SmartMS-ENABLED LC-MS SYSTEM FOR BIOTHERAPEUTIC DEVELOPMENT IN REGULATED/NON-REGULATED ENVIRONMENTS

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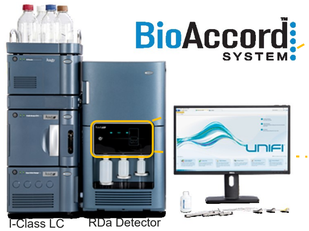
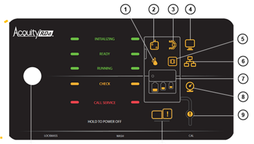
INTRODUCTION

- LC-MS is an essential tool in biopharmaceutical characterization, however it often requires high level of expertise and training before its deployment in laboratories across an organization for routine analysis.
- BioAccord System, an ACQUITY UPLC I-Class LC coupled with ACQUITY RDa Detector is a novel high-performance benchtop LC-MS system.
- It is an easy-to-operate UPLC-TOF MS system with intuitive software, standard operating modes and automated workflow methods.
- It has streamlined workflows for automated data acquisition, processing and reporting to assist scientists with different levels of expertise to perform routine intact/subunit, peptide mapping and released glycan analyses.



I-Class LC RDa Detector



Instrument front panel provides real-time status updates

METHODS

Intact protein analysis (Native and RP LC-MS):

- The cysteine-conjugated ADC in formulation at 10 mg/mL was diluted to 1 mg/mL in 50mM NH_4OAC before analyzed by native SEC-UV-MS using 50 mM NH_4OAc with isocratic elution (10 uL injections). An ACQUITY UPLC Protein BEH SEC column (p/n 186008471) was used for this application.
- The mAb 2 sample (1 mg/mL) was prepared in 50 mM NH_4OAC and analyzed by SEC-UV-MS.
- The instrument performance study was performed using Humanized mAb mass check standard (p/n 186008927) using an ACQUITY UPLC Protein BEH C4 column (p/n 186004495).

Peptide analysis:

- Forced degradation samples were prepared by exposing mAb 1 samples to light for 11 days. The samples were digested using trypsin and analyzed using the BioAccord system. A Waters XSelect CSH C18 column (p/n 186006727) was used for this experiment.

Data Acquisition:

- Intact Protein: ESI+, Full scan mode (*m/z* 400-7000)
- Peptide analysis: ESI+, MS with fragmentation mode (*m/z* 50-2000)

Informatics solution: UNIFI Scientific Information System V 1.9.4

- UNIFI is a compliance-ready software and has automated workflow methods for data acquisition, processing and reporting
  - Intact mass analysis: Intact protein (MS-RT window based) workflow method
  - Peptide analysis: Peptide Map MS (for Exact Mass MS data) workflow method

RESULTS

Intact Protein Analysis

The native intact analysis was performed for ADC samples using SEC-MS to determine drug-to-antibody ratio (DAR). The DAR values were compared to the data generated in-house for the same ADC samples.

Native SEC-MS analysis of intact ADC

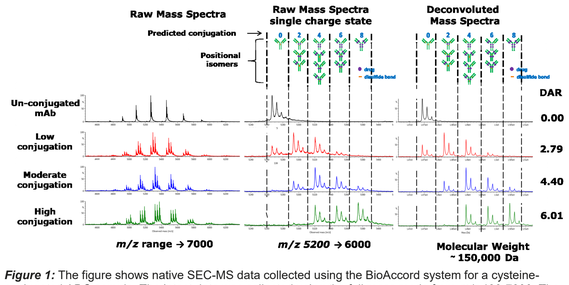


Figure 1: The figure shows native SEC-MS data collected using the BioAccord system for a cysteine-conjugated ADC sample. The intact data was collected using the full mass mode from *m/z* 400-7000. The automated intact protein workflow method was used to determine the DAR ratios based on deconvoluted mass spectra. The data presented DAR of 0, 2, 4 and 6.

Comparable performance with BioAccord intact MS and in-house UV and MS methods

Cysteine-conjugated ADCs drug loading distribution and DAR

	Low				Moderate				High			
	HIC	QTof1	QTof2	Tof	HIC	QTof1	QTof2	Tof	HIC	QTof 1	QTof 2	Tof
ADC 2	0.81	0.74	0.64	0.68	0.38	0.41	0.35	0.36	0.07	0.09	0.05	0.05
ADC 4	1.14	1.17	1.37	1.36	1.67	1.57	1.81	1.82	1.23	1.11	1.19	1.15
ADC 6	0.75	0.60	0.64	0.65	1.61	1.45	1.51	1.47	1.72	1.72	1.86	1.85
ADC 8	0.12	0.21	0.05	0.10	0.78	0.97	0.70	0.75	2.95	3.05	2.98	2.96
DAR	2.83	2.72	2.70	2.79	4.44	4.40	4.37	4.40	5.97	5.97	6.07	6.01

QTof 1 deglycosylated samples, run on Xevo G2-S in 2014
QTof 2 non-deglycosylated samples, run on Vion in 2017
Tof non-deglycosylated samples, run on BioAccord in 2018

Table 1: The table contains DAR values calculated based on the BioAccord, HIC and two in-house QTof systems. As shown here all methods show comparable DAR values for ADC samples.

Intact mass analysis for trisulfide bonds

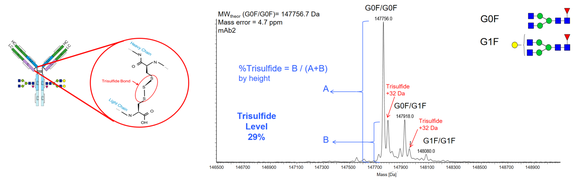


Figure 2: The trisulfide bond analysis of mAb 2 was performed by denatured SEC-UV-MS analysis. The deconvoluted MS spectrum shows the target trisulfide bond peaks which have an abundance of 29% relative to the most abundant N-glycosylated intact protein peak (G0F/G0F). The data shown here is in agreement with the data collected by the in-house SEC-UV-MS method (from a different vendor).

Peptide Analysis: Forced Degradation of mAb 1

Control and light-exposed mAb 1 samples were analyzed by peptide mapping. The unmodified and modified peptide peaks were validated using fragmentation spectra prior to their relative abundance measurements using extracted ion chromatograms (XIC).

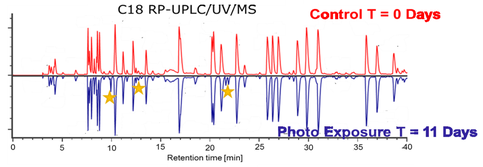


Figure 3: Using UNIFI binary sample comparison feature, chromatograms for control and light-exposed samples were visually inspected for any changes at peptide level. As shown here, the light-exposed sample exhibits increased levels of several peptide attributes as labeled in the chromatogram.

Peptide attribute monitoring

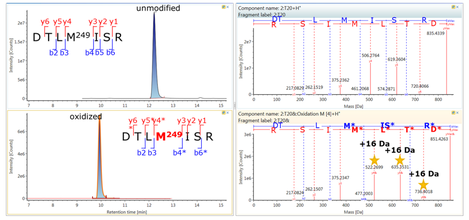


Figure 4: The relative quantification of peptide attributes can be performed based on the MS responses for any selected pair of unmodified and modified peptides. The MS response is determined using the XIC for each peak using automated data processing. For high confidence measurements, the method can be modified to only include sequence verified peptides using high energy fragmentation data. The annotated fragmentation spectra for DTLMISR and $DTLM_{(ox)}ISR$ peptides are shown here are an example.

Peptide %modification level comparison using optical, RDa TOF and Exactive+ MS

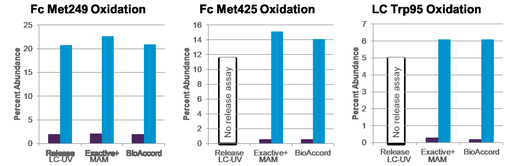


Figure 5: The data generated on the BioAccord system for a selected group of peptide attributes of mAb 1 were compared to data generated in-house using optical only and Exactive Plus MS systems. The summary plots show highly comparable %abundance levels across different platform methods.

Instrument performance

A set of intact protein data was used for instrument performance evaluation. The %modification levels of most abundant glycoforms of Humanized mAb mass check standard (G0F/G0F, G0F/G1F, G1F/G1F, G1F/G2F and G2F/G2F) were measured using 6 BioAccord systems for inter-system and intra system reproducibility over a period of 3 months.

Day-to-day reproducibility

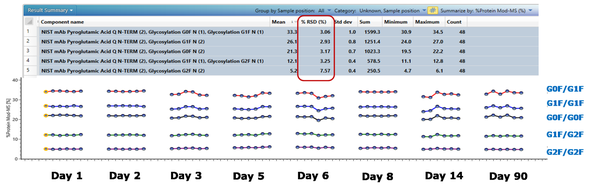


Figure 6: The data presents %protein modification levels for the five most abundant glycoforms of Humanized mAb mass check standard. The results reproducibility was determined using %RSD (%RSD <10%) calculated for multiple intact protein data sets collected over a period of 90 days on the same BioAccord system.

Instrument-to-instrument reproducibility

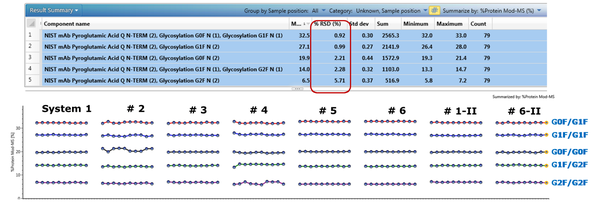


Figure 7: The figure shows %protein modification levels calculated for the five most abundant glycoforms of Humanized mAb mass check standard using 6 different BioAccord systems. The %RSD for these glycoforms indicate high data reproducibility with <10% %RSD value.

CONCLUSIONS

- The BioAccord system is a SmartMS-enabled LC-MS system applicable to many biotherapeutics laboratory settings due to:
  - small laboratory footprint
  - simplified system controls and design
  - automated workflow-driven methods
  - compliance-ready software features
- Both intact protein and peptide analysis case studies show high comparability of data generated on the BioAccord system to in-house platform methods using other detectors.
- The BioAccord system demonstrates high data reproducibility between systems as well on each system over time. All data points generated in the study show low %RSD at <10% for most abundant glycoforms at intact protein level.

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