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A High-Throughput Integrated HRAM-MS Method Enables IGF-1 Quantification, Targeted Variants Monitoring, and Untargeted Variants Screening in a Single Injection

Yu Zhou, Xiaolei Xie, Kristine, Van Natta, Bradley Hart, Shen Luan, Debadeep Bhattacharyya
Thermo Fisher Scientific, 355 River Oaks Parkway, San Jose, CA 95134

ABSTRACT

Purpose: To develop a fast, robust, and highly integrated HRAM data acquisition and data analysis workflow for serum IGF-1 on a modified Thermo Scientific™ Orbitrap Exploris™ mass spectrometer. With fast scanning and enhanced mass accuracy using EasyIC™, this novel method enables IGF-1 quantification, targeted variants identification, and unknown variants screening in a single injection. In addition, the integrated data analysis provides complementary information for improving data quality and confidence.

Methods: We utilized a two-step protein precipitation, combined with online sample extraction on a Thermo Scientific™ Vanquish™ Duo UHPLC. Analytes were detected on a modified orbitrap mass spectrometer. In a single method, data is acquired using a) multiplexed SIM scan mode for IGF-1 quantification, b) tMS^2 mode for targeted known variants confirmation, and c) full scan mode for unknown variants screening.

Results: The modified orbitrap shows good analytical sensitivity, linear dynamic range and excellent reproducibility for reliable serum IGF-I measurements. Intact IGF-1 was quantified at a LLOQ of 10 ng/mL with a standard curve range of 15.6–2,000 ng/mL (r^2>0.99).

INTRODUCTION

Insulin-like growth factor 1 (IGF-1) quantification has many clinical research and anti-doping applications. Differentiating IGF-1 variants is important for capturing mutations and correct interpretation of false results. High-Resolution Accurate-Mass (HRAM) MS methods have unique advantages for reliably measuring and differentiating IGF-1 species, attributes which might be missed by immunoassays and non-HRAM methods. Using the most abundant *m/z* values in the $[M+7H]^{7+}$ isotopic cluster as a quantifier ion, QTOF based HRAM methods have been successfully developed for IGF-1 quantification. However, this has practical challenges in a true routine context for differentiating IGF-1 and its variants due to limited mass resolving capability, compromised specificity and quantitative performance (using ±10 ppm extraction windows), and mass deviation issues.

MATERIALS AND METHODS

Sample Preparation

Calibrators were prepared in 20% acetonitrile in 1X phosphate buffered saline with 1% BSA. Internal standard N_{15}–labeled IGF-1 was purchased from Prospect International.

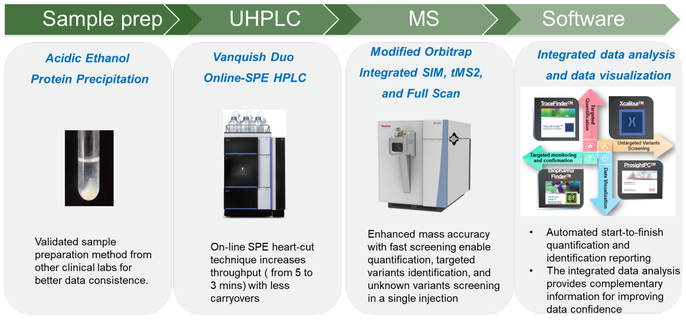
Test Method(s)

Internal standard (IGF1-N^{15}) were added to the serum samples which were first precipitated using acidic ethanol, and second cryoprecipitated after neutralization . Automated online SPE cleanup and chromatographic separation was performed on a Thermo Scientific™ Vanquish™ Duo UHPLC, followed by analyte detection on a modified Thermo Scientific™ Orbitrap Exploris™ mass spectrometer. A Thermo Scientific™ Hypersil Gold™ C8, 50 x 2.1 mm, 5 µm column was used for separation. Total run time was 3 minutes.

Data Analysis

Automated quantification of SIM data was performed by Thermo Scientific™ TraceFinder™ software. Top down and intact protein analysis were processed using Thermo Scientific™ BioPharma Finder™ and ProSight™ software.

Figure 1. Enhanced mass accuracy and fast scanning enable high-throughput integrated IGF-1 quantitation and its variants identification in serum



RESULTS

Figure 2. Mass extraction (±2.5 ppm extraction window) of a single isotope from IGF-1 in the 7+ charge state (*m/z* 1093.5225) was used to generate extracted ion chromatograms for accurate quantification

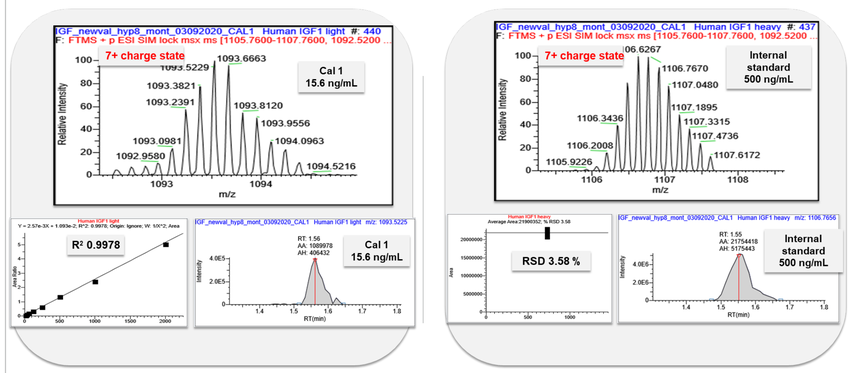
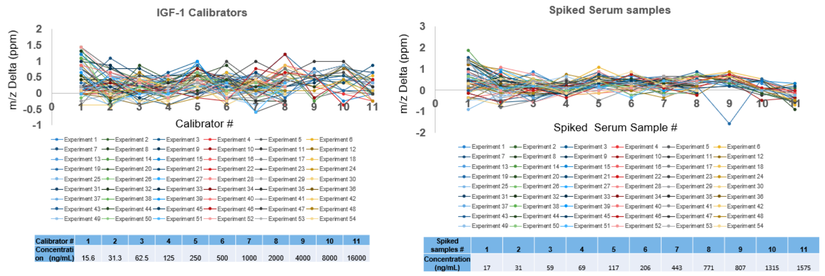


Figure 3. EASY-IC enable IGF-1 mass accuracy within ±0.8 ppm in Calibrators and ±1 ppm in serum samples across 130 hours of non-stop run time.



Calibrator #	1	2	3	4	5	6	7	8	9	10	11
Concentration (ng/mL)	15.6	31.3	62.5	125	250	500	1000	2000	4000	8000	16000

Spiked samples #	1	2	3	4	5	6	7	8	9	10	11
Concentration (ng/mL)	17	31	59	69	117	206	443	771	807	1315	1575

Figure 4. Overlay of 54 Calibration Experiments across 130 hours of non-stop run time for the IGF-1 quantification robustness.

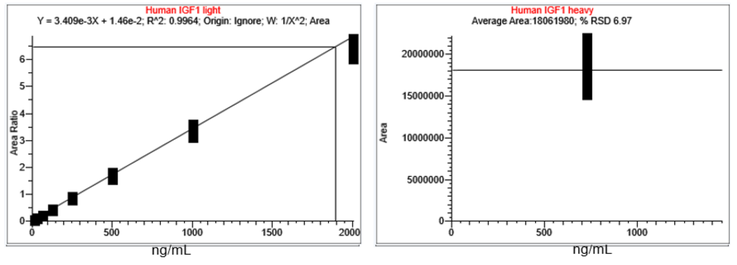


Table 1. Robustness statistics for replicates (n=54) of a calibration curve across 130 hours of non-stop run time for IGF-1.

Level (n=54)	Concentration	% Con Bias (Average)	% Ion Ratio	% CV
Cal1	15.6 ng/mL	-2%	pass	**7%**
Cal2	31.3 ng/mL	2%	pass	**4%**
Cal3	62.5 ng/mL	2%	pass	**4%**
Cal4	125 ng/mL	2%	pass	**3%**
Cal5	250 ng/mL	2%	pass	**4%**
Cal6	500 ng/mL	1%	pass	**3%**
Cal7	1000 ng/mL	-1%	pass	**3%**
Cal8	2000 ng/mL	-6%	pass	**3%**

Table 2. Robustness statistics for replicates (n=54) of 7 spiked serum samples across 130 hours non-stop run time for IGF-1.

Spiked sample # (n=54)	Concentration	% Recovery (Average)	% Ion Ratio	% CV
Sample 1	15.6 ng/mL	-4%	pass	**13%**
Sample 2	31.3 ng/mL	-5%	pass	**6%**
Sample 3	62.5 ng/mL	-7%	pass	**4%**
Sample 4	125 ng/mL	-7%	pass	**3%**
Sample 5	250 ng/mL	-16%	pass	**3%**
Sample 6	500 ng/mL	-12%	pass	**3%**
Sample 7	1000 ng/mL	-20%	pass	**3%**

CONCLUSIONS

- The Vanquish Duo UHPLC platform combined with the modified Orbitrap mass spectrometer provides a fast, robust, and highly integrated HRAM data acquisition and data analysis workflow for serum IGF-1.
- Normal-type, know variants and unknown IGF-1 variants can be measured in a single run.
- Based on accurate mass and signature isotope distribution, HRAM full scan mode allows identification of IGF-1 and its variants, including pathogenic, mutations, and post translational modifications. A great advantage to a full scan monitoring is the ability to re-analyze data when unknown mutations are suspected.
- The Vanquish Duo provided both samples clean-up and analytical separation in a 3-miniute method, enhancing the ability of the mass spectrometer to detect compounds.
- The modified orbitrap mass spectrometer provide excellent robustness of data over long run-times without maintenance.

REFERENCES

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TRADEMARKS/LICENSING

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