A New UHPLC Column for Polar Analyte Retention

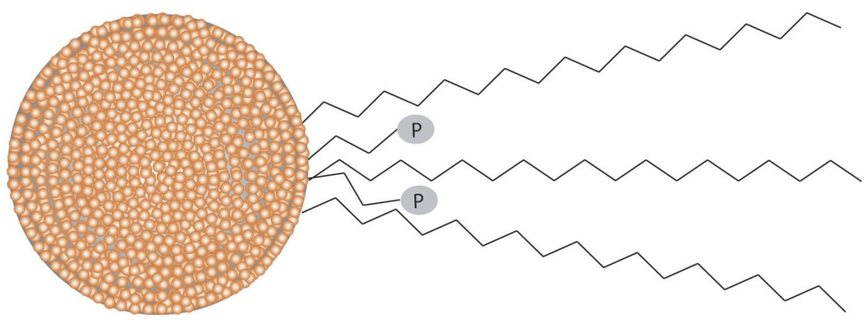
Ken Butchart, Mark Woodruff. • Fortis Technologies Ltd, 45 Coalbrookdale Road, Cheshire, CH64 3UG, UK

Introduction

Ultra High Pressure Liquid Chromatography (UHPLC) has been quickly adopted by the market allowing faster method development to be achieved in a shorter period of time. More efficiency and more speed are both sound parameters to be able to manipulate, however the strongest parameter in method development remains resolution.
There are many polar compounds that are difficult to retain on RP-18 stationary phases and in order to produce robust, accurate data sufficient retention of these analytes is crucial.

In this poster we discuss the use of a new stationary phase designed specifically for the retention and separation of these more hydrophilic molecules. We show that resolution can be achieved between analytes due to the mechanism of the stationary phase, exhibiting both hydrophobic and hydrophilic retention profiles. We highlight the applications that this new stationary phase can achieve. We also look at the critical factors that the analyst must consider such as scaleability and method transfer

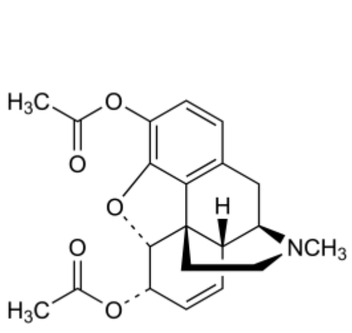
FIGURE 1. Structure of Fortis H2o



Separation

Small particles increase efficiency in our separations, aiding the sensitivity of analysis as well as improving both speed and resolution. However in order to gain selectivity between analytes we often require the ability to have available stationary phases with alternative selectivity even on small <2um particles. 1.7um Fortis H2o is a polar endcapped stationary phase, providing both hydrophobicity but also additional polar characteristics. This allows multiple retention mechanisms to aid in the retention of the more hydrophilic molecules, whilst also allowing the retention of the hydrophobic analytes.

FIGURE 2. Separation of Diamorphine



Column : **1.7µ Fortis H2o™ 50x2.1mm**
p/n : **FHO-020301**
Mobile Phase:
5:95% 0.1% formic: ACN in 7mins

Flow : **0.25ml/min**
Temp : **20ºC**
λ : **254nm**

Diamorphine

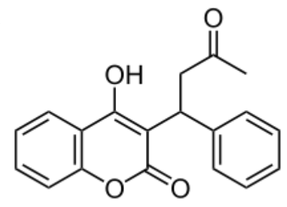
Figure 2. shows the resolution of Diamorphine, a synthetic opioid, at low levels, and retained sufficiently from the matrix, which is most often blood or urine. A fast run time is possible due to multi-modal separation characteristics and the sub 2µm particle size of the Fortis H2o stationary phase used.

Figure 3. Resolution away from any matrix effect of Warfarin, an anticoagulant, which in high throughput LC-MS is imperative in order to achieve accurate qualitative results.

FIGURE 3. Retention of Warfarin

Column : **1.7µ Fortis H2o ™ 50x2.1mm**
p/n : **FHO-020301**
Mobile Phase:
40:60 – 0.1% formic: ACN
Flow : **0.25ml/min**
Temp : **20ºC**
λ : **220nm**

Warfarin



Important Requirements

1.7um particles offer extra peak height and therefore sensitivity in LC and LC/MS, thus allowing the analyst to detect lower quantities of analyte, increase signal to noise ratio and improve quantitative results. If a method is developed on UHPLC then a requirement is to have fully scalable particles so that the move to analytical or preparative and worldwide method transfer is simplified

FIGURE 4. Scaleability of particle sizes

1.7µ 201,720p/m 313bar
3µ 122,980p/m 132bar
5µ 70,000 p/m 48bar

Column : **1.7µ Fortis H2o 50x2.1mm**
p/n : **FHO-020301**
Mobile Phase: **40:60 – H_2O: ACN**

Flow : **0.4ml/min**
Temp : **25ºC**
λ : **254nm**

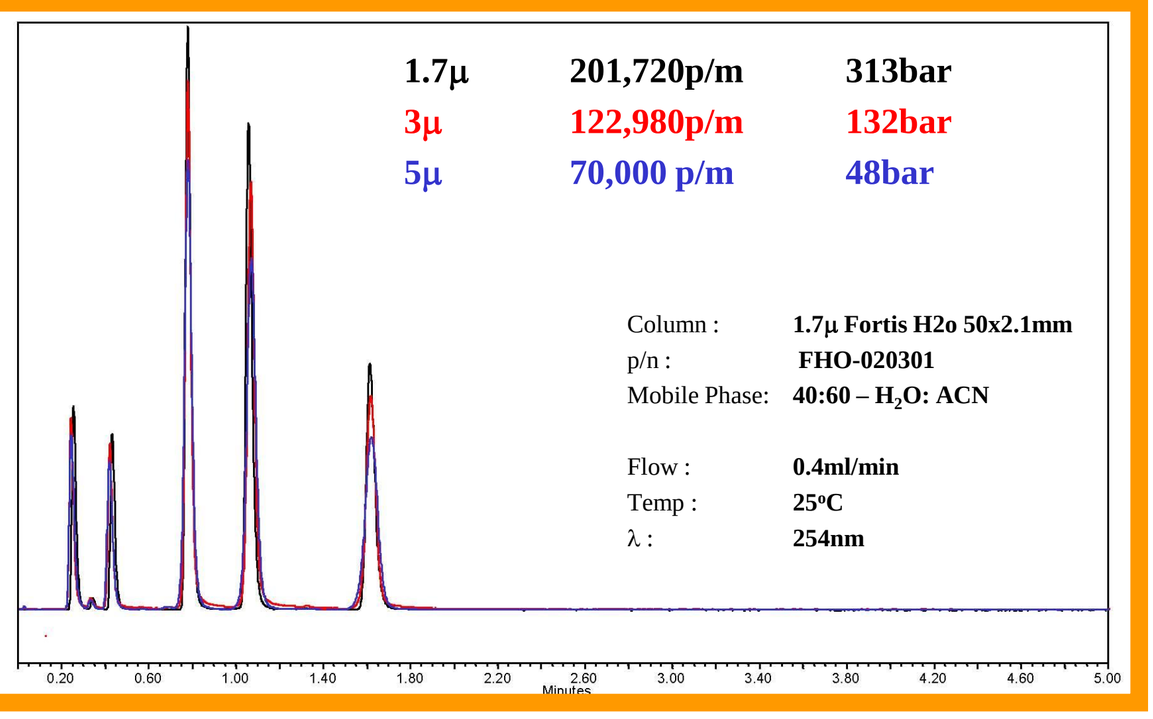
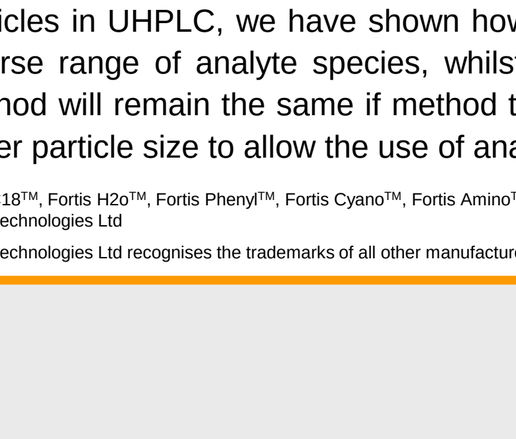


FIGURE 5. Selectivity Choices



1.7µ Fortis H2o
1.7µ Fortis C18
1.7µ Fortis Diphenyl
1.7µ Fortis Cyano
1.7µ Fortis C8
1.7µ Fortis HILIC
1.7µ Fortis HILIC DIOL
1.7µ Fortis Amino

Selectivity is of critical importance in UHPLC, even though most people to date have concentrated solely on the increased efficiency provided.
1.7um Fortis particles are available in 8 different phase chemistries, providing increased selectivity options to compliment the efficiency gains made.

Conclusion

Fortis H2o offers alternative and complementary selectivity when utilising small particles in UHPLC, we have shown how the selectivity can be used to retain a diverse range of analyte species, whilst highlighting how the selectivity of the method will remain the same if method transfer then needs to take place onto a larger particle size to allow the use of analytical or preparative chromatography.

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