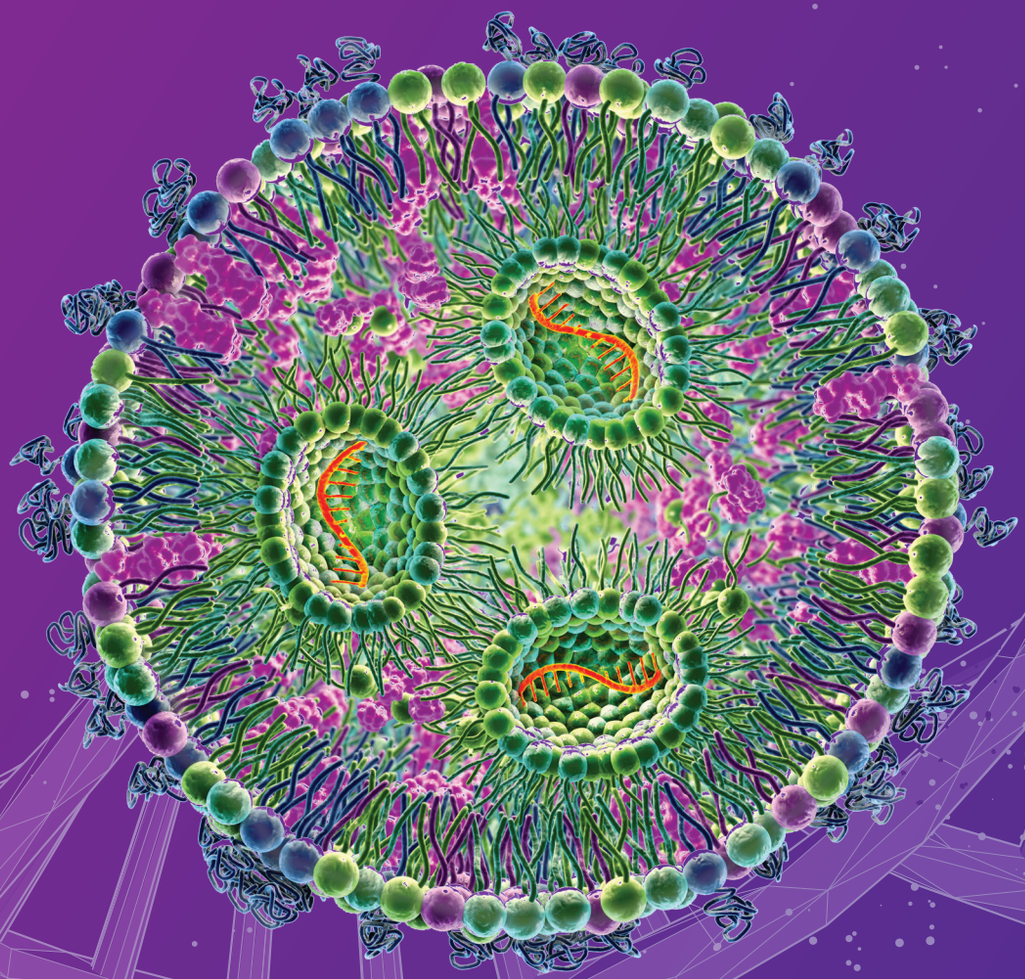
CONSUMABLES GUIDE BOOK

Characterizing LNP mRNA

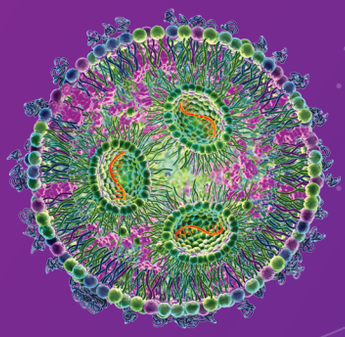
LC tools for purity, identity, integrity and concentration determining measurements



Waters™

A Changing Industry

mRNA based medicine helped address the COVID-19 pandemic. The viability of using an mRNA construct as a human drug substance has been demonstrated. When encapsulated into a lipid nanoparticle, mRNA can be efficiently delivered to patient cells to be expressed into vaccine antigens, enzyme replacements to bolster a patient's own gene expression, or someday a gene editing apparatus to alter a patient's somatic genome. The FDA approved mRNA vaccines have provided a successful formula for creating potent LNPs. With today's techniques, four types of lipids encase the mRNA. A synthetic ionizable lipid is combined with a zwitterionic phosphatidylcholine lipid, cholesterol and a PEGylated lipid to yield 700-800Å diameter nanoparticles. mRNA payloads are incorporated that range from 1,000 to up 10,000 nucleotides in length. Given their complexity, these LNP RNA drug products must be comprehensively characterized and tested. Chromatography can expedite the needed assays and thereby confirm physicochemical properties of the intact particle, check their compositions and raw materials for impurities, and confirm the molecular integrity and modifications of the mRNA drug substance.



Structure of lipid Nanoparticle

Molecular composition of an mRNA

Ionizable Lipid | *Cholesterol* | *Zwitterionic Lipid* | *PEG Lipid*

Chromatography and Method Options

Size Exclusion	**Anion Exchange**	**Poly A Tail LC**
Oligonucleotide Mapping	**5′ Cap Analysis**	**Lipid Testing**

Waters Peer Reviewed Articles

Waters scientists and collaborators are publishing on this subject. Make sure to frequent the Resource Tab on our **www.waters.com/GTx** website to keep up to date on the literature.

SEC to Measure Integrity of mRNA Drug Substance

Size Exclusion Chromatography is a widely employed separation technique for isolating species based upon differences in hydrodynamic radius. SEC of larger nucleic acids requires novel method development to test and report on critical quality attributes (CQA) as they relate to the safety and efficacy of the drug. An XBridge Premier GTx BEH SEC 450Å 2.5 µm column provides high resolution separations of small to medium sized nucleic acids species in various mobile phase conditions. New SEC separation capabilities with even wider pore diameter materials is underway for intact LNP and larger nucleic acid analyses.

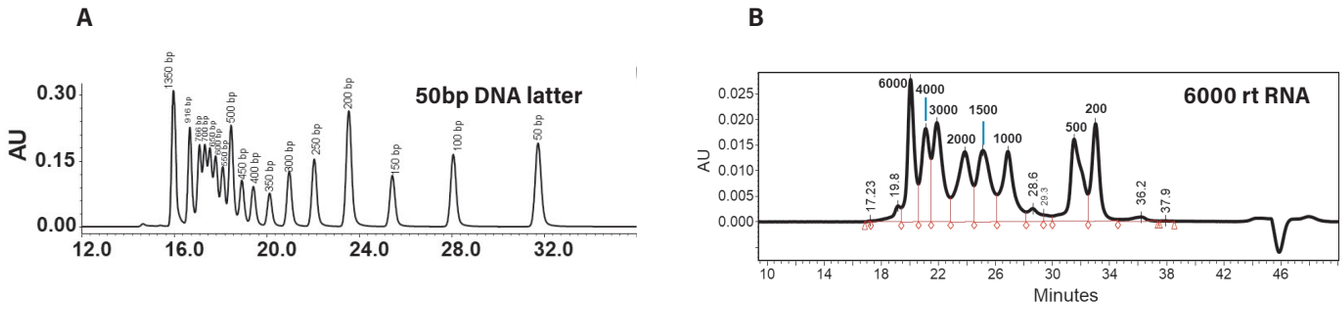


Figure 1. Components of 50 bp DNA ladder (A) and 6000 nt RNA ladder (B) were resolved using a Waters XBridge Premier GTx BEH 450Å SEC column as reported in Waters Application Note: 720008061.

AEX for Purity and Concentration Determining Measurements

Analytical anion exchange (AEX) analyses have proven to be a viable solution for heterogeneity assessment of negatively charged species including nucleic acids. Existing AEX columns for nucleic acid analysis are reported to have challenges in terms of low efficiency and recovery which has limited the development of robust and reliable AEX methods for CQA analysis. Protein-Pak HiRes Q and Gen-Pak FAX columns provide a set of strong and weak anion exchanger columns for the empirical optimization of new anion exchange techniques. Gen-Pak FAX columns have recently been used to provide higher recovery mRNA analyses as compared to an industry reference monolithic column.

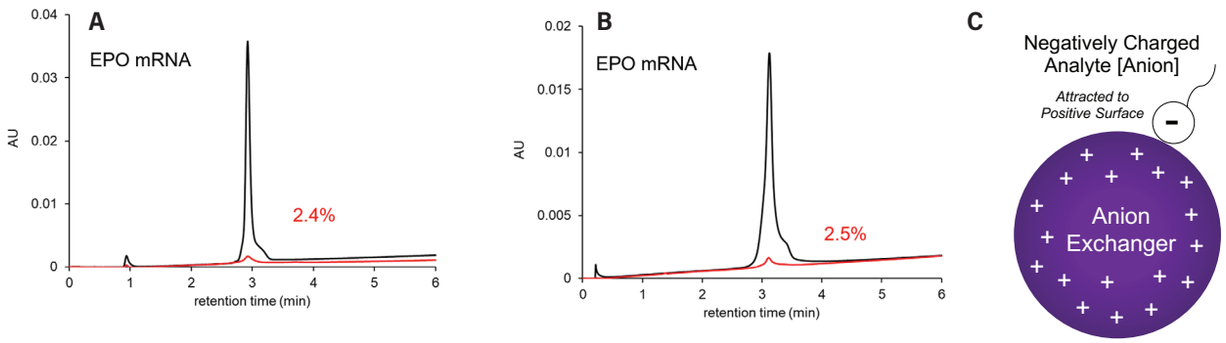


Figure 2. AEX of EPO mRNA using a Gen-Pak FAX (A) versus an industry monolithic column (B) utilizing a novel approach as reported in J Chrom Open, 2022, 2, 100031 (C) Schematic representation of an anion exchange stationary phase.

5′ Cap Analysis and Oligo Mapping Analysis

An mRNA's sequence and its modifications need to be confirmed to ensure the mRNA will reach its target efficacy. The 5′ cap can be characterized after sample prep with DNA probes and RNase H which is specific to DNA/RNA duplexes. Further sequence confirmation can be obtained with mRNA digestion leveraging residue specific endonucleases. Oligo batch tested BEH C_{18} sorbents provide rugged columns for each of the downstream separations. MaxPeak High Performance Surfaces ensure quick method starts by eliminating conditioning effects and improving analyte recovery.

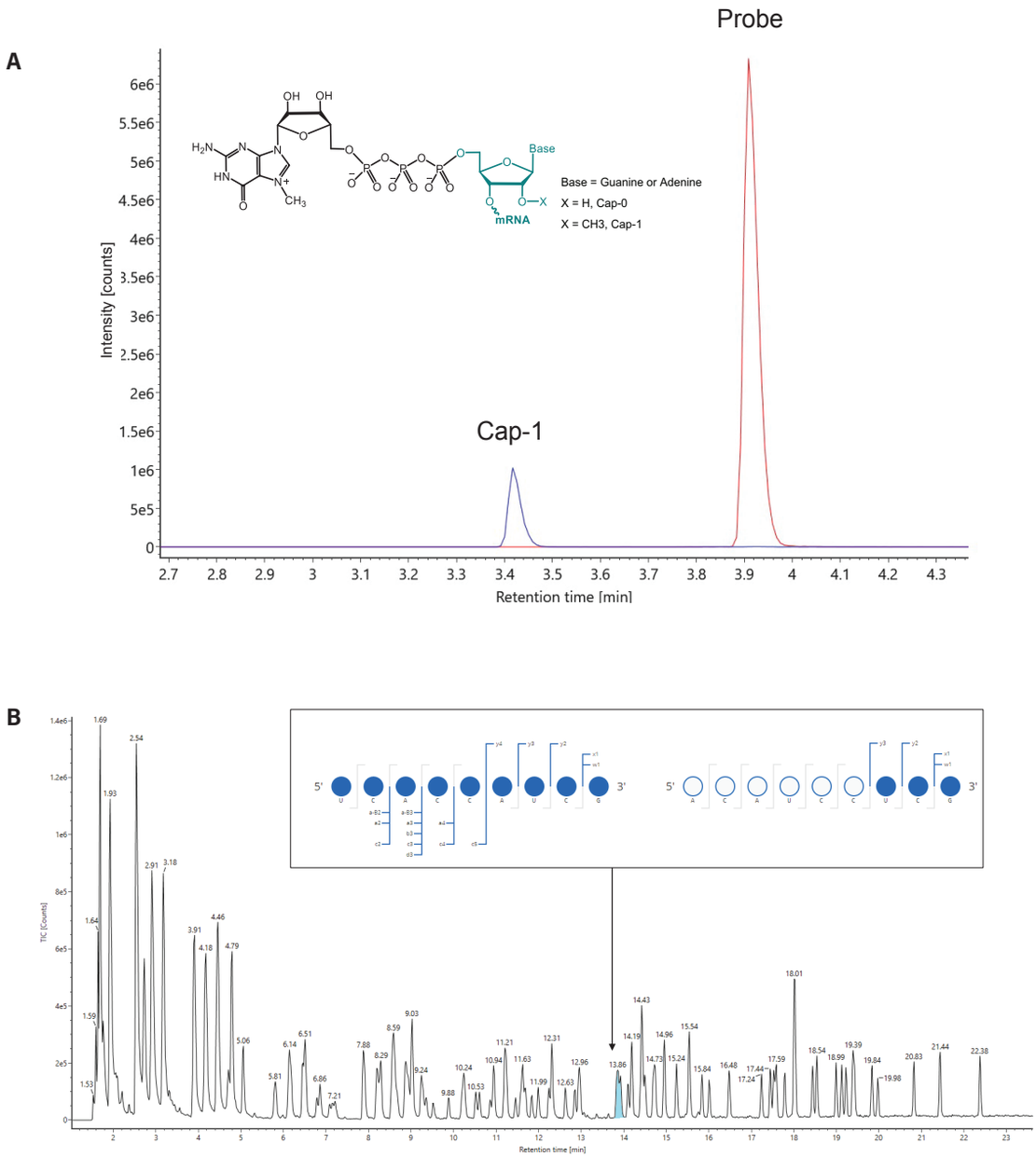


Figure 3. 5′ cap digestion product from RNase H cleavage (A) and and RNase T1 oligonucleotide map of a tail-less luciferase mRNA (B) as published in Waters Application Notes: 720007329 and 720007669.

Poly A Tail Analysis by RPLC

The length and structure of a 3′ poly A tail must be optimized to confer desired half life and ribosome binding affinity properties to an mRNA. RNase cleavage can be applied to digest an mRNA down to its poly A motif. An oligonucleotide batch tested widepore BEH 300Å C_{18} column provides a high resolution separation of the liberated poly A tail, and the use of strong ion pairing agents makes it possible to achieve single residue resolution.

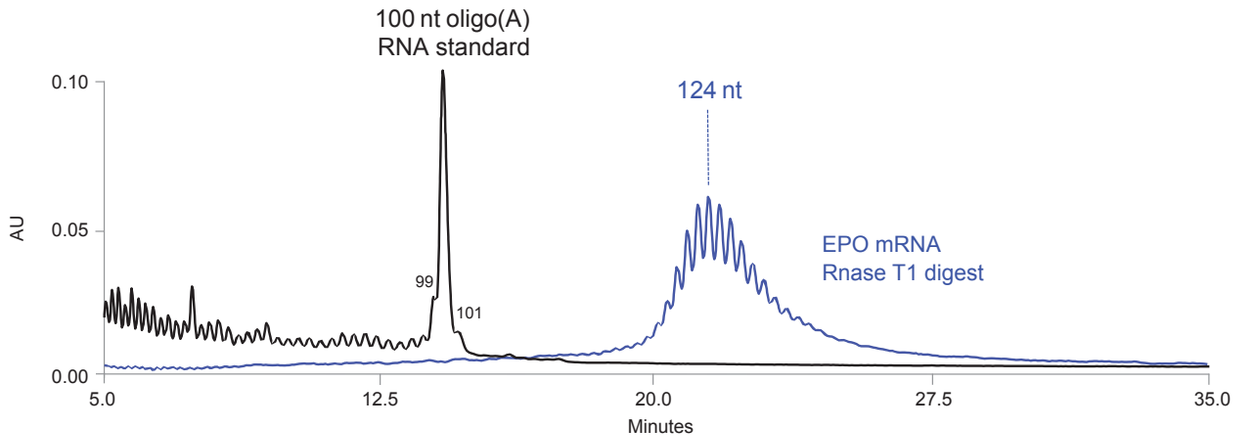


Figure 4. IP-RPLC chromatogram of a T1 digested mRNA sample and its constituent poly A tail as obtained with an ACQUITY Premier Oligonculeotide BEH 300Å 1.7 µm column (Waters Application Note: 720007873).

Lipid Raw Material and LNP Composition Analysis

Today's vaccines are prepared with a special, 4-component lipid formulation. An important critical quality attribute of an LNP is the relative abundance of each one of these species and related impurities of the raw materials such as oxidation and reaction related byproducts. A Charge Surface Hybrid (CSH) Phenyl Hexyl particle provides a unique repulsive effect against the ionizable cationic lipid found in these formulations and is recommended for these lipid separations because of the benefit it provides for peak shape.

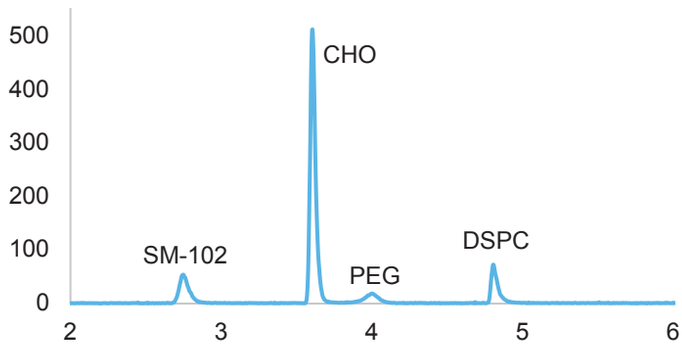


Figure 5. Evaporative light scattering chromatogram of lipids separated with an ACQUITY Premier CSH Phenyl Hexyl Column (Waters Application Note: 720007740).

Ordering Information

The columns, standards and reagents that can help you characterize LNP mRNA are provided below.

SEC

XBridge™ Premier GTx BEH SEC 2.5 µm 450Å Columns

	Dimension	150 mm	300 mm	Guard
Standard Column	4.6 mm	186010584	186010585	186010583
	7.8 mm	186010586	186010587	186010583

AEX

Anion Exchange Columns

	Dimension	P/N
Protein-Pak HiRes Q Column	4.6 x 100 mm	186004931
Gen-Pak FAX Column	4.6 x 100 mm	WAT015490

STANDARDS AND REAGENTS

Standards and Reagents

	P/N
dsDNA 50 to 1350 Ladder	186010778
ssDNA 10 to 60 Ladder	186009449
ssDNA 20 to 100 Ladder	186009448
ssDNA 20-mer LC-MS Standard	186009451

OLIGO RPLC

ACQUITY Premier Oligonucleotide BEH C_{18} 1.7 µm Columns

	Diameter	50 mm	100 mm	150 mm	50 mm	100 mm	150 mm
			130Å			300Å	
Standard Column	2.1 mm	186009484	186009485	186009486	186010539	186010540	186010541
VanGuard FIT Column	2.1 mm	186010685	186010686	186010687	186010754	186010755	186010756

OLIGO RPLC

XBridge Premier Oligonucleotide BEH C_{18} 2.5 µm Columns

	Diameter	50 mm	100 mm 130Å	150 mm	50 mm	100 mm 300Å	150 mm
Standard Column	2.1 mm	186009836	186009837	186009838	186010542	186010543	186010544
	4.6 mm	186009901	186009902	186009903	186010545	186010546	186010547
VanGuard FIT Column	2.1 mm	186010688	186010689	186010690	186010757	186010758	186010759
	4.6 mm	186010691	186010692	186010693	186010760	186010761	186010762

LIPID RPLC

ACQUITY Premier CSH Phenyl Hexyl 1.7 µm Columns

	Diameter	50 mm	100 mm 130Å	150 mm
Standard Column	2.1 mm	186009474	186009475	186009476
VanGuard FIT Column	2.1 mm	186009477	186009478	186009479

LIPID RPLC

XSelect Premier CSH Phenyl Hexyl 2.5 µm Columns

	Diameter	50 mm	100 mm 130Å	150 mm
Standard Column	2.1 mm	186009879	186009880	186009881
	4.6 mm	186009886	186009887	186009888
VanGuard FIT Column	2.1 mm	186009882	186009883	186009884
	4.6 mm	186009889	186009890	186009891

waters.com/GTx

Waters™

Waters Corporation
34 Maple Street
Milford, MA 01757 U.S.A.
T: 1 508 478 2000
F: 1 508 872 1990
waters.com