Quantitation Analysis of Human Insulin-like Growth Factor I (IGF1) in Serum by Using SPE-LC-MS/MS Workflow

Featuring SCIEX Triple Quad™ 4500 and QTRAP® 4500 Systems

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Human insulin-like growth factor 1 (IGF1) is a 70-amino acid polypeptide hormone. It is produced primarily in liver and serves as an endocrine (as well as paracrine and autocrine) hormone mediating the action of growth hormone in peripheral tissues such as muscle, cartilage, bone, kidney, nerves, skin, lungs, and the liver itself. IGF1 has been reported to be associated with multiple diseases and clinical symptoms including miscarriage, aging, cancers, stroke, dwarfism, etc. Therefore, accurate quantification of IGF1 in biological fluids has become an increased need for therapeutic research and development.

The IGF1 levels in biological matrix are between 15 and 750 ng/mL, its quantification is traditionally done by ligand binding assays (LBAs). Recently LC-MS has been increasingly adopted for peptide/protein quantitation serving as an orthogonal technology to the traditional LBAs. Compared to LBAs, LC-MS based workflows show significant advantages including short method development time, independency of antibodies, wide dynamic range, high sensitivity, excellent specificity, as well as multiplexing capability. Herein, a SPE LC-MS/MS workflow was developed to quantify IGF1 in serum by using SCIEX Triple Quad 4500 or QTRAP 4500 mass spectrometer. This method provides accurate quantification of IGF1 from 10 to 2430 ng/mL which well covers the IGF1 biologically relevant concentration range in biological matrix.

Key Feature of SCIEX Triple Quad and QTRAP 4500 systems for peptide quantification

- A wide mass range provides coverage from small to large peptides for precursor ion isolation and fragment ion selection for quantification
  - Triple quadrupole mass range (m/z): 5-2000
  - Linear Ion Trap mass range (m/z): 50-2000
- Ultra-low MRM dwell time (as low as 1 msec) to allow parallel quantification of multiple peptide targets in complex biological matrix or protein digest
- High pressure Q0 and QJet® ion guide and fast eQ™ electronics provide enhanced sensitivity

- The Turbo V™ ion source and Curtain Gas™ interface provide high robustness and ruggedness for high throughput, large scale sample analysis with minimum instrument maintenance requirement.

Methods

Materials and Reagents: Human IGF1 recombinant protein and human IGF1 partial recombinant protein were purchased from Abnova. The SPE plate (PAX 96-well plate 30 mg/2mL) was purchased from Agela.

Protein Precipitation: Due to the notable endogenous level of IGF1 in human serum, rat serum was used to prepare calibration curve. The IGF1 protein standard was spiked in 100 µL of rat serum with final concentrations at 10, 30, 90, 279, 810 and 2430 ng/mL. The blank human serum and human serum with IGF1 spiked in at 50 ng/mL served as unknown sample to evaluate the quantification accuracy. IGF1 partial recombinant protein was spiked in all samples as internal standard. The samples were mixed with 100 µL of 0.1% 3-((3-cholamidopropyl) dimethylammonio)-1-propanesulfonate (CHAPS) in water and incubated for 45 min at 37 °C, followed by incubating with 200 µL 95/5 (V/V) acetonitrile/acetic acid for 5 min at room temperature. After centrifugation at 15000 RPM for 5 min, the supernatants were collected and added with 900 µL 5% ammonia for solid phase extraction (SPE). The SPE condition are summarized in Table 1.

Table 1: SPE conditions for IGF1.

Plate/Step	Details
SPE plate	Agela PAX 96-well plate 30 mg/2mL
Activation	500 µL of methanol
Equilibration	500 µL of 5% ammonia
Sample loading	600 µL X 2
Wash 1	200 µL of 5% ammonia
Wash 2	200 µL of 1/5/94 (V/V/V) acetic acid/methanol/water
Elution 1	25 µL of 60/30/10 (V/V/V) methanol/water/acetic acid X 2
Elution 2	50 µL of water

Table 2: Chromatographic conditions for IGF1 analysis.

Parameter	Value
Stationary phase	Waters ACQUITY UPLC CSH C18 (2.1x100mm, 1.7 µm)
Mobile phase A	0.1% acetic acid in water
Mobile phase B	0.1% acetic acid in 3/1 (V/V) methanol/acetonitrile
Flow rate	300 µL/min
Column temperature	50 °C
Injection volume	20 µL

Time	Flow Rate (µL/min)	%A	%B
0	300	90	*10*
3	300	*20*	*80*
3.1	300	*5*	*95*
3.6	300	*5*	*95*
3.7	300	*90*	*10*
5	300	*90*	*10*

Table 3: MS conditions for IGF1 analysis.

Analyte ID	Q1	Q3	Dwell (ms)	DP	CE
IGF1-IS	973.5	473.5	80	60	48
IGF1	957.2	473.5	80	80	47

Source/Gas Parameter	Value	Source/Gas Parameter	Value
Curtain gas:	30	CAD gas:	Medium
Ion source gas 1:	55	Ion spray voltage:	5500
Ion source gas 2:	55	Source temperature:	550

LC-MS Conditions: The 2-step SPE eluents were combined and injected onto the QTRAP 4500 system coupled with Shimadzu Prominence HPLC system for LC-MS/MS analysis. Table 2 describes the chromatographic conditions. Table 3 describes the MS conditions.

Data Processing: Data was processed using MultiQuant™ Software 3.0.2.

Results and Discussion

IGF1 is a strong adhesive protein that tends to non-specifically bind to matrix molecules and lab apparatus during sample preparation, therefore effecting sample recovery. In order to minimize non-specific binding, low binding centrifuge tubes and CHAPS was used during sample preparation. Additionally, human IGF1 partial recombinant protein which is one amino acid different from IGF1 was chosen as an internal standard. Its similar physical and chemical characters to IGF1 made it an ideal internal standard to normalize any sample-sample inconsistency during preparation and LC-MS analysis.

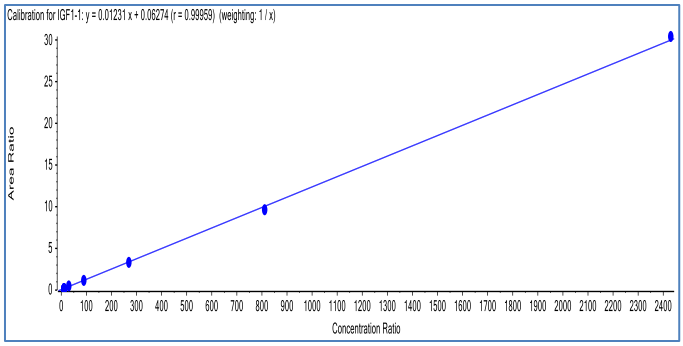


Figure 1. Calibration curve for quantitation of IGF1 in rat serum (10 ng/mL to 2430 ng/mL).

Index	Sample Name	Sample ID	Sample Type	IS	Component Name	IS Name	Actual Concentration	Calculated Concentration	Accuracy
24	C1_1		Standard		IGF1-1	IGF1-IS-1	10.00	9.99	99.89
31	C1_2		Standard		IGF1-1	IGF1-IS-1	10.00	9.12	91.15
38	C1_3		Standard		IGF1-1	IGF1-IS-1	10.00	9.69	96.88
45	C1_4		Standard		IGF1-1	IGF1-IS-1	10.00	10.41	104.08
52	C1_5		Standard		IGF1-1	IGF1-IS-1	10.00	10.43	104.32
59	C1_6		Standard		IGF1-1	IGF1-IS-1	10.00	10.58	105.80

Group by Concentration for Standards — Calculated Concentration

Row	Component Name	Actual Concentration	Num. Values	Mean	Standard Deviation	Percent CV	Accuracy	Valu
1	IGF1-1	10.00	6 of 6	10.04	0.56	5.57	100.35	9.99

Figure 2. Summary of IGF1 Quantification Results.

With the optimized method condition, the presented LC-MS/MS assay achieved a LLOQ of 10 ng/mL for IGF1 quantification in rat serum. The calibration curve covered a concentration range of 10-2430 ng/mL and displayed a regression coefficient (r) of 0.999 using a weighting of 1/x (Figure 1). As shown in Figure 2, the assay accuracy is 91-106%. To evaluate the assay reproducibility especially for the low concentration samples, six individually prepared samples with IGF1 at 10 ng/mL were analyzed and reported with CV% as 5.57% (Figure 2 and 3).

The IGF1 levels of the two unknown samples, blank human serum and human serum spiked with 50 ng/mL IGF1 were reported as 81.9 ng/mL and 149.3 ng/mL. To evaluate the quantification accuracy, the 50 ng/mL IGF1 spiked human serum was set as quality control (QC) sample with theorical concentration at 131.9 ng/mL (81.9 + 50 ng/mL). The accuracy for the QC sample was calculated as 113.2% (Figure 4).

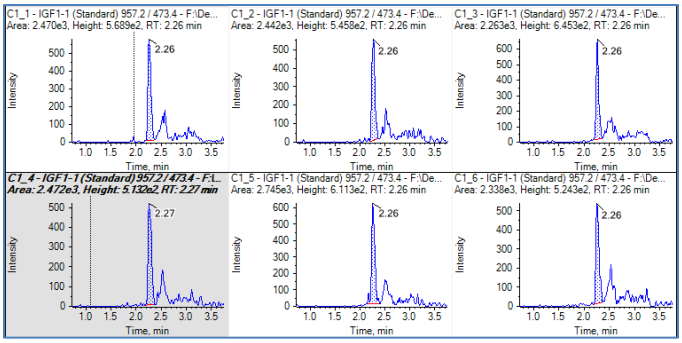
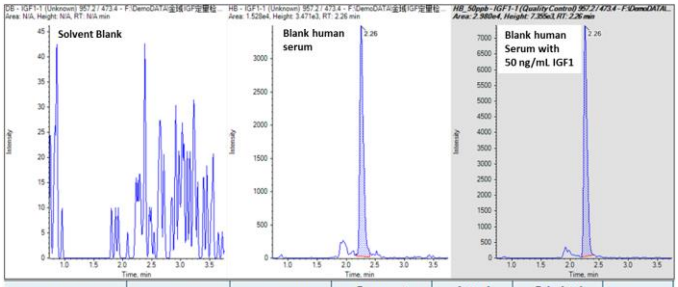


Figure 3. The extract ion chromatograms (XICs) of IGF1 from six individually prepared samples at 10 ng/mL.



Sample Name	Sample Type	Component Name	Component Group Name	Actual Concentration	Calculated Concentration	Accuracy
DB	Unknown	IGF1-1	IGF1	N/A	N/A	N/A
HB	Unknown	IGF1-1	IGF1	N/A	8.196e1	N/A
HB_50ppb	Quality Control	IGF1-1	IGF1	131.90	1.493e2	113.19

Figure 4. Extracted Ion Chromatograms for IGF1. Top: XIC of IGF1 from solvent blank, blank human serum and blank human serum spiked with 50 ng/mL IGF1 samples. Bottom: Quantification results of the QC sample (blank human serum spiked with 50 ng/mL IGF1).

Conclusion

An SPE LC-MS/MS method for quantifying IGF1 in serum was successfully developed. The optimized sample preparation efficiently reduced the protein loss due to non-specific binding. The QTRAP 4500 LC-MS/MS system provides reliable quantitation of IGF1 at 10 ng/mL level with high reproducibility, wide dynamic range coverage and high throughput. The presented work serves as a solid solution to quantify challenging peptide in biological matrix.

References

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2. Li *et al*., (2013) *J. Anal. Bioanal. Tech.* **S5.**

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