ADVANCING ATTRIBUTE CONTROL OF ANTIBODIES AND ITS DERIVATIVES USING HIGH RESOLUTION ANALYTICS

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OVERVIEW

- **This poster shows the performance of the new Vion IMS QTof mass spectrometer in intact mass analysis (including subunits), peptide mapping, and released glycan analysis for the characterization of monoclonal antibodies and ADCs.**
- **We also demonstrate the utility of an integrated high-resolution analytics platform, consisting of a Vion IMS QTof mass spectrometer and Unifi Scientific Informatics System, to understand the critical quality attributes of mAbs and Lysine-conjugated ADCs.**

INTRODUCTION

- The analytical characterization of biotherapeutics is challenging and requires the use of a plethora of orthogonal techniques such as chromatography and mass spectrometry.
- The analytical characterization of mAbs (ADCs, bispecific Abs) usually follows a multi-level workflow, where bio-pharmaceuticals are studied at the intact protein (top) level, after enzymatic digestion into smaller mAb subdomains (subunits, the middle-level), or after proteolytic digestion using a combination of enzymes to generate peptides (the bottom level) or the released glycans.
- The data presented in this poster demonstrates the performance of the Vion, when used in the multi-level workflow, for the characterization of mAbs and ADCs. It also highlights the integrated nature of the high-resolution MS with Unifi informatics system to cover data acquisition, data processing and reporting in a single analytical platform.

Figure 1. The Vion™ IMS QTof™ Mass Spectrometer and Unifi® Scientific Information System. Unifi provides a single platform for instrument control, data processing and reporting.

EXPERIMETNAL

Sample Preparation

Intact Mass Analysis
Waters mAb standard (1 mg) was dissolved in 25 mM NH_4HCO_3 to prepare a stock solution of 1.0 mg/mL. The stock solution was serially diluted with 25 mM NH_4HCO_3 to prepare mAb solutions with a range of concentrations for LC/MS analysis. The lowest concentration of the mAb solution was 1.0 µg/mL.

Subunit Analysis
Waters mAb standard was reduced with DTT at 37°C for 30 min, and then alkylated with iodoacetamide in dark for 45 min. An aqueous solution with 3% acetonitrile/0.1% FA was added to terminate the reduction/alkylation reaction. A solution of 0.1 µg/µL was used for LC/MS analysis.

Peptide Mapping Analysis
Trastuzumab sample was reduced with DTT at 37°C and then alkylated with IAM before trypsin digestion for 1hr. An aqueous solution with 10% acetic acid was added to terminate trypsin digestion. The solution was diluted to desired concentration for LC/MS analysis.

Released N-linked Glycan Analysis
The Waters GlycoWorks Sample Preparation Kit was used to generate RFMS labelled glycan from IgG1.

Instrumentation

LC: ACQUITY UPLC H-Class System
MS: Vion™ IMS QTof™ Mass Spectrometer

LC Conditions

Analysis Type	Columns	Column Temperature (°C)	Mobile Phases A	B	C	Gradients	Flow Rate (mL/min)	TUV Wavelength /FLR (nm)
Intact	ACQUITY UPLC Protein BEH C4, 300Å, 1.7 µm, 2.1 X 50 mm	80	H_2O	ACN	1% FA in H_2O	10% B to 90% B in 4 mins	0.3	280
Subunit	ACQUITY UPLC Protein BEH C4, 300Å, 1.7 µm, 2.1 X 50 mm	80	H_2O	ACN	1% FA in H_2O	27% B to 31 % B in 6.5 min; 31%B-40%B in 3 mins	0.3	280
Peptides	ACQUITY UPLC CSH C18, 130Å, 1.7 µm, 2.1 X 100 mm	65	1% FA in H_2O	1% FA in ACN	N/A	1% B to 33% B in 120 mins	0.2	215
Glycans	ACQUITY UPLC Glycan BEH Amide Column, 130Å, 1.7 µm, 2.1 X 150 mm	60	50 mM Ammonium Formate, pH=4.4	ACN	N/A	75% B to 54% B in 35 mins	0.4	265 (Fluorescence) 425 (Emission)

MS Conditions:

Analysis Type	Capillary Voltage (kV)	Cone (v)	Source Temperature (°C)	Desolvation Temperature (°C)	Desolvation Gas (L/h)	Acquisition Mode
Intact	3.0	150	150	500	800	Sensitivity
Subunit	3.0	150	150	500	800	Sensitivity /Resolution
Peptides	3.0	40	100	250	600	Sensitivity
Glycans	3.0	40	120	250	600	Sensitivity

Informatics System

Unifi® Scientific Information System

RESULTS (INTACT MASS ANALYSIS OF MABS AND ADCS)

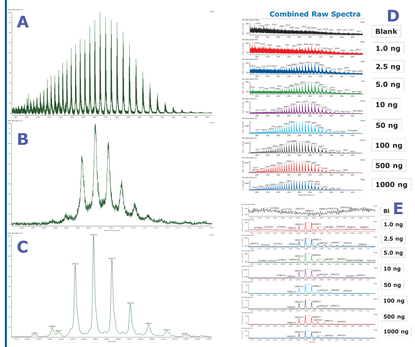

Figure 1. Mass spectra of an intact IgG1 antibody from the Vion IMS QTof mass spectrometer. (A) Raw spectrum from 50 ng of mAb on a 2.1x50 mm BEH C4 column; (B) Spectrum showing a single charge state from Panel (A); (C) Deconvoluted spectrum; (D) Raw mass spectra of an intact IgG1 antibody at different loading amounts (on-column), showing the enhanced sensitivity of Vion IMS QTof. (E). Deconvoluted spectra from Panel (D).

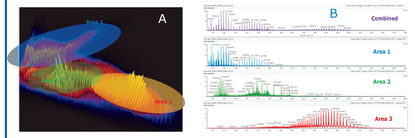

Figure 2. The analysis of an intact mAb by HDMS mode on a Vion IMS QTof. (A) A 3D-plot showing the orthogonal separation of the intact mAb from interference impurities. (B) Spectra from the impurities (smaller contaminants/protein fragments) as well as the mAb, demonstrating the IMS benefit for routine intact mass analysis.

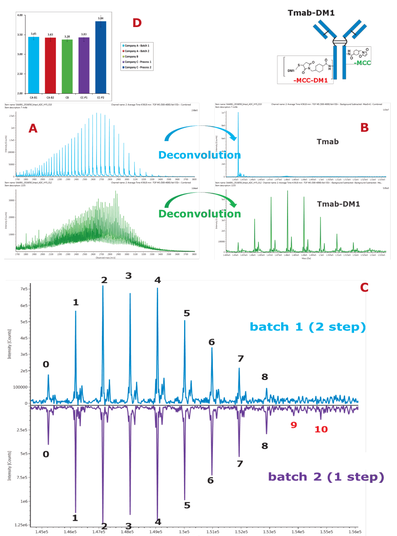

*Figure 3. LC/MS analysis of ADCs. (A) Combined MS spectra for Trastuzumab (Tmab) and ado-trastuzumab emtansine (Tmab-DM1); (B) Deconvoluted mass spectra of Tamb and Tmab-DM1; (C) Comparison of the deconvoluted Tmab-DM1 spectra from two different synthetic procedures (1-setp *vs.* 2-step); (D) Calculated DARs and CVs for the ADC samples from different manufacturers and/or synthesis methods.*

RESULTS (Subunits)

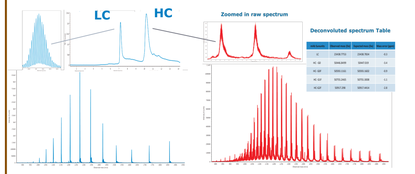

Figure 4. Analysis of mAb subunits showing the isotopic resolution (at the raw spectra level) of the light chain and the heavy chain of the mAb. Spectra from a charge state (insert) shows the enhanced resolution achieved by Vion IMS QTof mass spectrometer for the analysis of the light chain and heavy chain subunits.

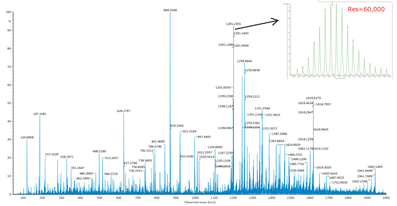

Figure 5. Middle-down fragmentation spectra (CID) of the light chain of Waters mAb standard on the Vion IMS QTof mass spectrometer. The insert shows the resolution of the instrument achieved for a large fragment from the light chain (>12 kDa).

RESULTS (PEPTIDES)

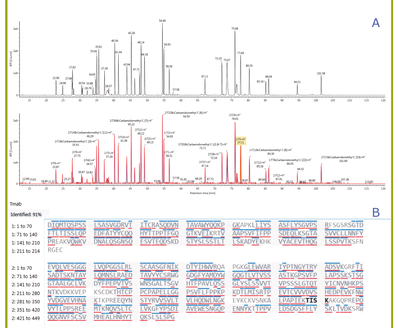

Figure 5. LC/MS^E peptide mapping of Trastuzumab showing the integration of Vion IMS QTof with Unifi Scientific Information System for automated data acquisition and processing. (A) BPI of a peptide map and annotation; (B) the sequence and fragmentation coverage from the MS^E peptide mapping analysis.

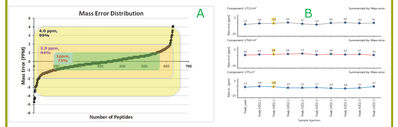

Figure 6. Vion IMS QTof achieves highly accurate mass measurement in the peptide mapping experiments. (A) Mass error distribution of peptides in HDMS^E analysis; (B). Stability of the mass measurement across a series of injections from the HDMS^E peptide mapping analysis.

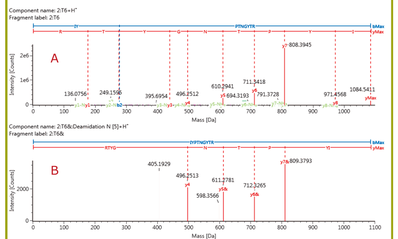

Figure 7. HDMS^E high-energy fragmentation spectra on a Vion IMS QTof for the identification of a low abundance deamidated peptide (~0.5%). (A) High-energy data of the native peptide (IYPTNGYTR) (B) High energy data of the deamidated peptides from the HDMS^E peptide mapping analysis.

RESULTS (RELEASED N-GLYCANS)

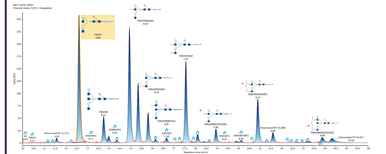

Figure 8. An annotated fluorescence (Ex 465 nm) chromatogram for the human IgG glycan standards from the Unifi glycan workflow (UPLC-FLR-HDMS). Based on Glucose Unit (GU) library searching and accurate mass measurement on the Vion in the positive HDMS mode, 25 mass confirmed glycans were identified (GU search tolerance: ±0.2 GU, mass accuracy <2 ppm).

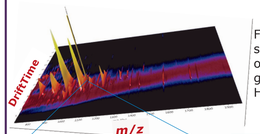

Figure 9. A 3D plot showing the analysis of human IgG glycans under the HDMS mode.

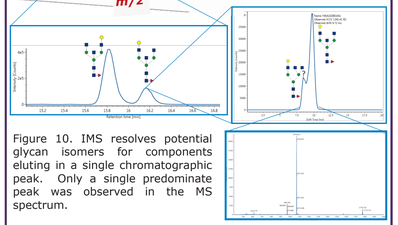

Figure 10. IMS resolves potential glycan isomers for components eluting in a single chromatographic peak. Only a single predominate peak was observed in the MS spectrum.

CONCLUSIONS

- **Increased sensitivity is achieved across a wide range of analytes including intact mAbs, ADCs and their subunits, peptides and glycans.**
- **The Vion IMS QTof QTof is capable of achieving near isotopic resolution of proteins with a MW of ~25 kDa, yielding low ppm mass accuracies results under the routine LC/MS conditions. The improved resolution provides greater confidence in the mass measurement of large peptide/small proteins, which is critical for effective middle-down fragmentation analysis.**
- **The IMS capability offered by the Vion IMS QTof allows the differentiation of glycan isomers in a routine LC-FLR-HDMS analysis, and provides better understanding for the glycan structures.**
- **The sensitivity gain with no reduction in resolution by the Vion IMS QTof enhances ion-feature definition that enables detection of minor components/glycoforms of proteins.**
- **The continued development by Waters in analytical technologies for biological molecules have permitted more in-depth characterization of mAb and ADCs.**

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