Mapping Critical Quality Attributes of a Biopharmaceutical *In Vivo* by Unique Affinity Purification and Advanced LC-MS Methods

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Abstract

Understanding the critical quality attributes (CQA's) of a biologic drug is essential in guiding its development and control strategies through bioproduction and clinical development. In this work, we have investigated the CQA's of a biopharmaceutical (MAB1) *In Vivo* using highly specific purification strategies and advanced LC-MS structure analyses of the MAB extracted directly from preclinical and clinical PK samples. Specifically, either an antibody with high specificity to human IgG Fc region and/or a receptor-Fc with specificity towards the CDR region of MAB1 was used to extract the MAB from animal or human sera. High recovery (~90%) with high specificity (little or no albumin or serum matrix protein) was achieved, and the entire MAB1 sequence, sequence modifications, glycosylation, and disulfides, were subsequently mapped by multiple enzyme digestion LC-MS strategies. The initial detection and quantitation levels were achieved using XIC (full MS) for initial quantitation, and PRM (MS/MS) was additionally used for identity confirmation and further quantitation as needed. Overall the affinity purification coupled with multi-enzyme digestion and LC-MS analysis achieved good linearity (R^2>0.95) for quantifying the MAB1 spiked into sera across a wide range of concentrations (1-800 µg /mL). Such approaches may be useful in MAB PK quantitation analysis. Further, CQA's including glycosylation, terminal truncation, correct and mismatched disulfides, and typical modifications such as deamidation, oxidation, pyroE, glycation, and hydroxylation, were all assessed including complete sequence coverage of the MAB1 directly from the *In Vivo* samples. In addition, the characterized *In Vivo* CQA's were further compared and assessed in serum stability studies (with MAB1 spiked in reference serum and incubated for different lengths of times), as well as in formulation solution stability studies in order to gain a better understanding of comparative degradation profiles of MAB1 in the PK samples *In Vivo*. Based upon such *In Vivo* CQA profiling a highly relevant assessment of the product quality attributes may be used to further guide development and control strategies through bioprocess and clinical development.