Characterization of Biologic Compounds with Differential Mobility and SWATH® Acquisition

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ABSTRACT

Full characterization of a therapeutic protein generally requires the use of strong chemical separation that can be challenging to achieve with liquid chromatography (LC) alone. In response to this challenge, we introduce DMS with SWATH® acquisition, a technique which combines differential mobility separation (DMS) with LC by leveraging SWATH® acquisition for the analysis of single-protein digests. This allows a significant improvement in MS/MS spectral quality and recovery, resulting in a 12-25% increase in MS/MS verified sequence coverage. DMS with SWATH® acquisition also resolves peptides which co-eluted and whose masses place them within the same SWATH® acquisition window. DMS with SWATH® acquisition is a powerful new means of untargeted data acquisition which we propose will have a large impact on the analysis of biopharmaceuticals like monoclonal antibody characterization.

INTRODUCTION

Full characterization of therapeutic monoclonal antibodies (mAbs) is an essential step in the production, validation and clinical use to verify safety and efficacy. These samples are typically evaluated by liquid chromatography in conjunction with mass spectrometry (MS) and/or tandem mass spectrometry (MS/MS). But, even the most advanced one-dimensional LC separation is not always sufficient. Additional separation methods must be employed and gas-phase separation methods like DMS are emerging as useful alternatives which do not add excessive time to an analysis like two-dimensional chromatography would.

DMS has been shown to be applicable in protein and peptide separations making it an ideal candidate to investigate.[1] In DMS, a radio-frequency alternating separation voltage (SV) is applied to two parallel plates.[2] Ions are pushed through the plates by a buffer gas, where they move perpendicularly to the gas flow in response to the SV and as a function of their mobility, toward one of the plates. A DC compensation voltage (CV) is applied to pull ions back toward the region between the plates, in line with the inlet of the mass spectrometer. Separation can be improved through the use of chemical modifiers.[3]

Here, we introduce DMS with SWATH® acquisition, a new method for combining LC with DMS for the analysis of protein digests. First, a 'DMS transparent' scan is performed to identify peptides from protein digests by their precursor ion. Then, SWATH® acquisition is employed in conjunction with DMS separation to capture MS/MS spectra of product-ion species from the survey scan. We use a large SWATH® acquisition window with DMS separation at a fixed SV while scanning CVs. In so doing, we trade the mass separation of the SWATH® acquisition window for the LC-orthogonal separation provided by DMS. This technique allows for an increase in MS/MS validation and spectral quality in a typical mAb digest over traditional data-dependent acquisition or SWATH analysis and it can effectively sort out co-eluting components of similar *m/z*.

MATERIALS AND METHODS

Samples

Standard trypsin digests of proteins (Waters mAb Standard, NIST mAb Standard, Fetuin, and Bovine Serum Albumin (BSA)) were prepared and diluted to 1 pmol/µL. KGAILKGAILR (KGAIL) was prepared at 1 pmol/µL for infusion analysis at a flow rate of 10 µL/min and it was also added to a sample of digested BSA for cluster analysis.

Mass Spectrometry

DMS experiments had fixed separation voltages (SV) of 3500 V with CV ranges of 5-16 V for analysis without ACN modifier and from -13-9 V with ACN modifier. Modifiers were introduced to the curtain gas by SelexION® technology at 1.5% (low) of the total gas flow.

LC-MS

LC-MS was performed using a Shimadzu Prominence LC system with a Phenomenex Aeris 2.6 µm PEPTIDE XB-C18, 100 x 2.1 mm column using a gradient elution of eluent A (Water/ACN/FA 97.9/2/0.1%) and eluent B (ACN/Water/FA 97.9/2/0.1%) over 20 minutes with 10 minutes of equilibration post run at a conserved flow rate of 0.5 mL/min. MS was mainly performed on a SCIEX TripleTOF® 5600+ with NIST mAb experiments performed on a TripleTOF® 6600 System (SCIEX).

Data Acquisition

Data were collected using four separate acquisition modes: information dependent acquisition (IDA), SWATH® acquisition (18 separate 50 *m/z* windows), 3 separate SWATH® acquisitions each at unique DMS CoV throughout, and DMS with SWATH® acquisition. Combined DMS and SWATH® analysis involved a standard SWATH method run with DMS enabled at three different CVs: 8, 12, and 16 V. These datasets were then combined into a single result. DMS with SWATH® acquisition (**Figure 1**) involves an MS-level survey scan followed by a ramping of CVs from 8-16 V over a 350-1250 m/z SWATH windows to produce MS/MS data. The data is then split into individual files using a custom plugin for PeakView® Software (v.1.2.2.0 research version) containing the survey scan and the MS/MS data from a single CV. All of these files are then analyzed simultaneously to determine the result. When operating on a TripleTOF 6600 system, the SWATH acquisition windows were increased to a maximum of 2250 m/z incorporating the increased Q1 width of the instrument. BioPharmaView™ Software v. 2.0r3682 was used to determine all sequence coverage values and to score the matched peptide spectra.

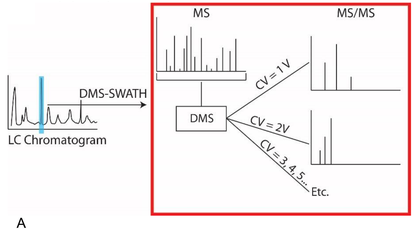

Figure 1: DMS with SWATH® acquisition eschews the mass selectivity of both IDA and SWATH® acquisition for selectivity and separation based on DMS.

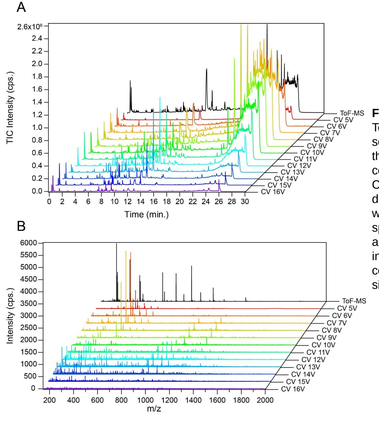

Figure 2: Each experiment consists of a ToF-MS survey scan (black) and 12 DMS separated MS/MS spectra obtained through SWATH® acquisition (multi-coloured), each at a unique CV **(A)**. Certain components are emphasized at different CV values. A 350-1250 *m/z* window from the survey scan mass spectrum (black) is separated by DMS across a CV range of 5-16 V (in 1 V increments) then fragmented by CID (multi-coloured) (**B**). Spectra are obtained at a single retention time: 10.9 min.

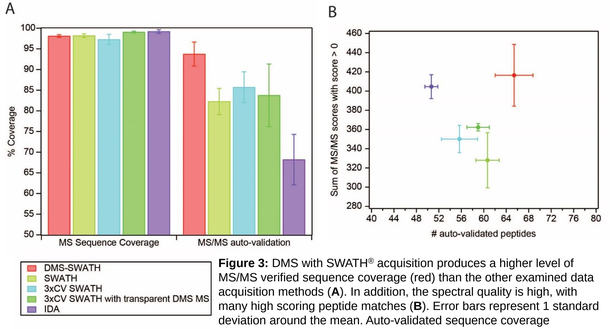

Figure 3: DMS with SWATH® acquisition produces a higher level of MS/MS verified sequence coverage (red) than the other examined data acquisition methods (**A**). In addition, the spectral quality is high, with many high scoring peptide matches (**B**). Error bars represent 1 standard deviation around the mean. Auto-validated sequence coverage improvements for both Fetuin and NIST mAb were 5%.

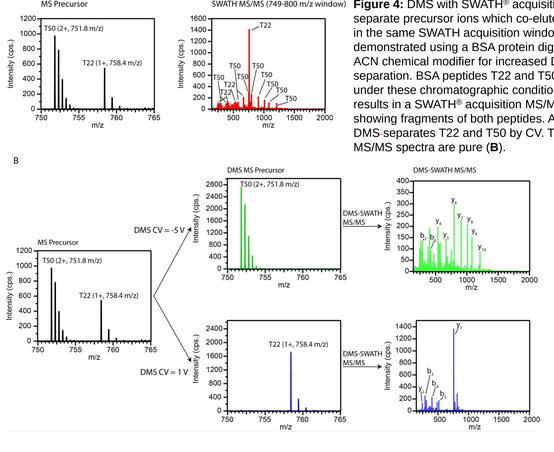

Figure 4: DMS with SWATH® acquisition can separate precursor ions which co-elute and exist in the same SWATH acquisition window. This is demonstrated using a BSA protein digest with ACN chemical modifier for increased DMS separation. BSA peptides T22 and T50 co-elute under these chromatographic conditions (**A**). This results in a SWATH® acquisition MS/MS spectrum showing fragments of both peptides. Applying DMS separates T22 and T50 by CV. The resulting MS/MS spectra are pure (**B**).

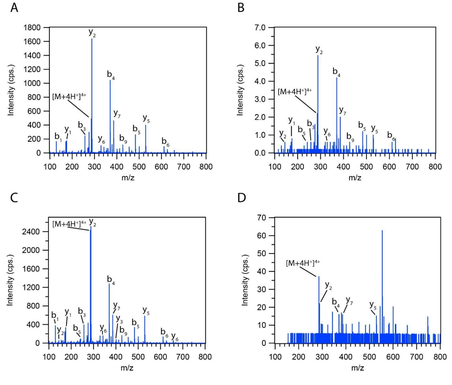

Figure 5: The used of gas phase modifiers in DMS can improve peak capacity. But, these modifiers can form unwanted, persistent clusters with analytes while exiting the cell. SWATH® acquisition based techniques can alleviate these clusters which prevent proper MS/MS isolation. The direct infusion MS/MS spectra of a model peptide (KGAILKGAILR, **A**) is not recoverable by isolating the expected precursor ion (285.6 *m/z*) when ACN is added to the DMS system (**B**). Utilization of SWATH® acquisition methods can recover the correct MS/MS spectra by using a SWATH® acquisition window (280-330 *m/z*) which encompasses the expected precursor and additional m/z values of ACN adducts (**C**). DMS with SWATH® acquisition enables recovery of some MS/MS fragments from the peptide spiked into a BSA digest sample and processed with LC (**D**).

CONCLUSIONS

DMS with SWATH® acquisition is a new method of untargeted analysis for protein digests. The combination of LC and DMS separation with SWATH® data acquisition allows for an improvement in MS/MS auto-validation as demonstrated by the evaluation of a model mAb digest. In addition, the inclusion of DMS separation can resolve co-eluting peptides which are found in identical SWATH® acquisition windows as well as providing a means of breaking clusters formed by chemical modifiers. The results reported here represent the first step in evaluating this method of untargeted analysis, focusing only on simple, single protein digests. Future work is necessary to explore the applicability of DMS with SWATH® acquisition for other types of analysis.

REFERENCES

1. Swearingen *et al. Mol. Cell. Proteomics* (2012), 11, 4, M111.014985.
2. Schneider *et al. Mass Spectrom. Rev.* (2016), 35, 6, 687-737.
3. Schneider *et al. Eur. J. Mass Spectrom.* (2010), 16, 1, 57.

TRADEMARKS/LICENSING

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