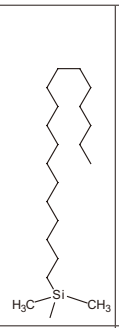
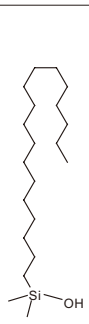
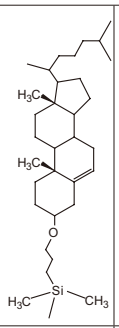
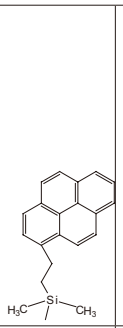
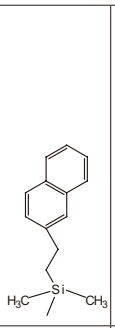
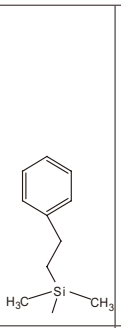
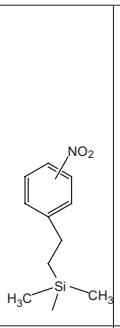
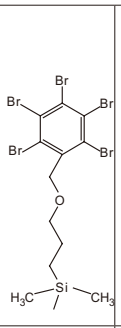
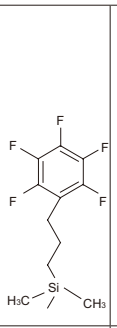
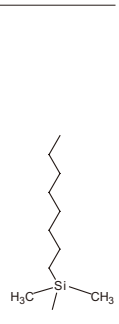
(7) Selectivity of Packing Materials in Reversed Phase Liquid Chromatography

Introduction

Reversed phase chromatography is the most commonly used method of HPLC because of its high theoretical plate number, excellent separation characteristics, reproducibility, and ease of use. Columns packed with octadecyl group-bonded silica gel (C_{18}, ODS) are the most widely used. However, C_{18} columns do not provide sufficient separation for compounds similar in hydrophobicity because their main separation mechanism is the hydrophobic interaction. Using longer columns, changing mobile phases or changing temperature may improve separation. However, in many cases, it is most effective to use different packing materials which retain compounds using a secondary interaction in addition to the hydrophobic interaction. Nacalai Tesque offers a variety of reversed phase packing materials. These are summarized in Table 1. Retention of compounds depends on the summation of all interactions. Therefore, comprehension of each interaction helps to select an appropriate column.

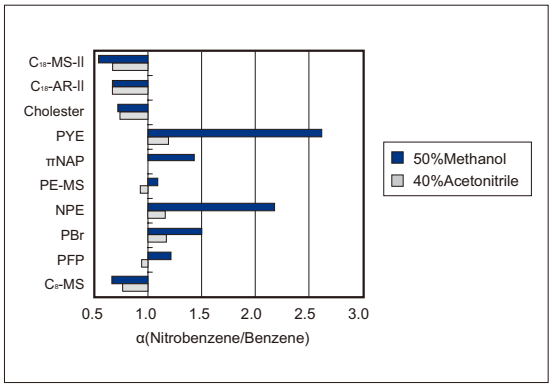
Table1. Stationary phase and interaction of packing materials

Packing Material	C_{18}-MS-II	C_{18}-AR-II	Cholester	PYE	πNAP	PE-MS	NPE	PBr	PFP	C_8-MS
Silica Gel	High Purity Porous Spherical Silica									
Particle Size	5 µm									
Pore Size	approx. 120 Å									
Specific Surface Area	approx. 300 m^2/g									
Bonded Phase										
	Octadecyl Group	Octadecyl Group	Cholesteryl Group	Pyrenylethyl Group	Naphthylethyl Group	Phenylethyl Group	Nitrophenylethyl Group	Pentabromo-benzyl group	Pentafluoro-phenyl group	Octyl Group
Bonding Type	Monomeric	Polymeric	Monomeric	Monomeric	Monomeric	Monomeric	Monomeric	Monomeric	Monomeric	Monomeric
Main Interaction	Hydrophobic Interaction	Hydrophobic Interaction	Hydrophobic Interaction Molecular Shape Selectivity	Hydrophobic Interaction π-π Interaction Dispersion Force Molecular Shape Selectivity	Hydrophobic Interaction π-π Interaction	Hydrophobic Interaction π-π Interaction	Hydrophobic Interaction π-π Interaction Dipole-dipole Interaction	Hydrophobic Interaction Dispersion Force	Hydrophobic Interaction π-π Interaction Dipole-dipole Interaction	Hydrophobic Interaction
End-capping Treatment	Near-perfect Treatment									
Carbon Load	approx. 16%	approx. 17%	approx. 20%	approx. 18%	approx. 11%	approx. 10%	approx. 9%	approx. 8%	approx. 10%	approx. 10%

1) Selectivity for Polar Functional Group

Selectivity

Selectivity for polar functional groups was evaluated by separation of benzene, nitrobenzene (nitro group), and anisole (methoxy group). The chromatograms below show separation of the three compounds on four COSMOSIL columns: C_{18}-MS-II, PE-MS, πNAP and PYE. Elution order on the aromatic columns is the reverse of the C_{18} column. Separation on the C_{18} column is based on only the hydrophobic interaction. The packing materials of the other three columns have aromatic rings and retain analytes by π-π interaction.

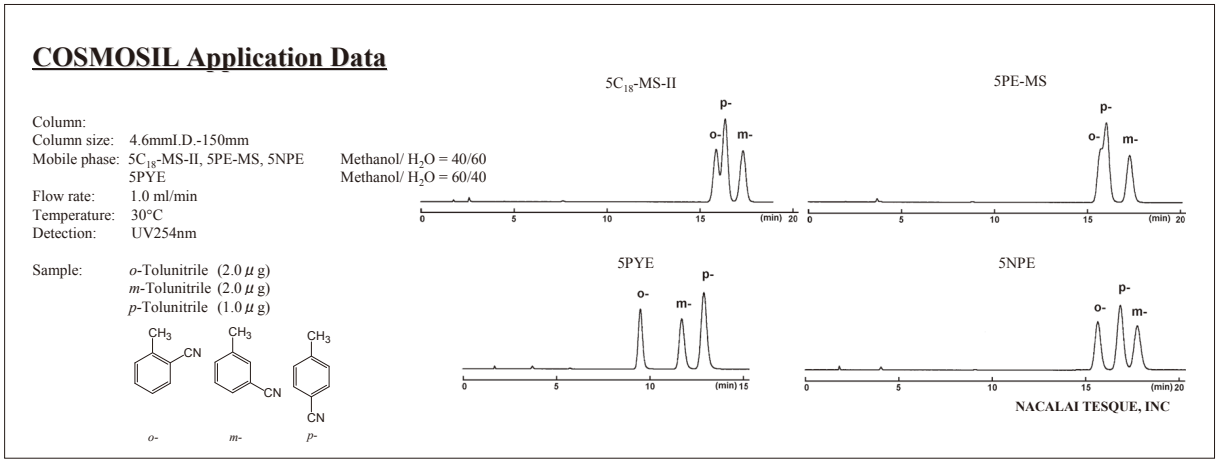


The graph of selectivity for polar functional group is shown above. Of the ten COSMOSIL columns, PYE and NPE columns displayed the highest separation factors. As a mobile phase, methanol is more effective than acetonitrile for separation using π-π interactions.

Application

● Separation of Tolunitrile Position Isomers

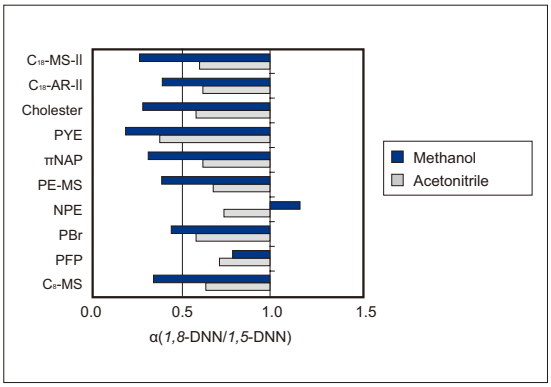
Tolunitriles have three position isomers. It is difficult to separate ortho and para isomers by C_{18} or phenyl columns because of weak π-π interaction. However, the isomers are well separated on PYE or NPE, which have strong π-π interactions.

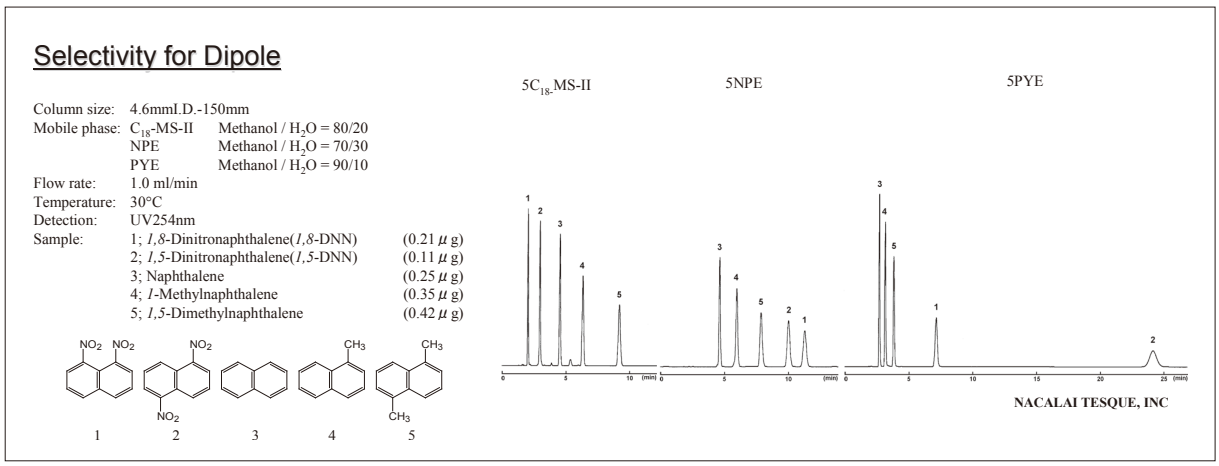


2) Selectivity for Dipole

Selectivity

Selectivity for dipoles was evaluated based on the separation of *1,5*-dinitronaphthalene and *1,8*-dinitronaphthalene. Dinitronaphthalenes (peak 1 and 2) were strongly retained on PYE and NPE compared to dimethylnaphthalenes due to the π-π interaction. However, there is a slight difference between these two columns. While *1,5*-dinitronaphthalene (peak 2) was preferentially retained on PYE, *1,8*-dinitronaphthalene (peak 1) was retained longer on NPE. The results with NPE indicate the presence of a strong dipole-dipole interaction. The two nitro group dipoles in *1,8*-dinitronaphthalene are aligned for a much greater dipolar coupling with the bonded nitrophenyl group in NPE than *1,5*-dinitronaphthalene.

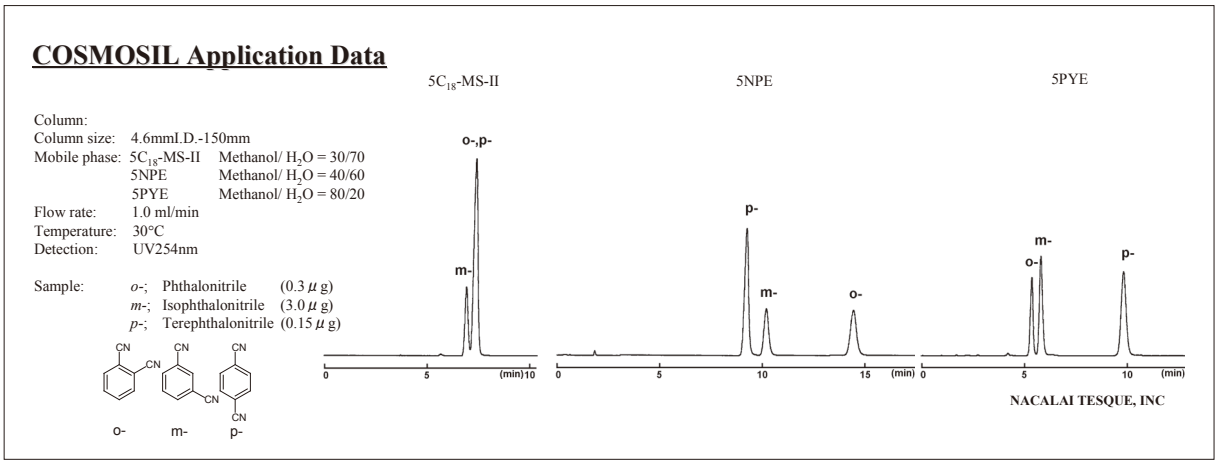




Application

● Separation of Phthalonitrile Position Isomers

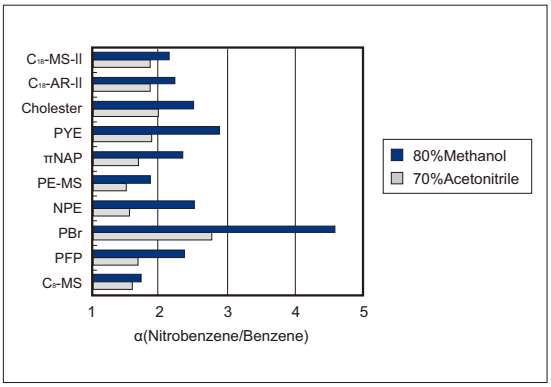
Phthalonitriles have three position isomers. NPE and PYE completely separate these compounds using π-π interaction. Furthermore, NPE strongly retains *o*-phthalonitrile due to dipole-dipole interaction.



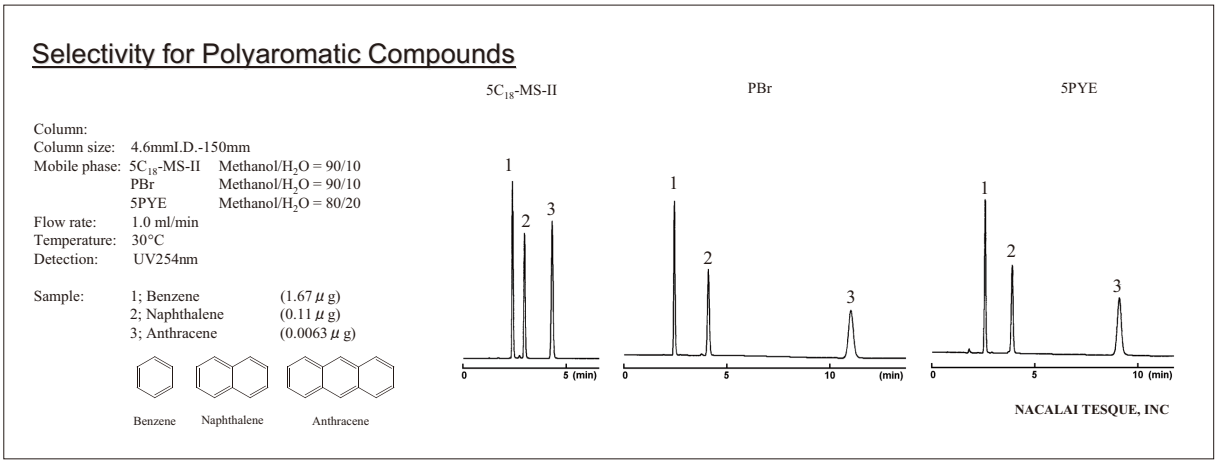
3) Selectivity for Polyaromatic Compounds

Selectivity

Selectivity for polyaromatic compounds is evaluated based on the separation of benzene, naphthalene and anthracene. The elution order in all columns is the same. Retention increases in all columns with increasing number of aromatic rings. In addition, highly dispersive packing materials, such as PBr and PYE, show much stronger retention for polyaromatic compounds due to their dispersion interaction.

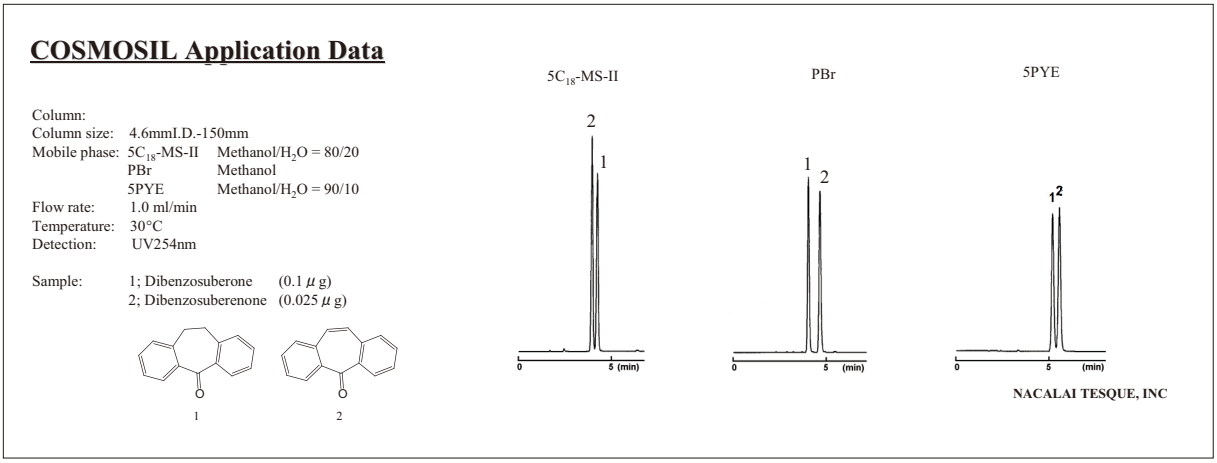


Application



- Separation of Dibenzosuberone and Dibenzosuberenone

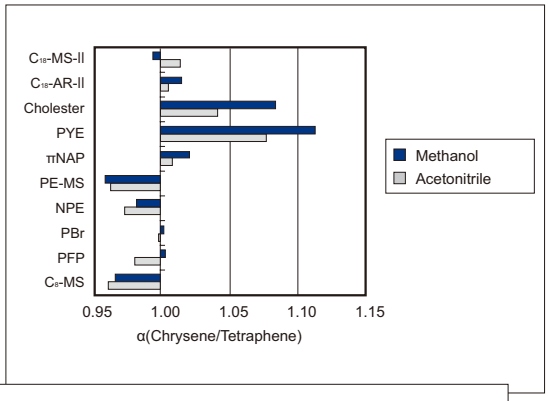
C_{18} retains dibenzosuberone (peak 1) longer than dibenzosuberenone (peak 2). On the other hand, PBr and PYE retain dibenzosuberenone (peak 2), which has a π−electron conjugated system, longer than dibenzosuberone (peak 1).

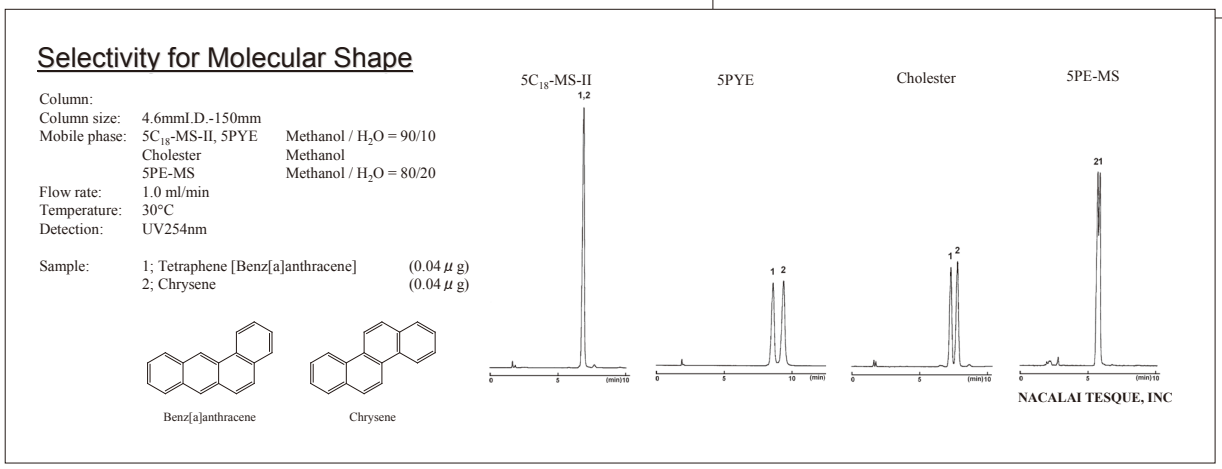


4) Selectivity for Molecular Shape

Selectivity

Selectivity for molecular shape is based on the separation of chrysene and benz[a]anthracene. These isomers, which consist of four benzene rings, are difficult to separate because of the similar hydrophobicity and aromaticity. However, the PYE and Cholester columns, which can recognize molecular shape, separate chrysene and benz[a]anthracene well.

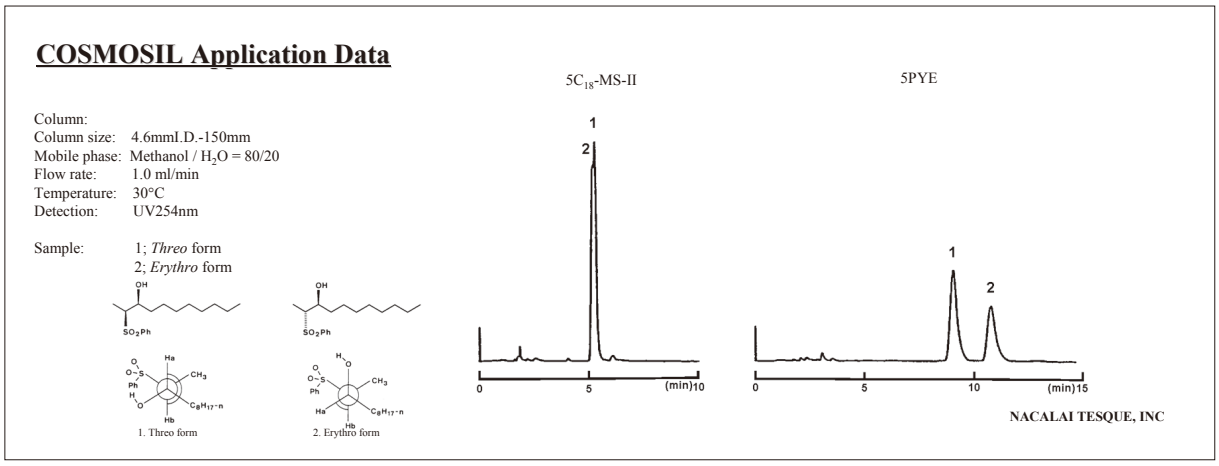




Application

- Separation of Diastereomers (*threo*- and *erythro*-)

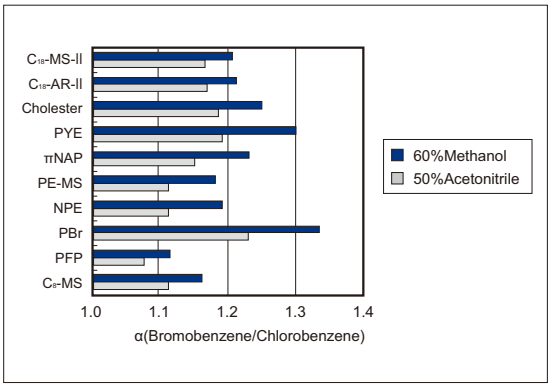
C_{18} cannot separate the threo and erythro forms. On the other hand, PYE retains the planar erythro form longer than the threo form.



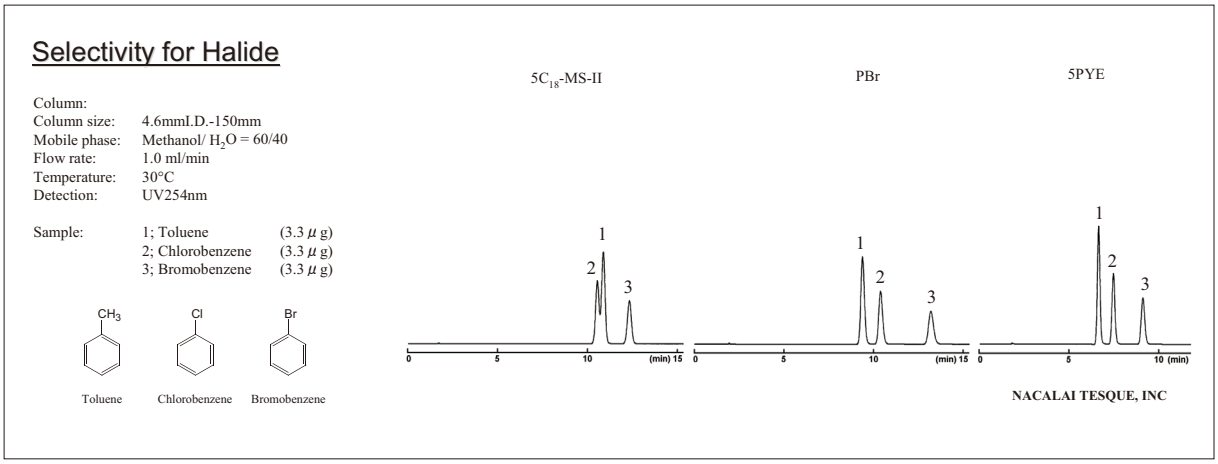
5) Selectivity for Halides

Selectivity

Selectivity for halides is evaluated by the separation of chlorobenzene and bromobenzene. PBr shows the highest separation factor due to the dispersion interaction of its five bromine atoms.

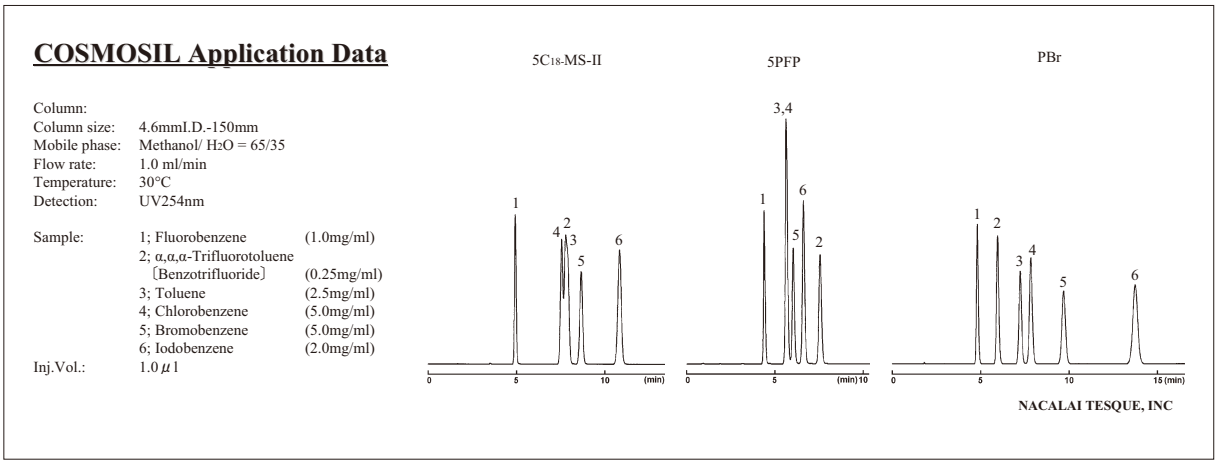


Application



● Separation of Trifluorotoluene and Phenyl Halides

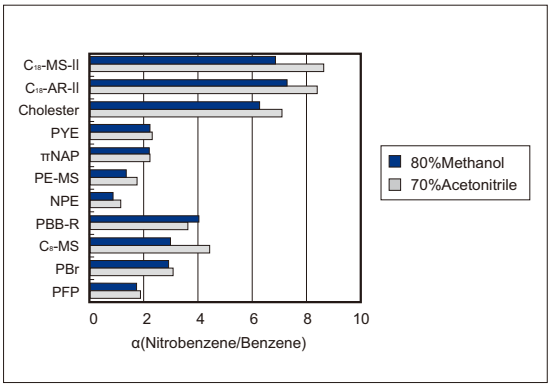
PBr elutes the halogens in order of atomic radius, with the largest retained the longest. As a result, it has excellent selectivity for halide compounds. C18 cannot completely separate some compounds that have similar hydrophobicity.

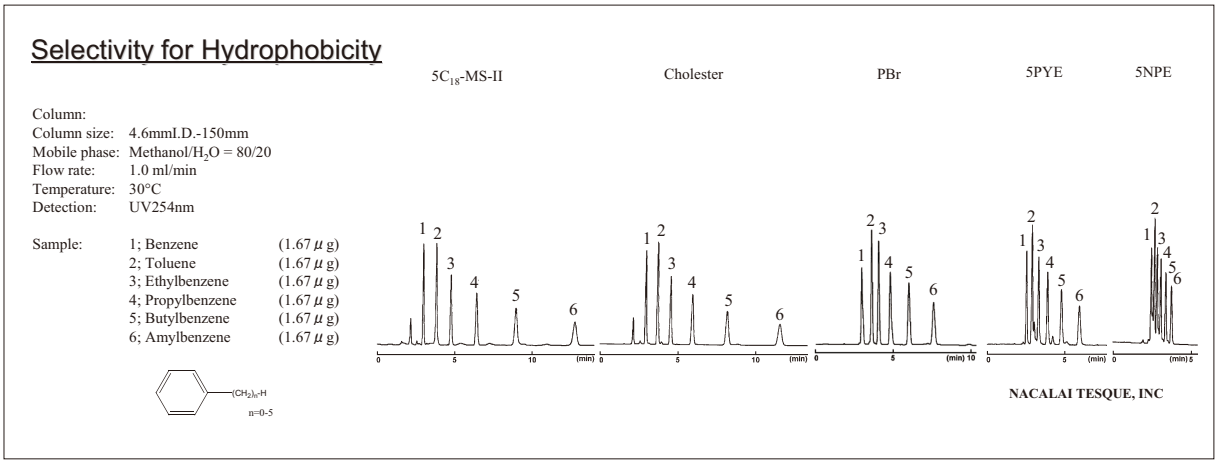


6) Selectivity for Hydrophobicity

Selectivity

Selectivity for hydrophobicity is evaluated by the separation of alkyl benzenes. C_{18} and Cholester, which have long carbon chains, both display high hydrophobicity. The other columns show less hydrophobicity compared to C_{18}.





Lower concentration of organic solvent in the mobile phase causes longer retention in reversed phase chromatography. For our NPE column, when the methanol concentration is reduced to 60%, retention increases to about the same as C_{18} with 80% methanol.