Complete characterization of biotherapeutic proteins by automated data processing on high resolution accurate mass spectrometry with SWATH® acquisition

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INTRODUCTION

Biotherapeutic proteins are a constantly growing market in the pharmaceutical industry. The rise of the biosimilars market has increased the number of laboratories requiring robust analytical capabilities. Confidence in data quality and reproducibility is vital for the successful characterisation of biotherapeutic proteins. Confidence is not only dependent on the data quality, but also the ease-of-use in which it is obtained to avoid mistakes and the need for reacquiring the data.

Biotherapeutic proteins carry more complexity than the traditional small molecule drugs. The heterogeneity assessments are vital to ensure product quality – drug efficacy and patient safety. Mass spectrometry techniques are a powerful tool to answer the critical questions: full amino acid sequence, post-translational modification (PTM) ratios, as well as possible process and storage derived modifications.

Mass spectrometry techniques are used to investigate the biotherapeutic proteins on the level of intact, subunit and peptide mapping. Single platform able to handle all levels of characterisation is desired. This approach has been taken in this study to characterise two commercially available monoclonal antibodies (mAbs; see figure 1.) Rituximab and Trastuzumab. The data acquisition has been performed with a quadrupole-time-of-flight (Q-TOF) instrument. Here we utilise for peptide mapping for the first time SWATH® acquisition, a data independent acquisition (DIA) technique, unique to SCIEX instrumentation The SWATH technique overcomes one of the issues associated with the default data acquisition methods: peptides (precursor ions) being irreproducible picked for MS/MS analysis. With SWATH acquisition all peptides are selected for MS/MS analysis – without instrument (data-dependent) or prior user (targeted) precursor ion selection.

SWATH acquisition has been previously used in the proteomics world to quantitatively analyse samples with high complexity (1). For this purpose, the data processing has relied on an ion library. With biotherapeutic proteins, the expectation is to have limited number of high abundant proteins in the samples – in which the low abundant proteoforms need to be assessed with confidence. With this in mind, the data processing for BioPharmaView™ software 2.0 has taken a different approach – using no ion library, but matching the theoretical MS/MS fragments.

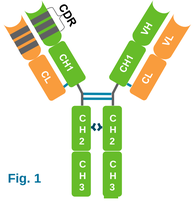


Fig. 1

Figure 1. Large numbers of biotherapeutic proteins belong to the class of monoclonal antibodies. These proteins carry two heavy and two light chains covalently bound by disulphide bonds. The usual characterization workflows include full intact analysis for the approximately 150kDa protein complex, subunit analysis achieved by cleaving the protein by the hinge region, and/or by reducing the disulphide bonds, and by peptide mapping after a digestion with a proteolytic enzyme.

MATERIALS AND METHODS

1) intact protein analysis; 2) subunit analysis; and 3) peptide mapping analysis

Sample Preparation:

mAb samples were prepared following standard procedures for sample preparation. Shortly:

1) mAb material was diluted with aqueous 0.1% Formic Acid solution to a concentration of 0.02µg/µl.

2) For the subunit analysis the material was digested with Promega IdeZ protease according to manufacturer's recommendation (37ºC – 1h). For Light chain – Heavy chain analysis the sample was reduced with TCEP (SCIEX protein digestion kit)

3) The tryptic digestion used octyl-β-D-glucopyranoside (OGS) as a denaturant, TCEP as a reducing agent, and MMTS as the alkylating reagent. All reagents were from an in-house SCIEX trypsin digest reagent kit and were used according to the instructions in the kit.

LC-UV-MS Conditions:
Two mAbs were analysed using as instrumentation a Shimadzu analytical flow instrument coupled to a quadrupole-Time-of-Flight accurate mass instrument. The instrument was operated in TOF MS, IDA and SWATH modes.

The chromatographic conditions used were standard for the respective workflows, utilising 1) and 2) Agilent C-8 Poroshell column with 15 to 20 min gradient run time, and 3) Phenomenex peptide Aeris C-18 column with 60 min runtime. The UV was collected at 214nm and 280nm.

Data processing
The data analysis (1)+ 2) data reconstruction and 3) peptide mapping) was performed with BioPharmaView™ software 2.0. System performance on mass accuracy, S/N levels and quantitative information on post-translational modifications (including glycosylation) were used to assess the reproducibility of the analysis.

Figure 2. Data processing was achieved with BiopharmaView software 2.0 which utilizes projects: in the project you specify the assay information (sequence, disulphide bonds and modifications) and characterize a golden standard. This processing is then utilized to number of samples in a batch to achieve fast and easy comparison studies of biotherapeutic samples (batches, stability study time points, biosimilarity assessments). The results can then be reviewed and a report generated.

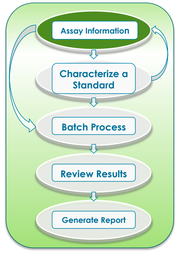
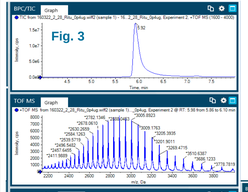


Fig. 2

RESULTS

The current platform performance was demonstrated on several levels of analysis: 1) Intact protein, 2) subunit analysis and by 3) peptide mapping. The workflows allow for different levels of information:

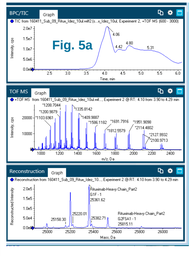


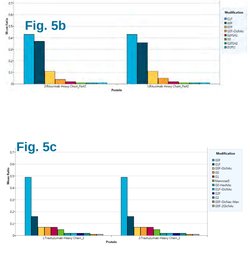
1) The intact protein analysis (fig 3 &4) provides a comprehensive fingerprint of the product, including the glycosylation pattern. We demonstrate the resolving power able to clearly separate the main features, mass accuracy with errors less than 1 Da on 150kDa on main features and reproducibility in acquisition and in processing.

Figure 3. Data processing (characterization of a standard and batch process) for the intact analysis of Rituximab. The resolving power enables the glycoforms to detect with confidence, and the dynamic range of the TOF detector allows for the lower abundant forms to be visible. The main glycoforms are calculated automatically and abundances as mean ratio to protein for each feature are shown.

Figure 4. Data processing (characterization of a standard and batch process) for the intact analysis of Trastuzumab. The automatic calculations for glycoform abundances (mean ratio to protein for each feature) for a batch process are shown.

2) At the subunit level we drill down to the smaller PTMs, where we show analysis on the level of oxidation and pyroglutamate formation, demonstrating the resolving power, mass accuracy and reproducibility.





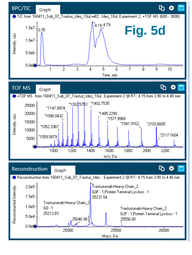


Figure 5. Subunit analysis of IdeZ digested Rituximab (5a and b.) and Trastuzumab (5c and d) show in higher definition the glycoforms and the calculations can be used for further verification of the findings from full intact level.

3) Peptide mapping reveals the position of the quality attributes that are need to be controlled for regulatory purposes. Here we demonstrate peptide mapping mass accuracy, providing greater than 98% sequence coverage (see figure 6) with processing ≤3.5 ppm error and MS/MS performance to elucidate the positional information of PTMs.

Figure 6. Peptide mapping analysis and sequence coverage for the two studies mAbs, Rituximab (6a and b) and Trastuzumab (6c)

The data acquisition in SWATH acquisition mode (See figure 7) enables all information to be collected, including the lower abundant features that could easily be missed by usual data-dependent acquisition modes. BioPharmaView processing (see figure 8.) provides automated PTM ratio calculations.

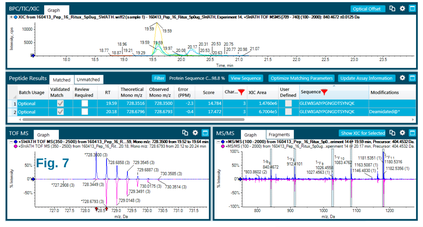


Figure 7. The SWATH processing in BioPharmaView software 2.0 allows for confirmation of all fragment information chromatographically, as the MS/MS is collected for all peptides continuously.

Figure 8. The automated calculations (8a) for the PTMs allows for easy review of the peptide main results. The ratios are calculated based on validated data (8b), and the user has easy access to see the information included and confirm the numbers calculated.

CONCLUSIONS

Characterization of biotherapeutic proteins is enabled by high data quality and designed-for-purpose software. The data demonstrates key characterization questions, such as glycosylation, oxidation and deamidation to be answered fast and with confidence. High data quality at the intact level allows for important process decisions to be made without delays. In Peptide mapping workflow the DIA acquisition mode SWATH enables high and low abundant-features to be included in all samples, in all data files without exceptions. It is also a permanent record of all peptides in the sample.

REFERENCES

1 A repository of assays to quantify 10,000 human proteins by SWATH-MS; Rosenberger, Koh, Guo, Röst, Kouvonen, Collins, Heusel, Liu, Caron, Vichalkovski, Faini, Schubert, Faridi, Ebhardt, Matondo, Lam, Bader, Campbell, Deutsch, Moritz, Tate, Aebersold; Sci Data. 2014 Sep 16;1:140031

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