Automated Optimization Methods for an Electron-Capture Dissociation Device

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Introduction

Electron-Capture Dissociation (ECD) is an increasingly important technique for the structural analysis of proteins and peptides, providing complementary data to traditional Collision-Induced Dissociation (CID). Modern research-grade mass spectrometers have significant flexibility, but this necessitates a degree of complexity which can make the optimization of experiment conditions challenging, both for initial settings and during ongoing studies. This study discloses an assessment of various automated approaches to the optimization of an ECD device within a Q-IMS-ToF mass spectrometer, determining performance metrics such as the speed of the optimization process and the reliability of the final conditions.

Methods

A Waters SELECT SERIES™ Cyclic™ IMS system fitted with an Electron-Capture Dissociation device, in the post-IMS position, was used for the development and assessment of several optimization routines. Performance was evaluated using an infusion of substance P (Merck), focusing on the c/z ions produced through the ECD process, while also minimizing the formation of b/y ions associated with CID fragmentation reactions. Example spectra of Substance P, under conditions where ECD only fragmentation is dominant, and under conditions where CID fragments are also produced, is shown in Figure 1.

An overview of the instrument is shown in Figure 2, with the location of the ECD cell highlighted. The potential energy arrangement of the device is also shown, indicating how the biases and gradients are applied.

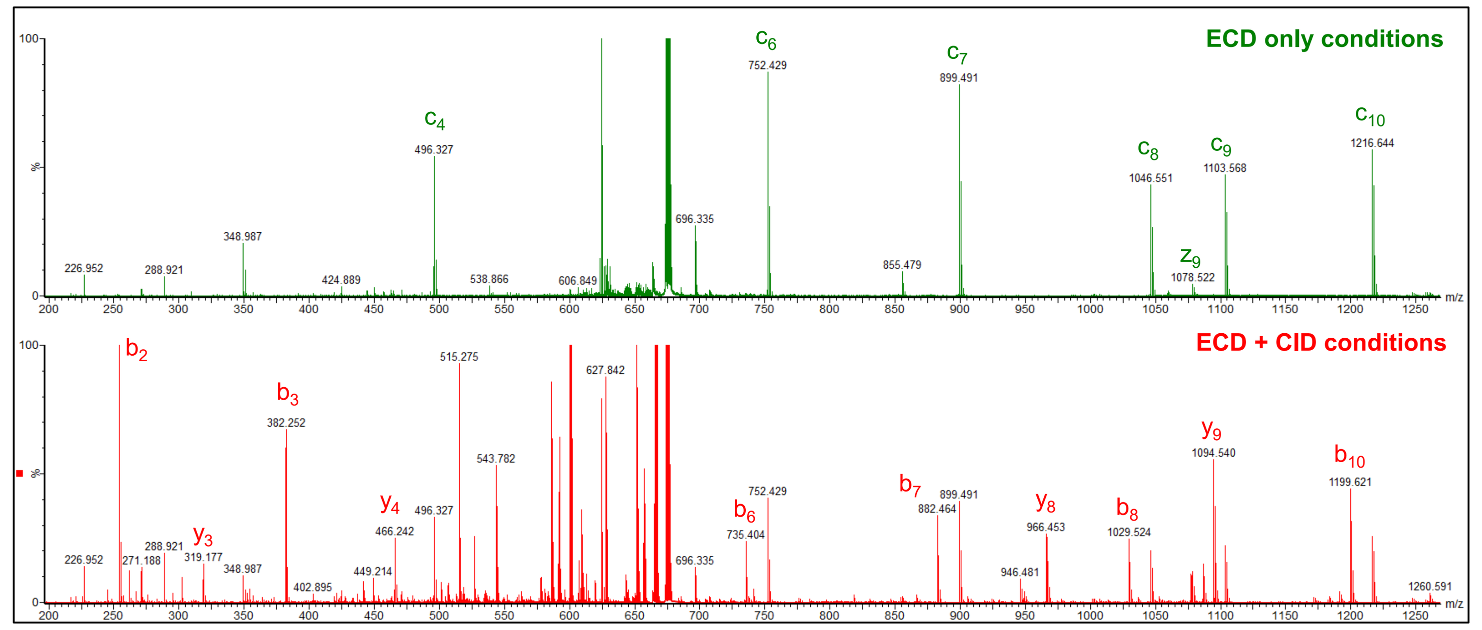

Figure 1. Mass spectra of Substance P, using tuning conditions which produce dominantly ECD only (c/z) fragments (upper) and conditions that result in additional CID (b/y) fragments (lower).

Experimental

A research application was written (using Microsoft Visual Studios C#) which allowed direct control of the mass spectrometer and the ECD device (Figure 3). The application analyses data as it is acquired and adjusts the ECD-related control voltages, to study their impact on the formation of the ECD ions.

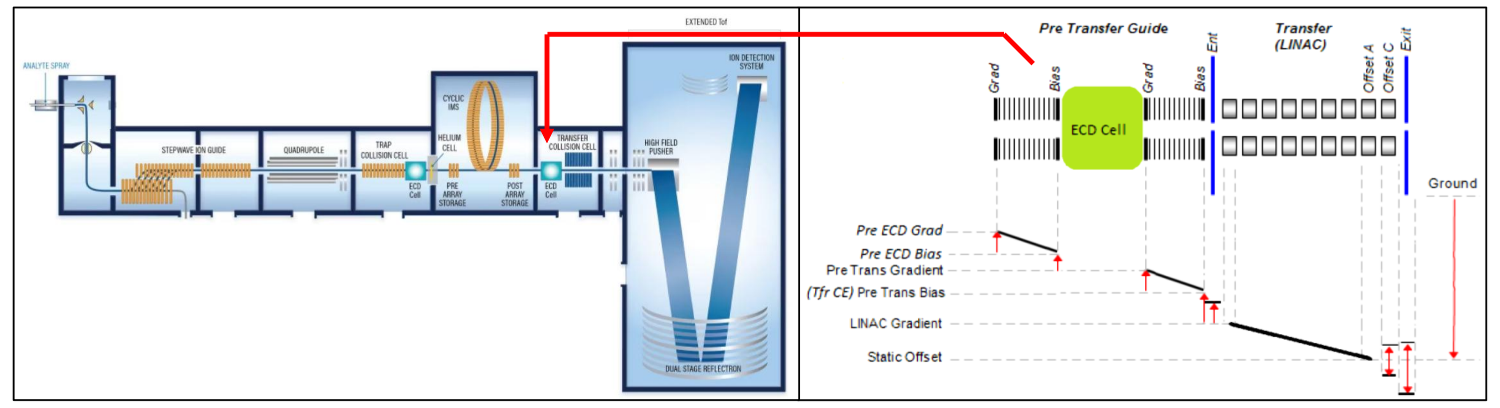

Figure 2. System schematic, and potential energy arrangement of the ECD device.

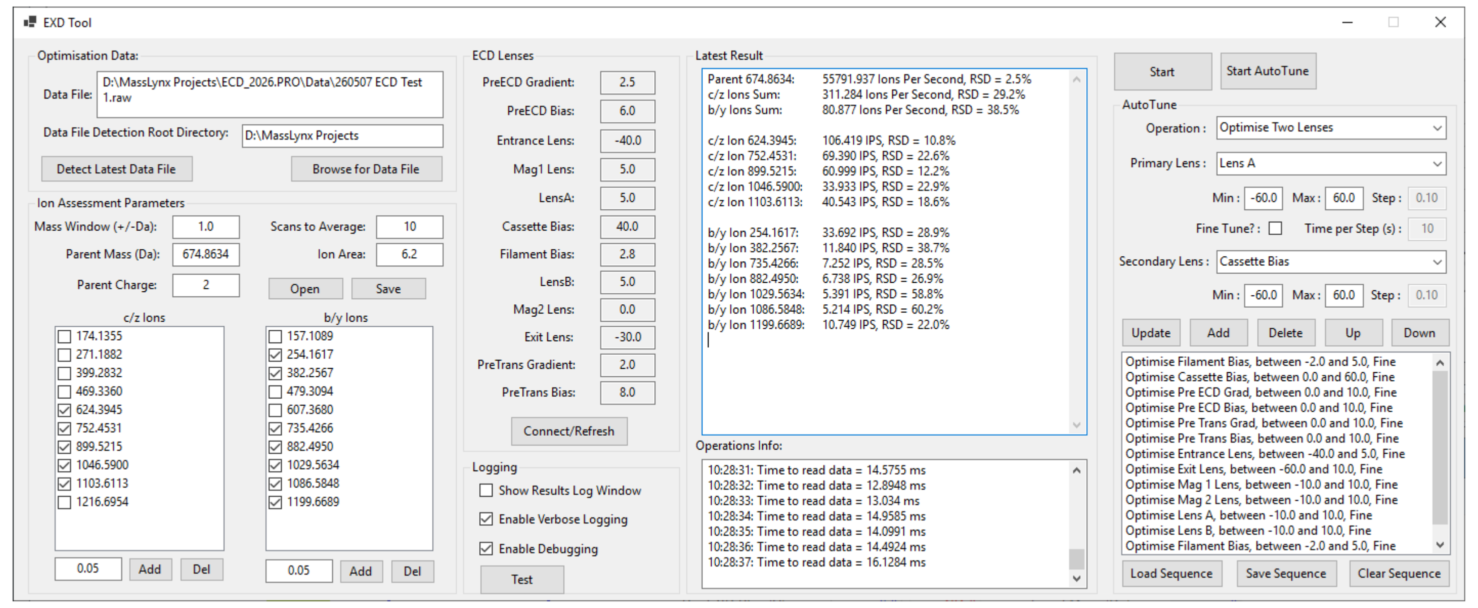

Figure 3. Screenshot of the research application, developed to control the ECD device and explore automated optimization techniques.

The application allows the ions of interest to be defined, both the favored c/z ions and the undesired b/y ions, which can be monitored as conditions are varied. It also facilitates the construction of an optimization schedule, with controls or control pairs being optimized in sequence with various parameters stipulated.

A further function of this program is to profile the intensity of the specified ions of interest with respect to the control voltage, allowing the determination of the useful range of the lens and its sensitivity to changes in voltage, which in turn can be used to maximize the effectiveness of the optimization process.

Furthermore, pairs of controls can be profiled together to map out their interdependency. This allows for more sophistication optimization processes to be developed, such that the optimum combination of control voltages can be determined with fewer iterative steps.

Results

The profiling of the ECD controls identified several which can have differing impacts on the formation of c/z and b/y ions, and their alignment to dominant parent ion signal. Examples of these are shown in Figure 4.

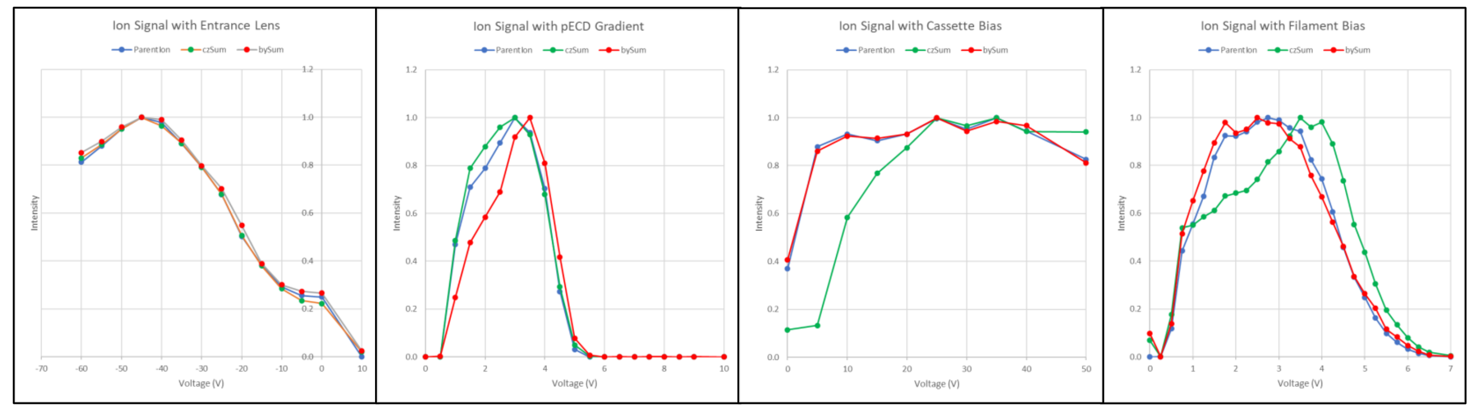

Figure 4. Intensity vs applied voltage profiles for selected controls. The Entrance Lens shows uniform responses for the parent ion signal and the c/z and b/y ions. The other controls shown exhibit differing responses for the c/z ions to the other ions monitored.

Where differing control voltage optima are observed for c/z ions, when compared to the other ions monitored, the optimization operation is adjusted to focus solely on these ions. For those controls where optima are aligned for all ions, the optimization can use the sum of all ions as its optimization metric, with higher precision due to greater ion flux.

Some controls exhibited interdependencies when profiled as pairs, as shown in the examples in Figure 5. The interaction maps allowed relationships between the controls to be established such that a virtual parameter can be used, which has a rotated axis aligned to the optima in these maps. A second virtual parameter is created which is orthogonal to the first. An optimization of both parameters allows the optimum combination to be determined in just two optimization operations.

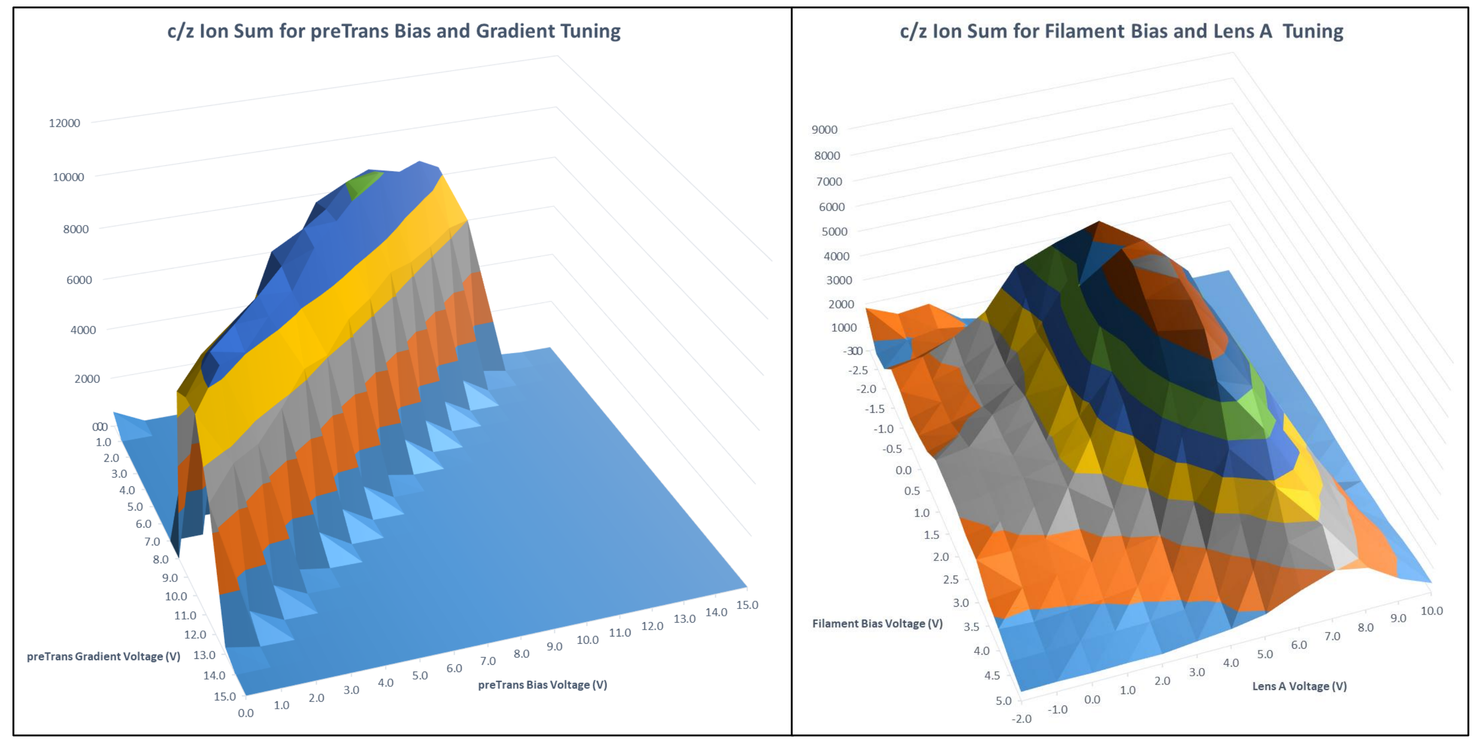

Figure 5. ECD controls interactions, demonstration the inter-dependency of certain combinations.

An example of the optimization process running during an infusion of Substance P is shown in Figure 6. The process took 26 minutes to run and resulted in a five-fold increase in the signal from the c/z ions. A second pass of the optimization process yielded little improvement, as did a manual re-optimization, demonstrating its effectiveness.

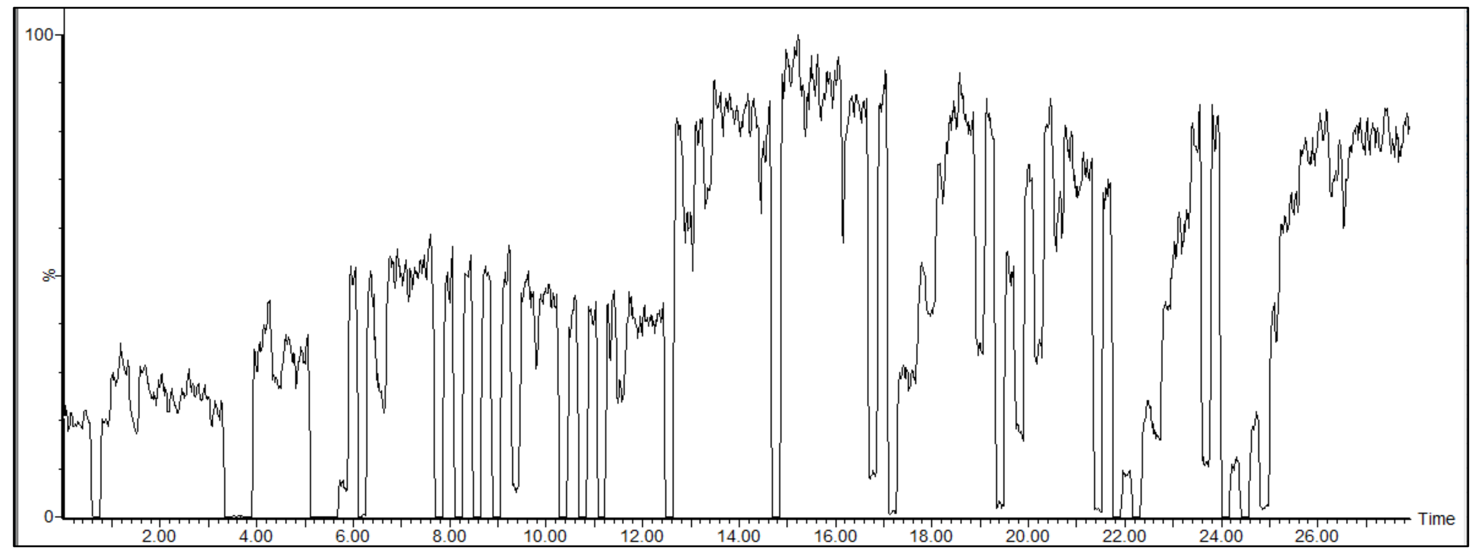

Figure 6. Chromatogram of the combined c/z ions' intensity during the automated optimization process

Conclusions and Future Work

A research application has been developed which facilitates investigation of the influence of the pertinent controls of an ECD device on a plurality of desirable c/z fragment ions, and provides a mechanism for their automated optimization. The speed of the optimization process is increased by optimizing certain controls as pairs, where a known interdependent relationship has been characterised and incorporated into the program. The process is faster than a manual optimization of these controls and can provide a higher degree of precision.

Further studies will be conducted to refine the optimization process and investigate if the user-facing controls of the device can be simplified by utilizing the relationships established between certain controls. Machine learning could also be of benefit to this study, to investigate other possible interdependent relationships between three or more of the controls.

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