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

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ABSTRACT

Given the increased focus on producing new protein and peptide therapeutics, there is a resulting demand for highly sensitive and robust quantitative bioanalytical techniques to ensure proper testing for their safety and efficacy. Bioanalysis of peptide therapeutics is often faced with analytical challenges such as inadequate sensitivity and complexity of the matrix resulting in poor selectivity. High-resolution accurate mass spectrometry (HRAMS) has been increasingly adopted in bioanalytical workflows as it provides high selectivity with narrow mass extraction windows. As part of this work, evaluation of enhanced duty cycle by a novel ion beam to time-of-flight (TOF) pulser efficiency was performed. The new quantitative enhancements were evaluated for peptide quantification in a complex biological matrix.

INTRODUCTION

- Traditional workflows for quantitative bioanalyses, such as immunological assays, have been displaced by LC-MS/MS analysis on a triple quadrupole mass spectrometer. Immunoassays often lack selectivity, specificity, and have a limited linear dynamic range
- While the triple quadrupole platform provides excellent sensitivity and quantitative performance, there can be some limitations with background interference based on the lower resolution considering the type of mass analyzer. Background interference is a common issue for workflows where analytes are present in a highly complex matrix. High-resolution accurate mass spectrometry (HRAMS) has been increasingly adopted for quantitative bioanalysis[1,2]
- The ZenoTOF 7600 system offers an exceptional combination of mass resolution, sensitivity, and acquisition speed for quantitative analysis. It also aids in more accurate and automated integration, the potential for less ion path tuning, the ability to change measured fragments post-acquisition, and improved reproducibility and LDR when interferences are mitigated
- These attributes complement the excellent sensitivity of a nominal mass triple quadrupole system for a biopharmaceutical lab which requires a full range of capabilities

MATERIALS AND METHODS

Sample preparation:
Digested universal proteomics standard (Sigma Aldrich, 48 human proteins) was spiked into rat plasma and serially diluted to generate a calibration curve. Samples were denatured using N-octyl-glucoside followed by reduction using dithiothreitol, and alkylation using iodoacetamide. Protease digestion was performed using Trypsin/Lys-C at 37ºC overnight, at an enzyme:protein ratio of 1:25. Digestion was stopped using formic acid. Supernatant was subjected to LC-MS/MS analysis.

LC conditions:
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 40ºC.

MS conditions:
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 software 2.0. 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 for both MS/MS and MS1 quantification.

RESULTS

- 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
- The Zeno trap ensures greater ion transmission by controlling the ion beam from the collision cell into the TOF accelerator (Figure 1)

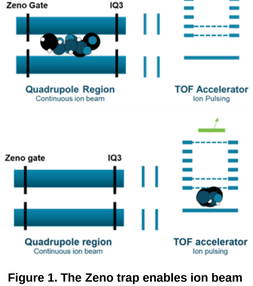

Figure 1. The Zeno trap enables ion beam control from the collision cell before entering the TOF accelerator.

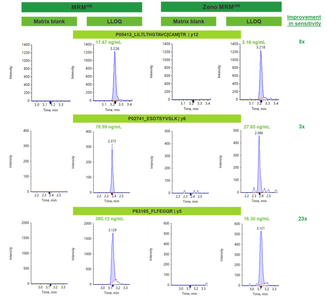

Figure 2. Greater sensitivity for peptide quantification was observed for Zeno MRM^{HR}.

- Significantly lower LLOQs were achieved using the Zeno trap
- The level of improvement in sensitivity varied across the peptide of interest (Figure 2)

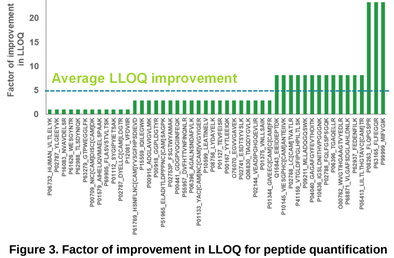

Figure 3. Factor of improvement in LLOQ for peptide quantification with Zeno MRM^{HR} compared to MRM^{HR}.

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

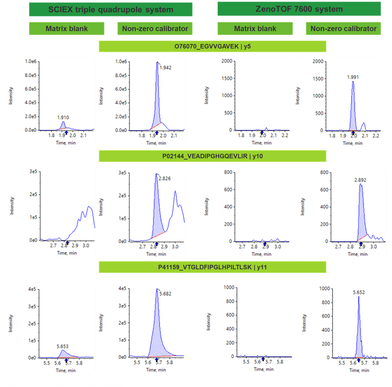

Figure 4. ZenoTOF 7600 system offered improved mass resolution between the target peptide and matrix-related components.

- Enhanced selectivity was observed when comparing the XICs of the matrix blank and non-zero calibrator between a triple quadrupole system and the ZenoTOF 7600 system (Figure 4)
- No matrix interferences were observed in the XIC of the matrix blank on a ZenoTOF 7600 system
- Improved mass resolution between target peptide and co-eluting components allowed for a more accurate and automated peak integration

- 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}
- A 3-fold improvement in LLOQ was achieved with quantification using summation of multiple dominant fragment ions (Figure 5)

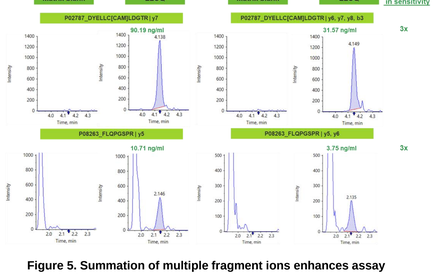

Figure 5. Summation of multiple fragment ions enhances assay sensitivity.

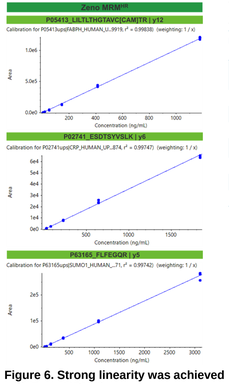

Figure 6. Strong linearity was achieved with Zeno MRM^{HR}.

Table 1. Accuracy and precision values per concentration level for Zeno MRM^{HR}.

P05413_LILTLTHGTAVC[CAM]TR y12			P02741_ESDTSYVSLK y6			P63165_FLFEGQR y5		
Conc. (ng/mL)	CV (%)	Accuracy (%)	Conc. (ng/mL)	CV (%)	Accuracy (%)	Conc. (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

- Strong linearity was achieved for all peptides analyzed (Figure 6)
- Excellent accuracy and precision was achieved using the Zeno trap (Table 1)
- 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

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
- A highly accurate and reproducible quantitative workflow for peptides was demonstrated using the ZenoTOF 7600 system
- Higher sensitivity, based on LLOQ levels, was achieved for peptide quantification using the Zeno trap by improving duty cycle through the accumulation of ions during each TOF pulse for enhanced duty cycle
- Greater selectivity was reached between target peptides and matrix-related components with the higher mass resolution offered by the ZenoTOF 7600 system
- 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
- Automated and accurate peak integration was easily attainable on the ZenoTOF 7600 system, with greater mass resolution ensuring overall data integrity

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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.

TRADEMARKS/LICENSING