Evaluation of XIC Peaks for Inclusion in Average Peptide Oxidation Calculations

FoxWare™ Data Processing Software uses the monoisotopic mass of an unmodified peptide and calculates the monoisotopic masses for modified peptides. Then the software generates the XICs for all peptides. Below is a description of how the FoxWare Software evaluates the XIC peaks to include them when calculating the average peptide oxidation.

Calculation of Average Peptide Oxidation

Once the XIC peaks are evaluated and selected, the FoxWare Software uses Equation 1 below to calculate the average peptide oxidation events per peptide.

Equation 1:

$$\frac{\sum_{n=1}^{MaxOxLevel}(XIC\ Peak\ Area\ for\ OxLevel_n)(n)}{(XIC\ Peak\ Area\ of\ Unmodified\ Peptide) + \sum_{n=1}^{MaxOxLevel}(XIC\ Peak\ Area\ for\ OxLevel_n)}$$

Unmodified XIC Peaks

The primary unmodified peak is chosen using an algorithm that searches for the most intense peak with the highest number of MS2 peptide identifications. To compensate for intermittent MS2 identifications, adjacent XIC peaks are evaluated and selected based on their MS1 spectra. Once the baseline is hit or the specified retention time limits are reached, the FoxWare Software stops selecting peaks to include in the total unmodified XIC peak area.

Modified XIC Peaks

When using reverse phase chromatography, the addition of oxygen(s) will shift the retention time of the peptide. Typically, this causes the peptide to elute immediately prior to the corresponding unmodified peptide. Due to this phenomenon, modified peptides are searched within a retention time window of four minutes prior and three minutes after the primary unmodified peak. To confirm the XIC peaks are from the modified peptide, each peak is evaluated using the MS1 spectra data at the XIC peak apex.

XIC Peak Evaluation

There are three MS1 evaluation methods available with the Foxware Software. The first method is feature detection, which is the most sophisticated and reliable evaluation method. The second method is MS1 isotope ratios which is used for evaluating lower intensity oxidation products where isotopic ratios may be more variable. The final method is the isotopic XIC ratios, the lowest confidence method for data in the noise, and it is recommended to always manually check the selected peptides. Any XIC Peak evaluation can be overrode by the user by manually excluded or included the peak. The following provides more information on the three XIC peak evaluation methods.

MS1 Feature Detection:

Always Applied to Unmodified & Modified XIC Peaks

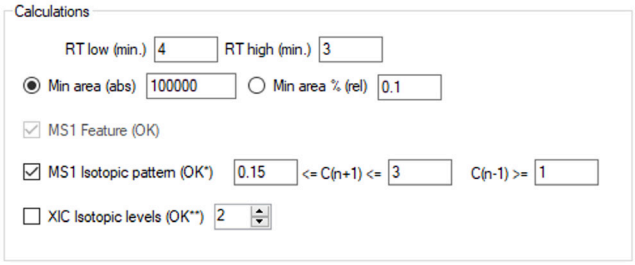
The MS1 Feature Finder examines the MS1 isotopic distribution of the peptide.

Using a sophisticated algorithm, the Feature Finder calculates the similarity of the isotopic distribution in the observed spectrum against the theoretical isotope patterns of averagine peptide models and determines a correlation score. If a peptide has a correlation score of 0.9 or greater, a Feature at the mass and charge state of the model is assigned. If a Feature is detected at the correct peptide mass and charge (within a user defined mass tolerance), that XIC peak is determined to be from the peptide as shown below.

Evaluation of XIC Peaks

MS1 Isotopic Pattern:

Users' Choice (recommended) Applied to Unmodified & Modified XIC Peaks

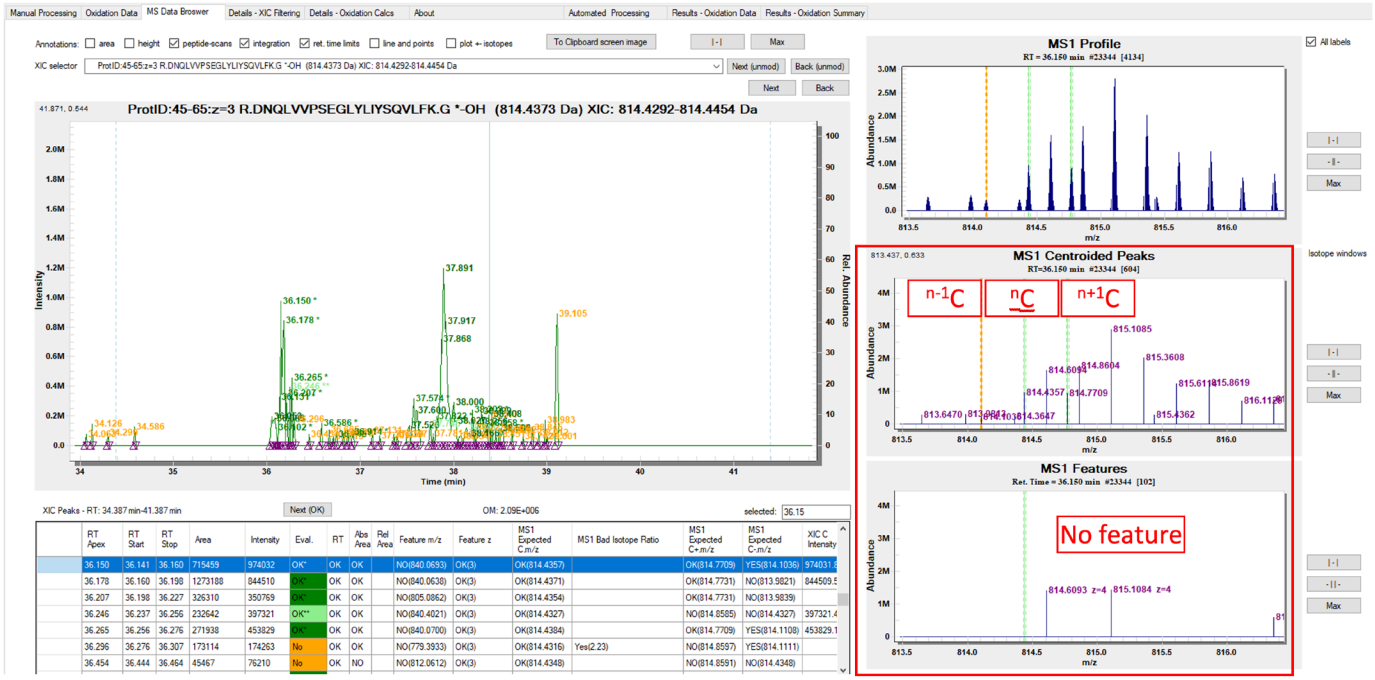


The MS1 Isotopic pattern is used when a Feature is not detected due to lower intensity or a complex mixture of peptides eluting together.

The FoxWare Software looks for ^{n}C, ^{n+1}C, ^{n-1}C isotopes within the defined mass tolerance. There are two criteria for the peptide to pass:

1. The ^{n}C isotope (e.g., 12C) and ^{n+1}C isotope (e.g., 13C) need to be detected, and the ratio (^{n+1}C /^{n}C) must be greater than 0.15 and less than 3 (defaults). For example: if the ^{n}C intensity is 1.35e6 and ^{n+1}C is 5.67e5 the ratio would be (5.67e5/1.35e6) or 0.42. This is within the set parameters.

2. If the ^{n-1}C ion was also detected, the ^{n-1}C to ^{n}C ratio must be below 1.0 (default). Typically, an isotopologue is less abundant than the parent ion, but for larger peptides (≥ 25 amino acids), the isotopologue can become more abundant. If the ^{n-1}C ion was detected, it is recommended checking a few of the more abundant OK* identifications and manually confirm the peptide identification.

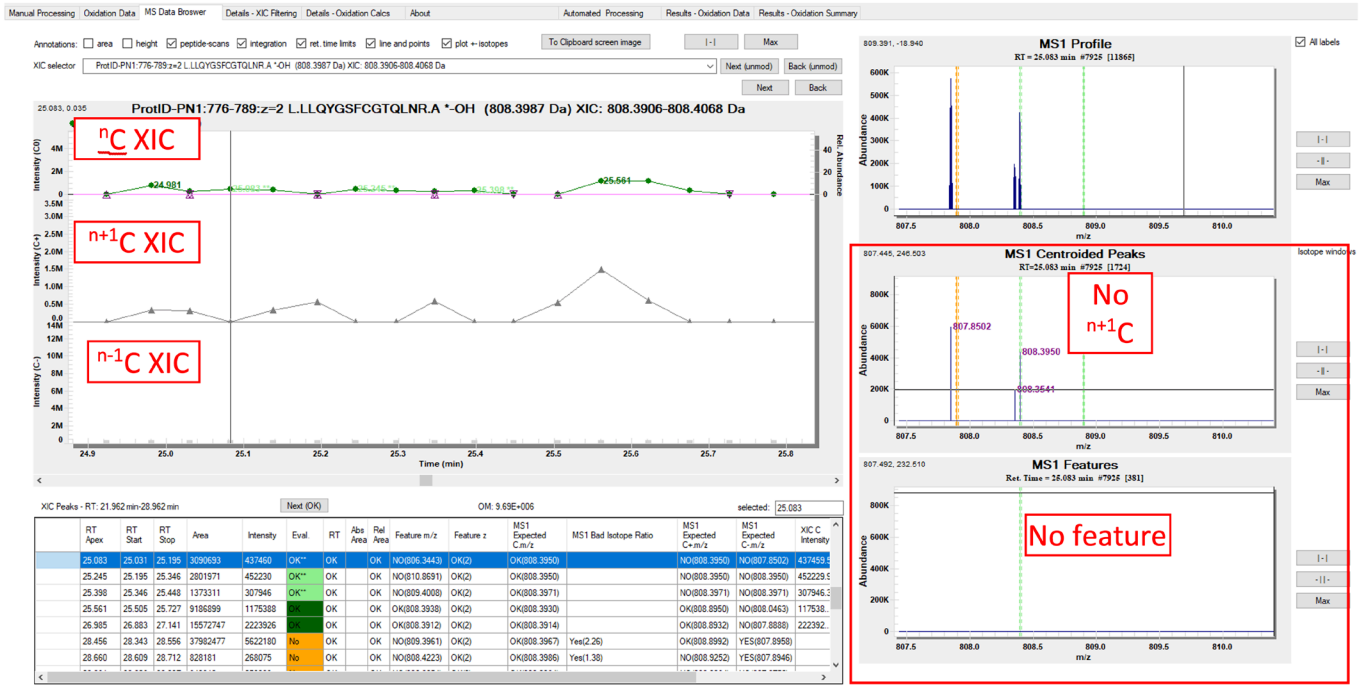


XIC Isotopic Levels (Low Level XIC):

Users Choice (Not Recommended) Applied to Modified XIC Peaks (Never Unmodified XIC Peaks)

Low level XIC, labeled OK** in XIC Peak table, was created for very low abundant ions that drift in and out of the detection threshold. Above are the XICs for the ^{n}C, ^{n+1}C, and ^{n-1}C ions (top to bottom, respectively). The points on the plot represent the intensity of the ion for that retention time.

As you can see, at time 25.083 the ^{n+1}C was not detected, but one scan to the right or left the ^{n+1}C ion is detected, so that peak was assigned OK**. It is recommended to limit the use of the low level XIC selection to the lowest intensity peptides.



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