Qualitative flexibility combined with quantitative power

Using the ZenoTOF 7600 system, powered by SCIEX OS Software

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 4-20x.[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.

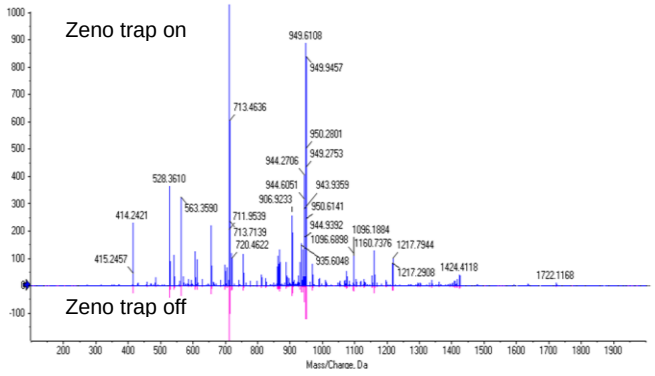

Figure 1. EAD MS/MS spectra of melittin with and without the Zeno trap activated. MS/MS spectra enhanced by using the Zeno trap (top, blue) shows 5-10x improvement in sensitivity across the mass range compared to the EAD MS/MS spectrum acquired without the Zeno trap activated (bottom, pink), with negligible changes to noise. EAD yields significant sequence coverage for structural elucidation.

Key innovations in the SCIEX ZenoTOF 7600 system

- Zeno trap provides an increase to ≥90% duty cycle across the entire mass range for MS/MS acquisition modes (Zeno IDA and Zeno MRM^{HR})
- MS/MS sensitivity improvements from 4-20x
- 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

Zeno trap

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. This is not usually an issue in the MS1 dimension, because the ion current as generated by modern sources (such as the Turbo V ion source), and transmitted by modern ion capture technology (such as the QJet ion guide), results in ion currents that need to be reduced to prevent saturation and to protect the longevity of TOF MS detectors. In the MS/MS dimension, however, an improvement in the duty cycle can lead to significant gains in sensitivity.

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. Previously, there have been many attempts to overcome this lack of synchronicity. It has only been achieved, however, either for narrow mass ranges or at low acquisition frequency.

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 2. Ions enter the ion trap and are contained with potential barriers on the ZG and IQ3 lenses, while subsequent packages

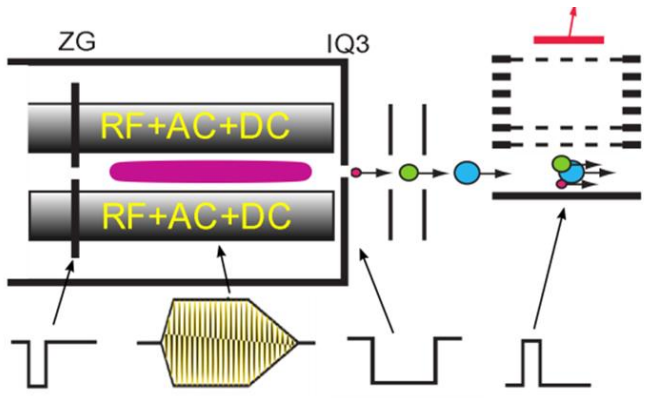

Figure 2: Timing diagram of gating voltages, AC ramp and TOF acceleration pulses. Ions are accumulated in a small ion trap at the exit of the collision cell, then released in reverse mass order to perfectly synchronize with each accelerator pulse.

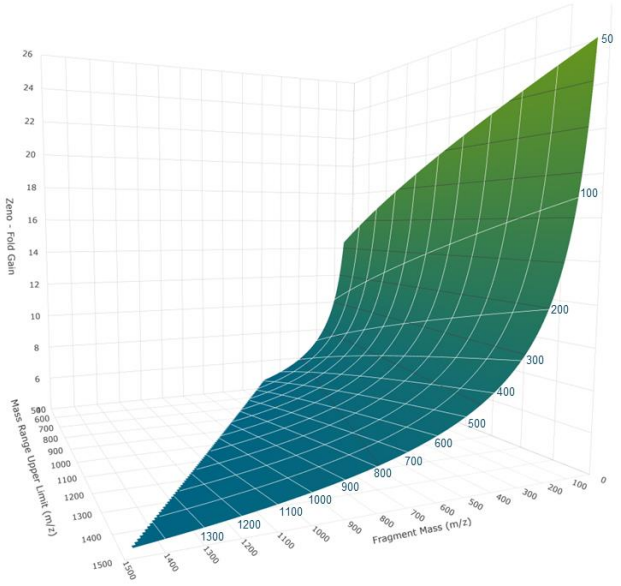

Figure 3: Theoretical sensitivity gains in MS/MS as a function of fragment m/z and acquisition mass range upper limit using the Zeno trap. Sensitivity gains are the result of the recovery of duty cycle losses that are a natural result of mating TOF analysis, a pulsed measurement technique, with the continuous 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.

of ions are accumulated in the LINAC collision cell, preventing ion loss. The trapped ions are left to energetically cool and are subsequently released based on potential energy resulting in an ordered release generally ranging from high m/z to low m/z. In this way, each ion across the mass range reaches the center of the TOF accelerator simultaneously.

This simple trapping and releasing mechanism leads to significant gains in MS/MS sensitivity, as highlighted in Figure 3. 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 Zeno 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 4, 5).

These improvements in MS/MS sensitivity not only have the ability to drastically improve LOQs for quantitative assays, but this additional sensitivity can be used to revolutionize entire workflows. With the Zeno trap activated, high-quality MS/MS spectra can be used for confirmation, identification, or library matching at much lower mass loading. This gives the ability to significantly dilute precious samples and improve ionization efficiency by minimizing matrix effects and improve instrumental

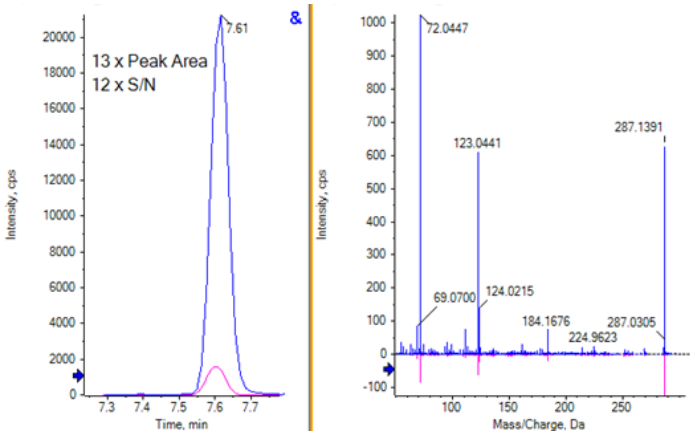

Figure 4: Sensitivity gains with the Zeno trap for difenoxuron with MRM^{HR} acquisition. Due to the selectivity afforded by MRM^{HR}, the gain in signal from the Zeno trap is accompanied by a minimal gain in noise. (Left) A 13-fold intensity gain results in a 12-fold signal to noise gain for the m/z = 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.

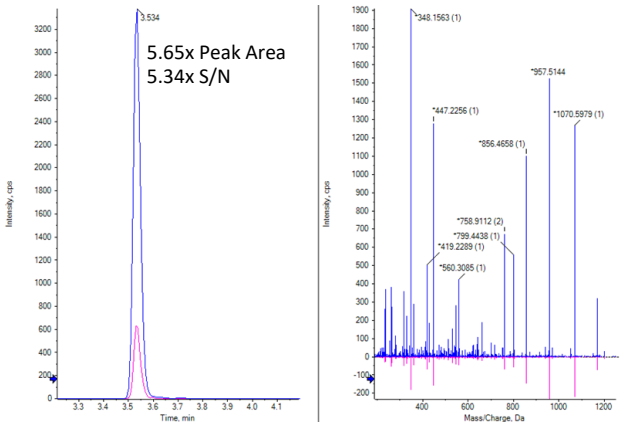

Figure 5: Sensitivity gains with the Zeno trap for SPYVITGPGVVEYK from PepCalMix with MRM^{HR} acquisition. (Left) A 5.65-fold gain in peak area for m/z = 1070.50 fragment ion with a 5.34-fold gain in S/N. (Right) Gain observed for higher m/z peptide fragment ions is 5-7 fold with the Zeno trap on across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

robustness with lower mass loading. At the same sample loading, MS/MS with the Zeno trap on yields greater confidence in identifications while allowing for the ability to discover new metabolites, peptides biomarkers and contaminants at lower concentrations than ever before.

Precisely tunable electron activated dissociation (EAD) cell

Tandem mass spectrometry is dominated by collisional induced dissociation (CID) that generates ion fragments for quantification and identification of molecular species. With CID, ions are generated in an accelerated cell filled with a neutral gas species promoting molecular collisions that result in bond cleavage, typically at the most labile sites. Although fast and efficient, CID can often result in few diagnostic fragments that are insufficient to elucidate structural information from unknown features or to differentiate isomeric species.

Electron activated dissociation (EAD), conversely, describes a family of free electron-based dissociation mechanisms characterized by the charge state of the precursor ion and the kinetic energy of the electron beam. EAD mechanisms are 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. With the EAD cell, the system has the ability to tune electron energies above zero, which opens up radical fragmentation to applications beyond biomolecules. Tunable electron kinetic energy from 0-25 eV provides access to different fragmentation regimes (Figure 6), 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. Also, at higher energies, reaction times are reduced allowing for these dissociation techniques to be used on a chromatographic timescale. It is the combination with the Zeno trap that allows EAD to now have the sensitivity and specificity needed for routine use.

ECD and hot ECD for multiply charged peptides, proteins and biotherapeutic molecules

Post-translational modifications (PTMs) are widely important for various protein functions, including protein conformation, signaling and activity. Some PTMs can be difficult to

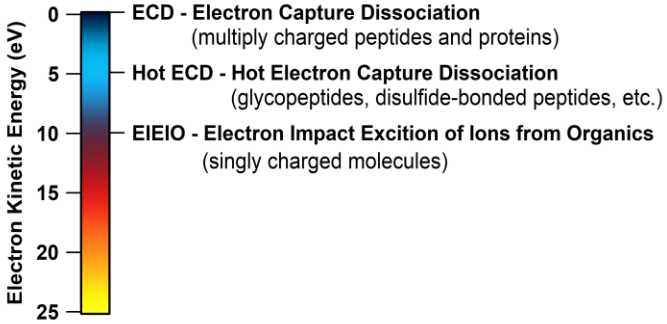

Figure 6: EAD family classified by precursor species and the kinetic energy of the electron beam. Common electron-based dissociation techniques and their typical applications are listed.

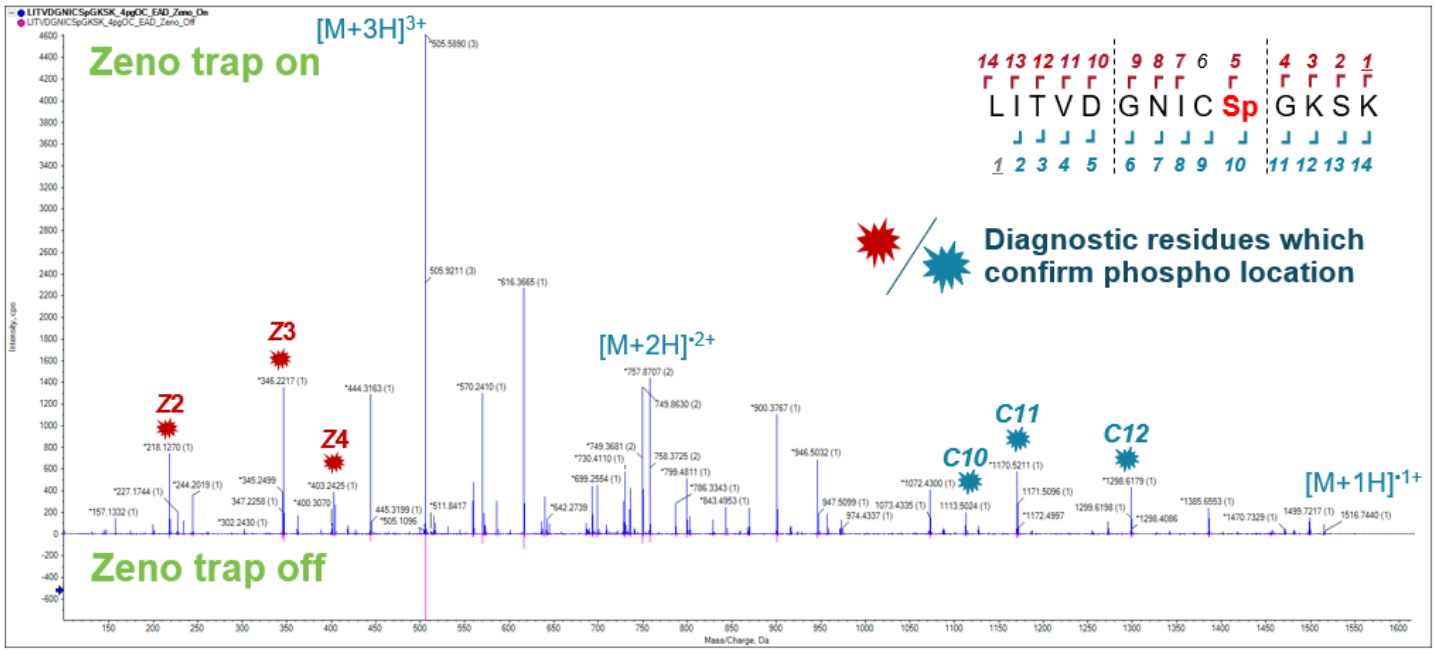

Figure 7. Phosphorylated peptide, LITV, analyzed using EAD-MS/MS. (Top) LITV EAD-MS/MS spectrum with Zeno trap on. (Bottom) LITV EAD MS/MS spectrum with Zeno trap off. MS/MS sensitivity is significantly enhanced with the Zeno trap activated. With the Zeno trap on, 100% sequence coverage is achieved with phosphorylation site location confirmed on multiple c- and z- ion series.

characterize using mass spectrometry, however, when they are labile, CID is used 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 7 shows the example of a phosphorylated peptide, LITV, using hot ECD (KE = 7 eV). Here, not only is nearly the entire peptide sequenced with hot ECD, but the location of the phosphorylation is maintained.[4]

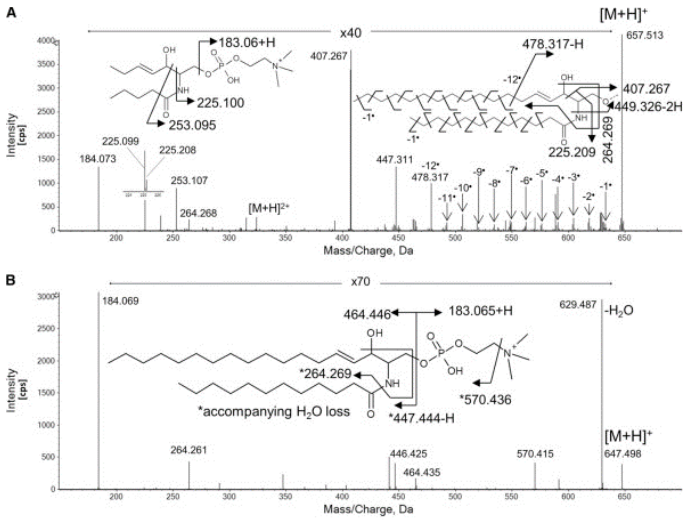

Figure 8: Comparison between dissociation product spectra by EIEIO (A) and CID (B). The sample was a synthesized standard SM, SM(d18:1,12:0).

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 8 shows the comparison between CID and EIEIO for the fragmentation of a sphingomyelin lipid species. EIEIO gives spectral information for nearly every bond to elucidate head group identity, backbone type, carbon chain lengths, double bond positions and double bond stereoisomerism. In a similar fashion, EIEIO can be used to differentiate between isomers of small molecules. Figure 9

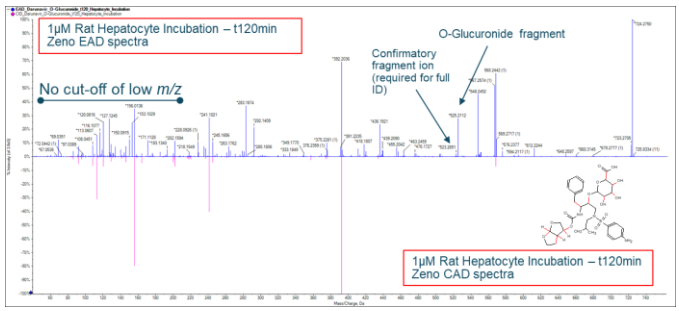

Figure 9: EAD (top) and CID (bottom) spectra of O-glucuronide conjugated darunavir. EIEIO creates unique fragments characteristic of the glucuronide conjugation positioning.

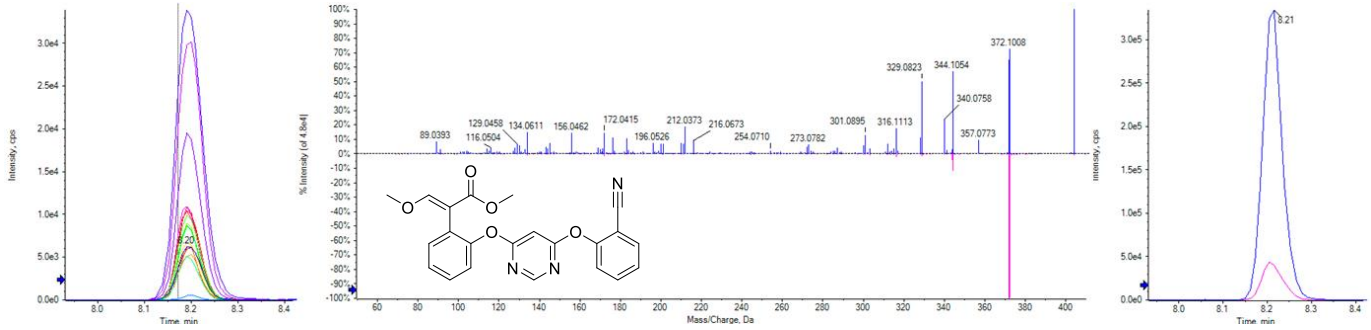

Figure 10: Comparison between CID and EAD MS/MS spectra for azoxystrobin. EIEIO (middle top, blue) creates roughly 100x the number of fragments with S/N >10 compared to CID (middle bottom, pink). The XICs for EAD (left) and CID (right) highlight the flexibility of EAD for ion ratio-based confirmation.

highlights the unique, characteristic fragment for O-glucuronide conjugated darunavir compared to its N-glucuronide isomer, allowing for more precise molecular information within metabolite identification workflows. EIEIO fragmentation also opens up additional specificity for non- targeted and suspect screening workflows. Figure 10 highlights the difference between EIEIO and CID for the fragmentation of azoxystrobin, a fungicide. 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 11: Linear dynamic range of the 4-channel NCP detector with ADC signal processing. Greater than 5 orders of inter-scan linear dynamic range can be achieved in both positive ion (top) and negative ion (bottom) modes, for both MS (left) and MS/MS (right) acquisitions.

Linear dynamic range (LDR)

LDR is important in many applications where the analyte concentration varies widely. The ZenoTOF 7600 system is equipped with a 4-channel MCP detector with ADC signal processing, resulting in greater than 5 orders of inter-scan linear dynamic range in both positive and negative ion modes, and for both MS and MS/MS acquisitions (Figure 11). Further, the ADC detector is capable of 4 orders if intra-scan linear dynamic range, allowing for the simultaneous detection of both high and low level analytes without a loss of data quality.

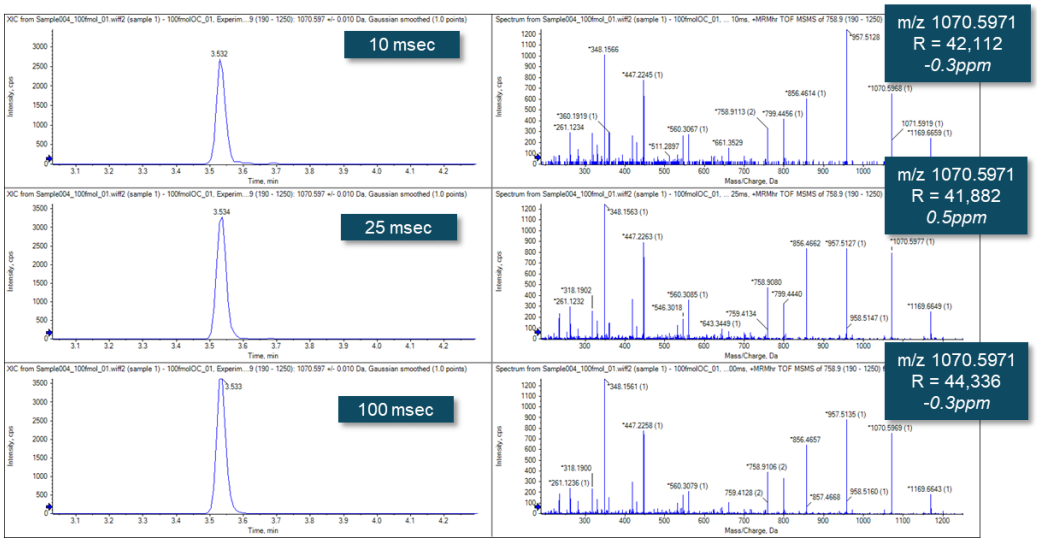

Figure 12: Peak intensity, mass resolution and mass accuracy across accumulation times. Faster accumulation times do not come at a sacrifice of mass resolution or mass accuracy with the ZenoTOF 7600 system. Mass resolution is consistent across the entire mass range.

Acquisition speed

The ZenoTOF 7600 system comes equipped with a high-speed LINAC collision cell that allows for fast acquisition rates, suitable for highly multiplexed experiments and rapid LC separations. Combined with the high speed of the MCP detector, the ZenoTOF 7600 system is capable of >100 Hz acquisition rates, with accumulation times as low as 5 msec, without sacrificing mass accuracy and resolution. Figure 12 highlights the maintenance of mass accuracy, mass resolution and peak intensity across a range of accumulation times.

OptiFlow Turbo V ion source for low flow chromatography

The ZenoTOF 7600 system has the option for the OptiFlow Turbo V ion source, which is designed for microflow and nanoflow chromatography (Figure 13).5 Probe and electrode combinations are pre-optimized for sensitivity and robustness from 0.1 to 200 µL/min. This means no manual adjustments are needed to maximize performance. SecurityLink tubing and fittings are used such that all fittings are finger tight, leak free and have zero dead volume. For microflow chromatography, the exit of the column connects directly to the electrospray probe, minimizing post-column broadening for enhanced S/N. The instrument comes configured with the OptiFlow interface, so switching between high flow or microflow to nanoflow is a toolless change, without the requirement of breaking vacuum on the system.

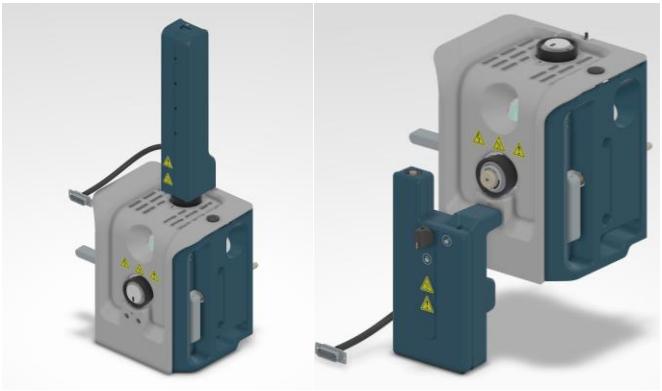

Figure 13: OptiFlow source configurations. (Left) Microflow configuration. (Right) Nanoflow configuration.

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