

A High Throughput and High Resolution LC-MS/MS Method to Measure IGF1 in Serum for Clinical Research

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ABSTRACT

Purpose:

To develop a high throughput and high resolution LC-MS/MS method to quantify insulin-like growth factor 1 (IGF1) in human serum for clinical research on a four-channel HPLC with online SPE and a high-resolution accurate-mass (HRAM) mass spectrometer (MS) powered by Orbitrap™ technology.

Methods:

Serum samples were precipitated by adding acidified ethanol. After a second step precipitation by tris base, supernatant was diluted and injected onto an online sample clean-up column. LC-MS/MS was performed on a four-channel HPLC system (Thermo Scientific™ Transcend™ II TLX-4 system) coupled with a high resolution mass spectrometer, Thermo Scientific™ Q Exactive™ Focus hybrid Quadrupole-Orbitrap™ MS. Intact protein analysis under high resolution was performed for quantitation and isotope ion ratio was used for confirmation.

Results:

High resolution LC-MS/MS can accurately quantify IGF1 in human serum with high sensitivity and selectivity. Our method was able to maintain the integrity of IGF1 protein by performing online sample clean-up. LLOQ was 7 ng/mL with ion ratio confirmation. The intra and inter-assay precision was good with CV less than 10%. Recovery was between 85% to 115%. By using a four-channel HPLC coupled with a HRAM mass spectrometer, we could analyze ~48 samples per hour.

INTRODUCTION

IGF1 is a 70-amino acid polypeptide (molecular weight 7.6kDa). IGF1 is the major mediator of the anabolic and growth-promoting effects of growth hormone (GH). Low IGF1 level is observed in GH deficiency or GH resistance. If acquired in childhood, these conditions result in short stature. Several immunoassays such as RIA, ICMA, or ECL have been developed to measure IGF1 level in serum. However, due to cross-reactivity, immunoassays can produce erroneously high values in some patients. LC-MS/MS removes cross-activity by direct measurement of IGF1 and thus does not produce the erroneously high results seen in immunoassays.

MATERIALS AND METHODS

Figure 1. From sample to knowledge IGF1 method

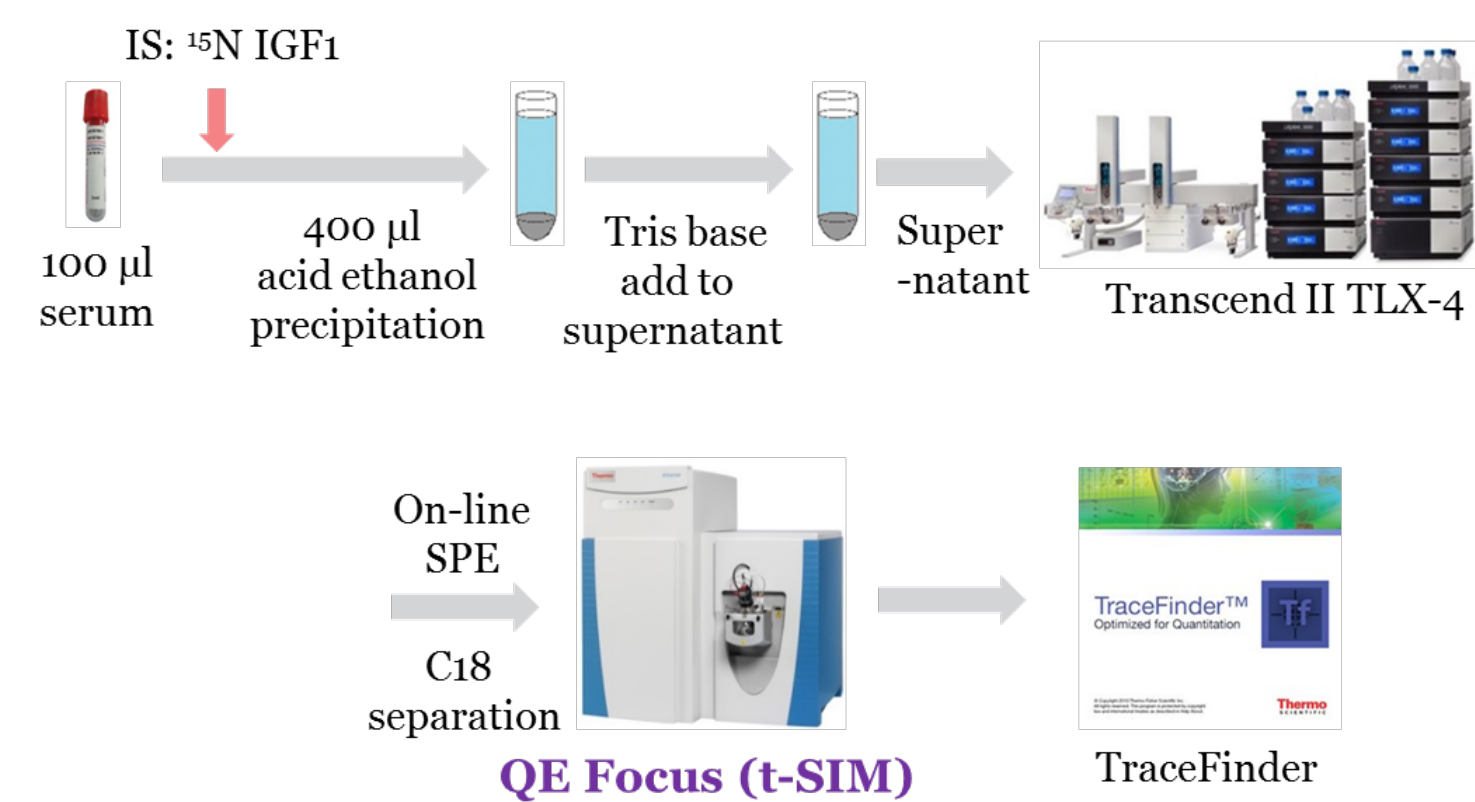


Figure 2. Transcend II TLX-4 HPLC online SPE method

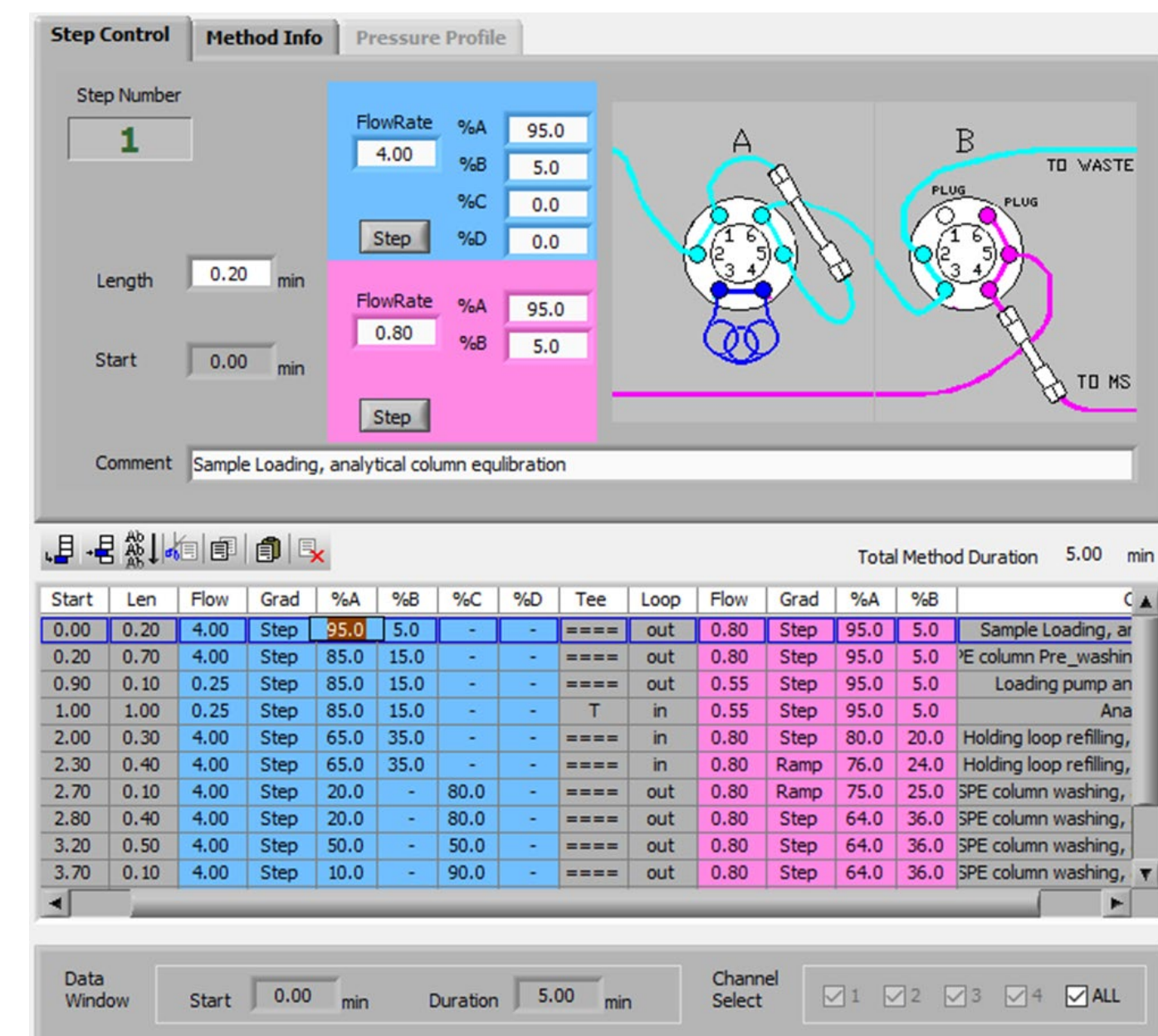
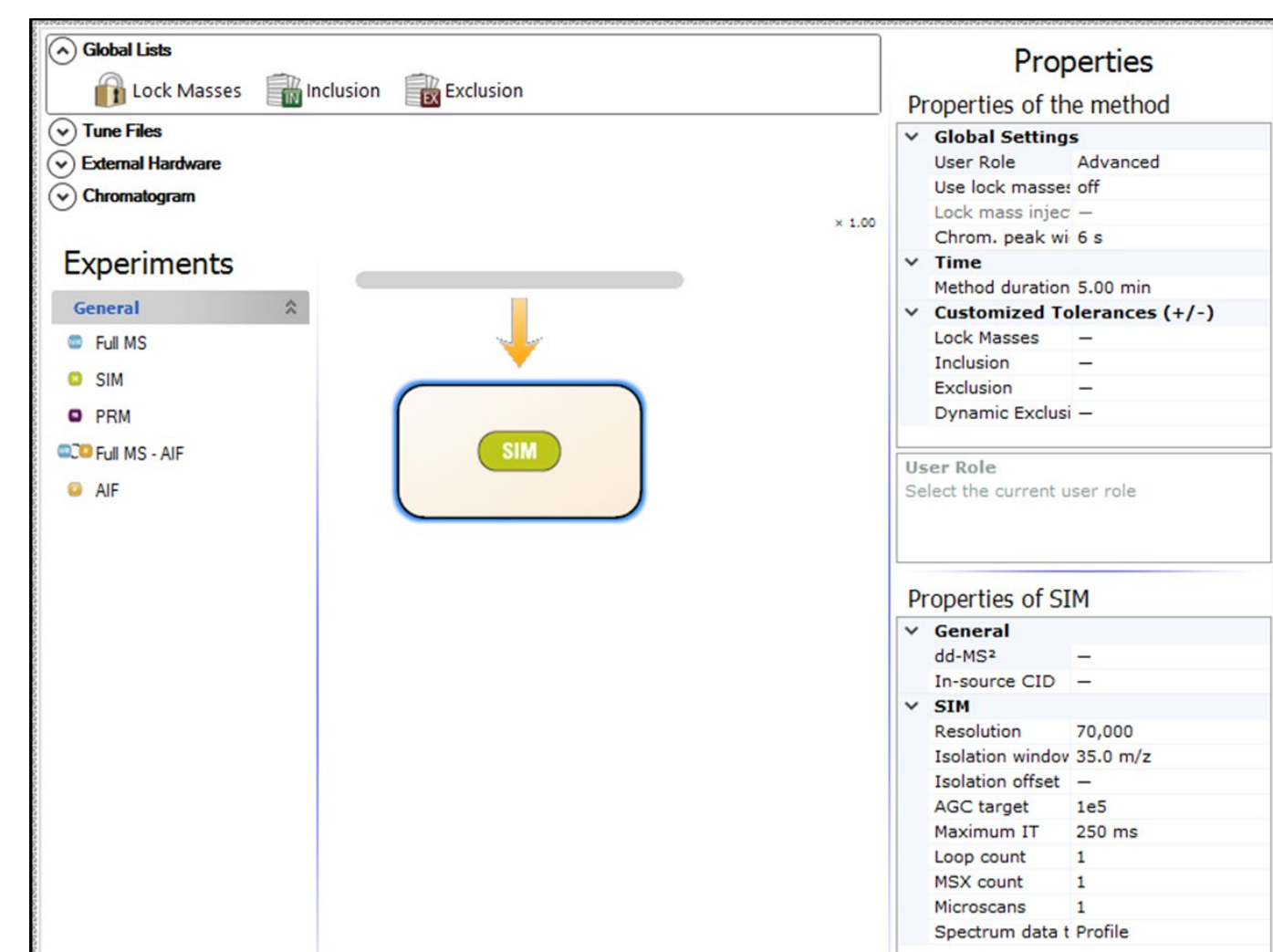


Figure 3. Q Exactive Focus MS method



RESULTS

Figure 4. Four-channel HPLC maximizes the productivity of MS

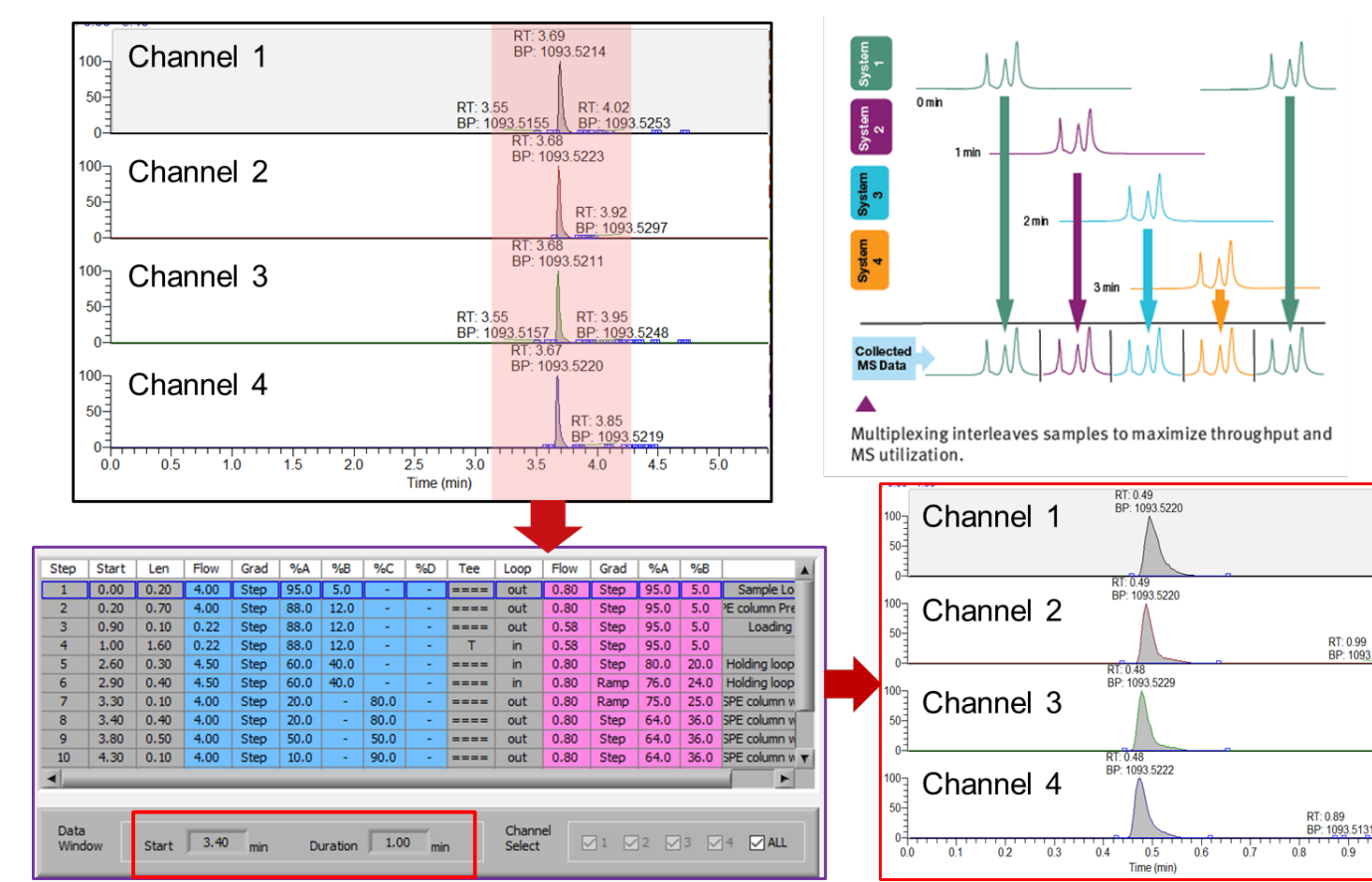


Figure 5. Calibration curve with good linearity (R²>0.999)

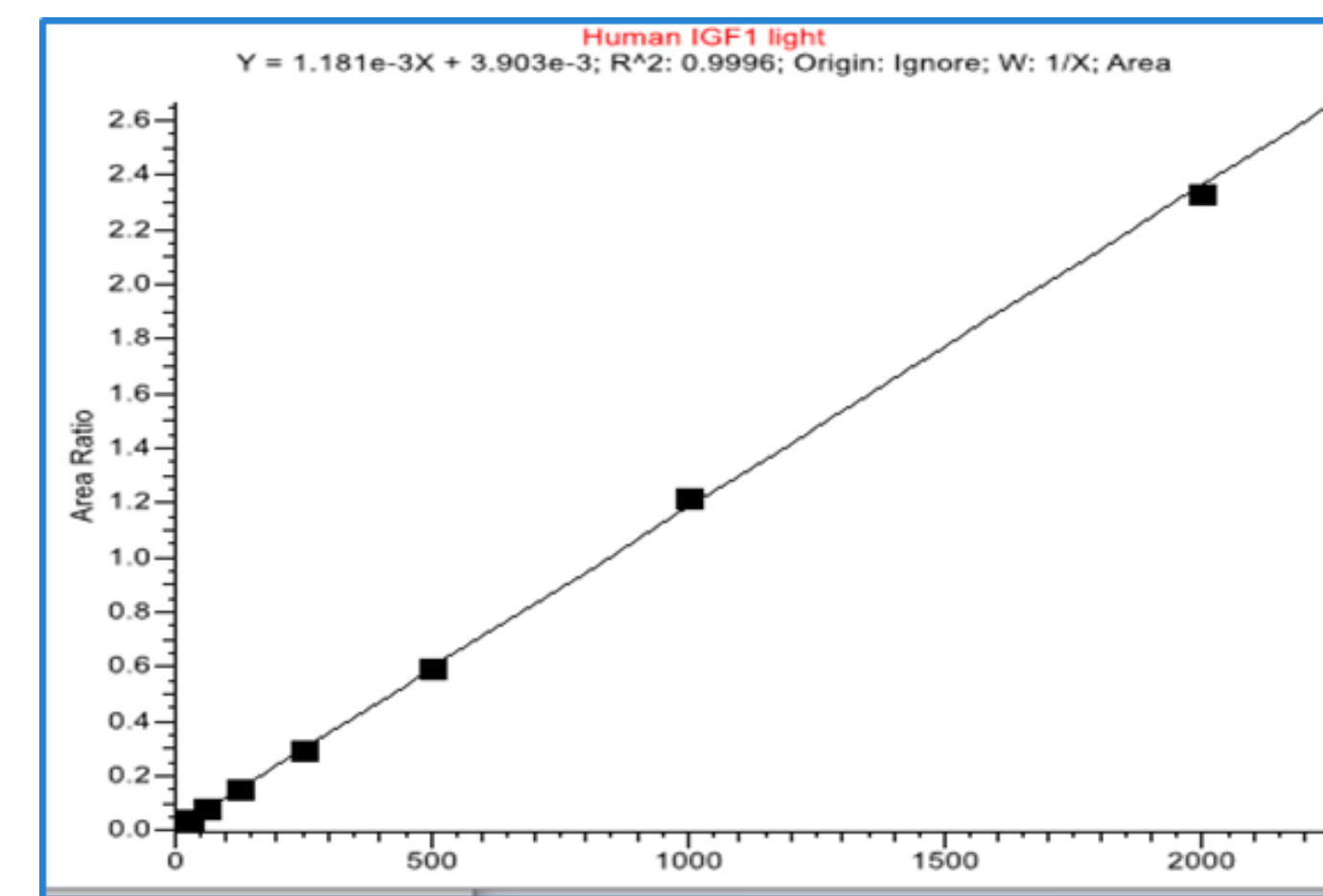


Figure 6. LLOQ: 7 ng/mL with ion ratio confirmation. Quantitation range: 7-2000 ng/mL

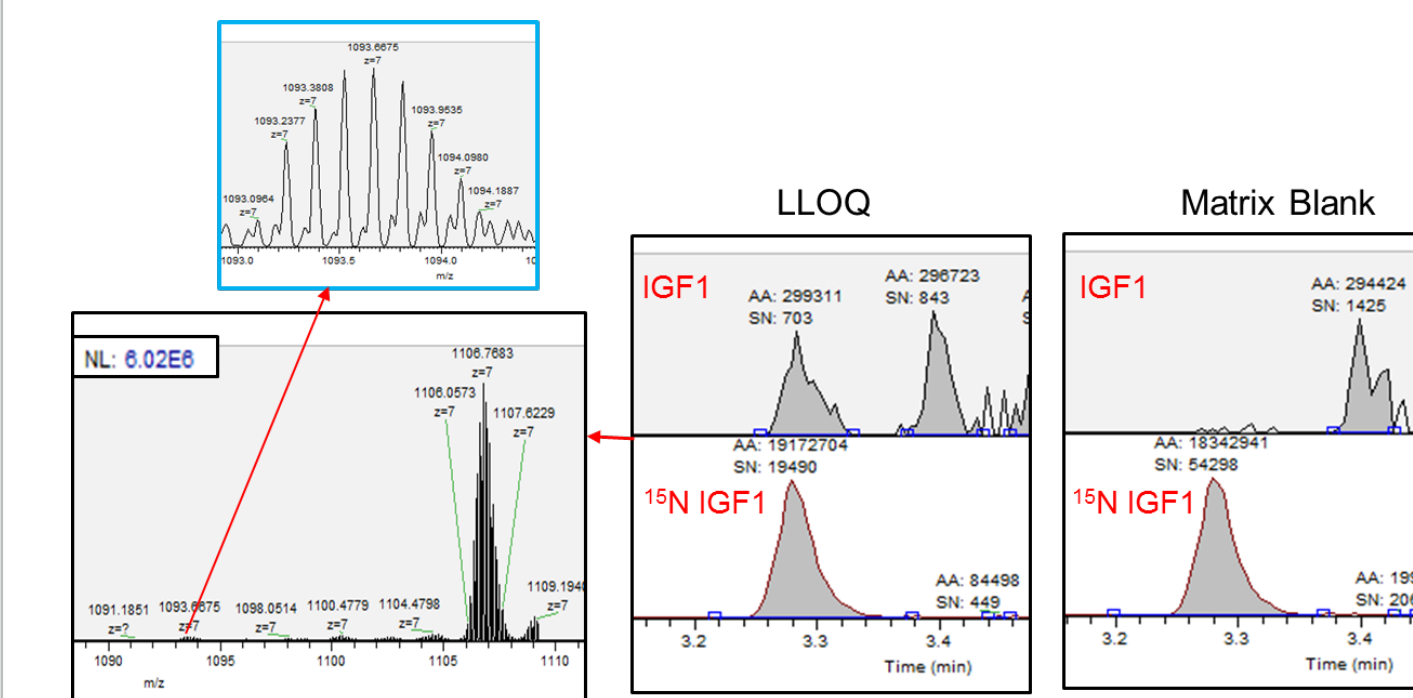
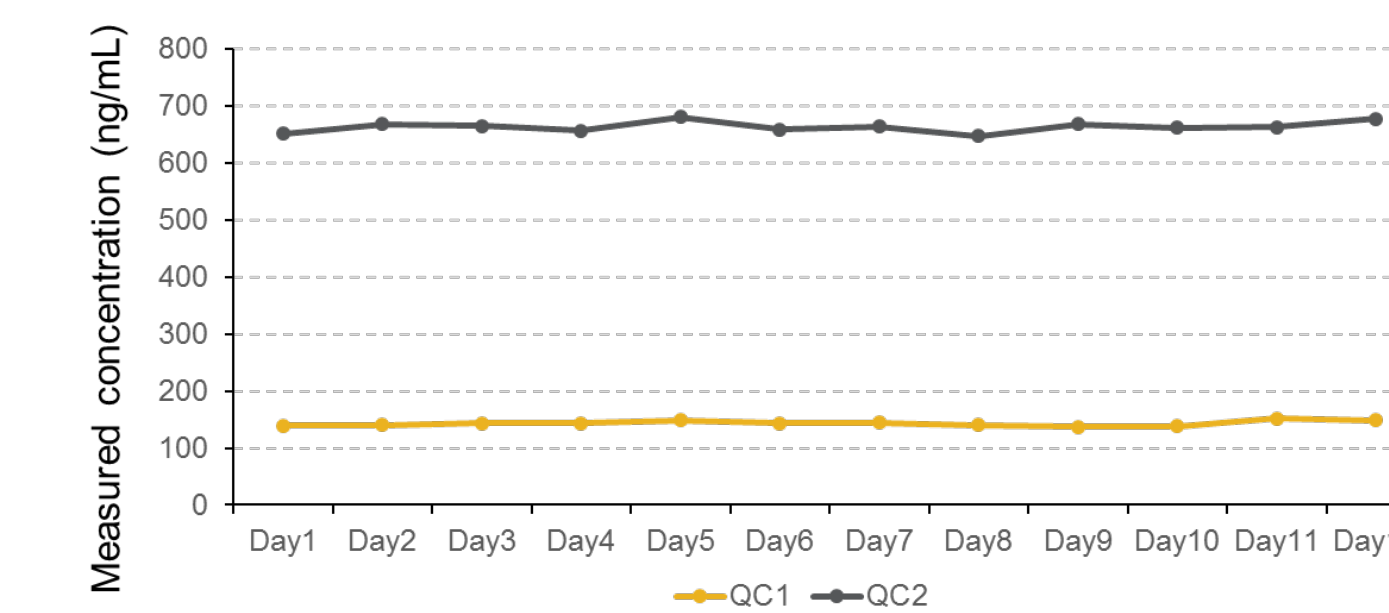


Figure 7. Intra-assay precision (CV<11%). Recovery between 85% to 115%

No.	Patient sample (ng/mL)	No.	pool-L (ng/mL)	pool-H (ng/mL)
Rep1	39.04	Rep1	54.88	124.67
Rep2	29.27	Rep2	79.81	122.43
Rep3	31.94	Rep3	61.66	117.96
Rep4	34.73	Rep4	58.32	116.79
Rep5	37.70	Rep5	58.31	117.55
Rep6	33.96	Rep6	56.44	119.06
Rep7	34.98	Rep7	60.17	126.31
Rep8	32.37	Rep8	57.70	122.79
Rep9	34.69	Rep9	60.51	124.74
Rep10	31.44	Rep10	58.34	125.81
Rep11	33.53	Rep11	59.50	122.40
Rep12	35.33	Rep12	62.91	131.09
Mean	34.08	Mean	60.71	122.63
SD	2.67	SD	6.40	4.25
CV	7.85	CV	10.54	3.46

Figure 8. Inter-assay precision (CV<4%)



QC1 Mean: 143.161 CV: 3.1%
 QC2 Mean: 663.230 CV: 1.4%

CONCLUSIONS

We developed a high resolution LC-MS/MS method for high throughput quantification of IGF1 in human plasma using Transcend II TLX-4 four-channel HPLC and Q Exactive Focus MS.

- Sample preparation
 - Simple and reliable, just two steps of protein precipitation, no speedvac needed.
- Online SPE
 - The focus mode on Transcend II TLX HPLC system enables easy and reliable online SPE method.
- Throughput
 - Transcend II TLX-4 HPLC enables four-channel online sample preparation, analyzing one sample in 1.25 min.
- Mass Spectrometry
 - Q Exactive Focus MS offers high selectivity and sensitivity
- Economical efficiency
 - Low cost for daily runs: the primary consumables are the internal standard, SPE column and analytical column.

TRADEMARKS/LICENSING

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