Quantitative Analysis of IGF-1 Using Online Digestion Coupled to the Triple Quad LCMS-8050

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Background:

Insulin-like growth factor 1 is a protein that is used in the screening of growth hormone deficiencies, as well as testing for abuse in professional sports. It is a 7.6 kDa, 70-amino acid protein that is produced in the liver and detected in serum in the range of 100 – 1,000 ng/mL[1]. Detection using an LC-MS approach is emerging as a preferred test to the standard immunoassay due to the improved reproducibility that LC-MS offers[2].

Methods:

Recombinant human IGF-1 standards were resuspended in Tris buffer, reduced (DTT) and alkylated (iodoacetamide) under standard conditions. Digestion time and temperatures were assessed from 1 – 8 minutes and 40 – 50 ˚C using an optimized digestion buffer on a Perfinity Workstation set up for digestion, desalting and reversed phase separation. MRMs were optimized (product selection and CE optimization) using Skyline predicted peptides and transitions, with a minimum of two transitions per precursor. Source conditions were adapted from previous peptide analyses using the LCMS-8050. A 10-minute LC gradient was utilized, resulting in a total run time of 20 minutes per sample. Following optimization, a standard curve was prepared in a neutral buffer. Standards were digested via Perfinity Workstation and directly analyzed using a Shimadzu LCMS-8050 triple quadrupole MS. Data were analyzed in either Skyline (qualitative) or LabSolutions (quantitative). Subsequently, acetonitrile extracted serum samples spiked with IGF-1 were subjected to analysis using the same criteria, along with a spiked unknown sample. Calibration curves were generated in the clinically relevant range of 100 – 1,000 ng/mL and replicate analyses were conducted.

Results:

Four peptides from IGF-1 were predicted and subsequently identified by LC-MS/MS and optimized for MRM analysis. Optimal digestion was found to occur at 4 minutes and 40 ˚C in Perfinity Digestion Buffer. A calibration curve showed good agreement and linearity throughout the analytical range (r^2 = 0.994 – 0.996 for the detected peptides from 100 – 1,000 ng/mL). CVs were below 10 % for the monitored transitions. The measurement of the unknown agreed with the spiked amount.

Conclusions:

This work demonstrates the feasibility of using automated digestion coupled online to a fast and sensitive triple quadrupole MS for the detection of IGF-1 at biologically relevant levels. The benefits of automation, including more rapid analysis times (20 minutes per sample) and reduced operator involvement, suggests that this is an attractive alternative to current immunoassays and manual sample preparation strategies.

References:

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2. Cox, H., Lopes, F., Woldemariam, A., Becker, J., Parkin, M. et al. Interlaboratory Agreement of Insulin-Like Growth Factor 1 Concentrations Measured by Mass Spectrometry. Clin Chem 2014 60: 541-548