AN INTEGRATED SUBUNIT LC-MS ANALYSIS FOR CORE-FUCOSYLATION ASSESSMENT OF MAB PRODUCTS

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INTRODUCTION
- MS-based approach is used to confirm and quantify multiple CQAs of the antibody and the technique is commonly used for antibody quantification
- Core fucosylation of the Fab portion is a CQA that influences antibody-dependent cell-mediated cytotoxicity (ADCC)
- We demonstrate the use of subunit-level analysis of MAM's using LC-ESI-MS/MS controlled by UNIFI Scientific Information System as a fast simple solution for CQA measurement
- This method measures the levels of fucosylated and aglycosylated Fab subunits as well as the aglycosylated form of it

METHODS
- Sample Preparation
  - Core fucose analysis: two Trastuzumab samples from different batches were digested with FabRICATOR/IdeS (Genovis, Cambridge, MA, USA) enzymes.
  - High mannose content was assessed based on the level of afucosylated scFc difference between the two digests.

RESULTS
- MS detection and identification

- Quantify high Manresse structures (via Endo S digestion)

CONCLUSION
- An MAM Workflow was developed to monitor the level of afucosylation, fucosylation/hybrid and mannose/hybrid type of the mAb products
- Ease to use: A fully automated UNIFI workflow with data acquisition and processing capability that can be easily deployed to monitor CQAs of the antibody (e.g. fucosylation levels) with minimal method development
- Quantification capability: UNIFI allows user defined formulas to be used in calculations. These calculations can be used to assess variability of both carbohydrates, such as high mannose, fucosylation containing, aglycosylated and aglycosylated
- Robustness of the method and instrumentation: UNIFI Scientific Information System and Visio INS-GTaf MS systems demonstrated that less than 10% RSD can be achieved for instrument-to-instrument variability for all specified modifications.

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