Zeno SWATH DIA

Harnessing the power of the Zeno trap for SWATH DIA

The Zeno trap brings significant sensitivity gains to MS/MS data acquisition. Now, Zeno SWATH DIA capitalizes on these gains, revealing tens, hundreds and even thousands more identified and quantified analytes in a shorter time and with higher precision than ever before. Reaching new depths of coverage, Zeno SWATH DIA delivers maximal information in minimal time.

SCIEX

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Executive summary

SWATH DIA (data independent acquisition) has emerged as an LC-MS/MS workflow that capitalizes on the speed and sensitivity gains that have been achieved with modern mass spectrometers. SWATH DIA can provide identification of vast numbers of proteins, peptides and metabolites in a more reproducible manner than conventional data-dependent acquisition (DDA) methods. With SWATH DIA, high-quality, high-resolution MS/MS data not only provide identification, but are also the secret behind highly accurate and precise quantification.

Now, Zeno SWATH DIA combines the sensitivity of the Zeno trap with the reproducibility and precision of SWATH DIA to deliver unprecedented levels of analyte identification and quantification. The 6-10x sensitivity gains in MS/MS mode that the Zeno trap provides through duty cycle improvements deliver up to 3x more identified proteins, and ~3-6x more quantified, at loads less than 20 ng. This leads to a more comprehensive understanding of underlying biological changes. With Zeno SWATH DIA, maximal information is obtained from each precious sample.

SWATH DIA has become the method of choice for large-scale proteomics and other studies. The sensitivity gains that Zeno SWATH DIA provides broaden the application space to include routinely performing these studies using microflow, and even high-flow, chromatography for more robust and rapid analyses.

Higher sensitivity also translates to higher quality data for lower levels of analyte. Nanoflow chromatography Zeno SWATH DIA provides reproducible qualitative and quantitative data at sample levels approaching single cell analysis. With single cell analysis, discrete cellular states and events can be separated from bulk proteomic discoveries, resulting in a better understanding of cellular heterogeneity. Accurate and reproducible quantification at these levels is crucial, but it is often more difficult or labor intensive to obtain these abundance measurements from profiling data or labeling techniques. Zeno SWATH DIA quantification using MS/MS data circumvents many problems by providing higher intensity spectra and high MS/MS scan rates with a wide dynamic range.

In this white paper, the technology behind Zeno SWATH DIA is reviewed along with the critical mass spectrometer performance attributes that make it possible. Examples are shown that reveal trace levels of analytes with higher throughput and precision than ever before.

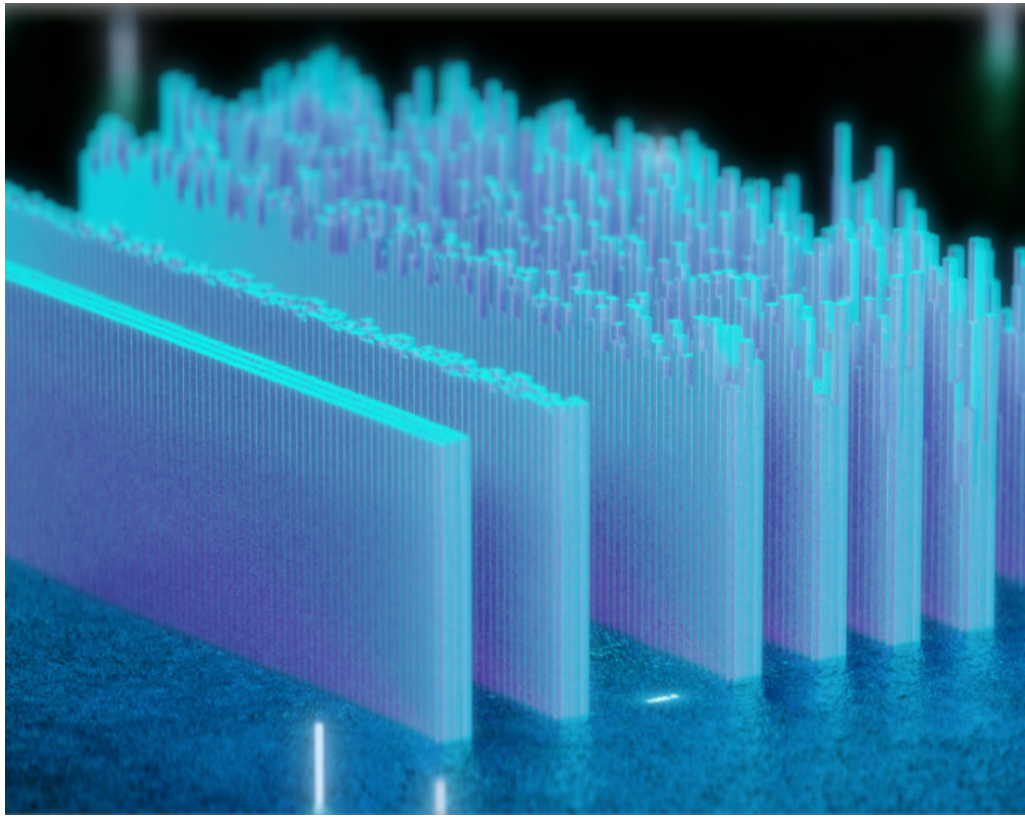
Data dependent acquisition (DDA)

Liquid chromatography tandem mass spectrometry (LC-MS/MS) is widely used for characterizing mixtures of unknown compounds. In the conventional approach, data-dependent acquisition (DDA) is used for acquiring MS/MS data on as many compounds as possible. DDA is well-established and in use for many applications, as it provides an untargeted sample analysis.

With DDA, quantification can be performed at the MS1 level using label-free approaches or at the MS/MS level using labeled techniques, with the latter requiring additional sample preparation protocols and cost.

One major drawback of DDA is that datasets can be incomplete since only precursor ions that match specific criteria are selected for MS/MS analysis. Additionally, detection reproducibility can suffer. Small shifts in retention times from run to run can affect the population of precursor ions entering the instrument during each cycle, and therefore the subset of compounds that are analyzed. Although the matrices remain as complex, the ability to ionize and detect has gotten better. But, the instrument does not have time to sample all the potential precursors entering the system. To counter this, a target list of precursors can be used, but this negates the benefit of a truly unbiased and global approach.

When comparing multiple DDA data sets, missing peaks and gaps are often observed, which can be particularly damaging for lower-level analytes and low replicate numbers.



Data independent acquisition (DIA)

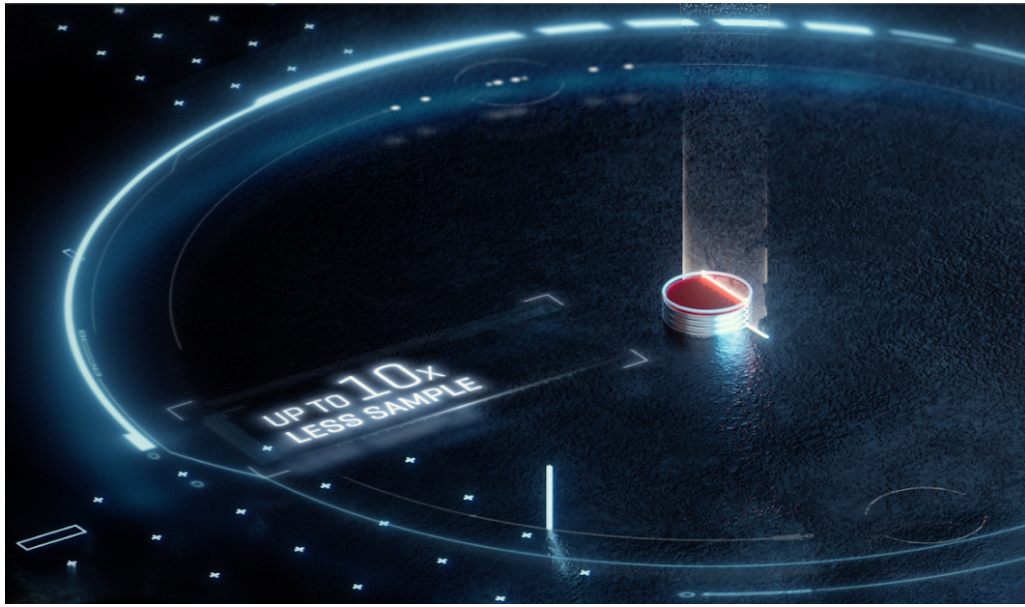
In 2012, the data independent acquisition (DIA) strategy known as SWATH DIA was published for the first time.[1] With SWATH DIA, all ionizable precursors are analyzed by MS/MS regardless of abundance or other criteria. This results in greater repeatability arising from a complete data set containing fragment data for all precursor ions. Although SWATH DIA was originally used for proteomics experiments, the workflow has been adopted for a wide variety of other applications, including metabolomics, environmental screening, food testing, forensics and pharmaceutical analysis.

With SWATH DIA, wider precursor selection windows are used for MS/MS that can allow multiple compounds through simultaneously. These windows are stepped across the entire precursor mass range such that all precursor masses are fragmented for every cycle. Compared with DDA, MS/MS spectra generated through SWATH DIA tend to be more complex.

To increase specificity, SWATH DIA uses a large number of windows to minimize the number of precursors within each window. Fast MS/MS scanning then allows iteration through all windows within each cycle while still covering a broad mass range.

The use of variable window widths further increases the selectivity of SWATH DIA. With variable windows, the width of each precursor window is adjusted according to the complexity of data found within that mass range. Very narrow windows are used where analyte density is greatest and wider windows are used where analytes are more sparsely populated. This increases the percentage of high-quality identified and quantified precursors.

The power of SWATH DIA was demonstrated in a landmark multi-laboratory proteomics study in 2017. In the study, 11 laboratories from around the world established that SWATH DIA can consistently detect and reproducibly quantify proteins for sensitive, reproducible and large-scale identification and quantification of complex proteomics samples.[2]



Zeno trap enabling Zeno SWATH DIA

Zeno SWATH DIA combines the power of the Zeno trap[3,4] with SWATH DIA. Similar to SWATH DIA, Zeno SWATH DIA identifies and quantifies analytes using MS/MS data. Thus, the rapid acquisition of high-quality MS/MS data across the entire precursor ion space is fundamental to operation.

In Zeno SWATH DIA, the Zeno trap, when activated, is used to increase the MS/MS sensitivity for each variable window acquired.[5] The Zeno trap provides a 4-20x gain in sensitivity for Zeno SWATH DIA, while also maintaining other key performance attributes.[6]

Fast scan rate

Zeno SWATH DIA uses the combination of fast scanning and detection to maximize the total number of high-quality MS/MS spectra generated per cycle. This enables a higher number of variable windows, increasing specificity and, therefore, confidence in the total number of identified and quantified analytes. Faster scanning also enables the use of shorter LC run times, greatly improving throughput and laboratory productivity.

Resolution and mass accuracy

Co-eluting isobaric analytes, contaminants and high background can interfere with the quantification of analytes, especially at the MS level, even when using very high-resolution instruments. Zeno SWATH DIA maximizes the accuracy and precision of quantification by utilizing the selectivity of MS/MS. When combined with the highest scan rates, these attributes (mass resolution and accuracy) are preserved to maintain the maximum number of identified and quantified analytes.

Dynamic range

Both intra-scan and inter-scan dynamic ranges are important for identification and quantification. Zeno SWATH DIA features a wide intra-scan dynamic range that allows low-level analytes to be detected in the presence of high-abundance analytes within the same scan, without peak distortion or saturation. Additionally, Zeno SWATH DIA possesses a wide inter-scan dynamic range (linear dynamic range, or LDR) allowing analytes that span a range of abundances to be detected and quantified within one run.

Zeno SWATH DIA

The 4-20x increase in MS/MS sensitivity that the Zeno trap provides, while maintaining all other critical performance specifications translates to more high-quality MS/MS spectra.

With Zeno SWATH DIA this translates from the raw MS/MS, to the MS/MS XICs (extracted ion chromatograms) and to the total peptide ion current.

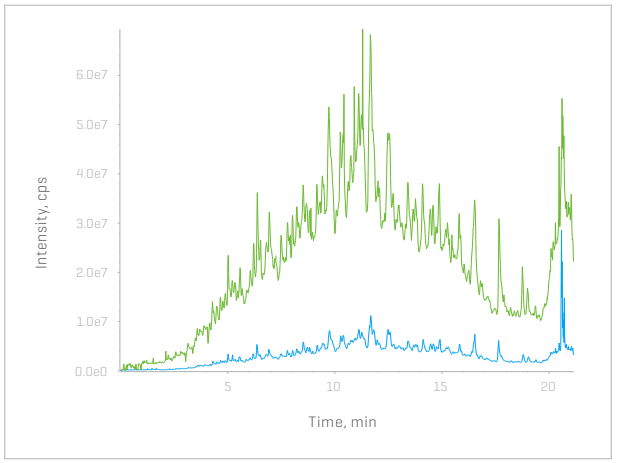


Figure 1. Total ion chromatograms (TICs) with and without the Zeno trap activated – SWATH DIA (bottom, blue) and Zeno SWATH DIA (top, green).

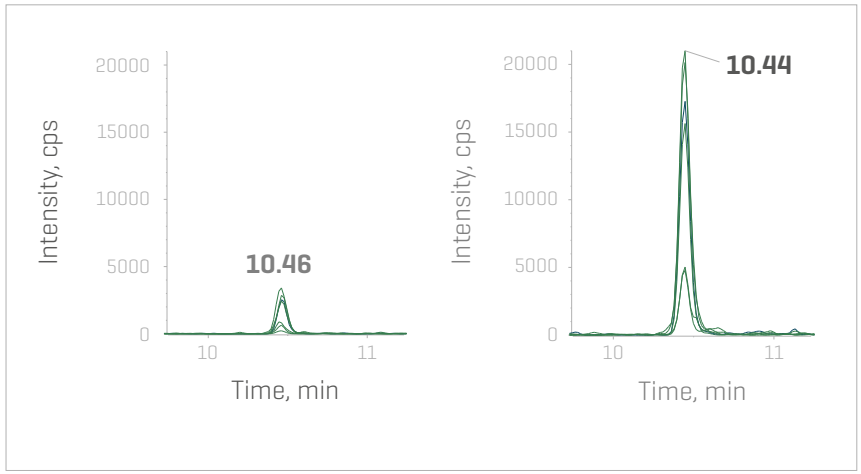


Figure 2. MS/MS XICs with and without the Zeno trap activated for peptide ITVTSEVPFSK (P35268) – SWATH DIA (left) and Zeno SWATH DIA (right).

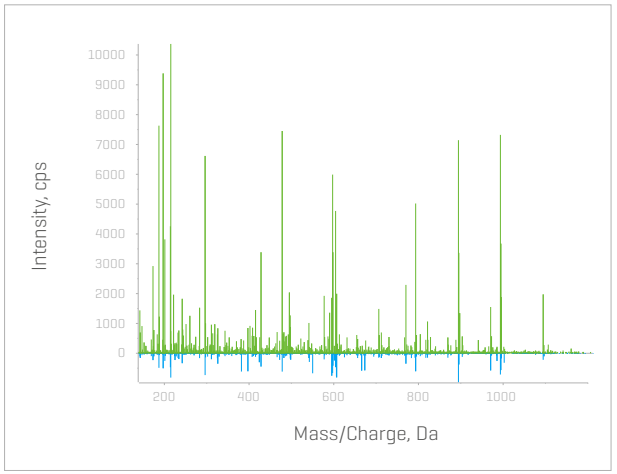


Figure 3. MS/MS spectra with and without the Zeno trap activated for peptide ITVTSEVPFSK (P35268) – SWATH DIA mirror (bottom) and Zeno SWATH DIA (top).

More proteins identified, more proteins quantified

The 4 -10x increase in MS/MS sensitivity for peptides that the Zeno trap provides, while maintaining all other critical performance specifications translates to more high-quality MS/MS spectra. With Zeno SWATH DIA, even more peptides and proteins can be identified and quantified with higher precision.[7]

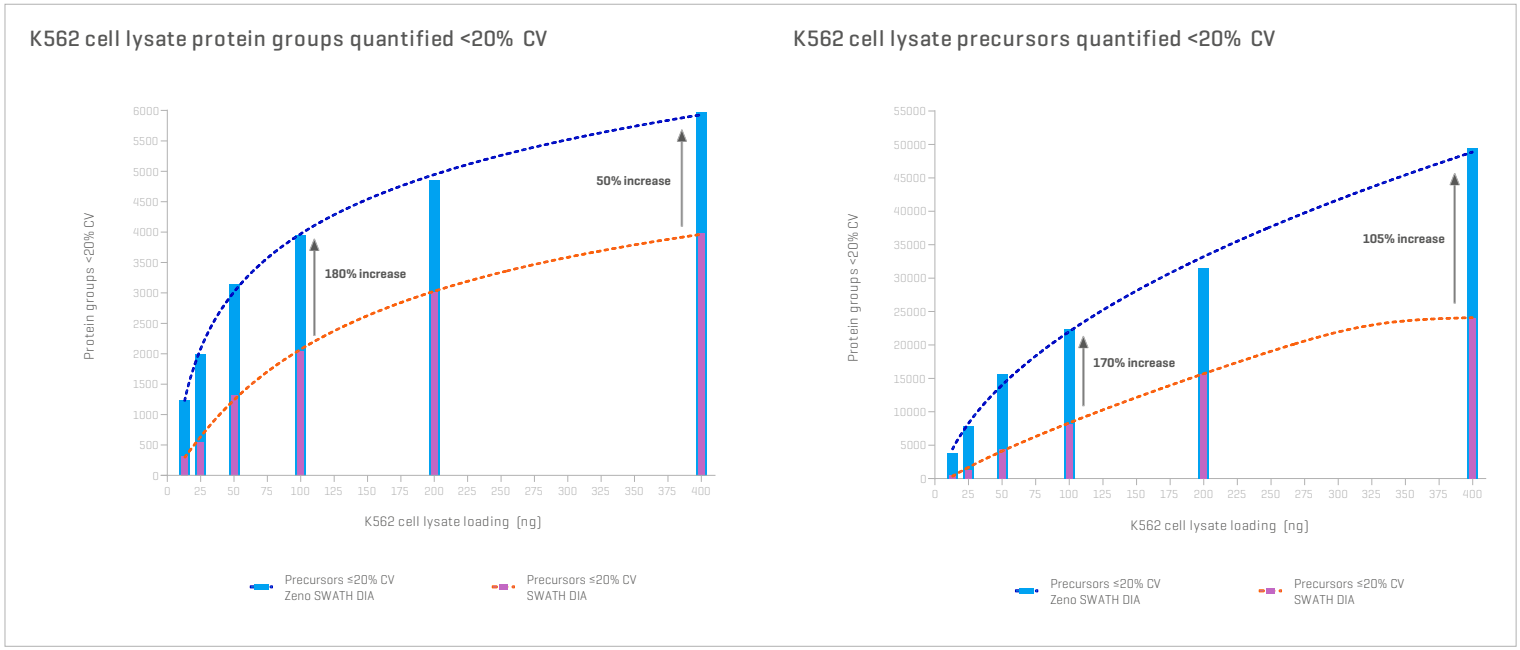


Figure 4. Protein and peptide precursor loading curves for 45 min gradient with and without Zeno MS/MS. Proteins and peptides quantified increases as sample loading increases.

Library free for protein ID

DDA uses a database search approach in which MS/MS fragment ions and precursor ions are algorithmically searched against FASTA files. In contrast, traditional SWATH DIA uses an MS/MS library for matching MS/MS data for peptide and protein identification. This library is experimentally generated in advance from multiple DDA experiments.

With recently developed algorithms, users now have the option to go "library free", by using an in silico generated library, thereby eliminating the preliminary experiments required to build the spectral library. Combining Zeno SWATH DIA data with the library-free approach in DIA-NN[8] enables a faster and more streamlined workflow for protein identification, with exceptional depth of coverage.[9]

Compared to searching an experimentally generated library, the library-free Zeno SWATH DIA approach identifies and quantifies a very large number of peptides and proteins. Using microflow chromatography, this was benchmarked against the experimental library approach using two different large libraries and very similar performance was observed. Furthermore, when examining the overlap in protein IDs, approximately 93% of the IDs are the same

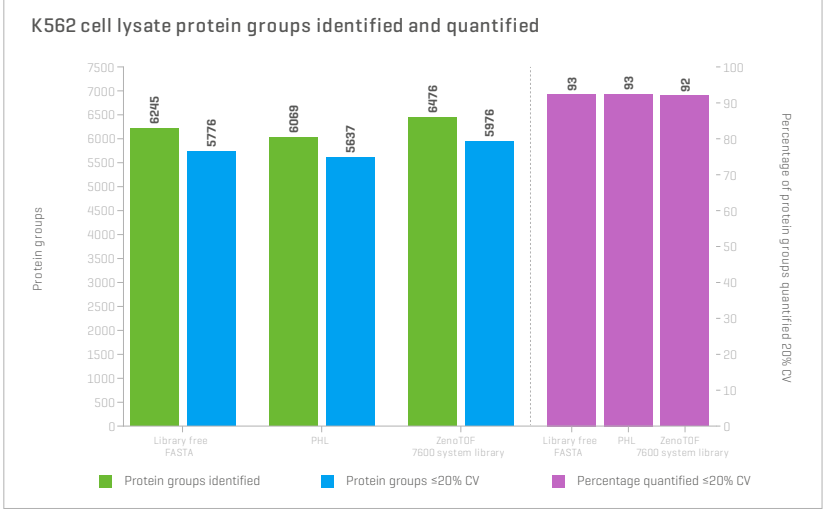


Figure 5. Comparing protein identifications using different approaches. Zeno SWATH DIA data were processed using two experimentally created libraries and a library-free approach. The number of proteins identified and quantified at ≤20% CV are very similar for all three approaches.

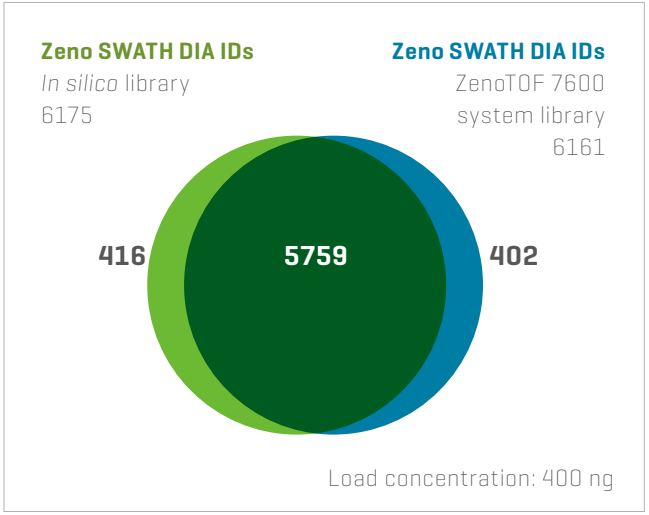


Figure 6. Similarity in protein identifications from Zeno SWATH DIA using an experimentally generated library and the library-free approach (in silico library). The overlap in protein identifications is very high, with nearly the same overall number identified by each.

Nanoflow Zeno SWATH DIA for ultimate sensitivity and coverage

Nanoflow chromatography is typically used when scientists want maximum sensitivity from their LC-MS/MS experiments. With Zeno SWATH DIA, the enhancements that the Zeno trap provides lifts these sensitivity gains to new heights.

Combining Zeno SWATH DIA with the very high sensitivity of nanoflow chromatography, thousands of proteins can be identified and quantified. At loadings of 12.5 ng of K562 cell lysate, over 5000 proteins can be identified, with 80% of those having a CV of less than 20%.

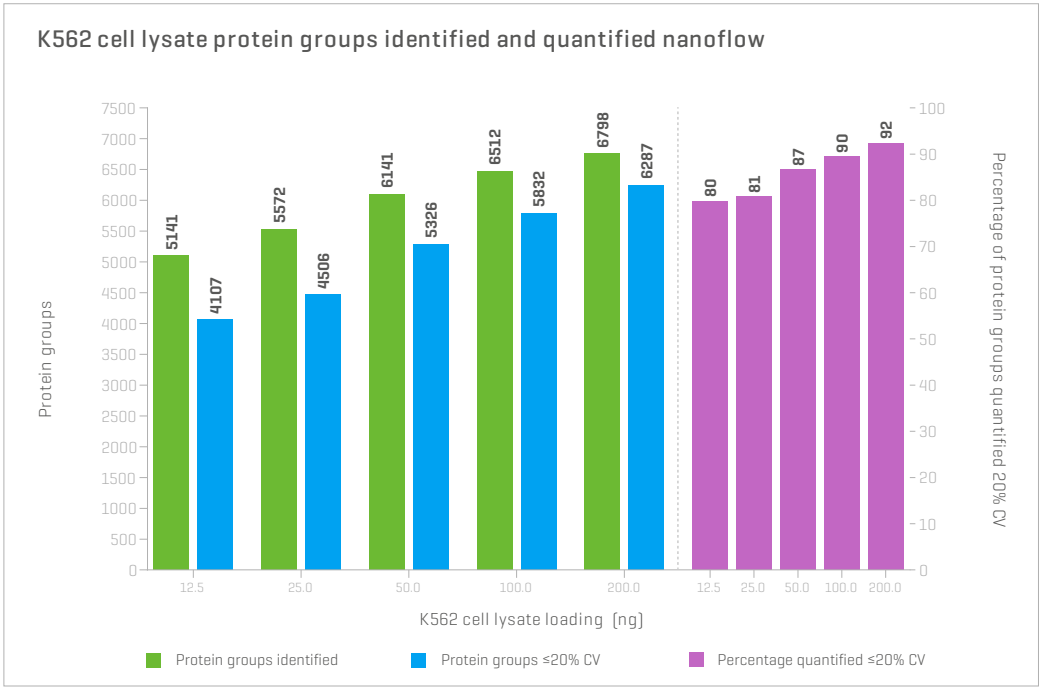


Figure 7. Proteins identified and quantified at different loadings of K562 digest for a 1-hour gradient. The number of proteins identified at <1% FDR and quantified at ≤20% CV using Zeno SWATH DIA across a range of loadings was studied. At all loads, >80% of proteins identified were also quantified.

Nanoflow Zeno SWATH DIA sensitivity – towards single cell analysis

Proteomic results derived from cellular extracts are representative of the entirety of all cellular states within the extract. But individual cells are dynamic biological entities often displaying immense variability in their molecular activities. Thus, increasingly, there has been a drive towards single cell analysis. There is a need to provide insight into different stages of individual cell growth and health, as well as discrete cell-to-cell interactions.

With Zeno SWATH DIA, ultra low-level analyses makes single cell analysis possible for many cell types. Zeno SWATH DIA identifies ~1000 proteins and quantifies ~400-500 proteins, for protein loads of only 250-500 pg of cell line.

Compared with SWATH DIA, Zeno SWATH DIA identifies ~3x more proteins and quantifies up to ~5x more, for a protein load of 500 pg K562 cell lysate.

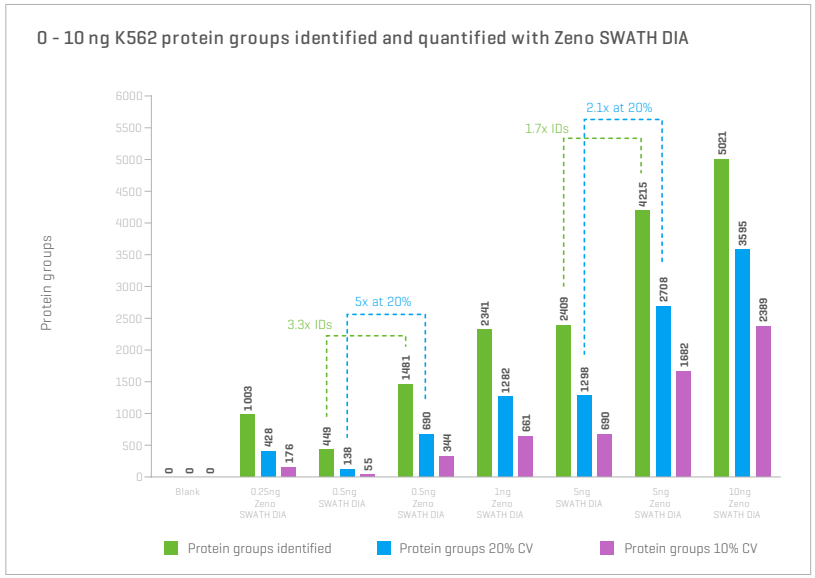


Figure 8. Proteins and peptides identified and quantified with Zeno SWATH DIA using nanoflow chromatography and low sample loadings. Even at the loading of a single cell (250-500 pg), Zeno SWATH DIA identifies and quantifies hundreds of proteins.

Nanoflow Zeno SWATH DIA reproducibility – towards single cell analysis

As sample loadings decrease, run-to-run variabilities in protein identification and quantification can become more extreme. But because of the data-independent nature of Zeno SWATH DIA, run-to-run reproducibility and repeatability is maximized.

Zeno SWATH DIA exhibits excellent sensitivity and reproducibility across multiple days, even for low sample loadings, with < 8% inter-day RSD for 500 and 1000 pg loadings.

At sample loads of only 250 pg, ~1000 proteins are reproducibly identified. Inter-day precision of quantification for all proteins is <20% CV. As shown in the inset, for a low abundant peptide it is quantified with <1% CV

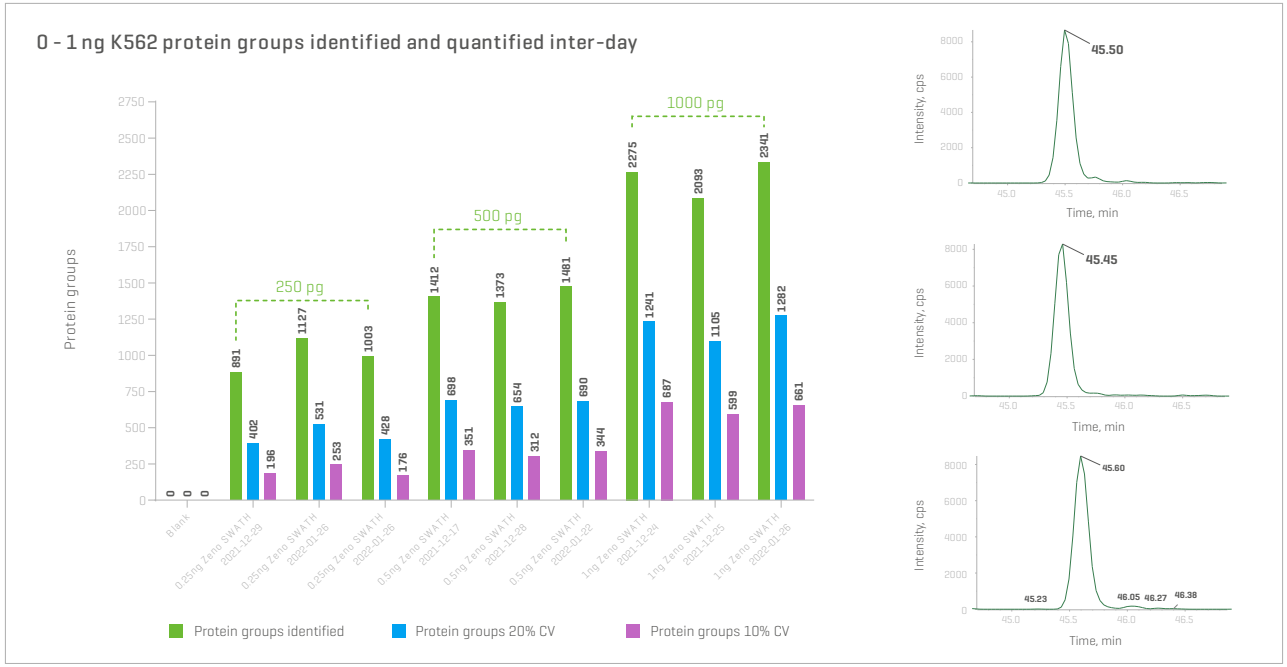


Figure 9. Proteins identified and quantified with Zeno SWATH DIA using nanoflow chromatography across multiple days. High precision and reproducibility are observed for multiple injections of the same sample over multiple days, even at very low sample loads. Inset: MS/MS fragment ion signals for 3 different injections, spanning multiple days, for a 250 pg sample load. A %CV of only 0.73% is observed for summed fragment ions

Higher flow rates, higher productivity

Because of its depth of proteome coverage and quantitative reproducibility, SWATH DIA has quickly become the method of choice for large scale proteomics studies. Zeno SWATH DIA takes this one step further with the added sensitivity gains that the Zeno trap provides. These gains can be leveraged to improve throughput and robustness by converting chromatography to higher flow rates.[7]

Zeno SWATH DIA drives throughput. With fast microflow gradients, Zeno SWATH DIA provides 3000-6000 quantified proteins, depending on the selected gradient and sample load.

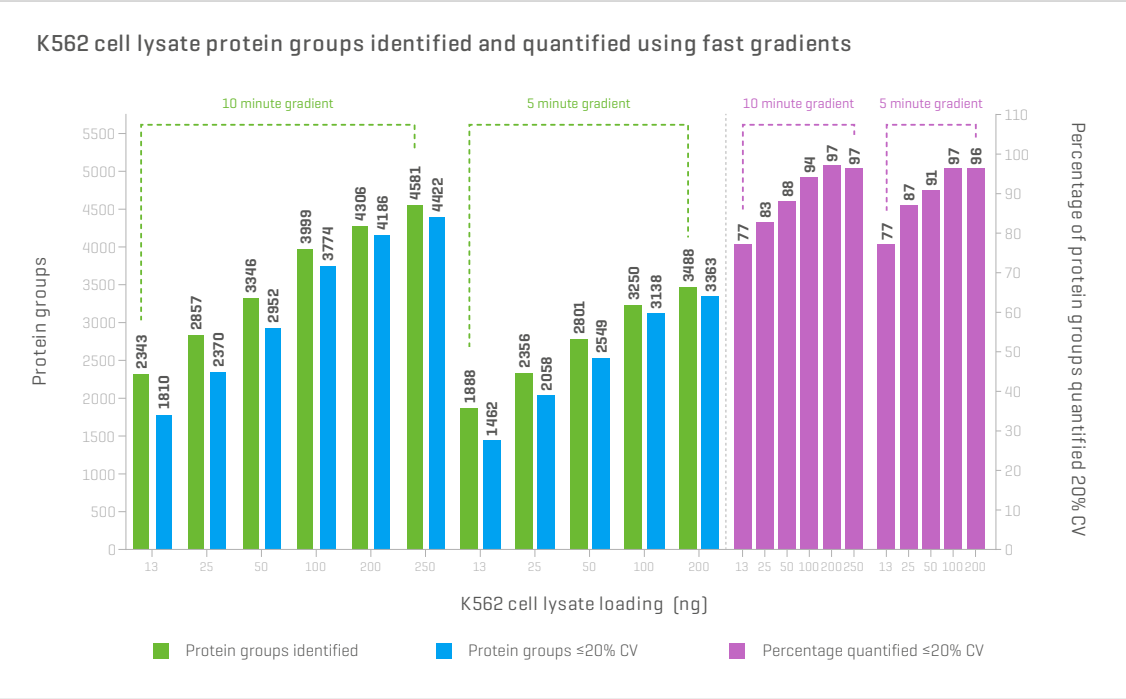


Figure 10. Comparing 10 min and 5 min gradients. The number of proteins identified at <1% FDR and quantified at <20% CV across a range of loadings was studied. 80-90% of proteins identified are also quantified with these fast gradients.

Faster analysis with lower sample amounts

In order to provide the same level of sensitivity as nanoflow experiments, high-flow LC-MS/MS experiments using robust, analytical flow rate chromatography typically require higher sample loadings. This sample requirement tends to limit the size and scope of any large-scale proteomics study using high flow rates. With its increased sensitivity, Zeno SWATH DIA now opens the door for fast and sensitive large scale proteomics studies using higher flow rates and lower sample loadings.

At high flow rates using fast, 5-minute gradients, Zeno SWATH DIA has been shown to provide the same level of results using an average of only 1/10 the amount of sample injected, when compared to SWATH DIA. Additionally, Zeno SWATH DIA significantly increases the number of proteins identified and quantified at high flow when injecting the same amounts, delivering hundreds and thousands more identified and quantified proteins.[5]

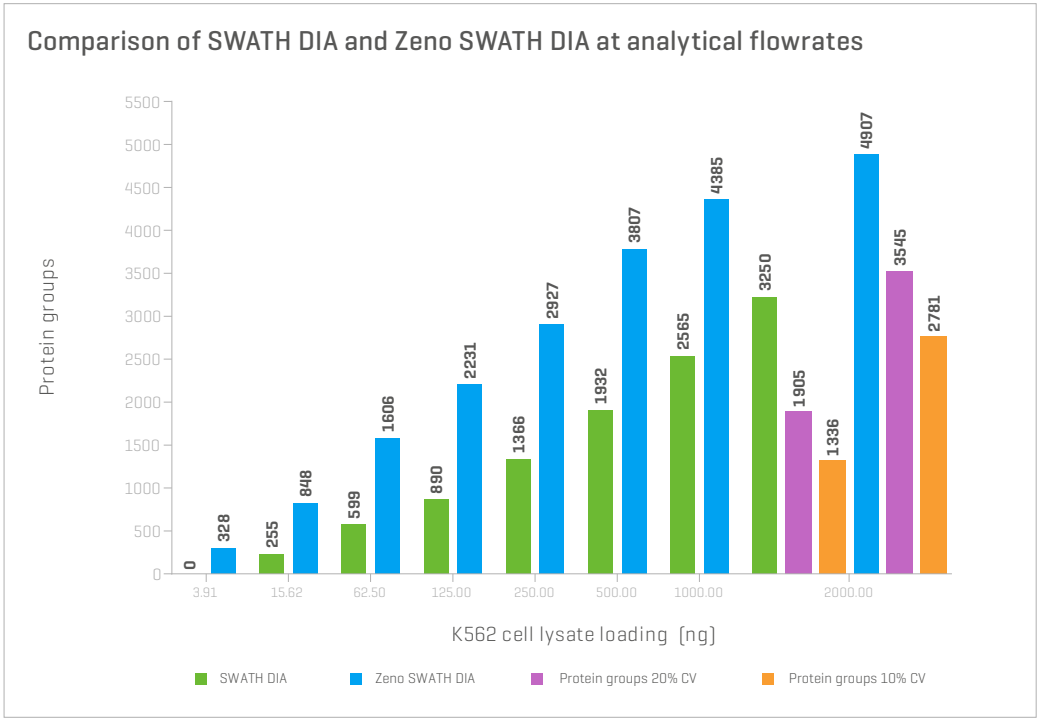


Figure 11. Comparison of SWATH DIA with Zeno SWATH DIA for high flow rate proteomics experiments (K562 using 800 µL/min, 5 min gradient). Left: Zeno SWATH DIA identifies and quantifies significantly more proteins and peptides than SWATH DIA across many different sample loading amounts. Right: Comparison of average number of IDs across 3 replicates (faint gray), number of consistent IDs (dark gray), quant < 20% CV (medium gray), quant < 10% CV (light gray).

High-throughput plasma proteomics

LC-MS/MS proteomics studies can reveal important biological insights, but it can be very challenging to collect deep proteome coverage at the scale needed to increase the size of a cohort study. Zeno SWATH DIA exceeds the performance of conventional, deep, untargeted, proteomics strategies in terms of depth, precision, and throughput.[10,11]

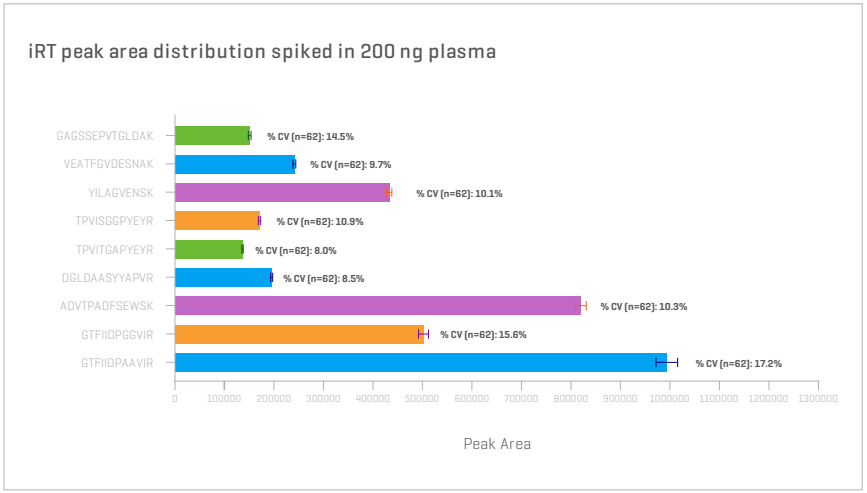


Figure 12. 200 ng non-depleted plasma spiked with iRT peptides. Run over two non-consecutive weeks to a total of 62 injections. iRT precision shows percentage CVs of less than 20%

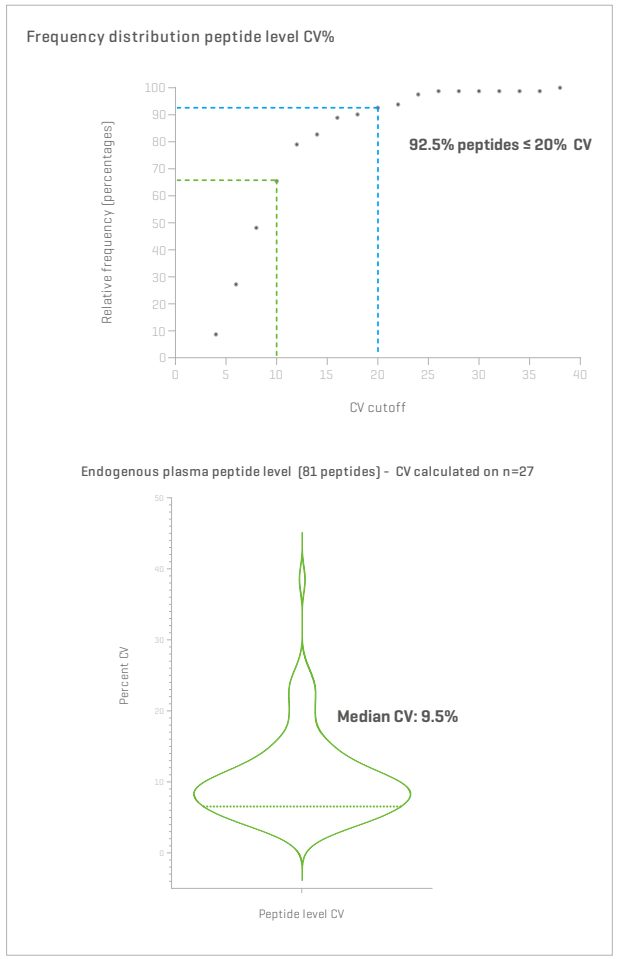


Figure 13. 200 ng non-depleted plasma spiked with SIL peptides for absolute quantification. Shown are the 81 endogenous light peptides with a measure of CV distribution, both absolute and median

Untargeted metabolomics

High confidence compound ID is undoubtedly the largest hurdle that researchers must overcome in untargeted metabolomics. In large due to the ever expanding metabolome that is yet to be annotated in our spectral libraries. Adding to this is the dynamic range of metabolites in a biological sample. The depth of metabolomics coverage was limited by sample size, MS/MS spectral richness and sensitivity to quantify low abundance metabolites. All three challenges have been addressed by ZenoTOF 7600 system, providing fast and deep metabolite profiling with the Zeno Trap and Orthogonal fragmentation offered by EAD DDA.

Zeno SWATH DIA serves as a powerful approach to marry the untargeted and targeted metabolomics through deep, comprehensive and quantitative metabolome coverage, Saving significant time and resources for metabolomics researchers in the pursuit of biomarkers.

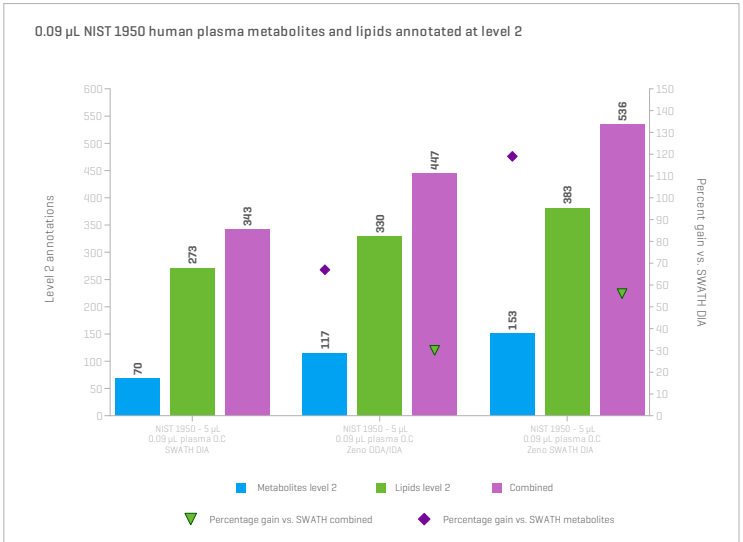


Figure 14. NIST 1950 plasma extracted with a simple methanol protein precipitation and analysed by reverse phase chromatography. Shown is a comparison of SWATH DIA, Zeno DDA/IDA and Zeno SWATH DIA

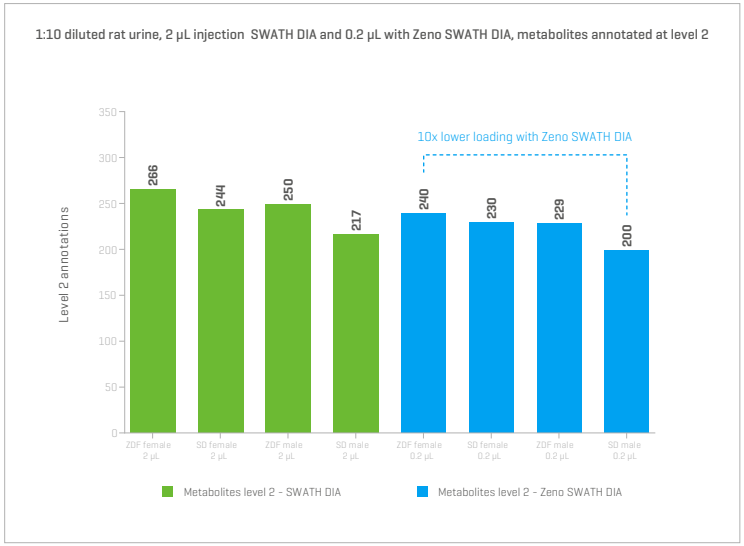


Figure 15. Zucker and Sprague-Dawley rat urine diluted 1 in 10. SWATH DIA loading 2 µL and Zeno SWATH DIA with 0.2 µL (1/10th the volume)

In-depth low-level drug metabolite identification

Drug metabolism is an integral part of the drug discovery process. Many advancements have taken place with respect to faster and more selective mass spectrometers but sensitivity in MS/MS mode has not been fully addressed, especially when using a data independent acquisition strategy. Detecting and identifying low-level metabolites is important, as not all metabolites will ionize in a similar manner and some of these metabolites can be very relevant for toxicity and pharmacokinetic purposes.

Use of the Zeno trap with SWATH DIA leads to a significant increase in sensitivity. The impact to the detection of metabolites under the conditions tested is a greater than 50% increase in coverage.

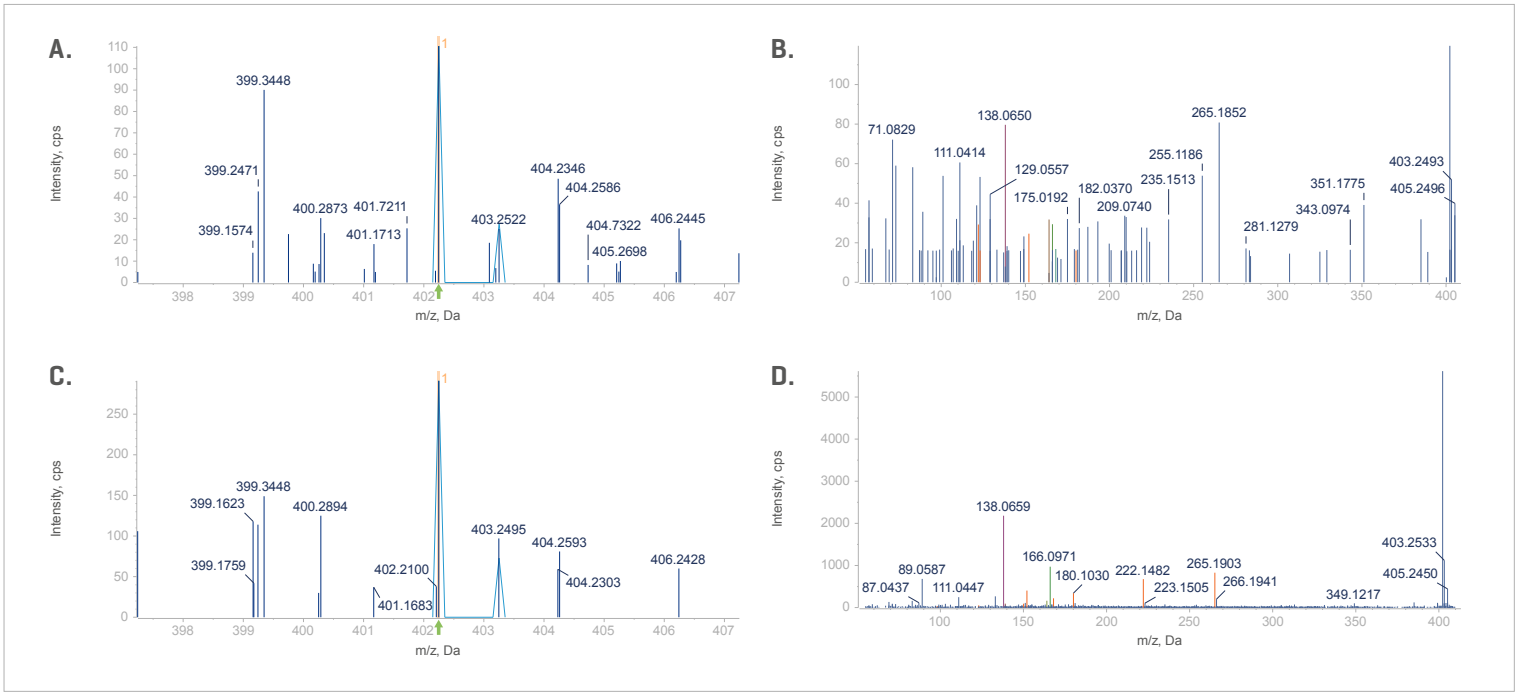


Figure 16. (Top) SWATH DIA for the fifth observed oxidation at the lowest abundance, showing TOF MS data (A) and the MS/MS data (B). (Bottom) Zeno SWATH DIA, for the fifth observed oxidation at the lowest abundance, shows TOF MS data (C) and the MS/MS data (D).

Summary

Zeno trap technology has expanded the reach of assays for many applications through sensitivity gains provided through MS/MS duty cycle enhancements.

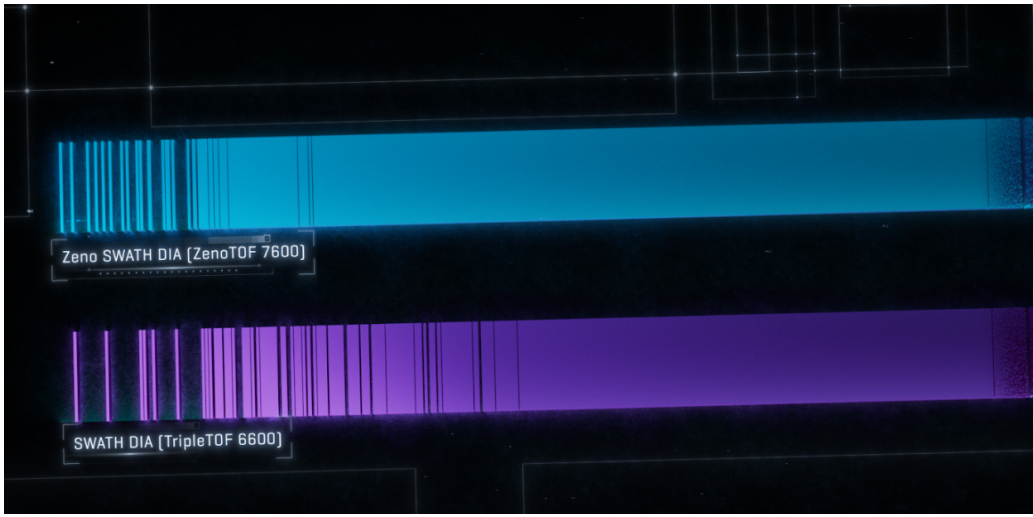
With the Zeno trap, nearly >90% duty cycles are now achievable for all ions regardless of mass, charge, or LC flow rate. This provides sensitivity gains of 4- to 20-fold without compromising other important performance specifications such as resolution, mass accuracy, dynamic range and speed.

The Zeno SWATH DIA workflow now capitalizes on these gains to enable truly widespread and comprehensive analyte coverage at new depths of detection and higher throughput. With Zeno SWATH DIA, higher sensitivity reveals more lower-level analytes, enables faster and more robust chromatography and the luxury of using much lower sample amounts.

For proteomics experiments, these gains now expose proteins across a broader and deeper range within plasma and translate to the potential of single cell analysis. Large scale high throughput proteomics studies are now possible using robust high flow chromatography but with much less sample consumed. The new “library-free” approach removes the effort of generating an experimental library and instead allows the creation of an in silico library, making library creation truly “effort free”.

Zeno SWATH DIA for metabolomics studies delivers metabolites and lipids at deeper levels within plasma. For drug metabolism studies, new metabolites are revealed that may be potentially toxic or can aid in a better understanding of pharmacokinetics and drug fate.

The reach of Zeno SWATH DIA is far and wide and continues to grow across different applications. With Zeno SWATH DIA, the synergy between Zeno technology and the SWATH DIA workflow continues to expand the boundaries of applications requiring sensitive and comprehensive, fast high-quality data for analysis.



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Note: Data comparisons made relative to SWATH acquisition on the TripleTOF 6600.

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