



PL-WCX & PL-SCX HPLC Columns



Information Installation Maintenance

The PL-WCX (weak cation) and PL-SCX (strong cation) exchange media have an optimized pore size and structure for the analysis of biological macromolecules.

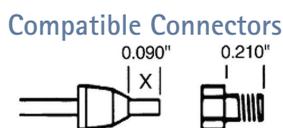
For optimum performance and lifetime please read this before commissioning and using the column.

INSTALLATION

Microbore, Analytical and Semi-Prep Columns up to 25 mm ID

1/16 in. stainless steel tubing is recommended for column connections, 0.002 in. ID for microbore, 0.010 in. ID for analytical work and 0.020 in. ID for preparative work. Connecting tubing lengths should be minimized to avoid excessive dead volume which will diminish system performance. Column connections should be made using Parker compatible 1/16 in. nuts and ferrules with special reference to compatibility of column connectors as illustrated in Figure 1. Connect the HPLC column in the flow direction indicated. The nut and ferrule should be tightened 1/4 of a turn past finger tight by applying the wrenches as shown in Figure 2.

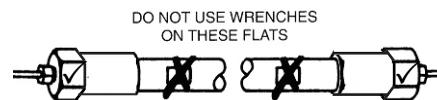
Figure 1



The distance 'X' for the standard PLRP-S column end fitting is 0.090 in. and a minimum male nut length of 0.210 in. is required. Some fittings from other manufacturers may not be compatible, eg Waters and Rheodyne. If unsure, please contact Varian, Inc.

To avoid loosening the endfittings and causing leaks, wrenches must be used on the endfitting adjacent to the connecting nut and NOT on the column barrel or the opposite endfitting.

Figure 2



Preparative Columns 50 & 100 mm ID

1/16 in. or 1/8 in. stainless steel tubing is recommended for column connections to the largest preparative columns. The size chosen should be appropriate to the flow rate used and the pressure of the column. Column connections should be made using fittings that are compatible with Vici Valco nuts and ferrules. 1/16 in. connections should be made using the reducing unions supplied with the column. Please ensure that the column is secured firmly to the bench before use.

SHIPPING ELUENT

PL-WCX and PL-SCX columns are supplied containing 0.1M Na₂SO₄ and 0.02% sodium azide. 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

It will be necessary to wash out the shipping solution and condition with the required counter ion prior to use. The following procedure is recommended at 180 cm/h (0.5 mL/min for a 4.6 mm ID column).

- Elute for 10 minutes with low ionic strength component of the

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- mobile phase, buffer A, for example 0.02M phosphate, pH 6.0.
- Exchange the counter ion by eluting with the high ionic strength component of the mobile phase, buffer B, for example 0.02M phosphate, 0.5M NaCl, pH 6.0. Continue with this eluent until a stable baseline is achieved at the required sensitivity, a minimum of 10 minutes.
 - Equilibrate with buffer A for a minimum of 2 column volumes prior to use.

MOBILE PHASES

The PL-WCX / PL-SCX matrix, being polymeric and macroporous, is stable in most polar mobile phases. The excellent chemical resistance of both the base polymer and cation exchange functionality enables the use of aqueous buffers in the pH range 1-13 without accelerated column degradation or loss of ionic capacity.

Both anionic and non-ionic detergents can be used. However, the column will need conditioning with the required detergent prior to use. As a cation exchange column, it is recommended that cationic detergents are not used.

The pre-packed columns are stable in alcohols (C₁-C₄), however when changing mobile phases, the miscibility and solubility of all eluent components must be examined.

FLOW RATE/PRESSURE

The maximum operating pressure for the PL-WCX / PL-SCX stainless steel HPLC column is 3000psi (207 bar). With low viscosity mobile phases this will enable flow rates of up to 4.0 mL/min to be used with 4.6 mm ID columns. If column pressures are high, due to mobile phase viscosity, or to improve sample solubility elevated temperatures, 80 °C can be used.

SAMPLE PREPARATION

The samples should be free from fat, which would otherwise contaminate the column, and be filtered (<0.5 µm). If turbid sample solutions are injected, even after being filtered, the lifetime of the column may be significantly reduced.

If possible the samples should be dissolved in buffer A, the low ionic strength component of the mobile phase. For interaction to occur with the strong cation exchanger the solutes must be positively charged at the analysis pH. In the case of proteins where the total nett charge is pH dependent, this will be below the pI, isoelectric point. The pH can be controlled by the use of any of the commonly

used anionic buffers, eg acetate, barbituates, or phosphate. The solutes can be eluted by increasing the ionic strength and/or changing the mobile phase pH.

COLUMN EFFICIENCY TESTING

Each column is provided with its own individual test certificate. It is recommended that the column be re-checked from time to time to monitor its performance.

COLUMN CLEAN-UP

If the column starts to exhibit signs of deterioration such as loss of resolution, increased back pressure or loss of solute recovery, then regeneration is required. When performance has deteriorated then the contamination will have occurred at the column inlet, therefore the flow through the column should be reversed during washing.

The excellent chemical stability of the PL-WCX / PL-SCX media enables washing with 1M acid, eg acetic or hydrochloric and 1M base, eg sodium hydroxide, to be accomplished. If the contamination is due to small hydrophobic molecules, eg fats, detergents and peptides, then the matrix should be washed with 95% methanol. The addition of 0.1% trifluoroacetic acid may be advantageous. After each washing sequence it is recommended that a high salt elution be carried out. After thorough cleaning, the column should be conditioned as detailed earlier.

STORAGE

On removing the column from the system the end plugs must be replaced to prevent the columns drying out by evaporation since disruption of the packed bed may occur. The end plugs should be finger tight only.

For long-term storage, the column should be washed with 1M solution chloride. After flushing with water the storage buffer of approximately 0.1 M Na₂SO₄ containing 0.2% sodium azide can be introduced.

WARRANTY

PL-WCX and PL-SCX columns are covered by warranty for 60 days following delivery. However, Varian cannot accept liability for improper handling/use. For a full warranty statement, please request our General Conditions of Sale.

Varian, Inc.

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