Improved Electron Transfer Dissociation (ETD) Duty Cycle and Spectral Signal to Noise Ratio in a Dual Cell Linear Ion Trap

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Overview

Purpose: Improve ETD Signal to Noise (S/N) ratio and scan duty cycle.

Methods: Thermo Scientific™ Orbitrap Fusion™ Tribrid™ mass spectrometer with the Thermo Scientific™ EASY-ETD™ ion source.

Results: Developed a methodology incorporating multiple fills of ETD products into a storage cell followed by a single *m/z* analysis leading to improved spectral S/N ratio and acquisition speed.

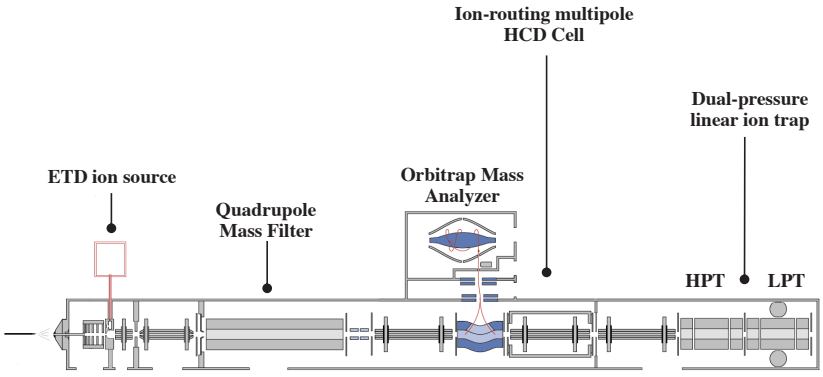
Introduction

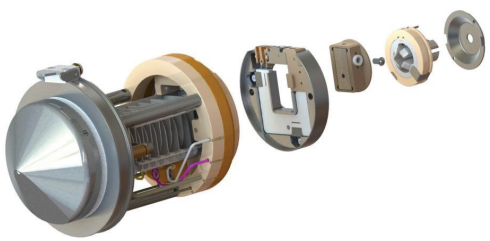
Electron transfer dissociation (ETD) has been demonstrated to be a useful tool for the analysis of polypeptide compounds including peptides with labile PTM's, peptides with many basic sites, and large proteins. One drawback of the approach is that it leads to a reduction of both scan duty cycle and total MS2 ion signal, compared to conventional resonant CID, due to necessity to perform the ion-ion reaction and the fact that the reaction consumes charge. This research explores the possibility to circumvent these limitations by performing multiple ETD reactions per *m/z* analysis in a 2D linear ion trap on a three-analyzer hybrid instrument based upon a mass resolving quadrupole, Orbitrap (OT), collision cell, and dual linear ion trap (Q-OT-LT) architecture.

Methods

The multi-fill per *m/z* analysis scan mode in the dual cell linear ion trap is accomplished by using the high pressure trap (HPT) as a reaction vessel, and utilizing the low pressure trap (LPT) as an accumulation and *m/z* analysis cell. In this fashion, the multiple fills ETD scan cycle becomes a loop over **n** (the number of fills requested) ETD reaction, transfer and storage cycles, followed by a single *m/z* analysis in either the low pressure trap of the dual cell or, after the appropriate transfer, in the OT mass analyzer. All experiments were performed on an Orbitrap Fusion Tribrid MS.

FIGURE 1. Schematic of the Orbitrap Fusion Tribrid mass spectrometer showing the location of the Easy-ETD reagent ion source within the overall ion optics path. The exploded view shows how the reagent ion source is incorporated into the S-Lens/Q00 region.



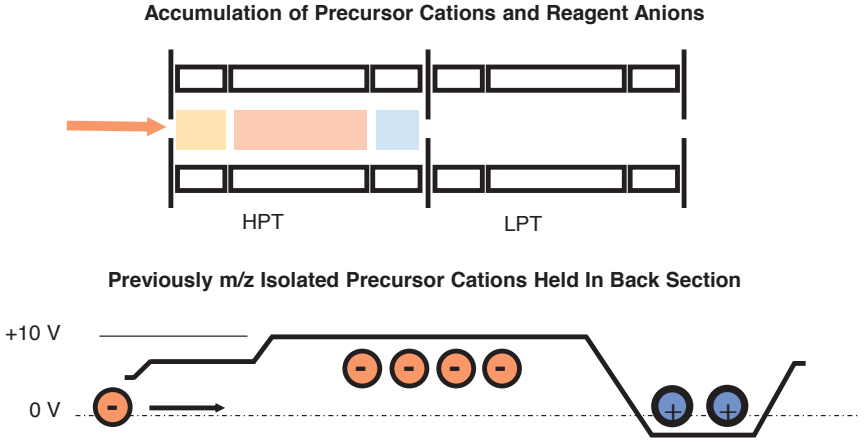


Results

Motivation for Employing Multiple Fills ETD

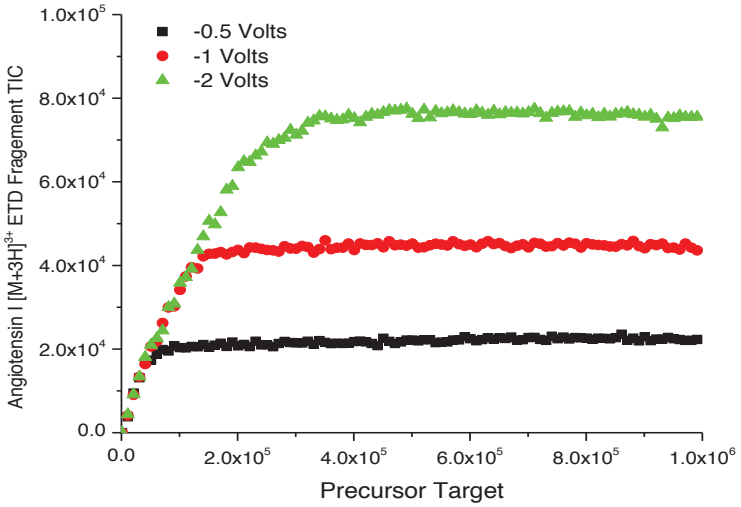
Performing the ETD reaction in the HPT of the Orbitrap Fusion Tribrid MS requires the cation precursor population to be sequestered to the back section of the HPT, so that the reagent anion species can be injected into the trap prior to the charge sign independent trapping (CSIT) and ETD reaction events. As a result, the maximum number of cation precursor charges that can be employed per ETD reaction is a function of the space charge capacity of the section employed for sequestration. Figure 2 shows the potentials employed during the cation sequestration/reagent injection events and the relative locations of the cationic and anionic species.

FIGURE 2. Cation sequestration and reagent injection conditions prior to the ETD reaction in the high pressure cell of the dual cell linear trap



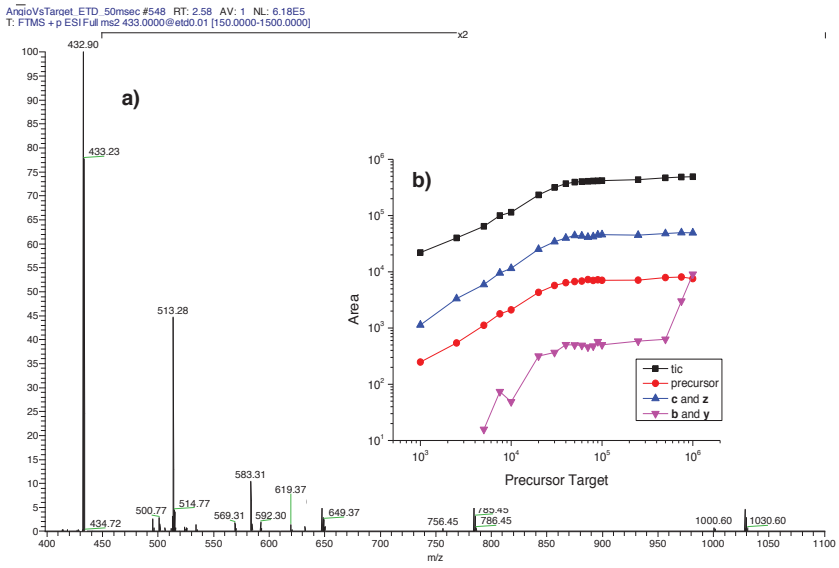
The charge capacity of the back section of the HPT is determined by the axial potential well and radial pseudopotential, and can be measured by a number of methods. We choose to examine the product yield from the ETD reaction of Angiotensin I as a function of the starting precursor ion population. Fragment TIC plotted as a function of the axial potential in the back section of the high pressure trap during the ion sequester event (Figure 3) is characterized by a linear region where ETD products increase with the initial precursor population, followed by a transition to a plateau region where additional precursor does not yield additional product species. The location of the plateau is dictated by the balance of space charge forces with that of the effective RF focusing forces, and has been well modeled in the literature.[1]

FIGURE 3. Angiotensin I ETD product ion yield vs. the starting precursor population for a single ETD reaction.



Unfortunately, certain combinations of axial well depth and RF pseudopotential can lead to ion excitation as the ions spend more time at greater radii. The increased kinetic energy in these situations causes fragmentation which generates significant amounts of b and y series ions[2], and contributes to overall lower signal to noise (S/N) ratio ETD spectra. We demonstrate that the precursor fragmentation is occurring during the sequester events by monitoring the fragment TIC in the absence of ion-ion reaction time, Figure 4.

FIGURE 4. a) Angiotensin I b and y ion formation observed at high precursor initial targets resulting from harsh sequestration conditions. b) Fragment yield versus precursor target demonstrating the onset of b and y ion formation.



ETD Multiple Fills Proof of Concept

The multiple fills ETD approach has been previously demonstrated[3,4] and is advantageous as it has the capability to provide higher S/N ratio ETD spectra, while providing facile ETD sequestration conditions. A flow diagram detailing the approach is presented in Figure 5. Figures 6, 7, and 8 compare and contrast the multiple fills approach to the scan averaging approach in terms of spectral S/N ratio scan acquisition speed.

FIGURE 5. Flow diagram describing the ETD multiple fills approach when the ETD reaction is conducted in the HPT and product accumulation is conducted in the LPT. Mass analysis can be conducted in either analyzer.

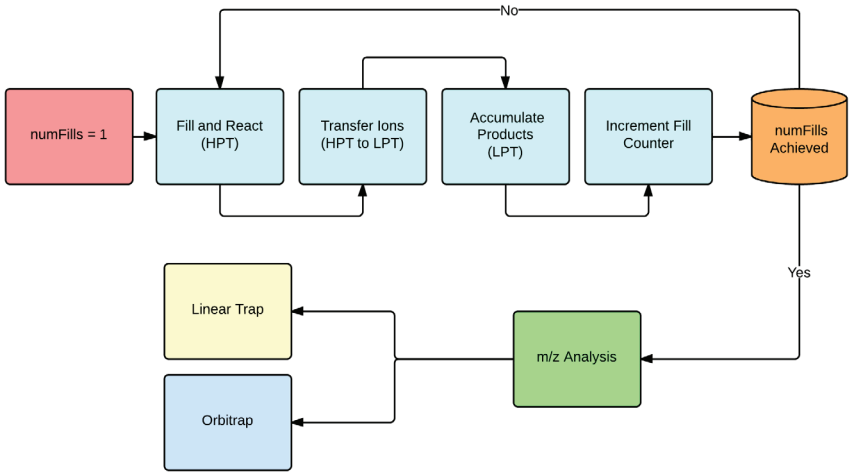
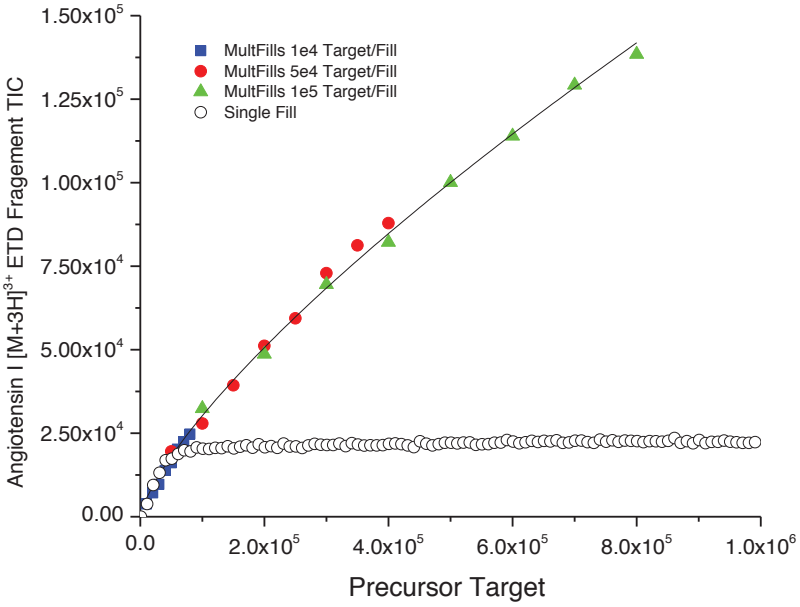


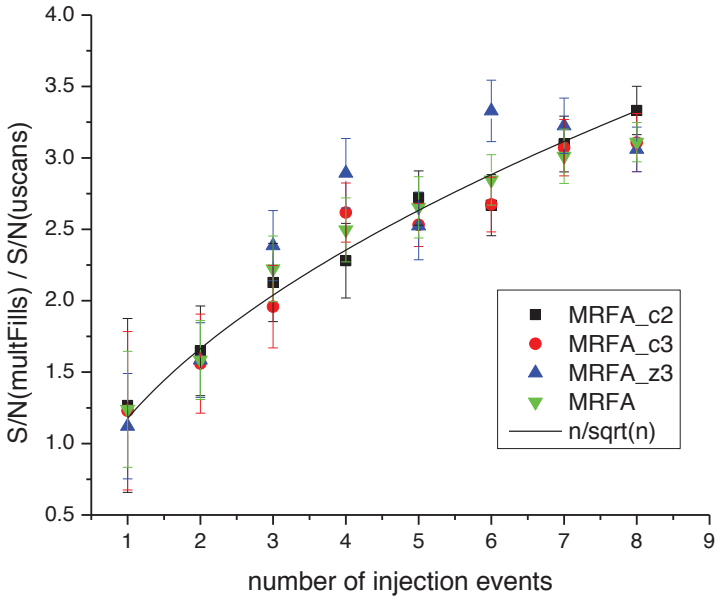
FIGURE 6. Comparison of the Angiotensin I ETD c and z ion fragment TIC for an up to 8-fills ETD approach versus a single fill. The multiple fill data was taken at three individual precursor targets per fill corresponding to 1e4 (blue squares), 5e4 (red circles), and 1e5 (green triangles) charges/fill.



It is apparent (Figure 6) that the working range using the multiple fills approach is much greater than that for a single fill, while preserving soft sequestration conditions.

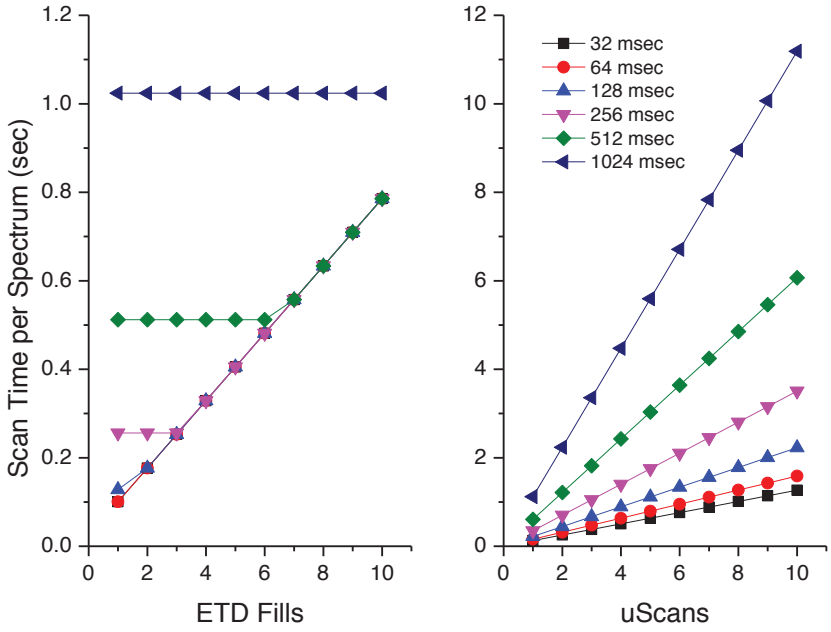
The fit to the data (Figure 7) represents the theoretical gains that could be realized if the S/N ratio increases linearly with the ion population and as a square root function for scan averaging.

FIGURE 7. Comparison of the S/N ratio of ETD c and z fragments from tetrapeptide MRFA $[M+2H]^{2+}$ after reaction for 100 msec versus the number of injection events. For the case of multiple ETD fills this represents the number of precursor fills/ETD cycles before *m/z* analysis; uScans represents the number of scans averaged.



The geometry of the Orbitrap Fusion Tribrid MS in conjunction with it's ability to run scans in a parallel/pipelined fashion affords a significant decrease in scan acquisition time per spectrum for the multiple fills approach (Figure 8) as the ETD fills are run in parallel with OT *m/z* analysis.

FIGURE 8. Scan acquisition time per spectrum for the ETD multiple fills experiment and the uScans approach taken at a variety of Orbitrap transient durations.



Conclusions

- Single fill ETD limited by space charge capacity of the back section of the ion trap in the Orbitrap Fusion Tribrid MS.
- The sequestration conditions during ETD need careful consideration in order to minimize trap-like collision induced dissociation.
- A multiple fills methodology has been presented based on ETD reaction in the high pressure trap followed by accumulation of ETD products in the low pressure trap.
- The geometry of the Orbitrap Fusion Tribrid MS in conjunction with the ability to run both analyzers in parallel allows for acquisition of high S/N ratio spectra in a much shorter time than can be done with scan averaging alone.

References

1. Tolmachev, A. V., Udseth, H. R., Smith, R.D., *IJMS* 222 (2003) pg 155-174.
2. Sannes-Lowery, K., Griffey, R.H., Kruppa, G.H., Speir, J.P., Hofstadler, S.A., *Rapid Comm in Mass Spec* 12 (1998) pg 1957-1961.
3. Rose, C.M. et al., *J Am Soc Mass Spectrom* 24 (2013) pg 816-827.
4. Earley, L. et al., Anal Chem 85:17 (2013) pg 8385-8390.

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