

Application Note

Use of Fortis™ HILIC stationary phases

Introduction

Hydrophilic interaction liquid chromatography or HILIC is a technique that has been around for quite some time, based somewhere between reversed-phase (RP) and normal-phase (NP) chromatography.

Understanding HILIC

A highly polar stationary phase, often bare silica, is used with largely a non-polar mobile phase system (water acting as a very strong solvent in HILIC) to provide retention of polar or hydrophilic molecules.

The mechanisms of HILIC are still being fully explored but it is quite well accepted now that the analyte partitions between the mobile phase and a water enriched layer at the surface of the stationary phase. The surface

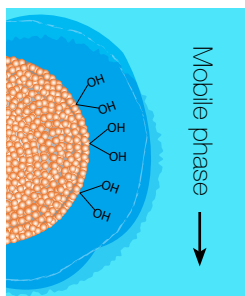


Figure 1. Water layer at the stationary phase surface

of the stationary phase when underivatized forms this layer with water due to the exposed silanols. Also thought to be part of the retention mechanism is ion-exchange and hydrogen bonding dependant upon the analyte in question.

For example, basic analytes with positive charge will partition into the water layer at the surface, but will also have a cation exchange mechanism with the silanols at the phase surface.

Method Development

Adjustment of the retention profile of hydrophilic compounds is made by several potential changes; adjustment of the organic:aqueous eluent, both ratio and organic solvent choice, concentration and

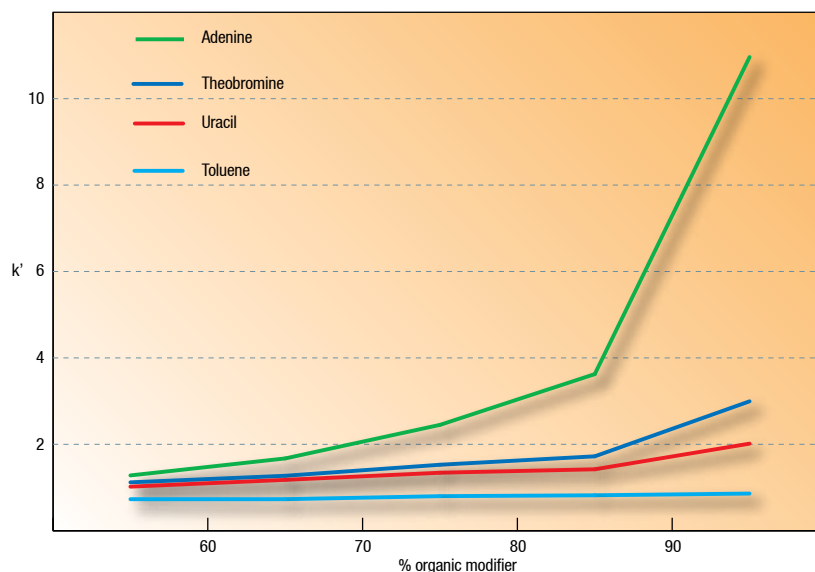


Figure 2. Role of organic modifier in HILIC

type of buffer used, and the pH of the mobile phase. Generally acetonitrile is the common choice for organic, with between 2% and 40% water added in order to elute the compounds in a suitable time frame. The steep curve of retention (figure 2) is a common theme in HILIC, where at a given point the organic:aqueous ratio is such that the compound suddenly exhibits strong retention where previously little was achieved. Figure 3.

Advantages of HILIC

HILIC chromatography provides retention of highly polar hydrophilic analytes that are difficult to retain on RP methods using simple combinations of mobile phase and stationary phase. Many RP options have been tried over the years; polar-endcapping and polar-embedded stationary phases, ion pair reagents and derivatization can all work to a greater or lesser extent. However all of them have drawbacks either in low retention, stationary phase "bleed" or lack of compatibility with detection techniques such as mass spectrometry (MS). HILIC provides a distinct advantage in this link up with MS, in that sensitivity is increased dramatically due to the

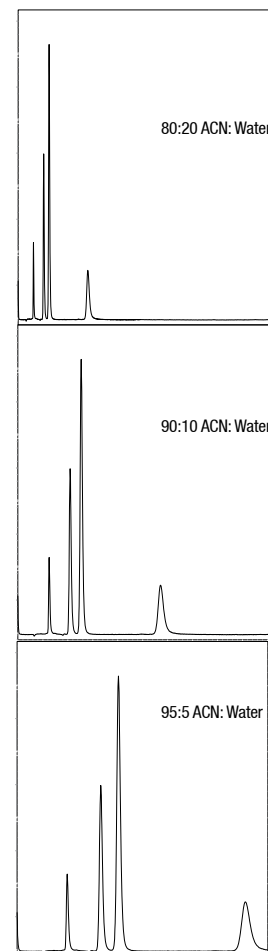


Figure 3. Role of Water as modifier in HILIC

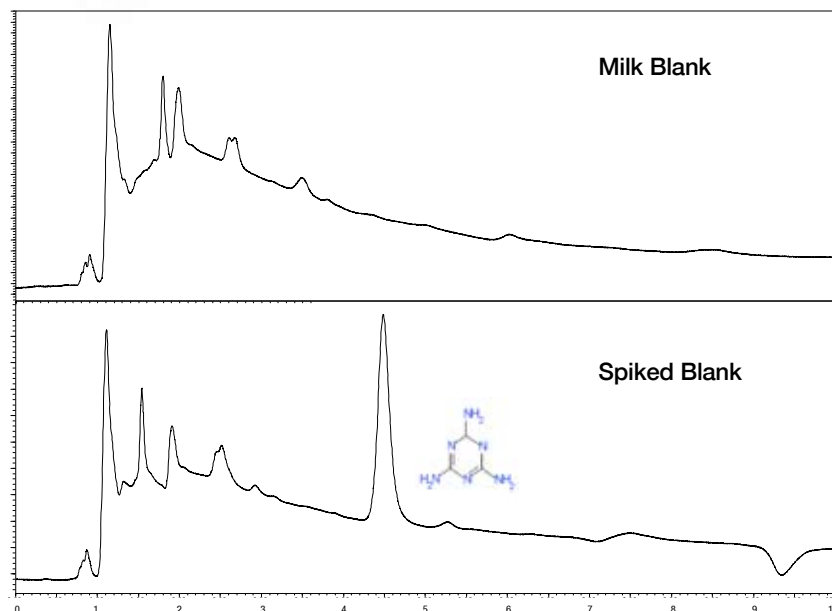


Figure 4. Melamine Contamination

Column: Fortis HILIC 100x2.1mm 3µ
p/n: FHI-020503
Mobile Phase: 90:10 ACN : 20mM NH₄OAc
Flow: 0.2ml/min
Temp: 20°C
Wavelength: 210nm

high organic concentration and this eases the ionization process during analyte infusion.

Hydrophilic retention

Most HILIC applications tend to centre around the analysis of small hydrophilic molecules such as carbohydrates, nucleosides and nucleotides, amino acids and organic acids. However use has been found with diverse molecules such as the application of melamine, figure 4, when there was a worldwide baby milk scare in recent years. Drugs of abuse, sugars, environmental herbicides/pesticides, small peptides and proteins are all applicable to the mechanisms that HILIC offers. The range is potentially limitless for small hydrophilic molecules.

Figure 5. highlights the ability of Fortis™ HILIC to separate and resolve a mixture (>150 compounds) of peptides, organic acids, sugars, phosphosugars and amino acids within a complex metabolite sample. The Fortis HILIC column is compared with another popular HILIC stationary phase, and exhibits better peak shapes, less co-elution,

full baseline resolution for species such as Leucine and Isoleucine. The amine, Spermidine proves difficult to elute with the ZIC-HILIC stationary phase under these gradient conditions, however the Fortis HILIC column provides good recovery and sensitivity.

UHPLC HILIC

UHPLC is the major talking point at this moment in time for most people in method development and those in chromatography with time or sample limitations. 1.7µm Fortis columns are available with two choices for UHPLC separations. Fortis HILIC an underivatized bare-silica stationary phase, and Fortis

HILIC DIOL; a bonded DIOL phase chemistry to aid in the retention and resolution of steroids, proteins and metabolites especially. The theory of basic analytes causing peak shape problems due to silanol activity is the norm in reversed phase chromatography, however in HILIC the current thought is that vastly superior peak shapes are achieved for basic analytes when the unbonded silica substrate is employed. The theory is that the silanols are now freely accessible to interact with the basic analytes instead of any steric hindrance compromising the peak shape. This improved peak shape for basic analytes is a major advantage with HILIC silica phases, leading ultimately to an improvement in sensitivity. The bare silica version of the Fortis particles have also recently been shown to exhibit excellent peak shape selectivity and resolution in SFC mode. This will be a major issue to those wishing to assess the suitability of “green techniques” within their laboratory.

Conclusion

In this application note we have shown the mechanisms and considerations that are important to those requiring the HILIC method of chromatography in order to retain the more polar analytes that can cause issues in retention and separation. The correct use of a HILIC column can provide additional selectivity and resolution, especially for hydrophilic analytes. As the understanding of HILIC mechanisms increases then the ability of this technique to

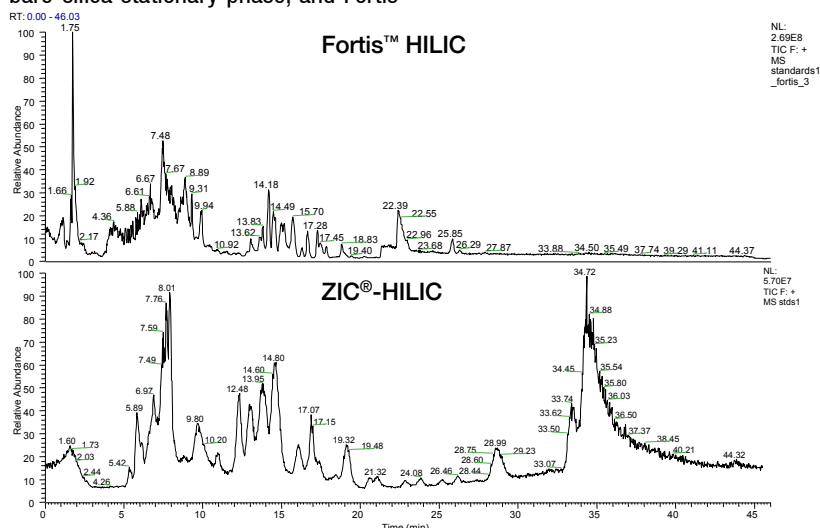


Figure 5. Selectivity of complex metabolite mixture. Data courtesy of ScotMet:Glasgow

grow will increase as sample types dictate. The new 1.7µm UHPLC offerings for HILIC chromatography (HILIC & HILIC DIOL) allow the analyst further gains in efficiency and speed. This will give the capability to develop rapid methods with high resolution of analytes. In HILIC with the power of MS detection, many analyte types can be resolved and we have highlighted a sample of 150 metabolomic species in a simple HILIC gradient system. The diversity of the sample could potentially cause problems but the sharp peaks shapes provided by the homogenous silica surface of the Fortis HILIC aid in the resolution achieved.

Fortis HILIC		Column Length					
		20	30	50	100	150	250
Column Diameter	2.1	FHI-0201xx	FHI-0202xx	FHI-0203xx	FHI-0205xx	FHI-0207xx	-
	3.0		FHI-0302xx	FHI-0303xx	FHI-0305xx	FHI-0307xx	-
	4.6		FHI-0502xx	FHI-0503xx	FHI-0505xx	FHI-0507xx	FHI-0509xx

Replace xx - 01 for 1.7µm - 03 for 3µm - 05 for 5µm - 10 for 10µm

Fortis HILIC DIOL		Column Length					
		20	30	50	100	150	250
Column Diameter	2.1	FDI-0201xx	FDI-0202xx	FDI-0203xx	FDI-0205xx	FDI-0207xx	-
	3.0		FDI-0302xx	FDI-0303xx	FDI-0305xx	FDI-0307xx	-
	4.6		FDI-0502xx	FDI-0503xx	FDI-0505xx	FDI-0507xx	FDI-0509xx

Replace xx - 01 for 1.7µm - 03 for 3µm - 05 for 5µm - 10 for 10µm

1.7µm Fortis™ is a trademark of Fortis Technologies. ZIC®-HILIC is a registered trademark of Merck Sequant AB. All columns are original manufacturers own.