Reproducible Peptide Mapping of Biotherapeutics with SWATH® Acquisition on the TripleTOF® 6600 System and BioPharmaView™ Software

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Peptide mapping is one of the most common workflows in the characterization of biotherapeutics. Peptide maps provide suitable data for confirmation of sequence, identification, and localization of modifications comparing to other workflows. In particular, when a peptide map separation is coupled with mass spectrometry, conclusive evidence at both the MS and MS/MS level is readily obtained to provide high confidence peak identification.

In this work, the use of the TripleTOF® 6600 system with BioPharmaView™ 2.1 processing software for peptide mapping of a monoclonal antibody is presented. Data was acquired using SWATH® acquisition to improve confidence in primary sequence characterization and for localization of targeted post-translational modifications. Data processing is accomplished using BioPharmaView software for primary sequence confirmation, identification, and quantification of targeted post-translational modifications. Overall, this solution provides a powerful workflow for rapid peptide map characterization, batch processing, and offers the necessary flexibility for more advanced workflows.

Key Features

- Using SWATH® acquisition, a data independent technique, captures complete MS/MS data for all detectable components in every sample for better sequence coverage
- Use of SWATH acquisition enables MS/MS confirmation of low level components often missed using data dependent analysis complete sequence coverage
- Streamlined data processing for targeted biotherapeutic characterization and batch processing of sample data

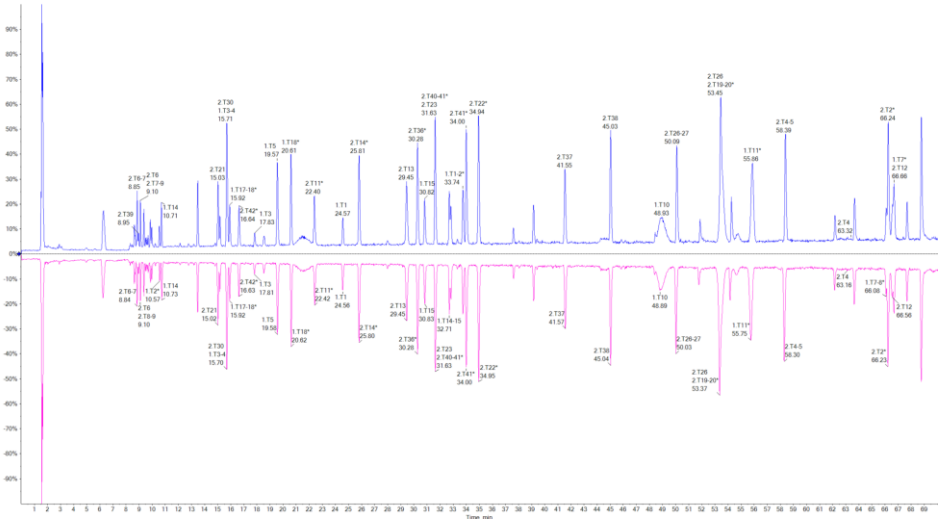

Figure 1. Peptide Mapping LC Traces. Mirror plot of standard (top) and test sample (bottom) peptide maps collected a SCIEX TripleTOF 6600 system and processed with BioPharmaView 2.1 software. Excellent agreement on retention time and peak identification observed between samples.

Experimental

Sample Preparation: NIST mAb standard (#RM8671) was purchased from NIST. An aliquot of 10 µl was taken and subjected to reduction with 10 mM DTT at room temperature for 30 minutes. The sample was then alkylated with iodoacetic acid at 20 mM for 20 minutes in the dark at room temperature. It was then digested with trypsin (Roche, sequence grade) for 30 min at 37 °C followed by quenching with TFA and used directly.

Chromatography: Separation was accomplished using an ExionLC™ system fitted with a 2.1×150 mm Agilent ZORBAX 300 SB-C18 , 1.8 µm column at 50°C with the gradient shown in Table 1. Mobile phase A was 0.1% formic acid in water and mobile phase B was 0.1% formic acid in acetonitrile.

Table 1. LC Gradient.

Time (min)	%A	%B
0	*99*	*1*
5	*99*	*1*
6	*90*	*10*
70	*65*	*35*
75	*10*	*90*
80	*10*	*90*
80.5	*99*	*1*
81	*99*	*1*
83.5	*90*	*10*
91.5	*55*	*45*
93	*10*	*90*
99	*10*	*90*
101	*99*	*1*
115	*99*	*1*

Mass Spectrometry: Mass spectrometric detection was accomplished with a SCIEX TripleTOF® 6600 system fitted with the DuoSpray™ ion source. MS analysis was performed using SWATH® acquisition. In this acquisition example, the instrument stepped through fixed 60 amu wide precursor window from m/z of 300-1800 and high-resolution MS/MS spectra were acquired from m/z of 100 -1800. The total scan time was 1.35 seconds. The MS instrument conditions are listed in Table 2.

Table 2. MS Parameters.

Parameter	Setting
Scan Mode	*Positive*
GS1	*60*
GS2	*60*
Curtain Gas	*55*
Temperature	*300 °*
Ion Spray Voltage	*5200 V*
Time Bins to Sum	*4*
Accumulation Time (MS)	*50 msec*
Accumulation Time (MS/MS)	*50 msec*
Declustering Potential	*50*
Collision Energy Spread	*5*

Discussion

Characterization of the peptide digest commenced with the analysis of the NIST antibody standard. As shown in Figure 2, a high-quality total ion chromatogram was obtained using SWATH® acquisition. The data was processed using BioPharmaView™ software which automates the identification and validation of assignments based on MS and MS/MS data collected during the experiment. Overall, the sequence coverage of the peptide map was greater than 99% as shown in Figure 3.

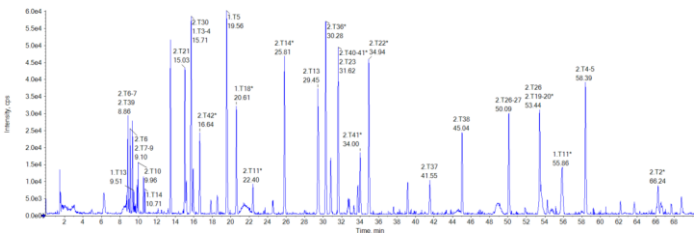

Figure 2. Peptide Map for Characterization of NIST Antibody. Standard data from TripleTOF® 6600 system processed with BioPharmaView software.

Following the initial characterization, analysis of a second tryptic digest was completed and batch analyzed using BioPharmaView 2.1 software. The two protocols were identical and the purpose of the study was to verify overall assay performance from digestion through data processing. As shown in Figure 1, there is very good agreement between the two samples with the alignment of the peak intensities and retention times for the two

Chain 1 - LC Sequence Coverage 98.6%

DIQMTQSPSTLSASVGDRVTITCSASSRVGYMHWYQQKPGKAPKLLIYDTSKLASGVPSRFSGSGSGTEFTLTISSLQ
DFATYYCFQGSGYPFTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNS
SVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Chain 2 - HC Sequence Coverage 100.0%

QVTLRESGPALVKPTQTLTLTCTFSGFSLSTAGMSVGWIRQPPGKALEWLADIWWDDKKHYNPSLKDRLTISKDTSKN
VLKVTNMDPADTATYYCARDMIFNFYFDVWGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT
WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELL
PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN
EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP
LDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Figure 3. Sequence Coverage for NIST Peptide Map from BioPharmaView 2.1 Software Processing.

different sample analysis. More importantly, overall sequence coverage and auto-validated results were nearly identical across both analyses.

Within a typical peptide map, the dynamic range required to detect components, particularly those that correlate to post-translational modifications, can easily span over 3 orders of magnitude in intensity. It is important to achieve suitable data quality at the MS and MS/MS level for all detectable components. To highlight the quality of the MS/MS data collected using SWATH acquisition, Figures 4 and 5 show mirrored fragment ion spectra for low and high responding peptides, respectively. The fragment ion spectra are annotated with drop lines indicating predicted b and y ions which correlate with the observed spectral peaks. Importantly, both the fragment ions present and their intensities are consistent between experiments.

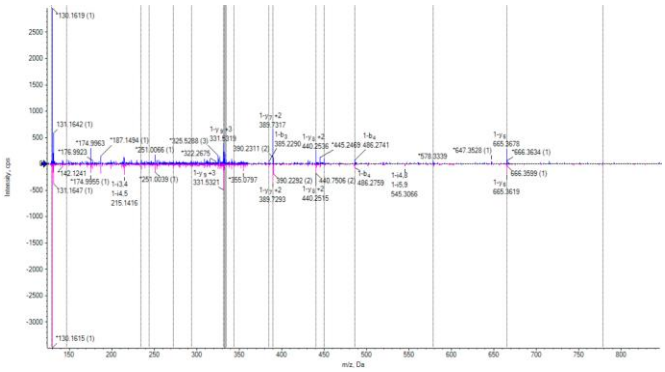

Figure 4. Fragment Ion Spectrum for Lowest Intensity Missed Cleavage Peptide (DRLTISKDTSK) identified in NIST Peptide Map Data.

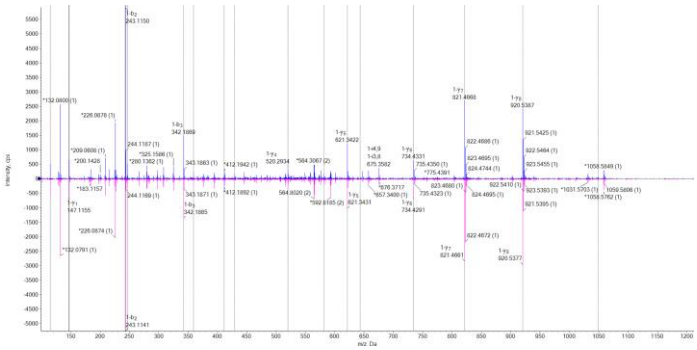

Figure 5. Fragment Ion Spectrum for Highest Intensity Peptide (NQVSLTCLVK) identified in NIST Peptide Map Data.

Conclusion

This study demonstrates the use of the TripleTOF 6600 system and SWATH acquisition coupled with BioPharmaView software processing for biotherapeutic peptide mapping analysis. High reproducibility between separate digests and acquisition sets coupled with high-quality MS and MS/MS makes it easy to unambiguously assign components with high sequence coverage for confidence in the results.

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