LC-MS/MS Analysis of Amyloid Beta Peptides in Artificial Cerebrospinal Fluid using the Xevo TQ Absolute for Clinical Research

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Introduction:

Alzheimer's disease (AD), the most common underlying cause of dementia, is a progressive neurodegenerative disorder associated with aging. Due to the extension of the average life span, the prevalence of AD is dramatically on the rise. Amyloid beta (Aβ) peptides are the most prominent biomarker compounds thought to be involved in AD pathogenesis, leading to increased interest in their quantification in clinical research studies. Historically, quantification of Aβ peptides in biological fluids has relied mainly on the use of immunoassays, such as ELISA. However, these techniques can suffer from cross-reactivity, contributing to batch-to-batch variation of the methods, which can impact confidence in results. In addition, for assessments that involve multiple biomarkers, an individual ELISA method is required for each peptide, increasing overall analysis time and cost. Therefore, a single robust method providing greater analytical selectivity would help overcome these challenges. Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) could be a beneficial technique in the clinical research of Aβ peptides due to the advantages it provides over traditional ligand-binding techniques. These benefits include improvements in analytical selectivity, and the capability of multi-analyte quantitative detection in a single run. Herein, we demonstrate the suitability of the ACQUITY™ Premier UPLC™ I-Class System with Xevo™ TQ-Absolute Mass Spectrometer as a tool for analytically sensitive and selective biomarker quantitation for the accurate quantitation of multiple Aβ peptides (1-38, 1-40, 1-42) extracted from 200 µL of artificial Cerebrospinal Fluid (CSF) within the concentration range of 0.1-10 ng/mL.

Methods:

Calibration and Quality Control (QC) working solutions at each level were spiked into the blank artificial CSF with 4% (w/v) Bovine Serum Albumin (BSA). An internal standard working solution was added to 200 µL of spiked samples, which was diluted with 200 µL of 5M guanidine-HCl and 200 µL of 4% (v/v) phosphoric acid. The samples were mixed at room temperature at 900 rpm for one hour. The incubated samples were loaded onto an Oasis™ MCX SPE µElution plate, washed and then eluted with 75:15:10 (v:v:v) acetonitrile:water:ammonia into a QuanRecovery™ 700uL 96-well Collection Plates with MaxPeak™ HPS. The 100 µL of eluate was evaporated with nitrogen to dryness at 50°C and reconstituted with 50 µL of 20:80:1 (v:v:v) acetonitrile:water:ammonia. The reconstituted samples were mixed at room temperature at 800 rpm for 15 min prior to injection onto the

LC-MS/MS system. Chromatographic separation was performed on an ACQUITY Premier UPLC I-Class FTN System using a ACQUITY UPLC BEH™ Peptide C18 300Å, 2.1 x 150 mm, 1.7 µm Column, using a 0.3% NH4OH/water/acetonitrile gradient. The detection was performed in positive electrospray ionization mode on a Xevo TQ Absolute Mass Spectrometer.

Results:
Analytical sensitivity of the lowest calibrator at 0.1 ng/mL was demonstrated with S/N (PtP) > 10 for all Aβ peptides (1-38, 1-40, 1-42) across the five analytical runs. We successfully demonstrated linearity from 0.1 – 10 ng/mL for the Aβ peptides, with r2>0.999 over five analytical runs. Total precision and repeatability across the Aβ peptides at the QC three concentrations (0.2, 1.0 and 7.5 ng/mL) respectively, with five replicates over five analytical runs (n = 25) was < 5% CV. The accuracy of the QCs compared to nominal concentrations ranged from 96.5 – 100.2% across the Aβ peptides.

Conclusions:
An LC-MS/MS method for the analysis of Aβ peptide biomarkers in CSF was developed for clinical research. Through the use of the ACQUITY Premier UPLC I-Class System and Xevo TQ Absolute Mass Spectrometer excellent inter-day linearity, analytical sensitivity, precision, and accuracy can be achieved, providing confidence in the results obtained for quantification of Aβ peptides in AD clinical research.

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