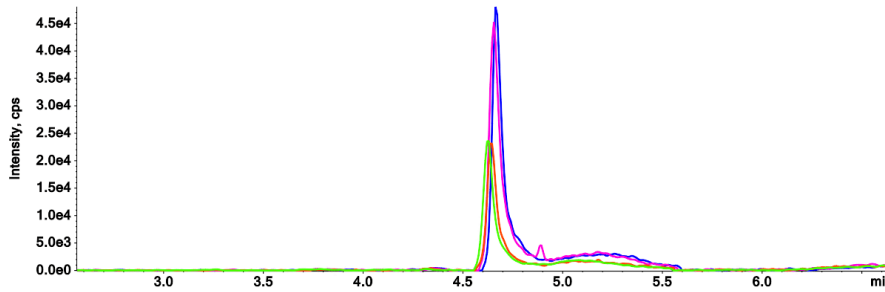


Spinraza on biozen Oligo : Serum load Optimization on micro-Clarity-OTX

Column: bioZen™ 2.6 µm Oligo, LC Column 50 x 2.1 mm, Ea
Dimensions: 50 x 2.1 mm ID
Order No: 00B-4790-AN
Elution Type: Gradient
Eluent A: Water + 15 mM N,N-diisopropylethylamine + 35 mM hexafluoroisopropanol
Eluent B: 90/10 methanol /water + 15 mM N,N-diisopropylethylamine + 35 mM hexafluoroisopropanol
Gradient Profile:

Step No.	Time (min)	Pct A	Pct B
1	0	80	20
2	5	60	40
3	5.5	10	90
4	7	10	90
5	7.5	80	20
6	10.5	80	20

Flow Rate: 0.25 µL/min
Col. Temp.: 70 °C
Detection: LC/MS/MS @ (400 °C)
Analyst Note: Clarity OTX 2mg micro elution plate



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ANALYTES:

- 1 Nusinersen
Retention Time: 4.5 min
- 2 Nusinersen
Retention Time: 4.5 min



Sample Preparation Details

for HPLC Application ID No.: 27195

Spinraza on biozen Oligo : Serum load Optimization on micro-Clarity-OTX

PRODUCT DESCRIPTION:

Clarity OTX, microelution 96-well plate, 2mg/well, Ea

Order No.: 8M-S103-4GA

SOLID PHASE EXTRACTION (SPE) PROCEDURE:

Note: The solvent volumes shown below are for a bed mass.

The solvent volumes will need to be adjusted for a smaller or larger bed mass.

Condition:

Load:

- 1.-Condition: 2x 200µl of methanol
- 2.-Equilibrate: 2x 200 µl of equilibration buffer
- 3.-Prep sample/Bind: in a 300 µl glass vial Lysis buffer + Oligo/Serum in a 1:1 Ratio. Up to 10 µL of Serum . Vortex and transfer to well.
- 4.-Wash/Bind: Add 100 µl of equilibration buffer to glass vial where sample was previously stored, transfer to well, use 2.5Hg to wash/bind.

Wash:

Dry:

Elute:

Final Prep and Analysis:

Prep sample right before adding to the well plate to avoid oligo loss to tube, if possible.

Inject: 20 µL on HPLC LC/MS/MS @ (400°C)

ANALYTES:	Spiked Conc. (ng/mL)	Log P	pKa	% Rec	%RSC (n=0)
1 Nusinersen	0				
2 Nusinersen	0				

Note: This method is designed as a convenient starting point for further investigation and can be tailored to meet your extraction goals.
Call your local Phenomenex Representative for assistance in method development and optimization techniques.

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For more information contact your Phenomenex Representative at support@phenomenex.com



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