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BETASIL Chromatographic Characterization

The BETASIL C18 phase is a high carbon load packing that offers up to twice the retention of other C18 packings with lower percent carbon loading. Figure 1 shows the increased retention of the BETASIL C18 column when compared to other Thermo Electron columns. The increased retention associated with the BETASIL column is directly attributed to the increased surface area of the particles, and higher carbon loading than both Hypersil BDS C18 and BetaBasic™ 18 columns. Where longer retention is required or there is a requirement to use a higher percentage of organic solvent in the mobile phase, e.g. to increase LC/MS sensitivity, the BETASIL C18 packing offers an excellent choice.
Stability at Low pH

All surface bonded chemistries belonging to the BETASIL family are highly stable under chemical attack. Hydrolysis of the silane ligand can occur under acidic conditions, resulting in a loss of retention and/or selectivity. The effect becomes more severe as ligand chain length is reduced, as is observed for the BETASIL C1 phase. Wettable stationary phases such as the BETASIL C6 packing can also show slightly less resistance to chemical attack, and consequently are recommended only for use between pH 2 and 8.

In general, BETASIL packings show superior stability to many other packings. Figure 2 shows BETASIL phases subjected to 25,000 column volumes of aggressive mobile phase at pH 1, 2, 18, and 50°C. The BETASIL C18 phase exhibits virtually no change in retention for the analysis of a sensitive drug mixture.

Phase Collapse Phenomenon

Mobile phases that contain a high proportion of water often are employed to retain highly polar compounds when using RP-HPLC. Many C18 columns will show a reversible loss of retention when exposed to highly aqueous mobile phase. The rate and degree of retention loss can vary greatly among different columns. A common explanation for this retention loss is that the hydrophobic alkyl chains of the stationary phase are not wettable and appear to “fold” down on the silica surface to avoid a highly aqueous, hydrophilic mobile phase (Figure 4).

Base Deactivated for Better Peak Shape with Basic Compounds

All BETASIL packings are base deactivated and show excellent peak shape for basic compounds. Figure 3 shows the analysis of a procainamide test mixture, a mixture that would typically have given rise to broad tailing peaks for the more basic compounds on traditional C18 silicas prepared on a Type A silica. In terms of silanol activity, the BETASIL packing is similar to other Type B silica based phases such as Betabasic and Betamax™ Neutral phases.

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Regeneration After Phase Collapse

In this folded or collapsed state, the alkyl chains are much less able to interact with solutes, resulting in a loss of retention. We use the term “chain folding” to refer to this reversible loss of retention induced by highly aqueous mobile phases, although the exact mechanism of reversible retention loss is not proven.

The BETASIL phase is a high carbon load, high density C18 and consequently shows the characteristics associated with chain folding, i.e. loss in retention. However, the situation is completely reversible and the column can be regenerated simply by flushing with 40% ACN / 60% H2O for 10 minutes (Figure 5).

BETASIL C18, 150x4.6mm
Eluent: 0.05M KH2PO4 + 0.03M H3PO4
Flow: 1.0 mL/min
Detector: UV @ 210

Figure 5. High Carbon Load C18 in 100% Aqueous Mobile Phase

Classical reversed phase retention, while the phenyl ring provides special selectivity for polar groups (Fig. 7).

BETASIL columns also offer choices for normal phase chromatography, including silica and diol phases in 4 different pore sizes from 60Å to 300Å, and cyano. Diol phases provide higher polar selectivity than cyano with less water sensitivity than bare silica.


Choices in Stationary Phase Chemistries

BETASIL columns are available in a wide range of reversed phase packings, including C18, C8, C6, Phenyl, Cyano, and Phenyl/Hexyl. The BETASIL Phenyl/Hexyl phase provides a combination of straight-chain C6 groups and phenyl groups, resulting in a mixed-mode separation. The C6 chain exhibits the phenomena of chain folding can be almost completely overcome by including trace amounts of organic solvent in the mobile phase. Figure 6 shows how just 0.3% n-propanol has been used to retard the loss in retention associated with chain collapse.

Figure 6. Trace Organics in Mobile Phase Retard Chain Folding

The phenomena of chain folding can be almost completely overcome by including trace amounts of organic solvent in the mobile phase. Figure 6 shows how just 0.3% n-propanol has been used to retard the loss in retention associated with chain collapse.

Figure 7. Polyphenols

The phenomena of chain folding can be almost completely overcome by including trace amounts of organic solvent in the mobile phase. Figure 6 shows how just 0.3% n-propanol has been used to retard the loss in retention associated with chain collapse.

Figure 7. Polyphenols

Classical reversed phase retention, while the phenyl ring provides special selectivity for polar groups (Fig. 7).

BETASIL Phenyl/Hexyl, 5µm, 150x4.6mm
Eluent: 5mM Acetic Acid
Flow: 1.0 mL/min
Detector: UV @ 280
Temp.: 25°C

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The phenomena of chain folding can be almost completely overcome by including trace amounts of organic solvent in the mobile phase. Figure 6 shows how just 0.3% n-propanol has been used to retard the loss in retention associated with chain collapse.

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BETASIL columns are available in a wide range of reversed phase packings, including C18, C8, C6, Phenyl, Cyano, and Phenyl-Hexyl. The BETASIL Phenyl(Hexyl) phase provides a combination of straight-chain C6 groups and phenyl groups, resulting in a mixed-mode separation. The C6 chain exhibits classical reversed phase retention, while the phenyl ring provides special selectivity for polar groups (Fig. 7).

BETASIL columns also offer choices for normal phase chromatography, including silica and diol phases in 4 different pore sizes from 60Å to 300Å, and cyano. Diol phases provide higher polar selectivity than cyano with less water sensitivity than bare silica.

**Organic Acids**

- **Ascorbic Acid**
- **Acetaminophen**
- **Benzoic Acid**
- **Salicylic Acid**

**Benzodiazepines**

- **Nitrazepam**
- **Oxazepam**
- **Temazepam**
- **Nordiazepam**
- **Diazepam**
- **Medazepam**

**BETASIL Phenyl(Hexyl), 5µm, 150x4.6mm**

- Eluent: 5mM Acetic Acid
- Flow: 1.0 mL/min
- Detector: UV @ 280
- Temp.: 25°C

**Sample:**
1. Pyrogallol
2. Hydroquinone
3. Resorcinol
4. Phenol

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**Back to Table of Contents**
All surface bonded chemistries belonging to the BETASIL family are highly stable under chemical attack. Hydrolysis of the silane ligand can occur under acidic conditions, resulting in a loss of retention and/or selectivity. The effect becomes more severe as ligand chain length is reduced, as is observed for the BETASIL C1 phase. Wettability stationary phases such as the BETASIL C18 phase can also show slightly less resistance to chemical attack, and consequently are recommended only for use between pH 2 and 8.

In general, BETASIL packings show superior stability to many other packings. Figure 2 shows BETASIL phases subjected to 25,000 column volumes of aggressive mobile phase at pH 1.8 and 50°C. The BETASIL C18 phase exhibits virtually no change in retention for the analysis of a sensitive drug mixture.

**Base Deactivated for Better Peak Shape with Basic Compounds**

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**Mobile phases that contain a high proportion of water often are employed to retain highly polar compounds when using HPLC. Many C18 columns will show a reversible loss of retention when exposed to highly aqueous mobile phase. The rate and degree of retention loss can vary greatly among different columns. A common explanation for this retention loss is that the hydrophobic alkyl chains of the stationary phase are not wettable and appear to “fold” down on the silica surface to avoid a highly aqueous, hydrophilic mobile phase.**

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BETASIL™ Columns

Specifications

<table>
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<tr>
<th>Phase</th>
<th>Particle size</th>
<th>Carbon Load</th>
<th>Pore Size</th>
<th>Endcapping</th>
<th>Silica type</th>
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<tbody>
<tr>
<td>BETASIL C18</td>
<td>3, 5, 10µm</td>
<td>20%</td>
<td>100Å</td>
<td>Yes</td>
<td>High purity, base deactivated</td>
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<tr>
<td>BETASIL C8</td>
<td>3, 5, 10µm</td>
<td>12%</td>
<td>100Å</td>
<td>Yes</td>
<td>High purity, base deactivated</td>
</tr>
<tr>
<td>BETASIL C6</td>
<td>3, 5µm</td>
<td>11%</td>
<td>100Å</td>
<td>Yes</td>
<td>High purity, base deactivated</td>
</tr>
<tr>
<td>BETASIL C1</td>
<td>3, 5µm</td>
<td>6%</td>
<td>100Å</td>
<td>Yes</td>
<td>High purity, base deactivated</td>
</tr>
<tr>
<td>BETASIL Plano</td>
<td>3µm</td>
<td>11%</td>
<td>100Å</td>
<td>Yes</td>
<td>High purity, base deactivated</td>
</tr>
<tr>
<td>BETASIL Phenyl</td>
<td>3µm</td>
<td>11%</td>
<td>100Å</td>
<td>Yes</td>
<td>High purity, base deactivated</td>
</tr>
<tr>
<td>BETASIL Cyan</td>
<td>3µm</td>
<td>7%</td>
<td>100Å</td>
<td>Yes</td>
<td>High purity, base deactivated</td>
</tr>
<tr>
<td>BETASIL Oct 100</td>
<td>7µm</td>
<td>6%</td>
<td>100Å</td>
<td>Yes</td>
<td>High purity, base deactivated</td>
</tr>
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<td>BETASIL Silica 100</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>High purity, base deactivated</td>
</tr>
</tbody>
</table>

Introduction

BETASIL columns from Thermo Electron are based on a highly pure, 100Å silica designed for small molecule analysis. All reversed phases are end-capped with a highly pure, 100Å silica. High surface area and phase loading result in excellent reproducibility and stability. BETASIL C18 columns offer excellent performance with acids, bases, and neutral compounds.

Characterization

The BETASIL C18 phase is a high carbon load packing that offers up to twice the retention of other C18 packings with lower percent carbon loading. Figure 1 shows the increased retention of the BETASIL C18 column when compared to other Thermo Electron columns. The increased retention associated with the BETASIL column is directly attributable to the increased surface area of the particles, and higher carbon loading than both Hypersil™ BDS C18 and BetaBasic™ C18 columns.

Where longer retention is required or there is a requirement to use a higher percentage of organic solvent in the mobile phase, e.g. to increase LC/MS sensitivity, the BETASIL C18 packing offers an excellent choice.

Common Analgesic

Sample:
1. Acetaminophen
2. Aspirin
3. Acetylsalicylic Acid
4. Benzoic Acid

BETASIL C18, 5µm, 150x4.6mm
Gradient: 50% ACN / 50% H2O, step to 60% ACN / 40% H2O at 12 min.
Flow: 1.0 mL/min
Detector: UV @ 254

Water-Soluble Vitamins

Sample:
1. Pyridoxine
2. Thiamine
3. Niacinamide
4. Cyanocobalamin
5. Riboflavin

BETASIL C18, 5µm, 150x4.6mm
Gradient: 65% ACN / 35% H2O
Flow: 1.0 mL/min
Detector: UV @ 254

Pesticides

Sample:
1. Dimethoate
2. Methyl Parathion
3. Malathion
4. Fenitrothion
5. Ethyl Parathion (DNTP)
6. Diazinon
7. EPN

BETASIL C18, 5µm, 150x4.6mm
Gradient: 65% ACN / 35% H2O
Flow: 1.0 mL/min
Detector: UV @ 254

Steroids by Normal Phase

Sample:
1. Progesterone
2. 20α-Hydroxy-4-pregnene-3-one
3. 17α-Hydroxyprogesterone
4. Deoxycorticosterone
5. 11α-Ketoprogesterone
6. Corticosterone
7. Cortisone Acetate
8. Hydrocortisone
9. Cortisone
10. 11α-Hydroxyprogesterone Acetate
11. Corticosterone

BETASIL Diol 60, 5µm, 150x4.6mm
Eluent: 60% isooctane / 14.7% EtOH / 0.3% H2O
Flow: 1.2 mL/min
Detector: UV @ 254

BETASIL Diol 100, 5µm, 150x4.6mm
Eluent: 60% isooctane / 14.7% EtOH / 0.3% H2O
Flow: 1.2 mL/min
Detector: UV @ 254

BETASIL Diol 300, 5µm, 150x4.6mm
Eluent: 60% isooctane / 14.7% EtOH / 0.3% H2O
Flow: 1.2 mL/min
Detector: UV @ 254
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