Applications of a new HILIC stationary phase

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Introduction

Hydrophilic Interaction Chromatography (HILIC) is an ever more popular and growing area of interest for the retention and separation of polar analytes that are not achievable on traditional reversed phase stationary phase systems. Moving towards the use of normal phase conditions HILIC chromatography offers the ability to retain very polar molecules without the need for complex mobile phase systems, ion-pair reagents or other buffers that weaken the ability to utilise the advantages of MS as a detection technology.

We discuss the use of a new HILIC stationary phase for the separation and retention of polar analytes, we compare this to other LC techniques for the retention of polar analytes, such as high pH and ion-pair chromatography, and we highlight the relative strengths and weaknesses.

Methodology

The theory of HILIC chromatography revolves around the ability of polar analyte compounds to retain as organic modifier concentration is increased. As more acetonitrile (ACN) is added extended retention is afforded by the partitioning, ion-exchange and hydrogen bonding that can occur on a HILIC stationary phase.

HILIC chromatography works in a similar way to normal phase chromatography. A polar stationary phase surface combined with a non-polar mobile phase, typically ACN, allows for partition of the polar analytes and hence retention and separation. Water is used in low concentration as the strong solvent in order to help elute the compounds.

FIGURE 1. Role of Organic modifier in HILIC

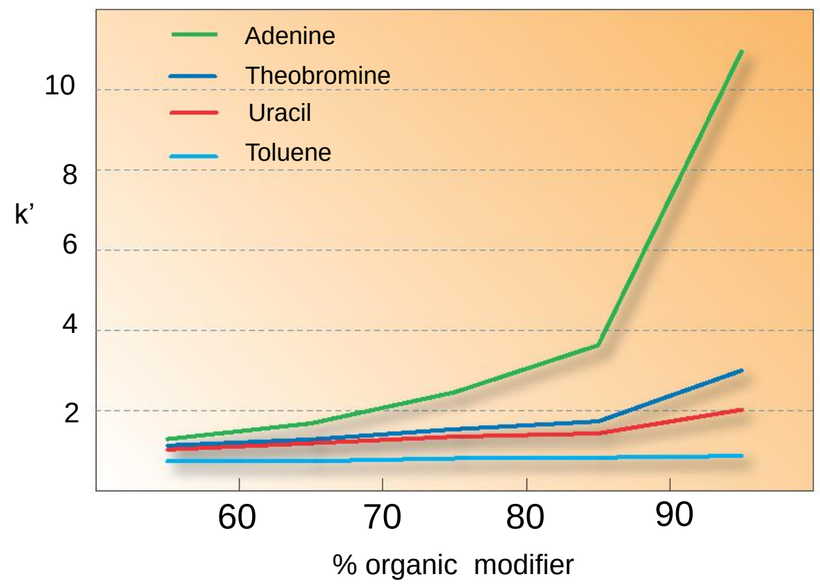
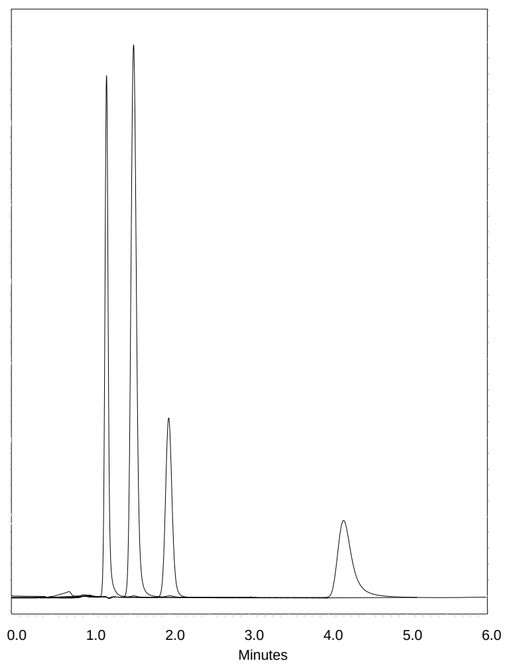


Figure 2 shows the separation of a range of polar species, Pyridoxine and in particular Uracil are very difficult to retain in reversed phase chromatography without the use of ion-pair reagents or similar complex mobile phase systems. Pyridoxine is part of the class of water soluble vitamins, which usually require either 100% aqueous mobile phases or ion-pair reagents in order to gain satisfactory retention for accurate quantitation and qualitation. If MS was the detection method of choice the use of highly aqueous or ion-pair mobile phases systems would be problematic.
One consideration with HILIC chromatography is that of the unretained peak (*t0*), generally measured by Uracil or a similar unretained peak, care must be taken since these compounds can now be retained.

FIGURE 2. Retention of Polar analytes



Column : **Fortis HILIC™ 50x3.0mm 5µ**
p/n : **FHI-030305**
Mobile Phase:
95:5 – ACN: Water

Flow : **0.3ml/min**
Temp : **Ambient**
λ : **200nm**

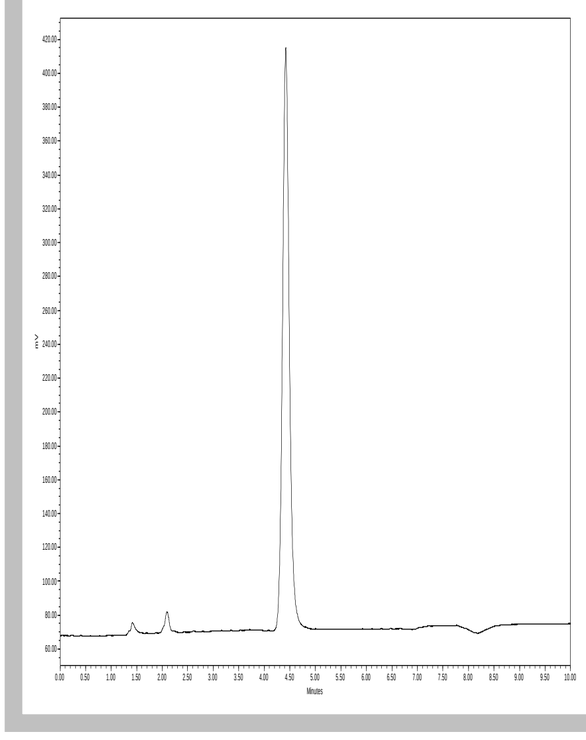
1. Fluorouracil
2. Uracil
3. Theobromine
4. Pyridoxine

Discussion

To retain polar analytes multiple options are available, all have positives and negatives:
1. ion-pair reagents : **pos +** good retention , **neg -** poor MS compatibility
2. High pH :**pos+** good retention, good MS results , **neg –** only works for polar basic species
3. Use of a polar embedded or polar endcapped stationary phase: **pos +** some RT of polars, **neg –** need very high water concentration, very polar analytes still have no retention.

HILIC offers the options to retain the very polar analytes in a more friendly MS mobile phase then ion-pair, retention of acids is also achievable.

FIGURE 3. Retention of Melamine

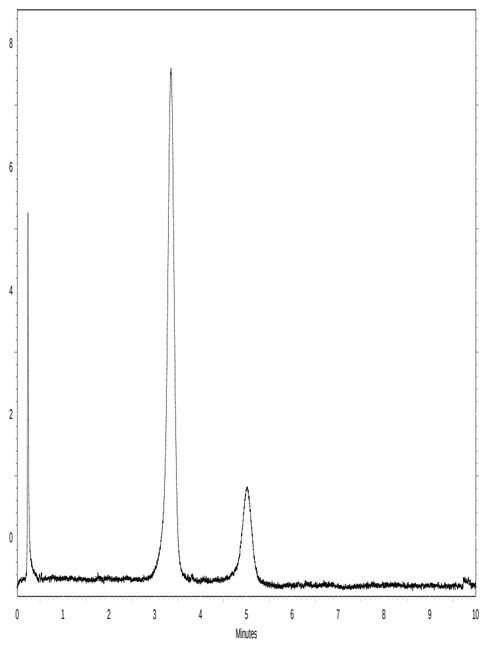


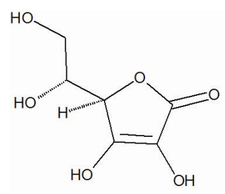
Column : **Fortis HILIC ™100x2.1mm 3µ**
p/n : **FHI-020503**
Mobile Phase:
90:10 – ACN: + 20mM NH_3OAc
Flow : **0.2ml/min**
Temp : **20°C**
λ : **210nm**

Melamine

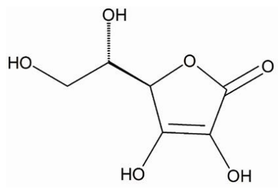
Figure 3 Shows the retention of Melamine, a much talked about compound due to its adulteration into baby milk and pet food. Its highly polar 1,3,5-Triazine structure means it is very difficult to retain in traditional RP-HPLC. The Fortis™ HILIC column leads to a simple UV methodology being developed with a good linear quantitation range even when present in a complex matrix of milk powder without the need for sample preparation.

FIGURE 4. Resolution of Vitamin C Isomers





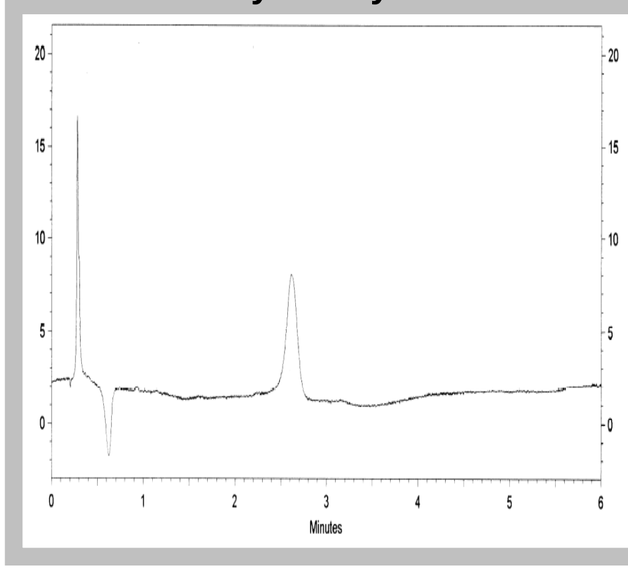
Isoascorbic acid



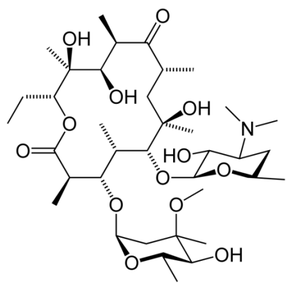
Ascorbic acid

Column : **Fortis HILIC™ 50x4.6mm 5µ**
p/n : **FHI-050305**
Mobile Phase:
90:10 – ACN:100mM HN_3OAc

FIGURE 5. Erythromycin - Antibiotic



Column : **Fortis HILIC™ 50x4.6mm 5µ**
p/n : **FHI-050305**
Mobile Phase:
90:10 – ACN:100mM HN_3OAc
Flow : **0.8ml/min**
Temp : **25°C**
λ : **200nm**



If we look at a more complex sample such as the antibiotic erythromycin (figure 5) we again can gain good retention of this highly polar analyte with the use of a HILIC stationary phase and mobile phase system.

Conclusion

We have shown the use of a HILIC stationary phase which allows the analysis of polar analyte species, water soluble vitamins, melamine, acids and basic species. The use of HILIC allows retention of species that would not normally retain in traditional RP HPLC. Good sensitivity in MS can be achieved due to the high organic concentrations utilised.
Whilst other options are available such as high pH or ion-pair reagents both have limitations. HILIC stationary phases give us another option to retain and therefore resolve very polar analyte species.

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All columns are original manufacturers packed columns

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