BIO_24 - Development of analytical method for charge variants determination of biosimilar monoclonal antibodies

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Introduction: Antineoplasic monoclonal antibodies (mAbs), such as pembrolizumab and nivolumab, dominate global pharmaceutical sales. Considering the Brazilian public health system and the competition for its limited resources which is aggravated by legal demands for access, the strengthening of the national production of biological medicines is crucial for health autonomy and strategic security. Demonstrating comparability in terms of quality, efficacy and safety with the biological product already registered in the regulatory authority based on the submission of a complete dossier is required for a biological product to obtain registration by the comparability pathway for development. Several variants of the drug substance may arise due to the biosynthetic production process and molecular characteristics of biotechnological products, derived from post-translational modification or formed during the manufacturing process and/or storage. Therefore, charge heterogeneity profile of therapeutic proteins, such as mAbs, should be characterized and monitored to ensure quality. Isoelectric focusing separates proteins according to their isoelectric point and may be used for charge heterogeneity determination.

Objectives: Develop a method for determination of charge variants by capillary isoelectric focusing (cIEF) in the mAbs produced by Bio-Manguinhos.

Methodology: Different batches of the commercially available drugs Keytruda® (pembrolizumab) and Opdivo® (nivolumab) were tested with the equipment iCE3™ from ProteinSimple after treatment with carboxypeptidase B (CPB). System suitability preparation included pI markers of values 7.05, 7.65 and 8.18, besides 6.14 (low marker) and 9.46 (high marker) used for all samples, including water blank and CPB control. Three combinations of carriers were tested with pharmalytes of pH values 3-10, 5-8 and 8-10.5. Pre-focusing occurred at 1500 V for 1 minute and focusing at 3000 V for 8 minutes. Data were processed using Empower 3 software.

Results: Separation of charge variants of mAbs by cIEF was achieved with reproducible profiles comparable to the ones described for pembrolizumab and nivolumab. The resolution was adequate to clearly identify at least five different variants for each mAb. The most adequate migration was obtained with three pharmalytes. The main peaks presented pI values 8.3 for pembrolizumab and 8.6 for nivolumab.

Conclusion: A cIEF method was developed for determination of charge variants in mAbs. After analytical validation, the proposed method might be used in characterization panel tests for registration submission and quality control of biosimilar mAbs produced by Bio-Manguinhos.

Keywords: Biosimilar; Monoclonal antibodies; Method development