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.

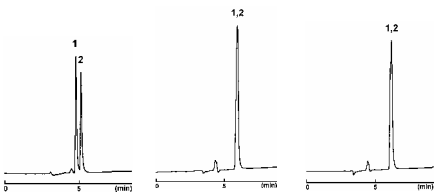
Columns Evaluated

Column	Manuf.	Particle size	Pore size	Stationary Phase
COSMOSIL HILIC	Nacalai Tesque	5 µm	120 Å	Triazole
COSMOSIL 5SL-II	Nacalai Tesque	5 µm	120 Å	Silica
Atlantis HILIC Silica	Waters	5 µm	100 Å	Silica

Experimental Results

Hydrophilic interaction 1

COSMOSIL HILIC α=1.20 **COSMOSIL SL-II** α=0.97 **Atlantis HILIC** α=0.98



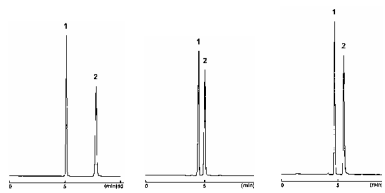
Column size: 4.6mmI.D.×250mm
Mobile phase: Acetonitrile / H_2O = 95/5
Flow rate: 1.0ml/min
Temperature: 30℃
Detection: RI
Sample: 1. Trimethylene glycol (10 mg/ml)
2. Ethylene glycol (10mg/ml)
Injection. Vol: 2.0 µl

1. Trimethylene glycol

2. Ethylene glycol

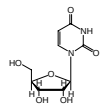
Hydrophilic interaction 2

COSMOSIL HILIC α=2.23 **COSMOSIL SL-II** α=1.42 **Atlantis HILIC** α=1.53



Column size: 4.6mmI.D.×250mm
Mobile phase: Acetonitrile / H_2O = 90/10
Flow rate: 1.0ml/min
Temperature: 30℃
Detection: UV254nm
Sample: 1. Uracil (0.1mg/ml)
2. Uridine (0.2mg/ml)
Injection Vol.: 1.0 µl

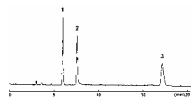
1. Uracil



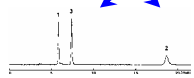
2. Uridine

Ionic interaction

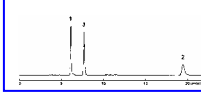
COSMOSIL HILIC



COSMOSIL SL-II



Atlantis HILIC



Cosmosil HILIC
α(Tris/Ery)=1. 48, α(Gly/Ery)=4.36

Cosmosil SL-II
α(Tris/Ery)=5. 35, α(Gly/Ery)=1. 53

Atlantis HILIC
α(Tris/Ery)=5.03, α(Gly/Ery)=1.47

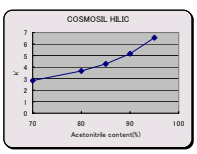
Column size: 4.6mmI.D.×250mm
Mobile phase: Acetonitrile/50mmol/l CH_3COONH_4 = 80/20
Flow rate: 1.0ml/min
Temperature: 30℃
Detection: ELSD
Sample: 1. meso-Erythritol (1.0mg/ml)
2. Tris (1.0mg/ml)
3. Glyceric Acid (2.0mg/ml)
Injection Vol: 3.0 µl

1. meso-Erythritol

2. Tris

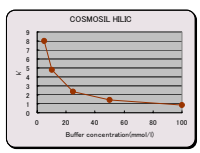
3. Glyceric Acid

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



Column: COSMOSIL HILIC
Column size: 4.6mmI.D. × 150mm
Mobile phase: Acetonitrile / H2O = *:*
Flow rate: 1.0ml/min
Temperature: 30℃
Detection: ELSD
Sample: meso-Erythritol

Higher acetonitrile concentration provides longer retention for polar compounds, which is opposite to the one usually encountered with hydrophobic interaction mode.



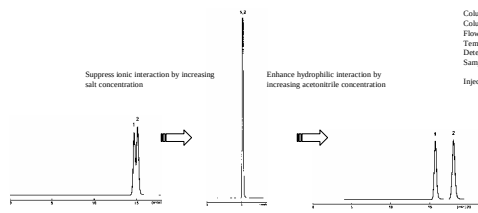
Column: COSMOSIL HILIC
Column size: 4.6mmI.D. × 150mm
Mobile phase: Acetonitrile / * mmol/l CH3COONH4 = 50/50
Flow rate: 1.0ml/min
Temperature: 30℃
Detection: UV220nm
Sample: Benzoic acid

Higher buffer concentration lowers the charge density, and reduces the ionic interaction for acidic compounds.

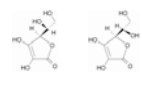
Effect of mobile phase 2 the retention can be controlled by changing the composition of mobile phase.

Suppress ionic interaction by increasing salt concentration

Enhance hydrophilic interaction by increasing acetonitrile concentration



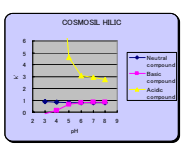
Column: COSMOSIL HILIC
Column size: 4.6mmI.D. × 250mm
Flow rate: 1.0ml/min
Temperature: 30℃
Detection: UV245nm
Sample: 1. Isoascorbic Acid (0.5mg/ml)
2. Ascorbic Acid (0.5mg/ml)
Injection Vol.: 2.0 µl

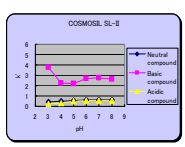


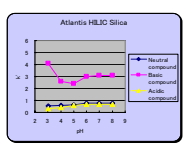
1. Isoascorbic Acid 2. Ascorbic Acid

Acetonitrile:10mmol/l CH3COONH4 =50:50
Acetonitrile:100mmol/l CH3COONH4 =50:50
Acetonitrile:100mmol/l CH3COONH4 =80:20

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







The retention of basic compounds decreases in lower buffer pH while acidic compounds are strongly retained on triazole stationary phase.

Column size: 4.6mmI.D. × 250mm
Mobile phase: Acetonitrile / 50mmol/l CH_3COONH_4 (pH *) = 70/30
Flow rate: 1.0ml/min
Temperature: 30℃
Detection: ELSD
Sample:
Neutral compound: meso-Erythritol
Basic compound: Tris(hydroxymethyl)aminomethane
Acidic compound: Glyceric Acid

1. meso-Erythritol

2. Tris

3. Glyceric Acid

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.