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VALIDATION OF A MFLC-MS/MS ASSAY FOR THE QUANTITATIVE ANALYSIS OF A NOVEL PEPTIDE, AT-01, EXTRACTED FROM K2EDTA PLASMA

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Abstract:

Since the synthesis of the first therapeutic peptide, insulin, in 1921, our understanding of the therapeutic potential of proteins and peptides has increased significantly. As our understanding expands, targeted biopharmaceutical candidates are being identified and explored at an increasing rate. As these treatments become more mainstream and effective, the need for robust, GLP-compliant quantitative assays is growing. Previously, the majority of analysis has been done utilizing ELISA assays or similarly intensive, expensive methods. While robust, these methods do not offer the ease and high throughput potential that can be achieved with a MFLC (micro flow)-MS/MS method. Additionally, many of these existing methods require a high sample volume, which can be limiting when it comes to regulated analysis. In support of two GLP studies featuring AT-01, a novel, synthetic amyloid-targeting peptide designed for imaging systemic amyloid deposits, GLP-compliant methods were developed in both rat and dog K_2EDTA plasma utilizing a straightforward MFLC-MS/MS method. Here AT-01 was extracted from plasma using a Phenomenex Strata XPro micro-elution plate and analyzed by MFLC-MS/MS. AT-01 was spiked into plasma and a 25µL aliquot was plated in a LOW-bind 96 well plate. Internal standard was added, and the sample was acidified with a 4% phosphoric acid solution. The total volume was loaded onto a pre-conditioned Strata XPro micro-elution plate, rinsed with water, and then eluted into a clean 96 well LOW Bind plate with two volumes of 1:74:25 trifluoroacetic acid/acetonitrile/water. A final dilution was done with water + 0.1% formic acid. The extract was analyzed on an API-6500 mass spectrometer equipped with an Optiflow source, operating in positive ESI mode. The MFLC system was a Waters M Class set to run a binary gradient method with a flow rate of 40µL/min. Chromatographic separation was achieved using a Kinetics Biphenyl column (1.7µm, 50 x 1.0mm) from Phenomenex. The methods were linear from 30.0 – 5000 ng/mL with an R-value of 0.9961 for rat and 0.9936 for dog. Matrix effects from six individual lots of plasma in each species were examined and found to be within ± 5.8% of nominal across the LQC and HQC for rat, and within -9.6 and +6.8% of nominal across the LQC and HQC for dog. Blanks were also examined using six individual lots of plasma for each species. In both cases, the assay was found to be selective across all lots. The methods were validated according to FDA guidance and met *a priori* acceptance criteria. These low sample volume methods were found to provide a selective and accurate quantification of this innovative peptide.