Complete Characterization of Biotherapeutic Proteins Using High Resolution Accurate Mass Spectrometry

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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 multiple levels including intact, subunit and peptide mapping. Here we present a single platform able to handle all levels of characterisation. This approach has been taken in this study to characterize two commercially available monoclonal antibodies: Rituximab and Trastuzumab. The data acquisition has been performed with SCIEX X500B QTOF system. We utilise for peptide mapping for the first time SWATH® acquisition, a information independent acquisition (IDA) technique, unique to SCIEX instrumentation.

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.

Figure 1: SCIEX X500B QTOF System coupled with SCIEX ExionLC™ AC system

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.

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:

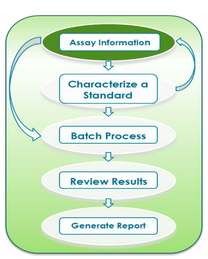
Two mAbs were analysed using as instrumentation a LC Stack coupled to SCIEX X500B QTOF system. The instrument was operated in TOF MS, IDA and SWATH modes. The system was calibrated with Calibrant Delivery System (CDS) for all the analysis.

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, or Waters UPLC Intact Mass Analysis Application Kit with 5 min gradient 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, disulfide 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.



RESULTS

This platform performance was demonstrated on several levels of analysis: 1) Intact protein, 2) subunit analysis and by 3) peptide mapping utilized features designed in SCIEX OS software .

1) Intact protein

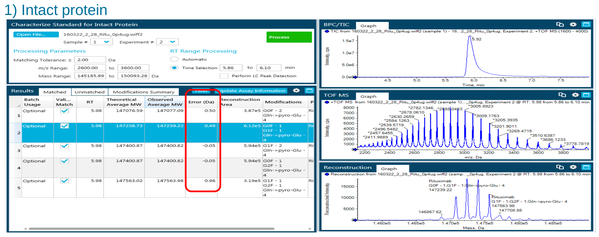


Figure 3. The intact protein analysis provides a comprehensive fingerprint of the product, including the glycosylation pattern. We demonstrate the resolving power able to clearly separate the main features, mass accuracy with errors less than 1 Da on 150 kDa on main features and reproducibility in acquisition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

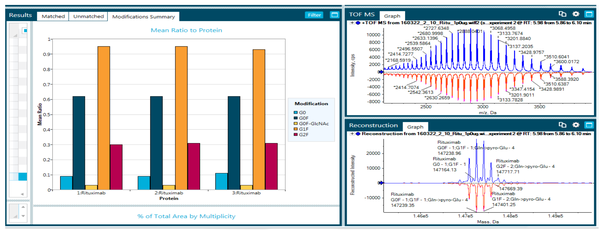


Figure 4. Data processing (characterization of a standard and batch process) for the intact analysis of Rituximab. The resolving power enables the glycoforms to detect with confidence, and the dynamic range of the TOF detector allows for the lower abundant forms to be visible. The main glycoforms are calculated automatically and abundances as mean ratio to protein for each feature are shown.

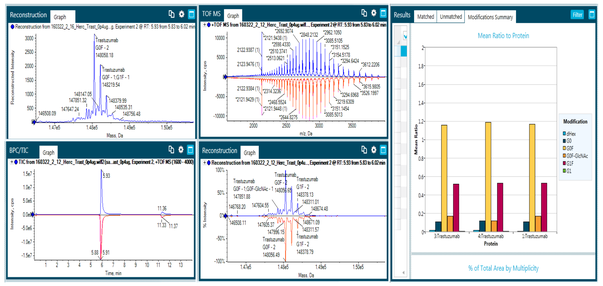


Figure 5. Data processing (characterization of a standard and batch process) for the intact analysis of Trastuzumab. The automatic calculations for glycoform abundances (mean ratio to protein for each feature) for a batch process are shown, and demonstrated a great reproducibility of the system.

2) Subunit Analysis

At the subunit level the more detailed information of mAb can be revealed, where we show light, heavy information as well as PTM analysis on the level of minor glycoforms and pyroglutamate formation, demonstrating the resolving power, mass accuracy and reproducibility.

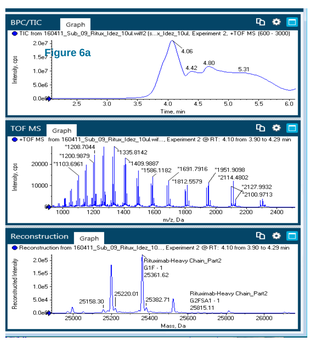
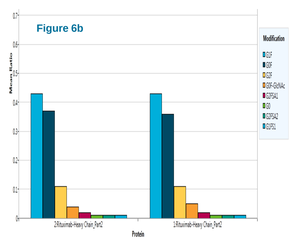


Figure 6. Subunit analysis of IdeZ digested Rituximab (6a and b.) show in higher definition the glycoforms and the calculations can be used for further verification of the findings from full intact level.



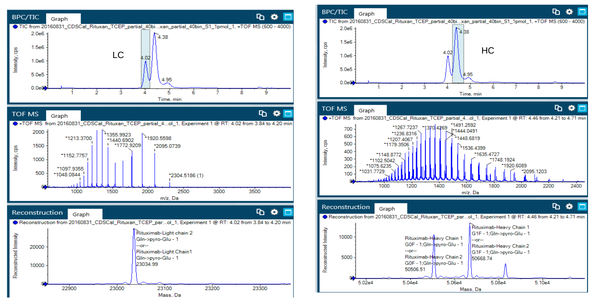


Figure 7. Subunit analysis can be performed in two different approaches, while IdeZ digest preserves inter disulfide binding, TCEP digest completely reduces inter and intra disulfide bindings. In some cases, intra disulfide binding will be partially reduced depending on the amount of TCEP introduced. These two approaches provides complementary binding information of chains.

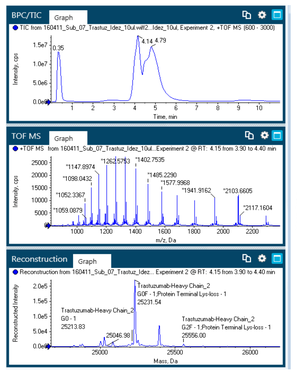


Figure 8. Subunit analysis of IdeZ digested Trastuzumab (8 a and b) show in higher definition the glycoforms and the calculations can be used for further verification of the findings from full intact level.

3) Peptide Mapping

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 9) with processing ≤3.5 ppm error and MS/MS performance to elucidate the positional information of PTMs.

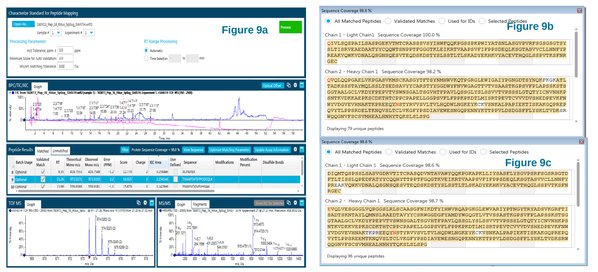


Figure 9. Peptide mapping analysis and sequence coverage for the two studies mAbs, Rituximab (9a and b) and Trastuzumab (9c)

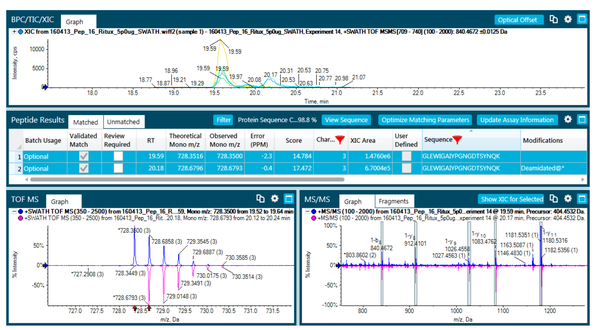


Figure 10. The SWATH processing in BioPharmaView software 2.0 allows for confirmation of all fragment information chromatographically, as the MS/MS is collected for all peptides continuously.

The data acquisition in SWATH acquisition mode (See Figures 10) 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 11.) provides automated PTM ratio calculations.

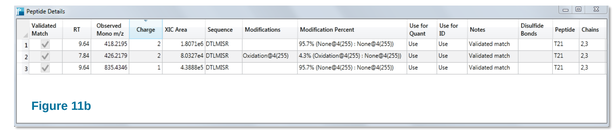


Figure 11. The automated calculations (11a) for the PTMs allows for easy review of the peptide main results. The ratios are calculated based on validated data (11b), and the user has easy access to see the information included and confirm the numbers calculated.

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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