Metabolomics

Orbitrap-based metabolomics workflows: Discover. Innovate. Exceed.

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Abstract

The field of metabolomics has been advancing at an impressive rate, inspiring key analytical innovations designed to keep pace with the biological needs of a study. Despite these advances, challenges remain around compound annotation and identification. Thermo Fisher Scientific is committed to advancing the field of metabolomics through intelligence driven data acquisition, streamlined software processing strategies and key hardware innovations that keep at pace and push beyond the current analytical demands of the field. Herein, we present a novel hybrid orbitrap mass spectrometer with key technological advances that directly address many common metabolomics pain points such as unintentional MS1 (in-source) fragmentation and the need to detect high abundant and low abundant species within the same chromatographic run and the same MS scan.

Innovation and Evolution

Thermo Scientific™ Orbitrap Exploris™ 480 mass spectrometer
Introduced in 2019

- Known for **robustness** and as a **workhorse** MS
- **Over 3000 publications** - the highest numbers of any commercially available instrument!

Thermo Scientific™ Orbitrap Excedion™ Pro hybrid mass spectrometer
Introduction at ASMS 2025

All the benefits, size and flexibility of Orbitrap Exploris 480 MS with new HW, higher performance and new capabilities!

- ✓ **Improved gentle source design**
- ✓ **Enhanced dynamic range (eDR)**

Orbitrap Excedion Pro mass spectrometer

The new quadrupole-Orbitrap mass spectrometer.

The new system was designed to increase the metabolome coverage, improve sensitivity for measurements of complex samples and enable deeper profiling of metabolites with optimal relative quantitation.

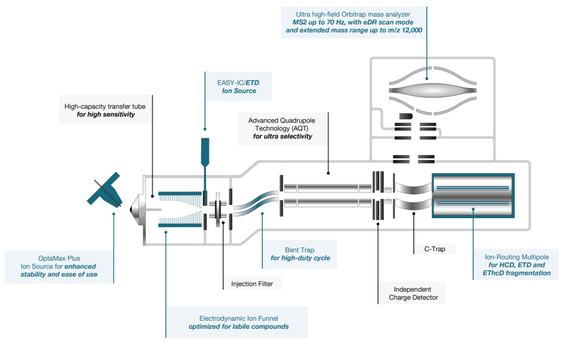


Figure 1. Orbitrap Excedion Pro MS and its schematics. The blue color indicates innovations implemented in this ins

Figure 2. Expanded view of the Thermo Scientific™ Optamax™ Plus ion source and electrodynamic ion funnel. Together, these hardware features provide enhanced stability and increased transmission of labile compounds.

Impact of Orbitrap Excedion Pro MS improved source design on unintentional fragmentation

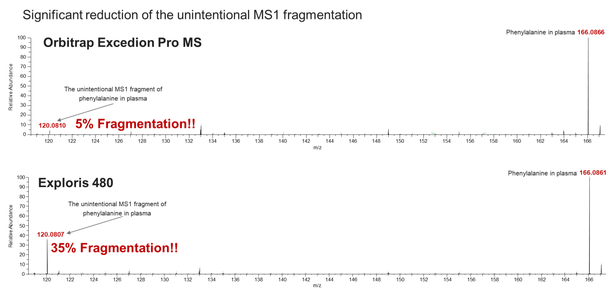


Figure 3.The above spectra represent the full scan of Phenylalanine in the SRM 1950 run via reversed phase chromatography on the Orbitrap Excedion Pro MS versus the Orbitrap Exploris 480 MS using the value of RF 50%. The softer ion source and electrodynamic funnel provide significantly less fragmentation on the Orbitrap Excedion Pro MS which translates to more sensitive MS1 level detection in complex mixtures.

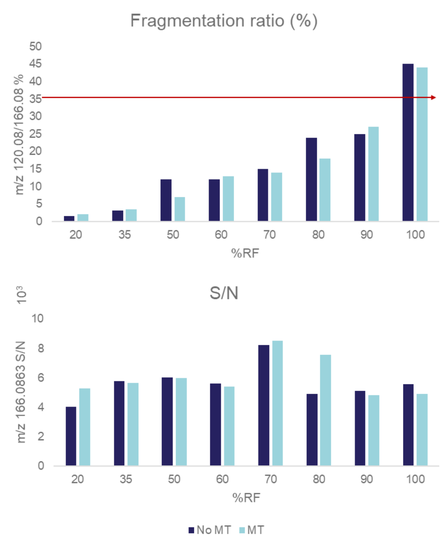


Figure 4. These two bar graphs represent the fragmentation ratio and signal-to-noise ratio of phenylalanine in SRM 1950 across multiple %RF values. Unlike other platforms where increasing the RF can lead to significant in-source fragmentation, higher RF values enhance the signal-to-noise ratio without causing dramatic fragmentation, ensuring cleaner, more reliable data.

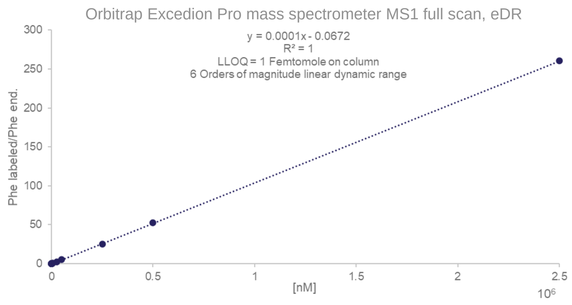


Figure 5. Unlike other platforms where increasing the RF value can lead to significant in-source fragmentation, higher RF values enhance the signal-to-noise ratio without causing dramatic fragmentation, ensuring cleaner, more reliable data.

eDR: Background

High-confidence detection and identification of low to medium abundant metabolites in complex matrices pose significant challenges in LC-MS-based metabolomics. Segmenting the mass range into narrower windows, known as the BoxCar or spectral stitching approach, has shown to improve spectral signal-to-noise ratios and increase the detection quality of low-abundance molecules[1,2,3,4,5]. We advanced these concepts by implementing intelligent MS1 multiplexing strategies with optimized injection times. This resulted in the eDR scanning mode on the Orbitrap Excedion Pro MS to increase metabolome coverage.

Basic Concept:

Gaining dynamic range on a spectral level to be able to detect low abundant compounds on a Full MS1 level

Expected Benefits:

1. Improved detection of low-abundant compounds
2. Reduced saturation effects for high abundant compound
3. Improved compound identification confidence
4. Lower LOQs/LODs in complex matrices and/or high matrix load (due to better %CVs)

Enhanced Dynamic Range: explanations and benefits

Extended dynamic range (eDR) is achieved by dividing the full scan mass range into two separate Orbitrap MS1 subscans, each encompassing alternating mass range windows. For each subscan, the quadrupole automatically isolates different m/z windows based on the user-defined settings, which are then transferred to the Orbitrap in a single injection.

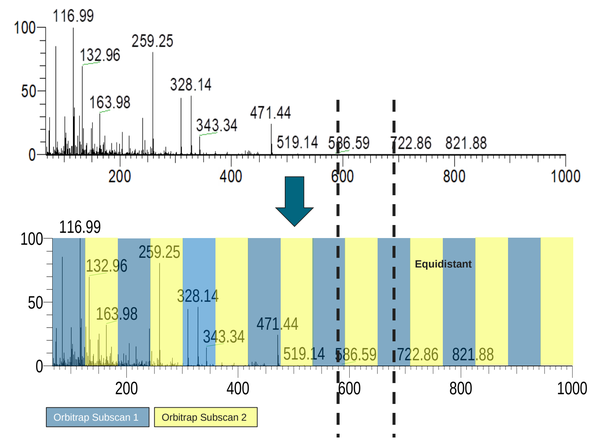


Figure 6. Visual representation of how eDR divides the mass range between windows and subscans.
Mass range: 67-1000 m/z, 16 eDR windows.

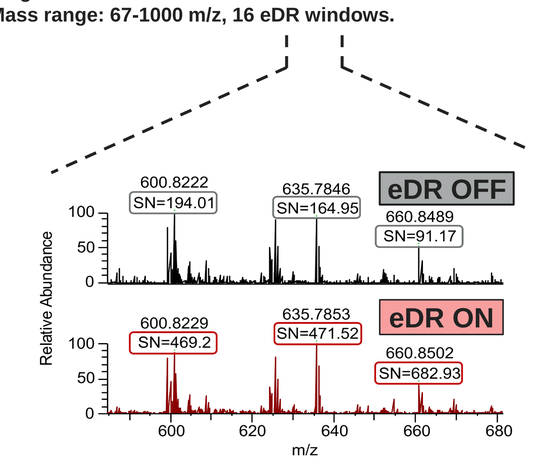
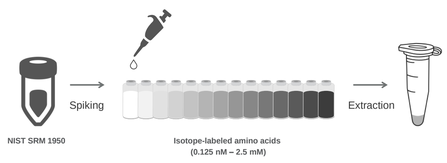


Figure 7. A zoomed in section of m/z range 580-680 showing the impact of eDR on the low abundant compounds in this range.

eDR: Increasing LOD in complex matrices

To investigate the impact of eDR on low abundant species in a complex matrix, we spiked in various levels of labeled amino acids into SRM 1950 and extracted the metabolites. This allowed us to evaluate the LOD of the labeled compound when co-isolated with its endogenous counterpart.



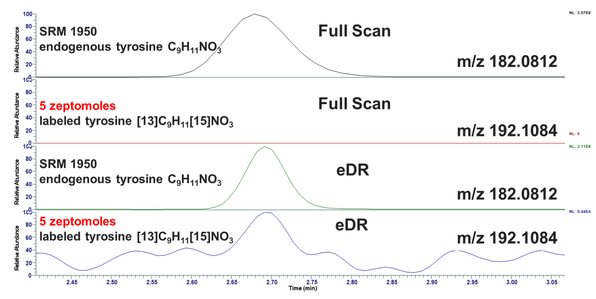


Figure 8. The extracted ion chromatograms for both the endogenous and labeled tyrosine show peaks for the endogenous compound in full scan and eDR while the 5 zeptomles of spiked labeled tyrosine is only detected when using the eDR feature.

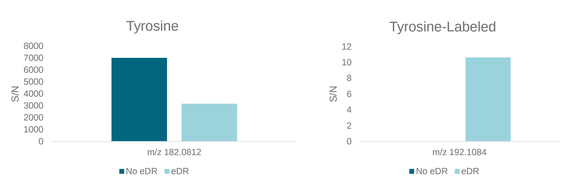


Figure 9. The bar graph for both endogenous and labeled tyrosine show how eDR reduces the S/N of the endogenous compound while allowing for more of the labeled species to be detected. This highlights the ability of eDR to reduce high abundant saturation while increasing the S/N of low abundant species.

eDR: Increasing depth of metabolome coverage

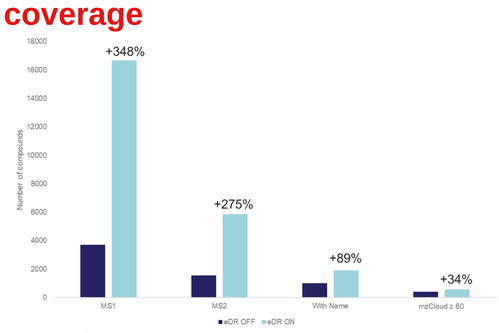


Figure 10. These bar graphs represent the number of compounds detected via Thermo Scientific ™ AcquireX™ Intelligent data acquisition and after processing with Thermo Scientific™ Compound Discoverer™ 3.4 software. MS1 indicates the number of detected compounds (after background removal) with an increase of ~3.5X while the number of annotated compounds also increases by 34%.

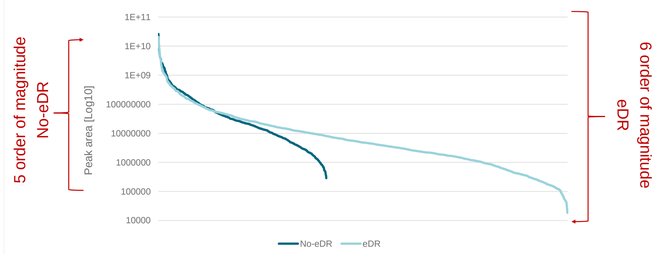


Figure 11. A regression of all compounds found in the above graph via MS1 are plotted with m/z on the X-axis and peak area on the Y-axis. This shows how eDR expands the compound dynamic range to 6 orders of magnitude.

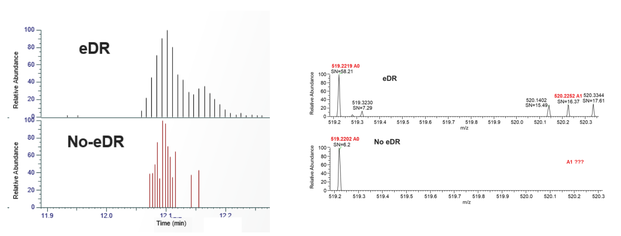


Figure 12. On the left is an extracted ion chromatograph of a compound detected confidently with eDR. On the right is the spectrum for this compound both with and without eDR. eDR clearly captures both the A0 and A1 peaks allowing for more confident detection. Furthermore, Thermo Scientific™ mzCloud™ mass spectral library identified the species as Sildenafil analogue III – m/z 519.22198 [M+H]+, an herbal supplement detected in the SRM 1950 extract.

Conclusions

The new Orbitrap Excedion Pro MS provides great benefits for metabolomics analysis compared to the legacy way of conducting such experiments, including:

- New ion source and electrodynamic ion funnel:
  - increased labile compound transmission with limited unintentional fragmentation
  - Increased sensitivity for MS1 level orbitrap quantitation (up to 10X observed)
- eDR function improves intrascan dynamic range:
  - Increased number of compounds that can found in the complex biological sample.
  - Increased S/N for low and medium abundant compounds in complex matrices

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