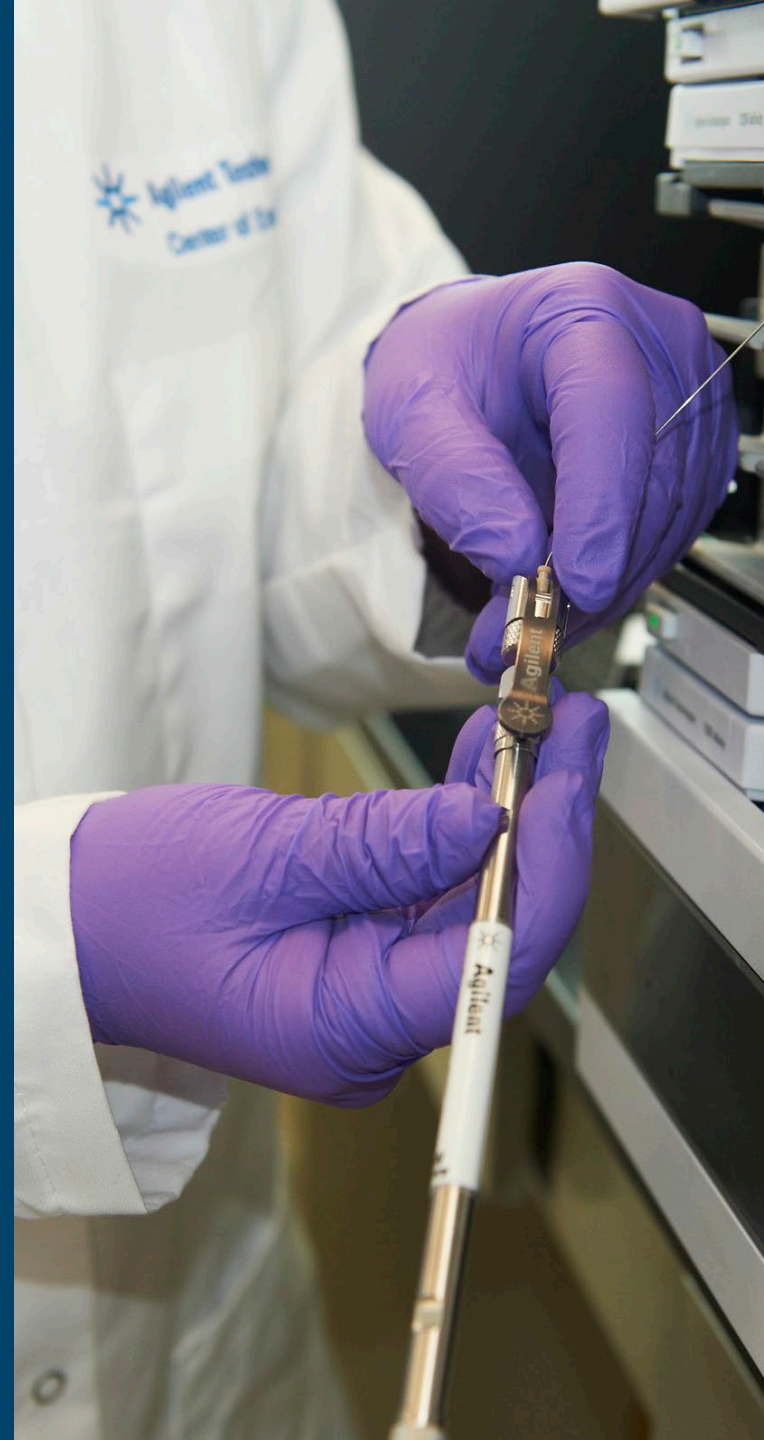


All About LC Connections – What to Do to Make A Great One

HPLC educational webinar:
Becoming a better chromatographer

Golnar Javadi
Applications Engineer
Chemistries and Supplies Technical Support



LC Connections

- Problems caused by improper connections
 - Leaks
 - Problems mistaken for chromatography issue
- Making connections can be user dependent
- Different manufacturers supply different types of fittings
- Capillary tubing choices
- Contribution to dispersion and extracolumn volume

Dispersion Reduces HPLC Performance

What is dispersion?

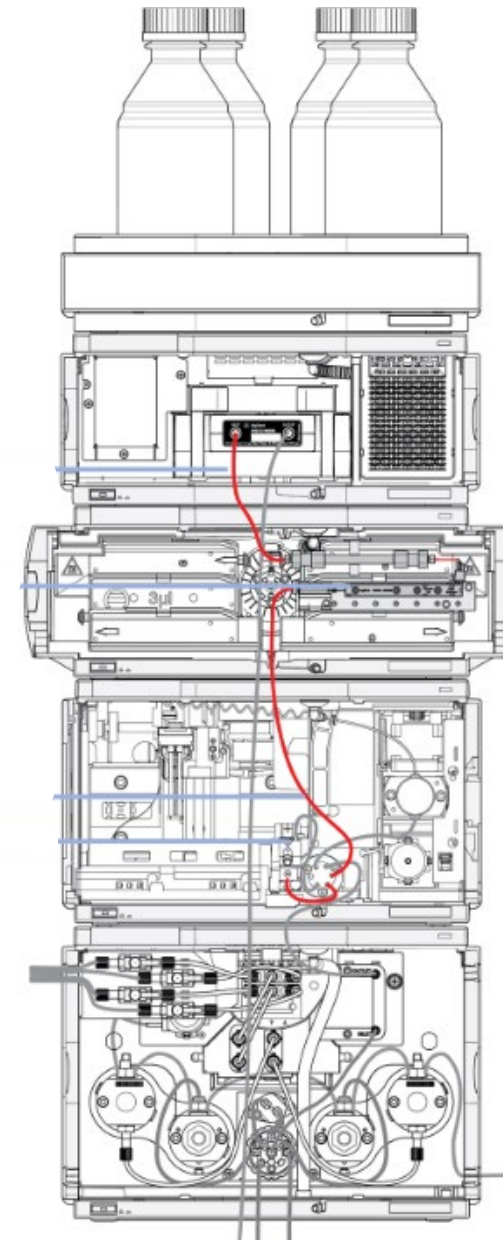
- Original sample concentration being diluted as it is carried through the system plumbing (extracolumn volume)

What increases dispersion?

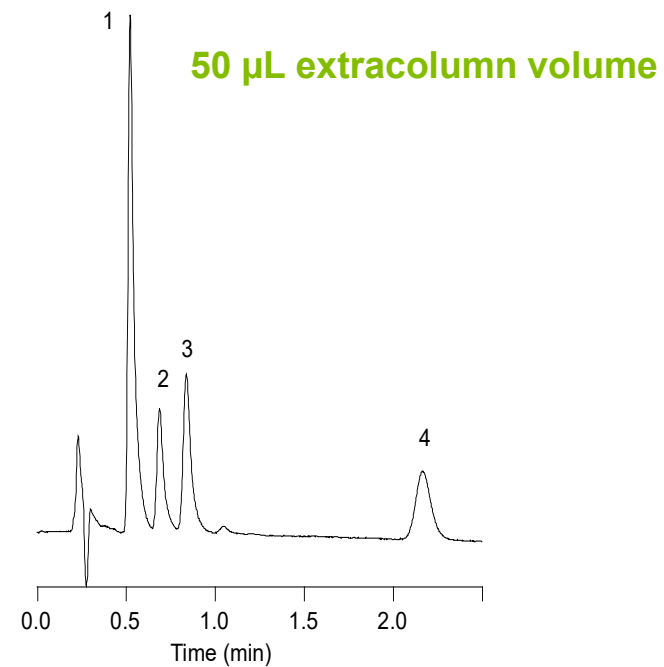
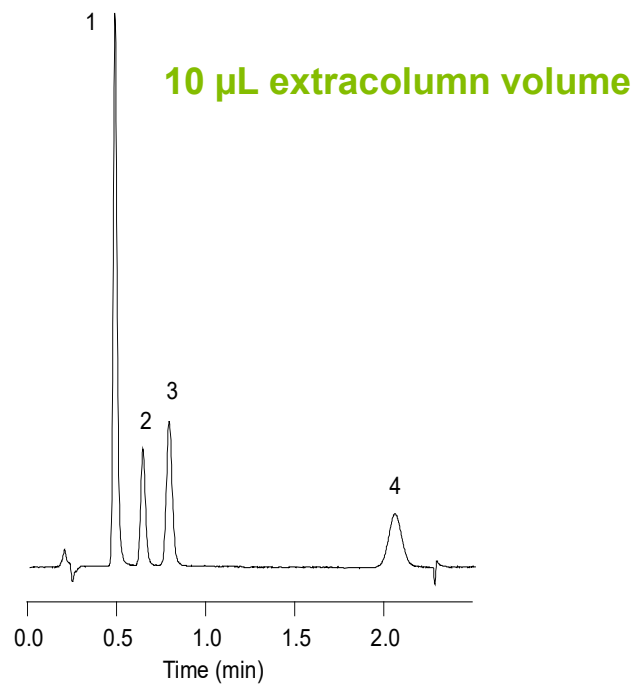
- Connecting tubing that is too long
- Connecting tubing that is too large in diameter
- Connections that have gaps and form small mixing chambers

Extracolumn Volume

- Flow cell
- Heat exchanger
- Switching valve
- Needle seat
- Connecting capillaries



Extracolumn Volume



Column: StableBond SB-C18, 4.6 x 30 mm, 3.5 μ m Mobile phase: 85% H₂O with 0.1% TFA : 15% ACN Flow Rate: 1.0 mL/min
Temperature: 35°C Sample: 1. Phenylalanine 2. 5-benzyl-3,6-dioxo-2-piperazine acetic acid 3. Asp-Phe 4. Aspartame

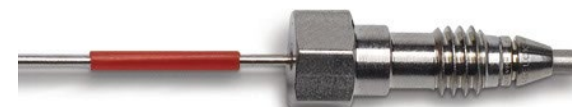
Aris-Taylor Equation: Peak Dispersion in Cylindrical Tubing

$$\sigma_{v,\text{ext}}^2 = \frac{\pi d^4 L_{cap} u}{96D_m}$$

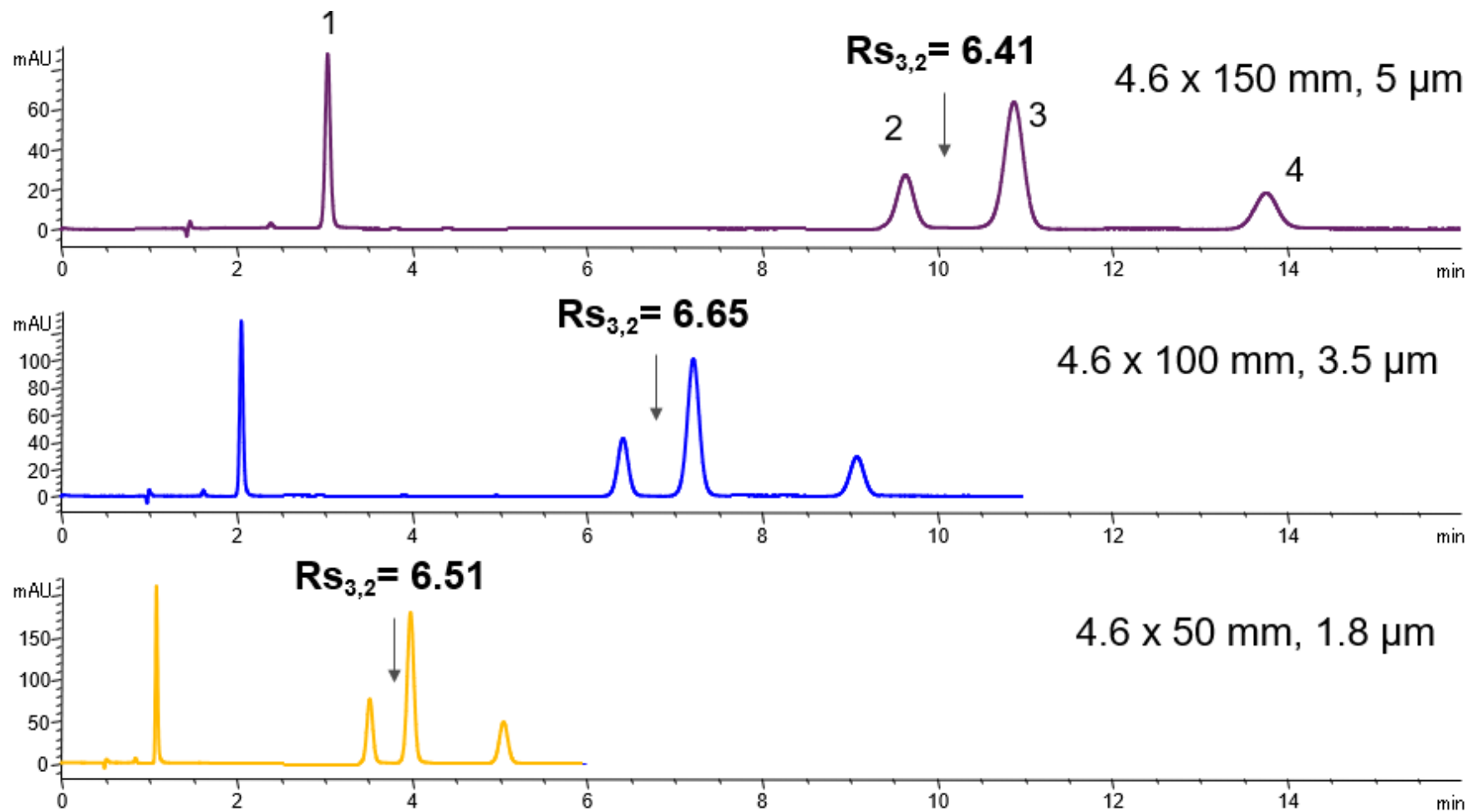
- $\sigma_{v,\text{ext}}^2$ is the volume variance
d is the tubing diameter
L is the tubing length
u is the linear velocity of the liquid
 D_m is the molecular diffusion coefficient

Tubing Volume

Tubing Length	10 mm	50 mm	100 mm	150 mm
Tubing id	Volume	Volume	Volume	Volume
0.17 mm (green)	0.227 μL	1.1 μL	2.27 μL	3.3 μL
0.12 mm (red)	0.113 μL	0.55 μL	1.13 μL	1.65 μL



As Efficiency Increases, Peak Width Decreases, System Dispersion Becomes More of a Factor

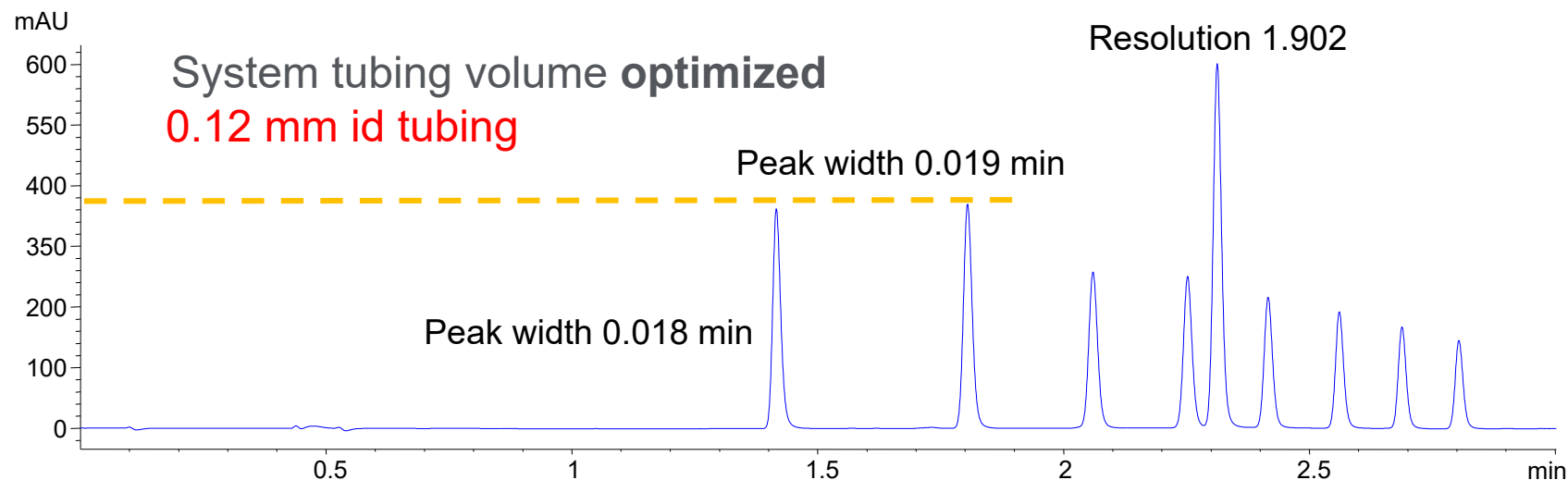
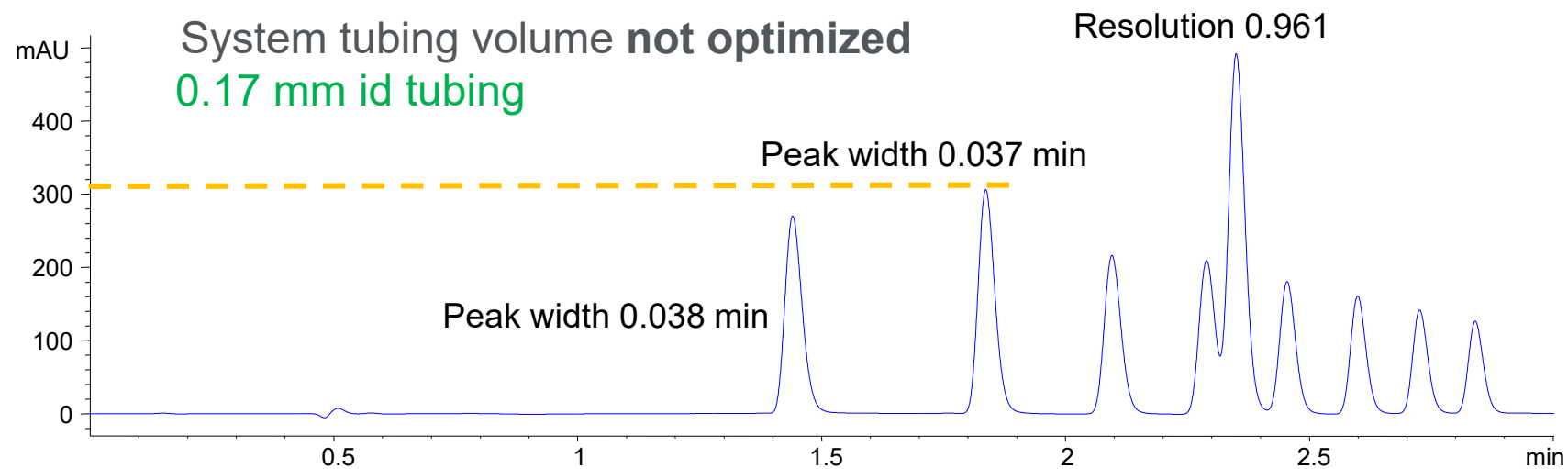


Columns: **Eclipse Plus C18**, as described above. Mobile Phase: A: water, B: MeOH, (15:85) Injection volume: 6 μ L
Temperature: 25° C Flow rate: 1 mL/min. Detection: 310, 4 nm, 0.5 s response time, semimicro flow cell, Sample: Sunscreens

Approximate Extra Column Volume Limits for This 4.6 mm id Column

	5 μm	3.5 μm	1.8 μm
Peak (1) volume in μL	181	136	45
Injection (1/10 P.V.)	18	14	5
Extra column (1/3 P.V.)	60	45	15
ECV minus injection volume	42	21	10
ECV estimate for 2.1 mm id column	8	4	2

Optimizing Connecting Tubing Volume For UHPLC columns



Stainless Steel vs. Polymeric Fittings

Stainless steel

- Agilent uses Swagelok type fittings with front and back ferrules
- Also available with a long nut

PEEK (< 400-bar system pressure)

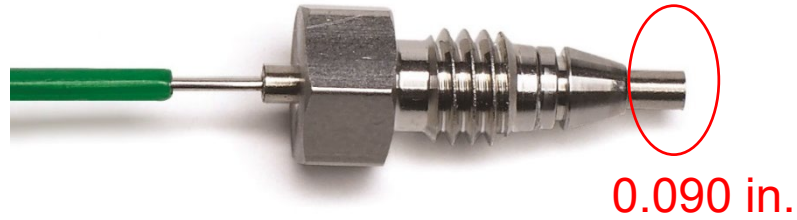
- Connections are changed frequently
- Connecting columns
- Pressure is less critical
- Fits on stainless steel or PEEK tubing

Polyketone

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

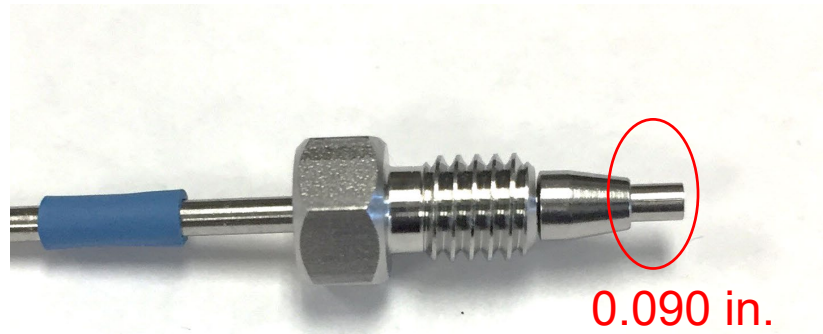


Types of Fittings



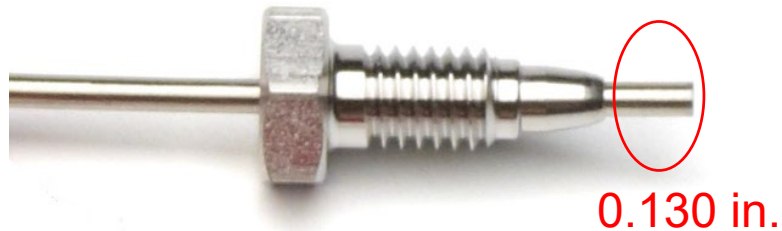
Swagelok

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



Parker

- One piece ferrule
- Short nut
- Very similar to Swagelok

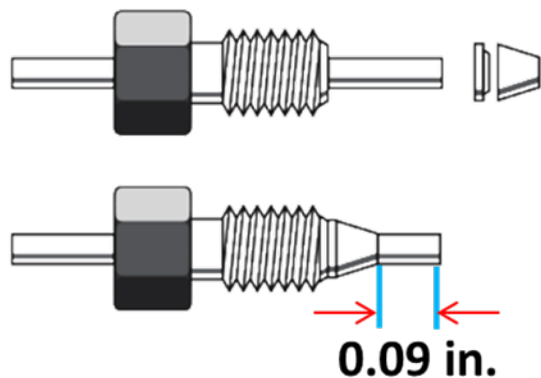


Waters

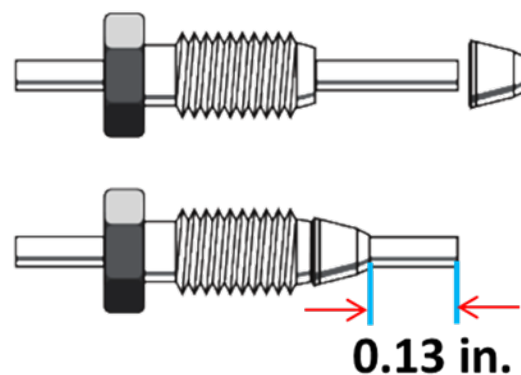
- Longer nut
- Used on Alliance systems

Types of Fittings

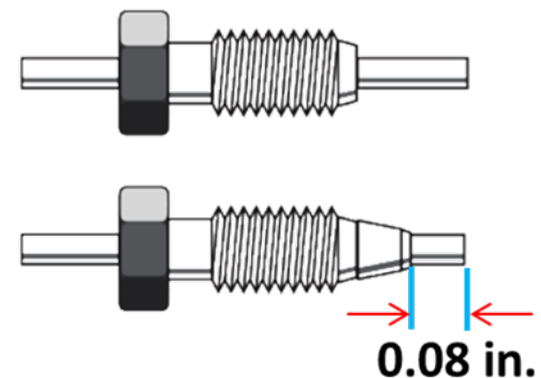
Swagelok



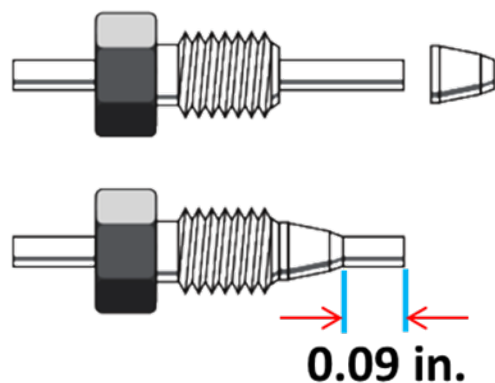
Waters



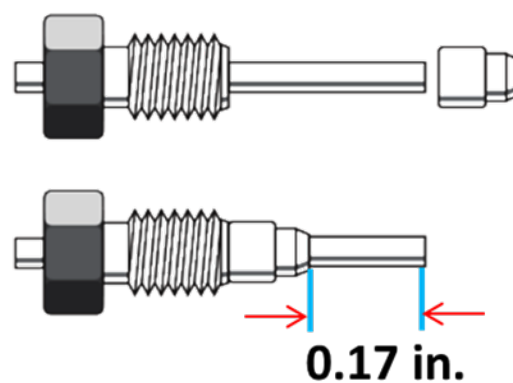
Valco



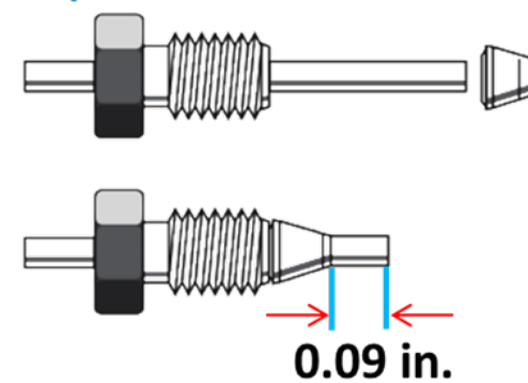
Parker



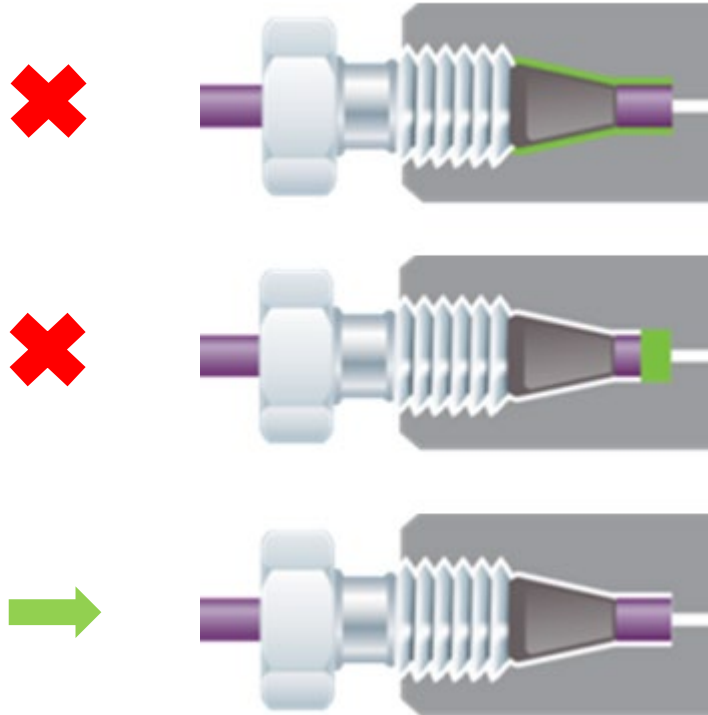
Rheodyne



Upchurch



Potential Fittings Issues

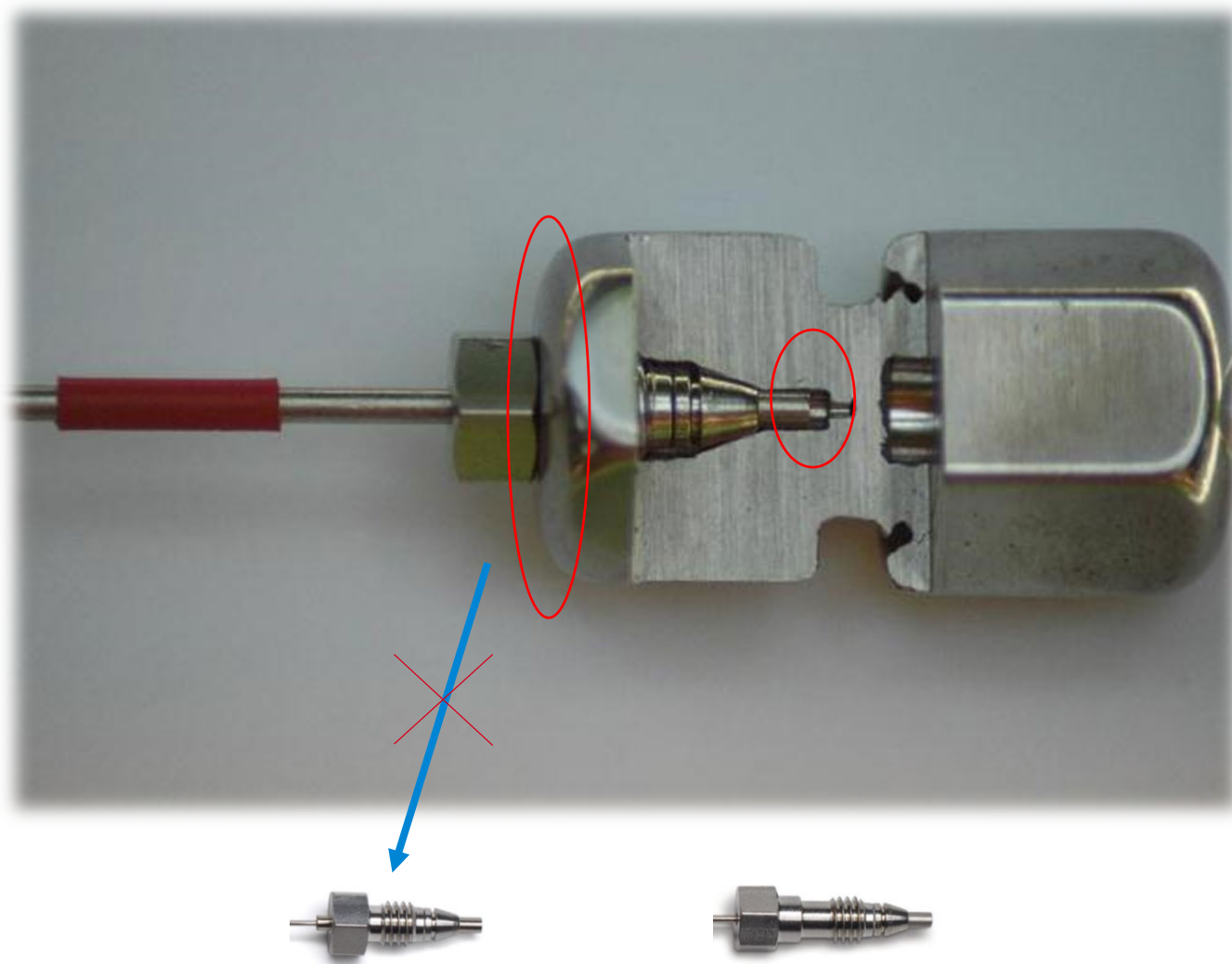


- Leak

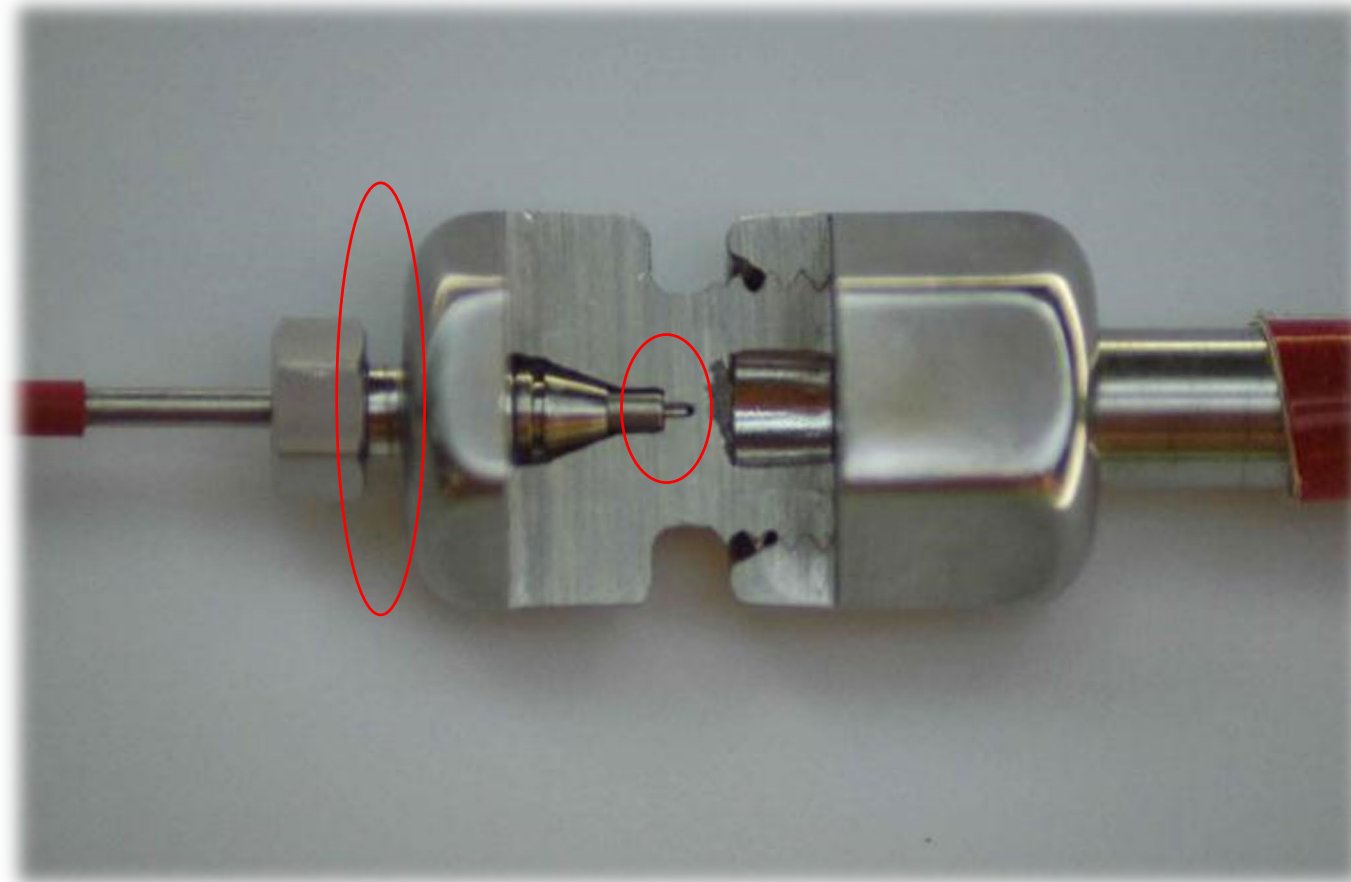
- Peak shape problem

- No dead volume

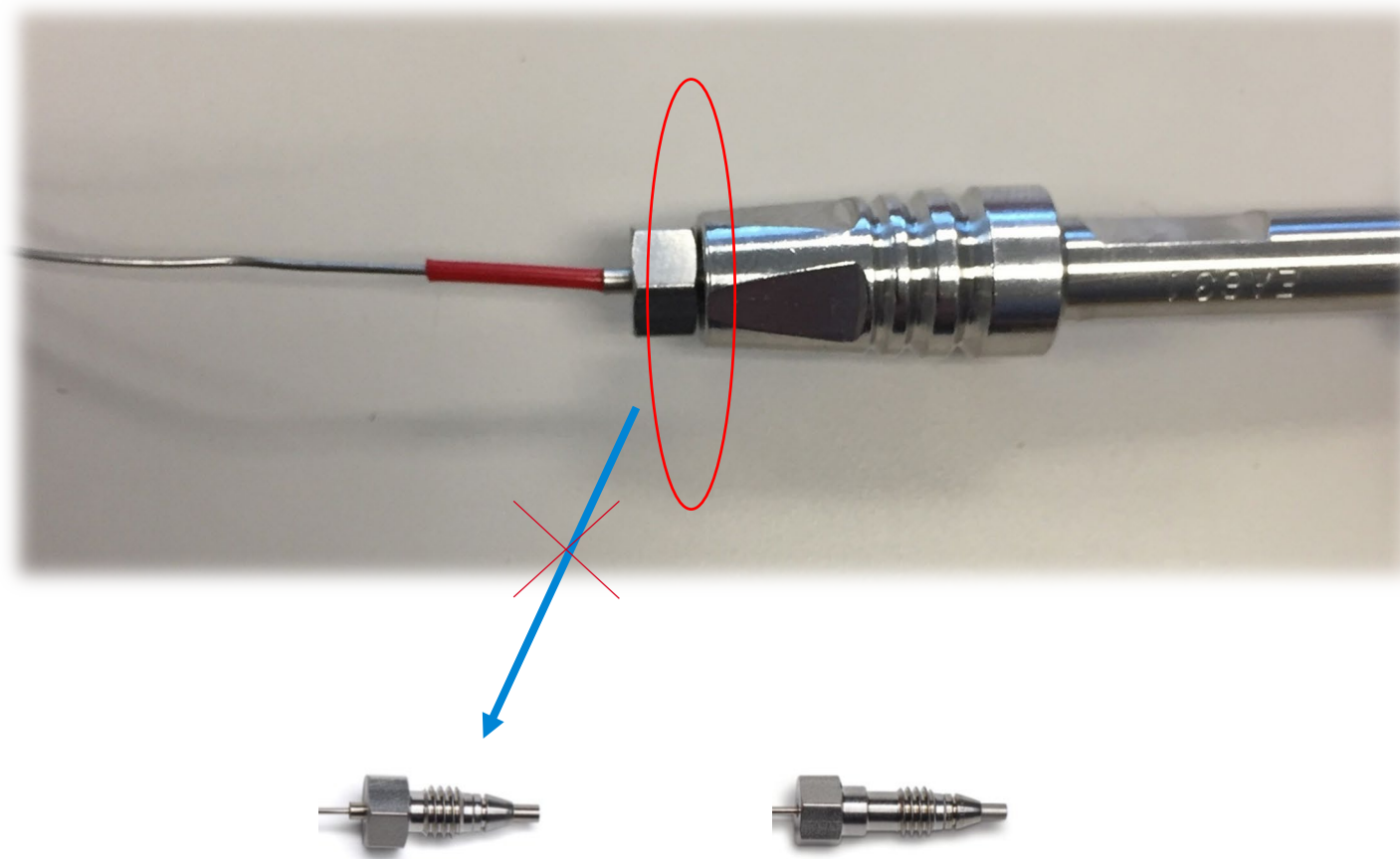
Fitting Mismatch



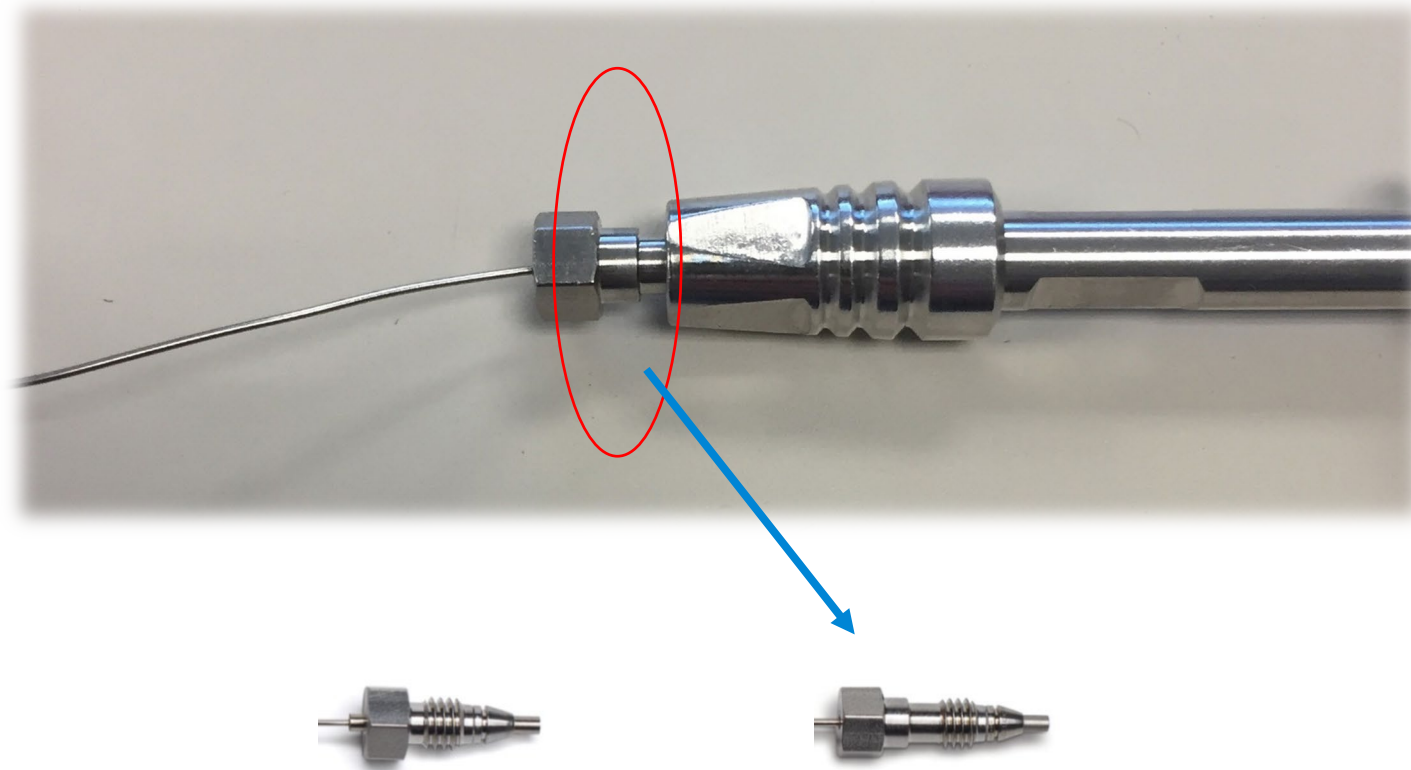
Proper Fit



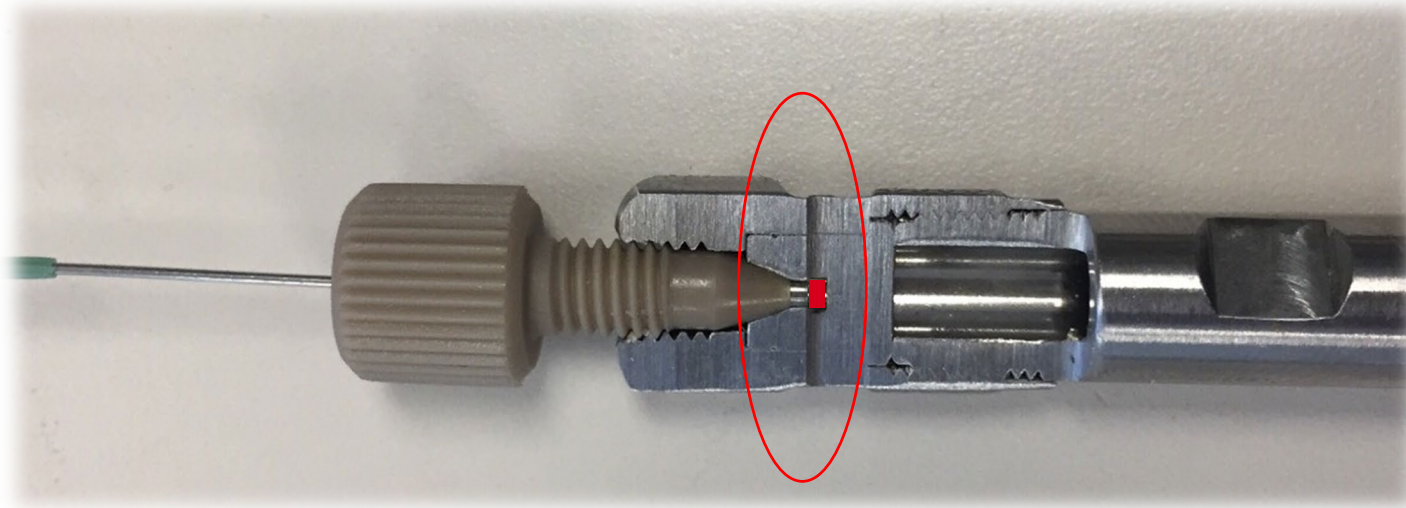
Fitting Mismatch



Proper Fit

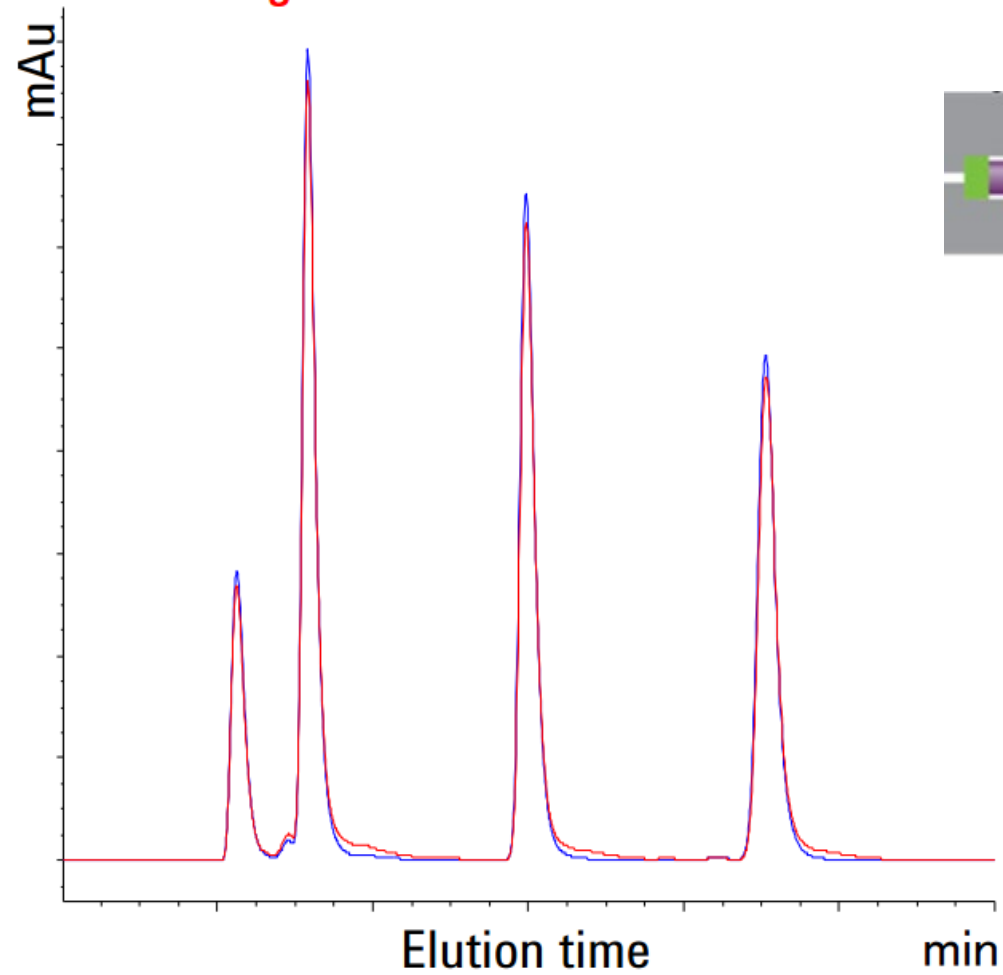


Polymeric Fittings

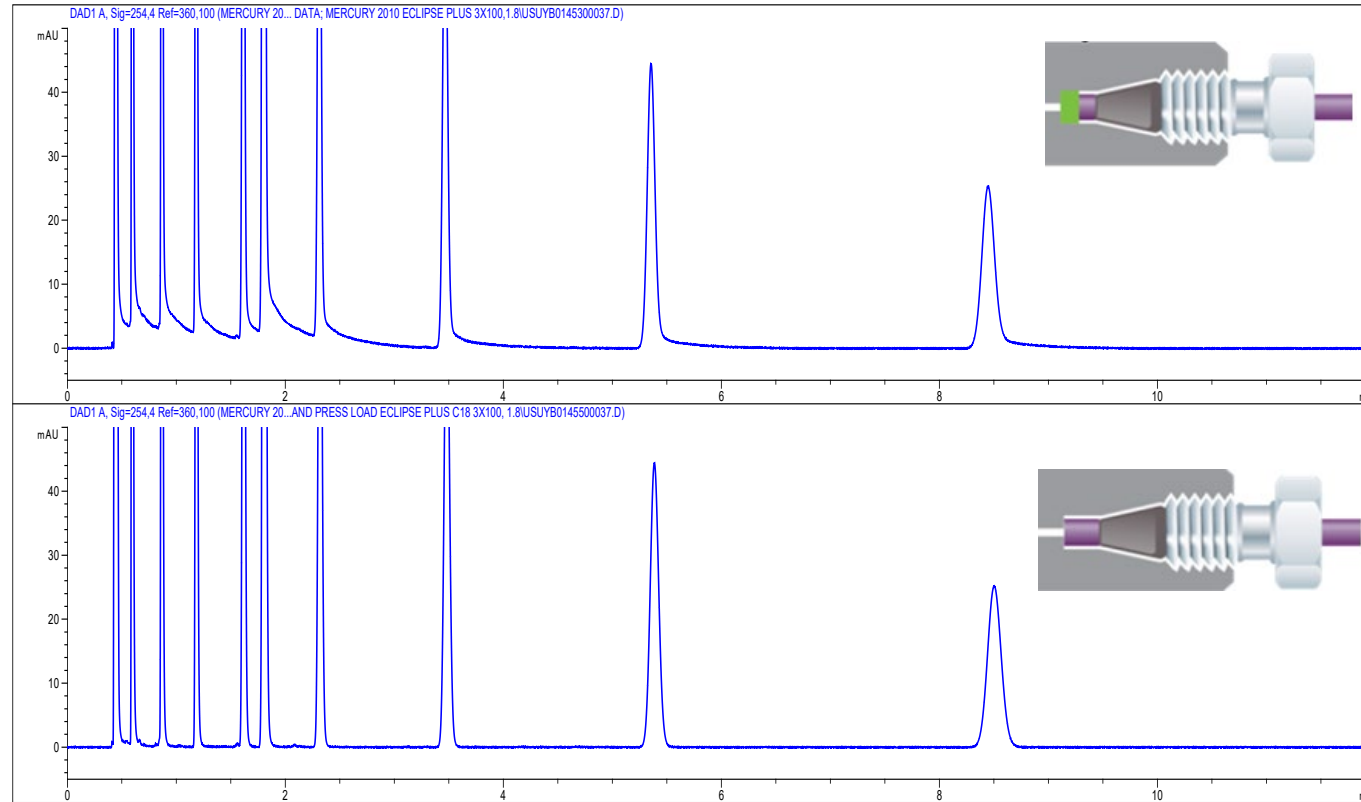


Peak Shape

— Zero-dead-volume fitting connection
— Fitting connection with dead volume



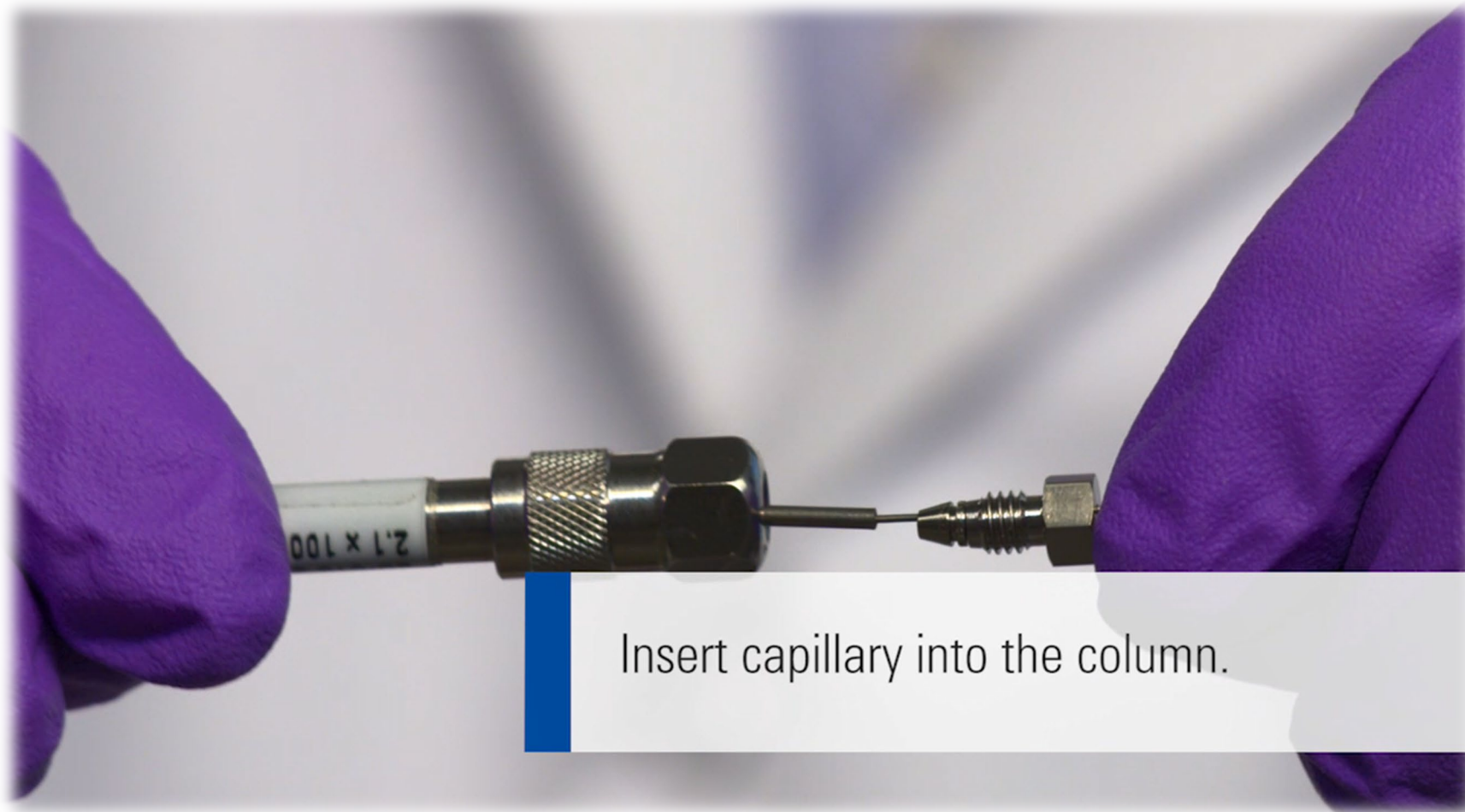
Peak Shape



Swaging Fittings

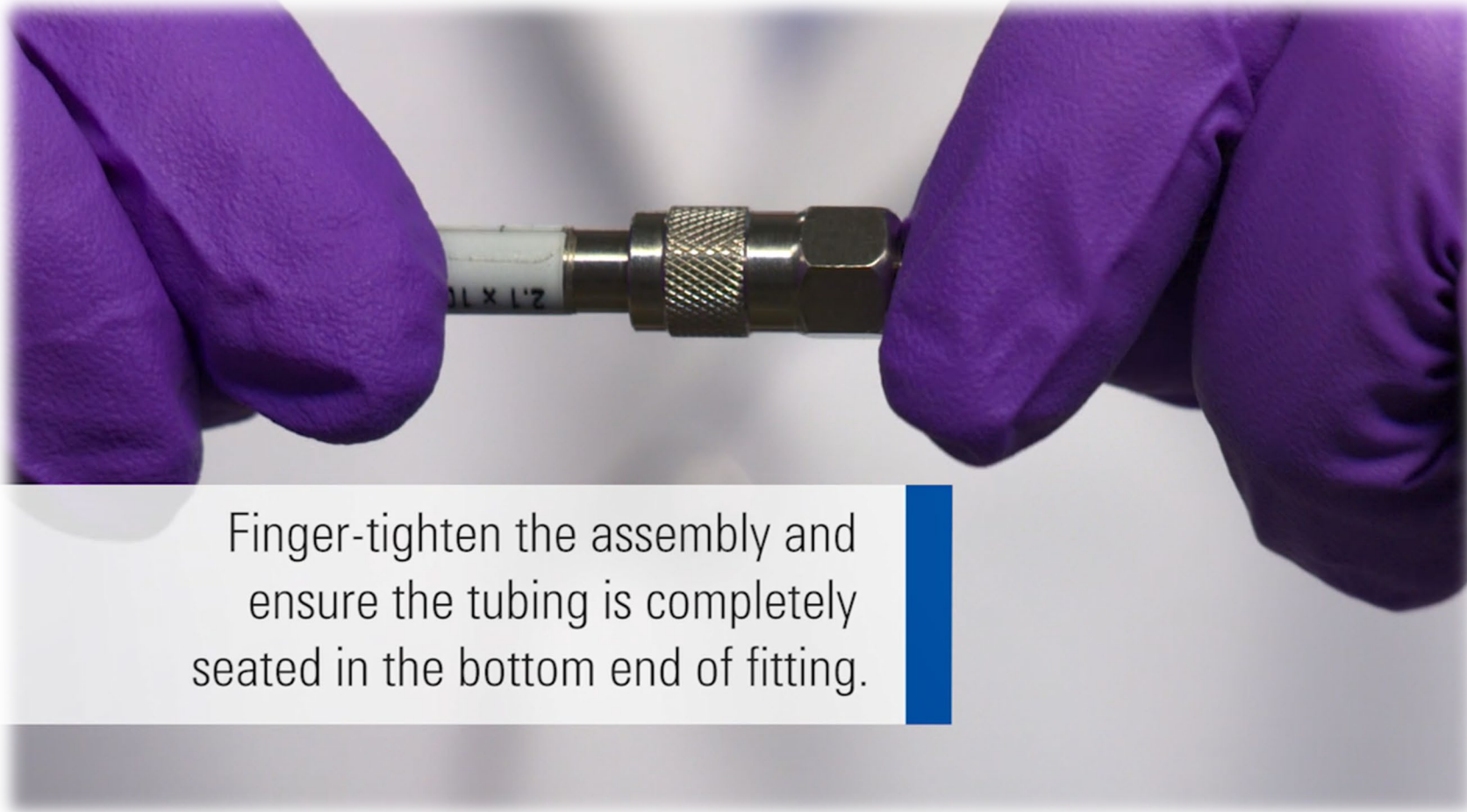


Swaging Fittings



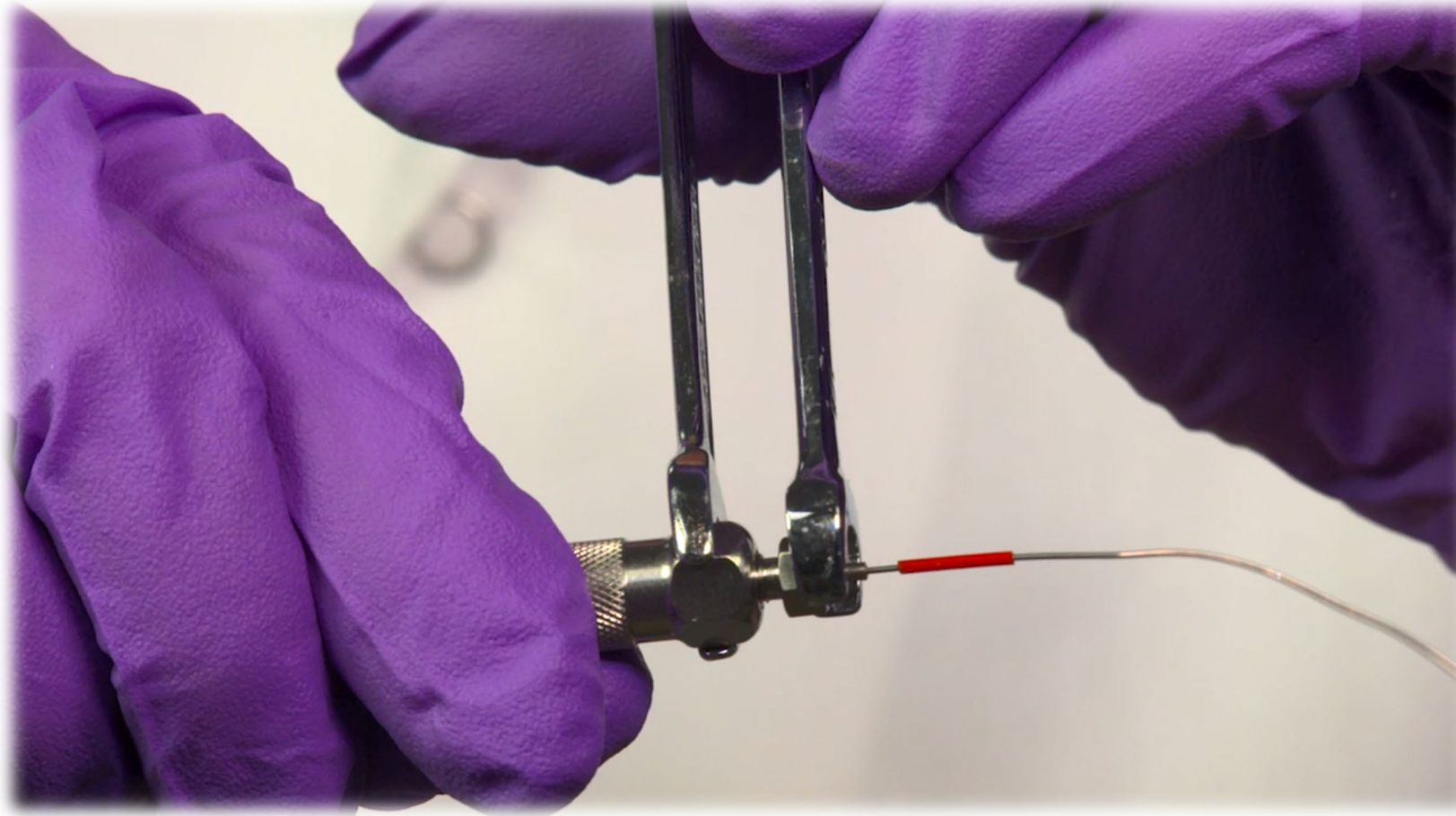
Insert capillary into the column.

Swaging Fittings

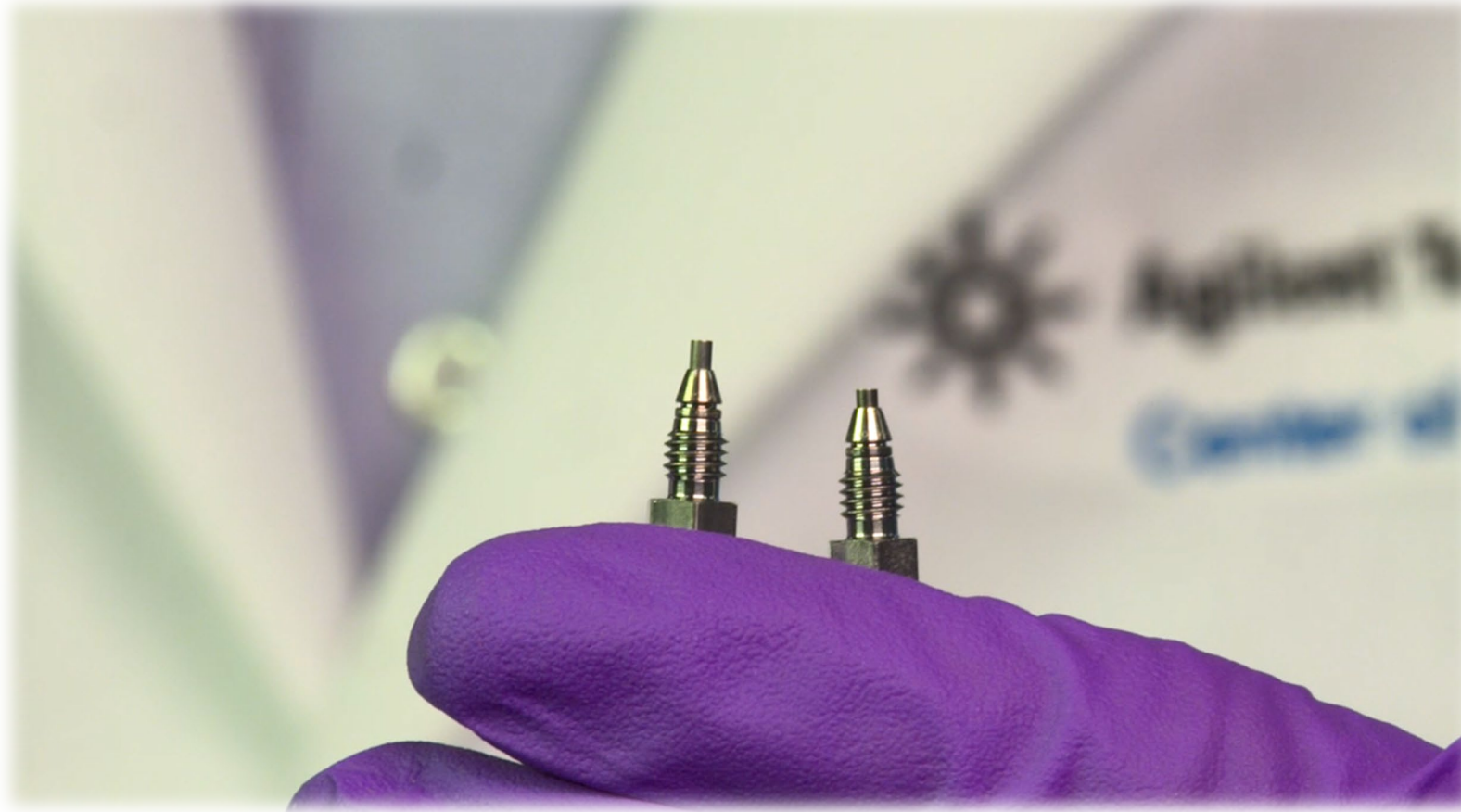


Finger-tighten the assembly and ensure the tubing is completely seated in the bottom end of fitting.

Swaging Fittings

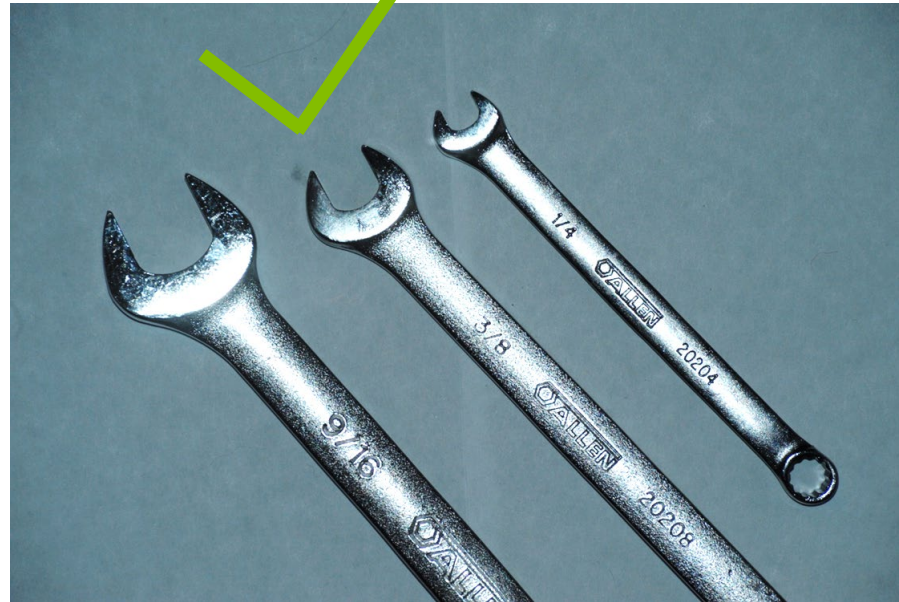
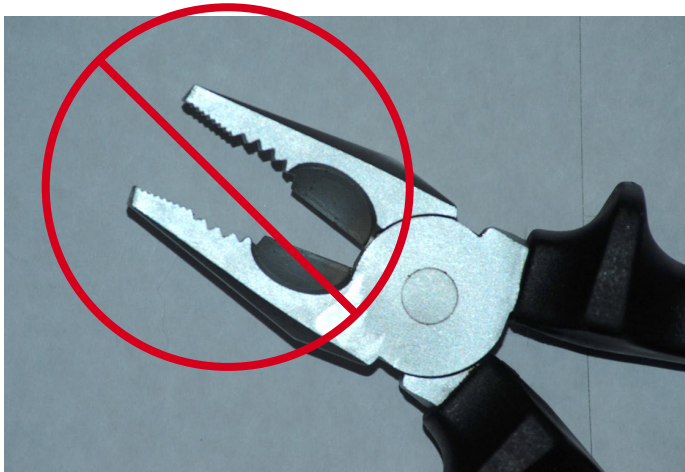
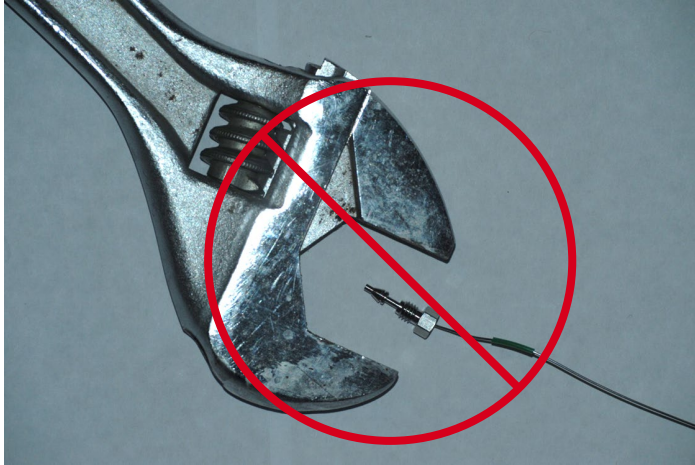


Inspect the Position of the Ferrule



[Video](#): How to properly swage stainless steel fittings onto stainless steel capillary tubing

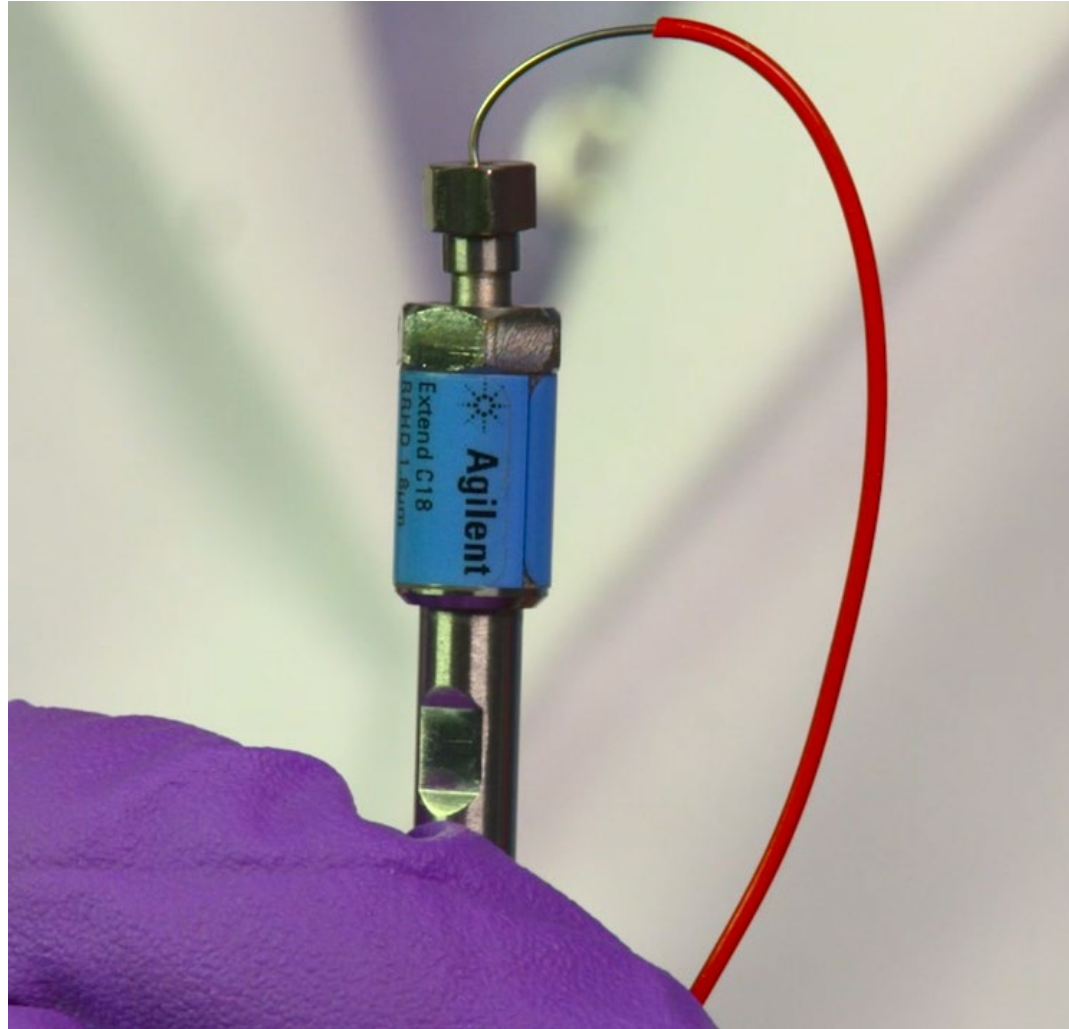
Tools



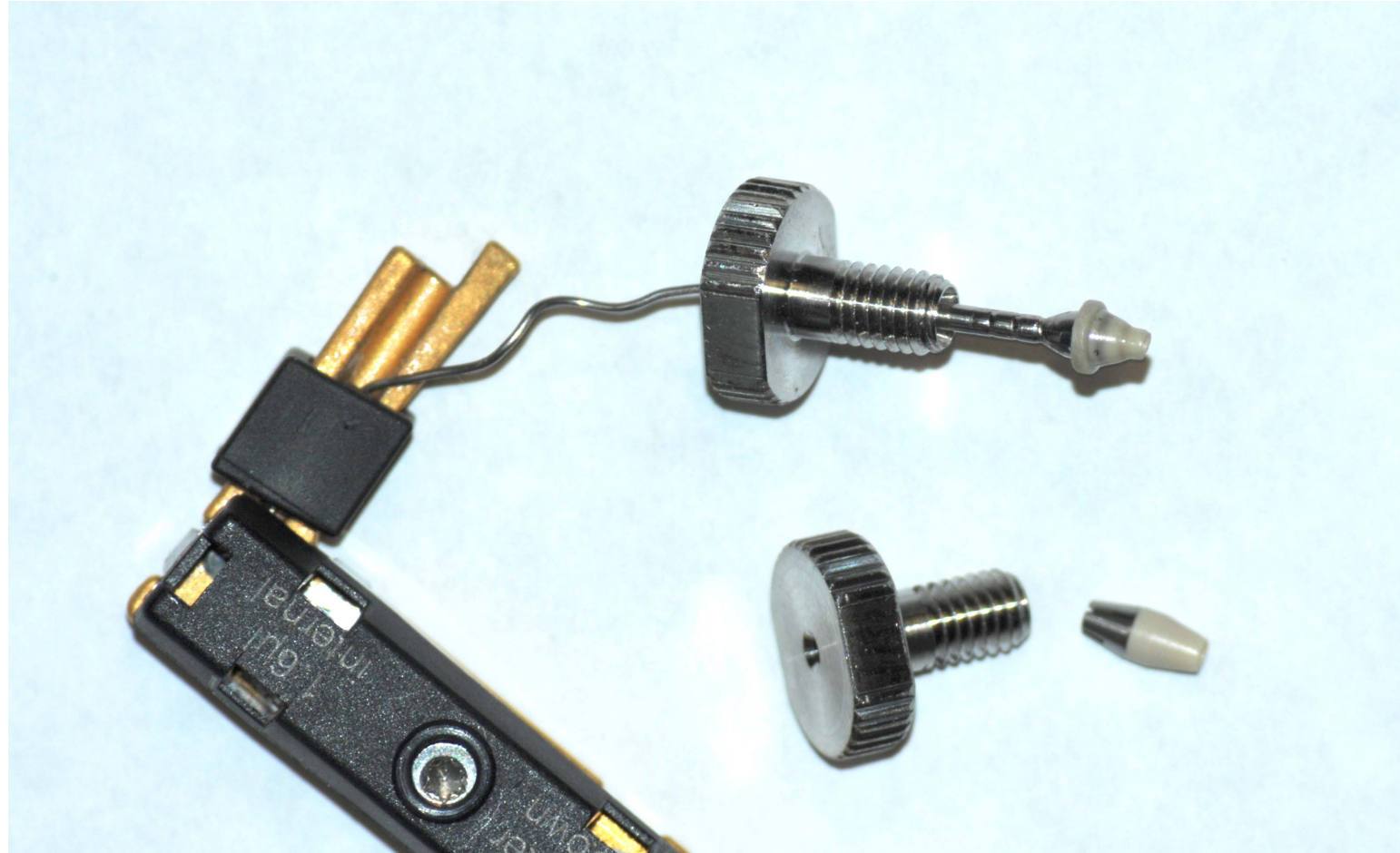
Tightening Fittings Into a Column



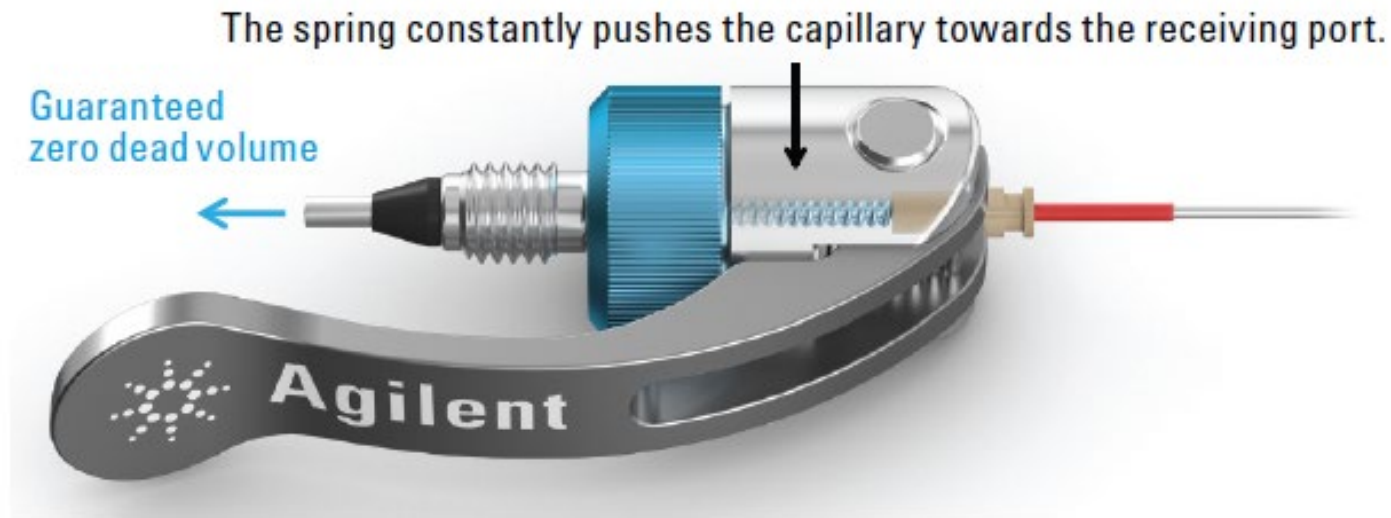
Overtightened Fittings



Overtightened Fittings



Agilent InfinityLab Fittings: Unique Spring-loaded Design



- The unique spring-loaded design applies a constant force to eliminate dead volume
- Finger tight to 1300 bar

InfinityLab Quick Connect Fitting



Connection problems can lead to:

- Downtime
- *High cost of operation*
- Poor chromatography results
 - Broad or tailing peaks
 - Loss of resolution
- Expensive maintenance cost
 - Over-tightening
 - Column damage
 - Leaks, added troubleshooting



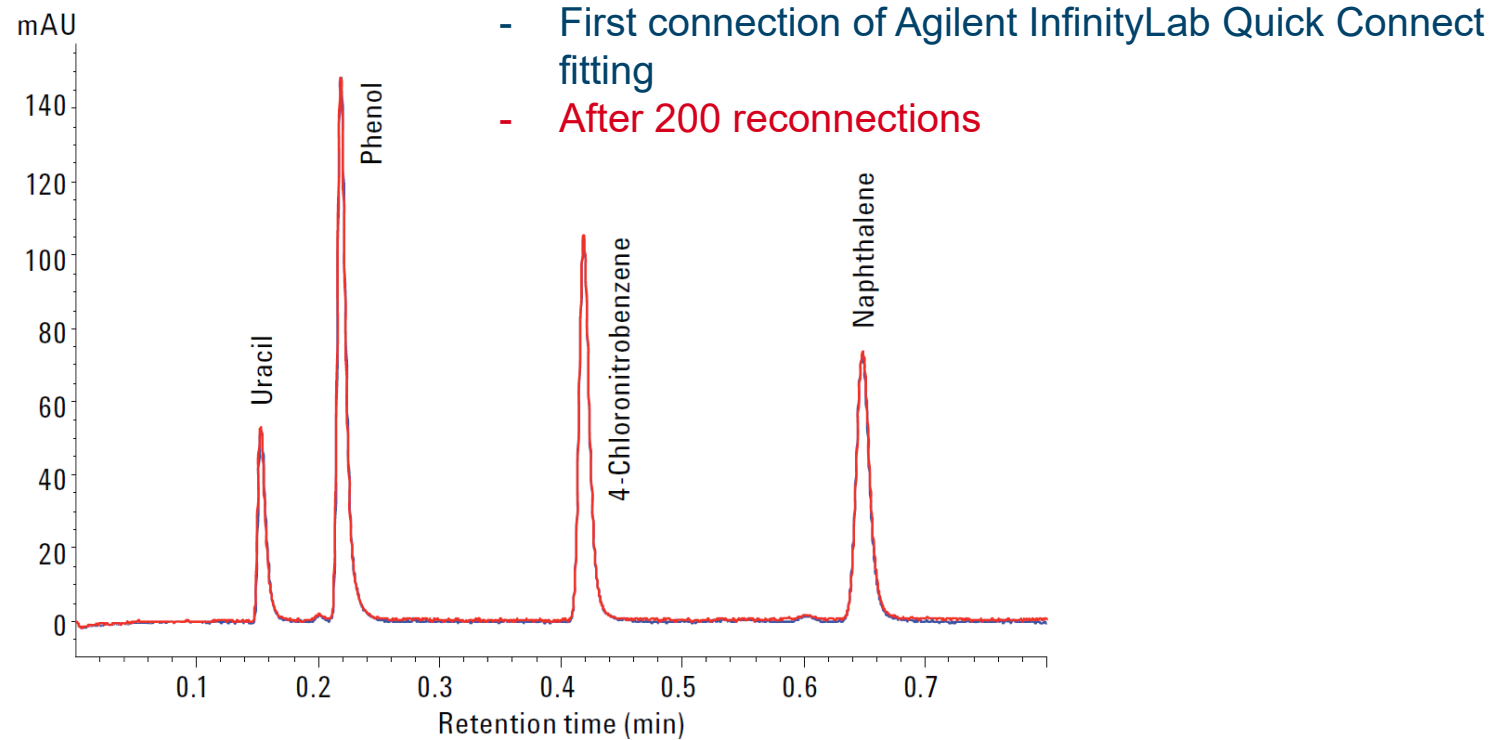
InfinityLab Quick Connect fitting



InfinityLab Quick Turn fitting

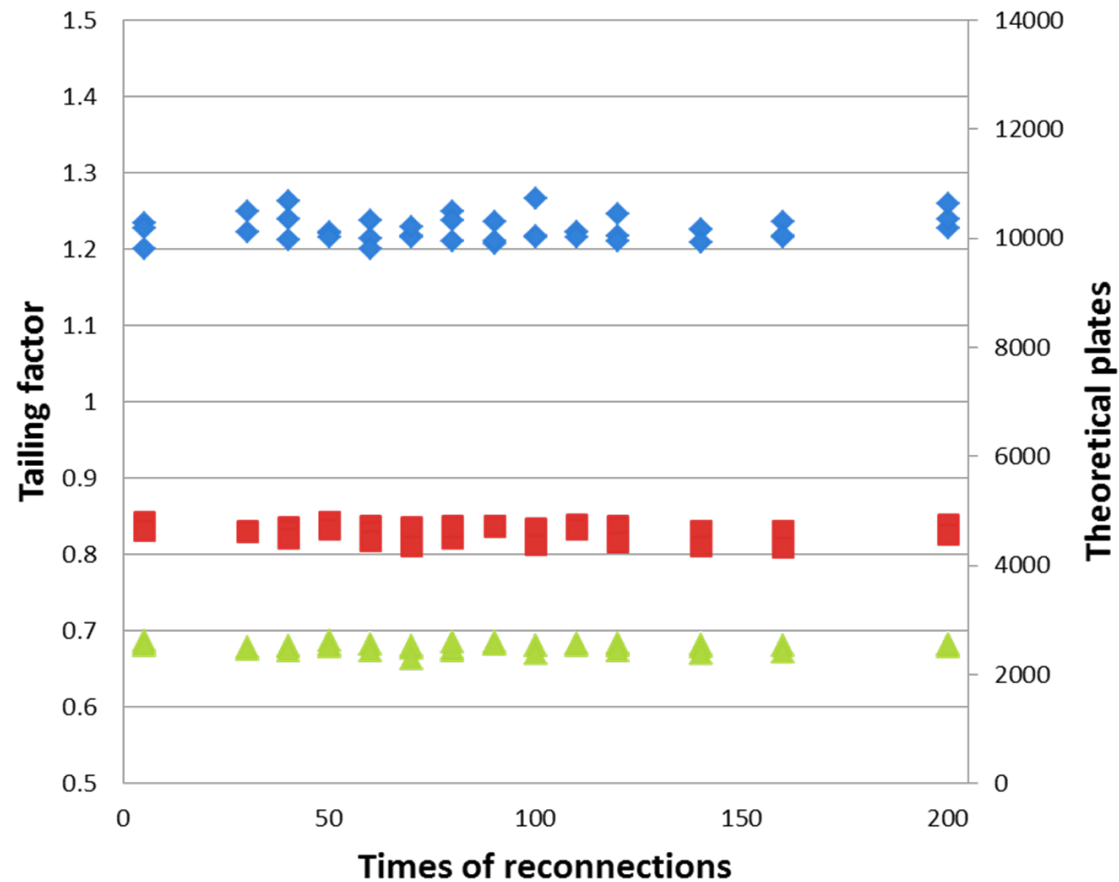
InfinityLab Fittings Last Longer

Chromatogram overlap before and after 200 reconnections



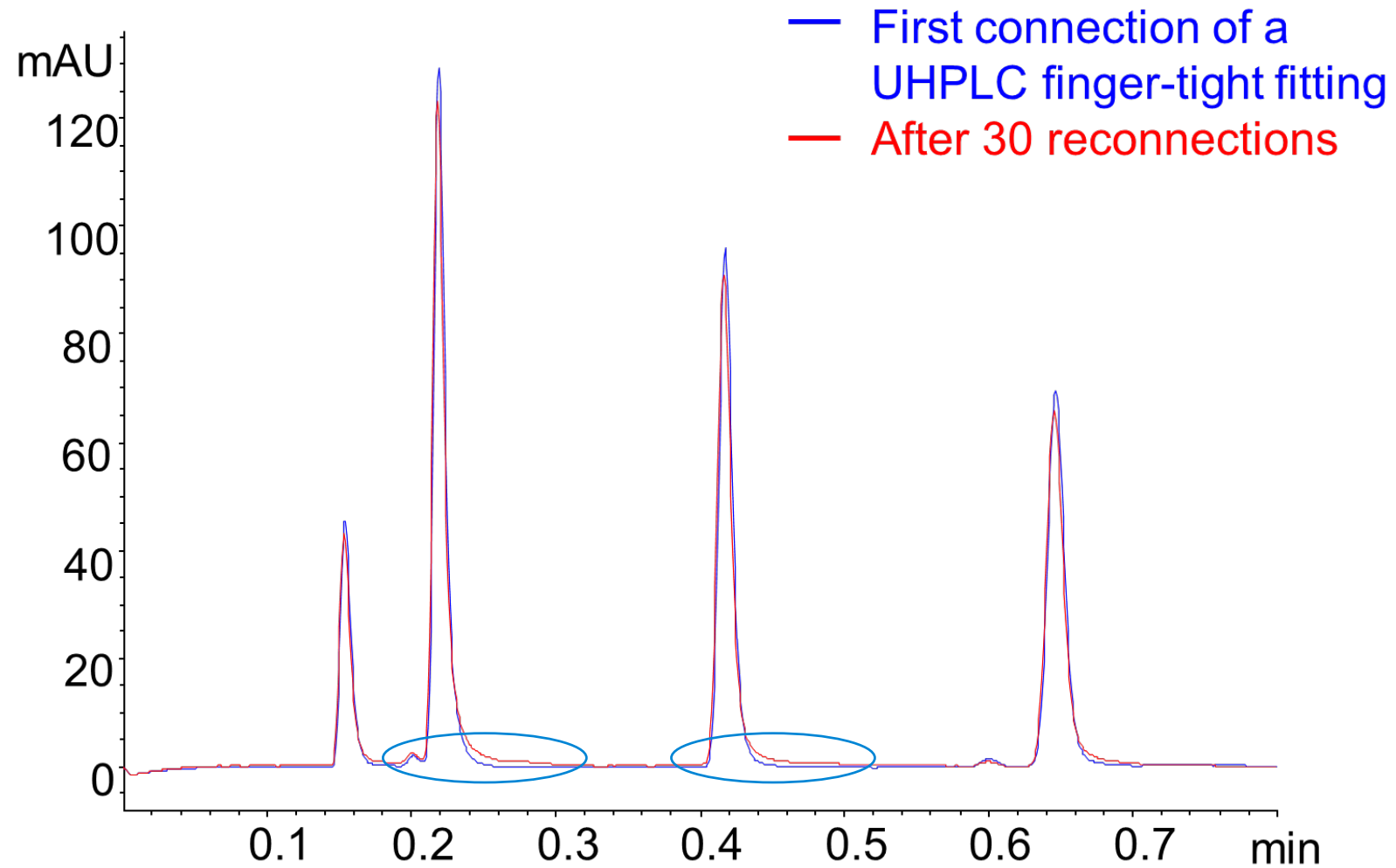
No visible change of chromatogram after 200 reconnections.

InfinityLab Fittings Last Longer

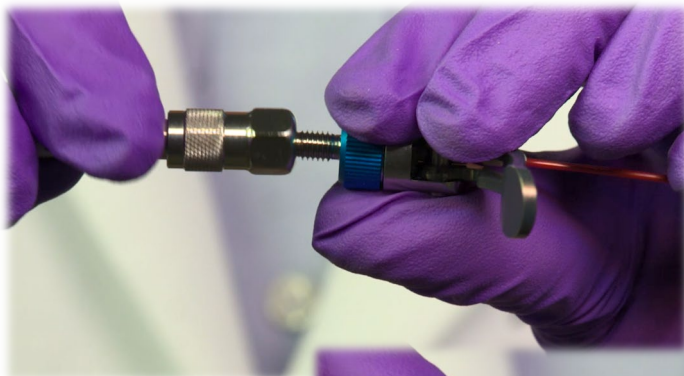


- Tailing factors and theoretical plates stayed constant within the experimental allowance through the reconnection procedures.

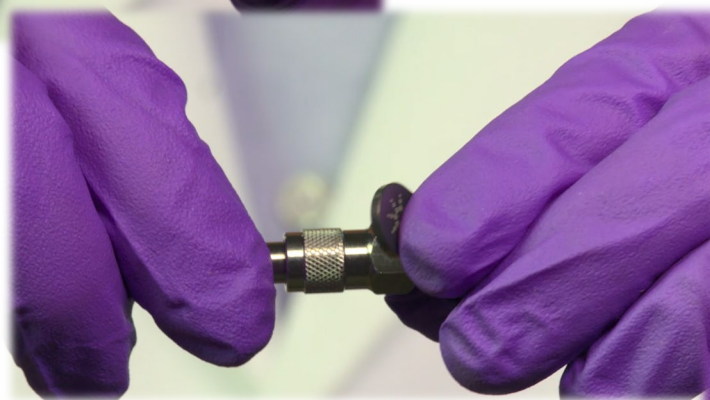
UHPLC Finger Tight Fitting from Another Vendor



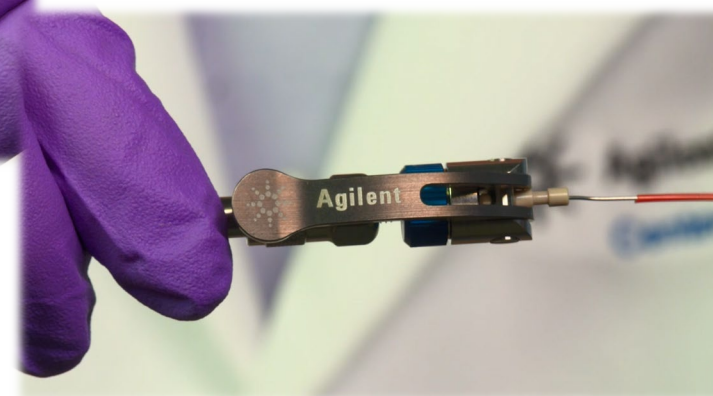
Using the InfinityLab Quick Connect Fitting



Finger tighten the fitting until you feel resistance, then close the lever.

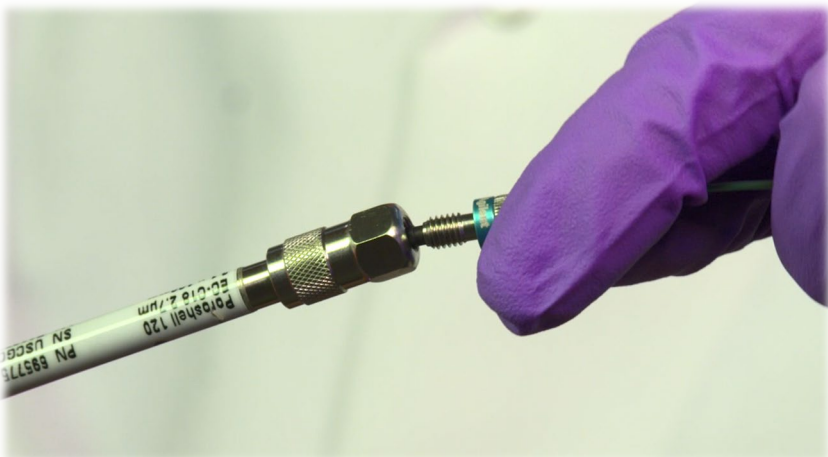


Leak tight to 1300 bar

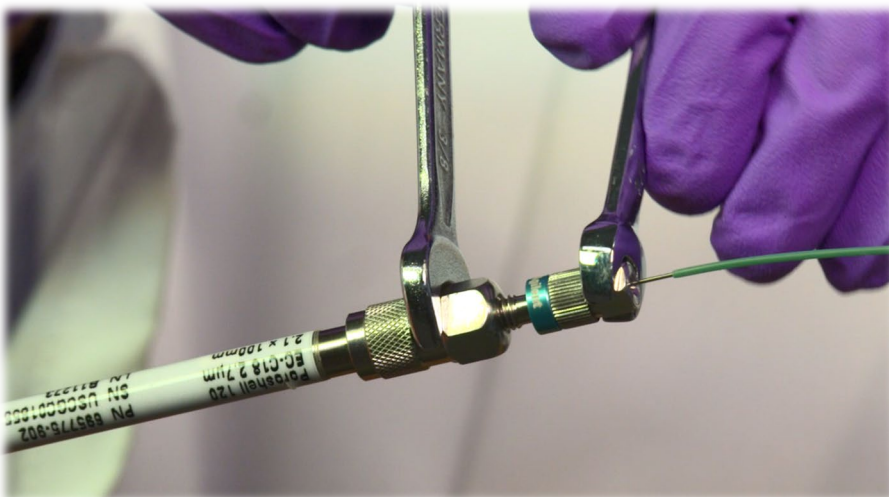


[Video](#): Making Great Connections – Less stress, more reliable fittings

Using InfinityLab Quick Turn Fitting



Finger tighten for 300–400 bar (user dependent)



Wrench/mounting tool tighten for 1300 bar



Mounting tool, p/n 5043-0915

InfinityLab Quick Connect/Turn Fittings Use Specific Capillaries



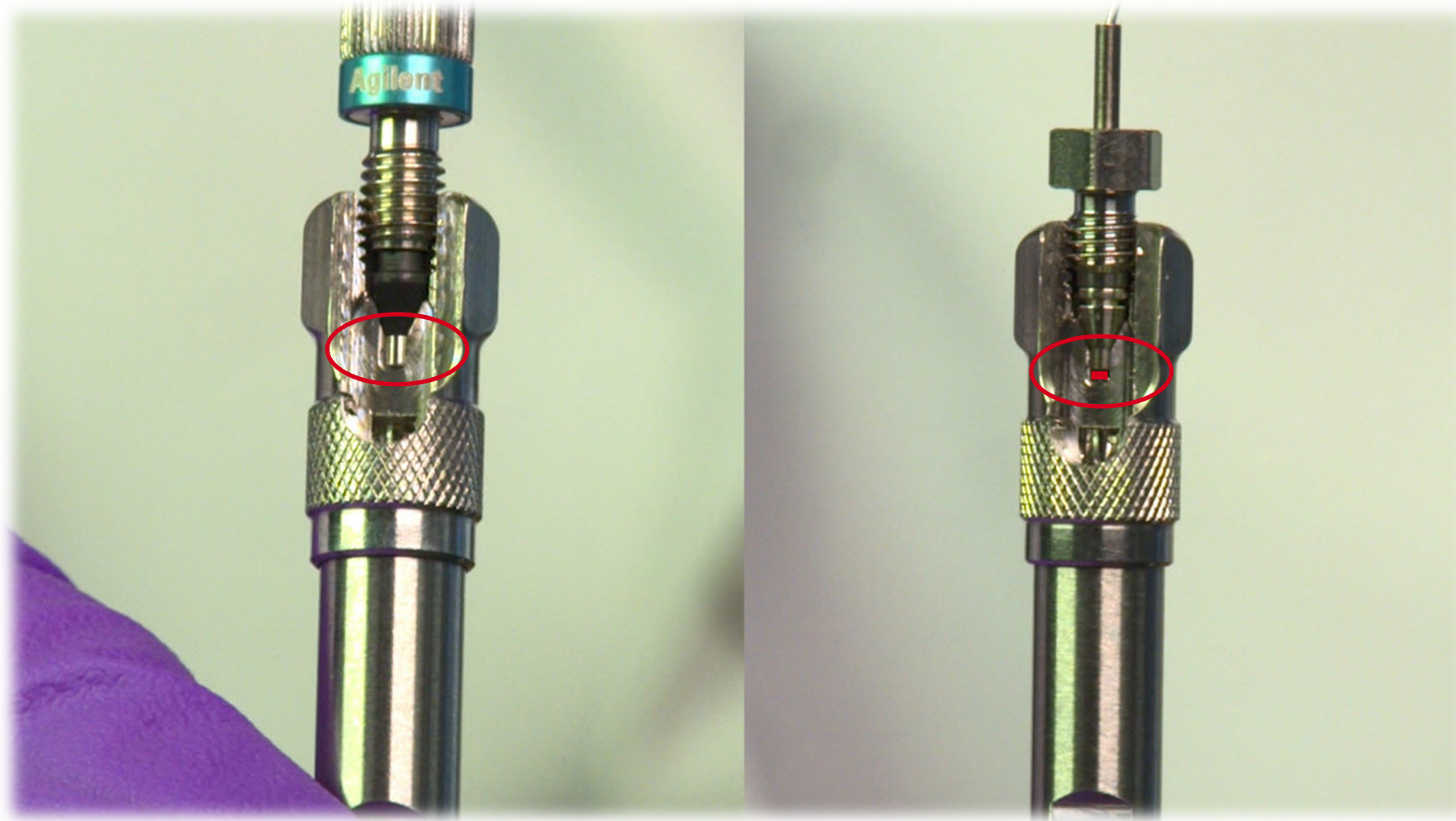
Quick Connect fitting requires capillary with spring and a PEEK component



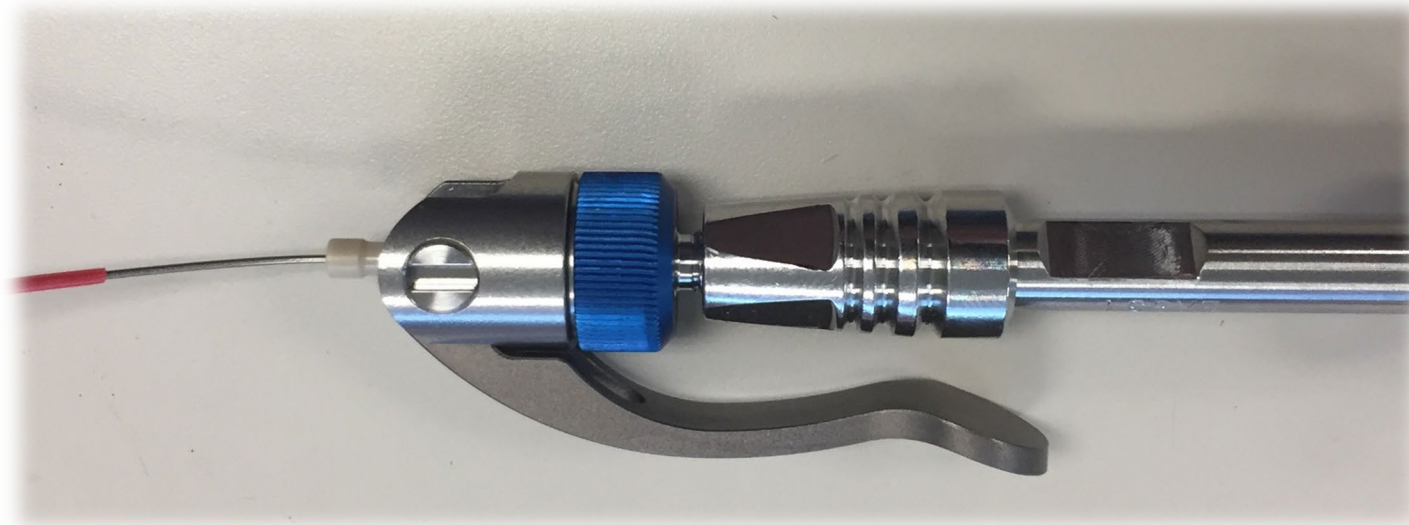
Quick Turn fitting needs capillary with long socket due to its internal spring action

A wide variety of capillary lengths and inner diameters are available to meet HPLC and UHPLC needs

Zero Dead Volume Connections



Not Just for Agilent Columns

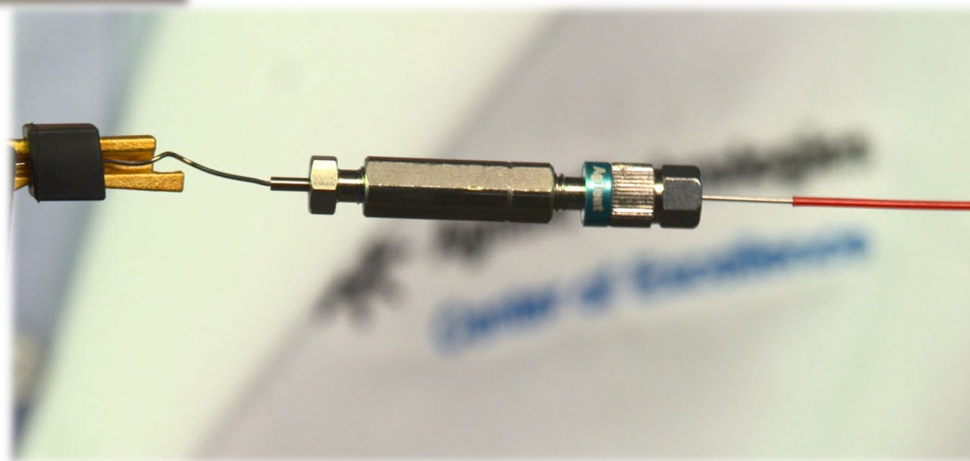


InfinityLab Fittings Tips



A ZDV union can be used to adapt to heat exchangers.

But....



InfinityLab Fittings in Action

Low dispersion heat exchanger,
p/n G1316-80022



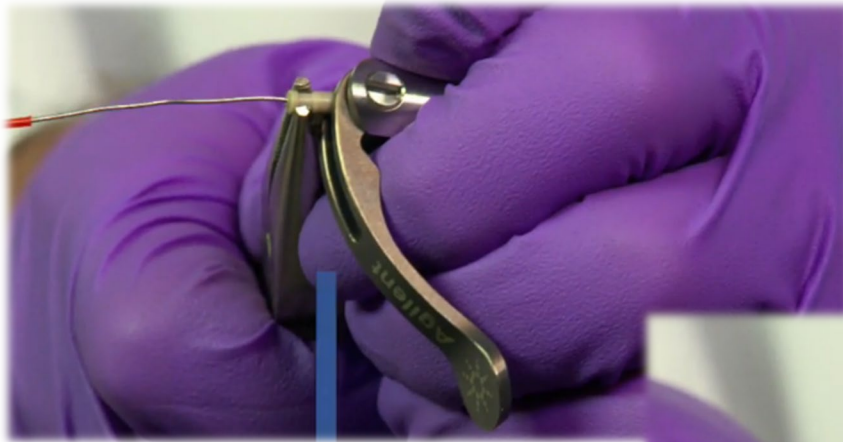
Works well with the latest low dispersion heat exchanger for G1315B/C TCC

InfinityLab Fittings in Action

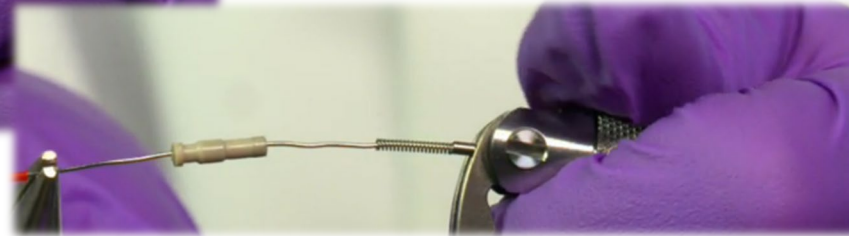


[Video](#): Making great Connections

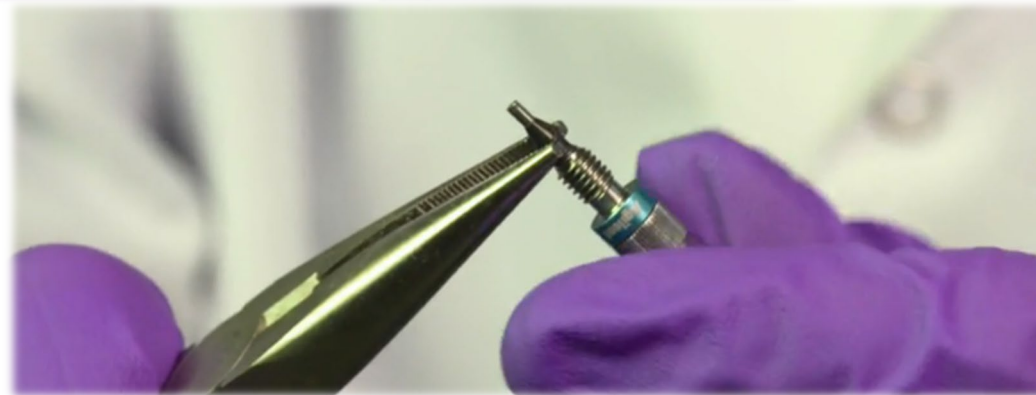
Replacing Capillaries and Ferrules



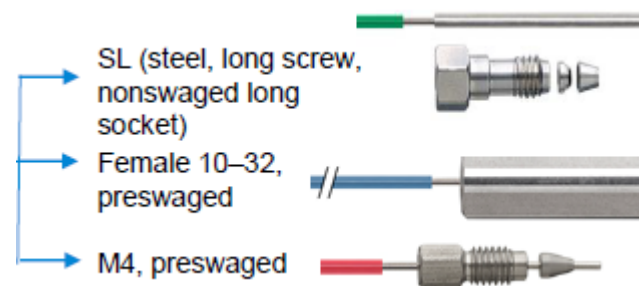
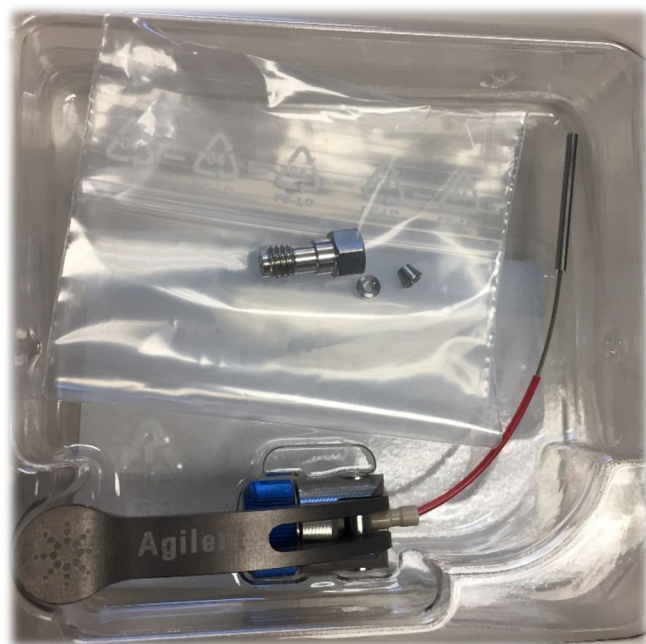
If the capillary is damaged, or another dimension is needed, just use a plier to remove the capillary and insert a new one.



The ferrules can also be replaced; just use pliers to remove the ferrule and insert a new one.



InfinityLab Quick Connect Fittings

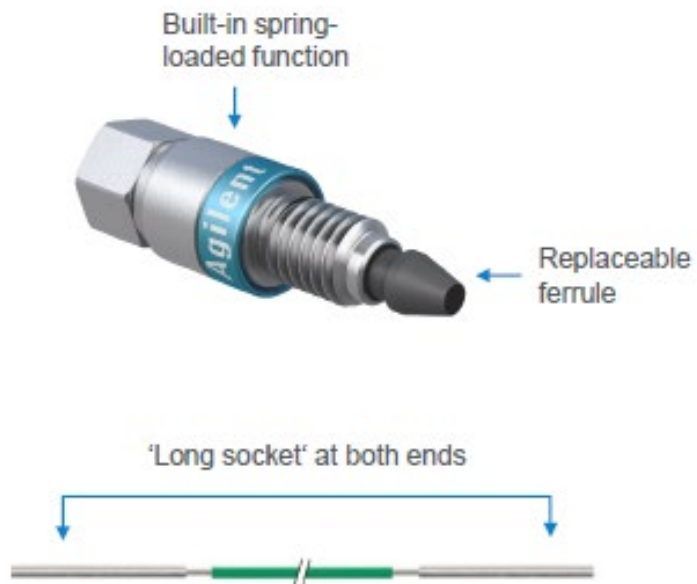


Fitting for the other end of the capillary

- Quick Connect fittings are sold as an assembly
- Come with a fitting for the other end
- Replacement capillaries are also available in stainless steel and bio-inert (PEEK/SST)
- Flyer: [5991-5164EN](#)

InfinityLab Quick Connect Fitting Description	Part number
Assemblies	
Stainless steel 0.075 x 105 mm	5067-5961
Stainless steel 0.075 x 150 mm	5067-6163
Stainless steel 0.075 x 220 mm	5067-6164
Stainless steel 0.075 x 280 mm	5067-6165
Stainless steel 0.12 x 105 mm	5067-5957
Stainless steel 0.12 x 150 mm	5067-5958
Stainless steel 0.12 x 220 mm	5067-5959
Stainless steel 0.12 x 280 mm	5067-5960
Stainless steel 0.17 x 105 mm	5067-6166
Stainless steel 0.17 x 150 mm	5067-6167
Stainless steel 0.17 x 220 mm	5067-6168
Stainless steel 0.17 x 280 mm	5067-6169
Fittings and ferrules	
Quick Connect fitting	5067-5965
Front ferrule	5043-0924
Capillaries	
Stainless steel 0.075 x 105 mm	5500-1174
Stainless steel 0.075 x 150 mm	5500-1175
Stainless steel 0.075 x 220 mm	5500-1176
Stainless steel 0.075 x 250 mm	5500-1177
Stainless steel 0.075 x 280 mm	5500-1178
Stainless steel 0.12 x 105 mm	5500-1173
Stainless steel 0.12 x 120 mm	5500-1247
Stainless steel 0.12 x 150 mm	5500-1172
Stainless steel 0.12 x 220 mm	5500-1171
Stainless steel 0.12 x 280 mm	5500-1170
Stainless steel 0.12 x 400 mm	5500-1179
Stainless steel 0.12 x 500 mm	5500-1180
Stainless steel 0.17 x 105 mm	5500-1181
Stainless steel 0.17 x 120 mm	5500-1248
Stainless steel 0.17 x 150 mm	5500-1182
Stainless steel 0.17 x 220 mm	5500-1183
Stainless steel 0.17 x 280 mm	5500-1230
Stainless steel 0.17 x 500 mm	5500-1231
Stainless steel 0.25 mm x 105 mm with a female connection	5500-1258
Stainless steel 0.25 mm x 150 mm	5500-1259
Stainless steel 0.25 mm x 400 mm	5500-1260
Stainless Steel 0.17 x 150 mm M4	5500-1291
Stainless Steel 0.12 x 150 mm M4	5500-1289
Stainless Steel 0.12 x 340 mm M4	5500-1416
PEEK/Stainless steel, bio-inert, 0.17 x 280 mm	5500-1276

InfinityLab Quick Turn Fittings



Quick Turn capillaries and fittings are sold separately

Flyer: [5991-5164EN](#)

InfinityLab Quick Turn Fitting Description	Part number
Fittings and ferrules	
Quick Turn fitting	5067-5966
Front ferrule	5043-0924
Capillaries	
Stainless steel 0.075 x 105 mm long socket	5500-1198
Stainless steel 0.075 x 150 mm long socket	5500-1232
Stainless Steel 0.075 x 250 mm long socket	5500-1206
Stainless steel 0.075 x 500 mm long socket	5500-1205
Stainless steel 0.12 x 105 mm long socket	5500-1188
Stainless steel 0.12 x 150 mm long socket	5500-1189
Stainless steel 0.12 x 180 mm long socket	5500-1233
Stainless steel 0.12 x 200 mm long socket	5500-1190
Stainless steel 0.12 x 280 mm long socket	5500-1191
Stainless steel 0.12 x 500 mm long socket	5500-1192
Stainless steel 0.17 x 105 mm long socket	5500-1193
Stainless steel 0.17 x 150 mm long socket	5500-1194
Stainless steel 0.17 x 180 mm long socket	5500-1234
Stainless steel 0.17 x 200 mm long socket	5500-1195
Stainless steel 0.17 x 280 mm long socket	5500-1196
Stainless steel 0.17 x 380 mm long socket	5500-1235
Stainless steel 0.17 x 400 mm long socket	5500-1236
Stainless steel 0.17 x 500 mm long socket	5500-1197
Stainless steel 0.17 x 700 mm long socket	5500-1237
Stainless steel 0.25 x 105 mm long socket with a female connection	5500-1261
Stainless steel 0.25 x 150 mm long socket	5500-1262
Stainless steel 0.25 x 400 mm long socket	5500-1263
Stainless steel 0.12 x 130 mm long socket M4	5500-1200
Stainless steel 0.12 x 150 mm long socket M4	5500-1288
Stainless steel 0.17 x 150 mm long socket M4	5500-1290

InfinityLab Fittings

Agilent InfinityLab fittings

Getting a perfect column connection, every time, by any operator.



InfinityLab Quick Turn fitting



InfinityLab Quick Connect fitting

Easy to use	Quick Connect is truly finger tight up to 1300 bar. Quick Turn, using a tool, can take pressures up to 1300bar
	Use a Quick Connect fitting for the column inlet and a Quick Turn fitting for the column outlet
	Compatible systems: Agilent portfolio - 1290, 1260, 1200, 1100 and the Waters Alliance systems
	Works on Agilent, Waters, Phenomenex, and Supelco columns – Great results!
	Fast column connection – make multiple column switches in seconds
Time and cost savings	Reliable and reusable multiple times – Capillary and ferrule are user changeable
	Eliminate leaks and avoid costly column damage
	Reduce time spent troubleshooting. Avoid costly downtime resulting from bad connections
Better chromatography	Zero dead volume connections
	Application Flexible - capillary length and the internal diameter is application dependent
	All typical lengths and internal diameters available for most connections

Who Can Use the InfinityLab Quick Connect and Quick Turn Fittings?

- Any lab with a range of users, including newer chromatographers who may not have the experience to do traditional fittings well
- Any lab that is changing columns often
- Labs who are seeing variation in chromatographic results – broad or tailing peaks, and are spending time troubleshooting issues
- Any lab looking to make chromatography easier for everyone.