

# EVALUATION OF NOVEL TRIAZOLE BONDED STATIONARY PHASE FOR HYDROPHILIC INTERACTION CHROMATOGRAPHY



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## Abstract

Hydrophilic Interaction Chromatography (HILIC) has become a useful alternative to reversed phase chromatography for separation of highly polar compounds, which are either weakly retained or not retained on traditional reversed phase columns. HILIC is a variation of normal phase chromatography where a polar stationary phase is used with a mobile phase which contains a high concentration of organic solvent and a low concentration of aqueous eluent. The main retention mechanism is the partitioning of the polar analytes between the polar stationary and the mobile phase containing a high concentration of water miscible organic solvent. It also shows a weak electrostatic interaction between the analytes and the stationary phase. Non-modified silica column is often used in HILIC mode. However, the separation and peak shape for acidic compounds are problematic. In this study, the novel triazole bonded stationary phase is evaluated in HILIC with several acidic and basic compounds. The positively charged triazole bonded stationary phase provides better separation for acidic compounds compared to a non-modified silica column.

## Introduction

This study demonstrated the differences in separation characteristics between non-modified silica column and the newly developed triazole bonded stationary phase in HILIC mode. The triazole bonded stationary phase provides higher polarity than non-modified silica column, therefore a stronger hydrophilic interaction is predicted. The positively charged triazole stationary phase also shows anion-exchange mechanism, thus acidic compounds can be strongly retained. Several parameters influence separation such as buffer pH, salt concentration and composition of organic solvent were evaluated.

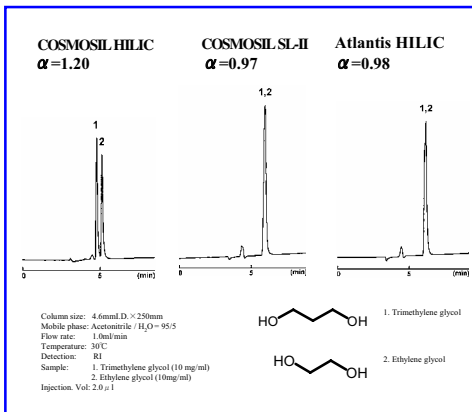
## Columns Evaluated

Column	Manuf.	Particle size	Pore size	Stationary Phase
COSMOSIL HILIC	Nacalai Tesque	5 $\mu\text{m}$	120 $\text{\AA}$	Triazole
COSMOSIL SSL-II	Nacalai Tesque	5 $\mu\text{m}$	120 $\text{\AA}$	Silica
Atlantis HILIC Silica	Waters	5 $\mu\text{m}$	100 $\text{\AA}$	Silica

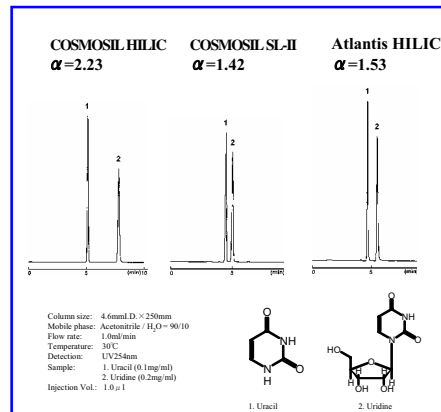


## Experimental Results

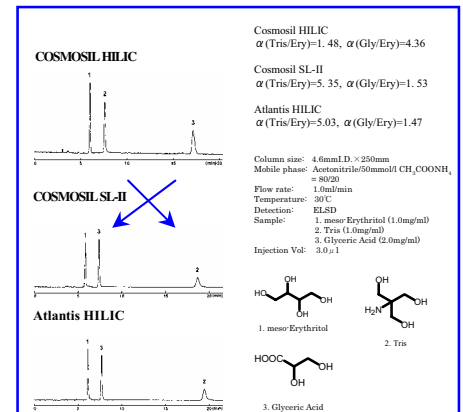
### Hydrophilic interaction 1



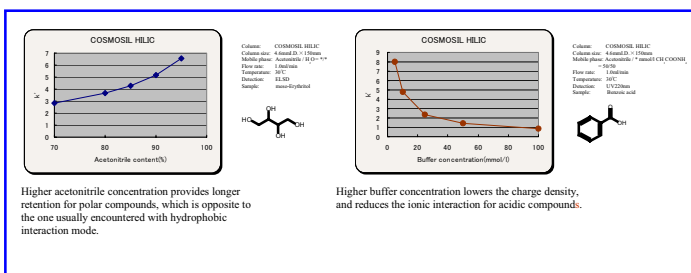
### Hydrophilic interaction 2



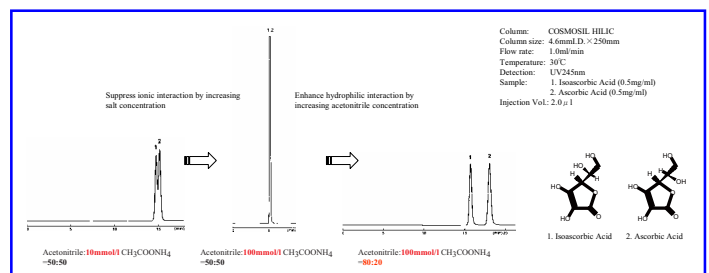
### Ionic interaction



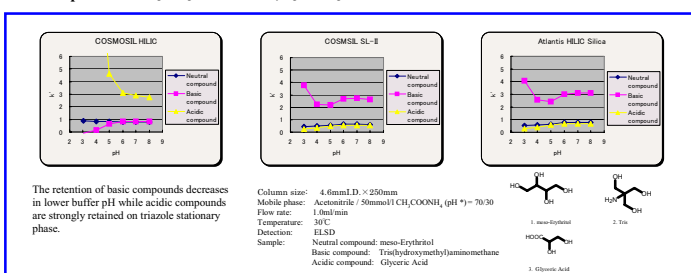
### Effect of mobile phase 1 the concentration of organic solvent and the buffer strength influence the retention.



### Effect of mobile phase 2 the retention can be controlled by changing the composition of mobile phase.



### Effect of pH the mobile phase pH is one of the key separation parameters.



## Conclusions

- The most important separation mechanism for HILIC mode, hydrophilic interaction, is greatly improved on triazole stationary phase compared to non-modified silica column.
- The positively charged triazole stationary phase shows unique anion-exchange mechanism, which provides greater retention for acidic compounds.
- Two separation modes -- ionic interaction and HILIC-- can be controlled by varying key mobile phase parameters such as pH, concentration of organic solvent and buffer ionic strength.