TOWARDS FAST MAPPING OF PROTEIN DISULFIDE BONDS: AN INTEGRATED WORKFLOW FOR AUTOMATIC ASSIGNMENT OF DISULFIDE PAIRING

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

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OVERVIEW

- An integrated workflow featuring high-resolution UPLC® separation, multiplexed MS data acquisition (MS^E), and automatic batch processing of data by BiopharmaLynx™, is developed for fast mapping of protein disulfide bonds
- Highly reproducible, fully annotated peptide maps for therapeutic proteins can be routinely acquired using the workflow
- The workflow enables automatic assignment of disulfide bonded peptides in the same run as peptide profiling

EXPERIMENTAL METHODS

Samples:
– RapiGest SF™ aided LysC digested IgG1

Instrumentation:
– Liquid Chromatography, ACQUITY UPLC®

– Mass spectrometers Xevo® G2 QTof or SYNAPT® G2

Columns:
– Peptide Separation Technology, ACQUITY UPLC® C4 BEH300, 1.7µm, 2.1×150 mm

Informatics:
– BiopharmaLynx™ v. 1.2 application manager

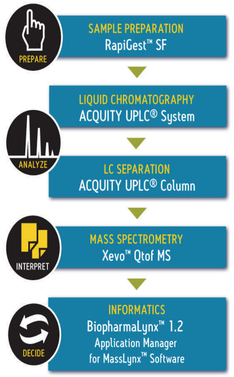
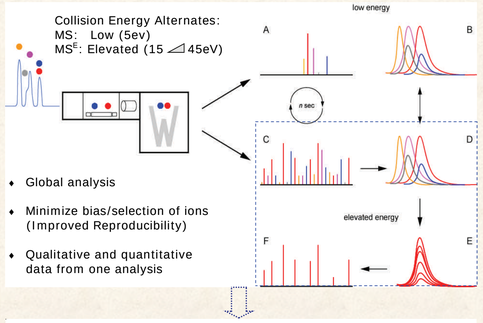
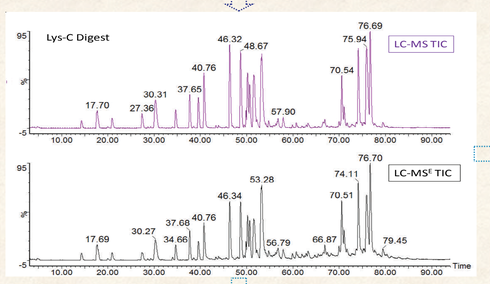


Figure 1. Waters biopharmaceutical LC/MS system: ACQUITY UPLC LC, Xevo G2 QTof, and BiopharmaLynx Informatics

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

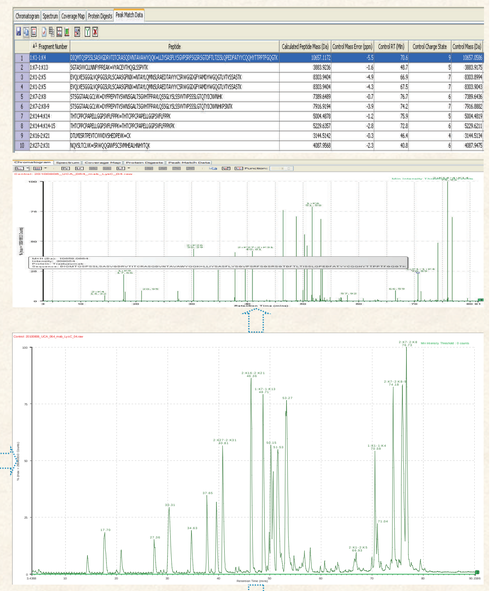


- Global analysis
- Minimize bias/selection of ions (Improved Reproducibility)
- Qualitative and quantitative data from one analysis

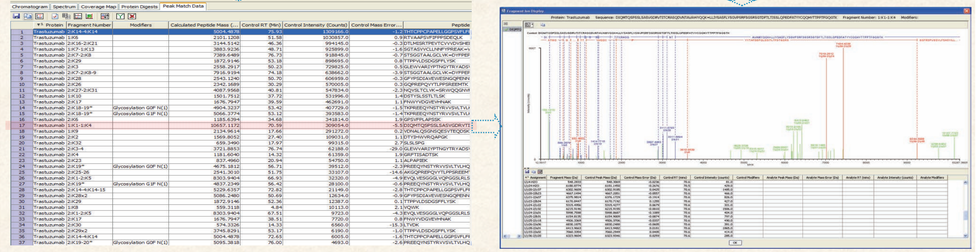


Xevo G2 QTof data processed by BiopharmaLynx displaying MS and MS/MS (MS^E) information. Fragment ions (b/y ions) are identified and listed in the Peak Match Data Table.

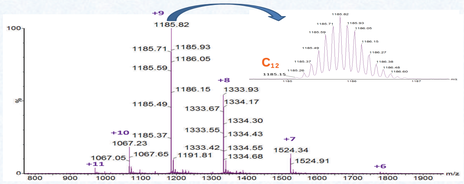
Identify and Confirm Disulfide Pairing using LC-MS^E Data: Peptide Table Showing the Assignment of S-S Linked Peptides



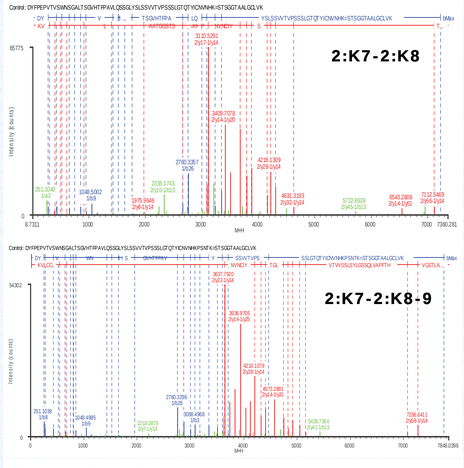
Fragment ions (b/y ions) are identified and displayed in BiopharmaLynx to validate MS peak assignment of disulfide pairing.



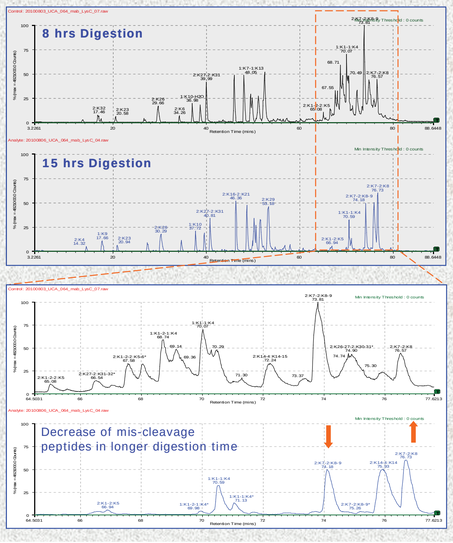
The combination of high resolution TOF MS with wide dynamic range enables the detection of larger disulfide bonded peptides



Automatic Annotation of the Fragmentation Peaks (MS^E) for Fast Confirmation of Disulfide Bond Linked Peptides



Influences of Sample Preparation on the Identities of Disulfide Bonded Peptides



CONCLUSIONS

- Fast mapping of disulfide linked peptides has been achieved using LC-MS^E methodology coupled with BiopharmaLynx informatics.
- Assignment of disulfide bonded peptides is automated, based on accurate MS measurement and confirmed by high-energy MS^E fragmentation data.
- Effective sample preparation in combination with robust analytical workflow facilitates routine analysis of disulfide bonded peptides within a non-reduced peptide map.
- Extension of this work for automatic detection of scrambled disulfide bonded peptides is in progress.

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