The Development and Validation of Two High Through-Put Methods for the Determination of Biomarkers IFG-1 and IGFBP-3 in Human Plasma

Sarah K. Roby, MA, Scientist
Richard Sukovaty, BSc, Senior Scientist
Curtis Sheldon, BSc, Associate Director, Bioanalytical Principal Investigator

Introduction

Insulin-like growth factor 1 (IGF-1) is a polypeptide protein hormone similar in molecular structure to insulin that forms a complex with IGF binding protein 3 (IGFBP-3). This complex prolongs the half-life of IGF-1 and changes its interactions with cell surface receptors.

Growth hormone stimulates the synthesis and secretion of IGF-1 by the liver. IGF-1 then stimulates systemic body growth, and has growth-promoting effects on almost every cell in the body. In addition to the insulin-like effects, IGF-1 can also regulate cell growth and development, especially in nerve cells, as well as cellular DNA synthesis.

Quantitative methods for IGF-1 and IGFBP-3 were required for pharmacodynamic assessment of samples for a first in man study comparing once daily and once weekly subcutaneous injections of human growth hormone.

Method

Diluted (IGFBP-3) or pretreated (IGF-1) plasma samples and corresponding calibration standards were pipetted into microtiter plates coated with the appropriate capture antibodies. The wells were washed to remove the unbound sample material and enzyme-labeled antibody was added. Unbound labeled antibody was removed and a chromogenic substrate was added to the bound labeled antibody. The development of the colored reaction product was directly proportional to the amount of analyte present in the sample and was detected using a colorimetric plate reader.

IGF-1 plasma samples were pretreated to release IGF-1 from binding proteins prior to analysis. This pretreatment resulted in a 100-fold dilution of IGF-1 matrix samples. IGFBP-3 matrix samples were diluted 100-fold prior to analysis.

Results

The IGF-1 and IGFBP-3 methods used a 4-parameter logistic regression weighted $1/y^2$ and $1/y$ over the analytical ranges 0.0900 – 6.00 ng/mL and 0.700 – 50.0 ng/mL, respectively. Due to the dilution of matrix samples prior to analysis, the detectable range is 9.00 – 600 ng/mL for IGF-1 and 70.0 – 5000 ng/mL for IGFBP-3.

The concentrations of IGF-1 and IGFBP-3 calibration standards were back-calculated from the regression equation determined using the experimental data. The coefficients of variation (C.V.) were less than or equal to 2.5% and 3.3%, respectively.

Inter-batch precision (% CV) of IGF-1 quality control samples between 0.0900 and 371 ng/mL was less than 7.1. Inter-batch accuracy (% Bias) of the same quality control samples was between -12.2 and +5.3. Inter-batch precision (% CV) of IGFBP-3 quality control samples between 0.700 and 1250 ng/mL was less than 5.8. Inter-batch accuracy (% Bias) of the same quality control samples was between -3.4 and +0.8.

Table 1. *IGF-1 Inter-Batch Precision and Accuracy*

IGF-1	LLOQ QC 0.0900 ng/mL	ULOQ QC 6.00 ng/mL	QC A 30.4 ng/mL	QC B 74.9 ng/mL	QC C 371 ng/mL
Inter-Batch Mean	0.0790	5.41	31.2	78.9	389
Inter-Batch SD	0.00312	0.208	2.22	4.09	21.3
Inter-Batch % CV	3.9	3.8	7.1	5.2	5.5
Inter-Batch % Bias	-12.2	-9.8	2.6	5.3	4.9
n	9	9	9	9	9

Table 2. IGFBP-3 Inter-Batch Precision and Accuracy

IGFBP-3	LLOQ QC 0.700 ng/mL	QC A 2.00 ng/mL	QC B 15.0 ng/mL	QC C 35.0 ng/mL	ULOQ QC 50.0 ng/mL	DF = 100 QC E 1250 ng/mL
Inter-Batch Mean	0.678	1.95	14.6	33.8	49.2	1260
Inter-Batch SD	0.0391	0.0761	0.292	0.471	1.70	62.1
Inter-Batch % CV	5.8	3.9	2.0	1.4	3.5	4.9
Inter-Batch % Bias	-3.1	-2.5	-2.7	-3.4	-1.6	0.8
n	9	9	9	9	9	9

Short-term stability in plasma was established for 5 hours for both IGF-1 and IGFBP-3 at ambient temperature.

Freeze and thaw stability in plasma was established for four and five freeze (-20°C) and thaw (ambient temperature) cycles for IGF-1 and IGFBP-3, respectively.

Long-term stability of matrix samples was established for 111 days when stored at -20°C for IGF-1 and 211 days when stored at -20°C for IGFBP-3.

Sample collection and handling stability was established in whole blood for 2 hours at 5°C under white light for both IGF-1 and IGFBP-3.

Table 3. Stability of IGF-1 During Sample Collection and Handling from Human Whole Blood (EDTA) at Ambient Temperature Under White Light Conditions

	Lot A		Lot B		Lot C	
	0 minutes	120 minutes	0 minutes	120 minutes	0 minutes	120 minutes
(ng/mL)	78.4	79.6	116	115	54.7	64.9
	78.6	77.6	114	113	56.1	60.6
	78.7	80.1	109	111	56.8	64.9
	79.2	74.7	111	104	61.8	63.7
	76.6	80.0	115	111	58.5	62.6
	80.2	79.7	112	118	57.1	62.6
Mean	78.6	78.6	113	112	57.5	63.2
% CV	1.5	2.7	2.3	4.2	4.3	2.6
% of Control		100.0		99.1		109.9
n	6	6	6	6	6	6

Table 4. Stability of IGFBP-3 During Sample Collection and Handling from Human Whole Blood (EDTA) at Ambient Temperature Under White Light Conditions

	Lot A		Lot B		Lot C	
	0 minutes	120 minutes	0 minutes	120 minutes	0 minutes	120 minutes
	1660	1690	3160	3040	1640	1500
	1710	1630	2750	3020	1640	1520
	1650	1650	2590	3020	1530	1470
	1710	1680	3170	3060	1640	1590
	1760	1740	3200	3240	1500	1500
	1760	1690	3120	3080	1640	1530
Mean	1710	1680	3000	3080	1600	1520
% CV	2.8	2.3	8.7	2.7	4.1	2.7
% of Control		98.2		102.7		95.0
n	6	6	6	6	6	6

The integrity of IGF-1 and IGFBP-3 hemolyzed samples was verified by comparing samples prepared in three lots of human plasma (EDTA) and sample prepared in the same lots of plasma with 5% whole blood. The IGF-1 hemolyzed samples quantitated between -1.0 and -5.2% of the control value while the IGFBP-3 hemolyzed samples quantitated between -1.5 and -2.9% of the control value.

Table 5. *Hemolyzed Sample Evaluation of IGF-1 in Human Plasma (EDTA)*

	Lot A		Lot B		Lot C	
	Control	Hemolyzed	Control	Hemolyzed	Control	Hemolyzed
(ng/mL)	83.6	81.2	39.7	39.3	40.3	38.2
% of Control		97.1		99.0		94.8

Table 6. *Hemolyzed Sample Evaluation of IGFBP-3 in Human Plasma (EDTA)*

	Lot A		Lot B		Lot C	
	Control	Hemolyzed	Control	Hemolyzed	Control	Hemolyzed
(ng/mL)	1710	1660	1150	1130	1300	1280
% of Control		97.1		98.3		98.5

An evaluation of dilution integrity demonstrated that a dilution factor of 300 can be applied to IGF-1 samples and a dilution factor of 490 can be applied to IGFBP-1 samples to dilute them into the quantifiable range.

The absence of a hook effect (an artifact causing samples with concentrations greater than the ULOQ to back-calculate within the analytical curve range) was demonstrated for both IGF-1 and IGFBP-3 by assaying a sample with a concentration higher than the ULOQ undiluted and at 3 dilution levels above the ULOQ. All samples assayed back-calculated with concentrations above the ULOQ.

Conclusions

The validated methods allow for rapid, selective, accurate and reproducible quantitation of IGF-1 and IGFBP-3 in human plasma samples for evaluation of pharmacodynamic response in clinical study participants.

The methods were used to analyze approximately 400 samples for both IGF-1 and IGFBP-3 in 3 days for 4 separate cohorts.

IGF-1 had a 96.6% batch acceptance rate and IGFBP-3 had a 97.3% batch acceptance rate.

Graph 1. *Participant 'X' Data*