High Resolution and High Efficiency Separations of mAbs and ADCs Using Proteomix HIC Columns

Stacy Shollenberger, Product Manager and Hillel Brandes, Principal R&D Scientist
stacy.shollenberger@sial.com

Introduction

A variety of products derived from monoclonal antibodies (mAbs), including mAb fragments and antibody-drug conjugates (ADCs), are being developed for the treatment of cancer and other diseases due to their increased potency combined with reduced toxicity. However, the efficacy of these molecules is highly dependent upon the target site-specificity and binding properties of the mAb, the linker stability, the potency of the drug, and both the distribution and number of drug species on the mAb.[1] These requirements highlight the importance of characterizing these highly heterogeneous products using appropriate analytical techniques in order to assess and monitor them during manufacturing and subsequent storage.

Hydrophobic interaction chromatography (HIC) is a technique for protein separations and has been commonly used as an orthogonal method to size exclusion chromatography (SEC) and ion exchange (IEX) chromatography for the characterization of mAbs. Here we introduce Proteomix® HIC columns which have been designed for high resolution and high efficiency separations of proteins, oligonucleotides, and peptides.

General Description

Utilizing proprietary surface technologies, Proteomix HIC-NP resin is made of non-porous polystyrenedivinylbenzene (PS/DVB) beads with narrow-dispersed particle size distribution. As shown in **Figure 1**, the PS/DVB bead is modified with alkyl groups or an aryl group that provides hydrophobic interaction with analytes. Proteomix HIC-NP resin is highly rigid and mechanically stable. In comparison to silica based HIC phase media, Proteomix HIC-NP phases have advantages for biomolecule separations with wide pH range (2-12) and high thermal stability. The nonporous structure and narrow particle distribution offer special selectivity, high resolution separation of proteins such as mAbs, ADCs, and related protein fragments, as well as DNA and oligonucleotides. Proteomix HIC-NP media are applicable at laboratory discovery, laboratory-scale purification, and process chromatography for the production of a few mgs to kilogram of proteins.

Figure 1. Structure of Proteomix HIC-NP5 Resin

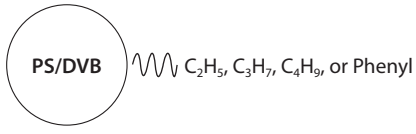


Table 1. Technical Specifications

Resin Matrix	Spherical, highly cross-linked PS/DVB
Pore Size	Nonporous
Particle Size	5 µm and 10 µm
Phase Structure	Ethyl, propyl, butyl, or phenyl
Separation Mechanism	Hydrophobic interaction (HIC)
pH Stability	2-12
Operating Temperature	Up to 80 °C
Operating Pressure	Up to 6,000 psi
Mobile Phase Compatibility	Compatible with aqueous solution, a mixture of water and acetonitrile, acetone, methanol, or THF

Featured Characteristics

- Highest capacity and resolution
- Consistent lot-to-lot reproducibility
- Improved protein recovery with intact biological activity
- Negligible non-specific interactions
- Ideal for separation and analysis of hydrophobic proteins, mAbs, and ADCs
- Suitable for separation and analysis of general biological samples

High Stability and Lot-to-Lot Reproducibility

Proteomix HIC columns are based on PS/DVB resin and all the surface coatings are chemically bonded onto PS/DVB support, which provides exceptionally high stability. The columns are compatible with most aqueous buffers, such as ammonium sulfate, sodium acetate, phosphate, Tris, and a mixture of water and acetone, methanol, acetonitrile and THF. When 25 mM sodium phosphate buffer, at pH 7.0, was used as the mobile phase to run the Proteomix HIC Butyl-NP5 column, 400 injections or 3 months of usage has negligible deterioration of the column.

Proteomix HIC columns provide high lot-to-lot consistency on ADC, mAb, and protein separations as shown in **Figures 2-4**.

(continued on next page)

Figure 2. Proteomix HIC Butyl-NP5 for Herceptin-cysteine ADC Separation-Lot Consistency Testing

column: Proteomix HIC Butyl-NP5, 4.6 × 35 mm, 5 µm (61864-U)
mobile phase: [A] 2 M ammonium sulfate in 0.025 M sodium phosphate, pH 7.0; [B] 0.025 M sodium phosphate, pH 7.0; [C] 100% IPA
flow rate: 0.8 mL/min
column temp.: 25 °C
detector: UV, 214 nm
injection: 10 µL
sample: ADC, 1 mg/mL in 1M ammonium sulfate

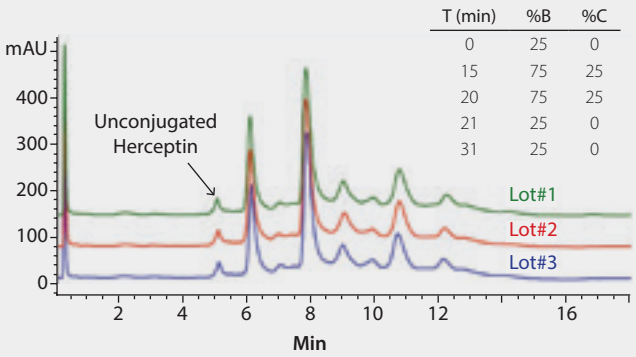


Figure 3. Proteomix HIC Butyl-NP5 for Herceptin-mAb Separation - Lot Consistency Testing

column: Proteomix HIC Butyl-NP5, 4.6 × 35 mm, 5 µm (61864-U)
mobile phase: [A] 2 M ammonium sulfate in 0.025 M sodium phosphate, pH 7.0; [B] 0.025 M sodium phosphate, pH 7.0; [C] 100% IPA
flow rate: 0.8 mL/min
column temp.: 25 °C
detector: UV, 214 nm
injection: 10 µL
sample: herceptin, 1 mg/mL in 1 M ammonium sulfate

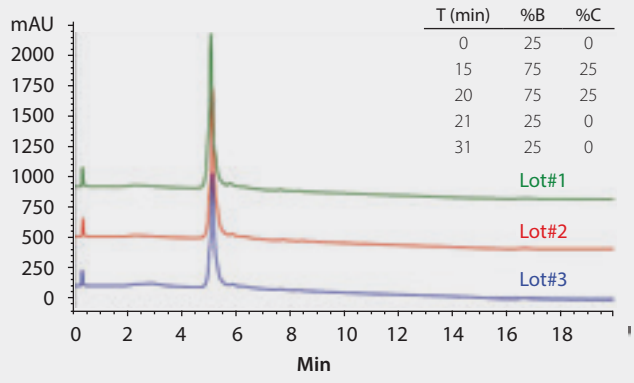
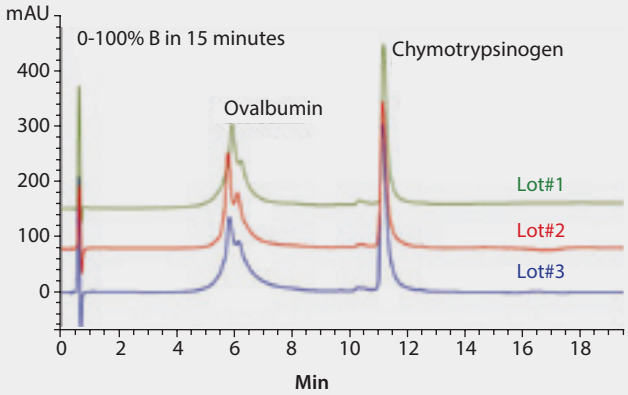


Figure 4. Proteomix HIC Butyl-NP5 for Protein Separation - Lot Consistency Testing

column: Proteomix HIC Butyl-NP5, 4.6 × 35 mm, 5 µm (61864-U)
mobile phase: [A] 2 M ammonium sulfate in 0.1 M sodium phosphate, pH 7.0; [B] 0.1 M phosphate, pH 7.0
flow rate: 0.4 mL/min
detector: UV, 214 nm
column temp.: 25 °C
injection: 4 µL
sample: ovalbumin 1.0 mg/mL, Chymotrypsinogen 0.5 mg/mL

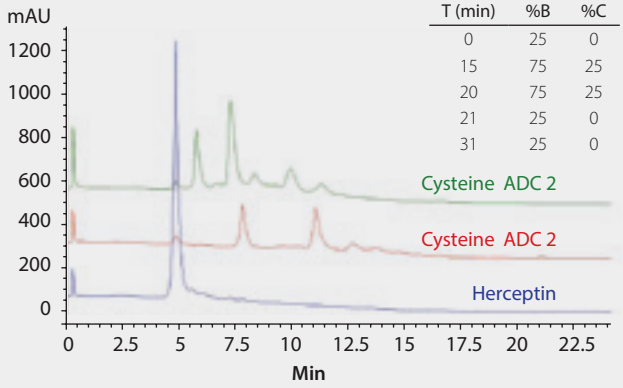


Additional Applications

In **Figure 5**, the *Proteomix* HIC Butyl-NP5 column was used for the characterization of the distribution of drug-linked species and the determination of the average drug to antibody ratio (DAR) after peak integration. Because HIC separates molecules based on their hydrophobicity, the Proteomix HIC Butyl-NP5 column is very effective for this type of separation due to the fact that hydrophobicity increases with the number of attached payloads.

Figure 5. Herceptin and its ADCs Separation on Proteomix HIC Butyl-NP5 Column

column: Proteomix HIC Butyl-NP5, 4.6 × 35 mm, 5 µm (61864-U)
mobile phase: [A] 2 M ammonium sulfate in 0.025 M sodium phosphate, pH 7.0; [B] 0.025 M sodium phosphate, pH 7.0; [C] 100% IPA
flow rate: 0.8 mL/min
column temp: 25 °C
detector: UV, 214 nm
injection: 10 µL
sample: herceptin/ADC1/ADC2, 1 mg/mL in 25 mM sodium phosphate



Reference

1. Wu, A. M.; Senter, P. D. Arming Antibodies: Prospects and Challenges for Immunoconjugates. *Nat. Biotechnol.* **2005**, *23(9)*, 1137-1146.

Featured Products

Description	Cat. No.
Proteomix HIC Butyl-NP5 Columns	
Proteomix HIC Butyl-NP5, NP, 5 cm × 2.1 mm, 5 µm	61862-U
Proteomix HIC Butyl-NP5 guard cartridge with holder, NP, 1 cm × 4 mm I.D.,5 µm	61863-U
Proteomix HIC Butyl-NP5, NP, 3.5 cm × 4.6 mm I.D., 5 µm	61864-U
Proteomix HIC Butyl-NP5, NP, 10 cm × 4.6 mm I.D., 5 µm	61865-U
Proteomix HIC Butyl-NP5, NP, 15 cm × 4.6 mm I.D., 5 µm	61866-U
Proteomix HIC Butyl-NP5, NP, 5 cm × 7.8 mm I.D., 5 µm	61867-U
Proteomix HIC Ethyl-NP5 Columns	
Proteomix HIC Ethyl-NP5 guard cartridge with holder, NP, 1 cm × 4 mm I.D.,5 µm	61868-U
Proteomix HIC Ethyl-NP5, NP, 3.5 cm × 4.6 mm I.D., 5 µm	61869-U
Proteomix HIC Ethyl-NP5, NP, 10 cm × 4.6 mm I.D., 5 µm	61870-U
Proteomix HIC Ethyl-NP5, NP, 5 cm × 7.8 mm I.D., 5 µm	61871-U
Proteomix HIC Phenyl-NP5 Columns	
Proteomix HIC Phenyl-NP5 guard cartridge with holder, NP, 1 cm × 4 mm I.D.,5 µm	61873-U
Proteomix HIC Phenyl-NP5, NP, 3.5 cm × 4.6 mm I.D., 5 µm	61874-U
Proteomix HIC Phenyl-NP5, NP, 10 cm × 4.6 mm I.D., 5 µm	61876-U
Proteomix HIC Phenyl-NP5, NP, 5 cm × 7.8 mm I.D., 5 µm	61878-U
Proteomix HIC Propyl-NP5 Columns	
Proteomix HIC Propyl-NP5 guard cartridge with holder, NP, 1 cm × 4 mm I.D.,5 µm	61879-U
Proteomix HIC Propyl-NP5, NP, 3.5 cm × 4.6 mm I.D., 5 µm	61881-U
Proteomix HIC Propyl-NP5, NP, 10 cm × 4.6 mm I.D., 5 µm	61883-U
Proteomix HIC Propyl-NP5, NP, 5 cm × 7.8 mm I.D., 5 µm	61884-U