AN INTEGRATED WORKFLOW FOR AUTOMATIC MAPPING OF DISULFIDE LINKAGES OF THERAPEUTIC PROTEINS USING HIGH-RESOLUTION LCMS, ETD FRAGMENTATION AND TARGETED INFORMATICS

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OVERVIEW

- Disulfide bond formation is critical for establishing three dimensional folding and maintaining proper function of therapeutic proteins.
- Localization and assignment of disulfide bonds are therefore an important aspect in protein structural analysis.
- In this study, non-reduced peptide maps were acquired and processed for several therapeutic proteins using Waters Biopharmaceutical System Solution with Unifi.
- ETD technique was used to induce both disulfide bond cleavage and backbone fragmentation
- This integrated approach, combining high performance LC-MS^E, ETD and targeted software, should be applicable for fast mapping and monitoring of disulfide linkages in the development of therapeutic proteins

Figure 1. Biopharmaceutical System Solution with UNIFI for disulfide bond mapping encompasses automated UPLC/MS^E Xevo G2-S QTof data acquisition, data processing, reporting and report sign-off tools.

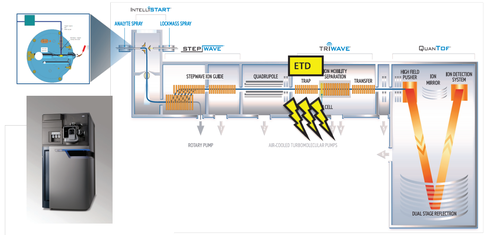


Figure 2. ETD spectra were collected using a Synapt G2-S with ETD capability

Methods

Waters Biopharmaceutical System Solution with UNIFI:

LC: Waters UPLC H-Class Bio

Column: ACQUITY UPLC BEH 300 C18, 2.1 x 150 mm, 1.7 µm

Mobile Phases:
A: 0.1% Formic Acid in Water
B: 0.1% Formic Acid in Acetonitrile

Column Temperature: 65˚C

TUV Wavelength: 214 nm

Gradient Table

Time	Flow Rate	Composition A	Composition B	Composition C	Composition D	Curve
0.00	0.200	89.0	1.0	10.0	0.0	Initial
60.00	0.200	48.0	42.0	10.0	0.0	6
61.00	0.200	10.0	80.0	10.0	0.0	6
64.00	0.200	10.0	80.0	10.0	0.0	6
65.00	0.200	89.0	1.0	10.0	0.0	6
85.00	0.200	89.0	1.0	10.0	0.0	6

MS: Waters Xevo G2-S QTof:

Data Acquisition: LC/MS^E
ESI + mode
Capillary voltage: 3.0 KV
Sample cone: 25 V
Source temperature: 100 ˚C
Desolvation temperature: 350 ˚C
Scan rate: 0.5 Sec, Mass Range: 100– 2000 m/z

Advanced Characterization of Disulfide bonds: Targeted ETD Analysis

LC: Waters ACQUTIY UPLC I-Class

Column: ACQUITY UPLC BEH 300 C18, 2.1 x 150 mm, 1.7 µm

A: 0.1% Formic Acid in Water
B: 0.1% Formic Acid in Acetonitrile

Column Temperature: 65˚C

MS: Waters Synapt^{TM} G2-S HDMS *:*

Data Acquisition: Targeted analysis
ESI + mode
Capillary voltage: 3.0 KV
Sample cone: 10-25 V
Source temperature: 100 ˚C
Desolvation temperature: 200 ˚C

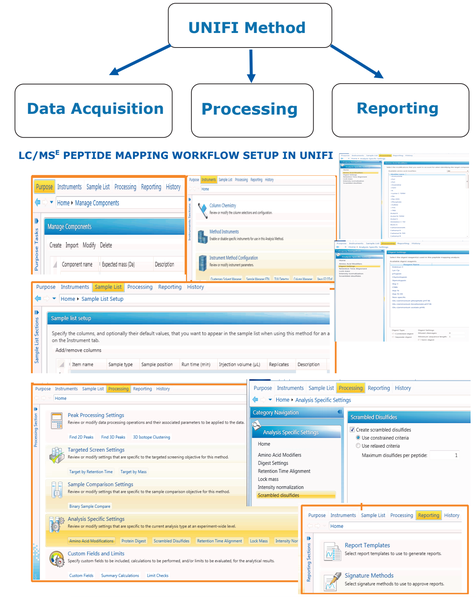
ETD settings:

Substance P (# 700005666)
Reagent: 1,3-dicyanobenzene (# 700005669)
Glow discharge current: 90 mA
Trap wave height: 0.2-0.25 V
Trap RF: 450-500 V

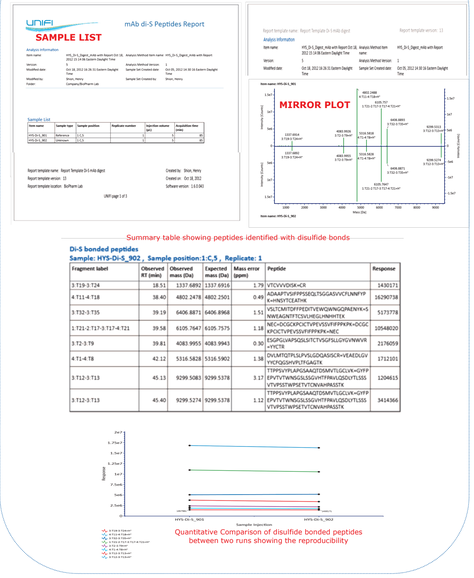
Sample Preparation:

Protein → Denature & Alkylate → Digestion Glu-C or Trypsin → Non-Reduced Digest

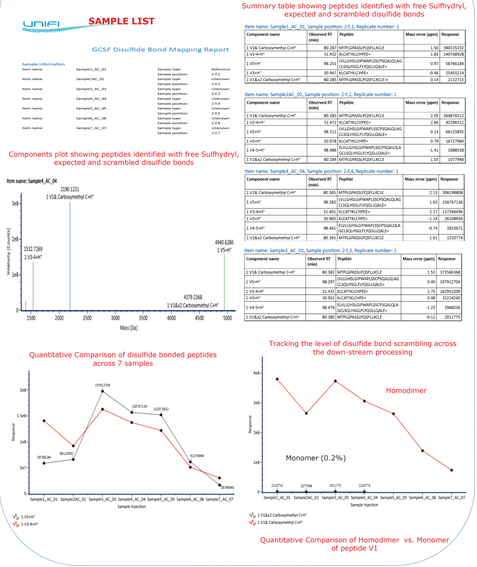
Disulfide Linkages Analysis of mAb



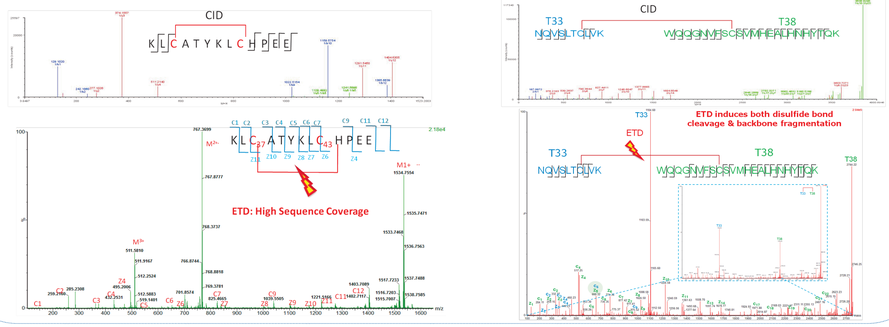
UNIFI Reporting on mAb S-S Bond Analysis



UNIFI Reporting on GCSF S-S Bond Analysis



Advanced Characterization: ETD Spectra of Disulfide Bonded Peptides



CONCLUSIONS

- The automated mapping of disulfide linked peptides (expected or scrambled) of GCSF and mAb has been achieved using Waters Biopharmaceutical system solution with UNIFI.
- The identity of disulfide bonded peptides is automatically assigned , based on accurate MS measurement and confirmed by high-energy MS^E fragment data.
- The UNIFI application workflow enables scientists in regulated or unregulated laboratory environments to acquire, process and report qualitative and quantitative information about disulfide linkages in biotherapeutics, with high confidence and minimal user intervention.
- ETD fragmentation cleaved disulfide bonds, allowing sequence information from both interchain and intrachain disulfide loop regions

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