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 ($\Delta L$) 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.

![Diagram](image-url)
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

Reversed-Phase Chromatography

StableBond
Diisopropyl
Diisobutyl bonding

SB-C18
Diisobutyl-C18

SB-CN
Diisopropyl-CN

Rx/SB-C8
Diisopropyl-C8

SB-Phenyl
Diisopropyl-Phenyl

Eclipse XDB
Dimethyl bonding
Double endcapping

Eclipse XDB-C18

Eclipse XDB-C8

Eclipse XDB-CN

Eclipse Plus
Dimethyl bonding
Double endcapping

Eclipse Plus C18

Eclipse Plus C8

Eclipse Plus Phenyl
Hexyl

Eclipse PAH

Extend-C18
Bidentate-C18
Double endcapping

Rx-C18
Dimethyloctadecylsilane

Varian
• Polaris
• Pursuit

Specialty products
Silica Particles Can Be Made by Different Methods

Silica Sol Aggregation

- 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 or suspension.

Xerogel
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 \(-\text{Si-O-Si}\)- 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
  – 400\(\mu\)mole/M\(^2\) for 80Angstrom Pore Particle
  – \(~180\)M\(^2\)/gram (\(~1.5\)gram 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

J.C. 268(1983) 1
J.C. 111(1975) 149

• Retention and selectivity can change dramatically when pH is changed.
Silica Particle Surface Chemistry

Non-Ideal Surface Re-Hydrolysis  Ideal Surface State

\[ \text{OH} \quad \text{HO} \quad \text{OH} \]
\[ \quad \text{Si} \quad \text{Si} \quad \text{Si} \]

Free Silanols  Geminol Silanols  Associated Silanols

decreasing acidity

\[ \text{+M} \quad \text{+M} \]
\[ \quad \text{Si} \quad \text{Si} \]

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

\[
\begin{align*}
\text{Si} & \quad \text{OH} \\
& + \\
& \quad \text{H}_3\text{C} - \text{CH}_3
\end{align*}
\]

\[
\begin{align*}
\text{Si} & \quad \text{X} - \text{Si} - \text{R} \\
& + \\
& \quad \text{H}_3\text{C} - \text{CH}_3
\end{align*}
\]

\[
\begin{align*}
& \quad \text{Si} - \text{O} - \text{Si} - \text{R} \\
& \quad \text{H}_3\text{C} - \text{CH}_3
\end{align*}
\]

- 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)

Eclipse PAH 4.6 x 100, 3.5 um
Column PAC1000, Batch NPA0737001

Column PAC1002, Batch B07060
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)fluoreanthene
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

Mobile Phase: 65% ACN: 35% 25 mM phosphate buffer (pH 7.4)

Eclipse Plus C18, 4.6 x 50mm

Tf = 1.20

Competitive C18, 4.6 x 50mm

Tf = 4.86

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

- Poroshell 120  Tf = 1.48
- Ascentis Express  Tf = 2.68
- Kinetex  Tf = 4.78
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

Selectivity Impacts Resolution Most
- Change bonded phase
- Change mobile phase

\[ R_s = \frac{N^{1/2}}{4} \cdot \frac{(\alpha-1)}{\alpha} \cdot \frac{k'}{(k'+1)} \]

<table>
<thead>
<tr>
<th>Plates:</th>
<th>5000</th>
<th>10000</th>
<th>15000</th>
<th>20000</th>
<th>25000</th>
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<tbody>
<tr>
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<td>1.10</td>
<td>1.35</td>
<td>1.60</td>
<td>1.85</td>
<td>2.1</td>
</tr>
<tr>
<td>k':</td>
<td>2.0</td>
<td>4.5</td>
<td>7.0</td>
<td>9.5</td>
<td>12.0</td>
</tr>
</tbody>
</table>
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

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

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

Rs 4,5=1.96
Rs 4,5=2.59
Rs 4,5=3.29

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

*Note: Diagrams of surface structures for each phase*
All C18 Phases Do Not Yield the Same Selectivity
Different Surface Chemistry/Structure—Different Selectivity!

Eclipse Plus C18

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

StableBond SB-C18

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

Eclipse XDB-C18

Extend-C18
Bonded Phase Surface Structure Alters Selectivity
Identical Silica, Different Phase Structure)

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

\[
\text{H}_2\text{N} \quad \text{COO(CH}_2\text{)}_3\text{N(C}_4\text{H}_9)_2
\]

Cinchonine

\[
\text{CH}_3 \quad \text{CH} \\
\text{N} \quad \text{H} \\
\text{CH}_3
\]

Lidocaine

\[
\text{CH}_3 \quad \text{NHOCH}_2\text{N(C}_2\text{H}_5)_2
\]

Procaine

\[
\text{H}_2\text{N} \quad \text{COOOCH}_2\text{CH}_2\text{N(C}_2\text{H}_5)_2
\]

Tetracaine

\[
\text{CH}_3\text{(CH}_2\text{)}_3\text{NH} \quad \text{COOOCH}_2\text{CH}_2\text{N(CH}_3)_2
\]
Selectivity Is Different, But No Gain in Speed or Overall Resolution

Eclipse XDB-CN

Eclipse XDB-Phenyl

Eclipse XDB-C8

Eclipse XDB-C18

Dimensions: 4.6x150mm, 5µm  Mobile Phase: 100% B in 18.8 min; A: 95:5 50mM NaH₂PO₄, pH 2.5:ACN; B: 47:53 50mM NaH₂PO₄, 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

- **SB-C18**
- **SB-C8**
- **SB-C3**
- **SB-Phenyl**
- **SB-CN**

- Columns: 4.6 x 250 mm
- Mobile Phase:
  - 0 - 100% B in 18.8 min
  - A: 50 mM NaH₂PO₄,
    - pH 2.5 in 95% H₂O/5% ACN
  - B: 50 mM NaH₂PO₄,
    - pH 2.5 in 47% H₂O/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% NaH₂PO₄, 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: NaH₂PO₄, pH 2.5
Flow Rate: 1.0 mL/min
Temperature: RT
Detection: UV 254 nm

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

Eclipse Plus C18
3 x 100 mm 3.5 um

Eclipse Plus Phenyl Hexyl
3 x 100 mm 3.5 um

Bonus RP
3 x 100 mm 3.5 um

G6240 TOF
1200 SL

June, 2011
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

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Salicylic acid</th>
<th>Coelution</th>
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<tbody>
<tr>
<td>20°C</td>
<td>20°C</td>
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<td>30°C</td>
<td>30°C</td>
<td>30°C</td>
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<td>40°C</td>
<td>40°C</td>
<td>40°C</td>
</tr>
<tr>
<td>60°C</td>
<td>60°C</td>
<td>60°C</td>
</tr>
<tr>
<td>90°C</td>
<td>90°C</td>
<td>90°C</td>
</tr>
</tbody>
</table>

Column: RRHT SB-C18
4.6 x 50mm, 1.8um
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
Antifungals on Pursuit XRs DP and C18

Columns: Listed on chromatograms
Dimensions: 150 x 4.6 mm, 5µ (all columns)
Mobile Phase: A: H₂O + 0.1% HCOOH, B: CH₃CN + 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 DP

Pursuit XRs C18
Pursuit PFP

- Novel selectivity – alternative to traditional C8/C18 as well as \( \pi-\pi \) /hydrophobic mechanism of DP
- Selectivity based on \( \pi-\pi \), 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
Separation of Nucleotides and Nucleosides on Pursuit PFP under high aqueous conditions

Column: Pursuit PFP
Dimensions: 150 x 4.6 mm, 5µ
Mobile Phase: H₂O + 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, 5 μm; Part Nr. A3050150X046
Mobile Phase: A: Water +0.1% Formic Acid
              B: Acetonitrile
Gradient:

<table>
<thead>
<tr>
<th>Time</th>
<th>%B</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>15</td>
<td>60</td>
</tr>
</tbody>
</table>

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
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

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!!

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

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

<table>
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<tr>
<th>Time (Min)</th>
<th>% B</th>
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<td>0.33</td>
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<tr>
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<tr>
<td>13.5</td>
<td>42</td>
</tr>
<tr>
<td>15</td>
<td>42</td>
</tr>
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</table>

Stop Time = 15.1

Temp. = 25°C

\[ R_s = 2.0 \]

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
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<td>5.2</td>
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<td>5.5</td>
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<td>Stop Time = 7.0</td>
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</table>
Temp. = 25°C
Rs = 2.2

Rs = 2.2 vs. 2.0 for 4.6 x 50mm, 3.5µm

Higher resolution is achieved in less time than with the 3.5µm particle size column
Vitamin D2/D3 - Use selectivity for the best potential for maximum resolution

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

Rs₂₃ = 1.60
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 1.8µm particles have similar efficiencies and are both good choices for UHPLC.
Analysis of 15 Analgesic Compounds on 3.5µm, 1.8µm Totally Porous and 2.7µm 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
Shorter columns with smaller particles allows faster analyses, maintains resolution of 15 analgesic compounds.

**ZORBAX Eclipse Plus C18**, 3 x 100 mm, 3.5 µm, 1 mL/min
- Average PW <sub>1/2</sub> = 0.025
- n<sub>c</sub> = 43

**ZORBAX Eclipse Plus C18**, 3 x 50 mm, 1.8 µm, 1 mL/min
- Average PW <sub>1/2</sub> = 0.0092
- n<sub>c</sub> = 39

**Poroshell 120 EC-C18**, 3 x 50 mm, 2.7 µm, 1 mL/min
- Average PW <sub>1/2</sub> = 0.0086
- n<sub>c</sub> = 43

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<sub>3</sub>CN in 1.5 min
- Stop Time: 2 min

50-mm gradient:
- 15-95% CH<sub>3</sub>CN in 0.75 min
- Stop Time: 1.05 min
Sub 2um vs. Poroshell 120
17 Amino Acid Analysis on 1290 Infinity

Poroshell 120 EC-C18
3.0 x 100mm, 2.7um
Pmax=331 bar
F=0.86 mL/min

RRHT Eclipse Plus C18
3.0 x 100mm, 1.8um
Pmax=474 bar
F=0.86 mL/min

RRHD Eclipse Plus C18
3.0 x 100mm, 1.8um
Pmax=528 bar
F=0.86 mL/min
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

Start Here
For time savings and improved resolution on any HPLC

Poroshell 120
- 1.7 μm particle size
- 250 mm × 4.6 mm, 4.6 mm × 2.1 mm sizes
- Robustness and reproducibility for high-throughput screening
- Unmatched performance for a broad range of analytes

Up to 90% less pressure than sub-2 μm
- Total lab productivity enhancer

IC-C18 (0.5 μm)
- Optimized for reversed-phase separations
- Superior column efficiency and selectivity

SB-C18 (0.5 μm)
- Enhanced peak capacity and selectivity
- Ideal for low polarity analytes

SB-C18 (2 μm)
- Excellent choice for high-speed separations
- Robustness and reproducibility

SB-C18 (3 μm)
- Faster elution times
- Ideal for routine analyses

SB-C18 (5 μm)
- Versatile choice for general use
- High robustness and reproducibility

ZORBAX Eclipse XDB
- Advanced particle technology for improved efficiency
- High resolution and exceptional selectivity

ZORBAX Eclipse Plus
- Advanced particle technology for improved efficiency
- High resolution and exceptional selectivity

ZORBAX Extend-C18
- Robustness and reproducibility for high-throughput screening
- Unmatched performance for a broad range of analytes

ZORBAX StableBond
- Robustness and reproducibility for high-throughput screening
- Unmatched performance for a broad range of analytes
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

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<tr>
<th>Part Number</th>
<th>Description</th>
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<td>ZORBAX Eclipse Plus PAH, 4.6 x 250mm, 5um</td>
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<td>959993-918</td>
<td>ZORBAX Eclipse Plus PAH, 4.6 x 150mm, 5um</td>
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<td>ZORBAX Eclipse Plus PAH Guard, 4.6 x 12.5, 5um (4 pk)</td>
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<td>ZORBAX Eclipse Plus PAH Guard, 2.1 x 12.5, 5um (4 pk)</td>
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- 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.
### 1.8μ Column Available

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