Comparison Between InertSustain C18 and Commercially Available ODS Columns
<table>
<thead>
<tr>
<th>Brand Name</th>
<th>Column Size (mm)</th>
<th>Particle Size (μm)</th>
<th>Surface Area (m²/g)</th>
<th>Pore Size (Å)</th>
<th>Pore Volume (mL/g)</th>
<th>Carbon Loading (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>InertSustain C18</td>
<td>2.1 × 150</td>
<td>3</td>
<td>350</td>
<td>100</td>
<td>0.85</td>
<td>14</td>
</tr>
<tr>
<td>Brand-A  CAPCELL PAK C18</td>
<td>2.0 × 150</td>
<td>3</td>
<td>260</td>
<td>100</td>
<td>0.91</td>
<td>15</td>
</tr>
<tr>
<td>MG III, Shiseido</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brand-B  YMC-Triart C18, YMC</td>
<td>2.0 × 150</td>
<td>3</td>
<td>—</td>
<td>120</td>
<td>—</td>
<td>Approx. 20</td>
</tr>
<tr>
<td>Brand-C  L-column2 ODS, CERI</td>
<td>2.1 × 150</td>
<td>3</td>
<td>340</td>
<td>120</td>
<td>1.10</td>
<td>17</td>
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<tr>
<td>Brand-D  Cadenza CD-C18, Imtakt</td>
<td>2.0 × 150</td>
<td>3</td>
<td>—</td>
<td>120</td>
<td>—</td>
<td>—</td>
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<tr>
<td>Brand-E  Gemini-NX 3u C18,</td>
<td>2.0 × 150</td>
<td>3</td>
<td>375</td>
<td>110</td>
<td>1.10</td>
<td>14</td>
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<tr>
<td>Phenomenex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brand-F  Luna 3u C18(2),</td>
<td>2.0 × 150</td>
<td>3</td>
<td>400</td>
<td>100</td>
<td>—</td>
<td>17.5</td>
</tr>
<tr>
<td>Phenomenex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brand-G  X SELECT CSH C18,</td>
<td>2.1 × 150</td>
<td>3.5</td>
<td>390</td>
<td>105</td>
<td>1.10</td>
<td>15</td>
</tr>
<tr>
<td>Waters</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brand-H  XBridge C18, Waters</td>
<td>2.1 × 150</td>
<td>3.5</td>
<td>185</td>
<td>135</td>
<td>0.74</td>
<td>18</td>
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<tr>
<td>Brand-I  ZORBAX Eclipse Plus C18,</td>
<td>2.1 × 150</td>
<td>3.5</td>
<td>160</td>
<td>95</td>
<td>—</td>
<td>8</td>
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<tr>
<td>Agilent</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inertsil ODS-3</td>
<td>2.1 × 150</td>
<td>3</td>
<td>450</td>
<td>100</td>
<td>1.05</td>
<td>15</td>
</tr>
<tr>
<td>Inertsil ODS-4</td>
<td>2.1 × 150</td>
<td>3</td>
<td>450</td>
<td>100</td>
<td>1.05</td>
<td>11</td>
</tr>
</tbody>
</table>
Explanation of Analytical Tests and Conditions

**Hydrophobicity Test**

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Eluent</th>
<th>Flow Rate</th>
<th>Col. Temp.</th>
<th>Detection</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CH₃OH/H₂O = 80/20</td>
<td>0.3 mL/min</td>
<td>40 °C</td>
<td>UV 254 nm</td>
<td>1: Uracil 2: Toluene 3: Ethylbenzene 4: Propylbenzene 5: n-Butylbenzene 6: n-Amylbenzene</td>
</tr>
</tbody>
</table>

**Basic Compound Test (1)**

Dextromethorphan is a strong basic compound. Severe tailing can be confirmed when the packing material contains residual silanol groups.

4. Berberine hydrochloride
5. Dextromethorphan

**Basic Compound Test (2)**

The following are antidepressants, which are also strong basic compounds. Poor endcapped columns will show tailing or some even may show different elution pattern. All compounds except for Hydroxyzine tends to elute later.

1. Chlorpheniramine
2. Triprolidine
3. Homochlorcyclizine
4. Hydroxyzine
5. Clemastine

**Basic Compound Test (2)**

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Eluent</th>
<th>Flow Rate</th>
<th>Col. Temp.</th>
<th>Detection</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CH₃CN</td>
<td>0.3 mL/min</td>
<td>40 °C</td>
<td>UV 220 nm</td>
<td>1: Chlorpheniramine 2: Triprolidine 3: Homochlorcyclizine 4: Hydroxyzine 5: Clemastine</td>
</tr>
</tbody>
</table>

Alkyl benzene is introduced to measure the retentivity of each column.

R: 2. Toluene —CH₃
3. Ethylbenzene —CH₂CH₃
4. Propylbenzene —CH₂CH₂CH₃
5. Butylbenzene —CH₃CH₂CH₂CH₃
6. Amylbenzene —CH₂CH₂CH₂CH₂CH₃
### Acidic Compound Test

Sharp peaks can be obtained when analyzing Phenol or Salicylic Acid. However, as Brilliant Blue FCF has three sulfonic groups in its chemical structure, tailing will occur when the surface of the packing material is slightly basic. 

**Conditions**

- **Eluent**:
  - A) CH₃CN
  - B) 0.1% H₃PO₄
  - A/B = 25/75
- **Flow rate**: 0.2 mL/min
- **Col. Temp.**: 40 °C
- **Detection**: UV 254 nm
- **Sample**: 1: Brilliant Blue FCF, 2: Phenol, 3: Salicylic acid

![Image of Brilliant Blue FCF](image.png)

1. Brilliant Blue FCF

### Chelating Compound Test

Hinokitiol is a strong chelating compound, which coordinately binds with the surface of residual trace metal impurities, resulting in severe tailing. However, the peak shape improves as the injection increases since the surface of the packing material of the adsorption active sites eventually become masked.

**Conditions**

- **Eluent**:
  - A) CH₃CN
  - B) 0.1% H₃PO₄
  - A/B = 25/75
- **Flow rate**: 0.2 mL/min
- **Col. Temp.**: 40 °C
- **Detection**: UV 254 nm
- **Sample**: 1: Brilliant Blue FCF, 2: Phenol, 3: Salicylic acid

![Image of Hinokitiol](image.png)

1. Hinokitiol

### Dewetting Test

When analyzing hydrophilic compounds under water rich mobile phase condition, once the pump is stopped, the hydrophobic bonded group pushes the aqueous mobile phase out off the pore in an irreversible fashion, in what has become known as the dewetting phenomenon.

**Testing Procedure:**

1. 100 % water is introduced into column over 20 minutes.
2. Conduct Analysis (Upper chromatogram in the following pages)
3. Stop flow for 15 minutes.
4. 100 % water is introduced again into column over 30 minutes.
5. Stop flow for 15 minutes again.
6. Conduct Analysis (Lower chromatogram in the following pages)

**Conditions**

- **Eluent**: 100% H₂O
- **Flow rate**: 0.2 mL/min
- **Col. Temp.**: 40 °C
- **Detection**: UV 254 nm
- **Sample**: 1: Cytosine, 2: Uracil, 3: Guanine, 4: Thymine, 5: Adenine
Hydrophobicity Test | Basic Compound Test (1) | Basic Compound Test (2) | Acidic Compound Test | Chelating Compound Test | Dewetting Test

**InertSustain C18**

1. Uracil
2. Pyridine
3. Phenol
4. Berberine hydrochloride
5. Dextromethorphan

1. Chlorpheniramine
2. Triprolidine
3. Homochlorcyclizine
4. Hydroxyzine
5. Clemastine

1. Brilliant Blue FCF
2. Phenol
3. Salicylic acid

1. Hinokitiol

Maintenance rate of retentivity: 82.4%

**X SELECT CSH C18**

1. Uracil
2. Pyridine
3. Phenol
4. Berberine hydrochloride
5. Dextromethorphan

1. Chlorpheniramine
2. Triprolidine
3. Homochlorcyclizine
4. Hydroxyzine
5. Clemastine

1. Brilliant Blue FCF
2. Phenol
3. Salicylic acid

1. Hinokitiol

Maintenance rate of retentivity: 95.9%

**XBridge C18**

1. Uracil
2. Pyridine
3. Phenol
4. Berberine hydrochloride
5. Dextromethorphan

1. Chlorpheniramine
2. Triprolidine
3. Homochlorcyclizine
4. Hydroxyzine
5. Clemastine

1. Brilliant Blue FCF
2. Phenol
3. Salicylic acid

1. Hinokitiol

Maintenance rate of retentivity: 76.8%

**ZORBAX Eclipse Plus C18**

1. Uracil
2. Pyridine
3. Phenol
4. Berberine hydrochloride
5. Dextromethorphan

1. Chlorpheniramine
2. Triprolidine
3. Homochlorcyclizine
4. Hydroxyzine
5. Clemastine

1. Brilliant Blue FCF
2. Phenol
3. Salicylic acid

1. Hinokitiol

Maintenance rate of retentivity: 27.1%
Hydrophobicity Test

Basic Compound Test (1)

Basic Compound Test (2)

Acidic Compound Test

Chelating Compound Test

Dewetting Test

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InertSustain C18

1: Uracil
2: Pyridine
3: Phenol
4: Berberine hydrochloride
5: Dextromethorphan

1: Chlorpheniramine
2: Tripolidine
3: Homochlorcyclizine
4: Hydroxyzine
5: Clemastine

1: Brilliant Blue FCF
2: Phenol
3: Salicylic acid
2

1: Hinokitiol

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Inertsil ODS-4

Dextromethorphan

1: Uracil 2: Pyridine
3: Phenol
4: Berberine hydrochloride
5: Dextromethorphan

1: Chlorpheniramine
2: Tripolidine
3: Homochlorcyclizine
4: Hydroxyzine
5: Clemastine

1: Brilliant Blue FCF
2: Phenol
3: Salicylic acid
2

1: Hinokitiol

---

Inertsil ODS-3

Dextromethorphan

1: Uracil 2: Pyridine
3: Phenol
4: Berberine hydrochloride
5: Dextromethorphan

1: Chlorpheniramine
2: Tripolidine
3: Homochlorcyclizine
4: Hydroxyzine
5: Clemastine

1: Brilliant Blue FCF
2: Phenol
3: Salicylic acid
2

1: Hinokitiol

100% aqueous mobile phase can be used by periodically flushing the column with an organic solvent.

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Maintenance rate of retentivity
82.4 %

Maintenance rate of retentivity
94.8 %

Maintenance rate of retentivity
98.2 %