Poster Reprint

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Streamlined Drug-to-Antibody Ratio Determination for Intact and Deglycosylated Antibody-Drug-Conjugates Using Automated Sample Preparation and an LC/Q-TOF Designed for Biomolecule Analysis

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Introduction

Antibody-drug-conjugate (ADC) comprises a monoclonal antibody (mAb) conjugated to small molecule drugs via synthetic linkers. The ratio of conjugated drug to mAb (drug-to-antibody ratio or DAR) is a critical quality attribute[1, 2]. Measurement of DAR in circulation by LC/MS requires tedious and error prone sample preparation procedures such as affinity capture of ADCs from complex matrices and deglycosylation. In this study, we automated affinity purification and deglycosylation to address the challenge of this complex sample preparation workflow. We demonstrate a streamlined ADC DAR workflow (Figure 1) using an easy-to-use automation platform, an LC/Q-TOF specifically designed for biomolecules, and DAR calculation software designed to simplify data analysis. This workflow is user friendly, highly reproducible, scalable, and minimizes hands-on time.

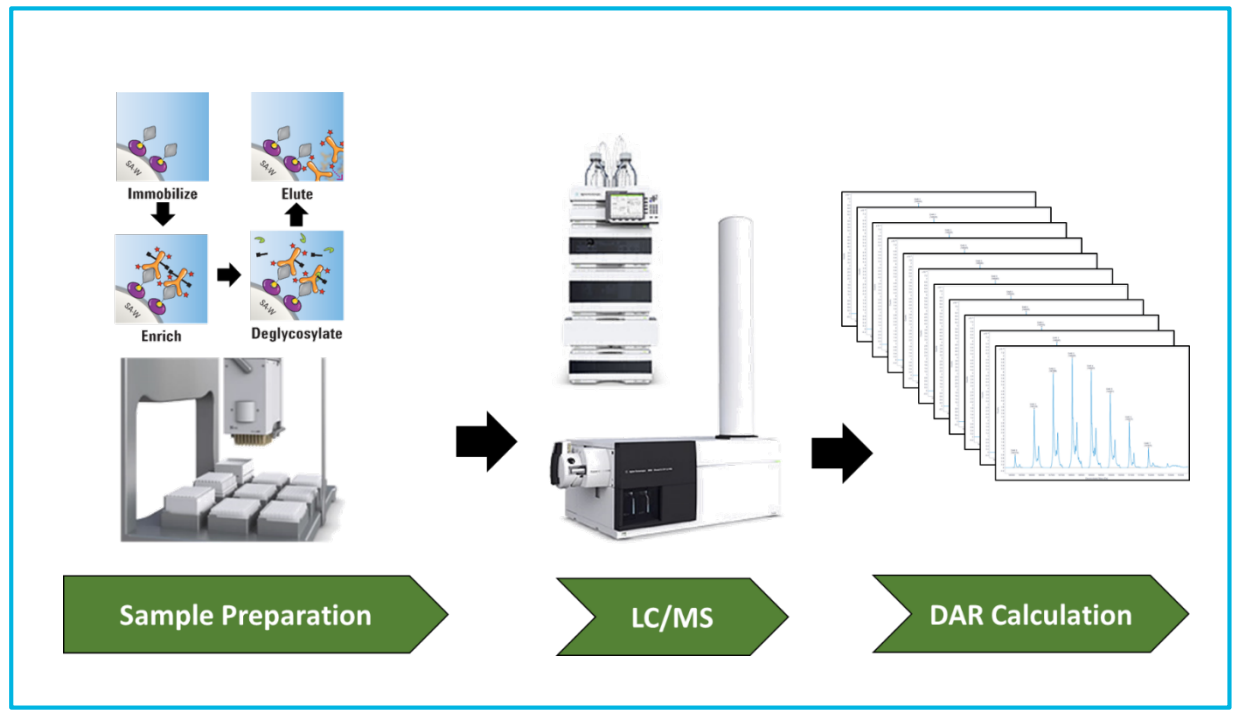

Figure 1. ADC DAR determination workflow.

Experimental

HER2 extracellular domain (ECD) was biotinylated and used as a bait for affinity purification of T-DM1 (Trastuzumab emtansine, an ADC that targets HER2 ECD) in serum. Various amounts of T-DM1 were spiked into clarified rat sera to obtain different concentrations of T-DM1 from 20 to 0.3125 µg/mL plus a no T-DM1 control (Table 1). The AssayMAP platform was used to immobilize biotinylated HER2 ECD on streptavidin (SA-W) cartridges, purify T-DM1 from serum with the HER2 ECD cartridges, and then treat the immobilized T-DM1 with buffer or PNGaseF (Figure 2). The purified samples were analyzed on an Agilent 6545XT AdvanceBio Q-TOF coupled with an Agilent 1290 Infinity II UHPLC system (Table 2). The MS data were analyzed by Agilent MassHunter software with DAR Calculator.

Plate layout for affinity purification and deglycosylation (ADC concentration in rat serum (µg/mL))						
	1	2	3	4	5	6
A	20	20	20	20	20	20
B	10	10	10	10	10	10
C	5	5	5	5	5	5
D	2.5	2.5	2.5	2.5	2.5	2.5
E	1.25	1.25	1.25	1.25	1.25	1.25
F	0.625	0.625	0.625	0.625	0.625	0.625
G	0.3125	0.3125	0.3125	0.3125	0.3125	0.3125
H	0	0	0	0	0	0
	Deglycosylation			No deglycosylation		

Table 1. Experimental design: plate layout for affinity purification and deglycosylation.

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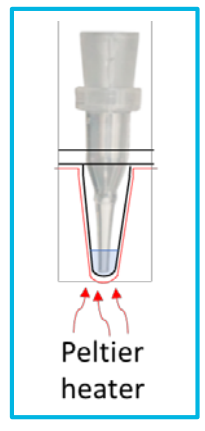

Figure 2. On-cartridge reaction

A

Parameter	Agilent 1290 Infinity II UHPLC System		
Column	Agilent PLRP-S 1000Å 8 µm 150 x 2.1 mm (PL1912-3802)		
Sample thermostat	5°C		
Mobile phase A	0.1% Formic Acid in Water		
Mobile phase B	0.1% Formic Acid in Acetonitrile		
Gradient (Segmented)	0-1	Min	25-25%B
	1-2	Min	25-37%B
	2-4	Min	37-37%B
	4-4.5	Min	37-50%B
	4.5-5.5	Min	50-50%B
	5.5-6	Min	50-25%B
	6-8.5	Min	25-25%B
Stop time	8.5 min		
Column Temperature	60°C		
Flow rate	0.4 mL/min		

B

Parameter	Agilent 6545XT AdvanceBio Q-TOF
Ion mode	Positive ion mode
Source	Agilent Dual Jet Stream
Drying gas temperature	350 °C
Drying gas flow	12 L/min
Sheath gas temperature	400 °C
Sheath gas flow	11 L/min
Nebulizer	60 psi
Capillary voltage	5,500 V
Nozzle	2,000 V
Fragmentor voltage	380 V
Skimmer	140 V
Oct RF Vpp	750 V
Acquisition parameters MS mode	High (30,000 m/z) Mass Range, Extended Mass Range (2GHz), MS only mode, Mass Range 1,000–5,000 m/z.

Table 2. LC and MS parameters. (A) LC parameters used to separate ADCs. (B) MS parameters used to acquire MS data for intact ADCs..

ADC Purification and Deglycosylation

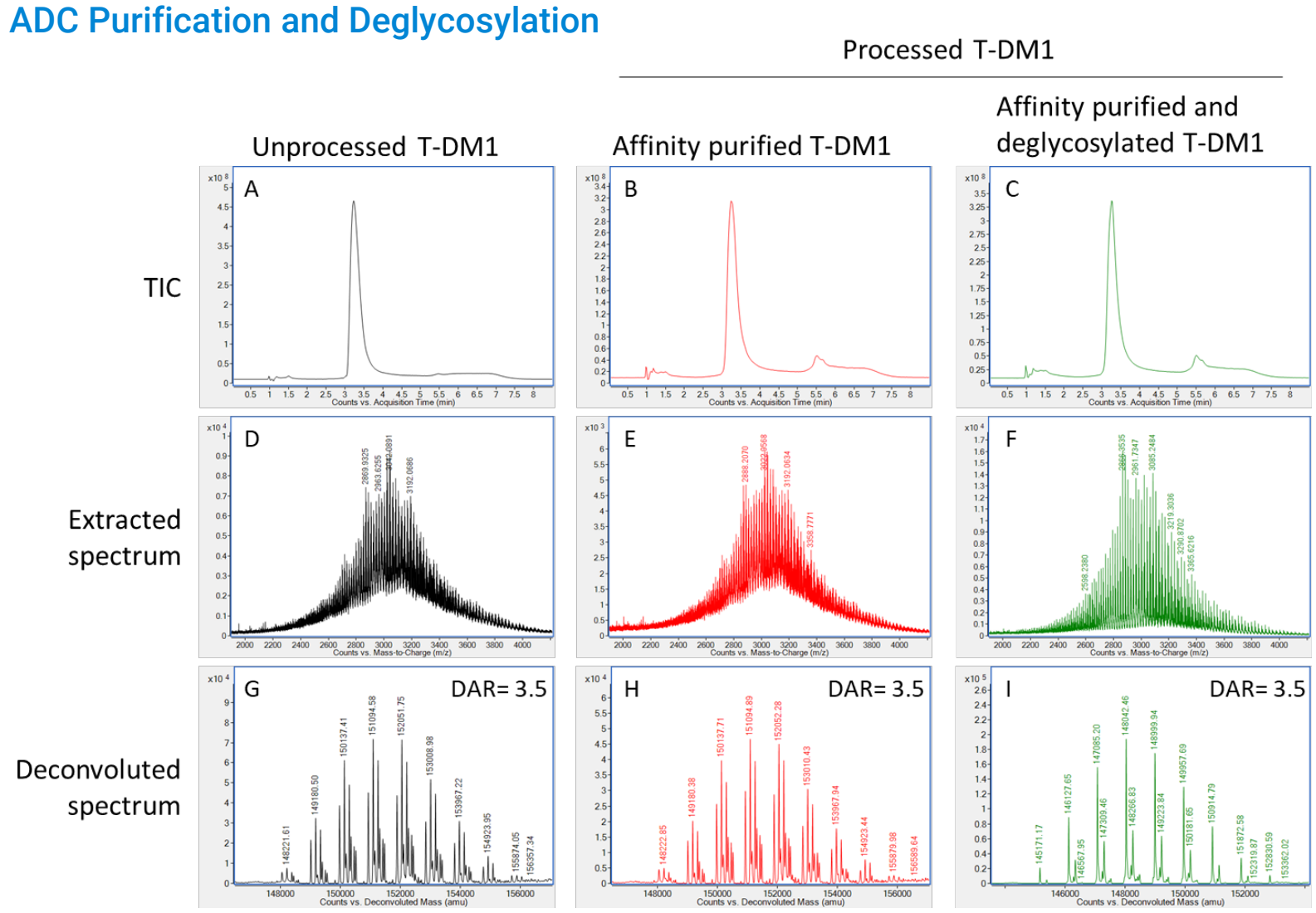

Figure 3. Representative total ion chromatograms (TICs), extracted spectra, and deconvoluted spectra from 200 ng of T-DM1 samples before and after sample preparation. TIC of unprocessed T-DM1 (A), affinity purified intact T-DM1 (B), affinity purified and deglycosylated T-DM1 (C); Extracted spectrum of unprocessed T-DM1 (D), affinity purified intact T-DM1 (E), affinity purified and deglycosylated T-DM1 (F); Deconvoluted spectrum of unprocessed T-DM1 (G), affinity purified intact T-DM1 (H), and affinity purified and deglycosylated T-DM1 (I).

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Workflow Reproducibility

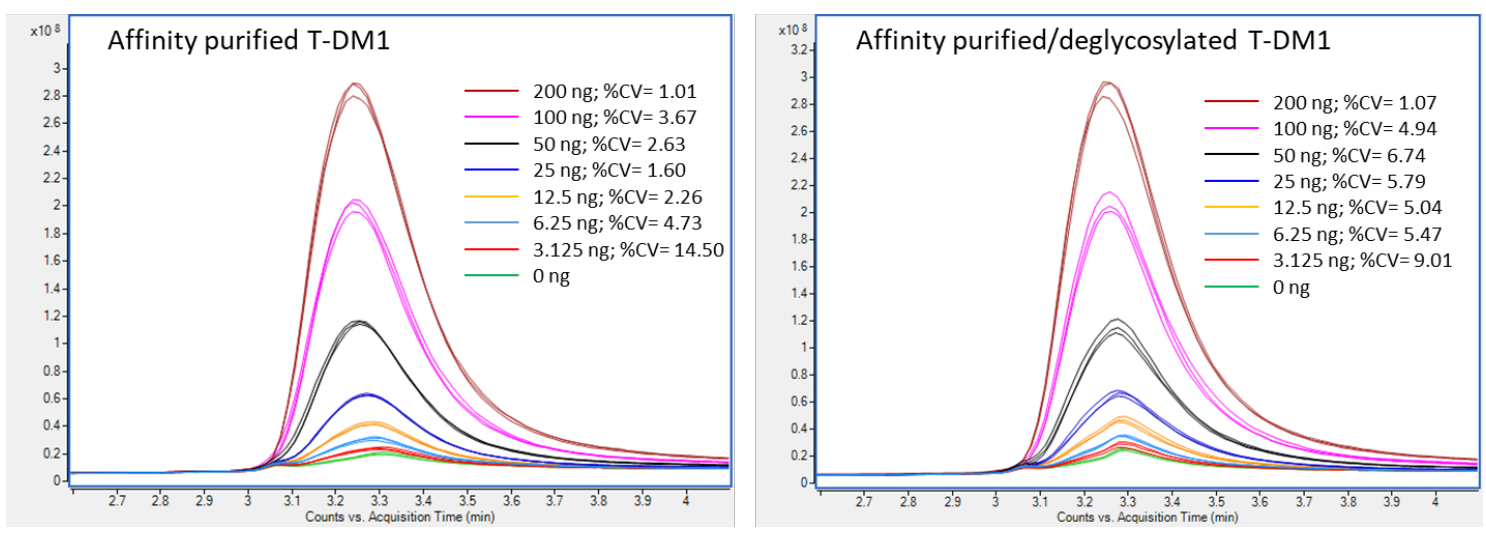
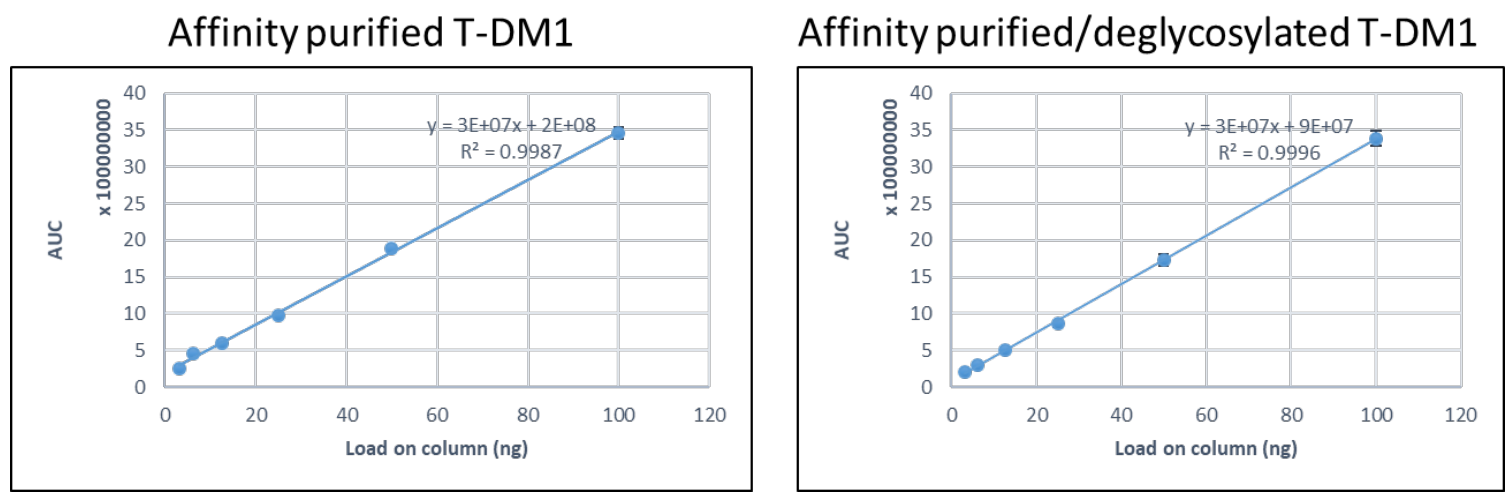


Figure 4. The workflow reproducibility evaluated by the extracted ion chromatograms (EICs) of the affinity purified T-DM1. The ADC T-DM1 was spiked in rat serum at concentrations of 20 to 0.3125 µg/mL and recovered by affinity purification with or without deglycosylation, followed by intact LC/MS analysis. The column loading masses of T-DM1, based on initial sample input, are indicated. The replicate traces are overlaid and shown in the same color. The %CV between replicates was calculated based on the integrated AUCs (n=3).

Linear Dynamic Range Analysis



Results and Discussion

DAR Determination using Agilent DAR Calculator

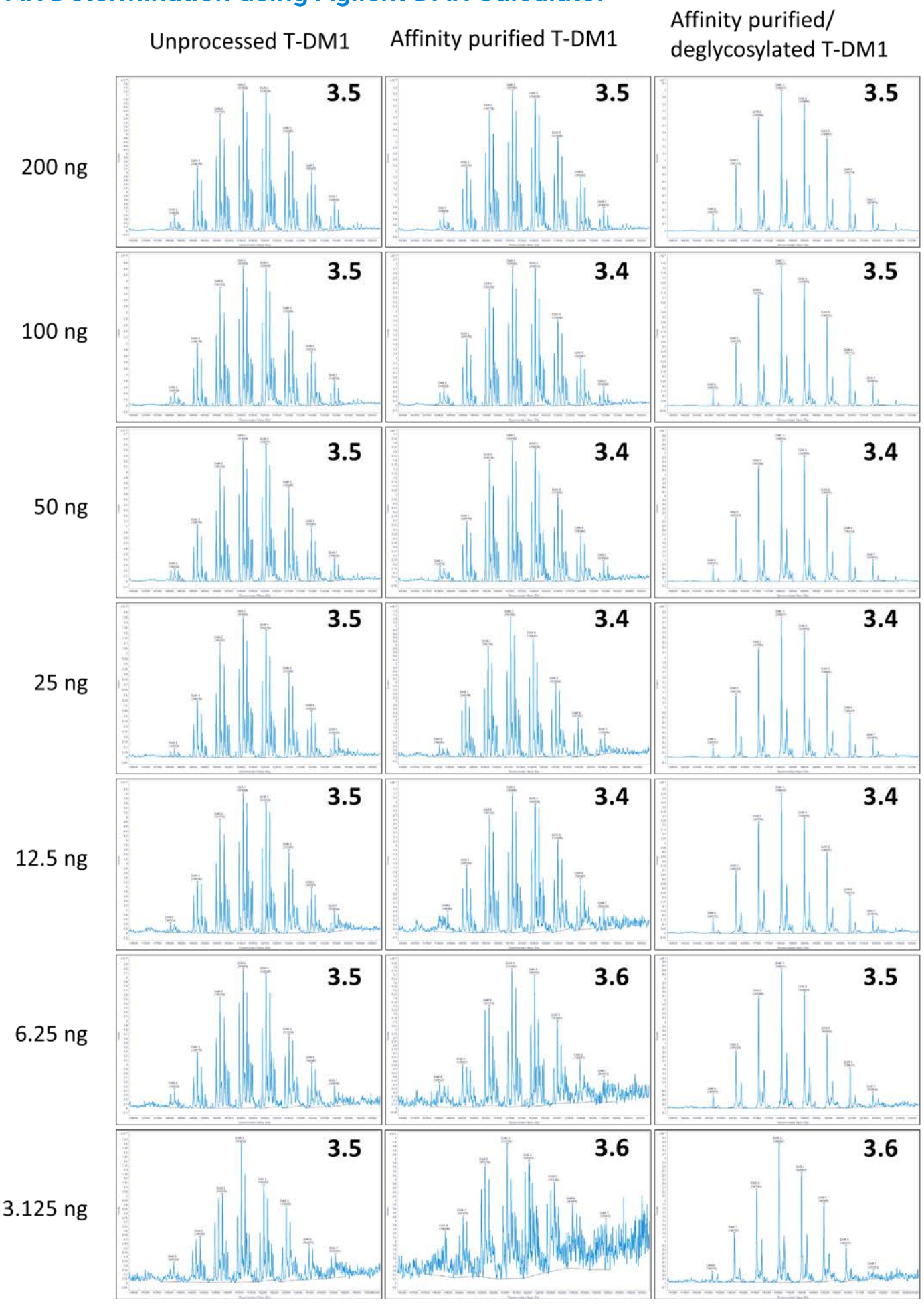


Figure 6. Representative deconvoluted spectra and average DAR values for unprocessed, affinity purified and affinity purified plus deglycosylated T-DM1. T-DM1 was spiked in rat serum at different concentrations over the range from 0.3125 to 20 µg/mL and recovered by affinity purification with or without deglycosylation, followed by intact LC/MS analysis. DAR values were determined from deconvoluted spectra using Agilent DAR Calculator. The on-column loading masses based on initial inputs are shown on left. The average DAR value averaged from replicates is shown in the upper-right corner in each spectrum (n=3).

Conclusions

- A complete solution was provided to address the challenges of *in vivo* ADC DAR analysis.
- A typical workflow to recover ADCs from serum followed by deglycosylation before LC/MS analysis was demonstrated with automated procedures using the AssayMAP Bravo platform.
- The automated antigen immobilization, affinity purification and on-cartridge deglycosylation could be completed in about 4.5 hours with excellent reproducibility, minimal hands-on time, and the ability to scale as needed.
- With the use of the Agilent 6545XT AdvanceBio LC/Q-TOF, the prepared samples can be analyzed seamlessly to yield high quality data for both qualitative and quantitative studies.

References

1. Peters C, Brown S. Antibody-drug conjugates as novel anti-cancer chemotherapeutics. Biosci Rep. 2015 Jun 12;35(4).
2. Beck A, Goetsch L, Dumontet C, Corvaïa N. Strategies and challenges for the next generation of antibody-drug conjugates. Nat Rev Drug Discov. 2017 May;16(5):315-337.

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