High resolution and sensitive LC-MS/MS method for the quantitation of oligonucleotides

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ABSTRACT

Antisense oligonucleotides (ASOs) and small interfering RNA (siRNA) are two examples of oligonucleotide therapies that are gaining ground in the treatment of previously incurable diseases. For pharmacokinetic analysis of oligonucleotides, high performance and robust quantitative approaches are required. In this work, a highly sensitive approach involving Liquid chromatography coupled to a unique QTOF mass spectrometer was created for the quantitation of oligonucleotides.

INTRODUCTION

While time-of-flight mass spectrometry (TOF MS) has been increasingly employed for quantitative purposes, the constraints in MS/MS sampling efficiency inherent to the mass analyzer design can make it challenging to reach the high sensitivity of a triple quadrupole system. Increased MS/MS sampling efficiency for MRM^{HR} investigations by as much as 10x is possible with the ZenoTOF 7600 system, which utilizes the Zeno trap to store fragment ions and optimizes the timing and release of the ions into the TOF accelerator. The time of flight (TOFs) based instruments have the advantage of providing high-resolution MS data. Shown in this study is a targeted, highly sensitive Liquid chromatography-Mass Spectrometry approach using a high-resolution and high speed TOF system for MRM (MRM^{HR}). The advantage of this workflow is that all product ions are detected, which enables the post-acquisition selection of the most suitable product ion(s) for quantification.

MATERIALS AND METHODS

Sample
Oligonucleotide ladder standard (15mer, 20mer, 25mer, 30mer, 35mer, and 40mer) was diluted with Milli Q water to make a calibration curve standard ranging from 0.06 nmole to 10000 nmole. 10 uL was injected for LC-MS analysis

Sequence Name	Sequence	MW (Da)
15mer	TTTTT TTTTT TTTTT	4500.6
20mer	TTTTT TTTTT TTTTT TTTTT	6020.6
25mer	TTTTT TTTTT TTTTT TTTTT TTTTT	7542.3
30mer	TTTTT TTTTT TTTTT TTTTT TTTTT TTTTT	9062.8
35mer	TTTTT TTTTT TTTTT TTTTT TTTTT TTTTT TTTTT	10584.0
40mer	TTTTT TTTTT TTTTT TTTTT TTTTT TTTTT TTTTT TTTTT	12106.6

Table 1. Sequence and molecular weights of the oligonucleotide ladder standard

HPLC
The oligonucleotides were chromatographically separated with the LC gradients described previously.[4-6] The separation was achieved using a Waters ACQUITY UPLC Oligonucleotide BEH C18 column (2.1 × 50 mm, 1.7 µm, 130 Å) at a flow rate of 0.4 mL/min. The column was kept at 60°C in the column oven of an Agilent 1290 LC system.

MS/MS
TOF MS (500 to 3000 m/z) and MRM^{HR} experiments were performed in SCIEX OS software using the ZenoTOF 7600 system. Two or 3 charge states were targeted per each oligonucleotide standard. The data were analyzed using Sciex OS software. An XIC peak width of 0.02 Da was used for quantification. A 1/X^2 weighing was used for the quantitation.

Analyte	Precursor ion (m/z)	Fragment ion (m/z)	DP (V)	CE (V)	CS Spread (V)
15mer	749.12	303.0399	-100	-35	15
20mer	1504.23	303.0399	-100	-70	15
25mer	753.21	303.0399	-100	-35	15
30mer	696.10	303.0399	-100	-35	15
35mer	704.57	303.0399	-100	-35	15
40mer	755.49	303.0399	-100	-35	15

Table 2. MRM^{HR} algorithm parameters and fragments for quantitation

RESULTS

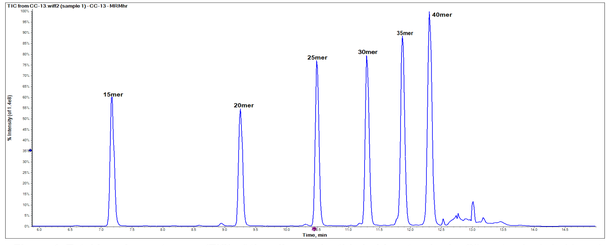

Figure 1. Total ion chromatogram (TIC) of the oligonucleotide ladder standards. Oligo standards ladder mix-15, 20, 25, 30, 35 and 40mer well separated

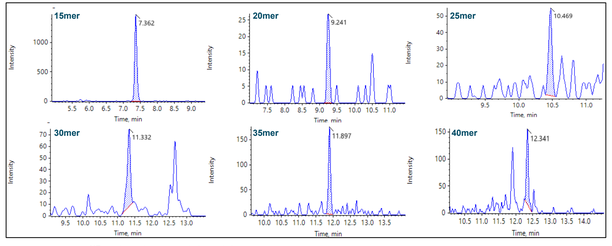

Figure 2. MRM^{HR} XICs of the LLOQs of Oligonucleotide ladder standards. 0.5, 0.1, 0.06,0.06, 0.1 and 0.1 nmole for 15mer, 20mer, 25mer, 30mer, 35mer and 40mer respectively.

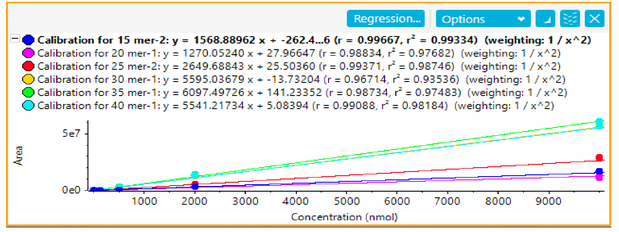

Figure 3. Calibration curves representing the linear range of oligonucleotide standards-MSMS fragment based MRM^{HR} algorithm quantitation. Overall, the linear dynamic range (LDR) was ~5 orders of magnitude.

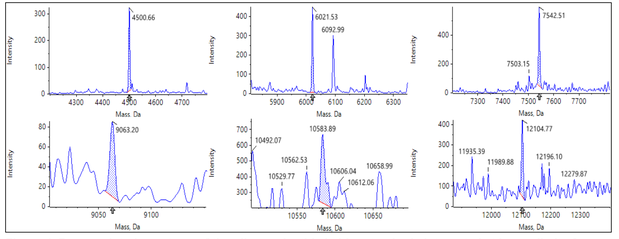

Figure 4. Deconvoluted spectrum of the oligonucleotide ladder standards integrated at Lower limit of quantitation (LLOQs)- 0.2 nM

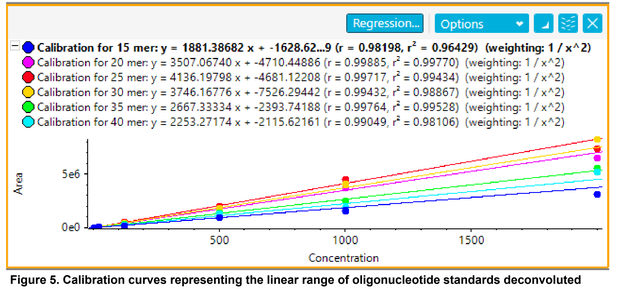

Figure 5. Calibration curves representing the linear range of oligonucleotide standards deconvoluted spectrum-based TOF-MS quantitation. Overall, the LDR was ~3.5 orders of magnitude.

Analyte	MRM^{HR}		TOF MS (Intact Quant)	
	Linearity Range (nM)	LDR	Linearity Range (nM)	LDR
15mer	0.5 to 10000	4.5	2 to 10000	3.5
20mer	0.1 to 10000	5	2 to 10000	3.5
25mer	0.06 to 10000	5.2	2 to 10000	3.5
30mer	0.06 to 10000	5.2	2 to 10000	3.5
35mer	0.1 to 10000	5	2 to 10000	3.5
40mer	0.1 to 10000	5	2 to 10000	3.5

Table 3. Comparison of the MS/MS fragment based MRM^{HR} algorithm quantitation with Deconvoluted spectrum- based TOF MS quantitation.

CONCLUSIONS

- Low-nM LLOQs for oligonucleotide ladder standards were achieved in MRM^{HR} mode using the Zeno trap function of the ZenoTOF 7600 system. This method can be applied for pharmacokinetic studies with limited sample availability that require quantification at very low levels.
- The TOF MS data acquired as part of the MRM^{HR} algorithm quantification workflow can be used for the quantification using Sciex OS Software intact quant feature.
- Generic DP and CE curves were used for acquisition. This allows the shortening of the time required for method optimization.

REFERENCES

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TRADEMARKS/LICENSING