# Improving peptide quantification using the on-demand operation of the Zeno trap

***Featuring peptide quantification with the ZenoTOF 7600 system***

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This technical note demonstrates a highly-sensitive workflow for quantifying peptides on an accurate mass spectrometer with improved MS/MS sampling efficiency (Figure 1). In this work, the lower limit of quantification (LLOQ) was enhanced by a factor of 6 by implementing the on-demand operation of the Zeno trap on the ZenoTOF 7600 system. With the Zeno trap, the linear dynamic range (LDR) was improved up to 0.8 orders of magnitude to enable the quantification of low-abundant peptides.

The development of peptide and protein therapeutics has increased dramatically over the past 2 decades, based on their high target specificity for disease treatment. During drug development, factors such as pharmacokinetics, metabolism and overall stability are critical factors that must be characterized using quantitative measurements. Biopharma development scientists supporting this work require highly sensitive and selective bioanalytical methods for quantification. While quantification of peptides in biological matrices is typically performed using nominal mass instruments, some analytical methods are now being performed using accurate mass instruments to improve selectivity.[1] However, sensitivity remains a critical challenge for quantification on accurate mass instruments, including time-of-flight (TOF) systems[2-3], because the duty cycle is typically less than 30%.

The ZenoTOF 7600 system demonstrates improved MS/MS sampling efficiency and therefore offers a robust and sensitive platform to support routine peptide and protein quantification. Here, the Zeno trap controls the ion beam from the collision cell, which facilitates greater ion transmission to the TOF accelerator, improving the duty cycle to ≥90% (classical TOF is below 30%). As a result, this enhances the overall MS/MS sampling efficiency to enable the ZenoTOF 7600 system to be highly advantageous for quantitative bioanalysis workflows that can benefit from the improvement in sensitivity using the Zeno trap.[4,5]

In this technical note, synthetic peptides in the range between *m/z* 400 to *m/z* 700 were selected as model analytes to evaluate the quantitative performance for peptide analysis using the Zeno trap on the ZenoTOF 7600 system.

## Key features of the peptide quantification workflow using the ZenoTOF 7600 system

- Achieve up to 6-fold improvement in LLOQ for peptide quantification in matrix using the ZenoTOF 7600 system
- Enhance the overall LDR for quantitative analysis of peptides up to 0.8 orders of magnitude by extending quantification at the lower end using the on-demand operation of the Zeno trap
- Ensure exceptional accuracy and precision for quantitative workflows using the ZenoTOF 7600 system
- Increase productivity with the user-friendly interface and integrated platform for data acquisition, processing and management in the SCIEX OS software

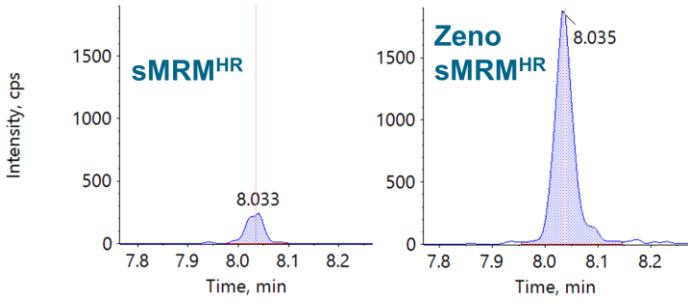


**Figure 1. Sensitivity improvement with the use of the Zeno trap for the peptide LDSTSIPVAK.** A concentration of 0.0655 fmol/µL was analyzed. A 7-fold gain in S/N was reached with Zeno sMRM$^{HR}$ when compared with sMRM$^{HR}$.

## Methods

***Sample preparation:*** Bovine serum albumin (BSA) tryptic digest (30 pmol/µL) was diluted in 5% acetic acid with 2% acetonitrile in water to a final concentration of 50 fmol/µL and was used as matrix. A 1 pmol/µL stock solution containing a mixture of isotopically labeled synthetic peptides was diluted into the matrix solution at various concentrations, ranging from 0.0017 to 500 fmol/µL.

***Chromatography:*** A NanoLC 425 system with a 1−10 µL/min microflow module was used to deliver a gradient consisting of 0.1% formic acid in $H_2O$ and 0.1% formic acid in acetonitrile at 5 µL/min (Table 1). An Eksigent ChromXP C18CL column was used for separation (150 x 0.3 mm, 3 µm, 120 Å). The column temperature was set at 40°C and 2 µL of the sample was loaded onto the column. Each concentration was analyzed in triplicate for this study.

**Table 1. Microflow gradient profile.**

| Time (min) | Mobile phase A (%) | Mobile phase B (%) |
|---|---|---|
| *0* | *97* | *3* |
| *0.5* | *97* | *3* |
| *6* | *75* | *25* |
| *6.5* | *75* | *25* |
| *7.5* | *20* | *80* |
| *9* | *20* | *80* |
| *9.5* | *97* | *3* |
| *15.5* | *97* | *3* |

***Mass spectrometry:*** A ZenoTOF 7600 system with the OptiFlow Turbo V ion source and a 25 µm electrospray ionization (ESI) electrode was used. Ion source conditions were optimized to obtain the best sensitivity (Table 2). A method based on the scheduled $MRM^{HR}$ ($sMRM^{HR}$) algorithm in positive ion mode was used for acquisition with the retention time window set at ±30 s. Precursor and fragment *m/z*, RT and CE for the peptides are listed in Table 3.

**Table 2. Ion source conditions.**

| Parameter | Value | Parameter | Value |
|---|---|---|---|
| *Curtain gas* | *30 psi* | *Source temperature* | *200°C* |
| *Ion source gas 1* | *20 psi* | *Ion source gas 2* | *40 psi* |
| *CAD gas* | *7* | *Ion spray voltage* | *5000 V* |

**Table 3. Peptide sequence and sMRM transition parameters.**

| Sequence* | Precursor (*m/z*) | Fragment (*m/z*) | RT (min) | CE (V) |
|---|---|---|---|---|
| *GAYVEVTA[K]* | *473.26020* | *136.07569* | *8.3* | *29* |
| *LDSTSIPVA[K]* | *519.79969* | *422.28529* | *8.8* | *31* |
| *AGLIVAEGVT[K]* | *533.32333* | *711.41268* | *9.6* | *32* |
| *AVGANPEQLT[R]* | *583.31360* | *753.41290* | *8.6* | *34* |
| *SAEGLDASASL[R]* | *593.80053* | *729.37651* | *9.0* | *34* |
| *VFTPLEVDVA[K]* | *613.34955* | *878.50731* | *10.7* | *36* |
| *VGNEIQYVAL[R]* | *636.35273* | *759.43872* | *10.1* | *37* |
| *YIELAPGVDNS[K]* | *657.34499* | *724.37154* | *9.7* | *38* |
| *DGTFAVDGPGVIA[K]* | *677.85827* | *764.43923* | *10.2* | *39* |
| *BSA (Internal standard)* | *582.31897* | *185.16483* | *11.1* | *34* |

**Mass shift is 10 Da or 8 Da for these peptides due to 13C and 14N labeling at their C-terminal R or K, respectively.*

The TOF MS mass range was 400–1,250 Da with a 100 ms accumulation time. The MS/MS mass range was 100–1,500 Da with a 50 ms accumulation time. The data were acquired with and without the use of the Zeno trap. The Zeno threshold was set at 20,000 cps during the Zeno trap acquisition.

The SCIEX OS software was used for data processing. Precursor and fragment masses of the peptides used for quantification are listed in Table 3. XIC peak widths of 0.04 Da and 0.08 Da were used for TOF MS and MS/MS intensity extraction, respectively. A $1/x^2$ weighting was applied to construct linear calibration curves for all peptides.

## Improving LLOQ by engaging the Zeno trap

The Zeno trap provides control of the ion beam from the collision cell into the accelerator. Ions are first accumulated in a short linear ion trap at the end of the collision cell, then are released based on their potential energy. Generally, higher m/z ions are released followed by lower m/z ions. Ions from a wide *m/z* range simultaneously arrive in the accelerator region and are subsequently collected, which enhances the overall MS/MS sensitivity.

Each calibration sample was analyzed in triplicate. Quantitative criteria required an accuracy value between 80 and 120% and a %CV less than 20% at the LLOQ level. For the concentrations greater than the LLOQ, an accuracy value between 85 and 115% and %CV less than 15% were required.

The sensitivity gains from the Zeno trap improved the LLOQs for peptide quantification. As an example, a LLOQ improvement of more than 6-fold was observed for the peptide, LDSTSIPVAK (Figure 2). Using Zeno sMRM$^{HR}$, a LLOQ of 0.0105 fmol/µL was achieved, whereas the LLOQ was 0.0655 fmol/µL when using sMRM$^{HR}$. For both quantitative modes, the %CV was less than 13% with accuracy within ±6% of the nominal concentration (Table 4). At the level of the LLOQ, the precision was less than 13% and accuracy was ±2% of the nominal concentration. As shown in Figure 3, the LDR was extended by 0.8 orders of

**Table 4. Calibration concentration, accuracy and precision for the peptide, LDSTSIPVAK, using sMRM$^{HR}$ and Zeno sMRM$^{HR}$.**

| | sMRM$^{HR}$ | | Zeno sMRM$^{HR}$ | |
|---|---|---|---|---|
| Concentration (fmol/µL) | %CV | Accuracy (%) | %CV | Accuracy (%) |
| 0.0105 | *N/A* | *N/A* | 13 | 102 |
| 0.0262 | *N/A* | *N/A* | 9.0 | 95.2 |
| 0.0655 | 0.8 | 102 | 5.7 | 103 |
| 0.164 | 4.3 | 94.4 | 6.1 | 99.1 |
| 0.41 | 4.8 | 102 | 1.7 | 99.8 |
| 1.02 | 6.0 | 95.9 | 3.9 | 102 |
| 2.56 | 6.3 | 105 | 3.3 | 96.7 |
| 6.40 | 2.2 | 99.3 | 2.8 | 98.2 |
| 16.0 | 3.1 | 99.6 | 1.6 | 105 |
| 40.0 | 2.5 | 101 | 2.1 | 103 |
| 100 | 1.5 | 102 | 1.4 | 101 |
| 250 | 1.7 | 102 | 3.4 | 101 |
| 500 | 0.7 | 96.9 | 2.0 | 96.1 |

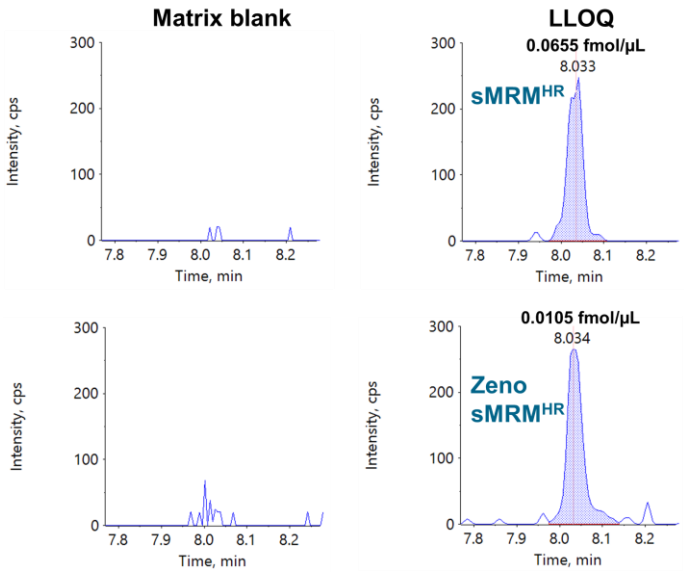


**Figure 2. Extracted ion chromatograms (XICs) of the matrix blank and LLOQ of the peptide LDSTSIPVAK.** Greater sensitivity was achieved using Zeno sMRM$^{HR}$ relative to sMRM$^{HR}$. The use of Zeno sMRM$^{HR}$ resulted in more than a 6-fold improvement in the LLOQ. No matrix interferences were observed in the blank.

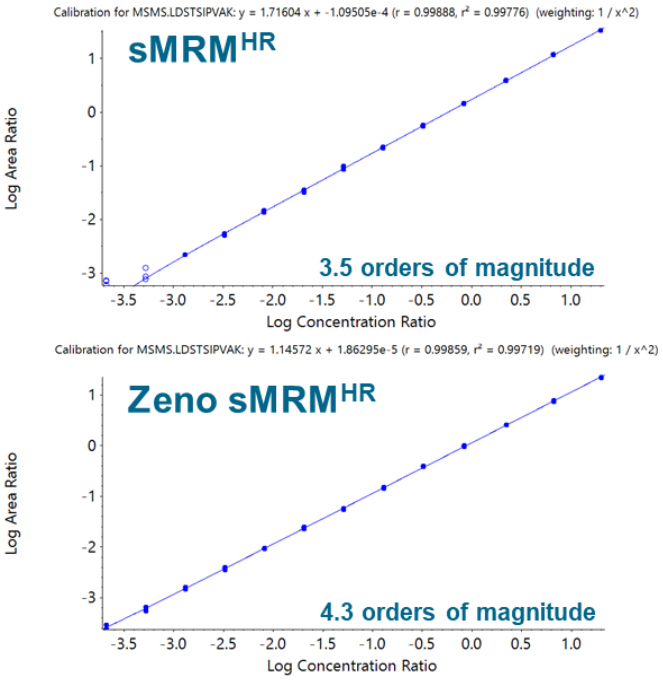


**Figure 3. Calibration curves obtained for the peptide LDSTSIPVAK.** The top calibration curve was generated with sMRM$^{HR}$ and the bottom curve was generated using Zeno sMRM$^{HR}$. The use of Zeno sMRM$^{HR}$ extended the LDR by 0.8 orders of magnitude enabling quantification at lower concentrations.

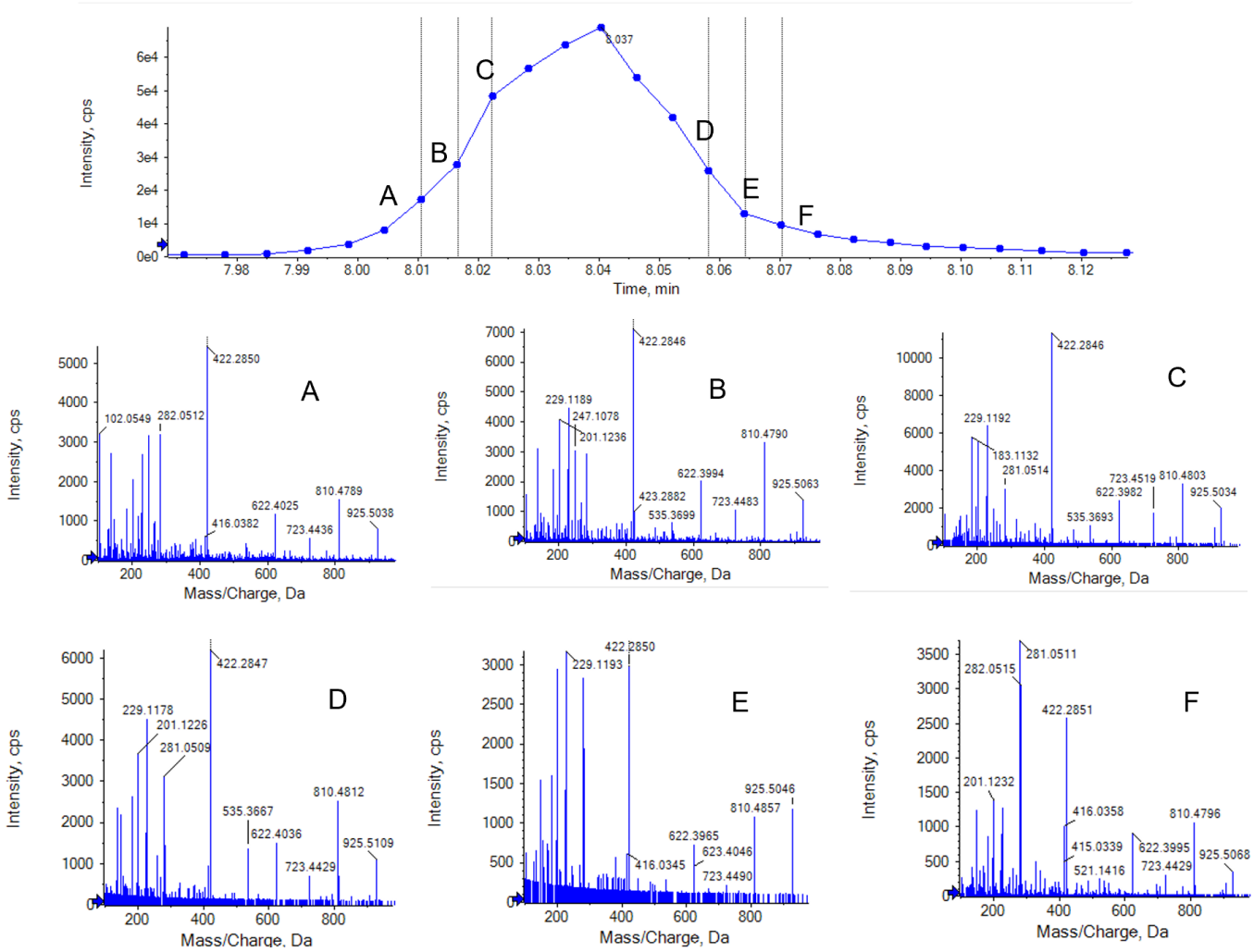


**Figure 4. On-demand operation of the Zeno trap.** The top panel shows a MS/MS XIC for the peptide LDSTSIPVAK at fragment *m/z* 422.28 Da. Panels A-F show MS/MS spectra of this peptide from 6 different MS/MS scans. The sum intensity of the fragment at *m/z* 422.28 was 17,000 cps for scan A. This increased to 28,000 cps for scan B, which was above the Zeno trap threshold of 20,000 cps. This caused the Zeno trap to be turned off for the next MS/MS scan (C). The sum intensity of *m/z* 422.28 then decreased from 27,000 cps for scan D to 13,000 cps for scan E, triggering the Zeno trap to be activated for scan F.

magnitude when using Zeno sMRM$^{HR}$, which therefore facilitated the quantification of low-abundant peptides.

## Preserving the ULOQ with on-demand operation of the Zeno trap

The Zeno trap is activated dynamically during acquisition using an intensity threshold to ensure that a broad dynamic range is achieved. When the ion intensity is below the intensity threshold, the Zeno trap is activated to increase sensitivity and improve the LLOQ. This intensity threshold can be adjusted to achieve the best quantitative performance.

During acquisition with the sMRM$^{HR}$ algorithm, the instrument switches the Zeno trap on and off based on the intensity of the most intense fragment ion in the previous MS/MS scan. Figure 4 illustrates how this process works with the Zeno trap threshold set at 20,000 cps.

Table 5 summarizes the MS/MS quantification results for all peptides using Zeno sMRM$^{HR}$ and sMRM$^{HR}$. Up to 6-fold lower LLOQs were reached for peptide quantification using Zeno sMRM$^{HR}$. The ULOQ was preserved at 1 fmol/µL with the on-demand operation of the Zeno trap. The LDR was extended by more than 0.4 orders of magnitude, achieving quantification at lower concentrations. This ensured quantification of low-abundant peptides with excellent accuracy, precision and linearity.

**Table 5. Sensitivity gains in LLOQ, ULOQ and LDR using the Zeno trap for peptide quantification.**

| Peptide | sMRM$^{HR}$ | | | Zeno sMRM$^{HR}$ | | | Gain with the Zeno trap | | |
|---|---|---|---|---|---|---|---|---|---|
| | *LLOQ (fmol/µL)* | *ULOQ (fmol/µL)* | *LDR (order)* | *LLOQ (fmol/µL)* | *ULOQ (fmol/µL)* | *LDR (order)* | *LLOQ (fmol/µL)* | *ULOQ (fmol/µL)* | *LDR (order)* |
| ***GAYVEVTAK*** | 0.0655 | 500 | 3.9 | 0.0105 | 500 | 4.7 | 6.25 | 1 | 0.8 |
| ***LDSTSIPVAK*** | 0.1638 | 500 | 3.5 | 0.0262 | 500 | 4.3 | 6.25 | 1 | 0.8 |
| ***AGLIVAEGVTK*** | 0.0655 | 250 | 3.6 | 0.0105 | 250 | 4.4 | 6.25 | 1 | 0.8 |
| ***AVGANPEQLTR*** | 0.0655 | 500 | 3.9 | 0.0262 | 500 | 4.3 | 2.5 | 1 | 0.4 |
| ***SAEGLDASASLR*** | 0.0655 | 500 | 3.9 | 0.0105 | 500 | 4.7 | 6.25 | 1 | 0.8 |
| ***VFTPLEVDVAK*** | 0.0655 | 100 | 3.2 | 0.0262 | 100 | 3.6 | 2.5 | 1 | 0.4 |
| ***VGNEIQYVALR*** | 0.0655 | 500 | 3.9 | 0.0105 | 500 | 4.7 | 6.25 | 1 | 0.8 |
| ***YIELAPGVDNSK*** | 0.0655 | 250 | 3.6 | 0.0105 | 250 | 4.4 | 6.25 | 1 | 0.8 |
| ***DGTFAVDGPGVIAK*** | 0.1638 | 500 | 3.5 | 0.0262 | 500 | 4.3 | 6.25 | 1 | 0.8 |

## Conclusions

- An average of 6-fold improvement in LLOQ was obtained for peptide quantification using the Zeno sMRM$^{HR}$ workflow
- The ULOQs were preserved while LDRs were extended up to 0.8 orders of magnitude to capture lower end quantification with the use of the on-demand operation of the Zeno trap
- A GLP level quantitative workflow for peptides quantification was demonstrated using the ZenoTOF 7600 system

## References

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