BIO_17 - Development of a Workflow for Therapeutic Antibody Characterization by LC-MS/MS

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Introduction: Inside the biopharmaceuticals class, monoclonal antibodies (mAbs) are prominent molecules with a large clinical application, including autoimmune diseases and different types of cancer. Is crucial to characterize their structural and physical-chemical properties, which can impact the product efficacy, safety, and compliance to regulatory requirements. LC-MS has been an essential tool in these evaluations, due its versatility, high sensibility, and precision. In the Brazilian scenario, the MS core facility of Fiocruz-Paraná, has offered shotgun proteomics analysis for a decade, and is now challenged by the increasing demand of therapeutical protein characterization. Here, we present sample preparation and LC-MS methods used to the characterization of mAbs including intact mass (IM), disulfide bond mapping (DBM) and peptide mapping (PM).

Objectives: Develop LC-MS/MS methods for characterizing mAbs to support the development of biopharmaceuticals in Brazil by providing a portfolio of analysis applied to therapeutic proteins.

Methodology: For the implementation of the analyses, the antibodies Opdivo, Keytruda, and the Reference Material 8671 NISTmAb were used. For PM and DBM analysis the antibodies were digested in urea with Lys-C, trypsin or Glu-C. For IM, the samples (untreated, reduced or deglycosylated) were diluted in 0.1% formic acid. Digested or undigested samples in the range of 200 to 1000 nanogram were injected into an Ultimate 3000 RSLC coupled to an Orbitrap Fusion Lumos (Thermo Scientific). The data were processed in Unidec, BioPharma Finder or Peaks DB softwares.

Results: In IM analysis, the deconvoluted spectra showed the intact molecule comprising the glycoforms, and the light and heavy chains (reduced samples). DBM analysis addressed all nine predicted disulfide bonds and PM confirmed the amino acid sequences. Besides the sample preparation and LC-MS analysis steps, the data processing showed to be an important step for obtaining reliable results and requires optimization as well.

Conclusion: To reach the presented results, different parameters were evaluated, enabling the core facility team to learn on analyzing mAbs by LC-MS. Other analysis should be implemented, such as glycan profiling and host cell proteins (HCP). Nonetheless, this work represents one step closer to strengthening competencies nationally, leading to the availability of a portfolio of analyses applied to monoclonal antibodies and support the development of innovators biopharmaceuticals and biosimilars.

Keywords: monoclonal antibody, LC-MS, Biopharmaceuticals