The Development and Validation of Two High Through-Put Methods for the Determination of Biomarkers IGF-1 and IGFBP-3 in Human Plasma Samples for Intra-Cohort Analysis Evaluation Prior to Dose Escalation

Sarah K. Roby, Richard L. Sukovaty, Sophie Corbeil, Curtis E. Sheldon
Celerion, Lincoln, NE

PURPOSE:

- 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.
- Methods for determining the concentrations of both IGF-1 and IGFBP-3 were required for pharmacodynamic assessment of samples from subjects given human growth hormone as part of a first in man study comparing once daily and once weekly subcutaneous injections.
- Commercially available IGF-1 and IGFBP-3 kits (R&D Systems) were adapted and the IGF-1 assay was validated to meet the FDA guidelines for validation of bioanalytical methods while the IGFBP-3 method met guidelines established for a fit-for-purpose biomarker method.

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 colormetric plate reader.
- IGF-1 plasma samples were pretreated to release IGF-1 from binding proteins prior to analysis.
- The IGF-1 assay takes approximately 3.5 hours to complete while the IGFBP-3 assay takes approximately 4.5 hours to complete.

RESULTS:

- The IGF-1 and IGFBP-3 methods used a 4-parameter logistic regression weighted $1/y^2$ and $1/y$ over the ranges 0.0900 to 6.00 ng/mL and 0.700 to 50.0 ng/mL, respectively.
- The concentrations of IGF-1 and IGFBP-3 calibration standards were back-calculated from the regression equation of 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.
- 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.
- The quantitative integrity of IGF-1 and IGFBP-3 samples prepared in three lots of hemolyzed matrix was verified.
- 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.

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

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

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

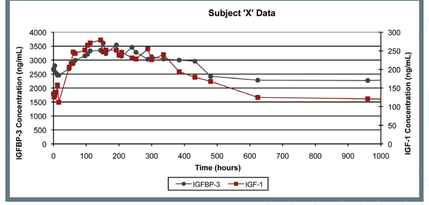
Table 7: IGF-1 Validation Summary

Validation Summary	
Analyte	IGF-1
Method Description	ELISA
Limit of Quantitation (ng/mL)	0.0900 ng/mL
Standard Curve Concentrations (ng/mL)	0.0900, 0.180, 0.400, 0.750, 1.25, 2.50, 4.00, 5.00, and 6.00 ng/mL
QC Concentrations (ng/mL)	LLOQ QC, ULOQ QC, 30.4, 74.9, and 371 ng/mL
QC Intra-Batch Precision Range (% CV)	1.3 to 9.3%
QC Intra-Batch Accuracy Range (% Bias)	-15.4 to 9.9%
QC Inter-Batch Precision Range (% CV)	3.8 to 7.1%
QC Inter-Batch Accuracy Range (% Bias)	-12.2 to 5.3%
Bench-Top Stability (Hrs)	Short-Term Stability: 5 hours at ambient temperature
Stock Stability (Days)	Long-Term Stability for Stock Solutions (Stock): 43 days in PBS-BSA (0.1%) at -80°C
Freeze-Thaw Stability (Cycles)	4 freeze (-20°C)-thaw(ambient temperature) cycles
Long-Term Storage Stability (Days)	Long-Term Stability: 111 days at -20°C
Dilution Integrity	Samples diluted up to 300-fold can be quantified
Matrix Effect	The matrix effect observed in the 10 human plasma (EDTA) lots that were fortified at low and high concentrations was consistent.
Assay Volume Required	0.0200 mL
Regression Type	$1/Y^2$ 4PL
Long-Term Stability for Stock Solutions (Substock)	55 days at 300 ng/mL in 0.1% bovine serum albumin in Dulbecco's phosphate buffered saline in a polypropylene container at -80°C
Short-Term Stability for Stock Solutions (Stock)	6 hours at 12.4 µg/mL in 0.1% bovine serum albumin in Dulbecco's phosphate buffered saline in a polypropylene container at ambient temperature under white light
Short-Term Stability for Stock Solutions (Substock)	6 hours at 300 ng/mL in 0.1% bovine serum albumin in Dulbecco's phosphate buffered saline in a polypropylene container at ambient temperature under white light
Stability of Analyte During Sample Collections and Handling	up to 120 minutes in human plasma (EDTA) in polypropylene tubes at ambient temperature under white light
Hemolyzed Sample Evaluation	no significant effect was observed in any of the 3 hemolyzed human plasma (EDTA) lots that were evaluated at an endogenous concentration

Table 8: IGFBP-3 Validation Summary

Validation Summary	
Analyte	IGFBP-3
Method Description	ELISA
Limit of Quantitation (ng/mL)	0.700 ng/mL
Standard Curve Concentrations (ng/mL)	0.700, 1.00, 3.00, 5.00, 10.0, 20.0, 30.0, 40.0, and 50.0 ng/mL
QC Concentrations (ng/mL)	LLOQ QC, 2.00, 15.0, 35.0, ULOQ QC, and 1250 ng/mL
QC Intra-Batch Precision Range (% CV)	0.2 to 6.8%
QC Intra-Batch Accuracy Range (% Bias)	-9.3 to 3.2%
QC Inter-Batch Precision Range (% CV)	1.4 to 5.8%
QC Inter-Batch Accuracy Range (% Bias)	-3.4 to 0.8%
Bench-Top Stability (Hrs)	Short-Term Stability: 5 hours at ambient temperature
Freeze-Thaw Stability (Cycles)	5 freeze (-20°C)-thaw cycles
Long-Term Storage Stability (Days)	Long-Term Stability: 211 days at -20°C
Dilution Integrity	Samples diluted up to 490-fold can be quantified
Assay Volume Required	0.0100 mL
Regression Type	1/Y 4PL
Stability of Analyte During Sample Collections and Handling	up to 120 minutes in human plasma (EDTA) in polypropylene tubes at ambient temperature under white light
Hemolyzed Sample Evaluation	no significant effect was observed in any of the 3 hemolyzed human plasma (EDTA) lots that were evaluated at an endogenous concentration

Graph 1: IGF-1 and IGFBP-3 Subject Data



CONCLUSIONS:

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