Qualitative flexibility combined with quantitative power using the ZenoTOF 7600 system with SCIEX OS software

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INTRODUCTION

The key to achieving robust analytical results lies in the combination of sensitivity, selectivity, and specificity. Sensitivity ensures there is plenty of signal to identify and quantify analytes of interest. Selectivity differentiates analyte signal from noise and interferences. Specificity ensures compound identifications are accurate and confident. The technological advancements in the ZenoTOF 7600 system combine qualitative flexibility and quantitative power for the most demanding sample types and workflows.

A hybrid collision cell is at the heart of the technological advancements in the ZenoTOF 7600 system. Previously, QTOF mass spectrometers have suffered from duty cycle losses as a result of mating time-of-flight (TOF) analysis, a pulsed measurement technique, with the continuous beam coming from the quadrupole ion path. A series of ion-staging events and reverse-mass sequential ion release, with high-capacity ion traps, allow for duty cycle losses to be mitigated and for MS/MS sensitivity gains of 4-25x.[1] The cell also has the ability to perform both collision induced dissociation (CID) and electron activated dissociation (EAD) experiments for high-resolution MS/MS flexibility. Electron kinetic energies can be precisely tuned from 0-25 eV without the use of chemical transfer reagents. This tunability means EAD can be performed on a wide array of analytes, from multiply-charged peptides to singly-charged small molecules.[2] The ability of the EAD cell to contain a high density of electrons allows for rapid reaction rates that keep up with fast chromatographic separations.

KEY INNOVATIONS IN THE SCIEX ZENOTOF 7600 SYSTEM

- Zeno trap technology, resulting in ≥90% duty cycle across the entire mass range for MS/MS acquisition modes (Zeno IDA and Zeno MRMHR)
- MS/MS sensitivity improvements from 4-25x
- Reagent-free and tunable, high-efficiency electron activated dissociation (EAD) fragmentation in the EAD cell, offering alternative fragmentation for both small and large molecules workflows
- New levels of specificity with various electron-based dissociation techniques
- Richer fragmentation for improved structural information.
- Greater than 5 orders of inter-scan linear dynamic range and 4 orders of intra-scan linear dynamic range in both MS and MS/MS modes
- Pre-optimized performance to easily switch between high flow, microflow and nanoflow rates with the OptiFlow Turbo V ion source

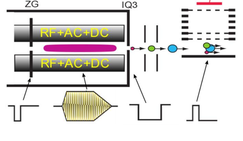

Figure 1: Timing diagram of gating voltages, AC ramp and TOF acceleration pulses. Ions enter the ion trap and are contained with potential barriers on the ZG and IQ3 lenses, while subsequent packages of ions are accumulated in the LINAC collision cell, preventing ion loss. The trapped ions are left to energetically cool and are subsequently ejected in order of high m/z to low m/z. In this way, each ion across the mass range reaches the center of the TOF accelerator simultaneously.

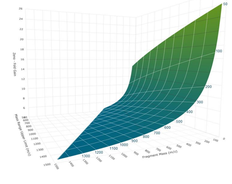

Figure 2: Theoretical sensitivity gains in MS/MS as a function of fragment m/z and acquisition mass range upper limit using the Zeno trap. Sensitivity gains are the result of the recovery of duty cycle losses that are a natural result of mating TOF analysis, a pulsed measurement technique, with the continuous beam coming from the quadrupole ion path. Greater ion losses occur as the upper limit of the MS/MS scan range increases. Zeno trap technology has the ability to recover >95% of these losses.

ZENO TRAP TECHNOLOGY

Quadrupole time-of flight instruments most commonly use the orthogonal injection of ions coming from a quadrupole collision cell into the flight tube region, because this configuration maximizes TOF resolution, mass accuracy, and sensitivity for an entire spectrum without the need for scanning. This type of ion pulsing, however, suffers from a relatively low duty cycle. Typically, only 5-25% of ions are ejected with each pulse of the accelerator, depending on the geometry and m/z range. The ion losses are a result of the drift region between the collision cell and the TOF accelerator. This region behaves as a crude TOF separation, where low m/z ions migrate faster than high m/z ions and, as a result, a significant fraction over or under migrate to the accelerator region and are lost with each pulse.

Use of the Zeno trap overcomes these technological barriers to recover duty cycle losses across the entire m/z at up to 100 Hz acquisition frequency. This is achieved using a linear ion trap, referred to as a Zeno trap, at the exit of the collision cell. The mechanism of trapping and releasing ions is highlighted in Figure 1.

This simple trapping and releasing mechanism leads to significant gains in MS/MS sensitivity, as highlighted in Figure 2. MS/MS with the Zeno trap activated results in a 4- to 15-fold (or greater) gain in signal, with increased gains at low m/z fragments. The ion trap efficiency combined with precise ion-release timing yields ≥90% of the theoretical gain across the entire mass range. Due to the degree of selectivity afforded with high resolution MS/MS data, these improvements in signal are combined with negligible changes to noise, resulting in spectral and chromatographic signal-to-noise on the order of the gains observed in raw signal (Figure 3, 4).

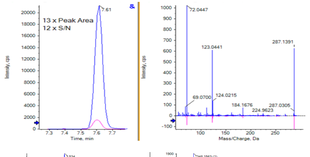

Figure 3: Sensitivity gains with the Zeno trap for difenoxuron with MRM^{HR} acquisition. Due to the selectivity afforded by MRM^{HR}, the gain in signal from the Zeno trap is accompanied by a minimal gain in noise. (Left) A 13-fold intensity gain results in a 12-fold signal to noise gain for the m/z = 72.044 fragment of difenoxurion. (Right) All peaks in MS/MS spectra show a sensitivity gain (6-13 fold) with use of the Zeno trap.

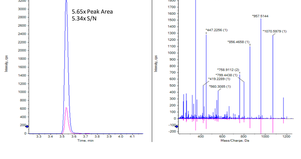

Figure 4: Sensitivity gains with the Zeno trap for SPYVITGPGVVEYK from PepCalMix with MRM^{HR} acquisition. (Left) A 5.65-fold gain in peak area for m/z = 1070.50 fragment ion with a 5.34-fold gain in S/N. (Right) Gain observed for higher m/z peptide fragment ions is 5-7 fold with the Zeno trap on across the MS/MS mass range.

PRECISELY TUNABLE ELECTRON ACTIVATED DISSOCIATION (EAD) CELL

Electron activated dissociation (EAD) describes a family of free electron-based dissociation mechanisms known to give complementary information to CID. The extent and location of bond cleavage differs for radical (EAD) and thermal (CID) techniques.

The ZenoTOF 7600 system features the EAD cell, a new electron beam optic design that simultaneously and independently traps precursor ions and free electrons for efficient radical fragmentation. Tunable electron kinetic energy from 0-25 eV provides access to different fragmentation regimes (Figure 5), including electron capture dissociation (ECD), hot ECD, and electron impact excitation of ions from organics (EIEIO). These advances enable fast, precise and quantitative dissociation of various analytes ranging from singly charged small molecules to multiply protonated proteins.

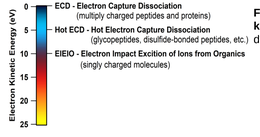

Figure 6: EAD family classified by precursor species and the kinetic energy of the electron beam. Common electron-based dissociation techniques and their typical applications are listed.

ECD AND HOT ECD FOR MULTIPLY CHARGED PEPTIDES, PROTEINS AND BIOTHERAPUTIC MOLECULES

Post-translational modifications (PTMs) are widely important for various protein functions, including protein conformation, signaling and activity. Some PTMs can be difficult to characterize using CID as the dissociation technique. Radical dissociation techniques have the ability to maintain these PTMs, which allows for peptide backbone mapping, while simultaneously elucidating the identity and location of the PTM. Figure 6 shows the example of a phosphorylated peptide, LITV, using hot ECD (KE = 7 eV).

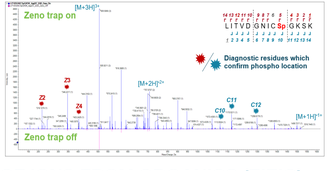

Figure 6. Phosphorylated peptide, LITV, analyzed using EAD-MS/MS. (Top) LITV EAD-MS/MS spectrum with Zeno trap on. (Bottom) LITV EAD MS/MS spectrum with Zeno trap off. MS/MS sensitivity is significantly enhanced with the Zeno trap activated. With the Zeno trap on, 100% sequence coverage is achieved with phosphorylation site location confirmed on multiple c- and z- ion series.

ELECTRON IMPACT EXCITATION OF IONS FROM ORGANICS (EIEIO) FOR SINGLY CHARGED MOLECULES

Having the ability to tune electron kinetic energies in the 5 to 15 eV range opens up EAD to the realm of singly charged molecules. Electron capture, resulting in neutralization, is reduced at this kinetic energy, allowing the electrons to induce dissociation through radical mechanisms. Figure 9 shows the comparison between CID and EIEIO for the fragmentation of a sphingomyelin lipid species. In a similar fashion, EIEIO can be used to differentiate between isomers of small molecules. Figure 8 highlights the unique, characteristic fragment for O-glucuronide conjugated darunavir compared to its N-glucuronide isomer. EIEIO fragmentation also opens up additional specificity for non- targeted and suspect screening workflows. Figure 7 highlights the difference between EIEIO and CID for the fragmentation of azoxystrobin, a fungicide.

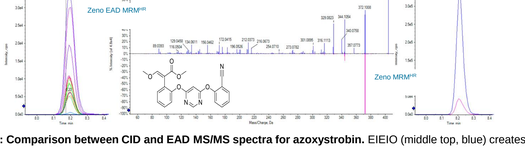

Figure 7: Comparison between CID and EAD MS/MS spectra for azoxystrobin. EIEIO (middle top, blue) creates roughly 100x the number of fragments with S/N >10 compared to CID (middle bottom, pink). The XICs for EAD (left) and CID (right) highlight the flexibility of EAD for ion ratio-based or library confirmation. The CID spectrum is dominated by two main fragments, whereas the EIEIO spectrum contains over 200 peaks with S/N >10, which allows for significantly improved confidence during library matching and structural elucidation.

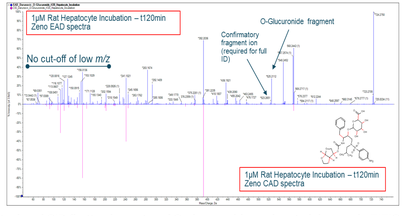

Figure 8: EAD (top) and CID (bottom) spectra of O-glucuronide conjugated darunavir. EIEIO creates unique fragments characteristic of the glucuronide conjugation positioning, allowing for more precise molecular information within metabolite identification workflows

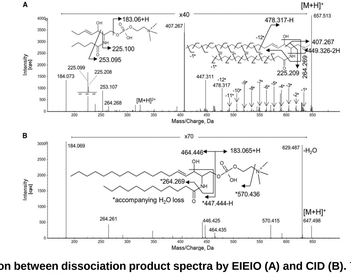

Figure 9: Comparison between dissociation product spectra by EIEIO (A) and CID (B). The sample was a synthesized standard SM, SM(d18:1,12:0). EIEIO gives spectral information for nearly every bond to elucidate head group identity, backbone type, chain lengths, double bond positions and double bond stereoisomerism.

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TRADEMARKS/LICENSING