Insulin-like growth factor 1 (IGF-1) is a polypeptide hormone similar in molecular structure to insulin that forms a complex with IGFBP-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 DNA repair, 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 (RAD Systems) were adopted by the IGF-1 assay was validated to meet the FDA guidelines for validation of bioanalytical methods while the IGF-3 method met guidelines established for a fit-for-purpose biomarker method.

Bench-Top Stability

Stock Stability

Freeze-Thaw Stability

QC Inter-Batch Precision Range

QC Intra-Batch Accuracy Range

QC Concentrations

Limit of Quantitation

ELISA Method Description

IGF-1

IGF-1 and IGFBP-3 methods used a 4-parameter logistic regression weighted 1/y² 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 (CV) were less than or equal to 2.5% and 3.3%, respectively.

IGF-1 had a 96.6% batch acceptance rate and 98.3% inter-batch accuracy (% Bias). 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 developmental and validation of two high throughput methods for the determination of biomarkers IGF-1 and IGFBP-3 in human plasma samples for incohort analysis evaluation prior to dose escalation

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

Celerion, Lincoln, NE

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.

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 Incohort Analysis Evaluation Prior to Dose Escalation

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.

The IGF-1 assay takes approximately 3.5 hours to complete while the IGFBP-3 assay takes approximately 4.5 hours to complete.

Sample collection and handling stability was established in white 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 400 can be applied to IGFBP-3 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 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.

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 (CV) were less than or equal to 2.5% and 3.3%, respectively.

The IGF-1 assay had a 96.6% batch acceptance rate and 98.3% inter-batch accuracy (% Bias).