STUDY OF ENHANCED π-π INTERACTIONS ON REVERSED PHASE HPLC COLUMNS.

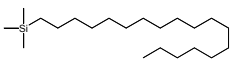
Toshi Ono[1], Jing Xu[1], Tsunehisa Hirose[2], Naoki Hiura, Kazuhiro Kimata[2]
[1]Nacalai USA, Inc., San Diego, United States, [2]Nacalai Tesque, Inc., Kyoto, Japan

Abstract

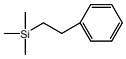
Separation of unsaturated compounds, which contain carbon-carbon π-bonds, on reversed phase chromatography is often problematic. Phenyl stationary phases are commonly used for these compounds because they form π-π interactions and show different selectivity from conventional alkyl phases such as C18 and C8. However, the π-π interactions on phenyl stationary phases are moderate, and the separations for some unsaturated compounds are still insufficient. We have recently developed a new stationary phase with naphthylethyl (NAP) group bonded silica material. The naphthylethyl group is composed of two fused aromatic rings and forms significantly stronger π-π interactions with unsaturated compounds than the phenyl stationary phase. In this study, the effect of π-π interactions on reversed phase HPLC is evaluated with unsaturated compounds such as tocopherols, steroids and sterols. Pyrenylethyl (PYE) group bonded stationary phase, which has much stronger π electrons than naphthylethyl group, is also evaluated.

Columns Evaluated

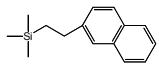
COSMOSIL 5C$_{18}$-MS-II
Stationary phase: Octadecyl group
Carbon content: 16.40%



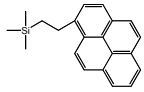
COSMOSIL PE-MS
Stationary phase: Phenylethyl group
Carbon content: 9.95%



COSMOSIL πNAP
Stationary phase: Naphthylethyl group
Carbon content: 10.80%

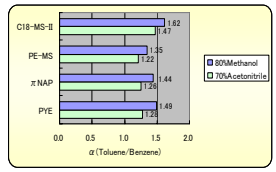


COSMOSIL PYE
Stationary phase: Pyrenylethyl group
Carbon content: 17.40%



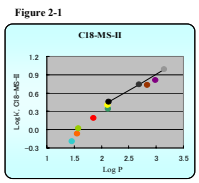
Experimental Results

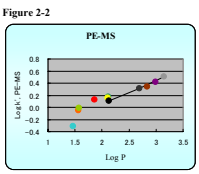
Figure 1 : Comparison of hydrophobic interaction

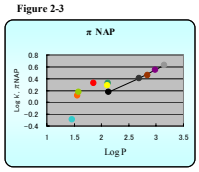


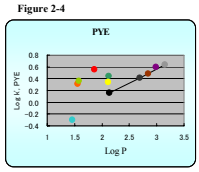
The degree of hydrophobic interactions was measured by the separation factor α (Toluene/Benzene).
C18 stationary phase shows the highest degree in hydrophobic interaction.

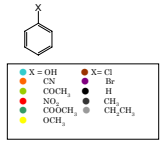
Figure 2 : Selectivity for mono-substituted benzene











The partition coefficient was used for the hydrophobic property.

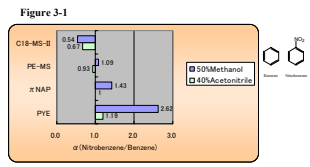
log P = log ([Solute] octanol / [Solute] water)
The larger the hydrophobicity, the larger the number of *log p*.

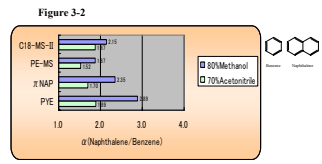
The hydrophobic functional groups, -H, -CH3 and -CH2CH3 were lined.

Figure 2-1 shows that the retentions on C18 depend on the degree of hydrophobicity.

Figures 2-2 to 2-4 show that the compounds which have π electron such as CN, COCH3 and NO2 were strongly retained on the other columns, of which the stationary phases also have π electrons.

Figure 3 : Comparison of π-π interaction strength





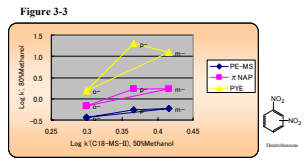


Figure 3-1 shows the nitrobenzene/benzene separation factors and Figure 3-2 shows naphthalene/benzene separation factors which indicate the strength of π-π interaction.

Both methanol and acetonitrile were used as mobile phases. The greater separation factors were observed when methanol was used. This indicates the π electron on acetonitrile itself interferes the π-π interaction between the samples and the stationary phase.

Figure 3-3 shows the selectivity of positional isomers.

The more number of aromatic rings, the stronger π-π interaction observed.

Figure 4 : Separation of tolunitrile isomers

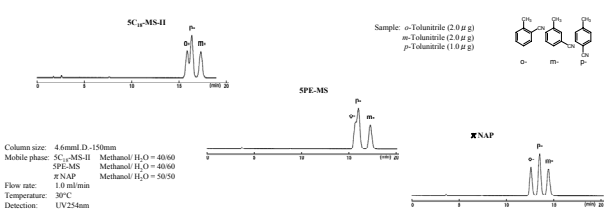


Figure 5 : Separation of steroids

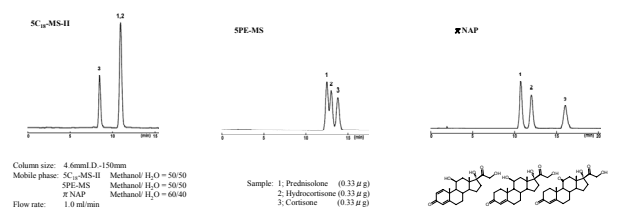
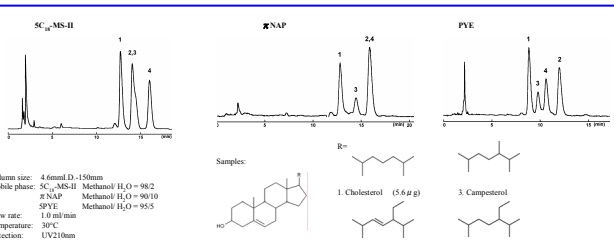


Figure 6 : Separation of sterols



Conclusions

- The more number of aromatic rings, the stronger π-π interaction observed.
- The stronger π-π interaction offers the greater selectivity for the compounds which are problematic to separate on a conventional phenyl stationary phase or a C18 column.
- In many cases, COSMOSIL π NAP column offers excellent separation for unsaturated compounds, however, COSMOSIL PYE column provides much stronger π-π interactions and better selectivity when the separation is still insufficient on π NAP column.