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A Universal Method for the Quantitation of Multiple Oligonucleotides from Plasma by MFLC-MS/MS

Chad Christianson, Ryan Schmich, and Jennifer Zimmer

Alturas Analytics, Inc.

CONTACT INFORMATION: Ann Hoffman, ahoffman@alturasanalytics.com
Lori Payne, lpayne@alturasanalytics.com

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PURPOSE

Bioanalysis of oligonucleotides by HPLC-MS/MS in support of regulatory submission has not been widely adopted. Several obstacles such as lack of sensitivity, adduct formation and analytical instrument dedication are barriers that many laboratories are unwilling to challenge. Traditional ELISA and qPCR bioanalysis of oligonucleotides may not have the selectivity to accurately quantify the therapeutic oligonucleotide from complicated biological matrix. HPLC-FL methods require a probe design that increases the method development time and cost of the assay. Additionally, the probe is not suitable for oligonucleotides smaller than 20 nucleotides. HPLC-MS/MS analysis is an alternative approach that provides better selectivity, increased dynamic range and the flexibility to 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.

METHOD(S)

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
- Add 0.5 mL water 1% formic acid, vortex 5 min, centrifuge briefly
- Inject onto the MFLC-MS/MS

HPLC Parameters

- Column: Phenomenex® Gemini®, 3 µm 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 85% A at 4 minutes

Mass Spectrometry

- SCIEX QTRAP® 5500
- Negative ion mode
- MRM

RESULT(S)

The developed methods resulted in a sensitive and selective assay that can be quickly transferred to any instrument.

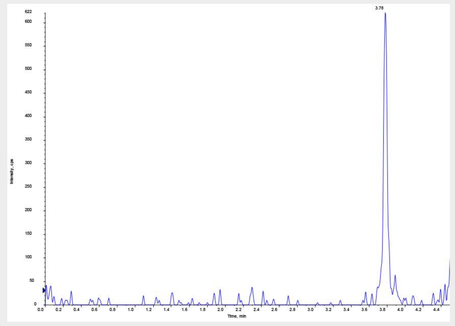

Figure 1: 2 ng/mL, Formivirsen, extracted human plasma

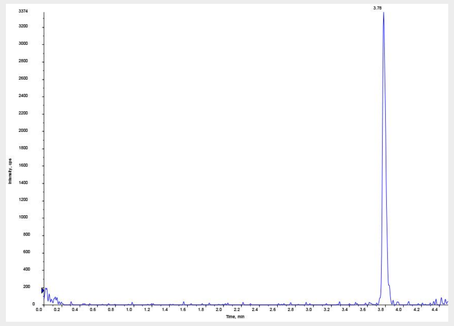

Figure 4: 2 ng/mL, 2'-MOE GapmerB, extracted human plasma

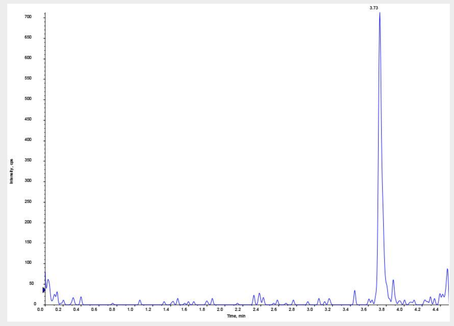

Figure 2: 2 ng/mL, Phospho B, extracted human plasma

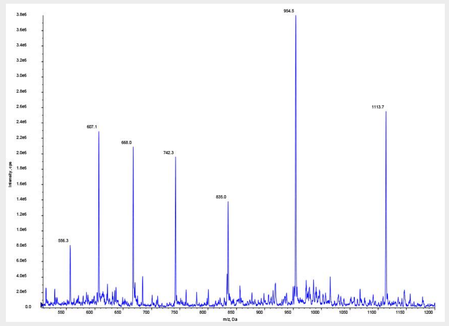

Figure 5: Q1 Scan 2'-MOE Gapmer, no adducts observed

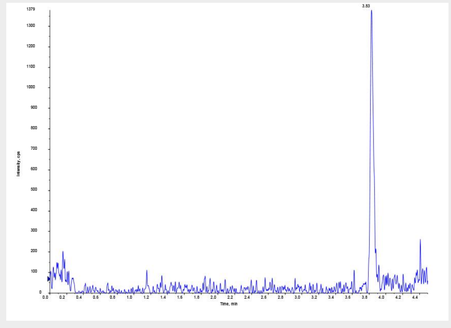

Figure 3: 1.0 ng/mL, 2'-MOE Gapmer, extracted human plasma

Oligonucleotide	Sequence	Molecular Weight (amu)	Q1/Q3
Formivirsen	G*C*G*T*T*T*G*C*T*C*T*T*C*T*T*C *T*T*G*C*G	6682.4	953.8/648.0
Phospho B	G*C*G*T*C*T*G*C*T*C*T*T*C*T *T* C*T*T*G*C*G	6667.3	951.3/319.2
2'-MOE Gapmer	mC*mG*mA*mC*mU*A*T*A*C*A*C*G* C*A*A*mU*mA*mU*mG*mG	6673.4	833.1/670.6
2'-MOE GapmerB	mC*mG*mA*mC*mU*A*T*A*C*G*C*G *C*A*A*mU*mA*mU*mG*mG	6689.4	954.3/669.7

Table 1: Oligonucleotide Properties

Charge State	1	2	3	4	5	6	7	8	9	10	11	12
Predicted Mass	6688	3344	2229	1671	1337	1114	955	835	742	668	607	556

Table 2: Q1 Scan 2'-MOE Gapmer, predicted masses

CONCLUSION(S)

1. A dedicated portable MFLC system allows for fast switching from typical LC-MS/MS compounds to oligonucleotides with no adduct formation.
2. MFLC provides more sensitivity when compared to conventional HPLC.
3. A single extraction method was utilized to analyze four separate oligonucleotides from human plasma.

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The LC-MS Experts