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Comparative Quantitative Analysis of Semaglutide, Tirzepatide & Retatrutide

Using HRMS & Triple Quad LC-MS/MS

Speaker Biography

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President & CEO



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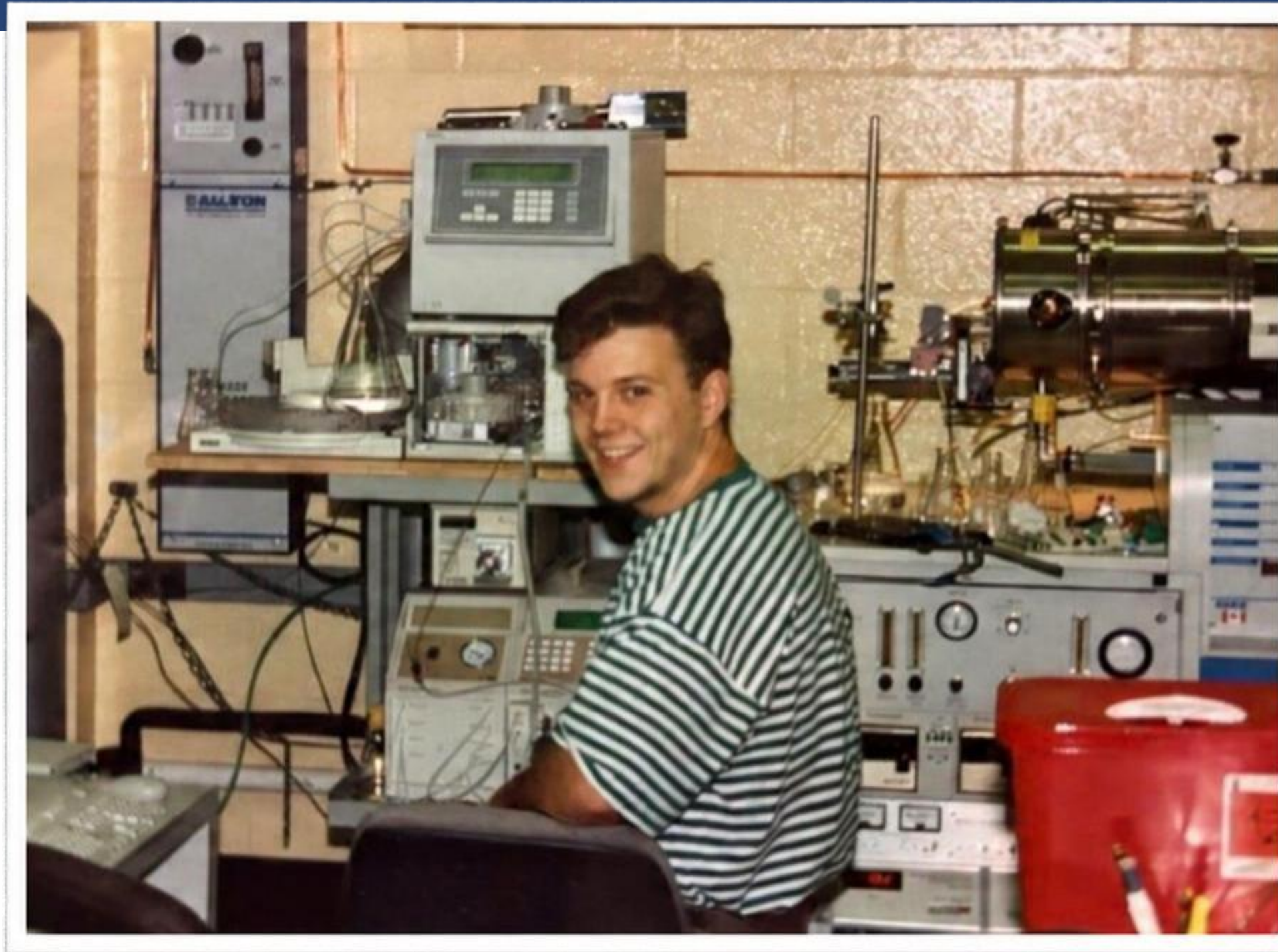
Velocity Labs

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



A Look Back: Working on SCIEX Serial No. 1



Complex peptides raise the bar for quantitative bioanalysis

GLP-1-based therapeutics are an increasingly important class of peptide therapeutics

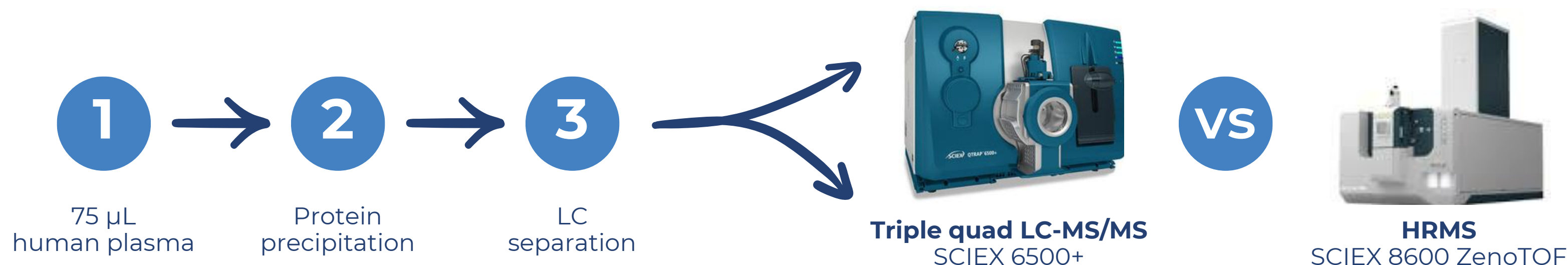
- Semaglutide, tirzepatide and retatrutide present analytical challenges requiring careful optimization of chromatography and MS conditions
- Quantitative methods must balance sensitivity, selectivity and robust quantitative performance
- Method-development speed also matters as peptide pipelines expand

Can HRMS deliver comparable quantitative performance while reducing method-development time?

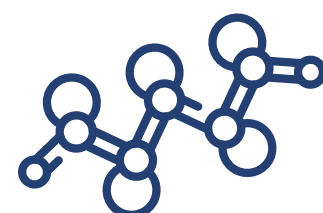
Head-to-head evaluation in human plasma



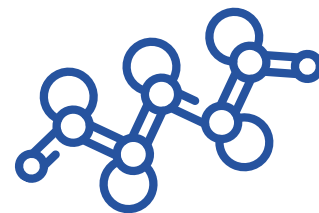
Study design & analytical workflow



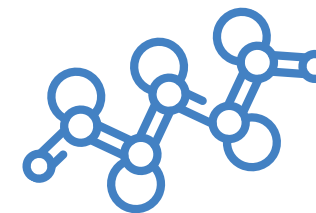
Three analytes from a single sample preparation and LC-MS/MS analysis



Semaglutide



Tirzepatide



Retatrutide

Internal standard: Stable isotope-labeled semaglutide

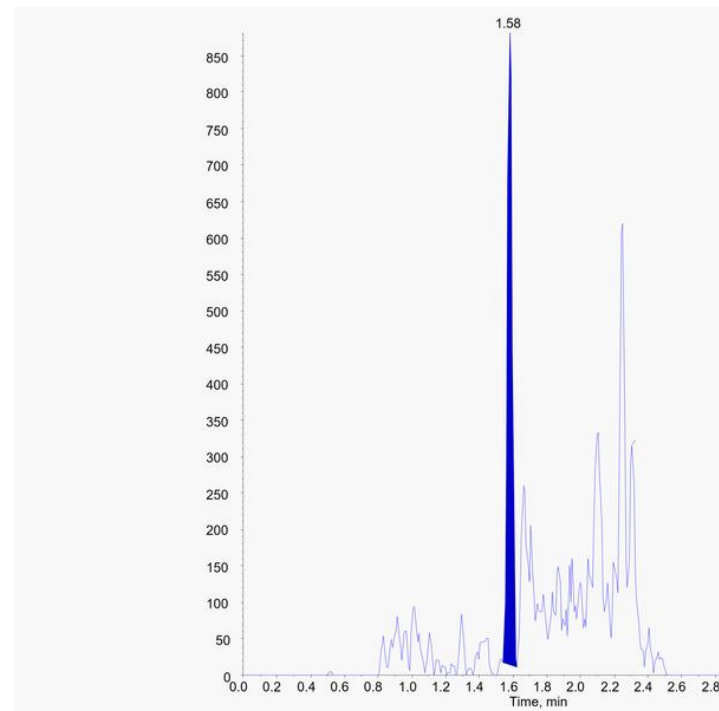
LLOQ comparison

HRMS maintained sensitivity across peptides



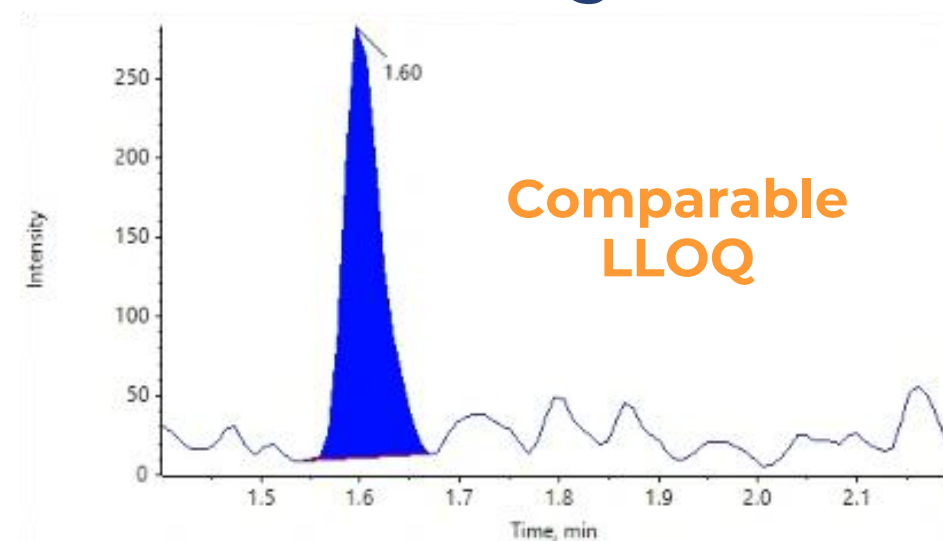
6500+
15 μ L

Semaglutide

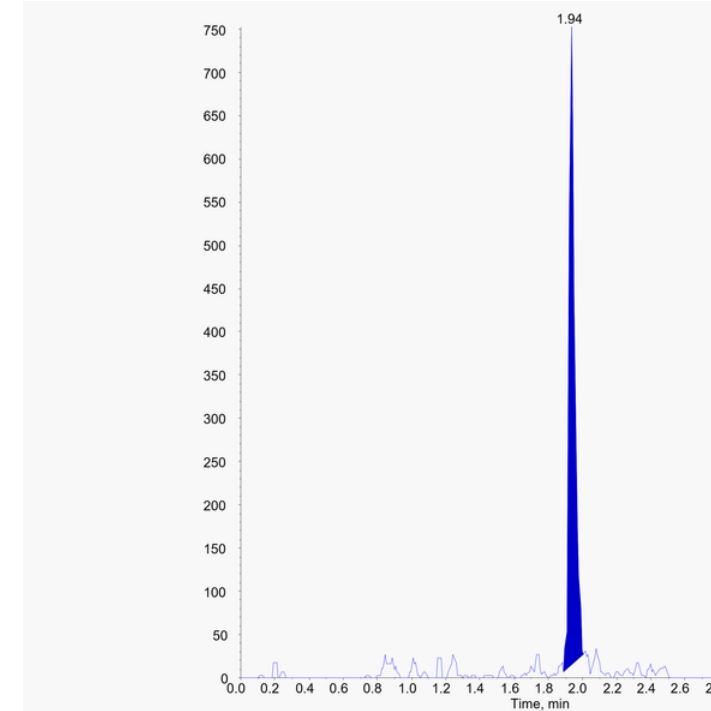


0.5 $\rightarrow$ 0.5 ng/mL

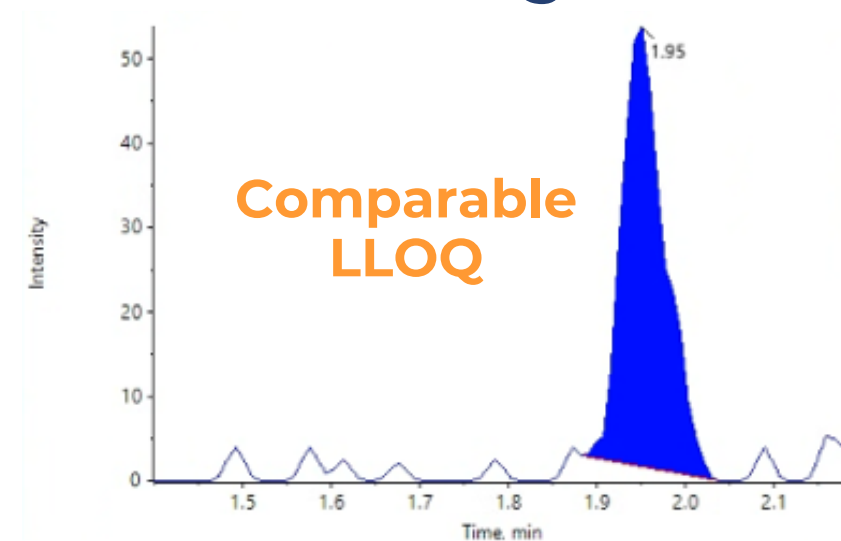
8600
5 μ L



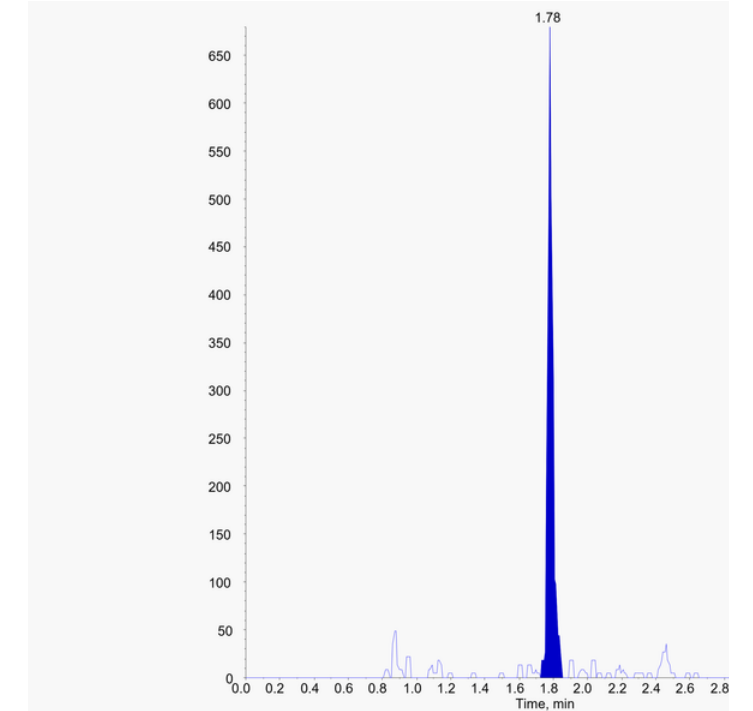
Tirzepatide



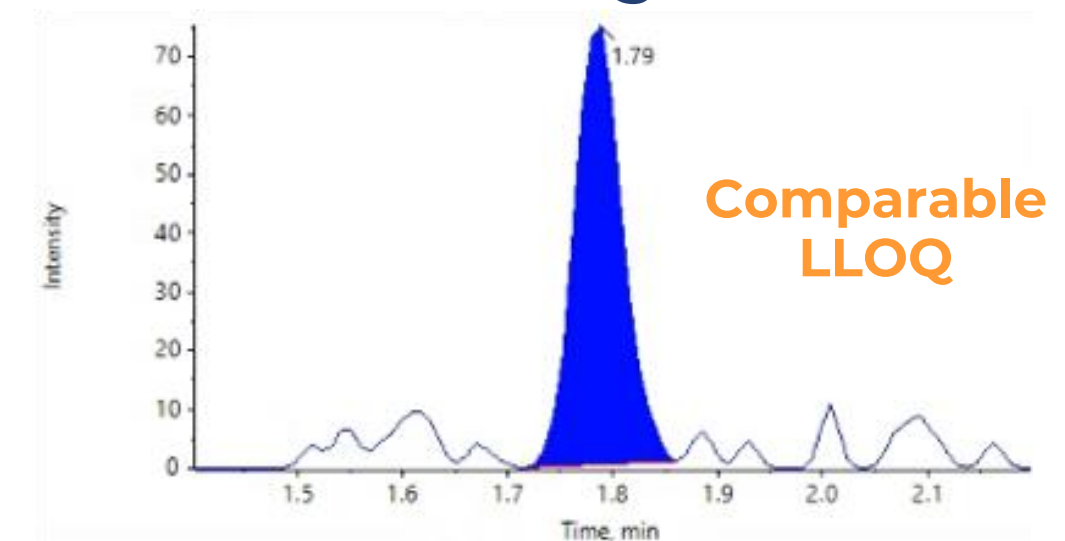
0.5 $\rightarrow$ 0.5 ng/mL



Retatrutide



0.5 $\rightarrow$ 0.5 ng/mL

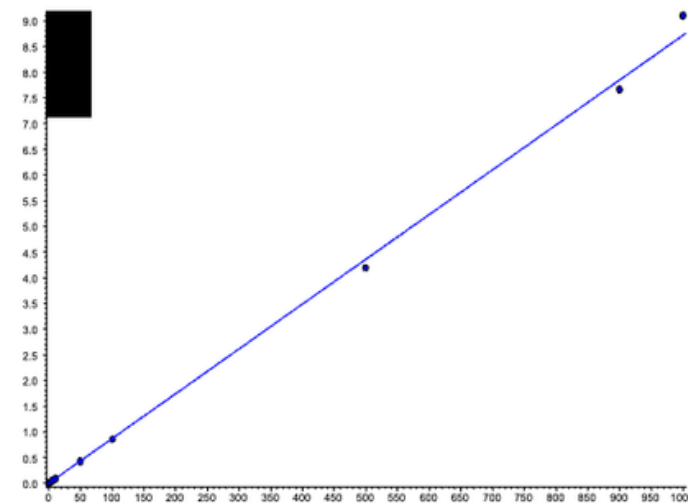


Calibration curve performance



Both workflows maintained linear dynamic range

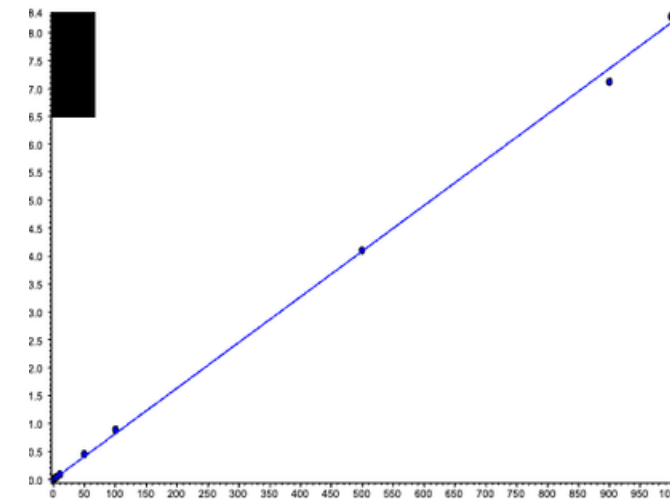
Semaglutide



6500+
15 µL

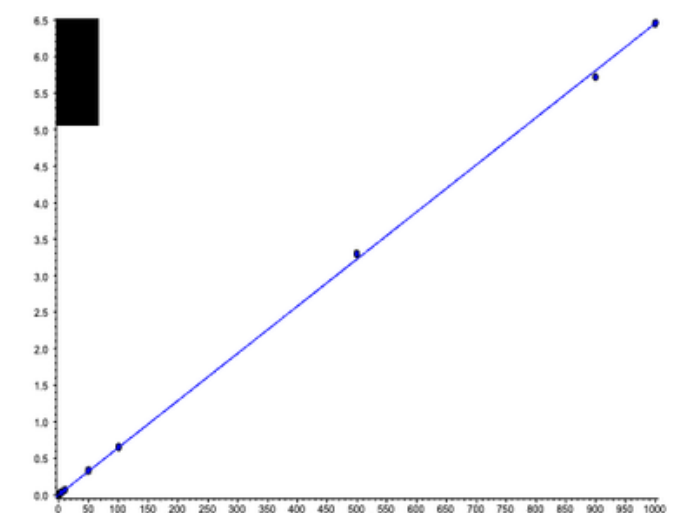
0.5–1,000 → 0.5–1,000 ng/mL

Tirzepatide



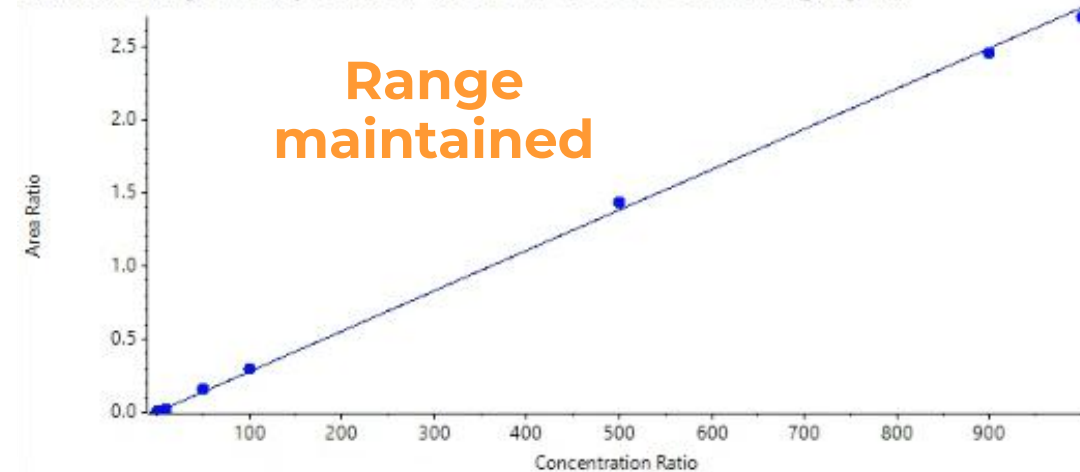
0.5–1,000 → 0.5–1,000 ng/mL

Retatrutide



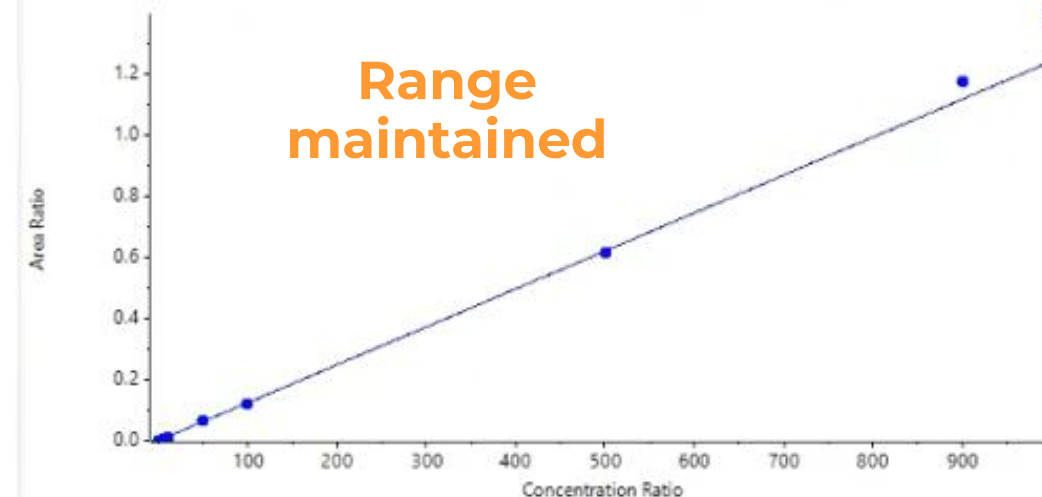
0.5–1,000 → 0.5–1,000 ng/mL

Calibration for Semaglutide_Sum: $y = 0.00276x + 3.59981e-4$ ($r = 0.99930$, $r^2 = 0.99860$) (weighting: $1/x$)

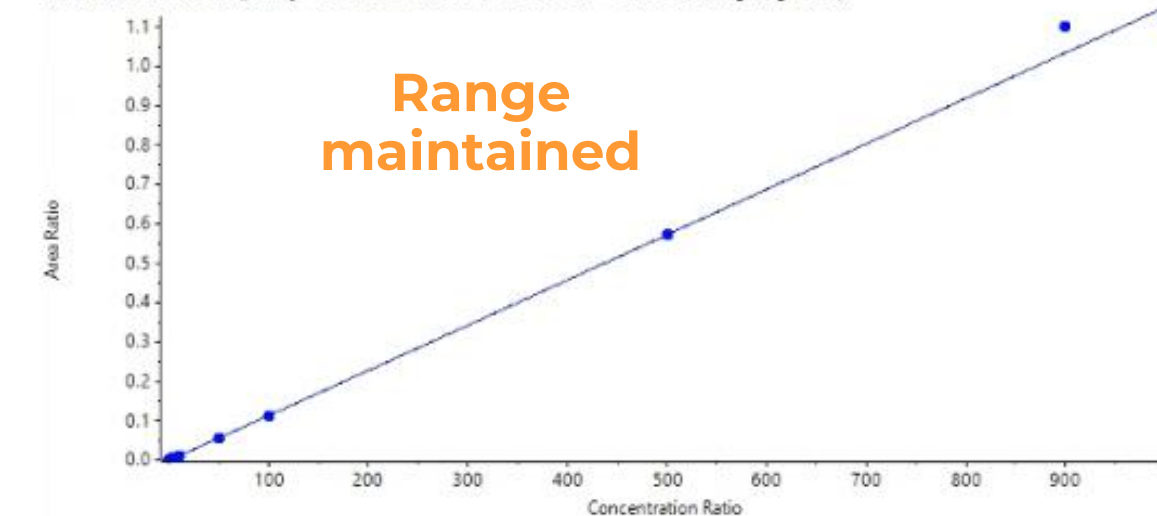


8600
5 µL

Calibration for Tirzepatide_1_Sum: $y = 0.00124x - 1.82903e-4$ ($r = 0.99649$, $r^2 = 0.99298$) (weighting: $1/x^2$)



Calibration for Retatrutide_Sum: $y = 0.00110x^{1.00597}$ ($r = 0.99876$, $r^2 = 0.99752$) (weighting: None)

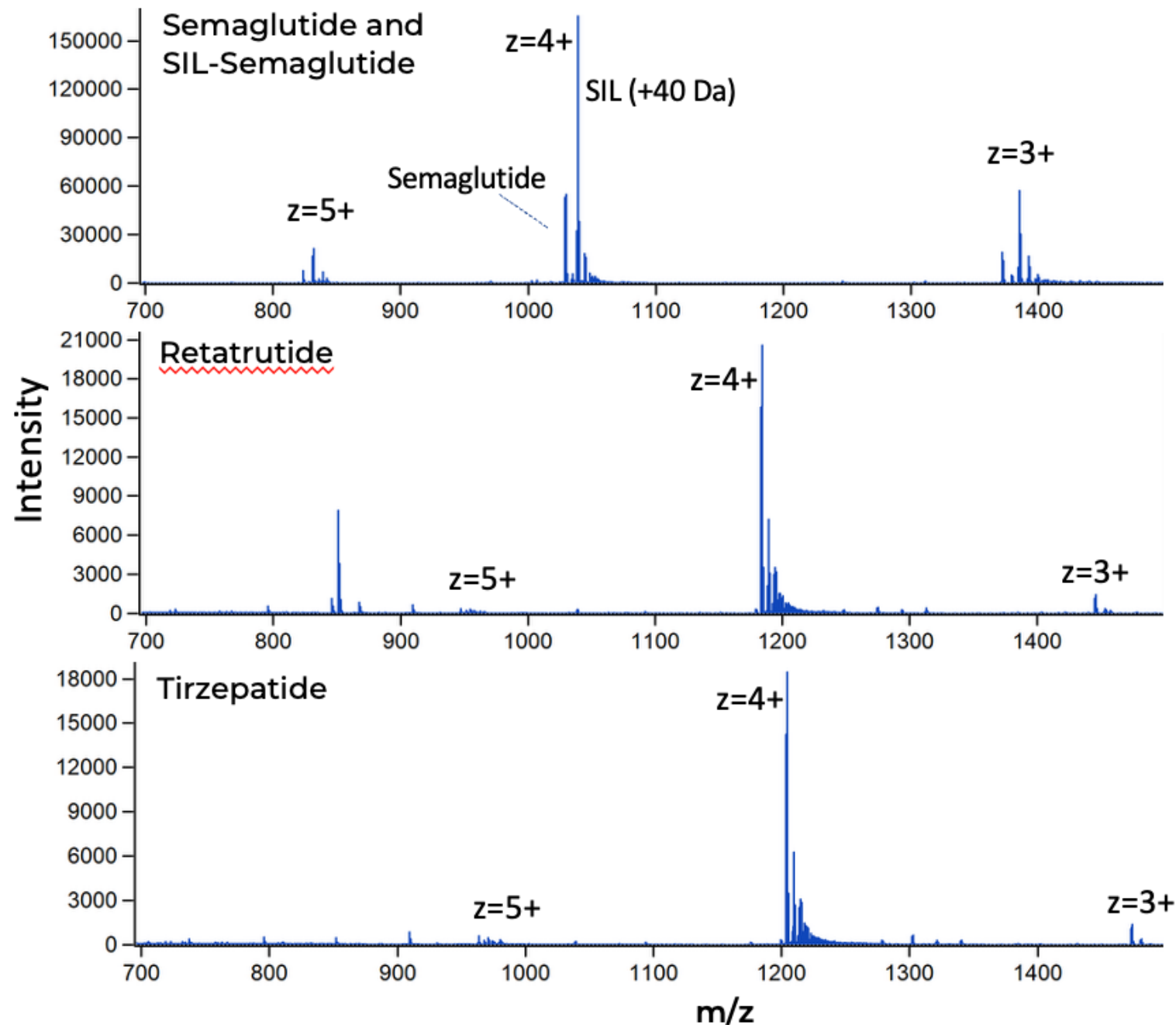


HRMS characterization

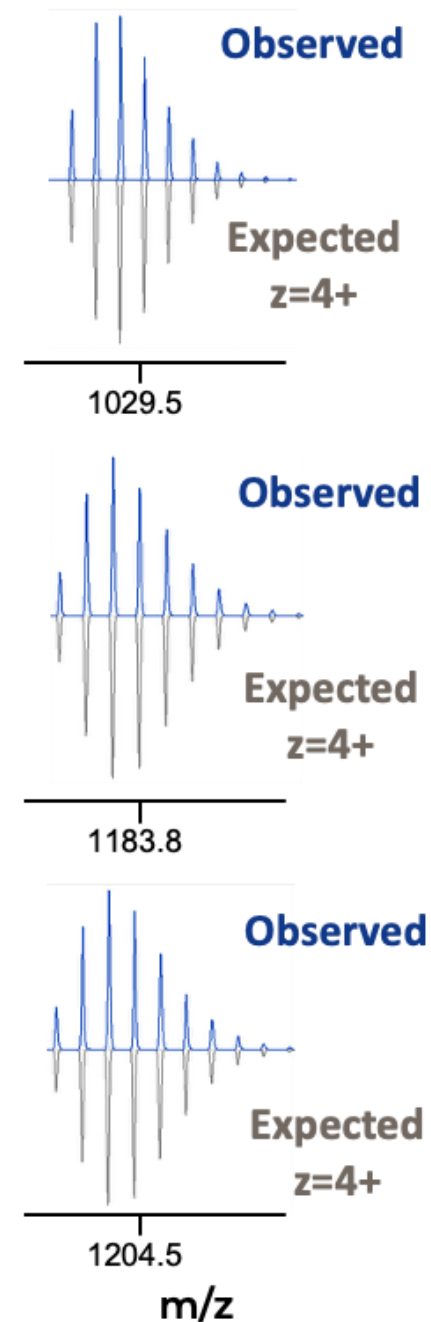


HRMS provides deeper insight while quantifying

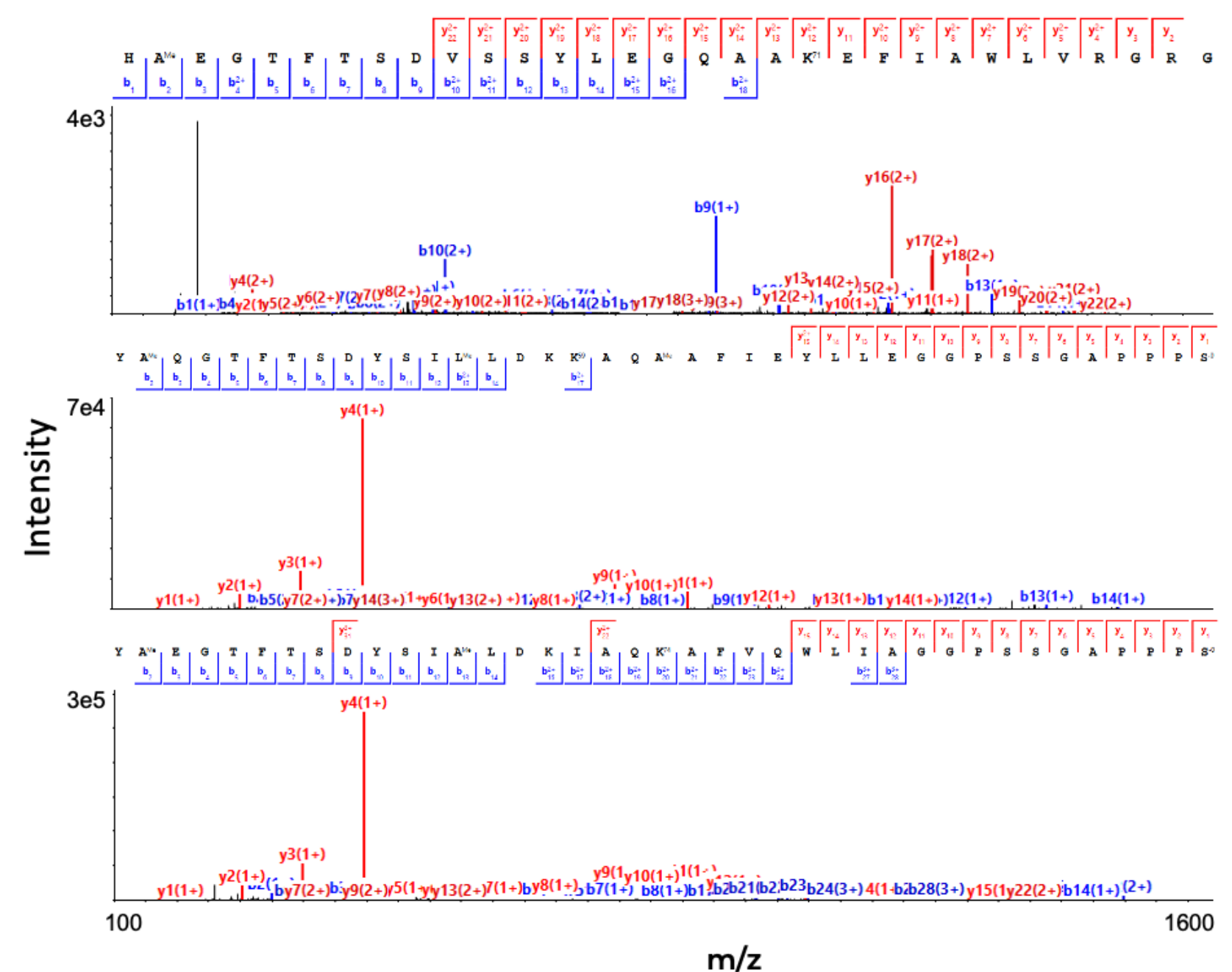
Intact Mass (MS1)



MS1 Spectral Match



Fragmentation (MS2)



Why HRMS changes method development



Quantitative performance tells only part of the story

Triple quad LC-MS/MS
SCIEX 6500+



- Select precursor/product-ion transition
- Optimize individual transition
- Evaluate quantitative performance
- Repeat as needed

HRMS
SCIEX 8600 ZenoTOF

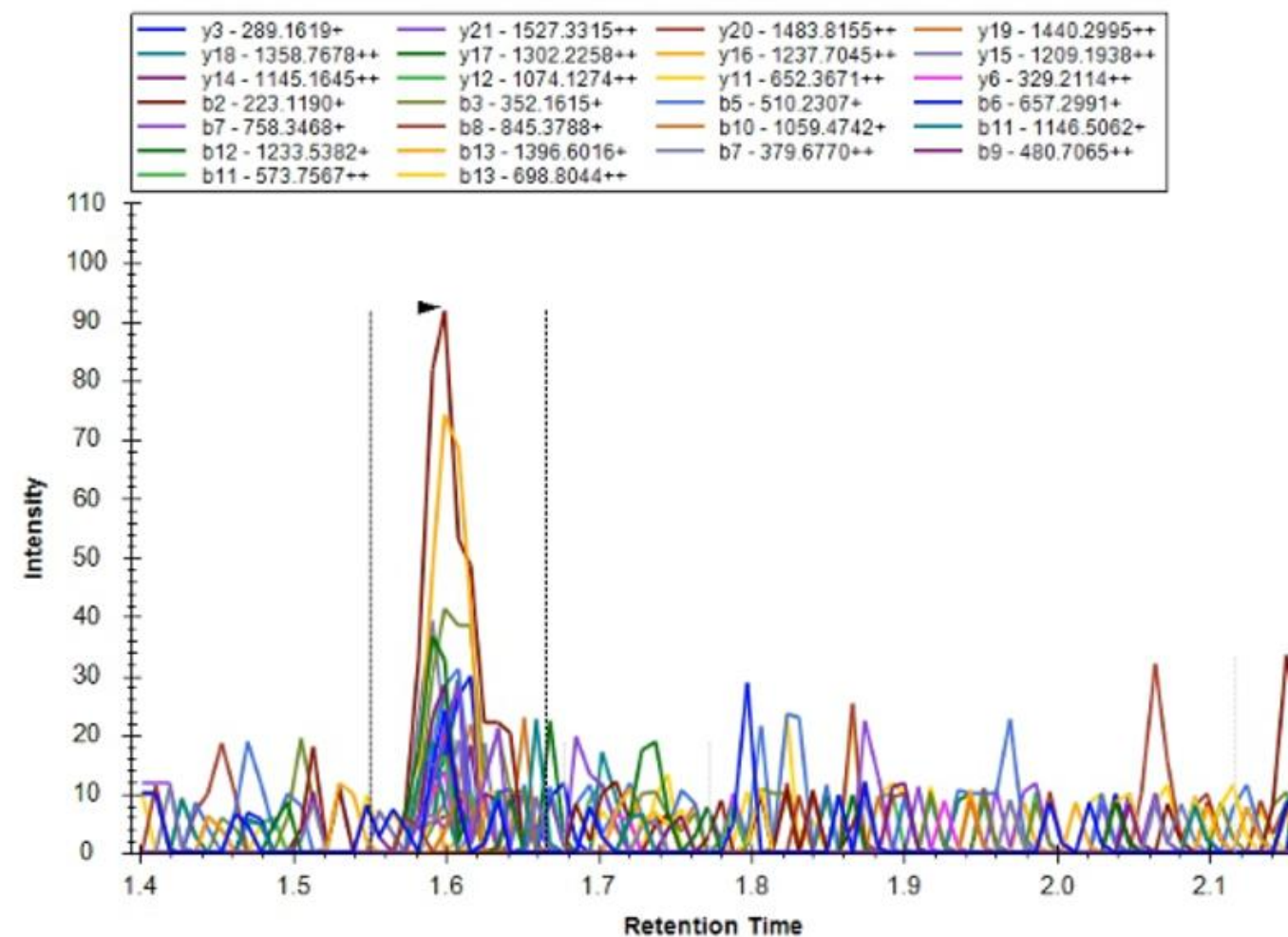


- Acquire comprehensive MS1/MS2 data
- Evaluate multiple product ions from the same acquisition
- Select informative fragments
- Combine/sum fragments when advantageous

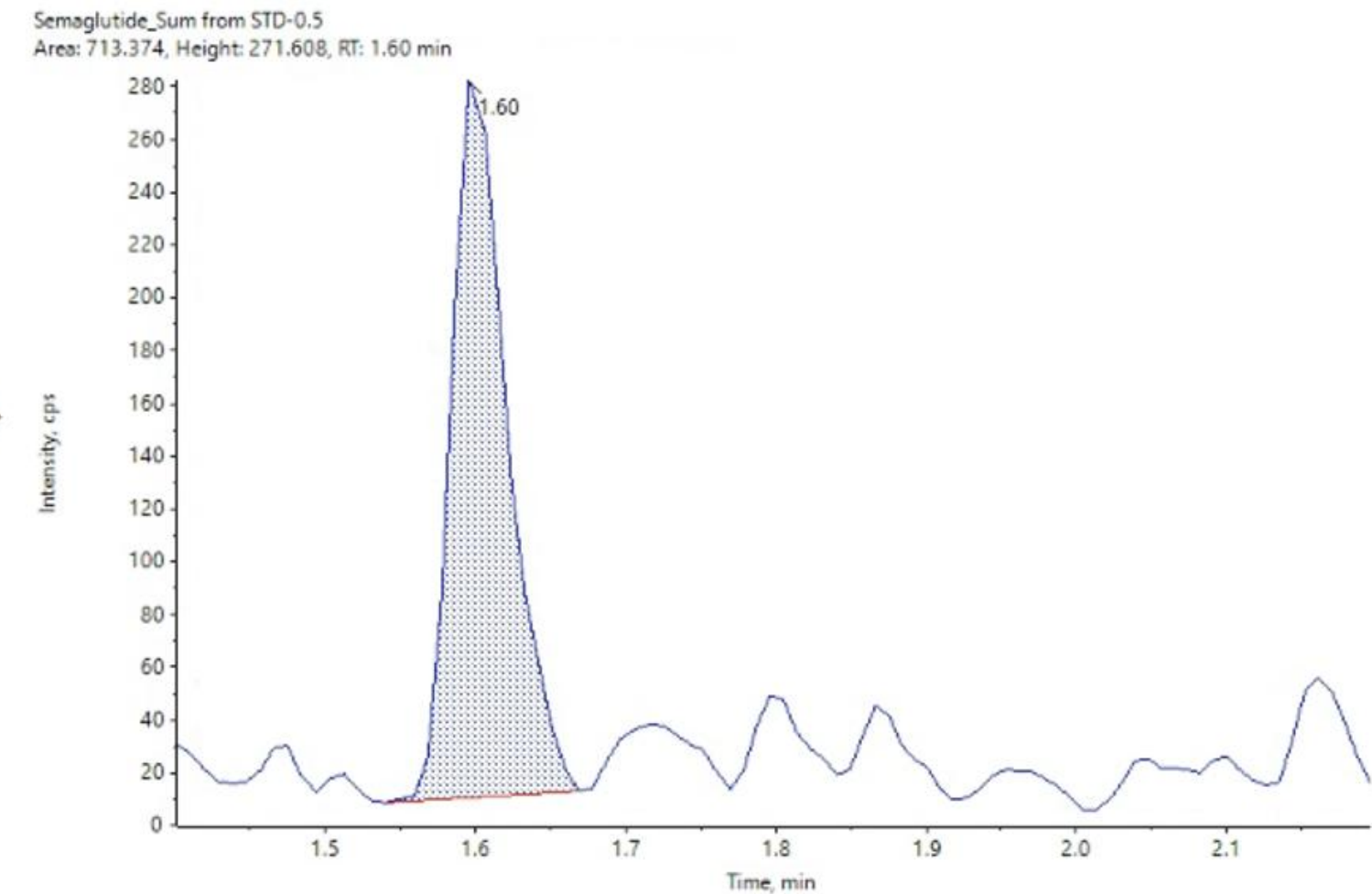
Greater information from each acquisition reduces iterative optimization
3-4x faster development with HRMS

Combining fragment signals improves semaglutide sensitivity

Semaglutide – 0.5 ng/ml (LLOQ)
Single fragment Chromatograms



Summed Fragment Ions



Summing informative fragments averages individual signal variability, improves peak shape and increases signal-to-noise.

Head-to-head findings



HRMS delivered comparable sensitivity & greater development flexibility

Measure	Triple Q
Semaglutide LLOQ	0.5 ng/n
Tirzepatide LLOQ	0.5 ng/n
Retatrutide LLOQ	0.5 ng/n
Linear dynamic range	Broad q
Product-ion information	Targeted
Fragment summing	Limited,
Relative method development	Baseline

The primary advantage was not simply lower LLOQ. HRMS provided substantially more analytical information and flexibility during method development while maintaining sensitivity across the three peptide assays.

Implications for increasingly complex peptide therapeutics



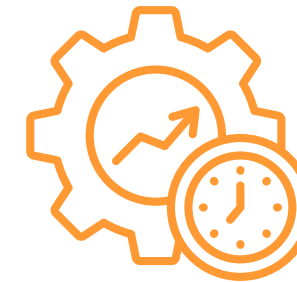
Comparable sensitivity

HRMS achieved comparable sensitivity across all three peptide assays.



More information per acquisition

Comprehensive MS1/MS2 data supported charge-state assessment, parent-mass confirmation and flexible fragment selection.



Faster development

Flexible product-ion selection and fragment summing supported approximately 3–4X faster method development.

For complex peptide bioanalysis, HRMS can complement established triple quad workflows by providing comparable quantitative sensitivity with greater selectivity, analytical insight and method-development flexibility.

Thank you & questions

Thank you!

With thanks to the Velocity Labs team members who contributed to this work:

- Chris Tudan, PhD
- Joel Stickling
- Matt Salske, MS
- Emily Northwick Darden, MS
- Henrique Seckler, PhD



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Questions?