

Analysis of Monoclonal Antibodies and Antibody-Drug Conjugates Using New Hydrophobic Interaction Chromatography (HIC) Columns

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Overview

Purpose: Demonstrate high resolution separation of mAbs and ADC mimics using Thermo Scientific™ MAbPac™ HIC columns.

Methods: Ammonium sulfate and sodium phosphate mobile phases were used. In some cases, addition of isopropanol to both mobile phases increased the resolution of the chromatogram.

Results: MAbPac HIC columns were developed using advanced surface bonding technology to achieve unique selectivity, high recovery and high efficiency. High resolution separation of proteins and various mAb samples were successfully carried out using MAbPac HIC columns.

Introduction

Various types of monoclonal antibody (mAb) products including intact mAbs, mAb fragments, engineered variants, and antibody-drug conjugates (ADCs) are being developed for the treatment of cancer and other diseases due to their excellent biocompatibility and high selectivity. The proliferation of monoclonal antibody therapeutics and their susceptibility to various biochemical modifications has highlighted the importance of characterizing these highly heterogeneous products for their safety and efficacy.

Hydrophobic interaction chromatography (HIC) is a technique for separation of proteins and has been widely used as an orthogonal method to size exclusion chromatography and ion exchange chromatography for the characterization of mAb variants.

Here we introduce a new family of HIC columns designed for the analysis of mAbs and related biologics. Three different ligand chemistries-polyamide, amide and butyl-were developed for the analysis of a wide range of mAb samples. Separation of mAb aggregates, mAb fragments, oxidized mAbs, and antibody-drug conjugates were successfully carried out with excellent efficiency and high recovery.

Methods

Samples

Monoclonal antibody samples and ADC sample were donated by biotech companies. Proteins and other chemicals were from Sigma-Aldrich®.

Columns

MAbPac HIC-10, 5 µm, 4.6 × 100 mm (P/N 088480)
MAbPac HIC-20, 5 µm, 4.6 × 100 mm (P/N 088553)
MAbPac HIC-20, 5 µm, 4.6 × 250 mm (P/N 088554)
MAbPac HIC-Butyl, 5 µm, 4.6 × 100 mm (P/N 088558)

Liquid Chromatography

HPLC experiments were carried out using a Thermo Scientific™ Dionex™ UltiMate™ 3000 BioRS system equipped with:

- SR-3000 Solvent Rack (P/N 5035.9200)
- LPG-3400RS Biocompatible Quaternary Rapid Separation Pump (P/N 5040.0036)
- WPS-3000TBRS Biocompatible Rapid Separation Thermostatted Autosampler (P/N 5841.0020)
- TCC-3000RS Rapid Separation Thermostatted Column Compartment (P/N 5730.0000)
- VWD-3400RS Rapid Separation Variable Wavelength Detector (VWD) equipped with micro flow cell (P/N 5074.0010)
- Chromatography was controlled by Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System .

Mobile phases

Formula 1

Mobile phase A: 2 M ammonium sulfate, 100 mM sodium phosphate, pH 7.0

Mobile phase B: 100 mM sodium phosphate, pH 7.0

Formula 2

Mobile phase A: 1.5 M ammonium sulfate, 50 mM sodium phosphate, pH 7.0 / isopropanol (95:5 v/v)

Mobile phase B: 50 mM sodium phosphate, pH 7.0 / isopropanol (80:20 v/v)

Method Development

Ammonium sulfate and sodium phosphate based mobile phases were used. For the applications with mAb and ADC mimics, mobile phases were optimized by either lowering the starting salt concentration by adjusting the gradient using formula 1 or adding isopropanol into both mobile phase A and mobile phase B (formula 2). For most applications, lower flow rate (0.5 mL/min) using longer gradient increased the resolution.

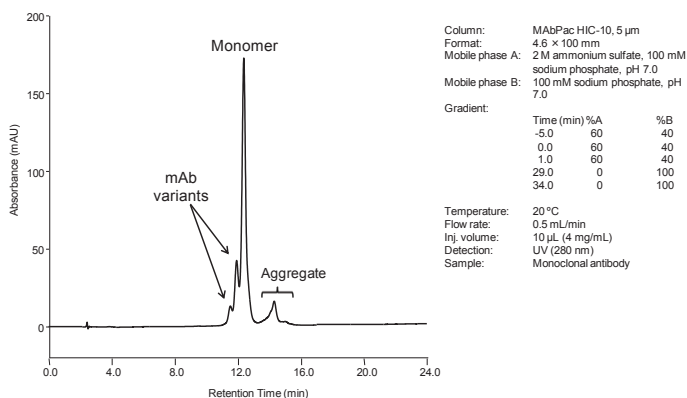
All three MAbPac HIC columns were screened to obtain the best separation.

Results

Analysis of mAb Aggregates

Protein and antibody aggregates are formed either during product expression in cell culture, downstream processing or storage. These aggregates may cause undesirable immune reactions which affect the safety of the drug. SEC is the most widely used technique for the detection and quantification of protein aggregates in biological drug products. However several researchers have reported the use of HIC for the removal of protein aggregates.^{1,2} Figure 1 demonstrates the separation of monoclonal antibody aggregates from the monomer form on the MAbPac HIC-10 column. In addition to the separation of aggregates, hydrophilic mAb variants were also detected using the MAbPac HIC-10 column.

FIGURE 1. Separation of mAb aggregates

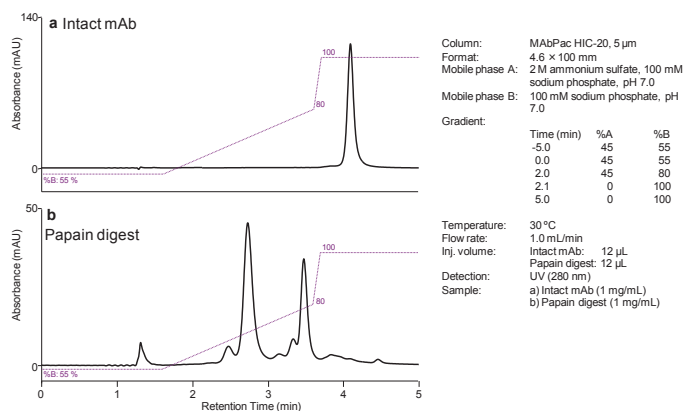


Analysis of mAb Fragments

Analysis of antibody fragments is important for both characterization of Fab or Fc based biotherapeutics and localization of the sources of heterogeneities on a monoclonal antibody molecule. HIC can provide the resolution required for the separation of Fab and Fc fragments and their hydrophilic or hydrophobic variants.³ Figure 2 shows a comparison of an intact mAb and its papain digest on the MAbPac HIC-20 column. Fast and high resolution separation of Fab and Fc fragment was achieved by simply optimizing the gradient method. The MAbPac HIC-20 column efficiently separated Fab and Fc fragments and further separated variants of these fragments. These variant peaks imply oxidation or other modifications in these fragments.

FIGURE 2. Separation of papain digested mAb

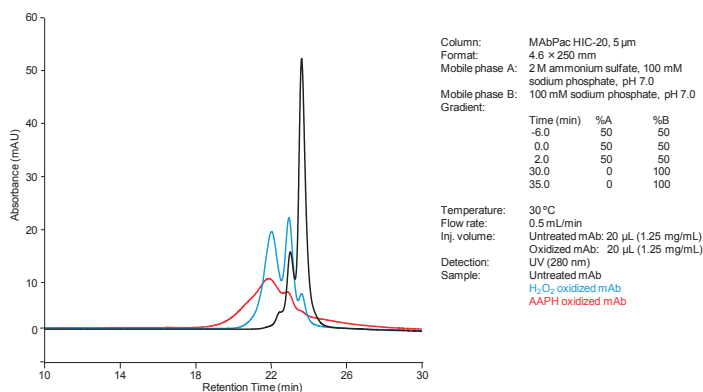
(a) Intact mAb, (b) Papain digested mAb



Analysis of oxidized mAb

Oxidation of therapeutic mAbs during production or storage is a common degradation mechanism and has become a major concern in mAb production. In many cases, oxidized mAbs have less to no potency compared to their native form.⁴ Oxidation of amino acid residues on a mAb can alter the hydrophobic nature of the mAb by either the increase in polarity of the oxidized form or the resulting conformational change. Hydrophobicity-based HPLC methods such as reverse phase chromatography and HIC are typically used to characterize oxidized mAb products. A mAb sample was oxidized using hydrogen peroxide or 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH). The MAbPac HIC-20 provided good resolution of oxidized mAb variants from unmodified mAb without fragmentation, or other sample preparation. The best result was obtained using a longer 250 mm column and lower flow rate.

FIGURE 3. Separation of oxidized mAb



Analysis of Antibody-Drug Conjugate Mimic

Hydrophobic interaction chromatography is often used for the separation of ADCs with different drug-to-antibody ratios (DARs) since attachment of cytotoxin alters the hydrophobicity of the antibody.⁵ The least hydrophobic unconjugated antibody elutes first and as the number of attached drugs increases the elution time of each ADC increases as well. Below is the separation of a cysteine-conjugated ADC mimic sample on the MAbPac HIC-Butyl column. The ADC mimics were conjugates between a drug mimic and mAb via the sulfhydryl group of interchain cysteine residues which results in a mixture of drug-loaded antibody species with 0 to 8 drugs (Figure 4a). The unmodified mAb and ADCs with DAR values ranging from 2 to 8 are well resolved by the MAbPac HIC-Butyl column (Figure 4b). The best peak shape and separation were achieved using 5% IPA in mobile phase A and 20% IPA in mobile phase B at 25 °C. Figure 5 compares the analysis of cysteine-conjugated ADC mimics with different drug loads assuming the UV absorption of the drug mimic is minimal at 280 nm. The mimic with high drug load showed no unconjugated mAb and a higher intensity of the 8-drug form while the low drug load sample contains a significant amount of unconjugated mAb, 2-drug form, and 4-drug form.

FIGURE 4. Separation of Cys-conjugated ADC mimic (a) Schematic representation of conjugation of drug mimic via interchain cysteine residues (b) Separation of Cys-conjugated ADC mimic on MAbPac HIC-Butyl

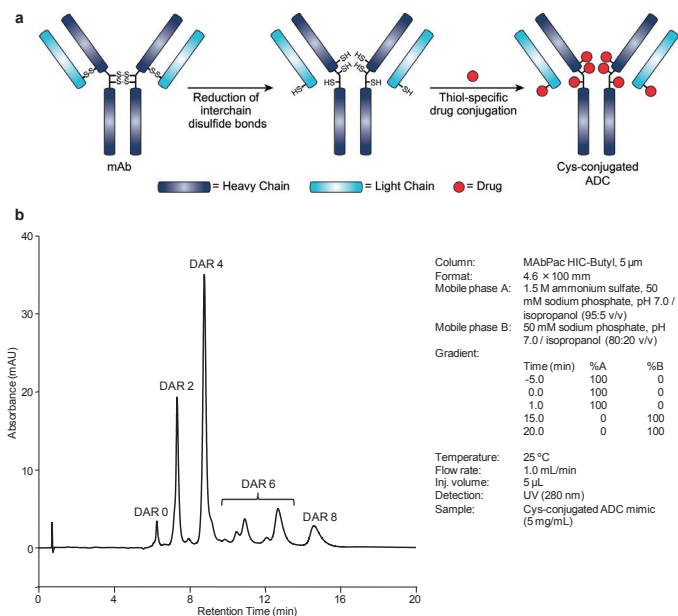
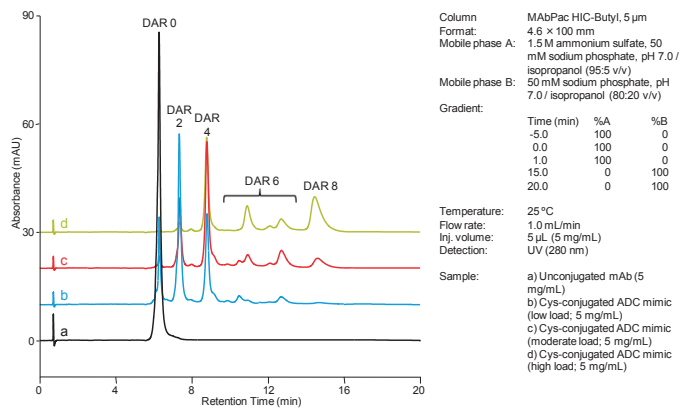


FIGURE 5. Comparison of Cys-conjugated ADC mimics with different drug loads



Conclusion

- MAbPac HIC columns with three different ligand chemistries were developed for the analysis of monoclonal antibodies (mAbs) and related biologics.
- High resolution separation of various mAb samples including mAb aggregates, mAb fragments, oxidized mAb, and ADC mimic were obtained using MAbPac HIC columns.

References

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