

Application Note

Correct Fitting of UHPLC Columns

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

“Variability in end fitting geometry can cause dead-volume which compromises UHPLC performance”

evaluating the most suitable column for the analyst's method, that a number of considerations regarding the system hardware and plumbing are taken into account.

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

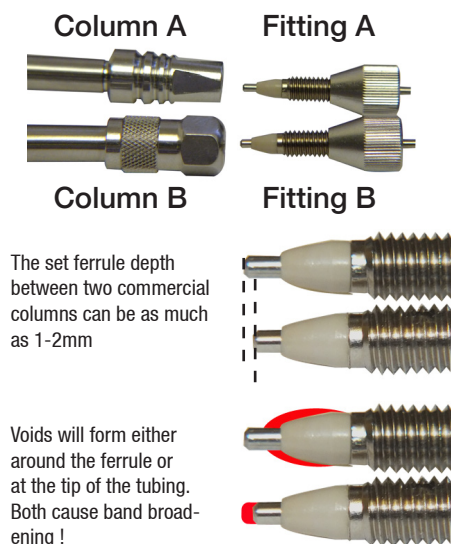


Figure 1. Difference in end-fitting depth

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 is created when the female and male fittings do not match.

Zero dead-volume Fittings

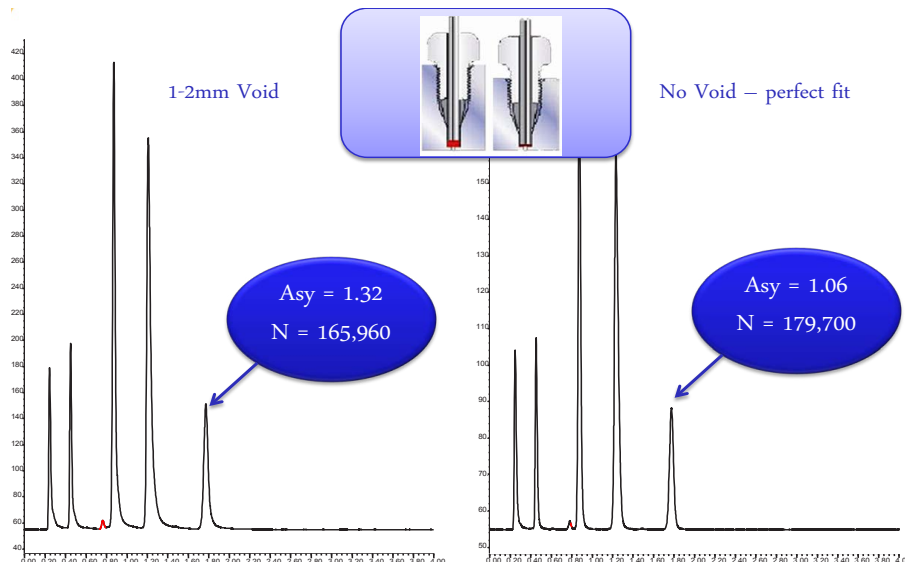


Figure 2. Losses due to fittings

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 the chromatography of figure 2.



Column Protection

Fortis® UHPLC filters are designed to protect the more expensive analytical UHPLC column from matrix, solvent and particulate matter. Designed with the same low dead-volume in mind as we have discussed for fittings, it

protects without compromising results and doesn't add any further retention so as to not add any more run time when we are looking for speed of analysis.

Conclusion

Fortis® UHPLC fittings allow for the correct fitting of any UHPLC column to any UHPLC system. Proper connections are critical to avoid introduction of dead-volume which has a negative impact on peak shape and plate count.

Adjustable connectors address the variability in end fitting geometry and offer the benefit of being finger tight.