

Optimization of Parameters for Performing Electron Transfer Dissociation on 2-Dimensional Linear Ion Traps

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Overview

Purpose: Characterization and optimization of parameters associated with performing electron-transfer dissociation (ETD).

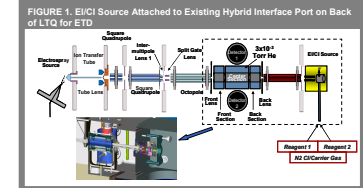
Methods: An electron impact/chemical ionization (EICI) source is attached to the existing hybrid interface port on the back of an LTO™. Parameters including those associated with forming reagent ions, storing the reagent and analyte ions simultaneously, and the ion-ion reaction conditions were systematically studied.

Results: By choosing optimum values for the various operating parameters, the information produced by ETD can be maximized even for large peptides. ETD fragmentation efficiency is optimized while the minimum time for performing ETD is maintained. Full automation and robustness are also achieved.

Introduction

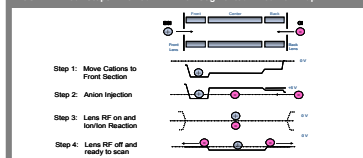
Electron transfer dissociation (ETD) on a linear ion trap has been demonstrated to be an extremely powerful dissociation technique for characterization of larger multiply charged peptide and protein¹. In implementing the ETD technique, there are many parameters which must be considered in order to optimize its performance and produce informative, interpretable data. These parameters include those associated with forming and delivering the reagent ions, storing the reagent and analyte ions simultaneously, and the ion-ion reaction conditions. These studies help to characterize and optimize many of these parameters.

Methods



An electron impact/chemical ionization (EICI) source from a Thermo Electron DSO™ GC-MS was attached to the existing hybrid interface port on the back of an LTO 2D-Linear ion trap as shown in Figure 1. This source is used for forming the negative radical anions of fluoranthene as the ETD reagent in these studies. Standard peptides such as Angiotensin I, Vasopressin, Intestinal Peptide 1-12, and ACTH 1-24 (Sigma-Aldrich), were used to study various parameters and their interactions. Figure 2 shows the steps involved in performing ETD on a linear ion trap. By storing the cations and anions in different segments of the linear ion trap, precise control of the timing of each step is maintained.

FIGURE 2. Four Steps Involved in ETD on Segmented Linear Ion Trap



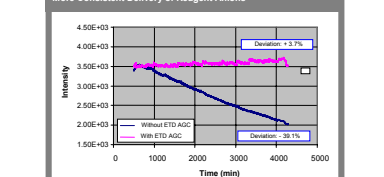
Results

Forming and Delivering Reagent Ions for Long Durations

To optimize the formation of the radical anion of fluoranthene and achieve a uniform delivery of the reagent over a long time period, a vial containing >40 mg of solid is heated to a temperature of 130 °C. The headspace is sampled using a carrier gas at a low flow rate of approximately 0.5 cc/min. Methane, Nitrogen, and Helium have been compared as chemical ionization gases. Although all three gases can be used for effective negative ion chemical ionization (NCI) of fluoranthene, somewhat higher pressures of N₂ and He are required to yield the same abundance of reagent anions. However, both N₂ and He were found to give significantly lower contamination rates versus Methane, resulting in considerably extended times between ion volume replacements and/or cleanings of the source. N₂ was chosen as the NCI gas since it requires somewhat lower operating pressures than He and is normally available on the system.

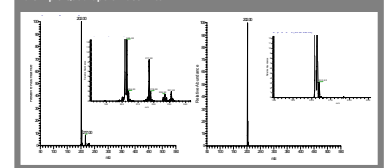
A second step, which significantly extends the time between ion volume replacements and/or cleanings of the NCI source, involves gating the electrons into the source only when they are needed. This requires reducing the filament offset voltage (electron energy) from -70 eV to -5 eV while keeping an intermediate lens at +65 volts. This gating scheme extends the time between ion volume replacement and/or cleanings of the source by more than ten-fold. Delivering a fairly constant number of reagent ions is critical to achieving reproducible results. Changes in the ion source sensitivity can be automatically compensated for by using an automatic gain control (AGC) for the ETD reagent ions. However, since the sensitivity of the NCI source changes only very slowly, an ETD scan is only required approximately once an hour. Figure 2 shows that the intensity of reagent ions remains within 3.7% with ETD AGC, whereas without ETD AGC it can vary 39.1% in 72 hours.

FIGURE 3. Automatic Gain Control (AGC) for ETD Provides a More Consistent Delivery of Reagent Anions



Mass selection of the reagent anions assures reproducibility, maximizes ETD efficiency, and allows real time control of the type of reagent desired. The combination of isolation waveforms and a simple quadrupole mass filter between the source and the trap enables the selection of the reagent ions before they enter the trap, thereby minimizing any unwanted reactions. The ability to reject background ions and select the reagent ions is demonstrated in Figure 4 and allows the use of multiple reagent ions for multiple reaction types.

FIGURE 4. Selection of a Particular Reagent Ion Before Entering the Trap Using a Simple Quadrupole Mass Filter



Storing the Analyte and Reagent Ions Simultaneously

As shown in Figure 2, during the ETD reaction process, analyte cations are stored in the front section of the trap while reagent anions are injected from the C2 source at the back of the instrument. During this process, it is important to use a trapping voltage which will store the cation while simultaneously allowing trapping of the injected anions. Figure 5 shows the dependency of these processes on the RF amplitude. Even a high m/z cation (16+ of Myoglobin) can be stored for ETD while the reagent anion at m/z 202 is injected.

FIGURE 5a. Effects of RF Voltage During Ion-Ion Reaction

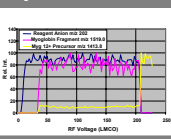
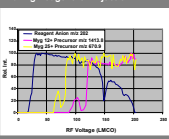


FIGURE 5b. Effects of RF Voltage During Reagent Ion Injection



During the ion-ion reaction, polarity independent trapping is accomplished by using RF voltages on the entrance and exit lenses. Both the frequency and amplitude of these voltages are important to trap all m/z's of interest. Figures 6a and 6b show the measured pseudo potentials established by an applied RF-voltage for both high and low m/z-ions. The frequency of the RF voltage was 600 kHz. 300 V_{pp} is found to provide a pseudo potential barrier of -9 Volts for m/z 195 and -2 volts for m/z 182 which is sufficient for efficient axial trapping.

FIGURE 6a. RF Lens Pseudo Potential for Ion at M/Z 195

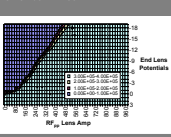
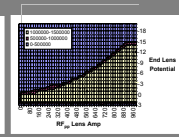


FIGURE 6b. RF Lens Pseudo Potential for Ion at M/Z 182



Ion-Ion Reaction Conditions

The effects on ETD fragmentation efficiency have been investigated with respect to parameters, such as reagent ion number, analyte ion number and ion-ion reaction time. One example of a multi-dimensional study is shown in Figure 7. This data is for the +4 charge state of Angiotensin I at m/z 325 using an MS1 target value of 1.0E4. The data shows the effects of both the ion-ion reaction time and the number of reagent anions on producing ETD fragments. The optimum number of reagent anions was approximately 3 to 5 times the analyte ion abundance. Greater numbers of anions did not increase the ETD efficiency but could allow some decrease in ion-ion reaction time to values of approximately 60 msec. Figure 8 shows a subset of the data indicating the fragment ion and charge reduced precursor ion intensity dependence on the reaction time.

FIGURE 7. Effects of Ion-Ion Reaction Time and Reagent Ion Intensity on ETD Fragment Ion Intensity for +4- Charge State of Angiotensin I

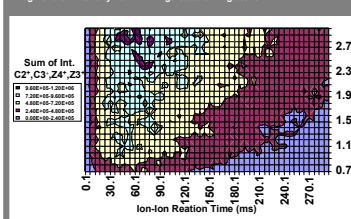


FIGURE 8. Effects of Ion-Ion Reaction Time on ETD Fragment Ion Intensity and Charged Reduced Precursor Ions for +4 Charge State of Angiotensin I

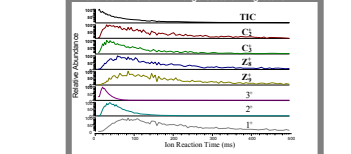
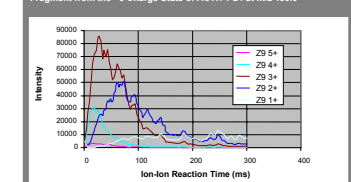


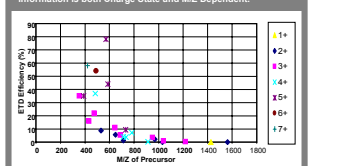
Figure 9 shows data for a larger peptide, ACTH 1-24, with a charge state of +6. This data indicates the dependence of the various charge states of the fragment ions on the reaction time. In this case, the highest intensity of the Z₆ sequence fragment is obtained at a reaction time of ~25 msec where it predominantly exists in the +3 and +2 charge states. The spectra can be amplified by allowing the charge states of the fragments to be reduced at longer reaction times, but with a loss in sensitivity.

FIGURE 9. Effect of Ion-Ion Reaction Time on the Charge State of the Z₆ Fragment from the +6 Charge State of ACTH 1-24 at m/z 499.0



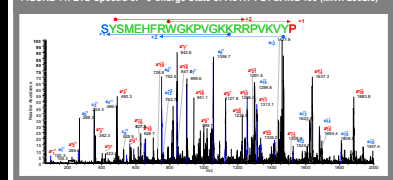
A study of many different peptides with various m/z and charge states was performed to evaluate the ETD efficiency of forming fragments without supplementary activation as a function of m/z and charge state. Figure 10 shows the data indicating that charge state alone does not assure good ETD performance. Having an appropriate m/z (between 350 and 600), along with sufficiently high charge, is required. Under these conditions, very high efficiencies for forming sequence ions can be obtained.

FIGURE 10. ETD Efficiency for Forming Sequence Ion Information is both Charge State and M/Z Dependent.



An understanding of the effects of each of the many parameters involved in performing ETD assures that the information content of ETD spectra can be optimized, and therefore provide substantial sequence coverage of large peptides and proteins. An example of 100% sequence coverage of the ACTH 1-24 peptide with molecular weight of 2932.6 is shown in Figure 11.

FIGURE 11. ETD Spectra of +6 Charge State of ACTH 1-24 at m/z 490 (M.W. 2932.6)



Conclusions

There are many parameters to consider when optimizing ETD performance.

- Robust production of ETD anion reagent:** Extension of the working time of the NCI source for producing ETD reagent ions by more than 20-fold is achieved by using N₂ instead of CH₄ as the CI gas, and also by gating the electrons into the source.
- Reagent anion purity:** Reagent ion selection for choosing reaction type and maximizing ETD efficiency can be achieved by using a simple quadrupole mass filter between the ion source and linear trap.
- ETD reaction efficiency:** Careful choice of trapping voltages must be made to trap both analyte ions and reagent ions simultaneously. Reagent ion abundance should be ~5 times the analyte ion intensity. Ion-ion reaction times will affect ETD efficiency and charge distribution of fragment ions, and is precursor charge state dependent. Best ETD efficiency is achieved when the m/z of the precursor is between 350 and 600, along with sufficiently high charge.

Controlling the many important parameters discussed above, in combination with the high performance of linear ion traps, makes an ideal system for ETD analysis.

References

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