Alturas Analytics, Inc.
The LC-MS Experts

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

Chad Christianson[1], Ryan Schmich[1] and Jennifer Zimmer[1]
[1]Alturas Analytics, Inc.

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 decreased method development time and resources. 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.

OVERVIEW

Purpose:

- Develop an Accurate/Precise MFLC-MS/MS method for the Quantitation of the Oligonucleotide Mipomersen from Human Plasma.

Methods:

- Mipomersen was extracted from human plasma and analyzed using MFLC-MS/MS.

Results:

- The developed method resulted in a sensitive/selective assay that can be utilized to analyze Mipomersen from human plasma samples with an LLOQ of 1.00 ng/mL.

METHODS

Extraction

- Add 100 µL STD + 100 µL Lysis-Loading buffer containing 2 mg/mL cysteine. Vortex 5 minutes
- Equilibrate Clarity OTX SPE with 1 mL MeOH
- Equilibrate Clarity OTX SPE with 1 mL Equilibration buffer
- Add entire sample volume
- Wash wells 1 mL Wash Buffer
- Elute with 0.5 mL Elution Buffer
- Dry wells using a Speed Vac
- Add 0.100 mL water, vortex 5 min, centrifuge briefly
- Inject onto the MFLC-MS/MS

HPLC Parameters

- Column: Phenomenex® Gemini®, 3µm C18, 50 X 0.3 mm
- LC Pumps: Waters™ ACQUITY UPLC®M-Class
- MP A: Water HFIP 100 mM,10 mM DIEA
- MP B: ACN HFIP 100 mM,10 mM DIEA
- Flowrate: 10 µL/min
- LC Gradient: 97% A to 70% A at 4 minutes

Mass Spectrometry

- SCIEX QTRAP® 6500+
- Negative ion mode
- MRM

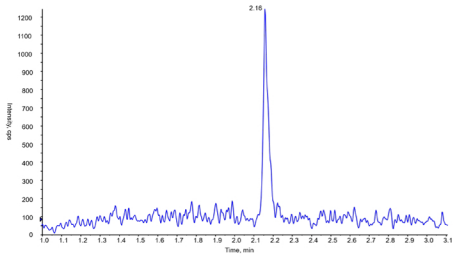

Figure 1: 1.00 ng/mL Mipomersen chromatogram, extracted from human plasma

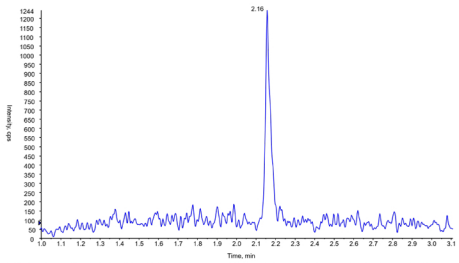

Figure 3: 1.00 ng/mL Mipomersen Qualifier MRM chromatogram, extracted from human plasma

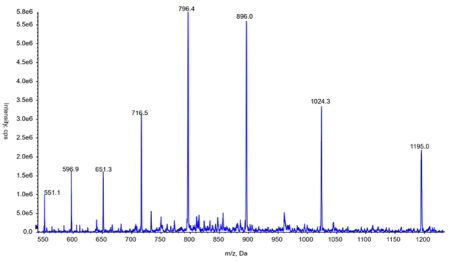

Figure 4: Q1 Scan Mipomersen, no adducts observed

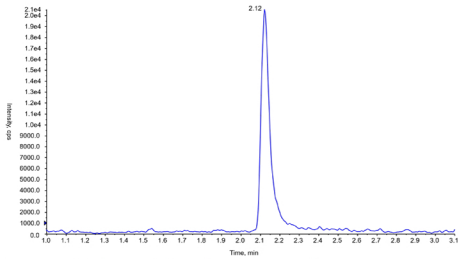

Figure 2: Internal Standard chromatogram, extracted from human plasma

Oligonucleotide	Sequence	MW (amu)	Primary Q1/Q3	Qualifier Q1/Q3
Mipomersen	5'-mG-mC*-mC*-mU*-mC*-dA-dG-dT-dC*-dT-dG-dC*-dT-dT-dC*-mG-mC*-mA-mC*-mC*-3'	7177	796.4/317.8	716.7/810.8
Internal Standard	5'-mT-mC*-mC*-mC*-mC*-dA-dC-dT-dC*-dC-dC-dT-dC-mG*-mC*-mA*-mC*-mC*-3'	7056	782.9/318.3	
	m : 2'-O-(2-methoxyethyl) nucleoside * : Substitution at 5-position of cytosine and uracil base with a methyl group d : 2'-deoxynucleoside (2'-deoxy)			

Table 1: Oligonucleotide Properties

Charge State	1	2	3	4	5	6	7	8	9	10	11	12	13
Predicted Mass	7176	3588	2391	1793	1434	1195	1024	896.1	796.5	716.7	651.5	597.1	551.1

Table 2: Q1 Scan Mipomersen Predicted Masses

Nominal QC Conc. (ng/mL)	AVG Calc. Conc. (ng/mL)	% Difference from Nominal	% CV
3.00	3.17	5.6	6.2
50.0	49.6	-0.9	6.0
800	763	-4.7	8.8

Table 3: QC Data n=6 Replicates Extracted from Human Plasma

CONCLUSION

- An accurate/precise method has been developed to extract and analyze Mipomersen from human plasma.
- An LLOQ of 1.00 ng/mL was achieved.
- A dedicated portable MFLC system provided more sensitivity with no adduct formation.