Enhanced sensitivity for peptide quantification in a complex matrix using high-resolution LC-MS/MS

Featuring the ZenoTOF 7600 LC-MS/MS system

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A significant 5-fold improvement in LLOQ for peptide quantification was achieved using the ZenoTOF 7600 system featuring the Zeno trap. Compared with traditional time-of-flight systems, the Zeno trap enables greater MS/MS sensitivity by enhancing the duty cycle. In addition, the versatility of TOF MS/MS data allows for the capability of post-acquisition decisions for the selection of fragment ion(s) for MRM^{HR}. For cases where multiple dominant fragment ions are generated from the target peptide, the sum of XICs enabled greater sensitivity. A 3-fold improvement in LLOQ was observed for peptides that leveraged the summing of multiple dominant fragment ions when MS/MS ion current was dispersed.

Traditional workflows for quantitative bioanalyses, such as immunological assays, have been displaced by LC-MS/MS analysis on triple quadrupole mass spectrometers. Immunoassays often lack selectivity and specificity, and have a limited linear dynamic range. While the triple quadrupole platform has been a key driver for most bioanalytical workflows, offering great sensitivity and quantitative performance, high-resolution accurate mass spectrometry (HRAMS) has increasingly been adopted for quantitative bioanalysis.[1,2] With the inherent advantage of greater selectivity with improved mass resolution, as well as the flexibility of TOF MS/MS data, the ZenoTOF 7600 system provides excellent quantitative performance in multiple dimensions.

High-resolution platforms, such as traditional time-of-flight systems, often lack sensitivity due to loss of ion transmission in between TOF pulses. The Zeno trap controls the ion beam from the collision cell which facilitates greater ion transmission to the TOF accelerator. Therefore, the duty cycle is improved to ≥90 %, which enhances overall MS/MS sensitivity.

The ZenoTOF 7600 system offers an exceptional combination of mass resolution, sensitivity, and acquisition speed for quantitative analysis. It also aids in the potential for: less ion path tuning, increased sensitivity with the Zeno trap, ability to change measured fragments post-acquisition and improved reproducibility and accuracy.

Key features of the ZenoTOF 7600 system for highly sensitive peptide quantification

- Demonstration of a 5-fold improvement in LLOQ for peptide quantification using the Zeno trap to accumulate ions during each TOF pulse for enhanced duty cycle
- Reach enhanced sensitivity by summing of multiple highly abundant fragment ions with availability of TOF MS/MS data
- Ensure exceptional accuracy and precision for quantitative workflows using the ZenoTOF 7600 system
- Easily acquire, process, and manage data on a single platform using the SCIEX OS Software

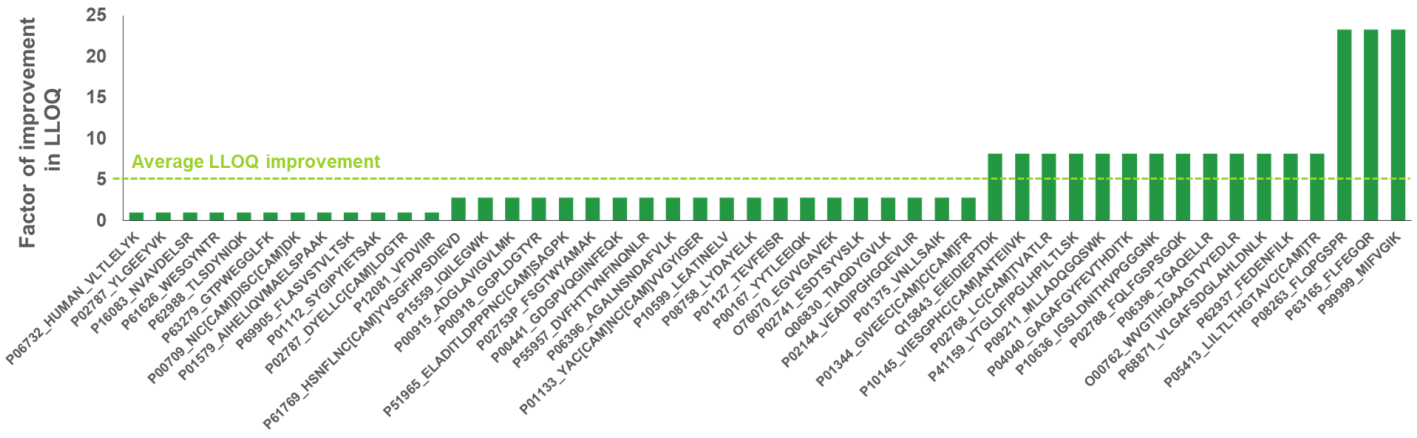

Figure 1. Factor of improvement in LLOQ for peptide quantification with Zeno MRM^{HR} compared to MRM^{HR}. On average, a 5-fold improvement in LLOQ for peptide quantification was observed with Zeno MRM^{HR} in comparison to standard MRM^{HR}.

Methods

Samples and reagents: Universal Proteomics Standard (UPS) was purchased from Sigma-Aldrich. Rat plasma (Sprague Dawley, K2 EDTA) was purchased from BioIVT.

Sample preparation: The calibration curve was prepared by spiking digested UPS into rat plasma digest followed by serial dilution.

Samples were denatured by incubating with N-octyl-glucoside (OGS), followed by reduction with dithiothreitol (DTT) and alkylation with iodoacetamide (IAM). A trypsin/Lys-C digestion was performed at 37 ºC overnight, with an enzyme-protein ratio of 1:25. Formic acid was spiked into the samples to abort digestion. The samples were centrifuged at a speed of 12,000 g and the supernatant was injected for LC-MS analysis.

As a note, proteins used for this study had limited starting concentrations. Therefore, the final LDRs were narrow for the peptides analyzed.

Chromatography: An ExionLC system was used for analyte separation. A volume of 20 µL was injected for analysis. Mobile phase A consisted of water with 0.1% FA in water, while organic phase B was composed of 0.1% FA in acetonitrile. For analyte separation, the operating flow rate was set to 0.5 mL/min using a Phenomenex Kinetex C18 column (3 x 50 mm, 2.6 µm, 100 Å). The column oven temperature was set to 40 ºC. Chromatographic conditions for analyte separation are shown in Table 1.

Table 1. Chromatographic conditions for analyte separation.

Time (min)	Mobile phase A (%)	Mobile phase B (%)
0.50	*98.0*	*2.0*
1.00	*88.0*	*12.0*
6.00	*68.0*	*32.0*
6.10	*10.0*	*90.0*
7.00	*10.0*	*90.0*
7.10	*98.0*	*2.0*
8.00	*98.0*	*2.0*

Mass Spectrometry: Samples were analyzed in triplicate. Method details such as source and gas parameters and MS conditions are summarized in Table 2. Sample analysis was performed using scheduled Zeno MRM^{HR} on the ZenoTOF 7600 system. The ZenoTOF 7600 system provides a scan speed of 133 Hz.

Data processing: MRM data were processed using SCIEX OS 2.0 software. Integration was performed using the MQ4 algorithm. Linear regression with 1/x weighting was used for quantification of all peptides. The XIC peak width was set to 0.05 Da.

Table 2. Source and MS conditions.

Parameter	Value	Parameter	Value
Curtain gas	*30 psi*	*Source temperature*	*550 °C*
Ion source gas1	*65 psi*	*Ion source gas 2*	*65 psi*
CAD gas	*12*	*Ion spray voltage*	*5500 V*
MS accumulation time	*40 ms*	*MS/MS accumulation time*	*20 ms*
TOF MS start mass	*350 Da*	*TOF MS stop mass*	*1500 Da*
TOF MS/MS start mass	*300 Da*	*TOF MS/MS stop mass*	*≥1000* Da*
ZOD threshold	*20,000 cps*		

**TOF MS/MS stop mass depends on peptide analyzed*

Greater sensitivity with Zeno trap

With traditional time-of-flight MS/MS, fragment ions arriving from the collision cell are often lost in transmission between TOF pulses due to differences in velocity. As a result, for standard time-of-flight MS/MS, duty cycle range is approximately between 5-25%. A decrease in sensitivity occurs as a consequence of loss in ion transmission. The Zeno trap ensures greater ion transmission by controlling the ion beam from the collision cell into the TOF accelerator (Figure 2). Ions exit the Zeno trap based on potential energy.

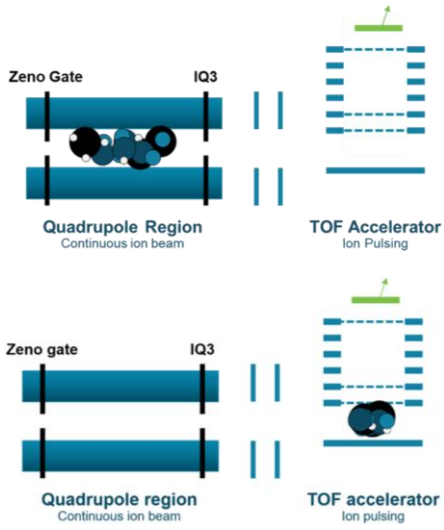

Figure 2. Zeno trap enables ion beam control from the collision cell before entering into the TOF accelerator. Gains in ion transmission improve overall MS/MS sensitivity.

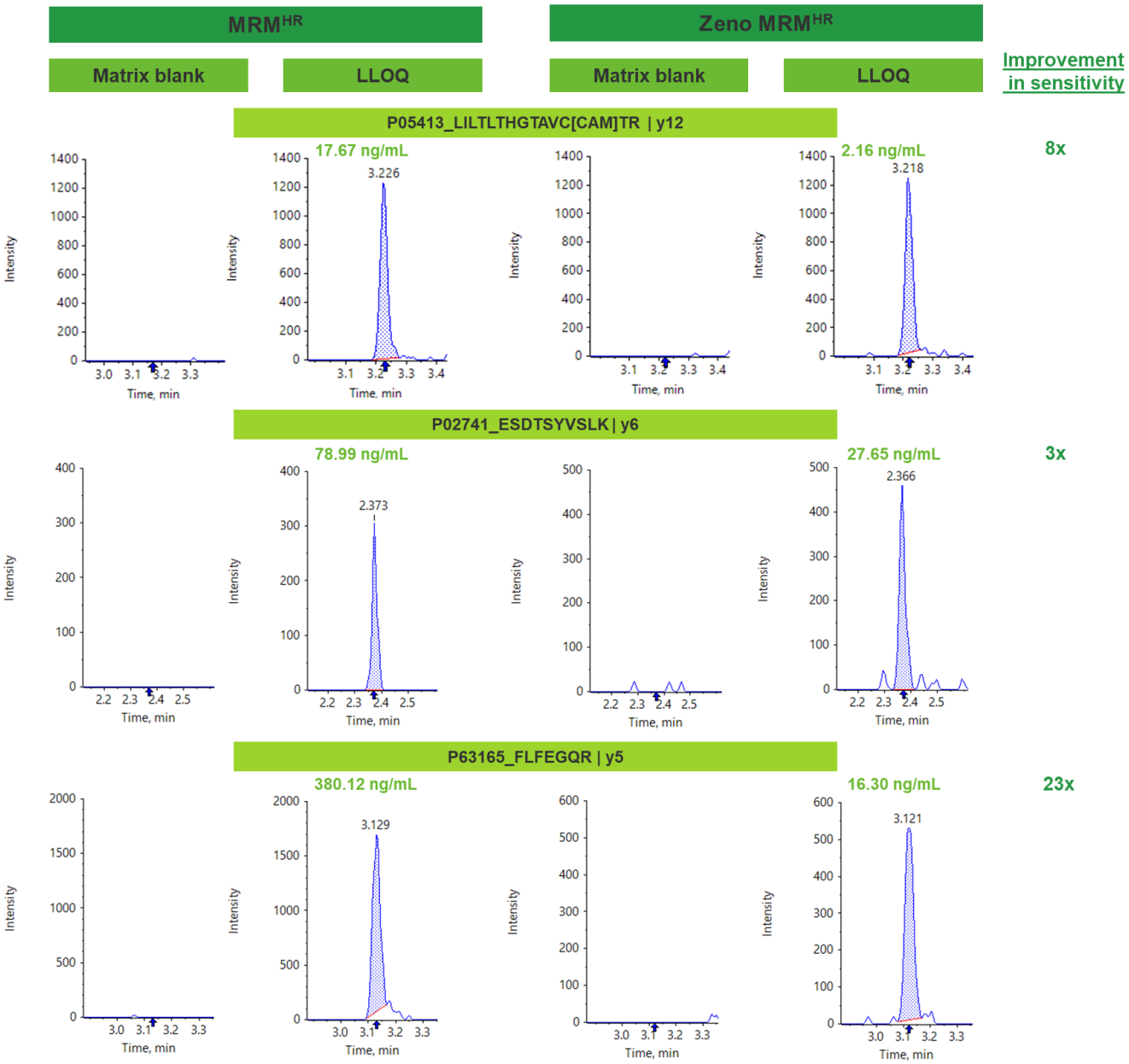

Figure 3. Greater sensitivity for peptide quantification was observed with Zeno MRM^{HR}. Significantly lower LLOQs were achieved using Zeno trap.

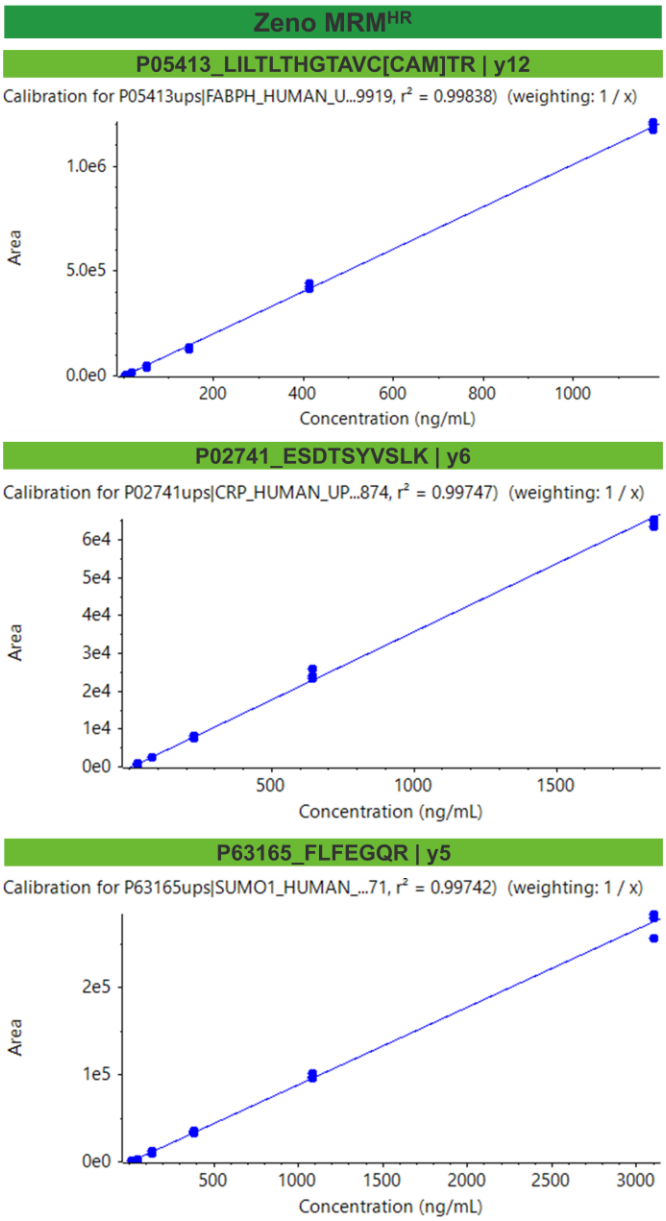

Figure 4. Strong linearity was achieved with Zeno MRM^{HR}.

The Zeno trap enabled significant improvements in MS/MS sensitivity. On average, 5-fold improvement in LLOQ was achieved using Zeno MRM^{HR} in comparison with standard MRM^{HR} (Figure 1). Out of 48 peptides measured, 75% of peptides showed ≥3-fold improvement in LLOQ.

Overall, XICs comparing LLOQs with MRM^{HR} and Zeno MRM^{HR} show significantly lower LLOQs were achieved with the Zeno trap (Figure 3). Strong linearity was achieved for all peptides analyzed (Figure 4). Accuracy at the LLOQ was within 80%-120%, while for all other non-zero calibrators, accuracy was within 85%-115% of the nominal concentration. Overall, precision was <15%, demonstrating high reproducibility (Table 3).

Summation of multiple fragment ions enhances sensitivity

The accessibility of TOF MS/MS data can be advantageous as post-acquisition data decisions can be made on which measured fragments can be utilized for MRM^{HR}. For MRM^{HR}, quantification can be performed using single fragment ion or by summing multiple dominant fragment ions. When multiple, high-abundant, fragment ions are generated from the target peptide, the sum of XICs can further enhance the assay sensitivity.

As shown in Figure 5, summing of multiple dominant fragment ions can achieve up to a 3-fold improvement in LLOQ. Strong linearity was achieved for quantification with single fragment ions and summed multiple fragment ions (Figure 6). Accuracy at the LLOQ was within 80%-120%, while for all other non-zero calibrators, accuracy was within 85%-115% of the nominal concentration. Overall, precision was <14%, demonstrating high reproducibility (Table 4).

As discussed earlier, the Zeno trap provides added sensitivity enhancement through improvements in duty cycle. The cumulative gain from the use of the Zeno trap and summation of highly abundant fragment ions enhances overall assay sensitivity (Figure 7).

Table 3. Accuracy and precision values per concentration level for Zeno MRM^{HR}. Excellent accuracy and precision was achieved using the Zeno trap.

P05413_LILTLTHGTAVC[CAM]TR y12			P02741_ESDTSYVSLK y6			P63165_FLFEGQR y5		
Concentration (ng/mL)	%CV	Accuracy (%)	Concentration (ng/mL)	%CV	Accuracy (%)	Concentration (ng/mL)	%CV	Accuracy (%)
1177.33	1.78	100.43	*1842.46*	*1.66*	*97.90*	*3103.01*	*5.27*	*99.24*
412.06	3.20	*102.60*	*644.86*	*5.54*	*106.65*	*1086.05*	*2.87*	*101.85*
144.22	*5.22*	*91.90*	*225.70*	*3.61*	*99.43*	*380.12*	*3.60*	*104.36*
50.48	*11.05*	*91.71*	*78.99*	*3.92*	*96.48*	*133.04*	*12.48*	*92.80*
17.67	*0.70*	*98.30*	*27.65*	*8.66*	*99.54*	*46.56*	*8.27*	*87.47*
6.18	*9.38*	*102.62*	*N/A*	*N/A*	*N/A*	*16.30*	*14.75*	*114.28*
2.16	*13.00*	*112.44*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*

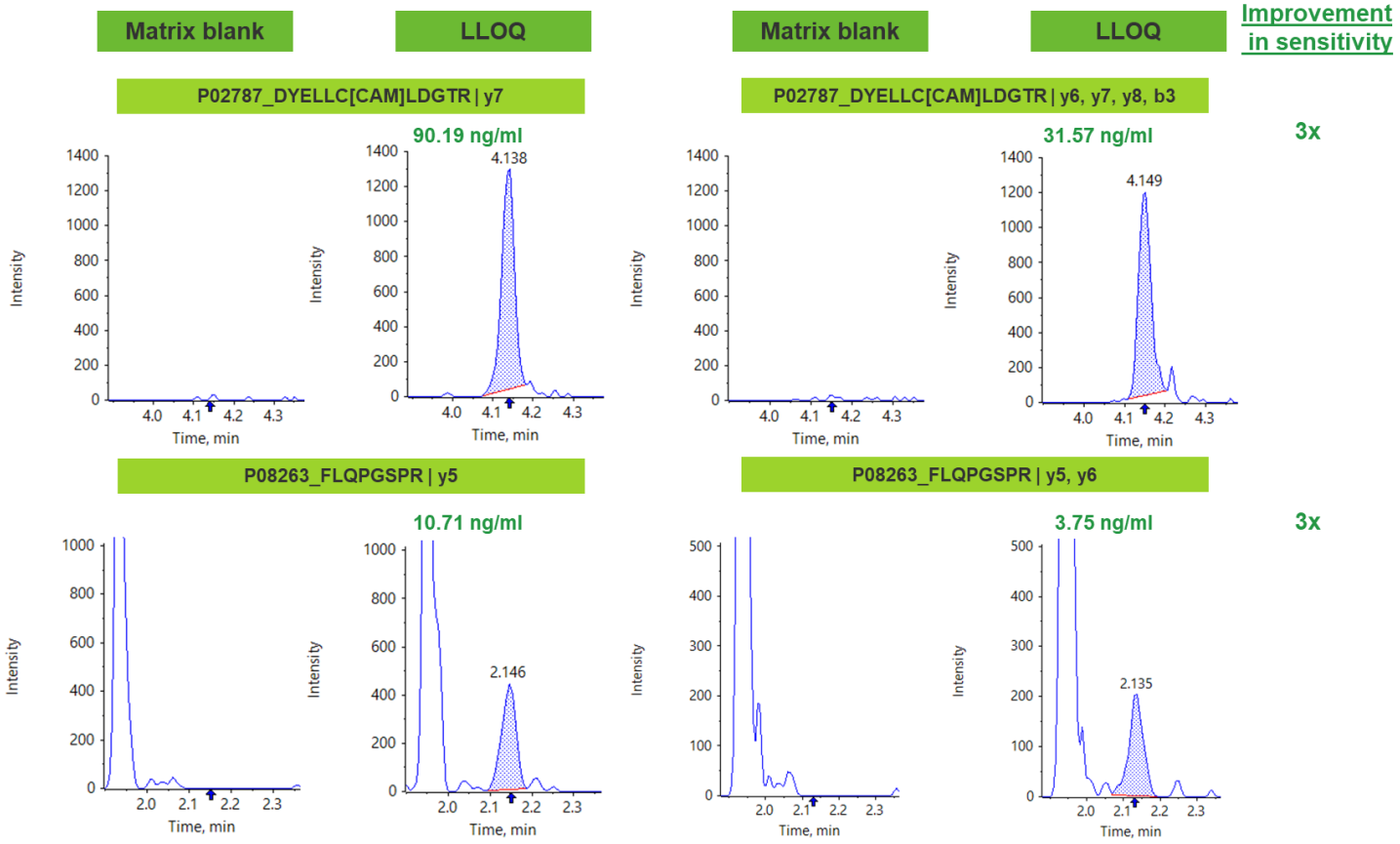

Figure 5. Summation of multiple fragment ions enhances assay sensitivity. A 3-fold improvement in LLOQ was achieved with quantification using summation of multiple dominant fragment ions.

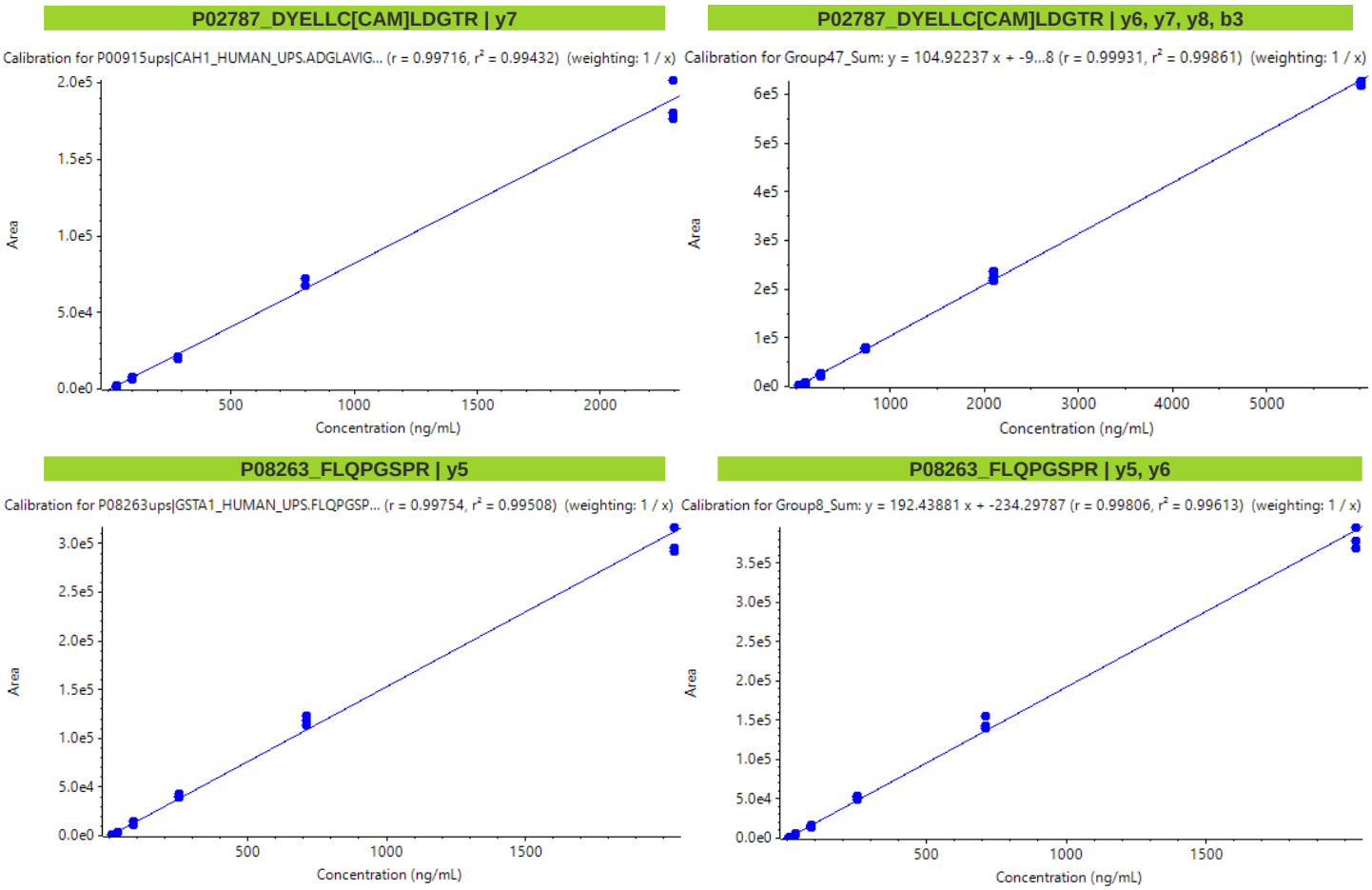

Figure 6. Strong linearity was achieved for all quantification using single fragment ion and summation of multiple fragment ions.

Table 4. Accuracy and precision values per concentration level. Excellent accuracy and precision was achieved for quantification using single fragment ion and summation of multiple fragment ions on the ZenoTOF 7600 system.

P02787_DYELLC[CAM]LDGTR y7			P02787_DYELLC[CAM]LDGTR y6, y7 , y8, b3		P08263_FLQPGSPR y5			P08263_FLQPGSPR y5, y6	
Concentration (ng/mL)	%CV	Accuracy (%)	%CV	Accuracy (%)	Concentration (ng/mL)	%CV	Accuracy (%)	%CV	Accuracy (%)
6010.25	1.19	99.07	*0.68*	*99.07*	*2038.56*	*1.75*	*95.78*	*3.44*	*97.21*
2103.59	3.50	*102.49*	*4.20*	*102.80*	*713.50*	*2.62*	*109.18*	*5.27*	*106.37*
736.25	*4.89*	*101.16*	*1.01*	*103.45*	*249.72*	*3.60*	*108.86*	*5.37*	*108.19*
257.69	*5.40*	*98.28*	*6.90*	*91.37*	*87.40*	*3.01*	*100.79*	*13.87*	*91.93*
90.19	*11.31*	*98.99*	*13.70*	*87.29*	*30.59*	*5.04*	*96.47*	*8.03*	*97.24*
31.57	*N/A*	*N/A*	*8.14*	*116.02*	*10.71*	*4.51*	*88.92*	*4.77*	*85.54*
N/A	*N/A*	*N/A*	*N/A*	*N/A*	*3.75*	*N/A*	*N/A*	*4.37*	*113.53*

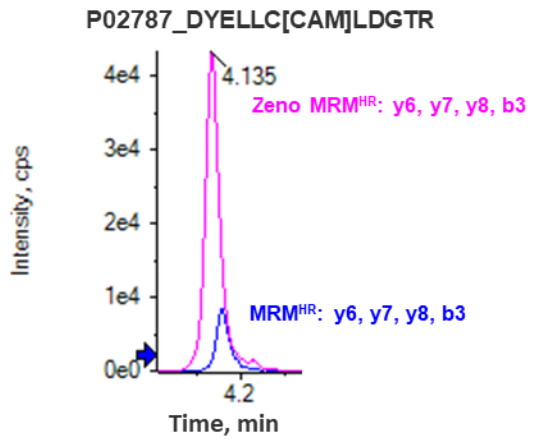

Figure 7. Cumulative gain in sensitivity from Zeno trap and summation of multiple abundant fragment ions. Summed XICs of multiple fragment ions for both Zeno MRM^{HR} and MRM^{HR} are displayed.

Conclusions

- An average of 5-fold in LLOQ improvement was achieved for peptide quantification in this sample set using the Zeno trap, which enhances the duty cycle through the accumulation of ions during each TOF pulse
- Improved LLOQs were reached by summing of multiple highly abundant fragment ions along with the availability of TOF MS/MS data, resulting in a 3-fold enhancement in LLOQ
- A highly accurate and reproducible quantitative workflow for peptides was demonstrated using the ZenoTOF 7600 system

References

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2. Yuan-Qing Xia, Jim Lau, Timothy Olah, Mohammed Jemal (2011). Targeted quantitative bioanalysis in plasma using liquid chromatography/high-resolution accurate mass spectrometry: an evaluation of global selectivity as a function of mass resolving power and extraction window, with comparison of centroid and profile modes. *Rapid Communications in Mass Spectrometry* **25**(19):2863-2878.

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