Comprehensive LC-MS Analytical Assays for Antibody-Oligonucleotide Conjugate (AOC) and siRNA Linker-Payload Characterization

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INTRODUCTION

Antibody-oligo conjugates (AOCs) are a similar class of biotherapeutics which build upon the fundamentals of antibody-drug conjugates (ADCs). The purpose of both AOCs and ADCs is the targeted delivery of a payload (oligonucleotide moiety or cytotoxic drug, respectively) using a full-length monoclonal antibody (mAb) or fragment thereof. AOCs will likely provide opportunities for targets thought to be inaccessible by other therapies.[1-2] mAbs themselves are large and complex, requiring extensive characterization and monitoring. Conjugated mAbs require the same characterization as mAbs, plus analysis of oligo-to-antibody ratio (OAR), characterization of linker-payload building blocks, as well as in-process monitoring. This study builds upon previous analysis with light scattering techniques, to incorporate high resolution LC-MS using the BioAccord™ System into the toolbox of approaches for AOC characterization.

METHODS

5 Different BioAccord System LC-MS Methods Utilized in AOC Analysis

1. RPLC-MS (ACQUITY™ Premier BEH™ C4 Column, 1.7 µm, 2.1 x 50mm) for free mAb
2. Native SEC-MS[3] (ACQUITY Protein BEH SEC 200 Å Column, 1.7 µm, 2.1 x 150 mm, with IonHance™ Ammonium Acetate Concentrate, pH 6.8) for AOCs
3. Native SCX-MS[5] (BioResolve™ SCX mAb Column, 3 µm, 2.1 x 100 mm, with IonHance CX-MS Concentrates A & B) for AOCs
4. Denaturing & Non-denaturing HILIC-MS[5-7] (ACQUITY Glycoprotein BEH Amide Column, 300Å, 1.7 µm, 2.1 x 50 mm) for siRNA confirmation
5. Denaturing IPRP-MS[3,8] (ACQUITY Premier Oligonucleotide BEH C18 Column, 130Å, 1.7 µm, 2.1 x 50 mm) for siRNA confirmation

LC-MS Instrument: BioAccord System (now available with Extended Mass Range (m/z 400-9000)[9]

Data Acquisition in UNIFI™ App in the waters_connect™ Informatics Platform

Data processed with either UNIFI App or INTACT Mass App v 1.9 with automatic OAR calculation[10] in the waters_connect Informatics Platform

Antibody-Oligonucleotide Conjugate Structure (siRNA Conjugate)

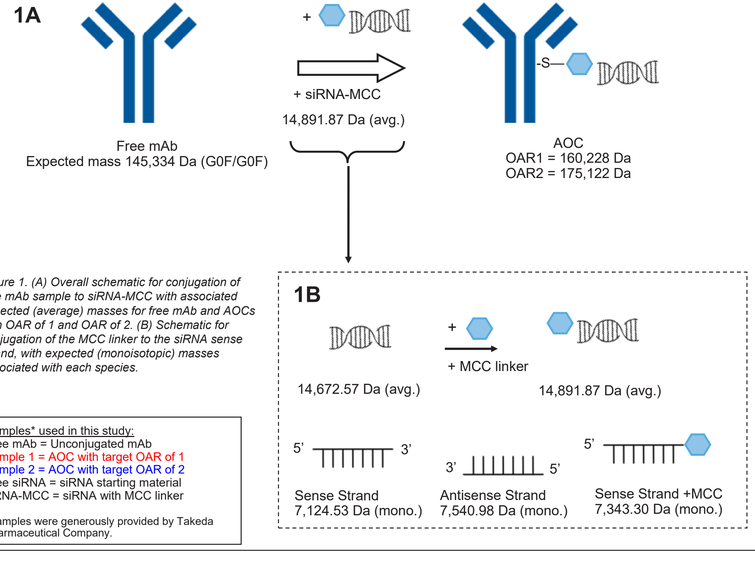

Figure 1. (A) Overall schematic for conjugation of free mAb sample to siRNA-MCC with associated expected (average) masses for free mAb and AOCs with OAR of 1 and OAR of 2. (B) Schematic for conjugation of the MCC linker to the siRNA sense strand, with expected (monoisotopic) masses associated with each species.

Samples* used in this study:
Free mAb = Unconjugated mAb
Sample 1 = AOC with target OAR of 1
Sample 2 = AOC with target OAR of 2
Free siRNA = siRNA starting material
siRNA-MCC = siRNA with MCC linker

*Samples were generously provided by Takeda Pharmaceutical Company.

RESULTS & DISCUSSION

Reagent Characterization

Free mAb Characterization by RP-MS (Denaturing) and SEC-MS (Non-denaturing)

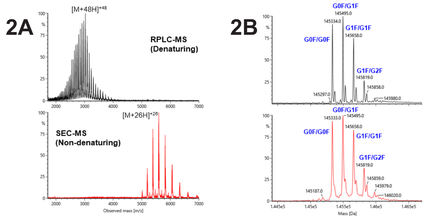

Figure 2. (A) Combined MS spectra for Free mAb sample analyzed via RPLC-MS (top) and SEC-MS (bottom). (B) De-convoluted mass spectra for RPLC-MS analysis (top) and SEC-MS (bottom), showing the either method may be used to assess the free mAb starting material prior to AOC conjugation.

siRNA Characterization by IPRP-MS (Denaturing)

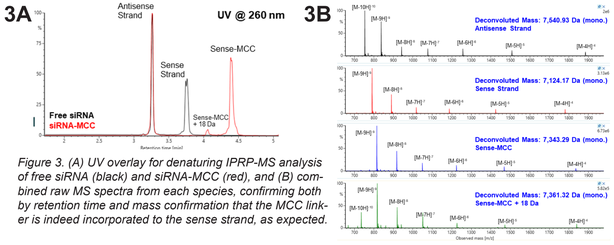

Figure 3. (A) UV overlay for denaturing IPRP-MS analysis of free siRNA (black) and siRNA-MCC (red), and (B) combined raw MS spectra from each species, confirming both by retention time and mass confirmation that the MCC linker is indeed incorporated to the sense strand, as expected.

siRNA Characterization by HILIC-MS (Non-denaturing & Denaturing)

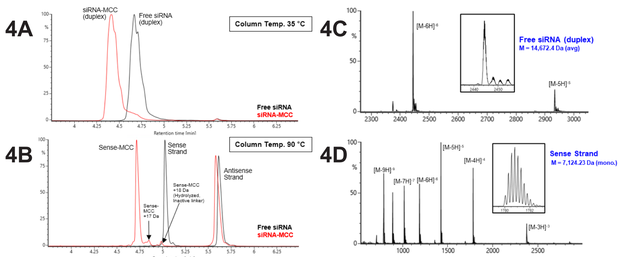

Figure 4. (A) UV overlay for non-denaturing HILIC-MS (column temp. 35 °C) for free siRNA (black) and siRNA-MCC (red). (B) UV overlay for denaturing HILIC-MS (column temp. 90 °C) for free siRNA (black) and siRNA-MCC (red). Example combined raw spectra for (C) duplex siRNA from non-denaturing HILIC-MS, (D) sense strand & (E) anti-sense strand from denaturing HILIC-MS.

- **LC-MS provides high quality chromatographic separation and MS confirmation (within 15 ppm of expected masses)**
- **Free mAb: mass confirmation of starting material by 2 orthogonal methods**
- **siRNA: MCC linker confirmed to be incorporated on the sense strand, as expected, both by RT shifts and mass confirmation by 2 orthogonal methods**

OAR and Distribution: Orthogonal Methods

OAR by SEC-MS (Non-denaturing)

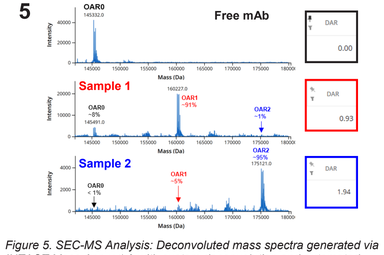

Figure 5. SEC-MS Analysis: Deconvoluted mass spectra generated via INTACT Mass App v 1.9 with custom deconvolution and automated OAR calculation, with OAR & relative % MS response for each species. SEC-MS is very useful as a HT method for OAR analysis.

SCX-MS (Non-denaturing)

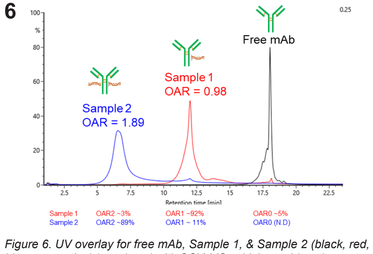

Figure 6. UV overlay for free mAb, Sample 1, & Sample 2 (black, red, & blue, respectively) analyzed with SCX-MS, which provides chromatographic resolution of OAR species and corresponding mass confirmation of species. OAR can be calculated via relative UV area or XIC area.

SEC-MALS (Non-denaturing)[11]

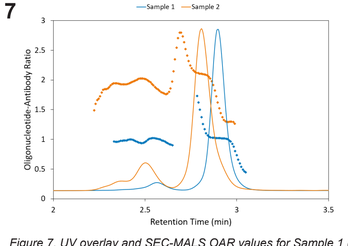

Figure 7. UV overlay and SEC-MALS OAR values for Sample 1 (blue) and Sample 2 (orange), as previously described.[11] OAR values are in good agreement with both SEC-MS and SCX-MS. SEC-MALS is another orthogonal technique for OAR analysis and is critical for high molecular weight (HMW) species investigation.

OAR and drug distribution via 2 orthogonal LC-MS methods, with comparable results to previous SEC-MALS data, for a total of 3 possible approaches to OAR assessment

Partial Site Localization (Subunit mAb SCX-MS)

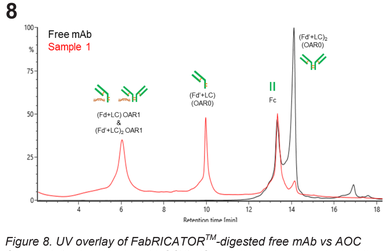

Figure 8. UV overlay of FabRICATOR™-digested free mAb vs AOC Sample 1 (red) analyzed with SCX-MS. Partial conjugation site localization is possible with this approach, by both RT shifts and corresponding mass confirmation of species. Fc species remains constant after conjugation, while new conjugation species are confirmed on the Fd'+LC species for Sample 1.

CONCLUSION

- **Successful characterization of AOC samples and starting materials utilizing 5 distinct orthogonal LC-MS methods, delivering both flexibility and high quality LC-MS data for these innovative new biomolecules.**
- **All five LC-MS methods, each tailored to address the diverse analytical needs of the AOC and siRNA samples, were developed on the BioAccord System, equipped with compliance-ready software tools for analysis and reporting, including INTACT Mass App v 1.9 for automatic OAR calculation.**
- **BioAccord System workflows are easily implemented from early research stages, through development and manufacturing.**

References

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