Characterization of Stable Isotope Labeled Insulin-Like Growth Factor-1 for Use as an Internal Standard in a Quantitative MS Workflow

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High-resolution mass spectrometry (HRMS) methods for quantitative analysis of intact proteins in clinical samples are becoming more widely adopted. The accurate quantitation of a plasma protein in clinical applications is enabled by early introduction of an internal standard that behaves identically to the native target protein throughout the analytical workflow. Surrogate proteins are typically used as internal standards in HRMS assays, but full length stable-isotope labeled (SIL) proteins provide a more ideal alternative.

Recombinant SIL-IGF1, expressed in the *E. coli* in the presence of ^{15}N labeled ammonium chloride, was provided for characterization. The purity was determined by HPLC. The protein was digested with trypsin and analyzed using LC-MS for isotopic incorporation. The isotopic incorporation was calculated by correlation analysis between the experimentally measured isotope distribution and a series of theoretical isotope distributions corresponding to incorporation rates from 95% - 100%. The best correlation between the experimentally and measured isotope was obtained to be 98.5% for IGF1. Intact mass analysis was used to evaluate the processing of the signal peptide and propeptides.

Recombinant IGF1 contains three intramolecular disulfide bonds. Different intramolecular disulfide arrangements have been shown previously to produce two steric isomers. This is critical in two cases; when the sample preparation procedure utilizes an immunoaffinity enrichment step or when LC-MS of intact IGF1 is used for quantification. An HPLC-UV-MS method was verified to resolve structural isomers of SIL-IGF1 and used to evaluate the structural integrity of the SIL-IGF1 produced internally and purchased from a commercial source.

We developed an LC-HRMS method for quantitation of intact IGF1 in human serum using full length SIL-IGF1 as an internal standard. The optimized LC-MS assay conditions and preliminary results will be presented.