Compliant-Ready Intact Biotherapeutic Protein Quantitation Using Reconstructed Masses

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INTRODUCTION

Bioanalysis of protein-based therapeutics widely focuses on the enzymatic digestion and quantitation of surrogate peptides. This approach can lead to the misinterpretation of the actual concentration due to unknown biotransformation in biologic matrices and the introduction of artifacts during extensive sample preparation. The quantitation of large proteins such as monoclonal antibodies (mAb) at the intact level using accurate mass spectrometry (MS) can circumvent these risks. SCIEX introduces a new workflow of quantification of proteins based on reconstructed masses within the SCIEX OS Software 1.7. With Good Laboratory Practice (GLP) and 21 CFR Part 11 compliance being needed quite often for the quantitative analyses in bioanalytical studies, the workflow presented can be set up to fully meet the required criteria.

KEY FEATURES

- SCIEX OS Software 1.7 enabling intact protein quantification based on peak height or peak area of reconstructed masses
- 21 CFR Part 11 compliance ready intact quantification workflow
- Ease-of-use of SCIEX OS for getting to answers in a time efficient manner

MATERIALS AND METHODS

Rat plasma spiked with humanized IgG mAb was processed using immunocapture with biotinylated anti-human-Fc-antibodies and streptavidin-coated paramagnetic beads. Generic conditions for intact protein analysis using denaturating conditions with reversed phase chromatography were used. An ExionLC™ system coupled to a SCIEX X500B System was used. Data was acquired using the intact protein mode turned on.

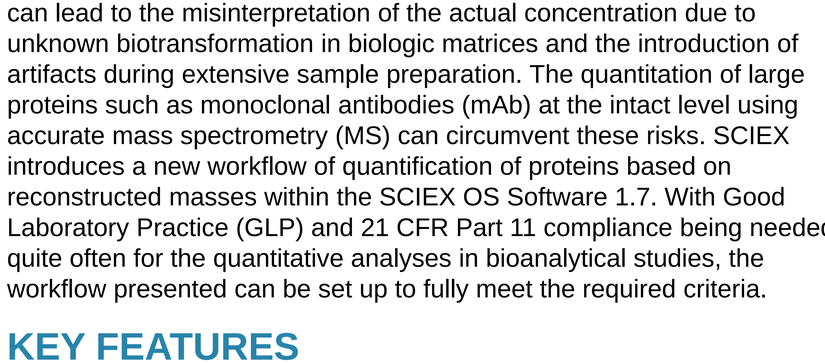

Figure 1. Overview of the intact quantification workflow based on XICs. Upon data acquisition using accurate mass time of flight instrumentation, the XIC or sum of XIC of one or multiple charge states or protein forms was used for integration and quantification in the SCIEX OS Software.

RESULTS

- Upon acquisition of the TOF-MS raw data, the SCIEX OS Software 1.7 offers two approaches for quantification: The first approach utilizes the XIC of a certain charge state of a protein or the sum of several XICs linked to either several charge states or to several protein forms or both (Figure 1). The second quantification approach is based on the reconstruction of masses, which is uniquely offered in SCIEX OS Software 1.7 (Figure 2).
- When analyzing matrix samples, the quantification using reconstructed mass information offers an additional advantage for quantification, since matrix proteins might interfere on spectral level, but not necessarily on reconstructed data level if differing in their molecular weight from the analyte of interest (Figure 4 and Table 1).
- SCIEX OS meets these criteria when being set up as a closed system including the requirement for records and signatures on an electronical basis (compliant-ready). The presented workflow is fully compliant, as a combination of SCIEX offering technical controls and the user's responsibilities (Figure 3).

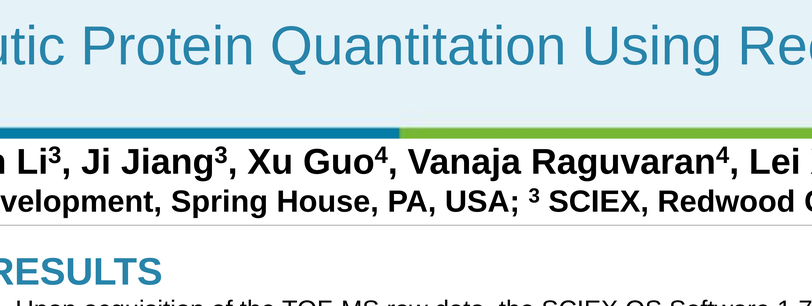

Figure 2. Overview of Intact Protein Quantification Workflow Using Peak Areas of Reconstructed Masses. Upon data acquisition using accurate mass time of flight instrumentation, the raw data was reconstructed, integrated and used for quantification in the SCIEX OS Software 1.7.

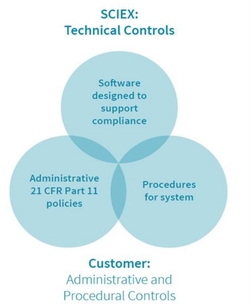

Figure 3. Three Types of Controls Required for Compliance According to 21 CFR Part 11.

Table 1. Quantification Results for G0F/G1F Using Reconstructed Masses.

Actual Conc. [µg/mL]	Reconstructed Peak Area G0F/G1F	Calculated Conc [µg/mL]	Accuracy [%]
100	*7.00E+06*	*88.2*	*88.2*
50.0	*4.03E+06*	*50.8*	*101.6*
10.0	*8.53E+05*	*10.7*	*106.6*
5.00	*4.23E+05*	*5.23*	*104.6*
1.00	*8.79E+04*	*1.01*	*100.9*
0.500	*4.57E+04*	*0.477*	*95.4*
0.200	*2.44E+04*	*0.208*	*104.1*
0.100	*1.57E+04*	*0.099*	*98.6*

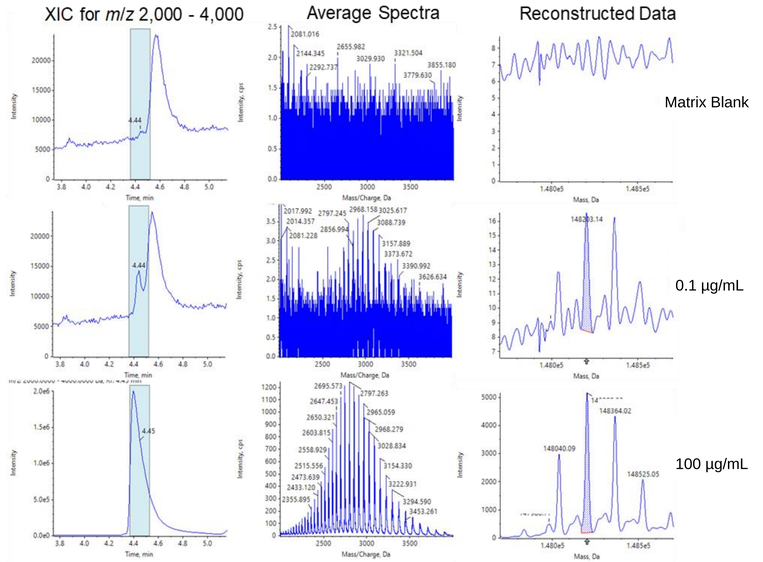

Figure 4: Overview of Quantification Results from SCIEX OS Software. XIC, average spectra and reconstructed data information for the matrix blank, the lowest concentration and the highest concentration used for quantification. The analyte protein is eluting at 4.44 min, the additional peak being visible in the matrix blank and the LOD at 4.55 min is related to the elution of matrix proteins.

CONCLUSIONS

- SCIEX OS Software 1.7 provides a comprehensive intact protein quantification workflow by offering the unique peak integration on reconstructed mass spectra, and reduces the complexity for LC-MS method development and cumbersome sample preparation
- The ease-of-use of the SCIEX OS Software gets new users up to speed quickly and the compelling data review options within the software speed up the time to get to correct answers
- Regulated environments can use the intact quantification workflow meeting GLP and 21 CFR part 11 criteria having the option to submit studies electronically to the FDA.

REFERENCES

1. Jian, W., Kang, L., Burton, L., Weng, N.: A workflow for absolute quantitation of large therapeutic proteins in biological samples at intact level using LC-HRMS. Bioanalysis 8 1679-1691 (2016).
2. White Paper, SCIEX OS LC/MS Software and 21 CFR Part 11 Regulations RUO-MKT-19-10018.

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