Off to a Fresh Start

HPLC Column Care

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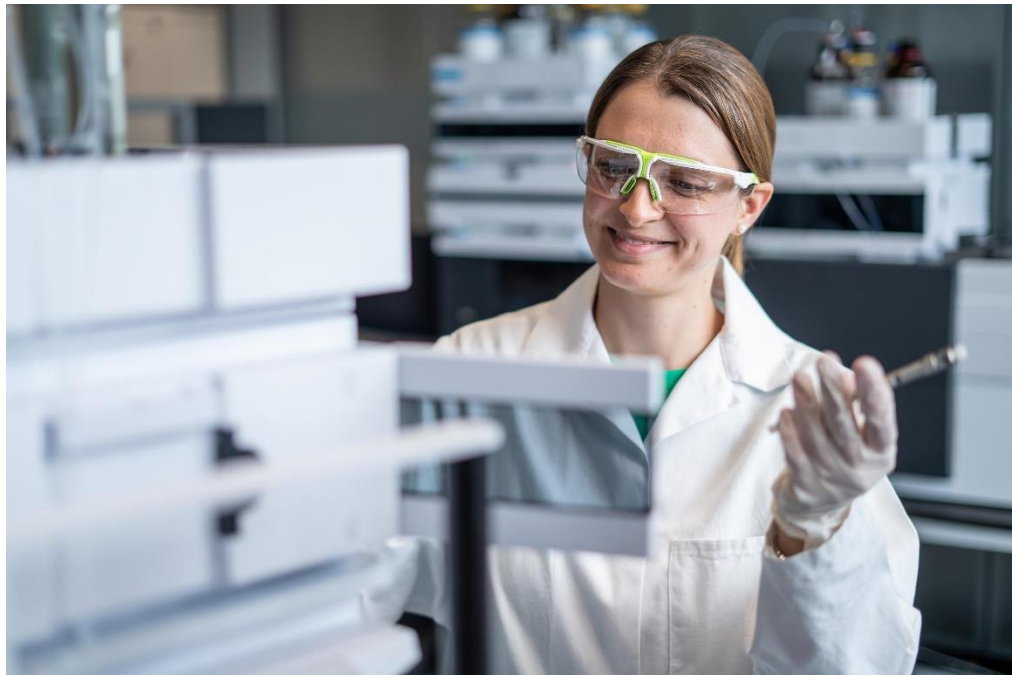
Columns and Supplies Technical Support
13 December 2022

Overview

Getting started with a new column

- Measure instrument pressure without the column
- Column pressure rating and pump settings
- Column installation
- Column equilibration
- Column benchmarking
- Column test report
- Column storage

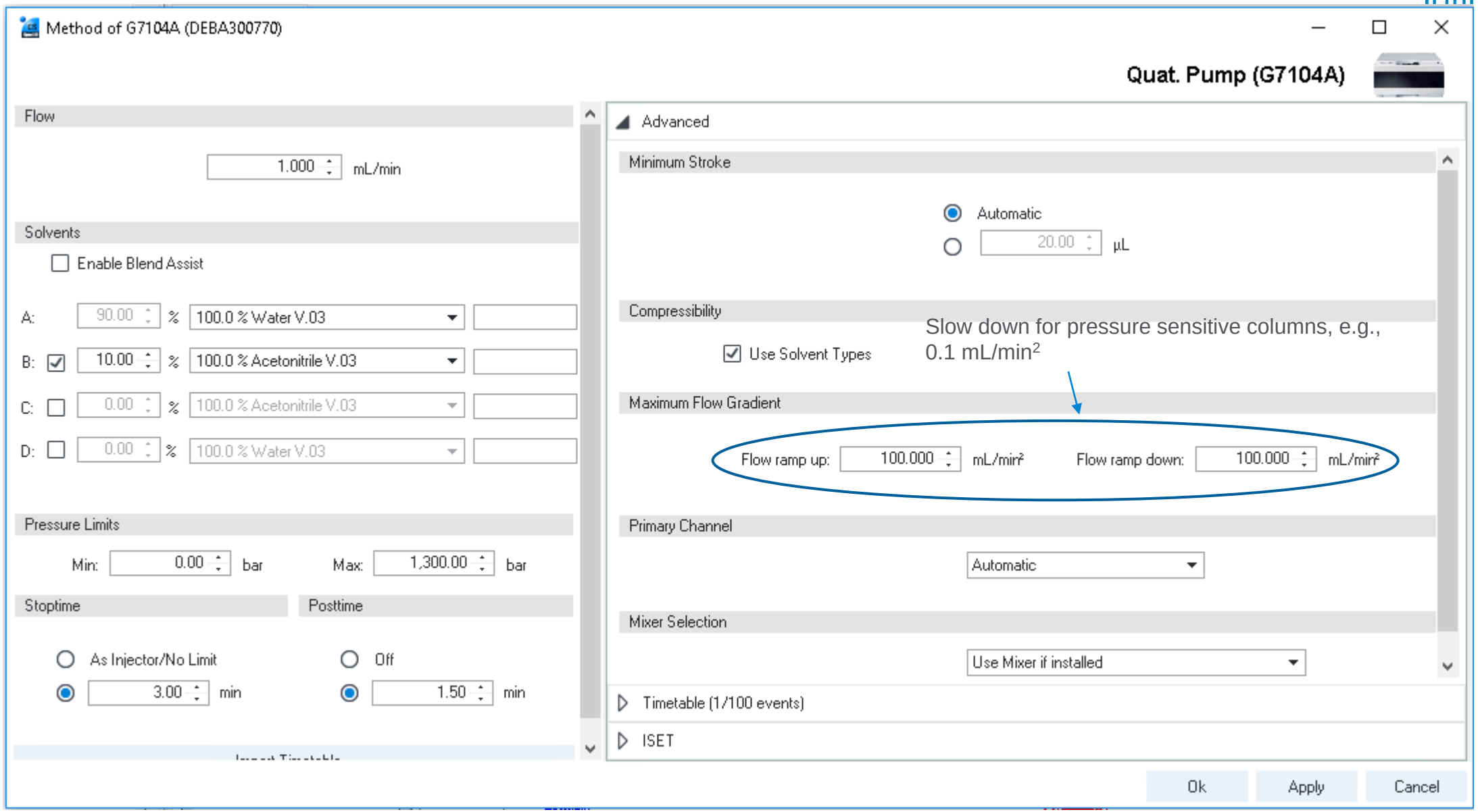


Check your Instrument Pressure

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

ZDV universal union, 5022-2184

Pump Setting



Column Connections

Stainless steel

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

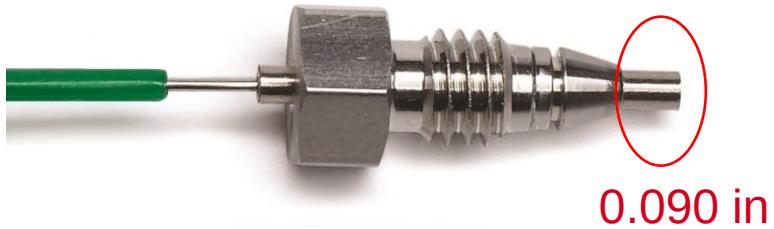
PEEK (< 400 bar system pressure)

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

Polyketone

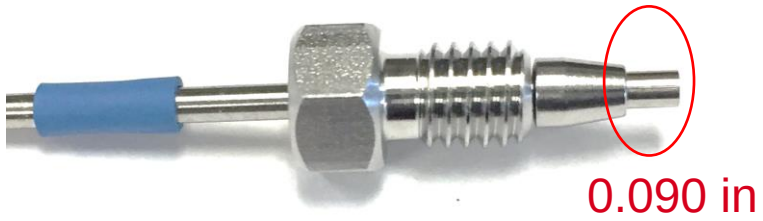
- Easy, hand tightened column connection
- Used up to 600 bar (p/n: 5042-8957)
- Best on SST tubing

Column Connections



Swagelok

- Two-piece ferrule
- Used on Agilent LCs
- Short nut
- Also available with long nut



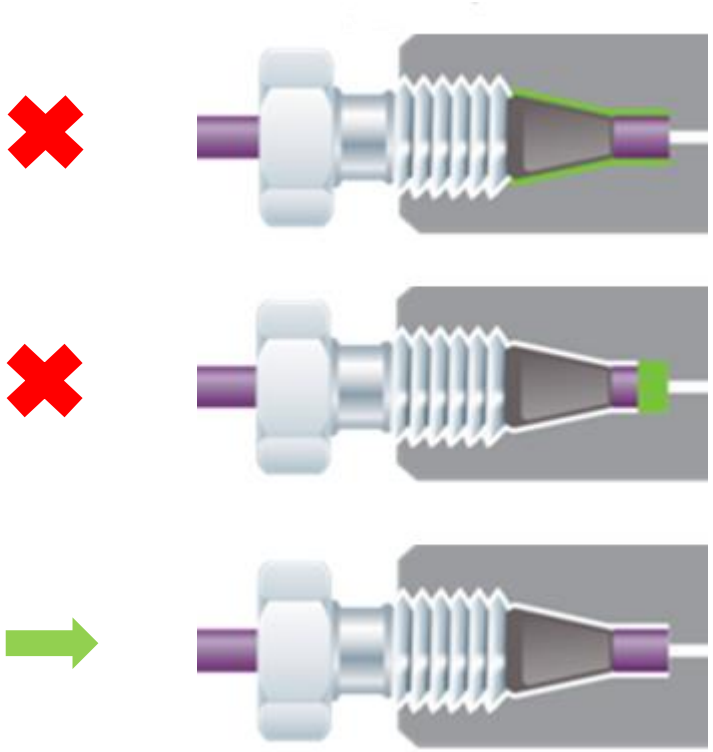
Parker

- One-piece ferrule
- Short nut
- Very similar to Swagelok
- Agilent GPC columns
- Waters Acquity systems

Waters

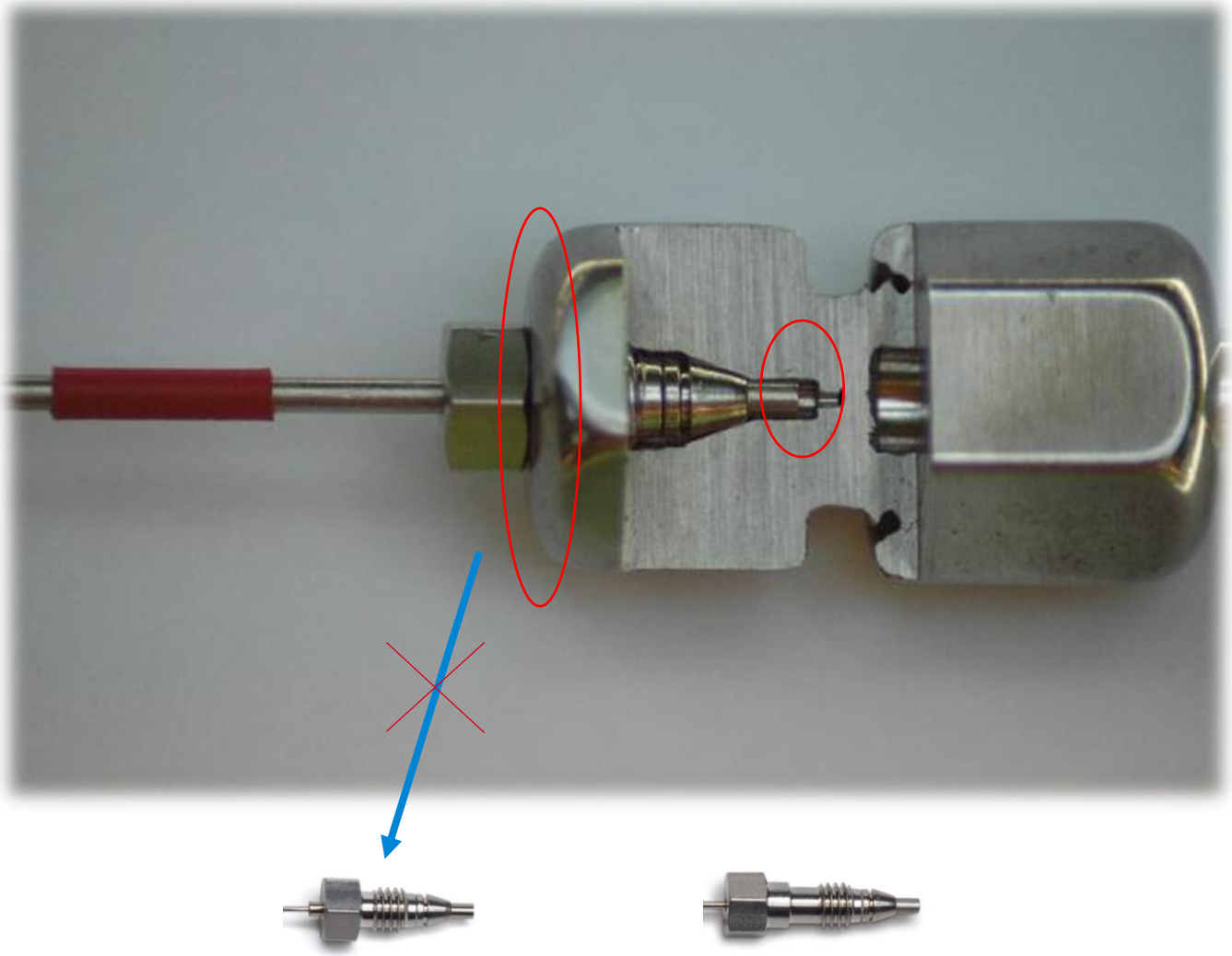
- Longer nut
- Used on Alliance systems
- Non-Acquity columns

Potential Issues with Fittings

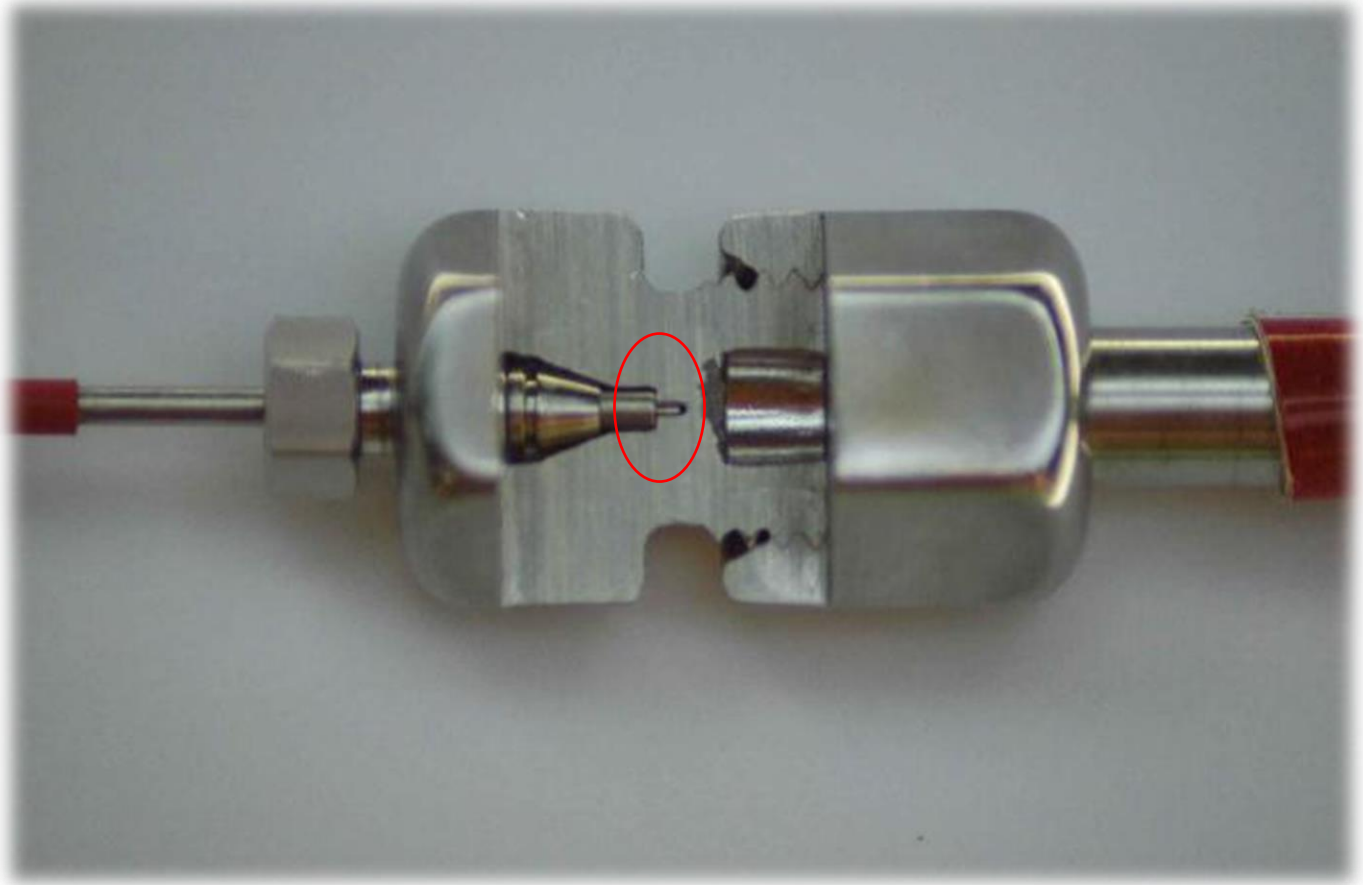


- Leak
- Peak shape problem
- No dead volume

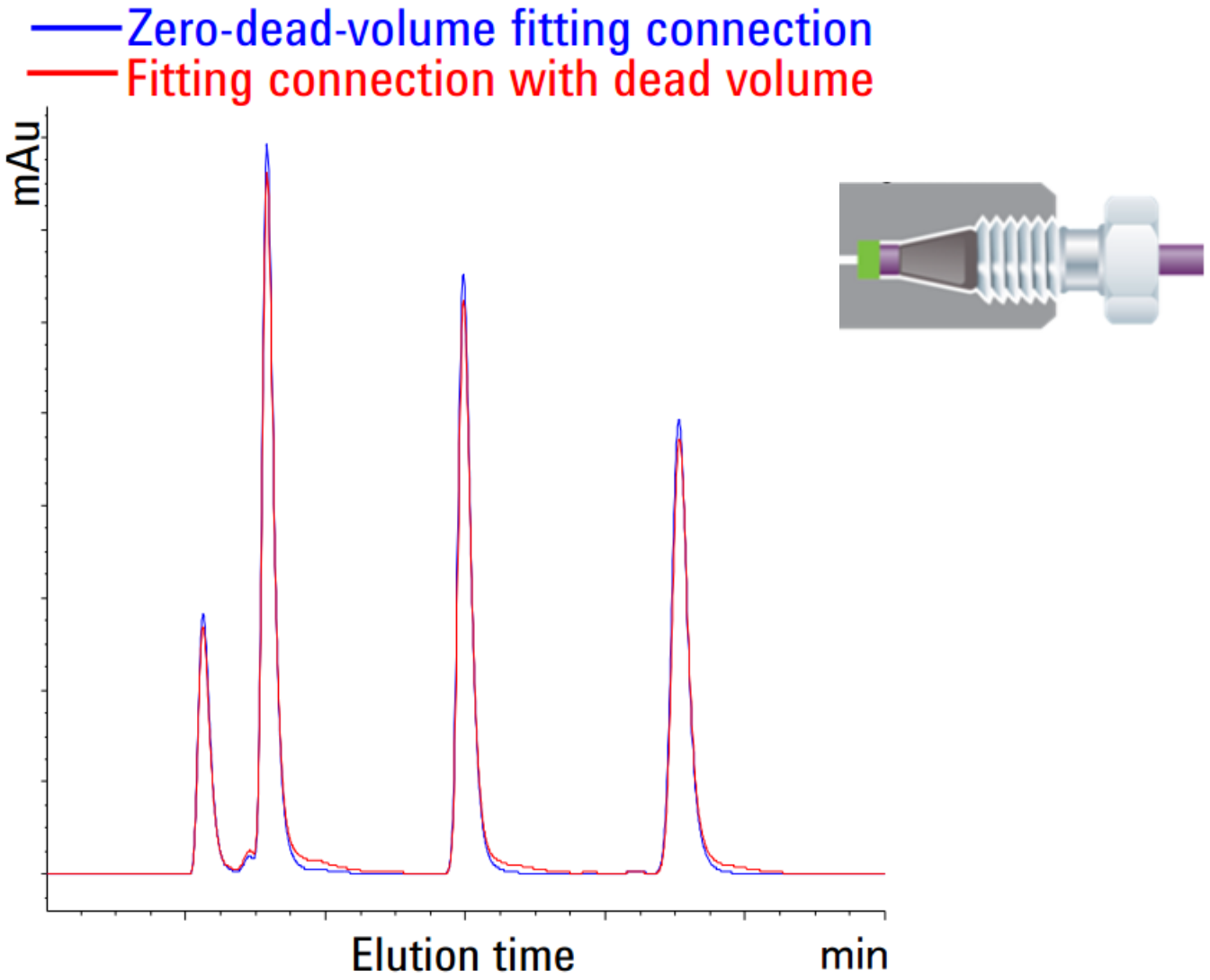
Fitting Mismatch



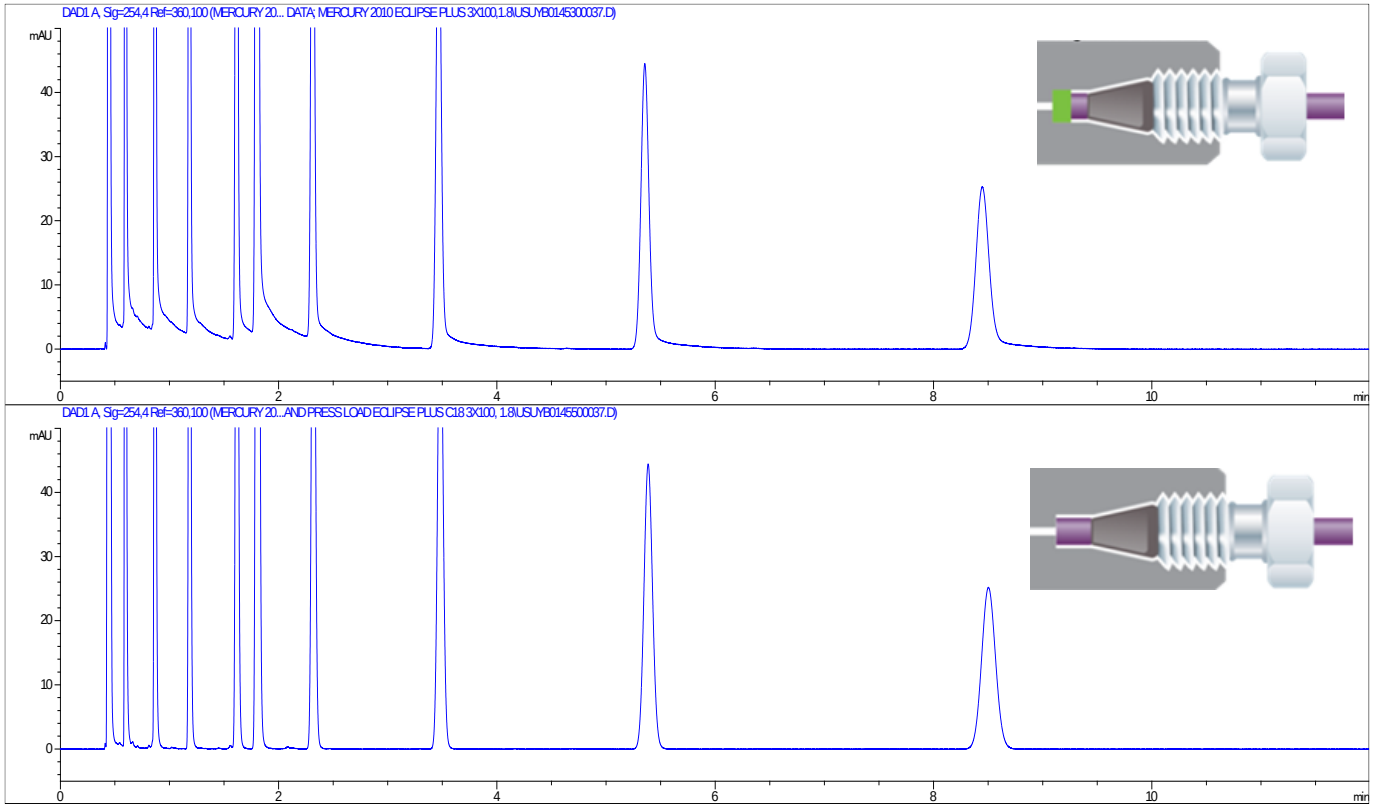
Proper Fit



Peak Shape


Zero-dead-volume fitting connection
Fitting connection with dead volume
mAu
Elution time
min

Peak Shape


DAD1 A, Sig=254,4 Ref=360,100 (MERCURY 20... DATA\MERCURY 2010 ECLIPSE PLUS 3X100,1.8\USUYB0145300037.D)
mAU
40
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min
DAD1 A, Sig=254,4 Ref=360,100 (MERCURY 20...AND PRESS LOAD ECLIPSE PLUS C18 3X100, 1.8\USUYB0145500037.D)
mAU
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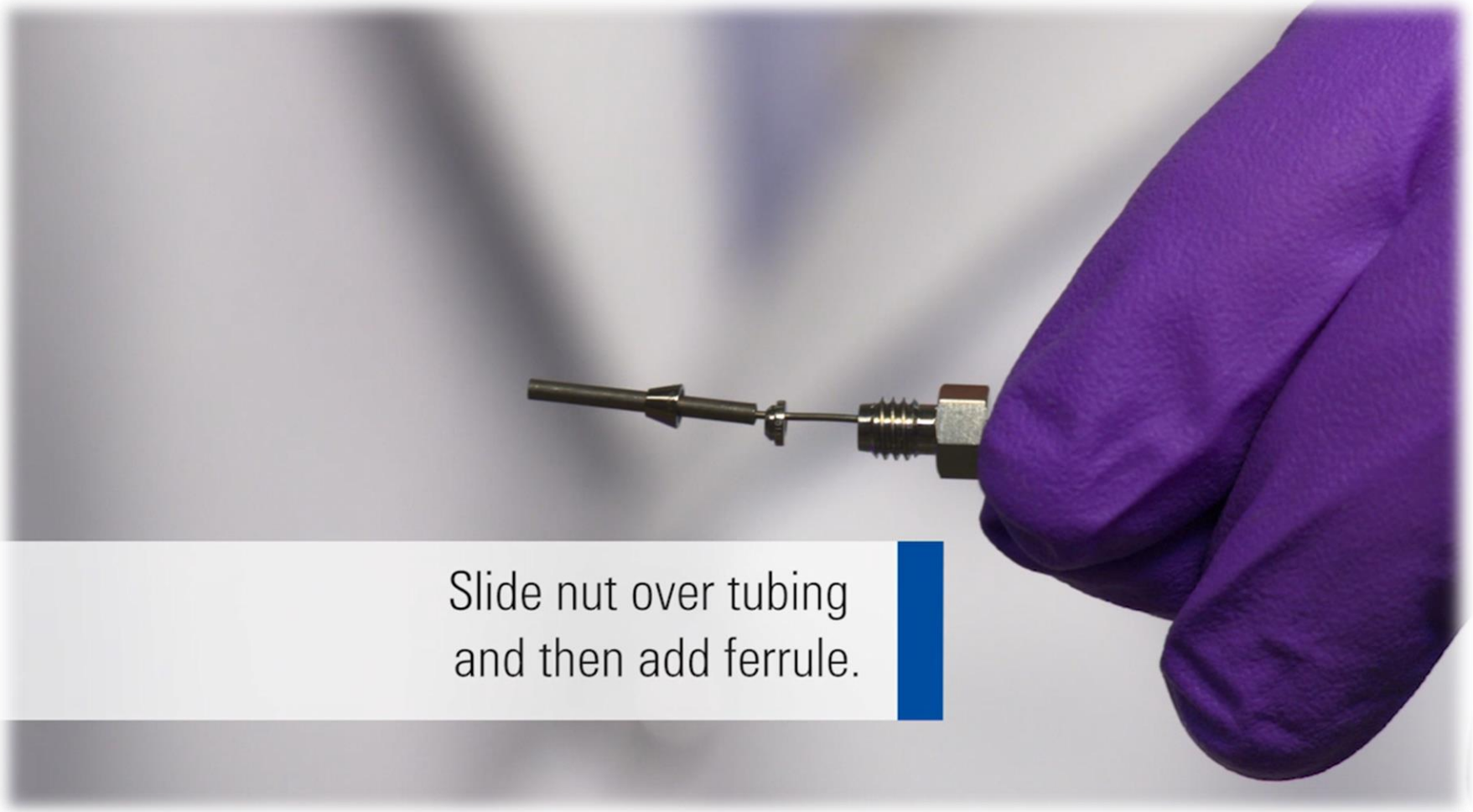
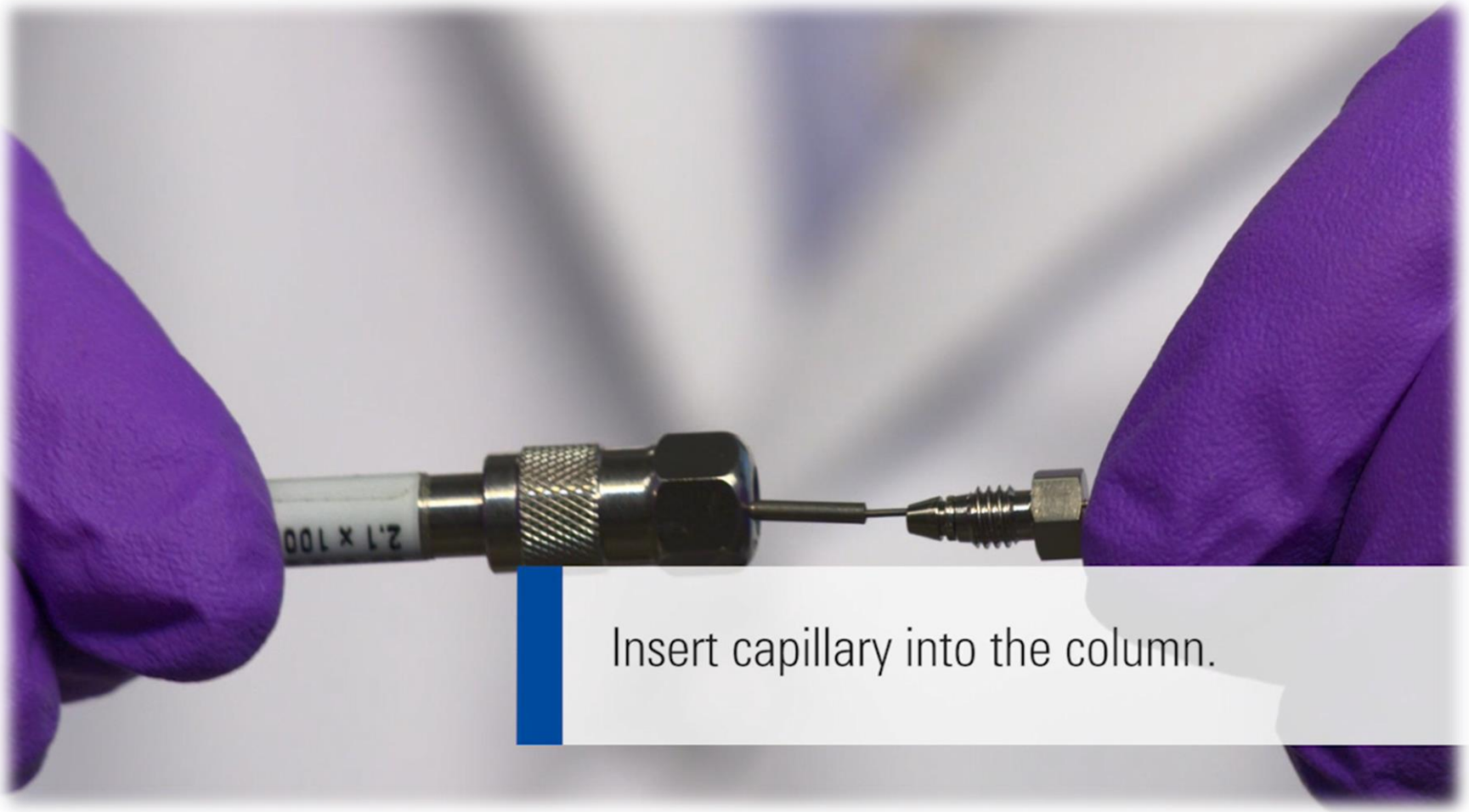
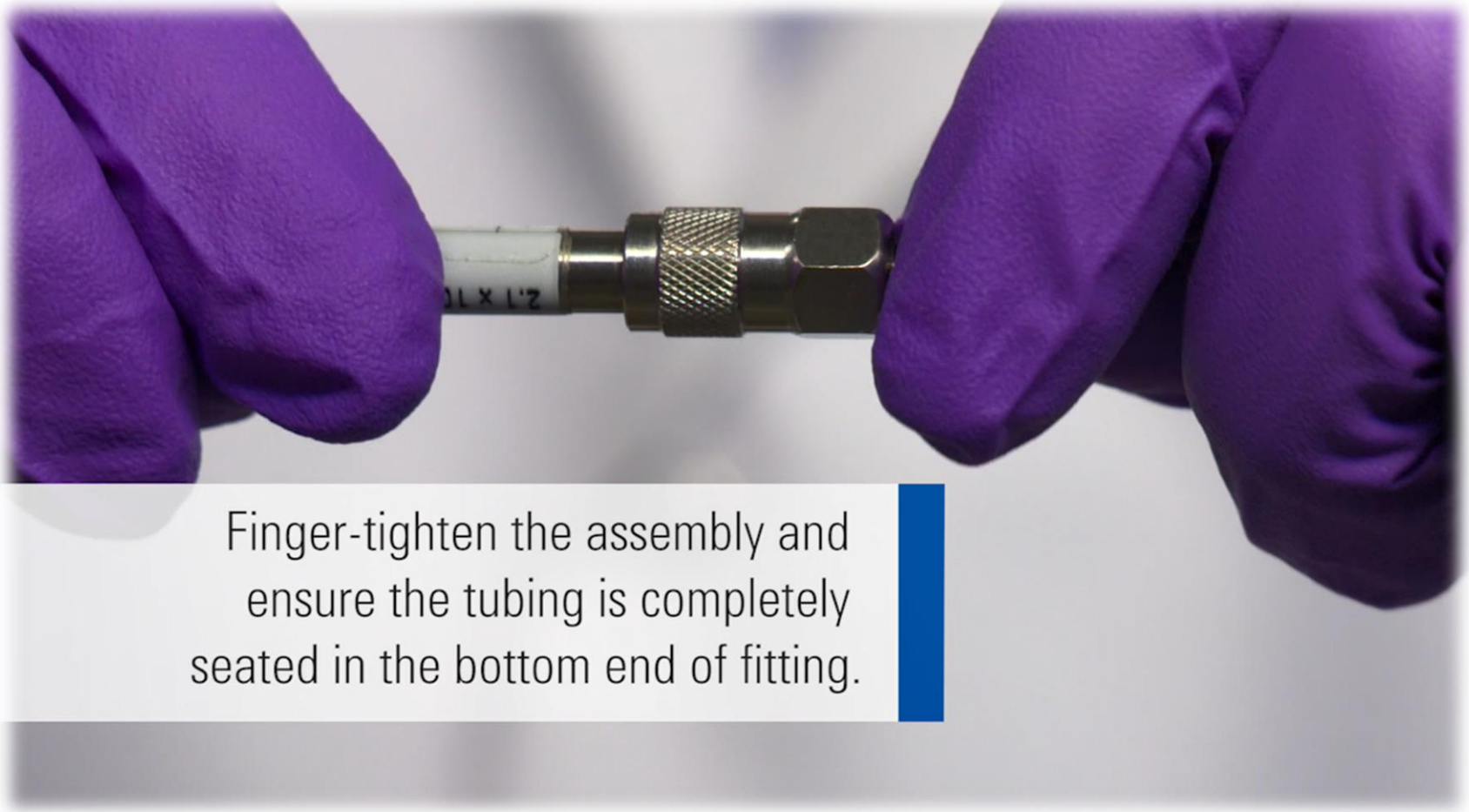
Swaging Fittings



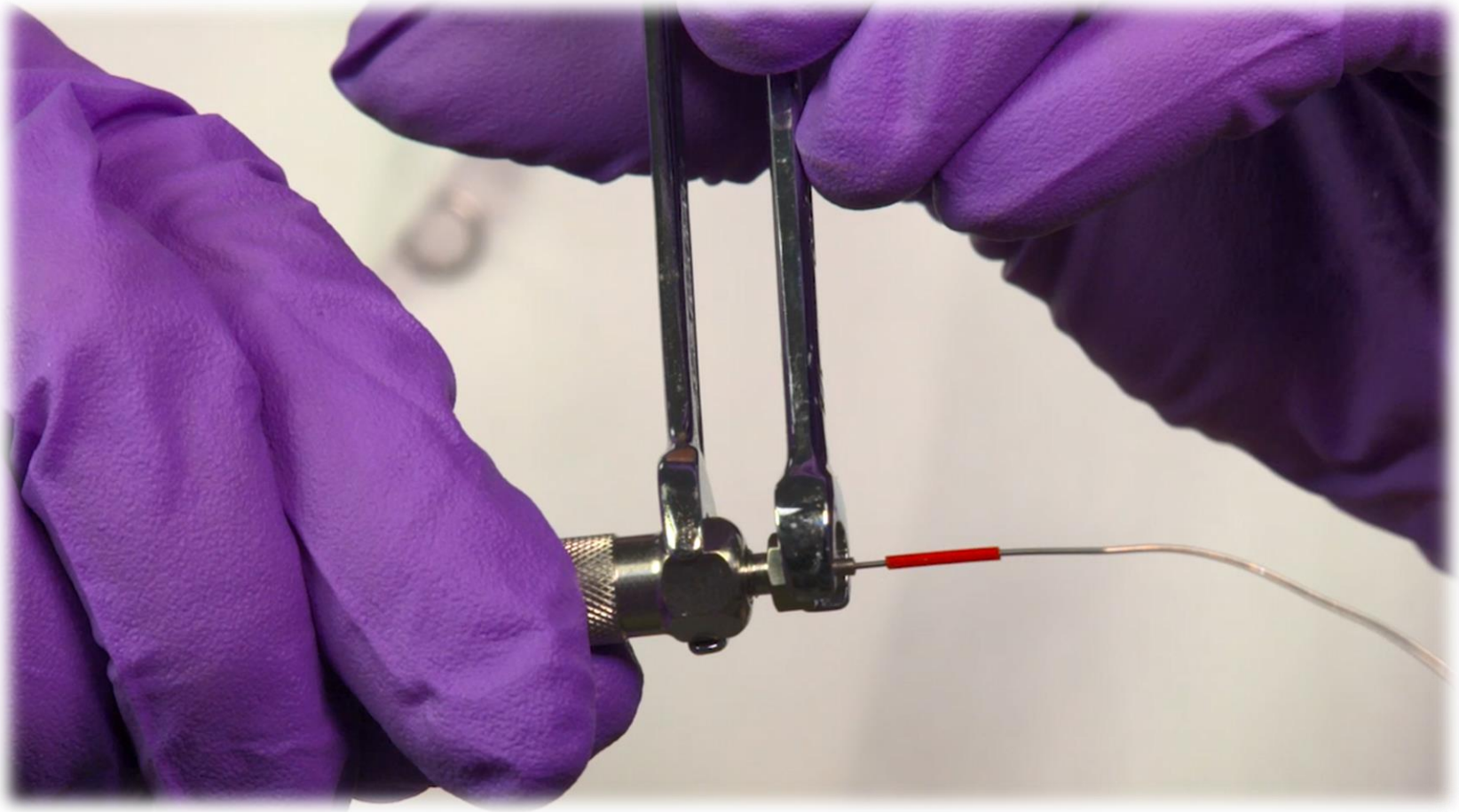
Swaging Fittings



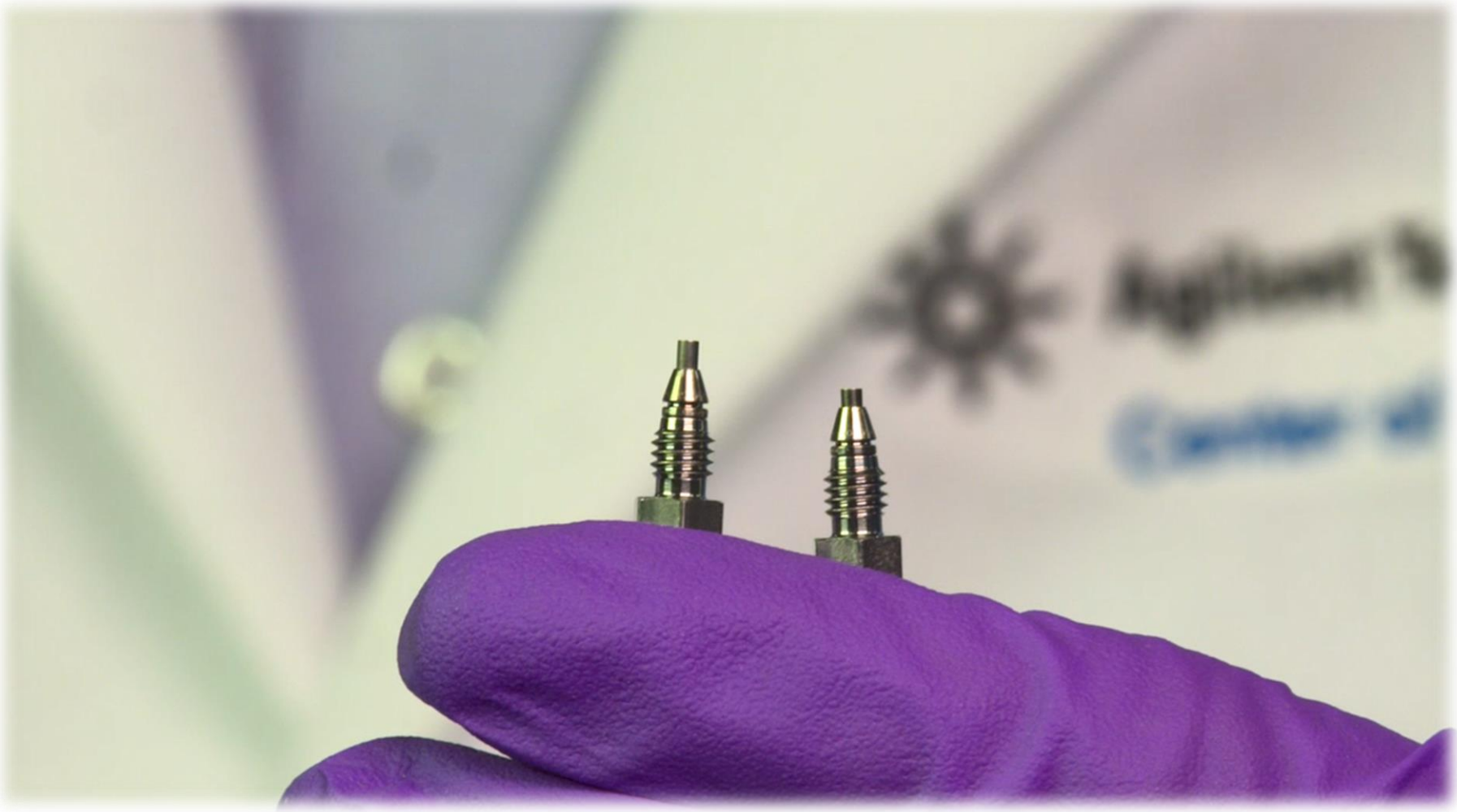
Swaging Fittings



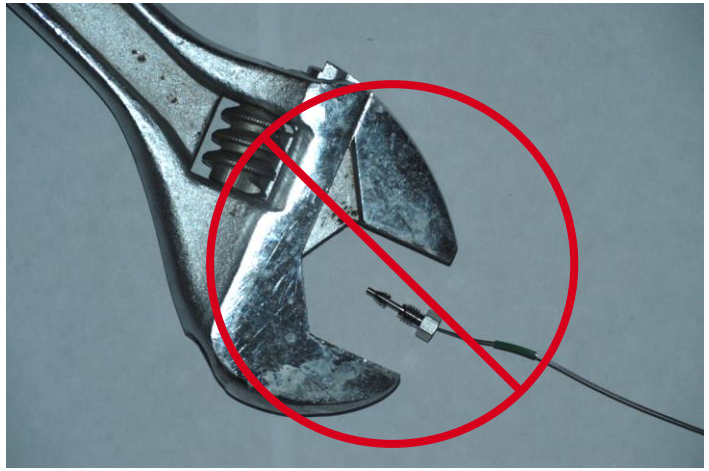
Swaging Fittings

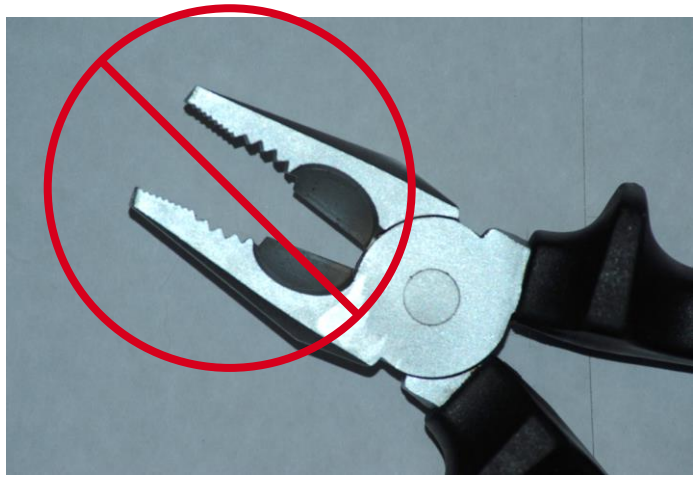


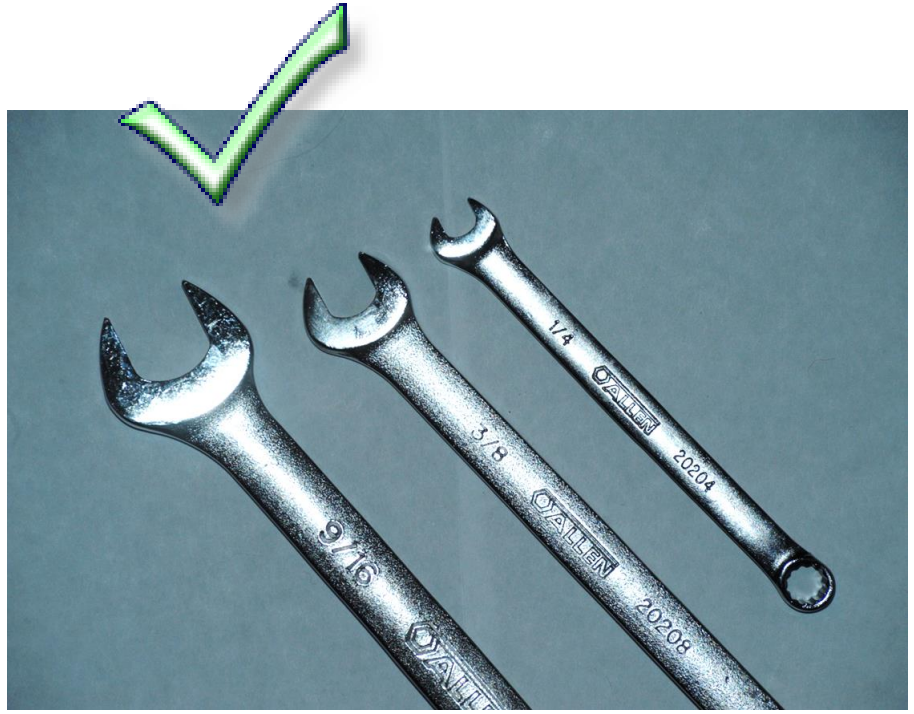
Inspect the Position of the Ferrule



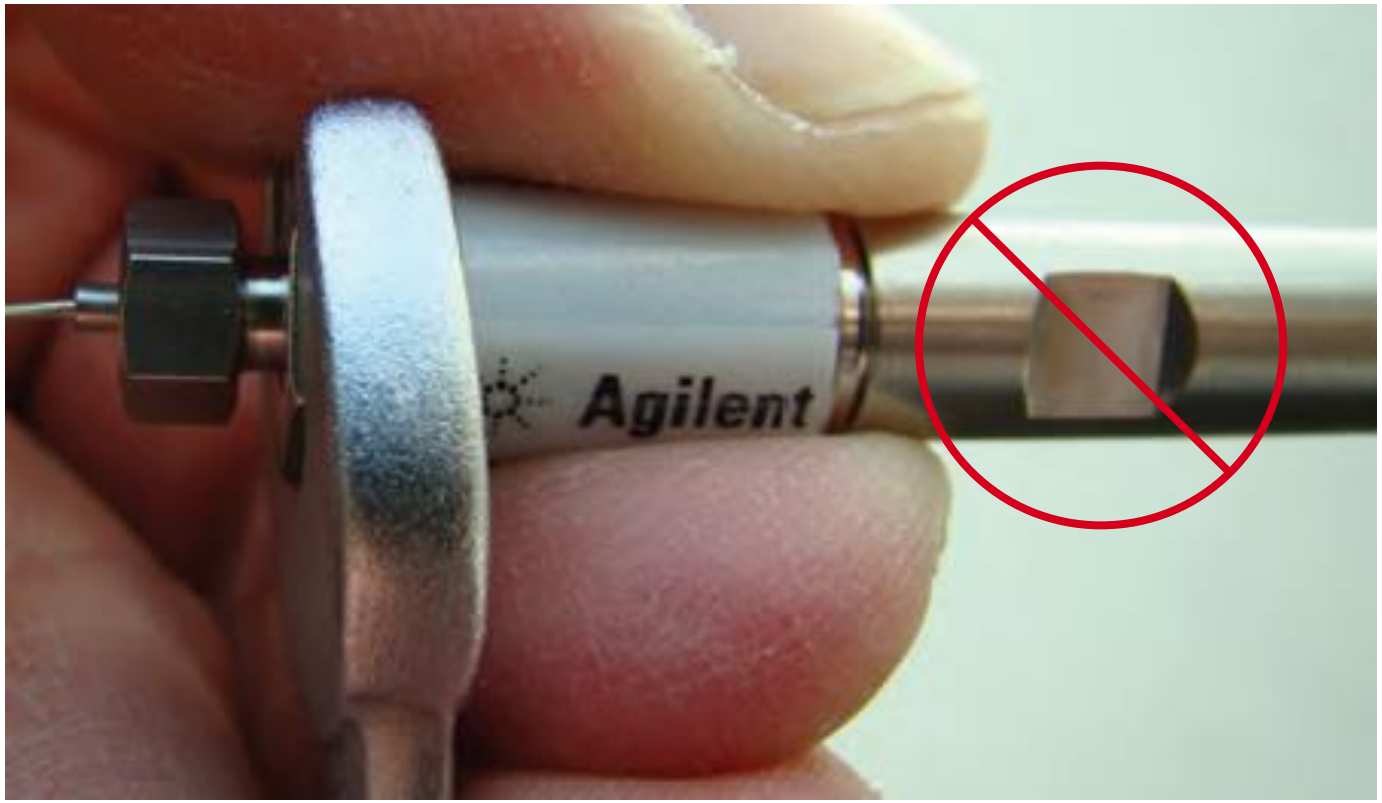
Tools







Tightening Fittings into a Column



InfinityLab Quick Connect and Quick Turn Fittings

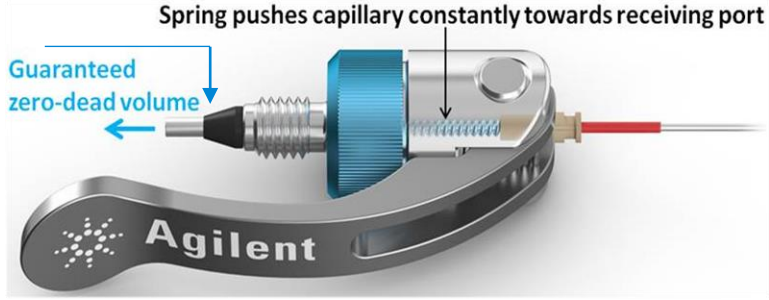
	Quick Connect fitting	Quick Turn fitting
Connects to	Columns (or inline filters)	Column, various receiving ports with 10-32 port geometry
Maximum pressure	1300 bar (finger-tight, by turning the lever)	To 400 bar (finger-tight, user dependent) 1300 bar (with mounting tool, 5043-0915)  Bio-inert mounting tool, 5043-0915
Features	• Spring-loaded function for zero-dead volume connections (special capillaries) • Replaceable ferrule and capillary • Capillaries in various lengths and diameters are available	• Spring-loaded function for zero-dead volume connections • Replaceable ferrule and capillary • Capillaries in various lengths and diameters are available
Wetted material	PEEK (ferrule)	PEEK (ferrule)

InfinityLab Quick Connect Fittings

It is important that Quick Connect fittings are only used with capillaries specially designed for them.

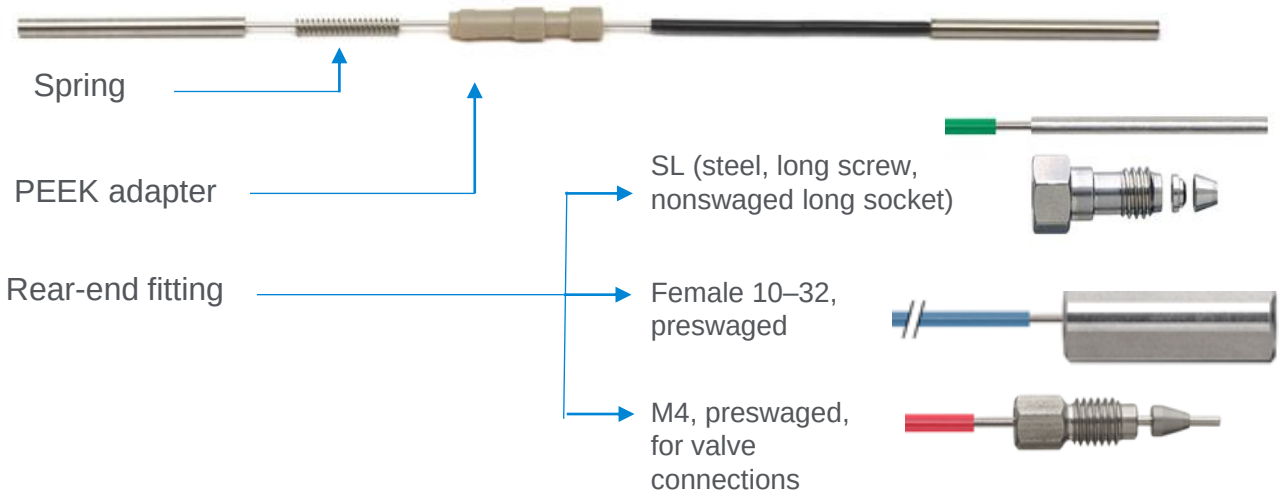
Quick Connect assembly

- Quick Connect fitting with premounted Quick Connect capillary



Quick Connect capillary

- Available in various combinations (length, id, rear-end fitting)
- Also available as a bio-inert (PEEK/SST) capillary

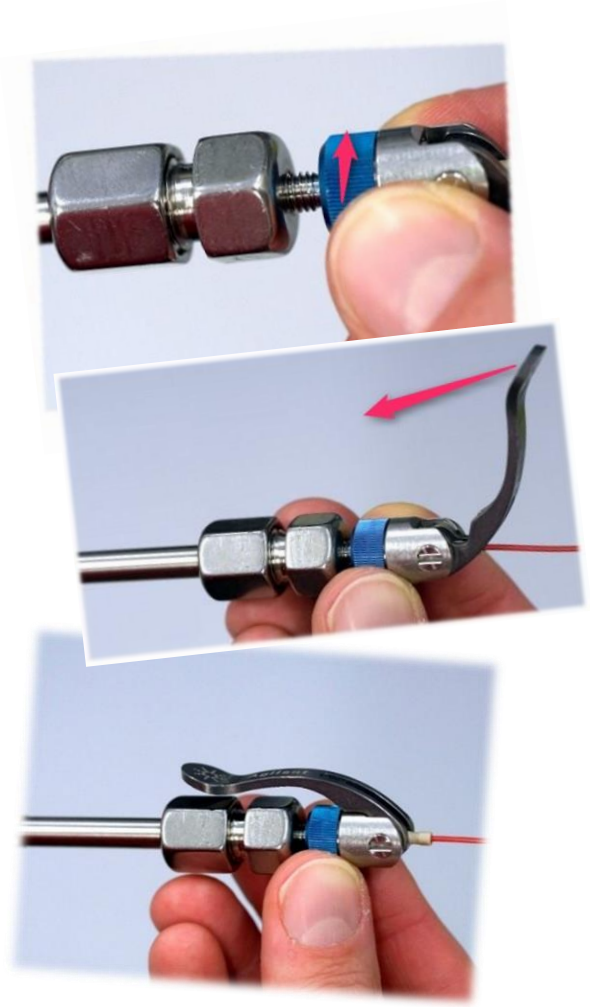


How to Use the InfinityLab Quick Connect Fitting

1. Screw the fitting (blue wheel) with the lever in the open position onto the column.

2. Stop when you can feel the **first resistance** and then close the lever.

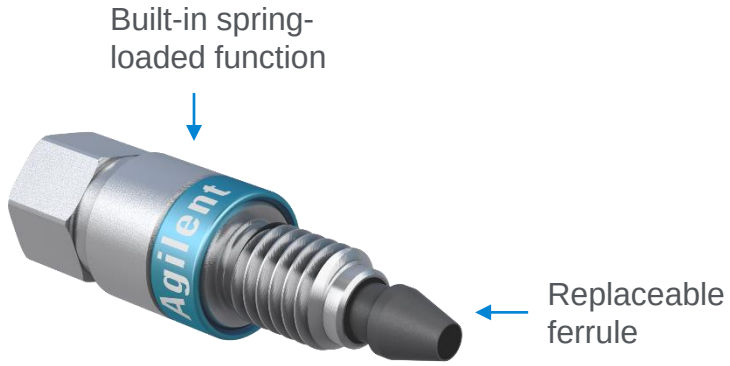
3. Finished – in seconds.



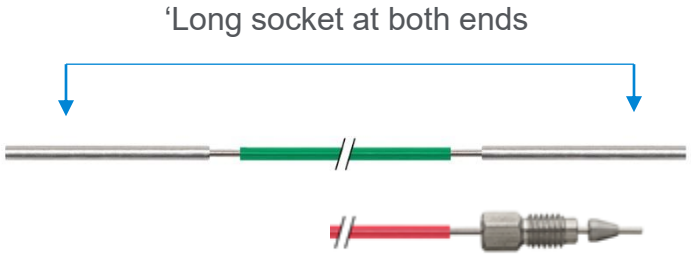
Quick Turn Fitting

Agilent InfinityLab

Quick Turn fitting capillaries are available in various lengths and inside diameters



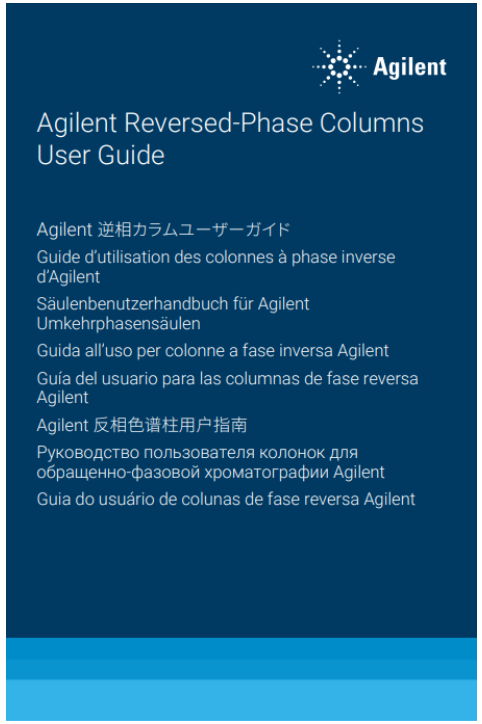
Bio-inert mounting tool, 5043-0915

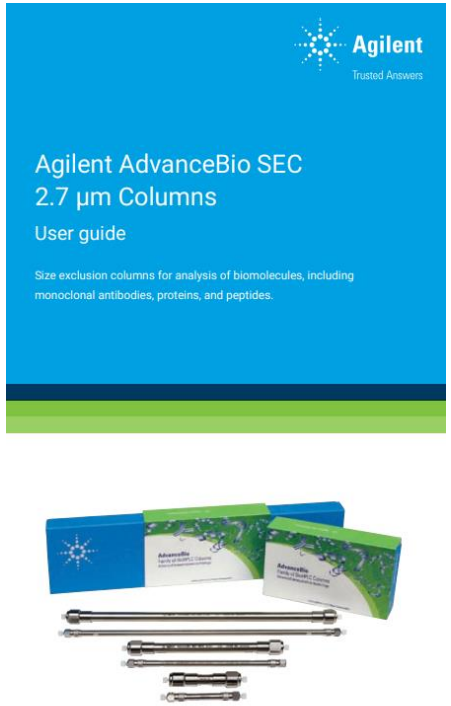


Quick Turn capillaries come without any fittings, except the ones with a single preswaged M4 fitting for the opposite end for valve connections

Getting Started with the New Column

Check the column user guide





[LC Column User Guides | Agilent]

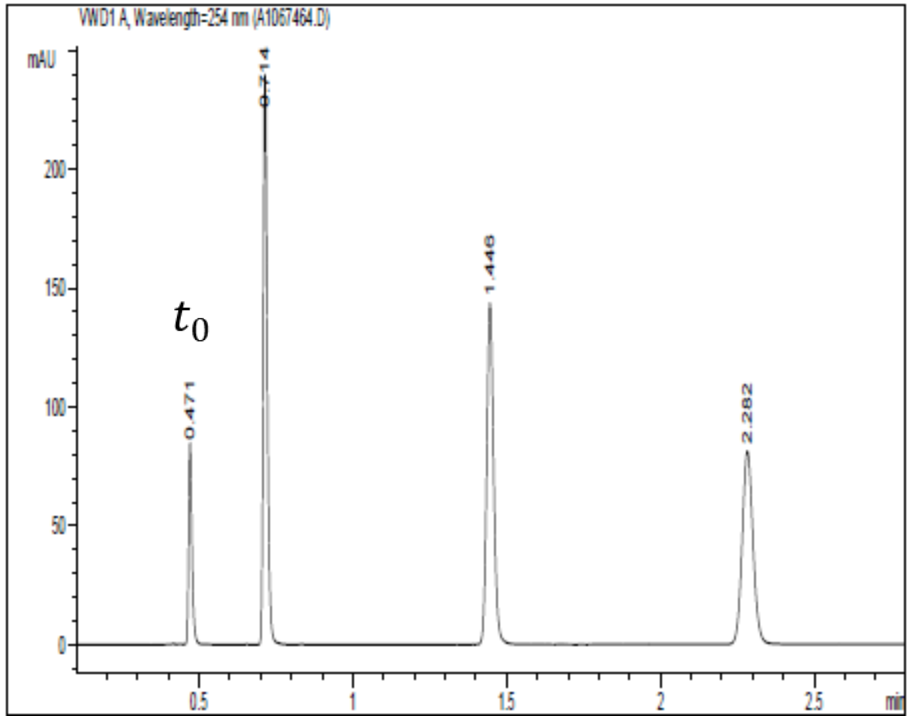
[Bio LC Column User Guides | Agilent]

Check the Column Test Report

PART NUMBER: 695975-302
COLUMN TYPE: Poroshell 120 EC-C18 3 x 100 mm, 2.7 µm

TEST CONDITIONS

MOBILE PHASE	=	60% Acetonitrile / 40% Water
COLUMN PRESSURE	=	274.8 Bar
COLUMN FLOW	=	0.80 ml / min
LINEAR VELOCITY	=	0.354 cm / sec
TEMPERATURE	=	AMBIENT (Nominally 23 °C)
INJECTION VOLUME	=	2 µl



$$t_0 * F \approx V_m$$

Sample components with concentrations diluted in mobile phase in the following elution order.

Peak #	Conc (ug/ml)	Sample Component
1	10	Uracil
2	400	Phenol
3	50	4-Chloro Nitrobenzene
4	80	Naphthalene

Column Volume

$$V_m = \pi \cdot r^2 \cdot L \cdot \sim 0.6$$

Column volume is calculated as the volume of a cylinder less the space occupied by the packing material. As an example, Agilent ZORBAX Eclipse Plus C18 packing material occupies 40% of the column, the remaining 60% of the cylinder is considered to be the column volume.

Quick rule of thumb that works for many (though not all) of our columns at Agilent: the void volume of a 4.6 mm id column is approximately 0.5 mL per 50 mm, and the void volume of a 2.1 mm id column is roughly 0.1 mL per 50 mm.

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
- If using a modifier like formic acid or TFA, you can then equilibrate with your mobile phase for 10–20 column volumes
- 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

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 watch for a flat baseline.
- Then equilibrate with the buffer at your isocratic conditions or your starting conditions for the gradient, again this make 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.

Equilibrating the Column

HILIC

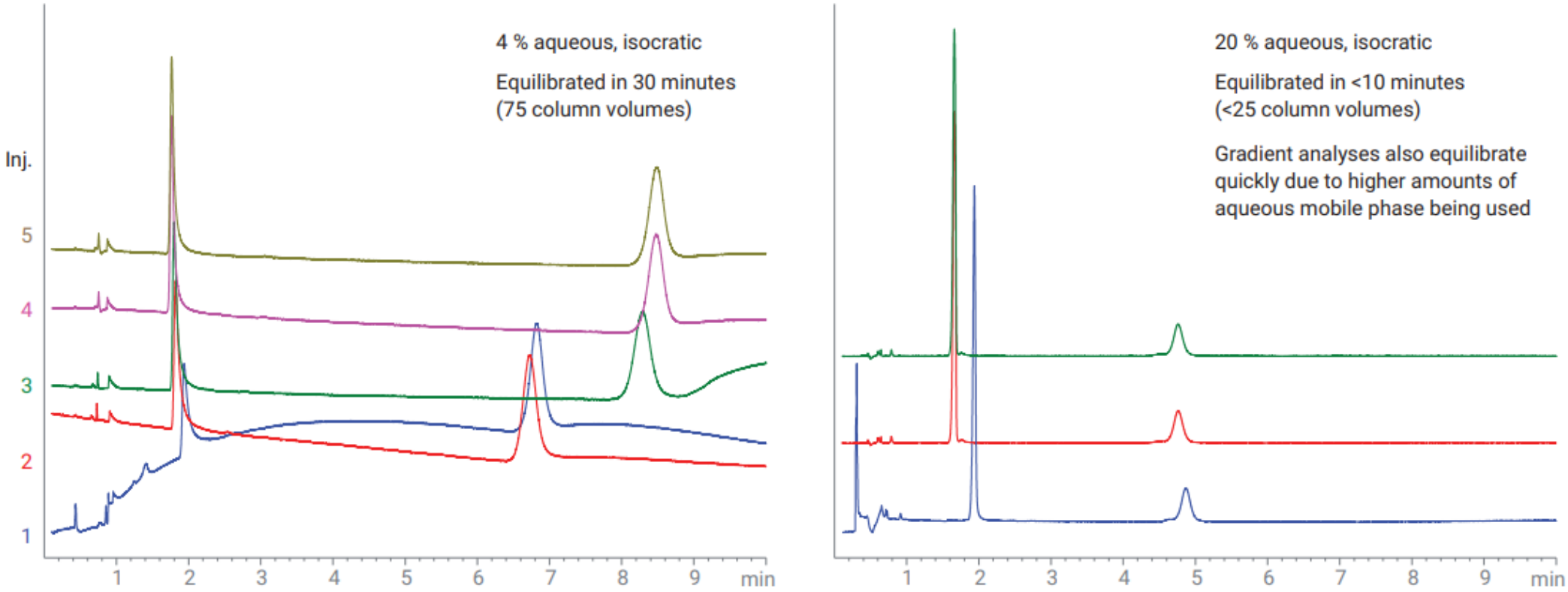


Figure 6. Separation of B vitamins using an InfinityLab Poroshell 120 HILIC-OH5, 2.1 × 100 mm, 2.7 µm column. Left: Column stored in 100 % acetonitrile prior to analysis. Mobile phase A: 100 mM ammonium formate, pH 3.0. Mobile phase B: acetonitrile. Isocratic conditions: 96 %B. Flow: 0.5 mL/min. Injection: 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% NaN_3.
- First 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/min^2. Equilibrate the column by flushing for a minimum of 10 column volumes or until the baseline is stable.

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.

Equilibrating the Column

Hi-Plex for carbohydrate analysis

Best practices:

- Hi-Plex columns must be run at elevated temperatures.
- The mobile phase should be run at 0.1 mL/min and the column heating device switched on. When the column reaches the desired operating temperature the flow rate may be increased gradually to the required level.
- Care should be taken to ensure that the maximum flow rate of the column is not exceeded. Hi-Plex columns should not be subjected to sudden changes in flow rate. It is recommended to adjust the maximum flow gradient setting.
- Under no circumstances should the column heating device be left switched on with no flow through the column.

Equilibrating the Column

Organic GPC columns, PLgel

Organic columns (PLgel; PlusPore; PL Rapide)		
Column supplied in ethylbenzene		
Transfer to low viscosity solvents e.g. THF, Chloroform, Dichloromethane	**Transfer to medium viscosity solvents** e.g. Toluene, DMF, DMSO, HFIP	**Transfer to high viscosity solvents** e.g. TCB, m-Cresol, NMP
Flush column with acetone at 0.5 mL/min for 2 column volumes	Flush column with acetone at 0.5 mL/min for 2 column volumes	Set column oven to 50 °C, flow at 0.1 mL/min
Flush with new eluent at 0.5 mL/min for 2 column volumes	Flush with new eluent at 0.2 mL/min for 2 column volumes	Flush column direct with new eluent** at 50 °C at 0.1 mL/min for 2 column volumes
Increase column temperature to 30 - 40 °C* as required for analysis at 1 °C/min	Increase column temperature to 50 - 80 °C* as required for analysis at 1 °C/min	Increase column temperature to 100 - 220 °C* as required for analysis at 1 °C/min
Operate column in new eluent at required flow rate	Operate column in new eluent at required flow rate	Operate column in new eluent at required flow rate

*Always ensure operating temperature is at least 10 °C below boiling point of solvent. **Always ensure miscibility. If unsure, use acetone at room temperature.

*Reduce the maximum flow gradient setting

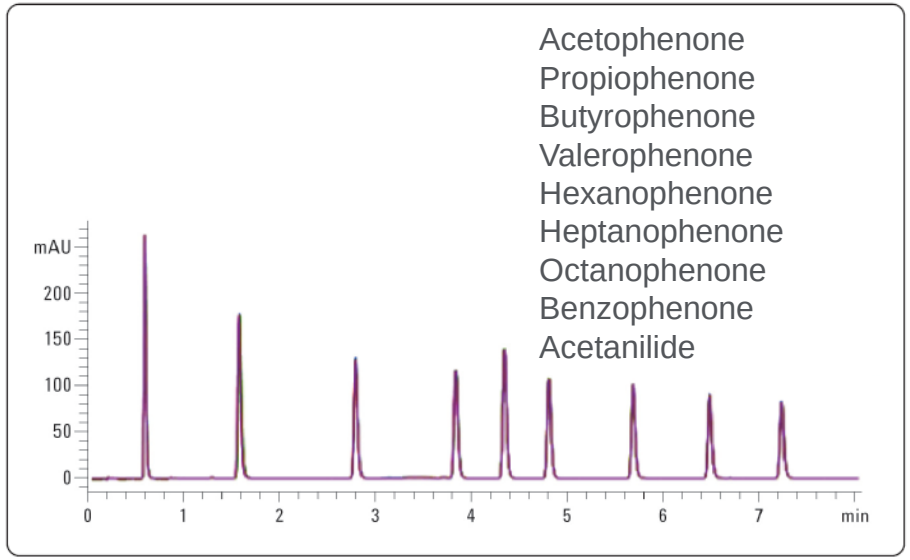
Benchmarking a New Column

Reversed phase

Agilent InfinityLab

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.

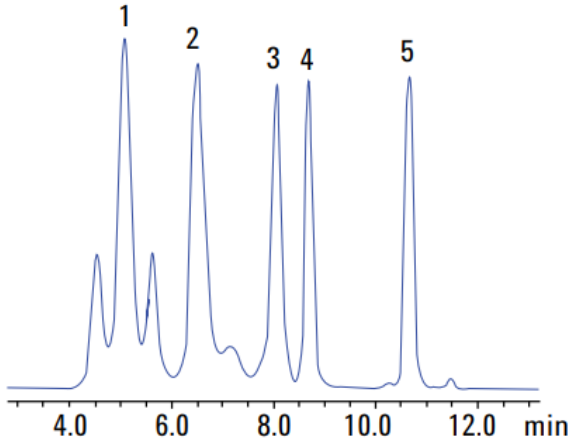


Chromatographic conditions

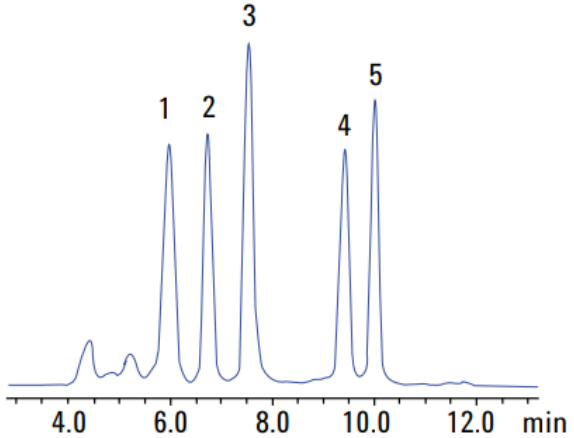
Sample:	RRLC Checkout sample (p/n 5188-6529)
Column:	Agilent Poroshell 120 EC C18, 3 mm × 50 mm, 2.7 µm
Mobile phase:	A = Water B = Acetonitrile
Gradient:	0 min 20% B 8 min 80% B
Flow rate:	1.2 mL/min
Stop time:	8 min
Post time:	4 min
Injection volume:	1 µL
Column temperature:	30 °C
DAD:	245/10 nm Ref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

Benchmarking a New Column

AdvanceBio SEC



AdvanceBio SEC 300Å Protein Standard separation on AdvanceBio SEC 300Å column



AdvanceBio SEC 130Å Protein Standard separation on AdvanceBio SEC 130Å column

AdvanceBio SEC 300Å Protein Standard (p/n 5190-9417, 1.5 mL vial)	
Analyte	**MW**
1. Thyroglobulin	670,000
2. γ-globulin	150,000
3. Ovalbumin	45,000
4. Myoglobin	17,000
5. Angiotensin II	1,000

AdvanceBio SEC 130Å Protein Standard p/n 5190-9416, 1.5 mL vial)	
Analyte	**MW**
1. Ovalbumin	45,000
2. Myoglobin	17,000
3. Aprotinin	6,700
4. Neurotensin	1,700
5. Angiotensin II	1,000

Column Test Reports

Performance report

SERIAL NUMBER: USDAZ01333

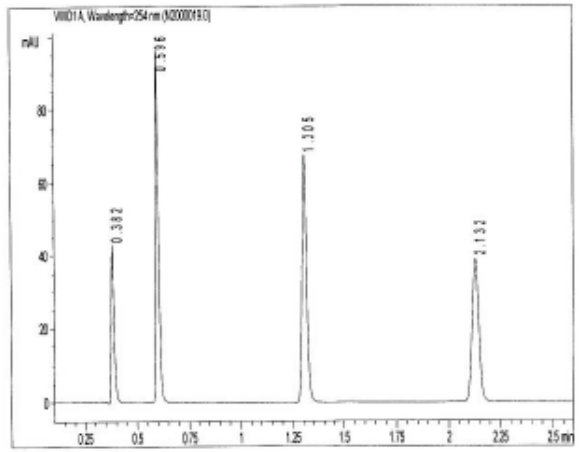
PART NUMBER: 959758-902
COLUMN TYPE: ZORBAX RRHD Eclipse Plus C18 2.1 x 100 mm, 1.8 µm
PACKING LOT #: B09089

TEST CONDITIONS

MOBILE PHASE	=	60% Acetonitrile / 40% Water
COLUMN PRESSURE	=	517.2 Bar
COLUMN FLOW	=	0.50 ml / min
LINEAR VELOCITY	=	0.436 cm / sec
TEMPERATURE	=	AMBIENT (Nominally 23 °C)
INJECTION VOLUME	=	1 µl

QUALITY CONTROL PERFORMANCE RESULTS FOR NAPHTHALENE

	TEST VALUES	SPECIFICATIONS
THEORETICAL PLATES =	22337	MIN = 21000
SELECTIVITY =	1.90	RANGE = 1.82 - 1.92
USP TAILING FACTOR = (@ 5% Peak Height)	1.08	RANGE = 0.98 - 1.20
k' =	4.58	



Sample components with concentrations diluted in mobile phase in the following elution order.

Peak #	Conc (ug/ml)	Sample Component
1	10	Uracil
2	400	Phenol
3	50	4-Chloro Nitrobenzene
4	80	Naphthalene

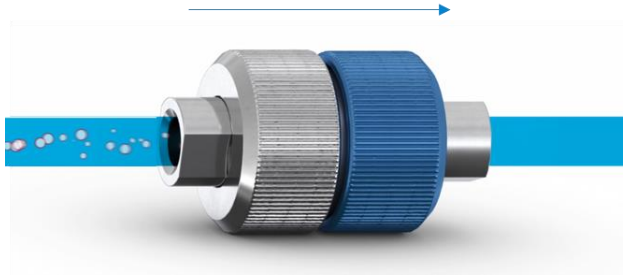
Manufacturing test chromatogram is done on a modified LC system to minimize extra column volume and will differ from a typical lab HPLC

- Do not expect to get the exact same result as the performance report
- Test column performance on your instrument to have as a reference

Protecting Your New Column

Inline filter

- Filters insoluble particulates
- Less expensive to replace
- One filter can protect many different types of columns
- Choose based on column diameter and particle size of the column

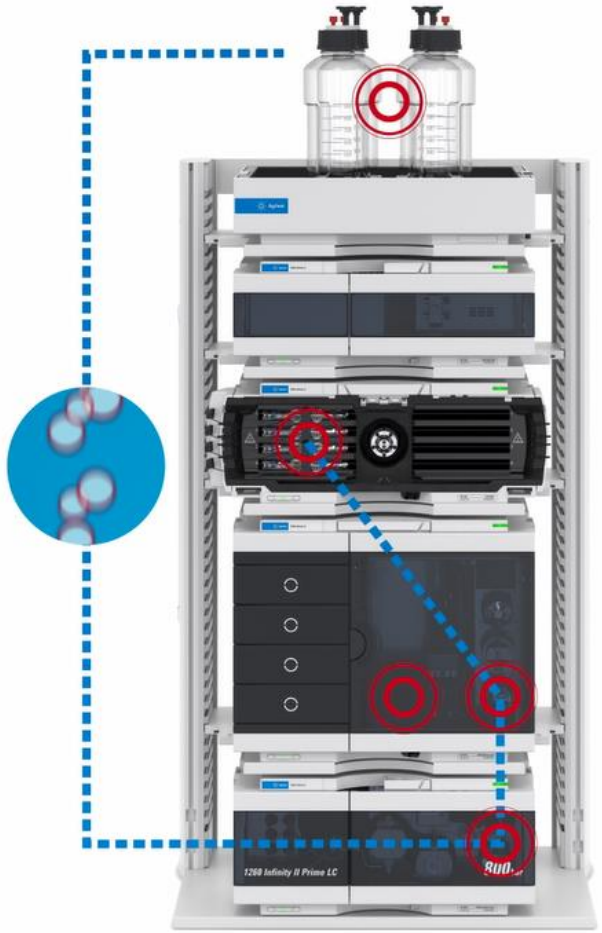


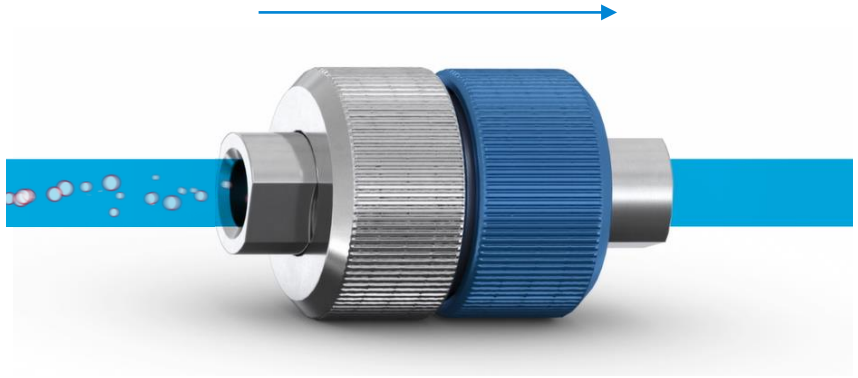
Guard column

- Filters insoluble particulates and soluble contaminants that may be highly retained by the column packing
- More expensive to replace
- Guard is matched to the analytical column
- Choose based on the specific column you are using

Why Use an Inline Filter?

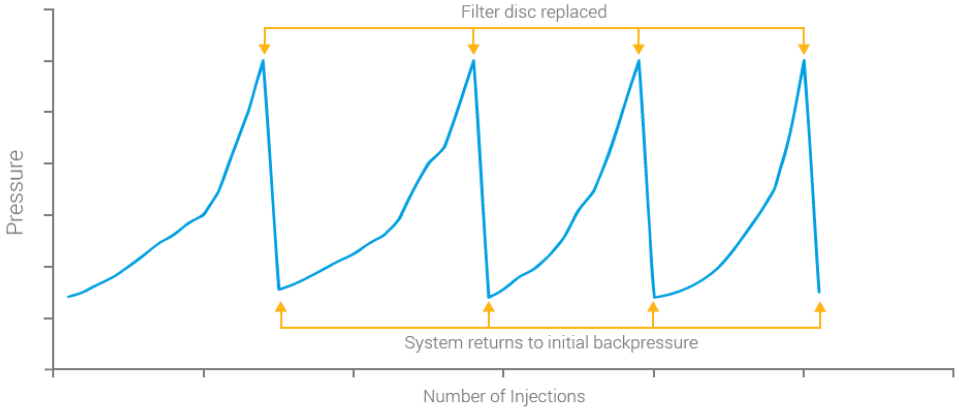
Particles lead to blockage





Filter particles to prevent column clogging

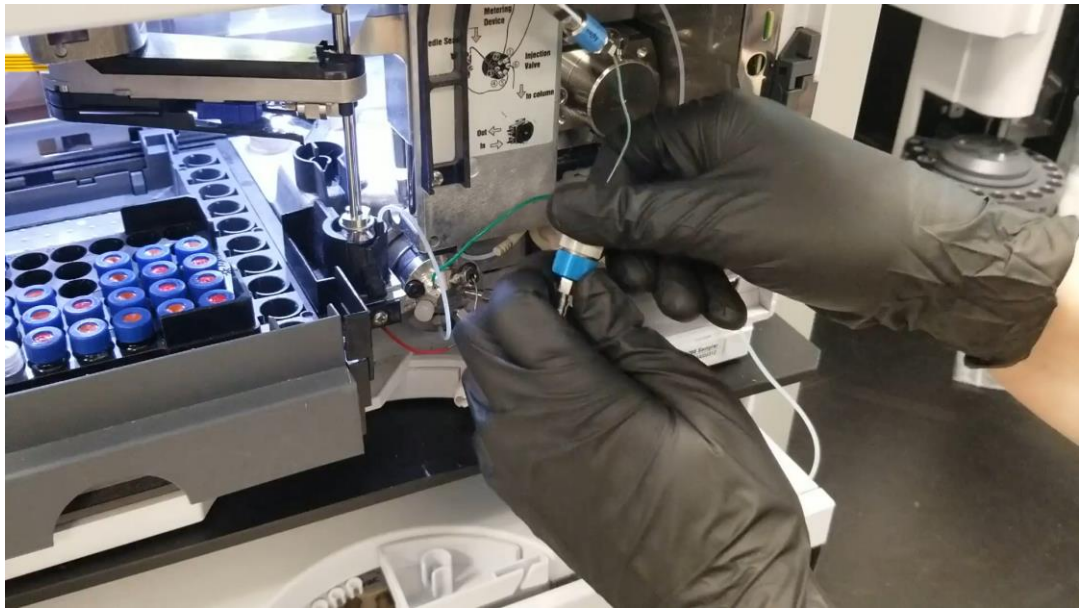
Extend column lifetime and reduce cost per sample



Accelerated lifetime test shows how inline filter removes particles

InfinityLab Quick Change Inline Filter

From

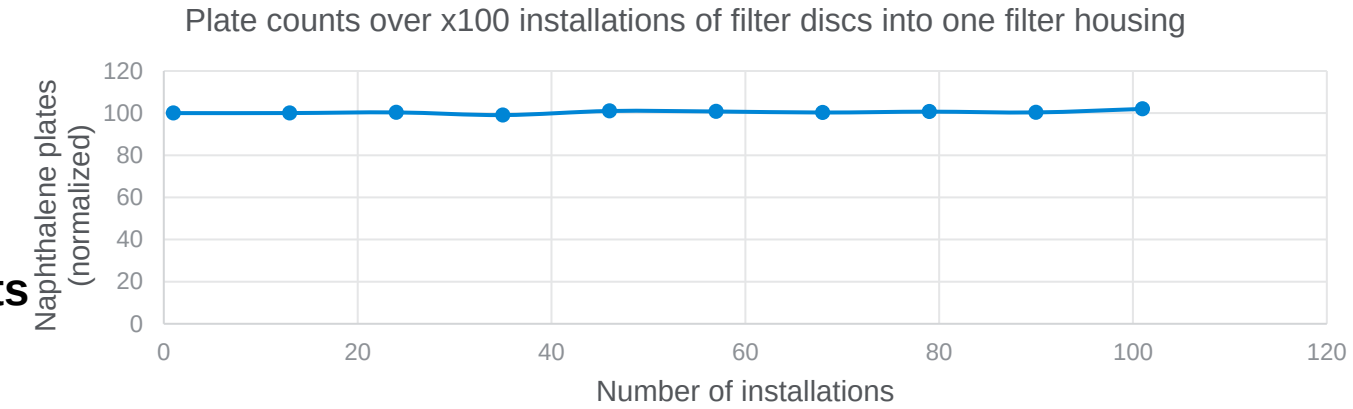


Ultimate ease-of-use

- **Finger-tight, tool-free** replacement of filter disc
- **Click and seal**: a click alerts users when the filter is tight up to 1300 bar, assuring no risk of over- or under-tightening

Robustness for low operational cost

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



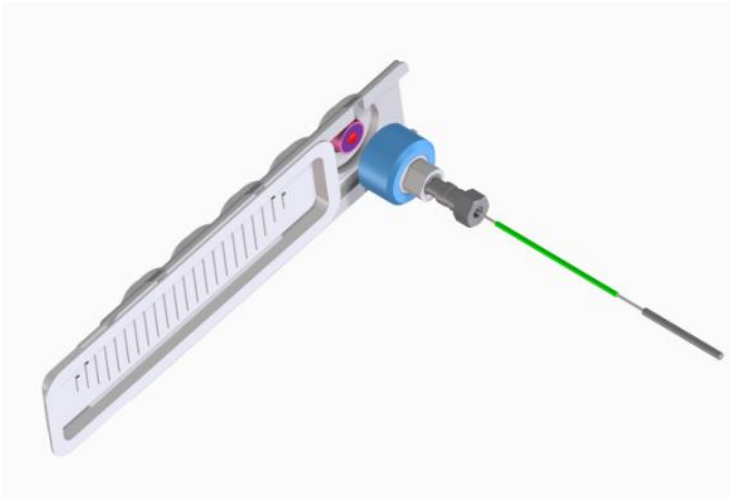
InfinityLab Quick Change Inline Filter – Filter Discs

High efficiency, easy-to-use filter discs

- **Various 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 specially designed packaging, you're able 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

Different dimensions and porosities of filter discs

Filter discs in touchless packaging



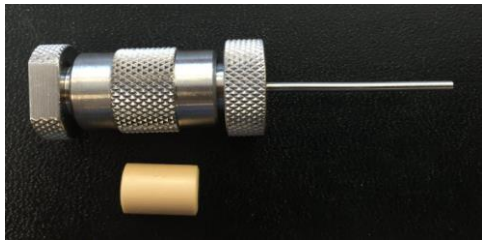
No-touch insertion of filter disc into filter housing

Protecting Your New Column

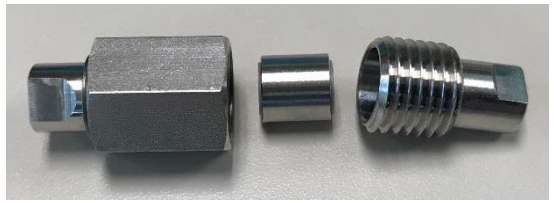
Guard column

- Match your guard column to the specific column you are using
- Choose based on the column phase, diameter and particle size
- Your results may vary but a general suggestion is to replace your guard column when the plate number, pressure, or resolution changes by more than 10%
- Guard column formats can differ: standard, cartridges, integrated connectors, etc.

High pressure Fast Guard
for sub-2 µm and
Poroshell columns



Zorbax guard cartridge hardware
for 3 and 5 µm columns



Cartridge hardware
for Hi-Plex and PLRP-S

Guard column for GPC

Storing the Column

Reversed Phase: InfinityLab Poroshell, ZORBAX

Best practices:

- Long-term storage of silica-based, bonded phase columns should be in a pure organic solvent such as acetonitrile.
- 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 organic solvent.
- Before storing, tightly cap with end-plugs.
- To protect equipment, is it best to remove salts from the instrument and column by purging with the same mobile phase without the buffer (for example using 60:40 ACN/H20 to remove a 60:40 ACN/0.02 M phosphate buffered mobile phase).

Storing the Column

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

Best practices:

- Acetonitrile:water (90:10) is recommended as the long-term storage solvent for HILIC columns.
- Columns may be safely stored for short periods in most HILIC mobile phases. However, to protect equipment, it is best to remove salts from the instrument and column by purging with the same mobile phase without the buffer (for example, using 90:10 ACN:H2O to remove a 90:10 ACN:0.01M formate buffered mobile phase).
- Before storing the column, tightly cap with the end plugs.

Storing the Column

Aqueous SEC for peptides and proteins: AdvanceBio SEC

Best practices:

- 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, pH ≤ 7, with or without 0.02% NaN3, 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.

Storing the Column

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

Best practices:

- 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. The column can be refrigerated or stored at room temperature (4 to 30 °C).
- 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).

Storing the Column

Hi-Plex for carbohydrate analysis

Best practices:

- Hi-Plex columns should ideally be run at reduced flow (0.1 mL/min) at the required operating temperature when left overnight.
- Hi-Plex columns are stable for shipping, day-to-day handling, and short-term storage at room temperature. However, the column's performance will deteriorate over the course of months or years if stored at room temperature.
- For long-term storage, the column should be flushed with HPLC grade water at the required operating temperature. The flow rate should be slowly reduced to 0.1 mL/min, and the column heating device switched off. When the column has cooled, it can be removed from the system, and the end plugs should be replaced.
- The columns should be refrigerated at approximately 4 °C, but under no circumstances should they be allowed to freeze.

Storing the Column

Organic GPC columns

Best practices:

- On removing the column from the system, the end plugs must be replaced to prevent the column from drying out by evaporation, since subsequent shrinkage of the gel and disruption of the packing will occur. The end plugs need only be applied finger-tight.
- All eluents mentioned previously are suitable for storage, but unstabilized THF and halogenated solvents should not be used.

Agilent Resources for Support

- Resource page http://www.agilent.com/chem/agilentresources
  - Quick reference guides, product catalogs
  - Online selection tools, “How-to” videos
  - Column user guides – www.agilent.com/chem/lc-columns-user-guides
  - Biocolumn user guides – www.agilent.com/chem/biocolumn-userguides
- Tech support: http://www.agilent.com/chem/techsupport
- InfinityLab LC Supplies catalog (5991-8031EN)
- Agilent University http://www.agilent.com/crosslab/university
- YouTube – Agilent Channel
- Your local product specialists
- Subscribe to Agilent Peak Tales podcasts at peaktales.libsyn.com

Contact Agilent Chemistries and Supplies Technical Support

Available in the USA and Canada 8-5 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

Option 5 for chemical standards

gc-column-support@agilent.com

lc-column-support@agilent.com

spp-support@agilent.com

spectro-supplies-support@agilent.com

chem-standards-support@agilent.com

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