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New innovations implemented on the Q Exactive HF mass spectrometer

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ABSTRACT

Purpose: Development of a new hybrid quadrupole-Orbitrap™ mass spectrometer with improved performance

Results: The new Thermo Scientific™ Q Exactive™ HF-X hybrid quadrupole-Orbitrap mass spectrometer is two times faster than its predecessor, with improved ion transmission.

INTRODUCTION

High resolution, accurate mass (HRAM) Orbitrap™-based mass spectrometers are increasingly being used for many different applications. Each application imposes special requirements on the mass spectrometer. Modern mass spectrometers have improved sensitivity, accuracy, high resolution, and/or increased scanning speed. These improvements directly result in significant benefits for applications such as proteomics, enviromental and food safety, metabolomics, lipidomics, and many more. Despite the impressive recent developments in Orbitrap technology, further technical improvements of next-generation mass spectrometers are desired by the mass spectrometry community. To address existing and new requirements from a broad field of applications, new technological developments and performance improvements on the existing Thermo Scientific™ Q Exactive™ HF instrument MS were undertaken. This poster presents the novel Q Exactive HF-X hybrid quadrupole-Orbitrap mass spectrometer and some applications demonstrating its capabilities.

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METHODS

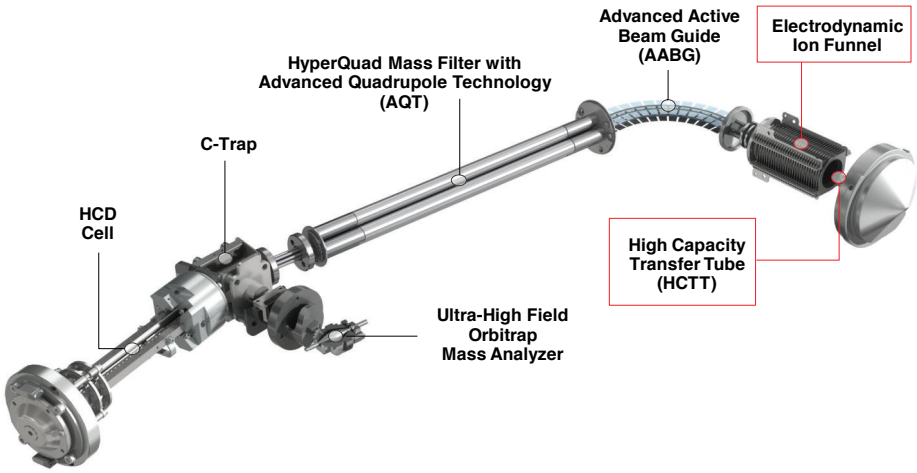
Mass Spectrometry

The novel Q Exactive HF-X mass spectrometer (figure 1) consists of:

- A high capacity transfer tube (HCTT) and an electrodynamic ion funnel for increased ion flux;
- A modified Advanced Active Beam Guide (AABG) to maintain operating pressure and minimize unwanted solvent cluster formation;
- Two fore-vacuum pumps, one with high pumping capacity, to accommodate both modifications on the source region;
- Further changes aiming at reducing the overhead time between scans combined with an improved charge state recognition.

This instrument has a maximum resolving power of 240,000 @ *m/z* 200 at a scan speed of 1.5 Hz and a minimum resolving power of 7500 @ *m/z* 200 at a scan rate of 40 Hz.

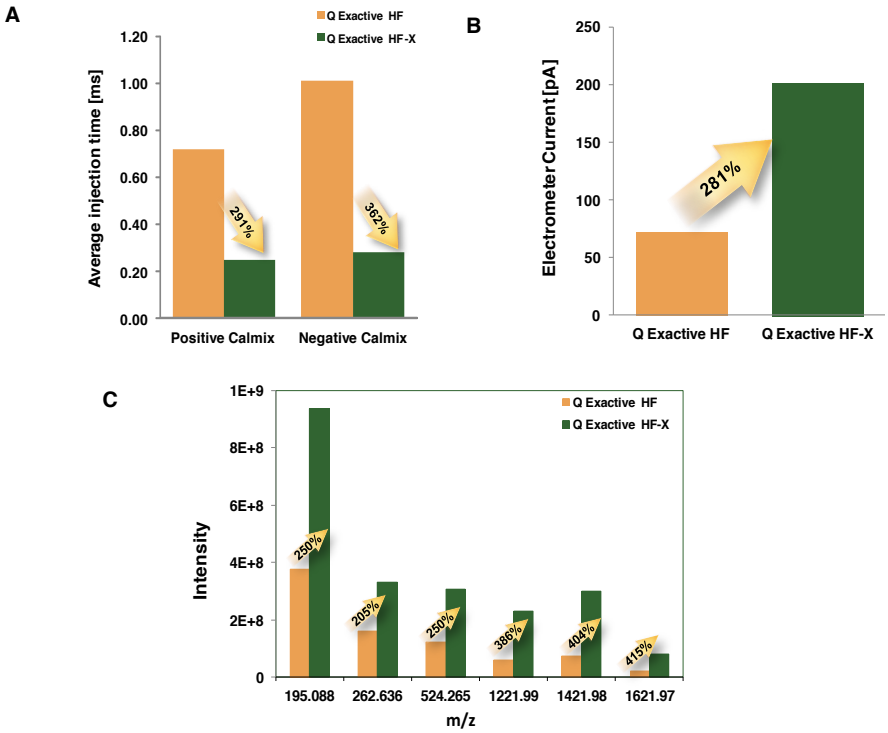
Figure 1. Schematics of the Q Exactive HF-X MS



RESULTS

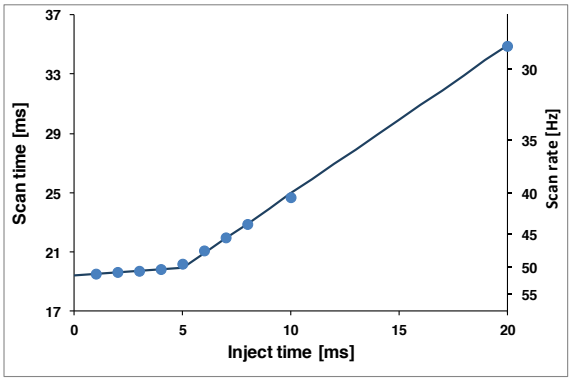
The advantage of the HCTT and the electrodynamic ion funnel is an increased ion flux into the instrument. To demonstrate the improvement in transmission, ion currents and injection times were recorded for the Thermo Scientific™ Pierce™ LTQ ESI Positive and Negative Ion Calibration Solutions (optimized flow rates: pos. 6 µL/min, neg. 8 µL/min) before and after modifying a Q Exactive HF instrument to a Q Exactive HF-X instrument. Figure 2A shows that the ion current is 2.8-fold higher after the modification. Figure 2B shows that the injection times in positive and negative mode have become approximately 3-5 times shorter upon modification, with correspondingly higher ion intensities throughout the mass spectrum for the Q Exactive HF-X MS (figure 2C).

Figure 2. Electrometer current (A), injection times (B), and ion intensities (C). Measurements were performed on a Q Exactive HF MS before and after its modification to Q Exactive HF-X MS.



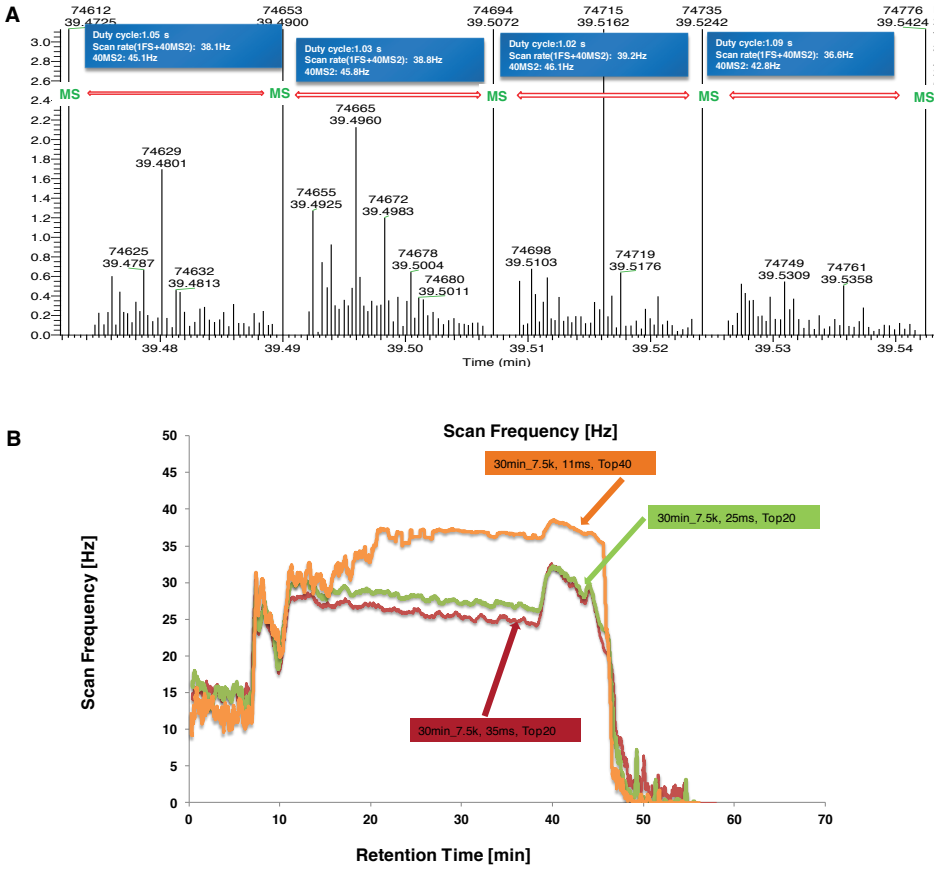
Another differentiator of the Q Exactive HF-X MS to the previous instruments is the rate at which the instrument scans. The achievable scan rate of the instrument is defined by the resolution setting and the injection time. At the highest resolution setting of 240,000 @ *m/z* 200 (512 ms detection time) the instrument runs at an MS/MS scan rate of 1.5 Hz, while at the lowest resolution setting of 7500 (16 ms detection time) @ *m/z* 200 it can reach up to 51 Hz, as shown in figure 3. For an injection time of 10 ms, which is common under real experimental conditions, the Q Exactive HF-X MS operates at a scan rate of 40 Hz, which is 2 times faster than the Q Exactive HF MS, without losing sensitivity. This is especially important for complex sample analysis using data dependent acquisition (DDA) or data independent acquisition (DIA) and targeted analysis.

Figure 3. MS/MS scan rate at resolution 7500 @ *m/z* 200 for the Q Exactive HF-X MS with respect to the ion injection time.



In real applications where information from the full scan is required, the scan rate of the instrument would vary based on the maximum injection time set for MS/MS. This is demonstrated in figure 4 for a complex LC-MS/MS experiment using 1 µg HeLa digest. For these measurements, the Q Exactive HF-X MS was operated in a DDA mode, selecting the Top-40 or Top-20 most intense ions for MS/MS. The Full-scan resolution was set to 60,000 and the MS2 resolution was 7500. The MS/MS maximum injection time was set to 11, 25, or 35 ms. The actual gradient was 30 min, but with column wash step the total run time was 58 min. Figure 4A shows a selected retention time window from the chromatogram. Under the conditions given above, the Q Exactive HF-X MS completed one full scan and 40 ms2 scans in a second, acquiring at a scan rate of approximately 38 Hz. Taking the acquisition rate over the whole gradient, an average of 35 Hz is achieved on the Q Exactive HF-X MS. Increasing the injection time to 25 or 35 ms, the overall scan rate drops to approximately 25 Hz (figure 4B), which is still considerably much faster than on the Q Exactive HF MS.

Figure 4. Performance for LC-MS/MS of HeLa digest on the Q Exactive HF-X MS: (A) scan rate for selected scan events; (B) Calculated scan rate throughout the whole gradient.

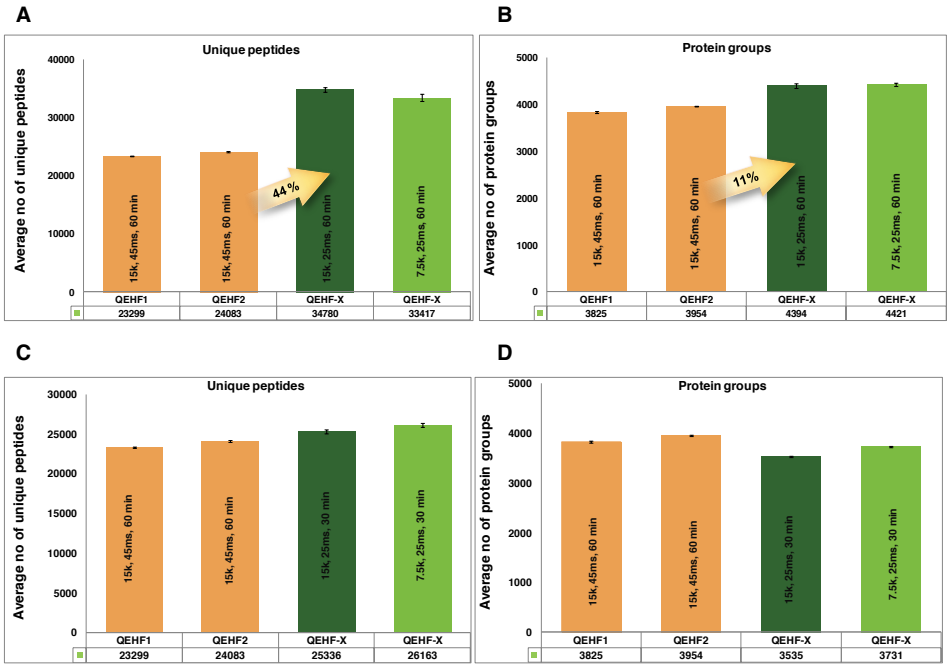


Application

Bottom-up Proteomics (Data Dependent Analysis)

Thermo Scientific™ Pierce™ Human cell line (HeLa) digest was separated on a 25 cm Thermo Scientific™ Acclaim™ PepMap™ 100 C18 column using the Thermo Scientific™ EASY-nLC™ 1200 system. The eluting peptides were measured on both the Q Exactive HF MS and Q Exactive HF-X mass spectrometer. The mass spectrometers were operated in a DDA mode using optimized methods for each instrument. The raw files were analyzed with Thermo Scientific™ Proteome Discoverer™ software version 2.1. Figure 5A and B show a comparison between the Q Exactive HF MS (orange) and the Q Exactive HF-X MS (green) for a 60 min gradient. No matter the resolution setting in MS/MS (15,000 represented in dark green bars and 7500 in light green bars), the Q Exactive HF-X MS showed a 44% improvement in peptide IDs. The number of protein groups increased by 11%, an indication that more peptides per protein are identified. This is especially important for PTM studies. In Figure 5C and D, a 30 min gradient on the Q Exactive HF-X MS was compared with a 60 min gradient of the Q Exactive HF. The Q Exactive HF-X MS identified an equivalent number of peptides and protein groups in only half of the analysis time (30min) when compared to the Q Exactive HF MS. This is attributable to the increased ion transmission of the HCTT and electrodynamic ion funnel in combination with the improvements in timing overhead and overall scan speed.

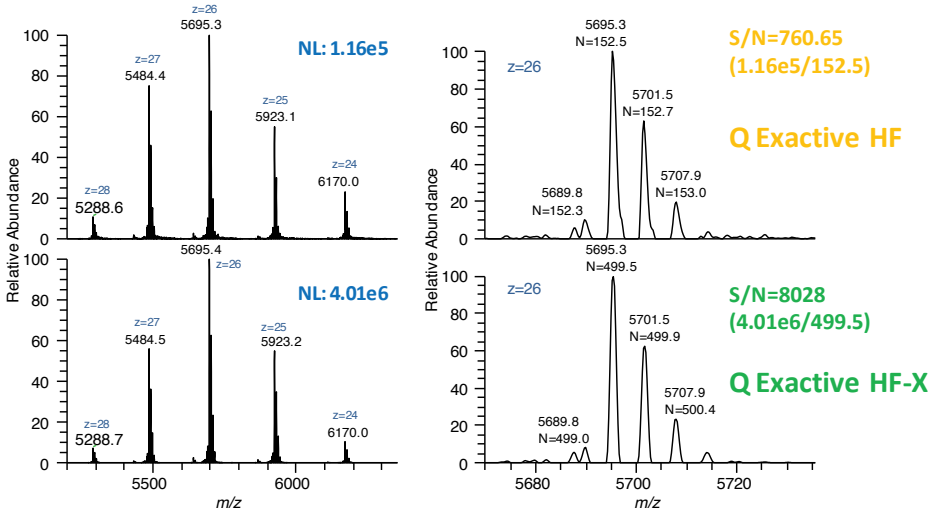
Table 5. Average number of unique peptides and protein groups identified from triplicate experiments on the Q Exactive HF MS (orange) and the Q Exactive HF-X MS (green) mass spectrometers. (A and B) 60 min comparison of the Q Exactive HF and the Q Exactive HF-X MS. (C and D) Results from a 60 min gradient on the Q Exactive HF MS compared to a 30 min gradient on the Q Exactive HF-X MS.



Intact Monoclonal Antibody (mAb) Analysis

MS analysis of antibodies at the protein and or peptide levels is a relevant step during the development and production of biopharmaceuticals. The compositions of current generation therapeutic proteins can be very complex because of the various modifications. Intact analysis of these antibodies by ESI-MS is usually performed under either denatured or native-like conditions. Compared to the denatured conditions, where the mAb are detected in high charge states with complex mass spectra, native-like conditions result in lower charge states and detection at higher m/z ranges with better spatial resolution. Figure 6 shows the performance gain of the Q Exactive HF-X MS compared to the Q Exactive HF MS in the analysis of trastuzumab intact monoclonal antibody by size exclusion chromatography (SEC-)LCMS. The S/N increased approximately 10-fold, which is attributable to the improved transmission of the HCTT and electrodynamic ion funnel.

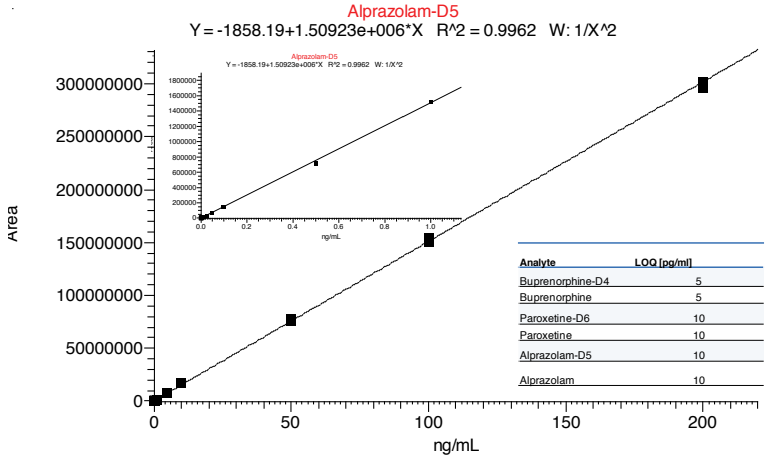
Table 6. SEC-LCMS analysis of intact trastuzumab monoclonal antibody using Thermo Scientific™ Acclaim™ SEC 300 column, 4.6 x 300 mm, 300 ml flow, 50%/50% 100mM ammonium acetate/water (50 mM). Full MS, HMR mode, m/z 2500–8000, resolution 30,000, 10 *µ*scans



Small molecule quantitation

In another application to estimate the linear dynamic range of the Q Exactive HF-X MS, different concentrations (0.01-1.00ng/ml) of 3 analytes (Alprazolam, Buprenorphine, Paroxetine and their deuterated standards) were spiked in plasma and analyzed using a t-SIM method. As exemplified on figure 7 using Aprozolam-D5, the Q Exactive HF-X MS was able to detect down to 10 pg/ml with CV below 15 %, of the injected components with 4 orders of linear dynamic range. The limit of detection was up to 5 times lower than on the Q Exactive HF MS.

Table 7. Example of a t-SIM analysis of small molecules on the Q Exactive HF-X MS, showing 4 order of linear dynamic range .



CONCLUSIONS

A new Q Exactive™ HF-X hybrid quadrupole-Orbitrap mass spectrometer and some applications demonstrating its capabilities have been developed and presented:

- The novel Q Exactive HF-X MS acquires MS/MS spectra at twice the speed compared to the Q Exactive HF MS with a maximum acquisition frequency of 40Hz.
- The implementation of a high capacity transfer tube and an electrodynamic ion funnel on the new Q Exactive HF-X MS significantly increases ion flux.
- In bottom-up proteomics, the Q Exactive HF-X MS yields up to a 44% increase in peptide identifications.
- The Q Exactive HF-X MS can identify an equivalent number of peptides and protein groups in just half of the analysis time compared to the Q Exactive HF MS.
- The Q Exactive HF-X MS shows up to 10-fold improvement in S/N ratio for the analysis of intact monoclonal antibody under native-like conditions.
- The Q Exactive HF-X MS shows up to 5 time lower LOQ than Q Exactive HF MS for small molecule applications

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