

# Automated Disulfide Bond Mapping in Comparing Innovator and Biosimilar mAbs Using UNIFI Software

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### Abstract

In this application note, we describe a streamlined disulfide bond mapping analysis workflow using the Waters Biopharmaceutical Platform Solution with UNIFI. An innovator biologic, infliximab, is compared with a biosimilar.

#### **Benefits**

This application note describes a UPLC-MS<sup>E</sup> analysis of monoclonal antibody (mAb) disulfide bond mapping using the Waters Biopharmaceutical Platform Solution with UNIFI. This study demonstrates that UNIFI – a compliance-ready software – is designed to support biopharmaceutical laboratories in performing fast peptide mapping and monitoring of mAb disulfide linkages.

## Introduction

Disulfide bond formation is critical for the structure, stability, and biological functions of therapeutic proteins.<sup>1</sup> Disulfide bond linkage assignment, localization, and monitoring are therefore of great importance to ensure process consistency and product integrity during biotherapeutic drug development. Regulatory agencies, including the EMA and FDA, have specific requirements for mapping the disulfide bonds in biotherapeutics, such

#### as monoclonal antibodies (mAbs).<sup>2,3</sup>

In this application note, we describe a streamlined disulfide bond mapping analysis workflow using the Waters Biopharmaceutical Platform Solution with UNIFI. It combines UPLC, UV, and QTof MS characterization technologies for automated data acquisition, processing, and reporting under the compliance-ready architecture of the UNIFI Scientific Information System. As shown in Figure 1, this workflow was used for the disulfide bond mapping analysis of the Waters Intact mAb Mass Check Standard, and for three batches each of infliximab innovator and biosimilar samples.<sup>4,5</sup> This approach provides biopharmaceutical laboratories with a complianceready tool for fast mapping and monitoring of the disulfide linkages of mAbs.



Figure 1. Disulfide bond mapping using the Waters Biopharmaceutical Platform Solution with UNIFI. The workflow encompasses automated UPLC-MS<sup>E</sup> data acquisition, processing, and reporting.

#### Experimental

#### LC conditions

| Column:       | ACQUITY UPLC BEH C18, 1.7 $\mu\text{m}$ , 2.1 mm x 100 mm |
|---------------|---|
|               | (P/N 186002352)   |
|               |   |
| Column temp.: | 65 °C   |

| Mobile phase A:                                   | water*                                |
|---|---------------------------------------|
| Mobile phase B:                                   | acetonitrile*                         |
| Mobile phase C:                                   | 1% formic acid in water*              |
| Mobile phase D:                                   | not used                              |
| Detection:  | ACQUITY UPLC Tunable UV (TUV), 280 nm |
| Total run time:                                   | 75 minutes                            |
| *LC-MS grade water, acetonitrile, and formic acid |                                       |

are highly recommended for mobile phase preparations.

## LC gradient table:

| Time | Flow rate | Composition |      |      |     | Curve   |
|------|-----------|-------------|------|------|-----|---------|
|      |           | А           | В    | С    | D   |         |
| 0.0  | 0.20      | 89.0        | 1.0  | 10.0 | 0.0 | Initial |
| 60.0 | 0.20      | 48.0        | 42.0 | 10.0 | 0.0 | 6       |
| 61.0 | 0.20      | 10.0        | 80.0 | 10.0 | 0.0 | 6       |
| 64.0 | 0.20      | 10.0        | 80.0 | 10.0 | 0.0 | 6       |
| 65.0 | 0.20      | 89.0        | 1.0  | 10.0 | 0.0 | 6       |
| 75.0 | 0.20      | 89.0        | 1.0  | 10.0 | 0.0 | 6       |
|      |           |             |      |      |     |         |

#### MS conditions

| Data acquisition mode:       | Positive sensitivity   |
|------------------------------|--|
| Capillary:                   | 2.5 kV   |
| Sampling cone:               | 50 V   |
| Source offset:               | 40 V   |
| Source temp.:                | 100 °C   |
| Desolvation temp.:           | 250 °C   |
| Cone gas flow:               | 0 L/Hr   |
| Desolvation gas flow:        | 800 L/Hr   |
| Data acquisition mass range: | m/z = 100 to 2000 amu  |
| Lockmass:                    | Glu Fibrinopeptide B at 100 fmol/µL in 50:50<br>water–acetonitrile, 0.1% formic acid |

#### Data management

Biopharmaceutical Platform Solution with UNIFI

#### Sample preparation

Three batches of the infliximab innovator product (REMICADE) were purchased from Janssen (Horsham, PA). The product was produced in a SP2/0 mouse cell line. Three batches of infliximab biosimilar samples produced by an alternative mammalian cell line (Chinese hamster ovary, CHO) were obtained from an outside collaborator. All of the samples were stored at -80 °C before analysis. The concentration of all the batched samples was at 21 mg/mL.

For the Waters Intact mAb Mass Check Standard, a 3 mg/mL stock solution was prepared by dissolving the mAb

in 1 M Tris (Sigma Aldrich P/N T2444, pH 7.5). The solution was then sonicated for five minutes before following the non-reduced protein sample digestion protocol as described next.

#### Non-reduced sample digestion protocol

For the infliximab and biosimilar candidate batches, 25  $\mu$ L of the stock solution (at 21 mg/mL) was mixed with 150  $\mu$ L of 1 M Tris and 325  $\mu$ L of 8 M GdnHCl (Sigma Aldrich P/N 50937) in order to prepare a 1 mg/mL mAb solution. For the Intact mAb Mass Check Standard, 175  $\mu$ L of the stock solution was mixed with 325  $\mu$ L of 8 M GdnHCl in order to prepare a 1 mg/mL mAb solution. The samples were denatured for one hour at 37 °C. After cooling down to room temperature, 7  $\mu$ L of 0.5 M iodoacetamide (Sigma Aldrich P/N I1149) was added and the samples were incubated in the dark for 30 minutes.

Next, the alkylated protein samples were buffer exchanged into a pH 7.5 digestion buffer (0.1 M Tris) using an illustra NAP-5 Column (GE Healthcare). The NAP-5 Column was equilibrated per the manufacturer's instructions (equilibration volume = 10 mL of digestion buffer; the NAP-5 Column holds 3 mL of solution at a time). 0.5 mL of the alkylated protein solution was transferred into the NAP-5 Column after the equilibration step, allowing the mAb solution to flow completely through the column. The flow was directed to a waste container. Only the desalted mAb collected was eluted with 0.50 mL of the digestion buffer. Solubilized trypsin (Promega P/N V5111, 20  $\mu$ g) was added to the buffer-exchanged mAb solution (about 500  $\mu$ g mAb, trypsin-to-protein ratio ~1:25). The samples were digested at 37 °C for four hours. The trypsin reaction was stopped by adding 0.5 mL of a quench solution (474  $\mu$ L water, 25  $\mu$ L acetonitrile, 0.5  $\mu$ L trifluoroacetic acid). The final concentration of the mAb was estimated at 0.5 mg/mL. Triplicate LC-MS analyses were performed for each mAb digest.

#### **Results and Discussion**

The Waters Biopharmaceutical Platform Solution with UNIFI supports a variety of analytical workflows designed for comprehensive biotherapeutic protein analysis. These workflows include intact protein, subunit, and peptide mapping analyses.<sup>5-9</sup> Disulfide bond analysis is completed using the peptide mapping workflow with disulfide bond searching parameters selected (Figure 2). There are two search criteria for the disulfide peptide mapping: constrained and relaxed. The "constrained" option in UNIFI "limits the generation of disulfide-bonded peptides to digestion products, missed cleavages, and semi-digestion products." The "relaxed" option "allows the consideration of all possible peptide products."<sup>4</sup> Users can link disulfide bonds in the protein sequence if there is prior knowledge. Without linked cysteines in the protein sequence, both search criteria will look for all scrambling situations. The possible disulfide peptide targets increase dramatically when no data search

restrictions are set – therefore increasing process time. The fastest processing with the most confident confirmatory assignments is achieved through use of "constrained" criteria with minimum PTMs and linking known C-C bonds in the sequence.

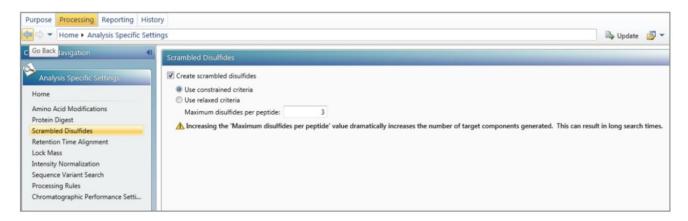


Figure 2. Disulfide bond searching parameters defined in a peptide mapping analysis method in UNIFI. Search results are displayed in a review panel after data processing. Figure 3 shows the processed results from the Intact mAb Mass Check Standard non-reduced tryptic digestion mapping experiment. Only disulfidecontaining peptides are displayed after application of a data filter. All seven unique disulfide bonds were found. There are many ways to display the result – e.g. in a sequence coverage map, component table, or component plot. The selected disulfide bond-containing peptide (1:T21-2:T17-3:T17-4:T2) is highlighted in all modes of display.

| ▼ 88 ▼ «  | Relcome to UNIFI   | C Explorer   |  | HYS_Di-S_Inf  | liximab Mar  | HYS_Di-S_Dige    | est_mAb Ja                   | HYS_DI-S                  | Digest_mAb: A             | Se                  | earch folders  | ٩ 🛙                                  |
|---|--|--|--|---|--|------------------|------------------------------|---------------------------|---------------------------|---------------------|--|--------------------------------------|
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| Review Results  |  |  |  |   |  |                  |                              | Ø Limi                    | t faults Proces           | C Edit              | • 🏟 Tools • 🚯  | File •                               |
| ctions and Components •   | 1 R Tray: 1:C,5  | Do HY  | S-Di-S Waters  | [1]   | A 1-T21.2-T17  | -3:T17-4:        | 111                          |                           |                           |                     | -  |                                      |
| cuons una component.  |  | 0  | J-DI-J_Waters  |   |  | -3.127-4         |                              |                           |                           |                     |  | Filters                              |
| Tray 1 👘 🌢  | Component Summary •  |  |  |   |  |                  |                              |                           |                           |                     | <b>*</b> #   |                                      |
| [ Limits ]  | Protein name   | Component name   | Sequence start   | Sequence end  | Observed mass (Da)   | Mass error (ppm) | Observed RT (m               | n) Response               | Observed m/z              | harge Ma            | tched 1st Gen Primary Io   |                                      |
| 1 2 3 4 5 6 7 8   | 1 Waters mAb sequence  | 2:T19-2:T24+H*   | 254  | 263   | 1337.6914  | -0.1             | 1                            | 8.48 1457961              | 669.3494                  | 2                   |  | 8                                    |
|   | 2 Waters mAb sequence  | 2:T2-2:T9+H*   | 6  | 38  | 4083.9926  | -0.4             | 3                            | 9.82 2397944              | 1021.7536                 | 4                   |  | 14                                   |
| 000000000   | 3 Waters mAb sequence  | 1:T11-1:T18+H*   | 114  | 147   | 4802.2488  | -0.3             | 3                            | 8.42 16833268             | 801.2142                  | 6                   |  | 13                                   |
| 00000000  | 4 Waters mAb sequence  | 2:T32-2:T35+H*   | 358  | 387   | 6406.8893  | -1.2             | 3                            | 9.22 5056749              | 916.1333                  | 7                   |  | 11                                   |
| 00000000  | 5 Waters mAb sequence  | 1:T1-1:T8+H*   | 1  |   | 5316.5818  | -1.6             | 4                            | 2.13 1845789              | 1064.1222                 | 5                   |  | 8                                    |
|   | 6 Waters mAb sequence  | 1:T21-2:T17-3:T17-4:T21+H  | 1:T24  | -2:T1≉-   | 3:T17-4  | T21 -0.1         | 3                            | 9.58 10971548             | 764.1010                  | 8                   |  | 12                                   |
|   | 7 Waters mAb sequence  | 2:T12-2:T13+H*   | 121  | 148   | 9299.5313  | -0.7             | 4                            | 5.37 3764492              | 1550.7613                 | 6                   |  | 11                                   |
|   | 8 Waters mAb sequence  | 2:T12-2:T13+H*   | 121  | 148   | 9299.5409  | 0.3              | 4                            | 5.11 1999133              | 1550.7629                 | 6                   |  | 8                                    |
|   |  |  |  |   |  |                  |                              |                           |                           |                     |  |                                      |
| Injections 💼 😤 🝷 🚔  | Coverage Map •   |  | Waters m   | Ab sequence   | 55 🎗 4x 🗖  |                  | Component                    | Plot -                    | View                      | by Mass             | - 💷 🐴 -  |                                      |
| Name Type   |  |  |  |   |  |                  |                              | S-Di-S_Watersm            |                           | -,                  |  |                                      |
| HYS-Di-S_WatersmAb1   | Identified: 26%  |  |  |   |  |                  | 1                            | 5-51-5_Watersin           |                           |                     |  |                                      |
| HYS-Di-S_WatersmAb2   | 1:1 to 70  |  | HSNGNTYLEW YLQ   | KPGQSPK LLIYK   | SNRF SGVPDRFSG   |                  | 1.75e7-                      |                           | 1:T11-1:<br>4802.3        |                     |  |                                      |
|   | 1 141 to 210 DIN V   | VKWKIDGSER QNGVLNSWTD  |  | LTLTKDE YER   | TSTSP  |                  |                              |                           |                           |                     |  |                                      |
|   | 1: 211 to 219 VKSFNRNEC  |  |  |   |  |                  | 1.5e7 -                      |                           |                           |                     |  |                                      |
|   | 2:1 to 70 QVQL<br>2:71 to 140 KD/SKSQVFL 1   | KMNSLQTDDT AK C APY  | QP PGQI<br>GKQYFAYWGQ GTL  | SLEWLMG IWGDGS  | TDYN SALKSRISI   |                  | T'JE'                        |                           | 1:T2                      | 1-2:T               | 17-3:T17-  | 4:T2                                 |
|   | 2: 141 to 210  | GCKPCICTVP EVSSVFIFPP  | EPAVLOSDLY ILS   | SVIVPS STMPS  | EVVIC INVALIDATION   |                  |                              |                           |                           |                     |  |                                      |
|   | 2 211 to 280 VDKKIVPRDC  |  |  |   |  |                  |                              |                           |                           |                     |  |                                      |
|   |  | GCKPCICTVP EVSSVFIFPP<br>FNSTFRSVSE LPIMHQDWLN   | GKEFKC VNS AAF   | PAPIEKT ISKTKO  | SRPKA PQVYTIPPP  |                  | 1.25e7-                      |                           |                           |                     | 1:T21-2:T17-3:T17-4:T  | 21+H-                                |
|   | 2: 15: 16: 420 FMAKDK  | CTUTOFFRE DUTYEMMENTE  | GRACHTANTQ PIM   | PAPIEKT ISKTKO<br>DTDGSYF VYSKLM  | SRPKA PQVYTIPPPI<br>NVQK <b>S SNEAGATET</b>  |                  | 1.25e7-                      |                           |                           |                     | 1:T21-2:T17-3:T17-4:T<br>6105.7570   | 21+H"                                |
|   | 1:T2000  | 7-3:T17-4:1  | T21  | SLEWLMG IWGDGS  | STDYN SALKSRIST  |                  | 1ts]                         |                           |                           |                     |  | 21+H*                                |
|   | 2.355 420 2.431 2.432 2.   | SLSHSP G<br>17-4:1<br>VAPSQLSI<br>VAPSQLSI<br>FPEPVTVTWN SGSLSSGVHT  | GROVENING PIN  | SLEWLMG IWGDGS<br>VTVSAAK TTPPSV<br>SSVTVPS STWPSI  | NYQKI SALKSRISI<br>YYPLA PGSAAQTDSI<br>TYYTC NVAHPASSTI  |                  | 1.25e7-                      |                           |                           |                     |  | 21+H*                                |
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|   | 2 350 420 FAACK<br>2 431 mass<br>3 1 m 50 KOULASSPE<br>2 71 m 50 KOULASSPE<br>2 71 m 50 KOULASSPE<br>2 14 4 50 VILGO NGY<br>2 211 5 20 VILGO NGY<br>2 211 5 5 COULASSPE<br>2 215 5 COULASSPE<br>2   | SLSHSP G<br>LAPSOLST AKYVCTRAPY<br>PREVITION SGSLSSGWT<br>GCKPCICTWP EVSSVFIEPP<br>FINSTFRSVSE LPIMHQOILIN<br>TCHITOFFPE DITVENQUAR  | GROVENING PIN  | DTDGSYF VYSKL<br>SLEWLMG IWGDG<br>VTVSAAK TTPPS<br>SSVTVPS STWPS<br>VTCVVVD ISKDD<br>PAPIEKT ISKTKG   | NQK SALKSRISI<br>YPLA PGSAAQTDSI<br>TVTC NVAHPASSTI<br>YEVQF SMFVDOVEVI<br>SRPKA PQVYTIPPPI  |                  | [stuno 1e7-                  |                           |                           |                     |  | 21+H"                                |
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| Components (HV: 💽 🖨<br>Status Name  | 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2  | С ВІЗКРУ 6<br>КАРОЗАТТ 17456741<br>КИРКІДТООТ АКУЧСТВАРУ<br>РРЕРУТУТИК SOSIJSSONIF<br>РОЗТРИТИК SOSIJSSONIF<br>РОЗТРИТИК ВОЗІЗСИНИ<br>ПУКІДОДАS ISCRSQVIV<br>SRVEADLIGY VICTOSINVO   | CRAMENTY PIM<br>CALL CALL CALL CALL CALL CALL CALL CALL  | DTDGSYF VYSKLI<br>SLEWLING IWGDGS<br>VTVSAAK TTPPSS<br>SSVTVPS STWPSI<br>VTCVVVD ISKDDF<br>PAPIEKT ISKTKK<br>DTDGSYF VYSKLI<br>KPGQSPK LLIYKV<br>ADAAPTV SIFPPS | INVECTION SALKSRIST<br>STDYN SALKSRIST<br>VYPLA PESAAQTDSI<br>PEVTC NVAHPASST<br>SERVA PQVYTIPPPI<br>INVES NHEAGNTFTO<br>SERVA PQVYTIPPPI<br>INVES SEVPDRFSE<br>SEEQL TSGGASVVCT |                  | Intensity [Counts]           |                           |                           |                     | 6105.7570<br>2:T32-2:T35+H*<br>6406.8893   |                                      |
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474567612<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АКУСТВИТ<br>АСУСТВИТ<br>АСУСТВИТ<br>АСУСТВИТ<br>АСУСТВИТ<br>АСУСТВИТ<br>АСУСТВИТ<br>АСУСТВИТ<br>АСУСТВИТ<br>АСУСТВИТ<br>АСУСТВИТ<br>АСУСТВИТ<br>АСУСТВИТ<br>АСУСТВИТ<br>АСУСТВИТ<br>АСУСТВИТ<br>АСУСТВИТ<br>АСУСТВИТ<br>АСУСТВИТ<br>АСУСТВИТ<br>АСУСТВИТ<br>АСУСТВИТ<br>АСУСТВИТ<br>АСУСТВИТ<br>АСУСТВИТ<br>АСУСТВИТ<br>АСУСТВИТ<br>АСУСТВИТ<br>АСУСТВИТ<br>АСУСТВИТ<br>АСУСТВИТ<br>АСУСТВИТ<br>АСУСТВИТ<br>АСУСТВИТ<br>АСУСТВИТ<br>АСУСТВИТ<br>АСУСТВИТ<br>АСУСТВИТ<br>АСУСТВИТ<br>АСУСТВИТ<br>АСУСТВИТ<br>АСУСТВИТ<br>АСУСТВИТ<br>АСУСТВИТ<br>АСУСТВИТ<br>АСУСТВИТ<br>АСУСТВИТ<br>АС 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| Components (HV: )   Status Name   ② 2:T19-2:T24+H*   ③ 2:T2-2:T9+H*   ③ 1:T11-1:T18+H*   ④ 2:T32-2:T35+H*   | 2 40 400<br>2 40 4 | С ВІЗКРУ 6<br>КАРОЗАТТ 17456741<br>КИРКІДТООТ АКУЧСТВАРУ<br>РРЕРУТУТИК SOSIJSSONIF<br>РОЗТРИТИК SOSIJSSONIF<br>РОЗТРИТИК ВОЗІЗСИНИ<br>ПУКІДОДАS ISCRSQVIV<br>SRVEADLIGY VICTOSINVO   | CARACTERINA PIN<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>C | DTDGSYF VYSKLI<br>SLEHLING INGDG<br>TVTSAAK TTPPS<br>SSVTVPS STHPSI<br>VTCVVD ISKDD<br>DTDGSYF VYSKLI<br>KPGQSPK LLIYKK<br>LTLTKDE YERHNE                       | INVECTION SALKSRIST<br>STDYN SALKSRIST<br>VYPLA PESAAQTDSI<br>PEVTC NVAHPASST<br>SERVA PQVYTIPPPI<br>INVES NHEAGNTFTO<br>SERVA PQVYTIPPPI<br>INVES SEVPDRFSE<br>SEEQL TSGGASVVCT |                  | [stuno_]<br>/kisuat<br>7.5e6 | 110.2124.14               | 2:12-2:19+#"              |                     | 6105.7570<br>2:132-2:135+H"<br>6406.8893<br>/ 2:1  | 12-2:T13+H*                          |
| Components (HV )<br>Status Name<br>2:119-2:124+H'<br>2:12-2:19+H'<br>2:12-2:13+H'<br>2:132-2:135+H'<br>2:112-1:18+H'  | 2 40 400<br>2 40 4 | С ВІЗКРУ 6<br>КАРОЗАТТ 17456741<br>КИРКІДТООТ АКУЧСТВАРУ<br>РРЕРУТУТИК SOSIJSSONIF<br>РОЗТРИТИК SOSIJSSONIF<br>РОЗТРИТИК ВОЗІЗСИНИ<br>ПУКІДОДАS ISCRSQVIV<br>SRVEADLIGY VICTOSINVO   | CARACTERINA PIN<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>C | DTDGSYF VYSKLI<br>SLEHLING INGDG<br>TVTSAAK TTPPS<br>SSVTVPS STHPSI<br>VTCVVD ISKDD<br>DTDGSYF VYSKLI<br>KPGQSPK LLIYKK<br>LTLTKDE YERHNE                       | INVECTOR SALKSRIST<br>MPLA PESAAQTDSI<br>ETVTC NVAHPASSTI<br>PEVQF SMFVDDUEVU<br>INVECS NHEAGNTFTE<br>SGVPDRFSG:<br>SSEQL TSGGASVECI<br>SYTCE ATHKTSTSPI                         |                  | 1e7-<br>24<br>7.5e6-<br>5e6- | T19-2:T24+H*<br>1337.6914 | 2:T2-2:T9+H*<br>4083.9926 |                     | 6105.7570<br>2:132-2:135+H"<br>6406.8893<br>/ 2:1  | 12-2:T13+H*                          |
| Components (HV )<br>Status Name<br>2:719-2:1724+H*<br>2:173-2:173+H*<br>2:173-2:173-H*<br>2:173-2:173-H*<br>2:1712-1:178+H*<br>2:172-2:1712-3:11  | 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2  | С ВІЗКРУ 6<br>КАРОЗАТТ 17456741<br>КИРКІДТООТ АКУЧСТВАРУ<br>РРЕРУТУТИК SOSIJSSONIF<br>РОЗТРИТИК SOSIJSSONIF<br>РОЗТРИТИК ВОЗІЗСИНИ<br>ПУКІДОДАS ISCRSQVIV<br>SRVEADLIGY VICTOSINVO   | CARACTERINA PIN<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>C | DTDGSYF VYSKLI<br>SLEHLING INGDG<br>TVTSAAK TTPPS<br>SSVTVPS STHPSI<br>VTCVVD ISKDD<br>DTDGSYF VYSKLI<br>KPGQSPK LLIYKK<br>LTLTKDE YERHNE                       | INVECTOR SALKSRIST<br>MPLA PESAAQTDSI<br>ETVTC NVAHPASSTI<br>PEVQF SMFVDDUEVU<br>INVECS NHEAGNTFTE<br>SGVPDRFSG:<br>SSEQL TSGGASVECI<br>SYTCE ATHKTSTSPI                         |                  | [stuno_]<br>/kisuat<br>7.5e6 |                           |                           | 1                   | 6105.7570<br>2:132-2:135+H"<br>6406.8893<br>/ 2:1  | 12-2:T13+H*                          |
| Components (HV/ I) Image   Status Name 2   2:113-2:173-4rt <sup>4</sup> 2 2:172-2:179-4rt <sup>4</sup> 2:172-2:179-4rt <sup>4</sup> 2:173-2:178-4rt <sup>4</sup> 2   2:173-2:173-4rt <sup>4</sup> 2:173-2:178-4rt <sup>4</sup> 2   2:172-2:172-4rt <sup>2</sup> -4rt <sup>2</sup> - | 2 - Chain 1 (65% coverage)   | С ВІЗКРУ 6<br>КАРОЗАТТ 17456741<br>КИРКІДТООТ АКУЧСТВАРУ<br>РРЕРУТУТИК SOSIJSSONIF<br>РОЗТРИТИК SOSIJSSONIF<br>РОЗТРИТИК ВОЗІЗСИНИ<br>ПУКІДОДАS ISCRSQVIV<br>SRVEADLIGY VICTOSINVO   | CARACTERINA PIN<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>CARACTERINA<br>C | DTDGSYF VYSKLI<br>SLEHLING INGDG<br>TVTSAAK TTPPS<br>SSVTVPS STHPSI<br>VTCVVD ISKDD<br>DTDGSYF VYSKLI<br>KPGQSPK LLIYKK<br>LTLTKDE YERHNE                       | INVECTOR SALKSRIST<br>APPLA PESAAQTDSI<br>ETVTC NVAHPASSTI<br>PEVQF SMFVDDUEVU<br>INVECS NMEAGNTFTE<br>SGVPDRFSG:<br>SSEQL TSGGASVECI<br>SYTCE ATHKTSTSPI                        |                  | [stuno_]<br>/kisuat<br>7.5e6 |                           |                           |                     | 6105.7570<br>2:132-2:135+H"<br>6406.8893<br>/ 2:1  | 12-2:T13+H*                          |
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Figure 3. Only disulfide bond-containing peptides are displayed using a simple filter in the UNIFI review panel. The disulfide bond-containing peptides are confirmed from the high collision energy spectra. Figure 4 shows the MS-MS spectrum of the highlighted peptide (1:T21-2T17-3:T17-T4:T21) from Figure 3.

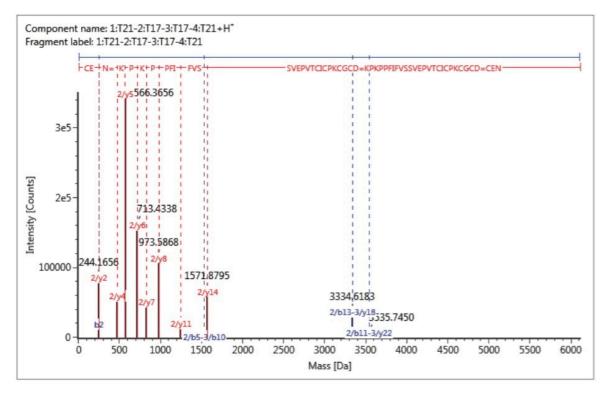


Figure 4. MS-MS spectrum of the disulfide bond-containing peptide of 1:T21-2T17-3:T17-T4:T21 from an MS<sup>E</sup> experiment. Because the mAb was not reduced, this particular peptide contains both interand intra-peptide disulfide bonds connecting duplicated tryptic peptides from heavy and light chains.

Because the peptide mapping workflow in UNIFI has a binary comparison ability for chromatograms or spectrum displays, we can now look at the innovator and biosimilar peptide maps in comparison mode. Figure 5 is a mirror image plot of the MS total ion chromatogram (TIC) comparison between an infliximab innovator batch and a biosimilar candidate batch. Figure 6 shows a mirror image plot of the confirmed disulfide-linked peptides comparing an infliximab innovator and biosimilar batch. After reviewing all of the peptide mapping results and carefully examining the data using the binary comparison tool, no differences were observed between any of the batches of infliximab and biosimilar candidates in terms of disulfide linkages.

8

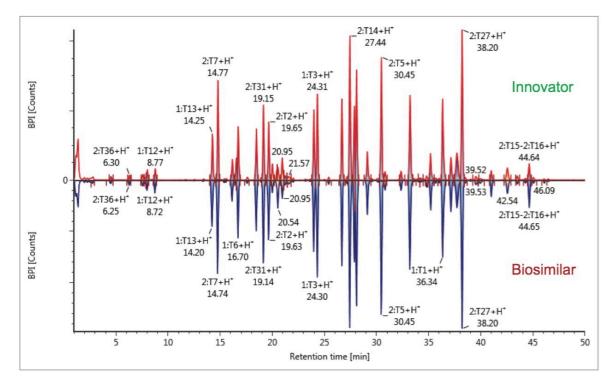


Figure 5. Mirror image plot of MS BPI chromatograms comparing an infliximab innovator and biosimilar sample. This plot demonstrates that no major differences in peptide level between the samples were observed.

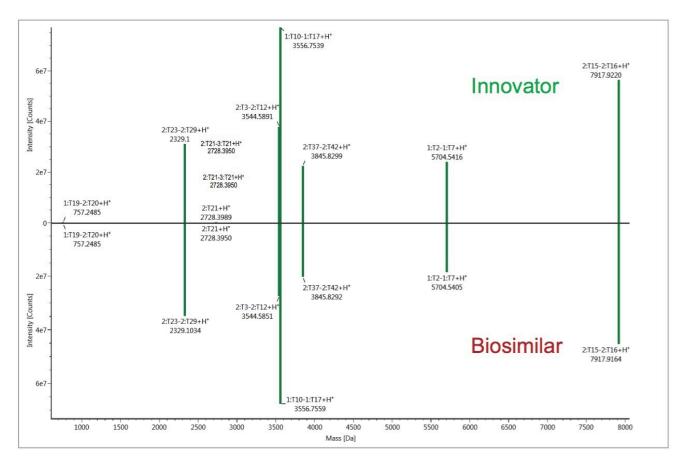
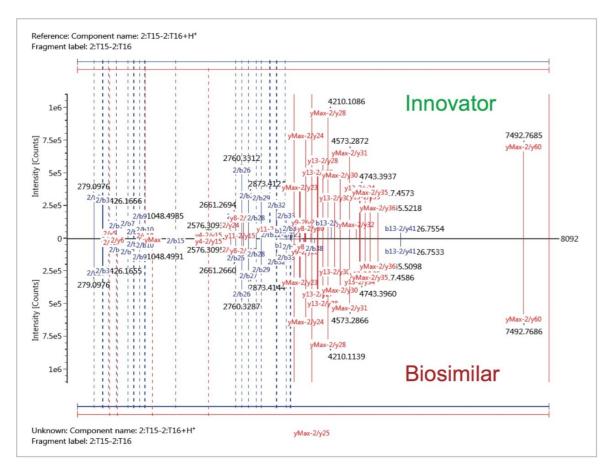
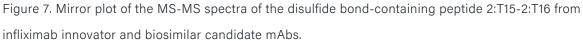


Figure 6. Mirror image plot of the confirmed disulfide-linked peptides comparing an infliximab innovator and biosimilar sample. No differences were found.

Figure 7 shows the mirror plot of the MS-MS spectra for the disulfide bond-containing peptide 2:T15-2:T16, which eluted at 44.64 minutes. No change in the fragmentation pattern was observed for the same disulfide bond-containing peptide from both infliximab innovator and biosimilar candidate.

10





The summary trending plot is another important and useful feature for data reviewing in UNIFI. Figure 8 shows the response trending plot of the disulfide bond-containing peptide 2:T15-2:T16. It provides an overview response comparison of the selected disulfide bond-containing peptides across all of the samples with triplicate injections. Elevated responses of peptides were observed in the innovator batches compared to the biosimilar batches. The plot also shows high reproducibility from injection to injection.

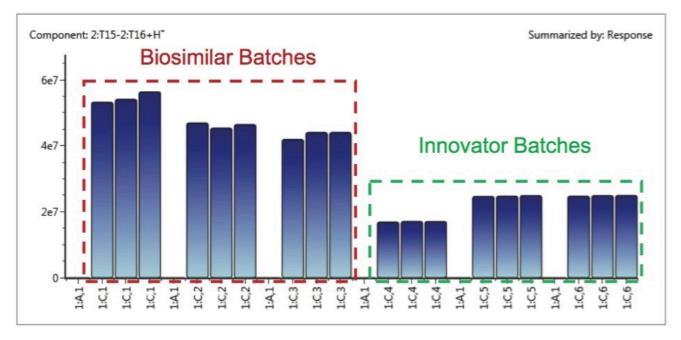


Figure 8. Trending plot of the disulfide bond-containing peptide 2:T15-2:T16. The total MS ion count response for this particular disulfide-linked peptide is plotted across all three sets of innovator and biosimilar samples, with triplicate injections.

Reporting is also a key feature of UNIFI. Customizable templates for automated report generation allow scientists to organize information in a fit-for-purpose fashion and communicate experimental results quickly and effectively. Key results from a typical disulfide bond mapping report are shown in Figure 9. In this instance, the report contains experimental information from the analysis, identified disulfide bonds, locations and linkages on the sequence map, examples of MS chromatograms, MS-MS spectra, and the trending plot of one of the identified disulfide bond-containing peptides.

12

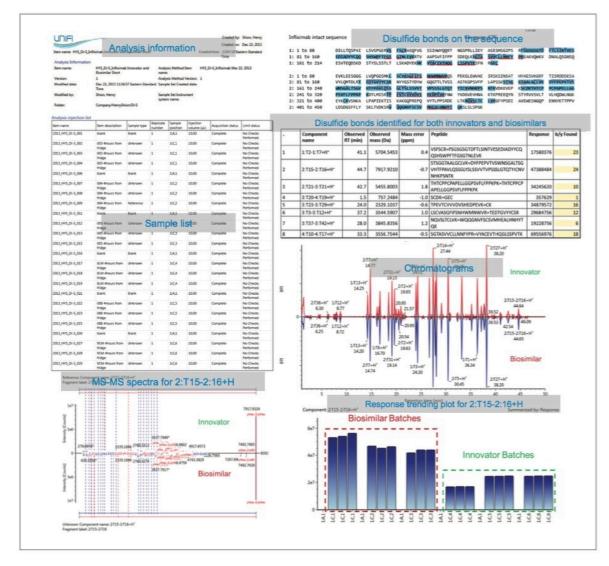


Figure 9. Example of a typical disulfide bond mapping report, which contains experimental information, identified disulfide bonds, locations and linkages on the sequence map, examples of MS chromatograms, MS-MS spectra, and the trending plot of one of the identified disulfide bond-containing peptides.

#### Conclusion

Biopharmaceutical organizations demand streamlined analytical platforms. These platforms need to increase the throughput of the characterization of biotherapeutics and to demonstrate batch-to-batch or innovator-to-biosimilar comparability in order to meet regulatory guidelines. Disulfide bond linkages are a key product quality attribute; however, mapping disulfide bonds using LC-MS techniques requires highly skilled scientists and is a

time-consuming process. The peptide mapping workflow in UNIFI is designed to lower the technical barrier for scientists. UNIFI addresses industry challenges and provides fit-for-purpose toolsets that effectively reduce analysis time. The described workflow streamlines the UPLC-MS/MS data acquisition, processing, reviewing, and reporting process for efficient disulfide bonds assignment, localization, and monitoring.

This application note demonstrated successful application of the described workflow to identify all expected disulfide bonds from the Waters mass check standards. Additionally, it demonstrated the comparability between innovator and biosimilar infliximab samples. Combined with the UNIFI compliance-ready architecture, this disulfide mapping workflow can help biopharmaceutical organizations overcome analytical challenges, and complete fast mapping and monitoring of disulfide bonds during the development and manufacturing of their biotherapeutic products.

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