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Sepax HPLC Columns for mRNA

Integrity of mRNA using SEC HPLC and/or RP HPLC

Size Exclusion Chromatography SEC has been utilized for its quick and reliable method in many applications such as purification or aggregate quantification. This SEC method allows a quick and reliable to quantify the percentage of aggregates of mRNA sample. Further characterization of each SEC peak can be performed using RP HPLC as an orthogonal method.

SEC HPLC

Column: SRT SEC-1000, 5 μ , 1000 \AA , 7.8x300mm (USP L33, L59)

HPLC Systems: Agilent Infinity II 1260

Mobile phase: 150mM Phosphate buffer, pH 7.0

Flow rate: 1.0mL/min

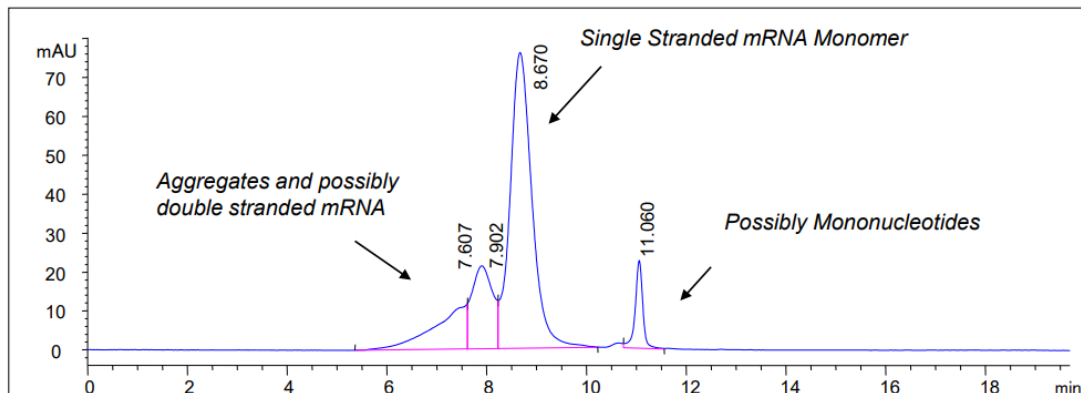
Detector: UV 260nm

Column Temperature: 25 $^{\circ}$ C

Sample: mRNA-1 (single-stranded, 1000 nucleotides, 300-600kDa) (0.5mg/mL)

Injection Volume: 5 μ L

Data Analysis: Agilent Chemstation



RT [min]	Height	Area	Area%	Plates	Tailing	Resolution
7.607	11	517.2	13.952	832	0.50	
7.902	21	623.2	16.812		-1.74	
8.670	76	2329.6	62.847	2055	1.23	
11.060	23	236.9	6.390	31503	0.78	4.71



RP HPLC

Column: Proteomix RP-1000, 5 μ , 1000 \AA , 2.1x100mm (USP L##)

HPLC Systems: Agilent Infinity II 1260

Mobile phase: A: 100mM TEAA, B: 100mM TEAA/ 25% ACN

Flow rate: 0.3mL/min

Detector: UV 260nm

Column Temperature: 50 $^{\circ}$ C

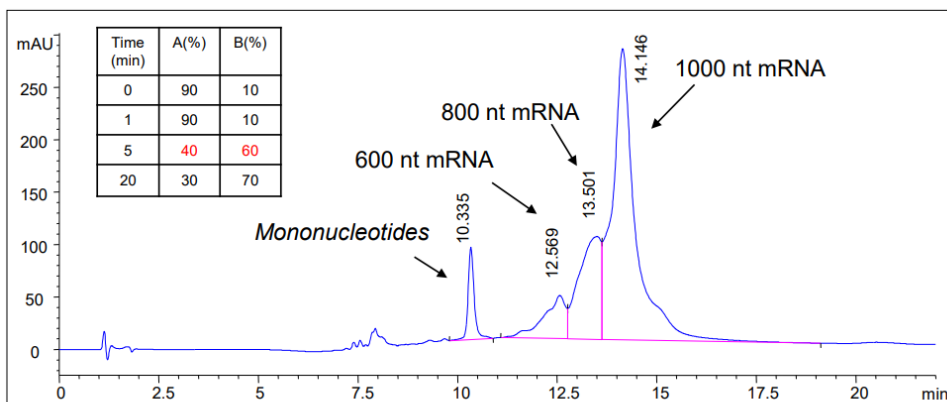
Sample: mRNA-2 (single-stranded, 1000 nucleotides, 300-600kDa)

Injection Volume: 10 μ L

Data Analysis: Agilent Chemstation

Gradient:

Time (min)	%A	%B
0	90	10
1	90	10
5	40	60
20	30	70



Compound Name	RT [min]	Height	Area	Area%	Plates	Tailing	Resolution
	10.335	88.1	940.2	5.32	25906	1.30	
	12.569	41.4	1590.2	9.00	2275	0.58	3.40
	13.501	98.2	3762.6	21.30	2173	-0.68	0.84
	14.146	278.1	11371.9	64.38	4812	1.70	0.65



Characterization of mRNA-LNP using SEC HPLC

SEC-HPLC can be used to rule out if tertiary mRNA structures (aggregates formation) causing any extra peak in RP-HPLC after mRNA-LNP preparation.

SEC HPLC

Column: Zenix SEC-300, 3 μ m, 300 \AA , 4.6x150mm (USP L33, L59)

HPLC Systems: Waters H-Class UPLC

Mobile phase: 100mM Tris acetate/ 2.5mM EDTA pH 8

Flow rate: 0.25mL/min

Detector: UV 260nm

Sample: e

Column Temperature: 25 $^{\circ}$ C

