Meeting the Challenges of Implementing Accurate-Mass Mass Spectrometry for Biotherapeutic Development in Regulated/non-Regulated Environments

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INTRODUCTION

- **Despite the widespread practice of exact-mass mass spectrometry (MS) analysis in the biotheropeutics development processes, experienced MS users are typically required to manage the instrument operation, data processing, and interpretation.**
- **There are major challenges for the adoption of MS by analysts who are more familiar with optical detection methods, such as the inherent complexities of MS technology, and the lack of compliance-ready informatics platforms that are capable of converting raw MS data into product quality attribute results seamlessly.**
- **In this study, we report the development of a new high performance bench-top orthogonal acceleration time-of-flight (oa-TOF) LC-MS system (BioAccord) with simplified and optimized operation modes to deliver automated, accurate, and reproducible mass measurements for proteins, peptides, and released glycans.**

Figure 1. *The BioAccord System comprised of an ACQUITY UPLC I-Class PLUS configured with an optical detector (TUV/FLR) coupled in-line to the ACQUITY RDa Detector.*

METHODS

Sample Preparation

Intact Mass Analysis

NIST mAb, Trastuzmab (T-mAb), and Kadcyla (T-DM1) were all diluted in 25 mM NH_4OAc to prepare a stock solution of 0.1 µg/µL. The T-mAb stock solution was serially diluted with 25 mM NH_4OAc to a range of concentrations for LC/MS analysis. The lowest concentration of the mAb solution was 1.0 ng/µL. Cysteine-conjugated ADCs were diluted to 2.5 µg/µL in 50 mM NH_4OAc before LC-SEC/MS analysis.

Subunit Analysis

Forced degraded (with H_2O_2) and standard NIST mAbs were digested with endopeptidase IdeS enzyme. The final diluted solution of 0.1 µg/µL were used for LC/MS analysis.

Peptide Mapping Analysis

NIST mAb sample was denatured and digested with trypsin followed by reduction and alkylation. The solution was diluted to desired concentration for LC/MS analysis.

Released N-linked Glycan Analysis

The Waters Glycoworks RapiFlour-MS Glycan kit was used to generate RFMS labelled glycan from the NIST mAb.

LC/MS:

The LC/MS System is comprised of a Waters ACQUITY™ UPLC™ system, with either the Tunable UV (TUV) Detector (intact, subunit and peptide mapping analysis) or the Fluorescence (FLR) detector (released glycan analysis), and the oa-TOF (Acquity RDa) MS instrument. The columns utilized in this study are:

Intact and subunit: ACQUITY BEH C4, 1.7 µm, 2.1 x 50 mm,
Native SEC/MS: ACQUITY BEH SEC 200 Å, 2.1 x 150 mm,
Peptide mapping: ACQUITY BEH 300 C18, 2.1 x 100 mm,
Released glycan with FLR labeling: ACQUITY BEH Glycan Amide 130Å, 1.7 µm, 2.1 x 150 mm.

The BioAccord system was operated in MS and/or DIA (data independent acquisition) modes. The automatic system data acquisition, processing and reporting were operated with the compliant-ready UNIFI platform.

RESULTS

NIST mAb intact mass analysis

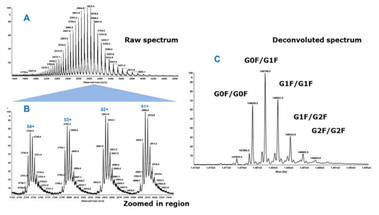
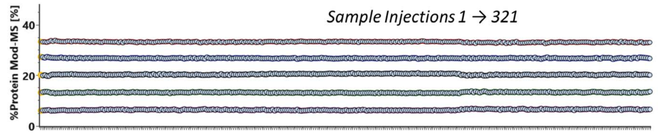


Figure 2. *NIST mAb RPLC-MS intact mass analysis. A-combined raw spectrum. B-Zoomed in region of A. C-Decovoluted spectrum with good mass accuracy. Same experiment was conducted with 0.1% TFA, or with IDC (intelligent data capture), both with excellent results (data not shown) .*

NIST mAb intact mass analysis reproducibility



	Mean	% RSD (%)	Std dev	Sum	Minimum	Maximum	Count
G0F/G1F	33.2	0.62	0.207	10663.0	32.5	33.9	321
G1F/G1F	20.4	1.41	0.287	6546.8	19.5	21.0	321
G0F/G0F	13.2	1.46	0.192	4234.7	12.8	13.8	321
G1F/G2F	26.8	0.54	0.146	8589.8	26.3	27.3	321
G2F/G2F	6.4	3.90	0.251	2065.8	6.0	7.4	321

Figure 3. *Spectrum quality and data consistency are the key factors of successful intact mass analysis. Relative quantitation of major glycoforms of the NIST mAb reference standard is displayed here. Across 321 injections, we were able to obtain consistent relative percentage of glycoforms. The RSD% is less than 4.0 % as highlighted in red.*

Antibody Drug Conjugates (ADCs)

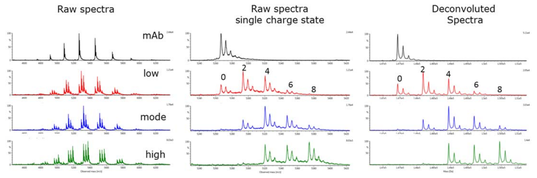


Figure 4. *Combined raw and deconvoluted spectra of cysteine-conjugated ADC samples (with increasing drug load) and the unconjugated mAb as a control. The raw spectra and the deconvoluted spectra show good consistency with the drug distributions as well as the glycoforms. The automatically calculated drug loading distributions and DARs are in good agreement with results from HIC analysis (data not shown)*

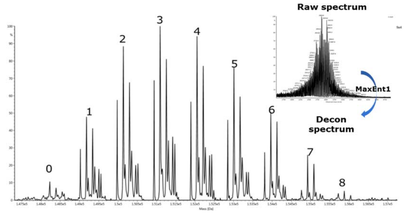


Figure 5. *Trastuzumab emtansine (T-DM1) is a lysine-conjugated ADC drug, the deconvoluted spectrum form the LC-SEC/MS experiment displays the detection of DAR 8 species with the calculated average DAR of 3.48, which is in good agreement with the expected average DAR of 3.50. The experiment was conducted without deglycosylation of the ADC drug.*

NIST mAb peptide mapping

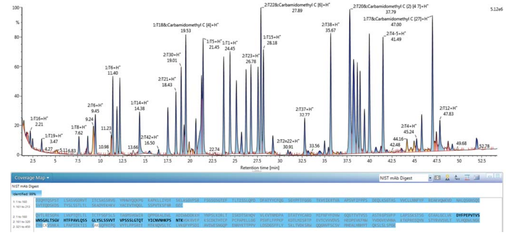


Figure 6. *NIST mAb peptide mapping chromatogram (top) with matched peptide labeled. High sequence coverage with good mass accuracy for both precursor ions and fragments demonstrate the high performance of the system for peptide mapping.*

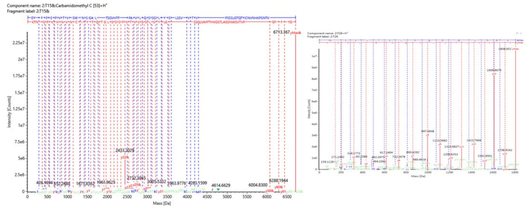


Figure 7. *Fragmentation spectrum examples (HC T15 and HC T26) from the NIST mAb peptide mapping experiment. The spectra shows high-energy fragmentation capability of the system with oa-Tof MS operated in the data-independent acquisition (DIA) mode.*

NIST mAb subunit analysis forced degradation study

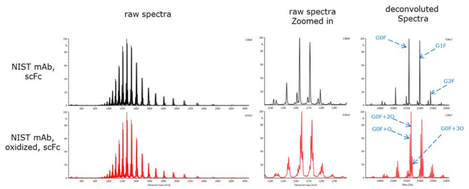


Figure 8. *NIST mAb IdeS digested scFc subunit before and after forced degradation (oxidation using H_2O_2) comparison. Up to 3 oxidized species were detected for all major glycoforms with good resolution and mass accuracy. For the F(ab) subunit, up to 3 oxidized species were also detected for the F(ab) peak and the glycation peak with good resolution and mass accuracy (data not shown)*

Released glycan analysis

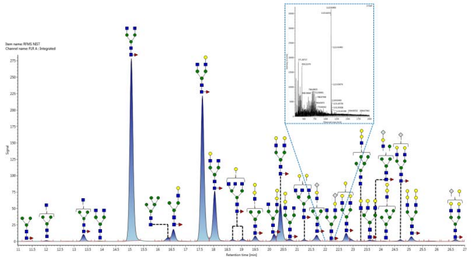


Figure 9. *Annotated FLR trace for NIST mAb glycans, 28 glycans were mass confirmed and 19 were determined to be present at % amounts greater than 0.1%. The sensitivity of the instrument is highlighted by the inset spectrum, which shows MS trace for F(6)A2[6]G(4)1Sg(6)1, a glycan that was present at 0.09 %Abundance. The signal-to-noise ratio for this glycan was estimated to be 30:1.*

CONCLUSION

The newly developed BioAccord LC/MS system underwent performance evaluation for the three key analysis workflow in the biotherapeutic drug development process: intact mass analysis, peptide mapping and released glycan analysis.

These experiment results are extremely promising and clearly demonstrate the potential of the BioAccord system to address many of the challenges in implementing accurate-mass mass spectrometry with qualities such as high system performance, easy of use, excellent reproducibility, automatic workflow for data acquisition, processing and reporting and compliance-ready.

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