Poster Reprint

ASMS 2024
Poster number MP 095

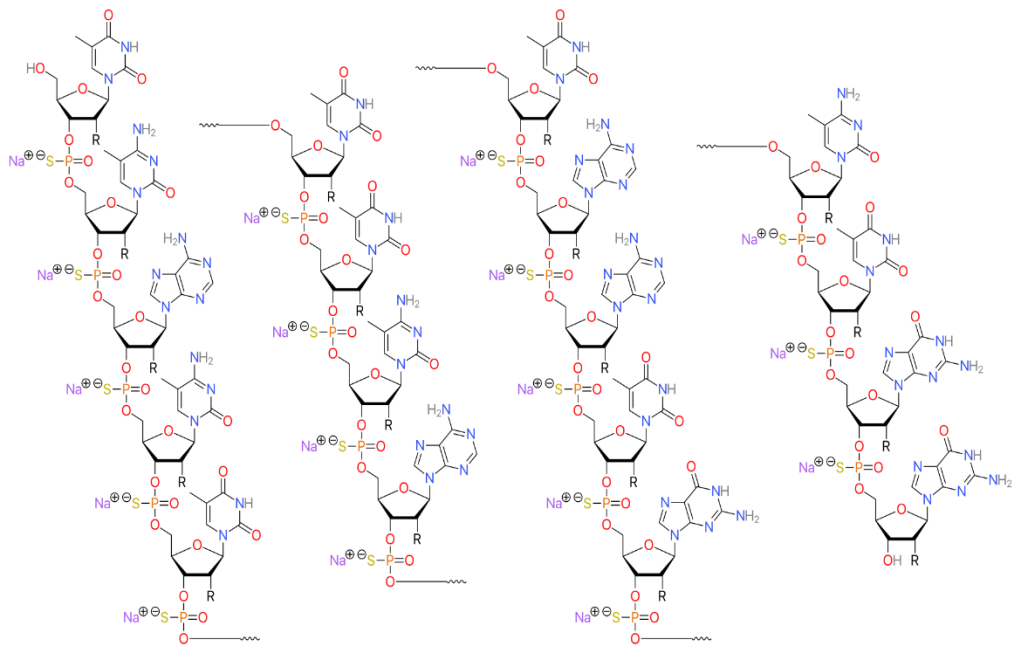
Sensitive Bioanalysis Method for Antisense Oligonucleotides using LC-MS/MS

Lee Bertram

Agilent Technologies, Inc., Santa Clara, CA, USA

Introduction

Antisense oligonucleotides (ASOs) have emerged as powerful and versatile therapies in recent years with the potential to treat a wide range of genetic disorders, neurodegenerative diseases, and various cancers. As oligonucleotide therapies expand, a need has been created for low-level detection and sensitive methods aimed at understanding the pharmacokinetic profile of the drugs. These methods will be crucial for optimizing the dosage regimens, predicting potential side effects and ensuring efficacy of future therapeutics. In this poster we describe a sensitive bioanalysis method utilizing an automatable sample preparation method combined with the high sensitivity and robustness of the 6495D triple quadrupole mass spectrometer for a 2'-O-2-methoxyethyl, fully phosphorothioated oligonucleotide in human plasma samples.



Experimental

Sample Preparation

A fully phosphorothioated single stranded oligonucleotide with the sequence U*/i2MOErC/*/i2MOErA/* /i2MOErC/*U*U* U*/i2MOErC/*/i2MOErA/* U*/i2MOErA/*/i2MOErA/* U*/i2MOErG/*C* U*/i2MOErG/*G was purchased from IDT. The calibration curve was prepared by spiking the oligonucleotide into extracted human plasma (K2 EDTA, Pooled, BioIVT).

The human plasma was extracted using Phenomenex Clarity OTX SPE plates and an Agilent Bravo liquid handler. The extraction procedure is listed in Table 1.

Experimental

Procedure	Solvent
Conditioning	Methanol
Equilibrating	Equilibration buffer (50 mM NH4OAc (pH 5.5))
Sample Loading	100 µL plasma + 100uL lysis buffer
Wash1	50 mM NH4OAc (pH 5.5)
Wash2	50 mM NH4OAc (pH 5.5) in 50%ACN
Elution	100 mM NH4HCO3 in H2O (pH 9.5)/ACN/THF (5:4:1, v/v/v)
Dry Down	SpeedVac drying
Reconstitution	Water with 20%MeOH

Table 1. Agilent Bravo extraction protocol.

Equipment

Sample separation was performed using the Agilent 1290 Infinity II Bio LC system consisting of the following modules:

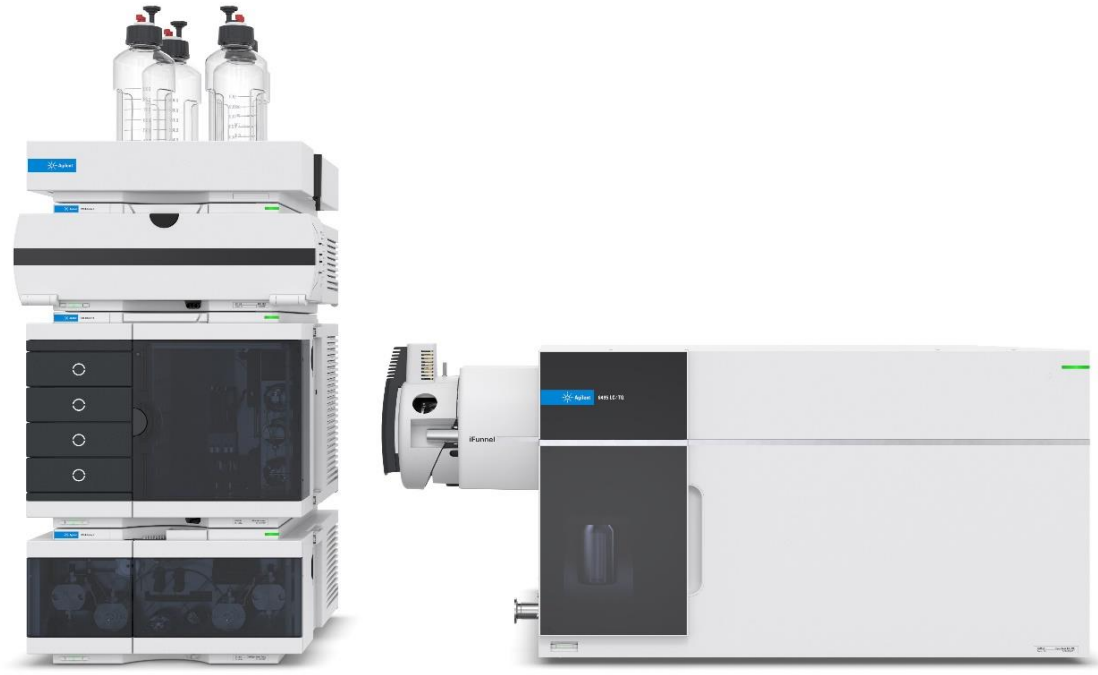
– Agilent 1290 Infinity II Bio high-speed pump (G7132A)

– Agilent 1290 Infinity II Bio multisampler with thermostat (G7137A)

– Agilent 1290 Infinity II multicolumn thermostat (G7116B)

The LC system was coupled to the Agilent 6495D triple quadrupole LC/MS (G6495D) equipped with the Agilent AJS ESI source. Agilent MassHunter Workstation software 12.1 was used for data acquisition and MassHunter Quant was used for data processing.

Sample preparation was done with an Agilent Bravo and Thermo Scientific SpeedVac.



Experimental

Methods

Liquid chromatography/mass spectrometry (LC/MS) conditions and parameters are provided in Tables 2 and 3. The multiple reaction monitoring (MRM) settings for the compounds are listed in Table 4.

Parameter	Value	
Column	Agilent AdvanceBio Oligonucleotide (p/n 659750-702)	
Sampler Temperature	4 °C	
Mobile Phase A	15 mM DIEA & 100 mM HFIP in water	
Mobile Phase B	Methanol	
Flow Rate	0.4 mL/min	
Injection Volume	10 µL	
Column Temperature	65 °C	
Gradient Program	Time (min)	%B
	0.0	20
	4.0	30
	5.0	60
	5.5	90
	6.5	90
	6.6	20

Table 2. Agilent 1290 Infinity II Bio LC method.

Parameter	Value
Ion Source	Agilent AJS ESI source
Polarity	Negative
Gas Temperature	290 °C
Drying Gas Flow	12.5 L/min
Nebulizer	30 psi
Sheath Gas Temperature	300 °C
Capillary Voltage	4000 V
Nozzle Voltage	1500 V
Scan Type	MRM
Detector Gain Factor (-)	10
LC Diverter to Waste	0 – 0.5 min (the remaining time diverter to MS)

Table 3. Agilent 6495D LC/TQ Parameters.

Compound name	Precursor m/z	Product m/z	Dwell (ms)	CAV (V)	CE (V)	Polarity
Oligo (z=7)	905.9	94.9	100	3	120	-
	905.9	305.0	100	3	35	-
Oligo (z=6)	1057.0	94.9	100	3	120	-
	1057.0	305.0	100	3	40	-

Table 4. Detailed multiple reaction monitoring settings.

Results and Discussion

Calibration Curve Analysis

To evaluate the quantification performance of the oligonucleotide in extracted plasma, the calibration curve was analyzed with concentrations ranging from 0.07 to 275 ng/mL (Figure 1). The results of the individual standards are shown in Table 5.

Figure 2, a representative total ion chromatogram, shows the peak shape and retention of the 18mer used for this study. With slight adjustments in the LC method gradient you can ensure separation and accurate quantitation of a variety of ASOs.

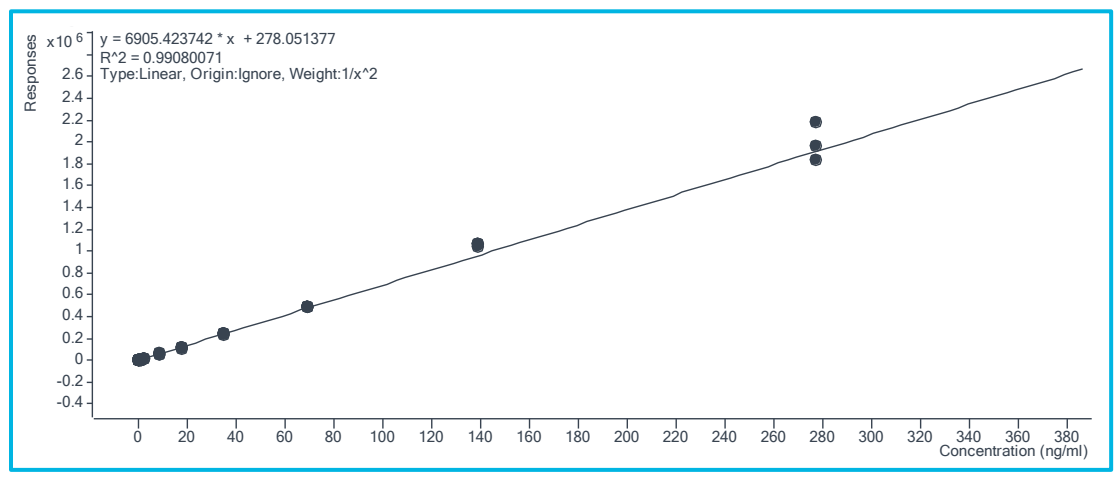


Figure 1. Calibration Curve of 18 mer.

Results and Discussion

Name	Response	S/N	RSD (%)	Accuracy (%)
Std 1	716	153	0.06	93.8
Std 2	1351	211	0.03	111.2
Std 3	2144	317	0.04	99.9
Std 4	3890	983	0.07	96.7
Std 5	6925	2085	0.05	89.0
Std 6	14668	3722	0.04	96.3
Std 7	57882	13417	0.07	96.4
Std 8	114380	25584	0.06	95.4
Std 9	242717	62103	0.02	101.4
Std 10	485153	66628	0.01	101.4
Std 11	1057180	141285	0.01	110.5
Std 12	1996025	316333	0.09	104.3

Table 5. Detailed calibration curve results.

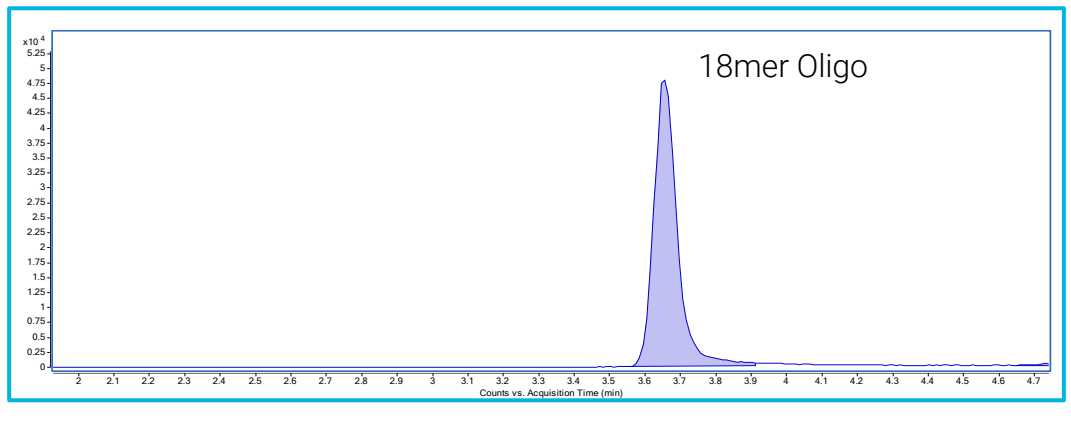


Figure 2. Total MRM chromatogram.

Quantification in Extracted Plasma

The results show that ASOs could be quantified with high confidence at 0.07 ng/mL in a plasma matrix. Excellent response, S/N, and reproducibility of the chosen ASO at the low concentrations in an extracted plasma matrix were demonstrated on the 6495D LC/TQ. Figure 3 shows the representative chromatograms of the matrix blank and 18 mer ASO at 0.07 and 275 ng/mL plasma matrix with the 6495D LC/TQ.

Retention time stability was also assessed for all injections and a consistent retention time was shown across all concentrations and replicates with a retention time % RSD of 0.003.

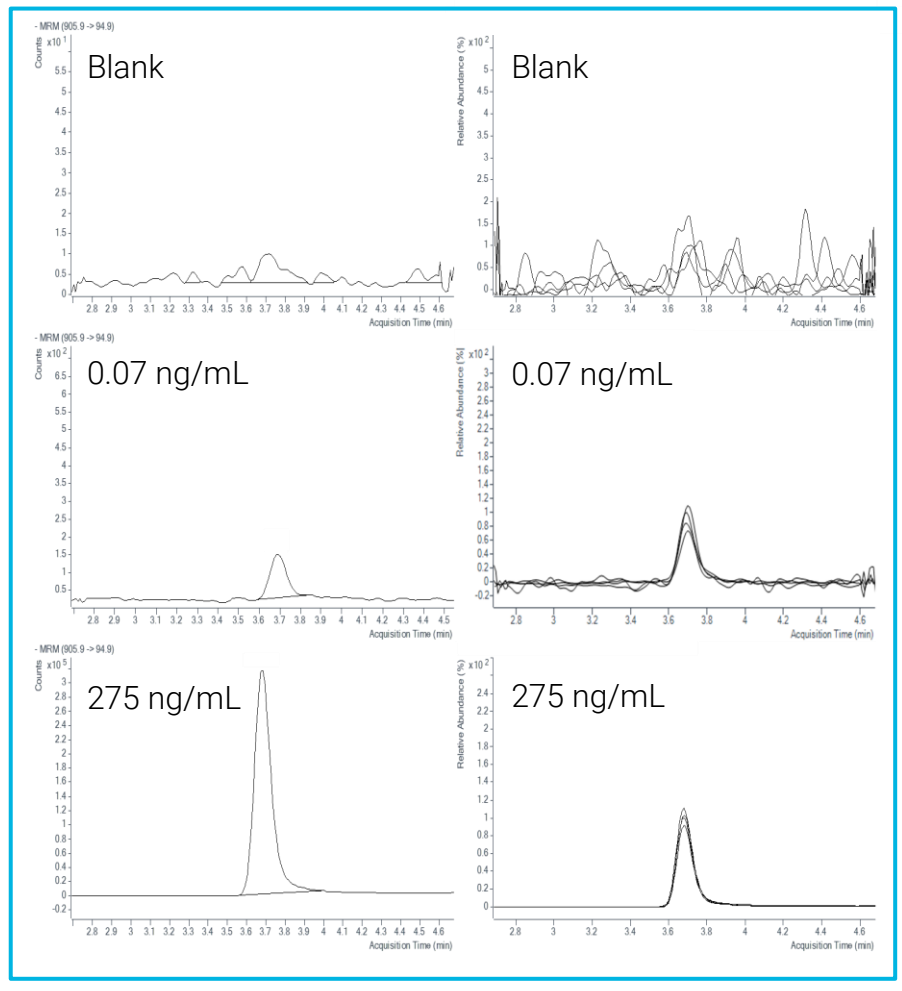


Figure 3. Representative chromatograms of the quantifier (left) and qualifier (right) ions for the 18mer ASO in extracted plasma matrix at low concentrations.

Conclusions

The method shown provides an end-to-end solution for low detection limits of quantitation for ASOs in human plasma by combing sample preparation via SPE on an Agilent Bravo liquid handler combined with a standard flow liquid chromatogram coupled with an Agilent 6495D LC/TQ.

The strategy applied in this experiment can be applied easily and adapted to varying oligonucleotides in complex matrices.

https://www.agilent.com/en/promotions/asms

RA45419.7200694444

Published in USA, May 31,2024

Agilent
Trusted Answers