Wolfe Laboratories — A Subsidiary Of — Pace Analytical® Life Sciences

High Resolution Mass Spectrometric Analysis of Complex Biotherapeutics

Introduction

Emerging classes of biotherapeutics, including ADCs and oligonucleotides, offer target specificity and reduced off-target effects; however, molecule complexity leads to many challenging development hurdles. These emerging classes of therapeutics are diverse in size and chemical composition resulting in new and unique regulatory challenges. The current limited guidelines for these new and complex therapeutics are evolving, as impurities, degradant identities and their associated clinical consequences become more well understood.

Regulatory Requirements

- **ADCs**: no regulatory guidelines specifically for development
  - FDA follows existing guidelines for small drugs and monoclonal antibodies
- **Oligonucleotides**: no regulatory guidelines specifically for development
  - Despite large size, synthetic oligonucleotide are treated as small molecules

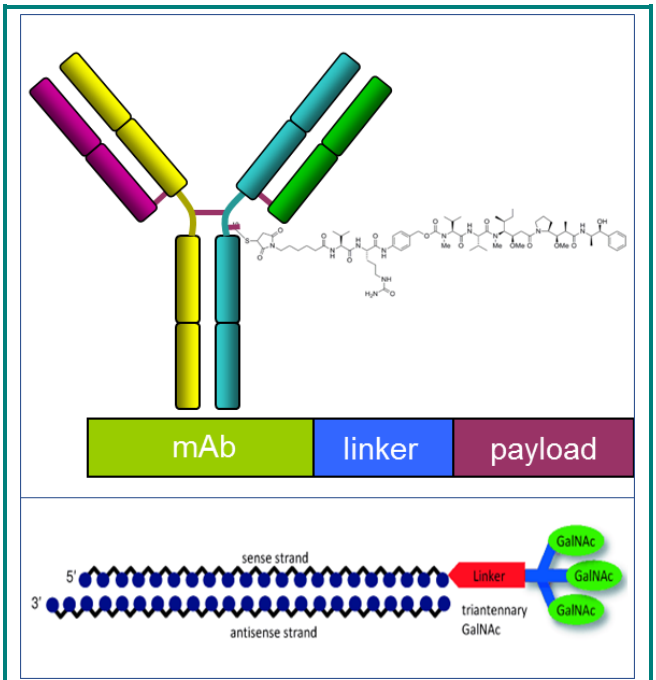

Figure 1: Schematic displaying components of an ADC and the of a GalNAc-conjugated siRNA (adapted from Rajeev KG et al. Chembiochem. 2015;16(6):903-8.

To de-risk the biotherapeutic development pipeline and increase successes, bioanalysis by high resolution mass spectrometry (HRMS) has become key due to large mass range, selectivity and unparalleled resolution. At Wolfe Laboratories, bioanalysis is performed using a state-of-the-art Waters Xevo G2-XS QToF platform coupled to a Waters Acquity H-Class Bio UPLC, which offers the resolution and sensitivity needed to analyze complex biologics and the flexibility to assess a diverse array of biotherapeutics classes.

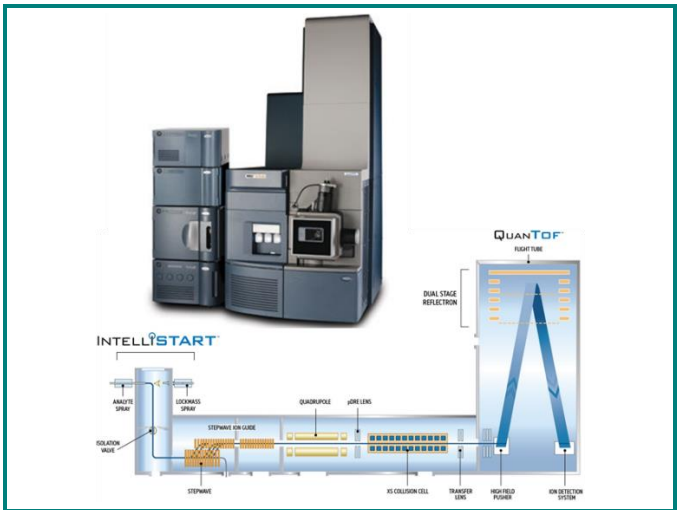

Figure 2: Waters Xevo G2-XS QTof instrument and internal components schematic.

Structural and Chemical Diversity

Biotherapeutics contain numerous complex chemical moieties that are produced through various means, including synthetic, cell culture and a combination approach. The diversity in chemical composition and need to analyze each component drives the popularity of HRMS characterization techniques.

- **ADCs** – numerous covalently linked components
  - mAb: IgG1, 2 or 4
  - Linker: cleavable and non-cleavable chemistries; cysteine, lysine or site-specific conjugation
  - Payload: tubulin inhibitors, DNA damaging agents, topoisomerase inhibitors, novel payload toxins
- **Oligonucleotides** – extensive variation in size, structure, chemical modifications and conjugations
  - Single stranded antisense
  - Double stranded siRNA
  - Aptamers
  - sgRNA, mRNA, and DNA plasmid

Impurity Characterization

HRMS methods are critical in confirming biotherapeutics identity and assessing low levels of impurities and degradants that are not accessible using conventional analytical tools. ADC characterization includes methods for both the mAb intermediate and ADC. These include confirmation of intended sequence, confirmation of identity, profiling of post translational modifications and quantifying process related and potential degradation impurities.

ADC Attribute	mAb Intermediate	ADC
Primary structure	Sequence by peptide mapping	drug-linker attachment sites
Identity	Intact mass	Intact mass and DAR
PTMs	Oligosaccharide profiling, sialic acid determination	Oligosaccharide profiling, sialic acid determination
Impurities	Degradation products, process-related impurities	Free drug, residual conjugation solvents, unconjugated mAb, degradation products

Table 1: HRMS Characterization methods that are critical to ADC development

Due to the broad mass range afforded by a QToF, HRMS analysis is possible for numerous aspects of protein biotherapeutics development, from characterization of intact 150 kDa proteins to quantification of 500 Da residual drug.

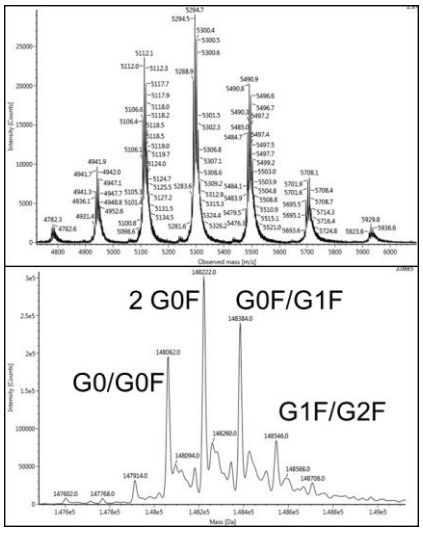

Figure 3: Intact mass analysis of mAb intermediate displaying glycan distribution using HRMS.

Peptide mapping by UPLC-MS/MS is used for protein sequence confirmation, characterization of side chain degradants/modifications and determination of drug and glycan loading, all of which have implications on protein stability, antigenicity and potency.

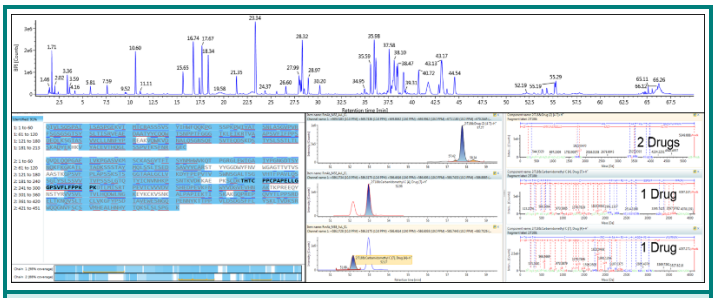

Figure 4: Trypsin digest peptide mapping of ADC to confirm sequence and characterize drug loading of specific cysteine residues.

Oligonucleotide therapeutics rely on HRMS UPLC-MS/MS methods to characterize and quantify impurities that arise from solid phase synthesis, processing and storage degradation and chemical modification. Small quantities of impurities can lead to unwanted stability profiles or potency outcomes.

Oligo Attribute	*Oligonucleotide*
Primary structure	Sequence confirmation of short oligos by LC-MS/MS
Identity	Intact mass
Impurities	Synthetic impurities, process-related impurities, degradation products

Table 2: HRMS Characterization methods that are critical to oligonucleotide development.

The labile nature of oligonucleotide therapeutics has led to introduction of numerous stabilizing chemical modifications (e.g. phosphorothiote, 2'-OMe, 2'-fluoro, locked nucleic acid). With increasing lengths of oligonucleotide, the number of non-full length products increases, making characterization of all low-level impurities within a modified oligo of utmost importance.

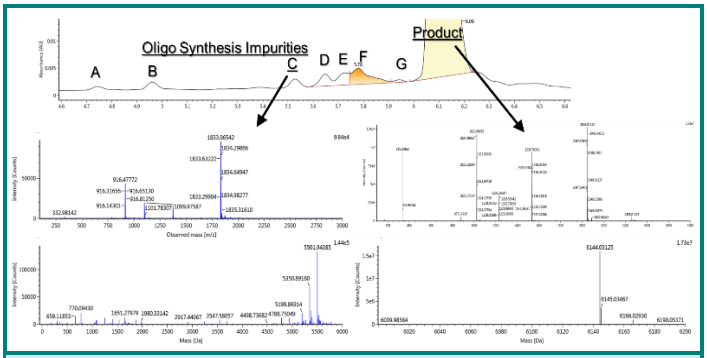

Figure 5: LC-MS/MS method for oligo impurity profiling and quantitation.