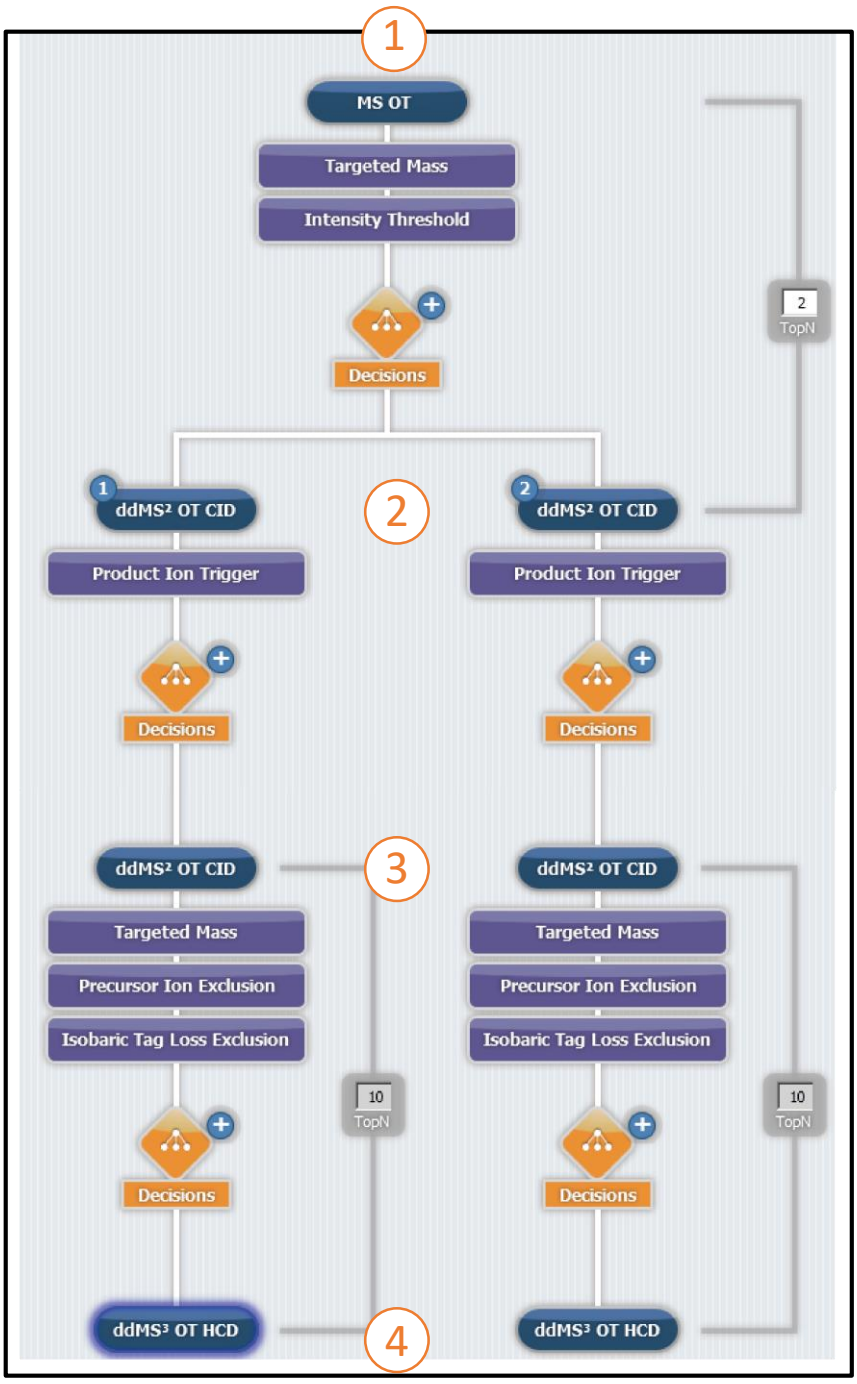
TOMAHAQ Method Construction

Triggered by offset mass accurate-mass high-resolution accurate quantitation (TOMAHAQ) can be performed in the standard method editor of the instrument, without modifications to the instrument code. In this mode, only two SPS fragment ion filters are missing: SPS fragment ion ratio and SPS isolation purity. While these features increase the accuracy of the method, a great deal of interference can removed by targeting the correct fragment ions during SPS-MS3 analysis.

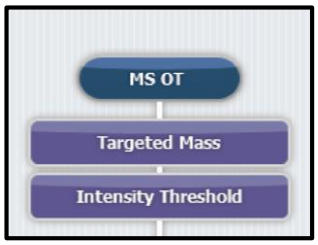


Method Overview

1. MS1 Scan
   - Targeted Mass Filter
   - Intensity Filter
2. Trigger MS2 Scan
   - Product Ion Trigger
3. Offset target MS2 Scan
   - Targeted Mass Filter
   - Precursor Ion Exclusion
   - Isobaric Tag Loss Exclusion
4. Offset target MS3 Scan

Example Peptides

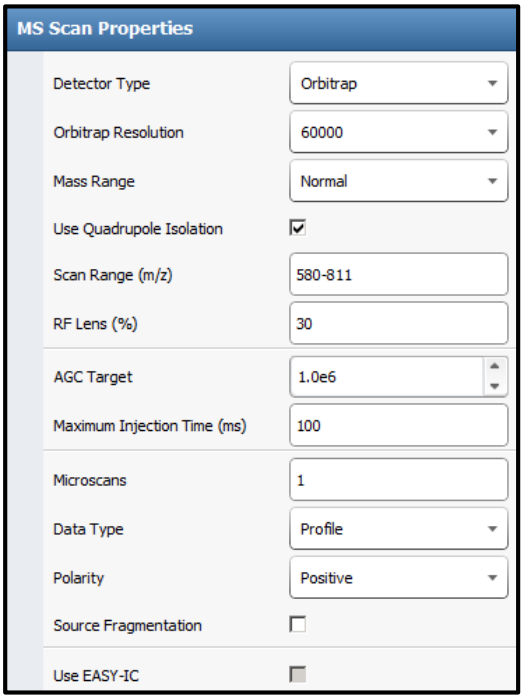
1. EIGGLTQVNK (+2)
2. NPDDITQEEYGEFYK (+3)



MS1 Setting and Filters

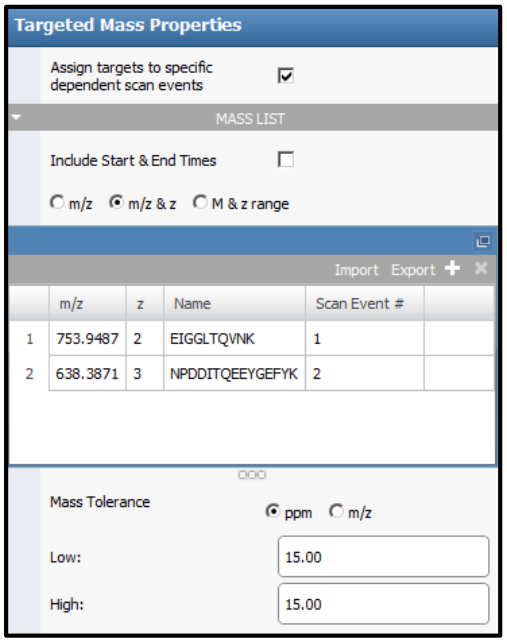
MS Scan Properties

- MS1 scan range is set +/- 50 m/z of the peptides that are being interrogated.
- Orbitrap resolution is set to 60,000 resolution to decrease the time used for FTMS analysis



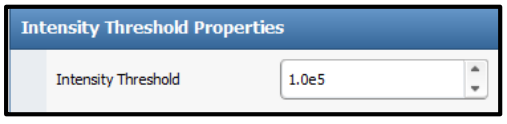
Targeted Mass Properties

- Targets are assigned to a specific scan event based on the specific isolation mass shift
    1. Isolation mass shift = 5.01 (+2 peptides w/ 2 tags)
    2. Isolation mass shift = 3.34 (+3 peptides w/ 2 tags)
- For this analysis start and stop times are not included; however, if you are using RT scheduling this is where the RTs would be entered
- Trigger peptides were analyzed only if the precursor m/z was within ±15 ppm of the theoretical value



Intensity Threshold Properties

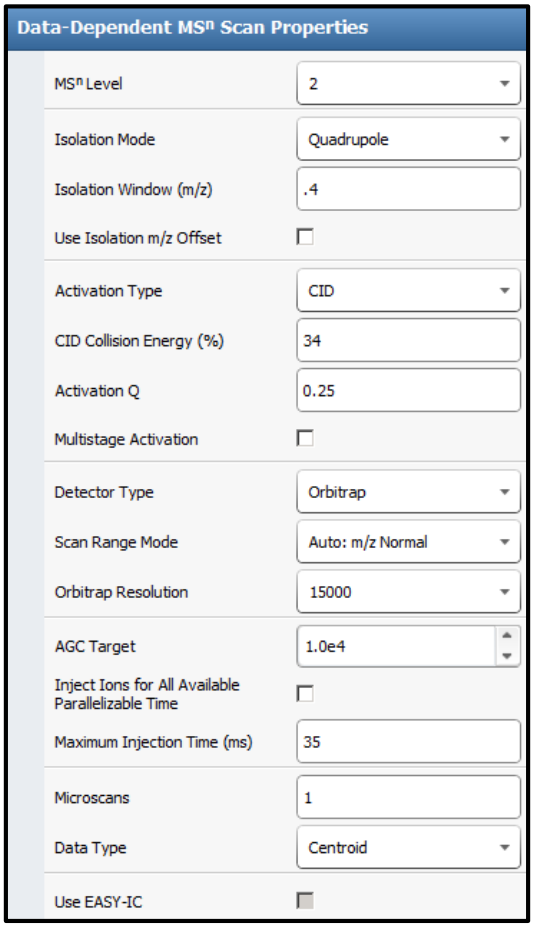
- The intensity threshold was set to 1e5. This value is higher than usual because the trigger peptide will be of sufficient abundance in the MS1 survey scan.



Trigger MS2 Setting and Filters

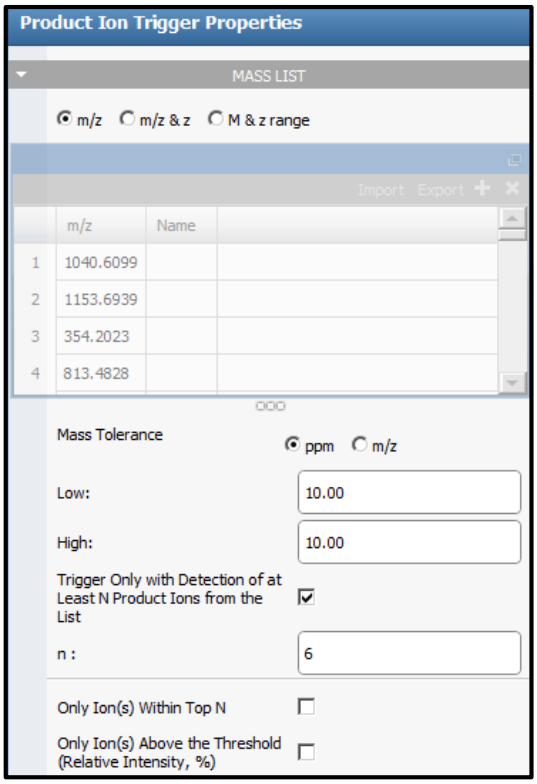
Data-Dependent MSn Scan Properties

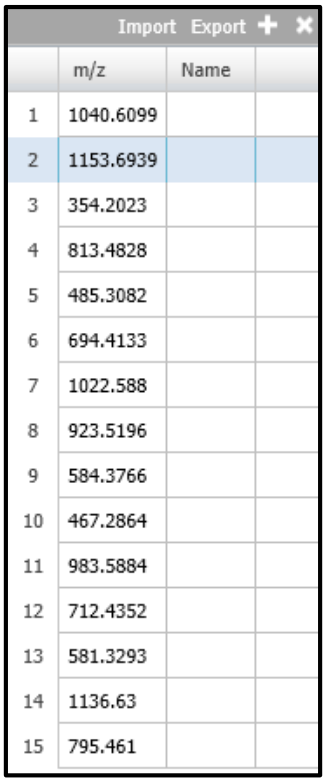
- Isolation is performed with the quadrupole at an isolation width of 0.4.
- A CID collision energy of 34 is used such that trigger and target scans can be differentiated in downstream analysis.
- FTMS analysis is used to ensure correct identification of trigger peptides, reducing the number of errant target MS2 scans that are performed.
- Trigger peptides will be abundant, so a low maximum injection time is used.

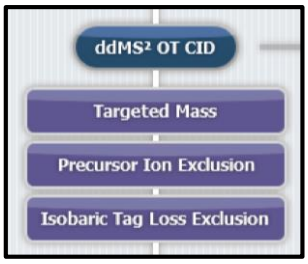


Product Ion Trigger Properties

- Online identification can be mimicked using product ion triggering.
- Fragment ion m/z values corresponding to the trigger peptide are loaded into a mass list and assessed at a tolerance of ±10 ppm.
- Here we require that at least 6 product ions are detected before triggering downstream scans.



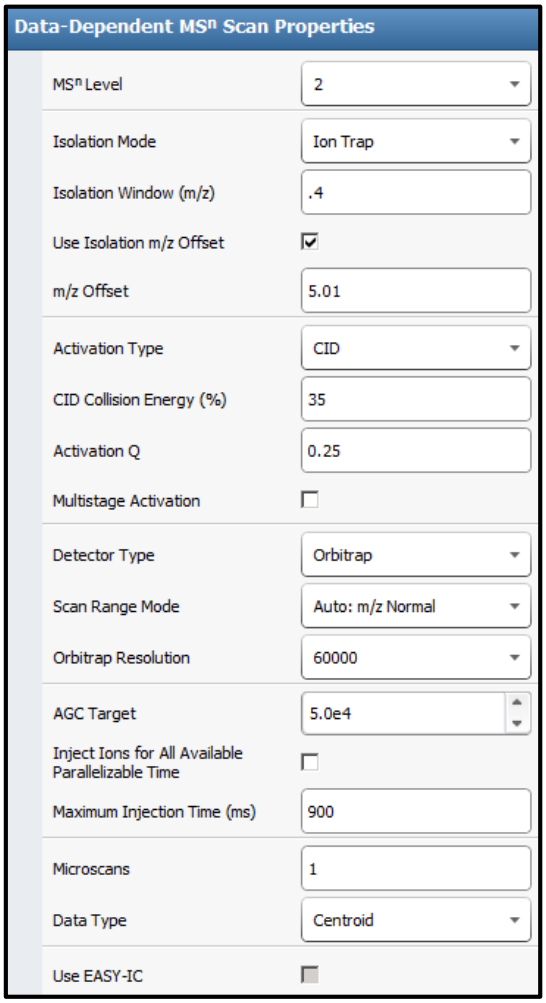




Target MS2 Setting and Filters

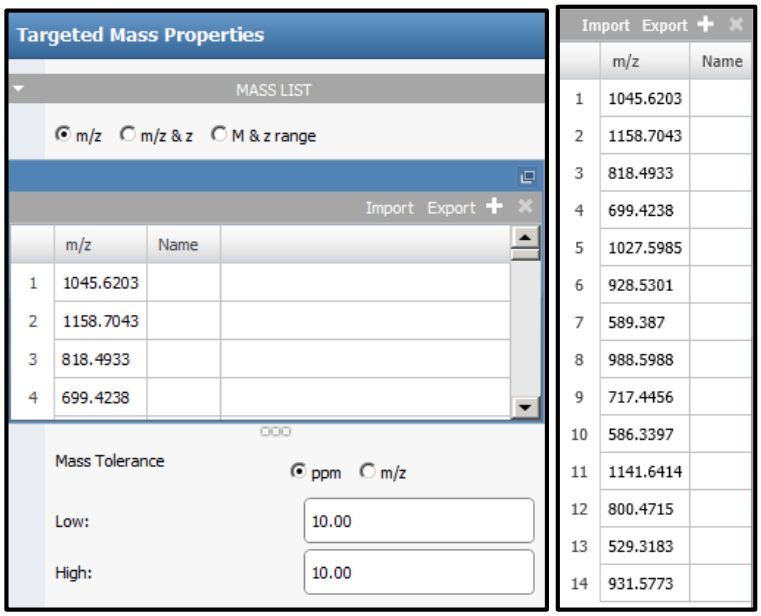
Data-Dependent MSn Scan Properties

- Isolation is performed with the quadrupole at an isolation width of 0.4. This removes many interfering peptides that are co-eluting.
- An isolation offset is used that corresponds to the number of isobaric labels on the peptide and the peptide change state.
  - Here the peptide has 2 tags and is shifted by +10.02 Da.
  - The peptide is a +2 charge state
  - 10.02 / 2 = 5.01 Da isolation shift
- A CID collision energy of 35 is used such that trigger and target scans can be differentiated in downstream analysis.
- FTMS analysis (60,000 resolution) is used to ensure correct identification of fragment ions and to enable detection of interfering fragment ions.
- Target peptides will not be abundant, so a high maximum injection time is used. Here we use 900 ms to ensure that we detect fragment ions from low-abundant target precursors.



Targeted Mass Properties

- Fragment ion m/z values corresponding to the target peptide are loaded into a mass list and assessed at a tolerance of ±10 ppm.
- Targeting SPS ions removes mostly all interference, even with low abundant precursors.

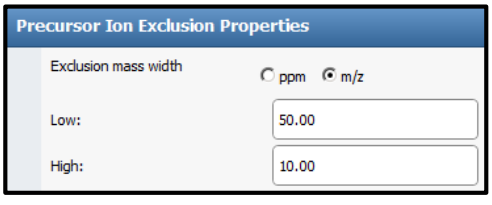


Precursor Ion Exclusion Properties

- CID fragmentation produces many neutral loss species around the precursor ion. These ions can lead to interference in the quantitative channels. To avoid this we exclude below (-50 Da) and above (+10) the precursor m/z.

Isobaric Tag Loss Exclusion Properties

- Isobaric labels produce “complement” ions that should not be selected for MS3 analysis.



Target MS3 Settings

Data-Dependent MSn Scan Properties

- Isolation is performed with the quadrupole at an isolation width of 0.4. This removes many interfering peptides that are co-eluting.
- Synchronous precursor selection (SPS) is enabled and up to 10 precursors can be included for MS3 analysis.
- An isolation offset is carried through subsequent scans and does not need to be applied to the MS3 scan.
- An HCD collision energy of 55 is used to efficiently convert peptides bearing isobaric labels into reporter ions.
- FTMS analysis (60,000 resolution) is used to enable analysis of 10-plex TMT, which uses isotopologues to increase multiplexing capabilities.
- A high AGC target is used, with the expectation that the peptides that will be interrogated will potentially represent a small percentage of the ion population isolated.
- Target peptides will not be abundant, so a high maximum injection time is used. Here we use 2500 ms to ensure that we create sufficient report ion signal for quantitation.