



Zeno trap

Defining new levels of sensitivity

The Zeno trap heralds a new era of sensitivity for accurate mass spectrometers.

The Zeno trap enables significant sensitivity gains, giving richer, more comprehensive breadth and certainty to MS/MS data, without compromise.

[SCIEX WHITE PAPER](#)



The Power of Precision

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Executive summary

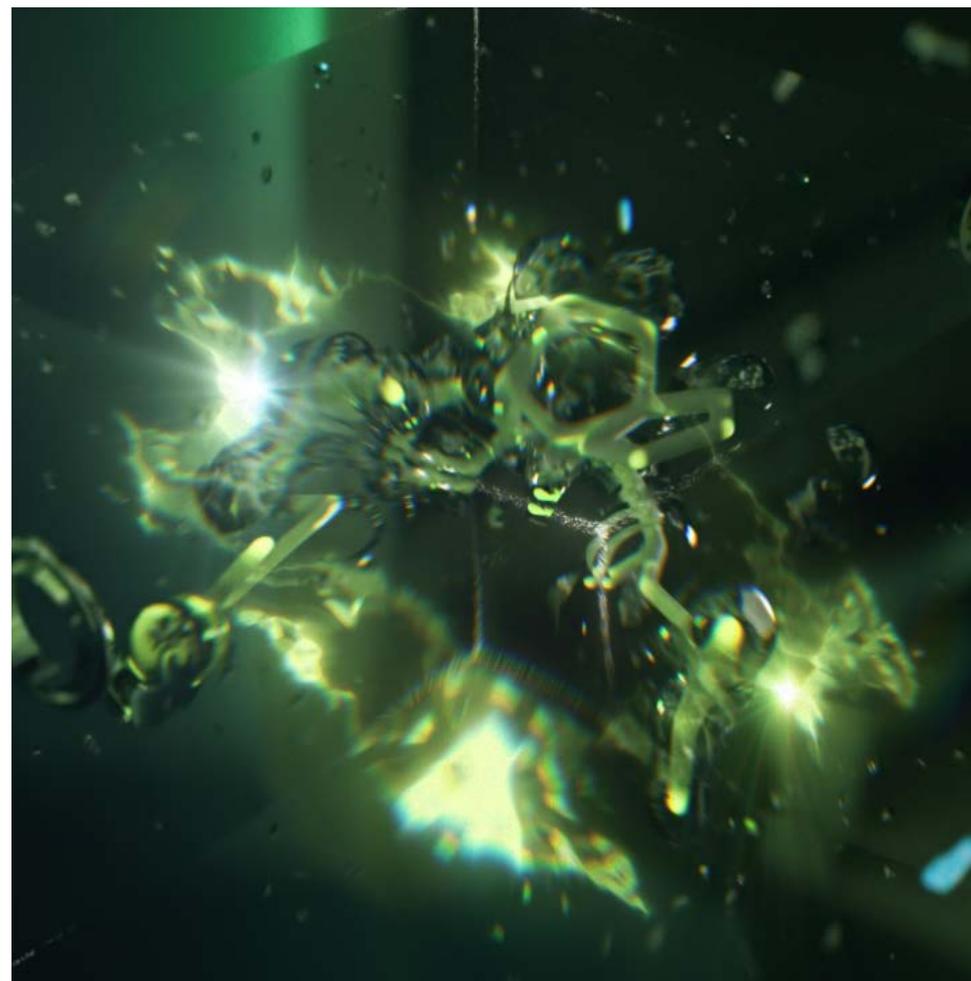
A consistent demand on the performance of mass spectrometers is high sensitivity. The fact that accurate mass instruments lose sensitivity because of their lower duty cycle is often overlooked.

The Zeno trap on the SCIEX ZenoTOF 7600 system increases duty cycle to >90% across the entire mass range when performing MS/MS. This can equate to sensitivity gains in the range of 4-20x. Importantly, this comes with no compromises on other performance specifications.

SCIEX recognizes the importance of providing highly sensitive mass spectrometers, and has continued to evolve this capability since the first commercial introduction of an atmospheric pressure ionization liquid chromatography tandem mass spectrometer [LC-MS/MS] over 30 years ago.¹

Duty cycle enhancements that improve sensitivity demonstrate our continuous drive toward innovative ways to advance the sensitivity and workflow performance of our accurate mass instruments.

This white paper provides an overview of the importance of high sensitivity and the instrument performance specifications that are essential for high-quality quantification. Examples show the power of the Zeno trap for lowering limits of quantification [LOQ], lowering required sample amounts and enabling quantitative assays in complex matrices where sensitivity challenges can greatly hinder assays from being developed.



Problem statement

There are many characteristics that define the ultimate performance of a mass spectrometer, but perhaps the most fundamental is sensitivity. In particular, high sensitivity is often demanded for liquid chromatography tandem mass spectrometry (LC-MS/MS) experiments to improve the detection, characterization and quantification of low abundance analytes. The benefits of high sensitivity can be realized directly by making the analysis of important analytes possible where no method previously existed, or indirectly by increasing laboratory efficiency and productivity through faster sample workup and analysis. With higher sensitivities:

- Low level compounds, impurities and contaminants can be quantified, even at trace levels
- Less material can be used for analysis, allowing the same levels of detection and quantification
- Sample preparation can be simplified with extensive extraction, derivatization and concentration protocols minimized or omitted completely
- Faster chromatographic separations can be utilized, shortening analysis run times

Today, quantification using mass spectrometry is in widespread use for a diverse range of applications spanning routine testing to groundbreaking research. Liquid chromatography coupled with electrospray ionization (ESI) and triple quadrupole-based instruments using multiple reaction monitoring (MRM) are the workhorses for these applications.

High-resolution accurate mass instruments have inherently better resolving power and provide higher mass accuracies than triple quadrupole instruments. Workflows such as information dependent acquisition/data dependent acquisition (IDA/DDA)

and data independent acquisition (DIA, SWATH acquisition) are now the standard for identifying as many components as possible from complex sample mixtures. Quantification is often performed during the same analysis or by using targeted scan types such as MRM^{HR}.

Although the above-mentioned workflows can provide good quantitative data, they typically exhibit lower sensitivities compared with MRM on a triple quadrupole system. Lower duty cycles on accurate mass platforms are one of the main culprits. For example, QTOF duty cycles typically range between 5-25% and thereby lose 75-95% of the detectable ions with each scan in the MS region where the quadrupole ion path mates with the pulsed time-of-flight region. Other accurate platforms exhibit even lower duty cycles. While the overall design makes them highly sensitive outright, even further gains in sensitivity could be realized by tackling the duty cycle issue.

Here, a novel trapping/releasing technology offers >90% duty cycle over an accurate mass platform.² As demonstrated for the ZenoTOF 7600 system, the Zeno trap can provide sensitivity gains of 4-20x.

Importantly, these enhancements come without compromising other vital performance specifications, such as speed, resolution, mass accuracy and dynamic range. In practice, this means that fast scanning, high-resolution, accurate mass MS/MS experiments for both qualitative and quantitative purposes are possible with even greater sensitivities and lower limits of quantification than before.



Considerations for quantification - scan rate

Many factors can influence the quality of quantification. The design philosophy of SCIEX QTOF instruments has been to deliver precise quantification of all molecule types without compromising on the key fundamentals. Namely, the goal is to deliver fast scanning without sacrificing resolution or accuracy in either MS or MS/MS, wide intra- and inter-scan dynamic range [linear dynamic range, LDR] and high sensitivity without compromising other performance specifications.

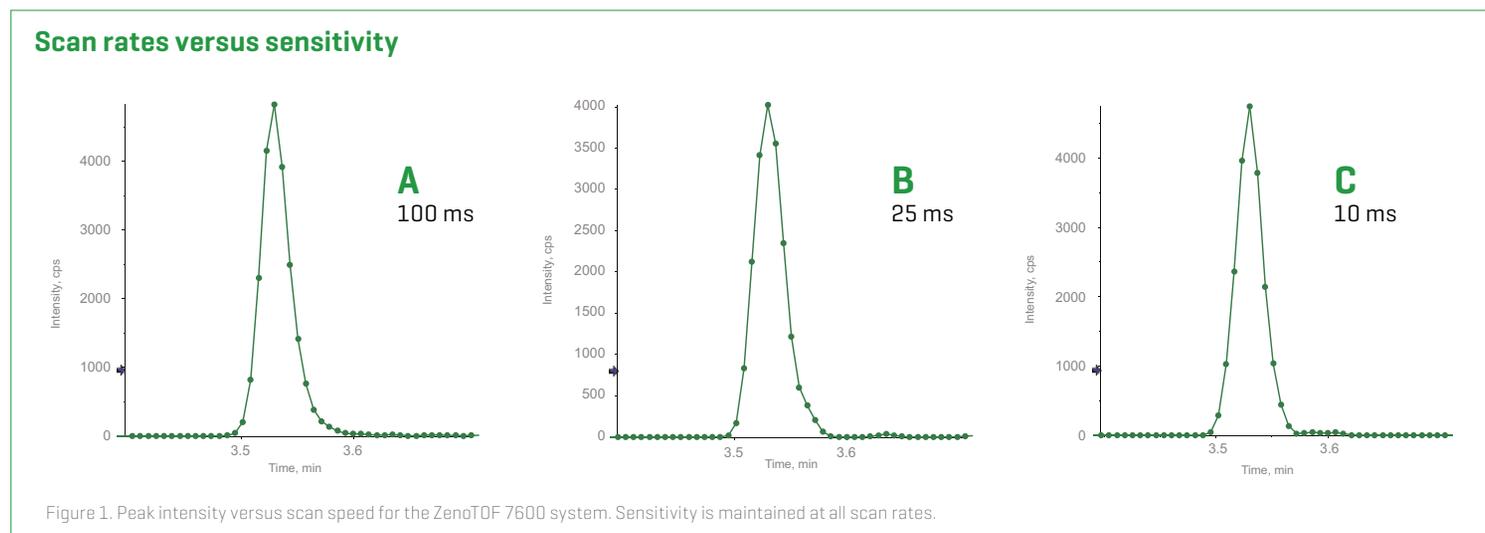
With the Zeno TOF 7600 system, the last and obvious area to look at is to overcome limitations of QTOF duty cycle, which SCIEX has now addressed to deliver a new accurate mass sensitivity revolution.

Speed

The wide adoption of quantitative LC-MS/MS today by so many different laboratories around the world can be traced back to the enabling technologies of electrospray and atmospheric

pressure ionization. These two fundamental developments more than 30 years ago allowed LC to be coupled to mass spectrometry in a direct and robust manner.^{1,3-5}

To provide good quantification, the mass spectrometer should scan fast enough so that each analyte is sampled a minimum of 10 times across its LC peak. As sample complexity increases, and/or chromatography runs shorten, the probability of co-elution becomes greater. Thus, higher scan speeds are required to provide the minimum number of data points for every analyte. Some accurate mass instruments can operate at fast scan speeds, and many do so by sacrificing other performance characteristics such as MS/MS resolution and sensitivity. An advantage of SCIEX QTOF instruments is that mass resolution, mass accuracy and sensitivity are independent of scan speed. As shown in Figure 1, as scan speed is increased and scan times are shortened from 100 ms to 10 ms, peak intensities are maintained.



Considerations for quantification - resolution and accuracy at speed

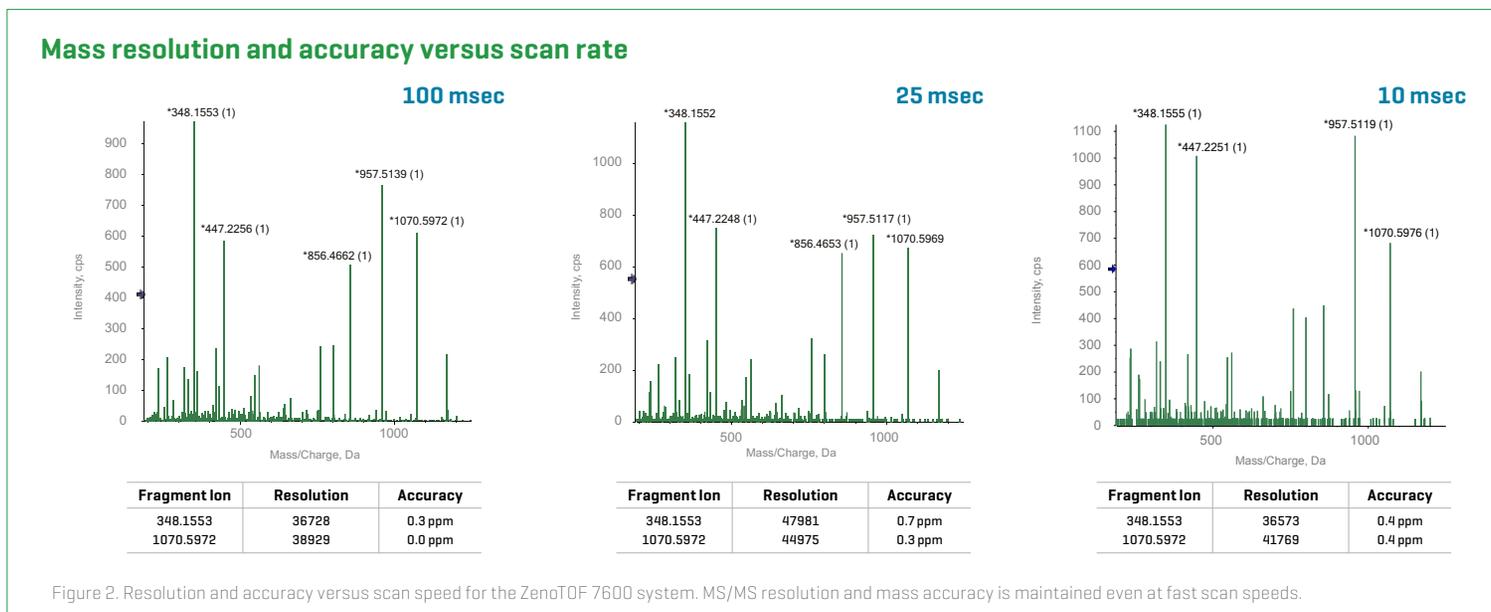
Resolution

Accurate mass instruments have typically used the intact precursor masses found in MS full scan data for identification and quantification. Often, co-eluting isobaric analytes, contaminants and high background can interfere with extraction of analytes at the MS level, even when using very high resolution.

Using fragment ion data at the MS/MS level for peak extraction and integration can greatly improve the quality of the quantification, as the background and interferences can be virtually eliminated. Moreover, using MS/MS-level data relieves the burden of performing additional sample preparation steps or investigating longer, more protracted chromatographic gradients to clean up MS-level data.

Some accurate mass instruments, when operating at fast scan speeds, will sacrifice performance characteristics such as MS/MS resolution. As shown in Figure 2, as scan speed is increased and scan times are shortened from 100 ms, to 10 ms, mass resolution and mass accuracy are maintained on the ZenoTOF 7600 system.

As the sample complexity increases and/or faster chromatography is used, more compounds elute per given unit of time. This can further exacerbate any problems encountered at the MS level. Using high resolution at the MS/MS level allows high-quality extraction and integration of important fragment ions, even in the presence of interferences, enabling lower detection and quantification limits and higher quantitative accuracy.



Considerations for quantification - dynamic range

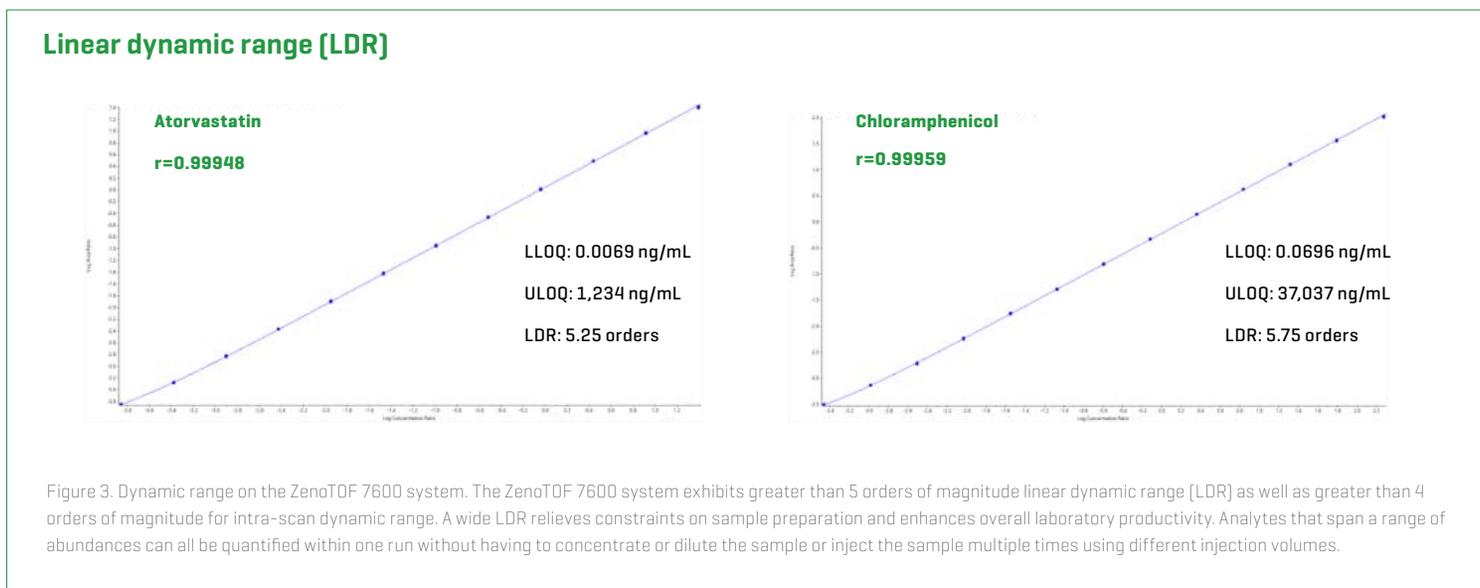
Dynamic range

Intra-scan and inter-scan dynamic range are important for quantification. A wide intra-scan dynamic range allows low level peaks to be detected in the presence of high abundance peaks in the same scan without peak distortion or saturation, which can ensure the accuracy and reproducibility of quantification. Inter-scan dynamic range or linear dynamic range (LDR) defines the concentration range over which an analyte can be quantified.

For positive mode ESI, as shown in Figure 3, an LDR of 5.25 orders of magnitude means that an analyte with a lower limit of quantification (LLOQ) of 0.0069 ng/mL can be accurately

analyzed up to an upper limit of quantification (ULOQ) 1,234 ng/mL. Accuracy is +/- 10% and precision is less than 2.5% for n=3 injections.

For negative mode ESI, as shown in Figure 3, an LDR of 5.75 orders of magnitude means that for chloramphenicol, a lower limit of quantification (LLOQ) of 0.069 ng/mL can be accurately analyzed up to an upper limit of quantification (ULOQ) of 37,037 ng/mL. Accuracy is +/- 10% and precision is less than 2.5% for n=3 injections.



Considerations for quantification - MS/MS duty cycle

Sensitivity and duty cycle

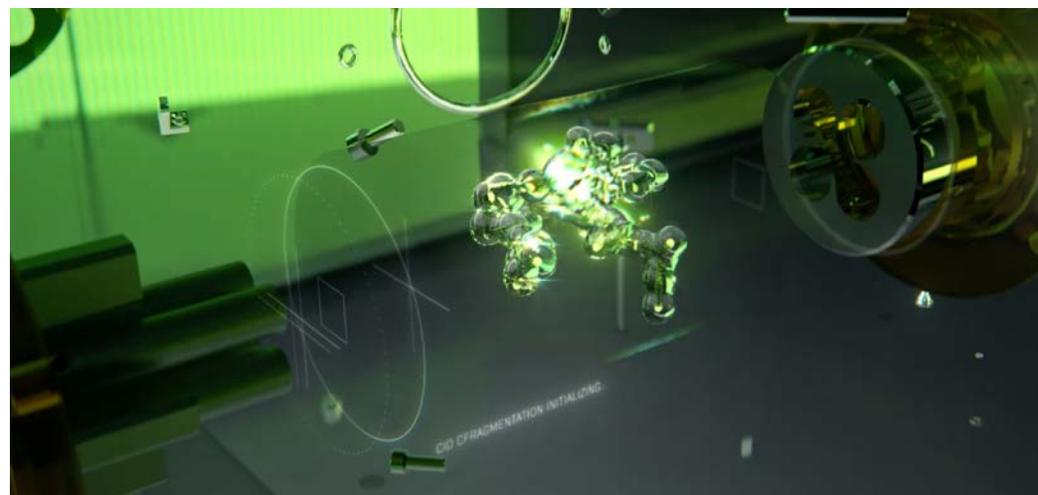
The sensitivity of any instrument will ultimately define the lowest levels of detection and quantification that are possible for any analyte. Sensitivity improvements can come from many areas within the instrument, including ionization and desolvation efficiencies of ion sources via electrode design, heat optimization, lower flow rates and spray orientations.

The focusing region behind the entrance aperture has used quadrupoles, tapered dodecapoles, dual stages and various lens elements to maximize the capture and focusing of ions from the source and into the vacuum region.⁶ In QTOF-based instruments, collision cells, TOF analyzers, mirrors and detectors have all been manipulated, redesigned, reconfigured and optimized.

The duty cycle, while having many definitions, is effectively a measure of efficiency of detection of ions and optimization of this efficiency drives much of the innovation in mass spectrometry. For the discussion here, duty cycle is defined as the percentage of fragment ions transitioning from the collision cell or electron activated dissociation (EAD) cell into the accelerator of the TOF.

To level set, a quadrupole instrument operating in single ion monitoring mode (SIM) theoretically has 100% duty cycle, as the continuous stream of ions generated at the source is well matched to the parked nature of the instrument in SIM mode.

Accurate mass instruments detect the entire spectrum at once and are typically much more sensitive in full spectrum mode versus scanning instruments such as triple quadrupoles. For example, those based upon QTOF platforms have orthogonal injection of ions into the TOF analyzer. This orthogonal injection suffers from losses with typically only 5-25% duty cycle observed and much of the ion beam lost at this stage.



Zeno trap

With the Zeno trap, each spectrum contains richer MS/MS information, particularly on low abundant species that were previously undetectable, introducing our customers to a new sensitivity revolution...

the Zeno trap revolution.



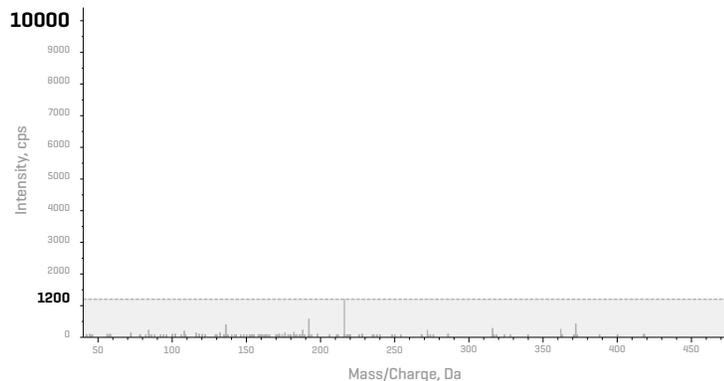
Zeno trap pulsing

To solve the duty cycle issue presently observed on QTOF instruments, SCIEX has introduced a new innovation, the Zeno trap.

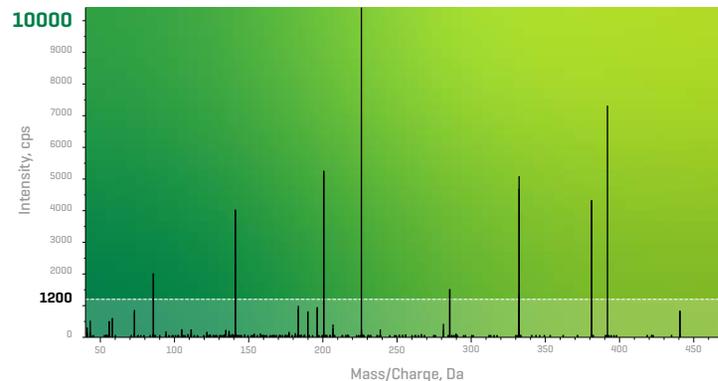
Ions are accumulated in the Zeno trap before being pulsed rapidly into the TOF, meaning up to 20x more ions can be detected. Consequently, each TOF MS/MS spectrum contains more useful fragmentation information, particularly on lower abundance species that were previously undetectable, introducing our customers to a new level of sensitivity.



Without Zeno trap pulsing



With Zeno trap pulsing



Over 40% more proteins identified using Zeno MS/MS

Utilizing high-throughput micro flow methodologies, the ZenoTOF 7600 system breaks through the 3,000 protein groups in 20 minutes for the first time. Unique Zeno trap pulsing provides significant gains in peptide and protein identifications for proteomics experiments. Up to ~ 45% improvements in the number of protein IDs and up to a 145% increase in the number of peptide IDs, compared to previous TripleTOF systems⁹.

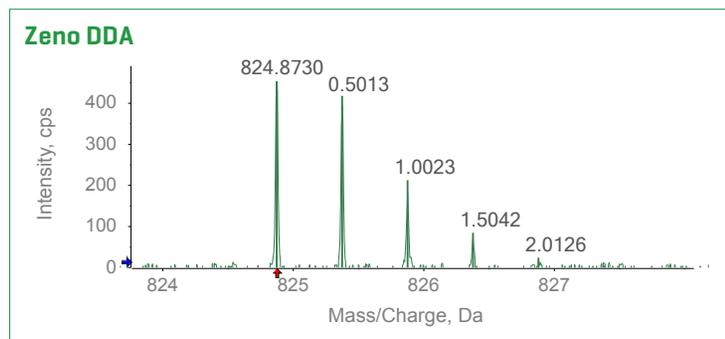


Figure 4. Impact of using Zeno trap for IDA. The TOF MS is shown for a peptide at 25 ng sample load which was triggered for the Zeno MS/MS. The isotopic fidelity is shown up to the M+4 isotope.

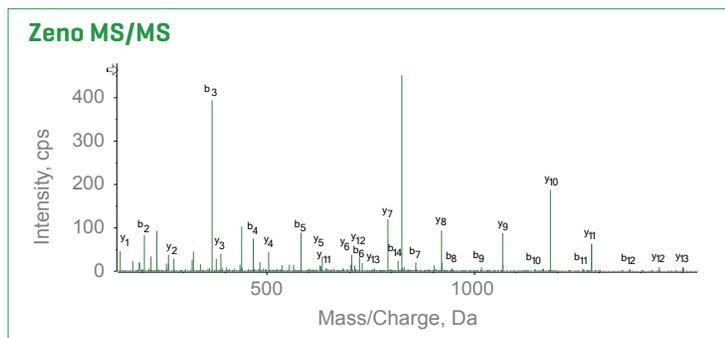


Figure 5. Impact of using Zeno trap for IDA. The Zeno CID MS/MS is shown for the precursor in Figure 6. For a low abundant precursor a high quality MS/MS for identification is acquired with near complete sequence coverage and excellent signal to noise.

Peptide and protein gains

	Gradient duration (min)	1000 ng
Peptide Gains	10	72%
	45	145%
Protein Gains	10	41%
	45	46%

Figure 6. The impact of the Zeno trap on CID IDA[DDA] over previous platforms is shown. Significant improvements in both peptide and protein numbers are observed at short and medium gradient lengths.

Peptide and protein IDs for HeLa at 500 ng on 60 SPD method*

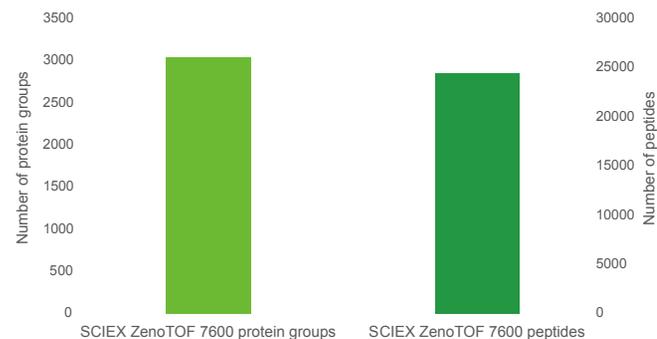


Figure 7. Routinely achieving high protein and peptide identifications at high throughput is difficult. The ZenoTOF 7600 system when coupled with a highly reproducible micro flow solution drives significant protein and peptide identifications breaking through >3,000 protein groups and >20,000 peptides using technical quadruplicates. * Evosep: Towards a Standardized Omics Platform with the 60 SPD: www.evosep.com/wp-content/uploads/2020/06/AN-008-20-06_60SPD.pdf



Highly multiplexed Zeno MRM^{HR} peptide quantification

The birth of proteomics research more than 20 years ago helped to fuel an immense interest in the use of mass spectrometry for protein quantification.

Today, mass spectrometry-based protein and peptide quantification assays are used for the quantification of proteins in biological fluids and tissues for research purposes, identification of protein-based biomarkers for disease and drug efficacy studies and the creation of assays for protein and peptide-based therapeutics.

Key to obtaining high quality quantitative results is the ability to acquire high resolution, high mass accuracy full scan MS/MS at high acquisition rates, as enabled by the Zeno trap. A large-scale targeted assay for 804 peptides in human plasma was developed on the ZenoTOF 7600 system using a Zeno MRM^{HR} assay.¹⁰

High precision peptide quantification

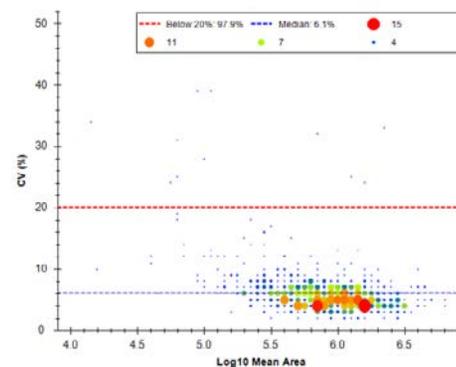


Figure 8. Precision of measurement for the 804 peptides from the PQ500 kit quantified in digested plasma matrix showing a median CV of 6.1% and 97% below 20% CV using microflow LC.

Zeno CID MRM^{HR} for peptide quantification

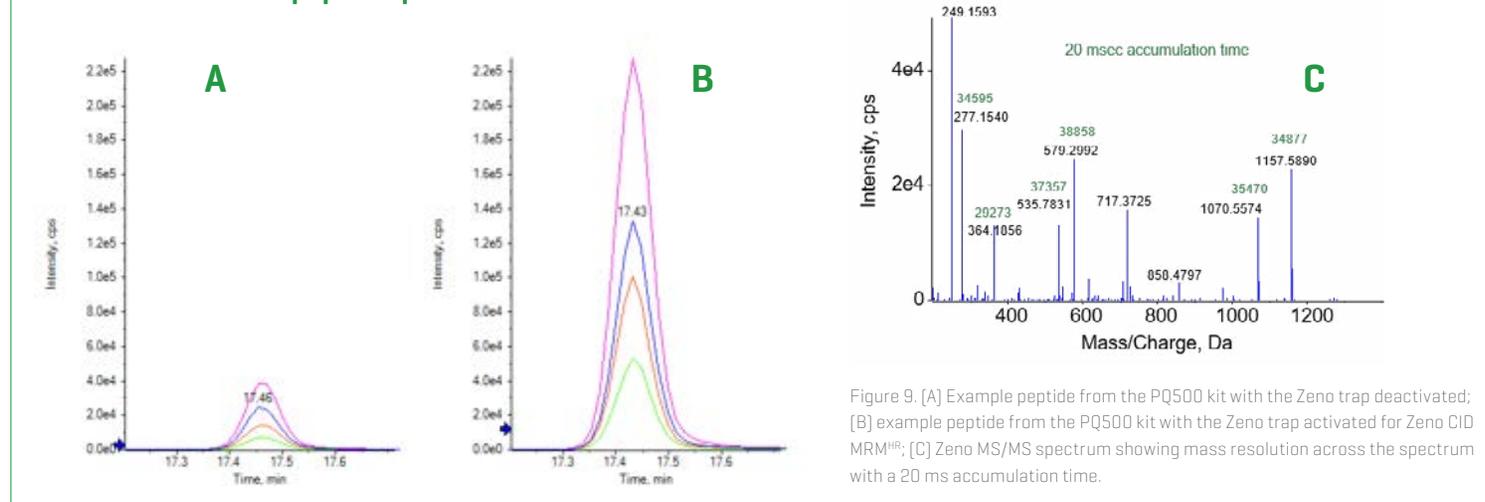


Figure 9. [A] Example peptide from the PQ500 kit with the Zeno trap deactivated; [B] example peptide from the PQ500 kit with the Zeno trap activated for Zeno CID MRM^{HR}; [C] Zeno MS/MS spectrum showing mass resolution across the spectrum with a 20 ms accumulation time.



Oligonucleotide quantification

Zeno trap pulsing on demand gives the ability to detect lower abundance ions at the same times as those in higher abundance, redefining the limits of quantification achievable with accurate mass. MRM^{HR} is a targeted approach that delivers high sensitivity. Oligonucleotide therapeutics are fast on the rise and pose a challenge for quantification via LC-MS/MS. With the introduction of the ZenoTOF 7600 system, new levels of selective quantification are achieved for accurate mass systems.

ANALYTE	LLOQ (ng/mL)	ULOQ (ng/mL)	LINEARITY (ORDERS)	CV AT LLOQ (%)	ACCURACY AT LLOQ (%)
20-MER PHOSPHOROTHIOATED AND 2'-O-METHYLATED ANTISENSE OLIGONUCLEOTIDE (ASO)	0.03	300	4	16.23	98.3
FOMIVIRSEN	0.03	300	4	11.2	96.3
NUSINERSEN	0.01	100	4	3.69	96.0
ELUFORSEN	0.03	100	3.5	18.71	95.7

Table 1. Zeno CID MRM^{HR} analysis of four oligonucleotide-based therapeutics. As shown, excellent limits of quantification are observed at or below 30 pg/mL, with good accuracy, precision and linear dynamic range.

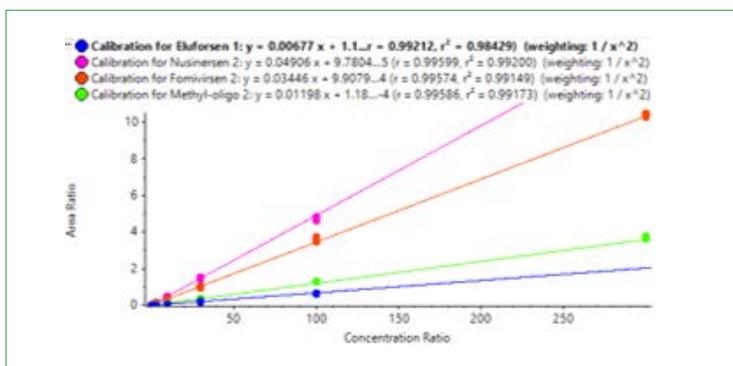


Figure 10. The calibration curves for the four analyzed oligonucleotides are shown. The low end of the curve is zoomed to show accuracy.

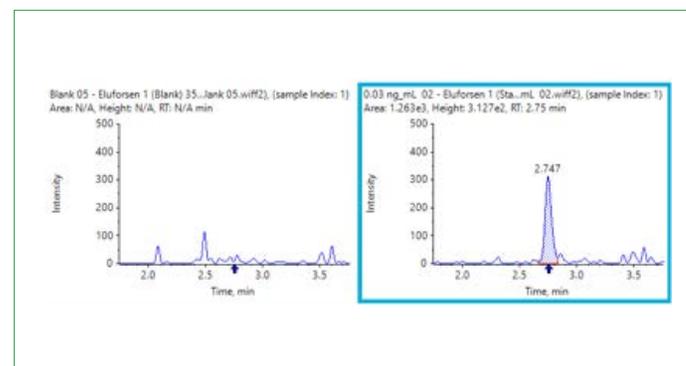


Figure 11. Shown is the blank and LLOQ for eluforsen analyzed with Zeno CID MRM^{HR}. It shows excellent selectivity and an LLOQ of 0.03 ng/mL.

New levels of sensitivity for library-based screening

Pesticides are used throughout the world to increase the quantity and quality of food production. While they can be extremely beneficial in decreasing or preventing the damage caused by weeds, insect infestation and other pests, certain pesticides and exposure levels can cause harm to human health and the environment. As a result, widespread testing of pesticide residues is now routine within food production chains.

The large number of pesticides in use today requires sensitive and selective methods for their detection and quantification. The methods must analyze multiple residues within one assay with speed, sensitivity, selectivity and quantitative accuracy. Figure 12 shows the analysis of 271 pesticides from an olive

oil extract using MRM^{HR} on the ZenoTOF 7600 system. Shown are the sensitivity improvements delivered for thiobencarb and phenthoate. With the Zeno trap enabled, the sensitivity has been improved by a factor of 10^{12} .

Furthermore, it is not just simply the quantity but the verification of the identity that is required. At low levels poor MS/MS quality can affect the match against library reference spectra. With the Zeno trap enabled, even for very low precursor intensities, unambiguous MS/MS is acquired and matched confidently against the library.

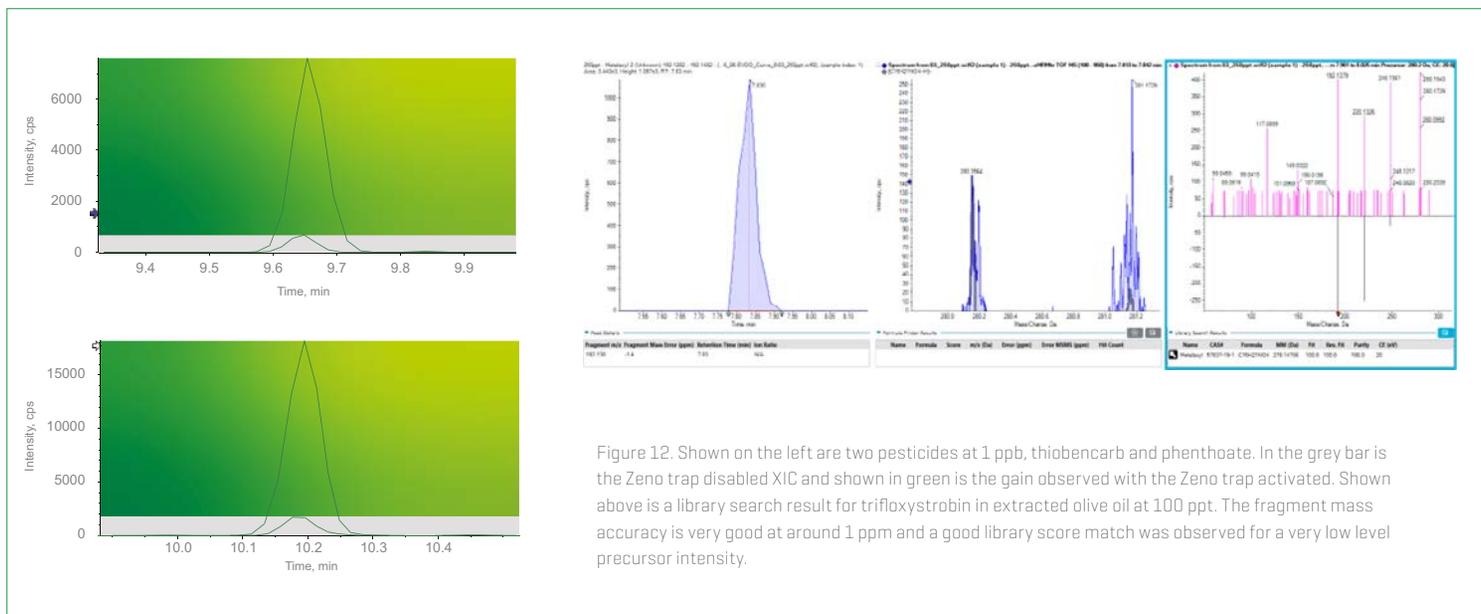


Figure 12. Shown on the left are two pesticides at 1 ppb, thiobencarb and phenthoate. In the grey bar is the Zeno trap disabled XIC and shown in green is the gain observed with the Zeno trap activated. Shown above is a library search result for trifloxystrobin in extracted olive oil at 100 ppt. The fragment mass accuracy is very good at around 1 ppm and a good library score match was observed for a very low level precursor intensity.



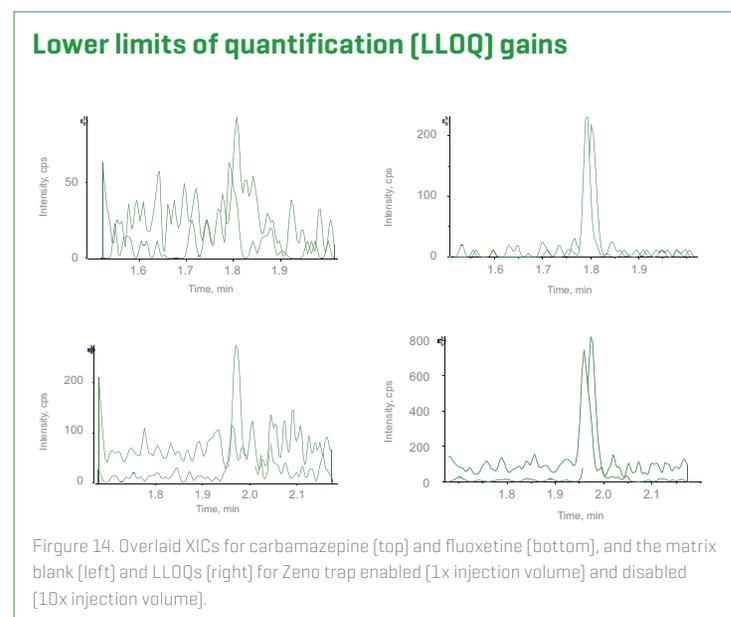
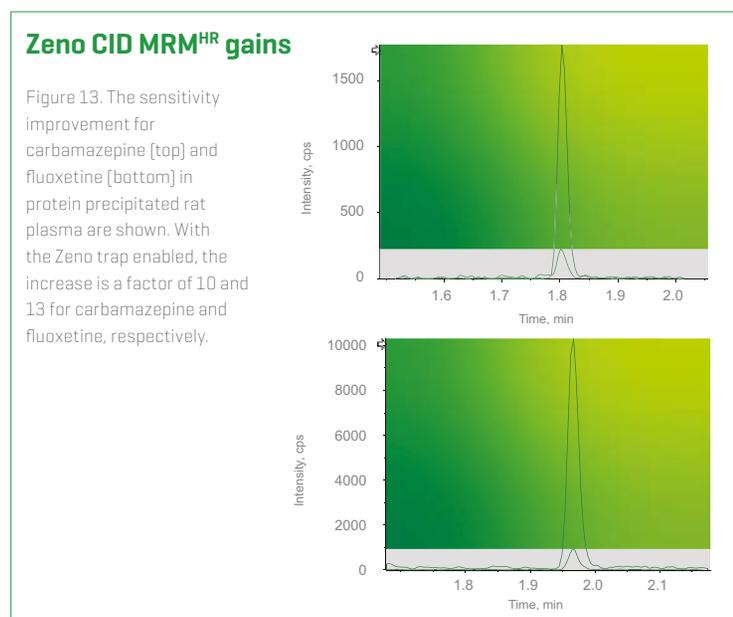
New levels of sensitivity for small molecule discovery quantification

Quantification of small molecule drugs is one of the most widespread applications in use today for LC-MS/MS. Most pharmaceutical and biotechnology companies need quantitative mass spectrometry not only during research and development, but also for clinical trials where assays are typically outsourced to contract research organizations.

The development of fast and sensitive assays is a top priority with dollars on the line for every day longer the assay development takes. As such, high sensitivity mass spectrometry can ease the burden of method development by alleviating additional sample preparation steps and long chromatographic run times in order to reach the limits of quantification required.

Figure 13 shows the increase in sensitivity observed when the Zeno trap is enabled. In the grey portion, the peak represents the signal observed with the Zeno trap disabled. With the green area, the peak is observed when the Zeno trap is enabled. As can be seen for both carbamazepine and fluoxetine, the signal gains are significant, with 10x and 13x observed, respectively.

Figure 14 (top) shows the blank and LOQ peak for carbamazepine detected in plasma using Zeno MRM^{HR} versus MRM^{HR} on the ZenoTOF 7600 system. Although the Zeno MRM^{HR} uses 10x less sample injected, similar signal-to-noise ratios are observed for both the Zeno MRM^{HR} and MRM^{HR}, resulting in a lower LOQ of 0.5 pg/mL for the Zeno MRM^{HR}. Additionally, the blanks are very similar in the amount and level of background observed.



New levels of sensitivity for any metabolites

The metabolism of any organism is constantly changing, using and producing various molecules – an extremely wide diversity of cellular processes. The incredible numbers and diversities of small molecule metabolites involved in these reactions makes metabolomics studies enormously challenging. Molecules differ by molecular weight, structure and chemistry. These differences can be subtle, such as the difference between cis and trans isomerism, or they can be large, such as the difference between a small polar amino acid and a large hydrophobic lipid.

Once analytes have been discovered using SWATH acquisition, targeted approaches can be used to provide better sensitivity for very low level quantification. Using MRM^{HR}, panels of metabolites of interest can be targeted to detect and quantify changing concentrations and uncover patterns of fluctuation for a given biological system.

In Figure 15, Zeno MRM^{HR} was used to quantify cAMP isomers. Compared with MRM^{HR} without the Zeno trap, the S/N is increased 12x and 9x for 2,3-cAMP and 3,5-cAMP, respectively, when the Zeno trap is used.

Sample volumes can often be very small and may be needed for multiple types of assays. Therefore, consuming less sample volume for any particular assay can be extremely advantageous.

As a further demonstration of the sensitivity of the Zeno MRM^{HR} assay, 10x less sample was loaded onto the column. As shown in Figure 16, Zeno MRM^{HR} provides similar peak area compared with MRM^{HR} without the Zeno trap, even with 10x less sample on column.

Small polar metabolites with Zeno CID MRM^{HR}

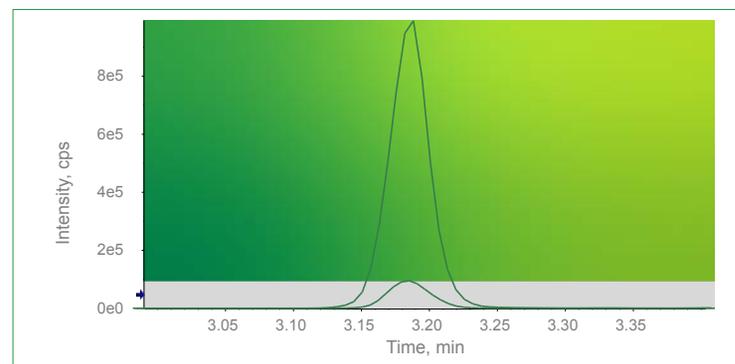


Figure 15. Targeted metabolite quantification. Significant sensitivity gains in MS/MS. Comparison of extraction ion chromatograms [XICs] for cAMP fragments obtained from MS/MS collected with Zeno trap on vs. Zeno trap off. Signal/noise ratio improved ~12.5 fold when using the Zeno trap.

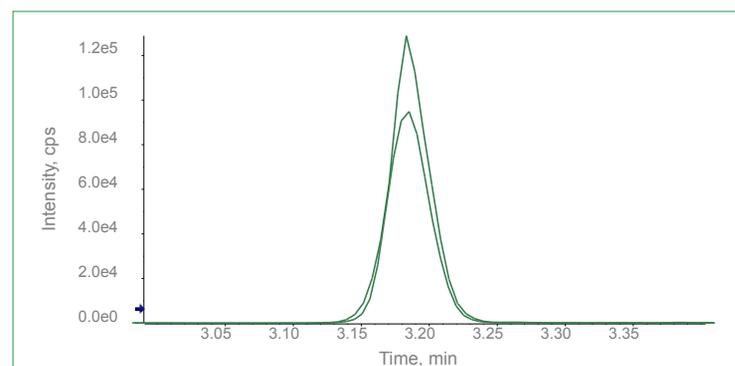


Figure 16. Better sensitivity in MS/MS with 10x less sample. Comparison of extraction ion chromatograms for cAMP fragments obtained from MS/MS collected with Zeno trap on [0.2 μL injection] vs. Zeno trap off [2 μL injection].



Executive summary

Sensitivity, especially MS/MS sensitivity, is a fundamental performance characteristic of a mass spectrometer. Increasingly, higher sensitivities are in constant demand in order to characterize and quantify with confidence an ever-decreasing abundance of trace analytes, impurities and contaminants.

Over the years, sensitivity improvements have come from many areas within the mass spectrometer, including hardware and software innovations as well as new workflows. These improvements have often been compound, mass or LC dependent.

The new Zeno trap on the ZenoTOF 7600 system provides sensitivity enhancements through duty cycle improvements in the orthogonal injection region into the TOF region.

Duty cycles of nearly 100% are now achieved for all ions, in this region of the mass spectrometer, regardless of the mass or charge or LC flow rate, opening up the possibility of being able to detect every ion created in the collision cell. In practice, this can provide sensitivity gains of more than an order of magnitude. Importantly, these gains do not come at the expense of other performance specifications. High resolution, accurate mass, fast scan speed and wide dynamic range are all maintained.

ZenoTOF 7600 system

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