Agilent VariTide RPC Columns

Data Sheet

VariTide RPC columns are physically and chemically stable across the complete pH range. This guide is intended to help maximize column performance and lifetime. The column is optimized as a universal solution for the analysis and purification of synthetic peptides.

Installation

Analytical and preparative columns

A 1/16 in stainless steel tubing is recommended for column connections, 0.010 in id for analytical work and 0.020 in id for preparative work using the 21.2 id column. 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 show in Figure 2.

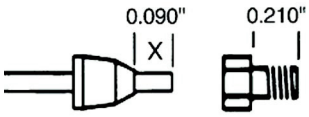

Figure 1. Compatible connectors.

The distance "x" for the standard HPLC 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, for example, Waters and Rheodyne. If unsure, please contact Agilent Technologies for advice.

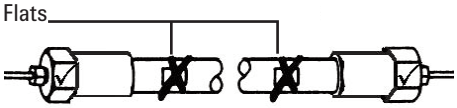

Figure 2. Don't use wrenches on the flats.

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.

Shipping Eluent

VariTide RPC columns are supplied containing 7:1 (w/w) acetonitrile/water. Columns are securely sealed with end caps which must ALWAYS be replaced when the column is disconnected from the HPLC system to prevent the column from drying out.

Column Conditioning

It will be necessary to wash out the shipping eluent and condition the column with the required mobile phase before use. Ensure the mobile phase components are soluble whenever changing eluent, and never use 100% aqueous eluents.

Mobile Phases

All eluents should be HPLC grade, they should be filtered (< 0.5 µm filter) and thoroughly degassed before use. The physical and chemical stability of VariTide RPC adsorbents allows buffered mobile phases across the complete pH range to be used. The maximum buffer salt concentration is determined by solubility.

Reversed Phase Chromatography

It is strongly recommended that a minimum of 1% organic modifier is maintained in the mobile phase. Column performance may be reduced when reintroducing organic modifier after use in 100% aqueous eluents. VariTide RPC absorbents can be used with all common organic modifiers in both isocratic and gradient elution modes.

Flow Rate/Column Pressure

The columns are packed to enable operating pressures, up to 3000 psi (207 bar) to be used. This pressure should not be exceeded. Optimum column performance will usually be found at flow rates of less than 360 cm/h (equivalent to 1.0 mL/min on a 4.6 mm id column). If column pressures are unusually high due to eluent viscosity, increasing the operating temperature will be beneficial. The increased temperature will reduce the eluent viscosity, which will result in a lower operating pressure and improved mass transfer.

Sample Preparation

Samples should be filtered (< 0.5 µm) to remove particulate matter which may otherwise foul the column inlet. In-line filters can be used to provide additional protection. Samples should be prepared in the starting eluent or in a solvent of lower strength, whenever possible, for maximum solute interaction.

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. 7:1 (w/w) acetonitrile/water is used, with acetone as the unretained test probe. System factors, such as dead volume, flow rate, temperature etc. can significantly affect the results obtained. It is important that these factors are taken into account when comparing the results with the test certificate data.

Column Clean Up

If the column begins to exhibit signs of deterioration then the following clean up procedures may be beneficial. Particulate matter blocking the inlet frit and causing excessive back pressure may sometimes be removed by gentle flushing in the reverse direction. The use of a higher strength organic modifier (such as acetonitrile or tetrahydrofuran) may remove hydrophobically bound contaminants. Peptide contamination may sometimes be removed by an aqueous acetonitrile gradient containing 1% v/v TFA. Strong acids and bases, including sodium hydroxide, can be used for cleaning in place and depyrogenation. All cleaning eluents must contain a minimum of 1% organic modifier.

Storage

On removing the column from the system, the end plugs must be replaced to prevent the column from drying, as this would disrupt the packed bed. The end plugs need only be applied finger tight. Long term storage in buffer should be avoided to prevent the risk of crystallization of buffer salts. A high organic content is recommended to inhibit bacterial growth.

Agilent Ordering Information

For more information on our products, visit our web site at www.agilent.com/chem/columns.

www.agilent.com/chem

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