Multi Attribute Monitoring Method for Process Development of Engineered Antibody for Site-Specific Conjugation

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Abstract

Antibody drug conjugates are a class of biotherapeutic proteins which have been extensively developed in recent years resulting in new approvals and improved standard of care for cancer patients. Among the numerous strategies of conjugating cytotoxic payloads to monoclonal antibodies, insertion of a cysteine residue at position 239 (C239i) achieves a tightly controlled, site specific drug to antibody ratio. Tailored analytical tools are required to direct the development of processes capable of manufacturing novel antibody scaffolds with the desired product quality. Here, we describe the development of a high throughput, 12-minute, mass spectrometry based method capable of monitoring four distinct quality attributes simultaneously: variations in the thiol state of the inserted cysteines, N-linked glycosylation, reduction of inter-chain disulphide bonds, and polypeptide fragmentation. When deployed, the method provided new insight into the properties of C239i antibody intermediate and its manufacturing processes. First, C239i forms exclusively oxidised thiol states within the bioreactor, of which a variant containing an additional disulphide bond was invariably produced and remained relatively constant throughout the fed-batch process; reduced thiol variants were introduced upon harvest. Second, close to twenty percent of N-linked glycans contained sialic acid, substantially higher than anticipated for wildtype IgG1. Lastly, previously unreported polypeptide fragmentation sites were identified in the C239i constant (C_H2) domain and the relationship between fragmentation and glycoform explored. This work illustrates the utility of applying a high-throughput liquid chromatography mass spectrometry (LC-MS) multi-attribute monitoring method to support the development of engineered antibody scaffolds.

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