Improve Sensitivity for Quantification of Antisense Oligonucleotides in Plasma Using Microflow LC-MRM Methodology

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INTRODUCTION

Oligonucleotide therapeutics are rapidly growing as a class of therapeutic compounds. An increase in the number of candidates entering clinical trials and in approvals has been observed, which triggers higher demands on bioanalytical assay development.

The orthogonal technology, mass spectrometry, has been routinely used for bioanalytical studies of oligonucleotides. However, the extent of its use to date has been limited because its sensitivity does not allow it to quantify the ultra-low analyte levels in matrices. Improvements in mass spectrometry instrumentation as well as the implementation of microflow chromatography have enabled sensitivity of the LC-MS assays to approach what was previously only achievable with hybridization ELISA. Presented in this work is the use of the QTRAP® 6500+ LC-MS/MS System and the OptiFlow™ Interface coupled with the M5 MicroLC System for improved sensitivity to quantify oligonucleotides in matrix.

KEY FEATURES

- The QTRAP 6500+ LC-MS/MS System provides the sensitivity and quantification power to analyze oligonucleotides in matrix
- The QTRAP 6500+ LC-MS/MS System is compatible with both high-flow and microflow HPLC analysis with minimum optimization required
- The OptiFlow Interface offers flexibility in column selection for challenging oligonucleotide separations, while requiring no probe or electrode position optimization
- The M5 MicroLC System provides accurate control of flow rates down to 1 µL/min with direct-inject and trap-elute capabilities for fast and large sample volume loading

MATERIALS AND METHODS

Samples and Reagents: The ion paring reagents HFIP and DIEA were purchased from Sigma Aldrich. The customized oligonucleotide standards were synthesized by Sigma, including an oligonucleotide that is designed to be structurally equivalent to fomivirsen and an internal standard (IS). Fomivirsen is a synthetic antisense oligonucleotide composed of 21 bases (5'G*C*G*T*T*T*G*C*T*C*T*T*T*C*T*T*C*T*T*G*C*G-3') linked by phosphorothioate bonds. The IS is an oligonucleotide composed of 23 bases (5'- ACGGCTACCTTGTTACGACTTCA-3'). The OTX Clarity SPE kit was purchased from Phenomenex.

Sample Preparation: 100 µL of rat plasma was extracted through the OTX Clarity SPE plate using the recommended protocol. The SPE plate was activated with 1 mL methanol and equilibrated with 1 mL equilibration buffer. After equilibration, rat plasma was loaded to the plate and washed with 1 mL washing buffer for 3 times and subsequently eluted with 1 mL elution buffer. The eluted samples were dried down with N_2 gas and reconstituted in 100 µL mobile phase A (100 mM HFIP and 15 mM DIEA in water) containing 100 µM EDTA. The calibration curve sample set with 0.1 ng/mL to 1000 ng/mL of fomivirsen was prepared by spiking fomivirsen into extracted rat plasma. The recovery rate of this sample preparation protocol is about 80% as evaluated.

LC-MS Conditions for Comparative Analysis: On-column MRM method development was performed because the Source parameters are highly dependent on the LC-MS conditions. In order to compare the sensitivity difference between analytical flow LC-MS and microflow LC-MS approaches, each sample was injected and analyzed in triplicate on the same QTRAP 6500+ System with the same MS dependent parameters coupled with either an ExionLC™ system and IonDrive™ Turbo V Ion Source, or an M5 MicroLC System and an OptiFlow® Turbo V Ion Source. For full details please refer to the associated technote

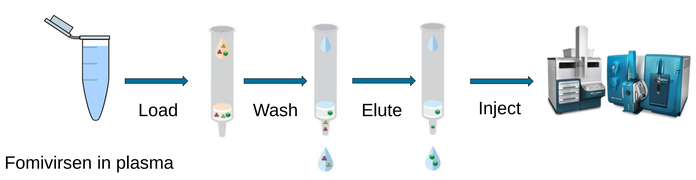


Figure 1. LC-MS workflow for quantitation of fomivirsen in plasma

RESULTS

The microflow LC data show significant improvement of sensitivity, at an average of 5-fold for S/N

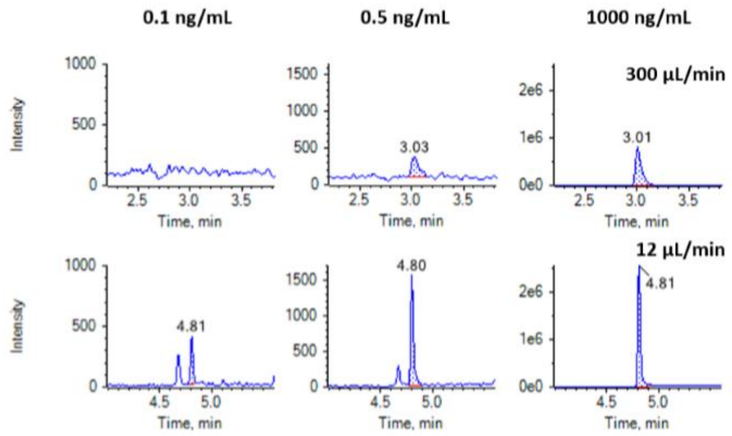
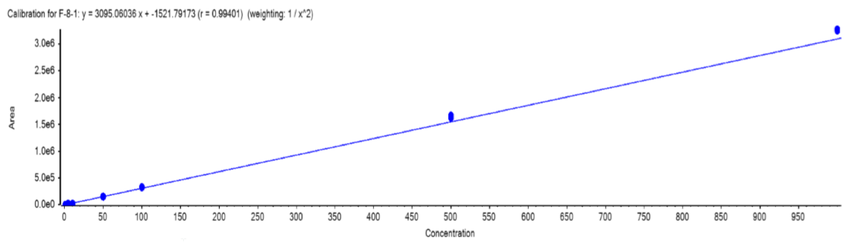


Figure 2. Comparison of High-flow (top) and Microflow (bottom) XICs of Fomivirsen at Selected Spiked-In Levels

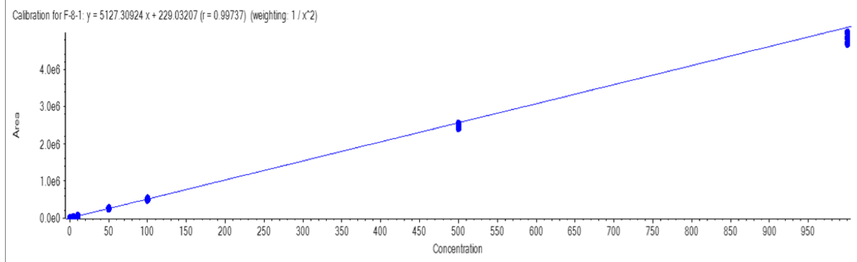
A calibration curve is obtained for quantification of fomivirsen from 1 ng/mL to 1000 ng/mL with high flow LC-MS



Component Name	Actual Concentration (ng/mL)	Mean	Percent CV	Accuracy
F-8-1	1.00	1.09	7.05	108.69
F-8-1	2.00	1.75	7.71	87.42
F-8-1	5.00	4.65	1.51	93.05
F-8-1	10.00	8.85	7.42	88.45
F-8-1	50.00	51.43	0.99	102.86
F-8-1	100.00	107.50	0.35	107.48
F-8-1	500.00	533.40	1.28	106.68
F-8-1	1000.00	1054.00	0.38	105.37

Figure 3. Statistics and Calibration Curve for Quantification of Fomivirsen Using High-flow Chromatography

A calibration curve is obtained for quantification of fomivirsen from 0.1 ng/mL to 1000 ng/mL with high flow LC-MS



Component Name	Actual Concentration (ng/mL)	Mean	Percent CV	Accuracy
F-8-1	0.10	0.10	16.33	98.01
F-8-1	0.20	0.20	11.45	101.75
F-8-1	0.50	0.52	4.45	103.58
F-8-1	1.00	1.04	3.01	103.93
F-8-1	2.00	1.98	8.54	98.81
F-8-1	5.00	5.14	2.72	102.77
F-8-1	10.00	10.11	0.94	101.11
F-8-1	50.00	49.92	1.27	99.83
F-8-1	100.00	98.74	2.46	98.74
F-8-1	500.00	485.00	2.54	97.01
F-8-1	1000.00	944.60	3.00	94.46

Figure 4. Statistics and Calibration Curve for Quantification of Fomivirsen Using Microflow Chromatography

CONCLUSIONS

- An ultra-sensitive microflow LC-MS/MS workflow for quantifying antisense oligonucleotides in matrix
- A 5-fold S/N improvement is achieved compared to the high-flow LC method
- Improved instrument robustness with less ion pairing reagents introduced

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