Enhancing Purity of an Emtansine Biosimilar ADC through Mixed-Mode Chromatography

[1] Scott Conrad McCarthy, [1] Chris Weinert, [2] Sharon Bola, [2] Young Li, [2] Erik Verona
[1] Pacifica Immuna Celkem, 2304 Jefferson Avenue, Tacoma, WA 98402
[2] Bio-Rad Laboratories, Inc., 6000 James Watson Drive, Hercules, CA 94547

Introduction

Antibody-drug conjugates (ADCs) are transforming the landscape of biotherapeutics by harnessing the precision of monoclonal antibodies and the power of cytotoxic drugs. Ensuring the purity of ADCs is essential, as product-related impurities—such as high molecular weight aggregates and variants with elevated drug-to-antibody ratios (DARs)—can compromise both safety and efficacy. Trastuzumab emtansine (T-DM1) is a humanized HER2 antibody covalently linked to MCC-DM1 drug linker. As an ADC It has been used in early-stage breast cancer. Here we demonstrate the process purification of T-DM1 on Nuvia aPrime 4A Media followed by polishing on Bio-Rad CHT™ XT Media.

Materials and Methods

Antibody Preparation

Monoclonal antibody was purchased from TargetMol and prepared at 5.0 mg/mL in a total volume of 20 mL (100 mg total protein). The solution pH was adjusted to pH 8.0 by addition of 1 mL 200 mM sodium phosphate buffer (pH 9.0). Following adjustment, the antibody concentration corresponded to 36 µM.

Nuvia aPrime 4A Chromatography (Pre-Conjugation Polishing)

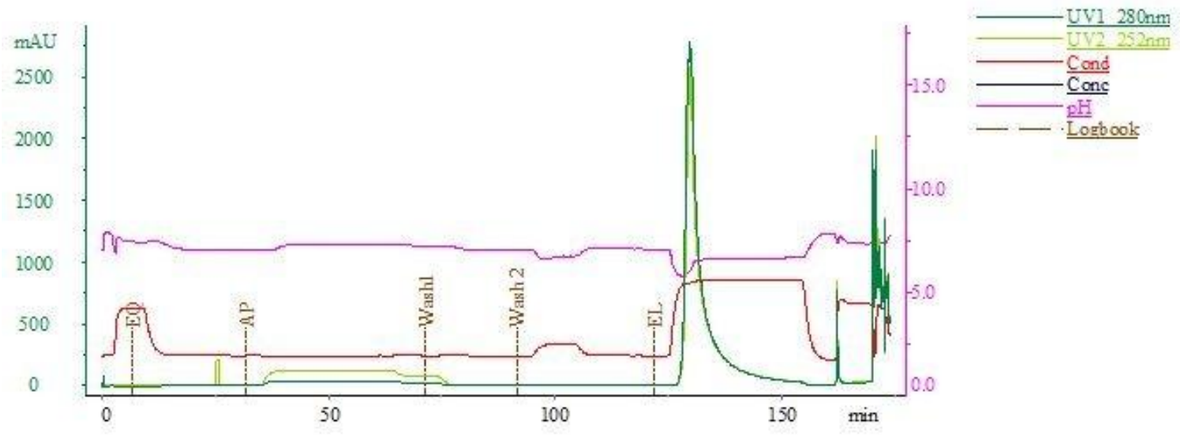
Pre-conjugation polishing was performed using a Foresight™ Nuvia™ aPrime 4A column (1 mL) operated at a flow rate of 1 mL/min. Antibody loaded in Tris pH 9, washed with Tris and phosphate buffers, and eluted at pH 5. Elution yield was 91% with 99% SEC monomer purity.

Drug-linker Preparation & Conjugation Reaction

The drug–linker was dissolved in dimethyl sulfoxide (DMSO) and added at an 8-fold molar excess relative to antibody (final drug concentration 288 µM). The drug solution volume was 578 µL in DMSO, and total DMSO content of the conjugation reaction was adjusted to 10% (v/v). The drug–linker solution was added slowly to the aqueous antibody under continuous mixing. The conjugation reaction was performed at 25 °C for 4 hours.

CHT XT Chromatography (Post-Conjugation Polishing)

CHT™ XT media was first evaluated using Foresight™ filter plates, followed by 1 mL column method development. The optimized conditions were subsequently scaled to a 5 mL Foresight™ column to assess recovery and product quality. The final chromatography conditions shown in figure 1., with performance evaluated by recovery, DAR, and SEC purity.



Step	Buffer	Column Volume
Pre-eq	400 mM PO4, pH 6.8	10
Eq	10 mM PO4, 10 mM NaCl, pH 6.8	15
Load	10 mM PO4, 10 mM NaCl, pH 6.8	20
Wash 1	10 mM PO4, 10 mM NaCl, pH 6.8	10
Wash 2	10 mM PO4, 75 mM NaCl, pH 6.8	5
Wash 3	10 mM PO4, 10 mM NaCl, pH 6.8	10
Step elution	10 mM PO4, 520 mM NaCl, pH 6.8	15
Step elution	400 mM PO4, pH 6.8	5
Water	Water	5
Cleaning	0.2M NaOH	10
Follow	10mM PO4, 10mM NaCl, pH 6.8	5

Fig. 1.. CHT XT Chromatography

Results

LC-MS Analysis of Drug-to-Antibody Ratio (DAR)

The drug-to-antibody ratio (DAR) distribution of T-DM1 following conjugation was characterized by liquid chromatography–mass spectrometry (LC-MS). Intact ADC species corresponding to different drug loads (DAR 0–8) were resolved and detected by mass analysis. Peak areas associated with each DAR species were quantified, and the relative abundance of each species was calculated as a percentage of total signal. The average DAR was determined by weighting each species by its relative peak area. (Figure 3A & 3B). Post-CHT™ XT LC-MS analysis revealed a clear shift in DAR distribution toward lower-DAR species, with the average DAR reduced from 4.5 to 3.6. Higher-DAR species were selectively reduced, while DAR 2–4 populations were enriched, demonstrating the ability of CHT™ XT to modulate DAR distribution in addition to removing payload-related impurities.

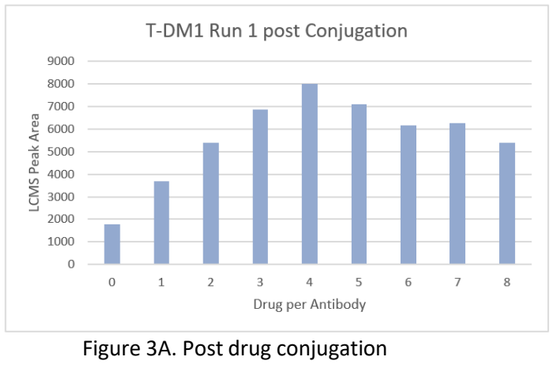


Figure 3A. Post drug conjugation

ADC conjugation

LCMS	Area	% Area	Drug Load	Expected	Difference	Expected
145172.3	1791	3.54	0	145172	0	0
146124.5	3673	7.25	1	146130	-964	958
147090.0	5399	10.66	2	147088	-1914	1916
148048.2	6864	13.55	3	148046	-2872	2874
149006.3	7994	15.78	4	149004	-3830	3832
149968.2	7097	14.01	5	149962	-4784	4790
150911.7	6166	12.17	6	150920	-5757	5748
151877.9	6269	12.38	7	151878	-6706	6706
152837.3	5397	10.66	8	152836	-7663	7664
	50650					

LCMS Avg DAR: 4.5
HIC
OD

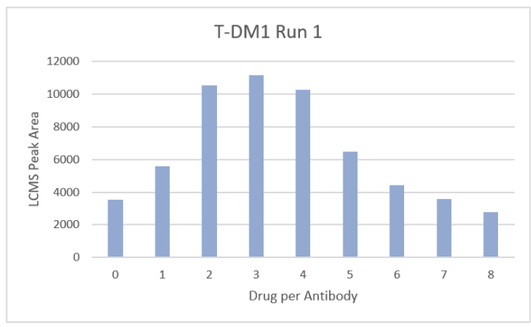


Figure 3B. Polishing step: CHT XT purification

ADC cV

LCMS	Area	% Area	Drug Load	Expected	Difference	Expected
145175.9	3540	6.07	0	145176	0	0
146131.3	5573	9.55	1	146134	-961	958
147089.7	10547	18.07	2	147092	-1918	1916
148048.6	11182	19.16	3	148050	-2875	2874
149006.1	10251	17.57	4	149008	-3834	3832
149964.0	6459	11.07	5	149966	-4792	4790
150922.9	4446	7.62	6	150924	-5749	5748
151846.8	3571	6.12	7	151882	-6741	6706
152846.8	2791	4.78	8	152840	-7657	7664
	58360					

LCMS Avg DAR: 3.6
HIC
OD

SEC Analysis

Size-exclusion chromatography (SEC) was used to assess monomer purity and aggregate content at key stages of the ADC workflow. SEC analysis was performed using an Enrich™ SEC column. (Figure 4A-D) SEC analysis across the workflow confirmed consistent improvements in monomer purity through purification, conjugation, and polishing steps, including after 5 mL scale-up, indicating robust and scalable process performance.

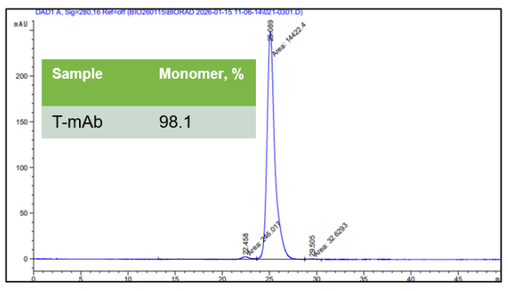


Figure A. T-mAb

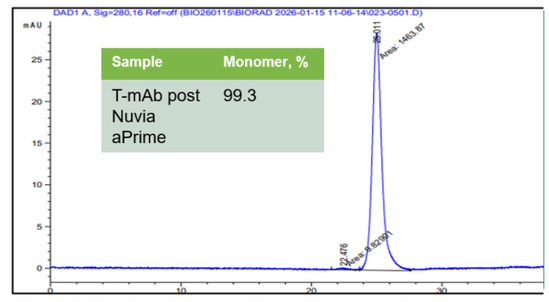


Figure B. T-mAb purification post Nuvia aPrime 4A capture step

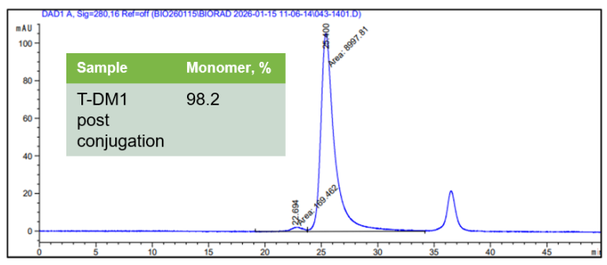


Figure C. T-DM1(ADC) post conjugation

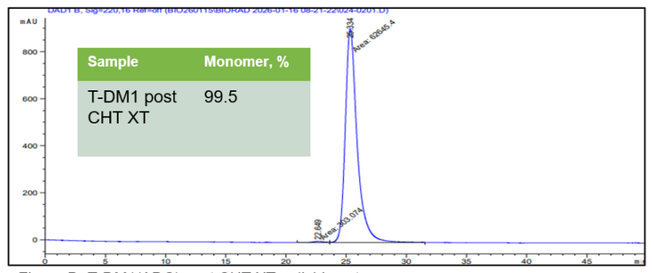


Figure D. T-DM1(ADC) post CHT XT polishing step

.

Conclusions

- Nuvia™ aPrime 4A effectively improves antibody monomer purity prior to conjugation.
- ADC conjugation produces a heterogeneous DAR distribution that requires downstream polishing.
- CHT™ XT provides high recovery (~96%) while effectively removing aggregates and payload-related impurities.
- CHT™ XT polishing shifts DAR distribution toward lower-DAR species, reducing average DAR from 4.5 to 3.6.
- High monomer purity (≥99.5%) is achieved after CHT™ XT polishing.
- The combined aPrime™ + CHT™ XT workflow is scalable and robust for ADC purification and DAR control.

BIO-RAD is a trademark of Bio-Rad Laboratories, Inc. in certain jurisdictions. All trademarks used herein are the property of their respective owner.