

## Sensitive Bioanalysis of Antisense Oligonucleotides of Various Lengths and Modifications

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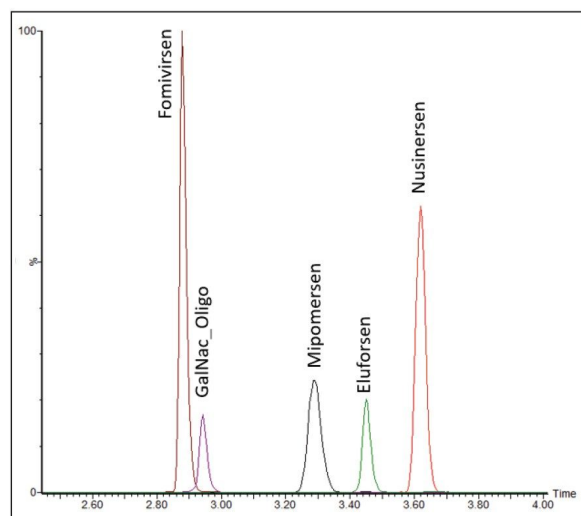
Waters Corporation

This is an Application Brief and does not contain a detailed Experimental section.

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### Abstract

This application brief demonstrates the sensitivity and suitability of the Xevo™ TQ Absolute Triple Quadrupole MS for bioanalysis of oligonucleotides in human plasma matrix with varying lengths (18 to 33 nucleotides), linkers, and modifications.



Left: Waters Xevo TQ Absolute System with Waters Acquity Premier UPLC;

Right: Representative MRM traces for 5 ASO compounds using 4 min bioanalytical LC-MS/MS IP method.

## Benefits

Coupled with the ACQUITY™ Premier UPLC System and ACQUITY Premier Oligonucleotide C<sub>18</sub> Column, the Xevo TQ Absolute MS demonstrates enhanced chromatographic recovery of oligonucleotides along with excellent (sub ng/mL) system sensitivity enabling support of challenging LC-MS/MS assays and PK studies.

## Introduction

High sensitivity and five orders of dynamic range performance were described previously using GEM91/Trecovirsen.<sup>1</sup> This work extends this methodology to antisense oligonucleotides (ASOs) of varying length and modifications.

## Experimental

Plasma samples were spiked at concentrations from 0.1 ng/mL to 1000 ng/mL with multiple ASOs containing 2'-MOE modified bases, GalNAc conjugate, or phosphorothioate linkers (Eluforsen, Fomivirsen, Mipomersen, Nusinersen, and a GalNAc conjugated oligonucleotide). 100  $\mu$ L were extracted using liquid-liquid extraction of plasma standards. GEM91 (100 ng/mL) was used as internal standard to quantitate all oligonucleotides. 100 mM hexafluoroisopropanol (HFIP) + 15mM N, N-diisopropylethylamine (DIPEA) in water and in 90% acetonitrile were used as mobile phase A and B, respectively.

For complete method details, please refer to Waters™ application note [720007574](#).

Name	Mol wt	Size (mers)	Linkers	Modifications	Parent (m/z)	Daughter (m/z)
GEM91	7776	25 nts	PO <sub>2</sub> S <sup>3-</sup>	N/A	597.2	319.1
Fomivirsen	6682	21 nts	PO <sub>2</sub> S <sup>3-</sup>	N/A	741.4	319.1
Nusinersen	7127	18 nts	PO <sub>2</sub> S <sup>3-</sup>	2'-MOE	889.8	393.1
Eluforsen	11,469	33 nts	PO <sub>2</sub> S <sup>3-</sup>	2'OMe	673.7	335.2
Mipomersen	7177	20 nts	PO <sub>2</sub> S <sup>3-</sup>	2'-MOE ; 5-Me rC	716.6	319.0
GalNAc_Oligo	~8000	21 nts	PO <sub>3</sub> <sup>3-</sup>	3'-triantennary GalNAc	A	B

Table 1. Details of the oligonucleotides used in study.

## Results and Discussion

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

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

Name	Std conc (ng/mL)	Eluforsen		Fomivirsen		Mipomersen		Nusinersen		GalNAc_Oligo	
		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.

Method was developed with optimized transitions, which were evaluated across a broad range of parent charge states and resulting fragment masses. Enhanced negative ion mode detection capabilities enabled improved counts, S/N and detection limits of the assay. 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 is shown in Figure 1 with representative chromatograms of lowest calibration standards of all oligonucleotides.

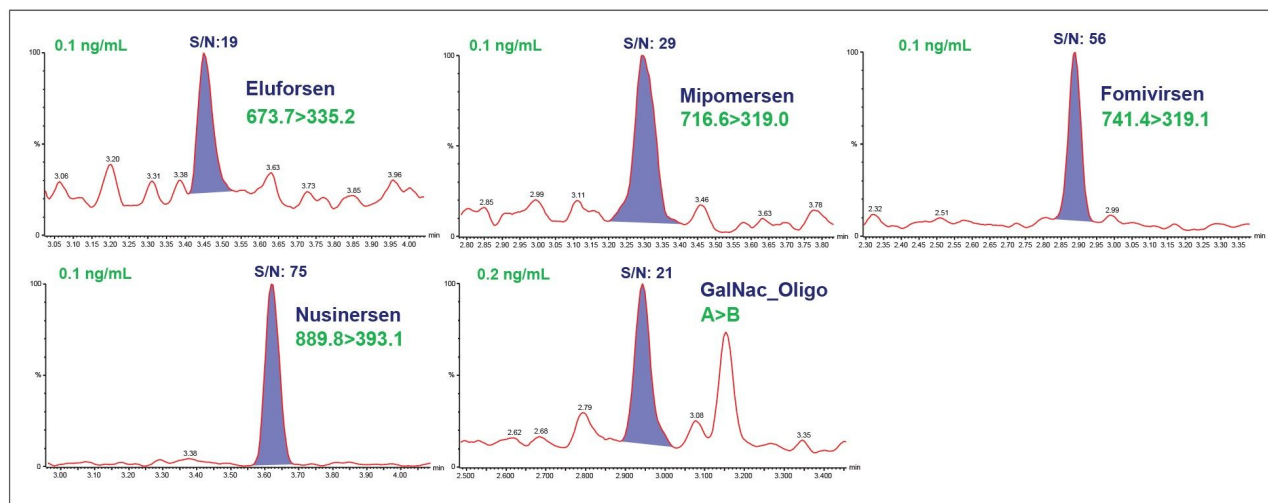


Figure 1. Representative chromatograms showing peaks at LLOQ levels.

## Conclusion

- 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.

## Acknowledgement

The authors thank Greg Jones and Alnylam Pharmaceuticals for donation of 21 mer GalNac oligonucleotide for our experiments.

This is the same as application note [720007418](#) by Mary Trudeau.

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## References

1. Suma Veeramachineni, Mark D Wrona, 'Sensitive LC-MS/MS Bioanalytical Quantitation of Antisense Oligonucleotides', Waters, Application Notes, [720007574](#), March 2022.

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