LC-MS based case-by-case assessment of bevacizumab charge variants on stability and bioactivity

Himanshu Malani, Sumit K. Singh, Deepak Kumar, Anurag S. Rathore*

Department of Chemical Engineering, Indian Institute of Technology Delhi, New Delhi, India

**Corresponding author Email: asrathore@biotechcmz.com*

Abstract: Analytical similarity between innovator and biosimilar monoclonal antibody products involves closely matching the product profile for all critical quality attributes. Conventional practice for charge heterogeneity specification is based on cumulative sum of acidic and basic variants. However, this practice is inadequate as any similar modification on two separate locations can have drastically different impact on biological function. In this study, we assessed the impact of charge heterogeneity on the structure, stability, and bioactivity of bevacizumab. Semi-preparative cation exchange chromatography employing linear pH-gradient was used to isolate one acidic and five basic variants of an in-house bevacizumab product with >85% purity. Intact and peptide level mass analysis confirmed that acidic variant was formed due to deamidation of asparagine residue (N84), oxidation of methionine (M258), and preservation of C-terminal lysine residue, located on the heavy chain, whereas basic variants were attributed to incomplete clipping of one or both C-terminal lysine residues in the heavy chain respectively. Using a battery of structural assessment tools such as Fourier Transform infrared, circular dichroism and time-resolved fluorescence spectroscopic interrogations, the basic variants were seen to possess a similar structural and stability profile to the main product while acidic specie had differences in tertiary structure. Although structurally and functionally similar, the basic variants were not functionally beneficial when spiked with the main species. Conformational differences exhibited by acidic variant resulted in a 62% reduction in bioactivity. This strongly suggests that charge alterations can have significant impact of target product profile. Biosimilar manufacturers need to incorporate this understanding to hasten development of safer and efficacious therapies in the QbD paradigm.