

# Evaluation of Anion-exchange Mechanism on The Unique Positively Charged Stationary Phase for Hydrophilic Interaction Chromatography



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## Abstract

In recent years Hydrophilic Interaction Chromatography (HILIC) has become a significantly important alternative to reversed phase chromatography for separation of highly polar compounds, which are either weakly retained or not retained on traditional reversed phase columns. Although the main retention mechanism is partitioning of polar analytes between the polar stationary and the mobile phase containing a high concentration of water miscible organic solvent, weak electrostatic interactions are also observed when ionic analytes are present. In this study, we evaluated the anion-exchange characteristic on the novel triazol bonded stationary phase in HILIC mode. Since non-modified silica based HILIC column is negatively charged, the positively charged triazol phase shows unique and different separation from non-modified silica based HILIC column. The separation characteristic is also different from the ones with zwitterionic type HILIC column. The effect of mobile phase pH, salt concentration and concentration of organic solvents is also discussed.

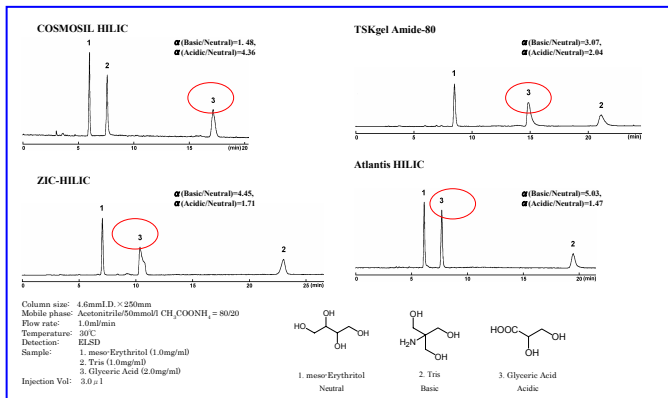
## Introduction

This study demonstrated the differences in ion-exchange characteristics among several stationary phases in HILIC mode. The positively charged triazole stationary phase shows anion-exchange mechanism, thus acidic compounds can be strongly retained. On the other hand, the negatively charged non-modified silica based column retains basic compounds more strongly than other columns. Interestingly, the anion-exchange characteristic was not observed on the zwitterionic type stationary phase. Several parameters influence separation such as buffer pH, salt concentration and concentration of organic solvent were evaluated.

## Experimental Results

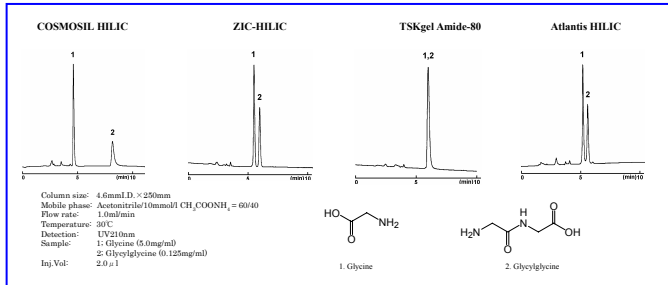
**Figure 1 : Separation of acidic, basic and neutral compounds**

Acidic (Glyceric Acid), basic (Tris) and neutral (meso-Erythritol) compounds were used for evaluation of anion and cation exchange characteristics. The separation factor  $\alpha$  (Acid/Neutral) indicates its anion-exchange capability and the factor  $\alpha$  (Basic/Neutral) shows its cation-exchange effect.



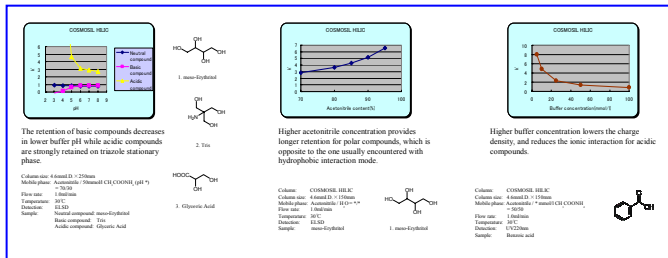
**Figure 3 : Separation of zwitterionic compounds**

The strong anion-exchange capability of COSMOSIL HILIC effects on the separation of zwitterionic compounds.



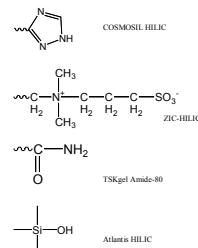
**Figure 5 : Effect of pH, composition of organic solvent and salt concentration**

Hydrophilic interaction was enhanced by higher concentration of organic solvent, and ionic interaction was decreased by higher salt concentration.



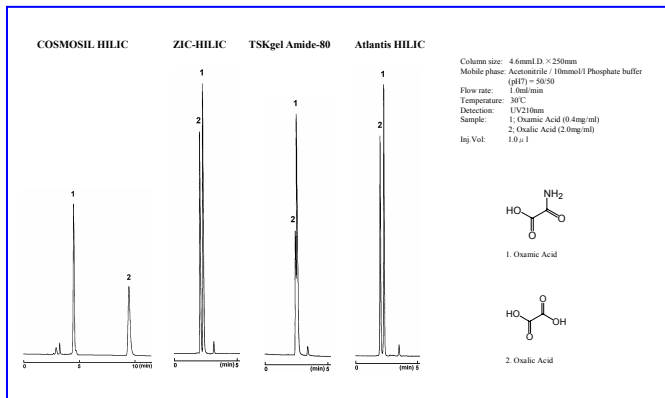
## Columns Evaluated

Column	Manf.	Particle size	Pore size	Stationary Phase
COSMOSIL HILIC	Nacalai	5µm	120Å	Triazol
ZIC-HILIC	SeQuant	5µm	120Å	Sulfobetaine
TSKgel Amide-80	TOSOH	5µm	80Å	Amide
Atlantis HILIC	Waters	5µm	100Å	Silica



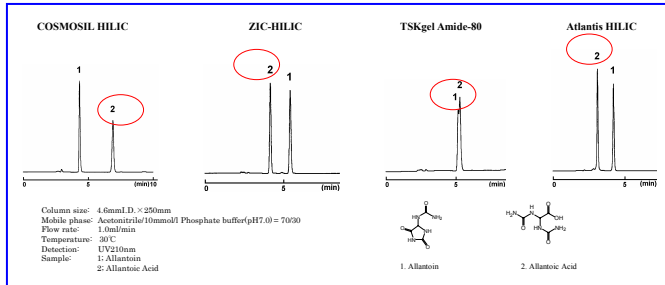
**Figure 2 : Separation of anionic compounds**

Anionic compounds were used to evaluate the anion-exchange capability. The only COSMOSIL HILIC showed strong selectivity of anionic compounds.



**Figure 4 : Separation of basic and zwitterionic compounds**

Allantoin and its oxidized compound Allantoinic acid were used for evaluation of ion-exchange characteristics. The elution was reversed between positively charged COSMOSIL HILIC and negatively charged Atlantis HILIC. ZIC-HILIC showed similar elution pattern as Atlantis HILIC. Non-ionic stationary phase of Amide-80 did not show selectivity for these compounds.



## Conclusions

- The positively charged triazol stationary phase showed strong anion-exchange characteristic, while the cation-exchange, due to a small amount of negatively charged residual silanol groups, was not observed.
- The zwitterionic type stationary phase (ZIC-HILIC) showed very weak anion-exchange effect, probably caused by a steric hindrance of its structure. ZIC-HILIC also showed a little weaker cation-exchange capability than non-modified silica stationary phase.
- A broad application of COSMOSIL HILIC can be expected when two separation modes – ionic interaction and HILIC – are controlled by varying two key mobile phase parameters – concentration of organic solvent and buffer ionic strength.