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Title:
Validation of an MFLC-MS/MS Assay for the Quantitative Analysis of a Novel Peptide, AT-01, Extracted from K_2EDTA Plasma

Abstract Topic:
Analytical

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Purpose:

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 analytical assays is growing. Previously, the majority of analysis has utilized ELISA assays or similarly labor 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 liquid chromatography)-MS/MS method. Additionally, many of these existing methods require a high sample volume, which can be limiting when it comes to regulated analysis.

Methods:

In support of two GLP studies of AT-01, a novel, synthetic amyloid-targeting peptide designed for imaging systemic amyloid deposits, GLP-compliant methods were developed for evaluation of both rat and dog K_2EDTA plasma utilizing a facile 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

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1:74:25 trifluoroacetic acid/acetonitrile/water solution. A final dilution was performed with aqueous 0.1% formic acid.

The extract was analyzed using an API-6500 mass spectrometer equipped with an Optiflow source, operating in positive ESI mode. The MFLC system was a Waters M Class running a binary gradient with a flow rate of 40µL/min. Chromatographic separation was achieved using a Kinetics Biphenyl column (1.7mm, 50 x 1.0mm; Phenomenex).

Results:

The methods were linear from 30.0 – 5000 ng/mL with an R-value of 0.996 and 0.994 for rat and dog plasma samples, respectively. Matrix effects from six individual lots of plasma from 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.

Conclusions:

This novel, low sample volume analytical method provided a selective and accurate quantification of this innovative peptide. This approach yields rapid, accurate, and facile identification and quantitation of exploratory peptides and protein in plasma samples, which will facilitate drug development of novel biological therapeutics and diagnostics.