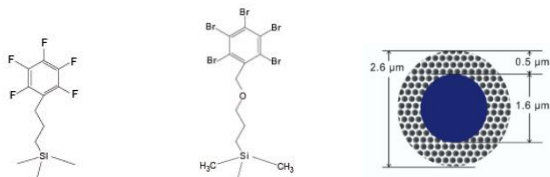


Ken Tseng¹ (ken@nacalaiusa.com), Toshi Ono¹, Tsunehisa Hirose²
¹Nacalai USA, Inc., San Diego, United States, ²Nacalai Tesque, Inc., Kyoto, Japan

Introduction

As pentafluorophenyl (PFP) and pentabromobenzyl (PBr) phases become popular in providing alternate selectivity to C18, it is imperative that we understand their separation mechanisms for better method development. In this poster, we compared 9 compounds on three PFP and one PBr core-shell HPLC columns. Both PFP and PBr phases are mixed-mode columns with π - π , dipole-dipole, dispersion (dipole-induced dipole), and ion-exchange interactions. In our experiments, PFP exhibited mainly reversed-phase and ion-exchange behavior, contributing to its characteristic U-shape retention profile with positively-charged compounds. Surprisingly, PBr showed reversed-phase separation with mostly π - π and dispersion interactions, but little or no ion-exchange. PBr retains non-charged compounds stronger regardless of the polarity when comparing to PFP (and C18). Consequently, PBr can be used as a robust alternative to HILIC for polar molecule analysis in reversed-phase. Further, PBr is useful in polar molecule prep-scale purification due to high sample loading capacity in water.



PentaFluoroPhenyl (PFP) PentaBromoBenzyl (PBr) 2.6µm Core-Shell Particle

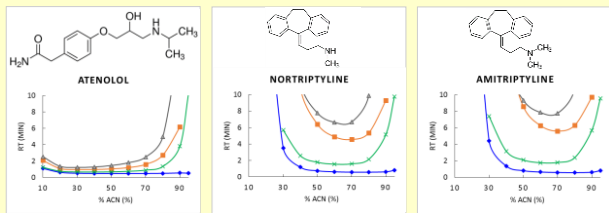
PBr vs. PFP Selectivity Comparison for each Compound

Retention time profile of three PFP and one PBr core-shell HPLC columns on 9 compounds.

Phase	End-Capped	Color	Core-Shell Silica HPLC Columns
PBr	Yes	Blue diamond	Cosmocore PBr, 90Å, 2.6µm, 2.1x100mm
PFP	No	Green cross	Raptor FluoroPhenyl, 90Å, 2.7µm, 2.1x100mm
PFP	Yes	Orange square	Ascentis Express F5, 90Å, 2.7µm, 2.1x100mm
PFP	Yes	Grey triangle	Sunshell PFP, 90Å, 2.6µm, 2.1x100mm

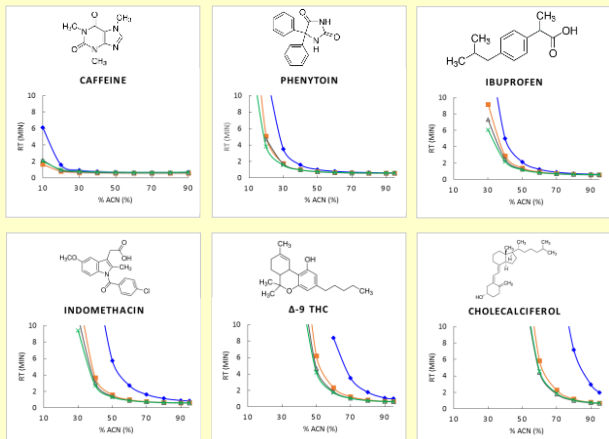
A: 0.1% formic acid in water B: 0.1% formic acid in acetonitrile
 0.4mL/min, 40°C, UV 254nm

Charged Compounds (basic)



All PFP columns exhibit the characteristic U-shape with positively-charged compounds. This is caused by dual separation modes of reversed-phase and ion-exchange in PFP. However, the retention time is markedly different for each brand. PBr lacks such strong ion-exchange mechanism, rather the reversed-phase interaction dominates.

Non-Charged Compounds (neutral and acidic)

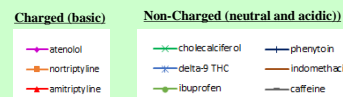


PBr retains non-charged compounds stronger than PFP in reversed-phase. It is, therefore, possible to retain very polar compounds in 100% aqueous mobile phase with PBr. All three PFP columns exhibit similar selectivity for non-charged compounds.

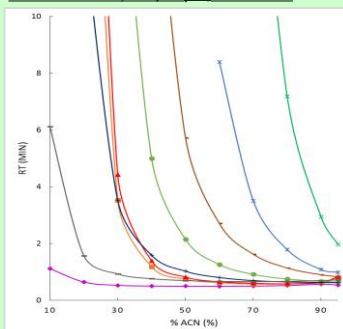
PBr vs. PFP Selectivity Comparison for each Column

Retention time profile of 9 compounds on three PFP and one PBr core-shell HPLC columns.

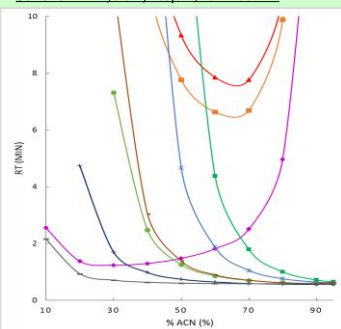
A: 0.1% formic acid in water
 B: 0.1% formic acid in acetonitrile
 0.4mL/min, 40°C, UV 254nm



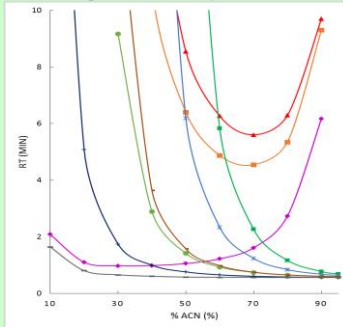
Cosmocore PBr, 90Å, 2.6µm, 2.1x100mm



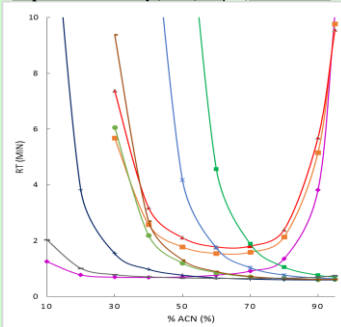
Sunshell PFP, 90Å, 2.6µm, 2.1x100mm



Ascentis Express F5, 90Å, 2.7µm, 2.1x100mm



Raptor FluoroPhenyl, 90Å, 2.7µm, 2.1x100mm



PBr retains non-charged compounds stronger than PFP in reversed-phase. All three PFP columns exhibit similar selectivity for non-charged compounds. The positively-charged compounds with their U-shape lines showed very different retention time in each PFP column tested. The difference could be due to ligand density, silica surface treatment, and type of linkers used.

Conclusions

- For **positively-charged compounds**, PFP columns exhibited both reversed-phase and ion-exchange separation, although each brand retains them very differently. The difference could be due to ligand density, bonding method, and silica surface treatment used. PBr showed mainly reversed-phase separation with little or no ion-exchange. PBr retains less of positively-charged compounds compare to PFP.
- For **non-charged compounds**, all three PFP columns produced almost identical retention. PBr showed consistently higher retention compare to PFP, sometimes significantly.
- Because PBr retains non-charged compounds much stronger than PFP and C18, it can be used for polar molecule analysis in reversed-phase mode, in high aqueous condition even in 100% water. This means PBr can compliment or replace HILIC for very polar compound analysis.
- PBr can also be useful in preparative-scale purification of very polar molecules by taking advantage of high sample solubility in aqueous solvent.
- More research needed to fully understand PFP and PBr separation mechanisms.