High-Resolution Separation of a Fusion Protein

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Key Words
MAbPac HIC-10 Column, Hydrophobic Interaction Chromatography (HIC), HPLC, Protein Drug, Isomer, Impurity

Goal
To develop a high-resolution HIC method for the separation of a fusion protein from a truncated version and a structural isomer

Introduction
Fusion proteins are proteins created through the joining of two or more genes that originated from separate proteins. Fusion proteins created through genetic engineering impart properties from each of the parent proteins. Fusion protein therapeutics (e.g., Fc-fusion proteins) have proven successful in clinical treatments.1

Because fusion protein drugs are protein therapeutics, they require high-resolution methods for characterization and quality control techniques that are significantly different and more challenging than those used for small molecule drugs. HIC provides unique separation power based on protein surface hydrophobicity. Compared to other commercially available HIC columns, the Thermo Scientific™ ProPac™ HIC-10 column has demonstrated superior separation power for discriminating post-translational modifications of monoclonal antibodies, especially those due to methionine oxidation.2 Here, a new HIC column, the Thermo Scientific™ MAbPac™ HIC-10, provides impressive separation of a fusion protein from both a truncated version and a structural isomer—resolutions that cannot be achieved using existing commercially available HIC columns.

Equipment
• Thermo Scientific™ Dionex™ UltiMate™ 3000 Dual Rapid Separation Liquid Chromatography (RSLC) system,* including:
  – DGP-3600AB Biocompatible Dual-Ternary Low-Pressure Proportioning Analytical Pump System (P/N 5037.0014) with SRD-3600 Integrated Solvent and Degasser Rack, 6 Channels (P/N 5035.9230)
  – WPS-3000TRS Wellplate Sampler, Thermostatted (P/N 5840.0020)
  – TCC-3000SD Thermostatted Column Compartment (P/N 5730.0010)
  – DAD-3000 Diode Array Detector, Without Flow Cell (P/N 5082.0010)
• Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System software, version 7.2 or higher
• Thermo Scientific™ Orion™ 2-Star Benchtop pH Meter
  * This application was successfully evaluated on the two UltiMate systems listed.
**Conditions**

Columns:  
- ProPac HIC-10 Analytical, 5 µm, 4.6 × 100 mm (P/N 063655)  
- MAbPac HIC-10 Analytical, 5 µm, 4.6 × 100 mm (P/N 088480)

Mobile Phases:  
A. 2.0 M ammonium sulfate, 0.1 M sodium phosphate, pH 7.0/isopropanol (93:7 v/v)  
B. 0.1 M sodium phosphate, pH 7.0/isopropanol (93:7 v/v)

Gradient:  
- Routine, 0–30 min, 5–100% B  
- Shallow, 0–5 min, 30% B; 5–30 min, 30–60% B

Flow Rate: 1.0 mL/min  
Temperature: 30 °C  
Detection: UV, absorbance at 214 nm

**Results and Discussion**

The fusion protein sample was donated by a customer and had three fractions: the fusion protein, the fusion protein minus several amino acid residues, and the fusion protein with a different tertiary structure. As shown in Figure 1, these three fractions were not resolved using a popular commercially available HIC column.

Figure 2 shows separations of the fusion protein sample using the ProPac HIC-10 and MAbPac HIC-10 columns under the routine gradient conditions. Three peaks were observed when using the ProPac HIC-10 column; their separation was further improved using the MAbPac HIC-10 column.

Experiments showed that a lower starting salt concentration and shallower gradient improved the separation. As shown in Figure 3 and Table 1, the three components were better resolved using the shallower gradient.

For complicated fusion protein drug samples, it is critical to obtain pure fractions for further characterization. These samples often contain structural forms of the protein. High resolution is also needed for quality control of therapeutic proteins. The MAbPac HIC-10 column provides another option for the separation of challenging fusion protein samples.

**Table 1. Peak resolution (Rs).**

<table>
<thead>
<tr>
<th>Column</th>
<th>Peak 1</th>
<th>Peak 2</th>
<th>Peak 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>ProPac HIC-10</td>
<td>—</td>
<td>NA*</td>
<td>NA*</td>
</tr>
<tr>
<td>MAbPac HIC-10 (Routine Gradient)</td>
<td>—</td>
<td>1.60</td>
<td>1.53</td>
</tr>
<tr>
<td>MAbPac HIC-10 (Shallow Gradient)</td>
<td>—</td>
<td>1.66</td>
<td>1.61</td>
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</tbody>
</table>

*NA = not applicable
References