Capillary-Scale Analysis of Nontoxic Antibody Drug Conjugate (ADC) Mimics

By Sam Foster, Milton Lee

Axcend, Lehi, Utah

Abstract

Antibody drug conjugates (ADCs) belong to a class of drugs featuring a monoclonal antibody conjugated to a toxic payload. The antibody allows for the delivery of this payload to specific antigen sites on cancer cells. Hydrophobic interaction chromatography (HIC) is often used during the manufacturing process to determine the drug antibody ratio (DAR) of the sample. Due to the highly toxic nature of commercially available ADCs, carefully designed analytical workflows are needed for their analysis to minimize laboratory worker exposure. This application note uses ADC conjugation workflows established in the literature to form nontoxic ADC mimics for analysis. The Axcend Focus LC was used to analyze both unconjugated and conjugated mAb samples using HIC. The total solvent consumption per run is ~60 µL, which reduces the generation of hazardous waste by orders of magnitude compared to existing analytical scale HPLC workflows, while maintaining excellent chromatographic resolution. This work demonstrates the viability of capillary scale HIC for the analysis of ADCs.

Introduction

Antibody drug conjugates (ADCs) comprise a drug modality featuring a monoclonal antibody (mAb) conjugated to a toxic drug payload. The specificity of monoclonal antibodies allows cancer antigens to be targeted with highly cytotoxic payloads. The toxic nature of these payloads introduces significant challenges for the manufacture and analysis of these drugs, typically requiring complete sealing of fume hoods and incineration of waste products. These strict safety requirements limit analytical instrument size and make solvent disposal costly. The compact Axcend Focus LC

addresses both safety requirements because it is small and uses minimal solvent. It operates at flow rates orders of magnitude below traditional analytical-scale HPLC separations (µL/min vs mL/min). These advantages make it an ideal instrument for deployment in high potency workflows. In this application note, the Axcend Focus LC is used to separate nontoxic ADC mimics using hydrophobic interaction chromatography (HIC), a common approach to determining drug antibody ratios (DAR).

Materials and Methods

Due to the highly cytotoxic nature of commercially available ADCs, their use in analytical method development is undesirable. In this work, nontoxic ADC mimics were produced using literature guidance [1]. NIST mAb reference standard was conjugated with a maleimide (MAL-83, CAS: 756487-18-0) previously described in the literature [1]. The NIST mAb was first diluted with 50 mM phosphate buffer to a pH of 7-7.2. Reduction of the mAb was performed using 2.1 equivalents of tris(2-carboxyethyl)phosphine (TCEP), and the solution was incubated at 37˚C for 1 h. During this time, a dilute (2 mM) maleimide linker solution was prepared in dimethyl sulfoxide (DMSO). After incubation, the reduced mAb was cooled to room temperature, and linker (3.0 equivalents) was added. The solution was left to react overnight at room temperature. Untreated mAb and ADC mimic samples were spiked to a final concentration of 0.75 M Ammonium acetate before analysis.

Chromatographic Conditions:

Column	150 x 0.15 mm column; 3 µm, 1000Å, PolyLC Polybutyl particles
Mobile Phase	A) Water with 1 M ammonium acetate buffer B) Water with 50% Acetonitrile
Flow Rate	2 µL/min
Injection Volume	250 nL
Temperature	Ambient
Detection Wavelength	275 nm
Method	Linear gradient from 0.5% B to 99.5% B over 20 min followed by isocratic hold at 99.5% B for 5 min

Results and Discussion

Hydrophobic interaction chromatography (HIC) is often used to determine the drug antibody ratio (DAR) of ADCs based on the ratio of peak areas and retention times. In this application note, a chromatogram of untreated mAb was first obtained, which gave a single peak (Figure 1). Following this, mAb treated according to the conjugation workflow was injected, and its resulting chromatogram is shown in Figure 2. This chromatogram shows multiple peaks formed from TCEP reduction and maleimide conjugation with retention times later than untreated mAb, which implies increased hydrophobicity due to reaction modifications. Although full structural characterization

was not performed, it is reasonable to assume these peaks represent ADC mimics of increasing DAR. This demonstrates the viability of capillary scale HIC for the analysis of ADCs.

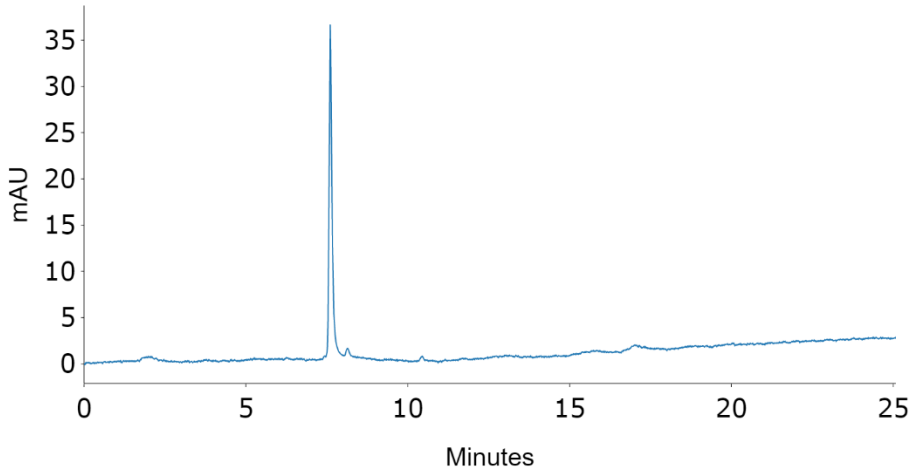

Figure 1. HIC chromatogram of untreated monoclonal antibody.

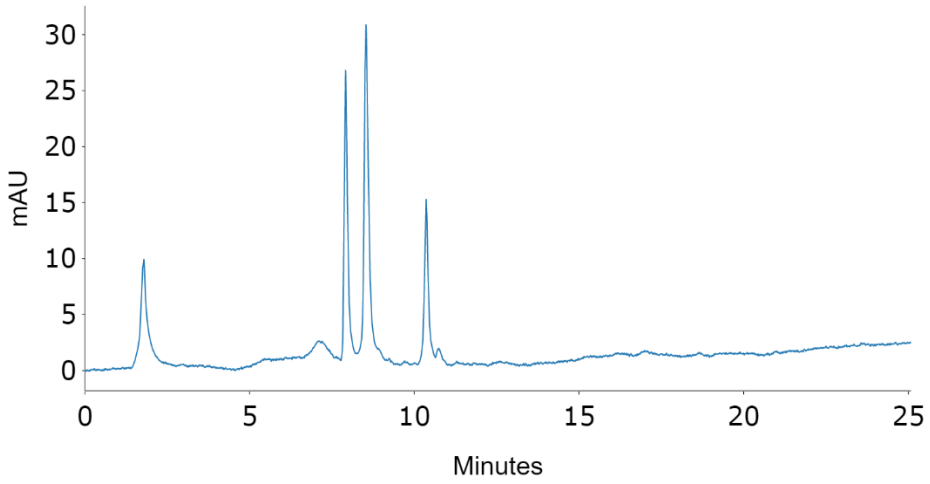

Figure 2. Chromatogram of monoclonal antibody and reaction products after TCEP reduction and addition of 3.0 equivalents of maleimide linker.

Conclusions

ADCs allow for highly specific delivery of cytotoxic payloads. Due to the highly toxic nature of these drugs, footprints of instruments used for the analysis of synthetic ADC reaction products must be small enough to fit within sealed fume hoods. Additionally, waste solvents resulting from chemical analysis of the reaction products are often incinerated to prevent contamination and, therefore, should be minimized as much as possible. The Axcend Focus LC offers small benchtop footprint due

to its compact size and it operates at low µL/min flow rates, satisfying both requirements. This positions it as a very attractive alternative to existing analytical HPLC workflows. In this application note, the production of a nontoxic ADC mimic by conjugating a NIST mAb standard with a maleimide linker is described. Capillary LC of the reaction products shows peaks of increasing hydrophobicity, demonstrating the application of capillary HIC to a relevant, critical analysis.

Reference(s)

[1] Emmert, M. H.; Bottecchia, C.; Barrientos, R. C.; Feng, Y.; Holland-Moritz, D.; Hughes, G. J.; Lam, Y.-H.; Regalado, E. L.; Ruccolo, S.; Sun, S.; Chmielowski, R.; Yang, C.; Lévesque, F.; Raymond, K.; Haley, M. "Build Your Own" ADC Mimics: Identification of Nontoxic Linker/Payload Mimics for HIC-Based DAR Determination, High-Throughput Optimization, and Continuous Flow Conjugation. *Organic Process Research & Development* **2024**, *28* (8), 3326–3338. DOI:10.1021/acs.oprd.4c00226.