Column Related

Column
- Column Choices
- New Column
  - Save Performance Report
  - Test mix (PN 01080-68704); 65/35 ACN/H2O or MeOH
- Installation
- Appropriate conditions
- Column Lifetime
  - Conditions for bad lifetime
  - Keep record/Column History
  - Store properly
- Method parameters, flow, inj, etc.
Different ZORBAX RRHT C18 Bonded Phases

1st choice
Best Resolution & Peak Shape

2nd choice
Good alternate selectivity due to non-endcapped

3rd choice
Good efficiency & peak shape
Resolution could be achieved

4th choice
Resolution not likely, Other choices better, for this separation.

Tip: Not all C18s are the same!
Trick: Test & find the best one for your application: Check out MD kits.
NSAID Separation with MeOH Gradient

Best resolution of all analytes with Poroshell 120 PFP

Poroshell 120 PFP

Poroshell 120 EC-C18

Poroshell 120 Bonus RP

Poroshell 120 Phenyl Hexyl

### Column Specifications

<table>
<thead>
<tr>
<th>InfinityLab Poroshell 120 column specifications</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>InfinityLab Poroshell Family</strong></td>
</tr>
<tr>
<td>Best all around</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Best for low-pH mobile phases</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Best for high-pH mobile phases</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Best for basic compounds at low pH</td>
</tr>
<tr>
<td>Best for polar compounds (HILIC)</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Best for alternative selectivity</td>
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<td></td>
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<tr>
<td>Best for Chiral separations</td>
</tr>
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<td></td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

Specifications represent typical values only.

<table>
<thead>
<tr>
<th>Particle</th>
<th>Pressure Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.9 µm</td>
<td>1300 bar</td>
</tr>
<tr>
<td>2.7 µm</td>
<td>600 bar*</td>
</tr>
<tr>
<td>4 µm</td>
<td>600 bar</td>
</tr>
</tbody>
</table>

* unless otherwise noted

- Method development kits – Selectivity, pH
- Method validation kit – 3 different lots
Column Documentation

Performance report

Data sheet or column guide

This booklet provides general information for all ZORBAX, Poroshell, Pursuit, and Polaris reversed-phase columns.

For additional detailed information about your specific phase or family, see: agilent.com/chem/columnchoices

Getting Started

A QC Column Performance Report, including a test chromatogram, is enclosed with every Agilent column. The QC test system has been modified from a standard system to minimize system dead volume, so it may vary from the system used in your lab. This allows a better evaluation of the column and assures a more consistent product. A properly configured LC system will generate similar results to the chromatogram on your QC Performance Report.

Modern columns are robust and are designed to operate for long periods under normal chromatographic conditions. You can maximize column performance by running it within specifications. Always review the specifications before putting in place a final method.

Manufacturing test chromatogram is done on a modified LC system to minimize ECV and will differ from a typical lab HPLC

- Don’t expect to get exactly the same result as the performance report
- Test column performance on your instrument to have as a reference
Column Documentation – Benchmark

Benchmark new column on your system

1. Test mixes
   1) Isocratic Standard (01080-68704)
   2) Checkout sample (5188-6529)
   3) QC reference material

2. Criteria like retention time, peak area, peak tailing, resolution, response, system pressure, etc.

3. Theoretical plates
   • Monitor column over time
   • Troubleshoot

Trick: Know what your column looks like on your instrument when it’s new.
Column Installation Recommendations

1. Purge the pumps (connections up to the column) of any buffered mobile phases. Flush at least 5 mL of solvent before attaching the column to instrument.

   **Goal:** Eliminate any dried out or precipitated buffer from the system so it doesn’t wash onto the column and plug the frit.

2. Flush your new column with your mobile phase (compatible with the solvents the column was shipped in) at an appropriate flow rate – start slowly at 0.1 mL/min for a 2.1 mm id column, 0.2 mL/min for a 3.0 mm id column, and 0.4 mL/min for 4.6 mm id.

   **Goal:** Avoid a pressure spike when the new mobile phase reaches the column. This occurs when the different solvents mix. The low flow rate allows this to happen without causing an unanticipated pressure change.

3. Increase the flow rate to the desired flow over a couple of minutes.

   **Goal:** Reach final operating pressure

4. Once the pressure has stabilized, attach column to detector

5. Equilibrate column and detector with 10 column volumes of mobile phase prior to use

   **Goal:** Reproducible chromatography from the start
Initial Column and System Equilibration*
Using Buffers Successfully

Insure HPLC has required mobile phase components
In appropriate vessel, test highest % organic(buffer ratio
• Verify buffer will not precipitate. With stirring, add organic to buffer first, not vice versa

Install column – Make a good connection
Equilibrate column with, in order:
• 100% organic modifier (if brand new)
• Mobile phase minus buffer
• Buffered MP w/highest % organic modifier (gradient)
• Buffered MP w/lowest % organic modifier (gradient)

Inject standard or sample several times until RTs stable
• For gradient, precede former with 1-3 blank gradients

*Check appendix for shutdown instructions
LC Columns Are Not Indestructible

Columns are packed using hydraulic pressure and can be damaged by it.

Silica dissolves (slowly)… higher pH

Acid hydrolysis of bonded phase at low pH

Column failure
- Void
- Contamination

Columns must be stored properly
- Check your user guide

Trick: Choose a mobile phase that is right for your column

Tip: Keep record/history of your column

| Columns:       | 4.6 x 150 mm, 5 µm |
| Purge:         | 50% ACN / 50% 0.02 M K₂HPO₄, pH 11 |
| Flow Rate:     | 1.5 mL / min |
| Temperature:   | 25°C |
| Detection:     | Silicate concentration by silicomolybdate color reaction |
LC Columns Are Not Indestructible

For maximum column lifetime, match column to pH of mobile phase

low pH and high temperature (pH 0.8, 90°C)

You Have Your Column, What’s Next?

Method conditions
- Flow rate
- Load
- Temperature
- Retention

What’s in your mobile phase?
- Organic
- Buffers
  - pH
  - Tables
- How to make

Tip: For good chromatography the trick is to match your conditions to your column choice.
## Column ID; Match Conditions

<table>
<thead>
<tr>
<th>Column id</th>
<th>Column volume</th>
<th>Peak volume, k=1</th>
<th>Typical injection volume.*</th>
<th>Typical injection volume range</th>
<th>Flow rate for equivalent ν**</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.6 mm</td>
<td>1500 µL</td>
<td>148 µL</td>
<td>20 µL</td>
<td>5–50 µL</td>
<td>1.0 mL/min</td>
</tr>
<tr>
<td>3.0 mm</td>
<td>640 µL</td>
<td>44 µL</td>
<td>10 µL</td>
<td>3–30 µL</td>
<td>0.42 mL/min</td>
</tr>
<tr>
<td>2.1 mm</td>
<td>320 µL</td>
<td>22 µL</td>
<td>2 µL</td>
<td>0.5–15 µL</td>
<td>0.21 mL/min</td>
</tr>
<tr>
<td>1.0 mm</td>
<td>70 µL</td>
<td>4 µL</td>
<td>0.5 µL</td>
<td>0.1–3 µL</td>
<td>47 µL/min</td>
</tr>
<tr>
<td>0.5 mm</td>
<td>15 µL</td>
<td>1 µL</td>
<td>150 nL</td>
<td>40–500 nL</td>
<td>12 µL/min</td>
</tr>
<tr>
<td>0.3 mm</td>
<td>6 µL</td>
<td>0.3 µL</td>
<td>50 nL</td>
<td>15–250 nL</td>
<td>4.2 µL/min</td>
</tr>
<tr>
<td>0.1 mm</td>
<td>700 nL</td>
<td>32 nL</td>
<td>10 nL</td>
<td>1–10 nL</td>
<td>472 nL/min</td>
</tr>
<tr>
<td>0.075 mm</td>
<td>400 nL</td>
<td>18 nL</td>
<td>2 nL</td>
<td>0.5–5 nL</td>
<td>266 nL/min</td>
</tr>
</tbody>
</table>

Column length = 150 mm, N = 13,000

### Tips

* **Typical injection volume = 10–30% of peak volume of first eluting peak**

** Maintain equivalent mobile phase linear velocity when scaling column diameter**
Effect of Temperature on Separation

Salicylic acid

- $20^\circ C$
- $30^\circ C$
- $40^\circ C$
- $60^\circ C$
- $90^\circ C$

Column: RRHT SB-C18
4.6 x 50mm, 1.8µm

Conditions: A: 0.1% formic acid B: ACN w/ 0.1% formic acid (65:15) Detection: UV 254 nm
**Separation Conditions That Cause Changes in Retention**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Change</th>
<th>Retention</th>
<th>Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow rate</td>
<td>+/- 1%</td>
<td>$t_R$</td>
<td>+/- 1%</td>
</tr>
<tr>
<td>Temperature</td>
<td>+/- 1 °C</td>
<td>$t_R$</td>
<td>+/- 1 to 2%</td>
</tr>
<tr>
<td>% Organic</td>
<td>+/- 1%</td>
<td>$t_R$</td>
<td>+/- 5 to 10%</td>
</tr>
<tr>
<td>pH</td>
<td>+/- 0.01%</td>
<td>$t_R$</td>
<td>+/- 0 to 1%</td>
</tr>
</tbody>
</table>


**Tip:** Minor changes in conditions can affect retention
Your Mobile Phase

• What's in your mobile phase?
  – Organic
  – Buffers
    • pH
    • Tables
  • How to make
  • Equilibration
  • Shutdown
Comparison of 25 Component Mixture using Methanol or Acetonitrile as the Mobile Phase

InfinityLab Poroshell 120 EC-C18 - good 1st choice

Organic choice considerations:
- Selectivity differences
- Methanol –
  - Higher pressure
  - In general, better peak shape with bases
  - Generally more miscible
  - Protic solvent
  - Weaker than ACN
- Acetonitrile
  - Lower pressure
  - Aprotic solvent
  - Wider UV window than MeOH

Tip: Consider a blend
Mobile Phase pH and Buffers

Why are they important in HPLC?

pH
• Silica surface of column
• Sample components of interest

Buffers
• Resist changes in pH and maintain retention
• Improve peak shape for ionizable compounds

Column lifetime
• Low pH strips bonded phase
• High pH dissolves silica
Selectivity and Resolution Can Change with pH

pH 2.7 0.1% Formic acid/ACN

pH 4.8 NH₄AC/ACN

pH 7 NaPO₄/ACN

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

1. procainamide
2. buspirone
3. pioglitazone
4. eletriptan
5. dipyridamole
6. diltiazem
7. furosemide
"I Don’t Have Time to Make Buffers or Adjust pH…!"

Mobile Phase:A: 20% H₂O  
B: 80% MeOH

Retention, poor peak shape (IEX interaction)

Column: SB-C18, 4.6 x 150 mm, 5 mm  
Flow rate: 1.0 mL/min.  
Temperature: 35 °C  
UV detection: 254 nm  
Sample: 1. Diltiazem  
2. Dipyridamole  
3. Nifedipine  
4. Lidoflazine  
5. Flunarizine

Mobile Phase:45% 25 mM NaH₂PO₄, pH 3.0  
55% MeOH

Trick: Know your sample  
Tip: Know if your detector is compatible with the buffer you choose
Buffer Preparation
Does It Make a Difference?

1. Dissolve salt in water using a 1 L or 2 L beaker. Use appropriate volume to leave space for pH adjustment. Equilibrate to RT for maximum accuracy.

2. Calibrate pH meter. Use 2-level calibration & bracket desired pH. Use appropriate audit solution to monitor statistical control (e.g., potassium hydrogen tartrate, saturated solution, pH = 3.56).

3. Adjust salt solution to desired pH. Minimize amount of time electrode spends in buffer solution (contamination). Avoid overshooting and re-adjustment (ionic strength differences can arise).

4. Transfer pH-adjusted buffer solution quantitatively to volumetric flask, dilute to volume, and mix.

5. Filter through 0.45 µm filter (discard first ~50 mL filtrate). Rinse solvent reservoir with small volume of filtrate and discard. Fill reservoir with remaining filtrate or prepare premix with organic modifier.
   - Agilent solvent filtration kit, 250 mL reservoir, 1000 mL flask, P/N 3150-0577
   - Nylon filter membranes, 47 mm, 0.45 µm pore size, P/N 9301-0895 (not for proteins!)

   Trick: For gradient methods, avoid buffer precipitation by testing the solubility of buffered mobile phase component with highest % organic used. Always add organic to buffer with stirring, not vice versa.

   Tip: Filtering is important - small particles in MP can permanently block capillaries in degasser.
## Buffer Options

### Nonvolatile

<table>
<thead>
<tr>
<th>Buffer</th>
<th>Chemical</th>
<th>pK&lt;sub&gt;a&lt;/sub&gt;</th>
<th>Buffer range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphate</td>
<td>H&lt;sub&gt;3&lt;/sub&gt;PO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>pK&lt;sub&gt;1&lt;/sub&gt; = 2.1</td>
<td>1.1–3.1</td>
</tr>
<tr>
<td></td>
<td>H&lt;sub&gt;2&lt;/sub&gt;PO&lt;sub&gt;4&lt;/sub&gt;&lt;sup&gt;-&lt;/sup&gt;</td>
<td>pK&lt;sub&gt;2&lt;/sub&gt; = 7.2</td>
<td>6.2–8.2</td>
</tr>
<tr>
<td></td>
<td>HPO&lt;sub&gt;4&lt;/sub&gt;&lt;sup&gt;-2&lt;/sup&gt;</td>
<td>pK&lt;sub&gt;3&lt;/sub&gt; = 12.3</td>
<td>11.3–13.3</td>
</tr>
<tr>
<td>Citrate</td>
<td>CH&lt;sub&gt;2&lt;/sub&gt;COOH</td>
<td>pK&lt;sub&gt;1&lt;/sub&gt; = 3.1</td>
<td>2.1–4.1</td>
</tr>
<tr>
<td></td>
<td>HOC&lt;sub&gt;2&lt;/sub&gt;COOH</td>
<td>pK&lt;sub&gt;2&lt;/sub&gt; = 4.7</td>
<td>3.7–5.7</td>
</tr>
<tr>
<td></td>
<td>CH&lt;sub&gt;2&lt;/sub&gt;COOH</td>
<td>pK&lt;sub&gt;3&lt;/sub&gt; = 5.4</td>
<td>4.4–6.4</td>
</tr>
<tr>
<td>Borate</td>
<td>H&lt;sub&gt;3&lt;/sub&gt;BO&lt;sub&gt;3&lt;/sub&gt;</td>
<td>pK&lt;sub&gt;1&lt;/sub&gt; = 9.2</td>
<td>8.2–10.2</td>
</tr>
</tbody>
</table>

### Volatile

<table>
<thead>
<tr>
<th>Buffer</th>
<th>Chemical</th>
<th>pK&lt;sub&gt;a&lt;/sub&gt;</th>
<th>Buffer range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trifluoroacetate</td>
<td>F&lt;sub&gt;3&lt;/sub&gt;CCOOH</td>
<td>pK&lt;sub&gt;1&lt;/sub&gt; = 0.5</td>
<td>xx–1.5</td>
</tr>
<tr>
<td>Formate</td>
<td>HCOOH</td>
<td>pK&lt;sub&gt;1&lt;/sub&gt; = 3.8</td>
<td>2.8–4.8</td>
</tr>
<tr>
<td>Acetate</td>
<td>CH&lt;sub&gt;3&lt;/sub&gt;COOH</td>
<td>pK&lt;sub&gt;1&lt;/sub&gt; = 4.8</td>
<td>3.8–5.8</td>
</tr>
<tr>
<td>Ammonium</td>
<td>NH&lt;sub&gt;4&lt;/sub&gt;&lt;sup&gt;+&lt;/sup&gt;</td>
<td>pK&lt;sub&gt;1&lt;/sub&gt; = 9.2</td>
<td>8.2–10.2</td>
</tr>
</tbody>
</table>

**Tip:** Make sure you know the buffering range of your buffer!
Change in Volatile Buffer Concentration and Shift in Retention Time and Peak Shape

Tip: Volatile – It’s definition is evaporating rapidly, passing off rapidly in the form of vapor…

Column: C18, 4.6 x 100mm, 5 µm
Flow rate: 2 mL/min
Detection: UV 210 nm
Temp: 25 ºC
Inj: 0.05 µg each compound (2 µL inj.)
Mobile Phase Preparation

- HPLC grade or better
- Buffer prep procedure
  - Be consistent
- Document process

Volume % of solvents can depend on preparation

Specified volume ACN added to a 1 L volumetric and made to volume with H₂O ≠
Specified volume H₂O added to a 1 L volumetric and made to volume with ACN ≠
500 mL H₂O added to 500 mL ACN

- Relative quantities of each affects degree of contraction
- Temperature

Tips
1. Small changes in mobile phase strength can have a large effect on retention
2. Immiscible solvent flow can cause high pressure and trigger system shutdown.
Mobile Phase Preparation
Effect on chromatography

Method used to prepare mobile phase can significantly affect elution pattern

Trick: Avoid this potential pitfall by being consistent.

Tip: W/w is more accurate than v/v.
Consider Your Sample

Type
• Ionizable?
• Compound type; acid, base, neutral

Solubility in mobile phase
• Test
  – If it’s not aqueous soluble and your starting mobile phase is 90%H2O:10%ACN, precipitation likely on inlet frit of your column

Size/MW
• Pore size

My sample is clean – or is it?
Is RP the best choice?
When Does pH Affect Resolution?
Compound type comparison

Tip: Ionizable compounds (acids and bases) can change retention and selectivity with changes in pH
I Don’t Have to Filter, My Sample Is Clean or… Is It?

A. PBS used as diluent
B. 2mg/ml egg albumin after GF filtration
   1) GF = glass over regen. cellulose
C. 2mg/ml egg albumin chilled overnight

Bright “sparkles” = insoluble debris and agglomerated proteins

CAUTION: Use appropriate safety precautions when using laser pointer for sample inspection!

Trick: Use a laser pointer to check sample and blank quality.
Is Reversed Phase the Right Choice?

Overlay of HILIC Separation of Catecholamines
(Poroshell 120 2.1 x 100, 2.7 µm), (Rx-Sil 2.1 x 150, 5 µm)

Poroshell 120 HILIC, 2.7 µm, 3 x 150 mm
0.84 ml/min
90/10 MeCN/100 mM pH 3.2 NH₄HCO₂H Buffer

Poroshell 120 EC-C18, 2.7 µm, 3 x 150 mm

1. Napthalene
2. Uracil

220 Bar

1. Dopamine
2. Epinephrine
3. Norepinephrine

224 Bar

“Hydrophobic Interaction Chromatography (HILIC) using Agilent Poroshell 120 HILIC”
William J. Long, Anne E. Mack, October 5, 2012, 5991-1242EN
Instrument

HPLC or UHPLC
• Pressure
• Particle size
• Column dimension
Dwell volume
Extra column volume
Column oven
Connections
• Use shortest length of tubing possible
• Quick connect and quick turn
Do you need an inline filter or guard column?

Tip: Attend upcoming webinar, November 17th @1 EST: "All About LC Connections – The Importance of Making a Great One"
Column Oven
Control the Temperature

Tip: Constant temperature = constant retention
Dwell Volume of HPLC System with Low Pressure Mixing
aka Gradient Delay Volume

Trick: Minimize it!
Tip: Check the appendix for instructions on determining your system’s dwell volume and document it.
Extracolumn Volumes in HPLC Sample Flow System

- Injection valve
- Sample volume in loop (a)
- Internal volume in valve from Sample loop (including fitting) (b)
- Connecting tube (c)
- Column end fittings including frits (d)
- Connecting tube (e)
- Internal flow lines in detector (f)
- Exit to waste
- Detector flow cell (g)

Tip: Effect of ECV – “Throw away plates!”
Column Protection

Inline Filters
- Extend column life
- Easy to change
- Not intended to replace sample cleanup

UHPLC options
RRLC, 0.2μm, max 600 bar
- 4.6mm frit id, 5067-1553
- 2.1mm frit id, 5067-1551
1290 Infinity LC, 0.3μm, max 1200 bar
- 5067-4638, replacement frits 5023-0271
1290 Infinity II, 0.3μm, max 1300 bar
- 5067-6189, replacement frits 5023-0271

Guard columns
Extend column life
Less expensive than analytical column
Match analytical column packing material
- Traps material that could bind strongly or irreversibly to analytical column
Inlet frit traps particulates

Cartridge format
340 bar, 200 bar
w/PEEK fitting

Individual guard column
600-1300 bar

Tip: Consider the cost vs. benefit
Remember Your

Column Choice
Method conditions
Mobile phase
Sample
Instrument

… all contribute to good chromatography. The more you know about each of them and how they can affect your chromatography, the better your chances of success are.
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*Some restrictions apply on supplies

October 17, 2019
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1-800-227-9770 Option 3, Option 3:

- Option 1 for GC/GCMS Columns and Supplies
- Option 2 for LC/LCMS Columns and Supplies
- Option 3 for Sample Preparation, Filtration and QuEChERS
- Option 4 for Spectroscopy Supplies
- Option 5 for Chemical Standards

- gc-column-support@Agilent.com
- lc-column-support@agilent.com
- spp-support@agilent.com
- spectro-supplies-support@agilent.com
- chem-standards-support@agilent.com
Determining the Dwell Volume of Your System

- Replace column with short piece of HPLC stainless steel tubing
- **Prepare mobile phase components**
  A. Water, UV-transparent
  B. Water with 0.2% acetone, UV-absorbing
- Monitor at 265 nm
- Adjust attenuation so that both 100% A and 100% B are on scale
- Run gradient profile 0 to 100% B/10 min at 1.0 mL/min
- Record
Measuring Dwell Volume ($V_D$)

- Intersection of the two lines identifies dwell time ($t_D$)
- Dwell volume is equal to product of the flow rate and the dwell time.

$$V_D = t_D \times F$$
Measure and Correct for Dwell Volume \((V_D)\)

If \(V_{D1} > V_{D2}\)

Compensate for longer \(V_{D1}\) by adding an isocratic hold to \(V_{D2}\), such that \(Hold + V_{D2} = V_{D1}\)

If \(V_{D1} < V_{D2}\)

Delay injection, such that \(V_{D2} - \text{delay} = V_{D1}\)
How to Estimate the Extracolumn Volume of Your System

One way:

• Remove HPLC column from instrument
• Join injector and detector tubing with zero-dead-volume (ZDV) union
• Inject (0.5–2 μL) of toluene in 100% acetonitrile
• Determine width of peak at base ($W_{\text{instrument}}$)
• Peak bandwidth follows:

$$W^2_{\text{tot}} = W^2_{\text{col}} + W^2_{\text{instrument}}$$

Make concentration about 1–5 mg/mL
Extra Column Volume and Peak Shape

Toluene in Acetonitrile

**Instrument #1**
**Low Volume System**

\[ w_{\text{instrument}} = 42 \ \mu\text{L} \]

**Instrument #2**

\[ w_{\text{instrument}} = 83 \ \mu\text{L} \]

\[ w_{\text{tot}} = w_{\text{col}} + w_{\text{instrument}} \]

*For peak having a \( k' = 2 \)*

\[ w_{\text{tot}} = (180)^2 + (42)^2 \]
\[ w_{\text{tot}} = 185 \ \mu\text{L} \]

\[ w_{\text{tot}} = (180)^2 + (83)^2 \]
\[ w_{\text{tot}} = 198 \ \mu\text{L} \]

\[ w_{\text{tot}} = (73)^2 + (42)^2 \]
\[ w_{\text{tot}} = 84 \ \mu\text{L} \]

\[ w_{\text{tot}} = (73)^2 + (83)^2 \]
\[ w_{\text{tot}} = 110 \ \mu\text{L} \]
## Method Development Kits

<table>
<thead>
<tr>
<th>Part No.</th>
<th>Kits</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>5190-6160</td>
<td>P120, USP method development kit, 3.0 x 100 mm</td>
<td>InfinityLab Poroshell 120 EC-C18, EC-C8, EC-CN columns, 3.0 x 100 mm</td>
</tr>
<tr>
<td>5190-6159</td>
<td>P120, USP method development kit, 4.6 x 100 mm</td>
<td>InfinityLab Poroshell 120 EC-C18, EC-C8, EC-CN columns, 4.6 x 100 mm</td>
</tr>
<tr>
<td>5190-6155</td>
<td>P120, Selectivity method development kit, 2.1 x 50 mm</td>
<td>InfinityLab Poroshell 120 EC-C18, Phenyl-Hexyl, Bonus RP columns, 2.1 x 50 mm</td>
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<tr>
<td>5190-6156</td>
<td>P120, Selectivity method development kit, 4.6 x 50 mm</td>
<td>InfinityLab Poroshell 120 EC-C18, Phenyl-Hexyl, Bonus RP columns, 4.6 x 50 mm</td>
</tr>
<tr>
<td>5190-6157</td>
<td>P120, Aqueous method development kit, 2.1 x 50 mm</td>
<td>InfinityLab Poroshell 120 SB-Aq, Phenyl-Hexyl, Bonus RP columns, 2.1 x 50 mm</td>
</tr>
<tr>
<td>5190-6158</td>
<td>P120, Aqueous method development kit, 4.6 x 50 mm</td>
<td>InfinityLab Poroshell 120 SB-Aq, Phenyl-Hexyl, and Bonus RP columns, 4.6 x 50 mm</td>
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<tr>
<td>5190-6153</td>
<td>RRHD Eclipse Plus method development kit, 2.1 mm id</td>
<td>RRHD Eclipse Plus C18, Eclipse Plus C8, Eclipse Plus Phenyl-Hexyl, 2.1 x 50 mm columns</td>
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<td>5190-6154</td>
<td>RRHD Aqueous method development kit, 2.1 mm id</td>
<td>RRHD SB-Aq, Bonus RP, and Eclipse Plus Phenyl-Hexyl column, 2.1 x 50 mm</td>
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<tr>
<td>5190-6152</td>
<td>RRHD pH method development kit, 2.1 mm id</td>
<td>RRHD StableBond SB-C18, Eclipse Plus C18, and Extend-C18 column, 2.1 x 50 mm</td>
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<tr>
<td>5190-6160</td>
<td>P120, USP method development kit, 3.0 x 100 mm</td>
<td>InfinityLab Poroshell 120 EC-C18, EC-C8, EC-CN columns, 3.0 x 100 mm</td>
</tr>
<tr>
<td>5190-6159</td>
<td>P120, USP method development kit, 4.6 x 100 mm</td>
<td>InfinityLab Poroshell 120 EC-C18, EC-C8, EC-CN columns, 4.6 x 100 mm</td>
</tr>
<tr>
<td>5190-6155</td>
<td>P120, Selectivity method development kit, 2.1 x 50 mm</td>
<td>InfinityLab Poroshell 120 EC-C18, Phenyl-Hexyl, Bonus RP columns, 2.1 x 50 mm</td>
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<td>5190-6156</td>
<td>P120, Selectivity method development kit, 4.6 x 50 mm</td>
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<tr>
<td>5190-6157</td>
<td>P120, Aqueous method development kit, 2.1 x 50 mm</td>
<td>InfinityLab Poroshell 120 SB-Aq, Phenyl-Hexyl, Bonus RP columns, 2.1 x 50 mm</td>
</tr>
</tbody>
</table>
Shutdown State and Instrument Flushing

Shutdown state

Next day use—using same buffers:
  • Pump the mobile phase slowly (for example, 0.01–0.1 mL/min).

When flushing column, or for longer term column storage:
  • Flush with 20/80 organic/water, then 80/20 organic/water or 100% organic.

Instrument flushing

Replace column with capillary tubing. Leave disconnected from detector.
Flush pumps with water, then connect capillary tubing to detector.
Inject water 2–3 times at maximum injection volume setting.
Flush all pumps with 100% organic for long-term storage.