How Do I Choose?

A guide to HPLC column selection

Mark Powell
Columns and Supplies Technical Support
16 December 2021
How do I choose?

- Efficiency
  - Particle size
  - Column length
- Selectivity
  - Bonded phase
  - Mobile phase
- Retention
  - Polar bonded phase
  - HILIC
Resolution Equation

\[ R_s = \frac{1}{4} \sqrt{N} \cdot \left( \frac{\alpha - 1}{\alpha} \right) \cdot \left( \frac{k}{1+k} \right) \]

**Efficiency**  **Selectivity**  **Retention**

Improve resolution by improving any of these parameters:

- **Efficiency** describes the separation power of the column.
- **Selectivity** has the highest influence on the resolution. Small changes in selectivity can lead to big changes in resolution.
- **Retention** has only a significant influence at small k values.
Efficiency and Retention Factor

Parameters influencing column efficiency:

- Column length (increasing column length increases efficiency)
- Particle size (decreasing particle size increases efficiency)

\[ N \propto \frac{L}{d_p} \]

\[ k = \frac{(t_R - t_0)}{t_0} \]

\( t_R \) = retention time for sample peak
\( t_0 \) = retention time for unretained peak

The retention factor measures the period of time that the sample component resides in the stationary phase relative to the time it resides in the mobile phase. It is calculated from the retention time divided by the time for an unretained peak.
Agilent’s Small Molecule LC Columns

### When to choose which product family

**InfinityLab Poroshell 120**

<table>
<thead>
<tr>
<th></th>
<th>HPLC</th>
<th>UHPLC</th>
<th>LD-UHPLC</th>
</tr>
</thead>
<tbody>
<tr>
<td>µm</td>
<td>4</td>
<td>2.7</td>
<td>1.9</td>
</tr>
</tbody>
</table>

**Features**
- Modern column technology that offers higher performance at similar backpressure
- or comparable performance at reduced backpressure
- Designed in with Agilent LC instruments and supplies
- Universal column platform with offerings for all separation modes, i.e., RP, NP, HILIC, SFC as well as chiral LC
- Modern, high-performance HPLC and UHPLC columns designed in for state-of-the-art instruments.

**ZORBAX**

<table>
<thead>
<tr>
<th></th>
<th>HPLC</th>
<th>UHPLC</th>
<th>LD-UHPLC</th>
</tr>
</thead>
<tbody>
<tr>
<td>µm</td>
<td>5, 3.5</td>
<td>1.8 (RRHT)</td>
<td>1.8 (RRHD)</td>
</tr>
</tbody>
</table>

**Features**
- Traditional, reliable columns that offer a vast amount of unique chemistries
- Higher overall retention, especially for early eluters, accepts larger amounts of strong solvent during injection
- Scalable phases that range from UHPLC to HPLC to research scale prep

**Special Phases**

<table>
<thead>
<tr>
<th></th>
<th>HPLC</th>
<th>UHPLC</th>
<th>LD-UHPLC</th>
</tr>
</thead>
<tbody>
<tr>
<td>µm</td>
<td>5, 3</td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>

**Features**
- Unique chemistries that help to solve non-standard applications from HPLC to Prep.

**Phases**
- High carbon load columns: Pursuit XRs, Pursuit XRs Ultra
- Analytical to Prep: Pursuit, Polaris
- Alternative selectivity for polar and non-polar: Polaris C18-Ether, C18 Amide, NH2
Making a Poroshell Particle

Strict monitoring of every production step ensures column performance and quality

**Step 1: Make the solid core**

Poroshell 120 column cores have a very smooth surface and a uniform particle size which contributes to a tight overall particle size distribution. As a result, you get a more tightly packed column bed and therefore a better lifetime.

**Step 2: Apply the porous shell**

In contrast to other manufacturers, Agilent applies the porous shell in one single step. This unique single-step process delivers better column-to-column reproducibility.

**Step 3: Apply the bonded phase**

Most of the Poroshell chemistries are bonded in a single step. This further increases batch-to-batch reproducibility and method scalability from 1.9 to 2.7 to 4 µm.
### Poroshell Particles

**Designed along with your LC instruments for highest performance**

<table>
<thead>
<tr>
<th>SPP particle</th>
<th>For</th>
<th>Maximum pressure</th>
<th>Typical pressure</th>
<th>Efficiency</th>
<th>Target system</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.9 µm</td>
<td>Highest UHPLC performance</td>
<td>1300 bar</td>
<td>Similar to sub-2 µm totally porous</td>
<td>~120% of sub-2 µm totally porous</td>
<td>1290 Infinity II</td>
</tr>
<tr>
<td>2.7 µm</td>
<td>UHPLC performance at lower pressures</td>
<td>600 bar / 1000 bar</td>
<td>50% of sub-2 µm totally porous</td>
<td>~90% of sub-2 µm totally porous</td>
<td>1290 Infinity II, 1260 Infinity II</td>
</tr>
<tr>
<td>4 µm</td>
<td>Improved HPLC performance</td>
<td>600 bar</td>
<td>Typically &lt; 200 bar</td>
<td>~200% of 5 µm totally porous</td>
<td>1260 Infinity II VL, 1220 Infinity II (VL)</td>
</tr>
<tr>
<td>Recommended product (Max pressure / bar)</td>
<td>Traditional HPLC</td>
<td>UHPLC 2.7 µm (SPP) / &lt; 2 µm (FPP)</td>
<td>Low Dispersion UHPLC &lt; 2 µm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------------------------------------</td>
<td>-------------------</td>
<td>---------------------------------</td>
<td>-----------------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Column length (mm)</td>
<td>50–300</td>
<td>Short: 30–50</td>
<td>Short: 30–50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Column id (mm)</td>
<td>3.0–4.6</td>
<td>2.1</td>
<td>2.1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Limitations**

- **V** – System volume (dispersion/delay)
- **P** – Pressure limits
- **V+P** – System volume and pressure
- **H/I** – if instrument is used for HPLC methods / ISET emulation

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**400 bar = 6000 psi**

**600 bar = 9000 psi**

**1200 bar = 17000 psi**

**1300 bar = 19000 psi**

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**Recommended**

**Limited Configurations**

**Not Recommended**

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**How Do I Choose?**

**16 December 2021**

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**DE15029082**
Particle Size and Dimension: P120

Disruption of 5 µm workflow possible?2
- No
- Yes

Interest in solvent savings?
- No
- Yes

Lowest solvent use / MS?
- No
- Yes

2.1 mm ID

Legend
- decision
- 1st choice
- alternative

1 ULD kit recommended (p/n 5067-5963)
2 not possible with regulated gradient methods, not recommended lab technicians that lack experience with UHPLC
3 Delay and dispersion volume. E.g., 0.17 mm ID tubing or bigger + 10 mm classic flow cell, valves, long tubing connections, old mixer design

<table>
<thead>
<tr>
<th>Particle Size</th>
<th>ID</th>
<th>Optimum Flow</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.9 µm</td>
<td>2.1 mm</td>
<td>0.4 – 0.5 mL/min</td>
</tr>
<tr>
<td>2.7 µm</td>
<td>2.1 mm</td>
<td>0.4 – 0.5 mL/min</td>
</tr>
<tr>
<td>4 µm</td>
<td>3.0 mm</td>
<td>0.8 – 1 mL/min</td>
</tr>
<tr>
<td>4 µm</td>
<td>4.6 mm</td>
<td>1.5 – 2 mL/min</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Column length</th>
<th>Recommended Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>High speed</td>
</tr>
<tr>
<td>100</td>
<td>High resolution</td>
</tr>
<tr>
<td>&gt;=150</td>
<td>Ultra-high resolution</td>
</tr>
</tbody>
</table>
Scalability

Traditional ZORBAX chemistries are aligned with InfinityLab Poroshell chemistries to offer simplified method transfer from fully porous particles to superficially porous particle columns.

### InfinityLab Poroshell Chemistries
- InfinityLab Poroshell 120 EC-C18
- InfinityLab Poroshell 120 EC-C8
- InfinityLab Poroshell 120 Phenyl-Hexyl
- InfinityLab Poroshell 120 SB-C18
- InfinityLab Poroshell 120 SB-C8
- InfinityLab Poroshell 120 SB-Aq
- InfinityLab Poroshell 120 Bonus-RP
- InfinityLab Poroshell 120 EC-CN
- InfinityLab Poroshell 120 HILIC

### Aligned Chemistry
- ZORBAX Eclipse Plus C18
- ZORBAX Eclipse Plus C8
- ZORBAX StableBond SB-C18
- ZORBAX StableBond SB-C8
- ZORBAX StableBond SB-Aq
- ZORBAX Bonus-RP
- ZORBAX Eclipse XDB-CN
- ZORBAX HILIC-Plus

### Method Transferability Across Product Families

For more information on method transfer:
- Technical Overview 5990-6588EN

- For 66 compounds
- Two solvents (MeOH, ACN)
- At 3 pH values
- Pressure vs. linear velocity
## Analytes

1. Formaldehyde-2,4-dinitrophenylhydrazone
2. Acetaldehyde-2,4-dinitrophenylhydrazone
3. Acrolein-2,4-dinitrophenylhydrazone
4. Acetone-2,4-dinitrophenylhydrazone
5. Propionaldehyde-2,4-dinitrophenylhydrazone
6. Crotonaldehyde-2,4-dinitrophenylhydrazone
7. Methacrolein-2,4-dinitrophenylhydrazone
8. Butyraldehyde-2,4-dinitrophenylhydrazone
9. 2-Butanone-2,4-dinitrophenylhydrazone
10. Benzaldehyde-2,4-dinitrophenylhydrazone
11. Cyclohexanone 2,4-dinitrophenylhydrazone
12. Valeraldehyde-2,4-dinitrophenylhydrazone
13. o-Tolualdehyde 2,4-dinitrophenylhydrazone
14. m,p-Tolualdehyde 2,4-dinitrophenylhydrazone
15. Hexaldehyde-2,4-dinitrophenylhydrazone

### Mobile Phase

- **Run time**
  - 0-8.33 min: 57% ACN
  - 8.33-10 min: 57%- 75% ACN
  - 10-16.7 min: 75% ACN

- **Flow rate**: 1.5 mL/min
- **Injection volume**: 5 µL
- **Column Temp**: 50 C
- **Detector**: UV 360 nm

### HPLC (4 µm)

- **Run time**: 14 min
- **Response / injection volume**: 80 mAU / µL
- **Solvent consumption**: 21 mL
- **Samples per 8 h day**: 24

### UHPLC (2.7 µm)

- **Run time**: 8.75 min
- **Response / injection volume**: 113 mAU / µL
- **Solvent consumption**: 13.1 mL
- **Samples per 8 h day**: 48

### LD UHPLC (1.9 µm)

- **Run time**: 5.25 min
- **Response / injection volume**: 295 mAU / µL
- **Solvent consumption**: 3.36 mL
- **Samples per 8 h day**: 80

### Scalability

An example of scalability between particle sizes

**InfinityLab Poroshell 120 SB-C18, 4.6 x 250 mm, 4 µm**

**InfinityLab Poroshell 120 SB-C18, 4.6 x 150 mm, 2.7 µm**

**InfinityLab Poroshell 120 SB-C18, 3.0 x 100 mm, 1.9 µm**

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**How Do I Choose?**

DE15029082

16 December 2021
Scalability

Scaling Water-Soluble Vitamins on InfinityLab Poroshell 120 SB-Aq

<table>
<thead>
<tr>
<th>HPLC (4 µm)</th>
<th>Value</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Run time</td>
<td>8 min</td>
<td>--</td>
</tr>
<tr>
<td>Response / injection volume</td>
<td>83.3 mAU / µL</td>
<td>--</td>
</tr>
<tr>
<td>Solvent consumption</td>
<td>12 mL</td>
<td>--</td>
</tr>
<tr>
<td>Samples per 8 h day</td>
<td>48</td>
<td>--</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>UHPLC (2.7 µm)</th>
<th>Value</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Run time</td>
<td>4.5 min</td>
<td>- 44.8%</td>
</tr>
<tr>
<td>Response / injection volume</td>
<td>250 mAU/ µL</td>
<td>+200%</td>
</tr>
<tr>
<td>Solvent consumption</td>
<td>4.5 mL</td>
<td>-43.8%</td>
</tr>
<tr>
<td>Samples per 8 h day</td>
<td>80</td>
<td>+ 32</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>LD UHPLC (1.9 µm)</th>
<th>Value</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Run time</td>
<td>3.1 min</td>
<td>- 61.3%</td>
</tr>
<tr>
<td>Response / injection volume</td>
<td>800 mAU / µL</td>
<td>+900%</td>
</tr>
<tr>
<td>Solvent consumption</td>
<td>1.55 mL</td>
<td>-87.1%</td>
</tr>
<tr>
<td>Samples per 8 h day</td>
<td>145</td>
<td>+ 97</td>
</tr>
</tbody>
</table>
Increase Throughput with Ultrafast Separations

Modern columns help to increase the number of samples measured per day

High throughput UHPLC at 1150 bar and 60 °C
Increase Throughput with Ultrafast Separations

Increase the amount of samples analyzed per day

Traditional HPLC
- ZORBAX Eclipse Plus C18, 5 µm
- 36 min runtime

UHPLC
- Poroshell 120 EC-C18, 1.9 µm
- 3 min runtime (12 x faster)
- 96% less solvents used
- 95% less sample injected

Ultrafast UHPLC
- Poroshell 120 EC-C18, 1.9 µm
- 0.3 min runtime (120 x faster)
- 98% less solvent
Selectivity impacts the resolution most

\[ R_S = \frac{\sqrt{N}}{4} \left( \frac{\alpha - 1}{\alpha} \right) \cdot \frac{k'}{k' + 1} \]

Selectivity impacts resolution
- Stationary and mobile phase
- Temperature
- \( N \) is strongly influenced by \( \alpha \)

<table>
<thead>
<tr>
<th>Alpha</th>
<th>1.10</th>
<th>1.35</th>
<th>1.60</th>
<th>1.85</th>
<th>2.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>plates</td>
<td>5,000</td>
<td>10,000</td>
<td>15,000</td>
<td>20,000</td>
<td>25,000</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>
## The InfinityLab Poroshell 120 Portfolio

Agilent Poroshell columns are designed for multiple separation modes

<table>
<thead>
<tr>
<th>Best all around</th>
<th>Best for low pH mobile phases</th>
<th>Best for high pH mobile phases</th>
<th>Best for alternative selectivity</th>
<th>Best for more polar analytes</th>
<th>Chiral</th>
</tr>
</thead>
<tbody>
<tr>
<td>EC-C18 A</td>
<td>SB-C18 A</td>
<td>HPH-C18 A</td>
<td>Bonus-RP A,B</td>
<td>SB-Aq A,B</td>
<td>Chiral-V A,C,D</td>
</tr>
<tr>
<td>1.9 µm, 2.7 µm, 4 µm</td>
<td>1.9 µm, 2.7 µm, 4 µm</td>
<td>1.9 µm, 2.7 µm, 4 µm</td>
<td>2.7 µm</td>
<td>1.9 µm, 2.7 µm, 4 µm</td>
<td>2.7 µm</td>
</tr>
<tr>
<td>EC-C8 A</td>
<td>SB-C8 A</td>
<td>HPH-C8 A</td>
<td>PFP A,B,D</td>
<td>EC-CN A,B,C,D</td>
<td>Chiral-T A,C,D</td>
</tr>
<tr>
<td>1.9 µm, 2.7 µm, 4 µm</td>
<td>2.7 µm</td>
<td>2.7 µm, 4 µm</td>
<td>1.9 µm, 2.7 µm, 4 µm</td>
<td>2.7 µm</td>
<td>2.7 µm</td>
</tr>
<tr>
<td>Phenyl-Hexyl A</td>
<td></td>
<td>CS-C18 A</td>
<td>HILIC C,D,E</td>
<td>Chiral- CD A,C,D</td>
<td></td>
</tr>
<tr>
<td>1.9 µm, 2.7 µm, 4 µm</td>
<td></td>
<td>2.7 µm</td>
<td>1.9 µm, 2.7 µm, 4 µm</td>
<td>2.7 µm</td>
<td></td>
</tr>
</tbody>
</table>

### Legend
- **A** reversed phase
- **B** can be operated at 100% aqueous
- **C** Normal phase
- **D** SFC
- **E** HILIC

### How Do I Choose?

**Agilent Poroshell columns** are designed for multiple separation modes.

- **Best all around**
- **Best for low pH mobile phases**
- **Best for high pH mobile phases**
- **Best for alternative selectivity**
- **Best for more polar analytes**
- **Chiral**
The influence of stationary phase on selectivity and resolution

**Compounds:**

**Time** | **% Organic**
---|---
0 | 8
6 | 100
7 | 100
8 | 8

2mL/min 254 nm
## Stationary Phase Selection Guide

### Starting Recommendation

**Poroshell 120 EC-C18**

<table>
<thead>
<tr>
<th>Change Selectivity Slightly</th>
<th>Change Selectivity Significantly</th>
<th>For Many Early Eluters</th>
<th>No retention at 98%+ Aqueous in RP</th>
<th>Acidic Solvents (pH &lt; 2)</th>
<th>Basic Solvents (pH &gt; 6)</th>
</tr>
</thead>
</table>

### Top 3 to keep around (covers 95% of analyses)

- Poroshell EC-C18
- Poroshell HILIC-Z
- Poroshell PFP

### Recommended Solvent A (Weak)

1. 0.1% Formic Acid (pH ~2.7)
2. 10 mmol Ammonium Acetate (adj. pH 5)
3. 0.1% Ammonium Hydroxide (pH ~10)
4. 0.1% Trifluoroacetic acid (pH ~1.5, no MS)
5. 150 mmol Sodium Phosphate (adj. pH 3, no MS)

### Solvent B (Strong)

1. Acetonitrile
2. Methanol
3. Isopropanol
4. THF
5. Acetone

### Sugars (RI or ELSD)

- **Normal Phase**
  - 1. Poroshell HILIC-Z
  - 2. Poroshell EC-CN
  - 3. Polaris NH2

- **Chiral**
  - 1. Poroshell Chiral-V
  - 2. Poroshell Chiral-T
  - 3. Poroshell Chiral-CD
  - 4. Poroshell Chiral-CF
HPLC Chemistry Selection: Poroshell 120

1. C8 phases have nearly identical selectivity compared to C18. Suitable for faster analysis times when sufficient resolution and retention are achieved.

2. Bare silica column. Shipped in RP solvents. For NP use, please properly flush out shipping solvent before.

RP = reversed phase
NP = normal phase
Choosing Between C18s

<table>
<thead>
<tr>
<th>InfinityLab Poroshell 120</th>
<th>Chemistry</th>
<th>Pore Size</th>
<th>Endcapped</th>
<th>Carbon Load</th>
<th>Surface Area</th>
<th>Best For</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>EC-C18</strong> 1.9 µm, 2.7 µm, 4 µm</td>
<td></td>
<td>120 Å</td>
<td>Yes</td>
<td>10%</td>
<td>130 m²/g</td>
<td>General Purpose Excellent peak shape and efficiency for acids, bases, neutrals</td>
</tr>
<tr>
<td><strong>SB-C18</strong> 1.9 µm, 2.7 µm, 4 µm</td>
<td></td>
<td>120 Å</td>
<td>No</td>
<td>9%</td>
<td>130 m²/g</td>
<td>Low pH Excellent stability and peak shape in highly acidic conditions</td>
</tr>
<tr>
<td><strong>HPH-C18</strong> 1.9 µm, 2.7 µm, 4 µm</td>
<td></td>
<td>100 Å</td>
<td>Yes</td>
<td>Proprietary</td>
<td>95 m²/g</td>
<td>High pH capable Robust performance and long lifetimes</td>
</tr>
<tr>
<td><strong>CS-C18</strong> 2.7 µm</td>
<td></td>
<td>100 Å</td>
<td>Yes</td>
<td>Proprietary</td>
<td>95 m²/g</td>
<td>Alternate selectivity Improved peak shape and sample capacity for basic compounds with low ionic strength mobile phases High pH capable</td>
</tr>
</tbody>
</table>
A pH Change Can Strongly Affect Selectivity

Mobile phase pH is a powerful method development tool for separating ionizable compounds

- In RPLC mode, ionizable analytes are more retained in their neutral state
  - **Acids** are more retained at **low pH**
  - **Bases** are more retained at **high pH**
  - **Neutrals** are not impacted by mobile phase pH

![Graph showing retention times of different compounds at various pH levels.](image)

Mobile phase: 45% Methanol, 55% 20 mM Phosphate Buffer
Selectivity Can be Controlled by Changing pH

Agilent InfinityLab Poroshell HPH-C18 4.6 x 50 mm, 2.7 µm

<table>
<thead>
<tr>
<th>pH</th>
<th>Buffer</th>
<th>MeCN</th>
<th>Time</th>
<th>% Buffer</th>
<th>% MeCN</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>10 mM HCO₂NH₄</td>
<td></td>
<td>0</td>
<td>10</td>
<td>90</td>
</tr>
<tr>
<td>4.8</td>
<td>10 mM NH₄HCO₃</td>
<td></td>
<td>5</td>
<td>90</td>
<td>10</td>
</tr>
<tr>
<td>10</td>
<td>10 mM NH₄HCO₃</td>
<td></td>
<td>7</td>
<td>10</td>
<td>90</td>
</tr>
</tbody>
</table>

1. Procainamide
2. Caffeine
3. Acetyl Salicylic Acid
4. Hexanophenone Deg.
5. Dipyrimadole
6. Diltiazem
7. Diflunisal
8. Hexanophenone

Acids

Bases

1. Procainamide
2. Caffeine
3. Acetyl Salicylic Acid
4. Hexanophenone Deg.
5. Dipyrimadole
6. Diltiazem
7. Diflunisal
8. Hexanophenone
Agilent InfinityLab Poroshell 120 CS-C18 Gives Better Peak Shape for Basic Analytes with Formic Acid Mobile Phase than a Traditional C18

A: 0.1% formic acid or 0.2% trifluoroacetic acid in water; B: acetonitrile; 0.4 mL/min; isocratic: %B varies; 2.1 x 100 mm columns, 1 µL injection, 30 °C, LC/MS: ESI+, dMRM; Sample: 5 µg/mL of doxepin, desipramine, imipramine, nortriptyline, amitriptyline, trimipramine

Read more: Agilent application note: 5994-2095EN
InfinityLab Poroshell 120 CS-C18

Hybrid end-capped C18 phase

SPP particle modified to have a charged surface

InfinityLab Poroshell 120 CS-C18 for basic analytes with low ionic strength mobile phases

• High pH stable
• Alternate C18 selectivity

• Better peak shape for basic compounds
• Formic acid compatibility
• Reduced operating pressures
• Increased speed of analysis

Column dimensions
• 2.1, 3, 4.6 mm id x 50, 100, 150 mm length
• PEEK-lined options ★

★ PEEK-lined column options are rare in the reverse phase column market and help with challenging metal sensitive compounds.
A pH Change Can Strongly Affect Selectivity

CS-C18 is another high-pH compatible L1 stationary phases

Low pH: 0.1% Formic acid, pH 2.7

High pH: 10 mM Ammonium Formate, pH 10

Read more: Application Note 5994-2274EN

5-95% CH₃CN in 10 min, 4 min post run, mobile phase A varies, 0.4 mL/min, 2.1 x 100 mm, 2.7 µm AgilentInfinityLab Poroshell 120 CS-C18, 30 ºC, DAD: 254 nm, 80 Hz; Sample: uracil, amitriptyline, butyl paraben, dipropyl phthalate, acenaphthene
The Agilent InfinityLab Poroshell 120 CS-C18 Offers Alternative Selectivity to Other C18s to Facilitate Method development at Low pH

Veterinary Drugs
1. Ciprofloxacin
2. Oxytetracycline
3. Tetracycline
4. Enrofloxacin
5. Sulfamerazine
6. Sulfamethazine
7. Erythromycin
8. Penicillin-G
9. Oxacillin

Method Parameters:
A: 0.1% formic acid in water
B: acetonitrile
0.4 mL/min, 0-95% B in 15 min
0.05 µL injection
Sample: 0.1 mg/mL in water
Column: 30 °C, 2.1 x 100 mm, 2.7 µm
Detection: LC/MS, ESI+, dMRM
What is HILIC and When Should I Consider it?

**HILIC Complements RPLC**

<table>
<thead>
<tr>
<th>Reversed-phase LC</th>
<th>Hydrophilic interaction LC (HILIC)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Non-polar stationary phase (e.g., C18)</strong></td>
<td><strong>Polar stationary phase (e.g., silica)</strong></td>
</tr>
<tr>
<td>Polar mobile phase</td>
<td>Polar mobile phase</td>
</tr>
<tr>
<td>$\text{H}_2\text{O}/\text{CH}_3\text{OH}$, $\text{H}_2\text{O}/\text{CH}_3\text{CN}$</td>
<td>$\text{H}_2\text{O}/\text{CH}_3\text{CN}$</td>
</tr>
<tr>
<td><strong>Polar mobile phase</strong></td>
<td><strong>Retains hydrophilic (polar and ionized) compounds well and often reverses elution order vs RPLC</strong></td>
</tr>
<tr>
<td><strong>Decrease retention by decreasing polarity of mobile phase</strong></td>
<td><strong>Elution Order</strong></td>
</tr>
<tr>
<td>$\text{ddH}_2\text{O} \downarrow = \text{retention} \uparrow$</td>
<td>non-polar to polar</td>
</tr>
<tr>
<td>$\text{CH}_3\text{CN} \uparrow = \text{retention} \downarrow$</td>
<td></td>
</tr>
<tr>
<td><strong>polar to non-polar</strong></td>
<td></td>
</tr>
</tbody>
</table>

**How Do I Choose?**

- **Polarity**
  - Polar stationary phase (e.g., silica)
- **Mobile Phase**
  - Polar mobile phase
  - $\text{H}_2\text{O}/\text{CH}_3\text{CN}$
- **Gradient**
  - $\text{ddH}_2\text{O} \uparrow = \text{retention} \downarrow$
  - $\text{CH}_3\text{CN} \downarrow = \text{retention} \uparrow$

**What is HILIC and When Should I Consider it?**

- Retains hydrophilic (polar and ionized) compounds well and often reverses elution order vs RPLC.
- Complements RPLC.
HILIC Method Development

InfinityLab Poroshell 120 HILIC column options

**HILIC**
- Bare silica chemistry
- For very simple mixtures, low column bleed

**HILIC-Z**
- Proprietary zwitterionic chemistry, high pH stable
- **Most modern and robust column – start method development here**
- PEEK-lined version available

**HILIC-OH5**
- Brushed fructan chemistry
- Alternative selectivity

Best for polar analytes

InfinityLab Poroshell HILIC
1.9 µm, 2.7 µm, 4 µm

InfinityLab Poroshell HILIC-Z
1.9 µm, 2.7 µm, 4 µm

InfinityLab Poroshell HILIC-OH5
2.7 µm
Mobile Phase A (Strong phase, H₂O):

- Typical buffer concentration: 5-30 mM
  - 10 - 20 mM is most common
- Ammonium Formate, pH 3
- Ammonium Acetate, pH 4–5
- Ammonium Acetate, pH ~7
  - Ammonium acetate solution is near pH 7, before adjusting with other modifiers
  - Not a true buffer, but still commonly used at mid-pH
- Ammonium Acetate or Formate, pH 9–10
  - Can be formate or acetate because the ammonium ion is buffering
  - HILIC-Z only!
- Ammonium Hydroxide, pH 10–11
  - HILIC-Z only!
- Phosphate buffers are not recommended *

Mobile Phase B (Weak phase, CH₃CN):

- Buffer concentration should match Mobile Phase A for improved reproducibility
- Adding 10% water in ACN generally recommended for improved solubility and faster re-equilibration
- Pure MeOH is too strong a solvent for most HILIC separations. Mixed with ACN in small quantities (<15%), it can be used to change selectivity slightly.

Example of mobile phase preparation:
Stock: 200 mM ammonium formate adjusted to pH 3 with formic acid
A: 900 mL water + 100 mL stock
B: 900 mL acetonitrile + 100 mL stock

*Note: Phosphates have low solubility in high % ACN (1-30 mM). Always test solubility before running. Never run in >80% ACN to avoid precipitation.
In HILIC mode, ionizable compounds are better retained when they are ionized

- Acids at high pH
- Bases at low pH

Once the analyte is fully ionized, retention should stabilize

- Note: if other retention mechanisms are occurring, this may not be true

**Biotin pKa = 4.5**

**Nicotinic acid pKa = 4.8**

**Pantothenic acid pKa = 4.3**
Analysis of Amino Acids (and Isobars) in Plant Tissue with LC-MS/MS

**LC/MS system**
1260 Infinity Binary LC + 6470 Triple Quadrupole LC-MS

**Flowrate**
0.5 mL/min

**Column**
InfinityLab Poroshell HILIC-Z, 2.7 µm, 2.1 x 100 mm (Part no. 685775-924)

**Column temperature**
25 ºC

**Mobile phase**
Stock: 200 mM Amonium formate adjusted to pH 3 with formic acid
A: 900 mL Water + 100 mL stock, B: 900 mL Acetonitrile + 100 stock

**Gradient**
10–70% B in 11.5 min

1 µL of 500 ng/mL standard
Agilent Pub # 5991-8922
Reversed Phase versus HILIC

Mobile Phase A | H₂O
---|---
Mobile Phase B | CH₂CN
Mobile Phase D | 200 mM ammonium acetate + 0.2% acetic acid, pH = 5.3
Flow Rate | 0.5 mL/min
Gradient | 0% B for 1 minute, 0 to 25% B in 8 minutes, hold 5% D constant throughout analysis, 3 minutes post-run
Injection | 0.5 μL of 0.4 μg/mL vitamin standard in H₂O
Column | 25 °C, Agilent InfinityLab Poroshell 130 Phenyl-Hexyl, 2.1 x 100 mm, 2.7 μm
Detection | Agilent Ultra 100/5 MS ESI+ dMRM, E20 Sg = 260 nm, 80 Hz

Mobile Phase A | H₂O
---|---
Mobile Phase B | CH₂CN
Mobile Phase D | 200 mM ammonium acetate (no pH adjustment), pH = 6.7
Flow Rate | 0.5 mL/min
Gradient | 95 to 65% B in 10 minutes, hold 5% D constant throughout analysis, 5 minutes post-run
Injection | 0.5 μL injection of 0.4 μg/mL vitamin standard in CH₂CN
Column | 25 °C, Agilent InfinityLab Poroshell 120 HILIC-C18, 2.1 x 100 mm, 2.7 μm
Detection | Agilent Ultra 100/5 MS ESI+ dMRM (parameters above), E20 Sg = 260 nm, 80 Hz
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  - Online selection tools, “How-to” videos
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- InfinityLab LC Supplies catalog ([5991-8031EN](5991-8031EN))
- Agilent University [www.agilent.com/crosslab/university](http://www.agilent.com/crosslab/university)
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