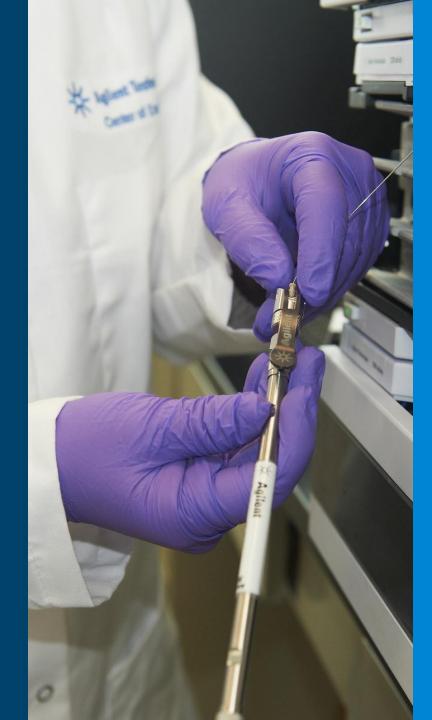


Golnar Javadi Applications Engineer Chemistries and Supplies Technical Support December 12, 2024





Agenda

- Why is it important to use the right connecting capillaries?
- Dispersion contribution from capillaries and fittings
- Types of capillaries and fittings
- Connecting capillaries for each LC system





Why Is It Important to Use the Right Capillaries and Fittings?

Problems caused by poor/wrong connections

- Leaks
- Dispersion due to the volume of the capillaries (extracolumn dispersion)

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- Mixing/dispersion due to use of the incorrect fitting
- Secondary interactions between the analytes and the body of the capillaries and fittings

Dispersion Reduces HPLC Performance

What is dispersion?

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

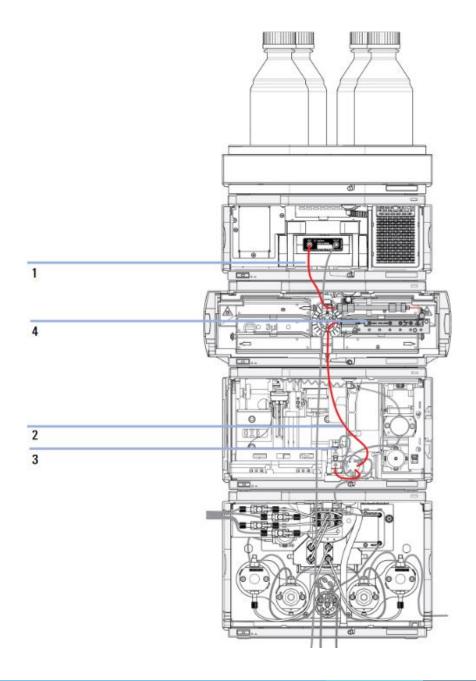
What increases dispersion?

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

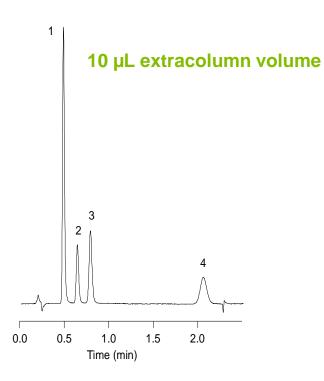


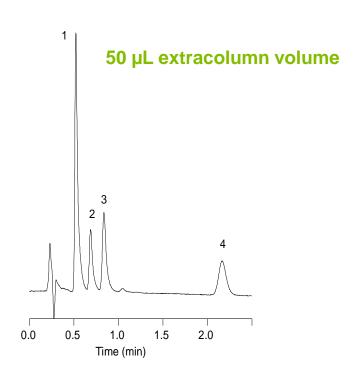
Extracolumn Volume

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



Extracolumn Volume





Column: StableBond SB-C18, 4.6 x 30 mm, 3.5 µm, 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.



Taylor-Aris Equation: Peak Dispersion in Cylindrical Tubing

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

 $\sigma_{\rm v,ext}^2$ 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



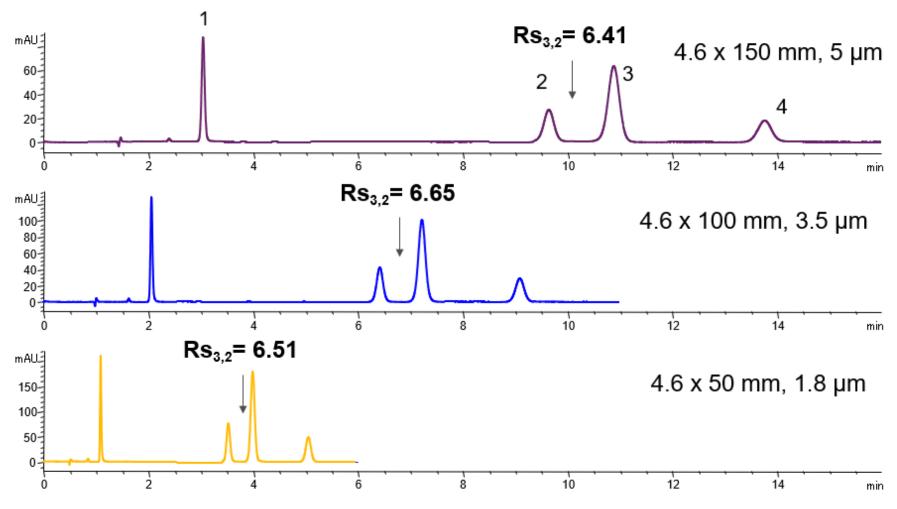
Tubing Volume

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



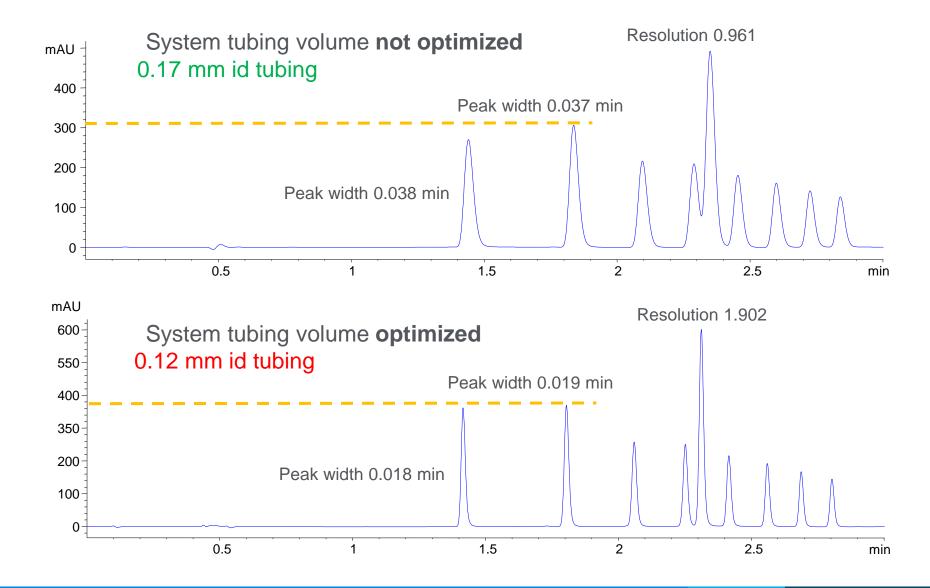


As Column Efficiency Increases, Peak Width Decreases, and System Dispersion Becomes More of a Factor



Columns: **Eclipse Plus C18**, as described above. Mobile phase: A: water, B: MeOH, (15:85), injection volume: 6 µL Temperature: 25 °C, flow rate: 1 mL/min. Detection: 310, 4 nm, 0.5 s response time, semimicro flow cell, sample: sunscreens.

Optimizing Connecting Tubing Volume For UHPLC columns



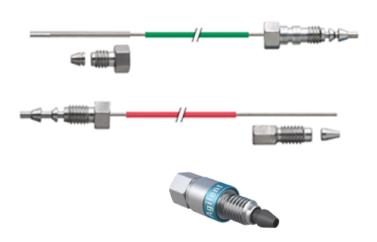


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Types of Capillaries and Fittings

What to consider

- Material
- Inner diameter and length
- Rigid or flexible
- Socket
- Compatible fitting types
- LC instrument







Types of Capillaries and Fittings

Material of capillaries

Material	Internal Diameter	Application	Maximum Pressure	Instrument Model
Stainless steel (SST)	0.075 – 0.94 mm	Standard HPLC, UHPLC, GPC, SFC, preparative HPLC	1300 bar	1100 through 1290 Infinity II
PEEK	0.12 – 0.5 mm		250 bar	All
PEEK-coated fused silica (FS/PEEK)	25 – 100 μm	Microflow HPLC	400 bar	1100 through 1260 capillary/nano LC series
PEEK-lined stainless steel (PEEK/SST)	0.17 mm	Bio-inert	400 bar	1260/1260 Infinity II bio-inert system
Titanium (Ti)	0.17 mm	Bio-inert*	1300 bar	1260/1260 Infinity II bio-inert system
MP35N	0.075 – 0.17 mm	Bio-compatible	1300 bar	1290 Infinity II bio LC

^{*}Only from pump to sampler.



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Types of Capillaries and Fittings Color coding of capillary inner diameters

Internal diameter	in	Color code
0.015		Orange
0.025		Yellow
0.05		Beige
0.075		Black
0.075	MP35N	Black with orange stripe
0.1		Purple
0.12		Red
0.12	MP35N	Red with orange stripe
0.17		Green
0.17	MP35N	Green with orange stripe
0.20/0.25		Blue
0.20/0.25	MP35N	Blue with orange stripe $\mathbb{O}_{\!\scriptscriptstyle{\mathtt{T}}}$
0.3		Grey
0.50		Bone White



Types of Capillaries and Fittings

Inner diameter for standard HPLC/UHPLC

0.17 mm:

- Standard for 1100, 1200, and 1260 series
- On 1290 Infinity and 1260/1290 Infinity II only used for pump-to-sampler connection

0.12 mm:

- Low-dispersion alternative for 1100, 1200, and 1260 series
- Standard on 1200 Series rapid resolution LC system
- Used as standard on the 1290 Infinity and 1260/1290 Infinity II for connections beyond the point of injection

0.075 mm:

Ultralow dispersion kit, optional to optimize system for low dispersion



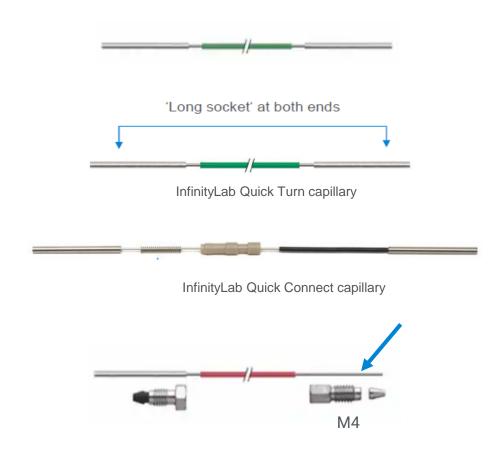


Types of Flexible Capillaries and Fittings Socket

Standard socket: 1.6 mm od, 17 mm long

Long socket: 1.6 mm od, 21 mm long

No socket: 0.8 mm od



Types of Fittings

Nonremovable (permanent) fittings

Advantages

- Simple and familiar handling
- Robust
- **Economical**

Disadvantages

- Nonremovable
- Can't be re-adjusted
- Require a wrench













Stainless steel fittings (S), 5062-2418



5065-4454



Stainless steel extra long fitting (SX), 5065-9967





Typical preswaged (pilot) distance: 2.1 mm (0.090 in.)

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Types of Fittings

Removable (re-adjustable) fittings



Finger-tight PEEK fitting (SPF), 0100-1516



PEEK long fittings (SPFL), 5062-8541



Finger-tight PEEK fittings (SPF), 5065-4426



Double winged fitting (SPF), 5042-6500



Finger-tight polyketone fitting (SPF), 5042-8957



1200 bar removable fitting (SV), 5067-4733



1200 bar removable long fitting (SLV), 5067-4738



1200 bar removable extra long fitting (SXV), 5067-4739



UHP-FF fitting, bio-inert (RLO), 5067-5695

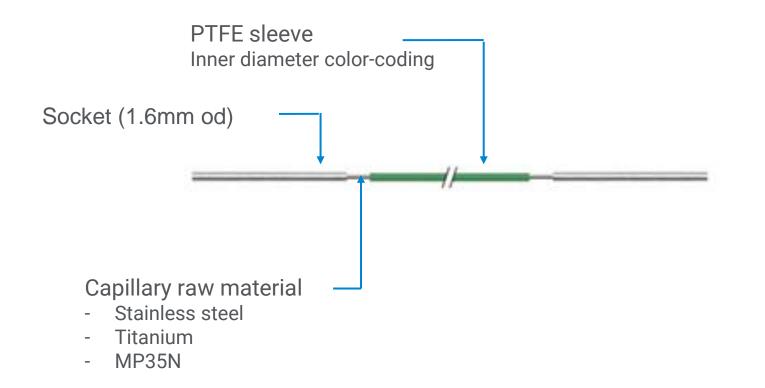


InfinityLab Quick Turn fitting, 5067-5966



Structure of a Standard Capillary

Compatible fittings



Suitable fittings:

- 1.6 mm (1/16 inch) id
- 10-32 coned thread (Swagelock)

Examples:



Stainless steel fittings (S), 5062-2418



Stainless steel long fittings (SL), 5065-4454



Stainless steel extra long fitting (SX), 5065-9967



Finger-tight polyketone fitting (SPF), 5042-8957



Finger-tight PEEK fitting (SPF) 0100-1516



PEEK long fittings (SPFL), 5062-8541



Finger-tight PEEK fittings (SPF), 5065-4426



Double winged fitting (SPF), 5042-6500

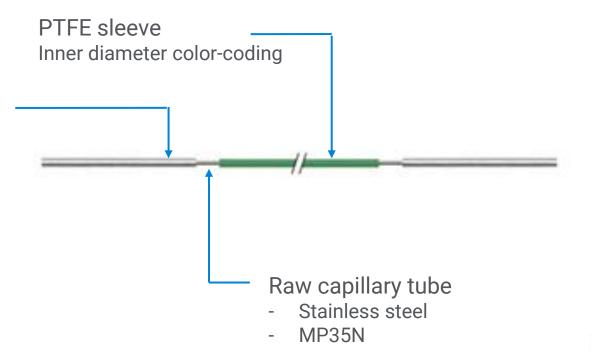


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Structure of a Quick Turn Capillary

Compatible fittings

"Long socket" 1.6 mm od 21 mm in length



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Finger-tight polyketone fitting (SPF), 5042-8957

Suitable fittings:

- InfinityLab Quick Turn fitting
- All "standard" capillary compatible fittings





Stainless steel fittings (S), 5062-2418



Stainless steel long fittings (SL), 5065-4454



Stainless steel extra long fitting (SX), 5065-9967



1200 bar removable fitting (SV). 5067-4733



200 bar removable long fitting (SLV), 5067-4738



200 bar removable extra long fitting (SXV), 5067-4739



Finger-tight PEEK fitting (SPF)



PEEK long fittings (SPFL), 5062-8541



5065-4426

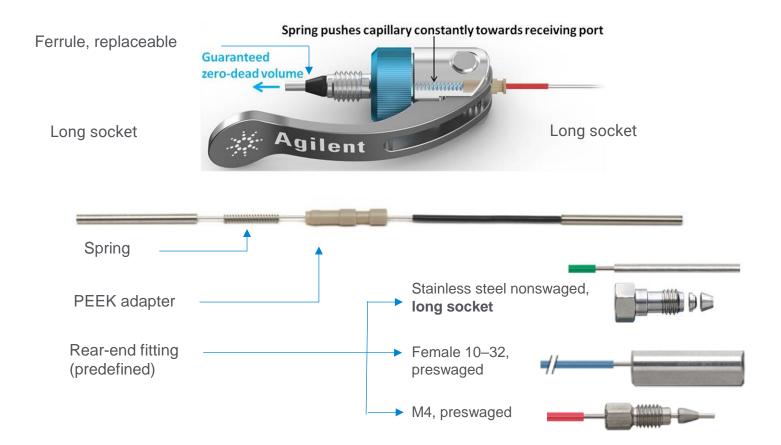


Double winged fitting (SPF),



Structure of a Quick Connect Capillary

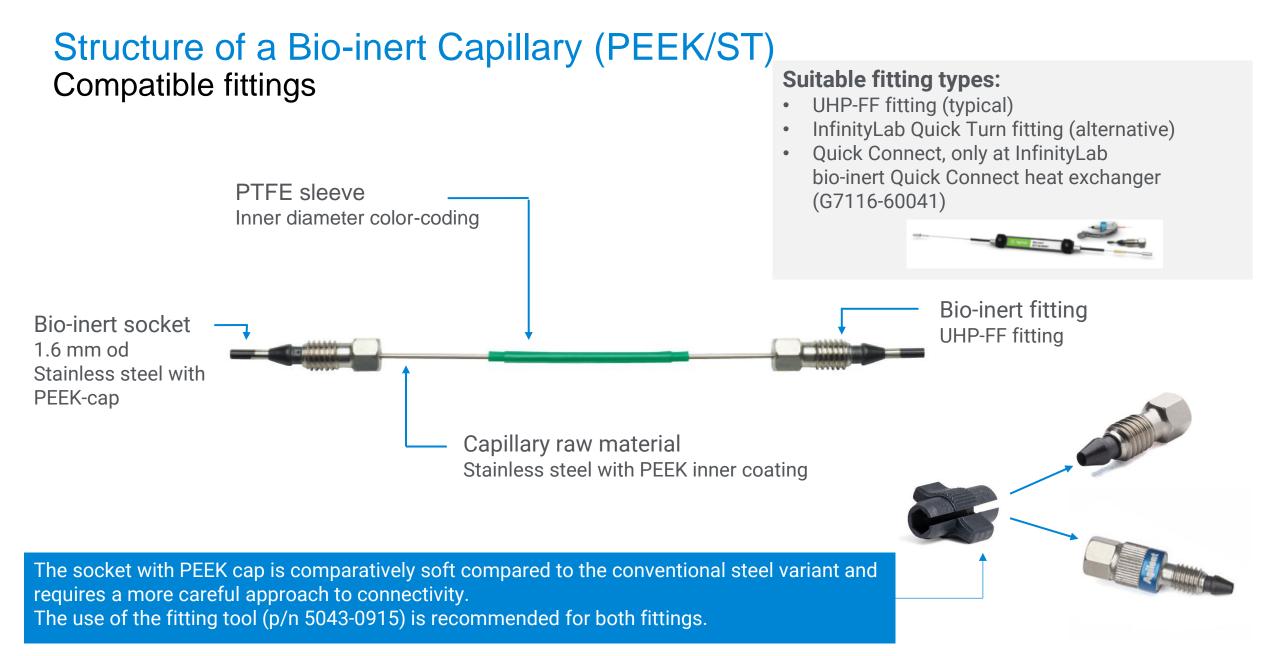
Compatible fittings



One side is always intended for use with a Quick Connect fitting. The opposite side is preconfigured with various fittings, typically with a nonpreswaged steel fitting. SX for capillary lengths of up to 150 mm, above with SL fitting.

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Structure of Titanium Capillaries

Compatible fittings

A few Titanium (Ti) capillaries are for use <u>only</u> with the 1260 Infinity and 1260 Infinity II bio-inert series, as they are only for connections within the pump and between the pump and the autosampler.

These capillaries are used with SwageLok fittings with a gold ferrule, and with removable (SV) fittings.



5005-0067: Gold-plated front ferrule, 1/16 in, 10/pk







Structure of MP35N Capillaries

Compatible fittings

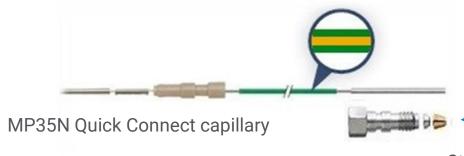
- System capillaries (predefined with fittings)
- Quick Turn capillaries (capillaries without fittings)
- **Quick Connect capillaries**

Suitable fittings:

- **UHP-FF** fittings
- "Standard" stainless steel fittings with gold ferrule
- **Quick Turn fitting**
- Quick Connect fitting with use of MP35N Quick Connect capillaries



MP35N long socket Quick Turn capillary



Stainless steel fittings with gold ferrule

All MP35N free-end capillaries can easily be used with a Quick Turn fitting, unless they are defined as an "M4" fitting.

Part numbers:

5005-0067: Gold-plated front ferrule, 1/16 in, 10/pk 5005-0442: Stainless steel fitting set, includes stainless steel nut, stainless steel back ferrule, and gold-plated front ferrule, 1/16 in, bio, 10/pk



PEEK-coated Fused Silica Capillaries

Compatible fittings

- Especially for micro and nano LC systems
- Most capillaries have a dedicated connection and come with the necessary fittings.
- Outer diameter: 0.8 mm
- Sensitive handling risk of breakage when bending



Suitable fittings:

- 0.8 mm id PEEK fittings
- Depending on the connection goal, a stainless steel 10-32 or M4 thread fitting is necessary



Stainless steel fittings, male (G), 5063-6593



Ferrule and stainless steel lock ring (W), 5065-4423



PEEK fittings, plugs (MP), 5065-4410



Double winged PEEK nut and ferrule (WPF), 5065-4422



PEEk fitting, long (WPFL), 5022-6536

Color code

15 µm id : black

25 um id: yellow

50 um id: green

75 um id: blue

100 um id: black

125 um id: red

Capillaries with M4 Fitting Connections

- To connect to the corresponding multicolumn selection valves or microvalves
- Outer diameter at M4 end: 0.8 mm
- Stainless steel and MP35N available



Capillaries for M4 fittings are marked accordingly with an M, for example, 5067-5110 capillary SST 0.17 x 90 SV/**M**

Labeling of Capillaries and Fittings

	ves some indication on the pri- on, like a loop or a connection	Material The mate material i	erial indicates which raw is used.	The t	ng left/fitting right fitting left/right indicate h fitting is used on both ends e capillary.
Key	Description	Key	Description	Key	Description
Capillary	Connection capillaries	ST	Stainless steel	W	Swagelok + 0.8 mm Port id
Loop	Loop capillaries	Ti	Titanium	S	Swagelok + 1.6 mm Port id
Seat	Autosampler needle seats	PK	PEEK	М	Metric M4 + 0.8 mm Port id
Tube	Tubing	FS/PK	PEEK-coated fused silica ¹	Е	Metric M3 + 1.6 mm Port id
Heat exchanger	Heat exchanger	PK, /ST	Stainless steel-coated PEEK ²	U	Swagelok union
		PTFE	PTFE	L	Long
		FS	Fused silica	Х	Extra long
		MP35N	Nickel-cobalt-chro- mium-molybdenium alloy	Н	Long head
				G	Small head SW 4
				N	Small head SW 5
				F	Finger-tight
				٧	1200 bar
				В	Bio
				Р	PEEK
				1	Intermediate

Fused silica in contact with solvent

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Examples:



Capillary ST 0.12 x X mm SL/M ps/ps



Capillary ST 0.17 x X mm S/S ps/ns

ps: preswaged ns: nonswaged



Stainless steel-coated PEEK

Styles of Fittings







Swagelok

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

Parker

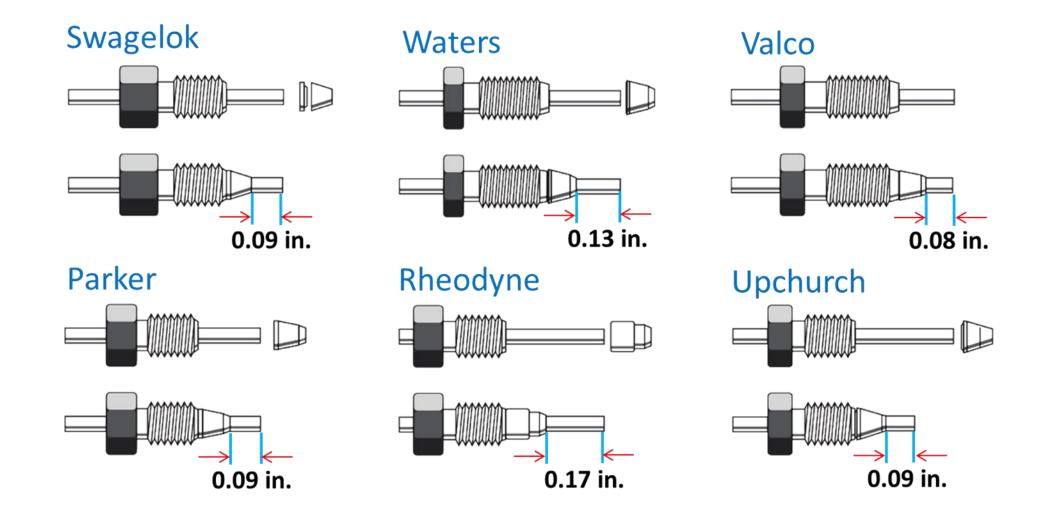
- One-piece ferrule
- Short nut
- Very similar to Swagelok

Waters

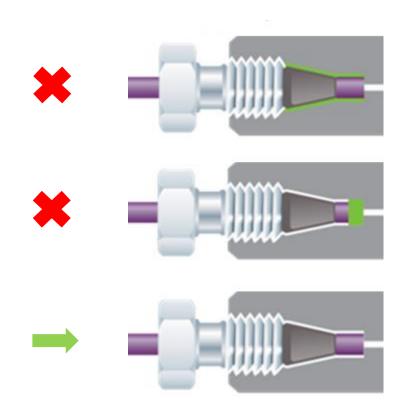
- Longer nut
- Used on Alliance systems



Styles of Fittings



Potential Issues with Fittings

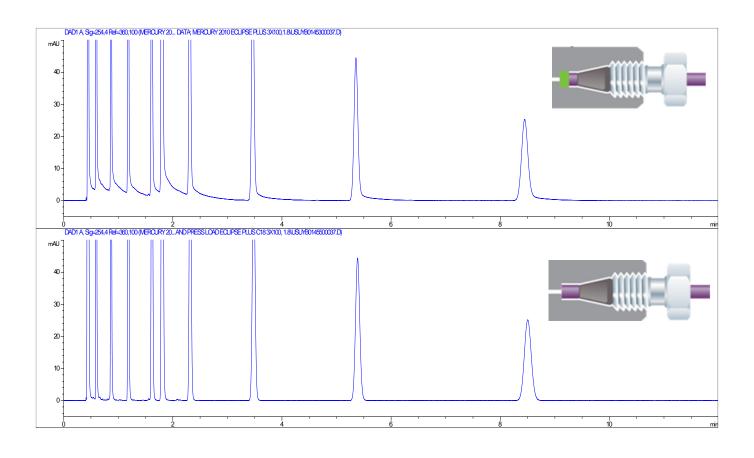


Leak

Peak shape problem

No dead volume

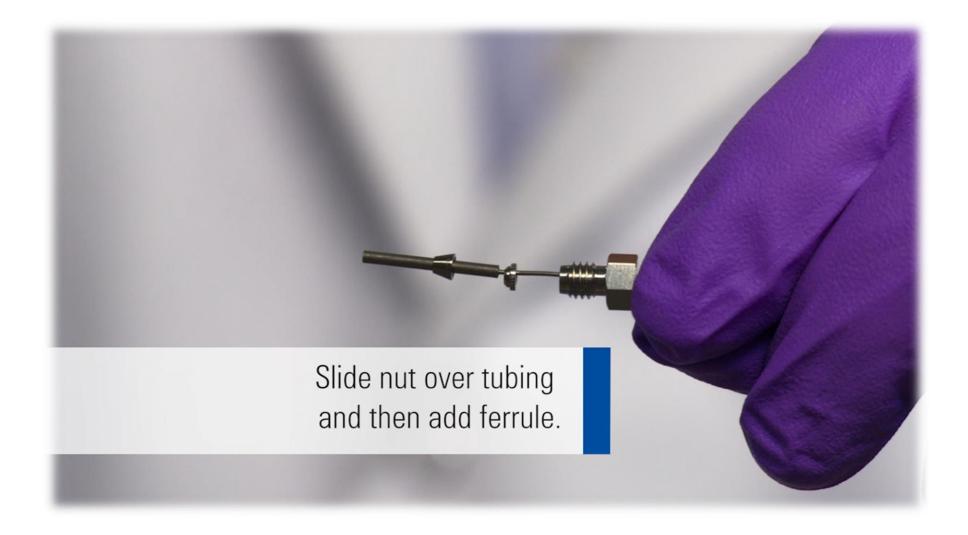
Peak Shape



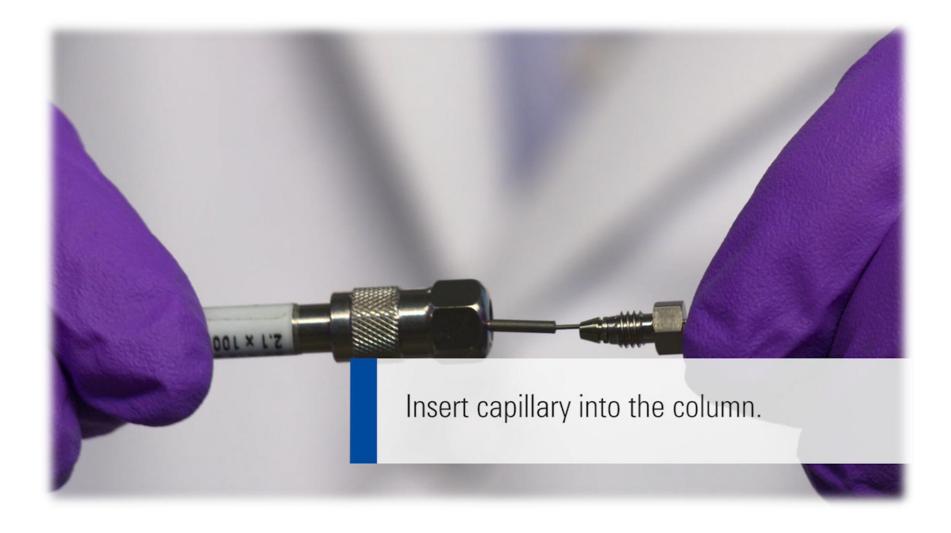


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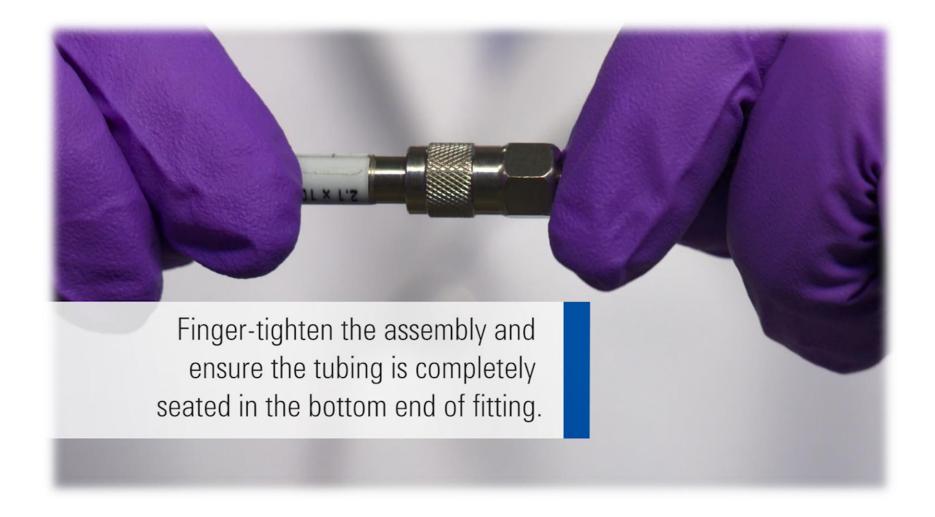
Swaging the SwageLok Fittings



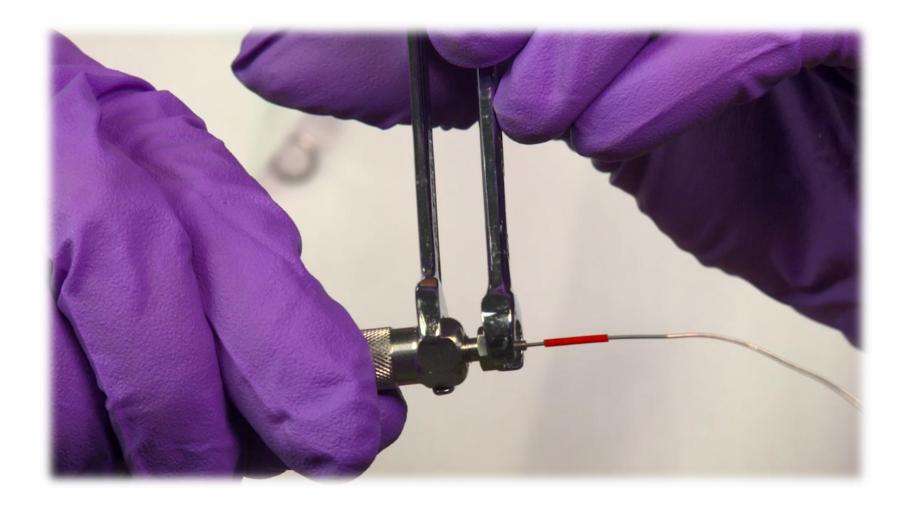
Swaging Fittings



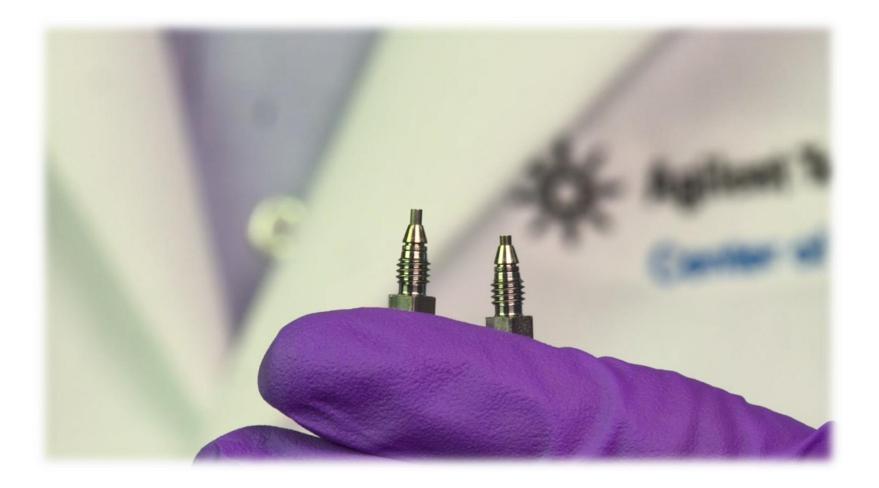
Swaging Fittings



Swaging Fittings

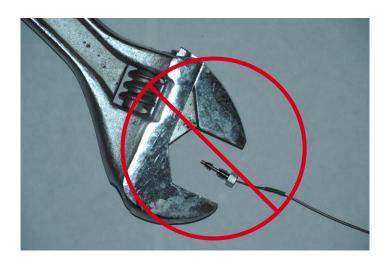


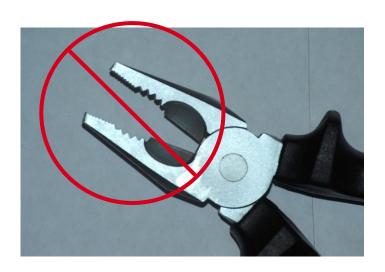
Inspect the Position of the Ferrule



<u>Video</u>: How to properly swage stainless steel fittings onto stainless steel capillary tubing

Tools



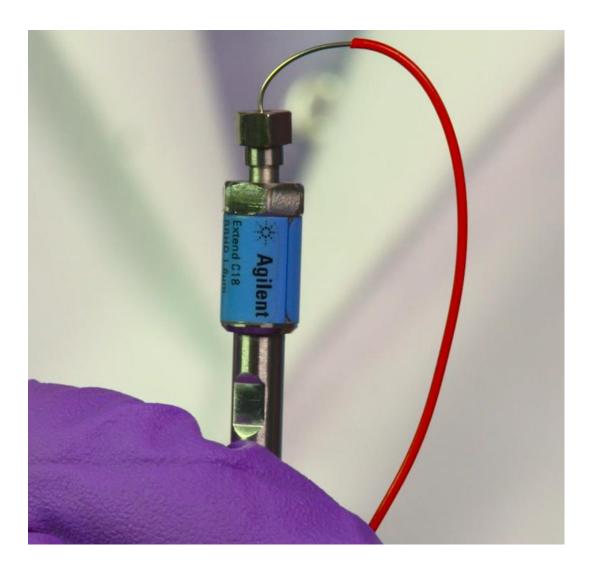




Tightening Fittings into a Column



Overtightened Fittings



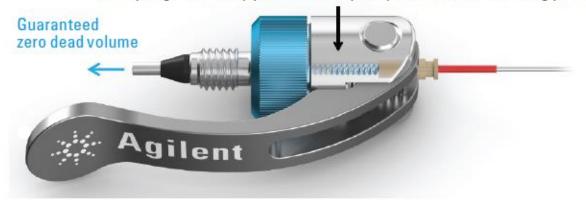


Agilent InfinityLab Fittings: Unique Spring-loaded Design





The spring constantly pushes the capillary towards the receiving port.



InfinityLab Quick Connect fitting, 5067-5965

Quick Connect and Quick Turn

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



InfinityLab Quick Turn fitting, 5067-5966

400 bar (finger-tight, user-dependent) 1300 bar (tightened with a mounting tool (5043-0915)



Flyer: 5991-5164EN



InfinityLab Quick Connect and Quick Turn Fittings

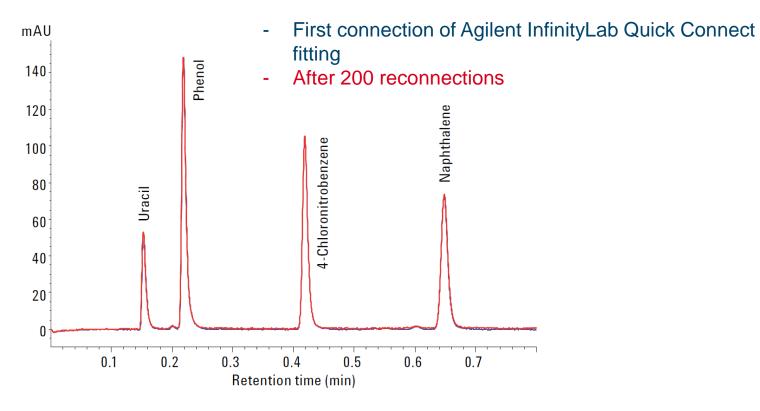




	Quick Connect Fitting	Quick Turn Fitting
Connects to	Columns (or inline filters)	Column, various receiving ports with 10-32 port geometry
Maximum pressure	1300 bar (finger-tight, by turning the lever)	To 400 bar (finger-tight, user-dependent) 1300 bar (with mounting tool, 5043-0915) Bio-inert mounting tool, 5043-0915
Features	 Spring-loaded function for 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
Wetted material	PEEK (ferrule)	PEEK (ferrule)

InfinityLab Fittings Last Longer

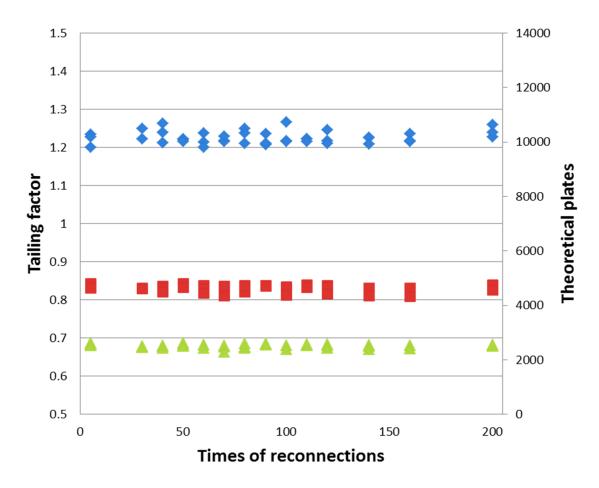
Chromatogram overlap before and after 200 reconnections



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.

Installing the InfinityLab Quick Connect Fitting



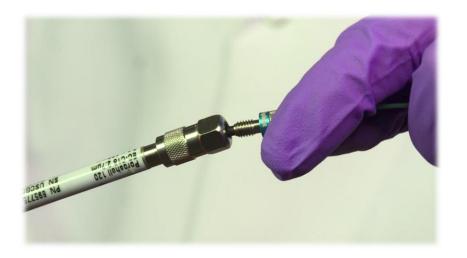
Finger tighten the fitting until you feel resistance, then close the lever.

Leak tight to 1300 bar

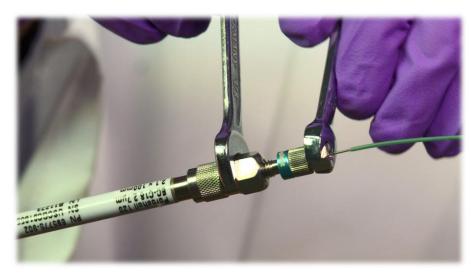
Agilent C

<u>Video</u>: Making Great Connections – Less stress, more reliable fittings

Installing InfinityLab Quick Turn Fitting



Finger tighten for 400 bar (user-dependent)



Tighten using a wrench/mounting tool for 1300 bar



Mounting tool, p/n 5043-0915

Not Just for Agilent Columns



Connecting Capillaries for Each LC System



1100/1200/1260 Series System

Connection	p/n	Description	
Solvent bottle to vacuum degasser	G1311-60003	Bottle head assembly for screw bottle (GL45), with glass filter, 20 µm (5041-2168)	5
Degasser to pump	G1322-67300	Tubing kit degasser, 300 mm tubing, 4/pk	١
Pump to autosampler	G1312-87303	Capillary, 0.17 mm x 400 mm	(
Pump (purge valve) to waste	5062-2461	PTFE tube, 5000 mm	ı
Autosampler to column compartment	G1313-87305 G1313-87304	Capillary, 0.17 mm x 180 mm Capillary, 0.12 mm x 180 mm	(
Thermostatted ALS to column compartment	01090-87306 01090-87610	Capillary, 0.17 mm x 380 mm Capillary, 0.12 mm x 280 mm	
Column compartment to column	G1316-87300 01090-87611	Capillary, 0.17 mm x 90 mm Capillary, 0.12 mm x 105 mm	
Column to VWD (std flow cell)	5062-8522	Inlet tubing assembly PEEK, 0.17 mm x 600 mm	
Column to DAD/MWD	G1315-87311 G1315-87312	Capillary, 0.17 mm x 380 mm (S/S, ps/ns) Capillary, 0.12 mm x 150 mm	,
VWD to waste	5062-8535	Waste accessory kit	
DAD to waste	5062-2462	PTFE tubing 0.7 mm id, 1.6 mm od, 5 m	

0.17 mm id capillaries	Standard setup	
0.12 mm id capillaries	Rapid Resolution LC setup	

Solvent cabinet

Vacuum degasser

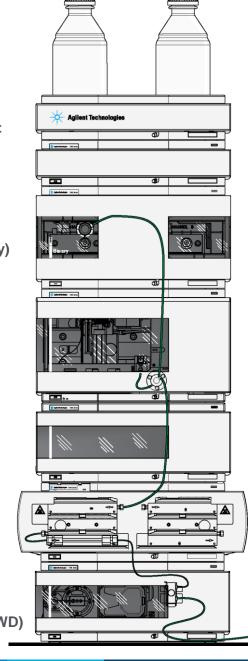
Pump (Iso/quat/binary)

Autosampler

Sampler thermostat

Column compartment

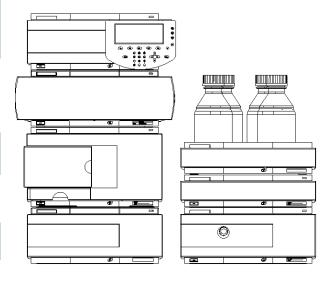
UV-detector (DAD/MWD/VWD)



1100/1200/1260 Infinity Series System

Dual – stack configuration with cooled ALS

Connection		p/n	Description	
Solvent bottle to vacuum degasser	G1311-60003		Bottle head assembly for screw bottle (GL45), with glass filter, 20 µm (5041-2168)	
Degasser to pump	G13	22-67300	Tubing kit degasser, 300 mm tubing, 4/pk	
Pump to autosampler	G13	12-87304	Capillary, 0.17 mm x 700 mm	
Pump (purge valve) to waste	5062	2-2461	PTFE tube, 5000 mm	
Thermostatted ALS to column compartment		90-87309 90-87610	Capillary, 0.17 mm x 380 mm Capillary, 0.12 mm x 280 mm	
Column compartment to column	G1316-87300 01090-87611		Capillary, 0.17 mm x 90 mm Capillary, 0.12 mm x 105 mm	
Column to VWD (std flow cell)	5062-8522		Inlet tubing assembly PEEK, 0.17 mm x 600 mm	
Column to DAD/MWD	G1315-87311 G1315-87312		Capillary, 0.17 mm x 380 mm Capillary, 0.12 mm x 150 mm	
VWD to waste	5062-8535		Waste accessory kit	
DAD to waste	DAD to waste 5062-2462		PTFE tubing, 0.7 mm id, 1.6 mm od, 5 m	
0.17 mm id capillaries Standard setu		Standard set	ир	
0.12 mm id capillaries Rapid Resolu		Rapid Resolu	ution LC setup	

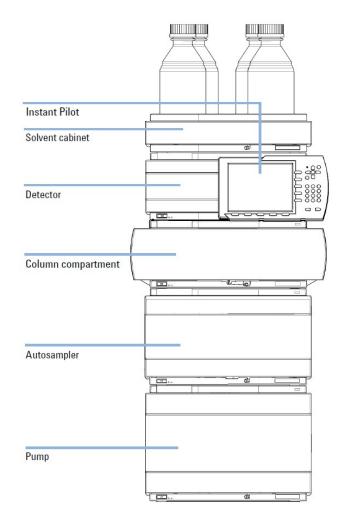




1290 Infinity Series System

Connection	p/n	Description
Solvent bottle to pump	G7120-60007	Bottle head assembly for screw bottle (GL45), with glass filter 20 µm (5041-2168)
Pump to autosampler	5067-4657	Capillary, 0.17 mm x 300 mm
Autosampler to column compartment	5067-4659	Capillary, 0.12 mm x 340 mm
Column compartment to column	5500-1188	Capillary, 0.12 mm x 105 mm (capillary comes without fittings, use Quick Turn fittings or SST fittings)
Column to DAD	5067-4660	Capillary, 0.12 mm x 280 mm
DAD to waste	5062-2462	PTFE tubing, 0.7 mm id, 1.6 mm od, 5 m

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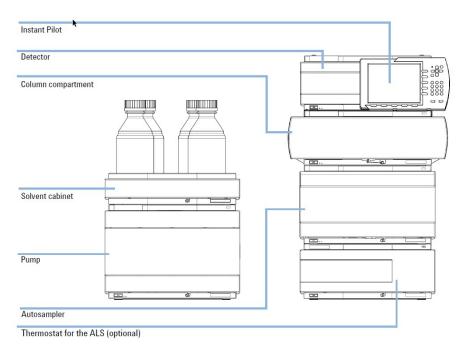




1290 Infinity Series System

Dual – stack configuration with cooled ALS

Connection p/n		Description	
Solvent bottle to vacuum degasser	G7120-60007	Bottle head assembly for screw bottle (GL45), with glass filter, 20 µm (5041-2168)	
Pump to autosampler	5500-1217	Capillary, 0.17 mm x 900 mm	
Autosampler to column compartment	5067-4659	Capillary, 0.12 mm x 340 mm	
Column compartment to column (low dispersion heat exchanger double)	5500-1188	Capillary, 0.12 mm x 105 mm (capillary comes without fittings, use Quick Turn fittings or SST fittings)	
Column to DAD	5067-4660	Capillary, 0.12 mm x 280 mm	
DAD to waste	5062-2462	PTFE tubing, 0.7 mm id, 1.6 mm od, 5 m	



1260/1290 Infinity II Series System

Single stack with multisampler and MCT

Connection	p/n	Description	
Solvent bottle to pump	G7120-60007	Bottle head assembly for screw bottle (GL45), with glass filter, 20 µm (5041-2168)	
Pump to multisampler	5500-1246	Capillary, 0.17 mm x 500 mm (SI/SI, ps/ps)	
Multisampler to MCT	5500-1157	Capillary, 0.12 mm x 500 mm, (SI/SI, ps/ns)	
Heat exchanger to column	5067-5957	Quick Connect assembly, 0.12 mm x 105 mm	
	5500-1173	Quick Connect capillary, 0.12 mm x 105 mm	
	5067-5965	Quick Connect fitting	
Column to DAD	5500-1191	Quick Turn capillary, 0.12 mm x 280 mm (comes without fitting)	
	5067-5966	Quick Turn fitting	
DAD to waste	5062-2462	PTFE tubing, 0.7 mm id, 1.6 mm od, 5 m	

DE-003199

Solvent cabinet

DAD

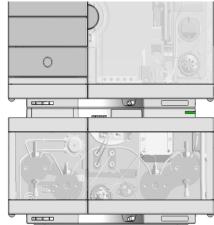


Multicolumn thermostat (MCT)

Multisampler



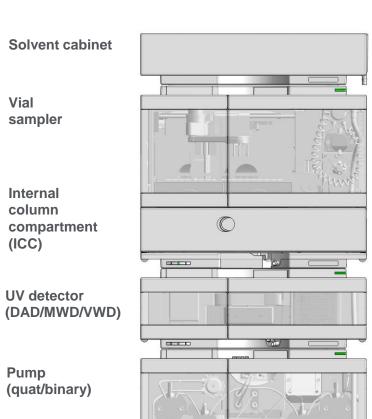
Pump (flex/high speed)



1260 Infinity II Series System

Single stack with vialsampler and internal column compartment

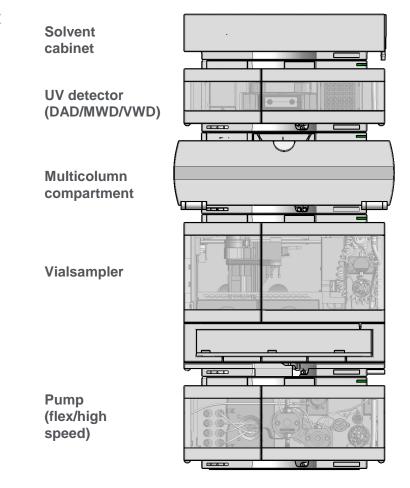
	Connection	p/n	Description
	Solvent bottle to pump	G7120-60007	Bottle head assembly for screw bottle (GL45), with glass filter, 20 µm (5041-2168)
S	Pump to vialsampler	5500-1217	Capillary, 0.17 mm x 900 mm (SI/SX, ps/ps)
SI	 Sampler to 3 µL heat exchanger 6 µL heat exchanger	5500-1249 5500-1250	Capillary, 0.12 mm x 120 mm, (SL/SL, ps,ns) Capillary, 0.17 mm x 120 mm (SL/SL, ps,ns)
SL	 Heat exchanger to column		
SX	 3 μL heat exchanger 6 μL heat exchanger	5500-1238 5500-1240	Capillary, 0.12 mm x 105 mm (SL/SL, ps,ps) Capillary, 0.17 mm x 105 mm (SL/SL, ps,ps)
M4	 Column to DAD	5500-1191	Quick Turn capillary, 0.12 mm x 280 mm (comes without fitting)
	DAD to waste	5062-2462	PTFE tubing, 0.7 mm id, 1.6 mm od, 5 m



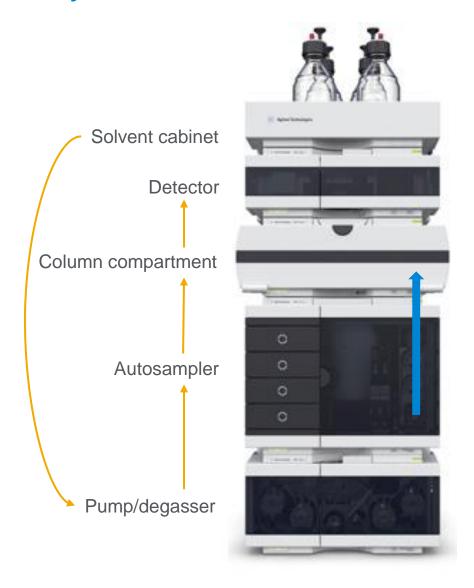
1260/1290 Infinity II Series System

Single stack with vialsampler and multicolumn thermostat

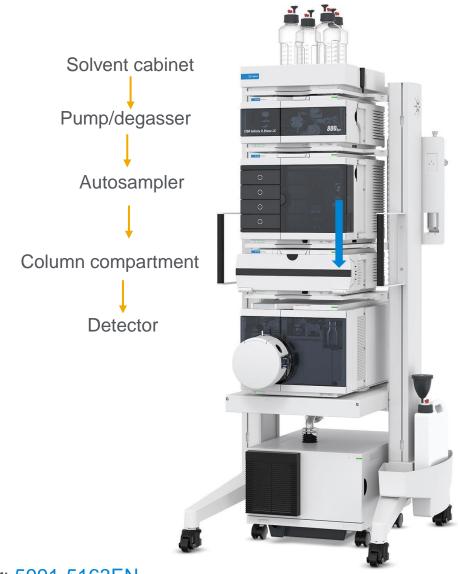
	Connection	p/n	Description	
	Solvent bottle to pump	G7120-60007	Bottle head assembly for screw bottle (GL45), with glass filter, 20 µm (5041-2168)	
	Pump to vialsampler	5500-1245	Capillary, 0.17 mm x 400 mm (SI/SX, ps/ps)	
	Sampler to heat exchanger	5500-1157	Capillary, 0.12 mm x 500 mm, (SL/SL, ps,ns)	
	Heat exchanger to column	5067-5957	Quick Connect assembly, 0.12 mm x 105 mm	
-		5500-1173	Quick Connect capillary, 0.12 mm x 105 mm	
		5067-5965	Quick Connect fitting	
	Column to DAD	5500-1191	Quick Turn capillary, 0.12 mm x 280 mm (comes without fitting)	
		5067-5966	InfinityLab Quick Turn fitting	
	DAD to waste	5062-2462	PTFE tubing, 0.7 mm id, 1.6 mm od, 5 m	
		Solvent bottle to pump Pump to vialsampler Sampler to heat exchanger Heat exchanger to column Column to DAD	Solvent bottle to pump Pump to vialsampler 5500-1245 Sampler to heat exchanger Heat exchanger 5067-5957 to column 5500-1173 5067-5965 Column to DAD 5500-1191 5067-5966	



InfinityLab Flex Benches



LC Capillaries and Fittings



Flyer: <u>5991-5163EN</u>



Resources on the Web

InfinityLab fittings on Agilent.com

InfinityLab LC Supplies catalog

Product catalog

Less Stress. More Reliable Connections.

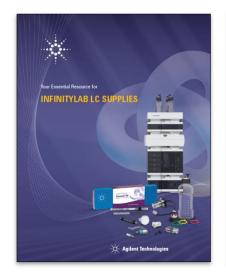
Product flyer

Agilent Bio-inert Capillaries and Fittings flyer

Product overview

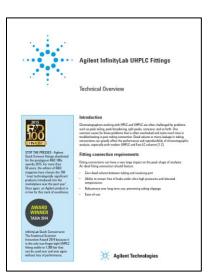
Agilent InfinityLab UHPLC Fittings

Technical overview











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

Option 4 for spectroscopy supplies

Option 5 for chemical standards

Option 6 for former Prozyme products

Available in the U.S. 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!

