Quantification of intact monoclonal antibodies by immunocapture and high-resolution mass spectrometry

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ABSTRACT

This technical note describes a streamlined LC-MS workflow for quantification of intact mAbs spiked in rat plasma using immunocapture and high-resolution accurate mass spectrometry (HRAMS). The lower limit of quantification (LLOQ) was determined to be 50 ng/mL for NISTmAb and 100 ng/mL for trastuzumab emtansine with excellent linearity and high accuracy.

INTRODUCTION

Traditionally, quantitative LC-MS bioanalysis of intact therapeutic proteins is performed using a bottom-up approach that employs surrogate peptides. Although the bottom-up approach offers high sensitivity, it introduces complexity and potential artefacts from enzymatic sample preparation. As a result, bottom-up approaches are unable to capture biotransformational changes at the intact level. By comparison, intact LC-MS assays require less sample preparation and can provide a better understanding of biologics to facilitate early drug discovery, particularly for pharmacokinetic studies where ultra-sensitivity is not required.

Intact protein quantification is often performed using the integrated peak area of extracted ion chromatograms (XICs) of one or multiple charge states of the protein. However, XIC-based quantification has limited sensitivity and is prone to interferences from matrix proteins. To overcome this challenge, a unique peak integration algorithm based on reconstructed masses was introduced in the SCIEX OS software. The SCIEX OS software also provides tools to meet 21 CFR Part 11 compliance requirements for bioanalysis.

In this work, NISTmAb and trastuzumab emtansine were spiked in rat plasma and prepared using immunoaffinity capture prior to LC-MS analysis. The data was analyzed using the Analytics tool within the SCIEX OS software. Figure 1 is an illustration of the workflow employed in this work.

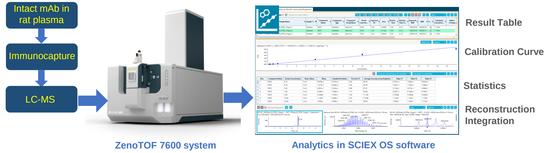

Figure 1. Illustration of intact mAb quantification using immunocapture and HRAMS. The ZenoTOF 7600 system provides excellent HRMS data while the utilization of the Analytics tool within SCIEX OS software enables confident intact quantification based on XICs or deconvolution in a compliant-ready environment.

MATERIALS AND METHODS

Sample preparation
The calibration standards of intact NISTmAb (NIST RM #8671) and trastuzumab emtansine were prepared in Sprague Dawley rat plasma, followed by immunocapture using streptavidinated magnetic beads conjugated with biotinylated goat anti-human IgG. The immunocaptured mAbs were eluted using trifluoroacetic acid (TFA).

LC-MS
The separation of intact mAbs was performed at a flow rate of 0.4 mL/min using an ExionLC system fitted with a BioResolve RP mAb polyphenyl column (450 Å, 2.7 µm, 2.1 x 50 mm, Waters). The data was acquired in TOF-MS positive mode using a ZenoTOF 7600 system with intact protein mode enabled.

Data analysis
LC-MS data was processed using Analytics within SCIEX OS software. The quantification was performed based on spectrum reconstruction and peak integration using the MQ4 algorithm with 1/x2 weighting.

RESULTS

The Analytics tool within SCIEX OS software offers a streamlined workflow and intuitive control for mass reconstruction, peak integration, protein quantification, and generation of the calibration curve and statistics summary (e.g. % CV and accuracy). Figure 2 shows an example of reconstruction of intact NISTmAb data using a resolution setting of 3,000.

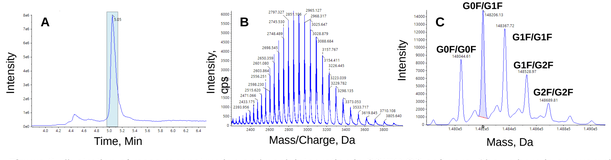

Figure 2. Illustration of mass reconstruction and peak integration in SCIEX OS software. Shown here is an example data of intact NISTmAb at 10 µg/mL. The data points selected in the TIC (A) provided excellent average (B) and reconstructed (C) spectra of intact NISTmAb. The major glycoform G0F/G1F was used for quantification of NISTmAb in this work.

The limit of detection (LOD) and lower limit of quantification (LLOQ) of NISTmAb (Figure 3) and trastuzumab emtansine (data not shown) were determined to 50 ng/mL and 100 ng/mL, respectively. This result is in good agreement with the data published previously.[1-2] The upper limit of quantification (ULOQ) was measured to be 10 µg/mL for NISTmAb and trastuzumab emtansine. The ULOQs were mainly determined by beads usage and capture antibody capacity during the immunocapture process.

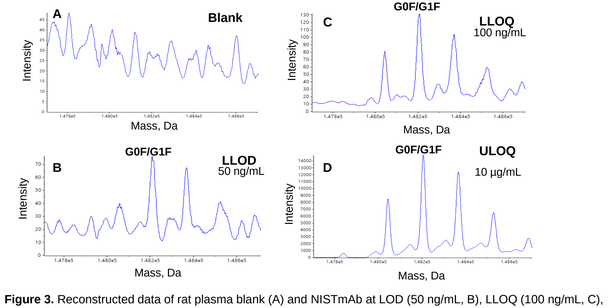

Figure 3. Reconstructed data of rat plasma blank (A) and NISTmAb at LOD (50 ng/mL, B), LLOQ (100 ng/mL, C), and ULOQ (10 µg/mL, D).

Excellent linearity was achieved for NISTmAb (Figure 4A) and trastuzumab emtansine (Figure 4C) in the ranges of 50 ng/mL–10 µg/mL and 100 ng/mL–10 µg/mL, respectively. The average accuracy from three replicate injections is within ±15%, with all %CV less than 10% (Table 1). These results demonstrate high sensitivity and high accuracy of intact protein quantification using the ZenoTOF 7600 system.

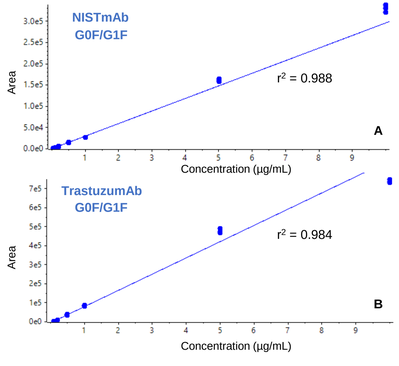

Figure 4. Calibration curves of the glycoform G0F/G1F of NISTmAb at 50 ng/mL–10 µg/mL (A and B) and trastuzumab emtansine at 100 ng/mL–10 µg/mL (C and D). Similar quantification results were obtained for other major glycoforms (data not shown).

Table 1. Summary of quantification statistics from the Analytics within SCIEX OS software. The results from the glycoform G0F/G1F of NISTmAb (A) and G0F/G1F of trastuzumab emtansine (B) are shown in this table.

	NISTmAb			Trastuzumab		
Acutal Conc. [µg/mL]	Calculated Conc. [µg/mL]	Percent CV [%]	Average Accuracy [%]	Calculated Conc. [µg/mL]	Percent CV [%]	Average Accuracy [%]
10	*11.15*	*2.67*	*111.5*	*8.72*	*1.22*	*87.2*
5	*5.43*	*1.93*	*108.6*	*5.65*	*2.35*	*113.0*
1	*0.903*	*1.15*	*90.3*	*1.06*	*2.63*	*105.7*
0.5	*0.477*	*4.86*	*95.4*	*0.500*	*5.93*	*100.1*
0.2	*0.184*	*5.23*	*92.1*	*0.179*	*1.55*	*89.5*
0.1	*0.0985*	*3.32*	*98.5*	*0.105*	*1.72*	*104.5*
0.05	*0.0526*	*9.50*	*105.3*	*n/a*	*n/a*	*n/a*

Figure 5. Three types of controls required for 21 CFR Part 11 compliance.

To meet the regulation outlined in 21 CFR Part 11, SCIEX OS software is designed as a closed system, including the requirement for records and signatures on an electronic basis. SCIEX OS software can open raw data files from any visible storage location, thereby offering the flexibility to work within a closed network using the designated processing workstations.

Figure 5 illustrates three types of controls required for 21 CFR Part 11 compliance. The presented intact quantification workflow is fully compliant as SCIEX provides:

1) Technical controls over hardware and software configuration
2) Security of network and operating system as well as policies
3) Procedures and user training

CONCLUSIONS

- The ZenoTOF 7600 system produces high-quality HRAMS data for intact protein quantification
- Strong linearity, low LOQ, and high accuracy were achieved for quantification of intact mAbs using the ZenoTOF 7600 system
- SCIEX OS software offers powerful tools for spectrum reconstruction, peak integration, and data visualization in a compliant-ready environment

REFERENCES

1. A new level of compliant-ready intact biotherapeutic protein quantitation using reconstructed masses. SCIEX technical note, RUO-MKT-02-10709-A.
2. Quantitation of intact therapeutic protein in plasma matrix by LC/MS. SCIEX technical note, RUO-MKT-02-8168-A.

TRADEMARKS/LICENSING