Quantitation of Human Insulin-Like Growth Factor-1 and 2 by MRM LC-MS/MS with Scheduled Ionization

Featuring SCIEX QTRAP® 6500+ and Triple Quad™ 6500+ Mass Spectrometer

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Insulin-like growth factor(IGF)-1 and 2 are ~7 kDa protein hormones that are monitored in the study of human growth disorders. In biological matrices, approximately 99% of IGF-1 in blood is bound to six IGF-1 binding proteins (IGFBPs), while IGF-2 binds and interacts with IGF-1 receptor, IGF-2 receptor, IGFBP3 and transferrin. Dissociating IGFs from IGFBPs or receptors and preventing the reformation of these complexes are crucial for quantitative analysis of IGF-1 and 2.

While traditional sample preparation methods using acidic alcohol[1] or sodium dodecyl sulphate (SDS)[2] meet significant challenges in obtaining optimal analyte recovery and sample cleanness, herein an MRM LC-MS/MS method coupled with mass spectrometry friendly sample preparation protocol utilizing CHAPS (3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate) is reported. The majority of CHAPS was removed from the samples during protein precipitation and SPE cleanup.[3] The remaining trace amount of CHAPS were base line separated from the analyte with the optimized HPLC condition and prevented from getting into the MS system by applying the scheduled ionization feature in Analyst® software 1.7 (Figure 1). With this method, IGF-1 and 2 were solidly quantified at 2 ng/mL level in serum samples by utilizing the QTRAP 6500+ LC-MS/MS system.

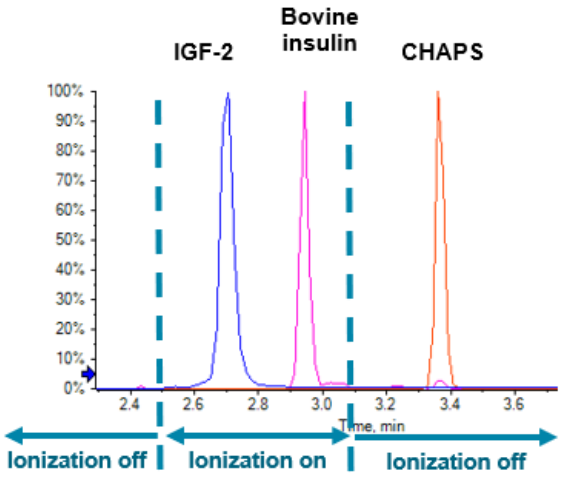

Figure 1. Chromatography. Baseline separation of CHAPS from analytes and scheduled ionization setup.

SCIEX QTRAP 6500+ system coupled with ExionLC HPLC system

Key Features of the MRM LC-MS/MS workflow for intact IGF-1 and 2 quantitation

- SCIEXQTRAP 6500+ and Triple Quad 6500+ mass spectrometer offers:
  - Superior quantitation with high sensitivity, speed, reproducibility and robustness for biological analytes in the most challenging matrices
  - Over five orders of linear dynamic range for enhanced breadth of applications
- Analyst software 1.7 offers scheduled ionization feature to reduce the mass spectrometer downtime by decreasing the risk of contamination.

Methods

Sample Preparation: IGF-1 and 2 (Cell Sciences, Inc.) were serial diluted with 30% methanol, 60% water and 10% acetic acid. Long acting human IGF-1 analog, human LR3 IGF-1 (Cell Sciences, Inc.), was used as internal standard for IGF-1. Bovine insulin was used as internal standard for IGF-2. IGF standards and internal standards were spiked into 100 µL of mouse serum to prepare calibration curve samples. Samples were protein precipitated and loaded on Phenomenex® Microelution plate Strata-X-A 33 µm polymeric strong anion 96-well plate (2 mg/well) for SPE cleanup. The detailed sample preparation procedure is demonstrated in Figure 2.

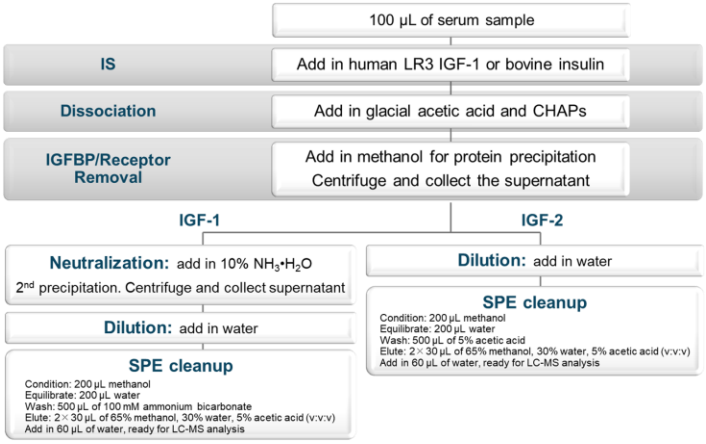

Figure 2. Sample Preparation Workflow.

LC-MS/MS Conditions: The processed serum samples were subjected to LC-MS/MS analysis using the SCIEX ExionLC™ AD system coupled to SCIEX QTRAP 6500+ system. The Phenomenex® Kinetic C18 column (2.6 µm, 50 x 3 mm) was used for LC separation. A linear gradient of buffer A (99.9% water, 0.1% formic acid) and buffer B (99.9% acetonitrile, 0.1% formic acid) was used at a flow rate of 700 µL/min, wherein the B% ramped from 5% to 45% in 3.5 min. The MRM parameters were summarized in Table 1. The data were processed using MultiQuant™ software 3.0.

Table 1. Summary of MRM parameters for IGFs quantitation.

ID	Q1	Q3	DP	CE	CXP
IGF1_1	957.0	1175.6	100	41	15
IGF1_2	957.0	473.3	100	47	20
IGF1_3	1093.5	1196.9	100	52	17
IGF1_4	957.0	1196.9	100	42	15
IGF1_5	1093.5	473.3	100	55	19
LR3 IGF1_1	1013.4	473.3	100	50	9
LR3 IGF1_2	912.1	473.3	100	45	18
IGF2_1	934.6	989.6	100	40	15
IGF2_2	934.6	1034.4	100	38	15
IGF2_3	934.6	1057.8	100	40	15
IGF2_4	1067.9	1065.4	100	46	15
Bovine insulin_1	956.6	1115.2	80	30	25
Bovine insulin_2	956.6	637.0	80	32	17
CHAPs	615.7	412.3	80	15	20

Sample Preparation Optimization

The analyte recovery of IGFs sample preparation highly depends on the dissociation efficiency between IGFs and their binding proteins and/or receptors. To optimize the dissociation process, various concentrations of CHAPs (1%, 5% and 10%) were evaluated for both human serum (Innovative Research, Inc.) and mouse serum (BioIVT) sample. The XIC peak areas of IGF-1 were used to evaluate the recovery rate. As shown in Figure 3, 5% and 10% CHAPS conditions provided the similar recovery which was ~2 folds higher than the 1% CHAPs condition. Therefore, 5% of CHAPs was used in the following work.

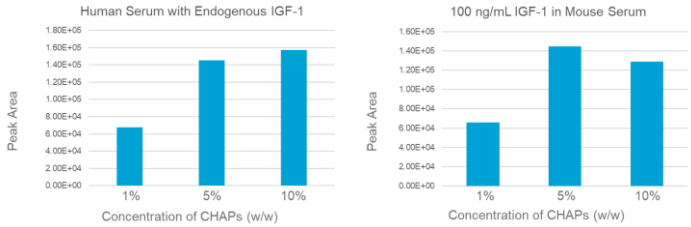

Figure 3. Sample Preparation Recovery Evaluation. The pooled normal human serum (left) and 100 ng/mL IGF-1 in mouse serum (right) were processed parallelly with 1%, 5% and 10% CHAPs then analyzed in LC-MS.

Scheduled Ionization to Minimize Instrument Contamination

As CHAPS is a zwitterionic detergent and can induce instrument contamination, additional method optimization practices were applied to minimize the amount of CHAPs that could enter the mass spectrometer. Firstly, HPLC conditions were well optimized to provide baseline separation between CHAPS and all analytes/internal standards (Figure 4). Secondly, the scheduled ionization feature in Analyst software 1.7 was turned on to apply ion spray voltage (ISV) only during the elution of analytes and internal standards. At the retention time of CHAPS, the ISV was set to zero (Figure 4), therefore no electrospray was form to allow CHAPs getting into the mass analyzer.

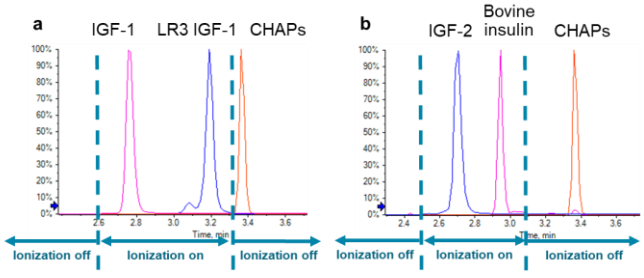

Figure 4. Retention time of components and scheduled ionization settings. a) IGF-1 assay; b) IGF-2 assay.

Quantitation Results

IGF-1 and 2 present endogenously in human serum, therefore mouse serum was used as a surrogate matrix to determine the linear dynamic range (LDR), limit of detection and quantitation (LOD and LOQ). The LDRs of IGF-1 and 2 were determined as 2-1000 ng/mL, with LOQ as 2 ng/mL and LOD as 1 ng/mL (Figure 5 and 6, Table 2 and 3). The pooled normal human serum was analyzed as an unknown sample and the concentrations of endogenous IGF-1 and 2 in this batch/lot of human serum were quantified as 119.4 and 397.6 ng/mL.

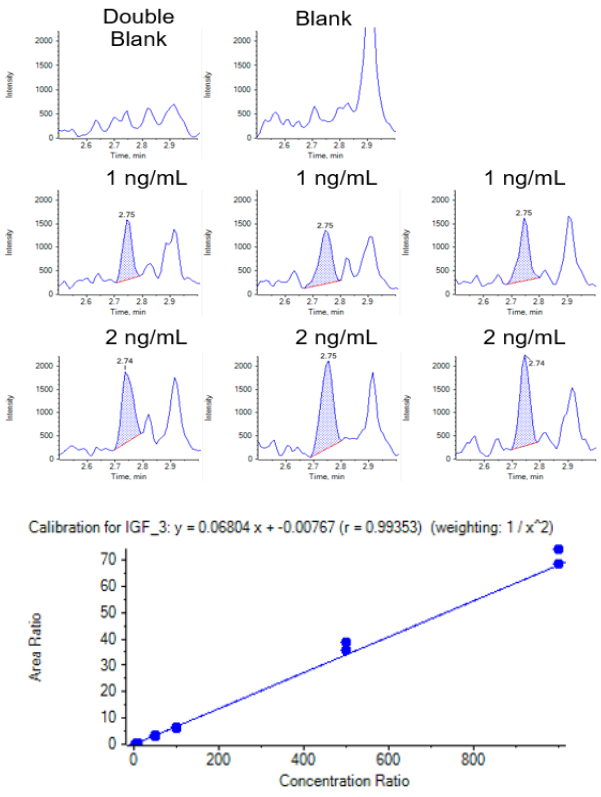

Figure 5. IGF-1 Quantitation Result in Mouse Serum. XICs (1093.5 / 1196.9) of double blank, blank, 1 and 2 ng/mL (top); calibration curve from 2 to 1000 ng/mL (bottom).

Table 2. Quantitation Summary for IGF-1.

Concentration (ng/mL)	Calculated concentration	Accuracy	%CV
2	2.036	101.78	15.83
5	4.641	92.81	7.71
10	11.01	110.05	1.61
50	48.48	96.97	6.75
100	92.05	92.05	5.91
500	547.2	109.44	5.01
1000	1034	103.39	4.53

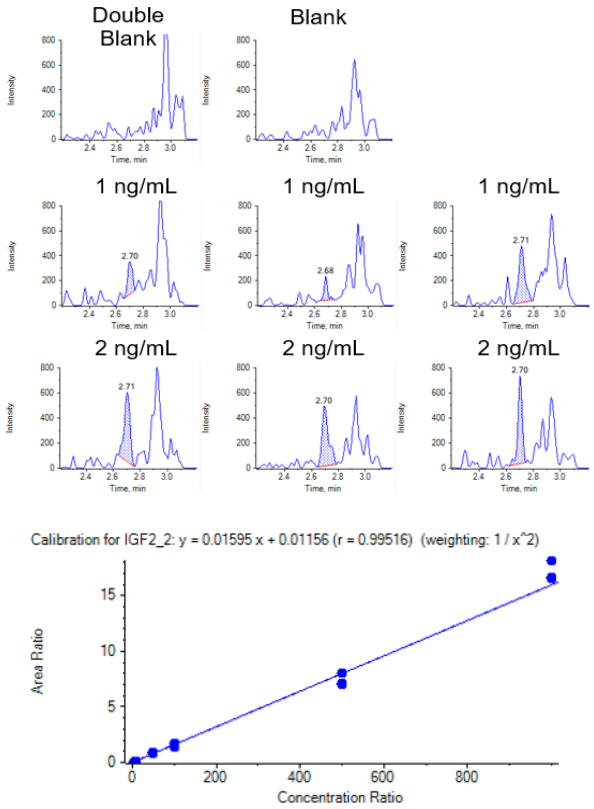

Figure 6. IGF-2 Quantitation Result in Mouse Serum. XICs (934.6/1034.4) of double blank, blank, 1 and 2 ng/mL (top); calibration curve from 2 to 1000 ng/mL (bottom).

Table 3. Quantitation Summary for IGF-2.

Concentration (ng/mL)	Calculated concentration	Accuracy	%CV
2	2.014	100.69	10.73
5	4.955	99.11	7.07
10	9.507	95.07	3.05
50	53.80	107.59	3.99
100	95.82	95.82	11.17
500	463.7	92.75	7.44
1000	1070	107.03	5.28

Conclusions

An MRM LC-MS/MS workflow for intact IGF-1 and 2 quantitation in serum samples was presented. Using the QTRAP 6500+ LC-MS/MS system, IGF-1 and 2 were quantified at 2 ng/mL level with high reproducibility. Meanwhile, the Scheduled Ionization feature in Analyst software 1.7 efficiently prevented instrument contamination from sample preparation detergent (CHAPS). This workflow offers simplicity, speed and robustness, aligning up with the need in large molecule bioanalysis.

References

1. Cote L, McCann K, Chu C, Determination of insulin-like growth factor-1 in serum by HRAM LC/MS for clinical research, 2014 MSACL.
2. Tanna N, Lame M, Wrona M, Chambers E, LC-MS/MS quantification of intact insulin-like growth factor 1 (IGF-1) in serum for clinical research, 2017.
3. Luo J, Guo L, Jin W, Xiong L, Quantitation analysis of human insulin-like growth factor 1 (IGF1) in serum by using SPE-LC-MS/MS workflow, SCIEX Technical Note, 2019, RUO-MKT-02-8964-A.

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