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Drug Discovery and Development

SCIEX

SWATH® –Data Independent Acquisition for Confidence in Peptide Mapping for Biotherapeutics Characterization

SWATH® Solution Featuring the SCIEX X500B QTOF System

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Key Challenges in Biotherapeutics Characterization

- Comprehensive coverage of target sample.
- Identification and confirmation of low abundant attributes.
- Quantification of slight changes in the sequence modification.

Key Features of the SCIEX SWATH® Acquisition

- Single generic method for all analytes.
- Unbiased acquisition of MS/MS data of all the possible *m/z.*
- Broader dynamic range of detected signals.
- Improved reproducibility and better sensitivity.
- Ability to identify and quantify low abundance modification with BioPharmaView™ software.

Introduction

Peptide mapping is now considered a routine analytical procedure when analyzing biologics. It allows in-depth characterization of large biomolecules, enabling identification of all wanted and unwanted post-translational
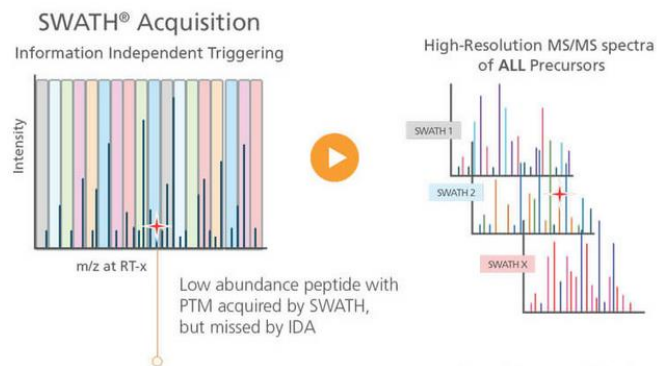

Figure 1: SCIEX SWATH® Acquisition Workflow for comprehensive coverage of sample

modifications to ensure that the product quality, safety and efficacy are not compromised. The importance of being able to observe and identify these low-level modifications is imperative to ensure that the product quality and safety is maintained.

Here, we present data pertaining to analysis of Trastuzumab using SWATH, the SCIEX data independent acquisition (DIA) solution. SWATH is an unbiased acquisition method which allows wider Q1 isolation windows across the entire m/z range, collecting full MS/MS spectra on all detectable analytes, ensuring full in-depth coverage of the sample. Unlike conventional data dependent acquisition (DDA), SWATH is not limited by the need to set filtering criteria for precursor ion selection and peak picking of co-eluting precursor ions during a peptide mapping workflow. This provides better detection and reproducible quantitation of low-level analytes in each sample.

Experimental

Sample Preparation:

Trastuzumab was purchased from Myoderm (Norristown, PA, USA). ProteaseMax™ and trypsin were purchased form Promega (Madison WI, USA). The antibody was denatured using ProteaseMax™ (Promega, Madison WI, USA) in 50 mM Tris pH 7.8 buffer. The samples were reduced and alkylated using DL-dithiothreitol and 2-iodoacetamide (Sigma Aldrich). Trypsin was added in the ratio 1:25 and samples were incubated at 37°C for 3hrs before acidifying with TFA. Samples were centrifuged to remove any particulate before putting them into autosampler vials for analysis.

HPLC Conditions:

An ExionLC™ system with a Phenomenex Kinetex 1.7 μm C18 100 Å 50 x 2.1 mm column at 40°C with mobile phase

A: water + 0.1 % formic acid and mobile phase B: acetonitrile + 0.1 % formic acid was used at a flow rate of 250 µl/min. A volume of 8 µl was injected and separated with a gradient of 5 to 40 % B over 25 min.

MS Conditions:

SCIEX X500B QTOF system with IonDrive™ source and Electrospray Ionization (ESI) probe was used. For SWATH® acquisition a 150 ms TOF-MS scan time was used followed by 28 SWATH windows of 50 ms.

Data Processing:

Data was processed using BioPharmaView™ software using the default parameters. Replicate injections were processed as a batch.

Results and Discussion

Trastuzumab was acquired in triplicate using SWATH acquisition under identical LC conditions. Overlay of all three chromatograms in Figure 2A shows the robustness and stability of the LC as well as the X500B QTOF system
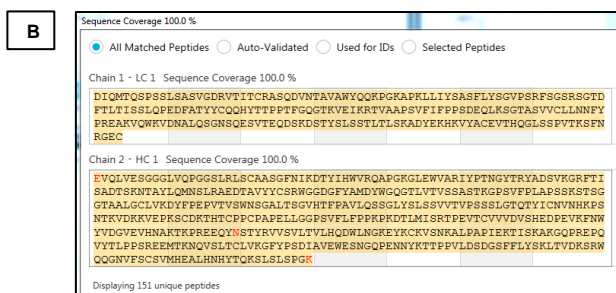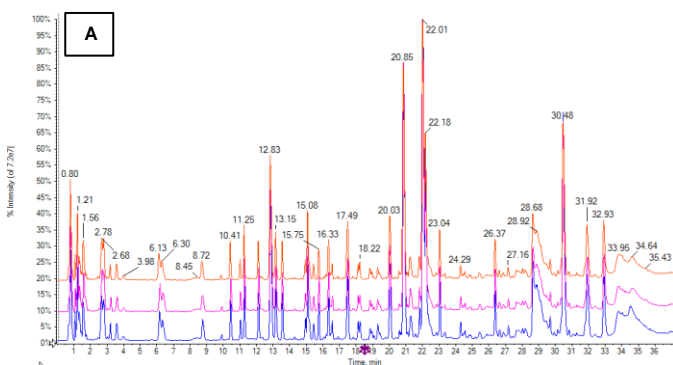
Figure 2. (A) Overlaid TIC spectra for the replicate injections of Trastuzumab. (B) Similar sequence coverage was obtained for the replicates.

All samples showed comprehensive sequence coverage of >99% and majority of the modifications frequently observed in tryptic digest of trastuzumab was identified within 5ppm mass tolerance (Figure 2B).

Data acquisition with SWATH enabled identification of low abundant peptides or modifications. For instance, deamidation on peptide EEQYNSTYR was identified at 2.44 min (Figure 3). The modified peptide was detected in all 3 samples with an average modification amount of 0.1% (Table 1). The identification of this modification is often challenging in DDA approaches due to the co-eluting high abundant glycoforms. The SWATH acquisition is not limited due to the cycle time, precursor intensity and co-eluting precursors, thus getting enough time to capture MSMS information on both high and low abundant precursors, which is essential for identification.
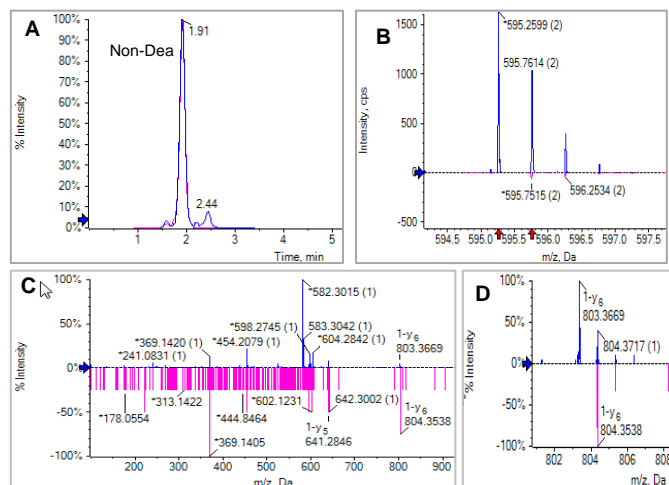

Figure 3 (A) XIC (B) SWATH MS (C) MS/MS and (D) y6 -fragment ion spectra for the non-deamidated (blue) and deamidated (pink) forms of the peptide EEQYNSTYR.

	Sample	% modification	Average	% CV
EEQYNSTYR	1	0.11%		
	2	0.12%	0.11%	5.1%
	3	0.11%		
FNWYVDGVEVHNAK	1	0.40%		
	2	0.45%	0.44%	8.2%
	3	0.47%		

Table 1: Relative Percent Deamidation levels for EEQYNSTYR and FNWYVDGVEVHNAK as detected by BiopharmaView software.

Another low-level of deamidation was identified on another peptide with the sequence FNWYVDGVEVHNAK using BioPharmaView (Figure 4).

on very low abundant attributes is critical for increasing the confidence in assigning the sequence identity as well as identifying potential critical quality attributes of a biomolecule.
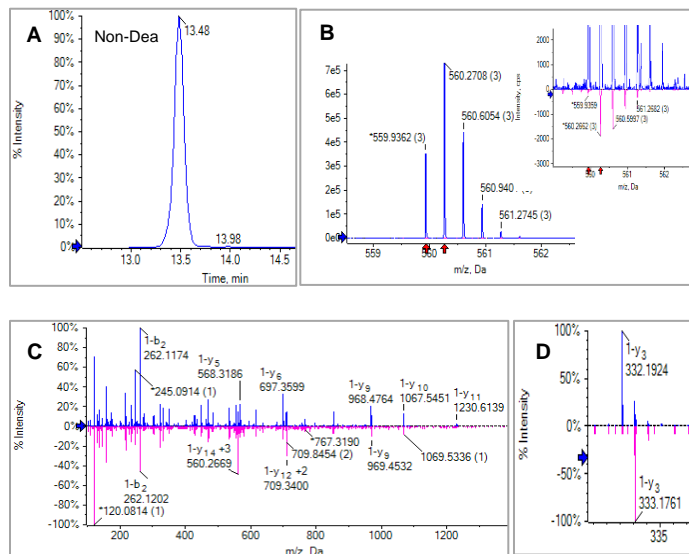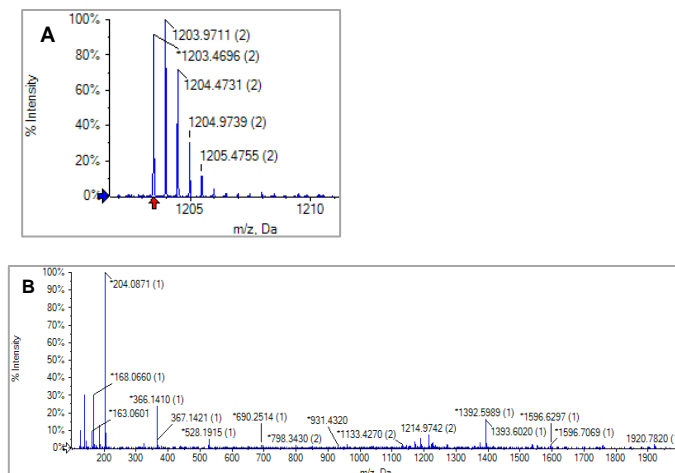

Figure 4: (A) XIC (B) SWATH –MS (C) MSMS acquisition for FNWYVDGVEVHNAK peptide showing non-deamidated (blue) and deamidated (pink) with (D) expanded spectra showing exact deamidation site.

Figure 5. (A)TOF-MS of doubly charged peptide of Mannose 5 modified EEQYNSTYR. (B) MS/MS data for the doubly charge peptide containing Mannose 5.

Next, complex glycosylation on EEQYNSTYR peptide was evaluated. Data processing through BioPharmaView, revealed low level mannose-5 glycosylation on the peptide. With SWATH acquisition confirmation of this glycosylation event was possible due to high quality of the MS/MS data (Figure 4B). The SWATH doesn't require any set fragmentation criteria and provides high quality MSMS data irrespective of the precursor intensity thus resulting in high confidence in the peptide identification and glysosylation profiling.

Conclusion

SWATH acquisition solution provides near complete sequence coverage for trastuzumab. SWATH yields high resolution XICs on fragment ions, reduced chance of interferences, provide greater degree of confidence in identification and confirmation of low abundant modifications. This ability to obtain high quality MS/MS data

References

1. MS/MSALL with SWATH™ Acquisition – Comprehensive Quantification with Qualitative Confirmation using the TripleTOF® 5600+ System. AB SCIEX Technical note, 3330111-03.

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