

Best Practices for HPLC Column Care and Maintenance

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LC Columns and Consumables Technical Support

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Easy Steps for HPLC Column Use

- **Purchase** your HPLC column for your application
- **Receive** your HPLC column
- **Remove** column from the packaging
- **Remove** the column's end plugs
- **Install** the HPLC column
- **Run** it

It's that simple, right?

Not so fast !



An Ounce of Prevention...Goes a Long Way

To keep your column in tip-top shape and maximize column lifetime, take into consideration...

1

Before the Run Starts

- Learn:
 - Technical specifications for your column
 - Column User Guides
 - Importance of connections
 - Why it's important to avoid solvent contamination

2

Operating Your Column

- Installing your HPLC column
- Equilibrating your column
- Testing your column
- Why and when to use inline filters and guard columns

An Ounce of Prevention

3

Common Problems Seen for Columns

- Increasing Back Pressure
- Peak Shape Issues
- RT Shifts

4

After the Last Run is Finished

- How to flush and clean HPLC columns
- How to properly store columns

Before the Run Starts



Getting Started With a New Column

Know the technical specifications for your column

- What is the column chemistry: C18, C8, HILIC, SEC, IEX, other
- Particle and pore size of packing
- Maximum back pressure
- Mobile phase considerations
- pH range
- Temperature range
- Flow rate range

Before Using Your Column

Know the technical specifications for your column

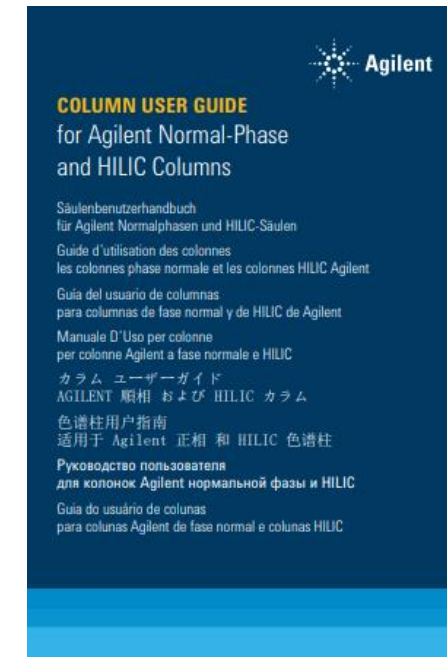
Refer to the column user guide

User guide provides information for:

- Shipping solvent
- Technical specifications
- Recommended fittings
- Column conditioning
- Mobile phases guidelines
- Recommended starting gradients
- Column care
- Tips for getting the best chromatography
- Troubleshooting
- Column cleaning and storage

[LC Column User Guides | Agilent](#)

[Bio LC Column User Guides | Agilent](#)



Column Connections

Stainless steel

- Agilent uses Swagelok type fittings with front and back ferrules (available with longer lengths)



PEEK (< 400 bar system pressure)

- Connections are changed frequently
- Connecting columns
- Pressure is less critical
- Fits on SST or PEEK tubing



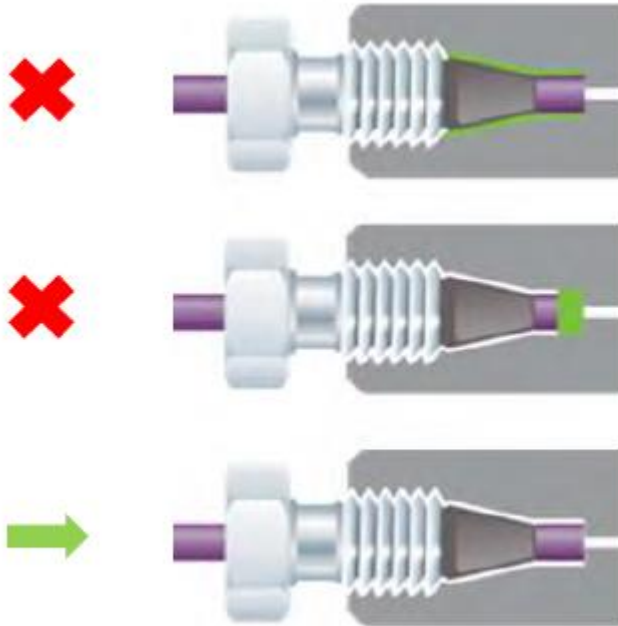
Polyketone

- Easy, hand-tightened column connection
- Used up to 600 bar (p/n: 5042-8957)
- Best on SST tubing

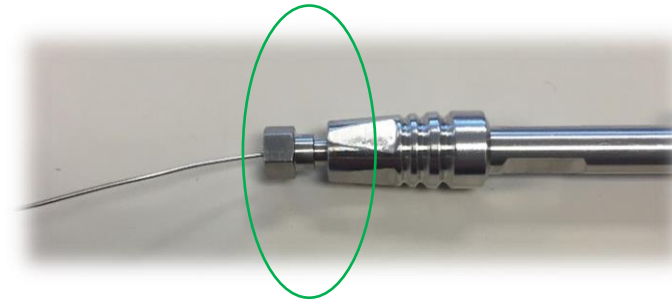
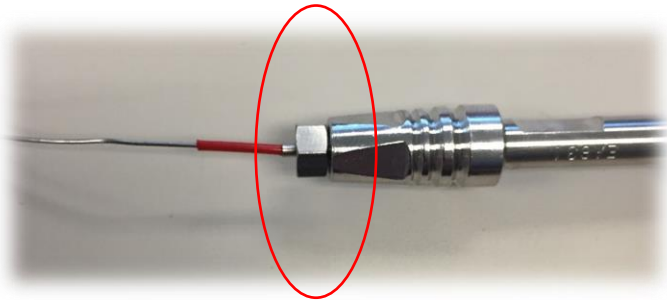


Importance of Column Connections

Potential fittings issues



- Leak
- Peak shape problem
- No dead volume



Connection problems can lead to:

Poor chromatography

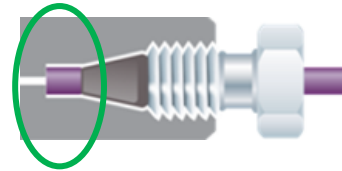
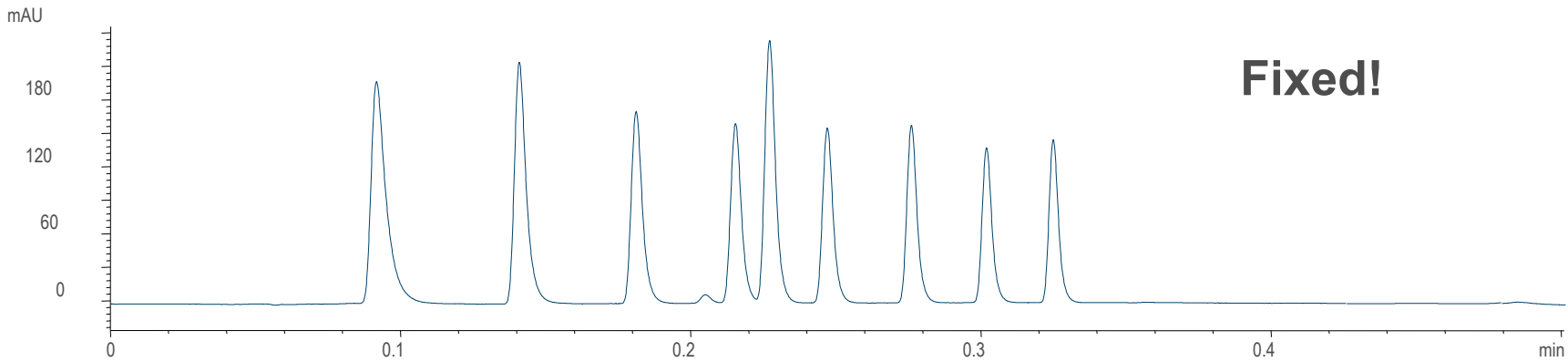
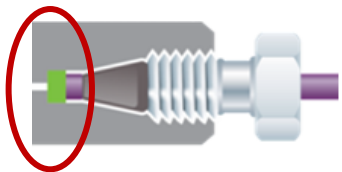
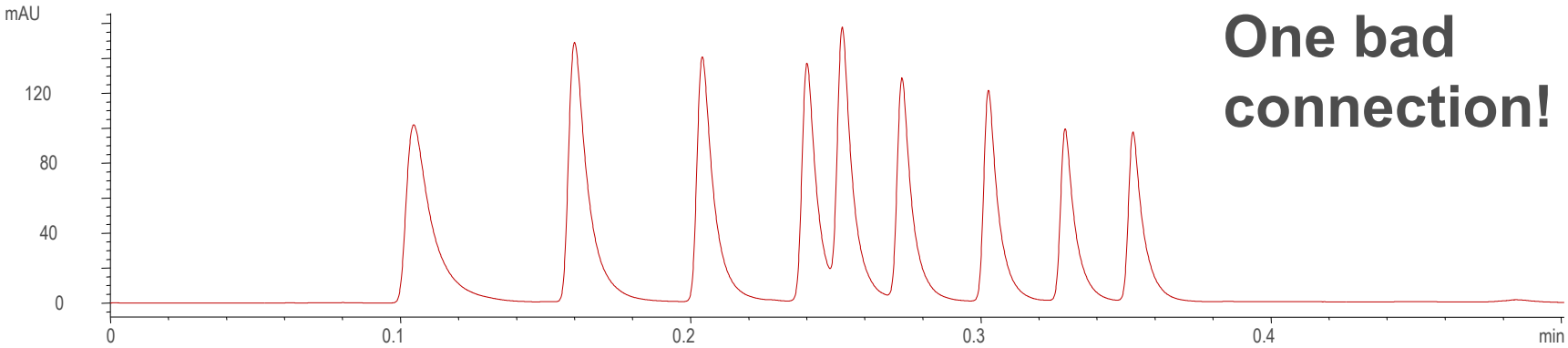
- Broad or tailing peaks
- Loss of resolution

Added maintenance costs

- Leaks, added troubleshooting
- Overtightening
- Column damage

What Can Result From a Poor Fitting Connection?

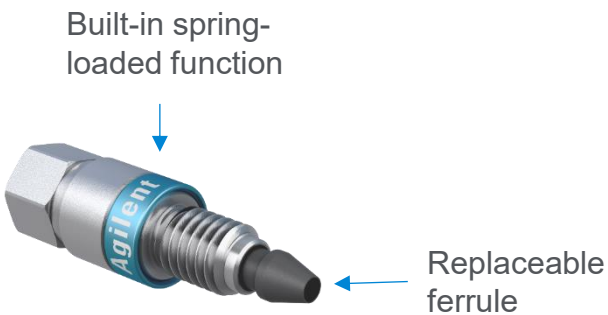
Peak shape issue



InfinityLab Quick Connect and Quick Turn Fittings

Correct connection every time

Agilent InfinityLab Quick Connect Fittings



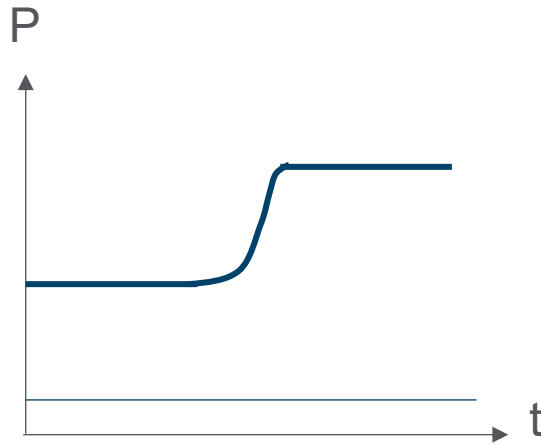
	Quick Connect Fitting	Quick Turn Fitting
Connects to	Columns (or inline filters)	Column, various receiving ports with 10-32 port geometry
Maximum pressure	1300 bar (finger-tight, by turning the lever)	400 bar (finger-tight, user-dependent) 800 bar (with mounting tool, 5043-0915) 1300 bar (with wrench, be careful to not overtighten)
Features	- Spring-loaded function for dead volume free connections (special capillaries) - Replaceable ferrule and capillary - Capillaries in various lengths and diameters are available	- Spring-loaded function for dead volume free connections - Replaceable ferrule and capillary - Capillaries in various lengths and diameters are available
Capillaries	Use Quick Connect capillaries	Use Quick Turn capillaries



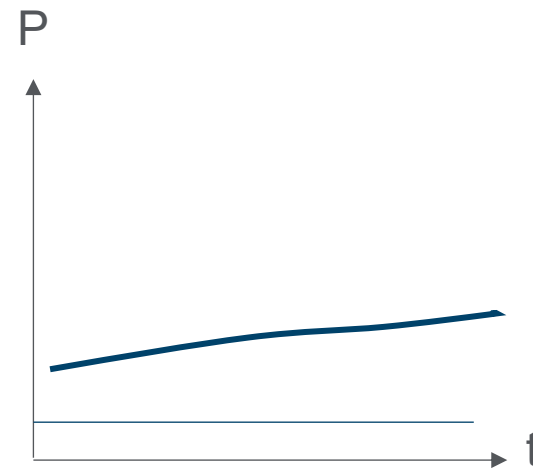
Protect Your Column Before a Run

Protecting your column from sources of particulates

Typical
Symptoms



Blockages: Instant pressure increase step



Clogging: Constant pressure increase over time

Instrument and solvents as a source of particulates

Sample as a source of particulates

Instrument as a Source of Particulates

Instrument and solvents as a source of particulates



InfinityLab Solvent
Filtration Assembly
PN 3150-0577

- Filter-buffered LC solvents with Agilent solvent filtration equipment to remove precipitated/undissolved salts
- Use Agilent inline filters in the pump to remove pump seal wear

Filter membrane types

- Regenerated cellulose 47 mm, 0.45 μm
- Nylon 47 mm, 0.45 μm
- PTFE 47 mm, 0.45 μm

Consumable parts for assembly
are sold separately

Solvent inlet filters



Glass filter, 20 μm pore size, p/n 5041-2168

Stainless steel filter, 10 μm pore size, p/n 01018-60025

Sample as a Source of Particulates

Sample as a source of particulates

- Filter sample with Agilent syringe filters to remove particulates
- Use Agilent inline filters and / or guard columns to protect from injector seal wear and sample compounds precipitating in gradient starting conditions



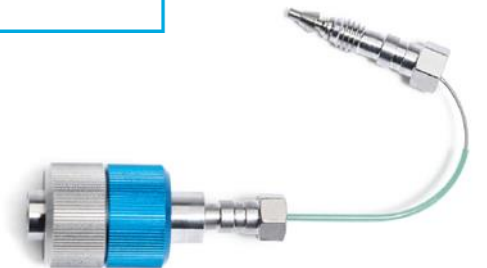
Agilent syringe filters



Captiva filter vials



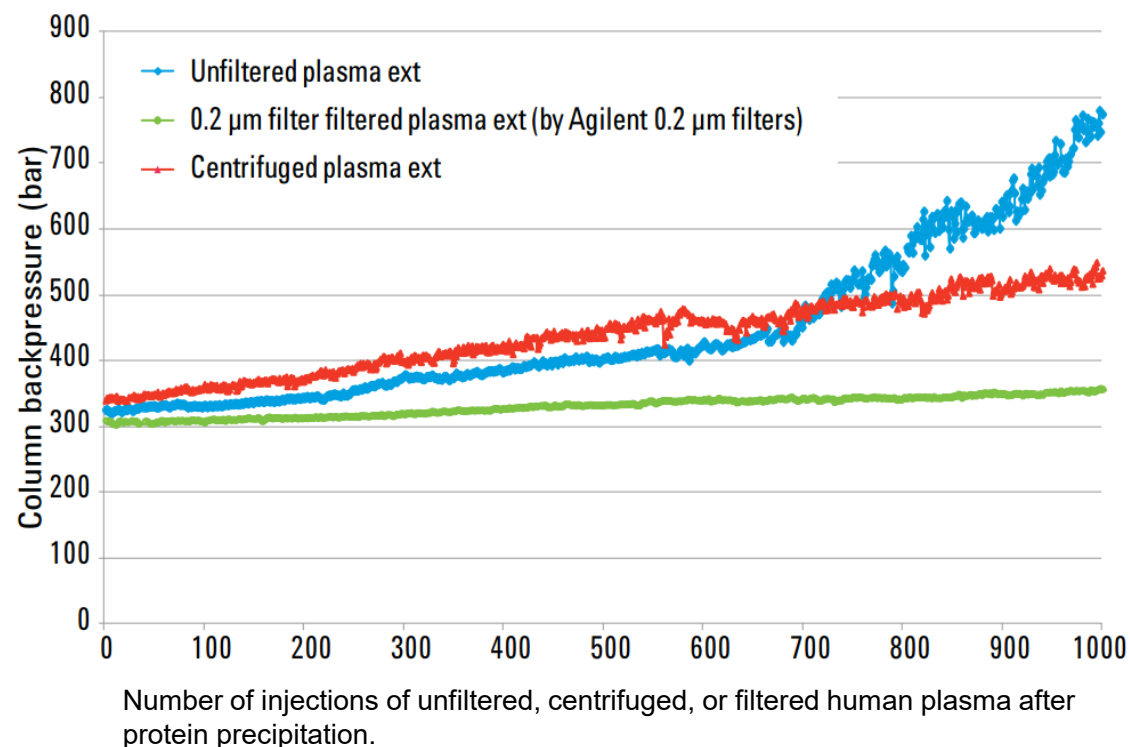
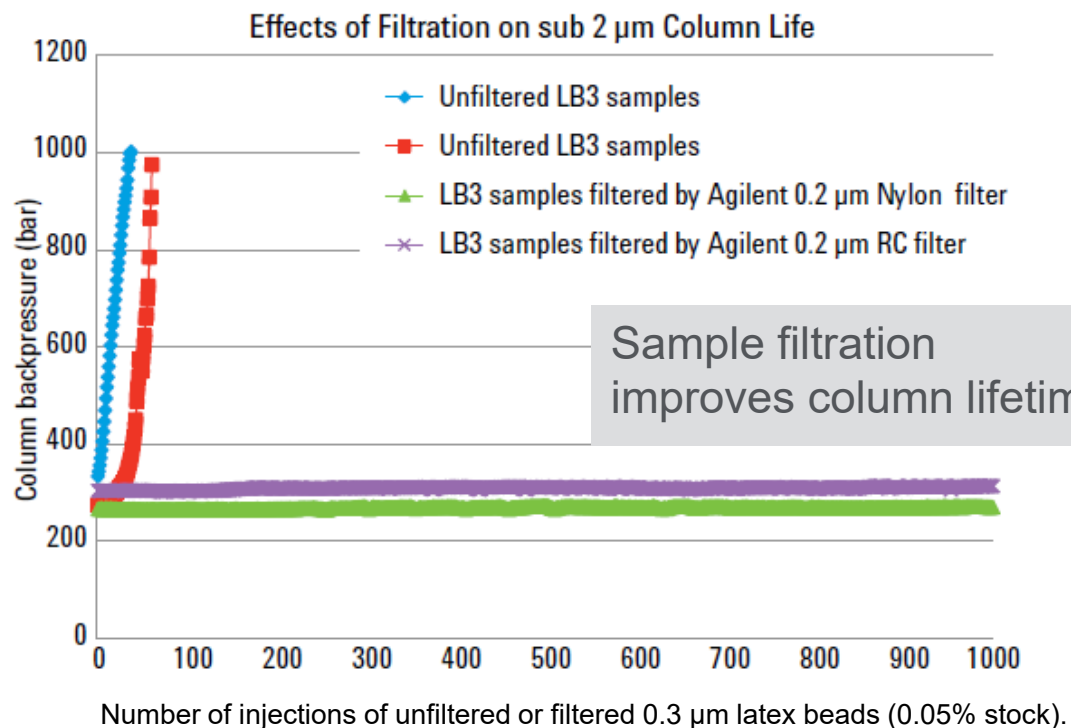
Agilent inline filters



Agilent Quick Change inline filters

Protect Your Column Before a Run

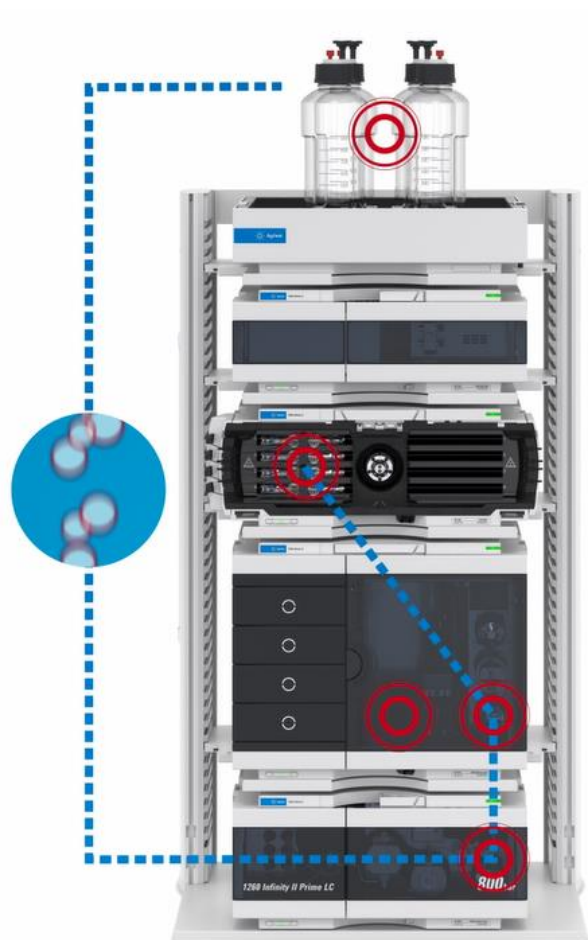
How particle and matrix components can block your LC column



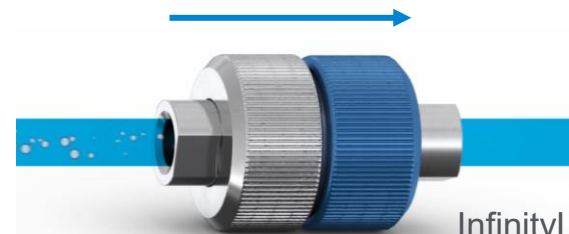
Agilent application note: 5994-1947EN

Why Use an Inline Filter?

Particles lead to blockage



1290 inline filter, p/n 5067-6189

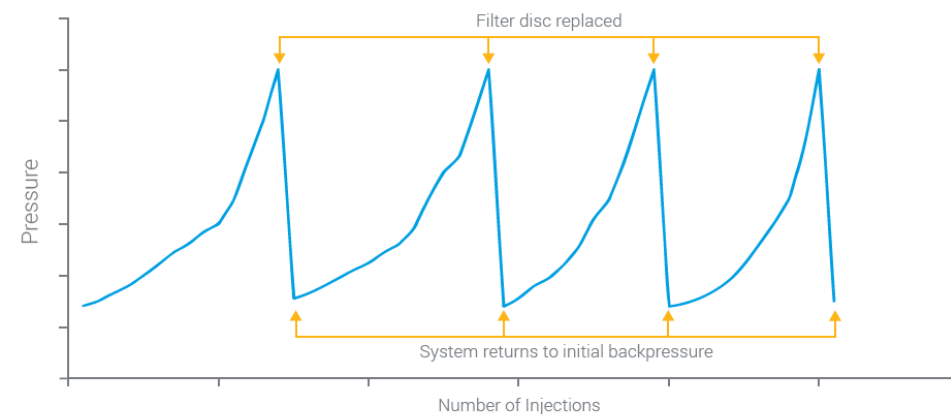


InfinityLab Quick Change inline filter

Filter particles to prevent column clogging



Extend column lifetime and reduce cost per sample



Accelerated lifetime test shows how inline filter removes particles

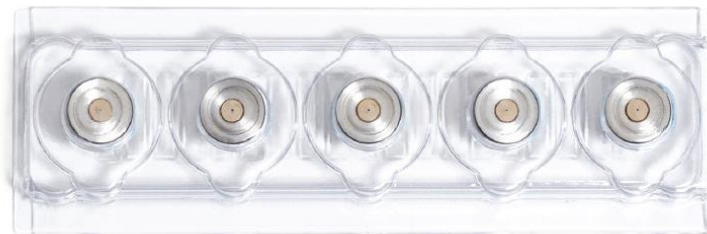
Agilent InfinityLab Quick Change Inline Filter – Filter Discs

High efficiency, easy-to-use filter discs

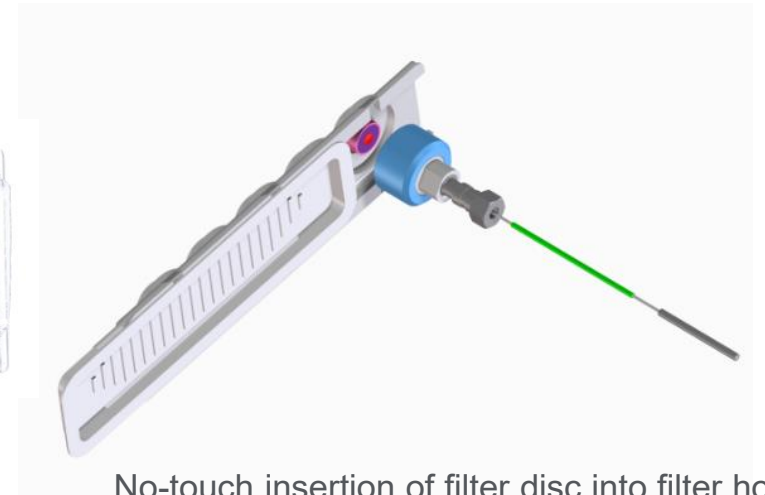
- **Various dimensions and porosities** –available in 2.1 mm and 4.6 mm inner diameters with different pore sizes. The filter housing is compatible with all types of filter discs.
- **Touchless packaging to avoid potential contamination** – specifically designed packaging allows you to insert the filter disc into the 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 when filter discs need replacing



Different dimensions and porosities of filter discs



Filter discs in touchless packaging



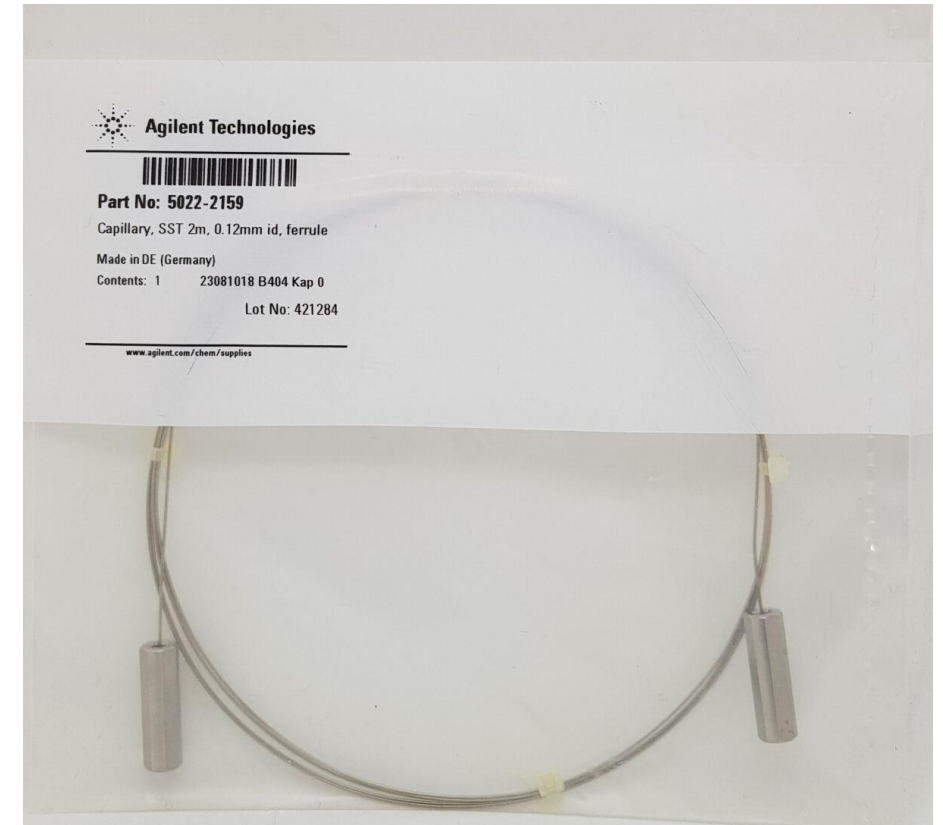
No-touch insertion of filter disc into filter housing

Check Your Instrument Pressure

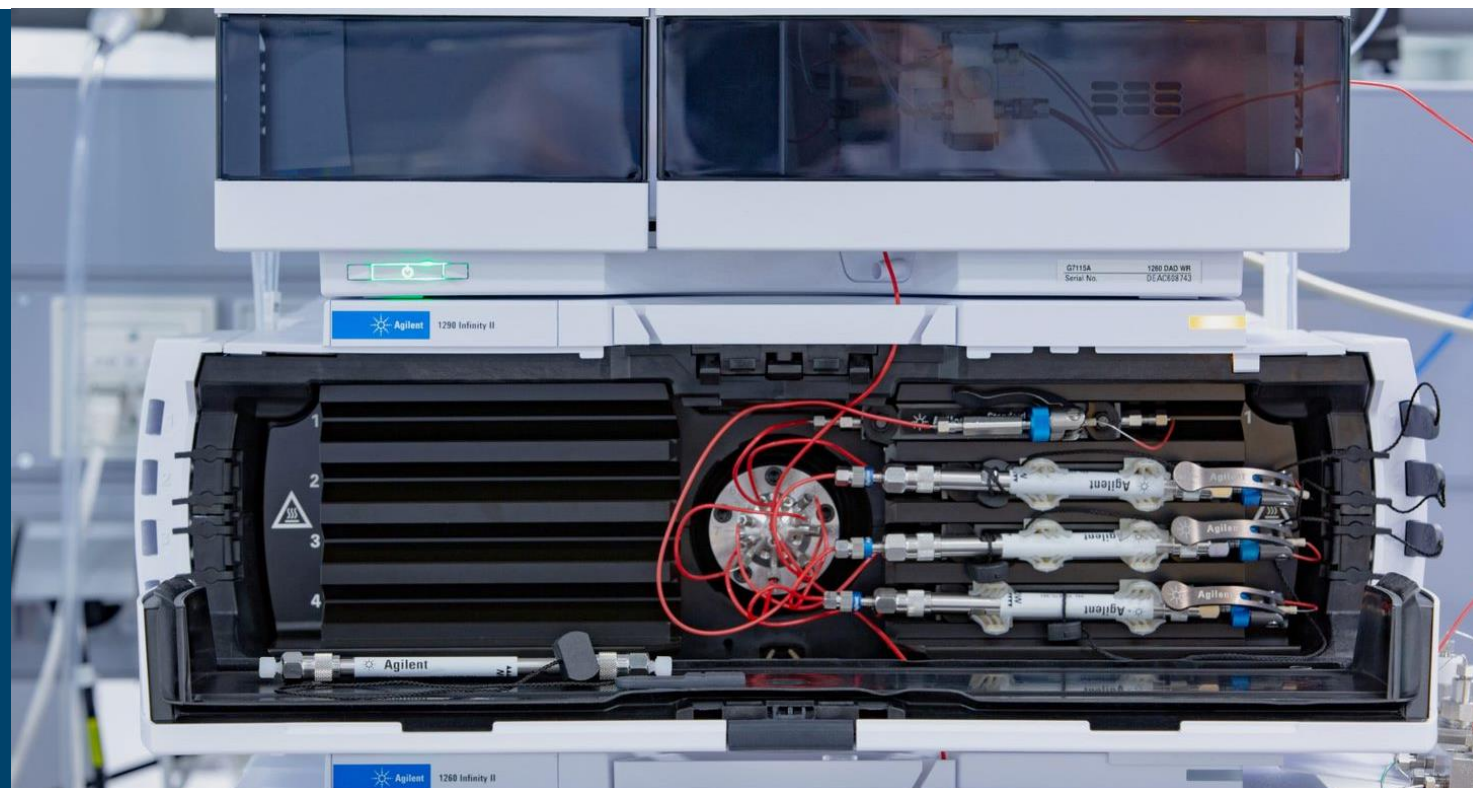
- Before installing your column, test your instrument pressure with no column
- Measure the system pressure under desired conditions
- Check the system pressure using just a union
- Install a restriction capillary (part number 5022-2159)



ZDV universal union, 5022-2184



Operating Your Column



Calculating Column Volume



1. Volume of a cylinder

$$V = \pi r^2 h$$

2. You'll need an estimation of the void volume of the column; 60% is considered reasonable

Example calculation – column size 4.6 x 150 mm

1. cm^3 ($\pi \times 0.23 \text{ cm} \times 0.23 \text{ cm} \times 15 \text{ cm}$), which is 2.49 cm^3 or 2.49 mL (remember $1 \text{ cm}^3 = 1 \text{ mL}$).
2. $2.49 \times 0.6 = 1.5 \text{ mL}$

Volume for this column – 1.5 mL

You can also determine this for your column on your system by running an unretained sample, like uracil for reverse phase columns.

[Calculating Column Volume - Wiki - Consumables - Agilent Community](#)

Take Care Installing Your Column

- Set maximum backpressure for your system which will be appropriate for your column
- Check to see what solvent the column was shipped in
- Know what mobile phase is currently running in your LC system
- Be careful not to pass any mobile phase through the column that may cause a precipitate to form – make sure it is compatible
- Flush the LC system with mobile phase through to the column inlet connection



Take Care Installing Your Column (cont.)

- Note the correct flow direction on the column when connecting it to the LC system
- If mobile phase additives are used (such as buffers or ion-pair reagents) it is advisable to do an intermediate flush with a mobile phase of the correct composition, but without these additions
- It is advised to introduce the mobile phase to the column at a reduced flow rate while monitoring backpressure
- Gradually increase the flow rate of your column to the working flow rate and allow for column equilibration



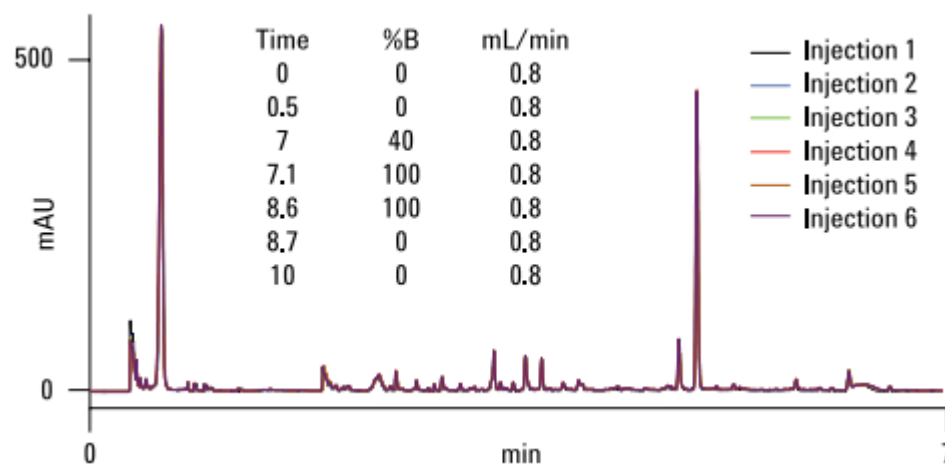
Equilibrating Your Column

Equilibration for HPLC columns is the process of preparing the column to ensure that the stationary and mobile phases are in a stable, balanced state before the analysis begins.

Goal of Equilibration:

- To ensure reproducible retention times
- Reproducible analyte interaction
- Stable baseline

HPLC: 1290 with DAD
Column: RRHD SB C18 1.8 μ m
2.1x 150 mm p/n 959759-902
Inj vol: 1 μ l of 0.2 μ m filtered grapefruit juice
Temp: 40C
Det: UV 276 nm
Solv A: Water
Solv B: ACN
Sample: Grapefruit juice



[Agilent LC-Handbook-Complete-2.pdf](#)

Equilibrating the Column

Reversed phase: InfinityLab Poroshell phases, ZORBAX

Best practices:

- Agilent columns ship in the solvent shown on the test chromatogram included with the column
- Connect the column inlet to the instrument and place the outlet in a beaker
- Flush the column for 10–20 column volumes with 100% acetonitrile or methanol as appropriate
- Connect the column outlet to the detector and continue to flush until a flat baseline is achieved
- If using a modifier like TFA, you can then equilibrate with your mobile phase for 10–20 column volumes. For using formic acid, a longer period of flushing is recommended.
- If using a buffered mobile phase, e.g. sodium phosphate, then equilibrate with mobile phase minus the buffer before introducing the buffered mobile phase to avoid buffer salt precipitation

LC column user guides: [LC Column user guides | Agilent](#)

Equilibrating the Column

HILIC: InfinityLab Poroshell HILIC, HILIC-Z, HILIC-OH5

Best practices:

- Remember, water is the strong solvent and acetonitrile is the weak solvent
- Connect the column inlet and place the outlet of the column in a beaker
- It is best to equilibrate the column with 30–40% water in acetonitrile before use
- Equilibration may require 20–50 column volumes
- Connect the column outlet to the detector and monitor for a flat baseline
- Then equilibrate with the buffer for your isocratic conditions or your starting conditions for the gradient. This may take longer than reversed phase.
- Column equilibration and re-equilibration are directly related to the water content of the mobile phase. Each run requires that the water layer be refreshed with high-aqueous mobile phase, after which the concentration of water in the mobile phase can be reduced to the starting conditions of the next run.

LC column user guides: [LC Column user guides | Agilent](#)

Equilibrating the Column

HILIC

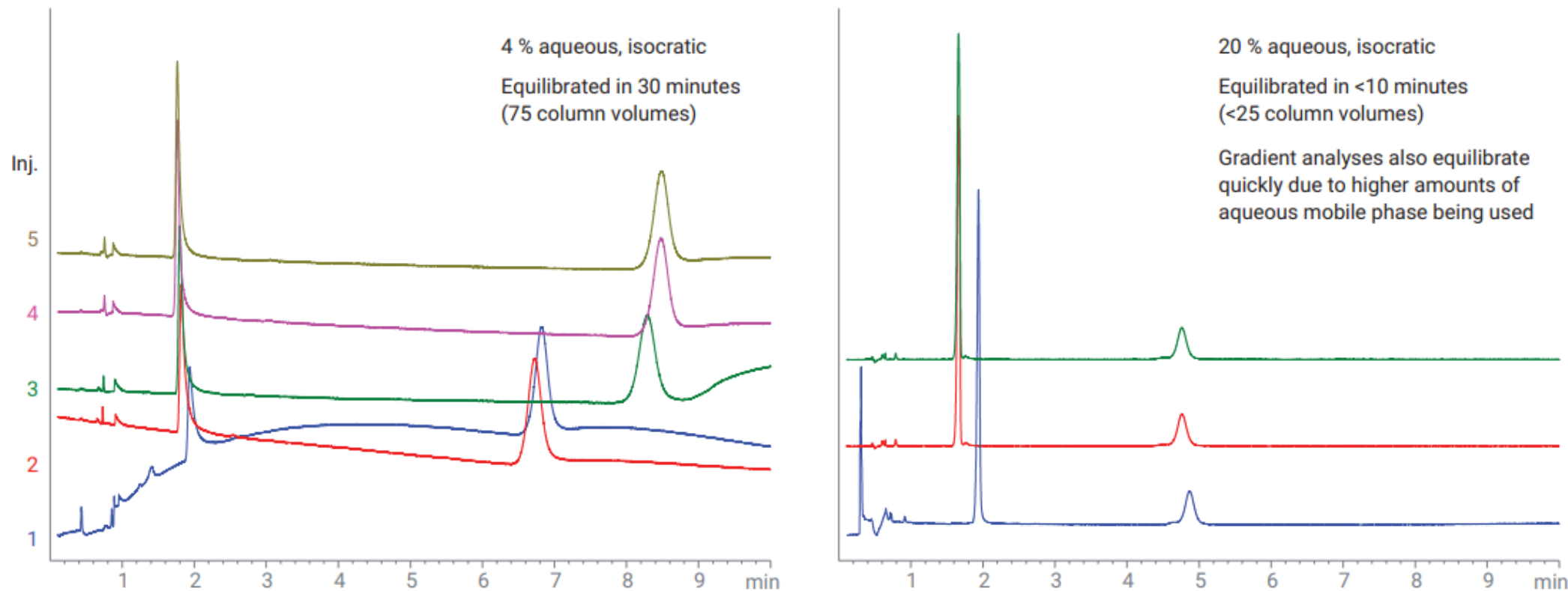


Figure 6. Separation of B vitamins using an InfinityLab Poroshell 120 HILIC-OH5, 2.1 × 100 mm, 2.7 µm column. Left: Column stored in 100 % acetonitrile prior to analysis. Mobile phase A: 100 mM ammonium formate, pH 3.0. Mobile phase B: acetonitrile. Isocratic conditions: 96 %B. Flow: 0.5 mL/min. Injection: 1 µL of B2+B6. Column temperature: 25 °C. Detection: 260 nm, 80 Hz. Right: Column stored in 100 % acetonitrile prior to analysis. Mobile phase A: 100 mM ammonium formate, pH 3.0, Mobile phase B: acetonitrile. Isocratic conditions: 80 %B. Flow: 0.5 mL/min. Injection: 1 µL of B9+B12. Column temperature: 25 °C. Detection: 260 nm, 80 Hz.

Equilibrating the Column

SEC for proteins and peptides; AdvanceBio SEC

Best practices:

- The columns are shipped in 100 mM sodium phosphate buffer, pH 6.7, containing 0.02% NaN₃.
- Connect the column inlet and place the outlet of the column in a beaker.
- Flush the column into the mobile phase required for your separation.
- Ramp up the flow rate slowly from 0.0 mL/min to the intended operating flow rate over a period of several minutes. If possible, the maximum flow gradient should be set at 0.1 mL/min².
- Equilibrate the column by flushing for a minimum of 10 column volumes
- Connect the column outlet to the detector and monitor for a flat baseline.

BioLC column user guides: [Bio LC Column user guides | Agilent](#)

Equilibrating the Column

Ion exchange for proteins: Bio IEX, PL-SAX, PL-SCX

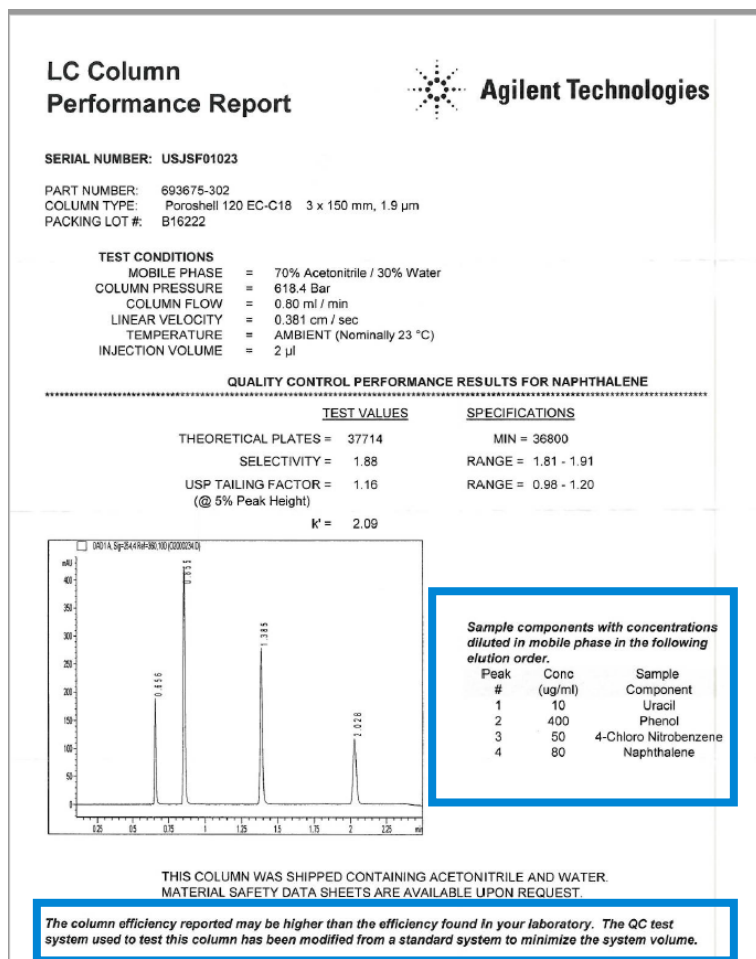
Best practices:

- Agilent Bio IEX columns are shipped in a 20 mM sodium phosphate buffer, pH 6.0 (for SCX, WCX), 20 mM Tris, pH 8.0 (for SAX, WAX).
- Start at a low flow rate (50% of the normal operating flow rate) and flush the column with mobile phase B (or a high salt concentration mobile phase) for 10 column volumes.
- Then equilibrate the column with mobile phase A until the baseline is stable.
- Gradually increase the flow rate to the desired operating flow rate.

BioLC column user guides: [Bio LC Column user guides | Agilent](#)

Before Starting Your Analysis

Benchmark your column



Performance verification based on Agilent checkout

- Run Agilent checkout before use
 - Record difference between your instrument and performance report (use as base value)
- Perform again if column seems to lose performance
 - Compare with results from first run

Performance verification based on in-house checkout

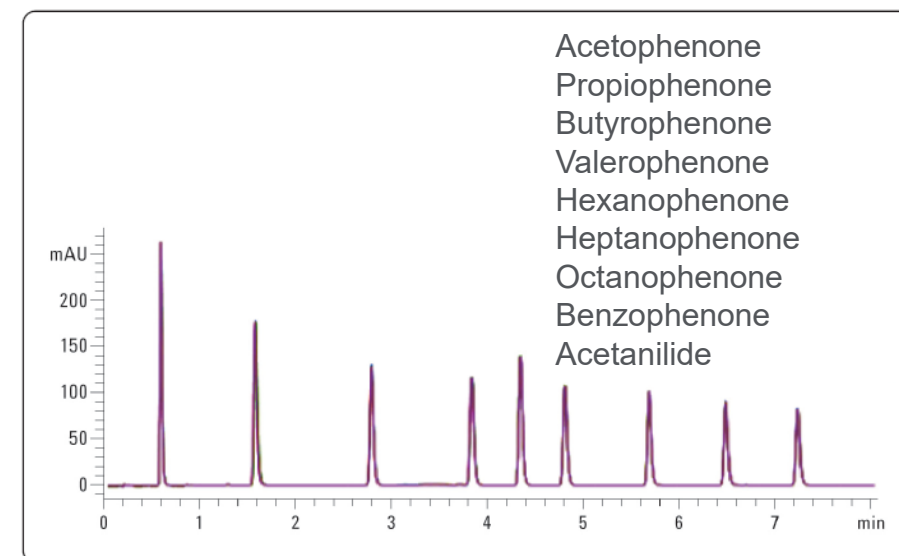
- Run in-house checkout before use
 - Record key specifications such as tailing factor, plates, and backpressure
- Perform again if column seems to lose performance
 - Compare with results from first run

Benchmarking a New Column

Reverse phase example

Benchmark new column on your system

1. Standard mix; test mix (5188-6529, 01080-68704; QC reference material)
2. Record criteria like retention time, peak area, peak tailing, resolution, response, and system pressure. Do a few injections to make sure it is reproducible.
3. Monitor column over time. Test again against this benchmark when you need to troubleshoot a problem.



Chromatographic conditions

Sample:	RRLC Checkout sample (p/n 5188-6529)
Column:	Agilent Poroshell 120 EC C18, 3 mm × 50 mm, 2.7 µm
Mobile phase:	A = Water B = Acetonitrile
Gradient:	0 min 20% B 8 min 80% B
Flow rate:	1.2 mL/min
Stop time:	8 min
Post time:	4 min
Injection volume:	1 µL
Column temperature:	30 °C
DAD:	245/10 nm Ref 400/100 nm
Flow cell:	10 mm
Peak width:	<0.025 min (10 Hz)

Column Test Reports: [eCertificates of Product Quality and Analysis | Agilent](#)

The Value of Guard columns

Guards protect your column in many ways

What They Do

- Provide protection for the analytical column
 - Filter particulates
 - Unwanted chemical contamination
 - Protect against pressure spikes
- Increase total “column length”
 - Increase retention
- Should increase efficiency

What They Don't Do

- Replace good sample cleanup
- Replace column hygiene



Agilent Guard Cartridge and holder

[Video: Fast Guards for HPLC and UHPLC | Agilent](#)

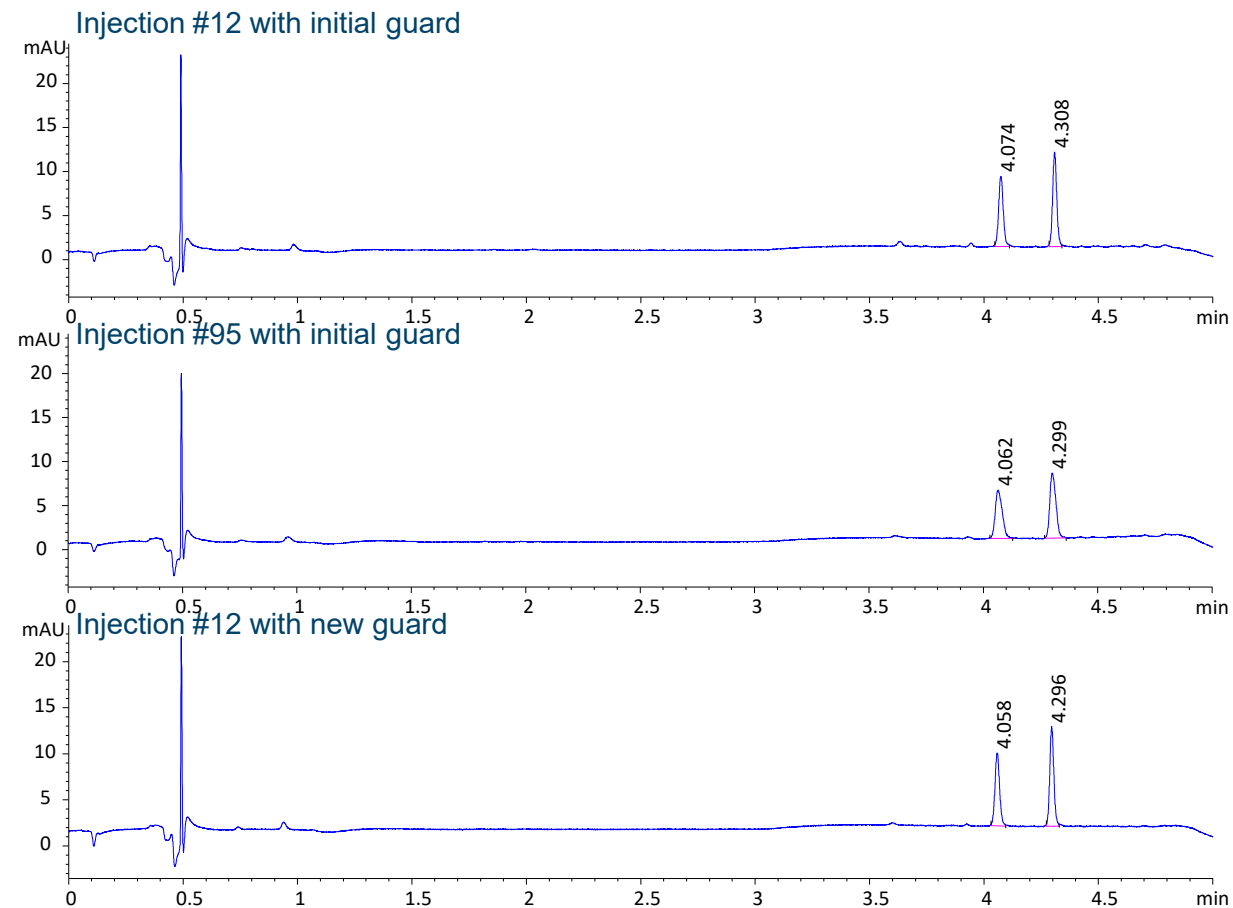
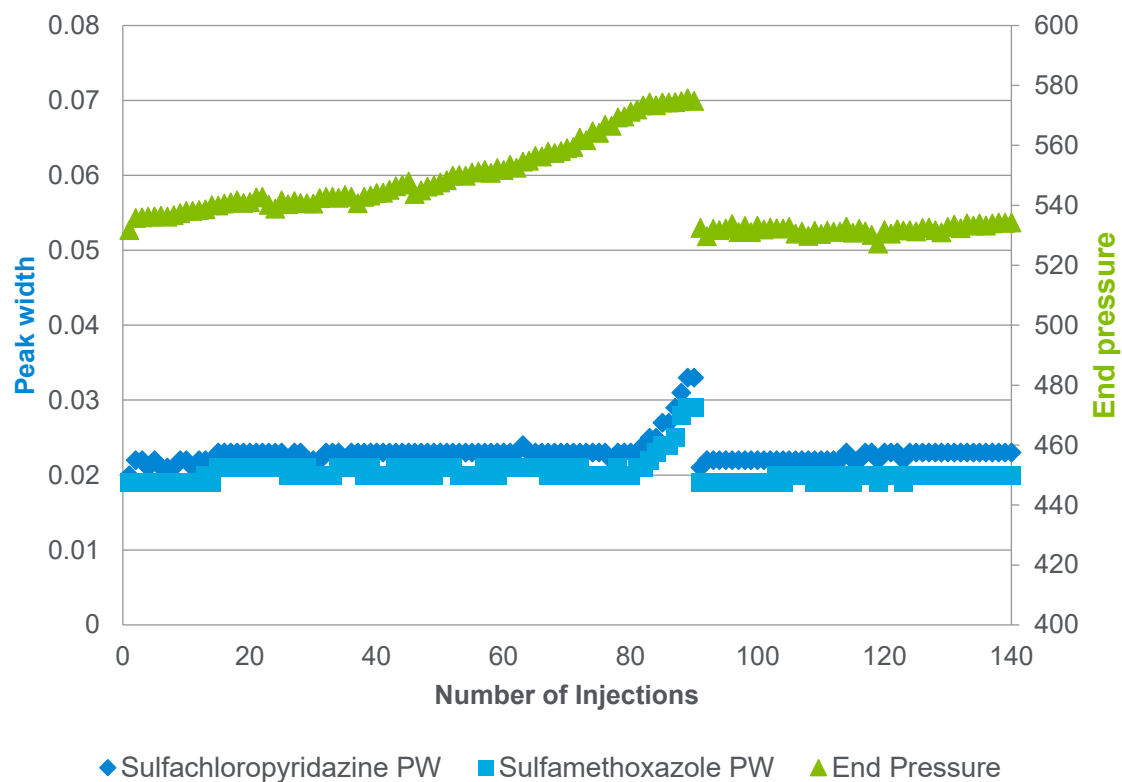


InfinityLab Fast Guard

Guard Columns

Guards protect your column in many ways

Poroshell Column + Poroshell Guard
Infant Formula* (1:300 in Water)



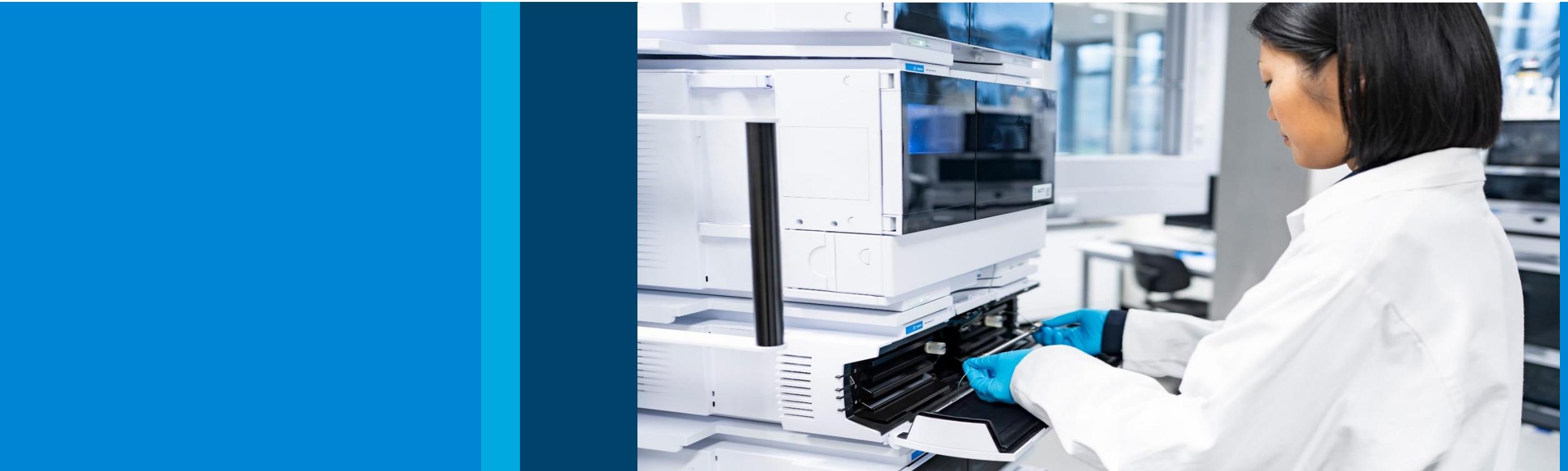
*Unfiltered infant formula including proteins and other precipitated ingredients.

Common Issues Seen for Columns

	Possible Cause	Possible Solution
High Column Backpressure	Column Blockage	Filter sample, sample cleanup, use guard column
	Mobile Phase viscosity too high	Use lower viscosity solvent, increase temperature
	Particle Size too small	Use larger particle packing
	Plugged inlet frit	Try cleaning; possibly reverse flush or replace column
Peak tailing	Unswept dead volumes	Minimize connections; ensure fittings are correct for your column and are properly seated
	Column performance	Change mobile phase; replace column
Ghost peaks	Peaks from previous injection	Flush column to remove contaminants; check with a blank injection
	Contamination; unknown interferences in sample	Proper sample cleanup
	Contaminated mobile phase	Check your mobile phase; be sure to filter
Retention Time Drifts/Shifts	Insufficient equilibration time w/gradient or change in isocratic mobile phase	Flush with at least 10 col volumes after sample run
	Inconsistent mobile phase mixing	Ensure system delivers constant composition; compare with manual preparation of mobile phase
	Variation in column temperature	Thermostat or insulate column; ensure constant lab temperature
	Contamination buildup	Occasionally flush column with strong solvent

After the Last Run is Finished

Best practices for properly flushing and storing LC columns



How to Flush Your LC Columns

A stepwise protocol can help to remove blockages and contaminations

Tip 1: Flush with stronger solvents than your mobile phase

Tip 2: Use at least 10 column volume of each solvent for analytical columns

Tip 3: Make sure the detector is taken out of the flow path

Tip 4: To remove blockages or contaminations, you can try to backflush your column (if supported – see your column user guide)



Reversed Phase Column Care – Best Practices

How to clean and store a Reverse Phase Column

Cleaning a Reverse Phase column:

Reversed-phase solvent choices in order of increasing strength:

- Mobile phase without buffer salts (water/organic)
- 100% organic (MeOH or ACN)
- Is pressure back in the normal range?
- If not, discard column or consider more drastic conditions: 75% acetonitrile:25% isopropanol, then
- 100% Isopropanol
- 100% Methylene chloride*
- 100% Hexane*

***Important to know** 

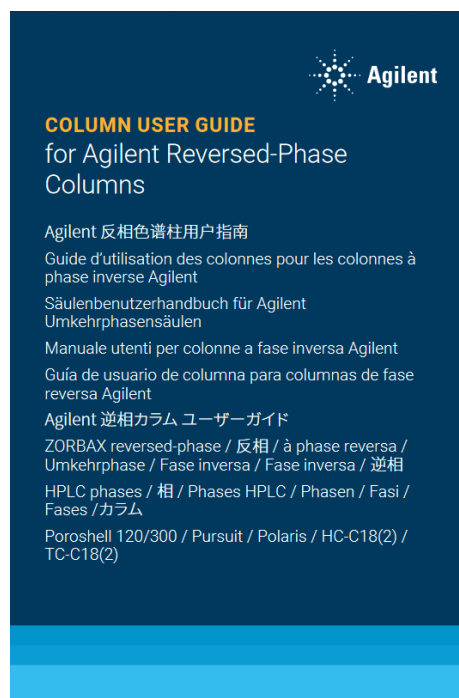
- When using either hexane or methylene chloride, the column must be flushed with isopropanol before returning to your reversed-phase mobile phase.
- Might also compromise instrument pump seal lifetime.

Reversed Phase Column Care (cont.)

How to clean and store a Reverse Phase Column

Storing a Reverse Phase Column:

User guides for Agilent columns are available on [Agilent.com](https://www.agilent.com) or by entering “Agilent LC Column user guide” into your search engine



[Agilent Reversed-Phase Columns User Guide](#)

1. Long-term storage of RP LC columns should be in a pure organic solvent. If the column has previously been used with a buffered mobile phase, the buffer should first be removed by purging the column with 20 to 30 column volumes of a 50:50 mixture of methanol or acetonitrile and water, followed by 20 to 30 column volumes of the pure solvent.
2. Before storing, end-fittings should be tightly capped with end-plugs to prevent packing from drying out.
3. To protect equipment, is it desirable to remove salts from the instrument and column by purging the column with the same mobile phase without the buffer.
4. Store columns at room temperature.

HILIC Column Care – Best Practices

How to clean and store a HILIC Column

Cleaning a HILIC column:

- Use a strong HILIC solvent to clean HILIC columns
- Flush HILIC columns with 100% water
- If that is insufficient, add in 100-500 mM salt
 - You can use a strong salt like NaCl, or if you prefer to avoid that, then you can use buffer salts, like ammonium acetate
- Increasing the temperature to 35-55 °C can also help with the cleaning efficiency
- Flush with about 30 column volumes per step
- Be sure that once you are finished flushing with high concentration salt, that you flush with pure water before reintroducing acetonitrile into the mobile phase!

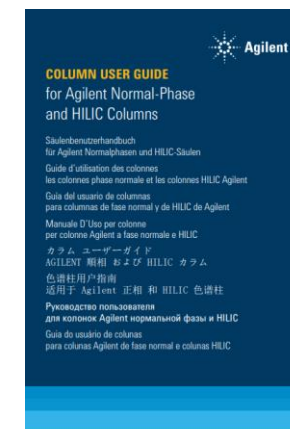
Storing a HILIC Column:

- Columns may be stored for short periods in most HILIC mobile phases

To protect equipment, it is best to remove salts from the instrument and column

Purge column with same mobile phase without the salt (ex. flush with 90:10 ACN:H₂O to remove a 90:10 ACN:0.01M formate buffered mobile phase).

Re-equilibration is faster with the original mobile phase when using this approach
- For long term storage, it is recommended to store in Acetonitrile:water (90:10)
- Store at room temperature



[Column User Guide for Normal Phase and HILIC Columns](#)

Aqueous SEC for peptides and proteins – Best Practices

AdvanceBio SEC

How to clean and store Aqueous SEC for peptides and proteins: AdvanceBio SEC

Cleaning an AdvanceBio SEC column:

- For strongly adsorbed contaminants:
 - ✓ High salt concentration at low pH (for example, 0.5 M Na₂ SO₄ , pH 3) or 0.5 M guanidine hydrochloride
 - ✓ Organic solvent for hydrophobic materials: up to 50% methanol, ethanol, or isopropanol
 - ✓ Acidic reagents for basic contaminants: 0.1% TFA, formic acid, or acetic acid in 15% acetonitrile
- Always flush the column in the direction of the flow arrow
- Lower the flow rate to keep the pressure below 200 bar for 2.7 µm columns or 400 bar for 1.9 µm columns.
- Rinse with at least five column volumes of ultrapure water before and after flushing with at least 20 column volumes of the cleaning solution.

Aqueous SEC for peptides and proteins: AdvanceBio SEC

How to clean and store Aqueous SEC columns: AdvanceBio SEC

- Short-term storage (less than two weeks): store the column in the mobile phase.
- Extended storage (longer than two weeks): store the column in filtered 100 mM sodium phosphate, $\text{pH} \leq 7$, with or without 0.02% NaN_3 , or 20% methanol in water.
- Flush the column with a minimum of 10 column volumes. To switch to or from 20% methanol, column flushing must be done at low flow rates to avoid overpressuring the column due to high viscosity. Starting at a lower flow rate, flush at no more than 0.1 mL/min for 4.6 mm columns, and no more than 0.2 mL/min for 7.8 mm columns, while also ensuring the pressure remains below 200 bar.
- Store columns at room temperature.



Agilent AdvanceBio SEC Columns

Size exclusion columns for analysis of biomolecules



Introduction

Agilent AdvanceBio SEC columns are designed and manufactured by Agilent for size exclusion chromatography of biomolecules. The innovative, high-porosity silica particles and unique hydrophilic bonding chemistry provide for exceptional stability with minimal nonspecific interactions.

AdvanceBio 2.7 μm SEC columns are available in four pore sizes: 130 Å for peptides and small therapeutic proteins; 300 Å for monoclonal antibodies (mAbs), antibody-drug conjugates (ADCs), and other proteins; a new 500 Å pore size for adeno-associated viruses (AAVs) and other large biotherapeutic proteins and oligonucleotides; and a new 1000 Å pore size for virus-like particles (VLPs) and other large biotherapeutics, such as oligonucleotides.

AdvanceBio 1.9 μm UHPLC SEC columns are optimized for high resolution, high-throughput separation, and characterization of size variants. They are offered in two pore sizes: AdvanceBio SEC 1.9 μm , 120 Å is best suited for the analysis of peptides and small therapeutic proteins, while AdvanceBio SEC 1.9 μm , 200 Å is designed for characterizing mAbs and ADCs.

[Agilent AdvanceBio SEC Columns User Guide](#)

IEX Column Care

Ion exchange for proteins: Bio IEX, PL-SAX, PL-SCX

How to clean and store a IEX Column

Cleaning an IEX column:

- Mild contamination:
Columns can be efficiently cleaned with 1.0 M NaCl in mobile phase A solution (equilibration buffer)
- Moderate contamination:
For SCX and WCX columns: The recommended cleaning buffer for the SCX and WCX columns is 50 mM phosphate buffer in 1.0 M NaCl, pH 10.
For SAX and WAX: The recommended cleaning buffer for the SAX and WAX columns is 150 mM potassium nitrate, pH 4.0.
- Severe contamination:
 - Columns can be washed in reversed direction with 25 mM NaOH for 15 CV at 0.25 mL/min. After, rinse the columns with at least 10 CV of deionized water. Next, rinse the columns with mobile phase B for 10 CV.
 - Finally, equilibrate the columns with equilibration buffer (such as mobile phase A or a mixture of mobile A and B) for 10 CV until the baseline is stabilized
- For Hydrophobic contaminants:
Columns can be washed using 50 to 75% ethanol or acetonitrile for at least 15 CV. Before using organics to clean the columns, ensure that the columns are flushed with water to remove most of the salt. After this organic wash, the columns should again be rinsed with 100% deionized water for 10 CV to remove organic solvents.

IEX Column Care (cont.)

How to clean and store a IEX Column

Storing an IEX Column:

- For short term column storage, flush the columns with mobile phase A at least 15 column volumes. Disconnect the columns from the LC system, and tightly seal both ends with the removable end plugs provided with the column.
- For extended storage, use 20% ethanol in mobile phase A. Flush the column with at least 20 column volumes at 0.25 mL/min. Disconnect the columns from the LC system, and tightly seal both ends with the removable end plugs provided with the column.
- The column can be refrigerated or stored at room temperature (4 to 30 °C).



Agilent Bio IEX Columns

Abstract

Agilent Bio IEX columns are packed with polymeric, nonporous, ion-exchange particles, and are designed for high resolution, high recovery, and highly efficient separations of proteins, peptides, oligonucleotides, and other biomolecules. The highly crosslinked and rigid nonporous poly(styrene divinylbenzene) (PS/DVB) particles are grafted with a hydrophilic, polymeric layer, virtually eliminating nonspecific binding, while increasing efficiency and recoveries. Highly uniform and densely packed ion-exchange functional groups are chemically bonded to the hydrophilic layer. The Agilent Bio IEX family offers strong cation exchange (SCX), weak cation exchange (WCX), strong anion exchange (SAX), weak anion exchange (WAX) phases. All phases are available in 1.7, 3, 5, and 10 μm nonporous particle sizes. The 10 and 5 μm particles are available in PEEK and stainless steel hardware. The 3 and 1.7 μm particles are available in stainless steel only.

Basic characteristics

Parameter	Value
Column Phases	SCX (strong cation exchange), WCX (weak cation exchange), SAX (strong anion exchange), WAX (weak anion exchange)
Packing	Nonporous, polystyrene-divinylbenzene (PS/DVB), grafted hydrophilic coating and bonded with a uniform, ion-exchange layer
Particle Size	1.7, 3, 5, and 10 μm
Pore Structure	Nonporous
pH Stability	2 to 12
Operating Temperature Limit	80 °C
Recommended Pressure Limit	275 bar (~4,000 psi) for 10 μm particles
Maximum Operating Pressure Limit	415 bar (~6,000 psi) for 5 μm particles
Maximum Operating Pressure Limit	550 bar (~8,000 psi) for 3 μm particles
Maximum Operating Pressure Limit	680 bar (~10,000 psi) for 1.7 μm particles
Mobile Phase Compatibility	Compatible with aqueous solution buffers, acetonitrile, acetone, methanol, ethanol, and water mixtures. Commonly used buffers: phosphate, Tris, HEPES, and acetate
Working Flow Rate	Typical range is 0.3 to 1.0 mL/min for a 4.6 mm column or 0.1 to 0.5 mL/min for a 2.1 mm column

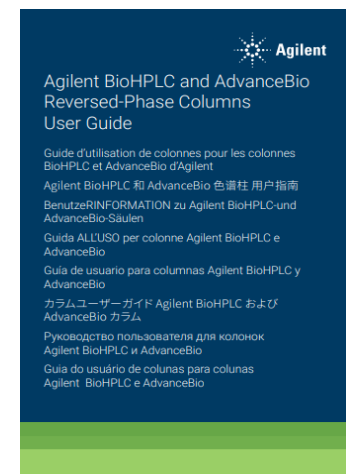
Note: To prevent sudden pressure increases, always start with a low flow rate and default to the maximum particle pressures. Gradually increase the flow rate to the designed operating flow rate condition and allow the baseline to flatten.

Review of Best Practices for HPLC Column Care and Maintenance

- ✓ Know the column packing technical specifications and limits. Read and be familiar with the USER Guide:
 - Know the packing chemistry, C18, C8, CN, HILIC, etc
 - Solvent and chemical compatibility
 - Temperature, pH range, flow rate range
- ✓ Use the proper fitting connections for your column
- ✓ Importance of mobile phase considerations; Use fresh mobile phase, filter buffers, filter samples
- ✓ Consider using inline filters for removal of mobile phase/sample particulates
- ✓ Take care when installing and equilibrating your column. Introduce mobile phase at low flow, check for miscibility, allow for proper equilibration time, etc.
- ✓ Test your column on first use to establish a 'benchmark'
- ✓ Use an appropriate guard column
- ✓ For cleaning, follow instructions provided in column user guide for your column chemistry. Ex: use stronger solvent such as Water for HILIC, stronger organic for RP
- ✓ To store column, follow instructions provided in column user guide for your column chemistry. Ex: purge buffers, and leave in appropriate storage solvent (ex; reverse phase in ACN)
- ✓ Avoid physically mishandling columns: banging, dropping, or overtightening fittings

LC Columns and Supplies Resources

- LC column user guides: [LC Column user guides | Agilent](#)
- BioLC column user guides: [Bio LC Column user guides | Agilent](#)
- InfinityLab supplies catalog: [InfinityLab LC Supplies \(agilent.com\)](#)
- LC troubleshooting poster: [LC Troubleshooting Guide \(Agilent.com\)](#)
- Column Test Reports: [eCertificates of Product Quality and Analysis | Agilent](#)
- eSelector Tools: [Selector Tools for Chromatography and Spectroscopy Lab Supplies | Agilent](#)
- App finder: [Application Finder | Agilent](#)
- Agilent Community: [Agilent Community](#)
- Consumables Community: [Agilent Community Consumables](#)
- Agilent University: [Agilent University](#)
- YouTube: [Agilent Channel](#)
- Your local product specialists
- Webinars, upcoming and recorded: [Webinars | Agilent](#)



Contact Agilent Chemistries and Supplies Technical Support

Available in the U.S. and Canada, 8am-5pm all time zones

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



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