

An Optimized Protocol for the 25 mg/well Clarity™ OTX™ Format Plates

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Overview

Oligonucleotides are becoming increasingly prominent in modern medicine. Due to their ability to modulate both gene expression and post-transcriptional splicing of introns and exons, oligos can be used in a variety of therapeutics. However, extraction of the oligo from biological samples can be challenging for good assay specificity, sensitivity and linearity. Because of their relatively low dosing, obtaining relevant lower limits of quantitation (LLOQ) and good linearity for oligo pharmacokinetic assays is particularly challenging. Experiments using several ASO and siRNA oligonucleotides spiked into plasma and serum reveal loading limits based on the amount of serum or plasma used, not the concentration of oligonucleotide present.

In this application note, we used Spinraza®, a typical 2'-MOE-Gapmer chemistry antisense oligonucleotide, mixed into different amounts of serum and loaded the samples onto a 25 mg/well Clarity OTX solid phase extraction (SPE) plate. Different amounts of oligo were spiked into 100 µL of serum to develop a calibration curve between 100 ng/mL and 1 ng/mL.

The Clarity OTX protocol is different from other SPE methods in that the sorbent not only binds the desired oligonucleotide analyte, but it also binds most other components in serum (proteins, lipids, oligos, etc.) before serially eluting them according to their chemical properties. Thus, any loading limit is based on the matrix (plasma, urine, tissue) protein and lipid concentration. Samples were analyzed by LC-MS/MS after extraction using a Biozen 2.6 µm Oligo LC column.

The results demonstrate that the Clarity OTX 25 mg/well format is a useful tool in the isolation, characterization, and quantitation of current antisense oligonucleotide therapeutics from mid-sized (100 µL or less) biological fluids like serum. Good linearity and sensitivity down to the single digit nanogram level were achieved when using the Clarity OTX 25 mg/well SPE plate.

Sample Preparation

Step	Description
Sample Pre-treatment:	In a glass vial, mix Lysis Buffer (Part No.: AL0-8579) and oligo/serum in a 1:1 ratio, up to 100 µL of oligo/serum. Vortex for 15 sec.
Condition:	Clarity OTX 25 mg/well, 96-well plate (Part No.: 8E-S103-CGA) with 1 wash of 1 mL Methanol.
Equilibrate:	Plate with 1 wash of 1 mL Equilibration Buffer (50 mM Ammonium Acetate, pH 5.5; from Buffer Extraction Kit, Part No.: KS0-9253).
Load:	Sample slowly onto plate using 2.5 in. Hg.
Wash:	Plate with 2 washes of 800 µL Wash Buffer (50 mM Ammonium Acetate, pH 5.5, and 50 % Acetonitrile; from Buffer Extraction Kit, Part No.: KS0-9253).
Elute:	Sample with 300 to 800 µL Elution Buffer (100 mM Ammonium Bicarbonate, pH 9.5, 40 % Acetonitrile, and 10 % Tetrahydrofuran; from Buffer Extraction Kit, Part No.: KS0-9253) into a Strata™ low binding, glass lined 96-well collection plate (part No.: AH1-7036).
Inject:	Sample as soon as eluted or lyophilize near dryness and reconstitute if higher concentrations are desired.

LC Conditions

Column:	Biozen™ 2.6 µm Oligo	
Dimensions:	50 x 2.1 mm	
Part No.:	00B-4790-AN	
Mobile Phase:	A: 15 mM N,N-Diisopropylethylamine and 35 mM Hexafluoroisopropanol, in Water B: 15 mM N,N-Diisopropylethylamine and 35 mM Hexafluoroisopropanol, in Methanol / Water (90:10, v/v)	
Gradient:	Time (min)	%B
	0	20
	5	40
	5.5	90
	7	90
	7.5	20
	10.5	20
Flow Rate:	250 µL/min	
Injection Volume:	20 µL	
Temperature:	70 °C	
Instrument:	Agilent® 1290	
Detection:	MS/MS	
Detector:	SCIEX® 7500 QTRAP®	

MS/MS Conditions

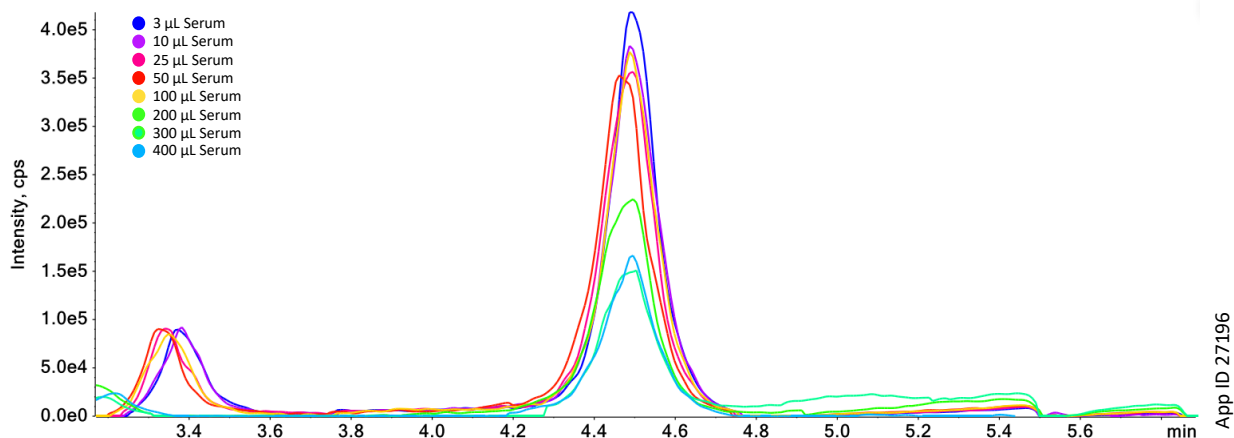
Ion Spray Voltage:	4000 V
Polarity:	Negative
Scan Type:	MRM
CUR:	40
GS1:	70
GS2:	70
EP:	-10
CE:	-38
CXP:	-23
QOD:	-2
Temperature:	400 °C

MRM Transitions

Analyte	Q1 (m/z)	Q3 (m/z)
Spinraza-1	890.1	402
Spinraza-2	890.1	393.2



Figure 1. Extracted Ion Chromatograms (EIC) Overlay of 10 ng/mL of Spinraza® Spiked into Different Volumes of Serum Loaded on the Clarity™ OTX™ 25 mg/well Plate.

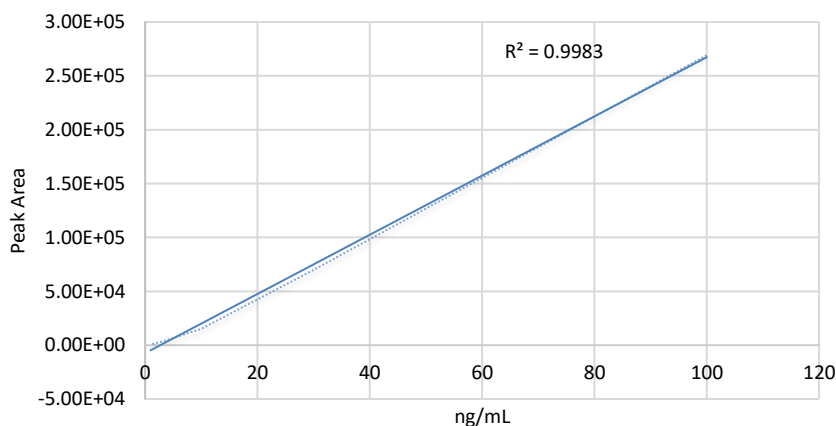


App ID 27196

Table 1. Percent Area Recovery of Spinraza in Different Volumes of Serum with Respect to Control and Respective Binding Capacities.

Serum (µL)	% Recovery	Proteins in Serum (mg)	% Binding Capacity
3	85.26	0.300	1
10	83.58	1	4
25	80.97	2.5	10
50	79.39	5	20
100	79.14	10	40
200	52.55	20	80
300	35.62	30	120
400	36.36	40	160

Figure 2. Peak Areas of Spinraza Spiked into Serum (Concentration Range 1 ng/mL to 100 ng/mL) on the Clarity OTX 25 mg/well plate.



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