

MAY  
11-14  
2026

aaps  
**National**  
Biotechnology  
CONFERENCE

SHERATON  
SAN DIEGO RESORT  
SAN DIEGO, CA



# Advancing Bioanalysis with HRMS-based Quantitation

Tuesday, May 12, 2026

2:30 PM – 3:15 PM

Shane Needham, PhD



# Session Description and Objectives

This session will provide a comprehensive introduction to HRMS-based quantitation for bioanalysis, focusing on strategies that enhance sensitivity and selectivity while meeting bioanalytical guidelines.

Case studies will reflect accurate quantitation of peptides and small molecules within complex biological matrices.

Attendees will also gain insight into achieving robust, reproducible and accurate results, ensuring confidence in both HRMS method development and routine analysis.

- Utilize MRM<sup>HR</sup> workflows for quantitation of peptides and small molecules in complex biological matrices
- Leverage HRMS for high selectivity capabilities to minimize interferences and reduce background for improved quantitative fidelity.
- Deliver robust quantitative performance with reproducibility and accuracy aligned with bioanalytical guidelines.

# Biography and Contact Information



**Shane Needham, Ph.D.**

President and CEO, Velocity Labs

[shane.needham@velocitylabs.com](mailto:shane.needham@velocitylabs.com)

Shane is a leader in bioanalytical mass spectrometry, with decades of experience advancing LC-MS workflows and drug development programs.

# Why HRMS for pharma applications?

Selectivity

Improved selectivity for complex assays with high background or matrix interference compared to nominal mass spectrometry-based analysis

Flexibility

Flexibility to choose a quantitation mode that is most selective and sensitive

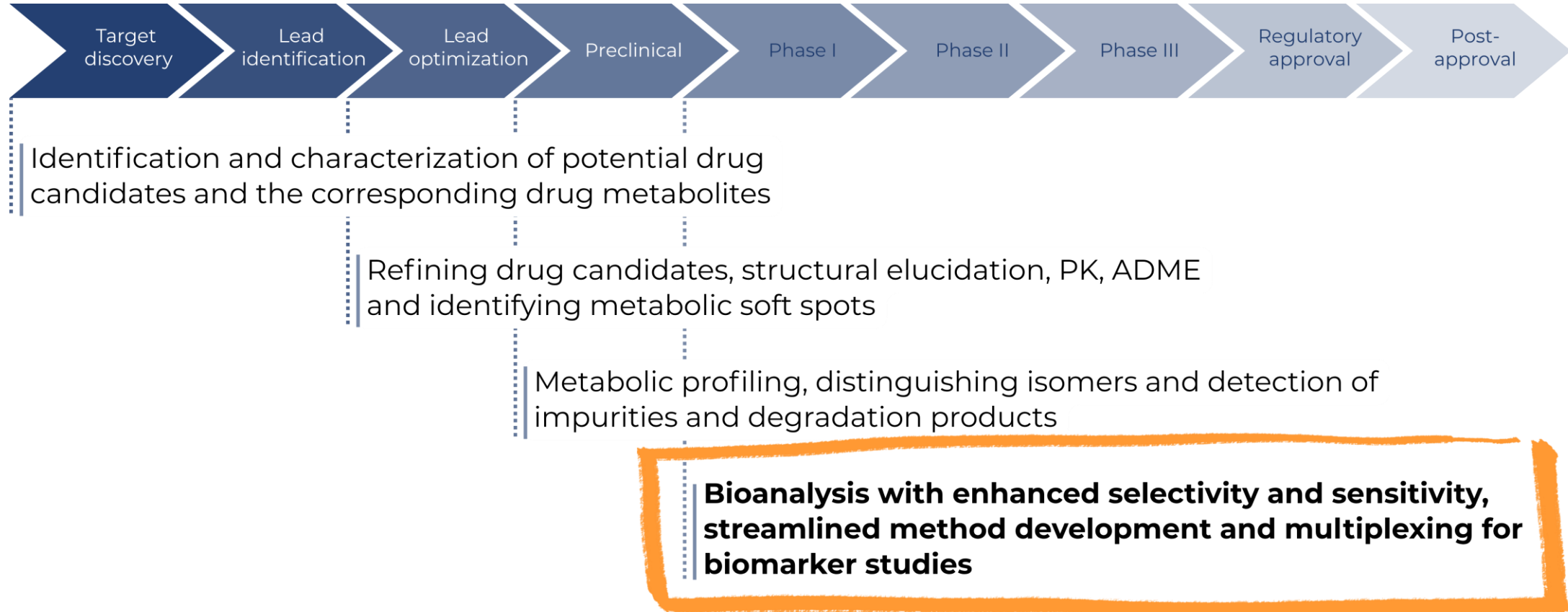
Versatility

Capability to perform both qualitative and quantitative workflows on one MS platform

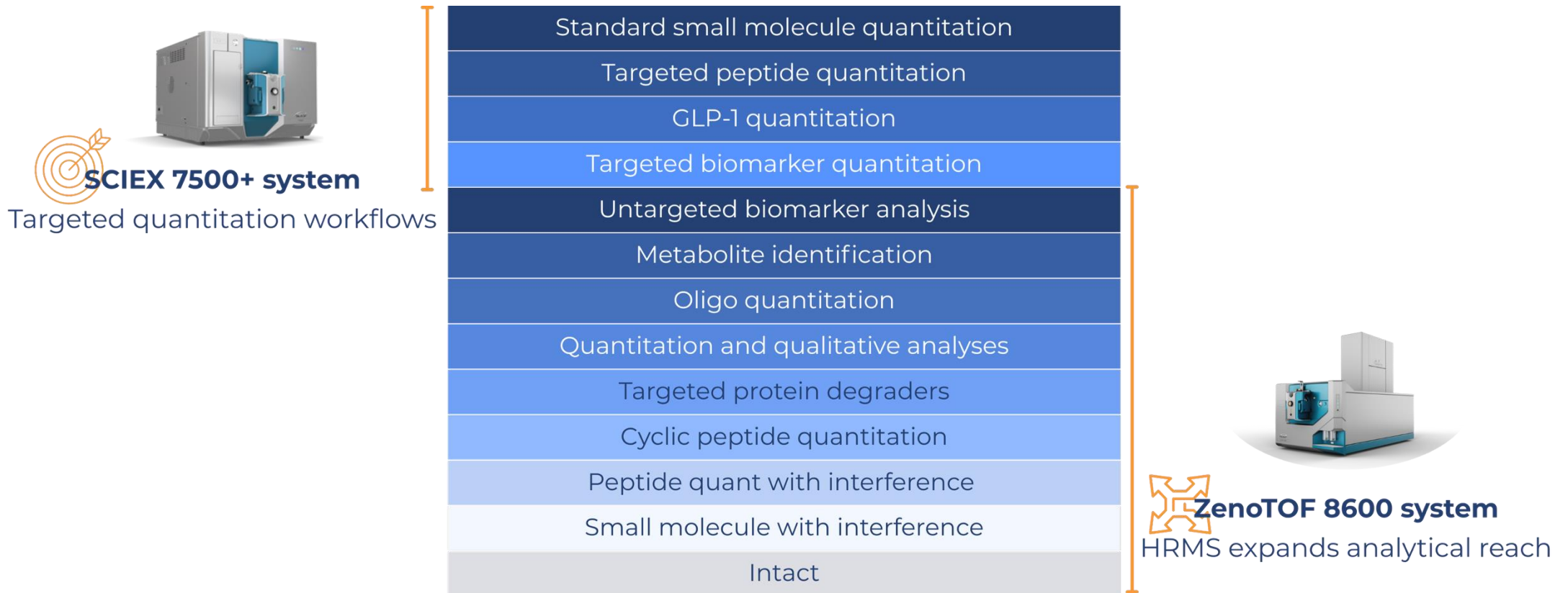
Efficiency

Reduction of method development time with less ion path tuning

# How HRMS is shaping the future of pharma

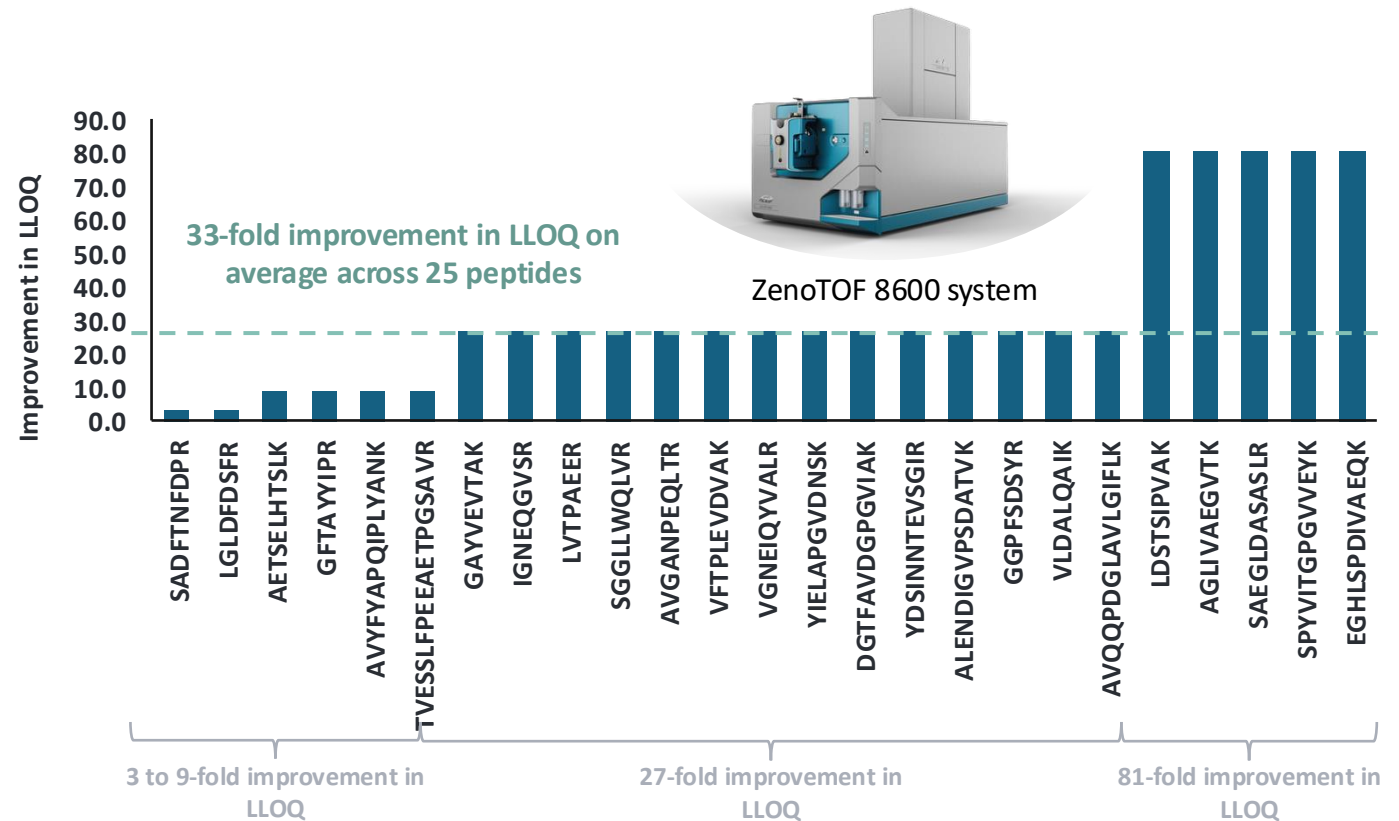


# Crushing complexity: HRMS-powered analytical breakthroughs



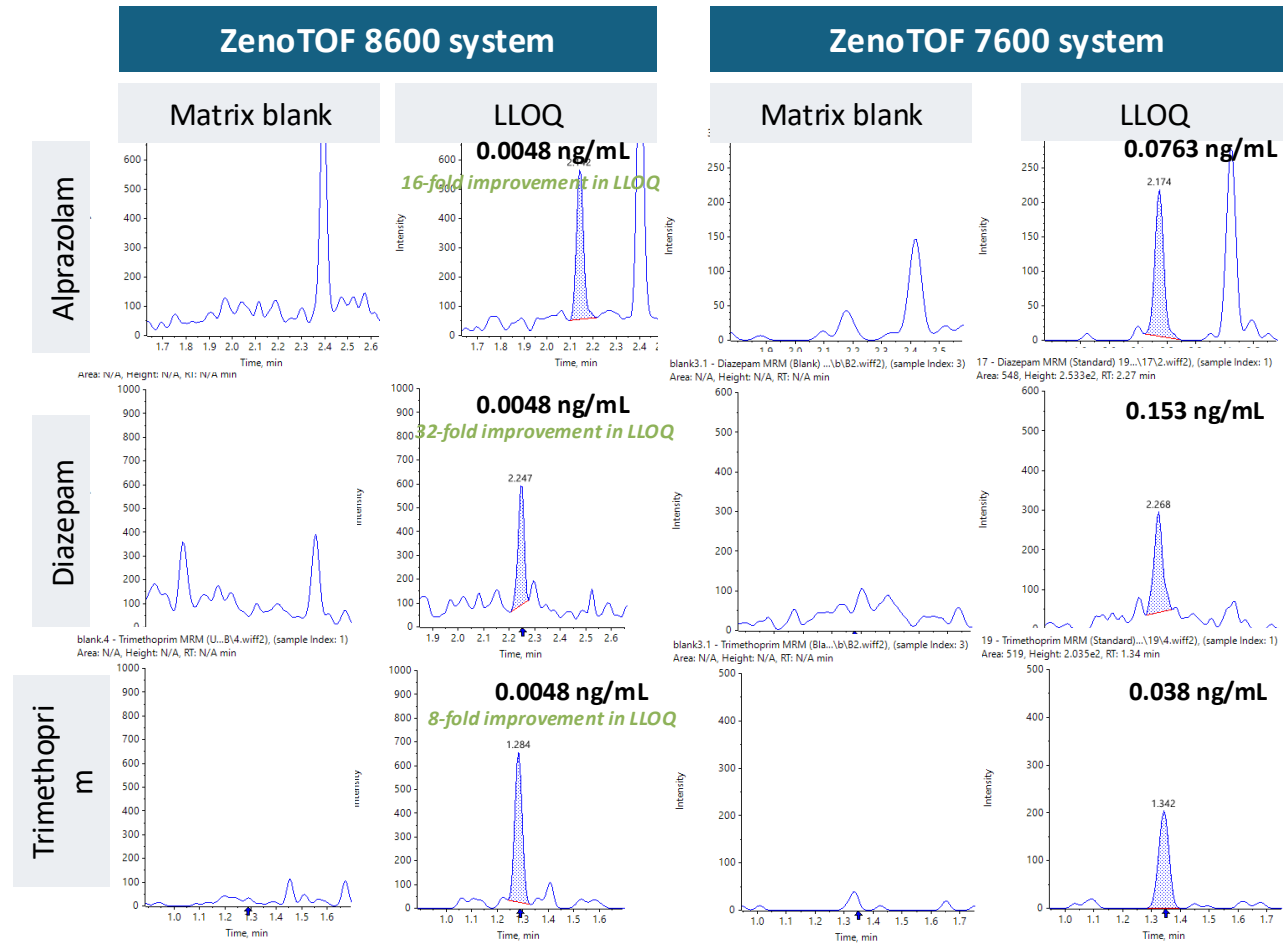
# Enhanced quantitative sensitivity for small molecule bioanalytical methods

- 6 small molecules were evaluated in extracted rat plasma using the ZenoTOF 8600 system compared to the ZenoTOF 7600 system
- Zeno MRMHR-based quantitation of each small molecule was performed using a single fragment ion
- On average, an 18-fold improvement in LLOQ was observed across 6 small molecules on the ZenoTOF 8600 system



# LLOQ XICs of representative small molecules highlight enhanced sensitivity

- A significantly lower LLOQ was achieved using the ZenoTOF 8600 system
- Alprazolam, diazepam and trimethoprim showed a 16-, 32- and 8-fold improvement in LLOQ, respectively
- No matrix interferences were observed in the blank XICs

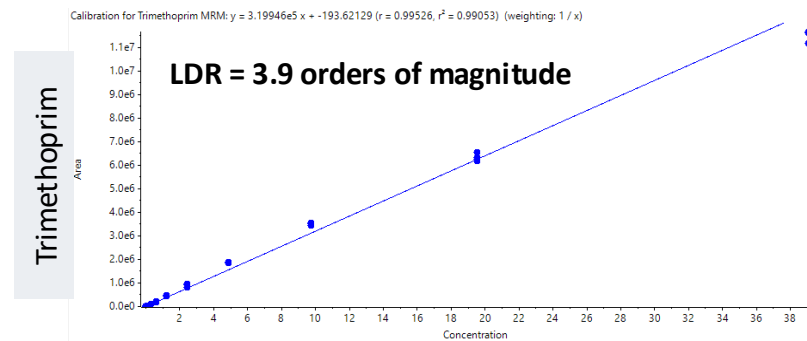
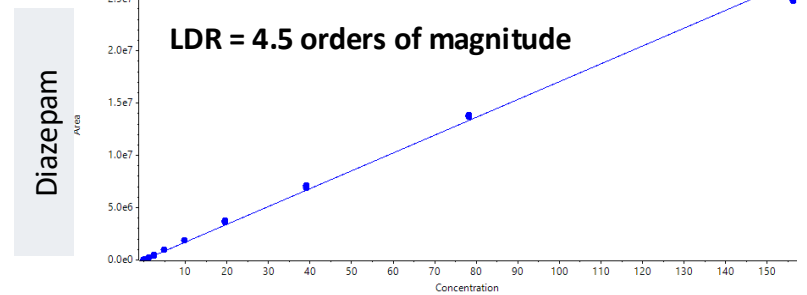
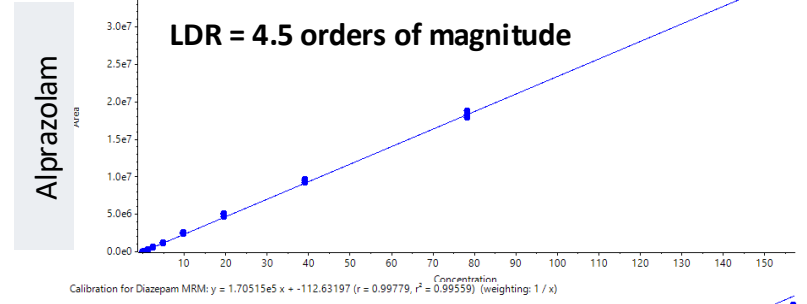


# Quantitative performance beyond the benchmark with Zeno MRM<sup>HR</sup>

Row	Component Name	Actual Concentration	Num. Values	Mean	Standard Deviation	Percent CV	Average Accuracy across Replicates
1	Alprazolam MRM	0.0048	4 of 4	0.01	0.00	12.3	107.
2	Alprazolam MRM	0.0095	3 of 4	0.01	0.00	9.93	103.
3	Alprazolam MRM	0.0191	3 of 4	0.02	0.00	8.04	84.5
4	Alprazolam MRM	0.0763	4 of 4	0.07	0.00	6.10	87.2
5	Alprazolam MRM	0.1526	4 of 4	0.15	0.01	7.93	95.8
6	Alprazolam MRM	0.3052	4 of 4	0.28	0.02	7.66	92.7
7	Alprazolam MRM	0.6104	4 of 4	0.59	0.01	1.92	96.7
8	Alprazolam MRM	1.2207	4 of 4	1.21	0.08	6.31	98.8
9	Alprazolam MRM	2.4414	4 of 4	2.57	0.09	3.46	105.
10	Alprazolam MRM	4.8828	4 of 4	5.24	0.20	3.88	107.
11	Alprazolam MRM	9.7656	4 of 4	10.76	0.33	3.05	110.
12	Alprazolam MRM	19.5313	4 of 4	21.22	0.81	3.83	109.
13	Alprazolam MRM	39.0625	4 of 4	40.33	1.02	2.53	103.
14	Alprazolam MRM	78.1250	4 of 4	78.24	1.73	2.21	100.
15	Alprazolam MRM	156.2500	4 of 4	151.77	2.03	1.34	97.1

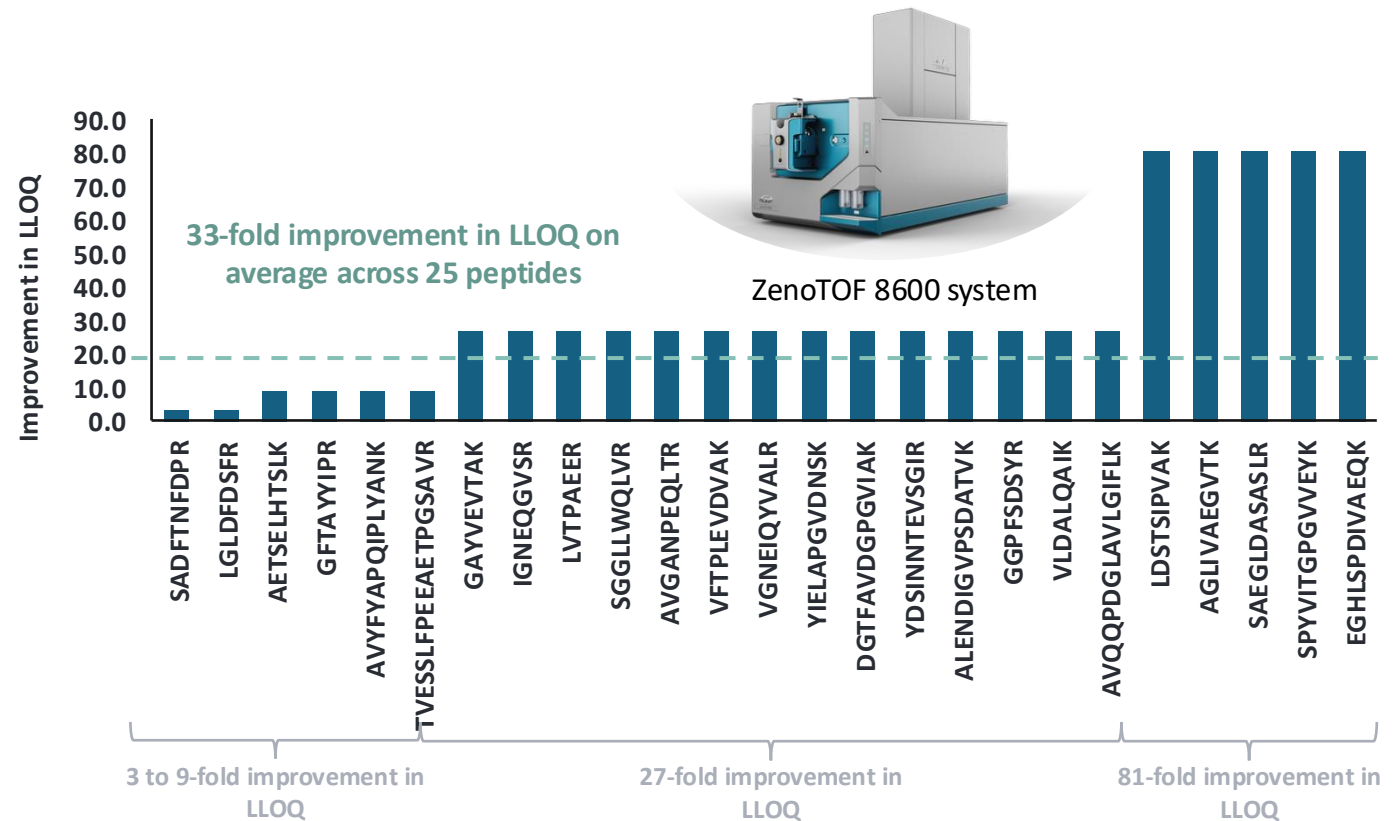
- Quantitative statistics from a representative small molecule, alprazolam is shown where assay accuracy was within  $\pm 15\%$  of the actual concentration and %CV was  $< 13$
- Calculated accuracy and %CV values were within the acceptance criteria at each concentration level (triplicate analysis)
- Wide range of concentrations were analyzed with an LDR up to 4.5 orders of magnitude

## ZenoTOF 8600 system



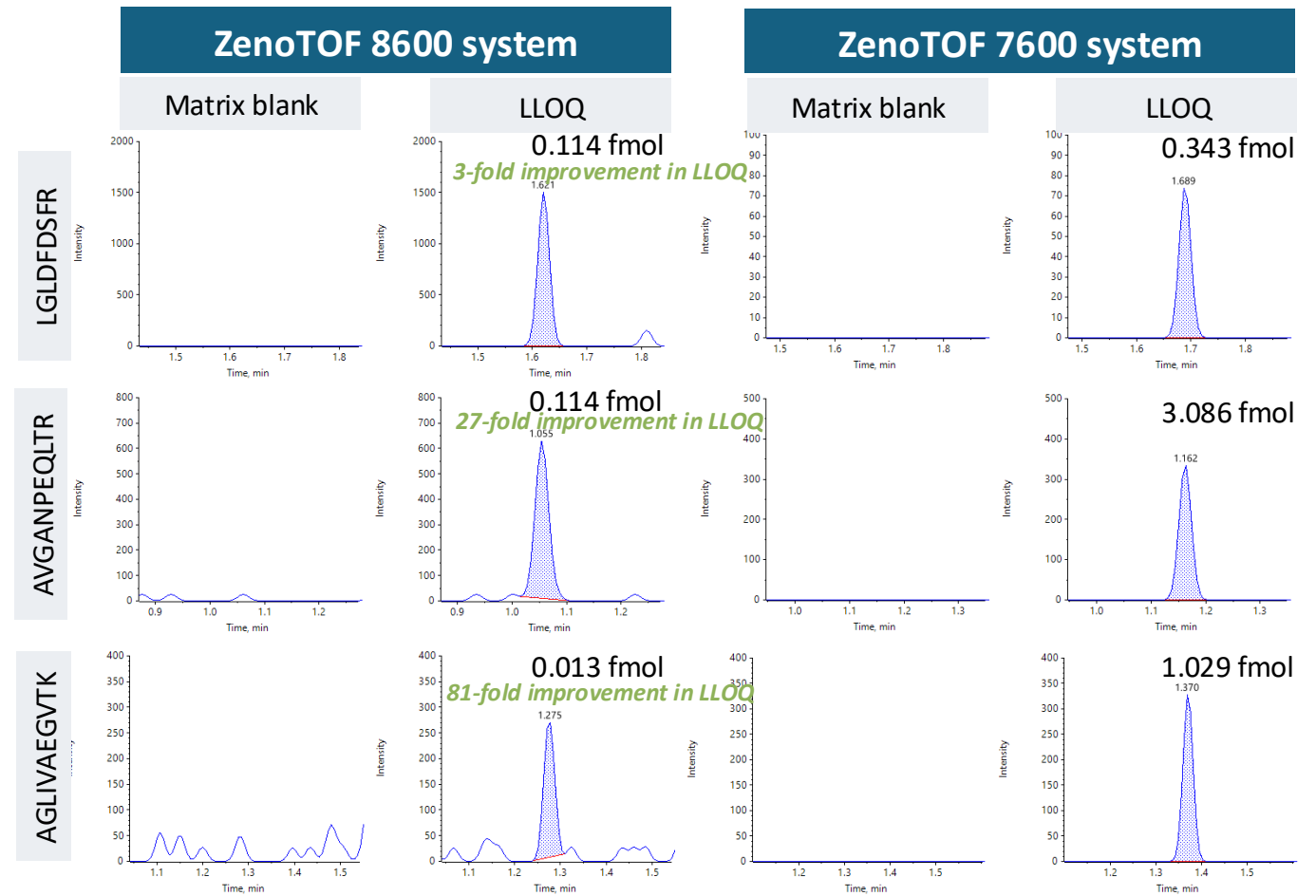
# Redefining sensitivity for targeted peptide quantitation

- 25 example peptides analyzed in extracted rat plasma using ZenoTOF 8600 system and compared with ZenoTOF 7600 system
- Zeno MRMHR approach was employed where 3 fragment ions were summed per peptide to evaluate the LLOQ
- On average, a 33-fold improvement in LLOQ was achieved across 25 peptides, with 3 examples demonstrating up to an 81-fold gain in LLOQ



# Significantly lower LLOQ observed for peptide quantitation in extracted rat plasma

- A sum of 3 fragment ions was applied for quantitation for all peptides on both MS platforms
- Peptides LGLDFDSFR, AVGANPEQLTR and AGLIVAEGVTK showed a 3-, 27- and 81-fold improvement in LLOQ, respectively
- No matrix interferences were observed in the blank XICs

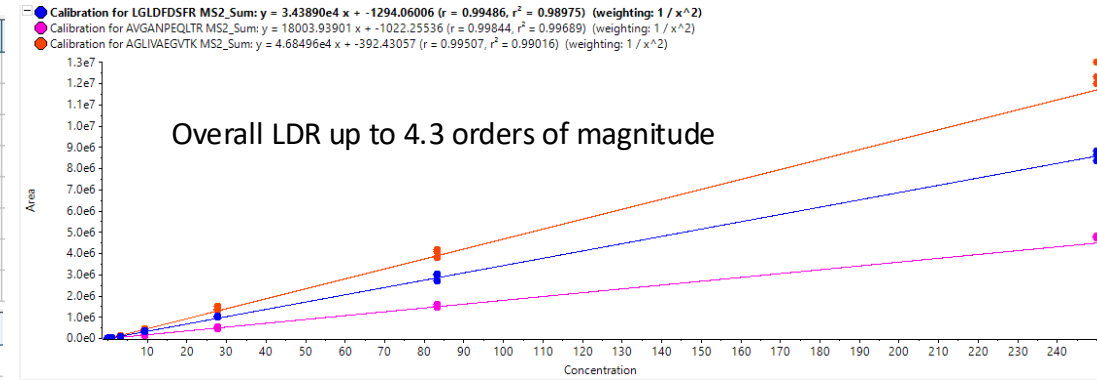


# Quantitative performance for peptide analysis on the ZenoTOF 8600 system

Row	Component Name	Actual Concentration	Num. Values	Mean	Standard Deviation	Percent CV	Average Accuracy across Replicates
1	LGLDFDSFR MS2_Sum	0.114	3 of 3	0.117	0.015	13.0	103.
2	LGLDFDSFR MS2_Sum	0.343	3 of 3	0.322	0.047	14.5	94.0
3	LGLDFDSFR MS2_Sum	1.029	3 of 3	0.948	0.112	11.8	92.1
4	LGLDFDSFR MS2_Sum	3.086	3 of 3	2.964	0.061	2.04	96.1
5	LGLDFDSFR MS2_Sum	9.259	3 of 3	9.522	0.668	7.02	103.
6	LGLDFDSFR MS2_Sum	27.778	3 of 3	30.562	0.747	2.44	110.
7	LGLDFDSFR MS2_Sum	83.333	3 of 3	84.640	4.515	5.33	102.
8	LGLDFDSFR MS2_Sum	250.000	3 of 3	251.092	6.996	2.79	100.

Row	Component Name	Actual Concentration	Num. Values	Mean	Standard Deviation	Percent CV	Average Accuracy across Replicates
1	AVGANPEQLTR MS2_Sum	0.114	2 of 3	0.117	0.003	2.21	103.
2	AVGANPEQLTR MS2_Sum	0.343	3 of 3	0.331	0.009	2.85	96.5
3	AVGANPEQLTR MS2_Sum	1.029	3 of 3	0.989	0.018	1.82	96.1
4	AVGANPEQLTR MS2_Sum	3.086	3 of 3	2.938	0.069	2.35	95.2
5	AVGANPEQLTR MS2_Sum	9.259	3 of 3	9.186	0.481	5.24	99.2
6	AVGANPEQLTR MS2_Sum	27.778	3 of 3	28.530	2.016	7.06	103.
7	AVGANPEQLTR MS2_Sum	83.333	3 of 3	84.993	3.170	3.73	102.
8	AVGANPEQLTR MS2_Sum	250.000	3 of 3	266.384	2.056	0.772	107.

Row	Component Name	Actual Concentration	Num. Values	Mean	Standard Deviation	Percent CV	Average Accuracy across Replicates
1	AGLIVAEGVTK MS2_Sum	0.038	3 of 3	0.040	0.003	7.98	104.
2	AGLIVAEGVTK MS2_Sum	0.114	3 of 3	0.104	0.011	10.2	90.8
3	AGLIVAEGVTK MS2_Sum	0.343	3 of 3	0.308	0.038	12.4	89.7
4	AGLIVAEGVTK MS2_Sum	1.029	3 of 3	0.993	0.062	6.29	96.5
5	AGLIVAEGVTK MS2_Sum	3.086	3 of 3	3.009	0.204	6.78	97.5
6	AGLIVAEGVTK MS2_Sum	9.259	3 of 3	9.450	0.493	5.22	102.
7	AGLIVAEGVTK MS2_Sum	27.778	3 of 3	30.397	2.133	7.02	109.
8	AGLIVAEGVTK MS2_Sum	83.333	3 of 3	86.075	3.823	4.44	103.
9	AGLIVAEGVTK MS2_Sum	250.000	3 of 3	265.772	11.144	4.19	106.



- Quantitative statistics from representative peptides (LGLDFDSFR, AVGANPEQLTR and AGLIVAEGVTK) were evaluated using a sum of 3 fragment ions
- Assay accuracy was within  $\pm 15\%$  of the actual concentration and %CV was  $<13$
- Calculated accuracy and %CV values were within the acceptance criteria at each concentration level (triplicate analysis)
- An LDR up to 4.3 orders of magnitude was reached, enabling measurement across a wide range of concentrations

# Streamlined automated selection of fragments for quantitation

Improved method development time by 5x

1 Provide peptide amino acid sequence and precursor ion

Process New Results with Optimized Fragments

Make sure that all of the information in the table is correct. An accurate precursor m/z gives the best results.

Find the retention time

Row	IS	Name	Precursor (Q1)	IS Name	Experiment Index
1		AETSEIHTSLK[R]	408.55		2 + TOF MSMS of 408.55 (100 - 1500)
2		GAVVEYFA[R]	473.26		3 + TOF MSMS of 473.26 (100 - 1500)
3		KINIGQVSR[10]	485.25		4 + TOF MSMS of 485.25 (100 - 1500)
4		LVSFRAER[10]	491.27		5 + TOF MSMS of 491.27 (100 - 1500)
5		LEDSFPAQR	519.8		6 + TOF MSMS of 519.8 (100 - 1500)
6		AGLHAGVYFA[R]	533.32		7 + TOF MSMS of 533.32 (100 - 1500)
7		LGLDFDFR[10]	540.27		8 + TOF MSMS of 540.27 (100 - 1500)
8		GFTRVPR[10]	549.29		9 + TOF MSMS of 549.29 (100 - 1500)
9		SGGLWQQR[10]	569.83		10 + TOF MSMS of 569.83 (100 - 1500)
10		AVGANPQLR[10]	583.31		11 + TOF MSMS of 583.31 (100 - 1500)
11		SAEGLDASLR[10]	593.8		12 + TOF MSMS of 593.8 (100 - 1500)
12		VTFRELVNKR[8]	613.35		13 + TOF MSMS of 613.35 (100 - 1500)
13		VGNKQVALR[10]	636.35		14 + TOF MSMS of 636.35 (100 - 1500)
14		YELAPGVNSKR	657.34		15 + TOF MSMS of 657.34 (100 - 1500)
15		DGTFVAGPQVAKR	677.86		16 + TOF MSMS of 677.86 (100 - 1500)
16		YDSINTEVSGVQR[10]	739.36		17 + TOF MSMS of 739.36 (100 - 1500)
17		SPYVIGGVVYK[R]	758.91		18 + TOF MSMS of 758.91 (100 - 1500)
18		ALENDKGVDSATVQR	768.9		19 + TOF MSMS of 768.9 (100 - 1500)
19		AVYVAPQPVVANKR	883.47		20 + TOF MSMS of 883.47 (100 - 1500)
20		TVESLPREATFPGVAFR[1]	964.98		21 + TOF MSMS of 964.98 (100 - 1500)
21					

2 Provide information on sample types

Process New Results with Optimized Fragments

Examine and identify sample types. A minimum of one sample must be a standard.

Row	File Name	Sample Name	Sample Type
1	1	20	Blank
2	2	20	Blank
3	3	20	Blank
4	1	11	Standard
5	2	11	Standard
6	3	11	Standard
7	1	10	Standard
8	2	10	Standard
9	3	10	Standard
10	1	9	Standard
11	2	9	Standard
12	3	9	Standard
13	1	8	Standard

3 Select whether a sum or individual fragments is preferred for quantitation

Process New Results with Optimized Fragments

Configure the settings to optimize fragment selection.

Retention time

Initial retention time tolerance: 0.1 min

Retention time drift tolerance: 0.02 min

Fragments criteria

Fragments to quantify: Best sum of individual fragments

Find fragments that match:

Quantitation:  Best sum of individual fragments

Fragments to give best:  LOQ  LDR

4 Perform fragment matching with MS/MS library spectra or theoretical fragments

Process New Results with Optimized Fragments

Configure the settings to optimize fragment selection.

Retention time

Initial retention time tolerance: 0.1 min

Retention time drift tolerance: 0.02 min

Fragments criteria

Fragments to quantify: Best sum of individual fragments

Find fragments that match:  Theoretical peptide fragments m/z

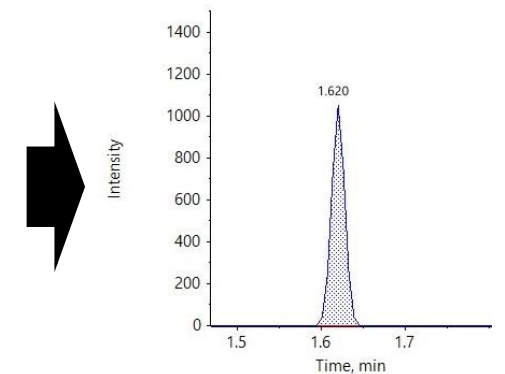
Component names must be visible:

MS/MS library spectra:

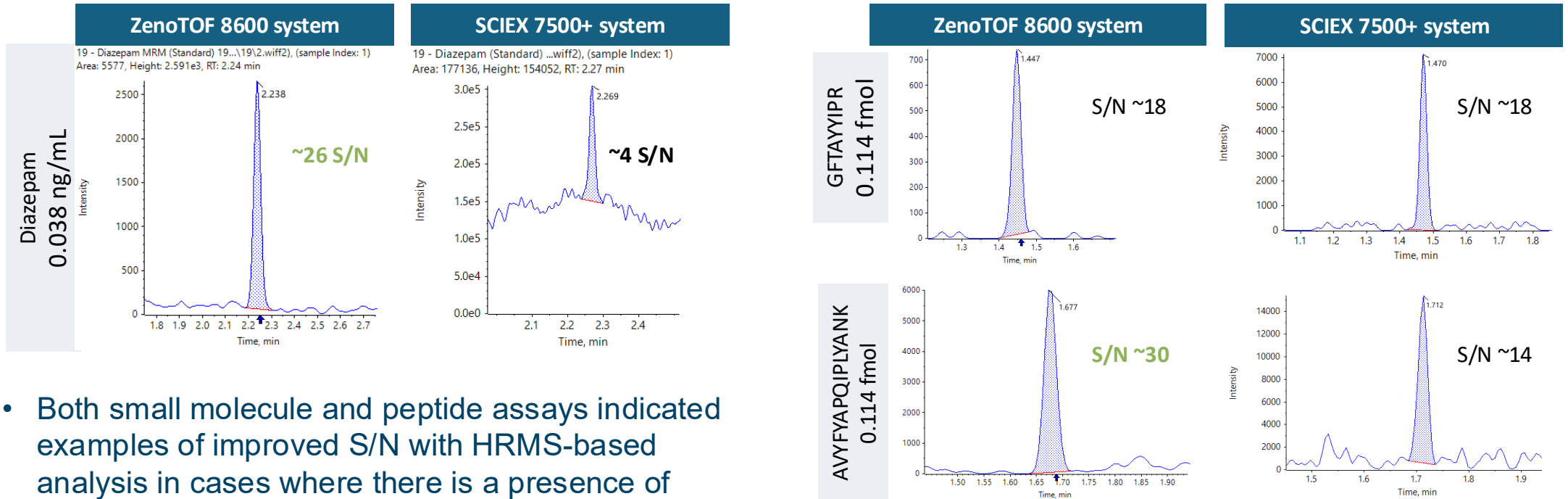
Quantitation:  Theoretical peptide fragments m/z

Fragments to give best:  LOQ  LDR

5 Summed XIC using automated fragment selection



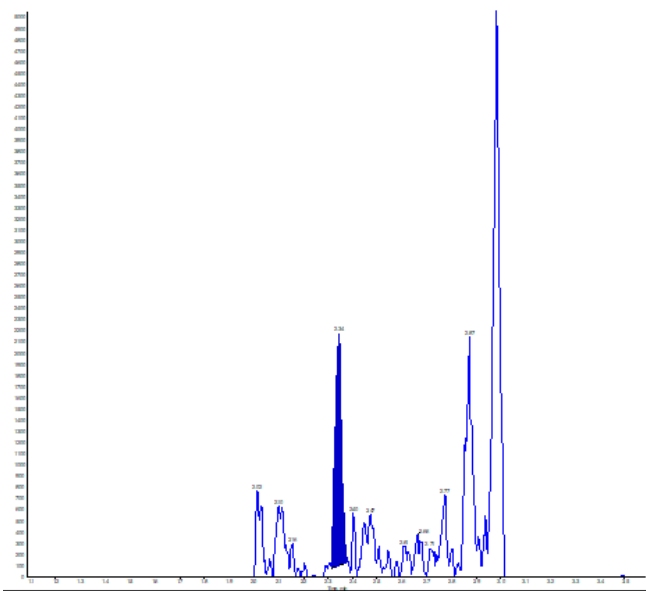
# Highly selective HRMS boost S/N and overall quantitative sensitivity



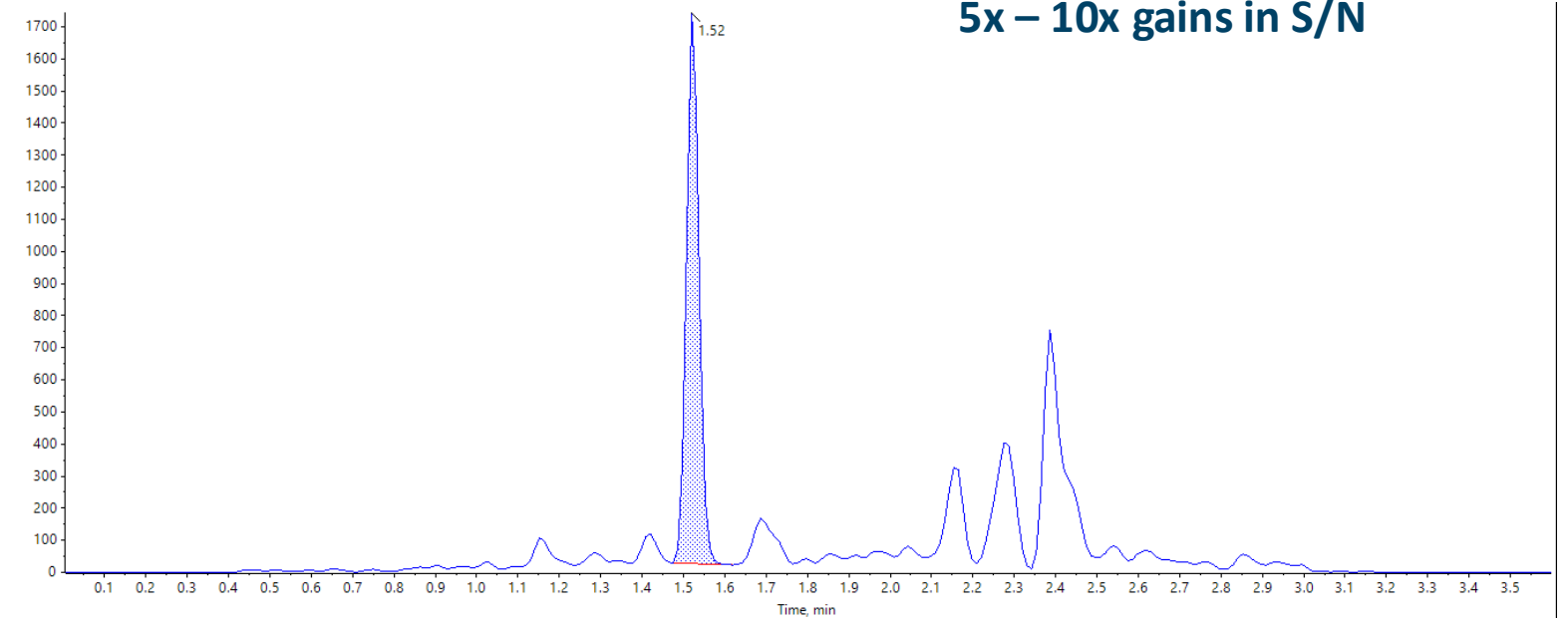
- Both small molecule and peptide assays indicated examples of improved S/N with HRMS-based analysis in cases where there is a presence of interfering peaks or high background
- Often, such cases can press for a need for additional method optimization or extensive sample preparation procedures

# 1.0 ng/mL human plasma semaglutide

SCIEX Triple Quad 6500+ system



ZenoTOF 8600 system



5x – 10x gains in S/N

# Thought leadership at Veloxity

- Submitting manuscript describing an LC-MS/MS method development and full validation for the quantitation of semaglutide in human plasma
- Following with manuscript comparing the sensitivity and selectivity of several GLP-1 peptides on the SCIEX Triple Quad 6500+ system versus the ZenoTOF 8600 system
- Paper: full validation of a GLP-1 peptide agonist on the ZenoTOF 8600 system
- April 30<sup>th</sup> published editorial with the European Society of Medicine describing the assessment of current gaps associated with clinical sample bioanalysis

# What's coming up?

Just released...

**AI quant!**

**Biomarker quant underway 10x S/N  
improvement from 6500+**

# Acknowledgments

Thank you to Eshani Galermo and the Global Marketing Team at SCIEX for their assistance.

Special thanks to Joel Stickling and Matt Salske of Velocity Labs for their help in data generation.

# Questions

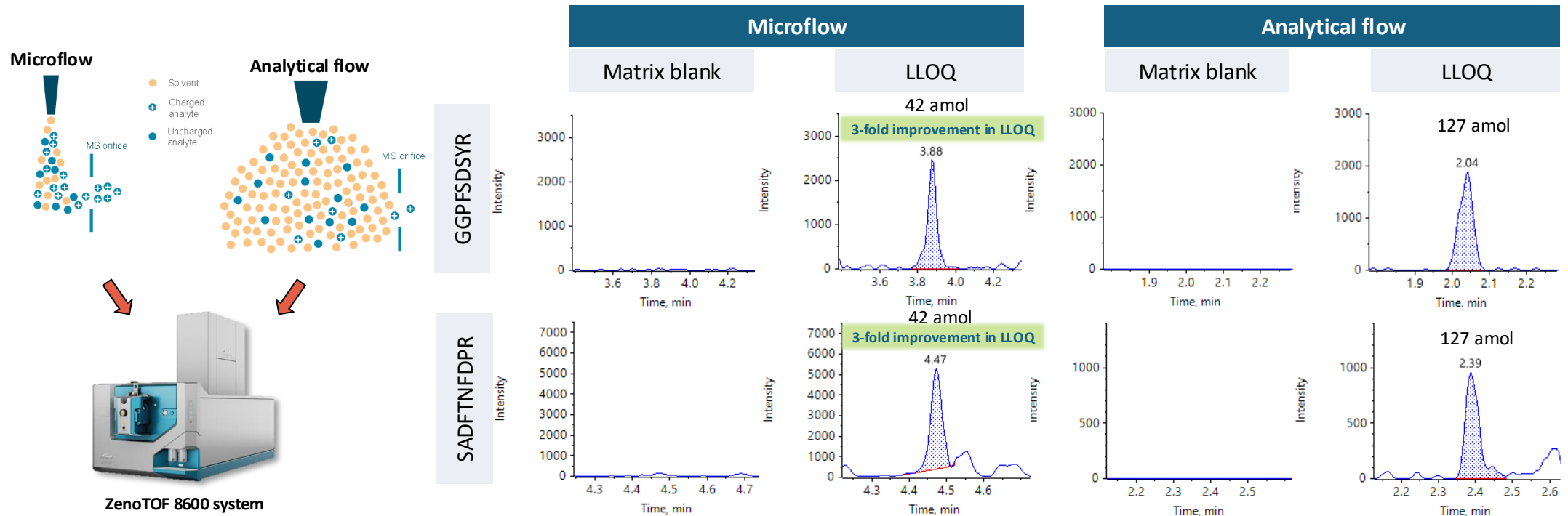


**Shane Needham, Ph.D.**

President and CEO, Velocity Labs

[shane.needham@velocitylabs.com](mailto:shane.needham@velocitylabs.com)

# Maximize speed and sensitivity for quantitative peptide bioanalysis using microflow



- Peptides SADFTNFDPR, EGHLSPDIVAEQK, ESDTSYVSLK, and NLSVEDAAR showed a 3-fold improvement in LLOQ compared with analytical flow LC.
- The observed gain in sensitivity was primarily attributed to improved ionization efficiency at a microflow rate.

# Enhance microflow peptide sensitivity with summation of multiple fragment ions

- A microflow-based result for the summation of highly abundant fragment ions is displayed, which showed improved signal-to-noise ratio (S/N) and increased assay sensitivity.
- Peptides EGHLSPDIVAEQK and NLSVEDAAR show a 3-fold improvement in LLOQ with summation of 3 fragment ions when compared to quantitation using a single fragment ion.

