User Guide

Agilent
Trusted Answers

Agilent PLRP-S, PL-SAX, and PL-SCX Preparative LC Columns

This document provides general information for Agilent preparative LC columns. For additional detailed information please visit the Agilent website: www.agilent.com.

Please read this document completely before using your preparative LC column.

Getting started

A QC Column Performance Report featuring a test chromatogram is included with every Agilent column. The instrument used to perform the QC test has been modified to minimize extracolumn dead volume and to ensure that the column performance is being measured without being compromised by the limitations of the LC instrument. This allows a better evaluation of column efficiency and ensures a more consistent product. Without optimization, the instrument may not match these results.

For specific questions, please contact the Technical Support team at www.agilent.com/chem/columnsupport.

Using the column

Column specifications

Each batch of stationary phase is quality controlled to ensure that it meets the Agilent specifications and performs as intended chromatographically. Each individual column is then tested to ensure that it has been correctly packed and meets specifications. The Column Performance Report is supplied with each column. It is recommended to regularly perform a similar test to monitor the performance of the column. Trying to replicate the conditions on the accompanying Performance Report may result in disparities that are likely due to instrument configuration differences.

Installation

Ensure that the LC instrument is configured correctly to minimize extracolumn band broadening, but to also ensure there are no pressure restrictions that could lead to excessive operating pressure. Agilent recommends choosing capillary tubing of the appropriate internal diameter (id):

Table 1. Recommended capillary internal diameter.

Flow (mL/min)	1.0 to 2.0	4.0 to 8.0	15 to 40	40 to 80	80 to 200
id (mm)	0.17	0.3	0.5	0.6	0.94

For more information about capillaries, please see www.agilent.com/chem/hplc-capillaries.

Before connecting the column, use a barrel connector and determine the backpressure contribution from the LC system. Identify any causes of high backpressure and rectify any problems prior to installing the column.

Note: Agilent 50 mm and 100 mm id PLRP-S, PL-SAX, and PL-SCX preparative columns are suitable for use with 1/8-inch outer diameter (od) tubing using Valco 1/8-inch nuts (PL1310-0038, 5/pk) and ferrules (PL1310-0038, 5/pk). Alternatively, 1/8-inch to 1/16-inch reducers are supplied with each column to enable 1/16-inch od tubing to be used where appropriate. The direction of flow is marked on the column.

Column conditioning

Column conditioning is best performed by first calculating the volume of the column (as a cylinder). For example, the calculation for a 21.2 × 250 mm column volume is:

$$\text{Volume, mL} = \pi \times (\text{radius, cm})^2 \times (\text{length, cm})$$

$$\text{Volume, mL} = 3.142 \times (\frac{2.12}{2})^2 \times 25$$

$$\text{Volume, mL} = 88.2 \text{ mL}$$

Ten column volumes for a 21.2 × 250 mm column is therefore 882 mL; so if the flow rate is 21.2 mL/min, 10 column volumes takes 41.6 minutes.

For a 50 × 150 mm with a column volume of 295 mL, operated at 60 mL/min, 10 column volumes (2,950 mL) takes 49.2 minutes.

Ten or 20 column volumes is the recommended minimum for flushing the column. Also check the UV detector trace and pressure reading to ensure that both are stable and that the column has equilibrated fully before continuing.

Important safety considerations

All points of connection in liquid chromatography systems are potential sources of leaks. Be aware of the toxicity and flammability of the mobile phase, particularly with the larger volumes required for preparative LC.

Agilent does NOT recommend that column end fittings are removed, or columns are disassembled. Column stationary phases contain small particles that can potentially be inhaled when dry.

Please adhere to the recommended operating conditions (see below) and to ensure that maximum operating pressures are not exceeded. Exceeding these limits will compromise column lifetime and could also be unsafe.

Ensure that large, heavy preparative columns are always safely secured.

Other operating tips

It is recommended that preparative LC columns are always started at low flow rate and gradually increased over time, as not to exceed the maximum pressure limit of the column.

Always use high-purity solvents and reagents to prepare the mobile phase; degas and filter solvents before use.

High flow rates can cause frictional heating of the mobile phase. This can result in thermal gradients across a column and may result in unwanted band-broadening. If thermal effects are observed, these can be minimized by maintaining a steady column temperature by immersing the column in a water bath. Mobile phase temperature can also be controlled in a similar fashion.

Maximum operating pressures

To avoid damage to the column, ensure that the maximum operating pressure is not exceeded. Where possible, set an upper pressure limit on the pump below that of the maximum operating pressure of the column.

Table 2. Recommended operating pressures.

PLRP-S		PL-SAX and PL-SCX	
8 µm, 10 µm	207 bar	8 µm, 10 µm	207 bar
10 to 15 µm, 15 to 20 µm, 30 µm	103 bar	30 µm	103 bar

Remember, mobile phase viscosity can change significantly during a gradient or when introducing a different mobile phase (particularly those containing water and organic modifiers). Sample solutions that are high concentration can also have higher viscosity and cause an increase in pressure during loading.

Mobile phase viscosity is also affected by temperature. When operating at elevated temperature, start with a lower flow rate and allow the temperature to increase and the viscosity to decrease. Then, safely increase the flow rate without risk of excessive operating pressure. To cool the column, first reduce the flow rate then allow the temperature to reduce, again ensuring that the maximum column pressure is not exceeded with the increase in mobile phase viscosity.

Column operating parameters

Always ensure that the instrument is flushed properly with the mobile phase, and that the flow rate is switched off before connecting the preparative LC column. Also ensure that the mobile phase is fully miscible with the storage solution contained in the column. Ensure that the column is installed with the flow in the direction of the flow arrow on the column. Before starting the pump, ensure that a suitable maximum pressure limit has been set to avoid damaging the column. Start the flow rate at a very low level and gradually increase the flow over a period of several minutes until the desired operating flow rate is reached. Refer to the guidelines in Table 3 for the recommended flow rates for the column but always ensure that the maximum operating pressure is not exceeded.

Table 3. Optimum flow rates by internal diameter.

Column id	Flow Rates Equivalent to 180 cm/hr	Flow Rates Equivalent to 360 cm/hr
4.6 mm	0.5 mL/min	1.0 mL/min
10 mm	2.3 mL/min	4.7 mL/min
21.2 mm	10.6 mL/min	21.2 mL/min
30 mm	21.2 mL/min	42.5 mL/min
50 mm	59 mL/min	118 mL/min
100 mm	236 mL/min	472 mL/min

The optimum flow rate for reversed-phase and ion exchange preparative LC columns is usually between 180 to 360 cm/hr (equivalent to 0.5 to 1.0 mL/min on a 4.6 mm id column).

Shipping solvents

Table 4. Shipping solvents.

Stationary Phase	Shipping Solvent
PLRP-S	7:1 (w/w) acetonitrile:water
PL-SAX / PL-SCX	0.1 M Na_2SO_4, 0.02% NaN_3

Mobile phase selection and operating temperatures

Reversed-phase columns are best used with water/acetonitrile gradients, with ion pair reagents as required. For example, use trifluoroacetic acid (TFA) or formic acid for peptide and protein separations, and triethylammonium acetate (TEAA) for oligonucleotide separations. It is suggested that a minimum of 1% acetonitrile is always present.

Remember that mobile phase viscosity will vary with differing mobile phase compositions. Ensure that column operating pressures are monitored carefully to prevent possible damage to columns due to exposure to excessive pressure.

Mobile phase viscosity will also vary depending on operating temperature. For separations performed at an elevated temperature, ensure that the operating flow rate is reduced when the column is at room temperature and only increased once the column has reached the desired operating temperature. Reduce the flow rate before allowing the column to cool.

Ion exchange separations are typically performed with a constant pH buffer and a salt gradient for elution. Suitable buffers are sodium phosphate for cation exchange separations (pH 6.5 to 7.5) and Tris-HCl for anion exchange (pH 7.5 to 8.5). Ensure that salt precipitation is avoided.

Recommended starting conditions

If possible, try to replicate the separation shown on the column performance report that was supplied with the column to ensure that the column and LC system are performing at an acceptable level. It may not be possible to achieve the same results where external factors such as extracolumn dead volume (from differences in capillary lengths, internal diameters, injection loop size, detector flow cell size) or even data collection rate may differ from those used by the manufacturer.

Regular use of a system suitability test such as this will ensure that any changes in performance are quickly identified and causes can be established and any corrective action taken.

Tips for getting the best chromatographic results

Wherever possible, optimize the separation using an analytical column packed with the same particles that are used in the preparative column, and use the same column length. These precautions prevent complications that can arise when changing particle size and column dimensions during scale-up.

Column care

Due to the large volumes of sample and high sample concentrations, together with increased levels of impurities, it is important to minimize the risk of blocking the column. Filtering sample solutions to remove particulates is strongly advised. Ensuring that the sample is soluble in the mobile phase and does not precipitate is also important.

It is better to use preventive techniques to remove impurities prior to injection than rely on column cleanup procedures to try to recover a contaminated column.

Each gradient run should include a section that contains high enough concentration of organic modifier (for reversed-phase columns) or salt (for ion exchange columns) to ensure that any strongly retained material is eluted from the column prior to re-equilibration and subsequent injection.

For example, if the optimum purification gradient has been found to be 5 to 25% acetonitrile, do not make up mobile phase A = 5% acetonitrile, mobile phase B = 25% acetonitrile and run the gradient from 0% to 100% B. Instead, ensure that mobile phase B contains strongly eluting components and extend the gradient to include a period of cleanup and re-equilibration (see Figure 1).

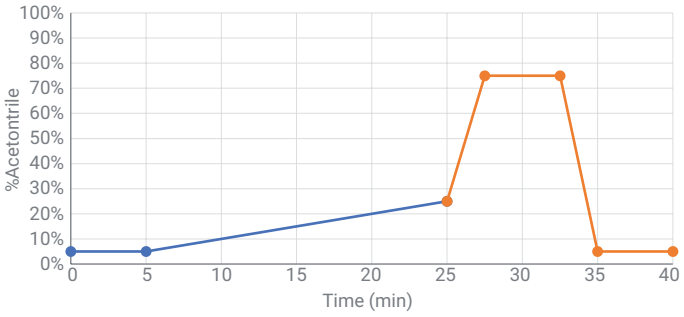

Figure 1. Gradient profile incorporating cleanup and re-equilibration. A minimum of five column volumes may be required for effective re-equilibration.

Cleaning the column and extending column life

The best cleaning procedure will depend on the kind of contamination that has been introduced. Choose the most appropriate method based on knowledge of the contaminants that have been introduced. Please do not simply use all cleaning procedures consecutively.

If the column pressure has increased due to a partially blocked inlet frit, run the column gently in the reverse direction to try to dislodge any particulate matter.

Ensure that all components are miscible. Introducing organic solvents into aqueous buffers can lead to precipitation. Flush columns to remove any salt prior to introducing organic solvents.

Potential cleaning solutions: PLRP-S columns

Acidic Cleaning Solution	1 M acetic acid or 1 M HCl
Basic Cleaning Solution	1 M NaOH
Hydrophobic Cleaning Solution	Acetonitrile or tetrahydrofuran

Potential cleaning solutions: PL-SAX columns

Acidic Cleaning Solution	1 M acetic acid or 1 M HCl
Basic Cleaning Solution	1 M NaOH
Hydrophobic Cleaning Solution	Propan-2-ol

Potential cleaning solutions: PL-SCX columns

Acidic Cleaning Solution	1 M acetic acid or 1 M HCl
Basic Cleaning Solution	1 M NaOH
Hydrophobic Cleaning Solution	95% methanol + 5% water

Storage recommendations

Where possible, flush the column and return to the same storage solution as the column was originally shipped in. It is not recommended to leave columns stored in solutions that contain high concentrations of buffers or salts.

If alternative storage solutions are required, ensure that there is no risk of bacterial growth occurring. Some storage solution combinations (such as aqueous alcohol) can be very viscous. To avoid pressure damage to the column only use these storage solutions at a reduced flow rate. Ensure that the end plugs are secure.

Agilent ordering information

To place an order, visit www.agilent.com/store.

www.agilent.com

DE03618256

Printed in the USA, April 4, 2022
5994-4723EN

Agilent
Trusted Answers