Development and Comparison of Two High Throughput LC-MS Methods for the Accurate Quantitation of IGF1 in Human Serum

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Introduction

Methods for quantification of plasma proteins by mass spectrometry demand a high level of analytical quality and the potential to be high throughput. To achieve a high level of analytical quality for IGF1, we developed a full-length stable-isotope labeled (SIL) internal standard and Certified Reference Material (I-033-1ML) standard which is traceable to the International System of Units. Early introduction of SIL 15N-IGF1 (MSST0063) mitigates any source of variation throughout the analytical workflow. Subsequent to reagents development, we have developed and compared two high-throughput plate-based LC-MRM formats for accurate quantification of IGF1. The first workflow incorporates immunoaffinity enrichment with anti-IGF1 immobilized onto a protein A/G plate followed by rapid, in situ trypsin digestion and analysis of a surrogate peptide pair. The second workflow utilizes protein precipitation followed by analysis of intact IGF1.

Methods

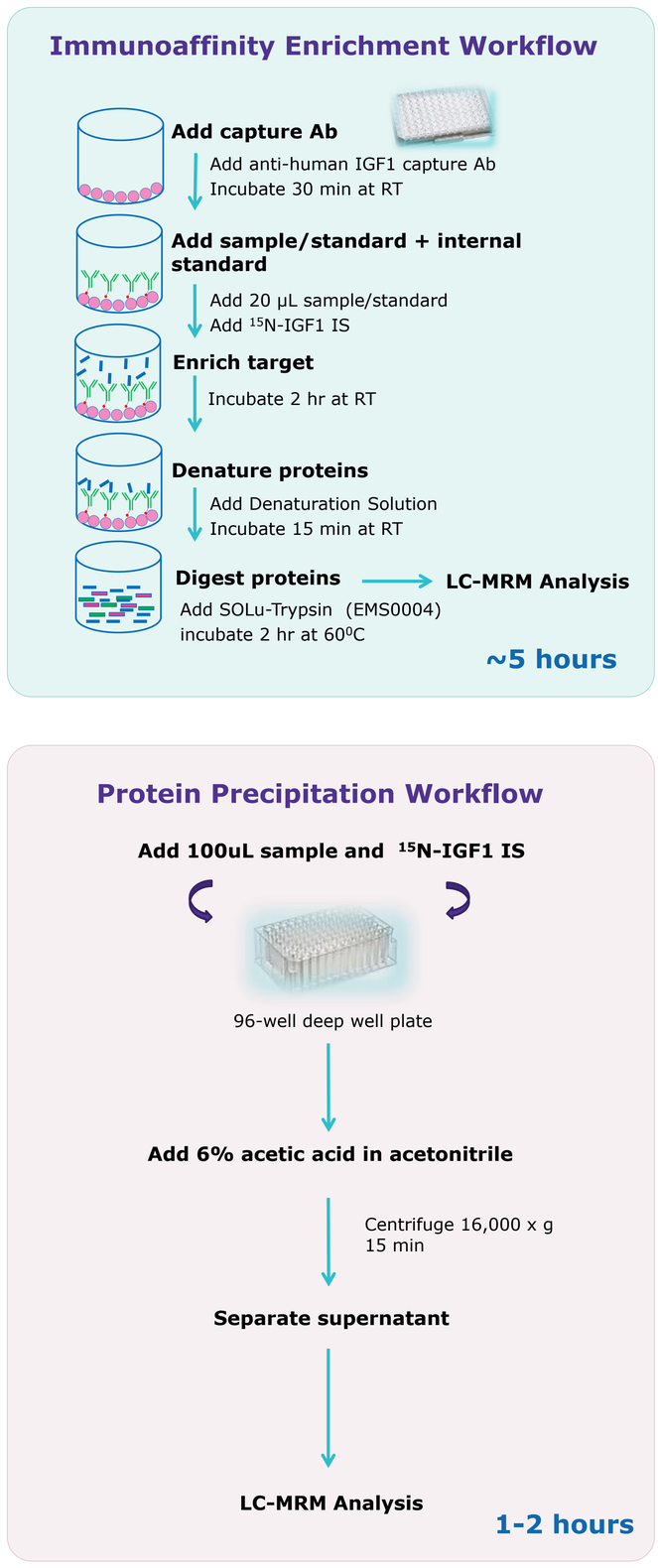
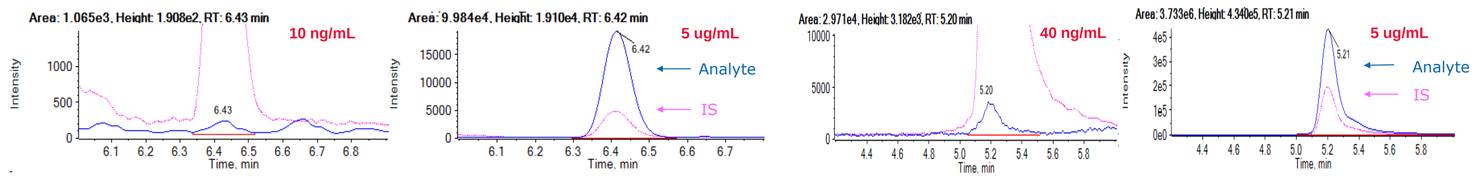
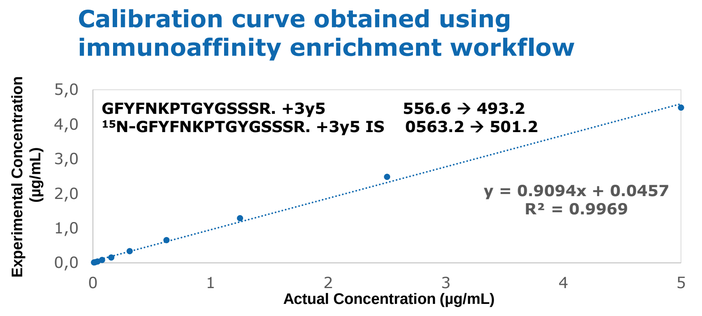
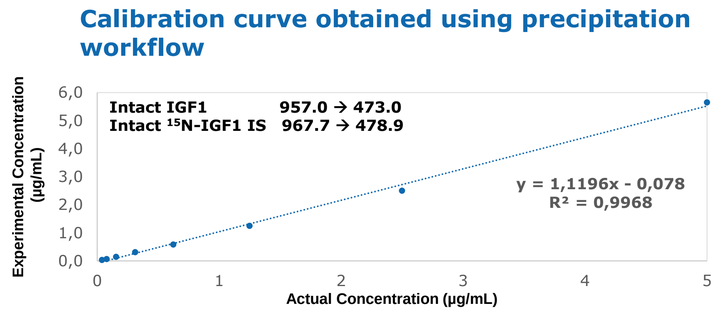


Figure 1
Immunoaffinity enrichment and precipitation workflows for quantification of IGF1

Results







Theo. Conc.	Num. Values	Exp. Conc.	% CV	Accuracy	Value #1	Value #2	Value #3
0.010	2 of 3	0.009	4.5	96.4	0.010	0.009	0.006
0.020	3 of 3	0.020	11.1	102.6	0.018	0.022	0.021
0.039	3 of 3	0.040	4.1	102.1	0.039	0.042	0.039
0.078	3 of 3	0.081	2.6	104.1	0.079	0.082	0.083
0.156	3 of 3	0.153	4.6	98.1	0.159	0.145	0.155
0.313	3 of 3	0.330	1.0	105.7	0.331	0.334	0.327
0.625	3 of 3	0.643	3.3	102.9	0.656	0.655	0.618
1.250	3 of 3	1.265	2.2	101.2	1.292	1.267	1.235
2.500	3 of 3	2.447	0.7	97.9	2.450	2.427	2.463
5.000	3 of 3	4.393	1.9	87.9	4.372	4.323	4.483

Theo. Conc.	Num. Values	Exp. Conc.	% CV	Accuracy	Value #1	Value #2	Value #3
0.040	3 of 3	0.034	15.3	102.1	0.034	0.041	0.047
0.078	3 of 3	0.065	12.1	92.7	0.065	0.070	0.082
0.156	3 of 3	0.142	5.5	90.5	0.142	0.149	0.133
0.313	3 of 3	0.309	4.0	95.0	0.309	0.286	0.295
0.625	3 of 3	0.581	5.7	95.4	0.581	0.635	0.572
1.250	3 of 3	1.249	3.3	102.7	1.249	1.332	1.272
2.500	3 of 3	2.499	3.2	102.4	2.499	2.501	2.618
5.000	3 of 3	5.649	1.9	115.4	5.649	5.863	5.802

Figure 2. XIC chromatograms, calibration curve and statistical values for standards of IGF1 (a) for IAE method over the concentration range of 0.01-5 µg/mL and (b) for protein precipitation method over the concentration range of 0.04-5 µg/mL.

Table 1. RPLC parameters:

Column	BIOshell™ A160 Peptide C18, 2.7 µm particle size, 10 cm × 500 µm (67096-U)
Gradient Peptide Intact	1% to 45% B in 9 min 10% to 35% B in 5 min
Flow Rate	25 µL/min
Column Temperature	40°C
LC Mobile Phases	Solvent A: 0.1% formic acid in H_2O Solvent B: 100% acetonitrile

Table 2. Comparison of IAE and precipitation methods.

Parameters	Immunoaffinity Enrichment	Protein Precipitation
Sample Volume	20 uL	100 uL
LLOQ	10 ng/mL	40 ng/mL
Capture Antibody	Yes	No
Digestion	Yes	No
# of Sample Prep Steps	6	4
Sample Prep Time	~5 hr	~2 hr

Conclusions

- A plate-based immunoaffinity enrichment kit (MSKT004) and a protein precipitation workflow were developed to enable high-throughput quantification of IGF1 by LC-MRM MS in less than five hours.
- 15N SIL-IGF1 (MSST0063) is an effective internal standard for both methods. IGF1 Certified Reference Material (I-033-1ML) which is traceable to NIST-SRM-2389a was used to prepare calibrators in Sigmatrix Synthetic Serum Diluent (D5322).
- The linear assay range was established from 0.01 to 5 µg/mL and 0.04 to 5 ug/mL using the IAE and protein precipitation methods, respectively, with regression coefficient of >0.99, CV values of <20%, and accuracies ranging from 80-120%.
- Future experiments will be performed in human serum.

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