Each column is individually packed and tested to ensure superior performance. The enclosed Quality Assurance Report and chromatogram is specific to your column and contains important information such as the column serial number and the lot number of the packing material. Retain this information as it may prove invaluable in troubleshooting.

**Column Hardware**
All column hardware is 316 stainless steel. The end fittings are compatible with fittings from Valco, Upchurch, Parker, Swagelok, and others. However, all connecting fittings should be assembled in your new column to ensure a proper fit.
Columns packed with 2.7 μm and 5 μm particles have 2 μm frits. Columns packed with 1.8 μm material have 0.5 μm frits.

**Connecting the Column to the HPLC system**
Loosen and remove the end plugs. Do not discard the end plugs—keep them in a safe place to use when storing the column. Ensure that the nuts and ferrules on the inlet and outlet column tubing are correctly seated before tightening. Improper seating can negatively affect column efficiency and make it difficult to remove the fittings from the column.

The column is shipped containing the mobile phase indicated on the enclosed test sheet. Be sure that your mobile phase is compatible with this. If not, you must flush with an intermediate solvent that is compatible with the storage solvent and your mobile phase. Be especially careful if you are using a buffer because most columns are shipped with more than 50% organic solvent, which could cause precipitation of the buffer and plugging of the column.

**Flow Direction**
The arrows on the column label indicate the flow direction. Begin by connecting the inlet end of the column to the injector or autosampler and gradually increasing the flow rate to its optimum value. Allow the mobile phase to flow from the outlet end of the column into a beaker for 10-15 minutes and then connect the column to your detector.

**Optimum Flow Rate**

<table>
<thead>
<tr>
<th>Column ID (mm)</th>
<th>1.8 μm dp</th>
<th>2.7 μm dp</th>
<th>5 μm dp</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.6 mm</td>
<td>-</td>
<td>1.6</td>
<td>1.0</td>
</tr>
<tr>
<td>3.0 mm</td>
<td>0.8</td>
<td>0.7</td>
<td>0.4</td>
</tr>
<tr>
<td>2.1 mm</td>
<td>0.4</td>
<td>0.3</td>
<td>0.2</td>
</tr>
</tbody>
</table>

*Optimal flow rates are mobile phase dependent. The table above is provided as a guide*

**Record the Operating Pressure**
Continuous monitoring of system pressure will alert you to changes that may require you to perform preventative maintenance such as column washing or replacement of a guard column or filter.

**Column Lifetime**
NexLeaf columns are packed with silica particles. The normal pH limitations are from pH 2.0 to pH 8.0. Extended use at either extreme of pH may shorten the lifetime of the column.
It is strongly recommended to use solvents designed specifically for HPLC that have been thoroughly filtered and degassed. Residue and chemical contaminants in non-HPLC grade solvents can cause alteration of selectivity and/or a significant increase in system pressure due to plugging of the inlet frit.

An upper temperature limit of 80°C is recommended. Elevated temperatures may improve efficiency by lowering solvent viscosity, but column lifetime may be compromised. Pressure limits are as follows:
- 1.8 μm - 15,000 psi (1034 bar)*
- 2.7 μm - 8700 psi (600 bar)
- 5 μm - 5800 psi (400 bar)

*For maximum lifetime, the recommended maximum pressure for 1.8 μm particle columns is 12,000 psi (830 bar).

Column Cleanup
Column lifetime can be extended considerably with routine column washes and proper storage. For maximum protection against particulate matter and sample contaminants, the NexLeaf Guard Column is recommended.

Columns should be flushed when not in use to remove buffers, acids, or bases. The ideal flushing solvent is a solution identical in composition to your mobile phase, minus the acid, base, and buffer. For Reversed phase columns, a more rigorous cleaning can be accomplished by flushing the column with the solvents below in the order listed at a partial flow (approximately one half of the optimal flow rate of your column):

- 95% Water:5% Methanol
- Methanol
- Isopropanol
- Hexanes
- Isopropanol
- Methanol
- Water
- Method initial mobile phase without any acids, bases, or buffers
- Method initial mobile phase without any acids, bases, or buffers
- OPTIONAL 50:50 Water:Acetonitrile
- Acetonitrile
- Isopropanol
- Hexanes
- Isopropanol
- Methanol
- Acetonitrile
- OPTIONAL 50:50 Water:Acetonitrile
- Method initial mobile phase without any acids, bases, or buffers

Column backflushing is not recommended.

Column Storage
For short-term storage, all reversed phase columns should be flushed with a solvent identical in composition to the most recently used mobile phase minus any acids, bases, or buffers and stored. For long-term storage, reversed phase columns should be stored in 100% organic solvent (i.e., acetonitrile or methanol).

All columns should be stored with their end plugs securely fastened to prevent the column from drying out. Because every HPLC system is unique, especially in gradient mode, your results may differ slightly from those obtained in our laboratory.

* The contents of this instruction sheet are subject to change without notice.

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