

## 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

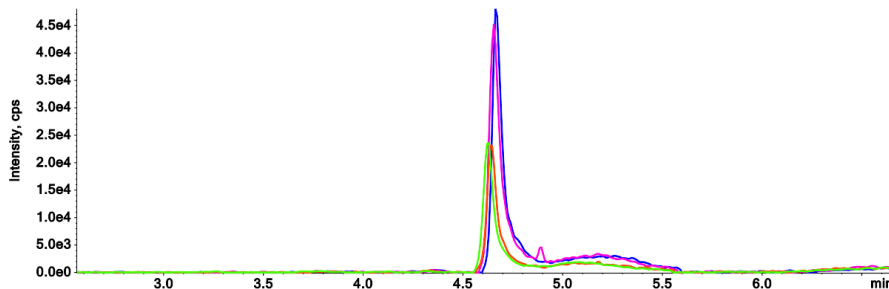
Gradient Profile:	Step No.	Time (min)	Pct A	Pct B
	1	0	80	20
	2	5	60	40
	3	5.5	10	90
	4	7	10	90
	5	7.5	80	20
	6	10.5	80	20

**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:

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### Load:

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- 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:

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### Dry:

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### Elute:

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### Final Prep and Analysis:

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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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