Development of an MS-Based Top-Down High-Throughput Approach for Characterization of New Biological Entities and Biosimilar Candidates

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Fast screening and assessment are essential during development and production of new biological entities (NBES) and biosimilar candidates. Top-Down mass spectrometry (TD-MS) analysis with high mass accuracy quickly provides detailed information on key quality attributes such as glycosylation, oxidation, deamidation and disulfide linkages.

We present a TD-MS screening method for monoclonal antibodies as well as NBES that uses the high mass accuracy of a Bruker maXis II ETD for reliable assignment and quantification of such quality attributes. Using commercially available mAbs we established a high-throughput workflow by combining fast analysis and automated data evaluation (Biopharma Compass 3.0). Thus, up to 50 product candidates can be screened per day with mass accuracies of <11 ppm (1.5 Da) for intact antibodies and <1 ppm (0.05 Da) for light and heavy chains of the reduced antibodies. This workflow allows determination of the relative abundance of a mAb’s modifications like different glycosylation forms, oxidation, pyro-glutamate formation and truncations. Notably, the high mass accuracy moreover enables quantification of deamidation and partially reduced disulfide linkages with mass shifts even below 1 Da averaged over all species present in a sample.

This TD-MS screening was further combined with TD-MS/MS using both CID and ETD. This approach not only delivers accurate molecular weight data but also allows to determine selected PTMs. Thus, the presented strategy provides fast and precise determination of critical quality attributes as required in initial screening of biotherapeutic candidates.

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