

# Comparative peptide mapping between two manufacturers of Trastuzumab using the X500B QTOF system

## Exploiting SWATH® acquisition workflows

Sibylle Heidelberger<sup>1</sup> and Sean McCarthy<sup>2</sup>

<sup>1</sup>71 Four Valley Dr. Concord, ON L4K 4V8, Canada

<sup>2</sup>500 Old Connecticut Path, Framingham, MA, 01701, USA

## Introduction

Consistency between batches and manufacturers is of vital importance in the process of drug development and production. Efficacy and potency can be significantly impacted by inconsistency of the drug product and so precise monitoring and characterization of any differences is critical. Trastuzumab, a recombinant IgG1 monoclonal antibody for the treatment of Her2 positive breast cancer was used to demonstrate the proposed SCIEX workflows designed for such analyses. Samples of Trastuzumab from two different manufacturing sources were obtained for comparative analysis using peptide mapping. This analysis illustrates what is required for biosimilar characterization, or for analysis of processing and production alterations that may affect the final product. Presented here are the results of the investigation into the differences between the two batches. Also discussed is the ability of the proposed workflows and technologies to rapidly and simply characterize and quantify differences between batches.

## Materials and methods

The two samples of biosimilar Trastuzumab therapeutic obtained from separate manufacturers were labeled, TRAST-1 and TRAST-2, arbitrarily and blindly for differentiation purposes. TCEP (tris(2-carboxyethyl)phosphine) and iodoacetamide were purchased from Sigma (St. Louis, MI, USA). ProteaseMAX™ and trypsin were purchased from Promega (Madison WI, USA). Samples were denatured using ProteaseMAX™, reduced with TCEP, alkylated with iodoacetamide and digested using trypsin per standard protocols. [1]

### High flow chromatography

A total of 2-4 µg of digested protein was injected onto the ExionLC™ and separated using a Kinetex® C18 100Å 1.7 µm, 2.1mm x 150mm column (Phenomenex, Torrance, CA, USA) held at 60°C throughout the analytical run. Standard mobile phases were used (Mobile Phase A: 0.1% formic acid in water, Mobile Phase B: 0.1% formic acid in acetonitrile) using a linear gradient of 5 to 40 over 120 min at a flow rate of 0.2 mL/min.

### Mass spectrometry

Analysis was performed on the new SCIEX X500B QTOF fitted with a Turbo V™ ion source. Additional electrospray parameters were as follows:

Curtain gas:	35
Ion source gas 1 (psi):	50
Ion source gas 2 (psi):	50
Temperature (°C):	500
Declustering Potential:	80

### SWATH®

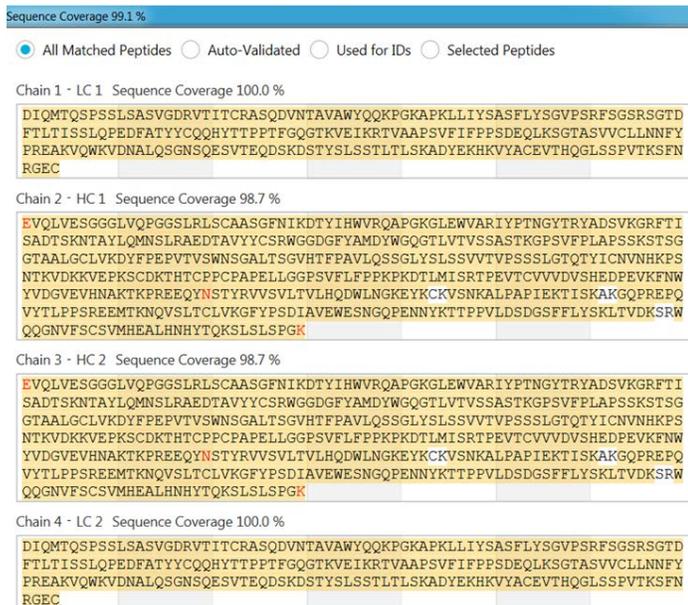
SWATH acquisition was setup with a TOFMS accumulation time of 150 ms and 28 variable SWATH windows covering TOF MS range of m/z 300-1800 with an MSMS range of m/z 100-1800. Each SWATH window was set to a duration of 50 ms, giving a total cycle time of 1.66 seconds.

### Data processing

Data processing was carried out in the dedicated SCIEX BioPharmaView™ Software. A standardized sample of digested trastuzumab was used as a reference.

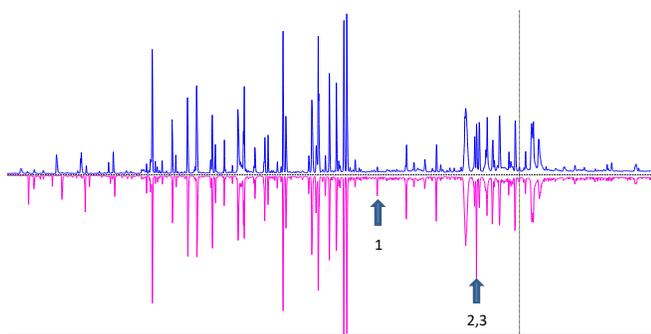
## Results and Discussion

Full coverage (100%) was obtained on the light chains while 98.7% coverage was obtained on the heavy chains. The coverage of TRAST-1 and TRAST-2 is highlighted in yellow in Figure 1, while the dipeptide sequence highlighted in grey was also identified a TRAST-2; likely resulting from incomplete digestion and a missed cleavage in that region. The undetected sequences are dipeptides which did not fall into the m/z range used for analysis.



**Figure 1: Total sequence coverage of trastuzumab TRAST-1 (99.1%) and TRAST-2 (99.4%).** Difference in sequence coverage is highlighted in grey.

Full coverage and a long gradient allows for an immediate comparison of chromatography between samples as a means of identifying significant differences between the two samples in question. Mirror plots of the chromatograms in Figure 2 are similar, with differences noted at the front of the chromatogram and slight changes in ratios of peaks later in the chromatogram.

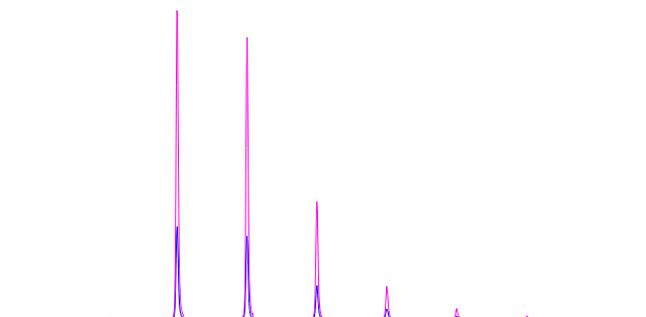


**Figure 2: Mirror plot comparison of two manufacturer trastuzumab. TRAST-1 (blue) and TRAST-2 (pink).** Differences are detected at 63 min and at 76.82 min, represented by arrows.

The small variations within the first 30 min of both chromatograms were identified as small molecules, none of which were peptidic in nature, and were likely a result of the differences in the formulation of the two manufacturers' batches of trastuzumab.

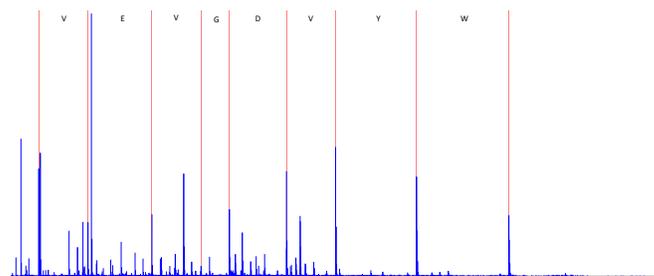
Three peaks were targeted for identification, labeled 1, 2 and 3 in Figure 2. The spectrum from peak 1 at 63 mins was extracted

and interrogated. A single distinct change in intensity at  $m/z$  682.82 was detected. This unknown is doubly charged, suggesting it is peptidic in nature. Comparative data mining showed a six fold increase in intensity in the TRAST-2 sample, as shown visually in Figure 3.



**Figure 3: Overlaid spectra of  $m/z$  682.82 from TRAST-1 (blue) and TRAST-2 (pink).**

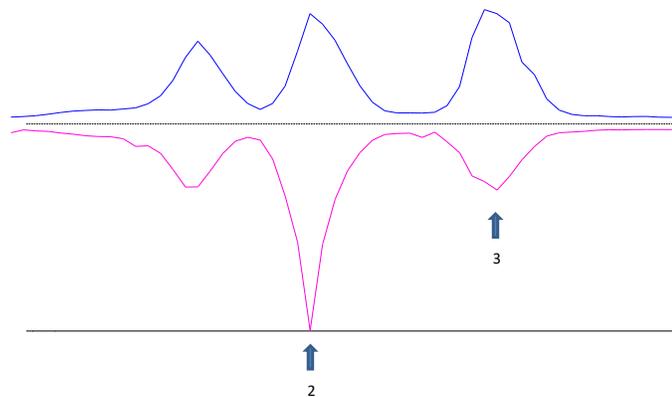
As samples were analyzed using a SWATH® Acquisition workflow, the MSMS data for this peptide was easily available. The SWATH® Acquisition program automatically generates a full MSMS fragmentation spectrum of every component in the sample, giving a wealth of information in a digital record able to be interrogated as necessary. Using this specific workflow and dedicated SCIEX software the data acquired using SWATH for this peptide was mined and the sequence was identified.



**Figure 4: Sequence of unknown peptide 682.82 from SWATH data.** The sequence was defined as \*WYVDGVEV\*, where \* is unknown on either end of the sequence.

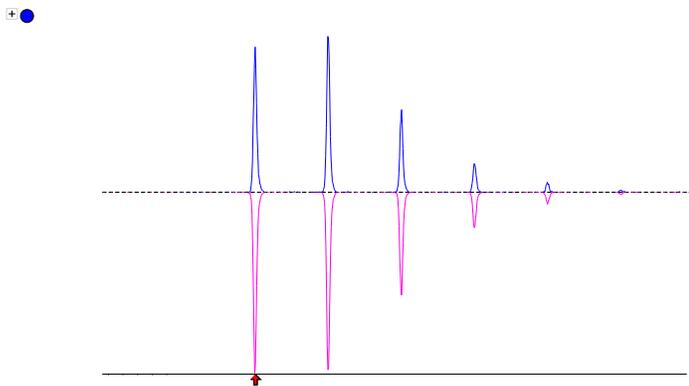
A comparison of the unknown sequence and the sequence of trastuzumab confirms that this peptide is from the heavy chain, and corresponds to a semi-tryptic internal fragment of K\*FNWYVDGVEVH\*K with a higher intensity in TRAST-2 compared to TRAST-1 (Figure 3). The origin for this semi-tryptic peptide is unclear as both samples were processed at the concurrently by the same protocol.

The second region of variability seen between the two samples, and interrogated was around 76 mins in the chromatogram. At this point, the relative intensities of a group of chromatographic peaks changes dramatically between the two samples. Figure 5 shows the magnified portion of the chromatogram in question. TRAST-1 shows almost identical intensities for each of the three peaks in question, while TRAST-2 has variability across the same three peaks.



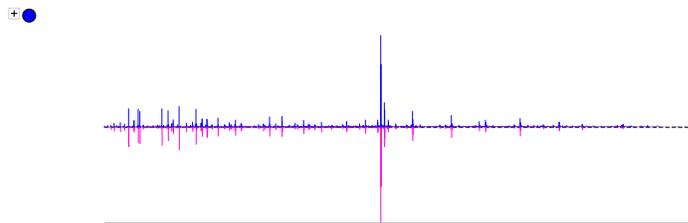
**Figure 5: Comparison of the region at 76 mins between TRAST-1 (blue) and TRAST-2 (pink).** Peak 2 at 76.8 mins and peak 3 at 77.2 mins are targeted for investigation.

Peak 2 from this region was extracted and investigated in the same manner as previously described. A single  $m/z$  of 904.55 was found to contribute to the changes in chromatography. The intensity of the peptide was 30% lower in TRAST-1 than TRAST-2. Figure 6 demonstrates this.



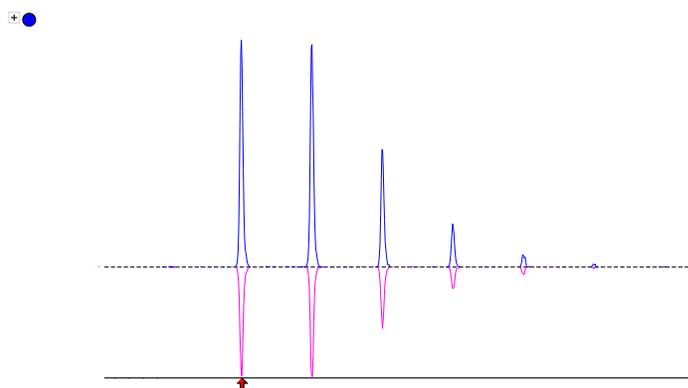
**Figure 6: Relative intensities of peptide VSVLTVLHQQDWLNGK at retention time of 76.8 mins.**

The data shows it is a multiply-charged species and the extraction of the MSMS data from the SWATH acquisition identified this peptide as originating from the trastuzumab sequence corresponding to VSVLTVLHQQDWLNGK, as shown in Figure 7.



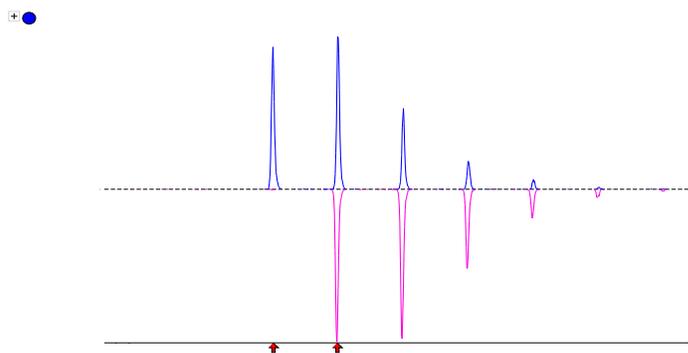
**Figure 7: SWATH acquisition data for  $m/z$  904.55 for TRAST-1 (blue) and TRAST-2 (pink)**

Peak 3 from Figure 5 was also sequenced and identified as the deamidated form of the sequence VSVLTVLHQQDWLNGK. The intensity differences are shown in Figure 8, with TRAST-1 showing approximately a 2-fold increase in intensity over TRAST-2.



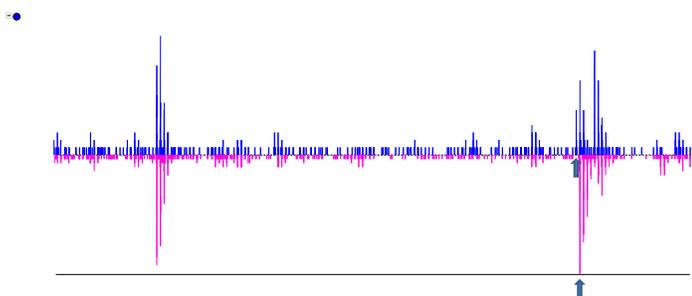
**Figure 8: Changes in intensity deamidated form of VSVLTVLHQQDWLNGK for TRAST-1 (blue) and TRAST-2 (pink).**

The changes in relative intensity of the peptide VSVLTVLHQQDWLNGK and the deamidated form of the peptide show differences between the two trastuzumab manufacturing lots, with TRAST-1 having a higher level of deamidated peptide than TRAST-2.



**Figure 9: Intensities of VSVLTVLHQQDWLNGK (blue) and the deamidated form of VSVLTVLHQQDWLNGK (pink) for TRAST-1 showing the differences between the masses of the  $[M+2H]^{2+}$ .**

Mirror plot of the peptide (Figure 9- blue) and the deamidated form (Figure 9 – pink) show 0.5  $m/z$  difference between peptide masses which is synonymous with a deamidation. The MSMS data from the same two peptides, confirms the shift of 1  $m/z$  within the sequence (Figure 10).



**Figure 10:** Expanded view of MSMS data obtained for VVSVLTVLHQDWLNGK(blue) and the deamidated form of VVSVLTVLHQDWLNGK(pink) showing the 1  $m/z$  difference between the masses. Arrows are pointing to an example of the 1  $m/z$  difference seen in the MSMS spectra.

Interrogation of the data behind the two chromatographic peaks (peak 2 and peak 3, Figure 5) has exposed a significant relationship between the two samples analysed. TRAST-2 shows a higher intensity of the peptide VVSVLTVLHQDWLNGK, while TRAST-1 shows a higher intensity of the deamidated form of VVSVLTVLHQDWLNGK.

**Table 1: Percent deamidation of TRAST-1 and TRAST-2**

Sample	Modification	Area	% Modification
TRAST-1	Peptide	1.17E+06	
	Deamidated	1.38E+06	54
TRAST-2	Peptide	1.47E+06	
	Deamidated	6.67E+05	31

Extraction of the percent deamidation using the doubly charged peptide from BioPharmaView™ confirms these findings - TRAST-1 has a higher intensity of the deamidated form than the non-modified peptide form while TRAST-2 has twice as much of the non-modified form to the deamidated form. This is shown in Table 1.

AB Sciex is doing business as SCIEX.

© 2017 AB Sciex. For Research Use Only. Not for use in diagnostic procedures. The trademarks mentioned herein are the property of AB Sciex Pte. Ltd. or their respective owners. AB SCIEX™ is being used under license.

Document number: RUO-MKT-02-5592-A

## Conclusion

Batch comparisons of biologics are of vital importance for the manufacturing process. To this end, enabling rapid, simple and facile workflows to compare manufacturers or product batch lines is vital to ensure that quality and efficacy of product is maintained. The benchtop X500B QTOF mass spectrometer was developed with this in mind, for routine analysis of biologics and fast batch comparison with the dedicated BioPharmaView software.

Shown here is the SCIEX dedicated workflow for such batch comparisons, utilizing the SWATH functionality of the X500B QTOF. This generates a fragmentation spectrum of everything within the sample analyzed, providing the user a digital library of all detected differences between samples, allowing rapid and simple mining of data, from a single injection. Complete MSMS coverage of the proteins is ideal for identifying change within batches or post-translational modifications. Relative differences in the chromatograms can be easily detected and data acquired using SWATH gives the user the ability to identify those differences and determine their importance.

In this comparison of two manufacturer batches of trastuzumab, several significant differences were discovered using this approach, including an internal semi-tryptic fragmentation as well as changes in levels of a peptide and its deamidated form, which can both be determined using the dedicated BioPharmaView software. These detected changes are significant for process scientists tasked with determining batch-to-batch variability in a routine environment.

## References

1. ProteaseMAX™ Surfactant, Trypsin Enhancer Technical Bulletin TB373