

A Sensitive Method for Quantitating Peptides on a New Orbitrap Mass Spectrometer

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Overview

Purpose: Absolute quantitation of large peptides using a new Orbitrap™ mass spectrometer.

Methods: Experiments were conducted on a benchtop Exactive mass spectrometer equipped with a heated electrospray ionization source (HESI).

Results: Sensitive detection and quantitation of large peptides can be simply achieved using high-resolution, accurate-mass full-scan mass spectra.

Introduction

Recent focus for peptide quantitation has almost exclusively employed the use of triple quadrupole mass spectrometers. Although triple quadrupoles generally provide the most sensitive and robust quantitation methods, poor fragmentation and multiple charge states of large peptides can result in weak selected reaction monitoring (SRM) signals.⁽¹⁾ Our approach is to utilize the novel Exactive Orbitrap benchtop mass spectrometer, in full-scan MS mode using accurate mass and high resolution for absolute quantitation. In this investigation, we demonstrate the accurate quantification of endogenous peptides in a biological matrix.

Methods

All experiments were conducted on a Thermo Scientific Exactive mass spectrometer with a HESI-II probe, a Thermo Scientific Accela liquid chromatograph, and a CTC PAL autosampler (Leap Technologies, Carrboro, NC). A linear gradient (15-52% B) over 10 minutes was conducted using a 100×1mm, 3µm particle size Thermo Scientific Hypersil GOLD column at a flow rate of 150 µL/min. The mobile phase was 0.1% formic acid (FA) in water (A) and acetonitrile (B). The Exactive™ LC-MS was optimized for peptides and operated in full-scan MS mode using the 100,000 resolution setting. Other instrumental parameters include heater temperature (360 °C), capillary temperature (275 °C), spray voltage (3.5kV), sheath gas (50), aux gas (10), sweep gas (5). The data were processed with Thermo Scientific QuanBrowser Xcalibur 2.1 software using narrow mass windows (3 ppm) as well as summing multiple charge states. Glucagon like peptide 1 human (GLP-1) (1-37) was purchased from Sigma (St. Louis, MO).

Results

GLP-1 is an insulinotropic hormone being investigated as a treatment for type 2 diabetes¹. Development of a simple and sensitive quantitation method to measure the peptide levels is necessary. The reported amino acid sequence of GLP-1 (1-37) is HDEFERHAEGFTSDVSSYLEGQAAKEFIALVKGRG, with MW at 4167.0081 Da.

Charge distribution of GLP-1

Figure 1 illustrates a chromatogram of GLP-1 molecular ions at charge states 6+, 5+, 4+. Masses are observed and extracted at *m/z* 695.5096, 834.4087, 1042.7588, respectively. Among these major charge states of the ion, [M+6H]⁶⁺ demonstrated the highest intensity. Summation of intensities of different charge states could lead to better sensitivity for quantitation. In Figure 2, the full-scan MS view of ions at each charge state was expanded. Both figures were processed from full-scan MS spectra obtained on the Exactive LC-MS at 100,000 resolution setting, 3,000,000 automatic gain control (AGC), 250ms injection time. In Figure 2, the mass accuracy was better than 1.5 ppm (external calibration). It also shows high-resolution results in well-resolved isotopic peaks of GLP-1 molecular ions in different charge states.

FIGURE 1. Chromatogram of GLP-1 molecular ions extracted by different charge states.

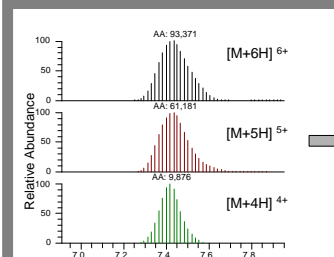
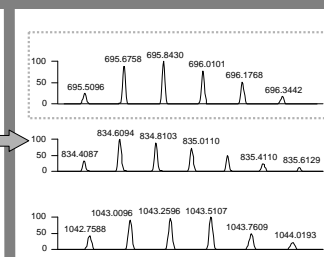


FIGURE 2. Full-scan mass profile of different charge states for GLP-1 peptide obtained at 100,000 resolution setting.



Isotope distribution of GLP-1

Figure 3 shows that isotopic peaks of GLP-1 [M+6H]⁶⁺ are a good match for the theoretical masses and isotope ratios. Figure 4 demonstrates chromatograms of major isotopes from GLP-1 [M+6H]⁶⁺ ions. For large peptides such as GLP-1, data here suggest better sensitivity is achievable by including all major isotope ions for quantitation.

FIGURE 3. Comparison of experimental and calculated spectra for isotope distribution. Top panel: Isotope distribution of 6+ charge state GLP-1 peptide acquired by Exactive LC-MS with accurate mass measurement; Bottom panel: Theoretical calculation of isotope distribution by QualBrowser Xcalibur 2.1.

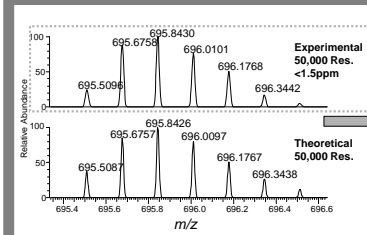
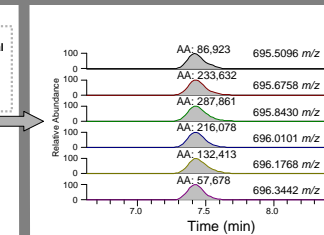


FIGURE 4. Chromatogram of isotopes for GLP-1 molecular ion at charge state 6+ with 3ppm mass tolerance. AA stands for area.



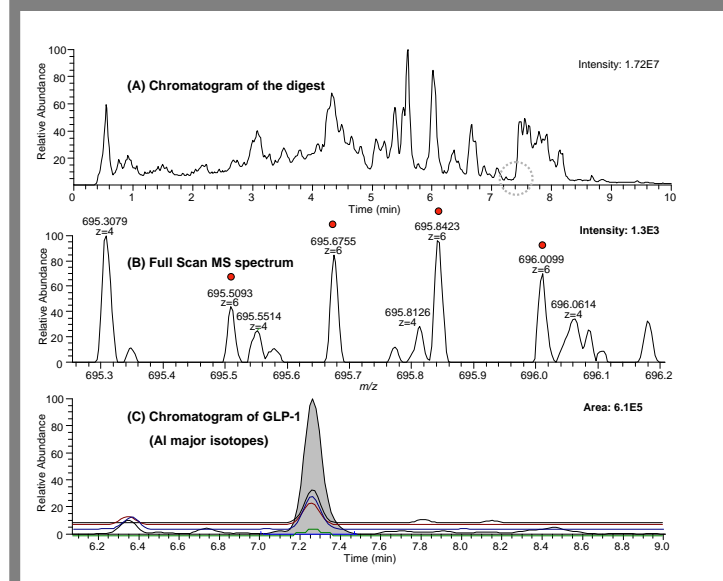
Limit of detection (LOD) in neat standards

For determination of LOD in neat standards, GLP-1 peptide was dissolved by 0.1% FA in 30% acetonitrile at a concentration of 30 amol/µL. GLP-1 molecular ion [M+6H]⁶⁺ was detected in duplicate injections.

Quantitation of GLP-1 in a complex mixture

Figure 5 indicates that the targeted GLP-1 molecular ions can be unambiguously identified, and thus quantitated in a complex mixture, such as human serum digests. The high-resolution and accurate-mass features of the Exactive LC-MS allow a sufficient separation for the isobaric peaks of highly charged GLP-1 ion from matrix inferences.

FIGURE 5. (A) Chromatogram of gradient-eluted analytes from a serum digest with GLP-1 peptide; (B) Full MS scan of 6+ charge state GLP-1 peptide at 1fmol/µL in serum digest acquired on an Exactive mass spectrometer at 100,000 resolution setting; (C) An ion current peak representing a summation of major isotopes of GLP-1 at charge states 4+ to 7+.



Limit of quantitation (LOQ) in serum digests

Figure 6 demonstrates calibration curves (250 amol/µL to 5 fmol/µL) for GLP-1 (a serum digest) obtained on an Exactive system. The left panel shows a curve using total areas of all major isotopes and the right panel illustrates one using the area of a major isotope. Figure 6 shows summation of areas from all isotopes (charge state 4+ to 7+) provides better sensitivity and linearity for quantitation and offers an LOQ at 250 amol/µL. Calculated concentration, %difference, %relative standard deviation (RSD) of each injection were presented in Table 1. In this experiment, matrix samples include 250 amol/µL to 5 fmol/µL GLP-1 in 1 mg/mL human serum digest.

Conclusions

High-resolution and accurate-mass capabilities of the Exactive mass spectrometer provide a sensitive method for quantitating peptides that are multiply charged and of low fragmentation efficiency. The summation of major isotopes from individual charge states could further increase assay sensitivity and quantitation accuracy. Using this method, analysis of GLP-1 provides an LOD of 30 amol/µL in neat sample and an LOQ of 250 amol/µL in matrix.

FIGURE 6. Calibration curves (250 amol/µL to 5 fmol/µL) for GLP-1 (serum digest matrix) obtained on an Exactive LC-MS system. All injections performed in duplicates. Left Panel: Area was calculated from all isotopes of major charge states; Right Panel: Area from a single isotope of [M+6H]⁶⁺.

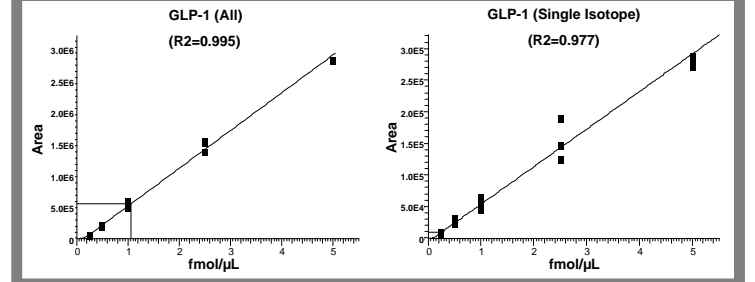


TABLE 1. Calculated concentration, %Difference, and %RSD generated from all major isotopes of individual charge states and %RSD from a single isotope mass of [M+6H]⁶⁺ for GLP-1 serum digest calibration curve.

Levels(fmol/µL)	Cal. Conc.(fmol/µL)	Area	%Diff	%RSD (All)	%RSD (Single)
0.25	0.241	54183	-4%	14.1%	22.3%
0.25	0.227	45735	-9%	14.1%	22.3%
0.25	0.251	60829	1%	14.1%	22.3%
0.50	0.442	177789	-12%	10.9%	15.4%
0.50	0.489	206640	-2%	10.9%	15.4%
0.50	0.513	221080	3%	10.9%	15.4%
1.00	1.155	614609	16%	10.4%	18.1%
1.00	1.065	559438	7%	10.4%	18.1%
1.00	0.966	498291	-3%	10.4%	18.1%
2.50	2.691	1555341	8%	6.2%	22.0%
2.50	2.439	1400704	-2%	6.2%	22.0%
2.50	2.714	1569044	9%	6.2%	22.0%
5.00	4.853	2879402	-3%	0.2%	3.0%
5.00	4.843	2873482	-3%	0.2%	3.0%
5.00	4.861	2884509	-3%	0.2%	3.0%

References

Shipkova P, Drexler DM, Langish R, Smalley J, Salyan ME, Sanders M. Application of ion trap technology to liquid chromatography/mass spectrometry quantitation of large peptides. *Rapid Commun Mass Spectrom.* 2008 May;22(9):1359-66.

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