Evaluation of Pentabromobenzyl Stationary Phase for Separation of Polar Compounds by Reversed-Phase Chromatography

Toshi Ono[1], Ken Tseng[1], Tsunehisa Hirose[2]
[1]Nacalai USA, Inc., San Diego, United States, [2]Nacalai Tesque, Inc., Kyoto, Japan

Abstract

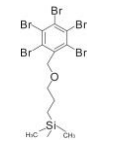
In recent years hydrophilic interaction liquid chromatography (HILIC) has become an increasingly popular method for separation of highly polar compounds. However, it is often difficult to develop a robust method due to less understanding of retention behavior in HILIC mode. Furthermore, high concentration of acetonitrile used in HILIC mobile phase makes it extremely sensitive to water concentration of sample solvation; injecting high water concentration of sample solvation often results in poor peak shape. This study demonstrates the advantages of a novel pentabromobenzyl bonded stationary phase (PBr), which shows dispersion force interactions, for separation of polar compounds in reversed phase conditions. We compare its performance to conventional C18 stationary phase as well as pentafluorophenyl bonded stationary phase (PFP). Excellent separations are demonstrated for various classes of compounds based on the hydrophobic interactions and the dispersion force interactions.

Introduction

A diverse range of HILIC stationary phases are commercially available. However, due to the complex retention mechanism of HILIC mode such as hydrophilic partitioning and electrostatic interactions, all HILIC columns would not retain polar compounds in a similar manner. This poses a common challenge in HILIC method development to identify the most suitable column to use. we recently developed a novel pentabromobenzyl stationary phase to separate highly polar molecules in reversed phase conditions. This column may offer more straight forward method development for separation of polar compounds.

Stationary phase characteristics

Silica Gel	High Purity Spherical Silica
Stationary Phase	Pentabromobenzyl Group
Average Particle Size	5 µm
Average Pore Size	120 Å
Surface Area	300 m²/g
Carbon content	8 %



Pentabromobenzyl Group

Experimental Results

Figure 1 : Comparison of two different HILIC stationary phases

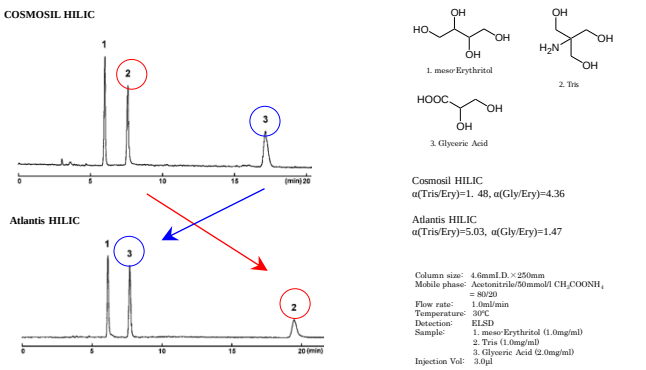


Figure 1 shows the separation of three polar compounds, neutral, basic and acidic, using two different HILIC stationary phases. The elution order of basic and acidic compounds was reversed between positively charged COSMOSIL HILIC stationary phase and negatively charged Atlantis HILIC stationary phase.

Figure 2: Effect of sample solvent on peak shape under HILIC conditions

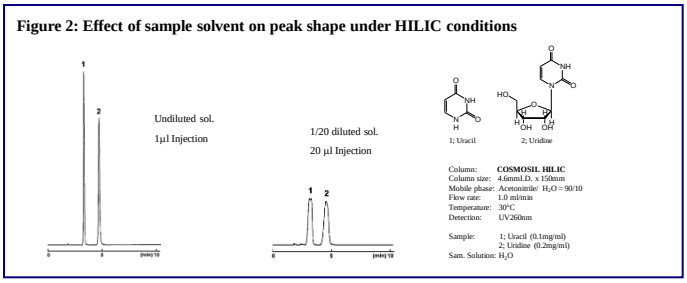


Figure 2 shows injecting high volume of aqueous solvent, which is high elution strength in HILIC mode, resulted in poor peak shape.

Figure 3: Aqueous sample solvent does not affect the peak shape on PBr stationary phase.

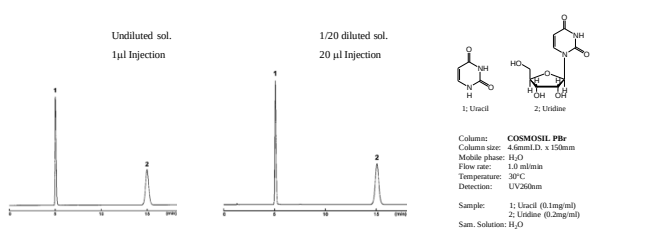
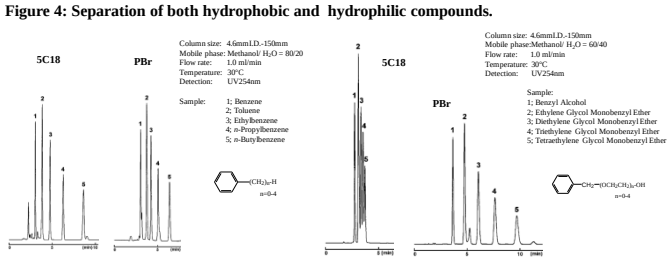


Figure 4: Separation of both hydrophobic and hydrophilic compounds.



Hydrophobic compounds are separated by both C18 and PBr stationary phases. PBr stationary phase can separate hydrophilic compound, which are not retained on C18 stationary phase.

Figure 5 : Separation of Triton-X surfactant.

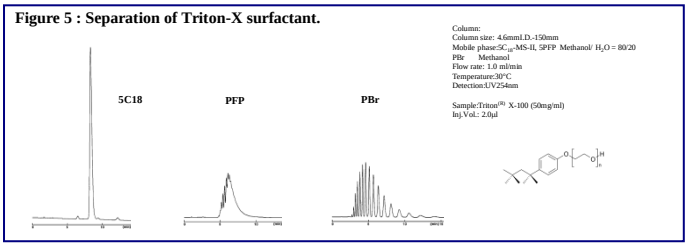
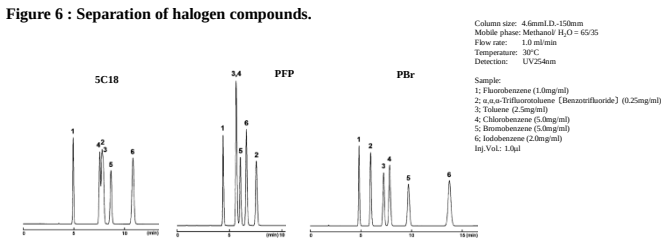


Figure 6 : Separation of halogen compounds.



Conclusions

The PBr and C18 HPLC columns have similar retention to hydrophobic compounds under the same reversed phase conditions. As would be expected for a reversed phase column, hydrophobic compounds are retained longer on the PBr colum.
The PBr column showed significant retention compared to C18 when separating compounds that exhibit dispersion forces;

- ✓ Compounds that can easily polarized, e.g., hydrophilic or polar compounds.
- ✓ Compounds containing high percentage of oxygen and nitrogen, e.g., Triton X-100.
- ✓ Compounds containing heavier and larger atoms, e.g., halogen compounds.