UHPLC of Biomolecules

Ken Butchart, Mark Woodruff. • Fortis Technologies Ltd, 45 Coalbrookdale Road, Cheshire, CH64 3UG, UK.

Introduction

UHPLC is rapidly becoming a routine method for the separation and qualification of analytes in pharmaceutical analysis. With the ever increasing trend to find new biomolecules either naturally or biosimilars there is a potential for UHPLC to be used to provide data on the trial drug molecule and any impurities quickly and accurately with high sensitivity.
With any new biopharmaceutical discovery, development and production process, four major separations are critical to bring a new chemical entity to market in a reasonable time period. The four separations are protein separation, peptide mapping, amino acid analysis and glycoprofiling. These methods will allow structural characterisation, identification of structural variations, location of individual modifications and demonstration of chemical equivalency of different batches. UHPLC will allow high sensitivity and high resolution within these separations to occur.

In this poster we show the use of a new UHPLC column specifically for the separation of biomolecules. This will provide us with good diffusion and mass transfer of biomolecules due to an optimised pore structure, whilst still maintaining the ability to operate at the elevated pressures required to utilise the small UHPLC particles. High peak efficiency with sharp narrow peak shapes will increase greatly the sensitivity of any low abundance impurities. We will look at the high resolution that should be provided for complex samples such as amino acids and tryptic digests.

Small Particle Columns for Bioanalysis

The move to 1.7um particles in Bioanalysis has not changed the fact that proteins and polypeptides will retain by an adsorption/desorption mechanism (Fig 1), but it has changed the efficiency of the columns in terms of the ‘separation power’ achievable. This is especially important with complex or low abundance samples such as peptide mapping, amino acid analysis and trypic digests. Higher resolution and higher sensitivity can both be achieved with the use of smaller particles.

Figuere 3. Highlights the use of a 1.7um UHPLC particle in comparison with a tryptic digest on a traditional 5um particle

FIGURE 1. Pore structure and adsorption/desorption

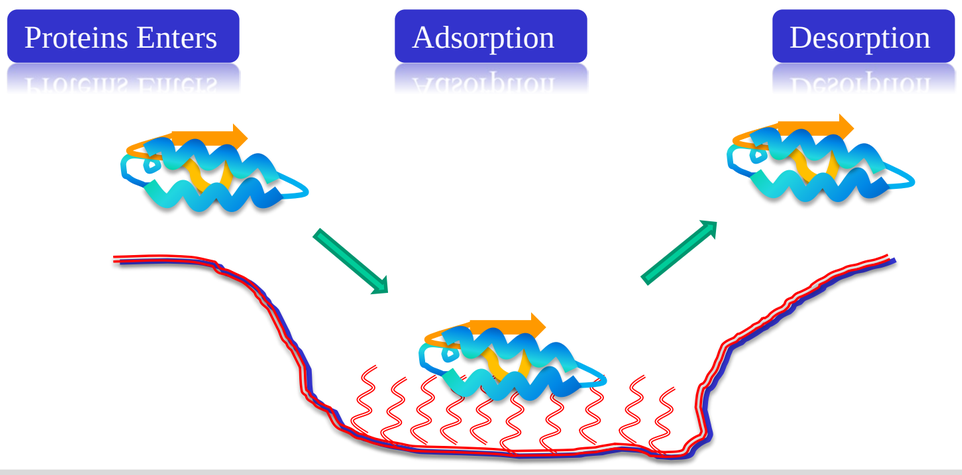


FIGURE 2. 5um vs 1.7um particles

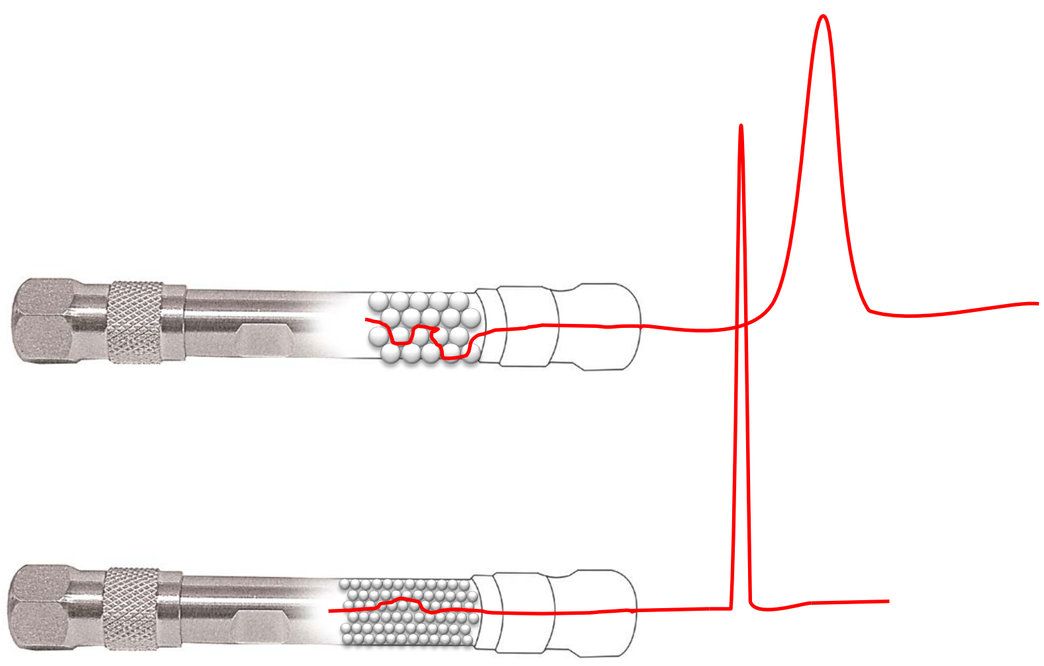
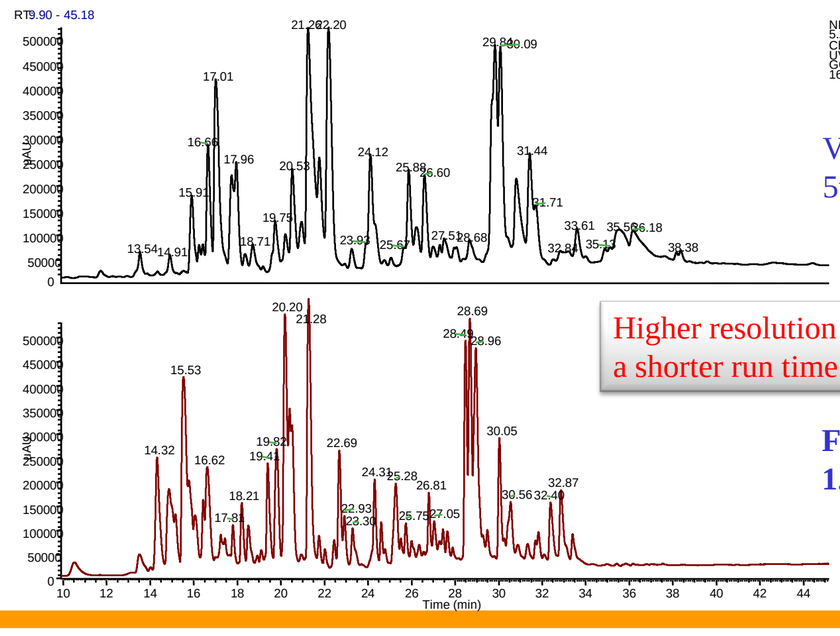


FIGURE 3. Tryptic Digest



Improved Results

It can be clearly seen how a faster run time is possible, whilst also gaining more resolution on the shorter small particle column. Both columns are silica based 300Å, but the extra efficiency of the small particle improves the chromatographic capability.

The small particle used is 1.7um FortisBIO C18 (300Å), due to being silica based it can withstand the 18,000psi option on the UHPLC systems.

FIGURE 4. Higher Resolution – Casein from Bovine

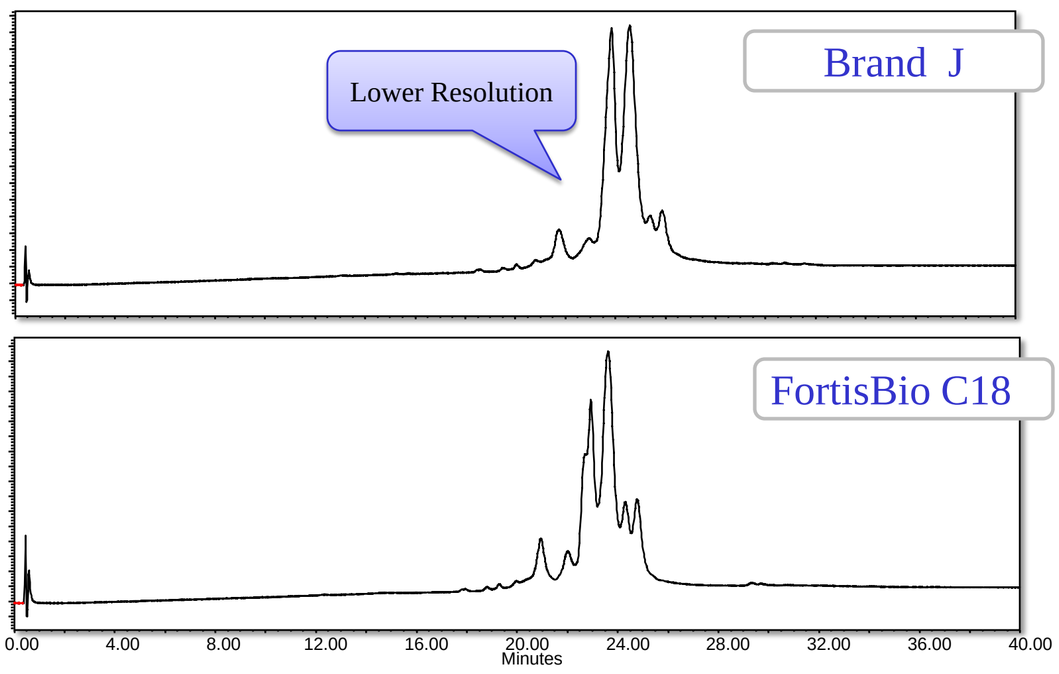
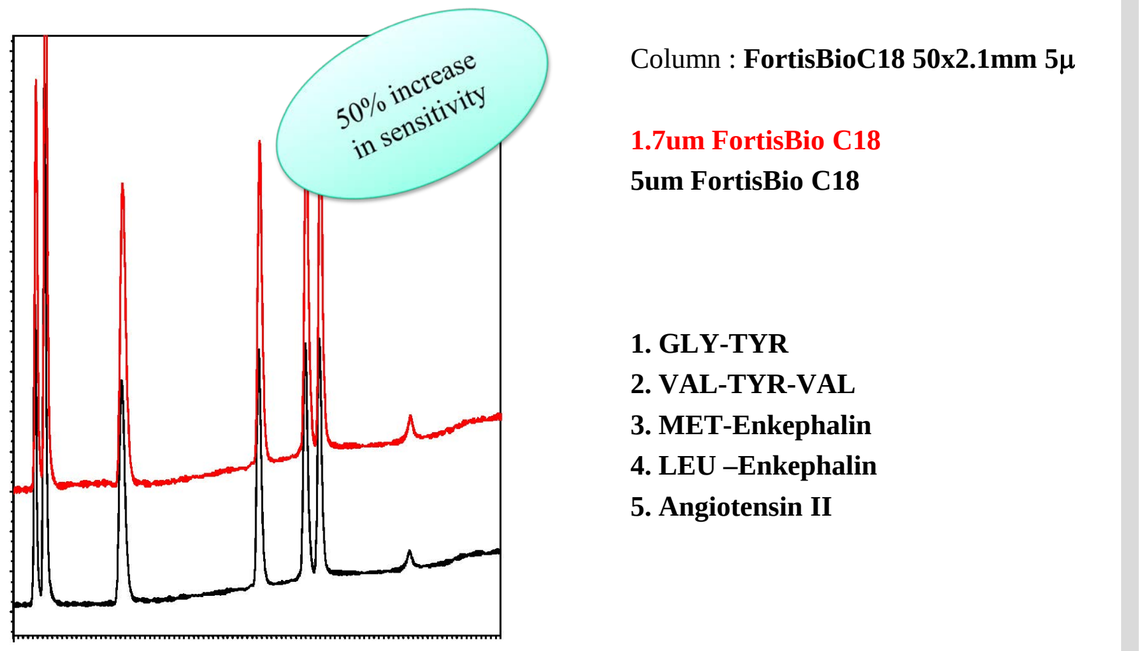


FIGURE 5. Higher Sensitivity - Peptides

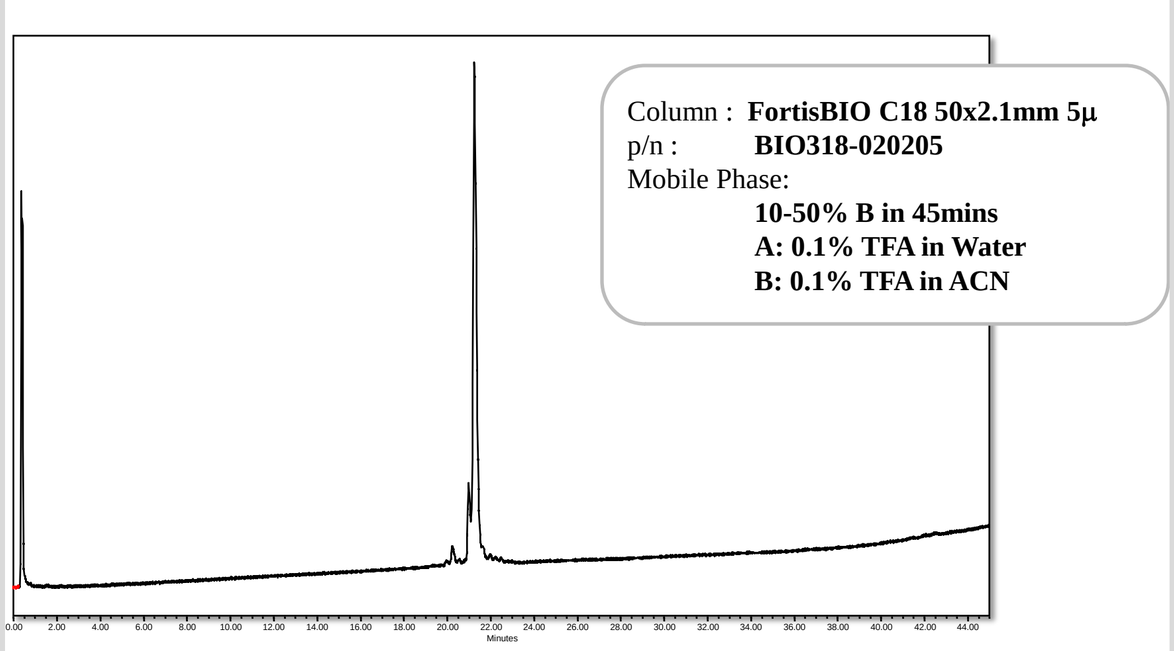


The higher sensitivity can be clearly seen on a range of peptides, upwards of 50% peak height and therefore sensitivity be achieved just by the use of the smaller UHPLC particle.

Scaleability

Another important factor in protein analysis is the ability to scale up to allow the purification of samples. Only using a silica phase where the surface area, pore size and carbon loading remain constant is the same separation achieved for process scale purification. Milligram to gram quantities of synthetic or recombinant polypetides for clinical trials or sales will be easier on a larger 5 or 10um particle size, but in order for the method to not have to be redeveloped then the phase characteristics need to remain identical as those of the smaller particle.

FIGURE 5. Higher Sensitivity - Peptides



Conclusion

In this poster we have shown the use of a new UHPLC column for the separation of biomolecules. We have shown how this provides increased efficiency, sensitivity and resolution for the molecules of interest. The UHPLC method can then be scaled up to a larger particle scale if all the physical characteristics are identical for purification methods.