COMPREHENSIVE PEPTIDE MAPPING FOR THERAPEUTIC PROTEINS BY A LC/MS^E APPROACH

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Weibin Chen and Asish B. Chakraborty
Biopharmaceutical Sciences, Waters Corporation, 34 Maple Street, Milford MA 01757, USA

OVERVIEW

- An integrated workflow featuring high-resolution UPLC separation, multiplexed MS data acquisition (MS^E), and automatic batch processing of LC/MS peptide mapping data by BiopharmaLynx, is developed for comprehensive protein characterization.
- Highly reproducible, fully annotated peptide maps for therapeutic proteins can be routinely obtained using the workflow. The performance of the workflow is demonstrated in the poster.
- The workflow enables efficient monitoring of low-abundance protein modifications in the same run as peptide profiling, therefore providing a comprehensive peptide mapping solution to the biopharmaceutical industry and reducing weeks of labor to a few hours.

EXPERIMENTAL METHODS

Samples:
– RapiGest SF™ aided Trypsin digested IgG

Instrumentation:
– Liquid chromatography, ACQUITY UPLC®
– Mass spectrometer, Xevo™ QTof or Synapt™ G2

Columns:
– Peptide Separation Technology, ACQUITY UPLC® C18 BEH300, 1.7µm, 2.1×150 mm

Informatics:
– BiopharmaLynx™ ver. 1.2 application manager

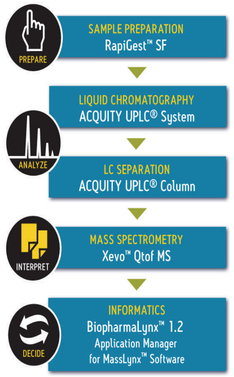
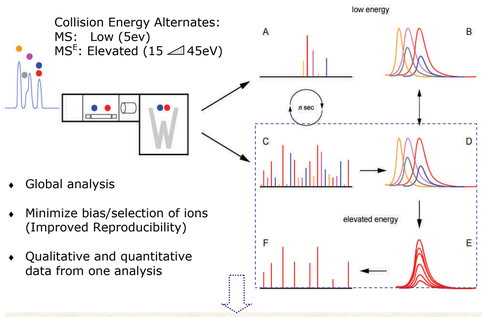
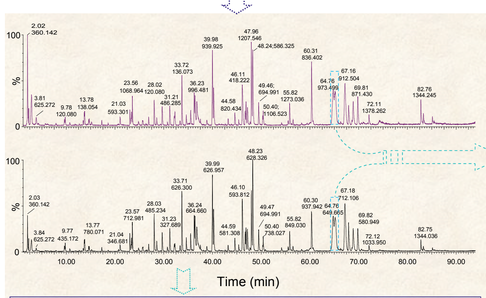


Figure 1. Waters biopharmaceutical LC/MS system: ACQUITY UPLC® LC, Synapt™ G2 MS, and BiopharmaLynx Informatics

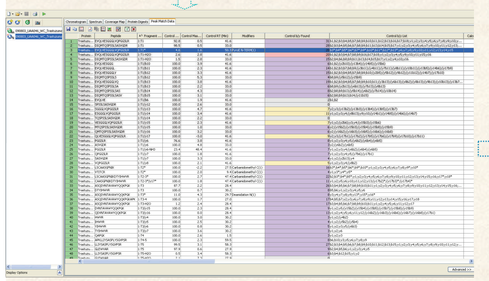
MS^E: An Alternate Scanning Methodology for Acquiring Peptide and Fragmentation Data



- Global analysis
- Minimize bias/selection of ions (Improved Reproducibility)
- Qualitative and quantitative data from one analysis

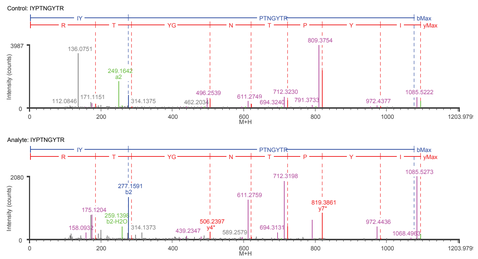


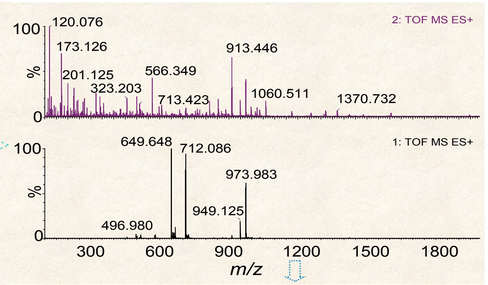
BiopharmaLynx processed Xevo QTof data displaying MS and MS/MS (MS^E) information. Fragment ions (b/y ions) are identified and listed in the Peak Match Data Table.



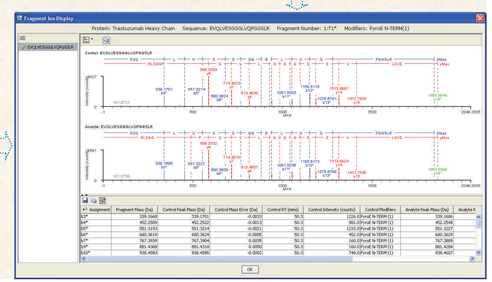
Identify and Confirm Low-Level Peptide Modifications by MS/MS (MS^E) Data:

Peptide fragmentation spectra from BiopharmaLynx confirm the presence of a deamidated form of peptide, IYPTNGYTR, at 0.5% level and reveal the modification site.



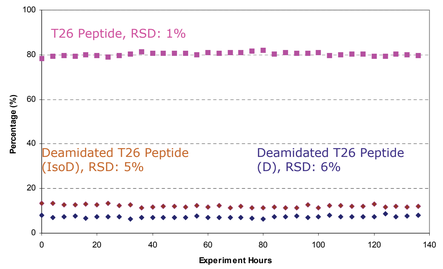


Fragment ions (b/y ions) are identified and displayed in BiopharmaLynx to validate MS peak assignment.

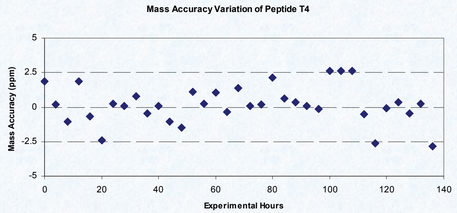


Highly Reproducible Peptide Mapping Results

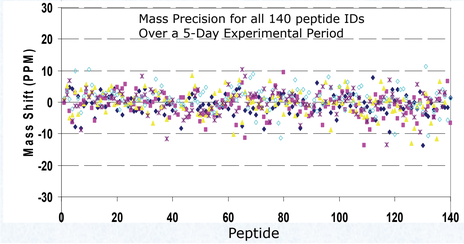
Consistent Measurement of the Abundance of Peptides and Their Variants



Steady and Accurate Mass Measurement for Peptides over 5-Day Experimental Time

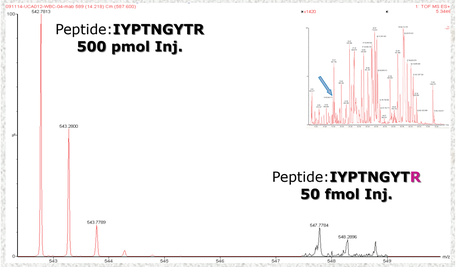


Peptide mass shifting from a Xevo QTof mass spectrometer for 140 peptide identifications over a period of 5 days. All the measurements were made based on a single MS instrument calibration. Masses for each of the peptides was repeatedly measured over the experiment time to track the changes of the detected peptide masses. On average, the measured peptide masses show less than 5 ppm variation during the course of the study.

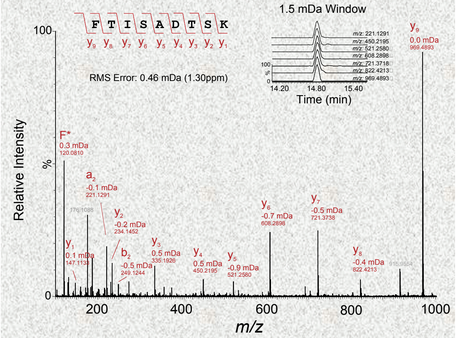


PEPTIDE MAPPING by SYNAPT G2

1. Over four (4) orders of magnitude in-spectrum linear dynamic range



2. Accurate Mass Measurement for MS/MS Data at High Data Acquisition Rate (10 spectra/sec)



CONCLUSION

– High-performance routine characterization of therapeutic proteins can be performed with an integrated workflow featuring
  - Waters ACQUITY UPLC® LC System
  - ACQUITY UPLC Columns (1.7µm particles)
  - Xevo™ QTof /Synapt G2 Mass Spectrometer
  - BiopharmaLynx™ informatics

– Peptide mapping by a LC-MS^E approach that is enabled with BiopharmaLynx offers a complete solution (workflow) for advanced characterization of biopharmaceuticals.

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