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Poster Reprint

ASMS 2020
WP 004

Mass Spectrometric Characterization of Antibody-RNA Conjugates using the Agilent 6545XT AdvanceBio LC/Q-TOF

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Introduction

Antibody-RNA Conjugates have drug-like properties comparable to antibodies and allow delivery of oligonucleotide payloads to non-hepatic tissues. The oligonucleotide payloads enable efficient treatment of previously undruggable targets. However, the use of such conjugates as therapeutic drugs is still under investigation and development. In this study, a LC/MS-based analytical method for identifying the intact antibody-RNA conjugates was developed and demonstrated. This workflow features various AdvanceBio columns for sample separation, and the 6545XT AdvanceBio LC/Q-TOF system with large molecule SWARM autotune feature and extended mass range of up to 30,000 *m/z* for sample analysis.

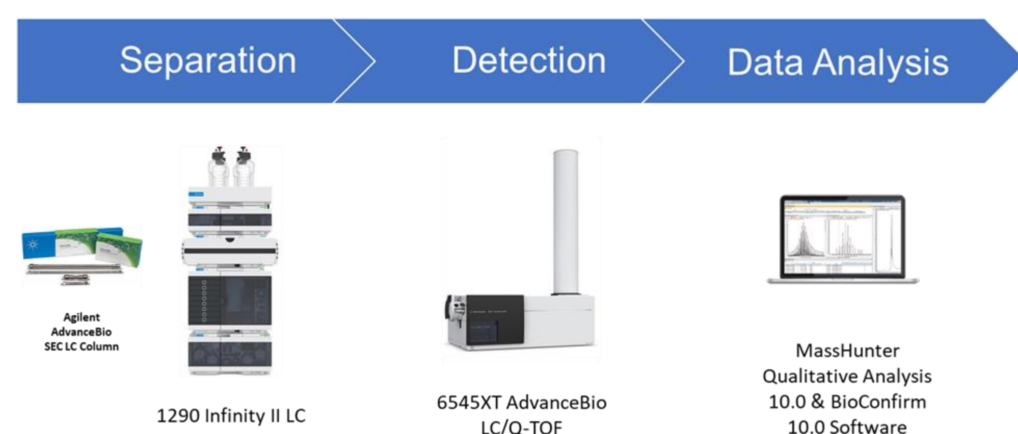

Figure 1. Analytical components of the native protein analysis workflow.

Experimental

The antibody was partially reduced with reducing agent and was then reacted with the activated RNA molecule (with linker). The unreacted free thiol groups (–SH) of mAb were capped with chemical reagent. The reaction mixture was further purified by ion exchange column. Unreacted antibody, DAR=1, DAR=2, and unreacted RNA were separated. The purified DAR=1 sample was then used for mass spectrometry analysis under denaturing and native conditions. Prior to the native MS analysis, sample desalting and buffer exchange with 100 mM ammonium acetate buffer (pH 7) were performed using the Bio-Rad Bio-Spin P-30 cartridge. Proteins were denatured under the traditional LC/MS analysis condition where organic and acid solvents were used.

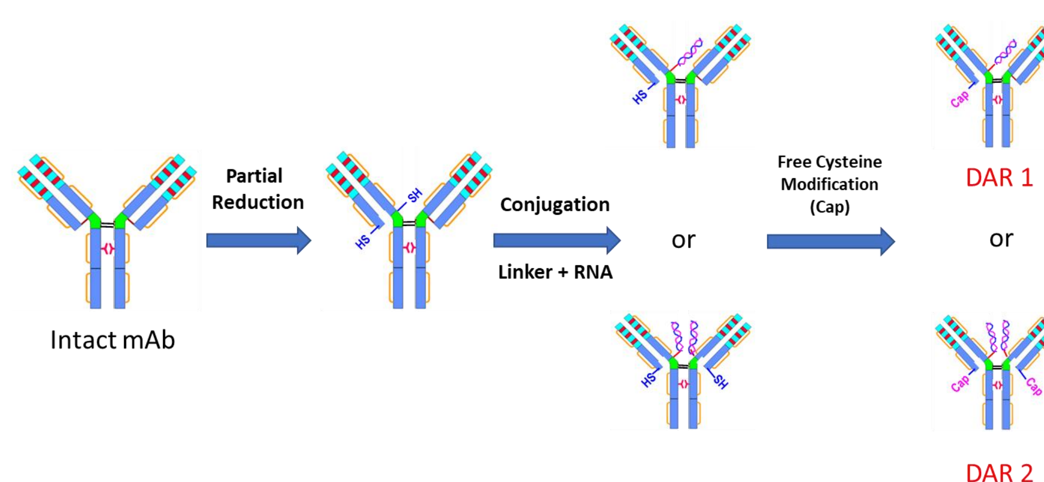

Figure 2. General Scheme of Antibody-RNA (mAb-RNA) Conjugate Synthesis.

Results and Discussion

LC/MS Analysis (Denaturing Condition) of Intact mAb (left) and mAb-RNA Conjugate (DAR1) (right):

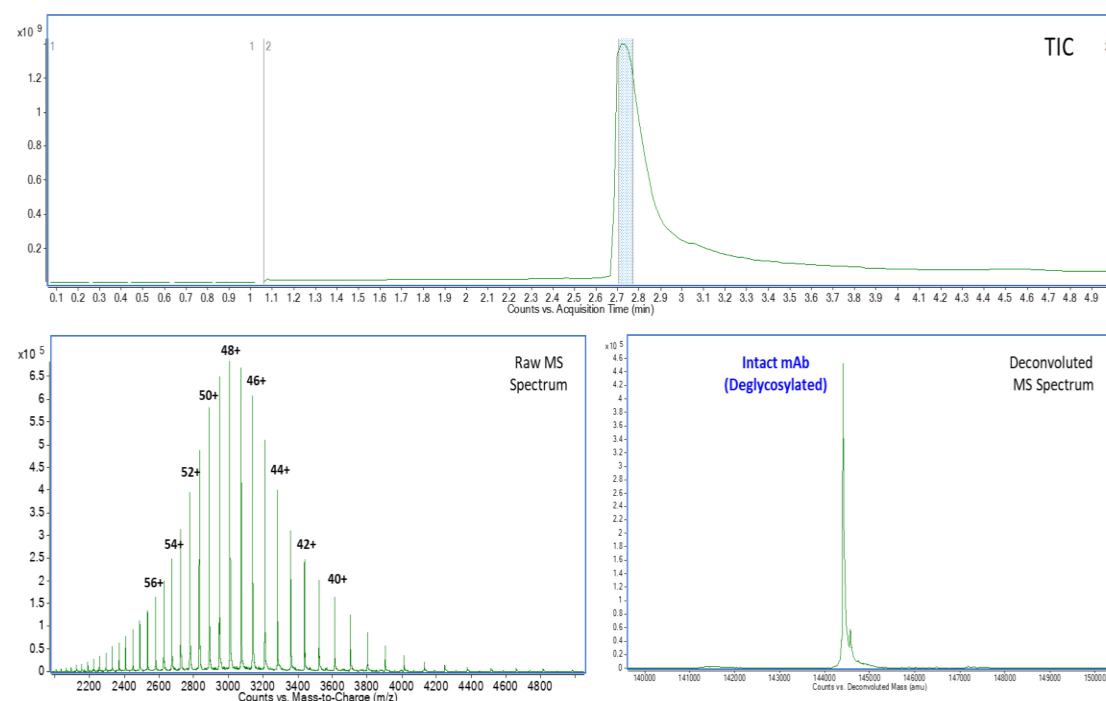

Figure 3. LC/MS analysis of intact deglycosylated mAb under denaturing condition (PLRP-S column was used). The charge state distribution of denatured mAb spanned in the mass range of *m/z* 2,000 to 5,000 (30+ to 75+).

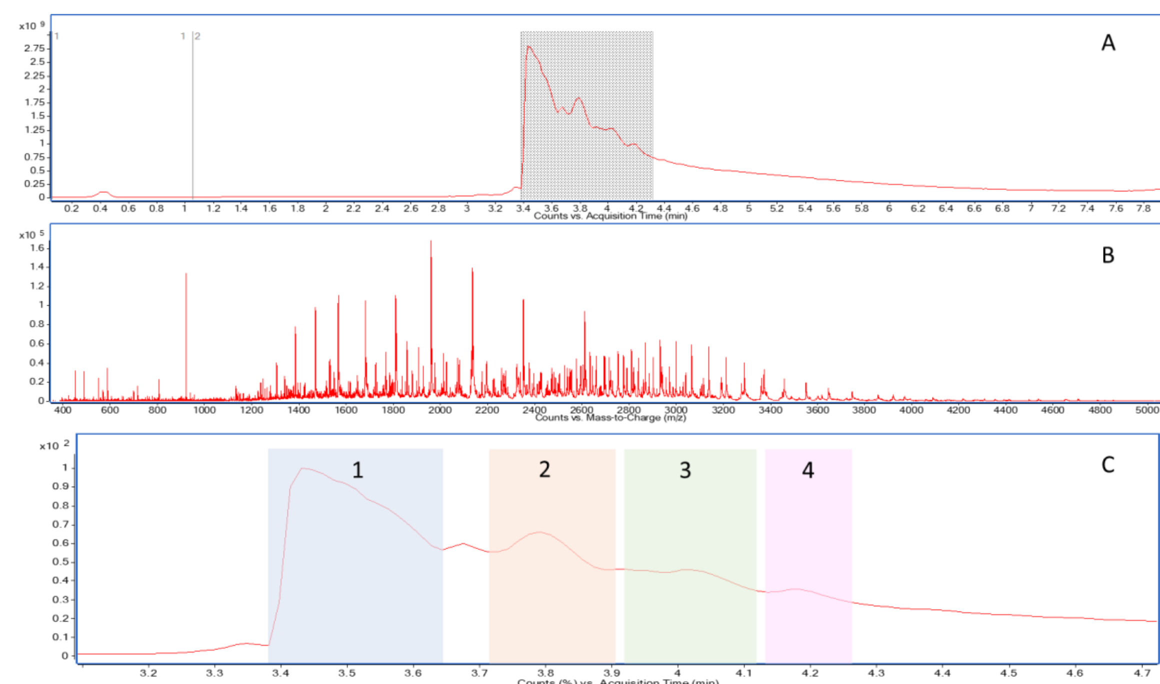

Figure 4. A) MS TIC of intact mAb-RNA conjugate (DAR1). B) Extracted Ion Chromatogram (EIC) of the chromatographic separated peaks over retention time of 3.4 – 4.3 min. C) Zoom-in chromatogram of the highlighted peaks (gray area in A). The MS data from each HPLC peaks (1-4) were deconvoluted and analyzed.

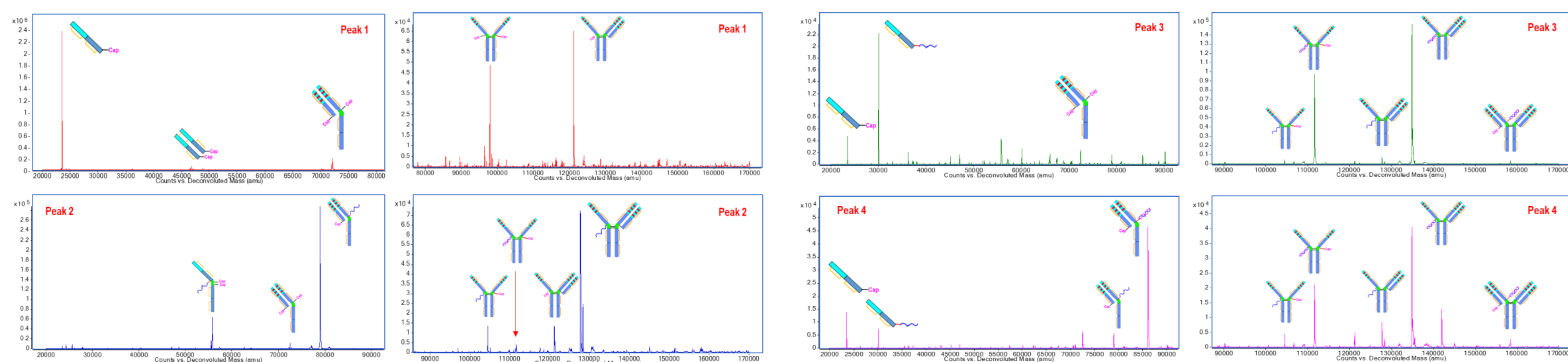

Figure 5. MS deconvoluted spectrum of HPLC peaks (1-4) of deglycosylated mAb-RNA sample (DAR1). LC/MS analysis was under denaturing MS conditions. Many dissociated molecules from mAb-RNA conjugate were observed in all 4 LC peaks mainly due to the weak electrostatic interaction of non-covalent mAb-RNA complexes. They were: mAb light chain (with Cap or RNA), mAb heavy chain (with Cap or RNA), half of conjugate, conjugates without 1 or 2 LCs, etc.

Native LC/MS Analysis of Intact mAb (top) and mAb-RNA conjugate (DAR1)(bottom):

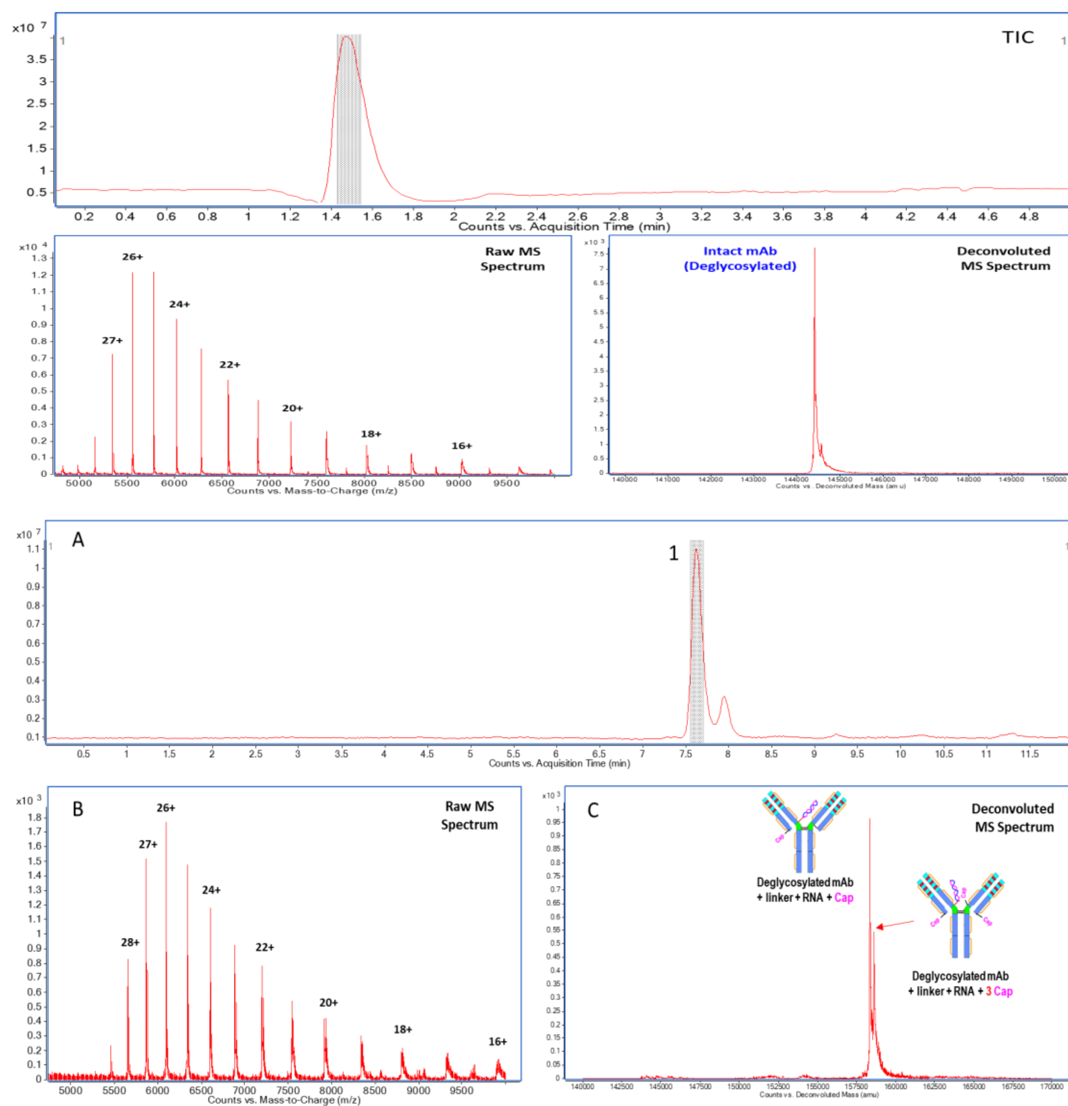

Figure 6. LC/MS analysis of intact deglycosylated mAb under native condition (SEC column was used). The native mAb had a charge envelope in the mass range of m/z 5,000 to 10,000 (15+ to 30+).

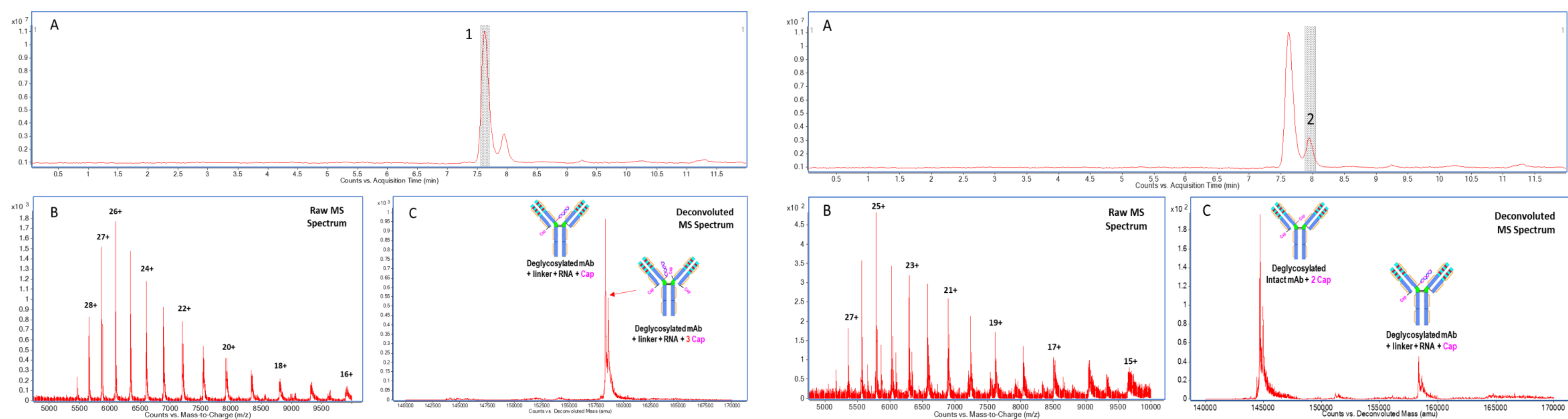

Figure 7. Native SEC LC/MS analysis of Antibody-RNA Conjugate (DAR1): A) HPLC chromatogram of SEC column separated conjugates. B) raw MS spectrum of intact mAb-RNA conjugates (peak 1 & 2). C) The deconvoluted MS spectra of intact mAb-RNA conjugates indicating two forms of conjugates were detected in peak 1: DAR1 with 1 or 3 cysteines modified by Cap, and unconjugated mAb with 2 Caps as well as DAR1 with 1 Cap in peak 2.

Conclusions

- Development of a novel method for characterization of mAb-RNA conjugates under native MS condition that overcomes the conjugate dissociation/stability issues caused by denaturing LC/MS condition.
- Native MS analysis of mAb-RNA conjugates can provide accurate mass information for conjugate structural assignment, and chromatographic separation enables relative quantitation on various types of mAb-RNA conjugates.
- Optimized workflow with the AdvanceBio SEC column, the 6545XT AdvanceBio LC/Q TOF, and MassHunter BioConfirm software.

References

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Published in USA, June 1, 2020

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