

Restek Liquid Chromatography (LC) Columns—Usage & Care

Column Certificate of Analysis

Each Restek liquid chromatography (LC) column is individually packed and tested to ensure superior performance. An LC certificate of analysis is generated for each column. It contains not only a chromatogram but also other important, column-specific information, such as the column serial number and the packing material lot number. Download your certificate at www.restek.com/documentation and retain this information for your records or troubleshooting if needed.

Column Hardware

All Restek column hardware is made from 316 stainless steel. Columns with 2.7 μm Raptor particles and columns with other particles greater than 3 μm in diameter have 2 μm frits. All other columns have 0.5 μm frits. Restek LC column end fittings are compatible with Valco, Parker, Upchurch, Swagelok, and other brands of 10-32 size fittings. To ensure a proper fit when assembling fittings into your new column, you may need to reset the nut and ferrule on both the inlet and outlet end of the column. Make sure that the column connectors are correctly seated before tightening; improper seating can negatively affect column efficiency and make it difficult to remove the column. Restek columns are shipped with PEEK end plugs; simply loosen and remove the plugs before installation. (Please refer to the LC system's operation manual for complete instructions on installing the column.)

Column Installation and Use

Mobile Phase

When shipped, the column contains the storage solvent listed on its LC certificate of analysis (download yours at www.restek.com/documentation). Make certain that your intended mobile phase is compatible with this solvent. If it is not, flush the column with an intermediate solvent that is compatible with both the storage solvent and your intended mobile phase. If you are unsure about solvent miscibilities, go to www.restek.com/solvent-chart for more information or contact Restek Technical Service. Be especially careful when using buffers; the storage solvent for most columns contains greater than 50% organic solvent, and contact with a buffer could cause a precipitate to form and plug the column.

Flow Direction and Flow Rate

The arrows on the column label indicate the recommended flow direction. Begin by connecting the inlet end of the column to the injector or autosampler and allow mobile phase to flow from the outlet end of the column into a beaker for 10–15 minutes. Gradually increase the flow rate to its optimal value (Table I). Then, stop the mobile phase flow and connect the column to your detector.

Because every LC system is unique, especially when used in gradient mode, your results may differ from those obtained in our laboratory. Restek Technical Service can assist you in optimizing your separations. Be sure to record the operating pressure before calling.

Equilibration

Prior to use, it is recommended to condition your column with at least 5-10 column volumes of mobile phase at the initial testing conditions. See below for some recommended resources for conditioning your specific column type. For additional information on column conditioning, please contact Restek Technical Service.

For more information related to conditioning and equilibrating a reversed-phase column, visit www.restek.com/reversedphaseconditioning

For more information related to conditioning and equilibrating a HILIC column, visit www.restek.com/HILICconditioning

For more information related to conditioning and equilibrating a Raptor EtG/EtS column, visit www.restek.com/EtGEtSconditioning

Column Maintenance

Continuous monitoring of system pressure will alert you to changes that may require you to perform maintenance, such as washing the column, replacing a guard column or filter, or cleaning the inlet frit. Increases in column back pressure indicate a plugged inlet frit or other problem. If system back pressure begins to rise during column use, backflushing the column may reduce the pressure by removing particle buildup from the inlet frit. (Note that backflushing is NOT recommended for any 1.9 μm Pinnacle DB column. Contact Restek Technical Service for help troubleshooting increased back pressure with these column types.)

If backflushing is appropriate, start by first turning off any flow and then disconnecting it from the injector and the detector. Reverse the column flow direction by connecting the outlet end of the column to the injector or autosampler. Flush the column with partial flow (approximately $\frac{1}{4}$ to $\frac{1}{2}$ of the optimal flow rate of your column) using a solvent compatible with your mode of separation. Allow the solvent to flow from the inlet end of the column into a beaker. *Do not backflush the column into your detector.* After rinsing, return to the normal flow direction and re-equilibrate the column with mobile phase. If back pressure lowers, consider using a guard column or precolumn filter for future analyses.

If peak shape deteriorates due to suspected contamination, flush the column with mobile phase starting with a high aqueous content and moving to a prolonged high organic solvent content, preferably using a gradient elution. After flushing the column, re-equilibrate the column with mobile phase and check for improved performance. If the problem persists, consider using a guard column.

Contact Restek Technical Service if you need more information on selecting flushing solvents or column maintenance.

Table I: Optimal Flow Rates Based on Particle Diameter and Column ID

Column ID (mm)	Optimal Flow Rate (mL/min)		
	1.9 μm d _p	3 μm d _p	5 μm d _p
4.6	–	1.50	1.00
3.2	–	0.73	0.50
3.0	1.12	0.65	0.40
2.1	0.55	0.31	0.20
1.0	–	0.07	0.05

Column Cleaning and Storage

Columns should be flushed prior to storage to remove buffers, acids, or bases with at least 10-20 column volumes of solution. For short-term storage, all columns should be flushed with a solvent identical in composition to the most recently used mobile phase minus any buffered, acidic, or basic components. For long-term storage, reversed-phase columns should be stored with 50% water/50% organic solvent (e.g., acetonitrile or methanol), and normal phase columns should be stored with a nonpolar solvent (e.g., hexane). If the long-term storage solvent is not compatible with the most recently used mobile phase, flush the column with an intermediate solvent that is compatible with both the most recently used mobile phase and the storage solvent. Store columns with end plugs securely fastened and be sure to include a description of the storage solvent.

Best Practices for Increasing Column Lifetime

- Routine column washes can significantly increase the lifetime of your column. The ideal flushing solvent is a solution identical in composition to the most recently used mobile phase minus any buffered, acidic, or basic components. When rinsing your column, we recommend using at least 10–20 column volumes of flushing solution.
- Extended use at extreme pH values is not recommended. Details about the pH limitations for your specific column can be found at restek.com.
- Do not exceed the column's upper temperature limit of 80 °C as elevated temperatures may compromise column lifetime.
- We strongly recommend using solvents prepared specifically for liquid chromatography that have been filtered and degassed prior to use.
- Column lifetime is also governed by the stationary phase type. Hydrocarbon phases, such as C18, are relatively chemically inert. However, polar phases, such as amino, require special care because they are chemically active. Mobile phases containing an aldehyde or ketone (e.g., acetone) will alter the retention of amino phases, and repeated injections will permanently alter the phase over time. Amino phase alteration progresses more quickly at pH 4.5 to pH 5.5, but no pH range is invulnerable.

LC Guard Column Systems

For maximum protection against particulate matter and sample contaminants, consider installing a guard column or filter frit in front of the column. For more information, visit www.restek.com/LC-guard or contact your local Restek representative.

Raptor Polar X Columns

There are some specific recommendations for Restek's Raptor Polar X LC columns as this phase has some unique considerations for best performance and lifetime.

Passivation:

- For chelating compounds, any steel in the sample flow path should be passivated.
- The column is delivered passivated by Restek, but if retention of chelating compounds starts to reduce, additional column passivation may be performed.
- Refer to Restek's LC Passivation Solution and instructions for guidance on system and column passivation at www.restek.com/LCpassivation

Usage:

- Condition the column with 5-10 blank injections prior to analysis.
- Avoid >90% aqueous conditions; using >90% water can reduce column lifetime.
- Avoid ammonium acetate buffer or acetic acid. Use of any of these could lead to reduced retention. Ammonium formate and formic acid are recommended alternatives.
- Avoid methanol. The use of methanol as a mobile phase component with Polar X columns has shown reductions in detector sensitivity and retention time variations. Acetonitrile is recommended.
- Less harsh conditions will increase lifetime. The use of $\leq 0.5\%$ acid modifier as well as $\leq 50^\circ\text{C}$ column temperature is recommended.

Cleaning and Storage:

- For best results, flush column at the end of the analytical set with the method organic mobile phase before shutting down.
- After use, it is recommended to store columns in 100% ACN.
- Static storage in buffered or acidified mobile phases is not recommended.
- If buffers and/or acid were used, it is recommended to flush column with 50:50 unbuffered H₂O/ACN for 5-10 column volumes; then, flush column with 5-10 column volumes of acetonitrile prior to storage.

Raptor FluoroPhenyl and Ultra IBD Columns

There are some specific recommendations for Restek's Raptor FluoroPhenyl and Ultra IBD LC columns for best performance and lifetime.

Cleaning and Storage:

- After use, it is recommended to store columns in 100% ACN.
- Static storage in buffered or acidified mobile phases is not recommended.
- If buffers and/or acid were used, it is recommended to flush column with 50:50 unbuffered H₂O/ACN for 5-10 column volumes; then, flush column with 5-10 column volumes of acetonitrile prior to storage.

Questions about this or any other Restek product?

Contact us or your local Restek representative (www.restek.com/contact-us).

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