

How to Optimise Your UHPLC Performance – Connect Properly!

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Introduction

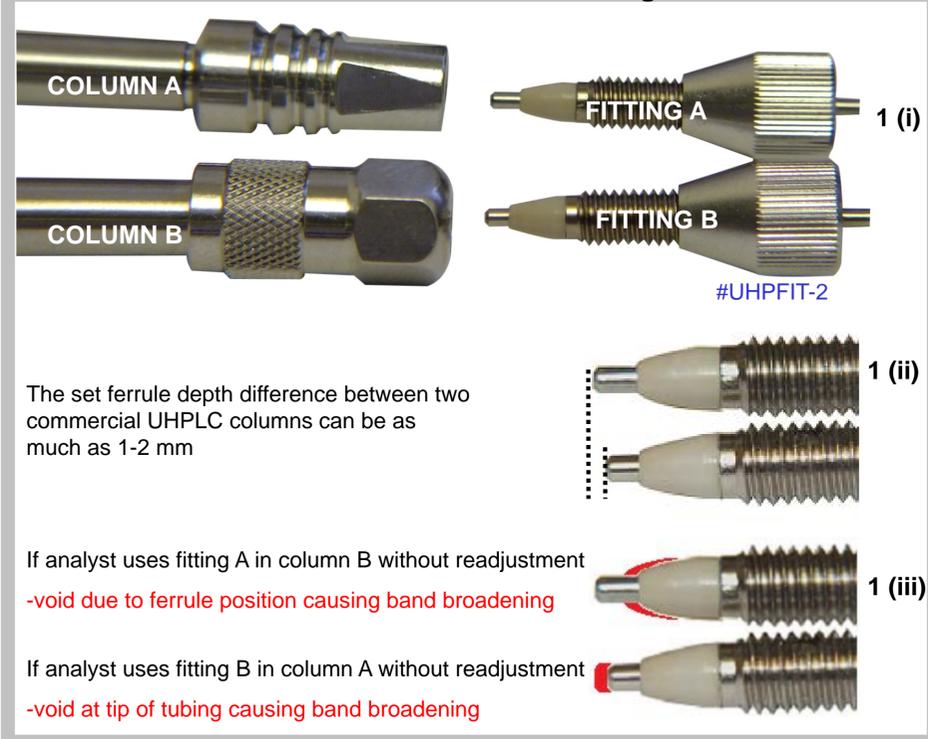
Ultra high-pressure liquid chromatography (UHPLC) is now an established technique allowing method development and analysis to be achieved in a shorter period of time. As a result an increasing number of UHPLC instrumentation has entered the marketplace alongside new UHPLC columns and chemistries from a number of companies, not all systems and column hardware are perfectly compatible! It is therefore important when evaluating the most suitable column for the analyst's method/method development, that a number of considerations regarding the system hardware and plumbing are taken in to account.

In this poster we discuss the variability between system and column plumbing and the contributing factors to poor chromatography. We show that by the correct selection of connections between the system and column that the true capability of UHPLC columns can be achieved. We highlight approaches to improving column lifetime and the implications for the resulting chromatography.

Column Fitting Issues

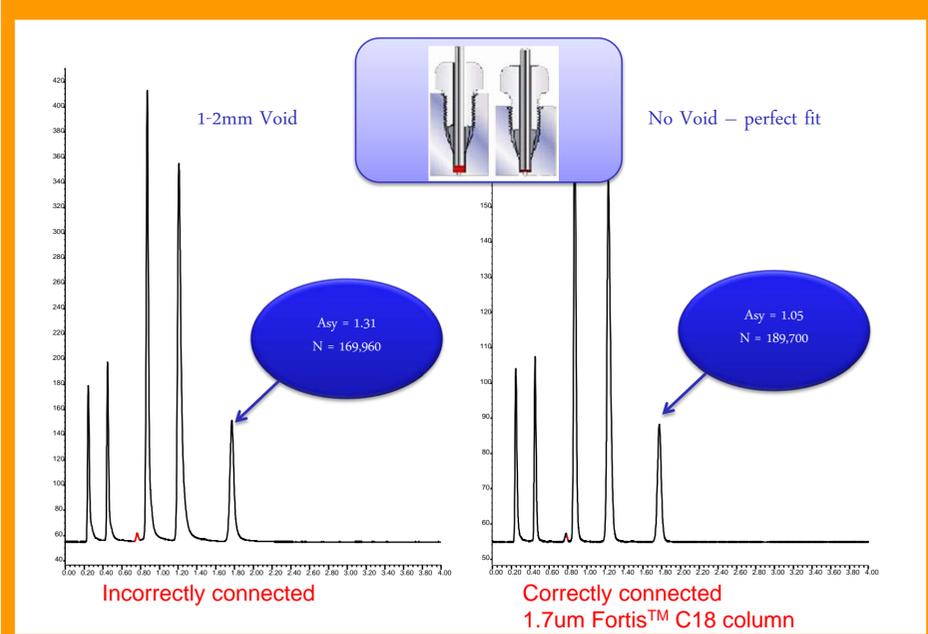
One of the key reason for loss of performance in UHPLC is band dispersion caused by excess system void volume. This void volume can arise from a number of areas such as hardware design, tubing connections and tubing volumes. Whereas most analysts have been made aware of the affects of tubing volumes, little has been mentioned of the issues associated with column hardware design and their connections. Figure 1 shows that dead volume can be created when the female and male fittings do not match.

FIGURE 1. Variation in Column Hardware Design



In order to account for the variability in end fitting geometry one has to either use dedicated connectors for each manufacturers' column or use an adjustable fitting. The Fortis™ UHPLC Fitting (seen in figure 1) is a 'finger tight' adjustable connector that is suitable for the extreme pressures associated with UHPLC. This connector allows the ferrule depth to be adjusted for different columns ensuring a zero dead volume connection regardless of column end fitting geometry. The benefits of this zero dead volume can clearly be seen in figure 2.

FIGURE 2. The Affect of Dead Volume on UHPLC Performance



Column Protection

With small particle UHPLC columns it is important to prevent frit blockage due to contamination. There are a number of commercial UHPLC column savers available that contain a low porosity frit to prevent contaminants reaching the column, once the UHPLC filter begins to block it is discarded and replaced with a new filter, meanwhile the UHPLC column remains uncontaminated/undamaged.

FIGURE 3. UHPLC Filter



The Fortis™ UHPLC Filter (figure 3) is a pre-column filter that is suitable for operation under UHPLC conditions. It contains a 0.2um porous frit and is designed to possess minimal dead volume, this ensures that it does not significantly contribute to any band broadening.

When compared with two commercially available filters the Fortis™ UHPLC Filter was seen to have a much reduced dead volume, this is reflected in the minimal loss of both peak shape and efficiency when a 1.7um Fortis™ C18 UHPLC column was tested with and without a filter in front of the column (see figures 4 and 5).

FIGURE 4. UHPLC Filter Use Affects – Change in Asymmetry

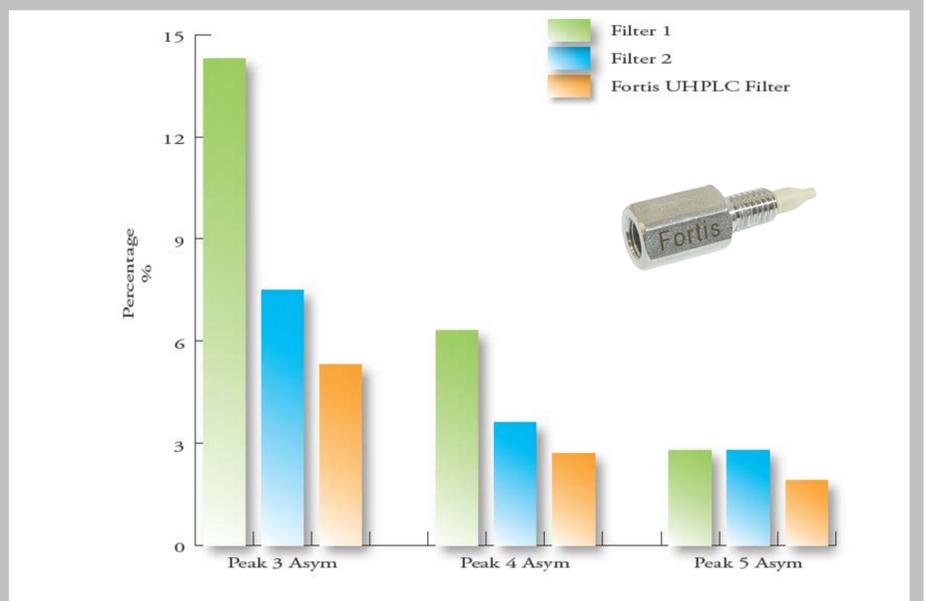
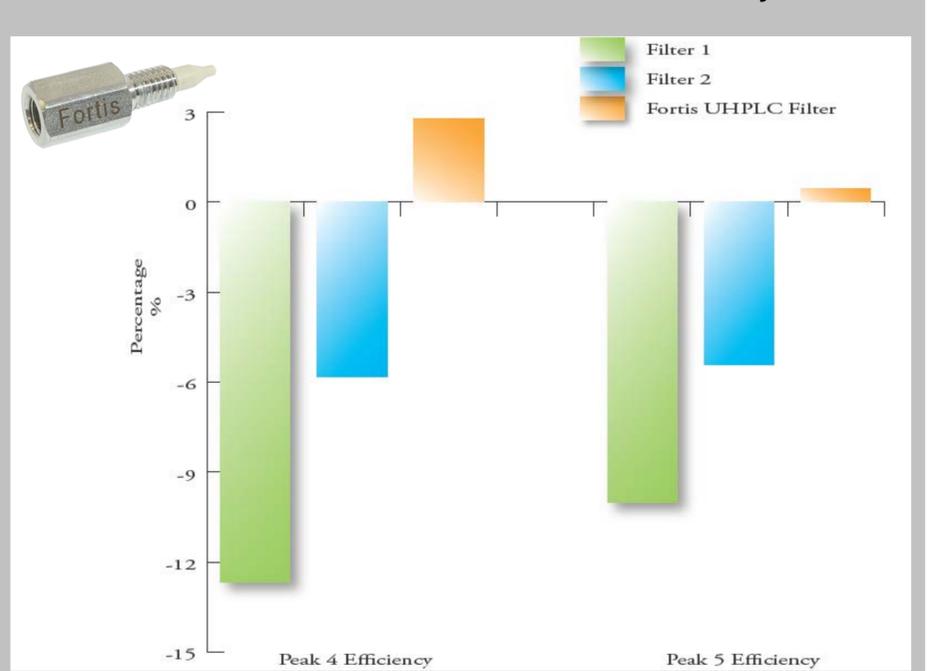


FIGURE 5. UHPLC Filter Use Affects – Loss of Efficiency



Conclusion

We have shown how proper connections in UHPLC are critical as these can lead to the introduction of dead volume which ultimately has a negative impact on peak shape and plate count.

Adjustable connectors address the variability in end fitting geometry and offer the added benefit of being finger-tight. The consistent pressure from connection to connection, end-user to end-user also contribute to a more reproducible connection and ultimately better chromatography.

When moving between column suppliers for UHPLC always ensure column connections are adjusted to suit the new column hardware.