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Quantitation of the Phosphorothioate Oligonucleotide Mipomersen from Human Plasma by MFLC-MS/MS

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Introduction

Mipomersen is a phosphorothioate oligonucleotide that is prescribed to treat homozygous familial hypercholesterolemia. This FDA approved (2013) oligonucleotide is a short, single-stranded synthetic DNA molecule that targets and complements an mRNA sequence responsible for coding apo B-100. Bioanalysis of oligonucleotides by HPLC-MS/MS in support of regulatory submission has not been widely adopted. ELISA, qPCR and HPLC-FL analysis is more widely used but these techniques may not have the required selectivity or the capability to analyze oligonucleotides smaller than 20 nucleotides. Additionally, the time and cost to develop these methods are typically much greater than the HPLC-MS/MS approach. HPLC-MS/MS analysis provides better selectivity, increased dynamic range and the flexibility to quickly analyze a vast array of oligonucleotides from biological matrix. In order to increase the sensitivity of the method, analysis was conducted using microflow HPLC (MFLC) conditioned for oligonucleotide analysis thus reducing adduct formation. A simple solid phase extraction (SPE) method was developed to extract the oligonucleotide from human plasma prior to the MFLC-MS/MS analysis.

Methods

SPE extraction followed by MFLC-MS/MS analysis was developed for the quantitative analysis of Mipomersen and a similar oligonucleotide that was used as the internal standard. Human plasma was spiked with the oligonucleotides and the mixture was extracted using Clarity OTX-100 mg 96 well plates and extraction solutions supplied by Phenomenex. Prior to SPE extraction 100 µL of plasma was mixed with 100 µL Lysis Loading buffer containing 2 mg/mL cysteine. The SPE plate was equilibrated with methanol then equilibration buffer. The entire sample volume was added and slowly eluted using a vacuum manifold. The wells were then washed with buffer then the oligonucleotides were eluted with elution buffer. The extracts were then dried using a ThermoFisher Scientific SPD120 Speed Vac and the dried sample was then reconstituted with 100 µL water. The sample was then analyzed by MFLC-MS/MS on an API-6500+ (Sciex) mass spectrometer equipped with an Optiflow source operating in negative ESI mode. The MFLC system was a Waters M-Class operating with a binary gradient method and a flowrate of 0.020 mL/min. Separation was achieved using a Phenomenex Gemini C18 column (5 cm x 0.3 mm, 3 µm). Mobile phase A consisted of water with 100 mM HFIB and 10 mM DIEA. Mobile phase B

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was prepared in acetonitrile containing 100 mM HFIB and 10 mM DIEA. In order to reduce adduct formation the MFLC system was conditioned by purging every component repeatedly with GC Resolv methanol. The MFLC system was then set to pump 50 µL/min overnight. The mass spectrometer was simply cleaned to Q_0 prior to injecting the oligonucleotides.

Results

The data indicates that with a simple SPE extraction and MFLC-MS/MS analysis Mipomersen can be accurately quantified with an LLOQ in the low ng/mL range. Additionally, no significant adducts were observed in the mass spectra for Mipomersen or the internal standard.

Conclusions and Discussion

A simple extraction and MFLC-MS/MS method has been developed to accurately quantify Mipomersen from human plasma.

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