Multiplexing SIMs on a Novel Benchtop Orbitrap MS with a Quadrupole Mass Filter to Increase Sensitivity for Peptide Quantitation

Louis Maljers, Kevin L. Cook Thermo Fisher Scientific, San Jose, California, USA

Thermo SCIENTIFIC

Overview

Purpose: To develop multiplexed selected ion monitoring (SIM) methods on a novel benchtop quadrupole-Orbitrap™ mass spectrometer for biopharmaceutical applications.

Methods: We employed a ten-minute LC method at 150 µL.min.$^{-1}$ Mass spectrometry analysis was performed using a quadrupole-Orbitrap LC-MS/MS systems equipped with a heated electrospray ionization (HESI) source. Wide SIM was used.

Results: A generic method development approach was used to analyze Insulin, Exendin-4 and GLP-1 peptides, between 10 pg.mL^{-1} and 10 µg.mL,$^{-1}$ with good linearity and reproducibility.

Introduction

In bioanalysis, triple quadrupole MS systems have been predominantly used for peptide quantitation. Although triple quadrupole systems generally offer the most sensitive and robust quantitation, the poor fragmentation and multiple charge states of large peptides can result in weak selected reaction monitoring (SRM) signals.

In this investigation, we demonstrate the accurate and precise quantification of peptides in a biological matrix suing a new approach that utilizes multiplexed SIMs capability provided on a novel benchtop quadrupole-Orbitrap mass spectrometer.

Multiplexed SIMs refers to multiple fills of the instrument's C-trap with quadrupole-filtered ions, prior to a Orbitrap mass analyzer scan.

Methods

Sample Preparation

Calibration curves were prepared in rat plasma.

Liquid Chromatography (Generic Separation)

A Thermo Scientific Accela UHPLC and Thermo Scientific Open Accela Autosampler were used to reproduce linear gradients on a 100×1mm, 1.9 µm particle size Thermo Scientific Hypersil GOLD column at 150 µL.min.$^{-1}$ The mobile phase was 0.1% formic acid in water and acetonitrile.

Mass Spectrometry

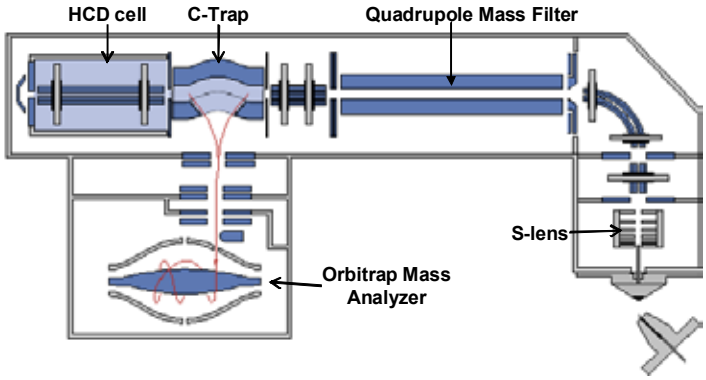
MS experiments were conducted on a Thermo Scientific Q Exactive high-performance benchtop quadrupole-Orbitrap system (Figure 1) operated in multiplexed SIM mode using 70,000 resolution (maximum 140,000 resolution at *m/z* 200). Targeted multiplexed SIM fills the C-trap with multiple packages of ions from the selected scan ranges. The C-trap collects the ions, and the Orbitrap mass analyzer analyses the ions previously collected. The isolation window used for the three peptides was 20 amu. To achieve maximum sensitivity, the AGC target values were optimized for each peptide.

Source conditions: heater temperature 200 ºC, capillary temperature 275 ºC, spray voltage 3.5kV, sheath gas 40, aux gas 10.

Data Analysis

The data were processed with Thermo Scientific LCQUAN quantitation software revison 2.7 using a 5 ppm mass window.

FIGURE 1. Schematic of the Q Exactive benchtop quadrupole-Orbitrap mass spectrometer.



Results

As shown in Figures 2 through 10, and Tables1 through 4, all three peptides showed excellent sensitivity and reproducibility, over large dynamic ranges.

Insulin

FIGURE 2. Spectra of the acquired mass peaks of human insulin at 70,000 resolution.

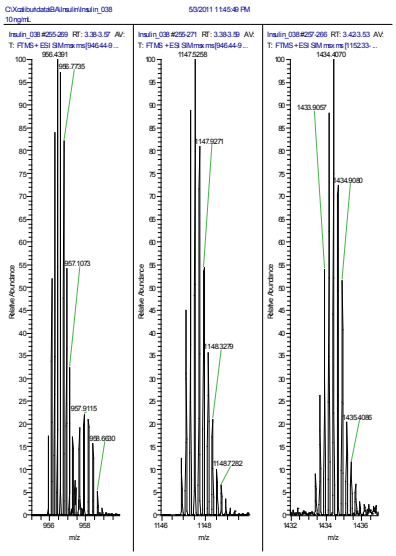


FIGURE 3. Chromatogram of 250 pg.mL^{-1} human insulin (LLOQ).

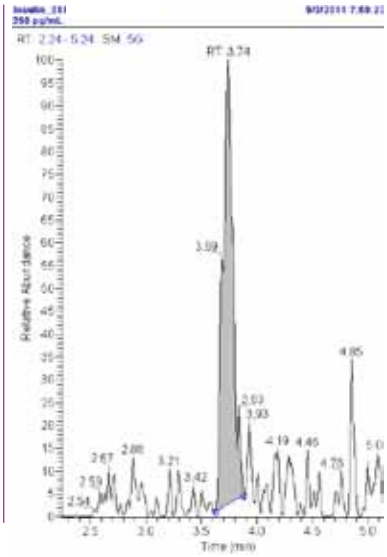
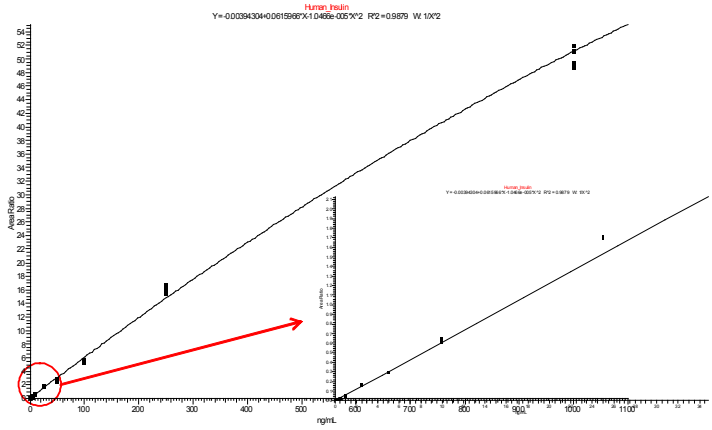


TABLE 1. Insulin summary table; dynamic range from 250 pg.mL^{-1} to 1000 ng.mL.$^{-1}$

Nominal Concentration (ng/mL)	Replicate #	Mean Calculated Concentration	Stdev	% CV
0.25	4	0.260	0.0300	11.5
0.5	4	0.434	0.0501	11.5
1	4	0.906	0.0540	5.96
2.5	4	2.81	0.0568	2.02
5	4	4.79	0.112	2.33
10	4	10.3	0.284	2.77
25	4	27.9	0.247	0.89
50	4	44.0	1.35	3.08
100	4	92.8	4.29	4.62
250	4	276	9.99	3.62
1000	4	979	36.7	3.75

FIGURE 4. Insulin calibration curve, dynamic range from 250 pg.mL^{-1} to 1000 ng.mL^{-1}



Exendin-4

FIGURE 5. Spectra of the acquired mass peaks of Exendin-4 at 70,000 resolution.

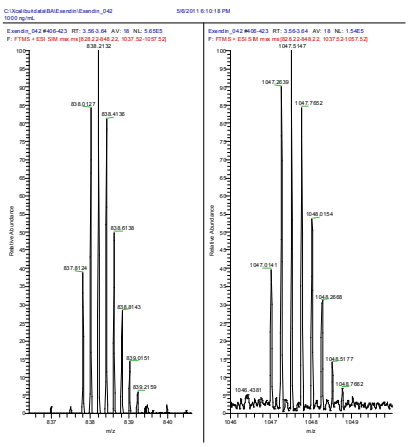


FIGURE 6. Chromatogram of 5 ng.mL^{-1} Exendin-4 (LLOQ).

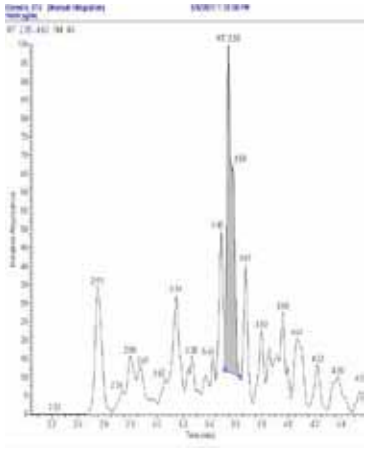
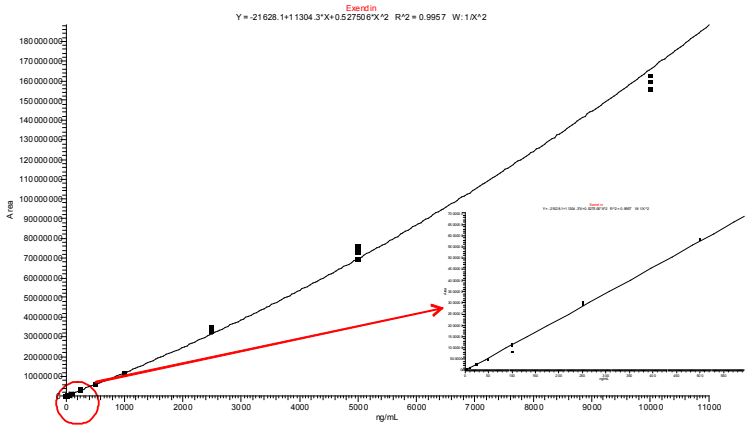


TABLE 2. Exendin-4 calibration table; dynamic range from 5 ng.mL^{-1} to 10000 ng.mL.$^{-1}$

Nominal Concentration (ng/mL)	Replicate #	Mean Calculated Concentration	Stdev	% CV
5	4	5.143	0.418	8.13
10	4	9.693	0.520	5.36
25	4	24.37	1.22	5.00
50	4	45.46	0.705	1.55
100	4	92.83	13.0	14.0
250	4	261.7	5.31	2.03
500	4	507.3	2.03	0.40
1000	4	966.0	25.2	2.61
2500	4	2675.6	60.7	2.27
5000	4	5205	169	3.24
10000	4	9740	150	1.54

FIGURE 7. Exendin-4 calibration curve, dynamic range from 5 ng.mL^{-1} to 10 µg.mL^{-1}



GLP-1:

FIGURE 8. Spectra of the acquired mass peaks of GLP-1 at 70,000 resolution.

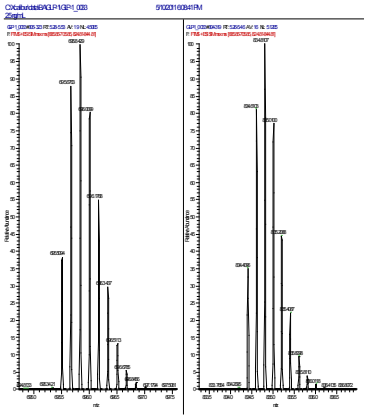


FIGURE 9. Chromatogram of 5 ng.mL^{-1} GLP-1 (LLOQ).

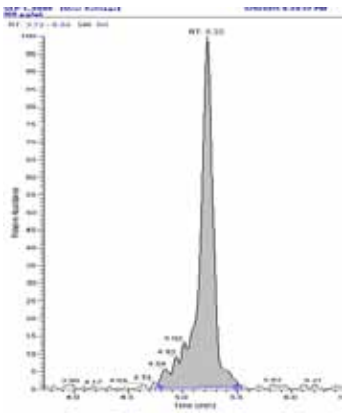
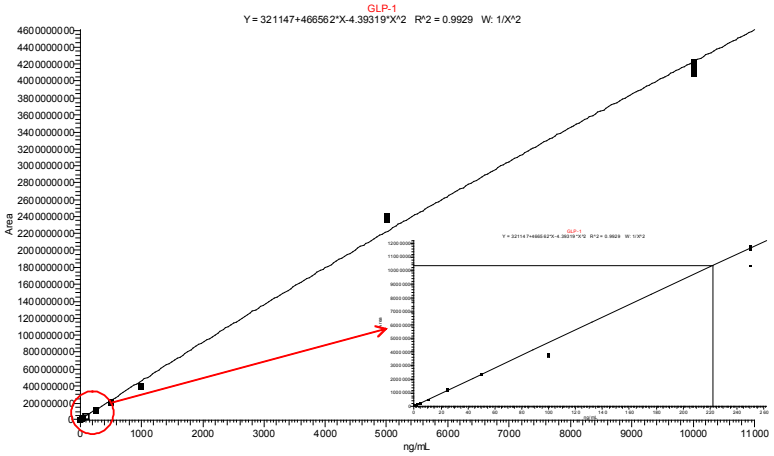


TABLE 3. GLP-1 summary table; dynamic range from 0.1 ng.mL^{-1} to 10000 ng.mL.$^{-1}$

Nominal Concentration (ng/mL)	Replicate #	Mean Calculated Concentration	Stdev	% CV
0.1	4	0.099	0.0098	9.91
0.25	4	0.251	0.0205	8.16
0.5	4	0.534	0.0337	6.31
1	4	0.905	0.0226	2.50
2.5	4	2.69	0.0578	2.15
5	4	5.36	0.126	2.36
10	4	9.99	0.120	1.20
25	4	26.6	0.664	2.50
250	4	50.6	0.562	1.11
100	4	80	0.692	0.86
250	4	243	14.2	5.86
500	4	462	15.5	3.35
1000	4	860	7.90	0.92
5000	4	5383	62.8	1.17
10000	4	9796	164	1.67

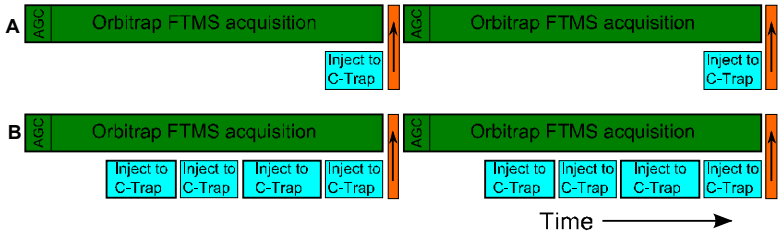
FIGURE 10. GLP-1 calibration curve; dynamic range from 5 ng.mL^{-1} to 10 µg.mL.$^{-1}$



Principle of Multiplexing

Figure 11 compares the standard operation mode with the spectrum multiplexing approach in the scan-to-scan AGC (automatic gain control) mode. In both modes, the instrument is operating in parallel mode. While one scan is acquired, the ions for the next scan are already collected. In the standard operation mode, only one precursor ion species is injected. With spectrum multiplexing, the idle time is used to inject several precursor ion species, resulting in higher throughput.

FIGURE 11. Standard operation mode (A) versus spectrum multiplexing (B).



Conclusion

- The Q Exactive™ high-performance benchtop quadrupole-Orbitrap mass spectrometer is a sensitive quantitation instrument that displays equal or better LOQs than the LOQs reported in the literature for high-end triple quadrupole instruments.[1] The Q Exactive mass spectrometer was found to be the instrument of choice for high-sensitivity quantitation with the following LOQs:
  - Insulin to an LOQ of 250 $pg.mL^{-1}$
  - Exendin-4 to an LOQ of 5 $ng.mL^{-1}$
  - GLP-1 to an LOQ of 0.1 $ng.mL^{-1}$
- The use of multiplexed SIMs is very effective for the quantitation of larger peptides such as insulin, exendin-4 and GLP-1. This technology makes the Q Exactive mass spectrometer highly selective.
- Because the instrument's multiplexing capability is coupled with advanced signal processing, its scan speed is fast enough to capture data across narrow UHPLC peaks. For example, 12 - 15 scans were recorded across the 6 s-wide exendin-4 chromatographic peak.
- These experimental results portend the applicability of the Q Exactive mass spectrometer for the quantitation of peptides, small proteins and oligonucleotides.

References

1. Quantification of Insulin at Micro dosing Levels Using LC-MS/MS, Montreux LCMS conference, Louis Maljers, Jonathan McNally & Rohan Thakur.
2. Application of DBS for quantitative assessment of the peptide Exendin-4; comparison of plasma and DBS method by UHPLC–MS/MS, Jonathan R Kehler, Chester L Bowen, Sharon L Boram & Christopher A Evans, Platform Technology and Science, Drug Metabolism and Pharmacokinetics, GlaxoSmithKline Pharmaceuticals, 709 Swedeland Road, King of Prussia, PA 19406, USA.
3. "Multiple C-Trap Fills as a Tool for Massive Parallelization of Orbitrap Mass Spectrometry- a new Concept for Targeted Mass Analysis." Oliver Lange; Jan-Peter Hauschild; Alexander Makarov; Ulf Fröhlich; Catharina Crone; Yue Xuan; Markus Kellmann; Andreas Wieghaus. Thermo Fisher Scientific, Bremen, Germany; ASMS 2011, Denver, CO, USA. Monday Poster #103.

Africa-Other +27 11 570 1840
Australia +61 2 8844 9500
Austria +43 1 333 50 34 0
Belgium +32 53 73 42 41
Canada +1 800 530 8447
China +86 10 8419 3588
Denmark +45 70 23 62 60
Europe-Other +43 1 333 50 34 0
Finland /Norway/Sweden +46 8 556 468 00
France +33 1 60 92 48 00
Germany +49 6103 408 1014
India +91 22 6742 9434
Italy +39 02 950 591
Japan +81 45 453 9100
Latin America +1 608 276 5659
Middle East +43 1 333 50 34 0
Netherlands +31 76 579 55 55
South Africa +27 11 570 1840
Spain +34 914 845 965
Switzerland +41 61 716 77 00
UK +44 1442 233555
USA +1 800 532 4752

Thermo SCIENTIFIC
Part of Thermo Fisher Scientific

ASMS11_M603_E 06/12S