# Agilent A-Line UHPLC Fittings

## Technical Overview

### Introduction

Chromatographers working with HPLC and UHPLC are often challenged by problems such as peak tailing, peak broadening, split peaks, carryover, etc. 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

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## 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).

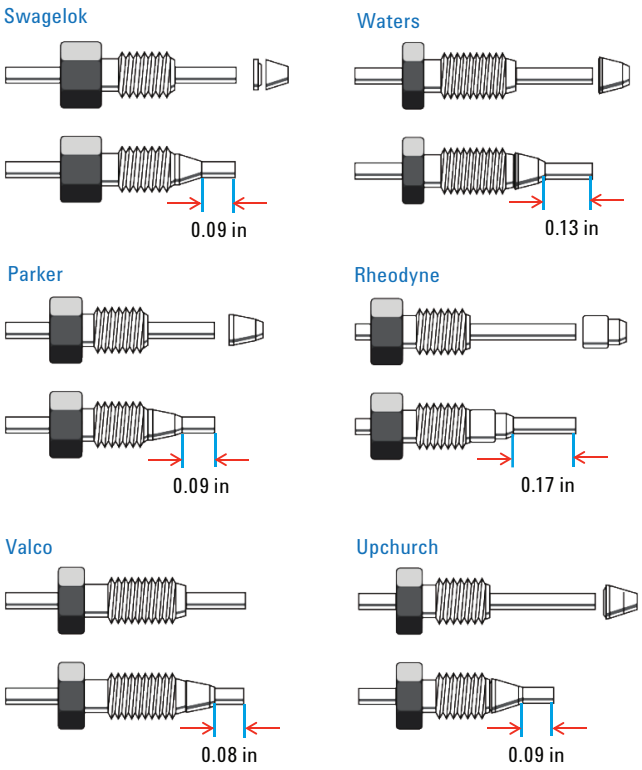


Figure 1. Common HPLC connectors from different manufacturers.

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.

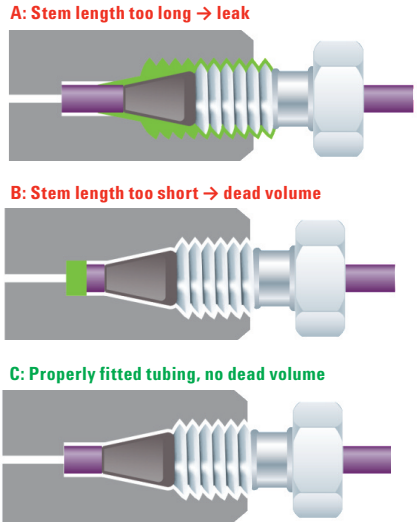


Figure 2. Comparison of correct and incorrect fitting connections.

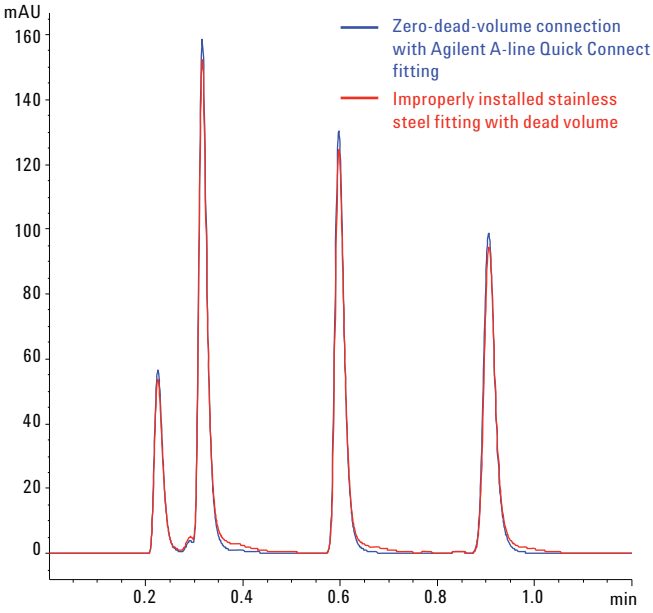


Figure 3. The Agilent A-Line Quick Connect fitting delivers zero dead volume with improved peak shape.

## 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:

- 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 creation of a dead volume

## Agilent A-Line fittings

Agilent A-Line UHPLC fittings avoid these drawbacks, enabling reproducible and leak-free column connection. There are two types of A-Line fittings. The A-Line Quick Connect fitting (Figure 4A) is for column connections with 1,300 bar sealability without the need for a wrench. The A-Line 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.

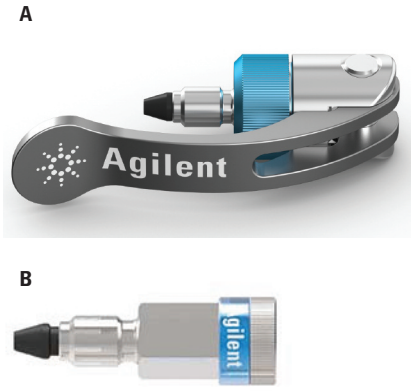


Figure 4. Agilent A-Line fittings. A) Quick Connect fitting, B) Quick Turn fitting.

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. Stem length is adjustable through the spring, which makes both fittings compatible with all types of LC columns. In addition, the A-Line 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 the need for any tools. One merely needs to tighten the nut by hand until feeling the first resistance, and then depresses the lever (Figure 6).

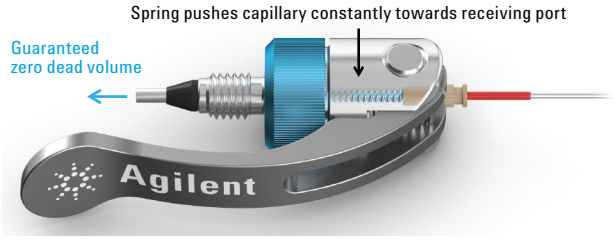


Figure 5. Unique spring-loaded design of Agilent A-Line fittings.

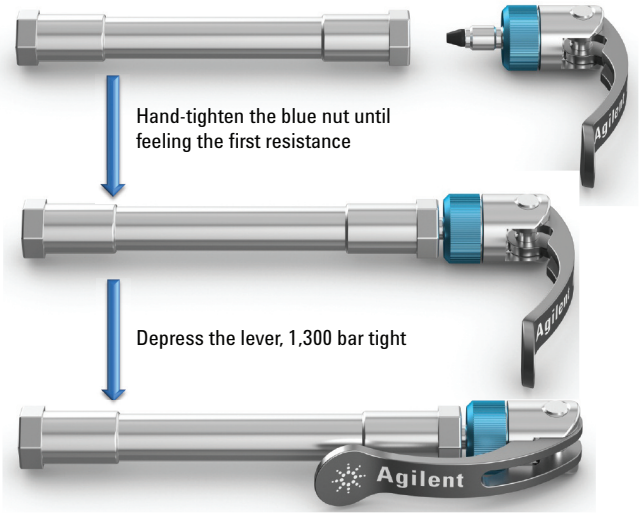


Figure 6. Installation of Agilent A-Line Quick Connect fitting.

## Experimental

The performance of Agilent A-Line fittings was assessed using an Agilent 1290 Infinity Binary LC. For robustness tests, the tested fitting (Agilent A-Line Quick Connect fitting or non-Agilent UHPLC finger-tight fitting) was connected at the column inlet. For compatibility testing A-line fittings with different column brands, an A-Line Quick Connect fitting was connected at column inlet, and A-line Quick Turn fittings were used at 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<br>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<br>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 Agilent A-Line 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, which indicated that the fitting connection remained free of dead volume and leaks after 200 reconnections.

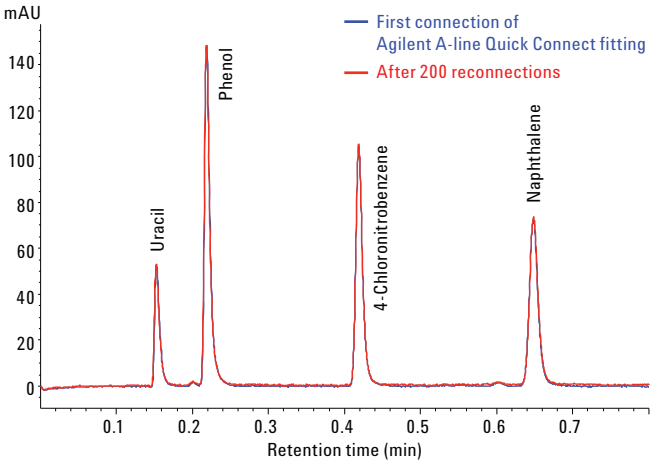


Figure 7. Comparison of chromatograms before and after 200 reconnections of an Agilent A-Line Quick Connect fitting.

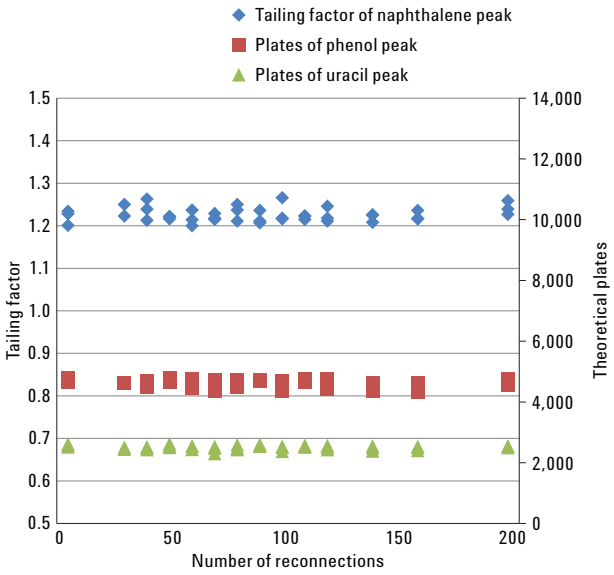


Figure 8. Monitoring tailing factor and the number of theoretical plates over 200 reconnections of the Agilent A-Line Quick Connect fitting.

## 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.

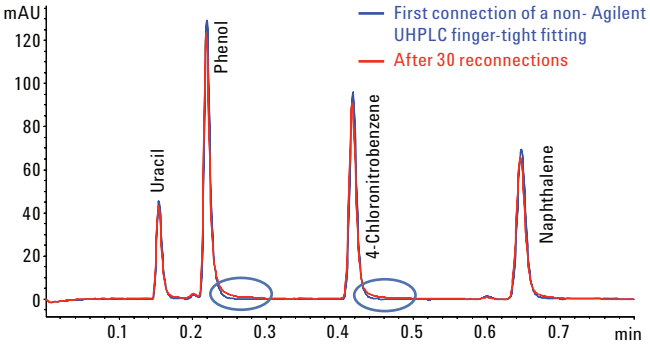


Figure 9. Comparison of chromatograms before and after 30 reconnections of a UHPLC finger-tight fitting from another vendor.

## 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 A-Line fittings with different column brands, columns from three column manufacturers, Waters, Phenomenex, and Supelco, were connected using Agilent A-Line fittings (Quick Connect fitting at the entrance and Quick Turn fittings at the exit and detector). The same set of A-line 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 Agilent A-Line fittings were compared to those with properly swaged stainless steel fittings in Figure 10. No visible difference was observed, which indicates that the A-Line fittings work perfectly with all the tested column brands.

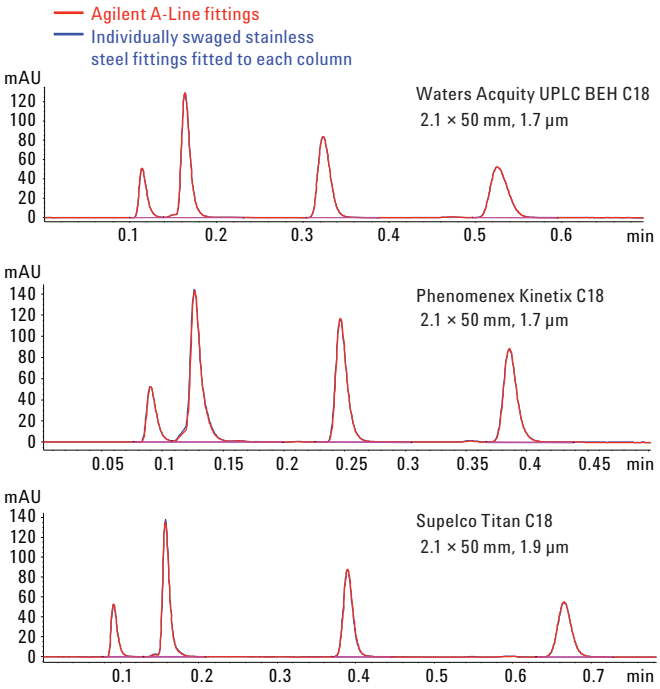


Figure 10. Compatibility of Agilent A-Line fittings with columns from different vendors.

## Conclusions

Agilent A-Line 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 A-Line Quick Connect fittings

**Assemblies***

| Part no. | Capillary connection length | Recommended for |
|---|---|---|
| 5067-5961 | 0.075 × 105 mm | Heat exchanger to column |
| 5067-5957 | 0.12 × 105 mm | Heat exchanger to column |
| 5067-5958 | 0.12 × 150 mm | Heat exchanger to column |
| 5067-5959 | 0.12 × 220 mm | Column to detector |
| 5067-5960 | 0.12 × 280 mm | Column to detector |

**Stand-alone parts**

| Part no. | Description |
|---|---|
| 5500-1174 | Capillary stainless steel, 0.075 × 105 mm, includes 1 non-swaged Swagelok fitting |
| 5500-1173 | Capillary stainless steel, 0.12 × 105 mm, includes 1 non-swaged Swagelok fitting |
| 5500-1172 | Capillary stainless steel, 0.12 × 150 mm, includes 1 non-swaged Swagelok fitting |
| 5500-1171 | Capillary stainless steel, 0.12 × 220 mm, includes 1 non-swaged Swagelok fitting |
| 5500-1170 | Capillary stainless steel, 0.12 × 280 mm, includes 1 non-swaged Swagelok fitting |
| 5043-0924 | Front ferrule** |
| 5067-5965 | Stand-alone Quick Connect fitting (pair with the appropriate capillary for your system) |

*All assemblies include one Quick Connect fitting attached to the appropriate capillary, and a non-swaged Swagelok fitting on the opposite end. Quick Connect fittings can only be used with the capillaries specified in this table.
**Agilent A-line Quick Connect and Quick Turn fittings are equipped with front ferrule.
Part no. 5043-0924 is a replacement part if the ferrule of the fitting is damaged.

## Ordering information for Agilent A-Line Quick Turn fittings

**Fittings and ferrules**

| Part no. | Description |
|---|---|
| 5067-5966 | Quick Turn UHPLC fitting |
| 5043-0924 | Front ferrule** |

**Capillaries**

| Part no. | Description |
|---|---|
| 5500-1198 | Stainless steel, 0.075 × 105 mm long socket |
| 5500-1188 | Stainless steel, 0.12 × 105 mm long socket |
| 5500-1189 | Stainless steel, 0.12 × 150 mm long socket |
| 5500-1190 | Stainless steel, 0.12 × 200 mm long socket |
| 5500-1191 | Stainless steel, 0.12 × 280 mm long socket |
| 5500-1192 | Stainless steel, 0.12 × 500 mm long socket |
| 5500-1193 | Stainless steel, 0.17 × 105 mm long socket |
| 5500-1194 | Stainless steel, 0.17 × 150 mm long socket |
| 5500-1195 | Stainless steel, 0.17 × 200 mm long socket |
| 5500-1196 | Stainless steel, 0.17 × 280 mm long socket |
| 5500-1197 | Stainless steel, 0.17 × 500 mm long socket |

Quick Turn fittings require the capillaries specified in this table

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Printed in the USA
Febrary 2, 2015
5991-5525EN

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