Differential Ion Mobility for Separation of Intact Monoclonal Antibody or Antibody-Drug Conjugates from Smaller Proteins, Free Drug, and Other Contaminants

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INTRODUCTION

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Methods for the analysis of intact proteins and antibody-drug conjugates by LCMS are often fast and simple, with the expectation that they provide a rapid answer confirming the molecular weight and/or drug-antibody ratio. Many times however, smaller proteins, fragments of the protein and/or small molecule contaminants (such as free drug, in the case of ADCs) confound this analysis through co-elution, adducting or interference. No amount of mass resolution helps under such circumstances if the issue is related to heterogeneity, sample complexity, salt adducts, or contaminant small molecules. Many days may be wasted trying to develop methods to resolve these issues . This time could otherwise be dedicated to productive science.
The mass spectrometry toolbox contains tools that are better suited to such tasks than just ever more powerful m/z-based separations. One recently developed tool is differential ion mobility mass spectrometry (DMS). DMS separates ions based on their dipole moment instead of by m/z. This provides an orthogonal technique for improving data quality in the quantitation and characterization of challenging samples requiring advanced analytical selectivity. We present here the capability to separate antibodies or antibody-drug conjugates from protein fragments, small molecule contaminants and smaller proteins using DMS on a modified Qq-TOF mass spectrometer. This technique can provide molecular weights accurately and quickly, even when the protein sample is confounded by other molecules. This technique is tunable to wide variety of molecular shapes and sizes. As such these experiments reveal a methodology that is likely to be generally applicable to rapid, intact molecular weight protein measurements of many proteins. The implications of this work are that organizations are likely to save days of method development time per protein, and have a better chance of accelerating assays that require intact protein molecular weight determination.

SelexION™ Technology for Orthogonal Separation of Proteins

- SelexION™ Technology is a planar differential mobility device (DMS) that separates ions based on differences in their chemical properties, prior to entering the instrument orifice, thus providing an orthogonal level of selectivity.[1,2]
- SelexION™ Technology has been successfully applied in studies of small molecules, including the separation of mixtures of isobaric compounds and isomers[3]
- In this study, SelexION™ Technology was utilized for the separation and characterization of proteins from other interfering ions/contaminants.

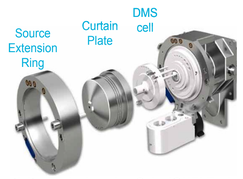

Figure 1. High Selectivity Analysis using SelexION™ Technology on a Modified Research TripleTOF® System. The SelexION™ Technology is an easy-to-install differential mobility separation (DMS) device that was installed on a research TripleTOF® System, attaching in front of the curtain plate. Gas draws the ions towards the orifice while a Separation Voltage (SV) in the form of an asymmetric waveform is applied to the flat plates, which alternates between high field, K(E) and low field, K(0). This moves the charged ion back and forth between plates, an ion will have net drift based on its high and low field mobility. A separation voltage (SV) is applied as the filtering voltage and the compensation voltage (CoV, a small DC offset between the plates) is applied as the restoring voltage, which can be tuned for the compound of interest. Other co-eluting species that tune with different compensation voltages will be filtered away.

MATERIALS AND METHODS

Sample Preparation: A mouse monoclonal antibody (Waters, USA) and an antibody-drug-conjugate (confidential) were dissolved in 10% ACN, 0.1% formic acid at a final concentration of 0.1 ug/µL. No additional cleanup was performed.

HPLC Conditions: Samples were analyzed using Eksigent ekspert™ ultraLC 100-XL System (Eksigent, USA). The peptides were directly loaded onto a 1 x 75 mm column (Zorbax Poroshell C8, 5 µm, Agilent) and an elution gradient of 10-90% acetonitrile (0.1% formic acid) over 15 min was used with a flow rate at 0.3 mL/min.

MS/MS Conditions: An AB SCIEX research TripleTOF® system equipped with Turbo V™ Source was used. The instrument was equipped with SelexION™ Technology for differential mobility separations. Compensation voltages (CoV) to isolate and transmit each protein were determined using on-column CoV mapping (Figure 2). The separation voltage (SV) was fixed at 2500 V, DMS temperature was set to low and no chemical modifier was introduced.

Data Processing: Results were analyzed using PeakView® Software. And Deconvolutions were performed using BioPharmaView™ Beta Software.

How SelexION™ Technology Separates Ions

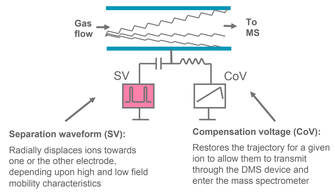

Figure 2. DMS SCHEMATIC. A separation waveform alternates between high field (higher mobility) and low field (lower mobility). A compensation voltage is tuned to allow your analyte of interest enabling its selective transmission through the cell.

RESULTS

Mouse Monoclonal Antibody – DMS Off

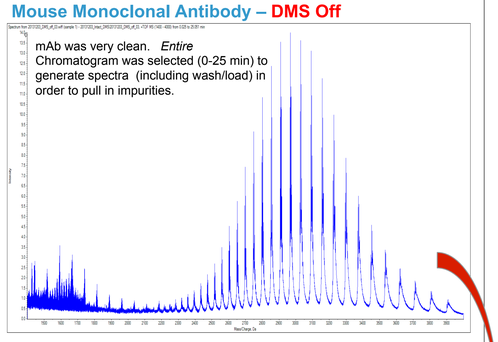

Figure 3. Intact mAb Spectrum with DMS Off. Note: this mAb was exceedingly clean, in order to demonstrate DMS separation, the entire chromatogram 0-25 min was selected to generate all spectra in Figures 3 and 4 to simulate DMS selectivity with a less pure sample.

Mouse Monoclonal Antibody – With DMS

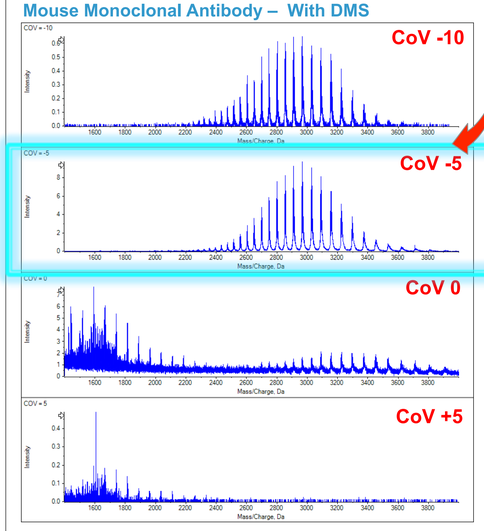

Figure 4. Intact mAb Specta at Four Separate DMS CoV Values. Note: Again, the entire chromatogram (0-25 min) was selected to generate all spectra in Figures 3 and 4. Spectra clearly demonstrate Pre-MS DMS benefit for orthogonal separation of mAb from impurities. CoV of -5 most selectively transmits the mAb through the SelexION™ device. All other instrument parameters were held constant for each of the above four spectra.

Mass Reconstruction of mAb from CoV -5 Spectrum

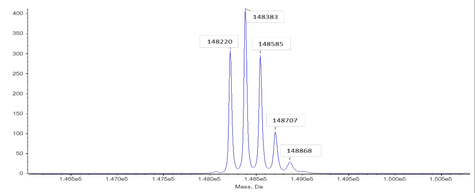

Figure 5. Deconvolution of MS spectra in Figure 4 panel 2. Use of DMS does not alter the relative abundance of glycoforms.

Antibody-Drug Congugate-A - DMS Off

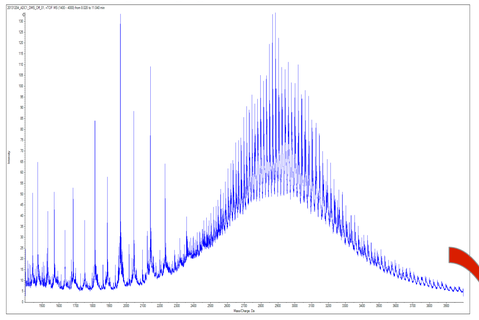

Figure 6. Intact ADC Spectrum with DMS off. This sample was shipped from a very distant collaborator and was held up in customs for two weeks. As such there are numerous breakdown products evident in the low *m/z* region of the above ADC spectra. This spectra was generated highlighting only the eluting peak.

Antibody-Drug Congugate A – With DMS

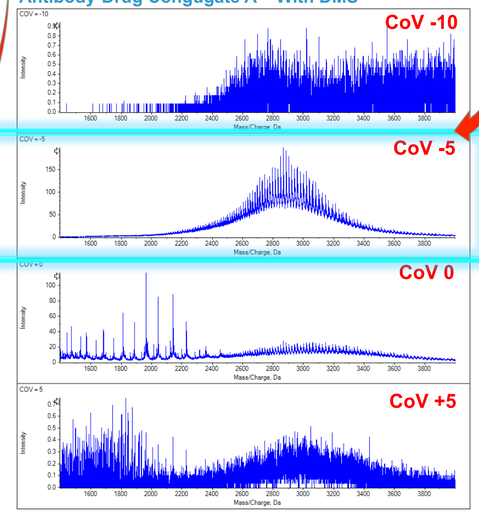

Figure 6. Intact ADC-A Spectra at Four Separate DMS CoV Values. This Lysine-linked ADC also selectively elutes from the DMS device at a CoV around -5. Smaller contaminants and contaminating fragments elute from the DMS device approaching positive CoV values. Retention time and all other instrument parameters were held constant for each of the four spectra.

Mass-Reconstruction of ADC-A from CoV -5 Spectrum

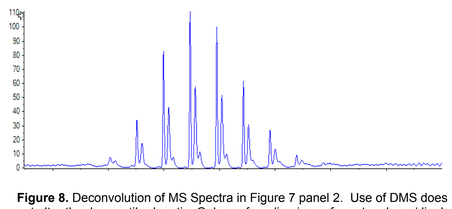

Figure 8. Deconvolution of MS Spectra in Figure 7 panel 2. Use of DMS does not alter the drug-antibody ratio. Only confounding ions of greater shape/dipole moment change are selectively transmitted apart from each other. Metadata and mass data obscured as this compound is still under development.

CONCLUSIONS

The mass measurement of intact proteins and their heterogeneity is important for organizations that wish to present a consistent and reliable protein drug product.

- Differential Mobility Separation (DMS) provides an orthogonal level of selectivity by separating components based on their chemical properties and mobility.
- Intact monoclonal antibody and antibody-drug conjugates may be separated rather dramatically from co-eluting smaller contaminating ions in the m/z range of small proteins, peptides and small molecule drug or drug-linker fragments.
- DMS settings will vary on a per-compound basis, however a trend is apparent by which very large proteins are transmitted by CoV around -5.
- Tuning DMS for each compound was very fast and easy.
- Implications of this work are that a CoV could be tuned to each protein therapeutic and that SelexION™ technology could be used to ameliorate the sample prep and method development hurdles to performing routine intact mass analysis on monoclonal antibody therapeutics and antibody-drug conjugates.

References

1. Schneider *et al*, (2010) Planar Differential Mobility Spectrometer as a Pre-Filter for Atmospheric Pressure Ionization mass Spectrometry, *Int. J. Mass Spectrometry*, **298 (1-3)**, 45-54.
2. AB SCIEX SelexION™ Technology: A New Solution to Selectivity Challenges in Quantitative Bioanalysis - Differential Mobility Separations Enhanced with Chemical Modifiers: A New Dimension in Selectivity. AB SCIEX Technical Note, Publication 2960211-01.
3. Schneider *et al.*, (2010) Chemical Effects in the Separation Process of a Differential Mobility/Mass Spectrometer System, *Analytical Chemistry*, **82**, 1867-1880.

TRADEMARKS/LICENSING

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