# Rapid, Sensitive, and Routine Intact mAb Quantification using a Compact Tof HRMS Platform

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## INTRODUCTION

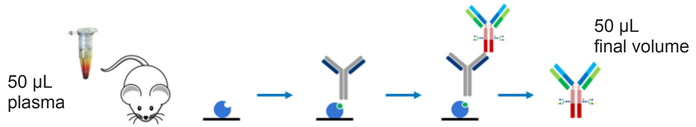
Intact level protein quantification by LC-MS is attractive as it offers the ability to monitor complex (heterogeneous) biotherapeutics over time. As an emerging technique, wide adoption of intact level protein quantification has been limited by factors such as access to and expertise with high end HRMS Platforms. The recently introduced BioAccord system is a compact and easy to use integrated LC-MS platform (ACQUITY RDa mass detector + I-Class UPLC) that does not require users to have advanced knowledge of HRMS theory and operation.

The general performance of the BioAccord system for intact level quantification of trastuzumab is described. Quantitative performance is reported under various conditions including mouse plasma with immunopurification.

## METHODS

*Sample preparation*

Trastuzumab was spiked into mouse plasma at concentrations from 0.01 µg/mL to 2.0 µg/mL. Samples were extracted using goat anti-human Fc antibody immobilized onto a streptavidin coated 96 well plate following the procedure published by J. Kellie et al.[1] After antibody capture and washing, the mAb was released using 5% acetic acid. BSA was then added to a final concentration of 0.1 mg/mL.




For extracted plasma, a matrix plate was prepared using the immunopurification procedure as described above but using blank plasma. Trastuzumab solution prepared in acetic acid was then added to the prepared plate followed by addition of BSA. Additional sample preparation details are described in application note.[2]

*UPLC-MS conditions*

The ACQUITY RDa mass detector was operated at full scan ESI+ with a mass range of 400-7000 *m/z*. Capillary voltage was 1.5 kV, cone voltage was 70 V, and desolvation temperature was 550°C. The ACQUITY I-Class was run using a generic gradient condition at flow rate of 0.2 mL/min.[2] Injection volume was 2 µL for formic acid (FA)/BSA and 10 µL for all other matrices. Total run time was 10 minutes.

**References**

1. Kellie, J, Kehler et al "Towards best practices in data processing and analysis for intact biotherapeutics by MS in quantitative bioanalysis, Bioanalysis 2017, 23, 1883.
2. Waters Application Note "High Sensitivity Intact Monoclonal Antibody (mAb) HRMS Quantification" (720006222EN).

## RESULTS AND DISCUSSION

Performance of intact trastuzumab quantification was assessed in four matrixes and are summarized in Table 1. Good sensitivity was observed with LOQs ranging from 0.025 µg/mL in surrogate matrix to 0.1 µg/mL of samples extracted from mouse plasma. Statistics for linear curve fitting are reported. The linear dynamic range ranges from 1.3 in mouse plasma to 2.4 across matrices. For mouse plasma samples the observed dynamic range reflects capacity of the plate solid support and protein binding kinetics.

QC data based on 6 replicates are excellent (Table 2). Calibration curve and chromatogram are shown in Figures 1 and 2 respectively. When the data was fitted using non-linear curving fitting such as log-log, or quadratic curve fitting, the dynamic range was extended further. For example, for extracted plasma samples, the dynamic range using linear curve fitting was 2.1, quadratic was 2.4, and log-log was 3.

Table 1. Summary of quantitation attributes in varying matrices (method validation criteria of 20% and 25% at LLOQ in % accuracy were used).

| Matrix | Final matrix | LOD µg/mL | LOQ µg/mL | ULOQ µg/mL | Dynamic range | R2 | Curve fitting |
|---|---|---|---|---|---|---|---|
| 50 mM ammonium acetate, 0.1 mg/mL BSA | 50 mM ammonium acetate, 0.1 mg/mL BSA | 0.025 | 0.05 | 6.25 | 2.1 | 0.99 | Linear $1/X^2$ |
| 0.1 %FA, 0.1 mg/mL BSA | 0.1% FA 0.1 mg/mL BSA | 0.025 | 0.025 | 6.25 | 2.4 | 0.99 | Linear $1/X^2$ |
| Extracted plasma matrix | 5% acetic acid, 0.1 mg/mL BSA | 0.025 | 0.05 | 6.25 | 2.1 | 0.98 | Linear $1/X^2$ |
| mouse plasma | 5% acetic acid, 0.1 mg/mL BSA | 0.1 | 0.1 | 2.0 | 1.3 | 0.98 | Linear $1/X^2$ |

Table 2. QC summary in mouse plasma.

| QC | n | Theoretical Conc. (µg/mL) | Mean Conc. (µg/mL) | Precision (%) | Accuracy (%) |
|---|---|---|---|---|---|
| QC Low | 6 | 0.5 | 0.5 | 10 | 102 |
| QC Mid 1 | 6 | 1.0 | 1.1 | 5.5 | 106 |
| QC Mid 2 | 6 | 1.4 | 1.4 | 6.2 | 99 |
| QC High | 6 | 1.75 | 1.5 | 4.9 | 87 |

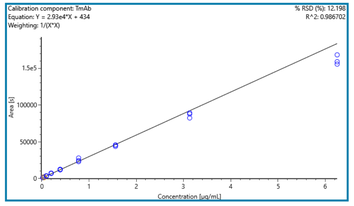



Figure 1. Representative calibration curve in FA/BSA.

Comparing Acquity RDa spectra to a spectrum obtained on a QTof operating at >50,000 resolution, a near identical mass spectrum with peak to valley profile is obtained (Figure 3). Under IDC mode of acquisition (Intelligent Data Capture, providing automatic noise and file size reduction), the peak valley decreases further. The detected charge states over the concentration range were remarkably consistent, aiding confident charge state selection and peak processing for quantification (Figure 4). Particularly for IDC enabled data, the data file size was significantly reduced, for a 96 samples run overnight with 10 min run time of each injection, the total file size was 980 Mb. This positively impacted overall system performance and utility for routine quantification.

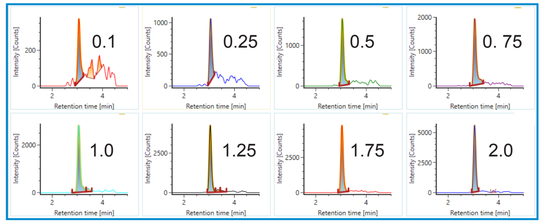



Figure 2. Representative chromatograms in mouse plasma (µg/mL).

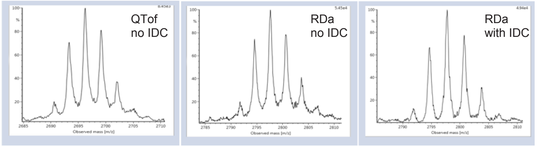



Figure 3. Comparison of mass spectra acquired with the ACQUITY RDa Mass Detector versus QTof with > 50,000 mass resolution.

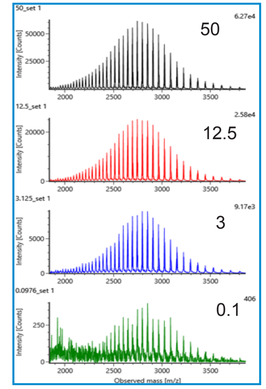



Figure 4. Overlaid mass spectra of sample in extracted plasma matrix (µg/mL).

## CONCLUSIONS

Intact trastuzumab was quantified with good sensitivity and good dynamic range. The quality of mass spectra used for intact protein analysis was excellent and comparable to other HRMS systems. Optimized file size and minimal charge state shift across large concentration ranges simplify its use. Representative bioanalytical statistics demonstrate that an easy to use Tof HRMS system can be fit-for-purpose for sensitive, routine intact protein bioanalysis.

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