High-Resolution Orthogonal Techniques for Separating and Detecting Impurities in Antisense Oligonucleotide Therapeutics

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Background

Oligonucleotide drugs pose promising candidates for gene therapy against treatment resistant conditions and rare diseases.

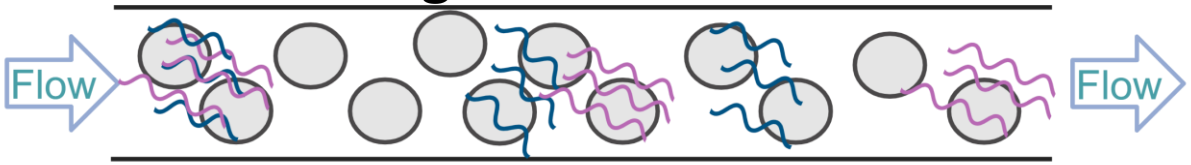
With any synthetic manufacture process, intrinsic impurities arise from the chemical synthesis.

A deeper understanding of RNA impurities must be gained to assess impurity impact prior to API singular use, bioconjugation or further incorporation within a drug delivery system.

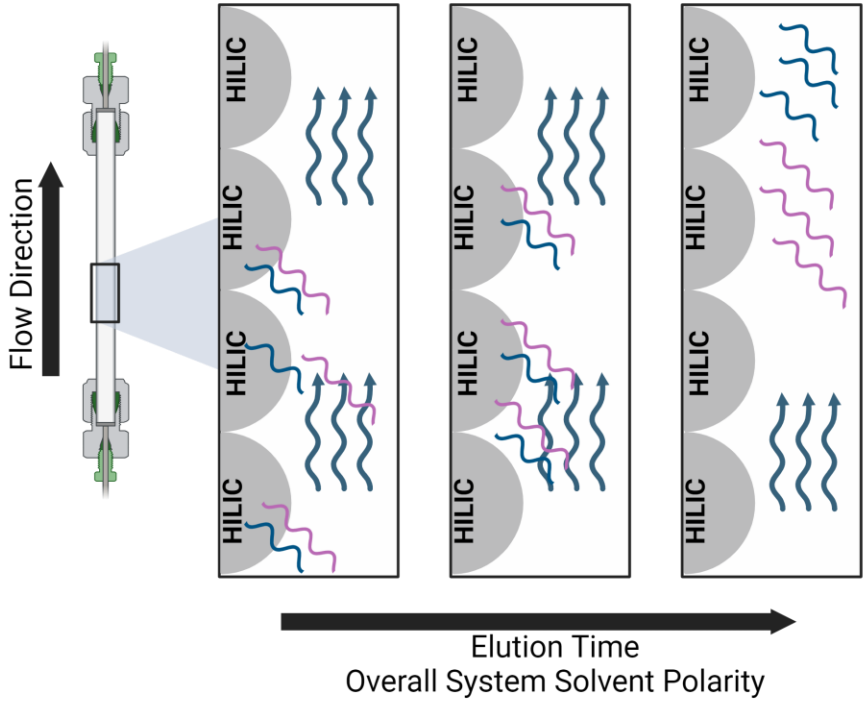
Background

Size Exclusion Chromatography

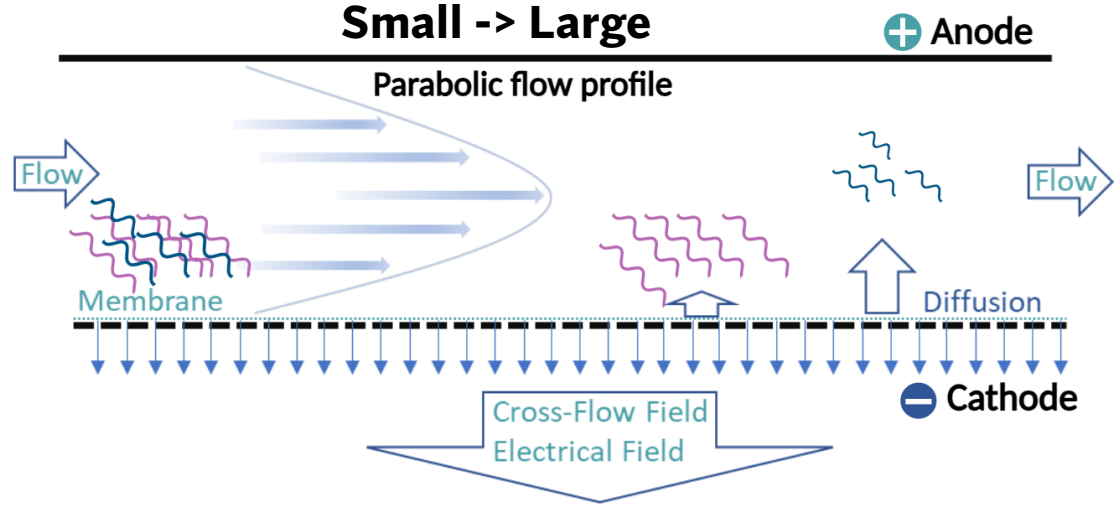
Large -> Small



Hydrophilic Interaction Chromatography



Small -> Large



Batch Mode
Inline Mode

Aims & Objectives

Aim

The aim of this study is to develop an orthogonal analytical pipeline for the analysis of manufacture associated impurities and RNA API critical quality attributes using Nusinersen.

Objectives

Develop and optimise hydrophilic interaction liquid chromatography.

Develop orthogonal analytical method pipeline to evaluate antisense oligonucleotide beyond the scope of routine techniques.

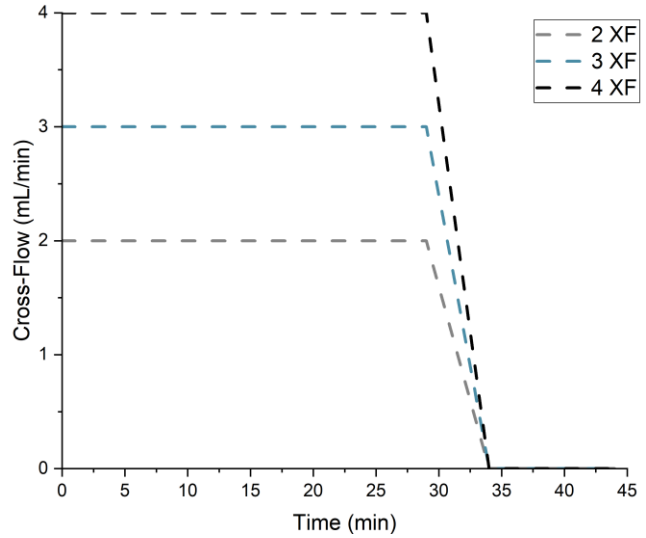
Compare results between orthogonal high resolution analytical techniques.

Methodology

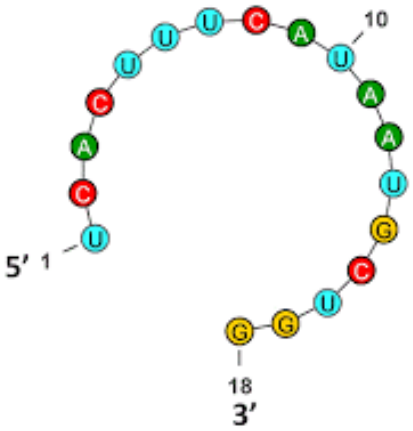
SEC Parameter	
Column (Temp)	Tosh mAb (25 °C)
Mobile Phase	PBS pH 7.4
Flow Rate	0.3 mL/min
Injection	0.50 mg/mL @ 2 µL
Inline Detectors	PDA

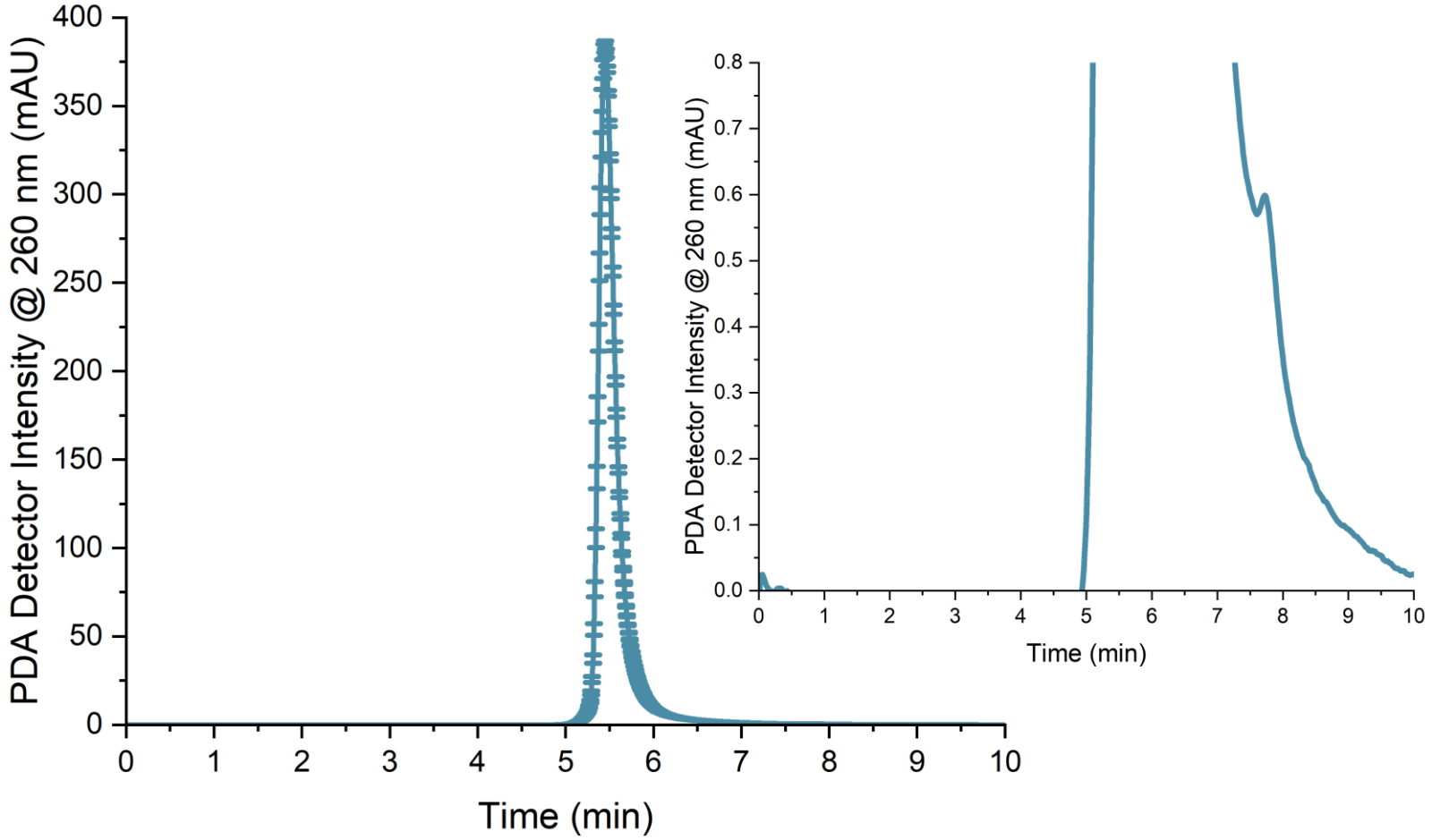
HILIC Parameter	
Column (Temp)	Waters BEH HILIC (40°C)
Mobile Phases (A/B)	ACN/Ammonium Formate pH 3
Flow Rate	0.4 mL/min
Injection	0.50 mg/mL @ 2 µL
Inline Detectors	PDA-MS
MS Range (m/z)	400-2000 m/z (+ve mode)

EAF4 Parameter	
Channel (Temp)	Conventional Electrical (25 °C)
Spacer	350 µm
Membrane	Regenerated Cellulose Amphiphilic (10 kDa)
Injection	0.05 mg/mL @ 20 µL
Eluent	0.5 mM phosphate pH 7.4
Cross-flow	2-4 mL/min
Detector Flow	0.5 mL/min
Inline Detectors	UV-MALS-DLS



Results – SEC

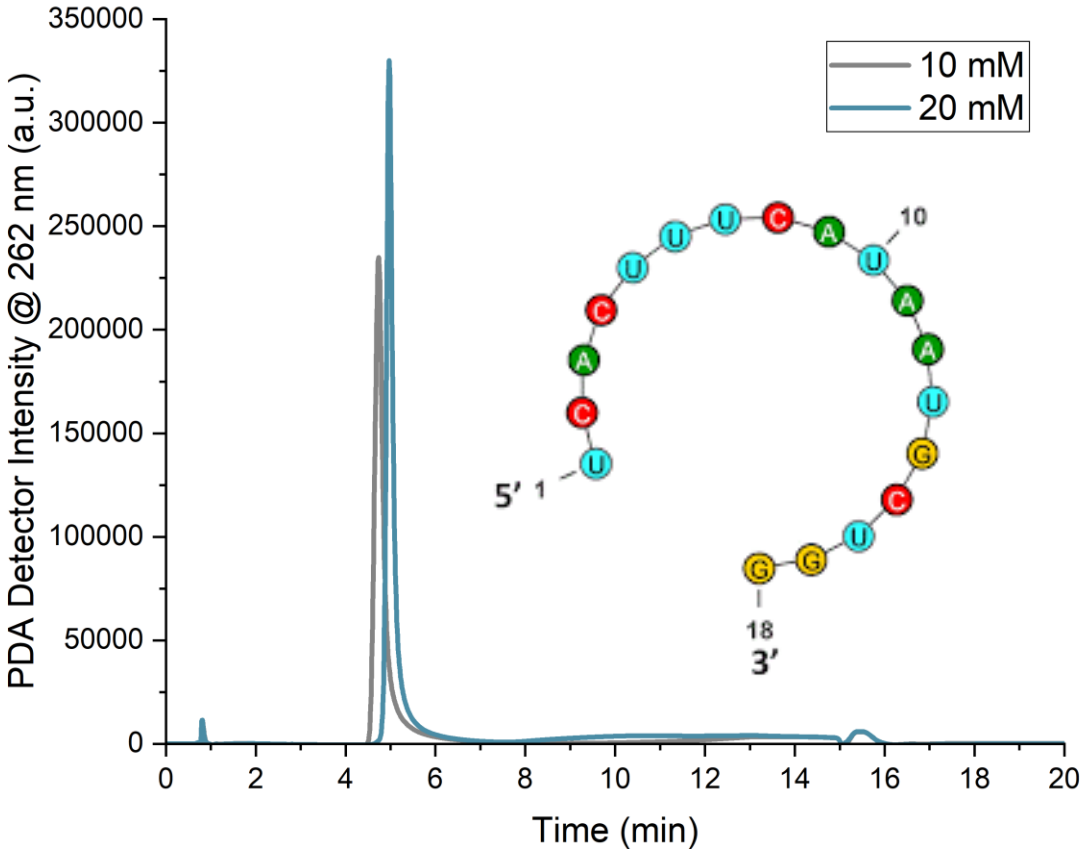




Main peak elution at 5.5 minutes, secondary shoulder peak ~ 8 minutes.

Main peak = 99.978%, shoulder = 0.022 % area.

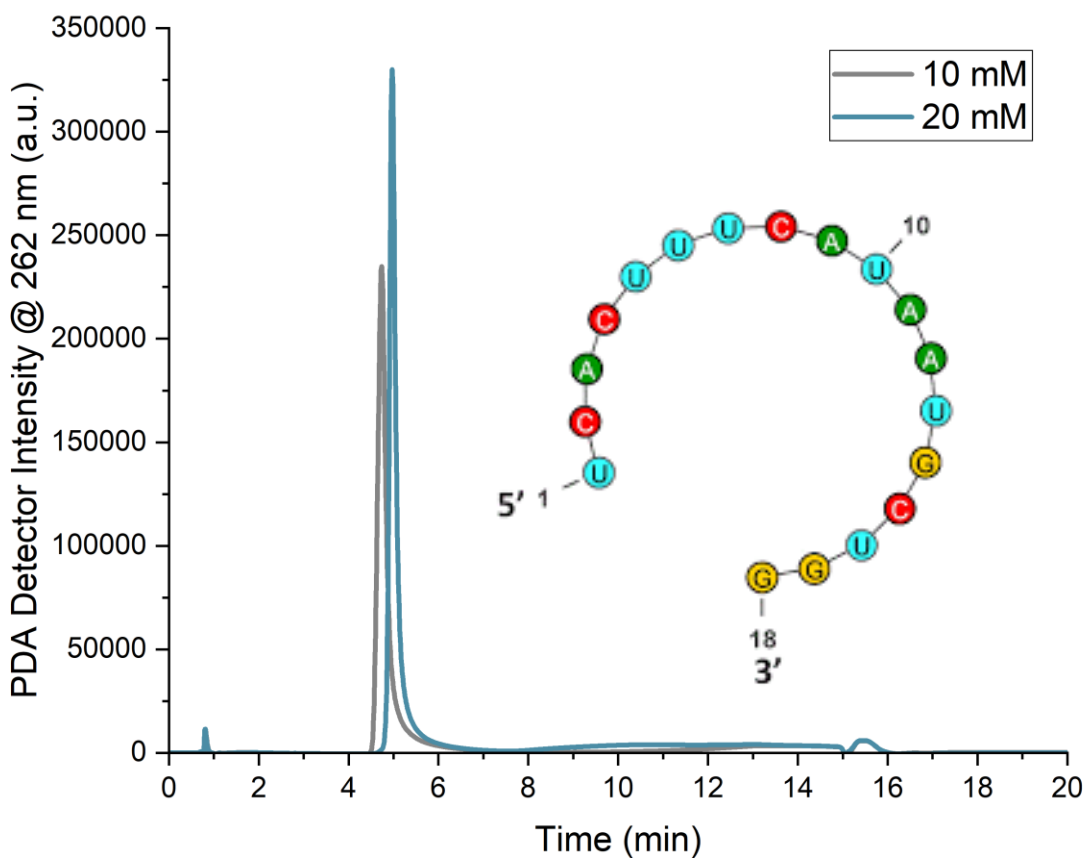
Results – HILIC

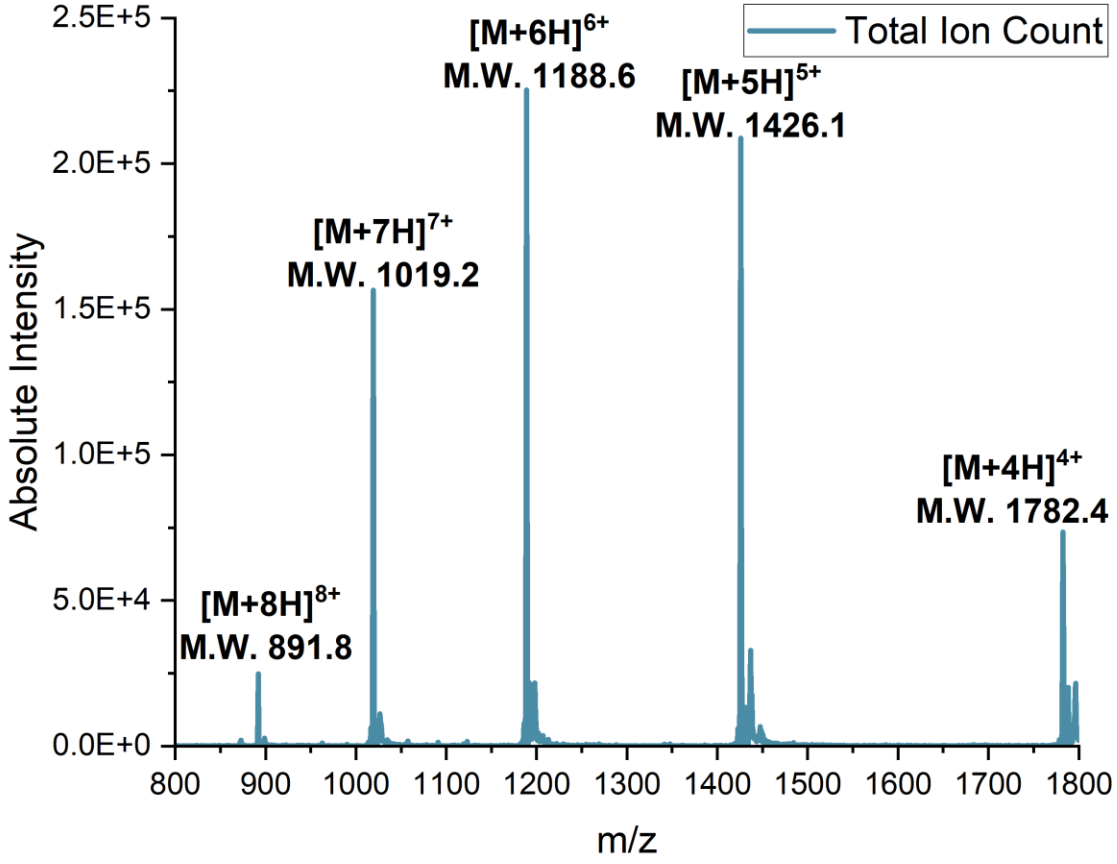


Maintained monodispersity highlighted through SEC to HILIC transition

Increased retention and increased PDA detector intensity (40 %) with increased salt concentration.

Results – HILIC

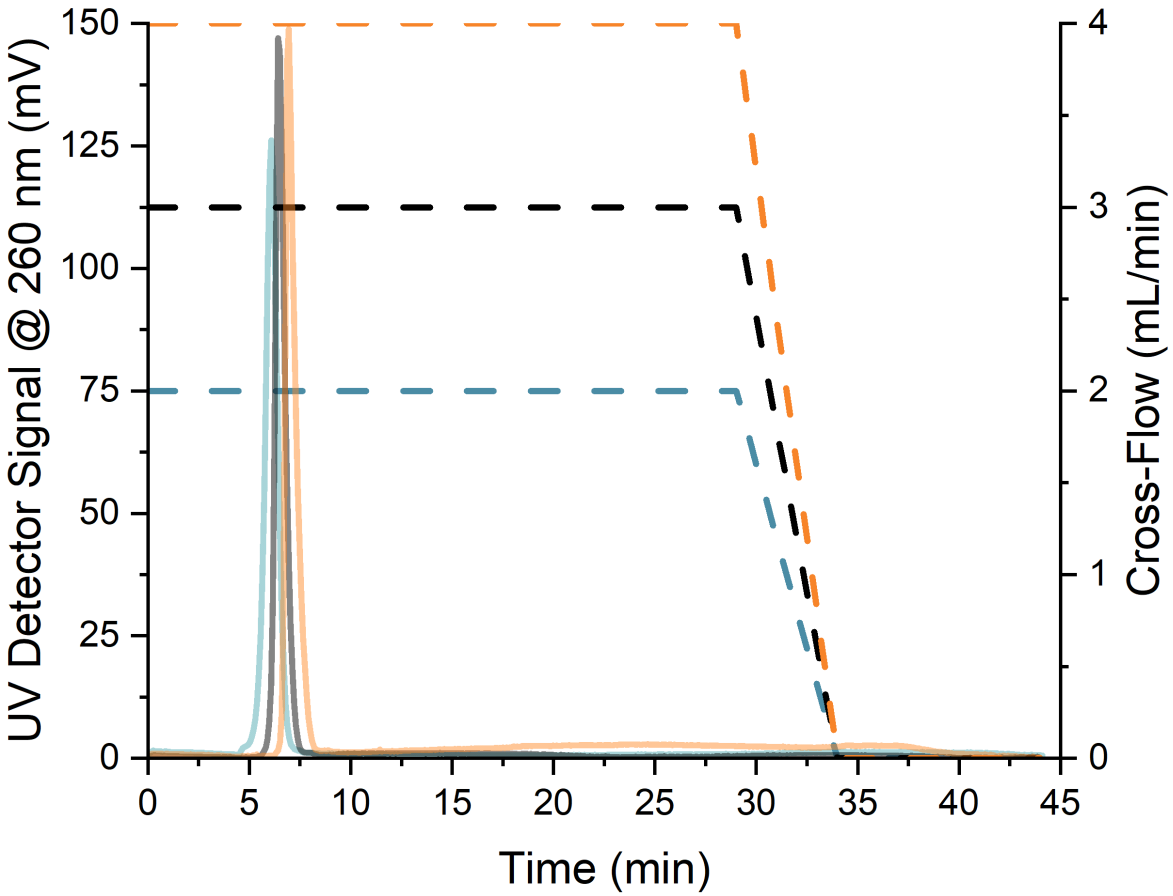




Increased retention and increased PDA detector intensity (40 %) with increased salt concentration.

Five protonated charge stated detected using MS with molar mass accuracy within 0.3 Da.

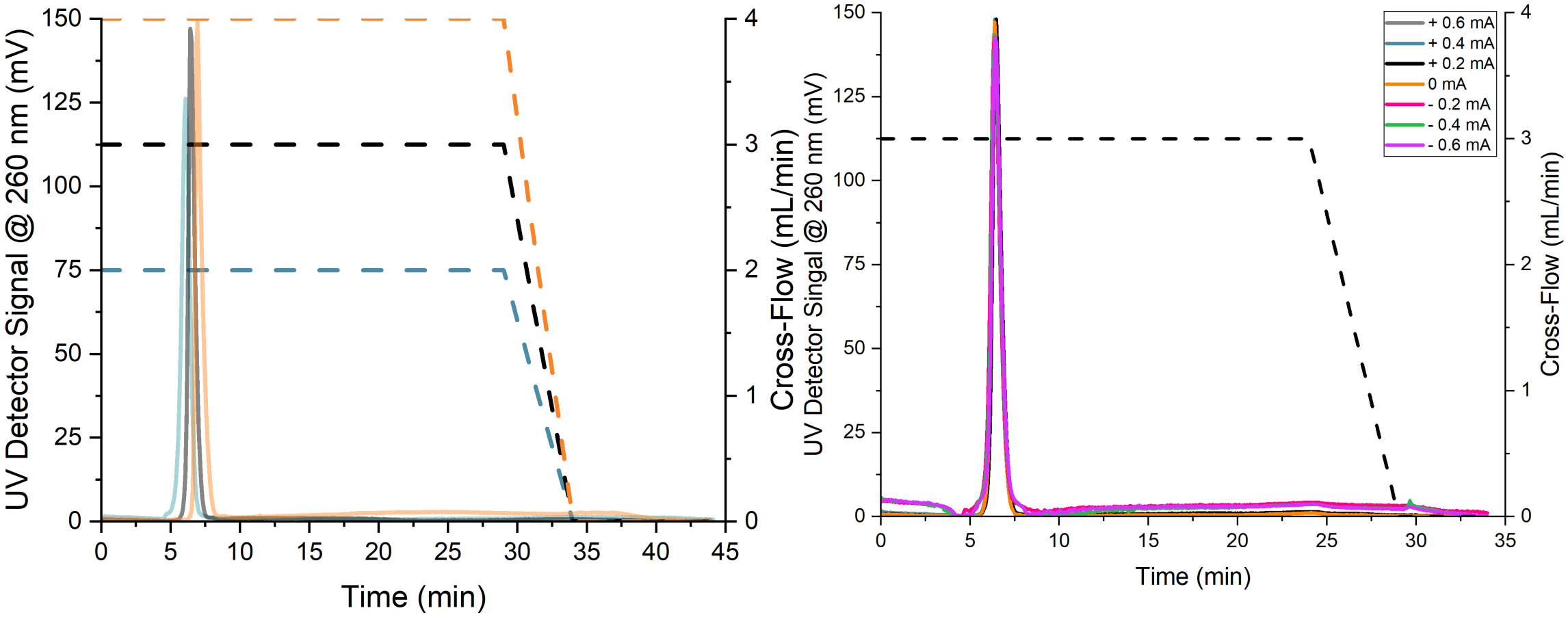
Results – AF4



Cross-flow dependant elution times, shift by 52 seconds increasing XF from 2-4 mL/min.

Applied currents within EAF4 did not change elution time, maintained separation based on size and not surface charge.

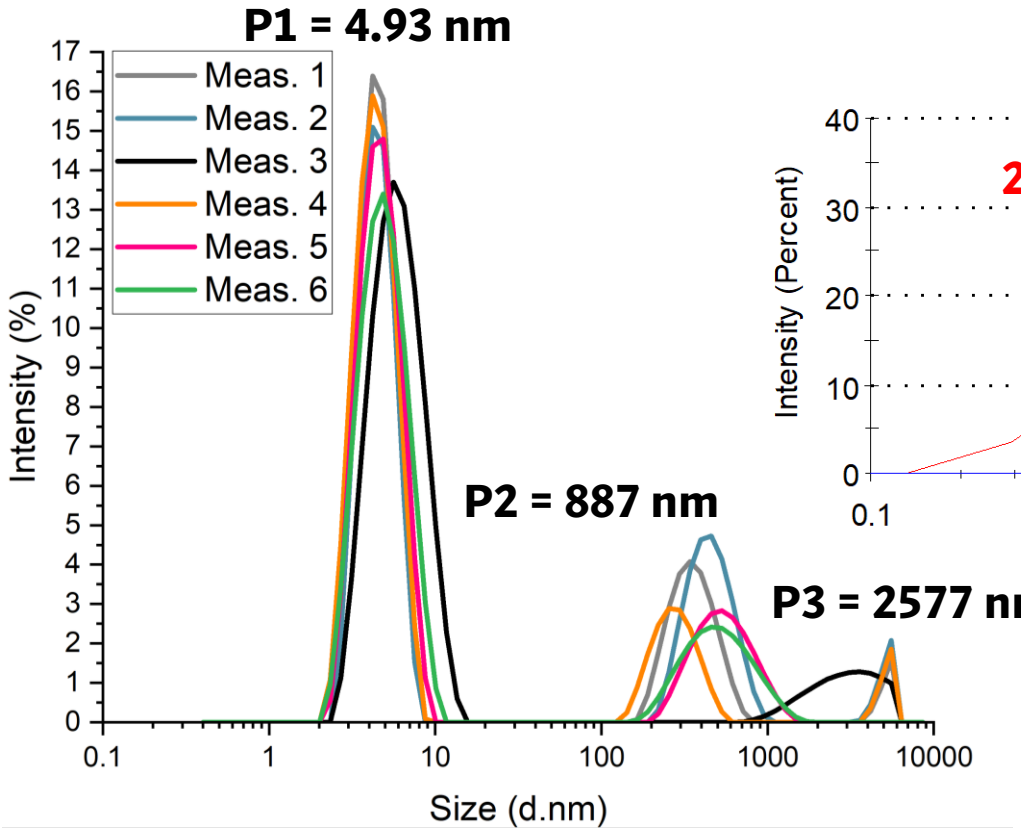
Results – EAF4

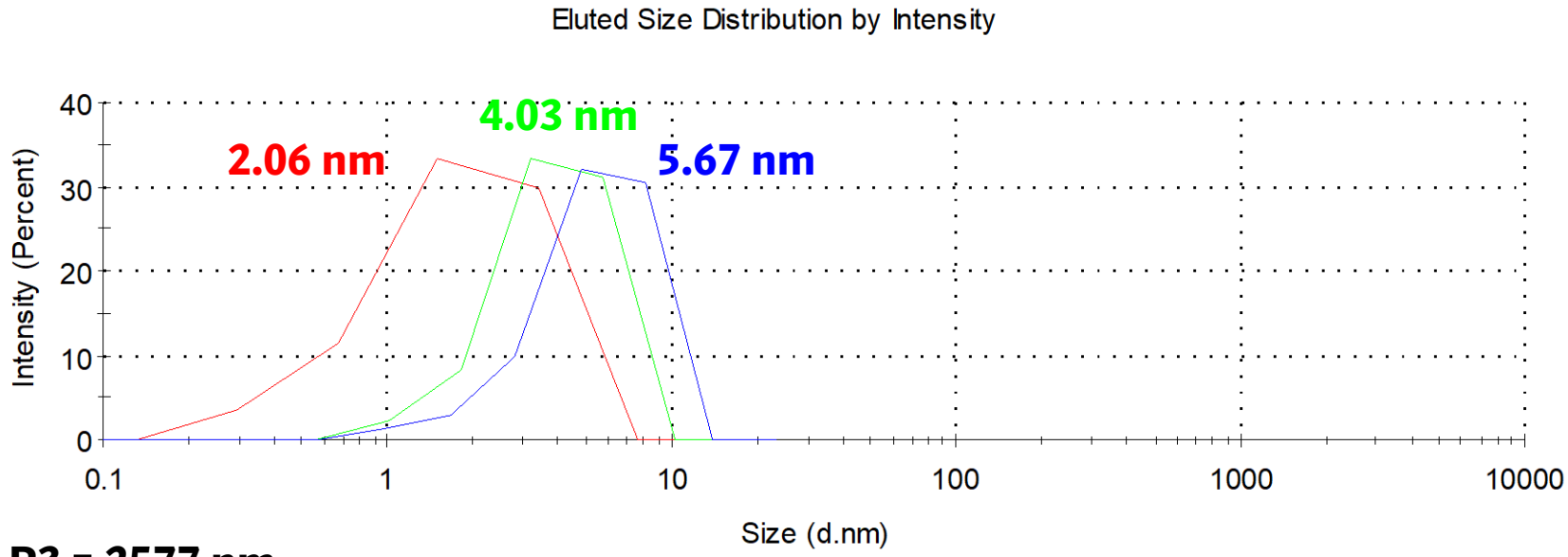


Cross-flow dependant elution times, shift by 52 seconds increasing XF from 2-4 mL/min.

Applied currents within EAF4 did not change elution time, maintained separation based on size and not surface charge.

Results – Light Scattering





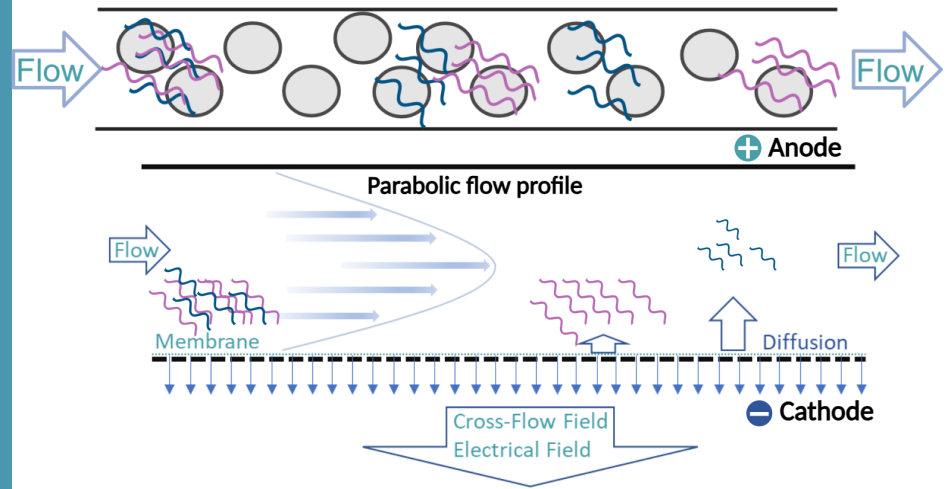
Light scattering is a function of Nusinersen concentration.

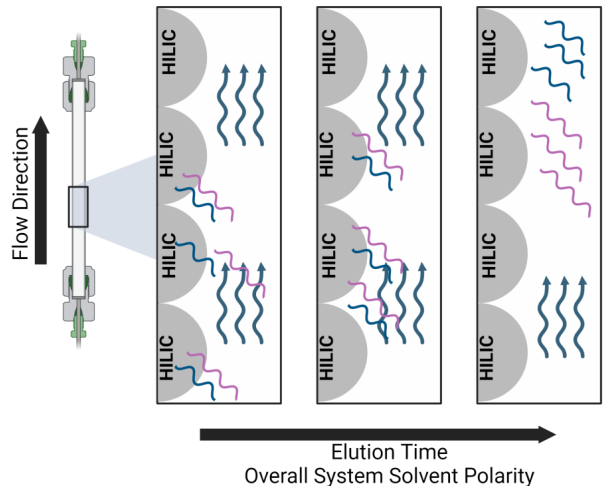
Batch mode producing variable results, whilst insufficient concentration for robust inline detection.

Results – Method Comparison

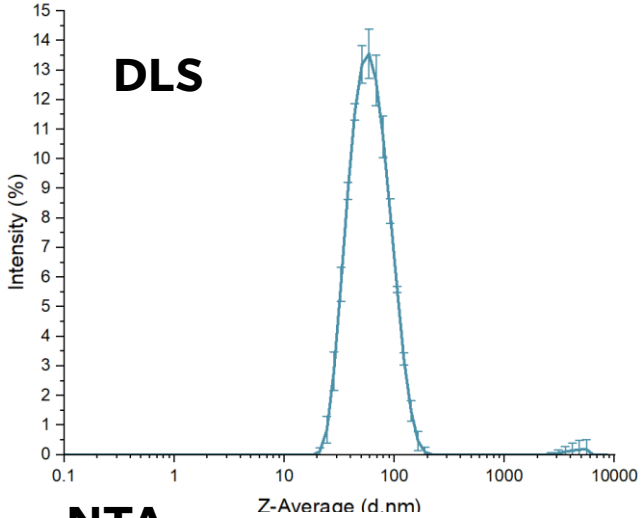
SEC, HILIC, LS, EAF4

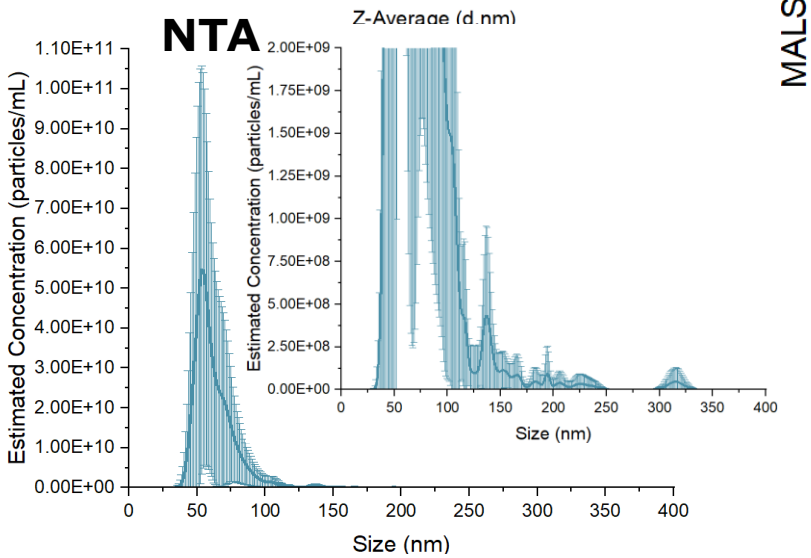
	SEC-PDA	HILIC-PDA-MS	Light Scattering	EAF4-UV
Dimensionality	3D	3D	3D	2D
Mode of Separation	Size	Polarity	Batch: none Inline: hyphenation	Size/Surface Charge
System pH	pH 7.4	pH 3.0	pH 7.4	pH 7.4
Detection	PDA	PDA-MS	LS	UV-MALS-DLS
Limitations	Resolution	Fragmentation	Low Scattering	High conc

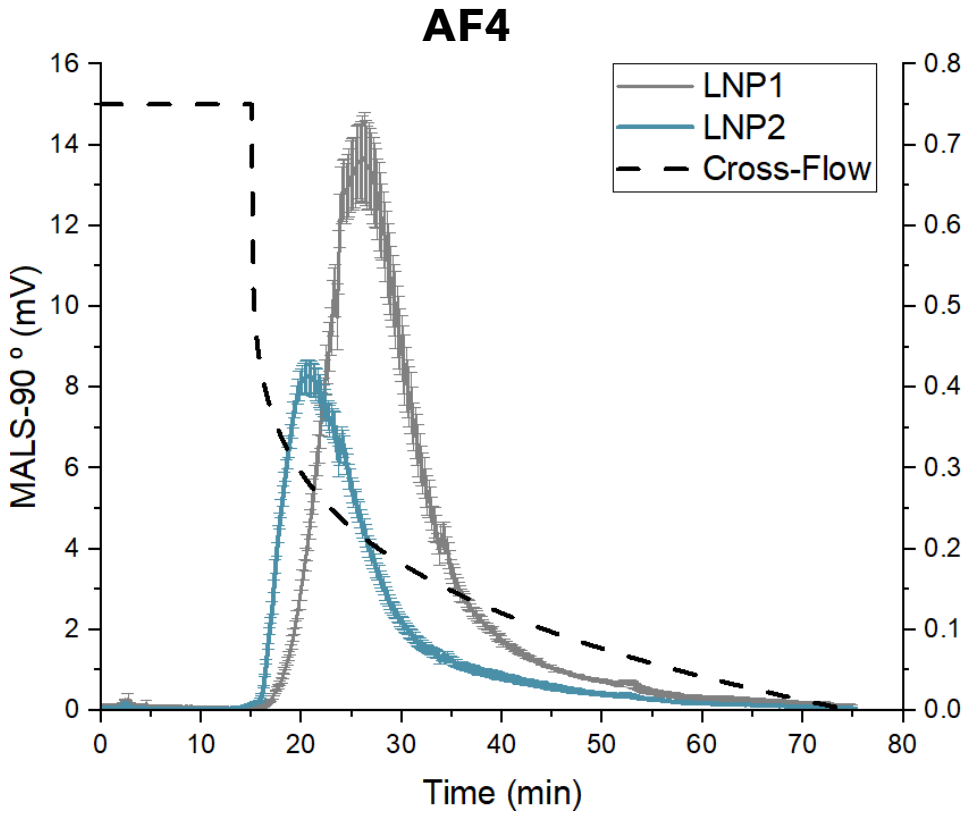


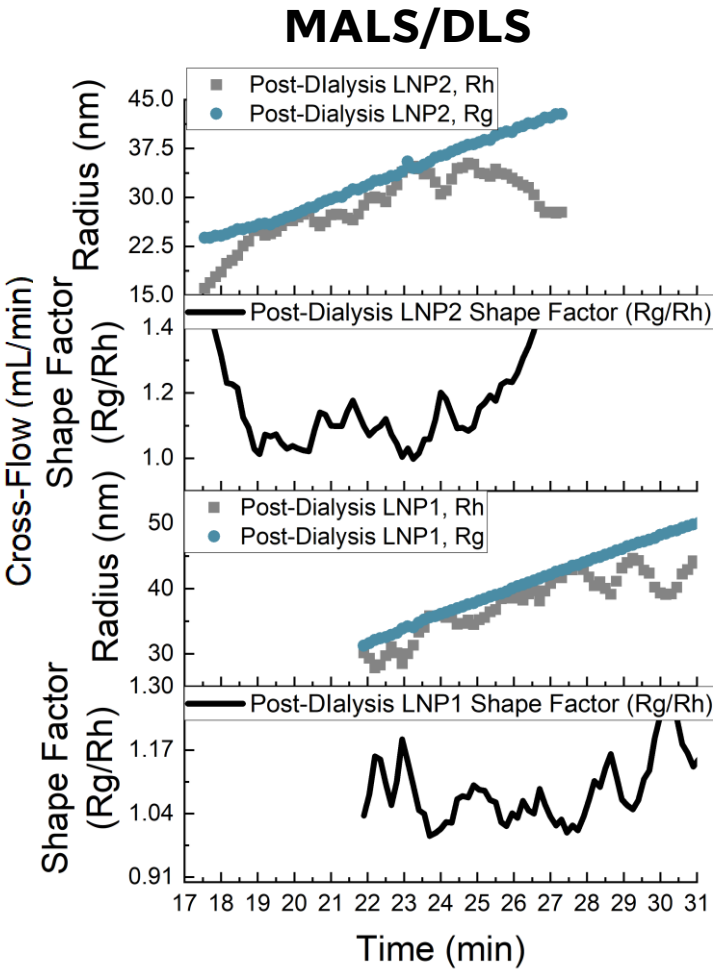


Results – RNA-LNP Model









RNA cargo further impacts drug delivery platform critical quality attributes.

Subpopulations formed due to heterogenous RNA cargo distribution

Conclusions

Aim

The aim of this study is to develop an orthogonal analytical pipeline for the analysis of manufacture associated impurities and RNA API critical quality attributes using Nusinersen.

Highlighted a range of orthogonal and complementary techniques for ASO quality evaluation.

Further optimisation required of methods to enhance separation and ASO quantification.

On-going work investigation the impact of buffer selection on different brand manufactured RNA using orthogonal and complementary framework.

Future work will include spiked impurity LOD and LLOQ, nuclease cleavage and conformation analysis.

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