New method filters for improved MS^n acquisition for small molecule and proteomics workflows

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ABSTRACT

Purpose: We describe the new filtering capabilities of the Thermo Scientific™ Orbitrap™ method editor software, which directs the instrument towards efficiently collecting more meaningful and informative tandem mass spectrometry (MS/MS) and higher-order MS^n spectra.

Methods: We developed and evaluated these filters using a combination of small molecule samples (human plasma and flavonoids) and a complex Tandem Mass Tag (TMT) labeled peptide sample.

Results: The AcquireX workflow guides the instrument towards efficiently collecting MS^n data at exhaustive breadth and depth, while the MS^n Quality Trigger informs the instrument of when it is appropriate to collect high-value and high-cost MS^n spectra.

INTRODUCTION

Complex hybrid and Tribrid™ mass spectrometers have been available for over a decade, and during this time the methods used on these instruments have grown to match the instrument's sophistication. Small molecule workflows often employ some of the most complex methods. For the sake of structural elucidation, these methods often delve to very high MS^n orders (>4), and they couple these ion manipulations with high resolution and mass accuracy *m/z* analysis. While these Orbitrap MS^n scan types can be incredibly information rich, there is a price paid when collecting them in terms of the spectral acquisition rate and sensitivity.

Herein, we describe new filters that are designed to guide the instrument towards efficiently collecting FTMS^n spectra at an exhaustive breadth and depth. The AcquireX workflow directs when the instrument should trigger MS^n analysis on a given MS1 precursor ion, while the MS^n Quality Trigger informs the instrument of when it is worthwhile to collect high-value and high-cost MS^n spectra.

MATERIALS AND METHODS

Mass spectrometer and instrument control

All the small molecule data presented in this poster were collected on the Thermo Scientific™ Orbitrap ID-X™ Tribrid™ mass spectrometer. This instrument combines the Tribrid architecture of the Fusion™ series platform with new instrument control software that has been optimized and streamlined for users who are focused on small molecule analysis. The proteomics data was collected on the Thermo Scientific™ Orbitrap Fusion™ Lumos™ Tribrid™ mass spectrometer.

Both the Orbitrap ID-X mass spectrometer and the Orbitrap Fusion Lumos mass spectrometer were running the latest versions of the Thermo Scientific™ Tune™ and Xcalibur™ instrument control software (versions 3.1 and 4.2, respectively).

Sample Preparation

The AcquireX workflow was tested on a commercially available human plasma sample (NIST SRM 1950). A custom mixture of flavonoid standards was analyzed using the MS^n library workflow: Luteolin 7-rutinoside, Kaempferol 3-O-ß –rutinoside, Luteolin 7-O-ß-D-glucoside, Kaempferol 3-O-D-galactoside. The TMT method was evaluated using a 2-proteome mixture of yeast and human peptides that were chemically labeled with the TMT 10-plex set of reagents.

Data Analysis

The small molecule data were analyzed using a combination of Thermo Scientific™ Compound Discoverer™ and Thermo Scientific™ Mass Frontier™ spectral interpretation software. The proteomics data were analyzed using Thermo Scientific™ Proteome Discoverer™ software.

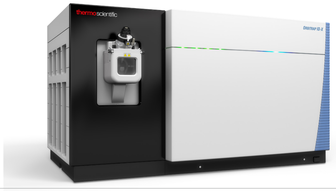


Figure 1. All the small molecule data presented in this poster were collected on the Thermo Scientific Orbitrap ID-X mass spectrometer.

RESULTS

AcquireX directs MS^n acquisition across a series of LS-MS analyses

A "typical" sequence of liquid chromatography–mass spectrometry (LC-MS) acquisitions consists of a series of injections/analyses, wherein each analysis is an independent "experiment". The results from the first LC-MS analysis do not impact how the mass spectrometer collects data during the second analysis.

AcquireX completely upends this "typical" workflow. Following the first LC-MS analysis, AcquireX processes the resulting data using LC-MS feature detection algorithms (Figure 1). AcquireX then reaches into the methods, via an advanced programming interface, and automatically updates the inclusion and exclusion mass filters accordingly.

There is a precedent for this type of iterative re-injection scheme, wherein the results of one analysis inform the next analysis.[1-3] However, this is the first time this functionality has been incorporated into the Xcalibur data-acquisition software.

Figure 2. Every AcquireX workflow consists of a series of LC-MS injections/analyses. The initial LC-MS analyses form the basis for the ion inclusion and exclusion lists, while the proceeding "ID" runs leverage these lists when deciding what ions to target for MS^n analysis. Following a given "ID" analysis, every ion interrogated with MS^n is moved from the inclusion list to the exclusion list.

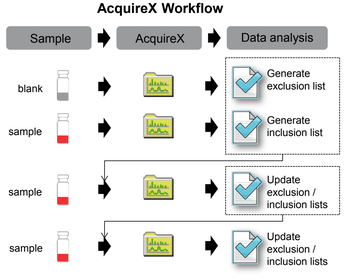
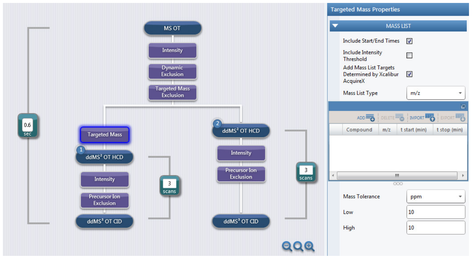


Figure 3. AcquireX reaches into the Tune method, and updates the "targeted mass exclusion" and "targeted mass inclusion" ion lists. The user can direct AcquireX to update specific filters by enabling the AcquireX checkbox. This functionality allows the user to build AcquireX logic into sophisticated branched methods. In the example below, the AcquireX targeted mass inclusion filter is located on a higher priority branch, while a second nearly identical branch is used for lower priority non-specific data-dependent MS^n.



AcquireX efficiently collects MS^n spectra to a greater depth than DDA

We analyzed a human plasma sample using an AcquireX-based method. For figures 4-7, we interrogated the MS1 precursors with FTMS2 only. For comparison, we analyzed the same sample with a traditional data-dependent FTMS2 workflow. For more details please visit **ThP 564**.

Figure 4. The AcquireX method dives deeper into a sample, interrogating more compounds by FTMS2 than the traditional data-dependent FTMS2 workflow.

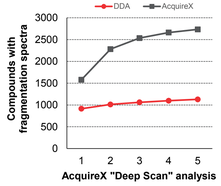
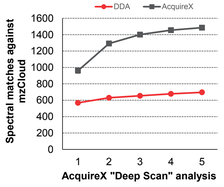


Figure 5. The improved FTMS2 coverage translates into more compounds with matches to the mzCloud library (exact+similarity).



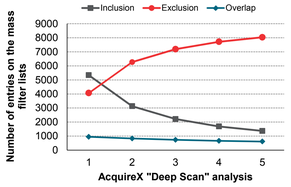


Figure 6. Following an AcquireX Deep Scan "ID" analysis, every ion interrogated with MS^n is moved from the inclusion list to the exclusion list. As the number of completed analyses increases, the proportion of entries on the inclusion list with corresponding entries on the exclusion lists increases (inclusion list size vs. overlap).

Figure 7. Across a series of injections, AcquireX avoids collecting replicate MS^n analyses in favor of interrogating new compounds.

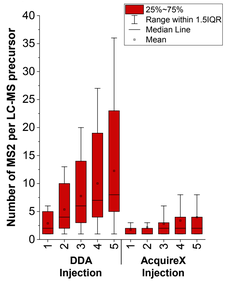
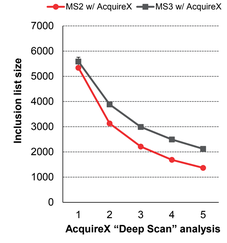


Figure 8. AcquireX provides excellent sample coverage, irrespective of the sample complexity or the acquisition rate of the MS^n method.



NEW FILTERS FOR BUILDING MS^n LIBRARIES

In-depth MS^n analysis using a "Library Builder" method

We analyzed a mixture of flavonoid standards using the "MS^n library builder" method. This method exhaustively interrogates a precursor using a combination of fragmentation mechanisms, MS^n levels, and *m/z* analyzers.

At high MS^n levels, the product ion signals can become quite weak. To address this concern, we developed an "MS^n Quality Trigger", which enables the user to trigger complementary ITMS^n scans if the corresponding FTMS^n scan S/N drops too low.

Mass Frontier 8.0 software can curate "MS^n library" data into MS^n "tree" libraries, and the software can search unknown MS^n data against this local library. For more details please visit **ThP 551**.

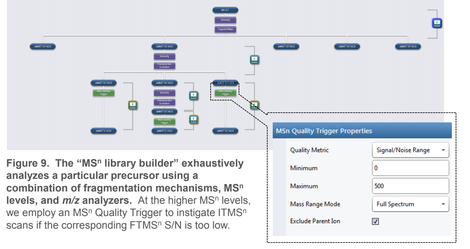


Figure 9. The "MS^n library builder" exhaustively analyzes a particular precursor using a combination of fragmentation mechanisms, MS^n levels, and *m/z* analyzers. At the higher MS^n levels, we employ an MS^n Quality Trigger to instigate ITMS^n scans if the corresponding FTMS^n S/N is too low.

Figure 10. Mass Frontier 8.0 can curate the data collected with the "MS^n library builder" method into local MS^n "tree" libraries. The MF 8.0 automatically removes bad quality spectra and noise peaks, while retaining the relevant ions and recalibrating the spectra.

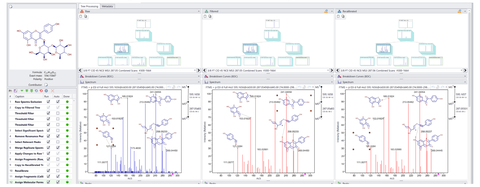
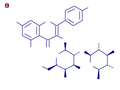


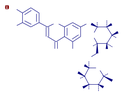
Figure 11. Two compounds that only produce unique fragmentation ions at the MS3 level were analyzed. A search against the MS2 "tree" produces identical scores, but MF 8.0 can distinguish the two compounds when it includes MS^n data in the "tree" search.

Library search conditions	LC-MS precursor	
	Luteolin 7-rutinoside	Kaemmpferol 3-O-ß –rutinoside
MS2 Tree: Luteolin 7-rutinoside	93.4	93.4
MS2 Tree: Kaemmpferol 3-O-ß –rutinoside	93.4	93.4
MS^n Tree: Luteolin 7-rutinoside	85.5	80.1
MS^n Tree: Kaemmpferol 3-O-ß –rutinoside	30.3	93.4

Kaempferol 3-O-ß -rutinoside



Luteolin 7-rutinoside



OTHER APPLICATIONS OF THESE NEW FILTERS

Applying the MS^n Quality Trigger to a TMT SPS-MS3 analysis

We analyzed a TMT labeled sample with a variant of the SPS FTMS3 method. This method utilized the MS^n Quality Trigger to initiate replicate FTMS3 scans when the reporter ions signals were too low.

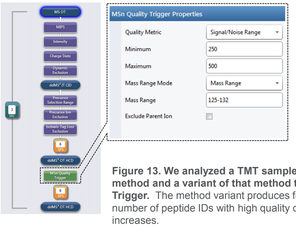
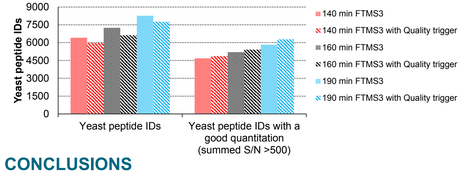


Figure 12. The MS^n Quality Trigger evaluated the summed FTMS3 signal in the TMT reporter ion region. If the summed reporter ion FTMS3 S/N was too low, the MS^n Quality Trigger queued up a replicate FTMS3 scan.

Figure 13. We analyzed a TMT sample using a standard SPS FTMS3 method and a variant of that method that included the MS^n Quality Trigger. The method variant produces fewer peptide IDs in total, but the number of peptide IDs with high quality quantitative measurements increases.



CONCLUSIONS

- AcquireX can expediently drive data acquisition towards more meaningful fragmentation spectra, while limiting the re-acquisition of MS^n spectra on the same set of precursors. AcquireX also allows the user to spread out higher-order MSn based analysis across multiple LC-MS injections/analyses, thereby removing some of the pressure to expediently collect MS^n spectra.
- The MS^n Quality Trigger provides specific guidelines to the instrument for when it should reacquire or reanalyze MS^n data to provide the highest possible data quality.

REFERENCES

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2. Hoopmann MR, Merrihew GE, Haller PD, MacCoss MJ. Post analysis data acquisition for the iterative MS/MS sampling of proteomics mixtures. J. Proteome Res. 2009, 8, 1870-1875.
3. Zhang Z. Automated precursor ion exclusion during LC-MS/MS data acquisition for optimal ion identification. J. Am. Soc. Mass Spectrom. 2012, 23, 1400-1407.

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TRADEMARKS/LICENSING

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