THE REGIS PINKERTON ISRP HPLC COLUMN

Its Nature, Care, and Use

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The Regis
Pinkerton ISRP
HPLC Column

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I. The Nature of the Pinkerton Internal-Surface Reversed Phase

The Pinkerton Internal Surface Reversed Phase (ISRP) concept, developed by Dr. Thomas C. Pinkerton, incorporates those desirable separation characteristics from both size-exclusion and conventional chromatography. In order to achieve this workable combination, it was necessary to develop a stationary phase with two distinctly different inner and outer surfaces. The outer surface, which is hydrophilic, embraces size exclusion characteristics to disallow the penetration of serum proteins into the smaller pores. Additionally, the serum proteins are neither retained nor denatured by the surface. Small particles, in contrast, are able to penetrate through to the inner surface where they are retained by the hydrophobic support in a manner that is common to conventional chromatography.

These unique surfaces are produced by bonding the tripeptide glycine-phenylalanine-phenylalanine (GFF) to the silica surfaces. The introduction of carboxypeptidase A, an exopeptidase of 35,000 D, results in the cleavage of the phenylalanine-phenylalanine portion from the outer surface, allowing the glycine to remain intact. However, this enzyme is unable to penetrate the silica pores to reach the inner surface, leaving the original tripeptide in place.
II. Comparison of GFF to GFF II

Since its introduction in 1988, the Pinkerton ISRP stationary phase has been effectively utilized in the resolution of a number of compounds. However, the original GFF was troubled by concerns for higher efficiency, better column performance, and batch-to-batch reproducibility.

As a result, an improved ISRP, called GFF II, was developed. This refined phase results from the bonding of the GFF peptide to the silica surface through a monofunctional glycidoxypropyl linkage rather than the original trifunctional linkage. The end result is a totally monomeric phase with consistent loading for increased retention and efficiency and batch-to-batch reproducibility.

However, while the phases are similar regarding changes in modifier, pH, and column temperature, the selectivity of the GFF II is not always identical to that of the GFF. This phenomenon is illustrated in Table 1.

<table>
<thead>
<tr>
<th>Drug</th>
<th>( k' ) GFF</th>
<th>( k' ) GFF II</th>
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</thead>
<tbody>
<tr>
<td>Toluic Acid</td>
<td>2.1</td>
<td>1.9</td>
</tr>
<tr>
<td>Caffeine</td>
<td>2.1</td>
<td>2.5</td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>3.4</td>
<td>4.1</td>
</tr>
<tr>
<td>Sulfinpyrazone</td>
<td>5.3</td>
<td>6.5</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>3.3</td>
<td>5.8</td>
</tr>
<tr>
<td>Methyl Salicylate</td>
<td>6.0</td>
<td>7.4</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>5.3</td>
<td>8.8</td>
</tr>
</tbody>
</table>

III. Mobile Phase Selection

The nature of ISRP analytes requires that mobile phases consist of a buffer with varying degrees of modification. Modifiers can include acetonitrile, methanol, isopropanol, and tetrahydrofuran but should comprise no more than a total of 25% of the mobile phase while proteins are on the column. This limitation is not determined by the nature of
the column, but rather by the sample. If the modifiers exceed the recommended concentration, the sample proteins denature and precipitate onto the phase. After proteins have eluted through the column, however, modifier concentrations are no longer subject to restrictions and may be increased as necessary.

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.

Suggested initial mobile phases:

1. 84% 0.1 M KH$_2$PO$_4$, pH 6.8
   10% isopropanol, 6% tetrahydrofuran

2. 80% 0.1 M KH$_2$PO$_4$, pH 6.0, 20% acetonitrile

IV. Care of the Pinkerton ISRP Column

Guard columns should be used at all times. The installation of such columns helps to prevent particle build up at the head of the column, thus allowing for increased column life.

If the Pinkerton ISRP columns is to be either set aside for an extended period of time or left connected to the chromatograph overnight, replace any water-containing mobile phase within the column with a pure organic
solvent. Columns used with buffered mobile phase should first be flushed with 20 mL of water, then 20 to 30 mL of pure methanol or acetonitrile.

The Pinkerton ISRP column is packed using Regis Technologies’ proprietary packing method. Therefore, each column has no specified flow direction and can be reversed. Periodic reversal of the column flow is recommended to prevent the buildup of fines and particles and retard back pressure increase. 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.

V. Testing Column Performance

The individual testing parameters, results, test chromatogram, and a care and use guide are supplied with each column. All Pinkerton ISRP columns are individually tested.

Due to the fact that the chromatographic results depend upon the configuration of the equipment, it is suggested that the column performance be characterized on the user’s instrumentation.
VI. Technical Service

Customer satisfaction is our goal.

If you have any questions concerning the performance or application of the column, contact the Regis Technical Support department by:
  Phone: (847) 967-6000
  Fax: (847) 967-5876
  Email: cservice@registech.com

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Regis has made every effort to present accurate information in this booklet. However, users are cautioned to use their own judgment and make their own determination of the suitability of any of the products, data, or procedures presented.

Note: The products presented are for research use only and are not intended for food or drug purposes; nor are they to be resold for such use. Due care should be exercised in their handling and use.