TP-455

INTERGRATING ION MOBILITY SEPARATION INTO PEPTIDE MAPPING FOR THERAPEUTIC PROTEIN CHARACTERIZATION, QUALITATIVE AND QUANTITATIVE ASPECTS

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INTRODUCTION

LC/MS peptide mapping is the primary technique for the comprehensive characterization of biotherapeutic proteins. Data dependent (DDA) and data independent acquisition, e.g., MS^E are both widely used to acquire detailed sequence information.

The unbiased MS^E approach ensures reproducible, accurate and precise quantitative results.

However, due to multiplex nature of MS^E, it often bears the risk of false positive or false negative identifications because of low abundant interference ions.

The post-ionization separation of ion mobility enables the alignment of fragment ions with drift-separated precursor ions to significantly reduce false positive and negative rate as well improve quantification accuracy and precision.

This study presents preliminary data using a Vion IMS QTOF MS system to enhance peptide mapping of mAbs.

METHODS

Sample Information

Denatured trastuzumab (1 mg/ml, 7 M guanidine chloride, 0.2 M Tris, pH 7.5) was reduced with 0.5 M DTT and alkylated with 0.5 M idoacetamide. Buffer exchange (0.1 M Tris, pH 7.5) over a NAP-5 column (GE healthcare) enabled efficient tryptic digestion for subsequent analysis by peptide mapping.

Hi3 PhosB peptide standard (Waters) were re-suspended by mixing with 5.5 pmol/µl trastuzumab tryptic digest. The resulting concentration was 100 pmol/µl for the Hi3 PhosB peptides. The mixture was diluted with 5 pmol/µl trastuzumab tryptic digest in series to 10000, 1000, 100, 10, 1 and 0.1 fmol/µl.

Instrumentation

LC Settings

System: ACUITY UPLC H-Class
Colum: ACUITY UPLC CSH C18, 2.1 x 100 mm, 1.7 µm
Column temp: 65 ºC
Mobile phase A: Water with 0.1% formic acid
Mobile phase B: Acetonitrile with 0.1% formic acid
TUV: 215 nm

Vion IMS QTOF MS Settings

Capillary Voltage: 3.0 kV
Sampling cone: 40 V
Source offset: 80 V
Source temp: 120 ºC
Desolvation temp: 250 ºC
DIA acquisition: MS^E and $HDMS^E$

Informatics

Unifi Scientific Information System

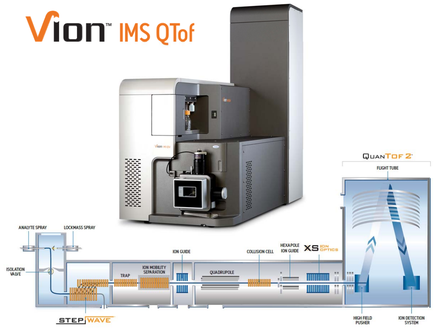

Figure 1. Vion IMS TOF MS schematics shows the layout of the instrument, with stepwave for robust, high sensitivity, an ion mobility separation device based on T-Wave technology upstream of the quadrupole, the XS ion optics device that delivers simultaneous high resolution and high sensitivity Tof spectra, and brand new QuanTof 2 that incorporats ADC ion detection electronics. It's clear that the geometry of this instrument is different compare to Synapt HDMS.

Consistent CCS Measurements for Peptides

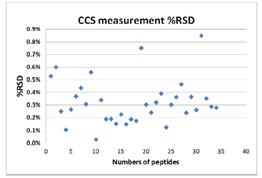

Figure 2. Vion IMS QTOF MS enables automated drift time and CCS measurements for peptide mapping using $HDMS^E$ mode of acquisition. The chart on the left shows reproducible CCS measurements for a total of 35 selected peptides from a mAb tryptic digest across 6 injections: the RSD% was <1% for these peptides.

Key Words:

1. IMS: Ion Mobility Separation
2. MS^E: MS with Elevated collision energy (typically refers to Data Independent Acquisitions, DIA)
3. $HDMS^E$: High Definition MS^E (or MSE with Ion Mobility Separations Upstream of the Quadrupole)
4. DT: Drift Time
5. CCS: Collision Cross Section

RESULTS

Detection Dynamic Range

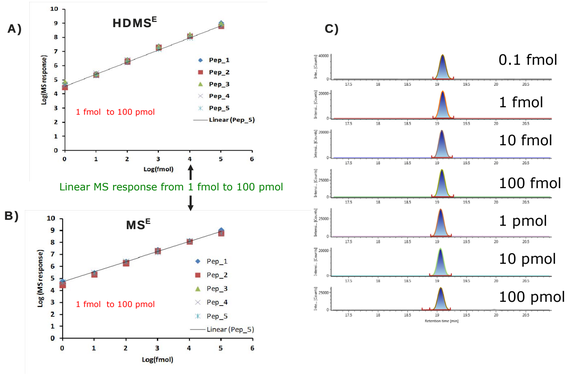

Figure 3. MS detection dynamic range was evaluated for both $HDMS^E$ and MS^E peptide mapping experimentations. Five peptides from the spiked-in Hi3 PhosB standards mixed with a fixed amount of transtuzumab tryptic peptides were prepared (Methods) for the measurement. We can measure from 1 fmol to 100 pmol in linearity for these 5 peptides in both $HDMS^E$ (A) and MS^E acquisition modes (B). The XICs of a representative peptide were displayed in panel (C) ranging from 0.1 fmol to 100 pmol (0.1 fmol data was not included in the dynamic range calculations).

IMS Enables Fast Peptide Mapping

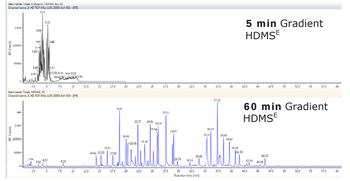

Figure 5. Two chromatograms and a table on the left show the sequence coverage results from a 5 min (A) a 60 min $HDMS^E$ peptide mapping LC methods (B). Both the short and long $HDMS^E$ analysis give comparable sequence coverage. The sample used are trypsin digested trastuzumab.

	A 5 min Gradient HDMS^E	B 60 min Gradient HDMS^E
Sequence coverage	85%	86%

Sequence coverage is calculated from 6 analysis

b/y > 3;
Δ mass< 5 ppm

IMS Improves the Detection of Low Abundant Peptide Fragment Ions

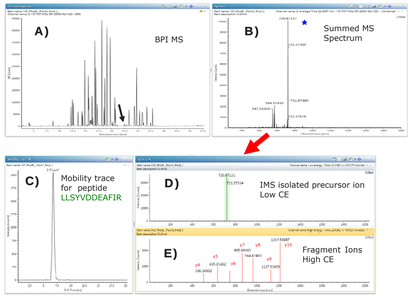

*Figure 4. A Hi3 PhosB standard peptide (LLSYVDDEAFIR) at 0.1 fmol mixed with 5.5 pmol trastuzumab tryptic digest was used to demonstrate the improvement in enhancing fragment ions by $HDMS^E$. A) BPI MS chromatogram of the mixture, the arrow points to the chromatographic peak for this Hi3 phosB peptide. B) Summed MS spectrum for the peak at 22.4 min (the * indicates the doubly charged Hi3 PhosB peptide). C) Mobility trace for the same peptide. D) Mobility isolated Hi3 phosB peptide spectrum; ions from trastuzumab peptides were separated in the drift tube. E) The annotated fragment ions from the mobility isolated peptide.*

Ion Mobility 3D Viewer

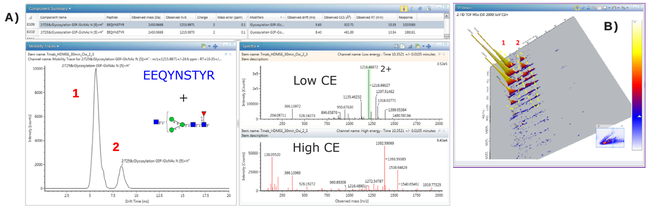

Figure 6. Screen captures from a UNIFI processed trastuzumab $HDMS^E$ data. (A) A mobility trace shows isobaric glycopeptides that have the same m/z and charge, but different DT and CCS values. The attached glycan is FA1 isoforms, the peptide is EEQUNSTYR (Heavy Chain). (B) Ion mobility viewer in 3D shows the mobility separated ion clusters for the two isobaric glycopeptides.

CONCLUSIONS

Vion IMS QTOF MS with UNIFI Scientific Information System improves peptide mapping analysis via $HDMS^E$ acquisition and processing.

- **Routine CCS measurement for peptides (with RSD% < 1%)**
- **Five order of MS detection dynamic range for MS^E and $HDMS^E$ acquisition modes**
- **Fast Peptide Mapping with good sequence coverage is achievable**
- **Higher specificity of fragmentation data was obtained via $HDMS^E$ for low abundant peptides**
- **Potential applications for isolating isobaric glycopeptides**
- **Improved data display for IMS chromatograms and processing results**

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