# “"Why Did That Happen?"”

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Agilent InfinityLab

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# "Why Did That Happen?"

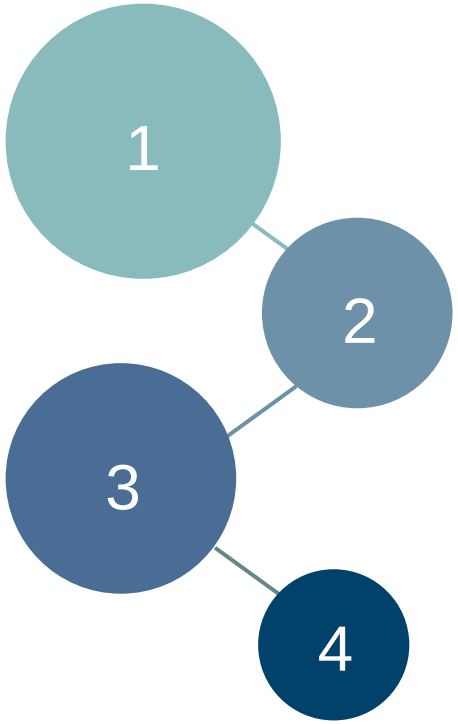
## Common chromatography problems

### Changes in System Pressure

- High pressure
- Low pressure
- Pressure fluctuations

### Changes in Separation

- Changing retention time
- Ghost peaks and carry over




### Changes in Peak Shape

- Tailing
- Broadening
- Fronting
- Peak splitting and doubling

### Changes in Detection

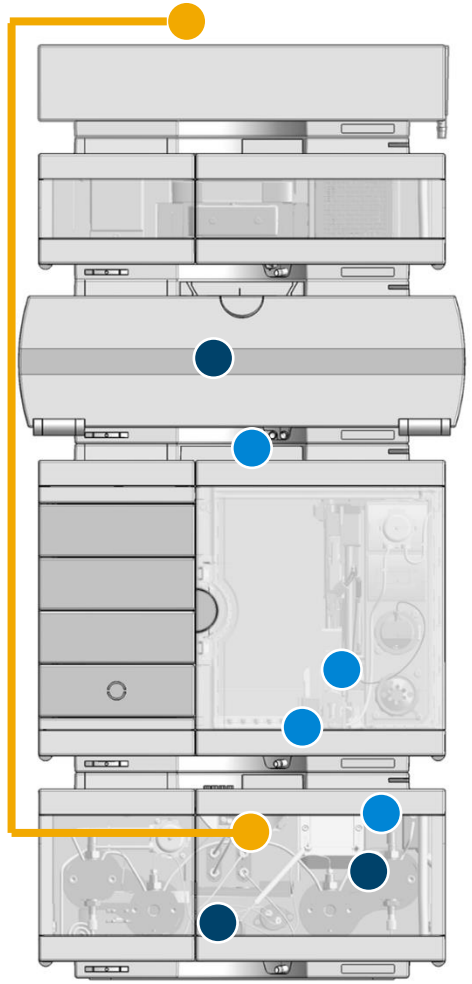
- Noisy baseline
- Reduced intensity or sensitivity

# Part 1: Changes in System Pressure

# Changes in System Pressure

## Increased pressure / overpressure and blockages

| | Potential Cause | Recommended Action |
|---|---|---|
| ● | Clogging of inline filter frit | • Identify the culprit by logical elimination process and replace affected part.<br>• Use HPLC grade solvents, filtered sample and buffers solution<br>• Prevent microbial growth in aqueous solvent bottle |
| ● | Plugging of capillaries, needle and needle seat | |
| ● | Partially clogged guard or column inlet frit | • Replace the guard<br>• Back flush the column, if recommended<br>• Check for correct mobile phase<br>• Check solvent reservoir and tubing connections<br>• Use filtered sample |



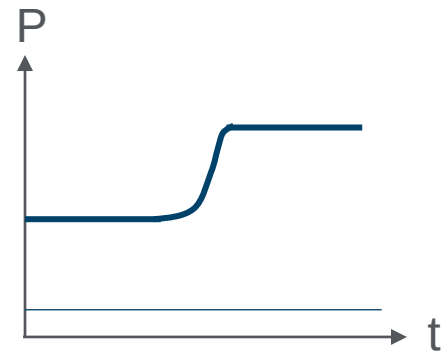
### Important to know

! Clogged filters and plugged flow paths always have a root cause. After identifying the problem, you should check for the root cause and eliminate it where possible.

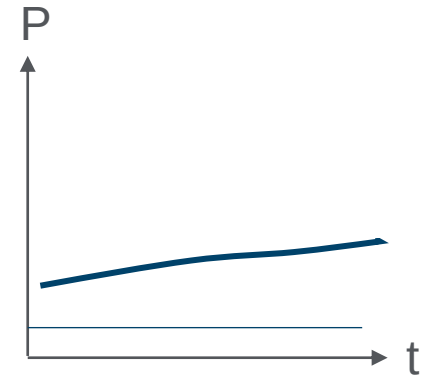
# Changes in System Pressure

## Increased pressure / overpressure and blockages

| Characteristics | |
|---|---|
| Parts affected | Blockages:<br>• Capillaries, needle, and needle seat<br>• Detector flow cells<br>Clogging:<br>• Filter frits (inline filter, column, purge valve) |
| Characteristic | |
| Possible root cause | • Debris from mechanically worn parts (needle seat material, rotor seal at injection valve)<br>• Coring of vial septa material |
| Identification | • Start by disconnecting the capillary at the column inlet<br>• Install test setup with restriction capillary<br>• Continue disconnecting capillaries, one-by-one, moving back toward the pump |
| Instant action / first aid | • Backflush affected part<br>• Replace part |
| Preventive measures | • Replace wear parts in time; apply proper preventive maintenance schedules<br>• Use high-quality septa<br>• Install inline filters/replace frit |




**Blockages:** Sudden pressure increase




**Clogging:** Gradual pressure increase over time

# Changes in System Pressure

## How to prevent clogging

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### Solvent Filtration

### Compatible filter membranes

| Part Number | Membrane Type |
|---|---|
| 5191-4336 | PTFE filter membrane, 47 mm diameter, 0.45 µm; 100/pack |
| 5191-4339 | PTFE filter membrane, 47 mm diameter, 0.20 µm; 100/pack |
| 5191-4338 | Nylon filter membrane, 47 mm diameter, 0.45 µm; 100/pack |
| 5191-4341 | Nylon filter membrane, 47 mm diameter, 0.20 µm; 100/pack |
| 5191-4337 | Regenerated Cellulose filter membrane, 47 mm diameter, 0.45 µm; 100/pack |
| 5191-4340 | Regenerated Cellulose filter membrane, 47 mm diameter, 0.20 µm; 100/pack |

5191-6776

Operational manual: 5994-1507EN
Technical Overview: 5994-1504EN

# Changes in System Pressure

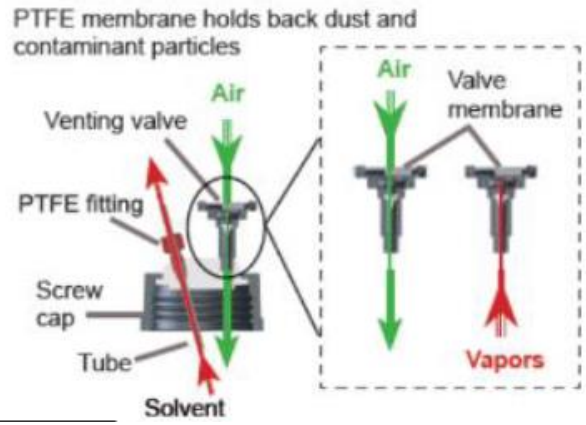
## How to prevent clogging

### Prevent Microbial Growth in Solvent Bottle

- Use high-purity solvents
- Use freshly made HPLC grade solvent and filtered buffer
- Use appropriate solvent bottle caps (Agilent InfinityLab Stay Safe caps)
- Replace solvent inlet filter as needed
- Always discard “old” mobile phase
- Do not add fresh mobile phase to old
- Use an amber solvent bottle for aqueous mobile phase
- If possible, add 5% organic to water to reduce microbial growth, or add a few mg/L sodium azide

# InfinityLab Stay Safe Caps

| Main Features | Advantages |
| --- | --- |
| *Venting valve for mobile phase*<br>*Charcoal filter for waste container*<br>*Time strip* | Eliminates harmful solvent vapors<br>Keeps solvent concentration constant<br>Keeps constant pressure in bottle<br>Prevents twisted tubing<br>Allows easy solvent refill<br>Allows easy tightening |

# Changes in System Pressure

## How to prevent clogging

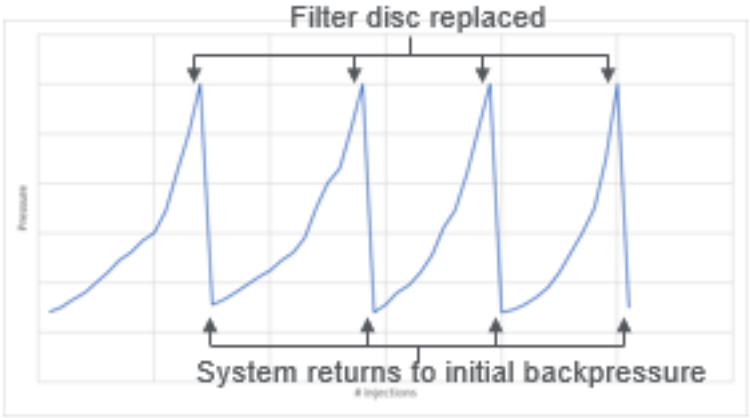
Agilent InfinityLab

### Inline Filters

### InfinityLab Quick Change Inline Filter

**Ultimate ease of use**

- **Finger-tight, tool-free** replacement of filter disc
- **Click & Seal**: a click sound tells you when the filter is tight up to 1300 bar, no risk of over-tightening or under-tightening

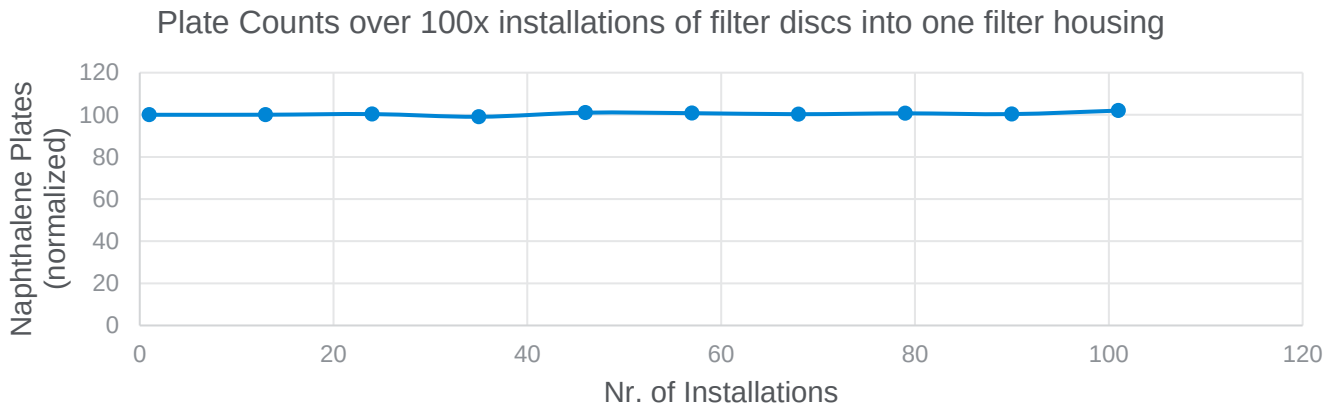



**Robustness for low operational cost**

Robust filter housing that enables **over 100 replacements** of filter discs without any damage

**Extend column lifetime**

Pressure increase caused by particles is quickly and easily eliminated by replacing the filter disk without damaging your valuable columns



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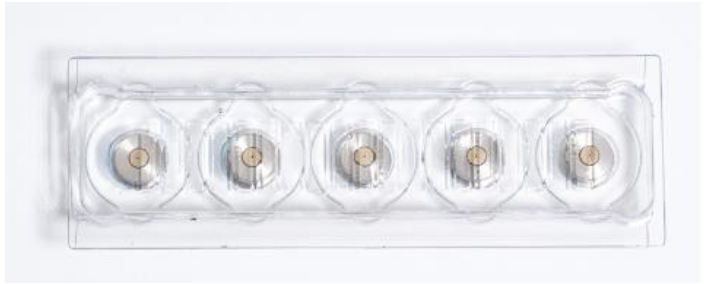
# InfinityLab Quick Change Inline Filter

## High efficiency, easy to use filter discs

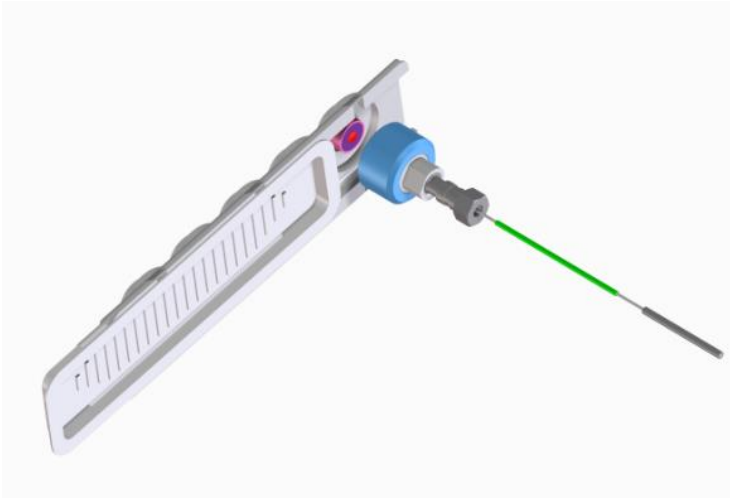
- **A variety of dimensions and porosities** – filter discs are available in 2.1 mm and 4.6 mm inner diameters with different pore sizes. The filter housing is compatible with all types of filter discs.
- **Touchless packaging to avoid potential contamination** – with the special designed packaging you're able to insert the filter disc into filter housing without touching it to avoid potential contamination
- **In-situ replacement** of filter disc – no need to disconnect the inline filter from the system
- **Smart alert** to remind users of replacing the filter discs – **coming soon**

| 2.1 mm | 2.1 mm | 4.6 mm | 4.6 mm | 4.6 mm |
|---|---|---|---|---|
| 0.2 µm | 0.5 µm | 0.2 µm | 0.5 µm | 2.0 µm |

Different dimensions and porosities of filter discs



Filter discs in touchless packaging



Insertion of filter disc into filter housing without touching

- Video
- Flyer: 5994-3028EN
- Installation instructions: 5994-2779EN

# InfinityLab Quick Change Inline Filter

| Inline Filter Assemblies | |
|---|---|
| InfinityLab Quick Change inline filter assembly for UHPLC (incl. 5 filter discs 2.1 mm id, 0.2 µm porosity), with 90 mm flexible capillary* | 5067-1603 |
| InfinityLab Quick Change inline filter assembly for HPLC (incl. 5 filter discs 4.6 mm id, 0.5 µm porosity), with 90 mm flexible capillary* | 5067-1602 |
| **Filter discs** | |
| Filter discs 2.1 mm id, 0.2 µm porosity, 5/pk, in touchless packaging | 5067-1610 |
| Filter discs 2.1 mm id, 0.5 µm porosity, 5/pk, in touchless packaging | 5067-1611 |
| Filter discs 4.6 mm ID, 0.2 µm porosity, 5/pk, in touchless packaging | 5067-1612 |
| Filter discs 4.6 mm id, 0.5 µm porosity, 5/pk, in touchless packaging | 5067-1613 |
| Filter discs 4.6 mm id, 2.0 µm porosity, 5/pk, in touchless packaging | 5067-1614 |
| **Replacement capillaries** | |
| Capillary stainless steel 0.12 mm id, 90 mm length, extralong fittings (2/pk), preswaged on one end, non-swaged on the other end, for inline filter for UHPLC | 5023-3344 |
| Capillary stainless steel 0.17 mm id, 90 mm length, extralong fittings (2/pk), preswaged on one end, non-swaged on the other end, for inline filter for HPLC | 5023-3343 |

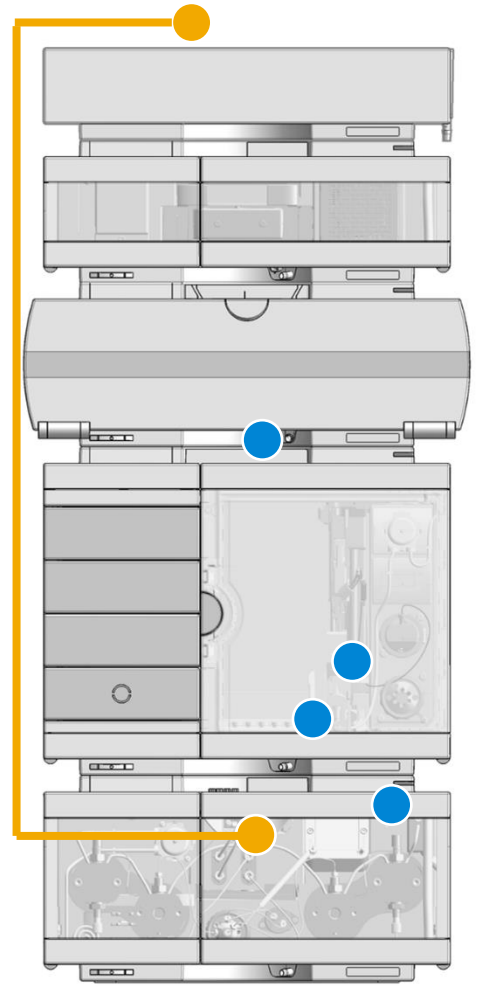
**Another version with rigid capillary for installation directly in front of column is due to be released soon*

InfinityLab Quick Change inline filter assembly
(a packaging insert with user instructions is also included)

# Changes in System Pressure

## Low pressure

| | Potential Cause | Recommended Action |
|---|---|---|
| ● | Leak in high-pressure flow path | • Visual inspection of flow path<br>• Instrument diagnostic tests |
| ● | Wrong mobile phase | • Check for correct mobile phase<br>• Solvent reservoir and tube connection |



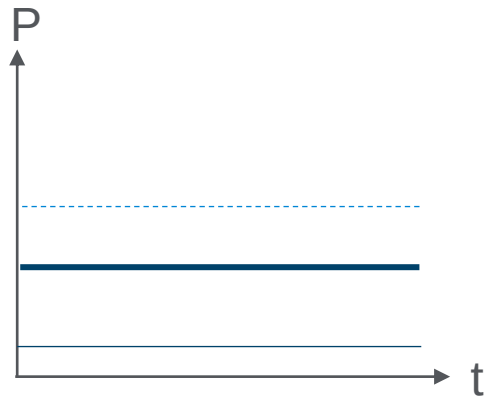
### Important to know

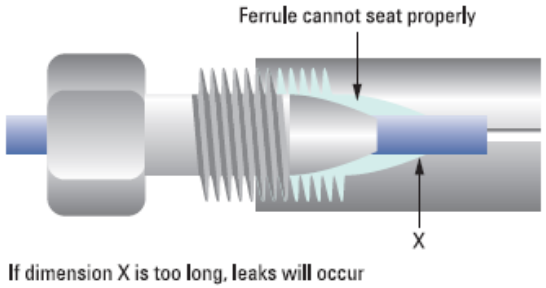
With its advanced diagnostic and maintenance capabilities, **Agilent Lab Advisor SW** helps you to keep your Agilent analytical instruments in top condition. Agilent Lab Advisor is independent of the chromatography software you are using.

# Changes in System Pressure

## Low pressure – Leaks

| Characteristics | |
|---|---|
| Parts affected | • Potentially all parts in the flow path<br>• High potential at frequently operated fitting connections (such as the column inlet) and parts with high mechanical stress (rotor seal, needle, and needle seat) |
| Characteristic | • Lower pressure<br>• Potentially impacting retention times and peak shape |
| Identification | • Drops of solvent or residue of salt<br>• System diagnostic tests |
| Possible root Cause | • Loose or bad fitting connections<br>• Cracked capillaries<br>• Worn needle and needle seat |
| Instant action / first aid | • Replace affected parts<br>• Renew or redo fitting connection |
| Preventive measures | • Use proper fitting connections<br>• Replace fittings and wear parts in time |



If dimension X is too long, leaks will occur

# Changes in System Pressure

## Low pressure – Leaks and the importance of proper fittings

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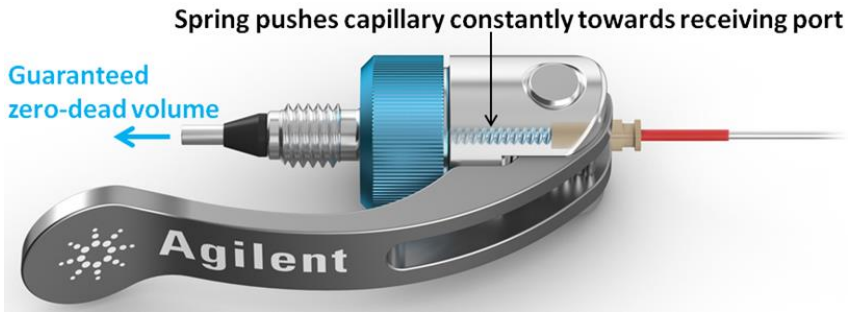
**InfinityLab Quick Connect and Quick Turn fittings**

- Spring loaded design
- Easy; no tools needed!
- Works for all column types
- Reusable
- Consistent ZDV connection




**Quick Connect fitting**

- Finger tight up to 1300 bar
- Hand tighten the nut, then depress the lever

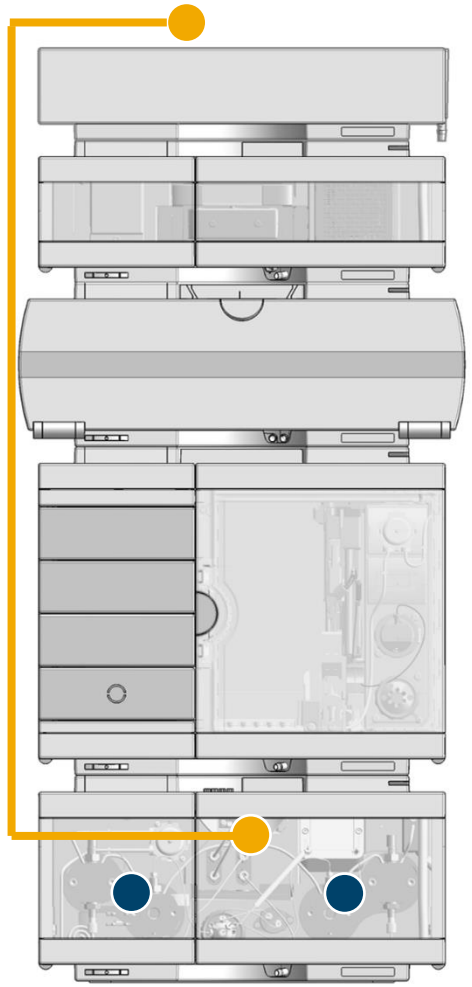

**Quick Turn fitting**

- Finger tight up to 400 bar
- Up to 800 bar with mounting tool
- Up to 1300 bar with a wrench
- Compact design, fits everywhere

# Changes in System Pressure

## Pressure fluctuations

| | Potential Cause | Recommended Action |
|---|---|---|
| ● | Air in the system | • Prime and flush instrument<br>• Check for sufficient solvent supply<br>• Check for correct plumbing (SSV/MCGV)<br>• Check for correct degassing |
| ● | Malfunctions at pump head | • Perform pump head diagnostic tests<br>• Replace defective parts<br>• Implement proper maintenance schedule |
| ● | Cavitation effects | • Check for flow restrictions (solvent bottle to pump head inlet)<br>• Clean or replace parts<br>• Verify that solvent supply is positioned above pump inlet |



### Important to know

! Pressure fluctuations typically also will impact the UV-signal due to refractive index effects.

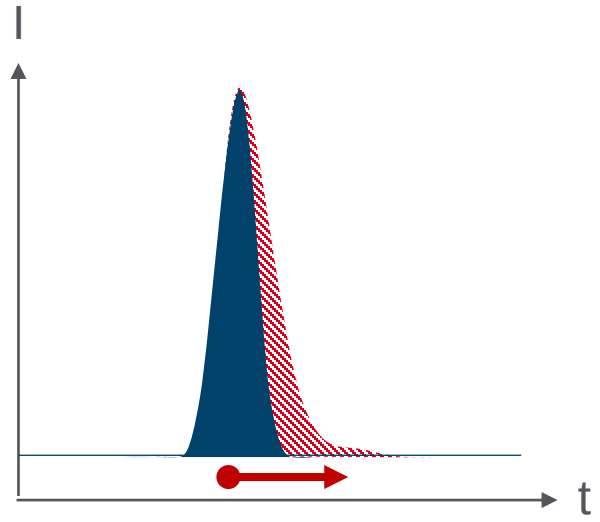
# Part 2: Changes in Peak Shape

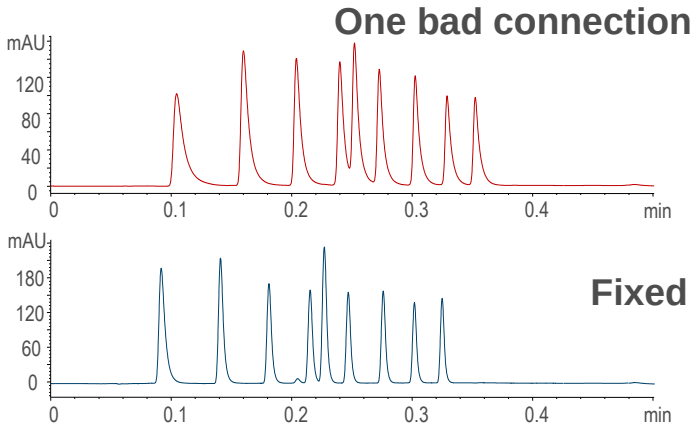
# Changes in Peak Shape

## Peak tailing

| If applicable to some peaks | Recommended action |
|---|---|
| Secondary interactions- could be due to improper pH of the mobile phase, column stationary phase, or contaminations from sample | • Change pH<br>• Change stationary phase<br>• Clean the column<br>• Sample cleanup |
| Small peak eluting on tail of larger peak | • Change selectivity (column, mobile phase).<br>• Switch to methods with higher resolution (UHPLC, 2D-LC) |

| If applicable to all peaks | Recommended action |
|---|---|
| Poor tubing connections; high dispersion volume/mixing chamber at connection sites; partially clogged column inlet frit | • Minimize number of connections<br>• Check connections / fitting condition and proper seat of fittings<br>• Use fittings with spring-load function<br>• Back flush the column (if recommended) |
| Silica based – column degradation at high pH | • Use specialty, polymeric, or sterically protected column |
| Silica based – interactions of basic analytes with stationary phase | • Use correct pH mobile phase or add appropriate base (for example, TEA) |



# About Columns

## Stationary phase selection guide

| Starting Recommendation | | | | | |
|---|---|---|---|---|---|
| Poroshell 120 EC-C18 | | | | | |
| **Change selectivity slightly** | **Change selectivity significantly** | **For many early eluters** | **No retention at 98+% Aqueous in RP** | **Acidic solvents (pH < 2)** | **Basic solvents (pH >6)** |
| 1. Poroshell Phenyl-Hexyl<br>2. Polaris C18-A<br>3. Poroshell EC-C8 | 1. Poroshell Bonus-RP<br>2. Poroshell PFP<br>3. Pursuit XRs Diphenyl | 1. Poroshell SB-Aq<br>2. Poroshell PFP<br>3. Poroshell HILIC-Z | 1. Poroshell HILIC-Z<br>2. Poroshell PFP<br>3. Poroshell HILIC-OH5 | 1. Poroshell SB-C18<br>2. Poroshell SB-Aq<br>3. PLRP-S<br>4. Poroshell SB-C8 | 1. Poroshell HPH-C18<br>2. PLRP-S<br>3. Poroshell CS-C18 |

| Starter Kit (covers 95% of analyses) | | |
|---|---|---|
| Poroshell EC-C18 | Poroshell HILIC-Z | Poroshell PFP |

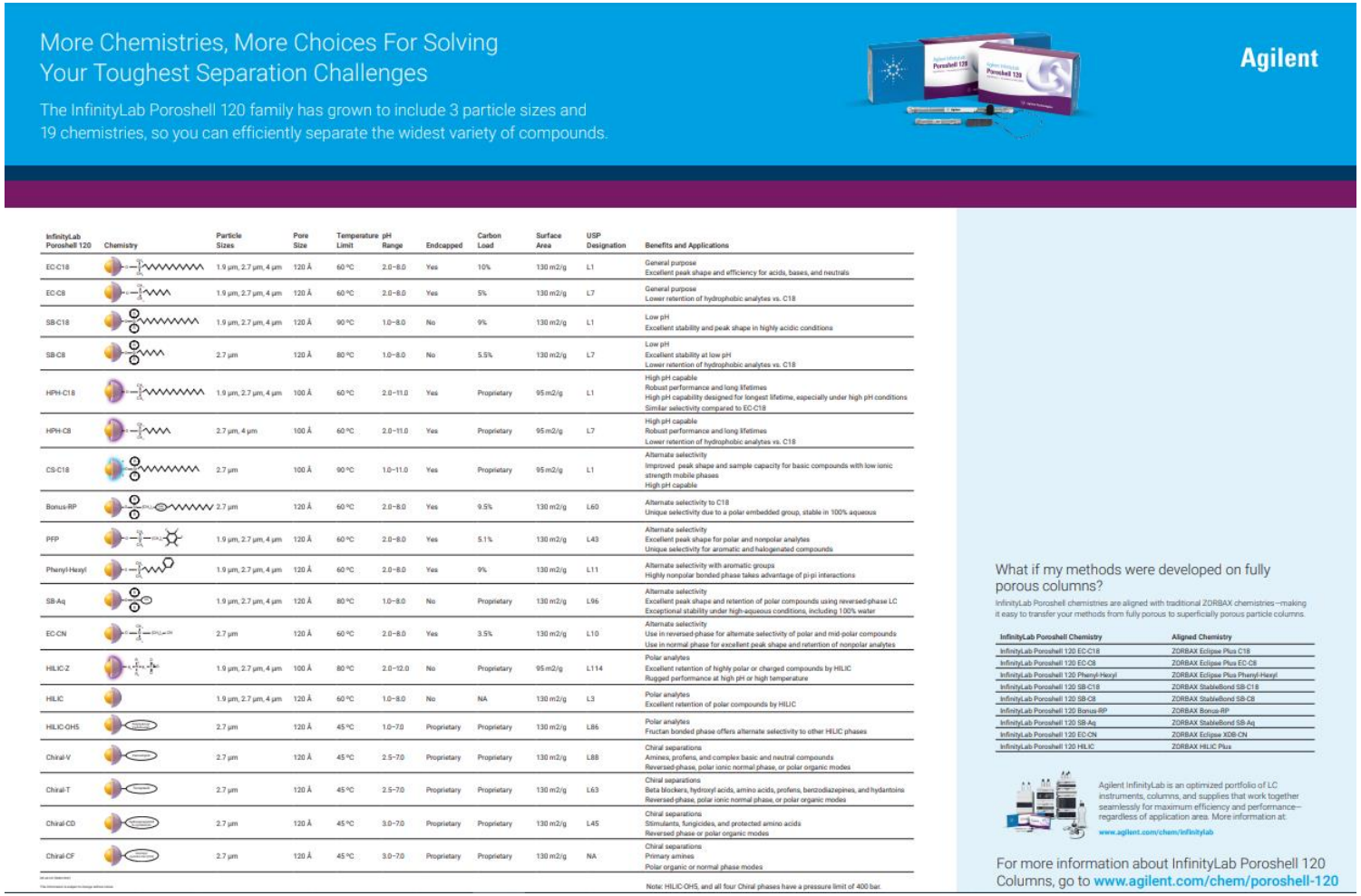
| Recommended Solvent A (Weak) | Solvent B (Strong) |
|---|---|
| 1. 0.1% formic acid (pH ~2.7)<br>2. 10 mmol ammonium acetate (adj. pH 5)<br>3. 0.1% ammonium hydroxide (pH ~10)<br>4. 0.1% trifluoroacetic acid (pH ~1.5, ~~*MS*~~)<br>5. 150 mmol sodium phosphate (adj. pH 3, ~~*MS*~~) | 1. Acetonitrile<br>2. Methanol<br>3. Isopropanol<br>4. THF<br>5. Acetone |

| USP L1 | USP L7 |
|---|---|
| 1. Poroshell 120 EC-C18<br>2. Polaris C18-A<br>3. Polaris C18-Ether<br>4. Pursuit XRs C18 | 1. Poroshell 120 EC-C8<br>2. Polaris C8-A |
| **USP L3** | **USP L8** |
| 1. Poroshell 120 HILIC<br>2. ZORBAX Rx-Sil<br>3. Pursuit XRs Si | 1. Polaris NH2<br>2. ZORBAX NH2 |
| | **USP L11** |
| | 1. Poroshell Phe-Hex<br>2. Pursuit XRs Diphenyl |

| Sugars (RI or ELSD) | Normal Phase | Chiral |
|---|---|---|
| 1. Poroshell HILIC-Z<br>2. Hi-Plex H<br>3. Hi-Plex Ca<br>4. Polaris NH2 | 1. Poroshell HILIC<br>2. Poroshell EC-CN<br>3. Polaris NH2 | 1. Poroshell Chiral-V<br>2. Poroshell Chiral-T<br>3. Poroshell Chiral-CD<br>4. Poroshell Chiral-CF |

# InfinityLab Poroshell 120 Column Specifications

Poster: 5991-9013EN



More Chemistries, More Choices For Solving Your Toughest Separation Challenges

The InfinityLab Poroshell 120 family has grown to include 3 particle sizes and 19 chemistries, so you can efficiently separate the widest variety of compounds.

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| InfinityLab Poroshell 120 | Chemistry | Particle Sizes | Pore Size | Temperature Limit | pH Range | Endcapped | Carbon Load | Surface Area | USP Designation | Benefits and Applications |
|---|---|---|---|---|---|---|---|---|---|---|
| EC-C18 | | 1.9 µm, 2.7 µm, 4 µm | 120 Å | 60 °C | 2.0–8.0 | Yes | 10% | 130 m2/g | L1 | General purpose<br>Excellent peak shape and efficiency for acids, bases, and neutrals |
| EC-C8 | | 1.9 µm, 2.7 µm, 4 µm | 120 Å | 60 °C | 2.0–8.0 | Yes | 5% | 130 m2/g | L7 | General purpose<br>Lower retention of hydrophobic analytes vs. C18 |
| SB-C18 | | 1.9 µm, 2.7 µm, 4 µm | 120 Å | 90 °C | 1.0–8.0 | No | 9% | 130 m2/g | L1 | Low pH<br>Excellent stability and peak shape in highly acidic conditions |
| SB-C8 | | 2.7 µm | 120 Å | 80 °C | 1.0–8.0 | No | 5.5% | 130 m2/g | L7 | Low pH<br>Excellent stability at low pH<br>Lower retention of hydrophobic analytes vs. C18 |
| HPH-C18 | | 1.9 µm, 2.7 µm, 4 µm | 100 Å | 60 °C | 2.0–11.0 | Yes | Proprietary | 95 m2/g | L1 | High pH capable<br>Robust performance and long lifetimes<br>High pH capability designed for longest lifetime, especially under high pH conditions<br>Similar selectivity compared to EC-C18 |
| HPH-C8 | | 2.7 µm, 4 µm | 100 Å | 60 °C | 2.0–11.0 | Yes | Proprietary | 95 m2/g | L7 | High pH capable<br>Robust performance and long lifetimes<br>Lower retention of hydrophobic analytes vs. C18 |
| CS-C18 | | 2.7 µm | 100 Å | 90 °C | 1.0–11.0 | Yes | Proprietary | 95 m2/g | L1 | Alternate selectivity<br>Improved peak shape and sample capacity for basic compounds with low ionic strength mobile phases<br>High pH capable |
| Bonus-RP | | 2.7 µm | 120 Å | 60 °C | 2.0–8.0 | Yes | 9.5% | 130 m2/g | L60 | Alternate selectivity to C18<br>Unique selectivity due to a polar embedded group, stable in 100% aqueous |
| PFP | | 1.9 µm, 2.7 µm, 4 µm | 120 Å | 60 °C | 2.0–8.0 | Yes | 5.1% | 130 m2/g | L43 | Alternate selectivity<br>Excellent peak shape for polar and nonpolar analytes<br>Unique selectivity for aromatic and halogenated compounds |
| Phenyl-Hexyl | | 1.9 µm, 2.7 µm, 4 µm | 120 Å | 60 °C | 2.0–8.0 | Yes | 9% | 130 m2/g | L11 | Alternate selectivity with aromatic groups<br>Highly nonpolar bonded phase takes advantage of pi-pi interactions |
| SB-Aq | | 1.9 µm, 2.7 µm, 4 µm | 120 Å | 80 °C | 1.0–8.0 | No | Proprietary | 130 m2/g | L96 | Alternate selectivity<br>Excellent peak shape and retention of polar compounds using reversed-phase LC<br>Exceptional stability under high-aqueous conditions, including 100% water |
| EC-CN | | 2.7 µm | 120 Å | 60 °C | 2.0–8.0 | Yes | 3.5% | 130 m2/g | L10 | Alternate selectivity<br>Use in reversed-phase for alternate selectivity of polar and mid-polar compounds<br>Use in normal phase for excellent peak shape and retention of nonpolar analytes |
| HILIC-Z | | 1.9 µm, 2.7 µm, 4 µm | 100 Å | 80 °C | 2.0–12.0 | No | Proprietary | 95 m2/g | L114 | Polar analytes<br>Excellent retention of highly polar or charged compounds by HILIC<br>Rugged performance at high pH or high temperature |
| HILIC | | 1.9 µm, 2.7 µm, 4 µm | 120 Å | 60 °C | 1.0–8.0 | No | NA | 130 m2/g | L3 | Polar analytes<br>Excellent retention of polar compounds by HILIC |
| HILIC-OH5 | | 2.7 µm | 120 Å | 45 °C | 1.0–7.0 | Proprietary | Proprietary | 130 m2/g | L86 | Polar analytes<br>Fructan bonded phase offers alternate selectivity to other HILIC phases |
| Chiral-V | | 2.7 µm | 120 Å | 45 °C | 2.5–7.0 | Proprietary | Proprietary | 130 m2/g | L88 | Chiral separations<br>Amines, profens, and complex basic and neutral compounds<br>Reversed-phase, polar ionic normal phase, or polar organic modes |
| Chiral-T | | 2.7 µm | 120 Å | 45 °C | 2.5–7.0 | Proprietary | Proprietary | 130 m2/g | L63 | Chiral separations<br>Beta blockers, hydroxyl acids, amino acids, profens, benzodiazepines, and hydantoins<br>Reversed-phase, polar ionic normal phase, or polar organic modes |
| Chiral-CD | | 2.7 µm | 120 Å | 45 °C | 3.0–7.0 | Proprietary | Proprietary | 130 m2/g | L45 | Chiral separations<br>Stimulants, fungicides, and protected amino acids<br>Reversed-phase or polar organic modes |
| Chiral-CF | | 2.7 µm | 120 Å | 45 °C | 3.0–7.0 | Proprietary | Proprietary | 130 m2/g | NA | Chiral separations<br>Primary amines<br>Polar organic or normal phase modes |

Note: HILIC-OH5, and all four Chiral phases have a pressure limit of 400 bar.

What if my methods were developed on fully porous columns?

InfinityLab Poroshell chemistries are aligned with traditional ZORBAX chemistries—making it easy to transfer your methods from fully porous to superficially porous particle columns.

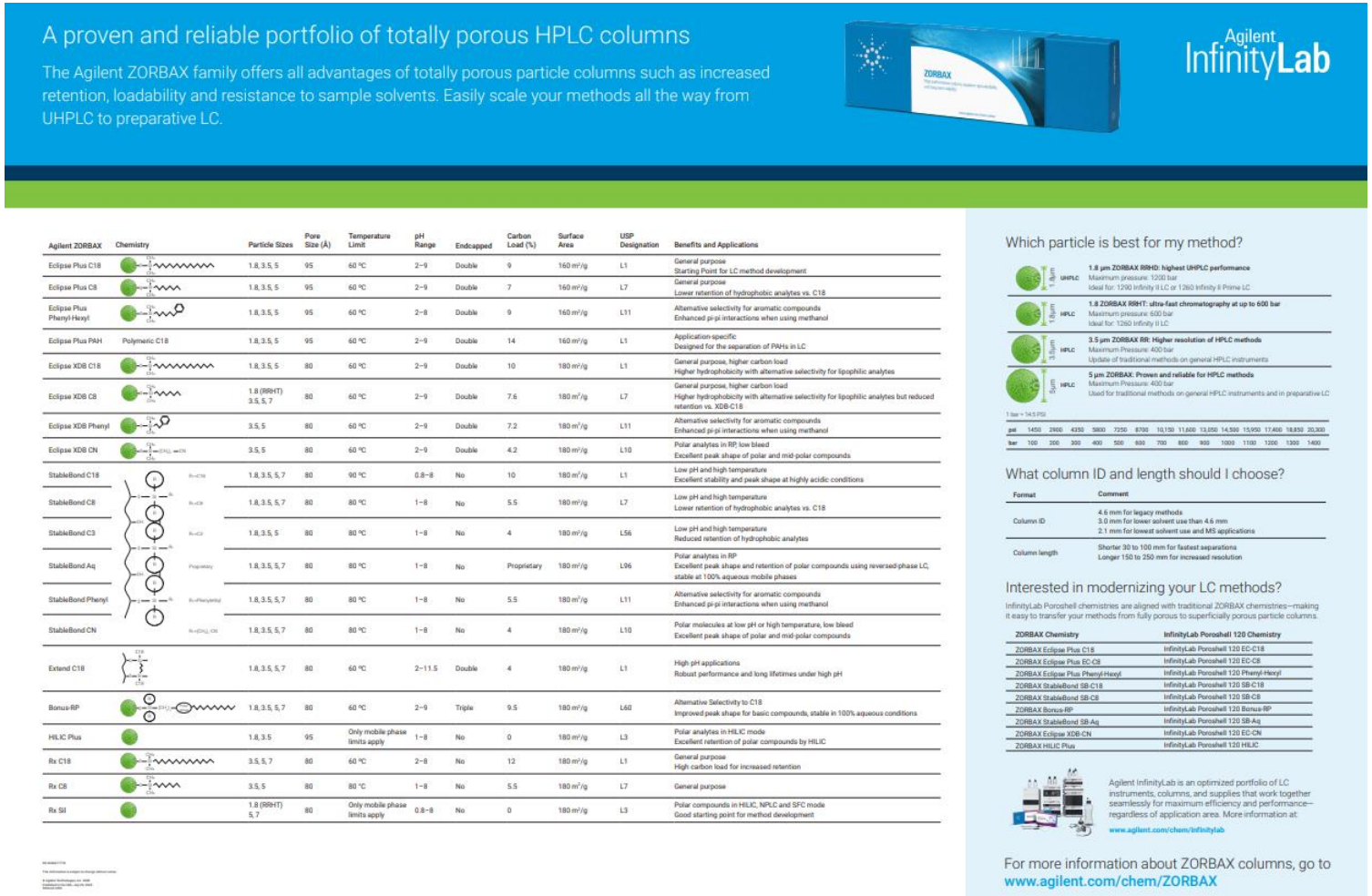
| InfinityLab Poroshell Chemistry | Aligned Chemistry |
|---|---|
| InfinityLab Poroshell 120 EC-C18 | ZORBAX Eclipse Plus C18 |
| InfinityLab Poroshell 120 EC-C8 | ZORBAX Eclipse Plus EC-C8 |
| InfinityLab Poroshell 120 Phenyl-Hexyl | ZORBAX Eclipse Plus Phenyl-Hexyl |
| InfinityLab Poroshell 120 SB-C18 | ZORBAX StableBond SB-C18 |
| InfinityLab Poroshell 120 SB-C8 | ZORBAX StableBond SB-C8 |
| InfinityLab Poroshell 120 Bonus-RP | ZORBAX Bonus-RP |
| InfinityLab Poroshell 120 SB-Aq | ZORBAX StableBond SB-Aq |
| InfinityLab Poroshell 120 EC-CN | ZORBAX Eclipse XDB-CN |
| InfinityLab Poroshell 120 HILIC | ZORBAX HILIC Plus |

Agilent InfinityLab is an optimized portfolio of LC instruments, columns, and supplies that work together seamlessly for maximum efficiency and performance—regardless of application area. More information at: www.agilent.com/chem/infinitylab

For more information about InfinityLab Poroshell 120 Columns, go to www.agilent.com/chem/poroshell-120

# ZORBAX Column Specifications

Poster: [5994-2212EN]



## A proven and reliable portfolio of totally porous HPLC columns

The Agilent ZORBAX family offers all advantages of totally porous particle columns such as increased retention, loadability and resistance to sample solvents. Easily scale your methods all the way from UHPLC to preparative LC.

| Agilent ZORBAX | Chemistry | Particle Sizes | Pore Size (Å) | Temperature Limit | pH Range | Endcapped | Carbon Load (%) | Surface Area | USP Designation | Benefits and Applications |
|---|---|---|---|---|---|---|---|---|---|---|
| Eclipse Plus C18 | | 1.8, 3.5, 5 | 95 | 60 °C | 2–9 | Double | 9 | 160 m²/g | L1 | General purpose<br>Starting Point for LC method development |
| Eclipse Plus C8 | | 1.8, 3.5, 5 | 95 | 60 °C | 2–9 | Double | 7 | 160 m²/g | L7 | General purpose<br>Lower retention of hydrophobic analytes vs. C18 |
| Eclipse Plus Phenyl-Hexyl | | 1.8, 3.5, 5 | 95 | 60 °C | 2–8 | Double | 9 | 160 m²/g | L11 | Alternative selectivity for aromatic compounds<br>Enhanced pi-pi interactions when using methanol |
| Eclipse Plus PAH | Polymeric C18 | 1.8, 3.5, 5 | 95 | 60 °C | 2–9 | Double | 14 | 160 m²/g | L1 | Application-specific<br>Designed for the separation of PAHs in LC |
| Eclipse XDB C18 | | 1.8, 3.5, 5 | 80 | 60 °C | 2–9 | Double | 10 | 180 m²/g | L1 | General purpose, higher carbon load<br>Higher hydrophobicity with alternative selectivity for lipophilic analytes |
| Eclipse XDB C8 | | 1.8 (RRHT) 3.5, 5, 7 | 80 | 60 °C | 2–9 | Double | 7.6 | 180 m²/g | L7 | General purpose, higher carbon load<br>Higher hydrophobicity with alternative selectivity for lipophilic analytes but reduced retention vs. XDB-C18 |
| Eclipse XDB Phenyl | | 3.5, 5 | 80 | 60 °C | 2–9 | Double | 7.2 | 180 m²/g | L11 | Alternative selectivity for aromatic compounds<br>Enhanced pi-pi interactions when using methanol |
| Eclipse XDB CN | | 3.5, 5 | 80 | 60 °C | 2–9 | Double | 4.2 | 180 m²/g | L10 | Polar analytes in RP, low bleed<br>Excellent peak shape of polar and mid-polar compounds |
| StableBond C18 | | 1.8, 3.5, 5, 7 | 80 | 90 °C | 0.8–8 | No | 10 | 180 m²/g | L1 | Low pH and high temperature<br>Excellent stability and peak shape at highly acidic conditions |
| StableBond C8 | | 1.8, 3.5, 5, 7 | 80 | 80 °C | 1–8 | No | 5.5 | 180 m²/g | L7 | Low pH and high temperature<br>Lower retention of hydrophobic analytes vs. C18 |
| StableBond C3 | | 1.8, 3.5, 5 | 80 | 80 °C | 1–8 | No | 4 | 180 m²/g | L56 | Low pH and high temperature<br>Reduced retention of hydrophobic analytes |
| StableBond Aq | | 1.8, 3.5, 5, 7 | 80 | 80 °C | 1–8 | No | Proprietary | 180 m²/g | L96 | Polar analytes in RP<br>Excellent peak shape and retention of polar compounds using reversed-phase LC, stable at 100% aqueous mobile phases |
| StableBond Phenyl | | 1.8, 3.5, 5, 7 | 80 | 80 °C | 1–8 | No | 5.5 | 180 m²/g | L11 | Alternative selectivity for aromatic compounds<br>Enhanced pi-pi interactions when using methanol |
| StableBond CN | | 1.8, 3.5, 5, 7 | 80 | 80 °C | 1–8 | No | 4 | 180 m²/g | L10 | Polar molecules at low pH or high temperature, low bleed<br>Excellent peak shape of polar and mid-polar compounds |
| Extend C18 | | 1.8, 3.5, 5, 7 | 80 | 60 °C | 2–11.5 | Double | 4 | 180 m²/g | L1 | High pH applications<br>Robust performance and long lifetimes under high pH |
| Bonus-RP | | 1.8, 3.5, 5, 7 | 80 | 60 °C | 2–9 | Triple | 9.5 | 180 m²/g | L60 | Alternative Selectivity to C18<br>Improved peak shape for basic compounds, stable in 100% aqueous conditions |
| HILIC Plus | | 1.8, 3.5 | 95 | Only mobile phase limits apply | 1–8 | No | 0 | 180 m²/g | L3 | Polar analytes in HILIC mode<br>Excellent retention of polar compounds by HILIC |
| Rx C18 | | 3.5, 5, 7 | 80 | 60 °C | 2–8 | No | 12 | 180 m²/g | L1 | General purpose<br>High carbon load for increased retention |
| Rx C8 | | 3.5, 5 | 80 | 80 °C | 1–8 | No | 5.5 | 180 m²/g | L7 | General purpose |
| Rx Sil | | 1.8 (RRHT) 5, 7 | 80 | Only mobile phase limits apply | 0.8–8 | No | 0 | 180 m²/g | L3 | Polar compounds in HILIC, NPLC and SFC mode<br>Good starting point for method development |

### Which particle is best for my method?

| | | |
|---|---|---|
| 1.8µm | UHPLC | **1.8 µm ZORBAX RRHD: highest UHPLC performance**<br>Maximum pressure: 1200 bar<br>Ideal for: 1290 Infinity II LC or 1260 Infinity II Prime LC |
| 1.8µm | HPLC | **1.8 ZORBAX RRHT: ultra-fast chromatography at up to 600 bar**<br>Maximum pressure: 600 bar<br>Ideal for: 1260 Infinity II LC |
| 3.5µm | HPLC | **3.5 µm ZORBAX RR: Higher resolution of HPLC methods**<br>Maximum Pressure: 400 bar<br>Update of traditional methods on general HPLC instruments |
| 5µm | HPLC | **5 µm ZORBAX: Proven and reliable for HPLC methods**<br>Maximum Pressure: 400 bar<br>Used for traditional methods on general HPLC instruments and in preparative LC |

1 bar = 14.5 PSI

| psi | 1450 | 2900 | 4350 | 5800 | 7250 | 8700 | 10,150 | 11,600 | 13,050 | 14,500 | 15,950 | 17,400 | 18,850 | 20,300 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| bar | 100 | 200 | 300 | 400 | 500 | 600 | 700 | 800 | 900 | 1000 | 1100 | 1200 | 1300 | 1400 |

### What column ID and length should I choose?

| Format | Comment |
|---|---|
| Column ID | 4.6 mm for legacy methods<br>3.0 mm for lower solvent use than 4.6 mm<br>2.1 mm for lowest solvent use and MS applications |
| Column length | Shorter 30 to 100 mm for fastest separations<br>Longer 150 to 250 mm for increased resolution |

### Interested in modernizing your LC methods?

InfinityLab Poroshell chemistries are aligned with traditional ZORBAX chemistries—making it easy to transfer your methods from fully porous to superficially porous particle columns.

| ZORBAX Chemistry | InfinityLab Poroshell 120 Chemistry |
|---|---|
| ZORBAX Eclipse Plus C18 | InfinityLab Poroshell 120 EC-C18 |
| ZORBAX Eclipse Plus EC-C8 | InfinityLab Poroshell 120 EC-C8 |
| ZORBAX Eclipse Plus Phenyl-Hexyl | InfinityLab Poroshell 120 Phenyl-Hexyl |
| ZORBAX StableBond SB-C18 | InfinityLab Poroshell 120 SB-C18 |
| ZORBAX StableBond SB-C8 | InfinityLab Poroshell 120 SB-C8 |
| ZORBAX Bonus-RP | InfinityLab Poroshell 120 Bonus-RP |
| ZORBAX StableBond SB-Aq | InfinityLab Poroshell 120 SB-Aq |
| ZORBAX Eclipse XDB-CN | InfinityLab Poroshell 120 EC-CN |
| ZORBAX HILIC Plus | InfinityLab Poroshell 120 HILIC |

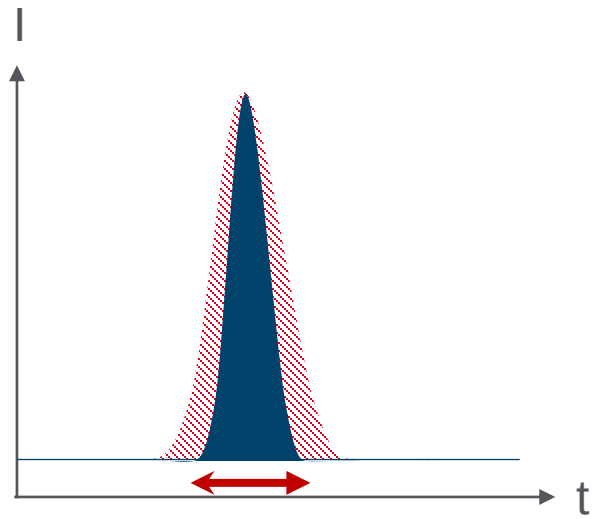
Agilent InfinityLab is an optimized portfolio of LC instruments, columns, and supplies that work together seamlessly for maximum efficiency and performance—regardless of application area. More information at:
www.agilent.com/chem/infinitylab

For more information about ZORBAX columns, go to
www.agilent.com/chem/ZORBAX

# Changes in Peak Shape

## Peak broadening

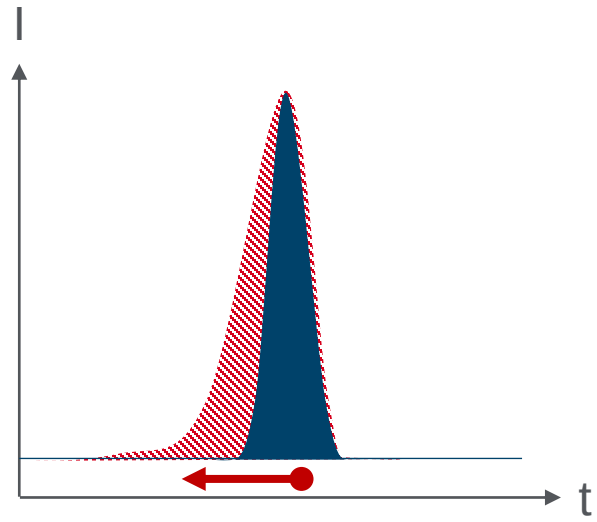
| Potential Cause | Recommended Action |
|---|---|
| Injection volume too large | • Decrease injection volume |
| Long retention times | • Use gradient elution or stronger mobile phase |
| Detector settings | • Check data collection rate:<br>• Adjust the detector setting and / or time constant to the fastest possible value without compromising signal-to-noise. |
| Viscosity of mobile phase too high | • Increase column temperature |
| Detector cell volume too large | • Use smallest possible cell volume |
| Improper fittings / connections | • Ensure that your fitting connections are made correct |
| Extra tubing volume on system | • Ensure that the tubing is narrow and as short as possible to avoid extra volume. |
| Sample solvent too strong | • Reduce diluent strength |

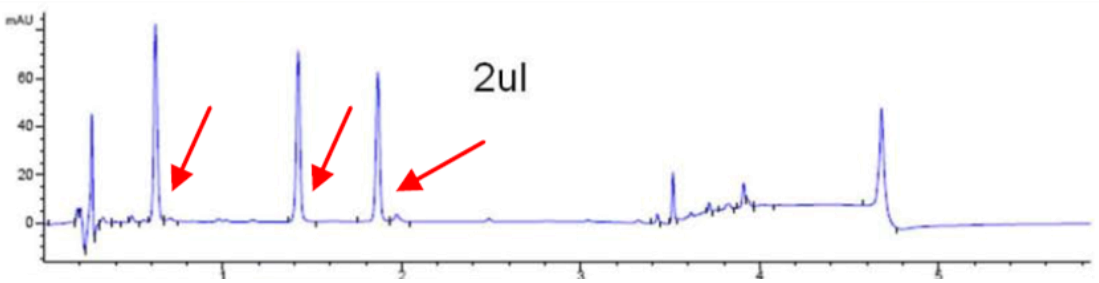


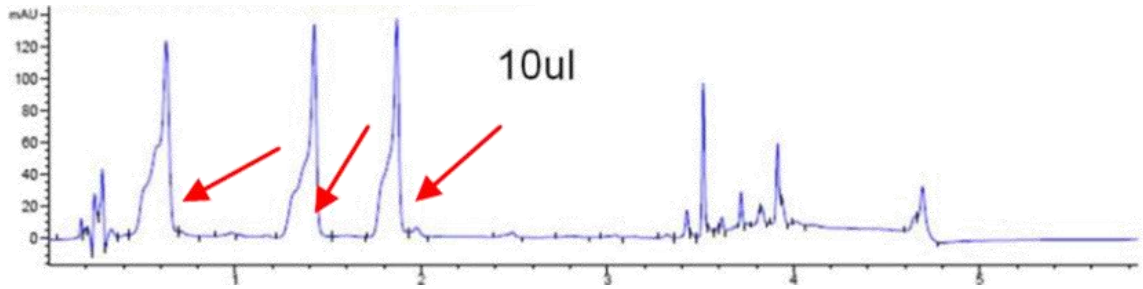
# Changes in Peak Shape

## Peak fronting

| Potential Cause | Recommended Action |
|---|---|
| Channeling in column | • Replace column<br>• Use guard columns |
| Column overload | • Use higher capacity column (increase length, diameter, or change to high-capacity material)<br>• Decrease sample amount |



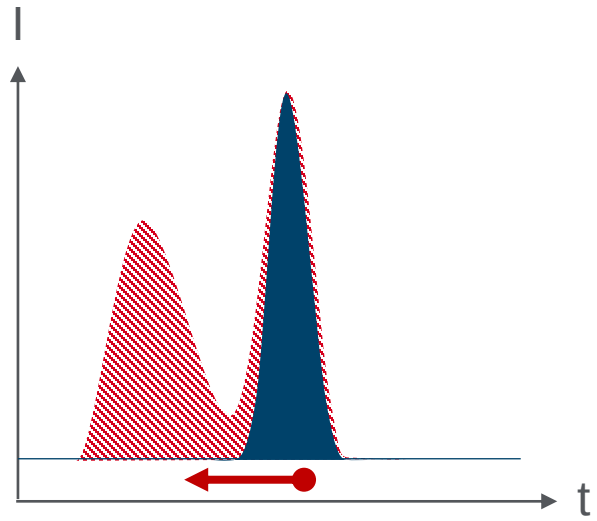



# Changes in Peak Shape

## Peak splitting / doubling

| Potential Cause | Recommended Action |
|---|---|
| Partially plugged column frit | • Backflush column (if applicable)<br>• Use inline filter<br>• Use guard column<br>• Cleanup sample |
| Column void | • Replace column<br>• Use guard column<br>• Use less aggressive mobile phase conditions<br>• Use a column with higher pH limit |
| Sample volume overload | • Use smaller injection volume |
| Sample solvent incompatibility with mobile phase | • Use mobile phase or weaker miscible solvent as the sample solvent<br>• If due to solubility/stability limitations you must use a stronger sample solvent, keep the injection volume small |
| Issues with injection valve | • Check injector valve parts<br>• Replace worn parts |

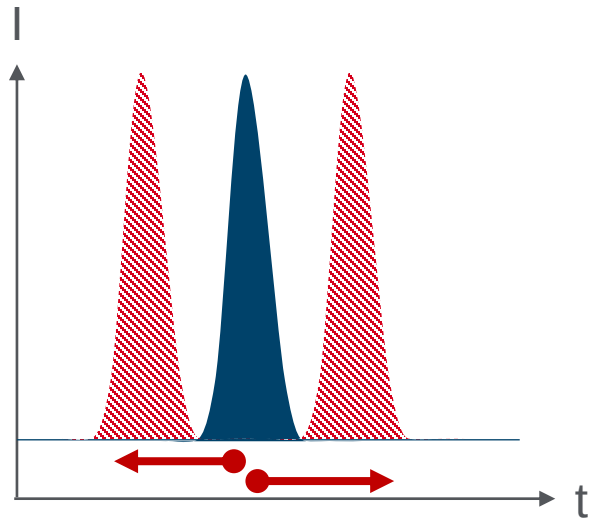


# Part 3: Changes in Separation

# Changes in Separation

## Retention time changing

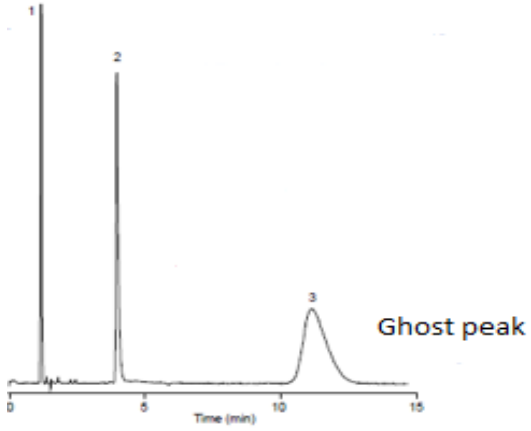
| Potential Cause | Recommended Action |
|---|---|
| Inconsistent online mobile phase mixing | Ensure gradient system delivering constant composition<br>Compare results with manual mobile phase preparation |
| Flow rate changing | Check 'Pressure fluctuation' |
| Column temperature varying | Thermostat column and<br>ensure constant lab temperature |
| Equilibration time insufficient with gradient run or change in isocratic mobile phase | Flush with at least 10 column volumes after solvent change or gradient conclusion |
| Selective evaporation of mobile phase component | Use appropriate solvent bottle cap<br>prepare fresh mobile phase |
| Buffer capacity insufficient | Use 20-50 mM concentration of buffer |
| Contamination buildup | Occasionally flush column with strong solvent to remove contaminants |
| First few injections – adsorption on active sites | Condition column by initial injection of concentrated sample |
| Column overloaded with sample | Decrease injection volume or concentration |
| Mobile phase composition changing | Follow ‘best practices’ |



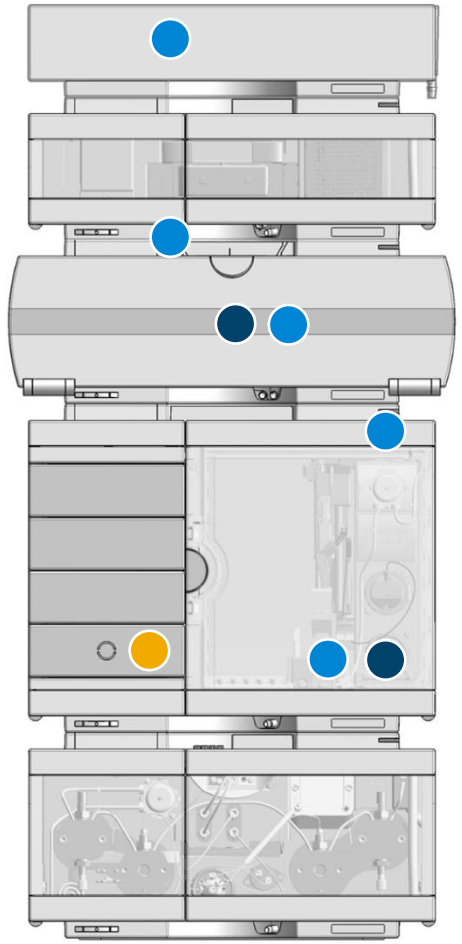
# Changes in Separation

## Ghost peaks, carry over

| | Potential Cause | Recommended Action |
|---|---|---|
| ● | Peaks from previous injection | • Flush column to remove contaminants<br>• Check with blank injection |
| ● | Specific interaction with metal surfaces | • Passivate instrument<br>• Use InfinityLab Deactivator Additive<br>• Use bio-inert LC instrument and column |
| ● | Contamination or unknown interferences and impurities in sample, mobile phase solvents | • Proper sample cleanup<br>• High purity solvents and additives |




BIO INERT

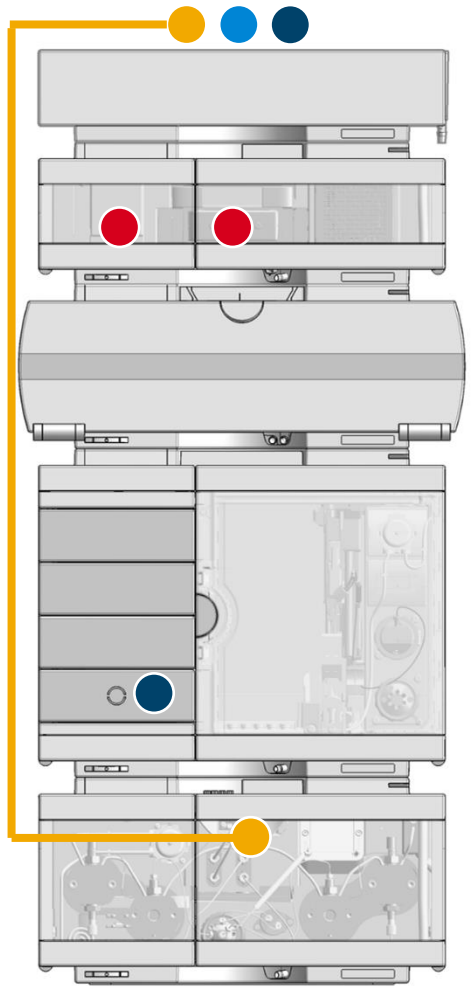


# Part 4: Changes in Detection

# Changes in Detection

## Noisy baseline and reduced intensity

| | Potential Cause | Recommended Action |
|---|---|---|
| ● | Gas bubbles in mobile phase | • Apply degassing<br>• Check degasser performance |
| ● | Low difference between sample and mobile phase absorbance | • Check absorbance values of sample vs. mobile phase |
| ● | Contamination | • Use degassed HPLC-grade solvents<br>• Flush system<br>• Clean up sample |
| ● | Detector optics | • Perform intensity test<br>• Check signal with flow cell removed if possible<br>• Replace lamp |
| | Pressure instability | • Check 'Pressure fluctuation' |



# More on Sample Related Problems and What to Do About Them

# Sample-related Problems – Physical Effects

- Sample solvent that is immiscible with mobile phase can cause early elution, peak distortion, low resolution, and precipitation of sample components due to low solubility in the mobile phase.
- Sample solvent that is stronger than the mobile phase can cause peak distortion, split/double peak, broad peaks, poor sensitivity, and shortening of retention time.
- Particulates in the sample can partially block the inlet frit of the column, guard, and inline filter causing split/double peaks and high pressure. We already talked about sample filtration in previous slides.

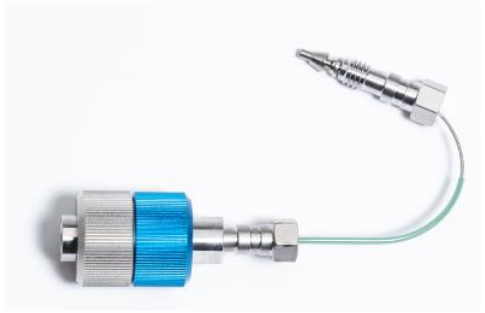
# Sample-related- Problems – Chemical Effects

- Chemical contamination/lipid buildup can cause secondary interaction and result in retention time variability, peak shape variability/tailing, selectivity changes, and (in some cases) increased backpressure.
- Lipids from the sample matrix can cause ion suppression with MS.
- Strong retention of interferences can result in ghost peaks and shouldering peaks in the following runs.
- With MS, salts can cause ion suppression.
- Interfering compounds from the sample matrix can coelute with target analytes and appear as split/shoulder peaks.

# What You Can Do

# Online Options for Sample Matrix Removal

InfinityLab Quick Change **inline filter**
Click & Seal. 2.1 mm and 4.6 mm id;
0.2 µm and 0.5 µm pore size filter;
max 1300 bar; touchless packaging



Agilent Fast **Guard**, 3/pk
RRHT, 600 bar
RRHD, 1300 bar
One piece pre-assembled, no cartridge or holder


Agilent Online **SPE**, Bond Elut PLRP-S
2.1 x 12.5 mm cartridge, 3/pk, 5982-1270
4.6 x 12.5 mm cartridge, 3/pk, 5982-1271
Cartridge housing, 820999-901

# Offline Options for Sample Matrix Removal

| | *More Specific* ← **Instrument Separation and Detection Specificity** ← *Less Specific* | | | | | |
|---|---|---|---|---|---|---|
| | *Less Specific* → ***Sample Preparation Specificity*** → *More Specific* | | | | | |
| **Sample Preparation Technique / Interference Removed** | **Filtration** | **Supported Liquid Extractions (SLE)** | **Protein Precipitation + Filtration** | **QuEChERS** | **Protein Precipitation + Filtration + Lipid Removal** | **Solid Phase Extraction** |
| Lipids | No | No | No | Yes | Yes | Yes |
| Oligomeric surfactants | No | No | No | No | Yes | Yes |
| Particulates | Yes | Some | Yes | Yes | Yes | Yes |
| Pigments | No | Some | No | Yes | No | Yes |
| Polar organic acids | No | Yes | No | Yes | No | Yes |
| Proteins | No | Yes | Yes | Yes | Yes | Yes |
| Salts | No | Yes | No | No | No | Yes |
| Suggested Agilent product | Captiva syringe filters Captiva filter vials | Chem Elut S | Captiva ND | Bond Elut QuEChERS with d-EMR-Lipid and other dispersive | Captiva EMR-Lipid | Bond Elut Silica and Polymeric SPE |

# Sample Filtration

## Captiva premium syringe filters

- Certified to be free of UV-detectable extractables on HPLC. PES and glass fiber also certified for LC/MS.
- Color-coded boxes for easy identification
- Comprehensive portfolio to meet all customers' needs

| Premium Syringe Filters | | | | | | |
|---|---|---|---|---|---|---|
| **Membrane** | Diameter/Pore Size | | | | | |
| | 4 mm | | 15 mm | | 25 mm (28 mm) | |
| | 0.2 µm | 0.45 µm | 0.2 µm | 0.45 µm | 0.2 µm | 0.45 µm |
| **PTFE** | ♦ | ♦ | ♦ | ♦ | ♦ | ♦ |
| **Nylon** | | | ♦ | ♦ | ♦ | ♦ |
| **PES** | ♦ | ♦ | ♦ | ♦ | ♦ | ♦ |
| **Regenerated cellulose** | ♦ | ♦ | ♦ | ♦ | ♦ | ♦ |
| **Cellulose acetate** | | | | | ♦ | ♦ |
| **Glass microfiber** | | | ♦ | | ♦ | |
| **Depth filters: glass/PTFE** | | | ♦ | ♦ | ♦ | ♦ |
| **Depth filters: glass/nylon** | | | ♦ | ♦ | ♦ | ♦ |

Captiva syringe filters guide 5991-1230EN

# Sample Filtration

## Captiva filter vials

| Description | Part No. |
|---|---|
| 0.45 µm PTFE filter vial, 100/pack | 5191-5933 |
| 0.20 µm PTFE filter vial, 100/pack | 5191-5934 |
| 0.45 µm Nylon filter vial, 100/pack | 5191-5935 |
| 0.20 µm Nylon filter vial, 100/pack | 5191-5936 |
| 0.45 µm RC filter vial, 100/pack | 5191-5939 |
| 0.20 µm RC filter vial, 100/pack | 5191-5940 |
| 0.45 µm PES filter vial, 100/pack | 5191-5941 |
| 0.20 µm PES filter vial, 100/pack | 5191-5942 |
| Vial closure tool | 5191-5943 |



www.agilent.com/chem/filtervials

Filter vials user guide: 5994-0814EN

# Sample Filtration – Targeted Filtration

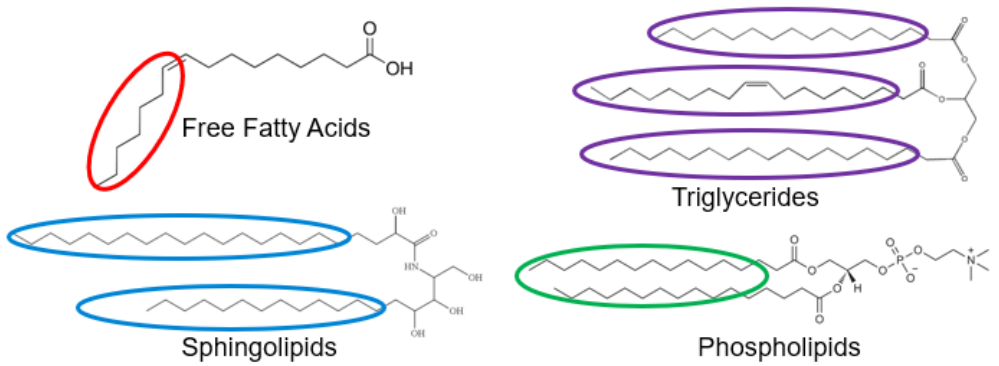
## Captiva EMR-Lipid

- One of the newest Agilent sample cleanup products with a 2-in-1 benefit of removing proteins and lipids.
- By removing lipids, it reduces ion suppression, increases analyte sensitivity, improves peak shape, and extends the lifetime of your analytical column.
- Simple pass-through, comes in 96-well plate and cartridge format
- Solvent-retention frit in 1 mL cartridge/96-well plate for in-well protein precipitation

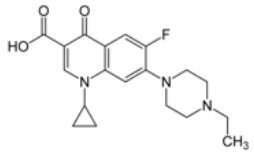
# Captiva EMR-Lipid
## Selective removal of lipids

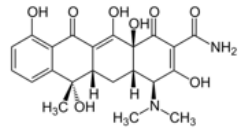
Agilent InfinityLab

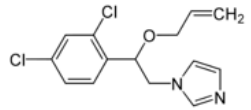
### Removes lipids

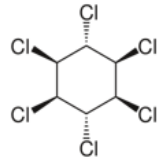


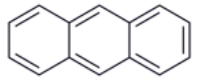

### Does not remove target analytes

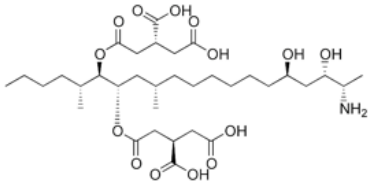

Fluoroquinolones


Tetracyclines


Imidazole pesticides


Organochlorine Pesticides

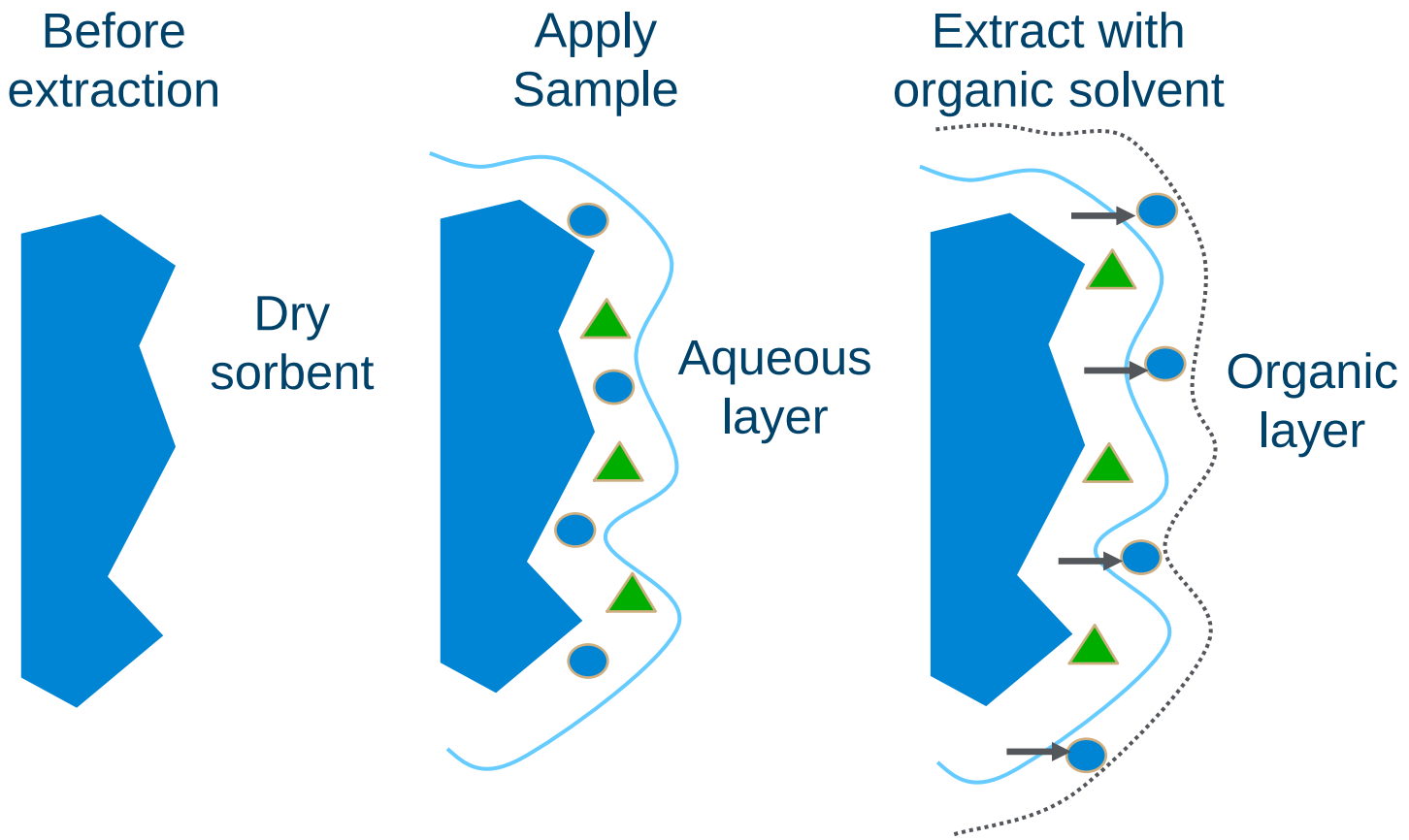

PAHs


Fumonisin B2

[Biological samples: Captiva EMR-Lipid method guide for 96 well-plate and 1 mL cartridge]

[Food samples: Captiva EMR-Lipid method guide for 3 mL and 6 mL cartridges]

# Supported Liquid Extraction (SLE)




- A thin layer of aqueous sample is formed on the surface of SLE sorbent.
- When the organic solvent passes through the SLE bed, analytes are extracted under the same principles as LLE.
- Increased contact area between the two phases allows efficient extraction without mixing.

# Supported Liquid Extraction (SLE)
## Chem Elut S

- Same extraction mechanism as in traditional liquid-liquid extraction (LLE)
- Cartridge and plate format, packed with proprietary synthetic sorbent– high surface area
- Simple method, gravity flow
- Smaller volume sample and solvent compared to LLE
- No emulsions

Cartridges for sample volumes 0.2 – 20 mL


Bulk Chem Elut S
1 kg and 4 kg

96-well plate for sample volumes 200 µL and 400 µL

# Agilent Bond Elut QuEChERS

## Quick Easy Cheap Effective Rugged and Safe

Initially developed for screening of pesticide residues in fruit and vegetables to make sample cleanup of food faster, simpler, less expensive, and more green.

Now, QuEChERS is used with other matrices and compound classes as well.

Consists of two steps, and two kits:

Step 1: Liquid extraction


Step 2: Dispersive SPE/ interference removal


# Bond Elut Dispersive SPE Kits

## Dispersive kit

Centrifuge tubes containing preweighed SPE sorbents

- C18: Removes residual fats and lipids
- PSA: ‘Primary secondary amine’ for removal of organic acids and sugars
- GCB: Graphitized carbon black, removes pigments
- EMR-Lipid: Removes unbranched hydrocarbon chains (lipids)

Dispersive SPE kits are available for different food types.

They are for both AOAC (US) method and EN (Europe).

QuEChERS is a nonselective technique and does not remove **all** the matrix, just enough.

Dispersive sorbents are also available as bulk material.

# Dispersive EMR-Lipid

## EMR-Lipid – What is it?

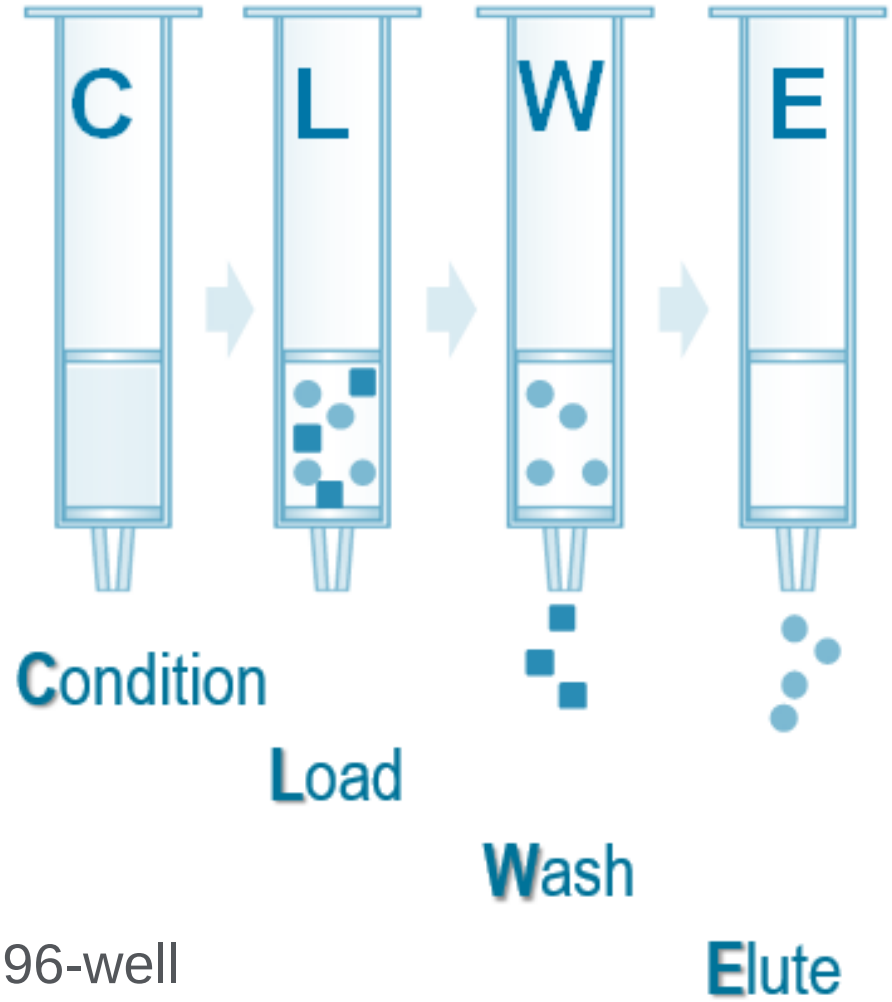


EMR-Lipid fits into current sample preparation workflows

# Solid Phase Extraction (SPE)

- Capabilities
  - Very selective
  - Highly clean samples
  - Concentrated samples
  - Wide range of applicability
  - Automation friendly

- Types of SPE
  - Nonpolar (reversed phase) SPE
  - Polar (normal phase) SPE
  - Cation exchange SPE
  - Anion exchange SPE
  - Mixed mode SPE
  - Specialty SPE




Bond Elut: Silica or polymer based, cartridge, and 96-well plate format

# Agilent SPE Offering

- Reliable SPE with a 30-year history
- Agilent offers the most comprehensive set of phases, sizes, and formats of any SPE provider (over 40 sorbent materials/phases available)
- Easy adoption of methods due to high number of publications and applications.
- Includes packed bed silica and polymeric phases, and monolithic silica phases.

**Bond Elut Silica SPE**

Bond Elut AccuCAT
Bond Elut $NH_2$
Bond Elut C1
Bond Elut C2
Bond Elut C8
Bond Elut C18 .....
..... **40 phases**

**Bond Elut polymer SPE**

**Plexa**

Bond Elut Plexa
Bond Elut Plexa PCX
Bond Elut Plexa PAX

**SampliQ SPE**

Multiple phases

**OMIX monolithic silica tip SPE**

OMIX C18
OMIX MP1
OMIX SCX

**SPEC monolithic silica disk SPE**

SPEC C2
SPEC C8
SPEC C18
SPEC C18AR
SPEC PH
SPEC NH2
SPEC CN
SPEC Si
SPEC PSA
SPEC SAX
SPEC SCX
SPEC MP1
SPEC MP3

# Bond Elut Plexa

- New generation of polymeric SPE
- Divinylbenzene-based polymeric sorbent with hydrophilic exterior, hydrophobic interior, and advanced polymeric architecture.
- Superior flow properties
- Great for extraction of a wide range of acidic, neutral, and basic analytes from different matrices
- Simple method
- Bond Elut Plexa, nonpolar
- Bond Elut Plexa PCX, mixed mode with strong cation exchange
- Bond Elut Plexa PAX, mixed mode with strong anion exchange
- Cartridge and 96-well plate format

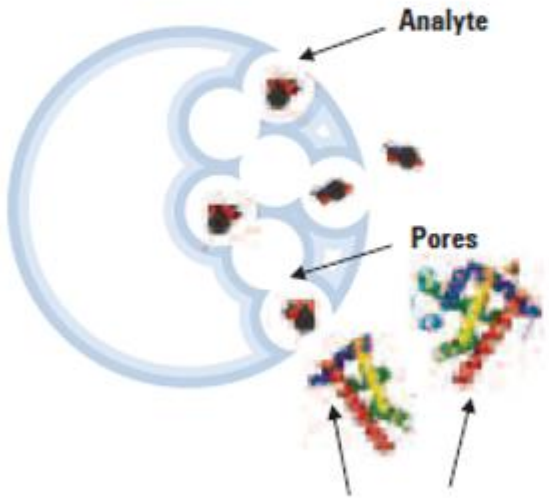
# Bond Elut Plexa

Agilent InfinityLab

## Advanced polymer architecture improves extraction performance

**LOAD:**

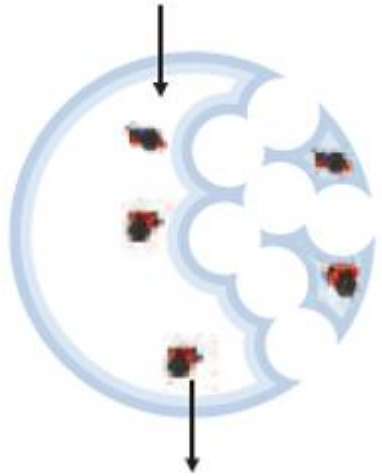
Water-rich, hydrophilic surface allows excellent phase transfer of analytes into the polymer core.




Large endogenous proteins do not bind to the surface of the polymer and cannot access pore structure.

**WASH:**

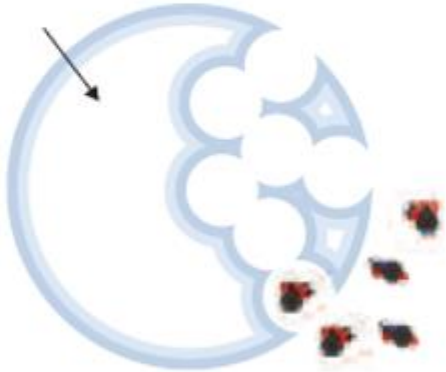
Analytes that have crossed the hydrophilic layers will remain tightly bound in the hydrophobic core.



Interferences wash away without leaching the analytes of interest.

**ELUTE:**

Specially engineered pore structure allows excellent mass transfer out of the polymer.



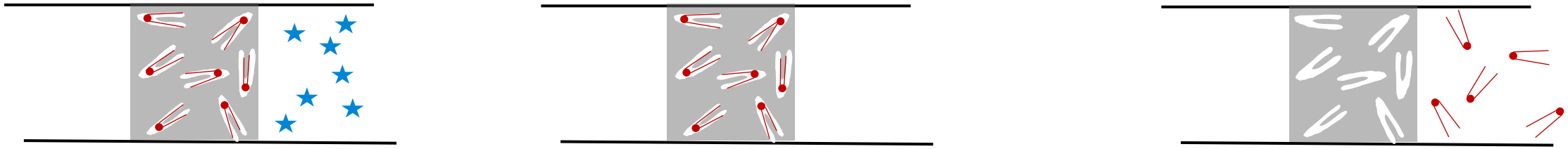
Clean extract with high recovery.

# Innovative Lipid Products

## Captiva EMR-Lipid – A pass through filtration

FLOW

## Bond Elut Lipid Extraction – An SPE like lipid isolation for lipidomics



"Captiva EMR like"
Lipid capture

Wash

FLOW

Lipids elution

[Method Guide for 96-well plate]

[Method Guide for 1 mL Cartridge]

# Manifolds for Processing Cartridges and 96-Well Plates

Captiva vacuum collar

Vac Elut 20 vacuum manifold

Vac Elut 12 vacuum manifold

SPS 24 vacuum manifold

96 well plate vacuum manifold

Positive Pressure Manifolds

# Summary

# Useful Parts

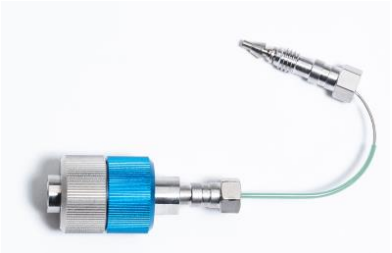
## Parts that address potential issues and help to ease your daily tasks

| Part description | Information | Part number |
|---|---|---|
| InfinityLab Stay Safe Caps | Prevent solvent evaporation; changes in mobile phase concentration; solvent contamination | Various: www.agilent.com/chem/staysafecaps |
| InfinityLab Quick Connect and Quick Turn Fittings | Spring-load function for optimized dead volume reduction | Various: www.agilent.com/chem/InfinityLabFittings |
| InfinityLab Quick Change Inline Filters | Capture particulates in mobile phase or sample; features click and seal, in-situ replacement, and touchless packaging | Various: www.agilent.com/chem/QuickChangeInlineFilter |
| Agilent Captiva Syringe Filters and Filter Vials | Solve issues like inlet clogging, increased backpressure, and retention time shift by filtering samples | Various: www.agilent.com/chem/filtration |
| InfinityLab Poroshell 120 Columns (see appendix) | High efficiency and high resolution; available in 18 chemistries; fitted with RFID tag | Various: www.aglient.com/chem/discoverporoshell |

InfinityLab Stay Safe Cap for solvent bottle


InfinityLab Quick Connect Fitting


InfinityLab Quick Turn Fitting



InfinityLab Quick Change Inline Filter

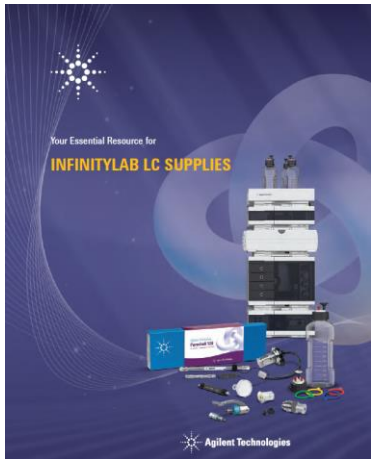
Captiva Syringe Filter and Filter Vials


InfinityLab Poroshell 120 Columns With column RFID tag

# Resources for Support

- LC troubleshooting poster (5994-0709EN)
- Tech support www.agilent.com/chem/techsupport
- Resource page www.agilent.com/chem/agilentresources
  - Quick reference guides
  - Catalogs, column user guides
  - Online selection tools, how-to videos
  - Application workflows (such as cannabis, PFAS, and more)
- InfinityLab LC Supplies catalog (5991-8031EN)
- LC handbook (5990-7595EN)
- Best practices for using an Agilent LC system (01200-90090)
- Your local FSE and specialists
- Agilent University www.agilent.com/crosslab/university
- YouTube – Agilent Channel (maintenance videos)
- Agilent service contracts





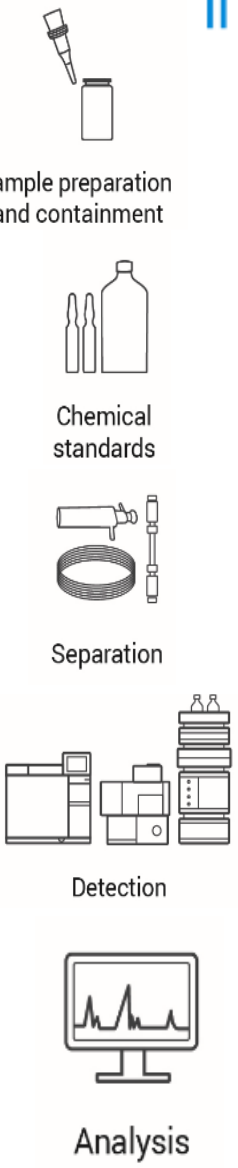

# Chemistries and Supplies Applications Workflows

1. Cannabis Pesticide/Mycotoxin kit (LC + GC)
   - P/n 5610-2050
   - P/n 5610-2051
   - P/n 5610-2052
   - P/n 5610-2053
2. Cannabis potency kit (LC) - p/n 5610-2036
3. Cannabis Pesticide/Mycotoxin bundle (LC + GC) – Pub no. 5994-1639EN
4. Cannabis potency bundle (LC)- Pub no. 5994-1639EN
5. Cannabis – Residual Solvents bundle (GCMS)- Pub no. 5994-1639EN
6. Cannabis – Terpenes bundle (GCMS)- Pub no. 5994-1639EN
7. Cannabis – Heavy Metals bundle (ICP-MS)- Pub no. 5994-1639EN
8. USP467 bundle (GC/MS) – Pub no. 5991-8659EN
9. Multi-class, Multi-residue Veterinary Drug Analysis in Food Matrices (LCMS) – Pub no. 5994-2085EN
10. Polycyclic Aromatic Hydrocarbons (PAH) in Food bundle (GCMS) – Pub no. 5994-2016EN
11. PAH in water bundle (GCMS) – Pub no. 5994-2060EN
12. Volatile Organic Compounds (VOC) in Water (GC/MS) – Pub no. 5994-0345EN
13. Semi-Volatile Organic Compounds (SVOC) (GC/MS) – Pub no. 5994-0932EN
14. Nitrosamine in water bundle (GCMS) – Pub no. 5994-2184EN
15. PFAS – EPA 533 (drinking water) (LCMS) - Pub no. 5994-2357EN
16. PFAS – EPA 537 (drinking water) (LCMS) -Pub no. 5994-2357EN
17. PFAS – EPA 8327 (Ground, surface and wastewater) (LCMS) - Pub no. 5994-2357EN
18. PFAS – ASTM D7979 (Sludge, Influent/Effluent and wastewater) (LCMS)- Pub no. 5994-2357EN
19. PFAS - ISO 21675:2019 (Non filtrated waters) (LCMS) - Pub no. 5994-2357EN
20. Analysis of legacy and emerging PFAS bundle (LCMS) - Pub no. 5994-2357EN
21. PFAS – MRM database method (LC/TQ) - Pub no. 5994-2357EN
22. Rapid Analysis of Pesticides in Food Matrices (LCMS) – Pub no. 5994-2909EN
23. Nitrosamine in pharmaceuticals (LCMS) – Pub no. 5994-2977EN
24. Nitrosamine in pharmaceuticals (GCMS) – Pub no. 5994-2979EN

**Where you will find them:**

Agilent Resource Center :www.agilent.com/en/agilentresources

Agilent Community Page: https://community.agilent.com/technical/consumables/w/wiki/3770/consumables-applications-and-workflows



# Contact Agilent Chemistries and Supplies Technical Support

1-800-227-9770 Option 3, Option 3:

Option 1 for GC and GC/MS columns and supplies

Option 2 for LC and LC/MS columns and supplies

Option 3 for sample preparation, filtration and QuEChERS

Option 4 for spectroscopy supplies

Option 5 for chemical standards

Option 6 for former Prozyme products

Available in the USA and Canada 8–5 all time zones

gc-column-support@agilent.com

lc-column-support@agilent.com

spp-support@agilent.com

spectro-supplies-support@agilent.com

chem-standards-support@agilent.com

advancebio.glycan@agilent.com

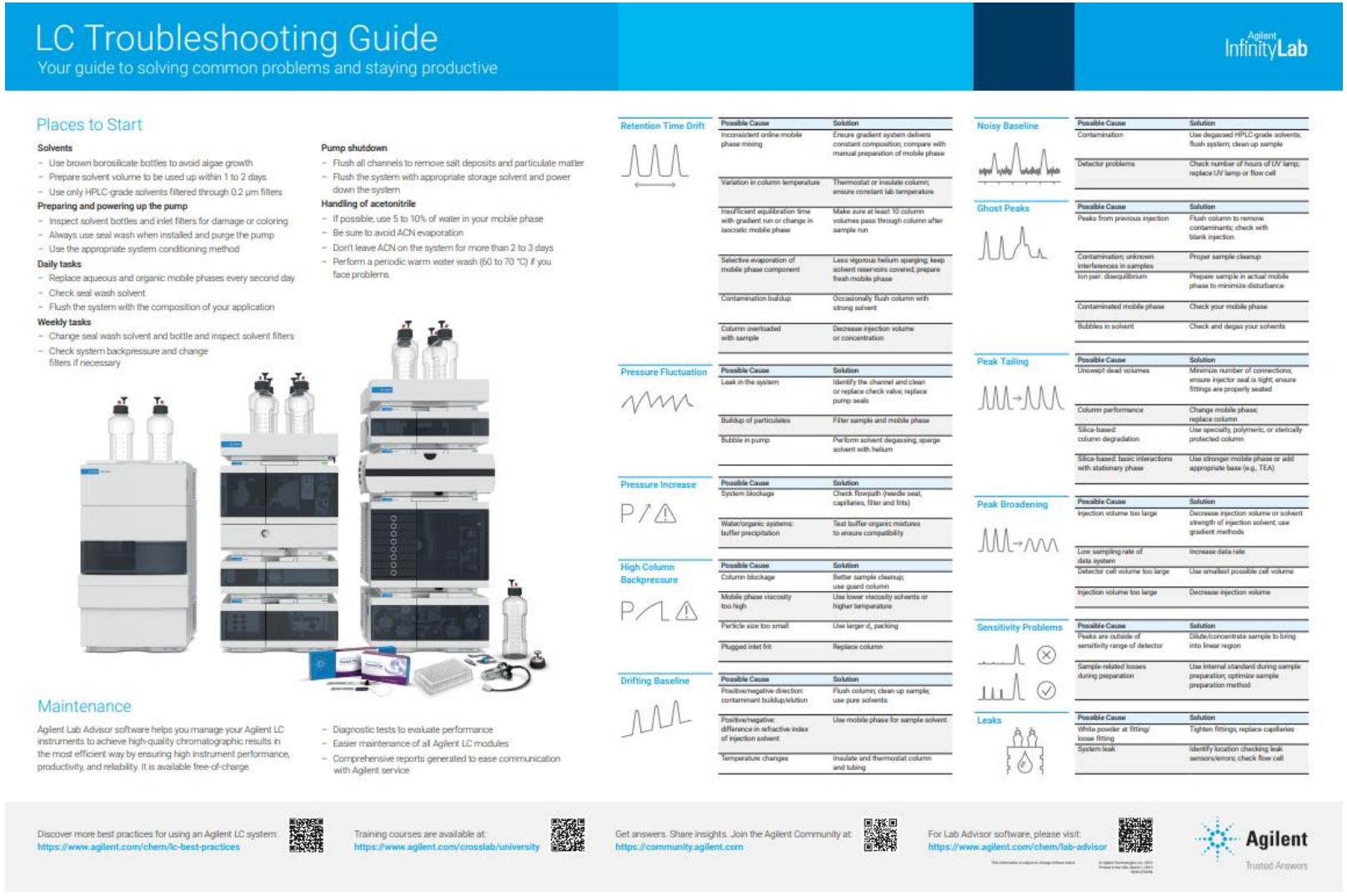
Web Chat: Product pages of agilent.com

# Thank You!

# Appendix

# LC Troubleshooting Poster Available

Poster: [5994-0709EN]



## LC Troubleshooting Guide

Your guide to solving common problems and staying productive

### Places to Start

**Solvents**
- Use brown borosilicate bottles to avoid algae growth
- Prepare solvent volume to be used up within 1 to 2 days
- Use only HPLC-grade solvents filtered through 0.2 µm filters

**Preparing and powering up the pump**
- Inspect solvent bottles and inlet filters for damage or coloring
- Always use seal wash when installed and purge the pump
- Use the appropriate system conditioning method

**Daily tasks**
- Replace aqueous and organic mobile phases every second day
- Check seal wash solvent
- Flush the system with the composition of your application

**Weekly tasks**
- Change seal wash solvent and bottle and inspect solvent filters
- Check system backpressure and change filters if necessary

**Pump shutdown**
- Flush all channels to remove salt deposits and particulate matter
- Flush the system with appropriate storage solvent and power down the system

**Handling of acetonitrile**
- If possible, use 5 to 10% of water in your mobile phase
- Be sure to avoid ACN evaporation
- Don't leave ACN on the system for more than 2 to 3 days
- Perform a periodic warm water wash (60 to 70 °C) if you face problems

### Maintenance

Agilent Lab Advisor software helps you manage your Agilent LC instruments to achieve high-quality chromatographic results in the most efficient way by ensuring high instrument performance, productivity, and reliability. It is available free-of-charge.

- Diagnostic tests to evaluate performance
- Easier maintenance of all Agilent LC modules
- Comprehensive reports generated to ease communication with Agilent service

**Retention Time Drift**

| Possible Cause | Solution |
|---|---|
| Inconsistent online mobile phase mixing | Ensure gradient system delivers constant composition; compare with manual preparation of mobile phase |
| Variation in column temperature | Thermostat or insulate column; ensure constant lab temperature |
| Insufficient equilibration time with gradient run or change in isocratic mobile phase | Make sure at least 10 column volumes pass through column after sample run |
| Selective evaporation of mobile phase component | Less vigorous helium sparging; keep solvent reservoirs covered; prepare fresh mobile phase |
| Contamination buildup | Occasionally flush column with strong solvent |
| Column overloaded with sample | Decrease injection volume or concentration |

**Pressure Fluctuation**

| Possible Cause | Solution |
|---|---|
| Leak in the system | Identify the channel and clean or replace check valve; replace pump seals |
| Buildup of particulates | Filter sample and mobile phase |
| Bubble in pump | Perform solvent degassing; sparge solvent with helium |

**Pressure Increase**

| Possible Cause | Solution |
|---|---|
| System blockage | Check flowpath (needle seat, capillaries, filter and frits) |
| Water/organic systems: buffer precipitation | Test buffer-organic mixtures to ensure compatibility |

**High Column Backpressure**

| Possible Cause | Solution |
|---|---|
| Column blockage | Better sample cleanup; use guard column |
| Mobile phase viscosity too high | Use lower viscosity solvents or higher temperature |
| Particle size too small | Use larger $d_p$ packing |
| Plugged inlet frit | Replace column |

**Drifting Baseline**

| Possible Cause | Solution |
|---|---|
| Positive/negative direction: contaminant buildup/elution | Flush column; clean up sample; use pure solvents |
| Positive/negative: difference in refractive index of injection solvent | Use mobile phase for sample solvent |
| Temperature changes | Insulate and thermostat column and tubing |

**Noisy Baseline**

| Possible Cause | Solution |
|---|---|
| Contamination | Use degassed HPLC-grade solvents; flush system; clean up sample |
| Detector problems | Check number of hours of UV lamp; replace UV lamp or flow cell |

**Ghost Peaks**

| Possible Cause | Solution |
|---|---|
| Peaks from previous injection | Flush column to remove contaminants; check with blank injection |
| Contamination; unknown interferences in samples | Proper sample cleanup |
| Ion pair: disequilibrium | Prepare sample in actual mobile phase to minimize disturbance |
| Contaminated mobile phase | Check your mobile phase |
| Bubbles in solvent | Check and degas your solvents |

**Peak Tailing**

| Possible Cause | Solution |
|---|---|
| Unswept dead volumes | Minimize number of connections; ensure injector seal is tight; ensure fittings are properly seated |
| Column performance | Change mobile phase; replace column |
| Silica-based: column degradation | Use specialty, polymeric, or sterically protected column |
| Silica-based: basic interactions with stationary phase | Use stronger mobile phase or add appropriate base (e.g., TEA) |

**Peak Broadening**

| Possible Cause | Solution |
|---|---|
| Injection volume too large | Decrease injection volume or solvent strength of injection solvent; use gradient methods |
| Low sampling rate of data system | Increase data rate |
| Detector cell volume too large | Use smallest possible cell volume |
| Injection volume too large | Decrease injection volume |

**Sensitivity Problems**

| Possible Cause | Solution |
|---|---|
| Peaks are outside of sensitivity range of detector | Dilute/concentrate sample to bring into linear region |
| Sample-related losses during preparation | Use internal standard during sample preparation; optimize sample preparation method |

**Leaks**

| Possible Cause | Solution |
|---|---|
| White powder at fitting/ loose fitting | Tighten fittings; replace capillaries |
| System leak | Identify location checking leak sensors/errors; check flow cell |

Discover more best practices for using an Agilent LC system: https://www.agilent.com/chem/lc-best-practices

Training courses are available at: https://www.agilent.com/crosslab/university

Get answers. Share insights. Join the Agilent Community at: https://community.agilent.com

For Lab Advisor software, please visit: https://www.agilent.com/chem/lab-advisor

Agilent Trusted Answers

# Reliability for Your Lab

Agilent InfinityLab

## InfinityLab Poroshell 120 columns are fitted with pre-installed and preprogrammed column RFID tags

1. **Usability -** Provides recommended column use parameters and column-specific details

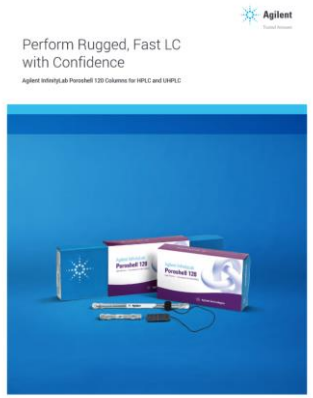
2. **Traceability -** Always know the column history, when it was installed, number of injections and operational parameters

3. **Security -** Data is not compromised as columns are replaced according to schedule


| Field | Example |
|---|---|
| Description | Poroshell EC-C18 |
| Length [mm] | 100 |
| Diameter [mm] | 4.6 |
| Particle size [µm] | 2.7 |
| Maximum pressure [bar] | 600 |
| Number of injections | [counter] |
| Product number | 695975-902T |
| Serial number | USABC12345 |
| Batch number | B12345 |
| Maximum temperature [°C] | 60 |
| Maximum measured temperature [°C] | [updated from instrument] |
| Minimum pH | 2.0 |
| Maximum pH | 8.0 |
| Void volume [mL] | 1.00 |
| First injection date | [updated from instrument] |
| Recent injection date | [updated from instrument] |

# More Information on Agilent LC Columns

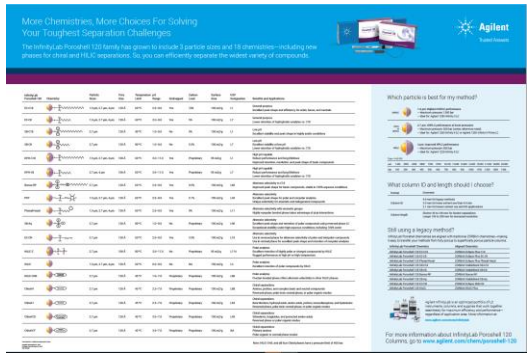
## 1 Brochures




Poroshell 120 Portfolio Brochure

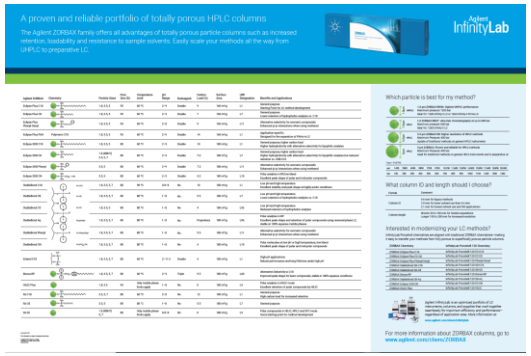
5991-8750EN

## 2 Posters




P120 Selectivity Overview

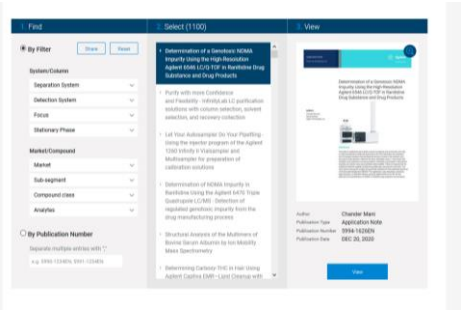
5991-9013EN




ZORBAX Selectivity Overview

5994-2212EN

## 3 Application Notes



Agilent Application Finder

**>200** P120 application notes

## 4 Agilent.com

Videos!
Web shop!
Collaterals!
Specifications!
And much more…