T1530
-09-54

Sensitive Bioanalysis of Antisense Oligonucleotides through Mitigation of Non-specific Binding and Improved LC-MS/MS Performance

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PURPOSE

Improving LC-MS/MS bioanalytical performance especially for ESI negative analytes has been of high interest especially for the challenging analysis of ONTs (oligonucleotide therapeutics) in biological matrices. Developing low/sub ng/mL oligonucleotide therapeutic quantitation methods requires special attention to recovery, removal of protein/interference and losses due to non-specific binding[1,2].

The high-performance tandem MS Waters™ Xevo™ TQ Absolute coupled with the Waters™ ACQUITY™ Premier UPLC system was used to evaluate quantitation of a panel of ASOs in human plasma.

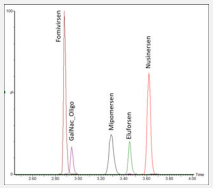
High sensitivity and 5 orders of dynamic range performance was described previously using GEM91/Trecovirsen as analyte[1], this work extends this methodology to ASOs of varying length and modifications.

METHOD(S)

Plasma samples 100µL (0.1 to 1000 ng/mL) were extracted using liquid-liquid extraction method with phenol:chloroform:isoamyl alcohol 25:24:1 followed by a second extraction with chloroform, +99%. Finally, all aqueous extracts were dried and reconstituted in 100uM EDTA solution. GEM91 (100 ng/mL) was used as internal standard to quantitate all other Oligonucleotides.

Waters™ MassPREP™ Oligonucleotide Separation Technology (OST) standards (p/n: 186004135) were also tested to ensure system performance over the characterized range of polythymidine oligonucleotide ladder standards 15-35mer prior to testing various Oligonucleotides (results not shown).

LC System	Waters ACQUITY Premier UPLC System (BSM)
Mobile phase-A	100mM Hexafluoroisopropanol (HFIP) + 15mM N, N-Diisopropylethylamine (DIPEA) in Water
Mobile phase-B	100mM Hexafluoroisopropanol (HFIP) + 15mM N, N-Diisopropylethylamine (DIPEA) in 80% Acetonitrile
Vials/plate	QuanRecovery with MaxPeak 700 µL Plate (p/n: 186009185) with Round Plug Pre-slit Silicone Cap-mat (p/n: 186006332)
Column(s)	Waters ACQUITY Premier Oligonucleotide C18 column, 1.7 µm, 2.1 x 50 mm (p/n: 186009484)
Column temp.	50 °C
Sample temp	8 °C
Injection vol	15 µL
Flow rate	0.5 mL/min



RESULT(S)

To demonstrate reproducibility, duplicates of calibration standards and six replicates of each QCs of ASOs (table 1) in each run were tested in three runs on 3 separate days. The calibration curves were linear with r^2 values >0.99 ($1/x^2$ weighting) with >75% non-zero calibrator levels and QCs met acceptance criteria in each run i.e., Non-zero calibrators and QCs should be ±15%, except at LLOQ where the calibrator or QCs should be ±20% of nominal concentrations in each run as shown in table 2 and 3.

Name	Mol Wt	Size (mers)	Linkers	Modifications
GEM91	7776	25 nts	O_3PS^{3-}	N/A
Fomivirsen	6682	21 nts	O_3PS^{3-}	N/A
Nusinersen	7127	18 nts	O_3PS^{3-}	2'-MOE
Eluforsen	11,469	33 nts	O_3PS^{3-}	2'OMe
Mipomersen	7177	20 nts	O_3PS^{3-}	2'-MOE ; 5-Me rC
GalNAc Oligo	~8000	21 nts	PO_4^{3-}	Triantennary GalNAc

Table 1. List of Oligonucleotides

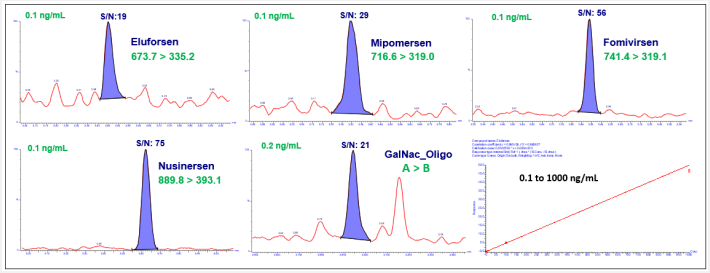


Figure 1. Representative chromatograms and calibration curve showing the run performance

		Eluforsen		Fomivirsen		Mipomersen		Nusinersen		GalNac_Oligo	
Name	Std Conc (ng/mL)	Accuracy (%)	RSD (%)	Accuracy (%)	RSD (%)	Accuracy (%)	RSD (%)	Accuracy (%)	RSD (%)	Accuracy (%)	RSD (%)
Cal Std-1	0.10	102.0	10.8	100.8	3.6	98.4	10.4	100.1	13.3	--	--
Cal Std-2	0.20	94.7	12.9	97.3	4.8	100.1	8.7	98.0	6.8	98.3	9.8
Cal Std-3	0.50	96.0	5.6	100.2	5.4	103.5	7.2	99.6	5.4	102.4	2.4
Cal Std-4	1.00	101.9	3.7	103.4	3.5	106.0	4.6	105.7	3.7	103.1	7.1
Cal Std-5	2.00	106.6	6.0	105.9	5.0	105.9	5.2	108.7	3.6	104.1	4.8
Cal Std-6	10.0	98.8	3.7	102.6	3.3	101.3	4.0	102.2	2.3	103.9	3.6
Cal Std-7	100	102.9	4.0	102.6	5.3	99.2	6.0	100.1	4.2	100.6	3.5
Cal Std-8	1000	94.9	2.8	89.3	2.9	89.0	4.5	86.6	1.2	90.1	2.9

Table 2. Statistics for calibration standards from 3 runs

		Eluforsen		Fomivirsen		Mipomersen		Nusinersen		GalNac_Oligo	
Name	Std Conc (ng/mL)	Accuracy (%)	RSD (%)	Accuracy (%)	RSD (%)	Accuracy (%)	RSD (%)	Accuracy (%)	RSD (%)	Accuracy (%)	RSD (%)
QC1	0.10	94.4	14.6	94.5	12.2	99.9	11.4	92.8	6.9	--	--
QC2	0.20	94.8	7.9	99.3	7.0	100.5	9.7	98.4	7.8	110.2	6.9
QC3	0.50	104.4	5.8	104.1	6.5	102.8	6.3	104.3	4.4	103.0	7.5
QC4	50.0	101.2	2.9	102.1	3.7	98.9	4.3	99.7	2.5	100.1	2.5
QC5	800	97.9	2.1	91.4	3.7	89.0	3.5	87.3	2.1	93.8	2.2

Table 3. Statistics for QC samples from 3 runs

The lower limit of quantification (LLOQ) of 0.1 ng/mL (0.2 ng/mL for GalNAc oligo) was achieved over a calibration range of 0.1 to 1000 ng/mL in human plasma and a representative standard curve is shown in figure 1 along with representative chromatograms of lowest calibration standards of all oligonucleotides.

CONCLUSION(S)

- Sub ng/ml levels of sensitivity, with good dynamic range performance was observed in human plasma for antisense oligonucleotides of different lengths (18 to 33 nts) and with a variety of linkers and modifications.
- MaxPeak™ HPS technology reduces nonspecific binding, metal absorption and enabled excellent sensitivity and low-level detection.
- With enhanced sensitivity for challenging negative ionization compounds, the Xevo™ TQ Absolute tandem MS can generate high quality data for routine LC-MS/MS based quantitation of antisense oligonucleotides in biological matrices.

REFERENCES

1. Suma Veeramachineni, Mark Wrona, 'Sensitive LC-MS/MS Bioanalytical Quantitation of Antisense Oligonucleotides', Waters™ Corporation, Application Notes, 720007574, March 2022.
2. Jennifer M Nguyen , Martin Gilar, Brooke Koshel, Michael Donegan, Jason MacLean, Zhimin Li & Matthew A Lauber, 'Assessing the impact of nonspecific binding on oligonucleotide bioanalysis', Future Science, BIOANALYSIS VOL. 13, NO. 16.
3. Guilherme J. Guimaraes, J. Michael Sutton, Martin Gilar, Michael Donegan, Michael G. Bartlett, 'Impact of Nonspecific Adsorption to Metal Surfaces in Ion Pair-RP LC-MS Impurity Analysis of Oligonucleotides', Journal of Pharmaceutical and Biomedical Analysis Volume 208, 20 January 2022, 114439

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