AN EFFICIENT WORKFLOW FOR MAPPING DISULFIDE BONDS OF THERAPEUTIC PROTEINS

Henry Shion, Liuxi Chen, Ying Qing Yu, Weibin Chen, and Asish Chakraborty

Waters Corp., Milford, MA, US

Waters
THE SCIENCE OF WHAT'S POSSIBLE.®

OVERVIEW

- Mapping disulfide bonds is essential for biotherapeutics development.
- An integrated disulfide bonds mapping solution, combining high performance LC-(HD)MS^E and ETD with Unifi informatics platform is presented.
- Etanercept non-reduced peptide mapping experiments were conducted with the Xevo G2-XS QTof, the Vion IMS QTof and Synapt G2-S*i* HDMS systems and data were processed using Waters Biopharmaceutical System Solution with Unifi 1.8.

METHODS

Disulfide Bond Mapping Solutions

1. Routine disulfide bond mapping (e.g. identification and confirmation) by LC-MS^E or LC-DDA with Xevo G2-XS QTof MS

2. Routine and IMS enabled advanced disulfide bond mapping (e.g. identification, confirmation and isobaric disulfide peptides differentiation by IMS) by LC-HDMS^E or LC-HD-DDA with Vion IMS QTof MS

3. Comprehensive disulfide bond mapping (e.g. identification, confirmation, isobaric disulfide peptides differentiation by IMS and sites specification) by LC-HDMS^E or LC-HD-DDA as well as LC-ETD MS with Synapt G2-S*i* HDMS system

Figure 1. Biopharmaceutical Platform Solution with UNIFI for disulfide bond mapping encompasses automated LC-MS data acquisition, data processing, and reporting with Xevo G2-XS, QTof or Vion IMS QTof; data collected from Synapt G2-Si HDMS via MassLynx can be processed and reported by Unifi

Sample preparation: non-reduced digestion

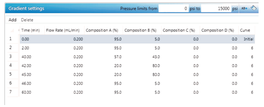
Etanercept (1 mg/mL) was denatured in PBS buffer containing 0.1 M phosphate, 0.15 M NaCl (pH= 7.2) with 0.1% RapiGest and 1.3 mM N-Ethylmaleimide (NEM) for 2 hours at room T (0.5mL total solution). The protein was then buffer exchanged into a pH 7.5 digestion buffer (0.1 M Tris, pH=7.5 with 1M Urea) using a NAP-5 column (GE Healthcare) and eluted (1 mL) directly into a Promega Lys-C/Trypsin vial (with 20 µg enzyme in vial) and incubated at 37 °C overnight. The protein was diluted to 0.4 mg/mL by 1% TFA and 5% ACN in H2O before LC-MS analysis.

Routine and advanced analysis of disulfide bonds by Xevo G2-XS QTof and Vion IMS QTof systems

LC: Waters UPLC H-Class Bio

Column: ACQUITY UPLC BEH 300 C18, 2.1 x 100 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

MS: Waters Vion IMS QTof and Xevo G2-XS QTof:

Data Acquisition: LC/MS^E
ESI + mode
Capillary voltage: 2.5 KV
Sample cone: 50 V
Source temperature: 100 °C
Desolvation temperature: 250 °C
Scan rate: 0.5 Sec, Mass Range: 100– 2000 m/z

Comprehensive analysis of disulfide bonds: targeted ETD analysis by Synapt G2-S*i*

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*i* 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:

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

RESULTS AND DISCUSSIONS

Xevo G2-XS QTof for automatic routine disulfide bond mapping

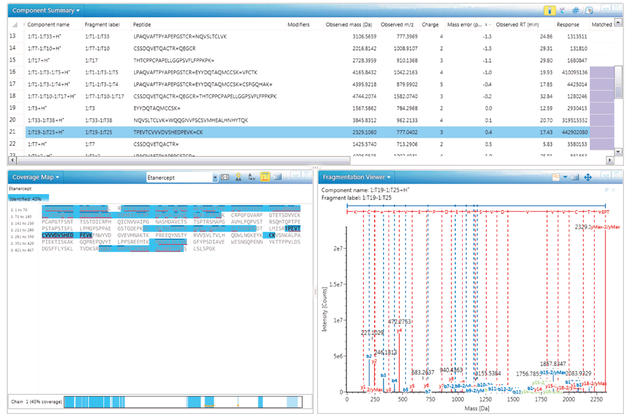


*Figure 2. Biopharmaceutical Platform Solution with UNIFI for disulfide bond mapping via UPLC-MS^E or HDMS^E with Xevo G2-XS QTof or Vion IMS QTof data acquisition, data processing, and reporting. The processed results in the review panel with identified disulfide bond peptides, Eatneracerpt sequence coverage map and identified disulfide bond peptide MS/MS fragmentation spectrum are presented here. In the current study, there were **29** unique disulfide bond peptides were identified.*

CONCLUSION

- Disulfide bond peptides from Etanercept were successfully mapped using Waters Biopharmaceutical Platform Solution.
- IMS is an enabling tool in mapping disulfide peptides in Etanercept. It facilitates to differentiate co-eluting disulfide isoforms and to enhance identification of alternative disulfide bond connections.
- ETD was important to induce both disulfide bond cleavage and backbone fragmentation to provide complementary information to CID approach. ETD can be particularly useful for sequencing peptides containing intact intrachain disulfide bonds.
- Additional IMS MS/MS experiments are needed to confirm some of the potential isobaric disulfide bonds discovered.

Vion IMS QTof for potential isobaric disulfide bond differentiating

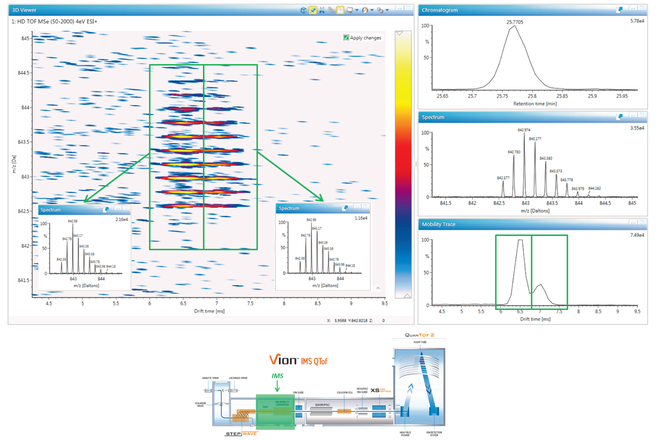
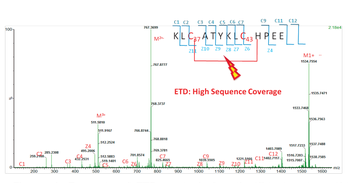


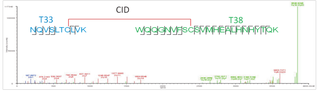
Figure 3. Example of disulfide bond peptide (1:T5-1:T7-1:T9-1:T10, SVFCTK=CSSDQVETQACTR=ICTCRPGWYCALSK=QEGCR) isoforms separated by IMS in UPLC-HDMS^E experiment with the new Vion IMS QTof system with the mobility cell shown (in green) in the lower portion of the Figure

Synapt G2-S*i* HDMS for comprehensive disulfide bond mapping with ETD

Intra-Disulfide Bond



Inter-Disulfide Bond



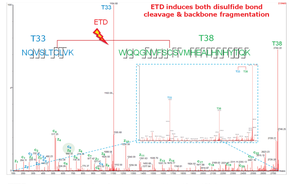


Figure 4. Example of ETD analysis for disulfide bond peptides. ETD was used to induce both disulfide bond cleavage and backbone fragmentation as a complementary technique to CID approach

TO DOWNLOAD A COPY OF THIS POSTER, VISIT WWW.WATERS.COM/POSTERS