Determination of Insulin-Like Growth Factor-1 in serum by HRAM LC/MS for clinical research

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Introduction

High Resolution Accurate Mass (HRAM) Liquid Chromatography Mass Spectrometry (LC/MS) is ideally suited for the rapid analysis of biomolecules. A highly sensitive and specific HRAM LC/MS method has been developed for the quantitation of Insulin-Like Growth Factor-1 (IGF-1) in serum. This method uses a simple sample preparation combined with an online sample cleanup procedure coupled to a high resolution accurate mass quadrupole time-of-flight mass spectrometer.

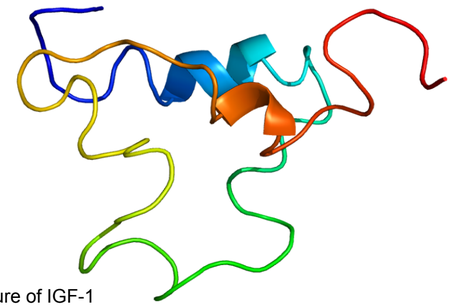

Figure 1. Structure of IGF-1

An efficient sample preparation procedure was developed for the extraction of IGF-1 in serum. Calibrators were created by spiking clean serum with various concentrations of IGF-1. The chromatographic system consists of a C8 extraction column coupled with a high resolution, 300 angstrom pore size analytical column and a mobile phase comprised of acetonitrile and water containing 0.2% formic acid. Quantifier and qualifier transitions were monitored and Rat IGF-1 internal standard was included to ensure accurate and reproducible quantitation. Standards and samples were kindly supplied by Mayo Clinic. Online sample cleanup, heart-cutting and chromatographic separation of a sample is achieved in less than three minutes. The separation of Rat IGF-1 and Human IGF-1 is especially critical; without proper separation by retention time, impurities present in both compounds can cause interferences with one another and lead to inaccurate quantitation.

Experimental

Sample Preparation

Pipette 100 µL of serum, 10 µL of ISTD and 400 µL of acid ethanol into a tube and vortex. Incubate at room temperature for 30 minutes and then centrifuge for 10 minutes at 14000 RPM. Transfer 350 µL of supernatant to a clean microcentrifuge tube along with 60 µL of 1.5 M Tris base. Chill at -20 °C for 30 minutes and then centrifuge for 10 minutes at 14000 RPM. Transfer supernatant and Speed Vac at 48 °C for 1 hour. Reconstitute with 100 µL of 0.1% TFA in 15% acetonitrile/85% H2O.

LC Method

Two Agilent 1290 HPLC binary pumps, well plate sampler with thermostat, temperature-controlled column compartment, 2 positions/6 ports switching valve.

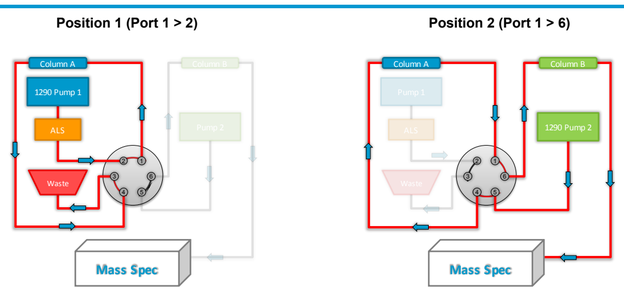

Figure 2. Back-Flush LC configuration for online sample cleanup and heart-cutting

Parameter	Value
Trapping Column	Poroshell 120 EC-C8, 3x5 mm, 2.7 µm
Analytical Column	Zorbax 300-SB-C18, 2.1x50 mm, 1.8 µm
Injection Volume	20 µl
Needle Wash	Methanol in Flush port for 15 seconds
Mobile Phase A	Water + 0.2 % Formic Acid
Mobile Phase B	Acetonitrile + 0.2 % Formic Acid
Switching Valve	Pos 1 (0.0 min), Pos 2 (0.5 min), Pos 1 (0.9 min)
Loading pump	At 2.0 mL/min, hold at 15% B for the first 0.9 min and then step up to 90% B at 0.91 min for the remainder of the run.
Analytical pump	At 0.5 mL/min, hold at 25% B for the first 0.9 min followed by a gradient to 40% B ending at 2 min. Step up to 90% B at 2.1 min and hold for the remainder of the run. Stop time at 3.0 min.
Post Time	90 seconds

Experimental

MS Method

Agilent 6550 iFunnel QTOF with JetStream Technology	
Ion Mode	Dual Agilent Jet Stream (Dual AJS)+
Drying gas	200 °C, 16 L/min
Nebulizer gas	30 psi
Sheath gas	200 °C, 12 L/min
Capillary voltage	4500 V
Nozzle voltage	500 V
RF funnel	High 210, Low 160
Mass Range	m/z 800-1700
Scan rate	3 spectra/sec
Reference mass	m/z 922.009798
Resolution mode	High 4 GHz

Results and Discussion

Narrow mass extraction of a single isotope from IGF-1 in the 7+ charge state was used to generate extracted ion chromatograms for quantitation. Three other isotopes of the same charge state cluster are used for qualifiers.

	Human IGF1	Rat IGF1
Chemical Formula	C331H512N94O101S7	C336H522N94O99S7
Average MW (da)	7648.788	7686.925
Charge state	7	7
Monoisotopic mass 1 m/z	1093.5209	1098.9622
Monoisotopic mass 2 m/z	1093.3778	1098.8190
Monoisotopic mass 3 m/z	1093.6641	1099.1053
Monoisotopic mass 4 m/z	1093.8051	1099.2484

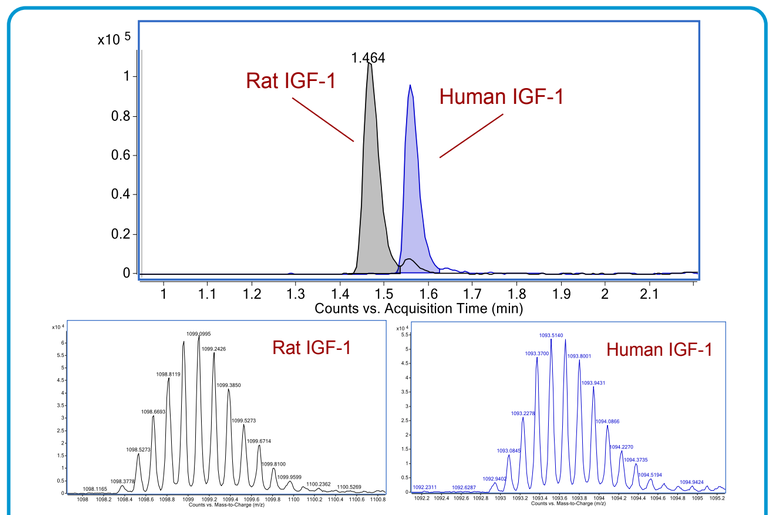

Figure 3. Narrow mass extracted chromatograms +/- 10 ppm for quantitation and high resolution mass spectra

Data review for HRAM LC/MS

MassHunter Quantitation software is used for data mining. Included with the basic quantitation information, high resolution and accurate mass scores as well as qualifier ratios are used to quickly assess data quality acceptance criteria. Graphical overlays for qualifiers and for isotopic distributions are also used to facilitate data review.

Figure 4. MassHunter Quantitation software tools for HRAM LC/MS data review

Results and Discussion

Calibration curve

Preparation of calibrants was done by first measuring the endogenous IgF-1 concentration in pool serum against a curve made in 0.1% BSA. The obtained value was 90 ng/mL. Lower levels were prepared by diluting down with 0.1% BSA and higher levels were prepared by spiking up with IgF-1 standard.

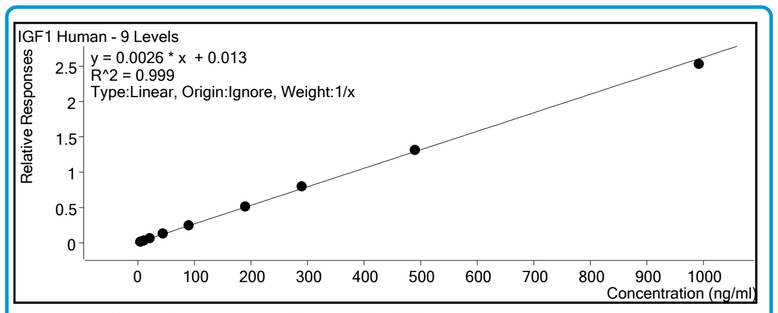

Figure 5. Calibration curve for IGF-1, 5.6 to 990 ng/mL

IGF1 Concentration (ng/ml)	Accuracy (%) n = 3	Intraday CV (%) n = 3
5.63	84.3	7.9
11.25	96.8	7.6
22.5	105.0	3.0
45	103.7	4.5
90	103.3	3.0
190	103.0	3.9
290	106.1	2.4
490	101.4	0.3
990	96.5	1.0

Accuracy and reproducibility

Separately prepared incurred samples were used to test the accuracy and reproducibility of this method. Measurements were repeated in triplicates and on three separate extractions to assess intra- and inter- day reproducibility and CVs were found to be below 8%. All measurements are in ng/mL.

	1st injection:	2nd injection:	3rd injection:	Intraday Ave:	Intraday CV%:
Sample 1-a	45.8	43.5	43.4	***44.3***	***3.1***
Sample 1-b	48.0	46.5	47.9	***47.5***	***1.8***
Sample 1-c	51.0	47.9	44.0	***47.6***	***7.4***
Inter-run Ave:	***48.3***	***46.0***	***45.1***		
Inter-run CV%:	***5.3***	***4.8***	***5.4***		
Sample 2-a	117.1	108.9	117.8	***114.6***	***4.3***
Sample 2-b	123.4	109.2	122.8	***118.5***	***6.8***
Sample 2-c	114.9	106.1	114.9	***112.0***	***4.5***
Inter-run Ave:	***118.5***	***108.1***	***118.5***		
Inter-run CV%:	***3.7***	***1.6***	***3.4***		
Sample 3-a	287.6	268.8	253.8	***270.1***	***6.3***
Sample 3-b	284.4	263.3	253.9	***267.2***	***5.8***
Sample 3-c	301.7	281.8	259.0	***280.8***	***7.6***
Inter-run Ave:	***291.3***	***271.3***	***255.6***		
Inter-run CV%:	***3.2***	***3.5***	***1.2***		
Sample 4-a	370.5	355.9	376.7	***367.7***	***2.9***
Sample 4-b	390.0	399.2	376.7	***388.6***	***2.9***
Sample 4-c	411.0	397.7	382.6	***397.1***	***3.6***
Inter-run Ave:	***390.5***	***384.3***	***378.7***		
Inter-run CV%:	***5.2***	***6.4***	***0.9***		

QTOF instrument models comparison

The same method was also evaluated on an Agilent 6530 QTOF (data not shown). The Agilent 6550 QTOF demonstrates a better signal to noise ratio at the 5.63 ng/mL level.

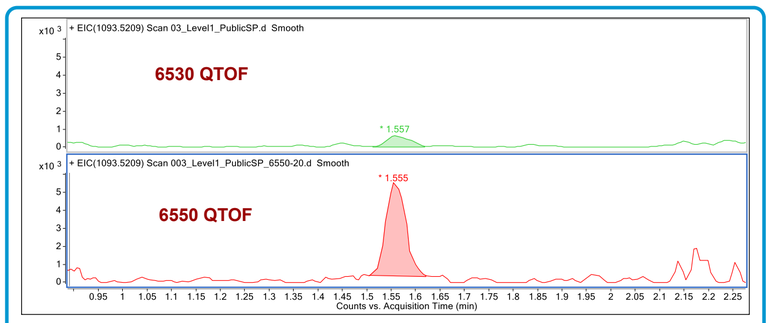

Figure 6. Human IGF-1, 5.63 ng/mL in serum

Conclusions

A robust method for quantifying Insulin-Like Growth Factor-1 in serum with excellent reproducibility and accuracy has been developed.

Bystrom, Cory E. "Narrow Mass Extraction of Time-of-Flight Data for Quantitative Analysis of Proteins: Determination of Insulin-Like Growth Factor-1." Anal. Chem. 2011, 83, 9005-9010.