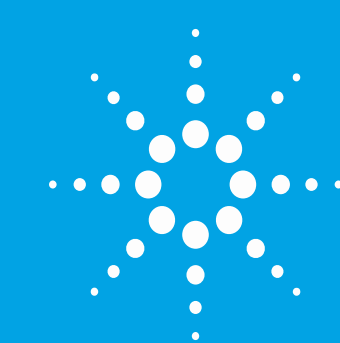


Optimizing HPLC and UHPLC Performance with Agilent A-Line Quick Connect Fittings

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Analytica 2014
Shanghai, China
24-26 September 2014



Agilent Technologies

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.

Fitting connection requirements

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

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

Existing products

Non-adjustable metallic fittings

Most commonly used fittings in UHPLC are non-adjustable 2-piece or 3-piece metallic fittings. Since different manufacturers of column hardware use different designs for column end fittings (Figure 1), it is recommended that a new set of tubing and fittings needs to be installed for every brand of column, to guarantee that the stem length, namely the length between the bottom of the ferrule and the end of tubing, fits the column end fitting. If the stem length is too short, a dead volume will be created, resulting in deterioration of peak shape, lower resolution, and carryover (Figures 2B and 3). If too long, the ferrule will not seat properly and leakage will occur (Figure 2A). In addition, since wrenches are used with traditional fittings and ferrules, they are often over-tightened resulting in the fitting getting stuck in the column permanently.

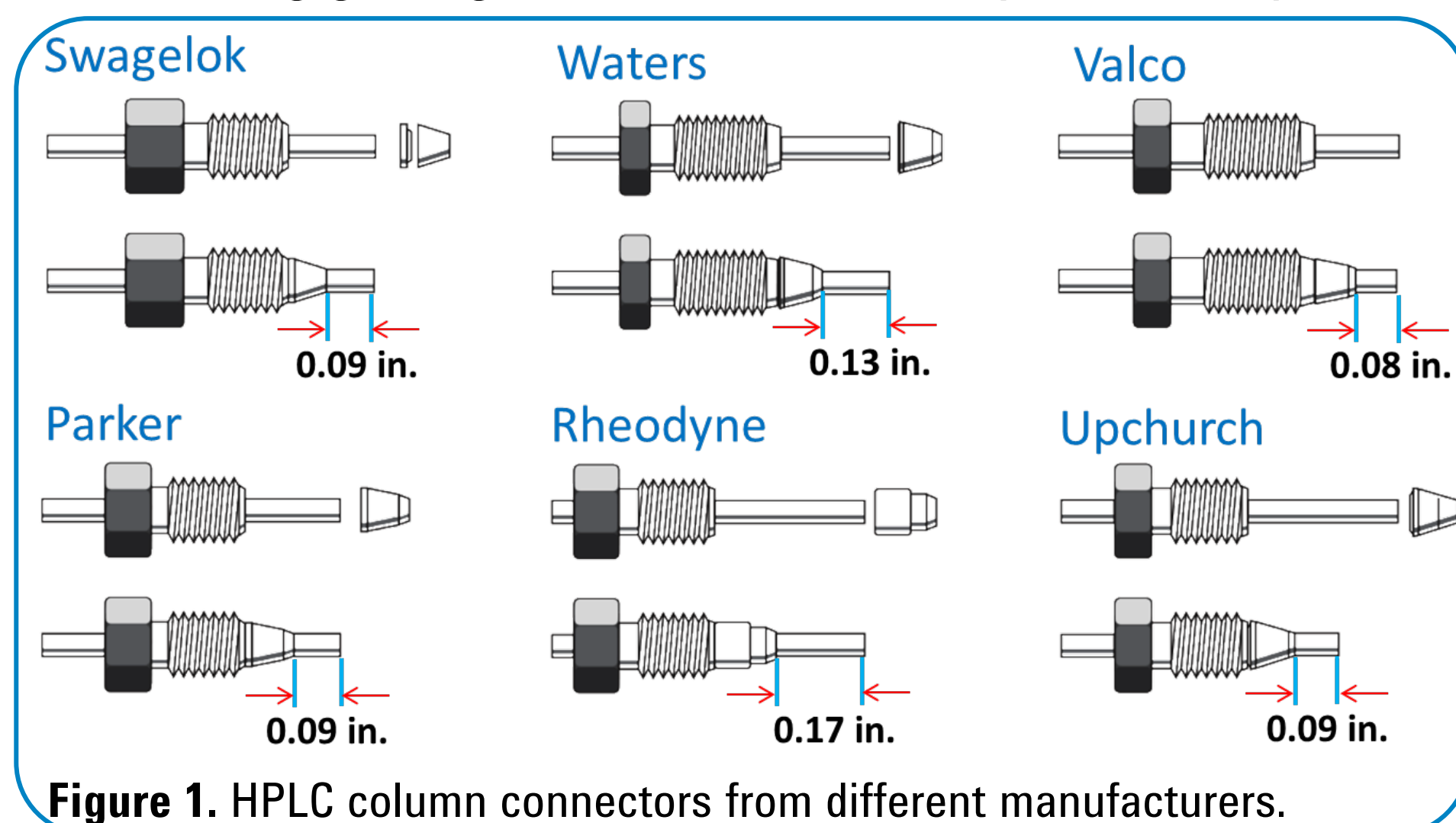


Figure 1. HPLC column connectors from different manufacturers.

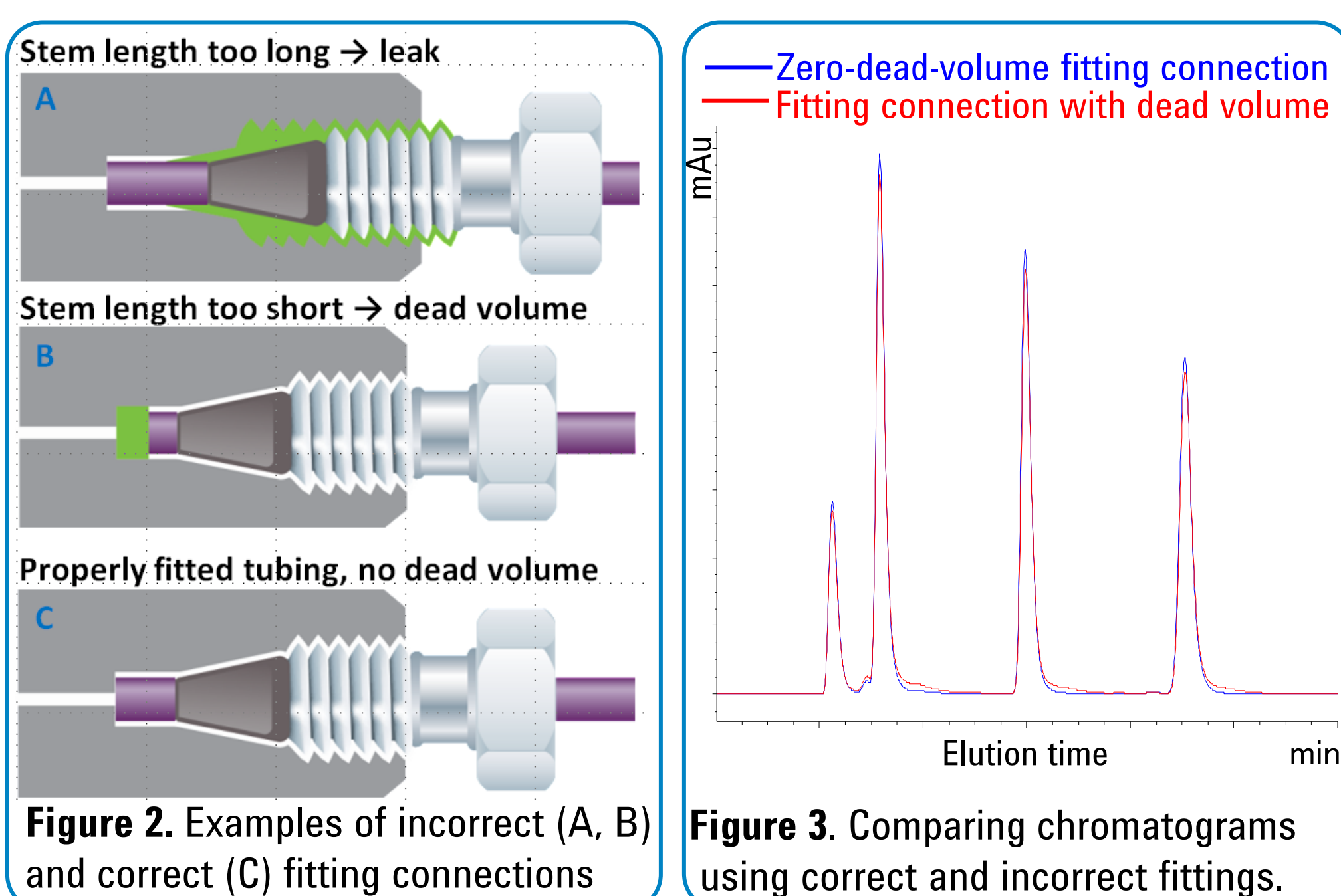


Figure 2. Examples of incorrect (A, B) and correct (C) fitting connections

Figure 3. Comparing chromatograms using correct and incorrect fittings.

Adjustable finger-tight fittings

Adjustable finger-tight fittings are available that are compatible with different columns. However, many of them still have some drawbacks, such as:

- Inability to reach 1300 bar ultra-high pressures without tools
- Strict instructions with exact range of turning angle that must be followed to avoid over-tightening
- Leakage needs to be checked every time after reconnection and the fitting often has to be re-tightened.
- Tubing could slip off the receiving port at high pressure leading to dead volume if the polymeric ferrule does not grip the tubing strongly

Agilent A-Line Quick Connect Fitting

The newly introduced Agilent A-Line Quick Connect Fitting avoids the drawbacks of other fitting types and enables a reproducible and leak-free column connection.

- The novel **spring-loaded design** (Figure 4) constantly pushes the tubing against the receiving port, delivering a reproducible connection with no dead volume for consistent chromatographic performance
- The spring assembly, including the lever, applies a constant force that presses the ferrule onto the tubing, so that **tubing slippage is avoided**
- Stem length is adjustable through the spring, which makes the fitting **compatible with all types of LC columns**
- **Little force** is required to tighten the fitting to 1300 bar (18,850 psi) without the need for any tools. All that is needed is to **hand-tighten** the nut until feeling the first resistance and subsequently depress the lever (Figure 5)

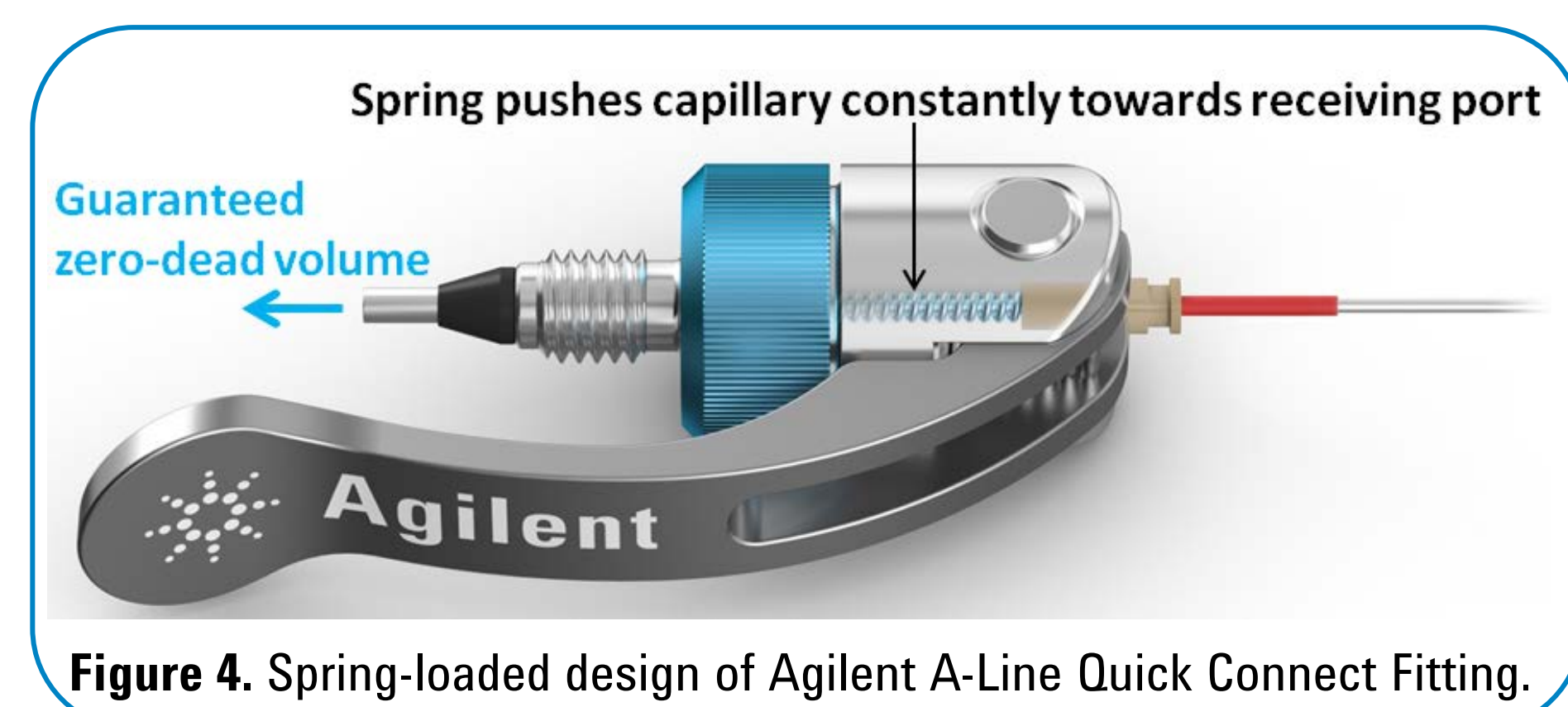


Figure 4. Spring-loaded design of Agilent A-Line Quick Connect Fitting.

Experimental

System: Agilent 1290 Infinity Binary LC
Column: Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 x 100 mm, 1.8 μ m (p/n 959758-902)
Mobile phase: A, water; B, acetonitrile; A:B 40:60 isocratic
Flow rate: 1.4 mL/min (system pressure 1100 bar)
Injection vol: 1 μ L
Column temp: 40 $^{\circ}$ C
DAD wavelength: 254 nm/4 nm, ref 400 nm/100 nm
Sample: Mixture of uracil 10 μ g/mL, phenol 200 μ g/mL, 4-chloronitrobenzene 25 μ g/mL, naphthalene 40 μ g/mL in water:acetonitrile 40:60 (v/v)

The finger-tight fittings were connected at column entrance.

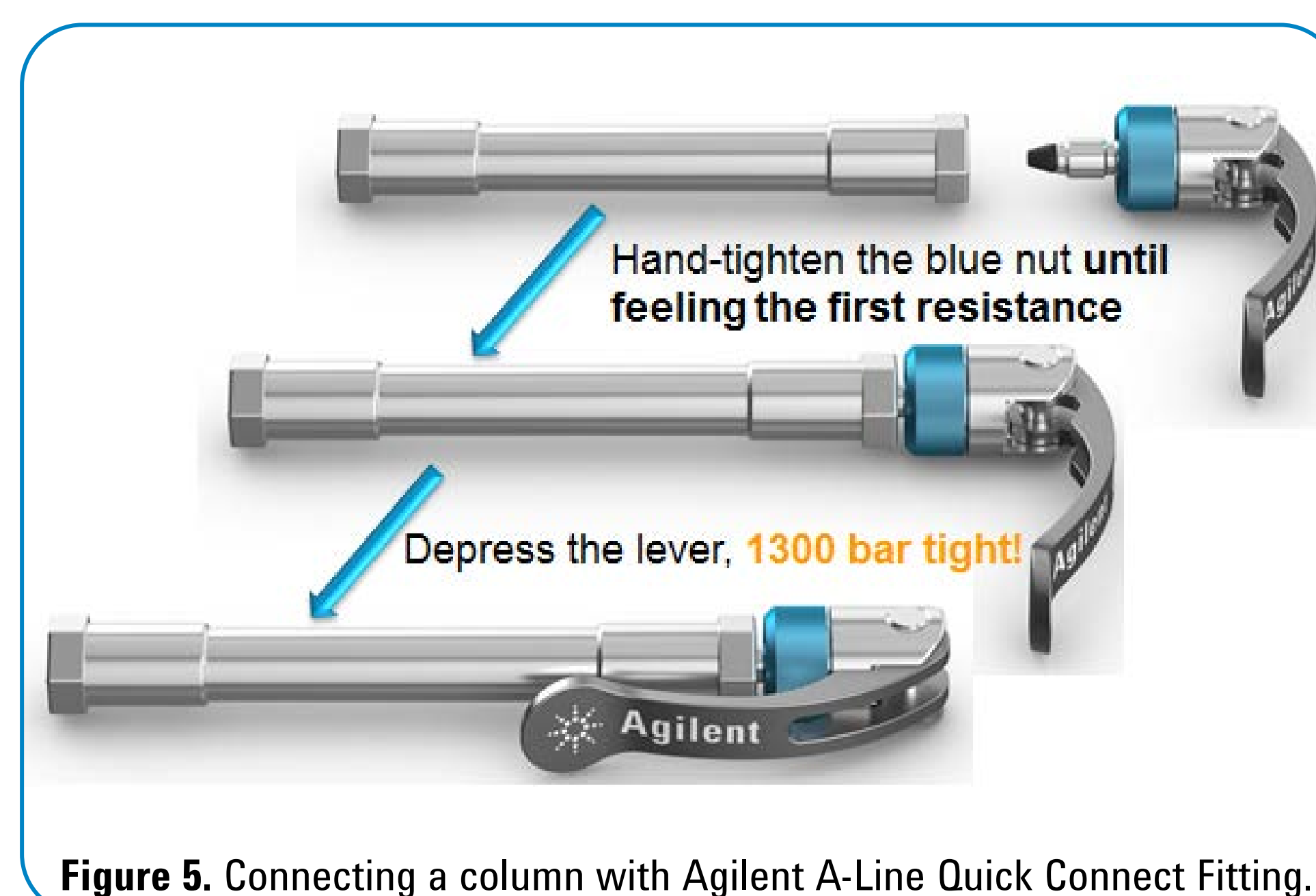


Figure 5. Connecting a column with Agilent A-Line Quick Connect Fitting.

Results and Discussion

Robustness over 200 reconnections

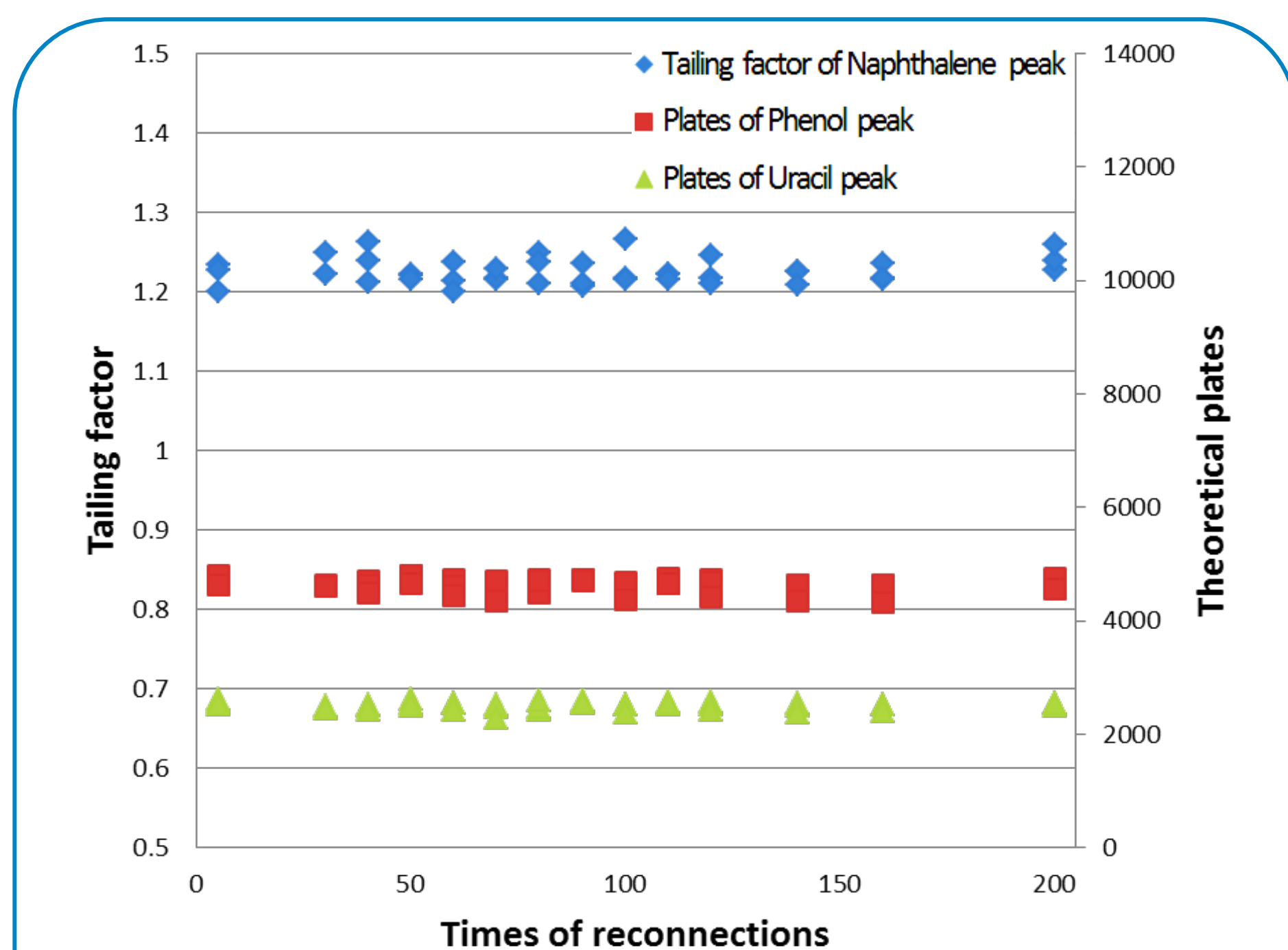


Figure 6. Monitoring tailing factor and theoretical plates number over 200 reconnections of the Agilent A-line Quick Connect Fitting.

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 7A. The chromatogram from a newly installed Swagelok stainless steel fitting was also compared, as a benchmark. It can be seen that there was no visible change in the chromatogram after 200 reconnections and it was still identical with that using Swagelok fitting. What's more, 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, because they are the most sensitive measure for peak-broadening effects through extra-column volume or micro-leakage. Figure 6 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.

Better chromatographic performance

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 a dead volume as shown in Figure 2B. This was also confirmed by comparing the chromatograms in Figure 7B, showing that a deterioration of peak shape, including larger peak tailing and lower peak height, was evident after 30 reconnections.

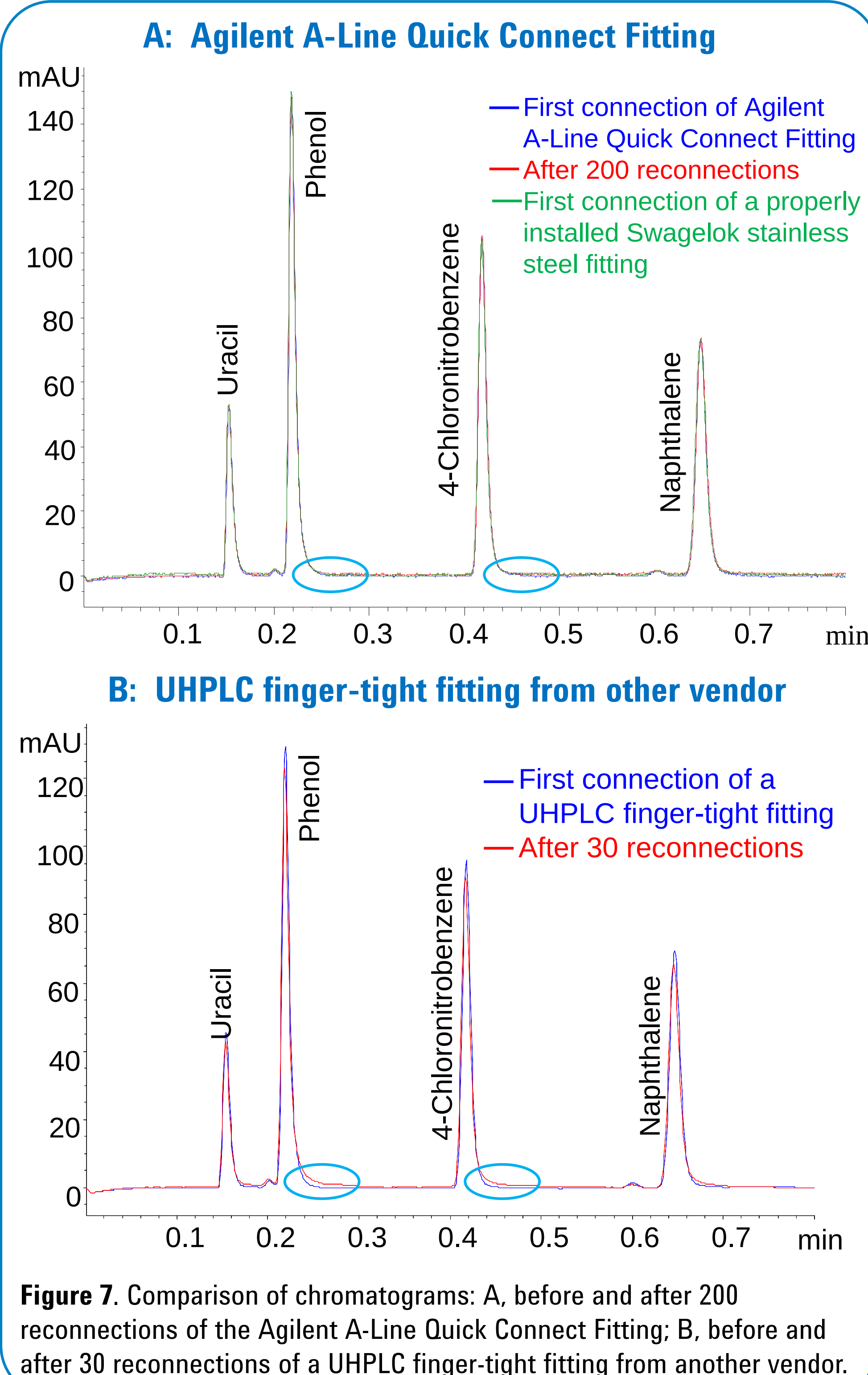


Figure 7. Comparison of chromatograms: A, before and after 200 reconnections of the Agilent A-Line Quick Connect Fitting; B, before and after 30 reconnections of a UHPLC finger-tight fitting from another vendor.

Conclusions

The newly introduced Agilent A-line Quick Connect fitting delivers usability, robustness, and UHPLC performance:

- 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 is guaranteed by the unique spring-loaded design.

For more information please visit:
www.agilent.com/chem/a-line