

Agilent Hi-Plex Ligand Exchange Columns

Installation column connections

Agilent Hi-Plex columns are supplied with industry standard end fittings. Only compatible nuts and ferrules should be used. Nuts should be tightened finger tight, then 1/4 turned using a wrench. To avoid loosening column end fittings, wrenches must be used only on the outer column end fitting, marked A in Figure 1.

Alternatively, the appropriate PEEK Fingertight fitting can be used, ensuring that there is no dead volume between the tube and the end fitting bottom.

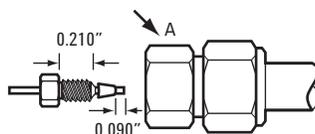


Figure 1. Wrench surface.

Hi-Plex guard columns

Hi-Plex guard columns are designed to be used in conjunction with the corresponding Hi-Plex analytical column type. The guard column should be connected prior to the analytical column to provide protection against contamination or blockage. It is an essential requirement to combine only guard columns in the matching ionic form together with the analytical column. Under no circumstances should a guard column with a different ionic form be used.

General guard column guidelines

- Eluents and samples should be filtered with a 0.45 µm filter before use.
- The same flow rate, pressure, and mobile phase restrictions that apply to the analytical column should remain for the guard column.

Shipping eluent

Hi-Plex columns are supplied containing UHP water. Columns are securely sealed with end caps which must ALWAYS be replaced when the column is disconnected from the system to prevent the column from drying out.

Column conditioning

Hi-Plex columns must be run at elevated temperatures (see Table 1). The mobile phase should be run at 0.1 mL/min and the column heating device switched on. When the column reaches the desired operating temperature the flow rate may be increased gradually to the required level. Care should be taken to ensure that the maximum flow rate of the column is not exceeded. Hi-Plex columns should not be subjected to sudden changes in flow rate.

Under no circumstances should the column heating device be left switched on with no flow through the column.

Mobile phases

All mobile phases used must be of high purity and should be filtered (< 0.5 µm filter) and degassed before use. The mobile phase should be degassed in the vessel used as mobile phase reservoir. Water is more effectively degassed at elevated temperature. Hi-Plex resins may be used with up to 5% v/v alcohol, or up to 30% v/v

Table 1. Description and recommended operating conditions (Hi-Plex Ca and Hi-Plex Pb).

Matrix	Hi-Plex Ca	Hi-Plex Ca	Hi Plex Ca (Duo)	Hi-Plex Pb
	Monodisperse, Sulfonated Styrene/Divinylbenzene			
Ionic Form	Calcium	Calcium	Calcium	Lead
Crosslink Content	8%	8%	8%	8%
Particle Size	8 µm	8 µm	8 µm	8 µm
Dimensions	250 × 4.0 mm 250 × 4.6 mm	300 × 7.7 mm	300 × 6.5 mm	300 × 7.7 mm 100 × 7.7 mm
Max Flow Rate	0.6 mL/min	1 mL/min	1 mL/min	1 mL/min
Typical Flow Rate	0.3 mL/min	0.6 mL/min	0.6 mL/min	0.6 mL/min
Temperature	80 to 90 °C	80 to 90 °C	80 to 90 °C	70 to 90 °C
Max Pressure*	50 bar	50 bar	50 bar	50 bar
Typical Pressure	12 bar	12 bar	27 bar	7 bar/6 bar
Eluent	Water or 30% acetonitrile	Water or 30% acetonitrile	Water	Water
Modifiers	< 5% alcohol, < 30% ACN			
Regeneration	0.1 M Ca(NO ₃) ₂	0.1 M Ca(NO ₃) ₂	0.1 M Ca(NO ₃) ₂ :0.1 M NaNO ₃ (1:49 v/v)	0.1 M Pb(NO ₃) ₂
Storage Eluent	Water	Water	Water	Water

* Remember to account for system pressures when assessing whether Pmax is being exceeded. Pmax can be calculated by subtracting the instrument pressure with no column installed from the instrument pressure with a column installed.

acetonitrile in the mobile phase. The system should be thoroughly purged with the mobile phase before attempting to connect the column.

Sample preparation

Sample solutions should be filtered prior to use. Solvent precipitation or extraction may be used to remove protein, lipid, and other biological contaminants. Ion exchange should be used to remove ionic contaminants where appropriate. Guard columns should be replaced on a regular basis to ensure contamination of the analytical column does not occur.

Maintenance column efficiency testing

It is recommended that a simple chromatographic test is used to monitor the performance of the column on a regular basis. Ideally, the test should replicate that of the enclosed Column Performance Report. Minor differences in peak shape or retention time may be due to variations in system configurations

and hold up volumes. In the case of major differences, the column should be re-tested with fresh mobile phase after checking the system thoroughly for possible sources of loss of efficiency such as excessive lengths of connection tubing, or injector faults.

Appropriate guard columns are strongly recommended in all cases. Extreme care should be taken during sample preparation to minimize the risk of introduction of particulate, ionic, or hydrophobic contaminants.

Hi-Plex columns are quite resilient and can sometimes be recovered following a loss in performance (see Column cleanup). Hi-Plex is a low backpressure column. System pressure should not be used as an indicator of column pressure. Performance, not pressure, is the primary indicator of column failure.

Column cleanup

Resin-based columns contaminated or damaged during use, can sometimes be regenerated.

Where the operating pressure has increased the column should be flushed with HPLC grade water in the reverse direction for at least 12 hours at the appropriate operating temperature. This may remove particulate contamination at the inlet of the column.

For strongly retained contaminants, the addition of a small amount of organic modifier to the mobile phase can be beneficial (< 30% v/v acetonitrile, < 5% v/v methanol or other alcohol).

Contamination with other counter ions is a more serious problem. Extensive flushing of the column with a 0.1 M solution of the appropriate nitrate salt (or 0.05 M sulfuric acid in the case of hydrogen forms) can help to regenerate the resin (see Tables 1 to 3).

Due to differences in swell of the resin in differing counter ion forms, it is not recommended to attempt to change the ionic form of a packed column.

Storage

Hi-Plex columns should ideally be run at reduced flow (0.1 mL/min) at the required operating temperature when left overnight.

Hi-Plex columns are stable for shipping, day-to-day handling, and short-term storage at room temperature. However, the column's performance will deteriorate over the course of months or years if stored at room temperature.

For long-term storage, the column should be flushed with HPLC grade water at the required operating temperature. The flow rate should be slowly reduced to 0.1 mL/min, and the column heating device switched off. When the column has cooled, it can be removed from

the system, and the end plugs should be replaced. The columns should be refrigerated at approximately 4 °C, but under no circumstances should they be allowed to freeze.

Warranty

Hi-Plex columns are covered by a warranty for 60 days following delivery. Agilent Technologies cannot accept liability for deterioration or loss of column performance as a result of improper handling or use.

Table 2. Description and recommended operating conditions (Hi-Plex K and Hi-Plex Na).

Matrix	Hi-Plex K	Hi-Plex Na (Octo)	Hi-Plex Na
	Monodisperse, Sulfonated Styrene/Divinylbenzene		
Ionic Form	Potassium	Sodium	Sodium
Crosslink Content	8%	8%	4%
Particle Size	8 µm	8 µm	10 µm
Dimensions	300 × 7.7 mm	300 × 7.7 mm	300 × 7.7 mm
Max Flow Rate	1 mL/min	1 mL/min	0.5 mL/min
Typical Flow Rate	0.6 mL/min	0.6 mL/min	0.3 mL/min
Temperature	80 to 90 °C	80 to 90 °C	80 to 90 °C
Max Pressure*	50 bar	50 bar	25 bar
Typical Pressure	22 bar	17 bar	16 bar
Eluent	Water	Water or 0.015 M NaOH	Water
Modifiers	< 5% alcohol, < 30% ACN		
Regeneration	0.1 M KNO ₃	0.1 M NaNO ₃	0.1 M NaNO ₃
Storage Eluent	Water	Water	Water

* Remember to account for system pressures when assessing whether Pmax is being exceeded. Pmax can be calculated by subtracting the instrument pressure with no column installed from the instrument pressure with a column installed.

Table 3. Description and recommended operating conditions (Hi-Plex H).

Matrix	Hi-Plex H	Hi-Plex H	Hi-Plex H
	Monodisperse, sulfonated styrene/divinylbenzene		
Ionic Form	Hydrogen	Hydrogen	Hydrogen
Crosslink Content	8%	8%	8%
Particle Size	8 µm	8 µm	8 µm
Dimensions	250 × 4.6 mm	300 × 6.5 mm	300 × 7.7 mm 100 × 7.7 mm
Max Flow Rate	0.4 mL/min	0.7 mL/min	1 mL/min
Typical Flow Rate	0.2 mL/min	0.4 mL/min	0.6 mL/min
Temperature	40 to 60 °C (acid) 60 to 70 °C (water)	40 to 60 °C (acid) 60 to 70 °C (water)	40 to 60 °C (acid) 60 to 70 °C (water)
Max Pressure*	50 bar	50 bar	50 bar
Typical Pressure	16 bar	25 bar	20 bar / 12 bar
Eluent	Water or 0.005 M H ₂ SO ₄	Water or 0.005 M H ₂ SO ₄	Water or 0.005 M H ₂ SO ₄
Modifiers	< 5% alcohol, < 30% ACN		
Regeneration	0.05 M H ₂ SO ₄	0.05 M H ₂ SO ₄	0.05 M H ₂ SO ₄
Storage Eluent	Water	Water	Water

* Remember to account for system pressures when assessing whether Pmax is being exceeded. Pmax can be calculated by subtracting the instrument pressure with no column installed from the instrument pressure with a column installed.

Agilent ordering information

For more information on our products, visit our website at:

www.agilent.com/en-us/products/liquid-chromatography/lc-columns

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