Development of an LC-MS/MS Method for Quantifying PGT 121.414.LS in Human Serum

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Introduction

Broadly neutralizing antibodies (bNAbs) have emerged as an alternative approach to HIV-1 prevention and treatment. To determine optimal dosing schedules, pharmacokinetic (PK) studies require methods for accurate and reproducible quantitation. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) provides an alternative to ligand-binding assays typically used for PK samples. Mass spectrometry offers distinct advantages to selectivity, making it a strong orthogonal approach to ligand binding assays for bioanalysis. In this study, we combined the purification of immunoglobulin Gs from human serum with bottom-up proteomics to generate characteristic tryptic peptides from PGT 121.414.LS. These unique tryptic peptides are generated from the variable region of the bNAb and are targeted with LC-MS/MS. Selected transitions from these peptides are monitored and used for quantitation.

Methods

Immunoglobulin Gs were isolated from 50µL human serum spiked with the bNAb PGT 121.414.LS using the Pierce Protein A IgG orientation kit. Waters Rapigest Sf was added to a final concentration of 1mg/mL and incubated at 60°C for 10 minutes to the antibody-containing fraction. The sample was reduced using dithiothreitol (5mM) and alkylated using iodoacetamide (10 mM). Post alkylation, MS grade trypsin was added to a ratio of 1:20 and incubated for 4 hours at 37°C. The digestion was quenched by adding 1% formic acid. LC-MS analysis was performed using a 100mm x 2.1mm, 1.7µm BioZen XB-C18 column (30°C) on an Orbitrap and triple quadrupole mass analyzers.

Preliminary Data

Tryptic peptide mapping of PGT 121.414.LS was performed and processed using Proteome Discoverer. Nine peptides derived from the variable region of PGT 121.414.LS were detected. The peptide SGDTNYSPSLK, derived from the complementary determining region was selected for quantitation. The selectivity of the quantitative peptide was determined by digesting IgGs purified from blank human serum and by BLAST analysis. Blank serum did not indicate detectable levels of the quantitative peptide. A 15-minute liquid chromatography method development was optimized for the quantitative peptide. Tryptic digestion was optimized by monitoring the peak area of the quantitative peptide across multiple trypsin ratios and times. The reproducibility of the digestion was analyzed by digesting the top 3 ratios and times in triplicate. A ratio of 1:20 and 4h was found to maximize the peak area of the quantitative peptide. Recovery of PGT 121.414.LS was estimated at three concentrations across the expected clinical range. Recoveries were estimated to be 71%-86% across the clinal range. The linearity and sensitivity of the quantitative peptide were evaluated across the clinical range on two mass spectrometers. Spiked serum standards from 0.5µg/mL to 500µg/mL were run on both mass spectrometers. The linear range on the TSQ Altis ranged from 0.5µg/mL to 100µg/mL with a calculated LLOD of 0.24µg/mL. The linear range on the Q Exactive Orbitrap ranged from 5µg/mL to 500µg/mL with a calculated LLOD of 1.44µg/mL.

Novel Aspect

A novel method for quantifying the broadly neutralizing antibody PGT 121.414.LS in human serum for pharmacokinetic studies.

Conflict of Interest Disclosure

The authors declare no competing financial interest.