7. Selectivity of Packing Materials in Reversed Phase Liquid Chromatography

Introduction

Reversed phase chromatography is the most commonly used method of HPLC, because of the high theoretical plate number, excellent separation characteristics, reproducibility, and ease of use. Columns packed with octadecyl group bonded type silica gel (C18, ODS) are the most widely used reversed phase chromatography. However, C18 columns provide insufficient separation for compounds similar in hydrophobicity because the main separation mechanism of C18 column is based on hydrophobic interaction. It may improve separation of columns with similar hydrophobicity by using longer columns, changing mobile phases or changing temperature. However, in many cases, it is probably most effective to use different packing materials which retain compounds base on a secondary interaction in addition to hydrophobic interaction.

At Nacalai, we offer a variety of COSMOSIL reversed phase packing materials. Summary of these packing materials and their respective retention mechanism are in Table 1. Retention of compounds in each stationary phase depends on summation of the interactions. Therefore, comprehension of each interaction leads to selection of an appropriate column.

<table>
<thead>
<tr>
<th>Packing Material</th>
<th>C18-MS-II</th>
<th>C18-AR-II</th>
<th>C8-MS</th>
<th>PE-MS</th>
<th>nNAP</th>
<th>PYE</th>
<th>NPE</th>
<th>PBB-R</th>
<th>Cholesteryl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silica Gel</td>
<td>High Purity Porous Spherical Silica</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average Particle Size</td>
<td>5 μm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average Pore Size</td>
<td>approx. 120 Å</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Specific Surface Area</td>
<td>approx. 300 m²/g</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bonded Phase</td>
<td>Octadecyl Group</td>
<td>Octadecyl Group</td>
<td>Octyl Group</td>
<td>Phenylethyl Group</td>
<td>Naphthylethyl Group</td>
<td>Pyrenylethyl Group</td>
<td>Nitrophenylethyl Group</td>
<td>Pentabromobenzyl Group</td>
<td>Cholesterol Group</td>
</tr>
<tr>
<td>Bonding Type</td>
<td>Monomeric</td>
<td>Polymeric</td>
<td>Monomeric</td>
<td>Monomeric</td>
<td>Monomeric</td>
<td>Monomeric</td>
<td>Monomeric</td>
<td>Monomeric</td>
<td>Monomeric</td>
</tr>
<tr>
<td>End-capping Treatment</td>
<td>Near-perfect Treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carbon Load</td>
<td>approx. 16%</td>
<td>approx. 17%</td>
<td>approx. 10%</td>
<td>approx. 10%</td>
<td>approx. 11%</td>
<td>approx. 18%</td>
<td>approx. 9%</td>
<td>approx. 8%</td>
<td>approx. 20%</td>
</tr>
</tbody>
</table>
1) Selectivity for Polar Functional Group

**Selectivity**

Selectivity for polar functional group is evaluated based on the separation of benzene, nitrobenzene, which has a nitro group, and anisole, which has a methoxy group. The chromatograms below show separation of the three compounds on four COSMOSIL columns: C18-MS-II, PE-MS, nNAP and PYE. Elution order on the C18 column is as following: nitrobenzene, anisole and benzene. Elution orders on the aromatic columns are reversed. Separation on the C18 column is based on hydrophobic interaction only. On the other hand, the packing materials on the other three columns have aromatic rings and reverse the elution order by π-π interaction.

The graph of selectivity for polar functional group is shown below. Among nine COSMOSIL columns, PYE and NPE columns have the highest selectivity factors for polar groups. As to mobile phases, methanol is more effective than acetonitrile for separation using π-π interaction.

![Selectivity for Polar Functional Group](image)

**Application**

- **Separation of Tolunitrile Position Isomers**
  Tolunitriles have three position isomers. It is difficult to separate ortho and para isomers by C18 or PE-MS column because of lack of poor π-π interaction. On the other hand, the isomers are well separated on PYE or NPE column which has strong π-π interaction.

![COSMOSIL Application Data](image)
2) Selectivity for Dipole

Selectivity

Selectivity for dipole is 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 because of π–π interaction compared with dimethylnaphthalenes. 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 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 or PYE completely separates these compounds due to 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 orders in all columns are the same: benzene, naphthalene and anthracene. Retention increases in all columns with increasing number of aromatic rings. In addition, highly dispersive packing materials such as PBB and PYE show much stronger retention for polyaromatic compounds due to dispersion interaction.

Application

- Separation of Dibenzosuberone and Dibenzosuberenone

C18 retains dibenzosuberone (peak 1) longer than dibenzosuberenone (peak 2). On the other hand, PBB-R 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 evaluated based on the separation of chrysene and benz[a]anthracene. The isomers of two polyaromatic hydrocarbons, which consist of four benzene rings, are difficult to separate because of the similar hydrophobicity or aromaticity. However, PYE and Cholesterol columns, which recognize molecular shape, enable them to separate chrysene and benz[a]anthracene.

Application

- Separation of Diastereomers (threo- and erythro-)

C18 cannot separate the threo and erythro forms. On the other hand, PYE retains the planar erythro form longer than the threo form.

COSMOSIL Application Data

<table>
<thead>
<tr>
<th>Column</th>
<th>Mobile phase</th>
<th>Flow rate</th>
<th>Temperature</th>
<th>Detection</th>
</tr>
</thead>
<tbody>
<tr>
<td>5C18-AR-II, 5PYE</td>
<td>Methanol / H2O = 90/10</td>
<td>1.0 ml/min</td>
<td>30°C</td>
<td>UV254nm</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>Methanol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SPE-MS</td>
<td>Methanol / H2O = 80/20</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Sample:
1; Tetraphene [Benz[a]anthracene] (0.04 μg)
2; Chrysene (0.04 μg)

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<th>Flow rate</th>
<th>Temperature</th>
<th>Detection</th>
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<tbody>
<tr>
<td>SPE-MS</td>
<td>Methanol / H2O = 80/20</td>
<td>1.0 ml/min</td>
<td>30°C</td>
<td>UV254nm</td>
</tr>
</tbody>
</table>

Sample:
1; Threo form
2; Erythro form
5) Selectivity for Halides

Selectivity

Selectivity for halides is evaluated based on the separation of chlorobenzene and bromobenzene. PBB-R shows the highest selectivity factor due to dispersion interaction of the five bromine atoms.

Application

- Separation of Halogen Exchange Reaction Products
  PYE and PBB-R retain dispersed iodine atom longer than bromine atom. As a result, PYE and PBB-R can separate the complicated bromine and iodine compounds that C18 cannot separate.

COSMOSIL Application Data

<table>
<thead>
<tr>
<th>Column</th>
<th>Column size: 4.6mmL.150mm</th>
<th>Mobile phase: 5C18-MS-II Methanol/ H2O = 90/10</th>
<th>Flow rate: 1.0 ml/min</th>
<th>Temperature: 30°C</th>
<th>Detection: UV254nm</th>
<th>Sample: AP-1029</th>
</tr>
</thead>
</table>

Sample courtesy of Dr. H. Yamamoto, RIKEN
Selectivity for Hydrophobicity

Selectivity for hydrophobicity is evaluated based on the separation of alkylbenzenes. Two C18 and Cholesterol show similar high selectivity for hydrophobicity. Other columns show less hydrophobic selectivity than C18.

Lower concentration of organic solvent in mobile phase leads to much retention in reversed phase chromatography. In case of NPE, when methanol concentration is reduced to 60%, the retention times increase to those similar to C18 with 80% methanol.