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Peptide Mapping Workflow for the Rapid Characterization of Biologics

BioPharmaView™ Software for facilitating assay development and automatic LC/MS processing of biologics

[1]Xu Guo, [2]Robert Deutschman, [2]Eric Johansen, [3]Byung-Hee Shin
[1]SCIEX Concord, ON, Canada; [2]SCIEX Foster City, CA, USA; [3]SCIEX Seoul, South Korea

INTRODUCTION

In the fast growing biopharmaceutical development space, mass spectrometry has become an important analytical tool in the characterization of biologics. During the development of novel protein biologics, LC/MS characterization needs to be performed to provide detailed structural information in order to address increasingly challenging regulatory expectations. LC/MS can be used to both localize and structurally characterize sites of both biological and artifactual modifications (e.g. acetylation, oxidation, deamidation, etc.). However, manual data analysis is inefficient, and typically requires significant levels of experience and expertise. Here, we describe LC/MS workflows using the SCIEX TripleTOF® family of instruments and an advanced, easy to use software solution, BioPharmaView™ Software, that allows for highly efficient characterization of biologics. In this work, we present a completed peptide mapping workflow for characterization of biologics and routine QC analysis with a focus on monoclonal antibodies (mAb).

MATERIALS AND METHODS

Samples: Intact mAb Mass Check Standard was purchased from Waters Corporation, 34 Maple Street Milford, MA 01757.

Chromatography: A Shimadzu Prominence XR system with a Thermo C18 Column, 2.1 x 150 mm, 3 μm at 35 ⁰C was used for separating mAb tryptic digest mixtures. The samples were eluted in a linear gradient from 4-35% acetonitrile and 0.1% formic acid in 45 min at a flow rate of 0.4 mL/min.

Mass Spectrometry: A SCIEX TripleTOF® 5600 system equipped with a TurboIonSpray® ion source was used for data acquisition.

Software: Analyst® TF 1.5 Software was used for instrument control and data acquisition using Information Dependent Acquisition (IDA). Pre-release BioPharmaView™ Software 1.0 was used for peptide mapping analysis and the QC workflow.

KEY BENEFITS

- BioPharmaView Software provides a complete peptide mapping workflow for biologics

- The software, in combination with the fast scan speed and high resolution of the TripleTOF® system, provides a powerful and easy to use solution for

  o characterization of biologics

  o routine QC analysis of biologics

RESULTS

BioPharmaView Software is structured around five key tasks: (1) entering assay information, (2) characterizing standards, (3) batch processing, (4) reviewing results, and (5) generating reports (Figure 1).
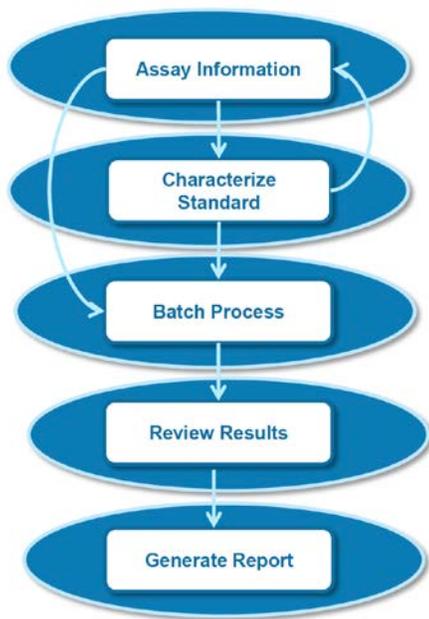
Figure 1: The steps in BioPharmaView Software are shown for characterizing a biologic standard, and then using that information for subsequent automated batch analysis of that biologic.

The workflow begins with the user entering the information about the protein which is then stored in the project. Once the assay information is entered, the software will subsequently process a

standard that has been enzymatically digested to evaluate potential target peptides in the LC/MS profile. The software automatically determines the accurate masses of the potential target peptides and assesses their applicability to the protein of interest. After the processing is complete, the user can sort the result table and review the spectra of the peptides of interest. The user can then choose to update the characterized information of the matched peptides and the processing parameters if desired. After characterizing the biological standard and setting up the comparison criteria, the user can then set up sample and control batches for routine QC analysis. Finally the user can generate a fully customizable report of the QC analysis. Each step is discussed in more detail below.

Assay Information: Assay information is a central place to store knowledge about the therapeutic protein which can be updated as more about the protein is learned. It contains three tabs: Sequence Features, Intact Protein, and Peptide Mapping. Sequence Features allows users to enter the protein sequence and any known modifications through a user friendly, searchable modification library (Figure 2). The software can calculate the molecular weight (M.W.) of the intact protein, or a partial sequence of interest. The Intact Protein tab features a summary of the intact protein M.W. The Peptide Mapping tab features alkylation, enzymatic digestion, maximum cleavage, and the generation of a digested peptide list.

Figure 2: Information for the Biologic is entered into the Assay Information section of BioPharmaView Software.

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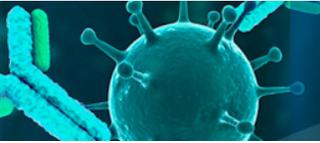
Characterize Standard: The user is able to characterize the standard data by simply choosing a file and clicking Process. The simplified processing parameters and appropriate default settings make it easier for the user to get started and focus on the results. The target peptides are compared with the in-silico digested peptides based on a user-defined mass to charge tolerance. The processed results are summarized in a tabular format that allows the user to easily navigate and visualize the various aspects of the data (Figure 3). The user has the option of overlaying the XIC with the TIC to aid in the navigation of the chromatography.
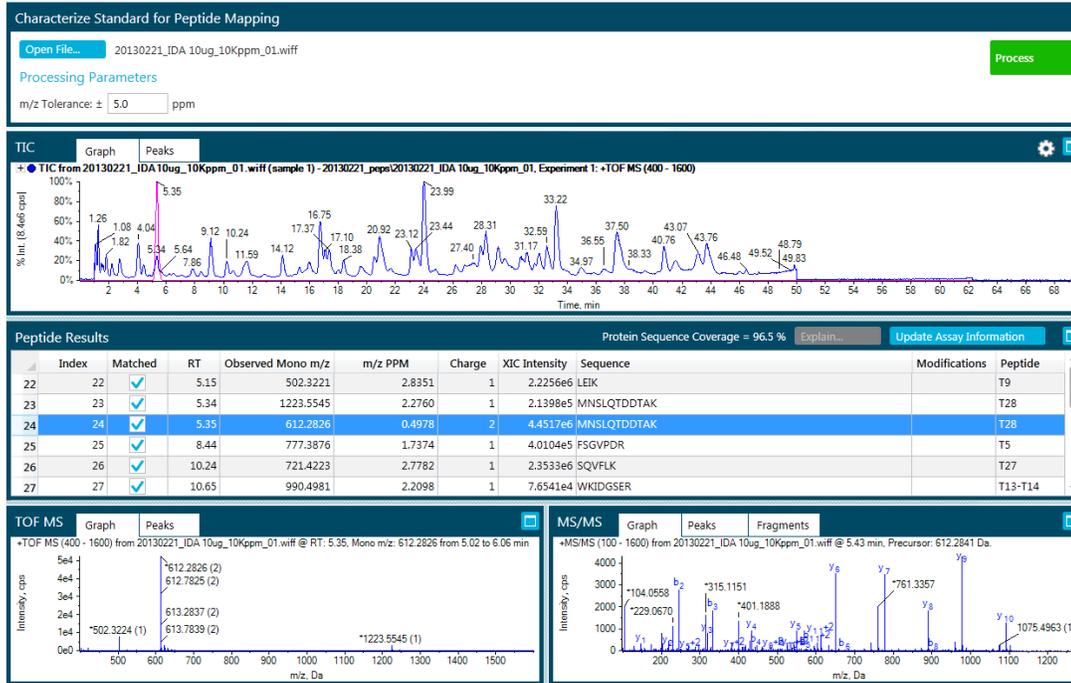

Figure 3: The TIC/XIC, peptide results, and MS and MS/MS data for the standard.

Using the tabular result, the user is able to easily navigate among the spectra to confirm the results. The MS/MS spectral information provides further sequence confirmation of the peptide. Once the review is complete, the user can update the characterized peptide information to the central assay information, i.e. peptide matched, intensity, and corresponding retention time. To set up a QC batch, the user can also define pass/fail criteria based on RT, intensity, sequence coverage and unique peptide matched. All information is saved in the project to be used for future QC analysis.
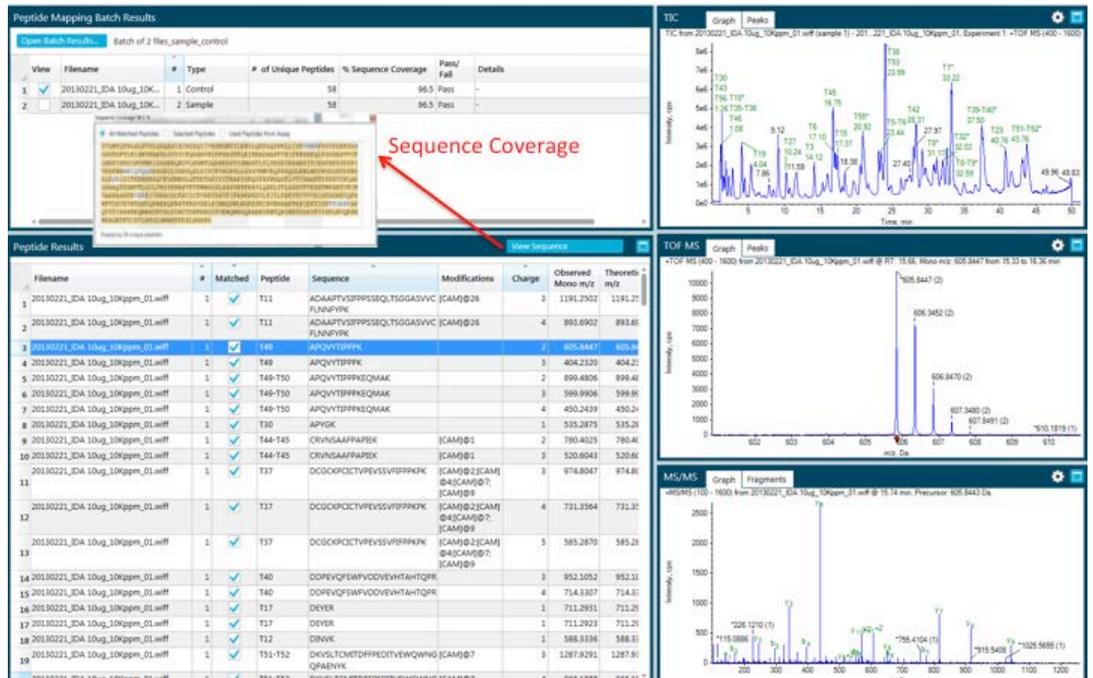
Batch Process: In the software, users can perform two types of batch processes: a sample-only batch and a sample-control comparison batch as a QC analysis. Overlaid TIC graphs can be used to pre-screen QC sample files prior to batch processing.

Review Results: The result review contains three types of information: a high level batch summary, detailed peptide results, and graphical views (Figure 4). The batch summary shows QC results and whether each sample passes or fails based on criteria defined in the assay information. The peptide result table reveals details including

Figure 4: Result review with Batch summary, peptide results, and graphical views.

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each peptide matched, sequence, charge state, PPM difference and RT and intensity information. The corresponding graph information allows users to review the TIC, TOF MS spectrum, and MS/MS spectrum along with the peptide information.

The TIC of both the sample and the control can be displayed as a mirror plot to allow users to examine and compare data visually (Figure 5). If there are differences in the TIC region of interest, the TOF MS and MS/MS spectra can be compared using mirror plots as well. In addition, XIC intensities of matches are reported in the peptide results table and the relative quantitation can be compared.
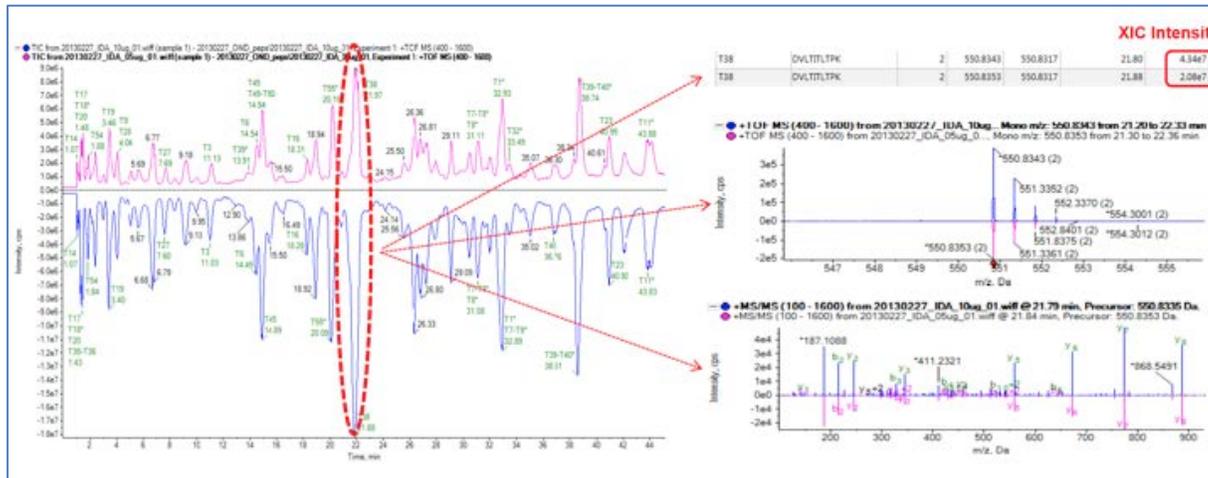

Figure 5: Mirror plots at the chromatographic and spectral levels enable easy comparison of samples with standard.

Generate Report: After the batch process is complete, you can create and generate QC reports (Figure 6). The user-changeable QC report template includes batch summary information, detailed peptide results, and graphical XIC, TOF MS, and MS/MS spectra for each of the matched peptides.

CONCLUSION

BioPharmaView Software provides complete characterization and analysis of biologics. The basic steps include assay development, characterization of standards, batch processing, result review, and report generation. When used in combination with the fast scan speed, high mass accuracy, and high resolution of the TripleTOF® system, the software provides a powerful and easy to use solution for the characterization of biologics and routine QC analysis.
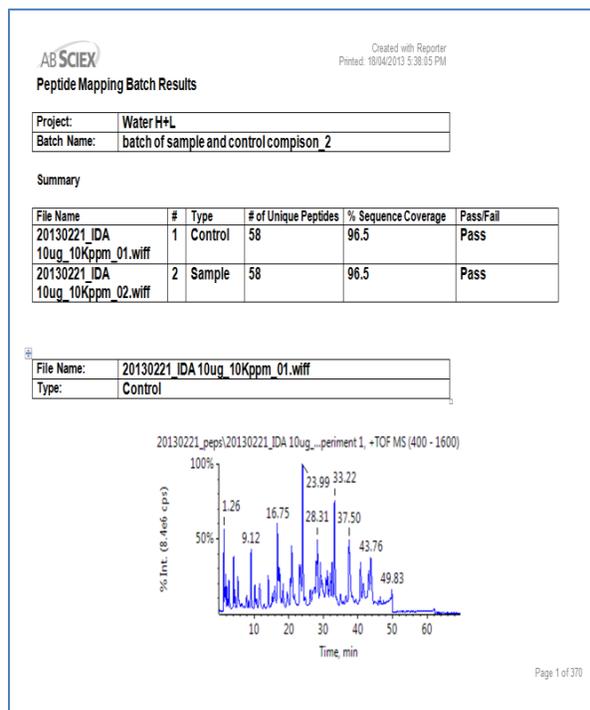

Figure 6: Example of one type of report view.

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500 Old Connecticut Path | Framingham, MA 01701 USA
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