

Optimizing UHPLC Fittings and Connections: A Case Study

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Abstract

We experienced persistent problems in achieving robust connections in UHPLC applications utilizing sub-3 micron particle size reversed phase chromatographic columns. Our application involved column testing, which involves frequent disconnection and re-connection of different columns into/from the flow path of the UHPLC system. In this paper we report our findings and recommendations.

Case Report

UHPLC abbreviates Ultra High Performance Liquid Chromatography. UHPLC is the next generation of liquid chromatographic separation, based on higher pressures of 800-1200 bar, while standard HPLC instrumentation was designed for pressure limits of 400-600 bar. The evolution of HPLC into UHPLC deeply impacted many different aspects of routine chromatography. UHPLC is not just an HPLC upgrade that reproducibly delivers mobile phases at higher pressure. First of all, UHPLC is designed to operate sub-3 μm particle size chromatographic columns and is optimized for transfer of narrow chromatographic peaks with minimal distortion to the detector [1-3].

Initial UHPLC systems utilized non-adjustable metallic fittings. Once a ferrule becomes compressed and locked, it cannot be moved (Figure 1). That design provided maximum ruggedness, although it is advantageous only if one specific column brand is used. Different column manufacturers have some differences in design of the column end-fittings. As a result, true zero dead volume connection might not be achieved and chromatographic peaks will be distorted [3,4]. In this case, brand new tubing and new set of fittings should be installed.

Adjustable and reusable fittings provide much more flexibility for chromatographers. There are different varieties of HPLC and UHPLC fittings that are available in the market. However the choice of the right fittings is not simple and it should be based on the application. Typically, metallic ferrules should be avoided with PEEK tubing, because during tightening of a metallic ferrule, softer polymeric tubing will be compressed. As a result, the tubing surface where the metallic ferrule grips will slightly

shrink; tubing internal diameter will be slightly decreased, causing a noticeable rise in backpressure (author unpublished observation). If the metallic ferrule is tightened and untightened several times, the back pressure may increase more. We have observed that some high pressure rated adjustable fittings perfectly hold pressure when they are newly installed. However, they do not hold pressure anymore after a few dis- and reconnections. Certain UHPLC fittings are more prone to be over-tightened and require more torque control during tightening; a serious problem can result where they can become stuck inside of the valve port or column. Such problems with connections rarely occur with standard HPLC applications where operational pressures are typically below 250 bar.

We purchased an Agilent 1290 UHPLC system (Wilmington, Delaware, USA), including the column thermostat model G1316C, equipped with low volume heat exchanger G1316-80003. The heat exchanger tubing (thin capillary) is connectable to the LC column by re-adjustable fittings equipped



Figure 1: Non-adjustable 2 piece ferrule UHPLC fitting. Once a ferrule becomes compressed and locked, it cannot be moved.



Figure 2: Agilent 1290 re-adjustable polymeric ferrule.

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with polymeric ferrules (Figure 2). The outlet of the heat exchanger was standard stainless steel 1.6 mm (1/16 inch) outside diameter, while inside of the heat exchanger it is a thin and soft capillary (Figure 3). During initial method development we tested various columns to find the best efficiency and selectivity in our application. Column testing requires frequent column exchange, and we observed that a column replacement is not a quick simple task as expected. Original end fitting requires at least a few adjustments (re-tightening by wrench) to assure absence of a leak from the fitting in the operation pressure range. After several column replacements, the ferrule becomes compressed and is either stuck inside a column or not maintaining high pressures anymore. The thin capillary heat exchanger tubing during tightening of a fitting often becomes severely bent and eventually broken. Once broken, the entire heat exchanger module has to be removed and replaced. Since the heat exchanger design appears to be less rugged and more fragile than expected, we added an extra union and short tubing between the column and heat exchanger outlet in order to protect the heat exchanger unit, the ferrule and to simplify tightening (Figure 4). However, such a design adds extra volume and changes the retention time (data not shown), which should be avoided. Many adjustable UHPLC fittings equipped with polymeric ferrules are optimal for polymeric tubing, but not ones that are made from stainless steel. This is because polymeric



Figure 5: Original fittings were replaced by EXP Hand-Tight Nut and Titanium Hybrid Ferrule.

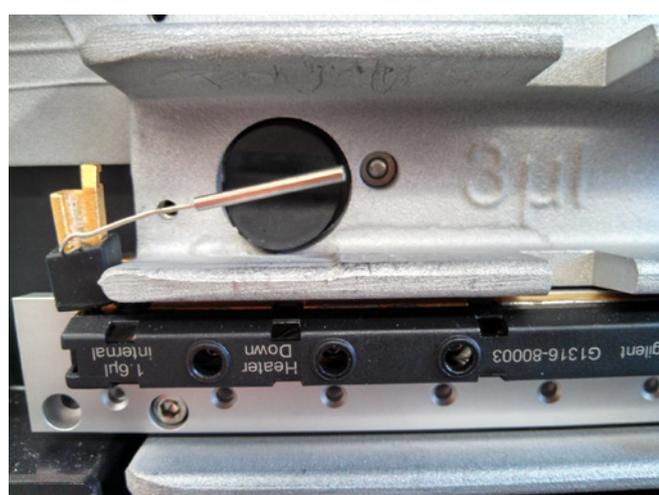


Figure 3: Heat exchanger outlet tubing design.



Figure 6: No mechanical effort in UHPLC column installation.

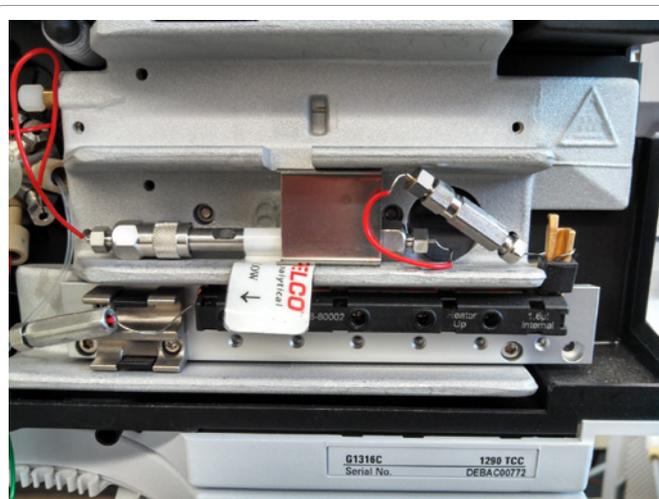


Figure 4: Union and additional short tubing was added to protect fragile heat exchanger tubing and polymeric ferrule.

ferrules are softer than stainless steel, and cannot provide sufficient grip strength around the tubing at UHPLC pressures (unlike HPLC). After many trials, and various connection combinations, we found a simple and complete solution. The original heat exchanger nut and polymeric ferrule were removed (Figure 3) and replaced by EXP H and-Tight Nut and Titanium Hybrid Ferrulekit (p#15-20-03888) available from Optimize Technologies (Oregon City, OR USA) (Figure 5). This fitting utilizes a novel sealing concept, and consists of both PEEK and titanium in a one piece ferrule. This unique hybrid design ensures the integrity of the seal at UHPLC pressures (above 1400 bar). This fitting allows hand tightening followed by only a half turn adjustment with the wrench. This protects the fragile heat exchanger capillary from mechanical stress load and potential damage during repeated column installation or removals (Figure 6).

Conclusion

In conclusion, we would like to mention there is no straightforward process for finding the optimal fitting because of the current variety of different products in the LC market. This necessitates detailed knowledge

of hardware. In addition, for any chromatographic system, there is no perfect design, and some optimization is always required.

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