The universal Orbitrap instrument - from metabolomics over targeted proteomics to native analyses

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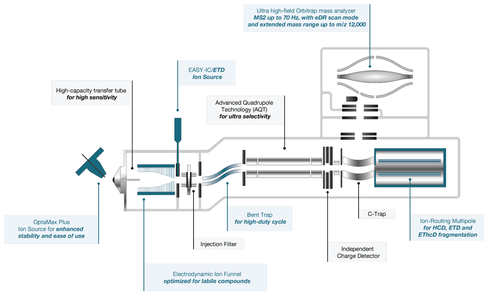
Abstract

The Thermo Scientific™ Orbitrap™ Excedion Pro™ hybrid mass spectrometer (MS) was developed to address the diverse needs of modern analytical laboratories. By combining cutting-edge Orbitrap technology with expanded capabilities, this instrument excels across a broad range of applications, including metabolomics, targeted proteomics, and native analyses. Its exceptional mass resolving power, accuracy, and sensitivity make it indispensable for researchers seeking to unravel complex biological systems. This poster will explore its technical capabilities and demonstrate its broad applicability. It focuses on instrumentation and provides usage examples and references to full use cases. Figure 1 shows the schematic of the Orbitrap Excedion Pro hybrid MS with new features highlighted in blue compared to the Thermo Scientific™ Orbitrap Exploris™ 480 hybrid MS.

Schematic

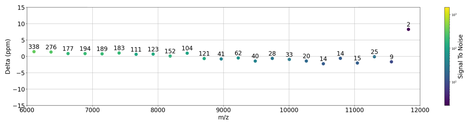
Figure 1. Schematic of the Orbitrap Excedion Pro instrument



Extended mass range[1]

Thermo Scientific™ Orbitrap™ Excedion Pro™ BioPharma MS has high-field Orbitrap technology covers a mass range up to *m/z* 12000. This is realized by a dedicated ion injection logic and specialized tuning for the intact protein mode. Mass accuracy better than 5 ppm up to *m/z* 12000 (signal-to-noise > 5) is achieved as shown in Figure 2.

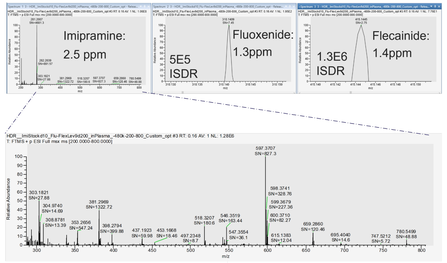
Figure 2. Plot of Cesium Iodide (CsI) cluster mass deviation in positive ion mode up to *m/z* 12000. The annotations indicate the signal-to-noise ratio of the individual peaks.



Enhanced Dynamic Range (eDR)[2]

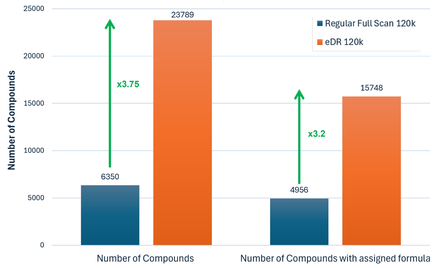
By multiplexing two Orbitrap subscans of a set of interlaced non-overlapping isolation windows, the space charge capacity of the Orbitrap instrument is fully used with ions of interest. The ion intensities are determined by intentional redundancy between the two subscans. More than five orders of magnitude intrascan dynamic range (ISDR) is observed for Flecainide/Fluoxenide and Imipramine spiked in crashed plasma in a flow injection experiment at 480k, mass range *m/z* 200-800, see Figure 3. (ISDR = signal intensity Imipramine divided by signal intensity Flecainide and Fluoxenide, respectively.)

Figure 3. Single scan mass spectrum with expanded regions showing ISDR of > 5E5 for Imipramine and Flecainide/Fluoxenide spiked in plasma sample



To boost the number of detected compounds in a complex plant extract, eDR was enabled on triplicate runs (MS1 only, R=120k). The data evaluation was conducted with Compound Discoverer™ 3.3 software and a CV<20% setting. This resulted in 3.7-fold more detected compounds and 3.2-fold more assigned formulas, see Figure 4.

Figure 4. Comparing the identified compounds of tea extract with regular operation versus eDR mode activated.

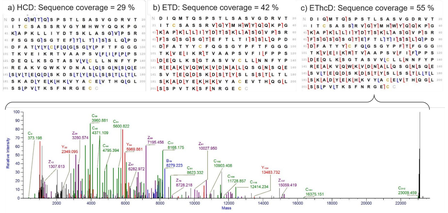


Electron transfer dissociation (ETD)[3,4,5]

The higher energy collisional dissociation (HCD) is extended with the electron transfer dissociation by the ETD option. Technically, the EASY-IC/ETD ion source is used to generate reagent anions, which are simultaneously trapped with the sample cations inside the ion routing multipole (IRM). By controlling the two ion clouds to interfere with each other, additional *c/z*-fragments are generated in addition to *b/y* fragments from HCD experiments. After the ETD reaction, an optional supplemental activation step can be done by re-injection of the ETD-fragments from the C-Trap intro the IRM – this is named EThcD.

ETD and EThcD offer complementary information to collisional activation approaches like HCD that is considered especially useful for top- and middle-down experiments, de novo sequencing, as well as identification and localization of labile post-translational modifications. Figure 5 demonstrates the additional sequence coverage obtained with ETD and EThcD for the middle-down analysis of the NIST monoclonal antibody light chain.

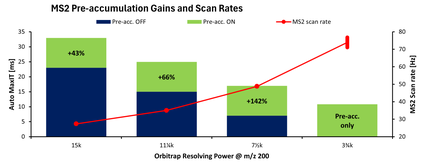
Figure 5. Sequence maps for the light chain of the NIST monoclonal antibody reference substance obtained using different fragmentation techniques (HCD, ETD and EThcD) including a deconvoluted mass spectrum example.



Bent trap[6]

Pre-accumulation of ions inside the bent trap allows to increase the duty cycle especially for low-resolution MS2 scans. A scan speed of 70 Hz for MS2 scans is possible with meaningful ion fill times as instrument overhead times can be utilized. The bent trap is extending the bent flatapole to ion trapping capabilities. While the c-trap is processing ions from the previous scan, ions are pre-accumulated already for the subsequent scan. To ensure correct quantification, the pre-trapping time is included into the AGC control of the instrument. Figure 6 shows the potential gains that can be achieved – the 70 Hz MS2 operation at resolving power 3750 becomes available.

Figure 6. Determination of extra accumulation time provided by the bent trap



Adaptive retention time correction[7,8]

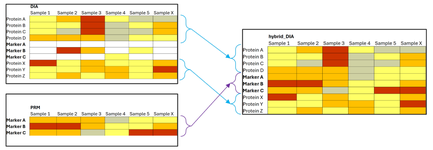
Automated real-time correction of retention time (r.t) windows to account for chromatographic fluctuations enables the selection of narrower scheduling windows during targeted applications. This allows for the inclusion of more targets in the assay and enhances sensitivity. As shown in Figure 7, incorporating an adaptive RT full scan into the method setup allows for the creation of a rtbin file. This file is used for the alignment of retention times of targeted peptides in a targeted MS2 experiment. During the alignment runs, the retention time is intentionally altered by changing the gradient to demonstrate that a new scheduled window for the targeted peptides is adjusted on the fly.

Figure 7. Targeted MS2 experiment with an adaptive full scan. MS2 peptide trace in the reference run and when inducing a change in r.t. Cross-correlation plot with light regions representing high correlation and dark regions representing low correlation. Cyan lines represent the correction on the left and right sides of the scheduled window.

Hybrid-DIA

Hybrid-DIA is the unique capability of combining global DIA scans with sensitive PRM (tMS2) scans. The use of the adaptive retention time correction feature makes this approach even more efficient, as narrow RT windows can be set.

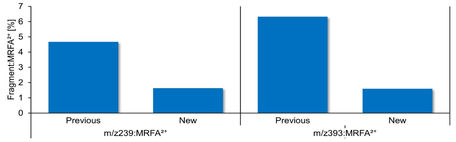
Figure 8. Combining discovery and validations using Hybrid-DIA



Electrodynamic ion funnel[9]

The ion funnel is extended and optimized in geometry for efficient but soft ion introduction into the mass spectrometer. Labile compounds benefit from this soft ion introduction, while in-source collisional dissociation capabilities are available on demand.

Figure 9. Characterizing the optimized electrodynamic funnel using the MRFA contained in Thermo Scientific ™ Pierce™ FlexMix™ calibration solution. The graph shows unintended fragmentation ratios – less is better here.



OptaMax Plus ion source

The optimized heat transfer design of the Thermo Scientific™ OptaMax™ Plus ion source allows higher temperatures for high-flow LC applications to boost sensitivity. The heater is line-voltage independent to enable full heater power under all line voltage conditions. Precise mechanical adjustment of the needle position is ensured by additional fine adjustment knobs.

Sustainability[10]

The entire instrument is designed for lower power consumption and operated with a dual-inlet dry fore vacuum pump. This reduces not only the direct power consumption, but also the air conditioning needs in the lab.

Conclusions

The presented extensions and features of the Orbitrap Excedion Pro MS make it an ideal universal instrument for (but not limited to) these application spaces:

- Metabolomics (Electrodynamic ion funnel, eDR, OptaMax Plus ion source)
- Proteomics (Hybrid-DIA, ETD, EThcD, eDR)
- Biopharma (Extended mass range, ETD, EThcD, eDR)

References

[1] ASMS 2025 Poster – Reiko Kiyonami, TP028
[2] ASMS 2025 Poster – Michał Kaczmarek, ThP439
[3] ASMS 2025 Poster – Peter Krüger, WP338
[4] ASMS 2025 Poster – Reiko Kiyonami, TP617
[5] ASMS 2025 Poster – Cong Wang, MP016
[6] ASMS 2025 Oral – Christian Hock, WOE am 8:30
[7] ASMS 2025 Poster – Markus Kellmann, MP402
[8] ASMS 2025 Poster – Qingling Li, ThP049
[9] ASMS 2025 Poster – Maciej Bromirski, ThP432
[10] https://www.thermofisher.com/uk/en/home/sustainable-design/act-label.html

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