

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 be 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

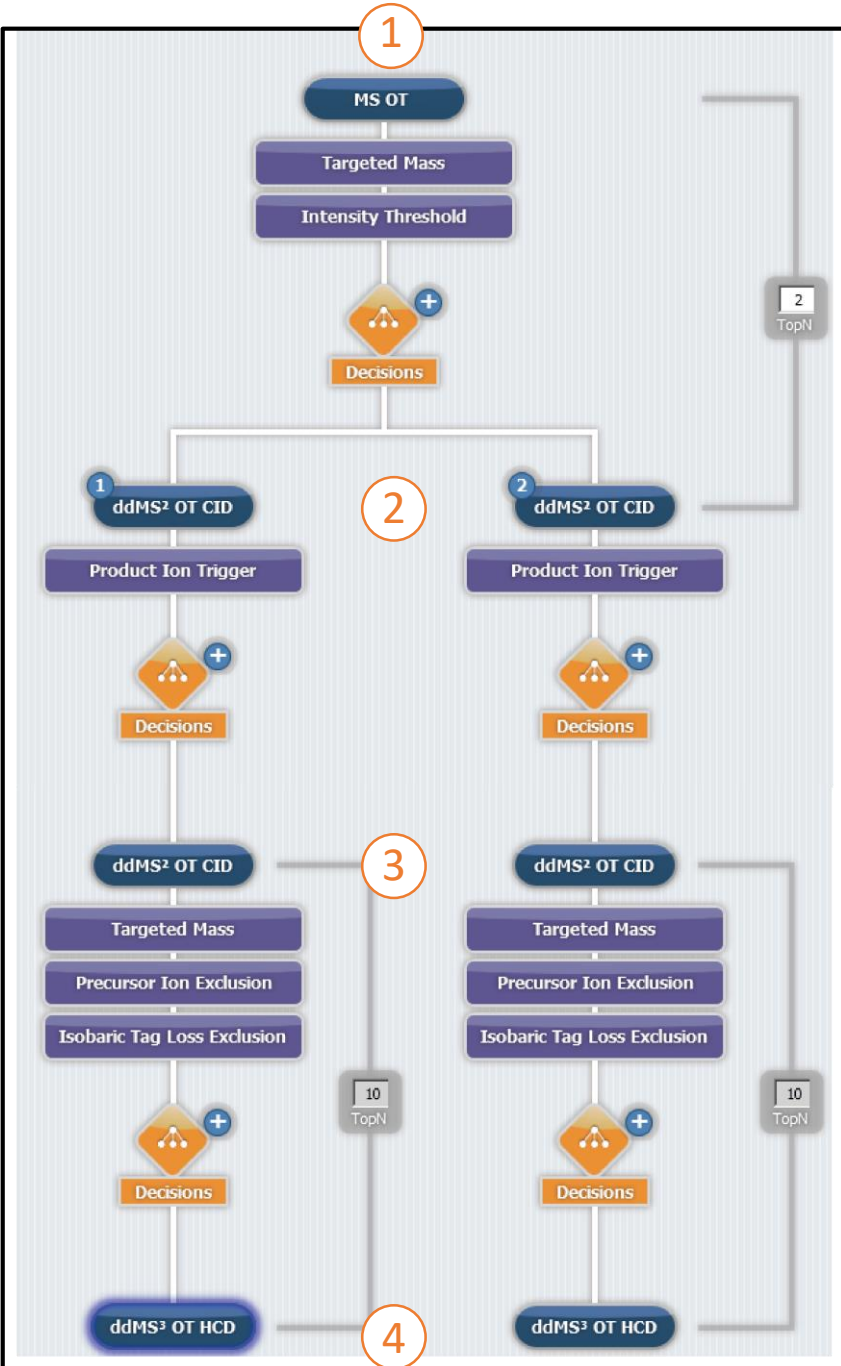
- Targeted Mass Filter
- Precursor Ion Exclusion
- Isobaric Tag Loss Exclusion

4. Offset target MS3 Scan

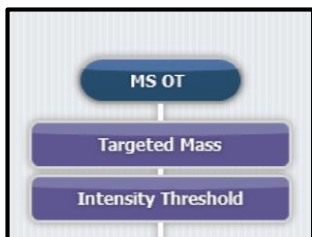
Example Peptides

1. EIGGLTQV NK (+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

MS Scan Properties

Detector Type	Orbitrap
Orbitrap Resolution	60000
Mass Range	Normal
Use Quadrupole Isolation	<input checked="" type="checkbox"/>
Scan Range (m/z)	580-811
RF Lens (%)	30
AGC Target	1.0e6
Maximum Injection Time (ms)	100
Microscans	1
Data Type	Profile
Polarity	Positive
Source Fragmentation	<input type="checkbox"/>
Use EASY-IC	<input type="checkbox"/>

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

Targeted Mass Properties

Assign targets to specific dependent scan events

MASS LIST

Include Start & End Times

m/z m/z & z M & z range

	m/z	z	Name	Scan Event #
1	753.9487	2	EIGGLTQVNK	1
2	638.3871	3	NPDDITQEEYGEFYK	2

Mass Tolerance ppm m/z

Low: 15.00

High: 15.00

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.

Intensity Threshold Properties

Intensity Threshold 1.0e5



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.

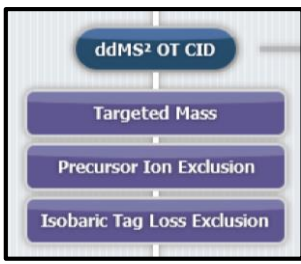
Data-Dependent MS ⁿ Scan Properties	
MS ⁿ Level	2
Isolation Mode	Quadrupole
Isolation Window (m/z)	.4
Use Isolation m/z Offset	<input type="checkbox"/>
Activation Type	CID
CID Collision Energy (%)	34
Activation Q	0.25
Multistage Activation	<input type="checkbox"/>
Detector Type	Orbitrap
Scan Range Mode	Auto: m/z Normal
Orbitrap Resolution	15000
AGC Target	1.0e4
Inject Ions for All Available Parallelizable Time	<input type="checkbox"/>
Maximum Injection Time (ms)	35
Microscans	1
Data Type	Centroid
Use EASY-IC	<input type="checkbox"/>

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.

Product Ion Trigger Properties	
MASS LIST	
<input checked="" type="radio"/> m/z <input type="radio"/> m/z & z <input type="radio"/> M & z range	
Import Export + X	
m/z	Name
1 1040.6099	
2 1153.6939	
3 354.2023	
4 813.4828	
ooo	
Mass Tolerance	<input checked="" type="radio"/> ppm <input type="radio"/> m/z
Low:	<input type="text" value="10.00"/>
High:	<input type="text" value="10.00"/>
Trigger Only with Detection of at Least N Product Ions from the List	<input checked="" type="checkbox"/>
n :	<input type="text" value="6"/>
Only Ion(s) Within Top N	<input type="checkbox"/>
Only Ion(s) Above the Threshold (Relative Intensity, %)	<input type="checkbox"/>

	m/z	Name	
1	1040.6099		
2	1153.6939		
3	354.2023		
4	813.4828		
5	485.3082		
6	694.4133		
7	1022.588		
8	923.5196		
9	584.3766		
10	467.2864		
11	983.5884		
12	712.4352		
13	581.3293		
14	1136.63		
15	795.461		



Target MS2 Setting and Filters

Data-Dependent MSⁿ Scan Properties

MS ⁿ Level	2
Isolation Mode	Ion Trap
Isolation Window (m/z)	.4
Use Isolation m/z Offset	<input checked="" type="checkbox"/>
m/z Offset	5.01
Activation Type	CID
CID Collision Energy (%)	35
Activation Q	0.25
Multistage Activation	<input type="checkbox"/>
Detector Type	Orbitrap
Scan Range Mode	Auto: m/z Normal
Orbitrap Resolution	60000
AGC Target	5.0e4
Inject Ions for All Available Parallelizable Time	<input type="checkbox"/>
Maximum Injection Time (ms)	900
Microscans	1
Data Type	Centroid
Use EASY-IC	<input type="checkbox"/>

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 charge 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.

Targeted Mass Properties

MASS LIST

m/z m/z & z M & z range

Import Export + X

	m/z	Name
1	1045.6203	
2	1158.7043	
3	818.4933	
4	699.4238	

Mass Tolerance ppm m/z

Low:

High:

	m/z	Name
1	1045.6203	
2	1158.7043	
3	818.4933	
4	699.4238	
5	1027.5985	
6	928.5301	
7	589.387	
8	988.5988	
9	717.4456	
10	586.3397	
11	1141.6414	
12	800.4715	
13	529.3183	
14	931.5773	

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.

Precursor Ion Exclusion Properties

Exclusion mass width ppm m/z

Low:

High:

Isobaric Tag Loss Exclusion Properties

Reagent

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.

Data-Dependent MS ⁿ Scan Properties	
MS ⁿ Level	3
Synchronous Precursor Selection	<input checked="" type="checkbox"/>
Number of Precursors	10
MS Isolation Window (m/z)	.4
MS2 Isolation Window (m/z)	3
Use Isolation m/z Offset	<input type="checkbox"/>
Activation Type	HCD
HCD Collision Energy (%)	55
Detector Type	Orbitrap
Scan Range Mode	Auto: m/z High
Orbitrap Resolution	60000
First Mass (m/z)	100
AGC Target	1.0e6
Inject Ions for All Available Parallelizable Time	<input type="checkbox"/>
Maximum Injection Time (ms)	2500
Microscans	1
Data Type	Centroid
Use EASY-IC	<input type="checkbox"/>