An Optimized Protocol for the 2 mg/well Clarity™ OTX™ Format Plates

Roxana Rangel-Eggleston, Michael McGinley, PhD, and Bryan Tackett, PhD
Phenomenex, Inc., 411 Madrid Ave., Torrance, CA 90501 USA

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 2 mg/well Clarity OTX solid phase extraction (SPE) plate. Different amounts of oligo were spiked into 10 µL of serum to develop a calibration curve between 0.03 ng/mL and 100 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 2 mg/well format is a useful tool in the isolation, characterization, and quantitation of current antisense oligonucleotide therapeutics from small (10 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 2 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 10 µL of oligo/serum. Vortex for 15 sec.
Condition:	Clarity OTX 2 mg/well, 96-well plate (Part No.: 8M-S103-4GA) with 2 washes of 200 µL Methanol.
Equilibrate:	Plate with 2 washes of 200 µL 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 glass vial with 100 µL of Equilibration Buffer and transfer to well.
Wash1:	Plate with 2 washes of 100 µL Equilibration Buffer.
Wash2:	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 30 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.

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
Q0D: -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 1 ng/mL of Spinraza® Spiked into Different Volumes of Serum Loaded on the Clarity™ OTX™ 2 mg/well Plate.

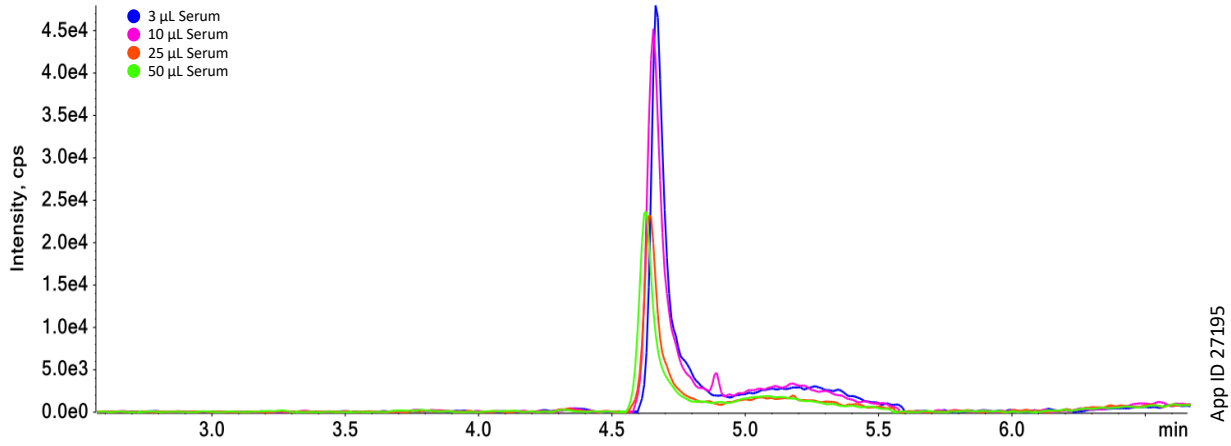
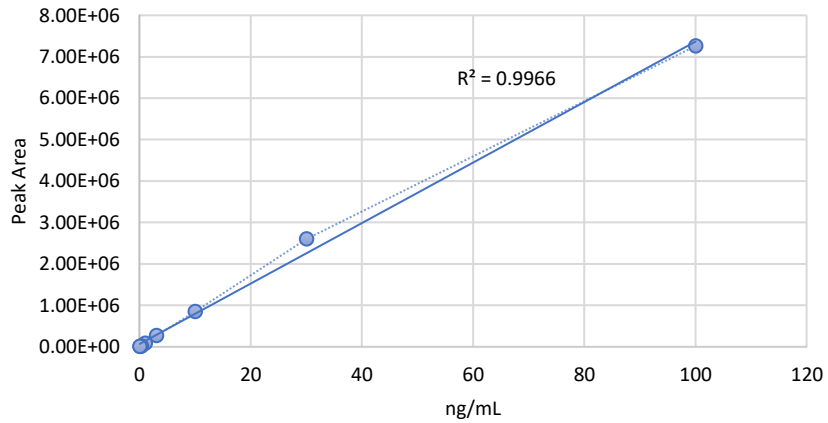


Table 1. Percent Area Recovery of Spinraza in Different Volumes of Serum with Respect to Control and Respective Binding Capacities.

Serum (µL)	% Recovery	Proteins in Serum (mg)	% Binding Capacity
3	62.20	0.3	15
10	64.14	1	50
25	31.70	2.5	125
50	27.43	5	250

Figure 2. Peak Areas of Spinraza Spiked into Serum (Concentration Range 0.03 ng/mL to 100 ng/mL) on the Clarity OTX 2 mg/well plate.



Need a different column size or sample preparation format?

No problem! We have a majority of our available dimensions up on www.phenomenex.com, but if you can't find what you need right away, our super helpful Technical Specialists can guide you to the solution via our online chat portal www.phenomenex.com/Chat.

Australia
t: +61 (0)2-9428-6444
auinfo@phenomenex.com

Austria
t: +43 (0)1-319-1301
anfrage@phenomenex.com

Belgium
t: +32 (0)2 503 4015 (French)
t: +32 (0)2 511 8666 (Dutch)
beinfo@phenomenex.com

Canada
t: +1 (800) 543-3681
info@phenomenex.com

China
t: +86 400-606-8099
cninfo@phenomenex.com

Czech Republic
t: +420 272 017 077
cz-info@phenomenex.com

Denmark
t: +45 4824 8048
nordicinfo@phenomenex.com

Finland
t: +358 (0)9 4789 0063
nordicinfo@phenomenex.com

France
t: +33 (0)1 30 09 21 10
franceinfo@phenomenex.com

Germany
t: +49 (0)6021-58830-0
anfrage@phenomenex.com

Hong Kong
t: +852 6012 8162
hkinfo@phenomenex.com

India
t: +91 (0)40-3012 2400
indiainfo@phenomenex.com

Indonesia
t: +62 21 5019 9707
indoinfo@phenomenex.com

Ireland
t: +353 (0)1 247 5405
eireinfo@phenomenex.com

Italy
t: +39 051 6327511
italiainfo@phenomenex.com

Japan
t: +81 (0) 120-149-262
jpinfo@phenomenex.com

Luxembourg
t: +31 (0)30-2418700
nlinfo@phenomenex.com

Mexico
t: 01-800-844-5226
tecnicomx@phenomenex.com

The Netherlands
t: +31 (0)30-2418700
nlinfo@phenomenex.com

New Zealand
t: +64 (0)9-4780951
nzinfo@phenomenex.com

Norway
t: +47 810 02 005
nordicinfo@phenomenex.com

Poland
t: +48 22 104 21 72
pl-info@phenomenex.com

Portugal
t: +351 221 450 488
ptinfo@phenomenex.com

Singapore
t: +65 6559 4364
sginfo@phenomenex.com

Slovakia
t: +420 272 017 077
sk-info@phenomenex.com

Spain
t: +34 91-413-8613
espinfo@phenomenex.com

Sweden
t: +46 (0)8 611 6950
nordicinfo@phenomenex.com

Switzerland
t: +41 (0)61 692 20 20
swissinfo@phenomenex.com

Taiwan
t: +886 (0) 0801-49-1246
twinfo@phenomenex.com

Thailand
t: +66 (0) 2 566 0287
thaiinfo@phenomenex.com

United Kingdom
t: +44 (0)1625-501367
ukinfo@phenomenex.com

USA
t: +1 (310) 212-0555
www.phenomenex.com/chat

All other countries/regions
Corporate Office USA
t: +1 (310) 212-0555
www.phenomenex.com/chat

www.phenomenex.com

Phenomenex products are available worldwide. For the distributor in your country/region, contact Phenomenex USA, International Department at international@phenomenex.com