Application of Amino Stationary phases for the Analysis of Carbohydrates

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Introduction

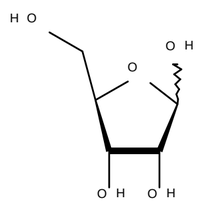
Carbohydrates are prevalent within biochemistry and are quite often broken down into four categories monosaccharides, disaccharides, oligosaccharides and polysaccharides. The first two groups are typically smaller and commonly referred to as sugars, whilst the latter two are larger polymer groups of sugars.
Carbohydrates carry out many functions in living organisms, playing key roles in the immune system, fertilization, preventing pathogenesis, blood clotting and development.
In food science the term carbohydrate can be used to mean any food that is rich in the complex carbohydrate starch, or more simple carbohydrates such as sugar in jams, sweets and desserts.

In this poster we discuss the application of Amino stationary phases in the retention and separation of carbohydrates. We look at the mobile phase requirements and the effect subtle changes can have on the resolution achieved.
Amino columns are not the only way to retain and separate carbohydrates, polymeric stationary phases are often used, but we will highlight why these will not work within UHPLC. Instead a new 1.7µm Amino silica based stationary phase will be shown to operate in the required pressure region.

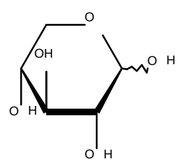
Simple Sugars

Simple sugars are broken into monosaccharide or disaccaride categories. If two monosaccharides form a glycosidic bond with the loss of a water molecule then these are classified as disaccharides. This linkage may continue to grow to produce oligosaccharides of great length.
Sugars have been associated with many health factors and risks, most notably, blood glucose levels (Diabetics), immune system suppression, obesity and cardivascular disease. Therefore their analysis is of great interest in clinical applications as well as in food analysis.

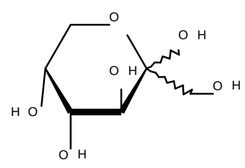
FIGURE 1. Sugars Mono and Disaccharide



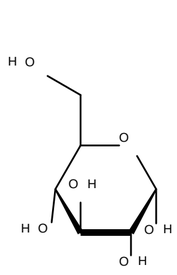
Ribose



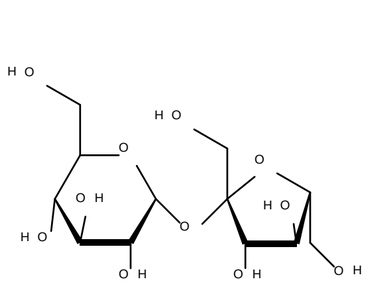
Xylose



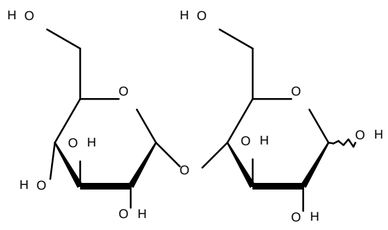
Fructose



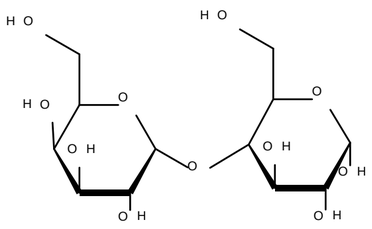
Glucose



Sucrose

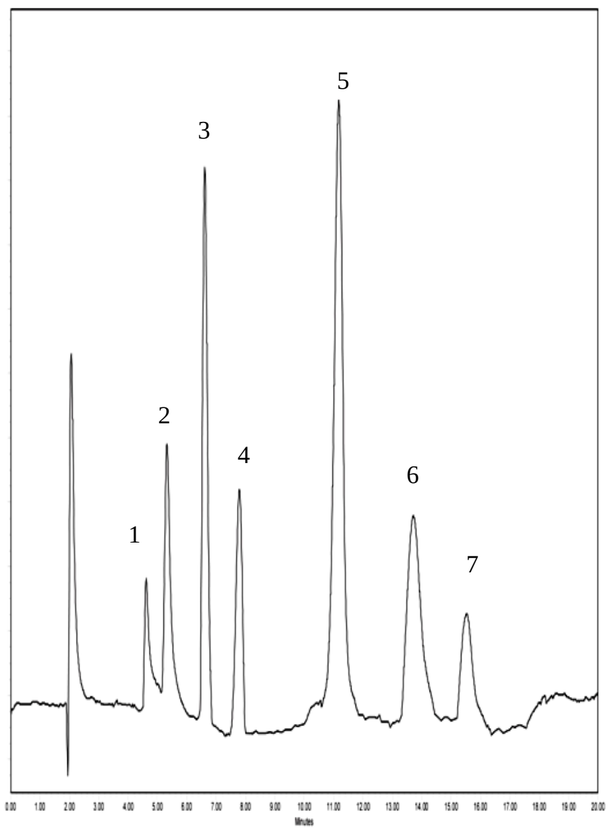


Maltose



Lactose

FIGURE 2. Separation of Carbohydrates



- Column : **Fortis Amino 150x4.6mm 5µ**
- p/n : **FNH-050705**
- Mobile Phase: **75:25 ACN : Water**
- Flow : **1.0ml/min**
- Temp : **30°C**
- λ : **RI**

1. **Ribose**
2. **Xylose**
3. **Fructose**
4. **Glucose**
5. **Sucrose**
6. **Maltose**
7. **Lactose**

Improved Results

Sugars can be difficult to detect due to their lack of chromophore, therefore refractive index detectors are typically employed. Elevated temperatures allow the rapid elution of the sugars. In the analytical method used, Figure 2. a range of seven mono and disaccharides are analysed and provide a suitable separation in a 20min run time.

If we then wish to speed up our analysis times we could change composition of mobile phase or we could increase the temperature further. The labile nature of some compounds can be an issue with temperature so it is probably not a variable used as much in method development as maybe it could be.

Transfer of the Method – HPLC to UHPLC

With the continued proliferation of UHPLC in laboratories the need for small particles for chromatography has grown. The higher efficiency of these particles allows for more sensitive and rapid methods to be developed however there is still a requirement for multiple stationary phase choices to be made available on these small particles allowing for a range of separation selectivities to be achieved.

1.7um Fortis Amino has the identical physical characteristics as its larger 3um and 5um analytical particles.

If this scaleability is available we know that we should be able to use simple calculations (Figure 3) in order to transfer the method. All of these calculations can usually be achieved quickly and simply with one of the many electronic calculators available online, such as:

www.uhplccolumns.com/UHPLC_Calculator.html

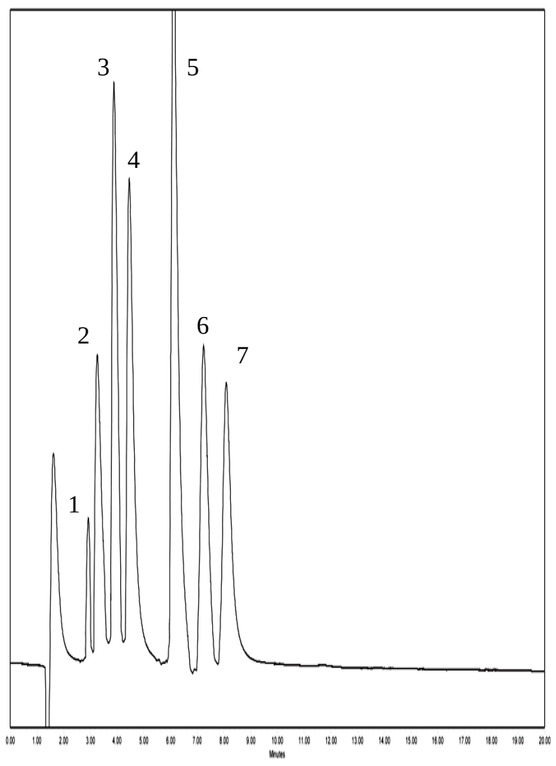
In this instance we have used the equation below to change flow rate to suit a smaller diameter column:

$$F_2 = F_1 \times (Dc_2 / Dc_1)^2 \qquad \text{(fig 3.)}$$

From this we would theoretically end up with an altered flow rate of 0.2ml/min from our original analytical 1.0ml/min.

Since we want to speed up the chromatography and we have sufficient resolution we can increase this to 0.3ml/min and increase the temperature by 10°C in order to achieve a faster throughput, with little or no loss in resolution. The other advantage of increased temperature in UHPLC mode is that the pressure of the system will be reduced. If we used a polymeric particle here we would struggle to utilise the pressure of UHPLC since the polymer will have a much softer physical nature.

FIGURE 4. UHPLC - Carbohydrates



- Column : **1.7µ Fortis Amino 150x2.1mm**
- p/n : **FNH-020701**
- Mobile Phase: **75:25 ACN : Water**
- Flow : **0.3ml/min**
- Temp : **40°C**
- λ : **RI**

1. **Ribose**
2. **Xylose**
3. **Fructose**
4. **Glucose**
5. **Sucrose**
6. **Maltose**
7. **Lactose**

Conclusion

In this poster we show how the separation of ‘simple’ sugars can be achieved with a new silica based Amino stationary phase providing good peak shape and high resolution.
We then highlight how this can be moved onto a UHPLC smaller 1.7um particle to speed up the run times while still retaining the resolution and selectivity. Run time is reduced from 20minutes down to < 9 minutes with a simple change to flow rate and a slight increase in temperature.

One of the main reasons that analysts have trouble in transferring methods, is the physical nature of many small particles changes from their analytical counterpart, meaning that selectivity then changes. If you wish to accurately transfer analytical methods then scaleability of the exact stationary phase on the exact same silica particle has to be paramount.