Poster Note 72101

New Integrated Informatics Solution for Protein Biotherapeutics Characterization

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Overview

Purpose: Integrated informatics solution for protein biotherapeutics characterization. Two software packages Thermo Scientific™ Protein Deconvolution and Thermo Scientific™ PepFinder™ software are integrated into one software platform.

Methods: Intact and sub-unit mass analysis and peptide mapping were performed to characterize trastuzumab. The new Thermo Scientific™ BioPharma Finder™ software was used for data processing.

Results: One integrated software solution was used for full characterization of trastuzumab. Intact protein mass was determined and all major glycoforms were identified using the intact protein analysis (Protein Deconvolution). Sub-unit analysis used a novel sliding window algorithm which improves peak detection in complex mixtures, to generate deconvoluted masses for the Fc/2, Fd and light chain molecules. A simulated stress study is automatically processed using the peptide mapping workflow (PepFinder software) where expected modifications are identified and a relative amount is determined automatically. New visualization for data mining and data interpretation enable both expert and beginner users to be successful with this new software while providing more confident results.

Introduction

Increasing requirements to fully characterize complex protein biotherapeutics for safety and efficacy place analytical scientists under pressure. In spite of this, the discovery and development of protein biotherapeutics continues to thrive and demands faster and better tools. Here we present a new, powerful software that can leverage chromatographic separations and High Resolution Accurate Mass (HRAM) analysis for the characterization of biotherapeutics.

Methods

Sample: Trastuzumab was used for both intact analysis and tryptic peptide mapping analysis.

LC: Thermo Scientific™ Vanquish™ UHPLC system

Column: Thermo Scientific™ MAbPac™ RP, (50 mm * 2.1 mm; 4 mm)

Thermo Scientific™ Accucore™, C18 (100mm * 2.1 mm; 1.7 mm)

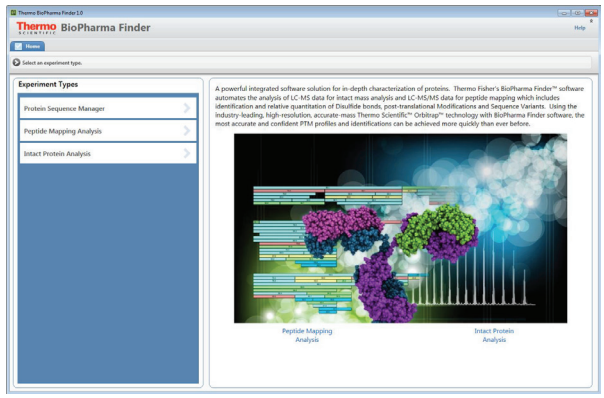
Mass Spectrometry: Thermo Scientific™ Orbitrap Fusion™ Tribrid™ MS

Data Analysis: Raw files were processed with Thermo Scientific™ BioPharma Finder software.

Results

BioPharma Finder software allows users to organize and store protein sequences with the Protein Sequence Manager and then the user can attach the sequence to the intact and peptide mapping methods (Figure 1). This simple interface allows users to easily navigate through the software seamlessly from one workflow to another.

FIGURE 1. BioPharma Finder software homepage



For the intact protein and sub-unit analyses, Trastuzumab was analyzed using the Orbitrap Fusion MS at 17K and 120K resolutions respectively. The deconvolution processing method used the ReSpect™ algorithm for the intact protein and Xtract™ algorithm was used for sub-unit study. MS scans were processed as “static” for the intact or “sliding window” for the sub-unit. All major glycoforms were identified and annotated. Average masses, sum intensities, matched delta masses and identification as well as abundances are reported in an exportable table. Sub-unit raw file was processed automatically using the sliding window tool eliminating the need to define manually the time range for each chromatographic peak.

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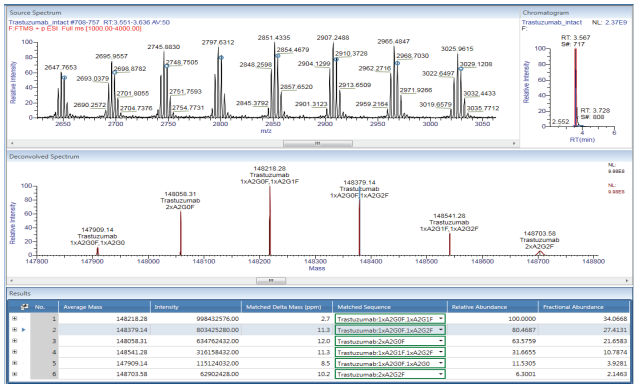
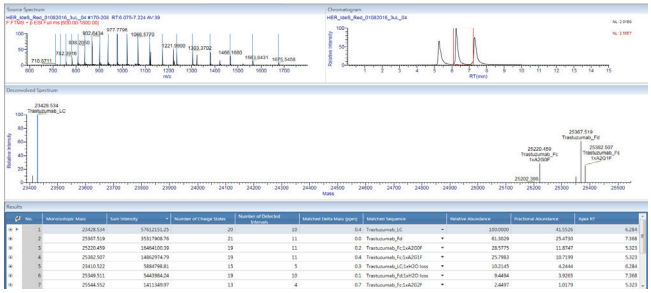
FIGURE 2. Intact mass analysis of Trastuzumab.



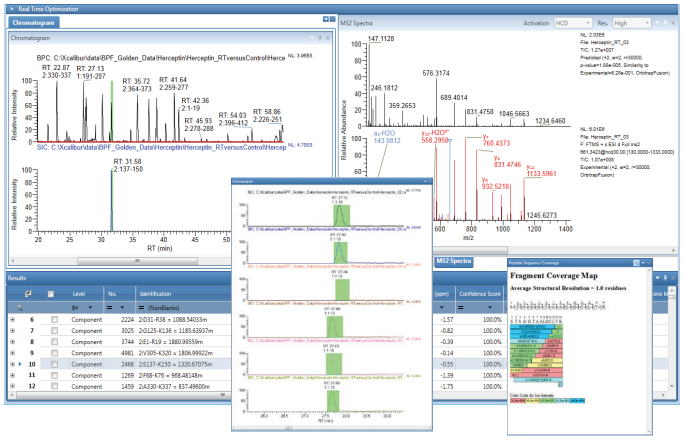
Displayed in the software is the source mass spectrum used for deconvolution with interactive graphics for manual interrogation of the results, chromatogram for reviewing elution profile, interactive deconvoluted spectrum and a results table which contains all of the relevant information.

FIGURE 3. Sub-unit analysis of Trastuzumab.



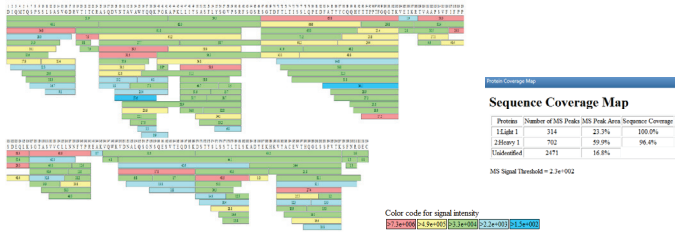
The width and the overlap between consecutive sliding window boxes for deconvolution is user defined. During processing the box will move across the chromatogram providing real time visualization.
All of the sub-units were identified with a delta mass error below 1ppm.

FIGURE 4. Peptide Mapping analysis of Trastuzumab. Main process & review page in BioPharma Finder software with interactive plots and tables for user friendly data mining.



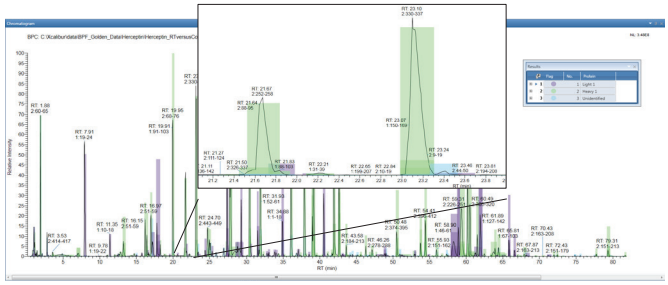
A “stress” sample was generated by storing at room temperature for 48 hrs a tryptic digest sample. For peptide mapping analysis, raw files were divided in two groups, control and stress. Data were acquired using an Orbitrap Fusion mass spectrometer. Data processing was performed using the peptide mapping workflow in BioPharma Finder software. The core algorithms for peptide mapping are from the PepFinder software. However, BioPharma Finder software has an updated user interface which provides a significantly improved user experience for data mining and data interpretation. Figure 4 shows the process & review page of the peptide mapping workflow. The main page has an interactive table, chromatogram plotting capability with 6 different types of plots and the ability to stack chromatograms from multiple raw files allowing the user to view peptides across samples. All of the plots and tables can be floated on the screen or moved to a second monitor for custom layout.

FIGURE 5. Sequence coverage map.



The sequence coverage map is automatically generated and provides a visual display of the depth of identification that is achieved in the peptide mapping workflow. Each peptide is colored based on abundance and a summary report is generated for each data file.

FIGURE 6. Novel chromatographic shading plot.



A new feature in BioPharma Finder software peptide mapping workflow is shown in Figure 6. The basic peak chromatogram is shaded using different colors based on the protein identification. The purple are peptides from the light chain, green are from heavy chain and blue are unidentified. This interactive plot provides the user an image where they can quickly see non-identified peaks, which peptides are the most abundant and displays co-eluting peptides.

FIGURE 7. Modification table for the oxidation on W420 of the heavy chain.

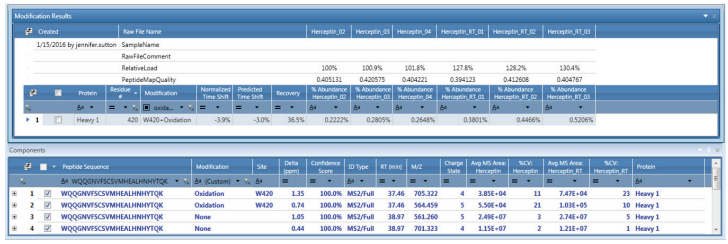


Figure 7 is a dispay of the new and improved modification summary report which was part of PepFinder software. This report is now an interactive report in which the user can select a specific modification on the top table and see the components used in the abundance calculation. Normalized time shift, a new feature, is the comparison of the modified and non-modified peptides retention times. The normalized time shift can be compared to the predicted time shift providing an extra level of confidence for the identification.

Conclusions

BioPharma Finder software provides:

- Confident deconvoluted molecule weight of proteins in denaturing and native conditions.
- Extra confidence in peptide identification by using a novel MS/MS predictive algorithm.
- Quantification of modifications
- Characterization of disulfide linkages
- Low level impurities - sequence variants identification
- Sequence alteration – stress samples, level of deamidation or oxidation

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