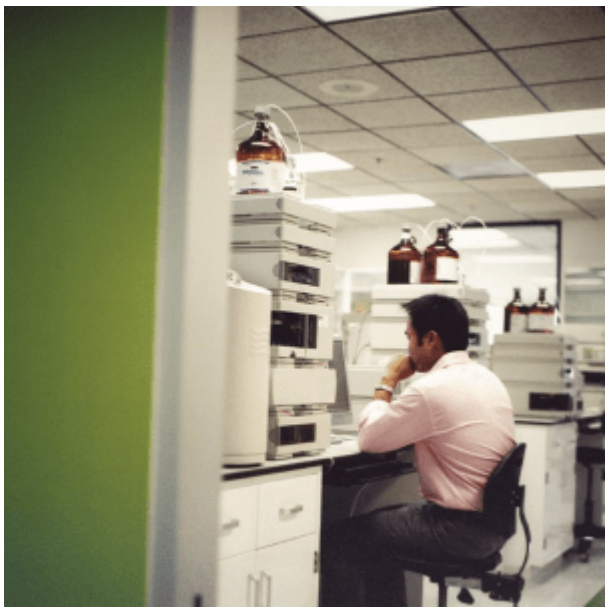
1. Why run a gradient from 5-95% organic solvent? Why not 0-100%?

When running a gradient, it is important to remember that the total time for each run includes both the run time, and the necessary re-equilibration before the next run. When running from 0-100% organic solvent, the re-equilibration step is far longer than for 5-95%—such that the majority of users sacrifice the small restriction in terms of method



flexibility for a much larger gain in productivity.

There is speculation on the mechanistic reason behind the lengthy equilibration time required for methods utilizing 0% aqueous and/or 100% organic solvent in the gradient. Phase wetting/dewetting and phase collapse have been proposed causes.

Either way, in practice, variations in retention can be observed with alkyl phases using 100% aqueous conditions. If necessary for retention of certain highly polar compounds, we recommend columns stable under such conditions.

2. What's the best way to dispose of my old HPLC columns?

We recommend consulting a chemical disposal company with the MSDS for the packing material and any other chemicals that might also be in the column. It may be possible to then flush the column, properly dispose of its contents, and recycle the hardware.

If your columns are gently used, consider donating them to academic institutions in the developing world. We love working with RORO (Recycling Organisation for Research Opportunities) in the UK who collects and distributes such columns (and instrumentation).

TUTORIAL: How HPLC Columns Work >>>

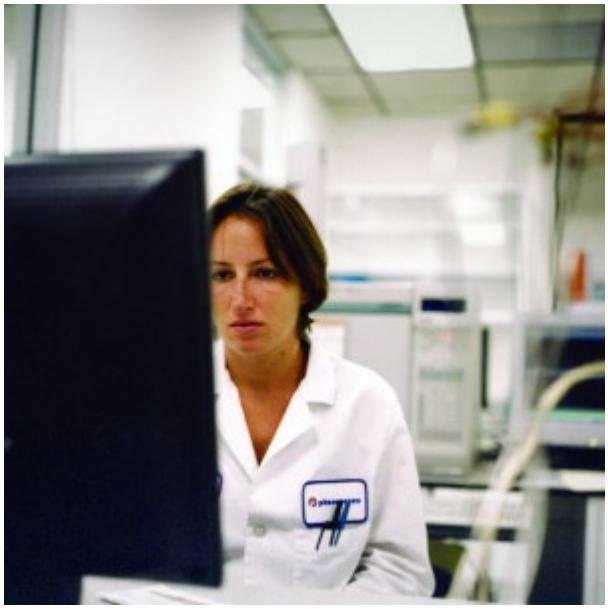
3. My LC column is leaking. What could be causing this? What can I do?

- Tighten your connective fitting, and ensure connective fittings and inlet threading have not been stripped.
- When appropriate, use finger-tight end-fittings to avoid both sealing problems *and* the need for wrenches.
- If using ultra-high performance fittings, ensure the fittings are swaged correctly by using two wrenches to tighten column to end-fitting until leak-free. This should be about ¼ turn. **Do not overtighten.** Ensure parts are not mismatched.

4. The pressure in my HPLC system is low and erratic, and the system keeps shutting down. How do I fix the problem?

First, check for leaks. If the system is in regular use, connections that are regularly made and broken (e.g. columns, guard columns, etc.) are potentially the weak link. If a leak is

suspected, disconnect the tubing from the leaking fitting, ensure that the end of the tubing and the connecting nut and ferrule, or finger-tight fitting, are free from damage. Then reconnect; don't be tempted to simply over-tighten as this will normally cause irreparable damage.



If there is no leak, then most likely cause of the problem is air within the system. Check that your mobile phase is adequately degassed when prepared, or ensure that your online degasser is switched on and is functioning correctly.

5. What is the best way to remove ion-pairing reagents from my HPLC column?
Washing the column with a strong organic solvent, such as acetonitrile, should remove a moderate amount of the ion-pair before column storage. However, because ion-pairing reagents can alter column selectivity, it is advisable to dedicate columns to ion-pairing methods to prevent problems with reproducibility.

VIDEO: How Unicorns Improve HPLC >>>

6. What factors affect HPLC column lifetime?
The following factors contribute to the degradation of chromatography and subsequent replacement of HPLC columns:

- Over time, the stationary phase backbone (i.e. silica) will begin to break down, resulting in the formation of column voids. This results in peak broadening and

splitting, and subsequently loss of sensitivity and resolution.
- The accumulation of fine particles can also cause an increase in pressure, which will further decrease lifetime.
- Even under neutral pH, the stationary phase ligand may be lost over time resulting in reduced retention and efficiency.

The injection of problematic samples and/or harsh running condition can significantly shorten column life (i.e. number of injections). To maximize column lifetime, especially with problematic samples, we recommend SecurityGuard and SecurityGuard ULTRA.

7. My LC method backpressure is cycling. What do I do? What could be causing this?

- Replace check valves if necessary.
- Replace pump seals, if necessary.
- Ensure there is no air in the pump; bleed air and use degassed solvents only.
- Check for leaks in the system.

If using gradient elution, viscosity changes could cause normal pressure fluctuations.

VIDEO: Agilent 1100 HPLC System Optimization >>>

8. What are the recommended long term storage conditions for HPLC columns?

Column storage conditions can affect column lifetime. As such, long term storage of columns containing buffers or ion-pairing reagents is not recommended. Before storing, flush with 5 column volumes of mobile phase without buffer to remove any buffers or salts.

Refer to the following table for recommended storage conditions based upon the chemistry of your column:

Column Type	Storage Solvent
Reversed Phase C18, C12, C8, C4, C2, C1, Phenyl, PFP	65% Acetonitrile/ 35% water
Normal Phase Silica, CN, NH2, PAC, Diol Alumina	Isopropanol or Hexane
Ion-Exchange SAX, SCX, WAX, WCX	Methanol

Size-Exclusion	0.05% NaN3 in water or
Diol	10% Methanol
HILIC	80% Acetonitrile/
Luna HILIC	20% water

9. I'm seeing extra peaks in my chromatogram. Help!

There are a couple of things to watch out for when you see extra peaks. Start by checking the purity of the mobile phase and ensure extra peaks are not simply other components in the sample such as impurities or contaminants.

Extra peaks may be late-eluting peaks from a previous injection. This is particularly common with isocratic runs and you may need to increase the run time or use a gradient to fix the problem.

It also helps to utilize sample vials. Though they may look alike, not all vials offer equivalent performance. Variations in product quality can lead to mystery peaks so be sure to use certified vials products.

10. What might be causing loss of peak resolution in my LC method?

- If the mobile phase has been contaminated or has deteriorated, this can cause loss of resolution.
- Obstructed guard can also cause loss of resolution; change out guard as necessary.
- If column inlet is obstructed, try and reverse flush as per our column protection guide.

Do you have other pressing HPLC questions? Leave them in the comments, or consult our Live Chat!

Related resources:

- HPLC Column Care Guide
- HPLC Troubleshooting Guide
- Easy Method Transfer from UHPLC to HPLC to Prep LC
- Enhancing Sensitivities and Peak Capacities for UHPLC-MS Fast Gradient Analyses
- Extend Your Analytical and Preparative HPLC/SFC Column Lifetimes
- Impact of pH on the Purity and Yield for Preparative HPLC Separations
- Reversed Phase HPLC Solutions for Proteins and Peptides
- Using pH-LC to Control Selectivity of Acidic and Basic Compounds by HPLC
- Fast Analysis of Sucrose, Glucose, and Fructose Composition in Fruit Juices and Processed Beverages Using Simplified HPLC Methodology
- Increased Speed of Analysis and Sample Throughput with Kinetex Core-Shell Technology