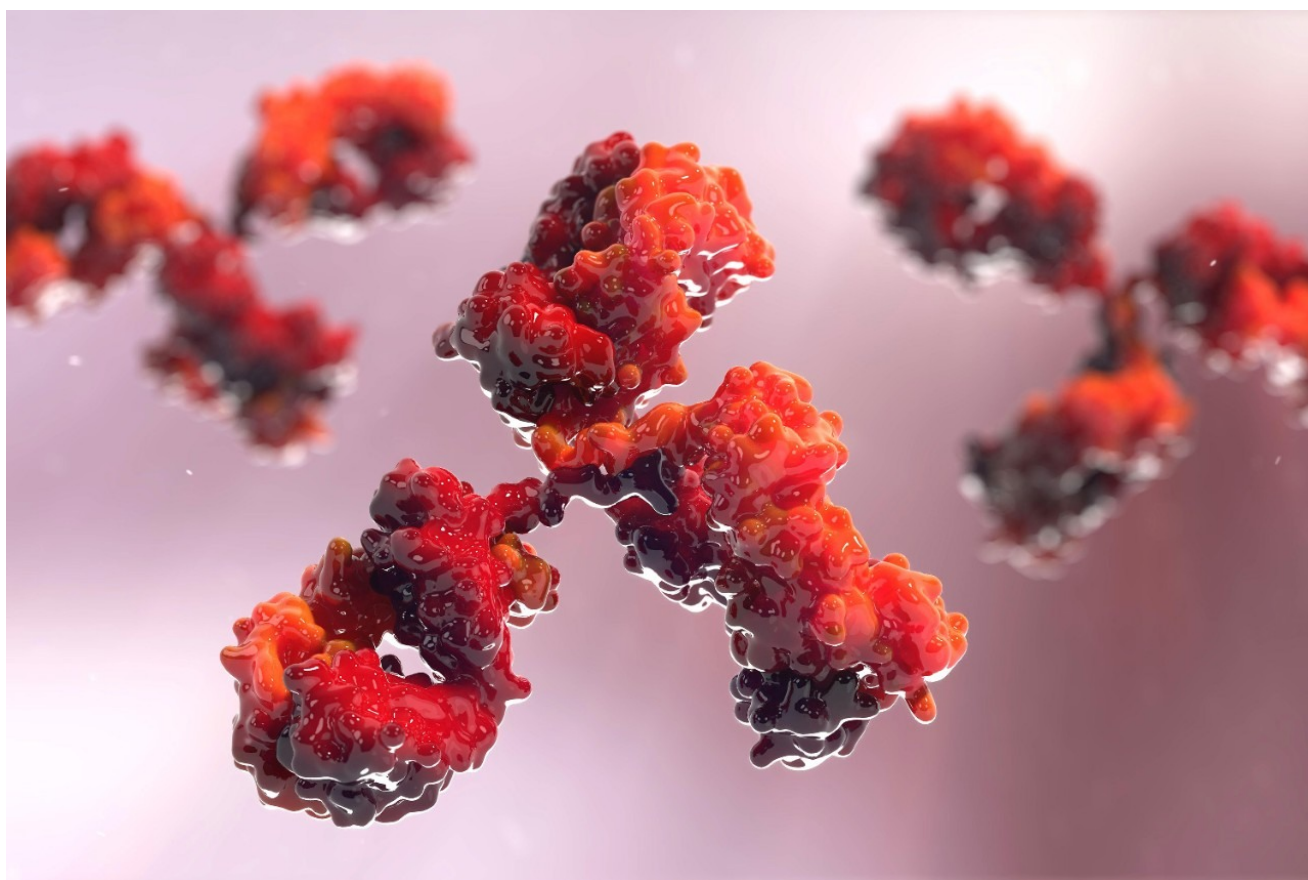


## Analysis of Antibody siRNA Conjugate Using BioAccord System

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Biopharmaceutical services at Waters Immerse Innovation and Research Center ([www.waters.com/immerse](http://www.waters.com/immerse) <<http://www.waters.com/immerse>>.) aim to perform method development for the analysis of emerging modalities.

This is an Application Brief and does not contain a detailed Experimental section.

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## Abstract

Short interfering RNA (siRNA) has emerged as a class of therapeutics with great promise. Due to the chemical nature of the molecule, however, a delivering vehicle is needed to carry the drug payload to targeted cells. A common approach is chemically linking the siRNA onto monoclonal antibody (mAb) molecules since the derivatized Antibody siRNA Conjugates (ARC) can effectively attach to the target cell for internalization.

For the ARC of interest in this study, the siRNA payload with a sense and an antisense strand is conjugated with the thiol group of the engineered cysteine residue of the FC glycosylation site on the carrier mAb. Using the BioAccord LC-MS System,<sup>1-3</sup> two methods for ARC molecule characterization were developed for this service project: 1) mass confirmation of siRNA sense and antisense strand using an Ion Pairing RPLC-MS method; 2) DAR calculation on the final ARC molecule using native SEC-MS. Both methods offer information on the product identity and potential impurities.

## Benefits

- The BioAccord System
  - Offers a wide range of application solutions (intact protein, peptide mapping, MAM, glycans, and oligonucleotides analysis)
  - Ease of use with robust system-to-system performance
  - Small footprint
  - Operates under a compliance ready informatics system, waters\_connect
- Two methods developed for the analysis of Antibody siRNA Conjugate (ARC)
  - An IPRP LC-MS method for mass confirmation of synthetic siRNA sense and antisense strand
  - A native SEC-MS method for DAR (Drug to Antibody Ratio) determination

## Introduction

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Short interfering RNA (siRNA) has emerged as a class of therapeutics with great promise. Due to the chemical

nature of the molecule, however, a delivering vehicle is needed to carry the drug payload to targeted cells. A common approach is chemically linking the siRNA onto monoclonal antibody (mAb) molecules since the derivatized Antibody siRNA Conjugates (ARC) can effectively attach to the target cell for internalization. There are many synthesis routes for ARC production, typically involving adding a linker at the siRNA 3' end for conjugate chemistry to occur with a functioning group on mAb molecule.

The ARC of interest in this study has the following conjugation chemistry: The Fc glycosylation site on the carrier mAb was engineered to a cysteine residue for conjugation reaction. The siRNA payload has sense and antisense strand, the sense strand has a fluorescent dye linked at the 5' end, while the 3' end has an amine group added for conjugation reaction with the thiol group of incorporated cysteine residue in the Fc domain of the carrier mAb via a linker (Figure 1). The aim of this service project is to develop two methods for ARC molecule characterization: 1) mass confirmation of siRNA sense and antisense strand using an Ion Pairing RPLC-MS method; 2) DAR calculation on the final ARC molecule using native SEC-MS. Both methods, which were developed on the BioAccord System (Figure 2), offer information on the product identity and potential impurities.

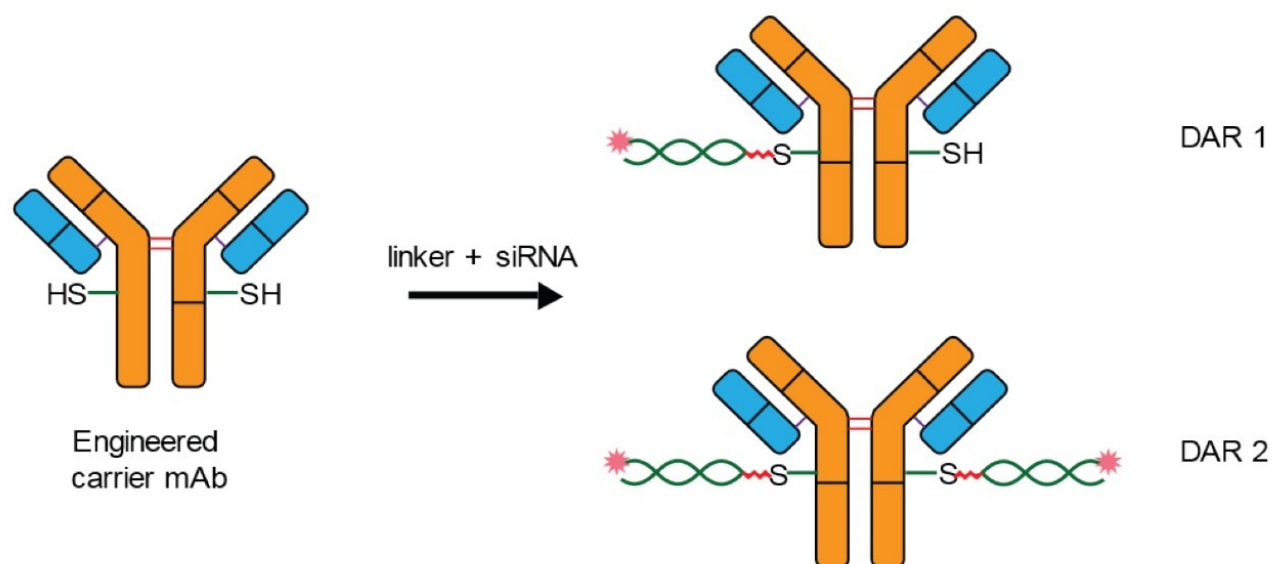


Figure 1. ARC (Antibody siRNA Conjugate).



Figure 2. BioAccord LC-MS System.

## Results and Discussion

In this study we reported two LC-MS methods that are optimized for mass confirmation of either the siRNA or the ARC. These methods are developed on BioAccord System, which is comprised of an ACQUITY UPLC I-Class PLUS System, a tunable UV detector, and a small footprint Time-of-Flight (ToF) mass spectrometry ACQUITY RDa Detector. This system is controlled and operated under the compliant ready waters\_connect informatics System (Figure 2). The BioAccord System is the “workhorse” for a variety of high-resolution mass spectrometry-based assays for biopharmaceuticals. These range from intact and subunit mass analysis, charge and size-based MS analysis, peptide mapping, and multi-attribute monitoring (MAM) to oligonucleotides and released glycan assays.

### Mass Confirmation of the Target siRNA (sense and antisense strand)

Reversed Phase Ion Pairing (IPRP LC-MS) performed in the negative ion mode, was used to confirm the accurate mass of the siRNA for both the sense strand and antisense strand (see Figure 3 for the charge deconvolute mass processed via MaxEnt 1 processing in waters\_connect). The experimental mass was in good agreement with the theoretical mass.

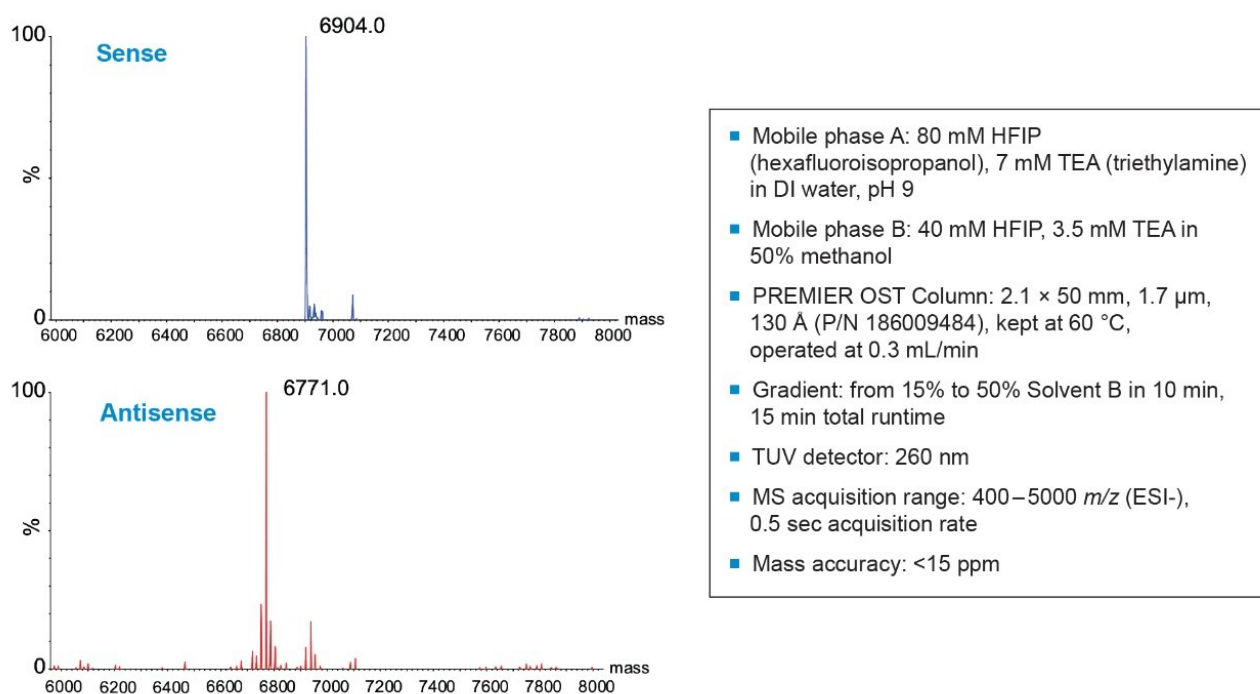


Figure 3. Intact mass of sense and antisense strand. MaxEnt 1 charge deconvolute was used to obtain the mass.

## ARC Characterization

The goal for ARC mass analysis focuses on identifying DAR1 and DAR 2 species. Traditional chromatography methods with optical detector (UV or FLR) alone is not sufficient to confirm their identity. In addition, LC-MS based assays are ideal for DAR measurement. RPLC-MS of the carrier mAb was performed initially to check the mAb molecule integrity (upon the replacement of an asparagine (N) residue with a cysteine (C) residue in Fc region). The major peak from the charge deconvoluted spectrum in Figure 4A shows the mass matched with the calculated mass of engineered mAb. The native SEC-MS experiment was performed to see if DAR 1 and DAR 2 species are present in the sample. The non-denaturing mobile phase helps the double stranded siRNA remain intact during the ESI+ MS process. From the MaxEn1 deconvoluted spectrum, we observed both DAR 1 and DAR 2 species. Additionally, we observed DAR1 and DAR2 without the antisense strand (Figure 4b).

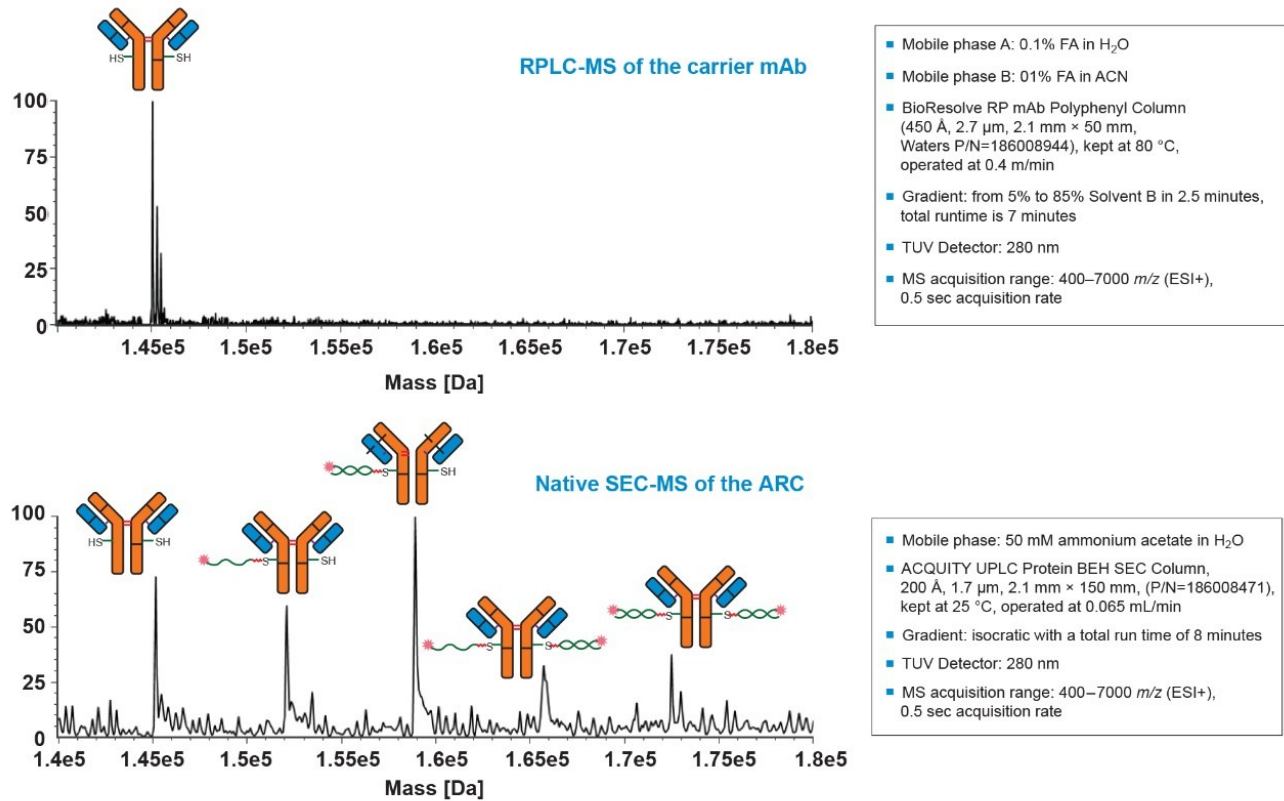


Figure 4. Top: Charge deconvoluted intact mass for the carrier protein is displayed. Data was obtained from RPLC-MS. The mass accuracy for the observed carrier protein is 10 ppm. Bottom: Charge deconvoluted DAR species from Native SEC-MS analysis is displayed. Peaks representing DAR1 and DAR2 were observed. In addition, single stranded (sense) DAR species were also detected as major byproducts.

## Conclusion

Two LC-MS methods were developed to confirm 1) the identity of the siRNA (sense and antisense strand) and 2) the distribution of antibody-siRNA molecules in a sample via native SEC-MS. An analytical scale ACQUITY UPLC I-Class PLUS with a bench top ToF mass detector (ACQUITY RDa) was used to develop and optimize both method and workflows. Both methods are simple to execute without much optimization on the BioAccord System. The compliant ready software used for method development adds additional benefits, such as improved efficiency and potential support of method transfer from development to QC lab.

## References

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## Acknowledgements

Henry Shion, Catalin E. Doneanu, Ying Qing Yu, Weibin Chen - Waters Corporation  
Ed Ha - Angiex, Inc.

## Featured Products

- [ACQUITY UPLC I-Class PLUS System <https://www.waters.com/134613317>](#)
- [ACQUITY UPLC Tunable UV Detector <https://www.waters.com/514228>](#)
- [BioAccord LC-MS System for Biopharmaceuticals <https://www.waters.com/waters/nav.htm?cid=135005818>](#)
- [waters\\_connect <https://www.waters.com/waters/nav.htm?cid=135040165>](#)
- [Biopharmaceutical Platform Solution with UNIFI <https://www.waters.com/waters/nav.htm?cid=10195515>](#)

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