



Agilent Reversed-Phase InfinityLab  
Preparative LC Columns  
User Guide

This booklet provides general information for all ZORBAX, Poroshell, Pursuit, and Polaris reversed-phase columns. For additional detailed information about your specific phase or family, see:

[www.agilent.com/chem/prepcolumns](http://www.agilent.com/chem/prepcolumns)

## Getting started


A QC Column Performance Report, including a test chromatogram, is enclosed with every Agilent HPLC column. The QC test system has been modified from a standard system to minimize system dead volume, so it may vary from the system used in your lab. This allows a better evaluation of the column and assures a more consistent product.

A properly configured LC system will generate similar results to the chromatogram on your QC Performance Report.

Modern columns are robust and are designed to operate for long periods under normal chromatographic conditions. You can maximize column performance by running it within specifications. Always review the specifications before putting in place a final method.

## General description

Any purification campaign centers around three objectives – yield, purity, and throughput. Agilent InfinityLab preparative LC columns are optimized to help you to achieve your goals. Available in a variety of media, such as Pursuit XRs with high loading capacity, ZORBAX with diverse selectivity, or Poroshell 120 with high speed and efficiency, these columns offer a suitable fit for your purification needs.



Agilent InfinityLab preparative LC columns are available in 21.2 and 30 mm internal diameters (id) and easily scale from analytical dimensions. Columns are loaded to a stable, uniform bed density using an optimized proprietary high-pressure slurry-loading technique to give maximum column efficiency and maintain column bed stability.

## Column characteristics

Agilent InfinityLab preparative LC columns are 21.2 or 30 mm id, 50, 100, 150, or 250 mm long, and are packed with Poroshell 120, ZORBAX, or Pursuit XRs high-performance chromatographic packings. The nominal average particle size of the packings used for InfinityLab preparative LC columns is either 4  $\mu\text{m}$  for Poroshell 120 or 5  $\mu\text{m}$  for ZORBAX and Pursuit XRs.

The packings used in the InfinityLab preparative LC columns are produced using the same particle and bonding technology employed in the production of analytical scale packings. The same thorough quality control procedures are used to monitor all products, including the measurement of surface area, pore size, and particle size of the base silica packing as well as elemental analysis of all bonded phases. This technology permits the direct scaleup of separations from analytical to preparative proportions with little or no modifications required in methodology. In general, to obtain equivalent separation on a preparative column, relative to those obtained using analytical column of the same packing, the mobile phase flow rate must be adjusted proportional to the square of the ratio of the column internal diameters (see Table 1).

Table 1.

Phase	Column id (mm)	Normalized Flow Rate (mL/min)
Poroshell 120	21.2	25
ZORBAX	21.2	21
ZORBAX	30	40
Pursuit XRs	21.2	21
Pursuit XRs	30	40

## Safety considerations

- Because of the larger volumes of mobile phase used with preparative columns, special awareness of solvent toxicity and flammability hazards is recommended.
- Please adhere to operating pressure limits noted for each column (see table). Exceeding these limits will compromise chromatographic performance and could be unsafe.
- Should a column be overpressurized and a tubing or fitting failure occur, the major result will be a large flow leak of mobile phase. Special caution is required in this regard for flammable or toxic solvents.

## Other operating tips

- The direction of flow is marked on the column.
- While not harmful to the column, reverse flow should be avoided except to attempt removal of clogged frit (see Column care).
- Always use high purity reagents and chromatography grade solvent to prepare your mobile phase. Degas and filter all mobile phase prior to use.
- Disassembling a column will degrade column performance.

- New columns contain a mixture of organic solvents and water. See your QC Performance Report for the solvent composition in your column. Initially, care should be taken not to pass any mobile phase through the column that may cause a precipitate to form.
- Agilent reversed-phase columns are compatible with water and all common organic solvents.
- The use of an inline filter is recommended to protect your column and increase its lifetime. To prevent the deposition of strongly retained sample components on the preparative column, precautions (such as recrystallization, distillation, sample filtration, and prefractionation of the sample using gravity-feed chromatography columns) should be taken to maximize column life and sample throughput.
- Columns should not be maintained at elevated pH or elevated temperature when not in use.
- Avoid use of this column outside of recommended pH ranges for column phase (see Column operating parameters: pH and temperature). Expect reduced lifetime when operating outside the recommended pH and temperature ranges.
- Prior to initial start-up of the preparative column, or for start-up after prolonged storage (e.g., greater than 5 days), it is recommended that the column be preflushed with 10 column volumes of 100% organic solvent (e.g. Methanol or Acetonitrile) followed by at least 10 column volumes of mobile phase to elute potential contaminations.

## Column operating parameters: pH and temperature

Phase	Recommended pH Range	Maximum Operating Temperature (°C)
Poroshell 120 HPH	pH 2.0 – 11.0	60
Poroshell 120 SB-C18	pH 1.0 – 8.0	90
ZORBAX SB-C18	pH 1.0 – 8.0	90
ZORBAX Eclipse Plus C18	pH 2.0 to 9.0	60
Pursuit XRs C18	pH 2.0 to 8.0	60

**Note:** All silica-based packings have some solubility in pH >6 aqueous mobile phases. When using silica-based columns at pH >6, best column lifetime is obtained at lower temperatures (40 °C max) using low buffer concentrations in the range of 0.01 to 0.02 M. Operating at extreme ends of pH and temperature ranges will have a significant impact on column lifetime.

## Maximum operating pressures – InfinityLab preparative LC Columns 21.2 – 30 mm id

Column Type	Particle Size (µm)	Pressure Limit
Poroshell 120	4	400 bar (6,000 psi)
ZORBAX	5	400 bar (6,000 psi)
Pursuit XRs	5	400 bar (6,000 psi)

## Column care

### Cleaning your column/extending column life

To backflush columns start with a stronger (less polar) solvent.

1. Disconnect column from detector and run wash solvents into a beaker.
2. Start with your mobile phase without buffer salts (water/organic). You Flush 10 to 20-column volumes through the column.
3. Next, use 100% organic (methanol or acetonitrile).

4. Check pressure to see if it has returned to normal. If not, move on to point 5.
5. Discard column or consider stronger conditions, for example, 75% acetonitrile/25% isopropanol.
6. Increase to 100% isopropanol, 100% methylene chloride or 100% hexane (if you use methylene chloride or hexane, you will need to flush the column with isopropanol prior to use and before returning to your reversed-phase mobile phase).

### Storage recommendations

Long-term storage of silica-based, bonded phase columns should be in a pure organic solvent such as acetonitrile. If the column has previously been used with a buffered mobile phase, the buffer should first be removed by purging the column with 20 to 30-column volumes of a 50:50 mixture of methanol or acetonitrile and water, followed by 20 to 30-column volumes of the pure solvent. Before storing, end fittings should be tightly capped with end-plugs to prevent packing from drying out. To protect equipment, it is desirable to remove salts from the instrument and column by purging the column with the same mobile phase without the buffer (for example using 60:40 ACN/H<sub>2</sub>O to remove a 60:40 ACN/0.02 M phosphate buffered mobile phase). Re-equilibration is rapid with the original mobile phase when using this approach and any danger of corrosion from the salts is eliminated.

Storage of unbonded silica columns in most other liquids is typically acceptable. Before storing the column, the end-fittings should be tightly capped with the original caps or end-plugs used for shipping the columns to prevent contamination or damage to the threaded column ends.

To avoid potential metal corrosion, long-term storage of any HPLC column in halogenated solvents (e.g., butyl chloride, methylene chloride, etc.) should be avoided.

## Preparative strategies

- The interested reader is referred to the primer **“Principles and Practical Aspects of Preparative Liquid Chromatography” (5994-1016EN)** for a good compendium on strategies for successful preparative separations.
- For best instrument performance, choose from Agilent InfinityLab preparative LC supplies. See **quick reference guide 5994-2810EN**.

## Agilent ordering information

For more information on our products and services, visit our web site at **agilent.com**

For technical support and local information, visit **agilent.com/chem/columnsupport**

To place an order, visit **agilent.com/chem/wheretobuy**

### **Agilent InfinityLab LC: reliable, efficient, always innovating for your best result**

Every component of the Agilent InfinityLab family is uniquely designed to work together, and to help you continuously improve your workflow, for efficiency gains that help you get more done and reduce operational costs.

**agilent.com/chem/InfinityLab**

DE44329.227708333

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Agilent Technologies, Inc. 2021  
Printed in Canada, May 13, 2021  
5994-3605EN