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Drug Discovery and Development

SCIEX

Studying Quality Attributes of Rituximab by Mass Spectrometry

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Introduction

Peptide Mapping with mass spectrometric detection is now one of the default techniques for biotherapeutic analysis, whether in development or for release assays. SCIEX has innovated to develop state-of-the-art software that facilitates the data analysis from accurate mass instruments to speed up data review and reporting at any stage in the process of developing biotherapeutics.

This article will describe some of the elements that allow users to rapidly adapt the processing without any need for specialist mass spectrometry knowledge.

The Utility of Accurate Mass QTOF Analysis

Accurate mass QToF instruments are the dominant mass spectrometry platforms for characterization of biologic's critical quality attributes (CQAs), assisting in process development analyses, and in troubleshooting. QToF technology dominates

this space, delivering high resolution, (above 30k, FWHM) at all mass ranges and fast acquisition rates. Additionally, there are no practical limitations for mass range, with TOF m/z ranges around 40kDa. Using SCIEX TripleTOF® platforms, therapeutic peptides and proteins of all sizes can be routinely analyzed. BioPharmaView™ Software is used to automate data processing, and reduces analysis time from days to minutes compared to manual techniques. Although automation avoids human error, users often wish to review data themselves to ensure the appropriate processing parameters, or investigate batch failures. There are a number of ways in which this type of review can be facilitated in BioPharmaView Software.

Smart filtering

A typical mAb peptide map might contain hundreds of peptides when considering all of the modifications, charge states and missed cleavages. Therefore, tools that allow smart filtering and multi-level filtering are always considered essential. The functionality displayed in Figure 1 shows how this is performed for
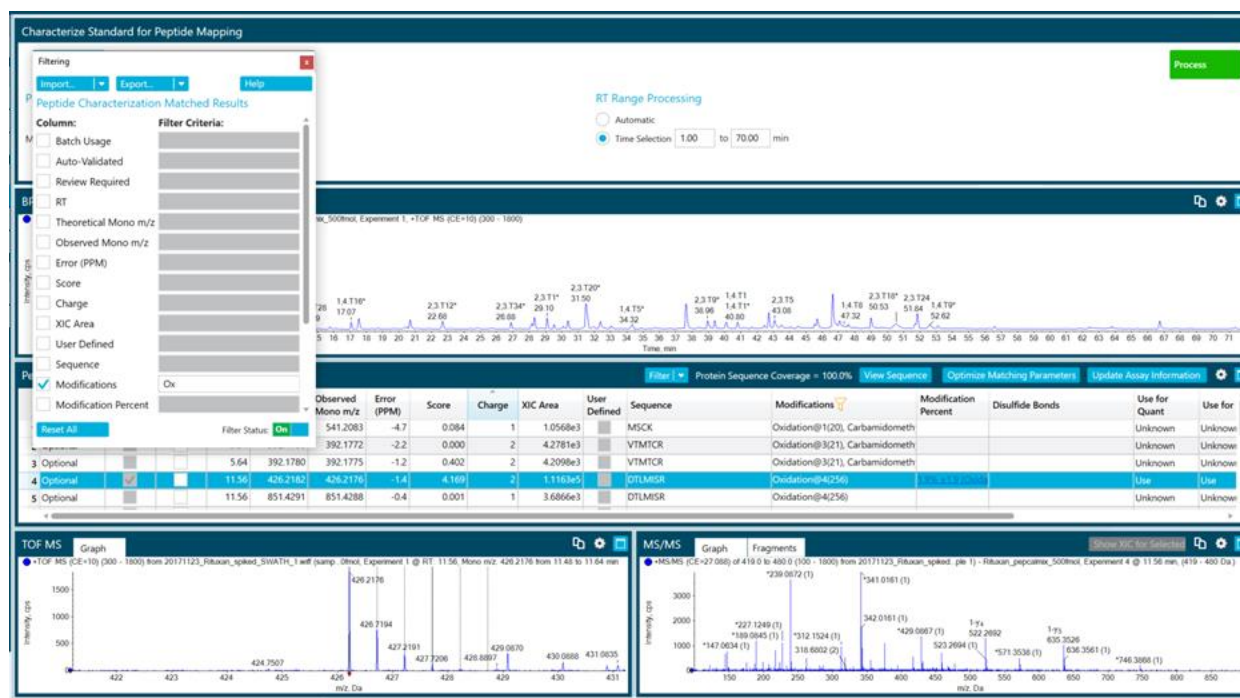

Figure 1: Screenshot of the filter function in BioPharmaView Software. On the left hand side is the pop out box with the options available to review, live-lined to the data in the review pans below. In this example, the peptides are filters for all oxidized species. The full list is not shown, which is in the middle pane. The red 'filter' symbol shows the columns where the multi-level filtering criteria have been applied.

a peptide map of IgG mAb rituximab, which is approximately 144 kDa and contains 1328 amino acids.[1]

BioPharmaView Software provides users with an exhaustive set of details regarding their biotherapeutics, but can simplify the information rapidly in the review stage. Figure 1. Shows the panels of information available where the chromatographic trace is displayed above the tabular and spectral data in separate panels below. At the base are two panels depicting the spectrum (MS) and the fragment ions (MS/MS) for that peptide. The analyst only needs to glance at the data to know whether further investigation is needed.

When more refined information is needed, a user can further filter the data in a manner to align data for each peptide in a simple table, and verify that no assignments are false positives. In Figure 2, this is illustrated with the use of an additional filter level that is applied instantly. Figure 2 shows the filter applied to screen for all peptides that are deamidated using the prefix "Ox" in the filter panel.

The filter is also applied to show only those matches contain an oxidation as well, a second peptide filter is set to exclude missed cleavages with a "NOT" Boolean logic operator applied for the "-" marker of missed cleavages in the peptide field.

The utility of the visual display is also apparent to signal when a filter is applied. The "on/off" toggle can remove all filters or re-

apply them with a simple click (Figure 3) allowing reviewers to see at a glance the effect of their filtering. Figure 4 is a zoom on the filter symbol which appears at the top of the relevant category (column) to show that a filter is being applied.
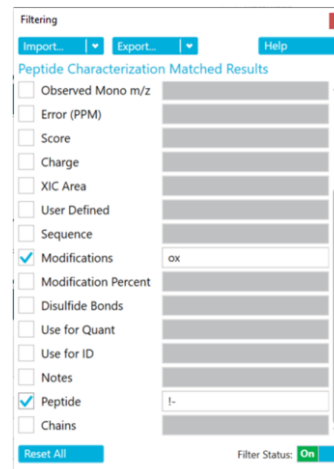

Figure 3:The "on/off" toggle switch for the filtering function in BioPharmaView Software.
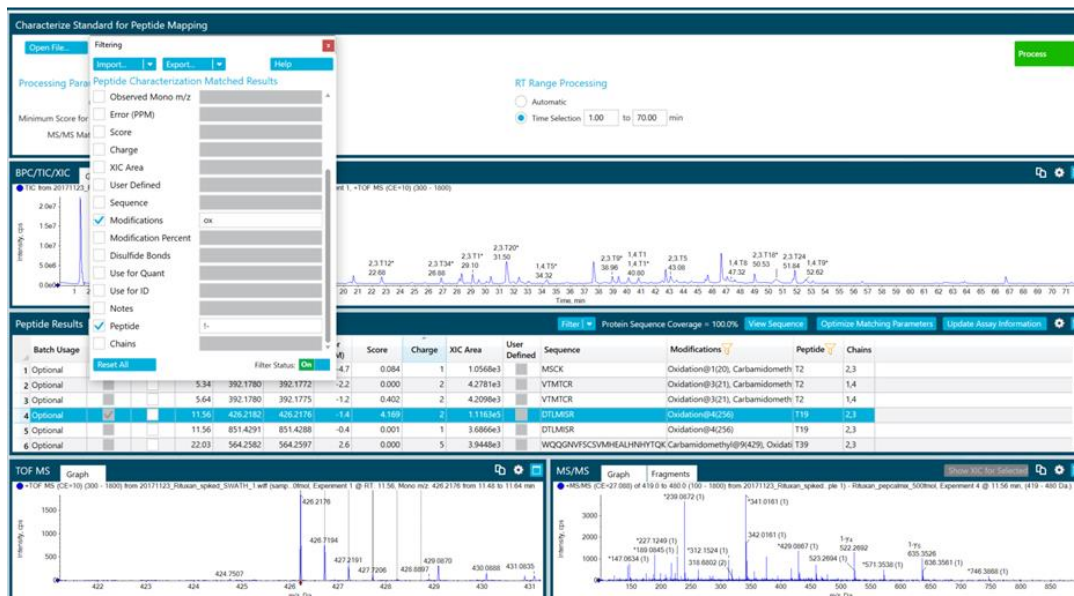

Figure 2: Smart filter applied to rituximab peptide map with filtering oxidation features and no missed cleavages that may be important quality attributes.

Figure 4: The "filter" symbol is shown in yellow at the top of each column category in BioPharmaView Software.

Conclusions

Efficient post-processing of peptide mapping data from TripleTOF® LC-MS systems is essential to providing SCIEX customers the rapid review capabilities they need. The flexibility of such a tool improves workflow efficiency by removing human bias, automating tedious procedures and allowing the in-depth analysis of a complex data set within seconds.

References

1 http:www.drugbank.ca/drugs/