Hi-Plex Ligand Exchange Columns

Information
Installation
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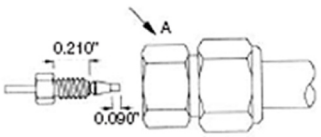
The Hi-Plex columns are a family of "soft" microporous sulfonated resins with a range of counter ions for carbohydrate, alcohol and organic acid analysis.

INSTALLATION
COLUMN CONNECTIONS

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



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

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 drying out.

COLUMN CONDITIONING

Hi-Plex columns should be run at elevated temperatures (see Table 1: Description and Recommended Operating Conditions for Maximum Temperatures and Flow Rates). 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. Due care should be taken to ensure that the maximum operating pressure 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 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 / 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 Test Certificate. 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 (excessive lengths of connection tubing, injector faults etc).

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 Clean-Up below).

COLUMN CLEAN-UP

Resin-based columns may be contaminated or damaged during use, but 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 0.1M solution of the appropriate nitrate salt (or 0.05M sulfuric acid in the case of hydrogen forms) can help to regenerate the resin. See Table 1: Description and Recommended Operating Conditions for further information.

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.

SYSTEM SHUTDOWN

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

For longer term storage, the column should be flushed with HPLC grade water at the required operating temperature. The flow rate should then be slowly reduced to 0.1 mL/min and the column heating device switched off. When the column has cooled it may be removed from the system and the end plugs replaced. Hi-Plex columns may be stored in a refrigerator (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. Polymer Laboratories, now a part of Varian, Inc., cannot accept liability for deterioration or loss of column performance as a result of improper handling or use.

Table 1: Description and Recommended Operating Conditions

	Hi-Plex Ca	Hi-Plex Ca	Hi-Plex Ca (Duo)	Hi-Plex Pb	Hi-Plex K	Hi-Plex Na (Octo)	Hi-Plex H	Hi-Plex Na
Matrix	Monodisperse, sulfonated styrene/divinylbenzene							
Ionic Form	Calcium	Calcium	Calcium	Lead	Potassium	Sodium	Hydrogen	Sodium
Crosslink Content	8%	8%	8%	8%	8%	8%	8%	4%
Particle Size	8 µm	8 µm	8 µm	8 µm	8 µm	8 µm	8 µm	10 µm
Dimensions	300 x 7.7 mm	250 x 4.0 mm	300 x 6.5 mm	300 x 7.7 mm 100 x 7.7 mm	300 x 7.7 mm	300 x 7.7 mm	300 x 7.7 mm 300 x 6.5 mm 100 x 7.7 mm	300 x 7.7 mm
Max Flow Rate	1 mL/min	0.6 mL/min	1 mL/min	1 mL/min	1 mL/min	1 mL/min	1 mL/min	0.5 mL/min
Typical Flow Rate	0.6 mL/min	0.3 mL/min	0.6 mL/min	0.6 mL/min	0.6 mL/min	0.6 mL/min	0.6 mL/min	0.3 mL/min
Temperature	85 °C	60 °C	85 °C	70 °C	85 °C	85 °C	65 °C	85 °C
Max Pressure	50bar	50bar	50bar	50bar	50bar	50bar	50bar	25bar
Typical Pressure	12bar	16bar	26bar	10bar / 5bar	35bar	35bar	20bar / 25bar / 12bar	11bar
Eluent	Water	Water or 30% acetonitrile	Water	Water	Water	Water or 0.015M NaOH	Water or 0.005M H_2SO_4	Water
Modifiers	<5% alcohol, <30% MeCN							
Regeneration	0.1M Ca $(NO_3)_2$	0.1M Ca $(NO_3)_2$	0.1M Ca $(NO_3)_2$: 0.1M $NaNO_3$ (1:49v/v)	0.1M Pb $(NO_3)_2$	0.1M KNO_3	0.1M $NaNO_3$	0.05M H_2SO_4	0.1M $NaNO_3$
Storage Eluent	Water	Water or 30% acetonitrile	Water	Water	Water	Water	Water	Water

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