



CARE & USE

RAM Internal-Surface Reversed-Phase (ISRP) and Semi-Permeable Surface (SPS) HPLC Columns

Column Certificate of Analysis

Every Regis column is tested to ensure performance and ships with a certificate of analysis. Column-specific information, such as material lot number, column serial number, a test chromatogram with operating conditions, and performance results are included with each report.

Recommended Conditions for Column Use

Guard columns should be used at all times to prevent contamination at the head of the column, thus allowing for increased column life.

Column life can also be extended by ensuring that prior to injection onto the column; all serum samples are either filtered through 0.2 μ filters or centrifuged at 5000 g.

Modifiers

Organic modifiers that are commonly used in reversed-phase chromatography may be used with the ISRP & SPS column. Acceptable organic modifiers include tetrahydrofuran (THF), acetonitrile (CH_3CN), isopropanol (IPA), and methanol (CH_3OH). In order not to precipitate the protein, the organic modifier concentration should be limited to 25% until the protein is off the column. After the protein is off the column, the concentration of organic modifier may be increased--by gradient, step gradient, or column switching without concern.

Buffer Concentration

A phosphate buffer is recommended. KH_2PO_4 concentration is generally in the range of 0.05-0.1 M.



pH Range

The pH of the mobile phase can be controlled to avoid protein denaturing and enhance selectivity. Within the optimal pH range of 6.0 to 7.5, both proteins and the glycine outer surface, which acts as a weak anion exchanger, take on a negative charge. As a result, the larger analytes are repulsed by the outer phase, and pass quickly through the column. Additionally, penetration and adsorption of the larger analytes can be enhanced by increasing the ionic strength of the buffer to decrease electrostatic repulsion with the outer phase. Thus with the decreasing pH, retention of the smaller analytes increases; potential ion-exchange interactions on the surface decrease.

Column Storage

When the column is to be taken out of service, it should be washed by reversing the column, then washing with 10 to 15 column volumes of water, followed by 10 to 15 column volumes of acetonitrile or organic modifier of choice (15 cm column: 20 mL of each; 25 cm: 30 mL of each). Each column is shipped with two removable endplugs to prevent the column packing from drying out. Save these plugs and reinstall them whenever the column is removed from the instrument.

Troubleshooting

Increased backpressure, changes in peak shape, and/or shifting retention times may indicate the need to perform column maintenance. Contact Regis' technical team for more support.

For full guide, visit registech.com/ram.



Visit registech.com for more technical resources and information on our full line of chromatography products.

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