Agilent InfinityLab UHPLC Fittings

Technical Overview

STOP THE PRESSES - Agilent Quick Connect fittings shortlisted for the prestigious R&D 100s awards 2015. For more than 50 years, the editors of R&D magazine have chosen the 100 "most technologically significant products introduced into the marketplace over the past year". Once again, an Agilent product is in line for this mark of excellence.

InfinityLab Quick Connect won The Analytical Scientist Innovation Award 2014 because it is the only true finger-tight UHPLC fitting stable to 1,300 bar that can be used over and over again without loss of performance.

Introduction

Chromatographers working with HPLC and UHPLC are often challenged by problems such as peak tailing, peak broadening, split peaks, carryover, and so forth. One common cause for those problems that is often overlooked and costs much time in troubleshooting is poor tubing connection. Dead volume or micro-leakage in tubing connections can greatly affect the performance and reproducibility of chromatographic analysis, especially with modern UHPLC and Fast LC columns [1-2].

Fitting connection requirements

Fitting connections can have a very large impact on the peak shape of analytes. An ideal fitting connection should feature:

- Zero dead volume between tubing and receiving port
- Ability to remain free of leaks under ultra-high pressures and elevated temperatures
- Robustness over long-term use, preventing tubing slippage
- Ease-of-use

Agilent Technologies

Existing products

Nonadjustable metallic fittings

Most commonly used fittings in UHPLC are nonadjustable 2-piece or 3-piece metallic fittings, which are permanent and nonadjustable after they have been assembled [3]. Since different manufacturers of column hardware use different designs for column end fittings (Figure 1), a new set of tubing and fittings should be swaged for every brand of column. This ensures that the stem length, namely the length between the bottom of the ferrule and the end of the tubing, is perfectly matched (Figure 2C).

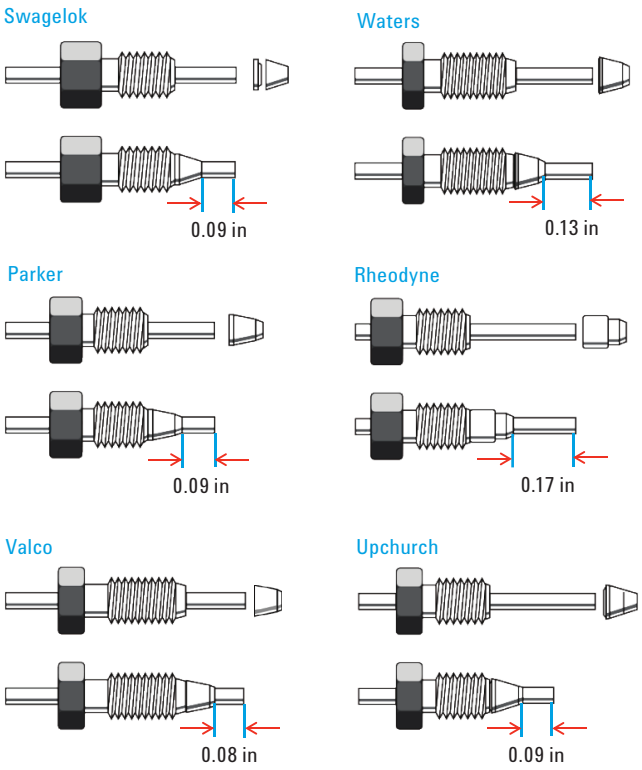

Figure 1. Common HPLC connectors from different manufacturers.

If the stem length is too short, a dead volume is created, resulting in deterioration of peak shape, lower resolution, and carryover (Figures 2B and 3). If the stem length is too long, the ferrule will not seat properly and leakage will occur (Figure 2A). In addition, conventional fittings and ferrules are often over-tightened when wrenches are used, resulting in the fitting getting permanently stuck in the column.

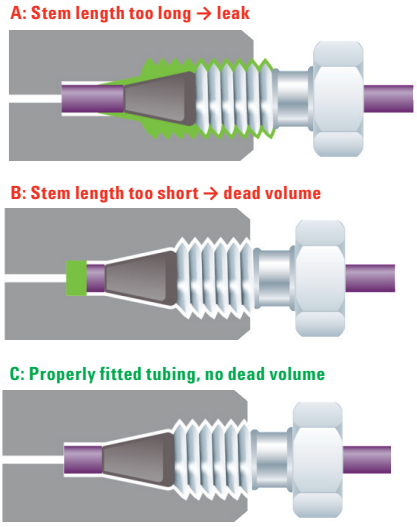

Figure 2. Comparison of correct and incorrect fitting connections.

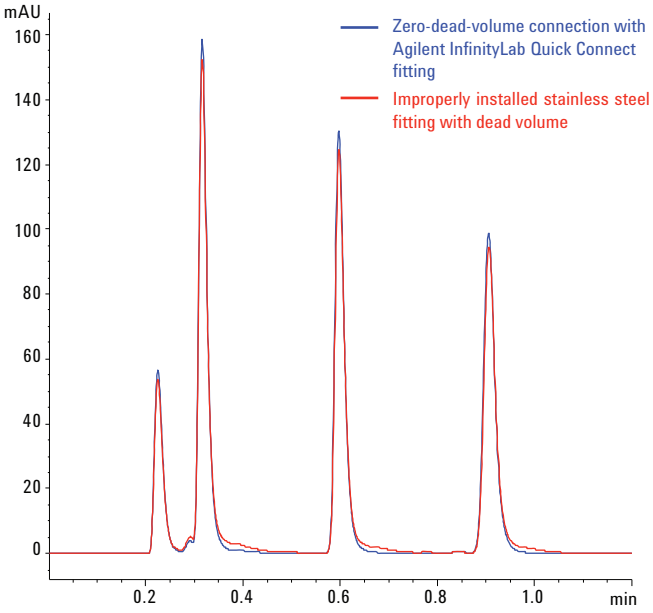

Figure 3. The Agilent InfinityLab Quick Connect fitting delivers zero dead volume with improved peak shape.

Adjustable finger-tight fittings

To solve the problems of conventional fittings, adjustable finger-tight fittings have been developed that are compatible with different columns. These fittings usually have polymer (for example, PEEK) ferrules, which make the fittings reusable because the ferrule is not permanently attached to the tubing. However, many of them still have some drawbacks, such as:

- The inability to reach 1,300 bar ultra-high pressures without tools
- The need to follow strict guidelines on the exact torque or range of turning angle to avoid over-tightening
- The need to check for leaks every time after reconnection
- The fitting often has to be retightened.
- If the polymeric ferrule does not grip the tubing strongly enough, it could slip off the receiving port at ultra-high pressures or pressure cycling, leading to the creation of dead volume.

Agilent InfinityLab fittings

Agilent InfinityLab UHPLC fittings avoid these drawbacks, enabling a reproducible and leak-free column connection. There are two types of InfinityLab fittings. The InfinityLab Quick Connect fitting (Figure 4A) is for column connections with 1,300 bar sealability without the need for a wrench. The InfinityLab Quick Turn fitting (Figure 4B) is for various flow path connections, including column inlet/outlet, valve, and other connections. This fitting seals up to 600 bar by finger tightening (depending on users and positions of connection) and to 1,300 bar with a wrench.

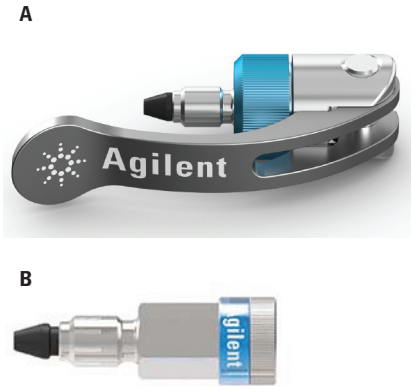

Figure 4. Agilent InfinityLab fittings.
A) Quick Connect fitting, B) Quick Turn fitting.

Both types of fittings have a novel spring-loaded design (Figure 5) that constantly pushes the tubing against the receiving port, delivering a reproducible connection with no dead volume for consistent chromatographic performance. The stem length is adjustable through the spring, which makes both fittings compatible with all types of LC columns. In addition, the InfinityLab Quick Connect fitting has a unique lever-actuated design, so that the spring assembly, including the lever, applies a constant force that presses the ferrule onto the tubing, avoiding tubing slippage. Little force is required to tighten the fitting to 1,300 bar (18,850 psi) without any tools. One merely tightens the nut by hand until feeling the first resistance, then depresses the lever (Figure 6).

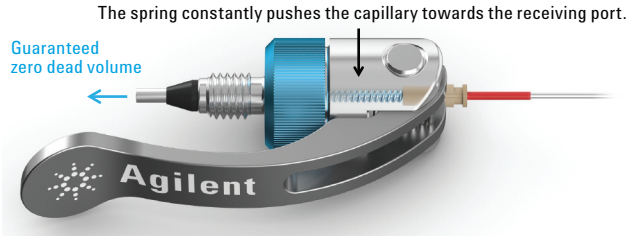

Figure 5. Unique spring-loaded design of Agilent InfinityLab fittings.

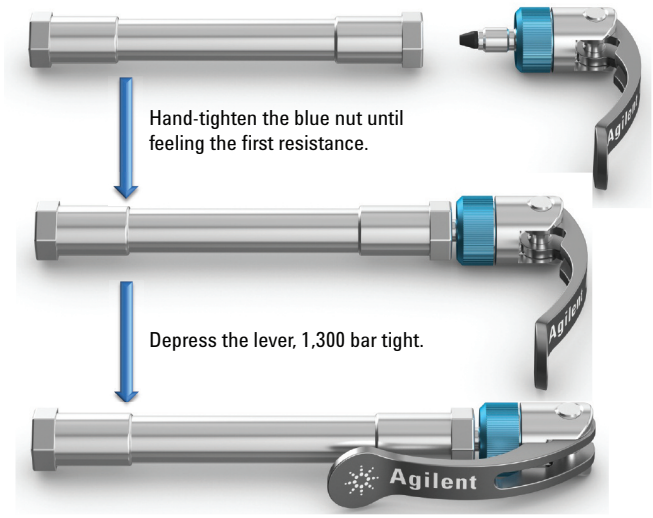

Figure 6. Installation of Agilent InfinityLab Quick Connect fitting.

Experimental

The performance of Agilent InfinityLab fittings was assessed using an Agilent 1290 Infinity Binary LC. For robustness tests, the tested fitting (Agilent InfinityLab Quick Connect fitting or non-Agilent UHPLC finger-tight fitting) was connected at the column inlet. For compatibility testing InfinityLab fittings with different column brands, an InfinityLab Quick Connect fitting was connected at the column inlet, and InfinityLab Quick Turn fittings (p/n 5067-5966) were used at the column outlet and detector. A mixture of uracil (10 µg/mL), phenol (200 µg/mL), 4-chloronitrobenzene (25 µg/mL), naphthalene (40 µg/mL) in water:acetonitrile 40:60 (v/v) was used as analyte.

Conditions to assess robustness and usability

Column:	Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 × 100 mm, 1.8 µm (p/n 959758-902)
Mobile phase:	A) Water B) Acetonitrile; A:B 40:60 isocratic
Flow rate:	1.4 mL/min (system pressure 1,100 bar)
Injection volume:	1 µL
Column temperature:	40 °C
DAD wavelength:	254 nm/4 nm, ref 400 nm/100 nm

Conditions to assess compatibility with different column brands

Columns:	C18 phase, 2.1 × 50 mm, sub-2 µm columns from different vendors
Mobile phase:	A) Water B) Acetonitrile; A:B 45:55 isocratic
Flow rate:	1.2 mL/min (system pressure 600 to 1,000 bar depending on column)
Injection volume:	1 µL
Column temperature:	25 °C
DAD wavelength:	254 nm/4 nm, ref 400 nm/100 nm

Results and Discussion

Robustness over 200 reconnections

To assess the reusability and robustness of the InfinityLab Quick Connect fitting, the fitting was disconnected and reconnected 200 times. Chromatograms before and after 200 reconnections are compared in Figure 7. It can be seen that there was no visible change in the chromatogram after 200 reconnections. The tailing factor of the peak with a long retention time (naphthalene) and theoretical plate numbers of the peaks with small retention factors (phenol and uracil) were monitored. They are the most sensitive measure for peak-broadening effects through extra-column volume or micro-leakage. Figure 8 demonstrates that the tailing factors and theoretical plates stayed constant within experimental variables. This indicated that the fitting connection remained free of dead volume and leaks after 200 reconnections.

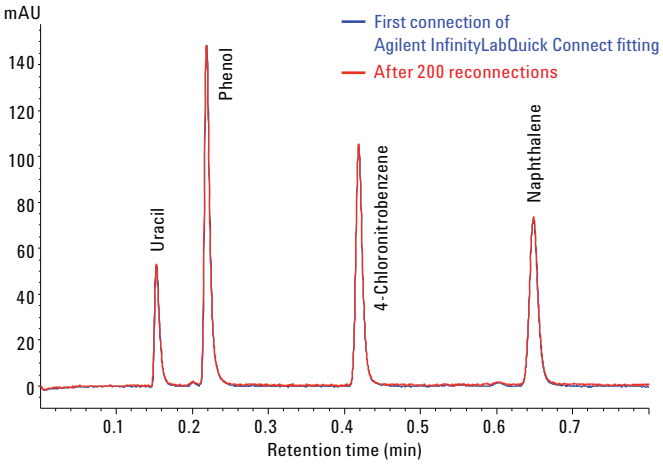

Figure 7. Comparison of chromatograms before and after 200 reconnections of an Agilent InfinityLab Quick Connect fitting.

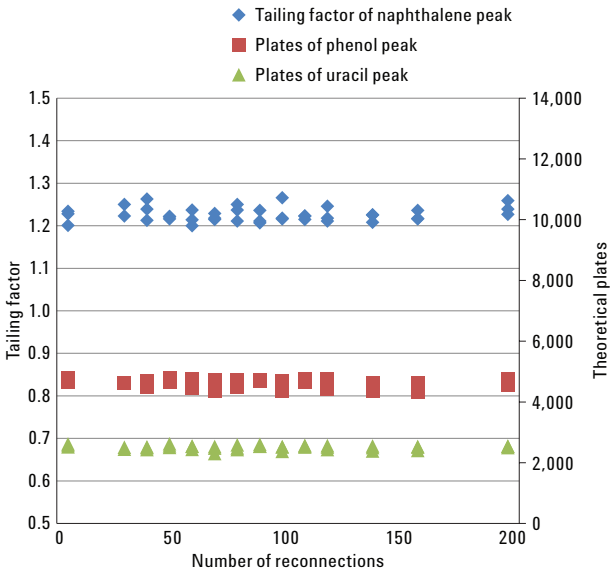

Figure 8. Monitoring tailing factor and the number of theoretical plates over 200 reconnections of the Agilent InfinityLab Quick Connect fitting.

Better robustness compared to fitting from another vendor

The same experiment was repeated using a reusable finger-tight UHPLC fitting with a polymeric ferrule from another vendor. After only 30 reconnections, the polymeric ferrule became locked onto the capillary and could not be adjusted, resulting in dead volume, as illustrated in Figure 2B. This was also confirmed by comparing the chromatograms in Figure 9, showing that deterioration of peak shape, including larger peak tailing and lower peak height, was evident after 30 reconnections.

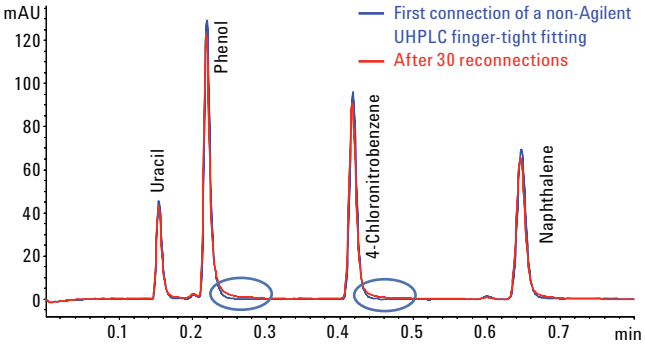

Figure 9. Comparison of chromatograms before and after 30 reconnections of a UHPLC finger-tight fitting from another vendor.

Compatibility with different column brands

Fitting connection design varies between different column manufacturers, and improper stem length of the fitting could cause leaks or poor peak shape. To evaluate the compatibility of Agilent InfinityLab fittings with different column brands, columns from three column manufacturers, Waters, Phenomenex, and Supelco, were connected using InfinityLab fittings (Quick Connect fitting at the entrance and Quick Turn fittings at the exit and detector). The same set of InfinityLab fittings and tubing was used for all columns. If common stainless steel fittings are used, a new set of fittings and tubing is needed for each column brand to fit the geometry of the column connection. The chromatograms with InfinityLab fittings were compared to those with properly swaged stainless steel fittings in Figure 10. No visible difference was observed, which indicates that the InfinityLab fittings work perfectly with all the tested column brands.

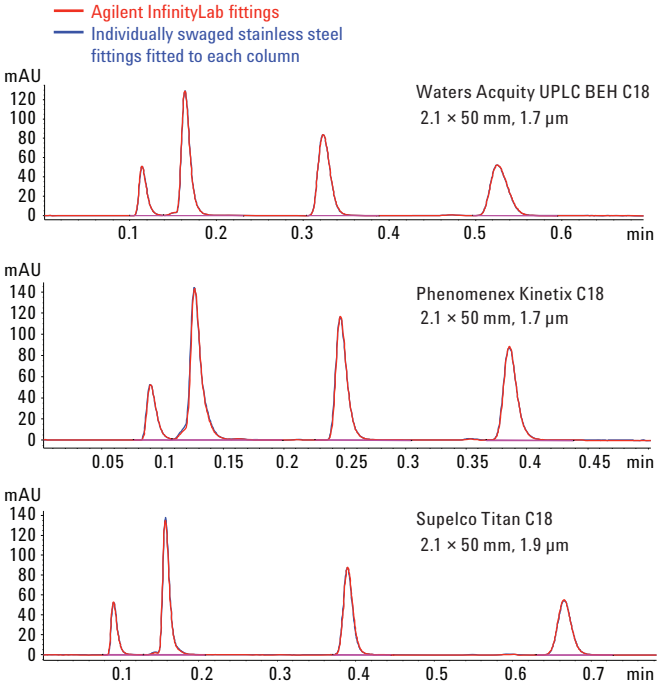

Figure 10. Compatibility of Agilent A-Line fittings with columns from different vendors.

Conclusions

Agilent InfinityLab fittings deliver usability, robustness, and UHPLC performance, with:

- Excellent chromatographic performance in terms of tailing factor, number of theoretical plates, and peak height that remained constant through 200 reconnections of the fitting
- Zero-dead-volume connection guaranteed by the unique spring-loaded design
- Compatibility with common column brands
- Ease-of-use

References

1. Rogatsky, E.;, Shaynah, B.; Cai, M.; Daniel, T. S. Optimizing UHPLC Fittings and Connections: A Case Study. *J. Chromatogr. Sep. Techniq*. **2013**, *4*, 193. doi: 10.4172/2157-7064.1000193.
2. Fountain, K. J.; Neue, U. D.; Grumbach, E. S.; Diehl, D. M. Effects of extra-column band spreading, liquid chromatography system operating pressure, and column temperature on the performance of sub-2-µm porous particles. *J. Chromatogr. A* **2009**, *1216*, 5979-5988.
3. Majors, R. E. Fittings and Tubing for Ultrahigh-Pressure Liquid Chromatography. *LCGC North America* **2014**, *32*, 840-853.

Please watch the product video for more details

http://www.chem.agilent.com/en-US/products-services/Columns-Sample-Preparation/LC-LC-MS-Columns/Pages/greatconnections.aspx

Ordering information for Agilent InfinityLab Quick Connect fittings

InfinityLab Quick Connect Fittings	Part no.
Assemblies	
Stainless steel, 0.075 × 105 mm	5067-5961
Stainless steel, 0.075 × 150 mm	5067-6163
Stainless steel, 0.075 × 220 mm	5067-6164
Stainless steel, 0.075 × 280 mm	5067-6165
Stainless steel, 0.12 × 105 mm	5067-5957
Stainless steel, 0.12 × 150 mm	5067-5958
Stainless steel, 0.12 × 220 mm	5067-5959
Stainless steel, 0.12 × 280 mm	5067-5960
Stainless steel, 0.17 × 105 mm	5067-6166
Stainless steel, 0.17 × 150 mm	5067-6167
Stainless steel, 0.17 × 220 mm	5067-6168
Stainless steel, 0.17 × 280 mm	5067-6169
Stainless steel, 0.25 × 105 mm with a female connection	5067-6210

NOTE: Each assembly is equipped with a Quick Connect Fitting, a capillary, and a Swagelok fitting, or with a female connection where specified.

Replacement parts	
Quick Connect LC Fitting	5067-5965
Front ferrule	5043-0924
Capillary stainless steel, 0.075 × 105 mm	5500-1174
Capillary stainless steel, 0.075 × 150 mm	5500-1175
Capillary stainless steel, 0.075 × 220 mm	5500-1176
Capillary stainless steel, 0.075 × 250 mm	5500-1177
Capillary stainless steel, 0.075 × 280 mm	5500-1178
Capillary stainless steel, 0.12 × 105 mm	5500-1173
Capillary stainless steel, 0.12 × 150 mm	5500-1172
Capillary stainless steel, 0.12 × 220 mm	5500-1171
Capillary stainless steel, 0.12 × 280 mm	5500-1170
Capillary stainless steel, 0.12 × 400 mm	5500-1179
Capillary stainless steel, 0.12 × 500 mm	5500-1180
Capillary stainless steel, 0.17 × 105 mm	5500-1181
Capillary stainless steel, 0.17 × 150 mm	5500-1182
Capillary stainless steel, 0.17 × 220 mm	5500-1183
Capillary stainless steel, 0.17 × 280 mm	5500-1230
Capillary stainless steel, 0.17 × 500 mm	5500-1231
Stainless steel, 0.25 × 105 mm with a female connection	5500-1258
Stainless steel, 0.25 × 150 mm	5500-1259
Stainless steel, 0.25 × 400 mm	5500-1260

NOTE: The InfinityLab Quick Connect fitting can only be equipped with an InfinityLab capillary specified in this table. The InfinityLab capillary is designed with a spring and a holder.

Ordering information for Agilent InfinityLab Quick Turn fittings

InfinityLab Quick Turn parts	Part no.
Fittings and ferrules	
Quick Turn UHPLC fitting	5067-5966
Front ferrule	5043-0924
Capillaries	
Stainless steel, 0.075 ×105 mm long socket	5500-1198
Stainless steel, 0.075 ×150 mm long socket	5500-1232
Stainless steel, 0.12 × 105 mm long socket	5500-1188
Stainless steel, 0.12 × 150 mm long socket	5500-1189
Stainless steel, 0.12 × 180 mm long socket	5500-1233
Stainless steel, 0.12 × 200 mm long socket	5500-1190
Stainless steel, 0.12 × 280 mm long socket	5500-1191
Stainless steel, 0.12 × 500 mm long socket	5500-1192
Stainless steel, 0.17 × 105 mm long socket	5500-1193
Stainless steel, 0.17 × 150 mm long socket	5500-1194
Stainless steel, 0.17 × 180 mm long socket	5500-1234
Stainless steel, 0.17 × 200 mm long socket	5500-1195
Stainless steel, 0.17 × 280 mm long socket	5500-1196
Stainless steel, 0.17 × 380 mm long socket	5500-1235
Stainless steel, 0.17 × 400 mm long socket	5500-1236
Stainless steel, 0.17 × 500 mm long socket	5500-1197
Stainless steel, 0.17 × 700 mm long socket	5500-1237
Stainless steel, 0.25 × 105 mm long socket with a female connection	5500-1261
Stainless steel, 0.25 × 150 mm long socket	5500-1262
Stainless steel,0.25 × 400 mm long socket	5500-1263

NOTE: InfinityLab Quick Turn fittings require the capillaries specified in this table.

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Printed in the USA
July 1, 2017
5991-5525EN

Agilent Technologies