

# Enabling the Use of Mass Spectrometry Throughout Biopharmaceutical Development

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## Abstract

The SCIEX X500B QTOF System with BioPharmaView™ Software enables routine characterization of biologics through all stages of discovery and development. The following study demonstrates these capabilities for the characterization of different manufactured lots of the biotherapeutic Trastuzumab. With streamlined workflows, SCIEX solutions simplify intact protein analysis, sub-unit analysis, peptide mapping, identification of modifications, and comparability analyses.

## Introduction

The development of biologics is now an integral part of many pharmaceutical programs. But because biologics are generally much larger and more complex than traditional small molecules, their characterization can be more time consuming and challenging. Consequently there has been an increasing demand for assays that can simplify their characterization and make comparability studies more straightforward.

SCIEX hardware and software solutions have been specifically developed to reduce the complexity of biologics characterization by streamlining routine tasks. Based on the X500B QTOF System and BioPharmaView™ Software, researchers can now perform full biologics characterizations and comparisons including batch analysis of multiple sample lots or products – all in a fraction of the time than what was previously possible.

## SCIEX Biologics Solutions

The X500B QTOF System is a next generation platform designed specifically for biotherapeutic characterization. This high resolution accurate mass system has the power to deliver consistent, reliable results that supports both research and development environments. It was engineered from the ground up as a benchtop instrument with robustness and ease-of-use in mind to make biologics characterization accessible to a wide range of scientists. The easy, point-and-click interface of SCIEX OS Software makes setup of biologics characterization workflows rapid and simple, even for novice users.

The ExionLC™ AC System provides the sensitivity of UHPLC in a modular workhorse design. This reliable, flexible and expandable solution gets from samples-in to results-out fast.

BioPharmaView Software provides powerful automated data processing tools for common biologics characterization workflows. The software delivers exceptional investigative capabilities such as intact and subunit mass analysis, peptide mapping, comparability studies with intuitive data visualization graphs and charts. With high throughput batch processing, BioPharmaView simplifies comparability assessments and quickly reveals product differences from sample to sample, site to site, or lot to lot.

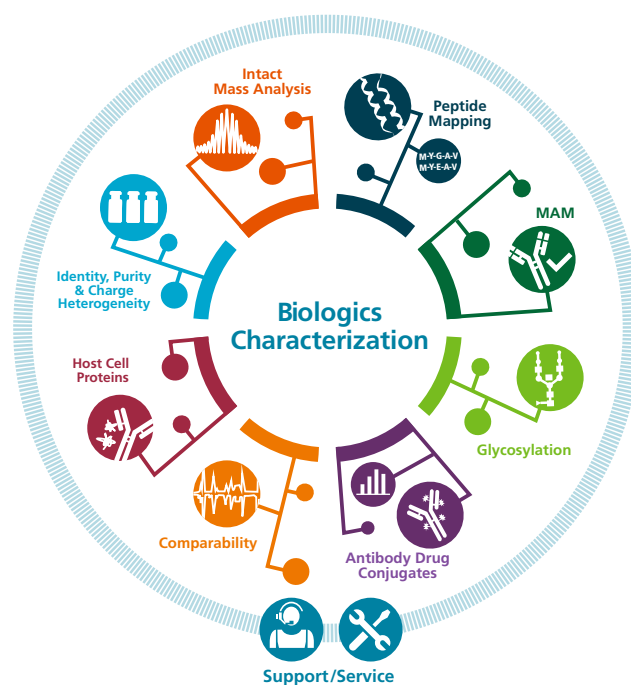


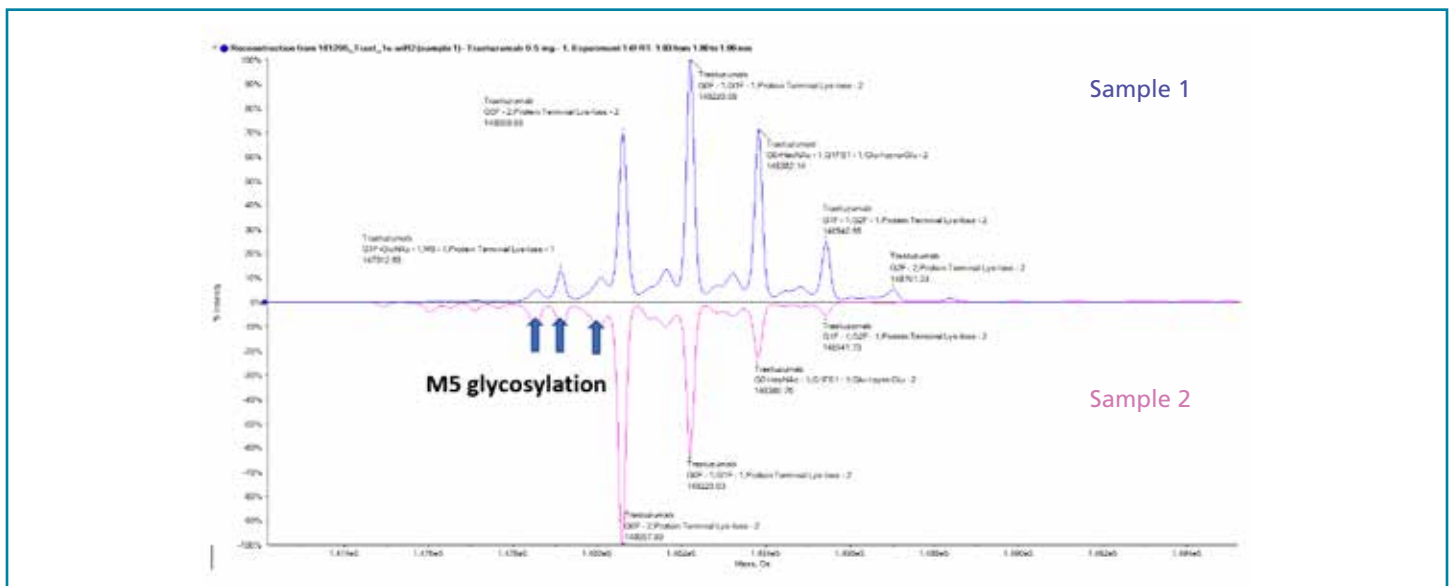
Figure 1: SCIEX MS and CE based biologics solutions provide streamlined workflows for the full characterization of biotherapeutics.

## Comparative Case Study

Trastuzumab is a recombinant IgG1 monoclonal antibody for the treatment of Her2 positive breast cancer. In this study Trastuzumab therapeutic samples from two different manufacturers ("Sample 1" and "Sample 2") were analyzed and compared using SCIEX Biologics Solutions and workflows.

### Intact Mass Analysis

Intact mass analysis is often the first assay performed in the characterization of a biologic and provides a rapid assessment of the mass of the molecule as well as the degree of heterogeneity. The high mass accuracy of the X500B QTOF system ensures correct protein mass assignments. The intact mass analysis of the two Trastuzumab samples, while similar in virtually all masses observed both in the raw and reconstructed data, were clearly different in the ratios of glycoforms present. One of the more subtle variations was within the ratios of mannose-5 (M5) glycoforms as shown in the mirror plot in Figure 2. Mirror plots are just one of the many features within BioPharmaView Software that greatly facilitate the comparison of different samples.

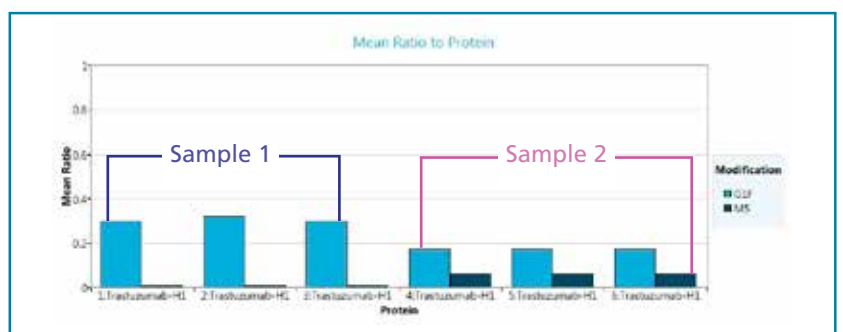


**Figure 2:** Mirror plot of reconstructed protein data showing differences in glycoform ratios between Sample 1 and Sample 2. The samples were generally consistent in mass but with observable differences in the ratios of glycoforms. The M5 glycoforms are emphasized with arrows.

### Subunit Analysis

In order to further assess and characterize the glycoforms distributions, a reduction of the Trastuzumab samples was performed to separate the heavy and light chains. Comparison of the light chains revealed good correlation both in charge distribution and mass position for both raw and reconstructed data indicating the two samples were highly similar and consistent with one another at the light chain level.

Comparison of the heavy chains showed that although the overall charge profile and distribution were largely consistent between the two samples, there were distinct differences in the relative abundances of the glycoforms observed. Some differences were obvious while others were less apparent. Bar charts of the relative ratios greatly aid in revealing any dissimilarities. As shown in Figure 3, the subtle M5 differences observed at the intact level were also visible within the heavy chain analysis.



**Figure 3:** Bar charts show the reproducibility and comparison of ratios for G1F and M5 glycoforms from 3 replicate injections of the heavy chain samples.

## Peptide Mapping Analysis

Peptide mapping allows more precise monitoring and characterization of biologics. With peptide mapping, the biologic is enzymatically digested into smaller peptides to confirm sequence and facilitate localization of any modifications. The two Trastuzumab samples were denatured, reduced, alkylated, and digested with trypsin and then analyzed by LC-MS/MS using SWATH® Acquisition on the X500B QTOF system. SWATH Acquisition is an extremely powerful and easy to set-up workflow that automatically generates MS and MS/MS fragmentation data for every component in the sample, thereby enabling quick confirmation of a peptide sequence and identification of any modifications. Because the technique is unbiased and all encompassing, every precursor in the sample is analyzed and a digital record is created that can be mined for future inquiries.

The LC-MS chromatograms of the Trastuzumab tryptic digests were very similar, with differences noted at the front of the chromatogram and slight changes in ratios of peaks later in the chromatogram (Figure 4). Automated analysis within BioPharmaView maps the peptides produced from Trastuzumab to the LC peaks. Most of the variations within the first 30 min of both chromatograms were identified as small molecules, none of which were peptide in nature, and were likely a result of the differences in the formulation of the two manufacturers' batches of Trastuzumab.

Comparison of the M5 modified peptides showed that the abundance was more than 3x greater in Sample 2 (Figure 5). This correlated well with the intact and subunit (heavy chain) findings. MS/MS data confirms the M5 peptide identification, and the ratio difference is observed at this level too. Even at the low level observed in Sample 1, good sequence coverage could still be obtained from the MS/MS data from SWATH acquisition.

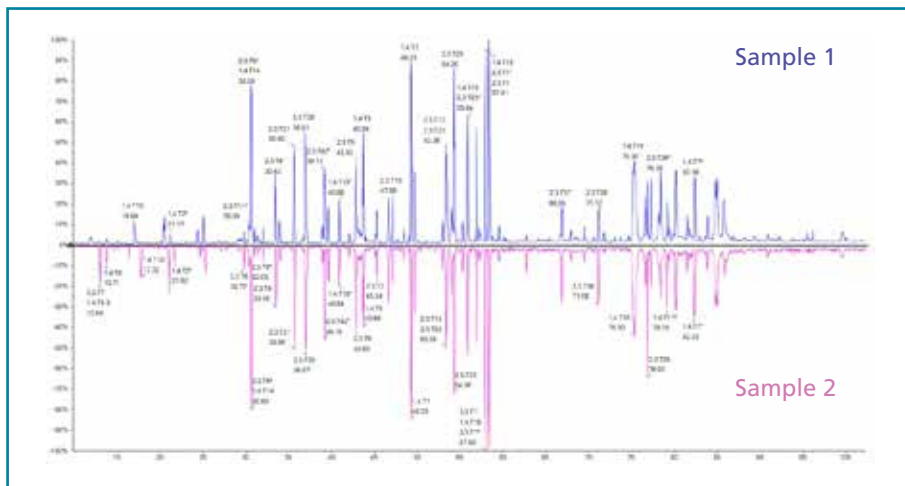


Figure 4: Mirror plot of LC-MS chromatograms of Trastuzumab tryptic digests. The chromatograms are highly similar with some differences noted in peaks before 30 minutes and after 60 minutes.

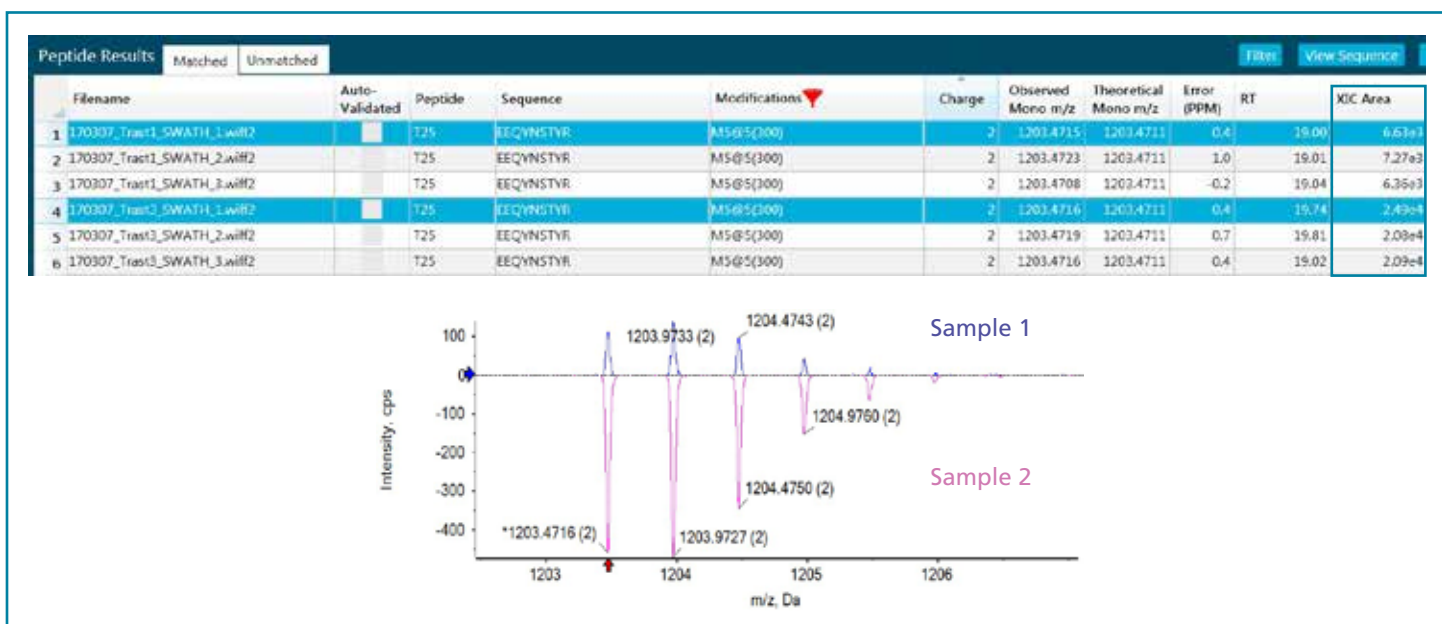


Figure 5: Top - BioPharmaView peptide data results showing more than 3x difference in abundance for M5 peptide from Sample 2 vs. Sample 1. Bottom - Raw MS data of M5 peptides (doubly-charged) from SWATH Acquisition.

Other variations between the two samples were also characterized. For example, around 77 minutes, the intensities of several LC peaks were inconsistent between the two samples. These turned out to be from the non-deamidated and deamidated forms of one of the tryptic peptides (T26). The SWATH Acquisition MS/MS data confirms the non-deamidated and deamidated forms and allows localization of the specific residue that is deamidated. More deamidated peptide was apparent in sample 1 with 54% deamidation compared with only 31% deamidation calculated for Sample 2 (Figure 6). BioPharmaView automatically makes this comparison and calculates the percentages for many types of modifications including custom modifications.

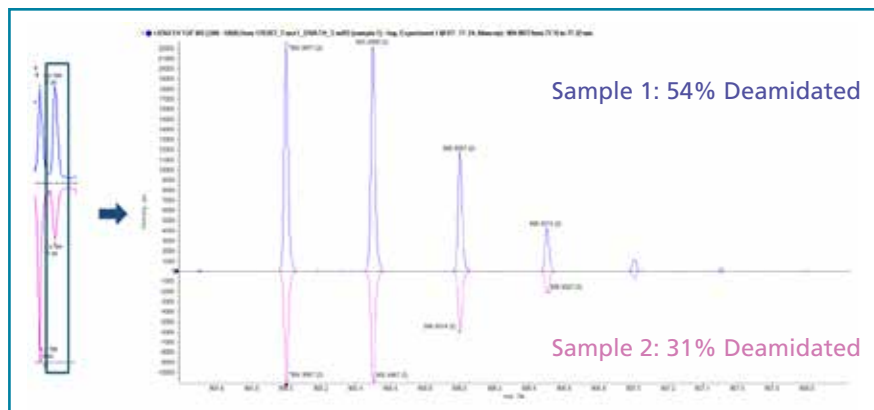


Figure 6: Mirror plot of the deamidated T26 peptides.

## Summary

With SCIEX Biologics solutions the complexity of biopharmaceuticals characterization is made routine. The powerful hardware of the SCIEX X500B QTOF in combination with SCIEX OS point-and-click software ensures that high quality data can be acquired with just a few button clicks. The ExionLC AC system provides a powerful LC solution for a wide range of biopharmaceutical characterization needs throughout the biologics development pipeline. And BioPharmaView Software simplifies and streamlines complex characterization tasks making them routine, with its straightforward “click-compare-report” format.

To read the full comparison study of the Trastuzumab samples with additional data, please download our technical notes listed in the references. To learn more about SCIEX Biologics Solutions please refer to our brochures in the references and visit our [website](#).

## References

**Technical Note:** [Routine Workflow for Comparability Assessment of Protein Biopharmaceuticals: Trastuzumab Intact Analysis using Benchtop X500B QTOF Mass Spectrometer](#), Sibylle Heidelberger and Sean McCarthy, SCIEX. Document number: RUO-MKT-02-5590-A

**Technical Note:** [Comparative Multi-Supplier Lot Analysis of Trastuzumab using Subunit Analysis on the X500B QTOF System](#): Trastuzumab Subunit Analysis using Benchtop X500B QTOF Mass Spectrometer, Sibylle Heidelberger and Sean McCarthy, SCIEX. Document number: RUO-MKT-02-5591-A

**Technical Note:** [Comparative Peptide Mapping Between Two Manufacturers of Trastuzumab Using the X500B QTOF System](#): Exploiting SWATH® Acquisition Workflows, Sibylle Heidelberger and Sean McCarthy, SCIEX. Document number: RUO-MKT-02-5592-A

**Brochure:** [Answers, Simple and Streamlined. Solutions for Biopharmaceutical Intact Mass Analysis.](#)

**Brochure:** [Simple Solutions to Complex Workflows. Innovation for Biopharmaceutical Peptide Mapping.](#)