Multiple C-Trap or HCD Fills as a Tool for Massive Parallelization of Orbitrap Mass Spectrometry – A New Concept for Targeted Mass Analysis

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Thermo Scientific Q Exactive LC-MS/MS

Overview

Purpose: The versatility and throughput of a benchtop hybrid quadrupole-Orbitrap™ mass spectrometer is drastically increased by detecting multiple analytes simultaneously.

Methods: Spectrum multiplexing is realized by the co-injection of several selected precursors into the ion storage device before they are simultaneously detected in a single Orbitrap acquisition.

Results: By analyzing several precursors together and using idle times of the instrument, the throughput is drastically increased, potentially up to more than 100 precursors per second. Collecting ions in the high energy collisional dissociation (HCD) cell allows the use of stepped collision energy scans without a loss in scan speed. By spectrum multiplexing, the analytical dynamic range is shown to be more than 320,000.

Introduction

Spectrum multiplexing is implemented on the bench-top Orbitrap instrument, the Thermo Scientific Q Exactive benchtop Orbitrap LC-MS/MS, and equipped with an S-lens ion source for improved ion transmission, a quadrupole mass filter to allow MS/MS experiments, a C-Trap, and a collision cell to provide HCD (Figure 2). A list of precursors of interest is either known, in the case of targeted analyses, or predicted from an overview mass spectrum (full MS). Several precursors are injected sequentially into the C-Trap or into the HCD cell after passing through the C-Trap before they are analyzed together in one Orbitrap detection. If a collision energy > 0 is chosen, the ions are stored in the HCD cell. It is possible to define a stepped collision energy range – in this case, the same precursor is injected into the HCD cell several times at different collision energies.

Methods

Principle of Operation

Figure 1 compares the standard operation mode with the spectrum multiplexing approach in the scan-to-scan AGC (automatic gain control) mode. The instrument is operating in parallel mode in both cases. While the previous scan is acquired, the ions for the next scan are already collected. In the standard mode, only one precursor ion species is injected. In the case of spectrum multiplexing, the idle time is used to inject several precursor ion species, resulting in a higher throughput. This is especially effective when fill times are short, which is greatly facilitated by increased sensitivity of the instrument due to the use of the S-lens (Figure 2).

FIGURE 1. Standard operation mode (A.) versus spectrum multiplexing (B.)

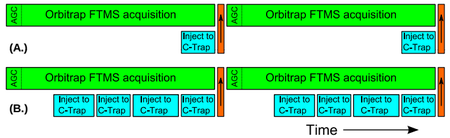
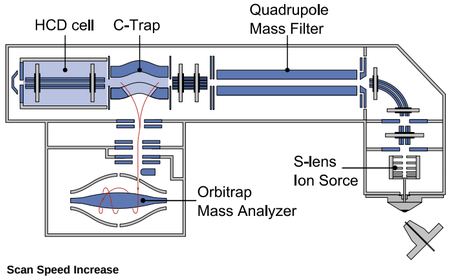


FIGURE 2. Q Exactive™ benchtop Orbitrap LC-MS/MS instrument layout



Scan Speed Increase

Figure 3 presents the resulting effective scan speed for different resolutions and multiplexing factors. It shows that spectrum multiplexing allows for the increase of the scan speed up to >100 precursors per second.

The useful number of ion injections for a single Orbitrap detection is limited by the sum of the individual inject times being lower than the time for the Orbitrap detection. Figure 4 shows the limit for the total inject time below which the instrument operates at full speed for given resolution setting including overhead times.

FIGURE 3. Scan speed: > 100 precursors/ second are possible in 8-plex at resolving power 17,500

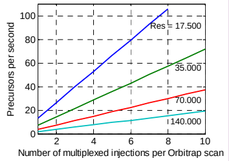
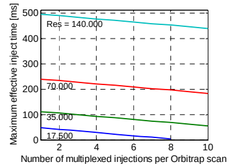


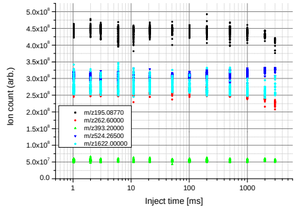
FIGURE 4. Maximum effective inject time at full scan speed



Checking Prerequisites

A precondition of this method is that the storage of the ion population remains independent on the storage time. Figure 5 shows an intensity of different ion species stored in the C-Trap as a function of storage time. The ion intensities stay constant for the entire time scale that proves the absence of any ion-molecule reactions.

FIGURE 5. C-Trap storage: No ion loss over a broad range of storage times.

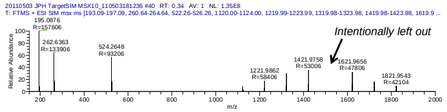


Results

Demonstration of 10-fold Spectrum Multiplexing

Figure 6 presents a spectrum of a calibration substance recorded with 10-fold multiplexing. Each precursor was filled to an AGC target of 1e5. The resulting spectrum, at a resolving power setting of 140,000, shows the expected spectral distribution, but with one abundant peak (U-mark, 1522) intentionally not injected.

FIGURE 6. Demonstration of 10-fold spectrum multiplexing



Targeted Analysis of a Pesticide Mixture

A pesticide mixture of 48 compounds was analyzed to demonstrate the performance of the multiplexing technology. A targeted analysis using 4-fold multiplexing showed consistent results compared to the standard operation mode. The scan speed increased to more than 48 precursors per second without compromising high signal-to-noise ratio characteristic for SIM scans. See Figure 7 for the experimental setup and Figure 8 for a snapshot of the resulting spectra.

FIGURE 7. Experimental setup of a 4-fold multiplexing configuration for targeted analysis

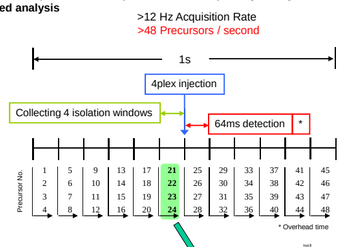
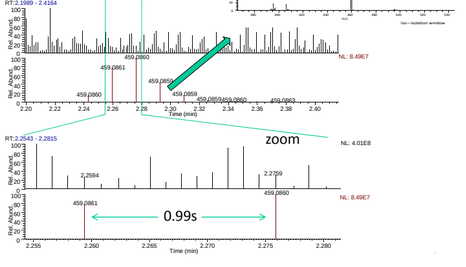


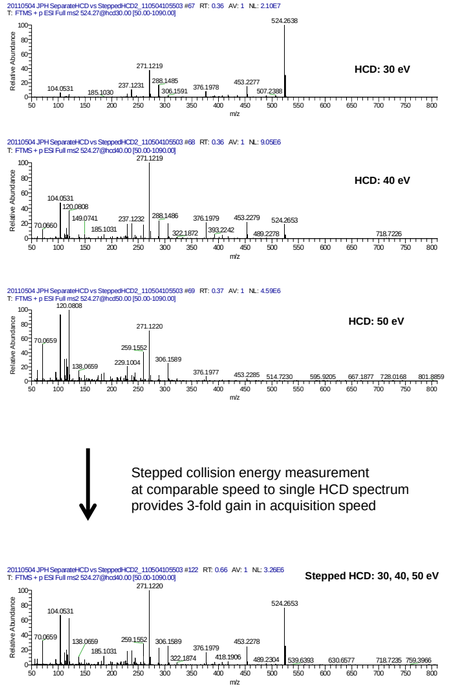
FIGURE 8. The plot on the left shows a 4-fold multiplexed spectrum taken from the entire measurement run, which shows a duty cycle of 0.99 s (corresponds to 48 precursors / s)



Stepped Collision Energy Increases Versatility

For fragmentation experiments, the ions pass through the C-trap and are directly injected into the HCD cell, typically with a predefined collision energy. If the fragmentation energy of the sample is unknown or a single energy setting does not cover all compounds of the sample, the spectrum multiplexing allows for the realization of a stepped collision energy. For this approach, several ion injections into the HCD cell are performed before a combined spectrum is acquired in the Orbitrap. Figure 9 shows the comparison between three single HCD spectra and one stepped collision energy spectrum of MRFA at the same scan speed.

FIGURE 9. Comparison of three single HCD spectra with a stepped collision energy spectrum. The fragmentation pattern is optimized by multiplexing three spectra into a single spectrum.



Stepped collision energy measurement at comparable speed to single HCD spectrum provides 3-fold gain in acquisition speed

OUTLOOK

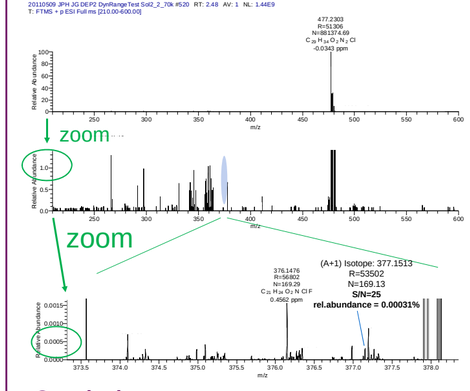
Pushing the Limits: Dynamic Range Boost by Spectrum Multiplexing

In many cases, spectra contain a significant amount of matrix or background ions or the sample, itself, covers a broad range of abundances. In these cases, the C-Trap space charge capacity can be more effectively used especially for the low abundant species. Figure 10 shows an example of a large amount of Loperamide (*m/z* 477) and a low amount of Haloperidol (*m/z* 376, with (A+1) isotope at *m/z* 377). Acquiring this mixture by overlapping multiplexed spectra shows the capability to deliver an analytical dynamic range of more than 320,000.

Two isolation windows were injected at fixed inject times, which were then used for scaling the ion abundances: [*m/z* 200..600]@0.1 ms and [*m/z* 374..378]@250 ms

The scan speed at a resolving power setting of 70,000 is 3.6 scans / second (no averaging).

FIGURE 10. Dynamic range boost by spectrum multiplexing: The analytical dynamic range in a single scan is 320,000 (!)



Conclusion

Spectrum multiplexing is a powerful feature to enhance the MS/MS capabilities of the Q Exactive instrument. Because it uses the idle times during parallel Orbitrap acquisition for filling several ion species for the next scan, spectrum multiplexing does not change the acquisition speed, but drastically increases the number of precursors per second. It is shown that up to 10 precursors can be acquired in the same Orbitrap acquisition without any impact on the C-Trap ion storage. Several examples propose the use of this feature:

- **Throughput:** The targeted analysis of a pesticide mixture containing 48 compounds showed that all precursors are acquired with less than a one-second cycle time. This is especially of interest for (ultra) fast chromatography in combination with coeluting compounds.
- **Analytical flexibility:** Stepped collision energy spectra are implemented without an impact on scan speed.
- **Research:** An analytical dynamic range of 320,000 in a single scan is demonstrated in a multiplexed spectrum. Further investigations will show the linearity and the limits of this approach.