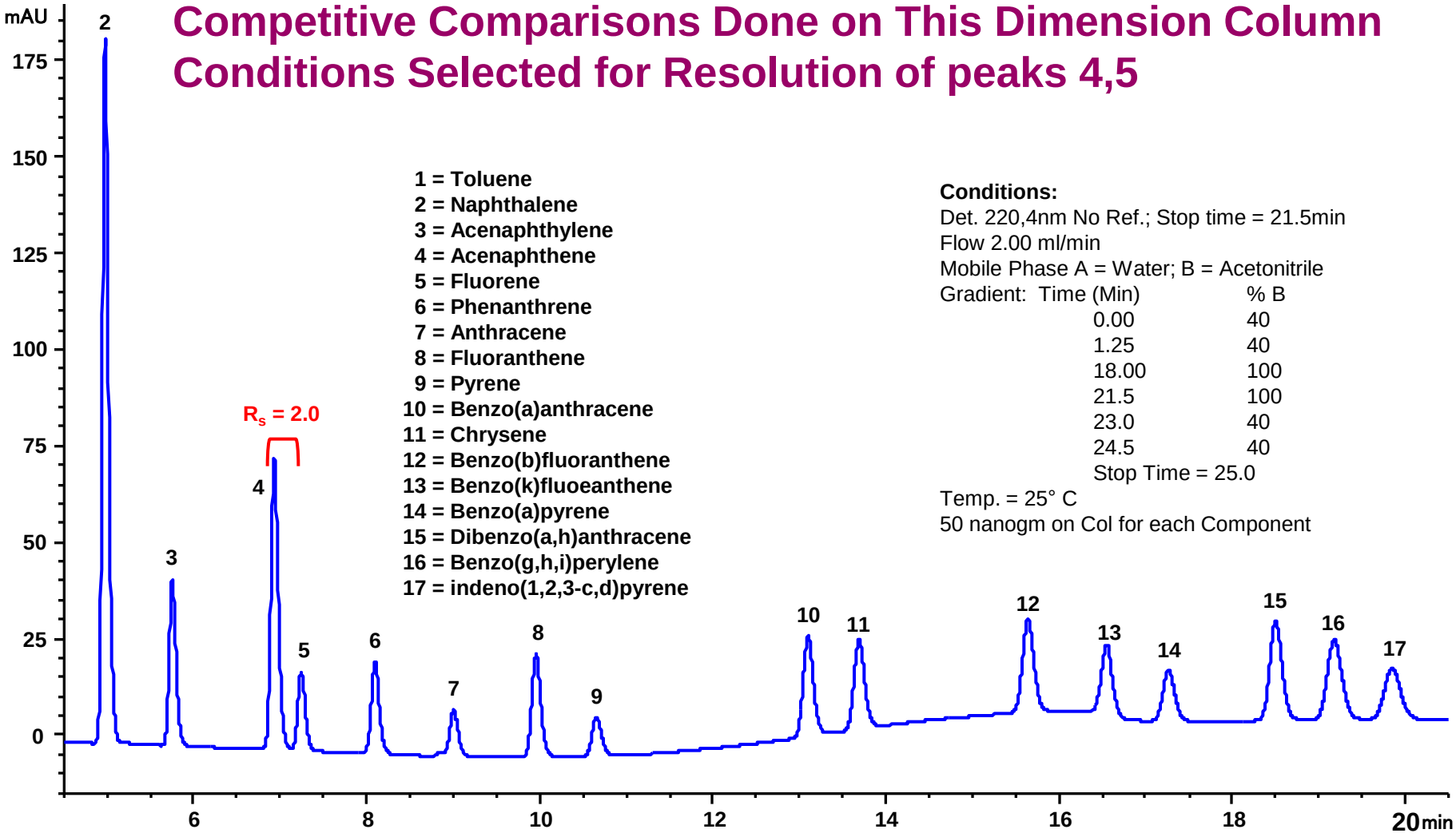


Phenanthrene



PAH 610 Mix on Eclipse PAH 4.6x150mm, 5.0µm Column – $R_s \geq 2.0$ for peaks 4,5

Competitive Comparisons Done on This Dimension Column Conditions Selected for Resolution of peaks 4,5



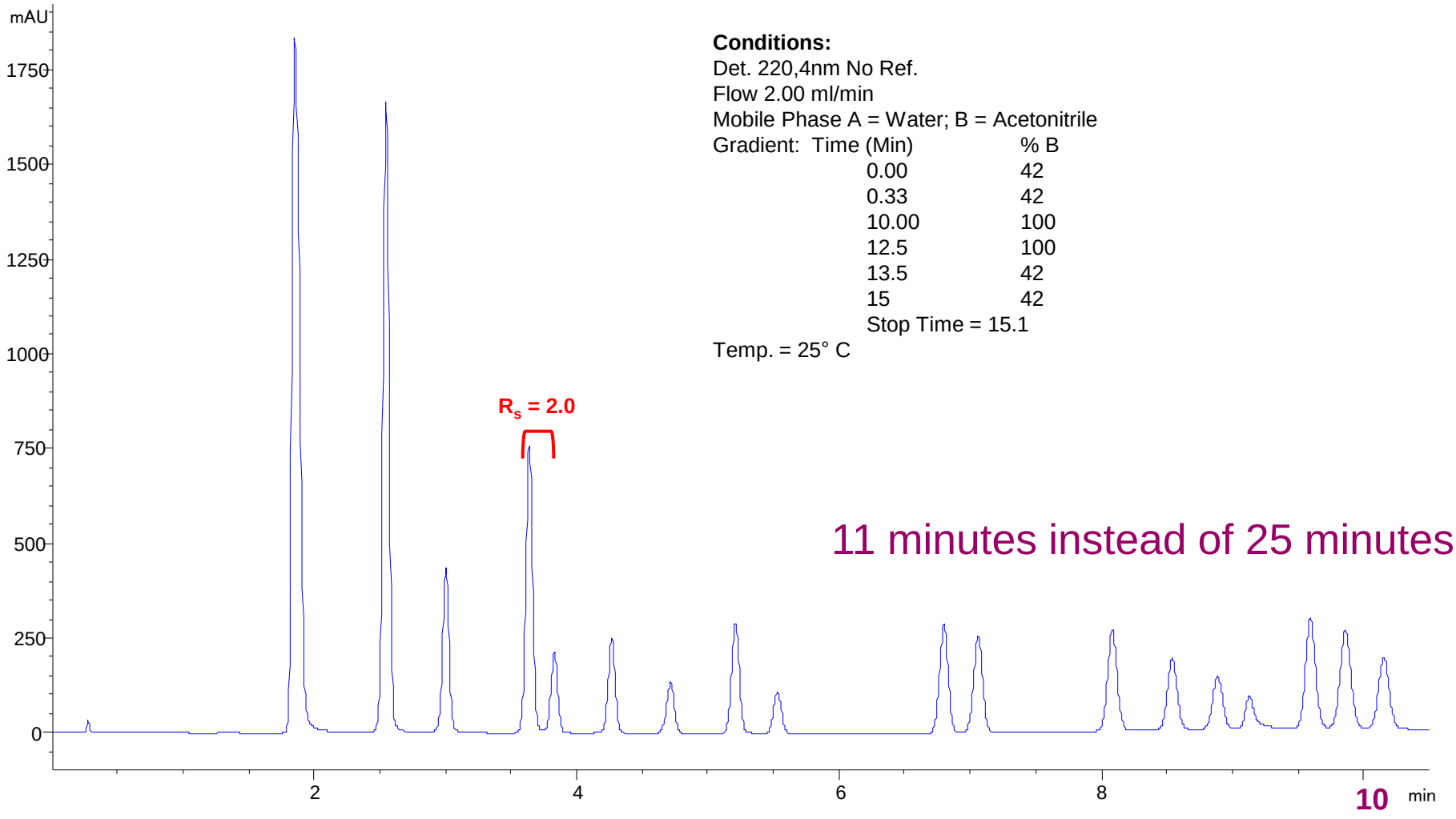
1 = Toluene
2 = Naphthalene
3 = Acenaphthylene
4 = Acenaphthene
5 = Fluorene
6 = Phenanthrene
7 = Anthracene
8 = Fluoranthene
9 = Pyrene
10 = Benzo(a)anthracene
11 = Chrysene
12 = Benzo(b)fluoranthene
13 = Benzo(k)fluoeanthene
14 = Benzo(a)pyrene
15 = Dibenzo(a,h)anthracene
16 = Benzo(g,h,i)perylene
17 = indeno(1,2,3-c,d)pyrene

Conditions:
Det. 220,4nm No Ref.; Stop time = 21.5min
Flow 2.00 ml/min
Mobile Phase A = Water; B = Acetonitrile
Gradient:

Time (Min)	% B
0.00	40
1.25	40
18.00	100
21.5	100
23.0	40
24.5	40

Stop Time = 25.0
Temp. = 25° C
50 nanogm on Col for each Component

PAH 610 Mix on 4.6x50mm, 3.5µm PAH Column – Fast with Excellent Resolution



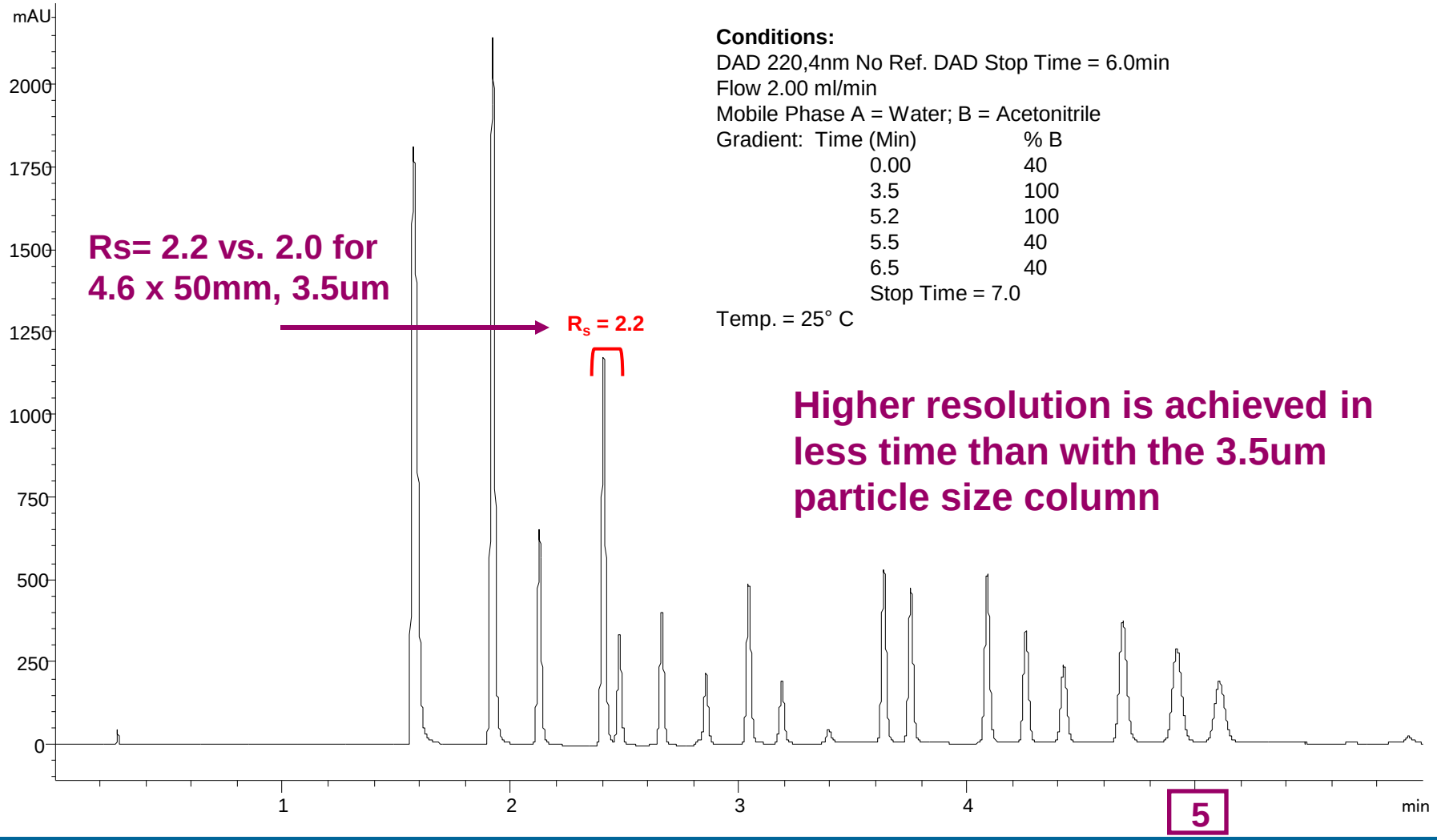
Conditions:
Det. 220,4nm No Ref.
Flow 2.00 ml/min
Mobile Phase A = Water; B = Acetonitrile

Gradient: Time (Min)	% B
0.00	42
0.33	42
10.00	100
12.5	100
13.5	42
15	42

Stop Time = 15.1
Temp. = 25° C

11 minutes instead of 25 minutes!

High Resolution and Fast Analysis on Rapid Resolution HT 4.6x50mm, 1.8µm Eclipse PAH Column



Conditions:
DAD 220,4nm No Ref. DAD Stop Time = 6.0min
Flow 2.00 ml/min
Mobile Phase A = Water; B = Acetonitrile

Gradient: Time (Min)	% B
0.00	40
3.5	100
5.2	100
5.5	40
6.5	40
Stop Time = 7.0	

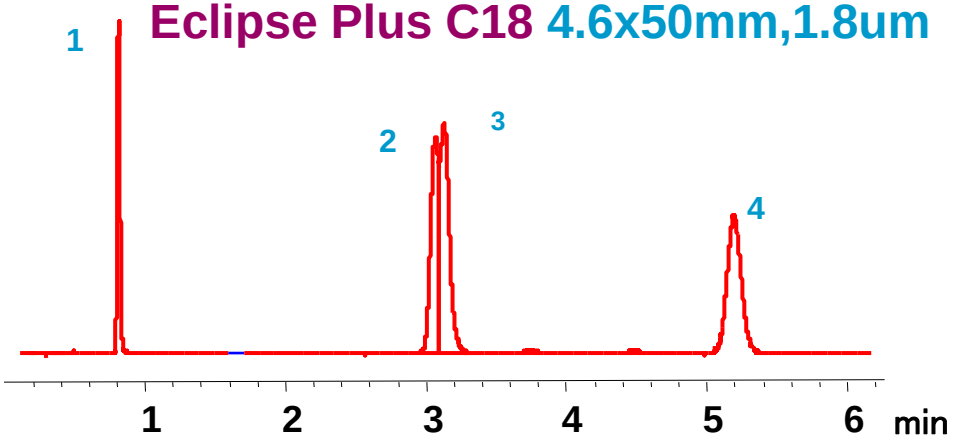
Temp. = 25° C

Higher resolution is achieved in less time than with the 3.5um particle size column

Vitamin D2/D3 - Use selectivity for the best potential for maximum resolution

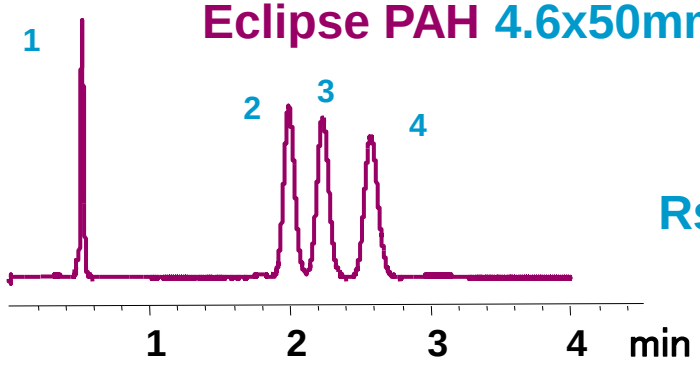
ZORBAX Rapid Resolution HT

Eclipse Plus C18 4.6x50mm,1.8um



ZORBAX Rapid Resolution HT

Eclipse PAH 4.6x50mm,1.8um



$Rs_{2,3}$=1.60

1 Vitamin A

2 Vitamin D2

3 Vitamin D3

4 Vitamin E (a-VE)

Temperature: 40 °C

Mobile phase: 92% MeOH, 8% water

Flow rate: 2 ml/min

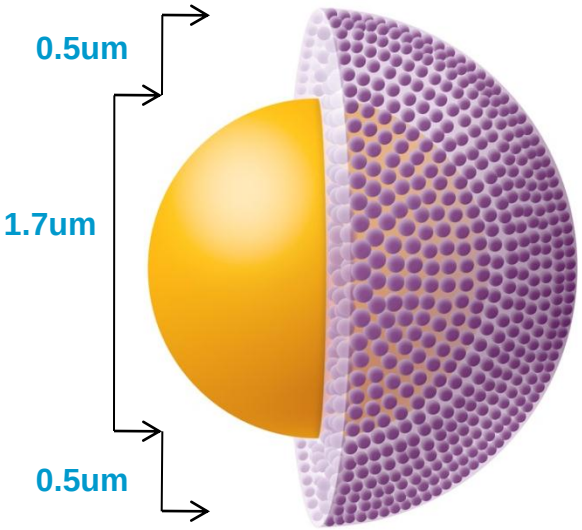
Wavelength: 325 nm for VA/280 nm for VD and VE

Poroshell 120 Columns for HPLC and UHPLC:

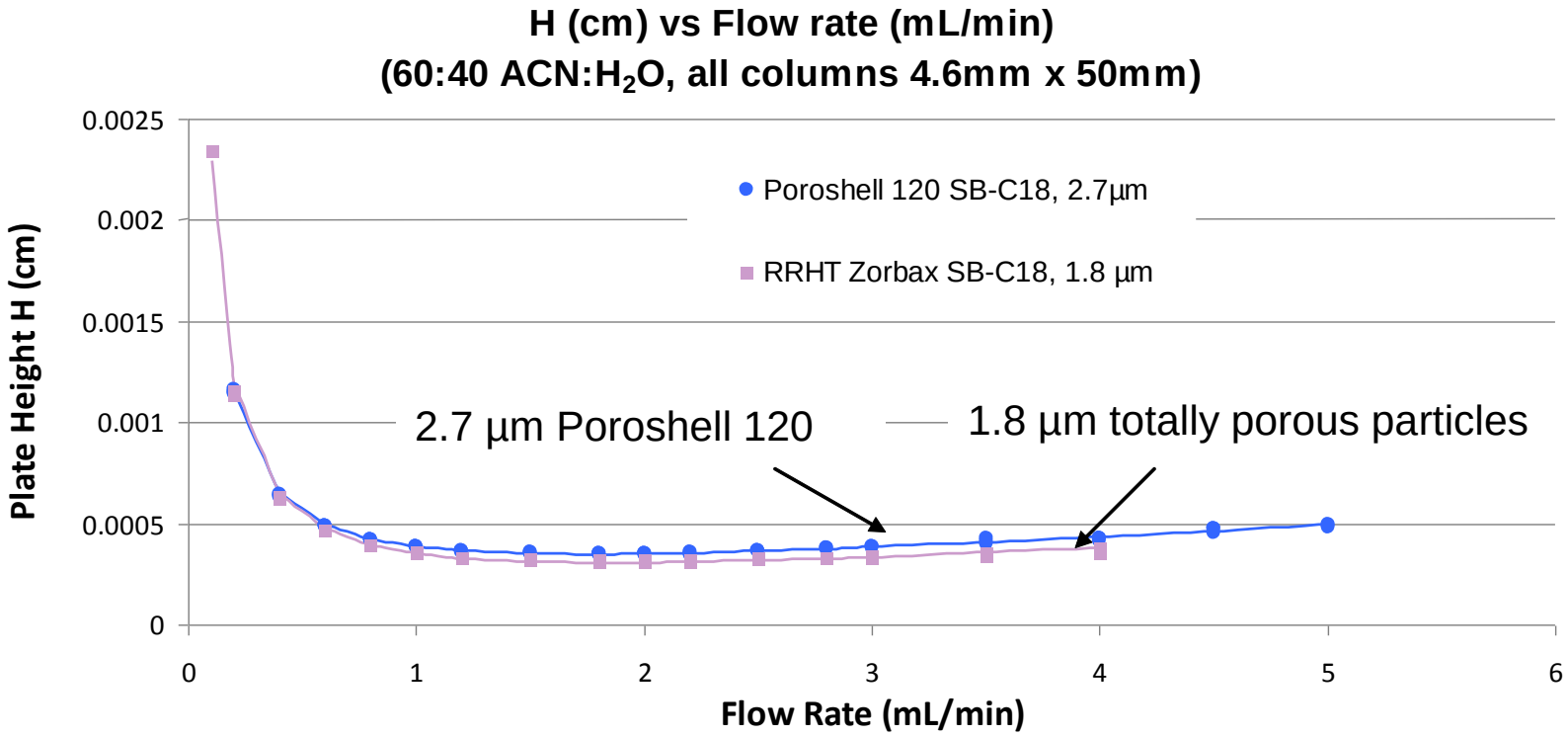
Poroshell 120 columns have:

- 80-90% efficiency of sub 2um
- At ~40-50% lower pressure
- 2X efficiency of 3.5um (totally porous)
- A 2.7um particle size
- A 2um frit to reduce clogging
- A 600 bar pressure limit

- The particle has a solid core (1.7um) and porous outer layer with a 0.5um diffusion path

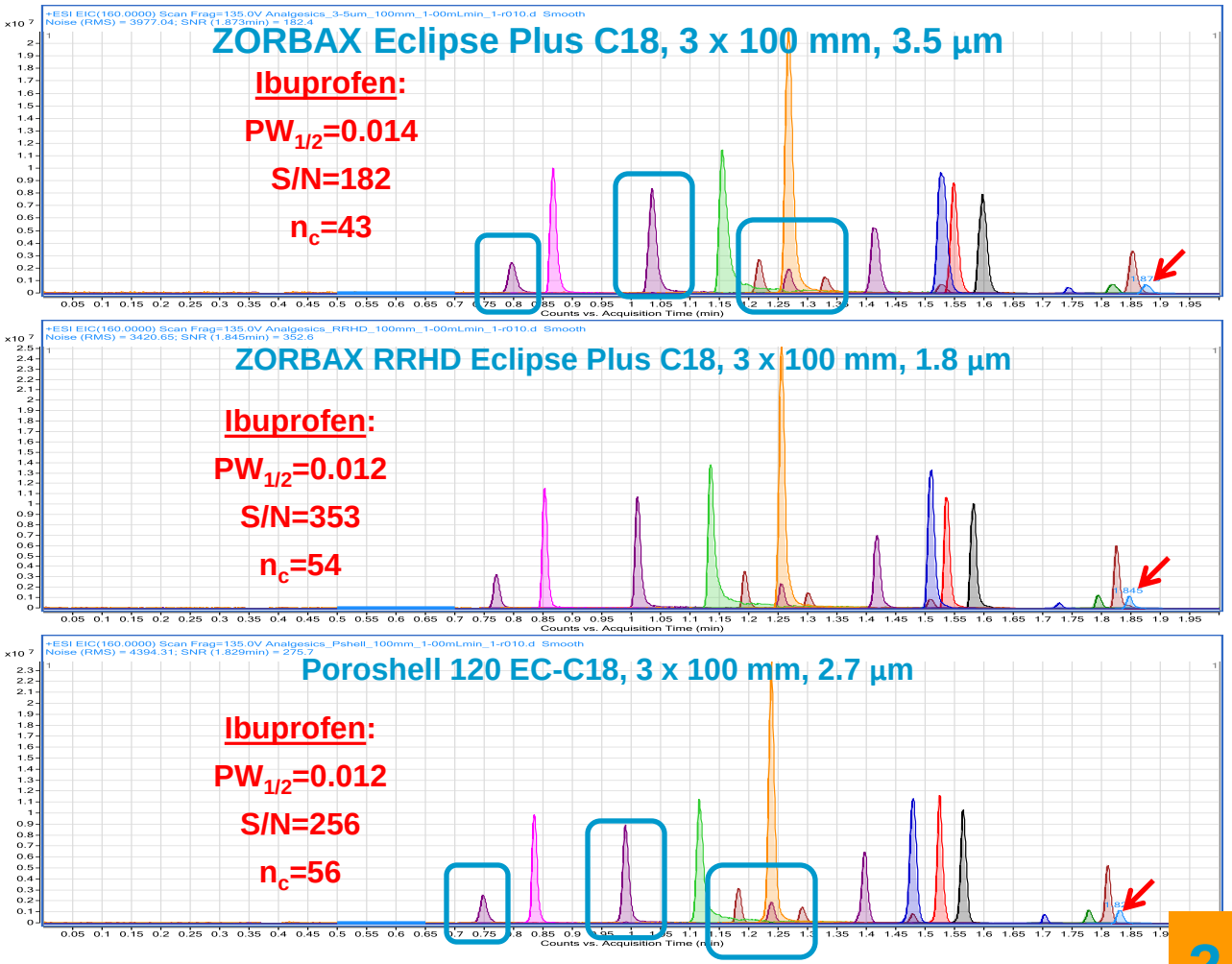


Similar Van Deemter Plot for Poroshell 120 and RRHT



Superficially porous particles and 1.8um particles have similar efficiencies and are both good choices for UHPLC.

Analysis of 15 Analgesic Compounds on 3.5um, 1.8um Totally Porous and 2.7um Superficially Porous Columns



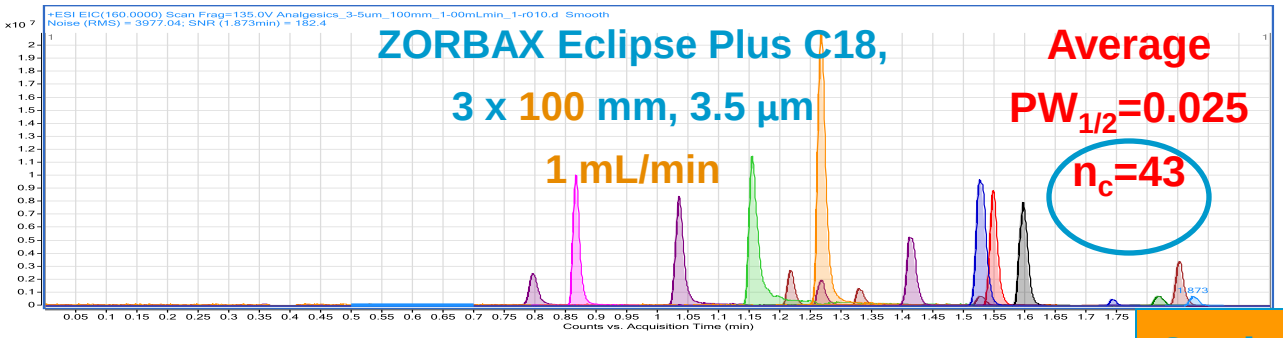
Same method can be used with all 3 columns due to similar selectivity

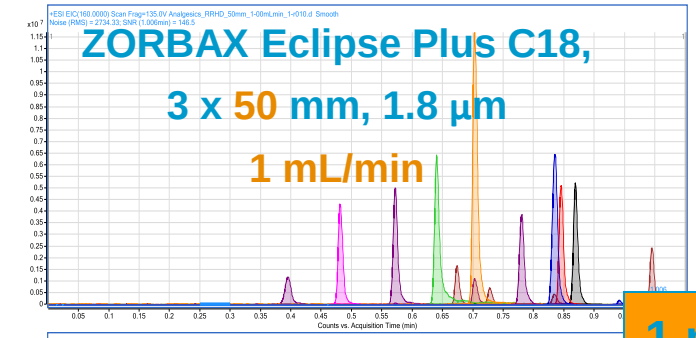
1.8 & 2.7 µm columns:

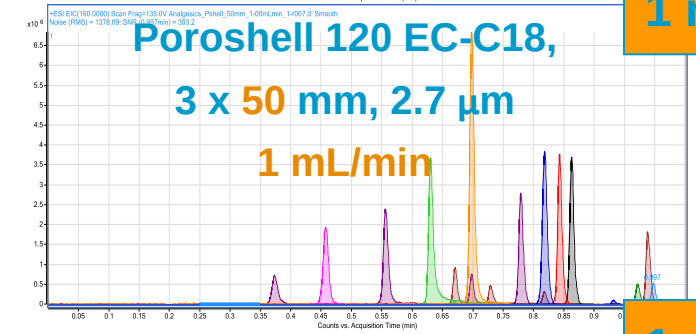
- taller, narrower peaks
- >40% more sensitivity, as noted by the S/N of ibuprofen
- Conditional peak capacity >20% higher than the 3.5 µm column

2 min

Shorter columns with smaller particles allows faster analyses, maintains resolution of 15 analgesic compounds





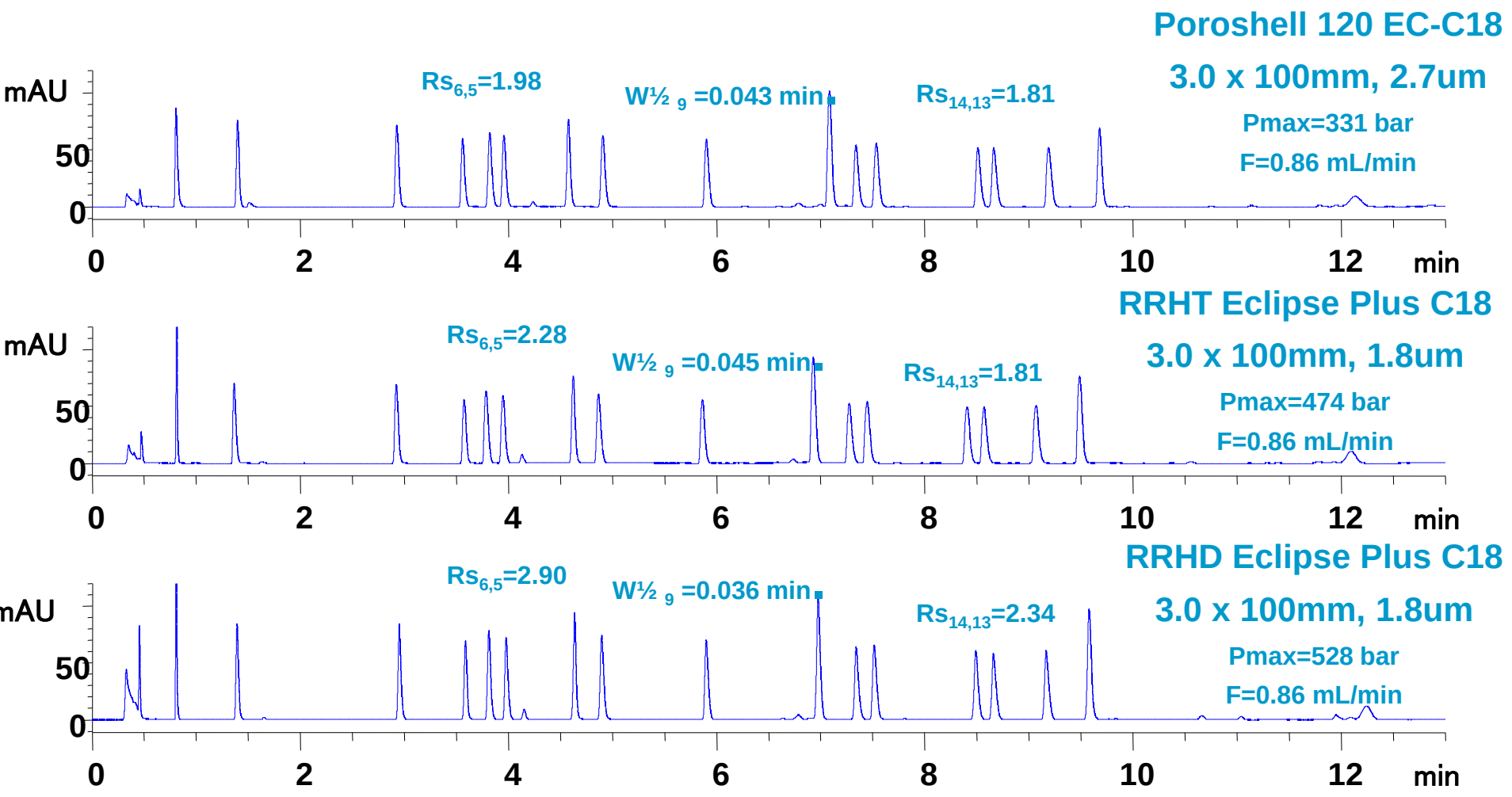


Conditional peak capacity same for: 50 mm 1.8 & 2.7 µm columns and 100 mm 3.5 µm column in half the analysis time,

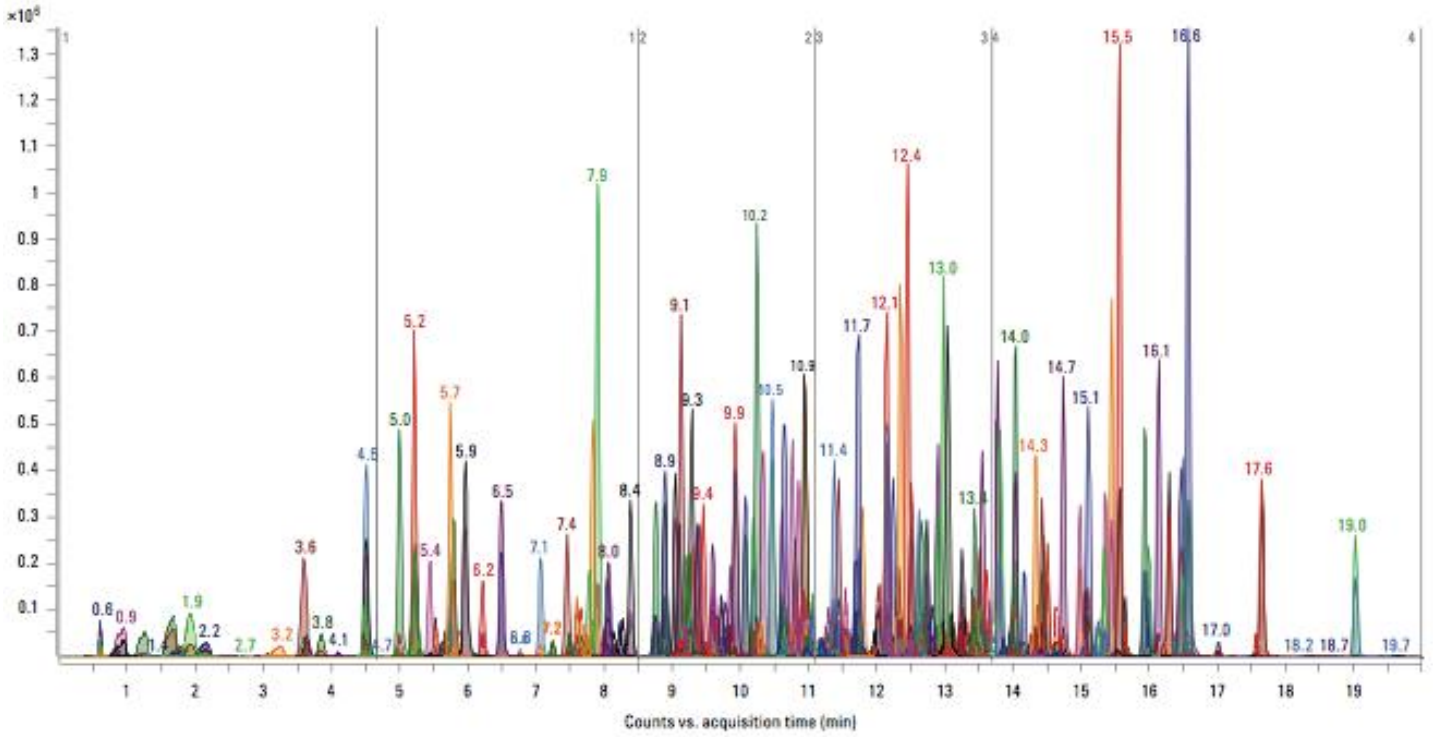
100-mm gradient:
15-95% CH_3CN in 1.5 min
Stop Time: 2 min

50-mm gradient:
15-95% CH_3CN in 0.75 min
Stop Time: 1.05 min

Sub 2um vs. Poroshell 120
17 Amino Acid Analysis on 1290 Infinity



300 Pesticides < 20 minutes, 1290 Infinity



Eclipse Plus C18 2.1 mm × 100 mm, 1.8 µm column at a flow rate of 0.5 mL/min.

A = 5 mM acetic acid in water B = 100% acetonitrile, Gr= 5-95% B

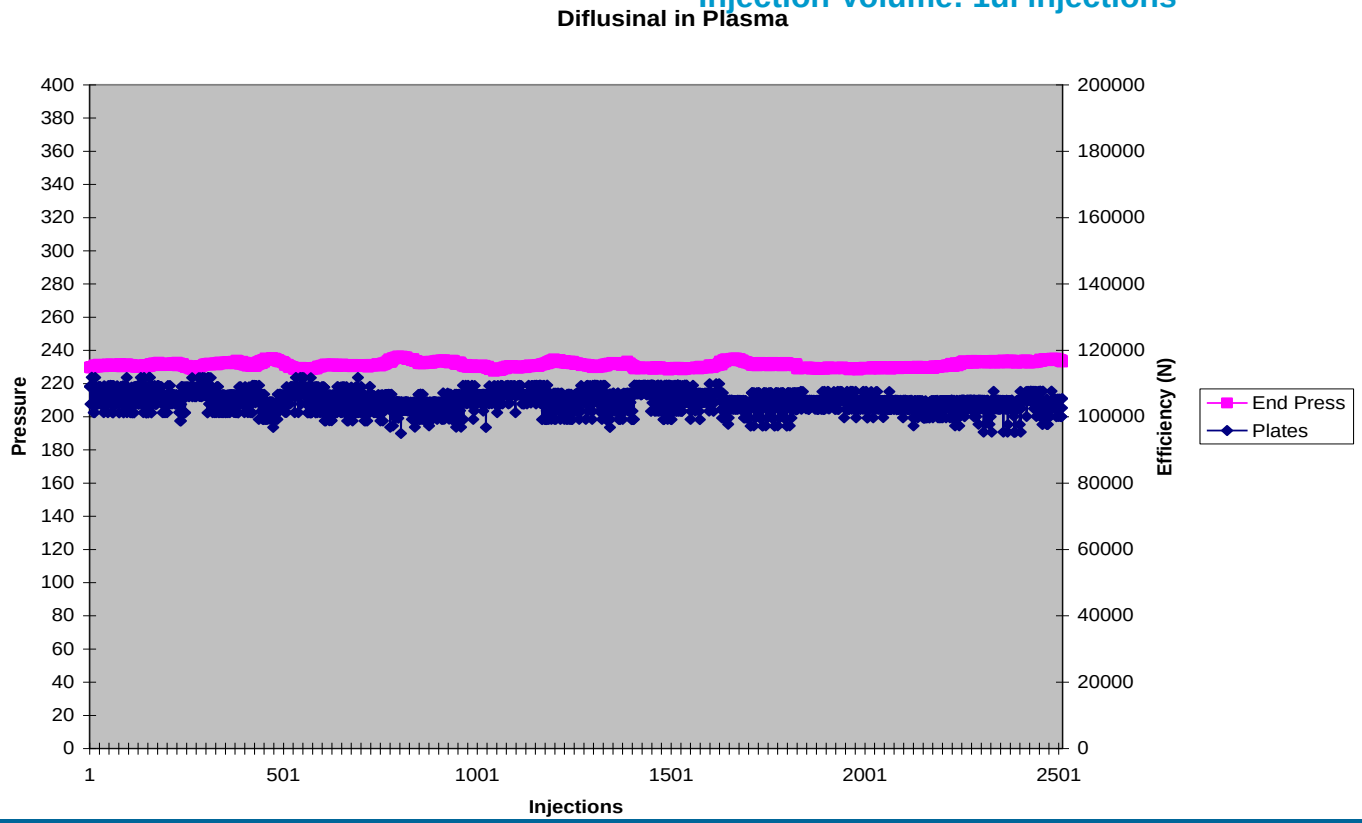
Poroshell 120 Resists Plugging with 2 um Frit Challenging Samples - Plasma

Column: Poroshell 120 EC-C18, 3.0 x 50mm, 2.7um LC: Agilent 1200 RRLC (SL)

Sample: Precipitated Plasma: 2 parts Plasma: 7 Parts 20/80 Water-MeCN w/0.1 % Formic Acid with 1 Part Diflusinal in 50/50 Water-MeCN 10 ug/ml (Final concentration Diflusinal 1 ug/ml) Shaken and allowed to settle 10 minutes

Not Centrifuged/ Not Filtered

Injection Volume: 1ul injections



Solvent A: Water w/0.1 % TFA

Solvent B: MeCN w/0.08 % TFA

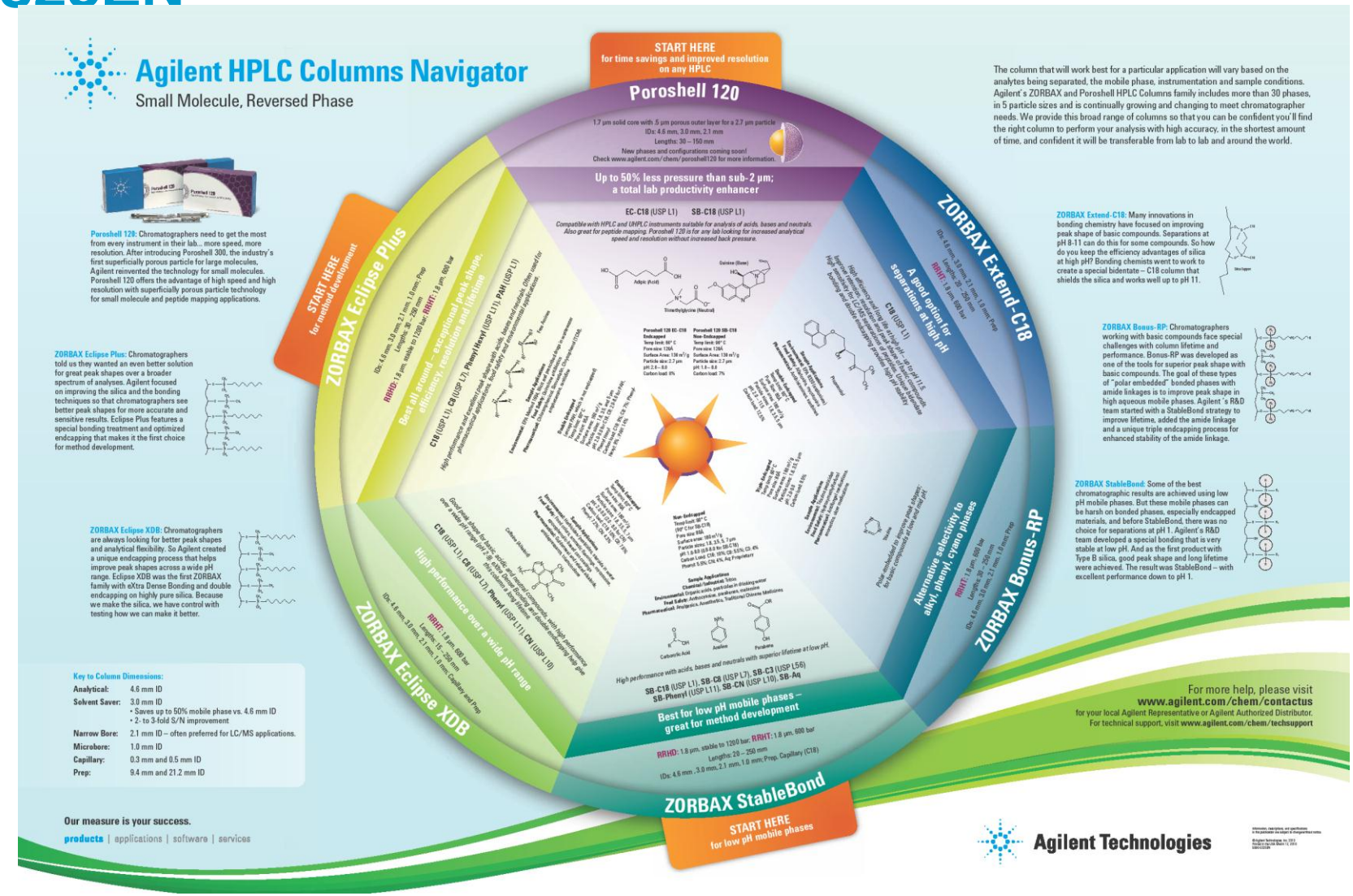
Flow Rate 1 ml/min 1 ul injection

Time	% B
0	20
0.5	90
0.6	90
1.1	20
2.5	20

Summary

- **Optimized Particle Chemistry Improves Reproducibility**
- **Bonded Phase Chemistry and Surface Structure Can Alter and Improve Sample Interaction**
- **Improving Sample Interaction Can Improve Resolution and Speed**
- **Tailoring Column and Particle Dimensions to Suite Laboratory Needs Can Further Improve Resolution and Speed**

HPLC Columns Navigator Poster – pub no. 5990-5325EN



Agilent Technologies

Benefits of Having a Range of Selectivities

Selectivity is the most powerful tool in achieving resolution

Achieve optimal resolution for any separation

Optimal bonded phase can minimize analysis time and improve productivity

Solvent change can change selectivity as well

Change separation mechanisms as needed

- i.e. Reversed-phase vs. HILIC

Eclipse PAH Columns – Part Numbers and Descriptions

Part Number	Description
959990-918	ZORBAX Eclipse Plus PAH, 4.6 x 250mm, 5um
959993-918	ZORBAX Eclipse Plus PAH, 4.6 x 150mm, 5um
959963-918	ZORBAX Eclipse Plus PAH 4.6 x 150mm, 3.5um
959996-918	ZORBAX Eclipse Plus PAH, 4.6 x 100mm, 5um
959961-918	ZORBAX Eclipse Plus PAH, 4.6 x 100mm, 3.5um
959964-918	ZORBAX Eclipse Plus PAH, 4.6 x 100mm, 1.8um
959943-918	ZORBAX Eclipse Plus PAH, 4.6 x 50mm, 3.5um
959941-918	ZORBAX Eclipse Plus PAH, 4.6 x 50mm, 1.8um
959931-918	ZORBAX Eclipse Plus PAH, 4.6 x 30mm, 1.8um
959990-318	ZORBAX Eclipse Plus PAH, 3.0 x 250mm, 5um
959790-918	ZORBAX Eclipse Plus PAH, 2.1 x 250mm, 5um
959701-918	ZORBAX Eclipse Plus PAH, 2.1 x 150mm, 5um
959763-918	ZORBAX Eclipse Plus PAH, 2.1 x 150mm, 3.5um
959793-918	ZORBAX Eclipse Plus PAH, 2.1 x 100mm, 3.5um
959764-918	ZORBAX Eclipse Plus PAH, 2.1 x 100mm, 1.8um
959741-918	ZORBAX Eclipse Plus PAH, 2.1 x 50mm, 1.8um
820950-939	ZORBAX Eclipse Plus PAH Guard, 4.6 x 12.5, 5um (4 pk)
821125-939	ZORBAX Eclipse Plus PAH Guard, 2.1 x 12.5, 5um (4 pk)

- **These part number choices represent those that are typically used for PAH analysis**
- **2.1 and 3.0 x 250mm columns are included, as well as exciting new 1.8um choices.**
- **The 4.6 x 30mm, 1.8um column makes the perfect choice for PAH screening as required in the EU.**
- **Other dimensions can be made available as needed.**

1.8u Column Available

Dimensions	Eclipse Plus C18	Eclipse Plus C8	Eclipse Plus Ph-Hex	Eclipse PAH	Eclipse XDB C18	Eclipse XDB C8	Extend-C18
4.6 x 150	959994-902						
4.6 x 100	959964-902	959964-906	959964-912	959964-918	928975-902	928975-906	728975-902
4.6 x 75	959951-902						
4.6 x 50	959941-902	959941-906	959941-912	959941-918	927975-902	927975-906	727975-902
4.6 x 30	959931-902	959931-906	959931-912	959931-918	924975-902	924975-906	724975-902
4.6 x 20					926975-902	926975-906	726975-902
3.0 x 150	959994-302						
3.0 x 100	959964-302	959964-306	959964-312		928975-302	928975-306	728975-302
3.0 x 50	959941-302	959941-306	959941-312		927975-302	927975-306	727975-302
3.0 x 30					924975-302	924975-306	724975-302
3.0 x 20					926975-302	926975-306	726975-302
2.1 x 150	959794-902						
2.1 x 100	959764-902	959764-906	959764-912	959764-918	928700-902	928700-906	728700-902
2.1 x 50	959741-902	959741-906	959741-912	959741-918	927700-902	927700-906	727700-902
2.1 x 30	959731-902	959731-906	959731-912		924700-902	924700-906	724700-902
2.1 x 20					926700-902	926700-906	726700-902

Dimensions	SB-C18	SB-C8	SB-Phenyl	SB-CN	SB-AQ	Rx-Sil	Bonus-RP
4.6 x 150	829975-902	829975-906	829975-912	829975-905	829975-914		
4.6 x 100	828975-902	828975-906	828975-912	828975-905	828975-914	828975-901	828668-901
4.6 x 75							830668-901
4.6 x 50	827975-902	827975-906	827975-912	827975-905	827975-914	827975-901	827668-901
4.6 x 30	824975-902	824975-906	824975-912	824975-905	824975-914		
4.6 x 20	826975-902	826975-906					
3.0 x 150	829975-302	829975-306	829975-312	829975-305			
3.0 x 100	828975-302	828975-306	828975-312	828975-305	828975-314	828975-301	828668-301
3.0 x 50	827975-302	827975-306	827975-312	827975-305	827975-314	827975-301	827668-301
3.0 x 30	824975-302	824975-306		824975-305			
3.0 x 20	826975-302	826975-306					
2.1 x 150	820700-902	820700-906	820700-912	820700-905			
2.1 x 100	828700-902	828700-906	828700-912	828700-905	828700-914	828700-901	828768-901
2.1 x 50	827700-902	827700-906	827700-912	827700-905	827700-914	827700-901	827768-901
2.1 x 30	824700-902	824700-906	824700-912	824700-905	824700-914		
2.1 x 20	826700-902	826700-906					