Improving peptide quantification using a novel accurate mass QTOF system

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ABSTRACT

The development of peptide and protein therapeutics has been rapidly and continuously growing every year. During the process of drug development, it is necessary to examine factors such as pharmacokinetics, metabolism and the overall stability of the therapeutics. Therefore, highly sensitive and selective quantification methods are needed. While quantification of peptides in biological matrices is typically performed using nominal mass instruments, some analytical methods are being transferred onto the accurate mass instruments to gain added selectivity. However, accurate mass instruments are still faced with critical challenges, including reaching the desired sensitivity and linear dynamic range (LDR). In this study, a novel accurate mass system was employed to achieve ultra-sensitive quantification of peptides in matrix.

INTRODUCTION

This novel QTOF instrument utilizes the Zeno trap to trap and release ions. Ions are first accumulated in a short linear ion trap at the end of the collision cell, then released based on their potential energy in a release of ions generally in sequence by their mass-to-charge ratio (*m/z*) from large to small. Ions from a wide *m/z* range are simultaneously collected in the accelerator region. This enhances MS/MS sensitivity due to improved duty cycle. The sensitivity gain is *m/z*-dependent (e.g., 14x-gain for *m/z* 100 Da and a 3x-gain for *m/z* 2000 Da).

During Zeno trap operation, a good portion of the TOF pulses are empty, causing the detector to saturate at an ion intensity about 10x lower than when the Zeno trap is not used. To ensure the widest dynamic range is achieved on the system for quantification, the Zeno trap is activated dynamically during acquisition based on an intensity threshold. When the ion intensity is below the intensity threshold, the Zeno trap is activated to increase sensitivity and the lower limit of quantification (LLOQ). When the ion intensity rises above the threshold, it is not activated to prevent detector saturation and extend the upper limit of quantification (ULOQ). This intensity threshold is called the Zeno trap threshold and it can be adjusted to achieve the best quantitative performance.

MATERIALS AND 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. In addition, 1 pmol/µL stock solution containing a mixture of isotopically labeled synthetic peptides was diluted into the matrix at various concentrations from 0.0017 to 500 fmol/µL.

HPLC conditions:
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). The column temperature was set at 40°C and 2 µL of the sample was injected on to the column.

Table 1. Microflow gradient.

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

MS/MS conditions:
A ZenoTOF 7600 system with the OptiFlow Turbo V ion source and a 25 µm electrospray ionization (ESI) electrode was used. Source conditions (i.e., GS1, GS2 and TEMP) were optimized. A method based on the Scheduled MRM^{HR} algorithm in positive ion mode was used for acquisition with the retention time window set at ±30 s (Table 2). The mass range in TOF-MS was 400–1,500 Da with a 100 ms accumulation time. The mass range in MS/MS was 100–1,500 Da with a 50 ms of accumulation time. The data were acquired with and without the use of the Zeno trap. The Zeno trap threshold was set at 20,000 during the Zeno trap acquisition.

Table 2. The method based on Scheduled MRM^{HR} algorithm, used for acquisition.

Compound	Adduct/ charge	Precursor (*m/z*)	CE (V)	Fragment (*m/z*)	Retention time (min)
GAYVEVTAK	$[M+H]^{2+}$	473.26020	29	136.07569	7.5
LDSTSIPVAK	$[M+H]^{2+}$	519.79969	31	422.28529	8.0
AGLIVAEGVTK	$[M+H]^{2+}$	533.32333	32	711.41268	8.8
AVGANPEQLTR	$[M+H]^{2+}$	583.31360	34	753.41290	7.7
SAEGLDASASLR	$[M+H]^{2+}$	593.80053	34	729.37651	8.2
VFTPLEVDVAK	$[M+H]^{2+}$	613.34955	36	878.50731	9.9
VGNEIQYVALR	$[M+H]^{2+}$	636.35273	37	759.43872	9.3
YIELAPGVDNSK	$[M+H]^{2+}$	657.34499	38	724.37154	8.9
DGTFAVDGPGVIAK	$[M+H]^{2+}$	677.85827	39	764.43923	9.3
BSA (Internal standard)	$[M+H]^{2+}$	582.31897	34	185.16483	9.7

RESULTS

During acquisition with the Scheduled MRM^{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 1 illustrates how this process works with the Zeno trap threshold set at 20,000. The ion intensity obtained when the Zeno trap is off is multiplied by the Zeno trap gain factor to help ensure a smooth chromatographic appearance.

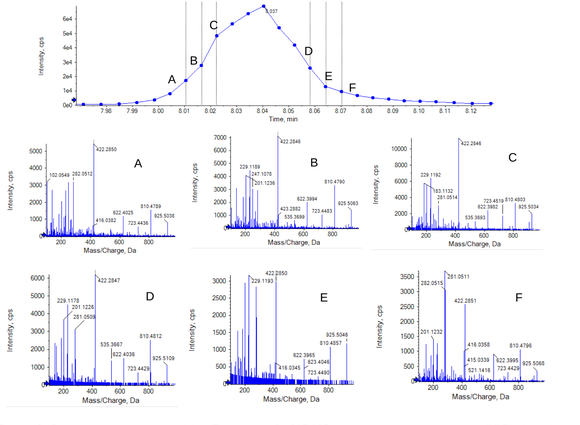
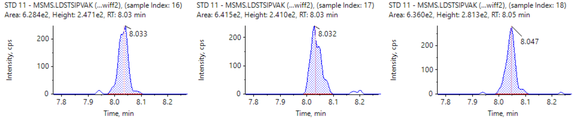
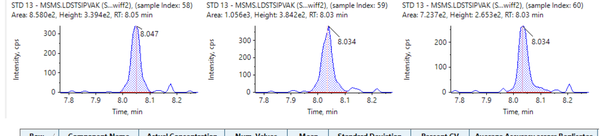


Figure 1. On-demand operation of the Zeno trap. An MS/MS extracted ion chromatogram (XIC) of peptide LDSTSIPVAK at fragment *m/z* 422.28 Da (top) and MS/MS spectra of this peptide at 6 different MS/MS scans (A to F). The sum intensity of the fragment at *m/z* 422.28 was 17,000 at scan A, and increased to 28,000 at scan B, which was above the Zeno trap threshold of 20,000, so the Zeno trap was turned off at the next MS/MS scan (scan C). The sum intensity of *m/z* 422.28 was 27,000 at scan D, and decreased to 13,000 at scan E, which is below the Zeno trap threshold, so the Zeno trap was back on at the next MS/MS scan (scan F).



Row	Component Name	Actual Concentration	Num. Values	Mean	Standard Deviation	Percent CV	Average Accuracy across Replicates
5	MSMS.LDSTSIPVAK	0.06554	3 of 3	6.692e-2	5.421e-4	0.81	102.10
6	MSMS.LDSTSIPVAK	0.16384	3 of 3	1.546e-1	6.605e-3	4.27	94.38
7	MSMS.LDSTSIPVAK	0.40960	3 of 3	4.172e-1	2.005e-2	4.81	101.86
8	MSMS.LDSTSIPVAK	1.02400	3 of 3	9.812e-1	5.890e-2	6.00	95.82
9	MSMS.LDSTSIPVAK	2.56000	3 of 3	2.678e0	1.686e-1	6.30	104.60
10	MSMS.LDSTSIPVAK	16.00000	3 of 3	1.592e1	4.936e-1	3.10	99.50
11	MSMS.LDSTSIPVAK	40.00000	3 of 3	4.033e1	9.881e-1	2.45	100.83
12	MSMS.LDSTSIPVAK	100.00000	3 of 3	1.018e2	1.509e0	1.48	101.79
13	MSMS.LDSTSIPVAK	250.00000	3 of 3	2.558e2	4.328e0	1.69	102.31
14	MSMS.LDSTSIPVAK	500.00000	3 of 3	4.841e2	3.299e0	0.68	96.81

Figure 2. An MS/MS XIC of 3 replicates of the LLOQ of LDSTSIPVAK (top), and the accuracy and percent CV of all standards (bottom) obtained without the Zeno trap.



Row	Component Name	Actual Concentration	Num. Values	Mean	Standard Deviation	Percent CV	Average Accuracy across Replicates
3	MSMS.LDSTSIPVAK	0.01049	2 of 3	1.072e-2	1.441e-3	13.44	102.24
4	MSMS.LDSTSIPVAK	0.02621	3 of 3	2.496e-2	2.248e-3	9.01	95.20
5	MSMS.LDSTSIPVAK	0.06554	3 of 3	6.750e-2	3.864e-3	5.72	103.00
6	MSMS.LDSTSIPVAK	0.16384	3 of 3	1.624e-1	9.830e-3	6.05	99.13
7	MSMS.LDSTSIPVAK	0.40960	3 of 3	4.088e-1	6.788e-3	1.66	99.81
8	MSMS.LDSTSIPVAK	1.02400	3 of 3	1.047e0	4.064e-2	3.88	102.20
9	MSMS.LDSTSIPVAK	2.56000	3 of 3	2.475e0	8.088e-2	3.27	96.67
10	MSMS.LDSTSIPVAK	6.40000	3 of 3	6.287e0	1.729e-1	2.75	98.23
11	MSMS.LDSTSIPVAK	16.00000	3 of 3	1.675e1	2.740e-1	1.64	104.67
12	MSMS.LDSTSIPVAK	40.00000	3 of 3	4.100e1	8.705e-1	2.12	102.50
13	MSMS.LDSTSIPVAK	100.00000	3 of 3	1.006e2	1.369e0	1.36	100.56
14	MSMS.LDSTSIPVAK	250.00000	3 of 3	2.512e2	8.502e0	3.38	100.47
15	MSMS.LDSTSIPVAK	500.00000	3 of 3	4.803e2	9.502e0	1.98	96.07

Figure 3. An MS/MS XIC of 3 replicates of the LLOQ of LDSTSIPVAK (top), and the accuracy and percent CV of all standards (bottom) with the Zeno trap.

Figure 2 and Figure 3 show the results of MS/MS quantification without and with the Zeno trap for one of the peptides examined. The LLOQ decrease of more than 6x, from 0.06554 fmol/µL to 0.01049 fmol/µL, was observed for this peptide when the Zeno trap was used.

Table 3 is the summary of the MS/MS quantification results for all peptides without and with the Zeno trap.

On average, 6x lower LLOQs were obtained for the 9 peptides included in this study. With on-demand operation of the Zeno trap, these peptides were able to achieve the same ULOQ levels reached when the Zeno trap was not used. Overall, the LDRs of these peptides were extended to the lower concentration end of the calibration curve for more than a half order.

Table 3. Gains in LLOQ, ULOQ and LDR using the Zeno trap in peptide quantification.

MS/MS	Without the Zeno trap			With the Zeno trap			Gain with the Zeno trap		
Peptide	LLOQ (fmol/µL)	ULOQ (fmol/µL)	LDR (order)	LLOQ (fmol/µL)	ULOQ (fmol/µL)	LDR (order)	LLOQ (fold)	ULOQ (fold)	LDR (order)
GAYVEVTAK	0.06554	500	3.88	0.01049	500	4.68	6.25	1	0.8
LDSTSIPVAK	0.16384	500	3.48	0.02621	500	4.28	6.25	1	0.8
AGLIVAEGVTK	0.06554	250	3.58	0.01049	250	4.38	6.25	1	0.8
AVGANPEQLTR	0.06554	500	3.88	0.02621	500	4.28	2.5	1	0.4
SAEGLDASASLR	0.06554	500	3.88	0.01049	500	4.68	6.25	1	0.8
VFTPLEVDVAK	0.06554	100	3.18	0.02621	100	3.58	2.5	1	0.4
VGNEIQYVALR	0.06554	500	3.88	0.01049	500	4.68	6.25	1	0.8
YIELAPGVDNSK	0.06554	250	3.58	0.01049	250	4.38	6.25	1	0.8
DGTFAVDGPGVIAK	0.16384	500	3.48	0.02621	500	4.28	6.25	1	0.8

CONCLUSIONS

The Zeno trap implemented in this novel QTOF mass spectrometer takes advantage of the sensitivity enhancements of the Zeno trap and overcomes early detector saturation with its on-demand operation, resulting in improved LLOQs without compromising ULOQs in peptide quantification.

REFERENCES

1. Chernushevich, I. et al. (2017) J. Am. Soc. Mass Spectrom., 28(10), 2143-2150.
2. Loboda, A. et al. (2009) J. Am. Soc. Mass Spectrom., 20(7), 1342–1348.

TRADEMARKS/LICENSING