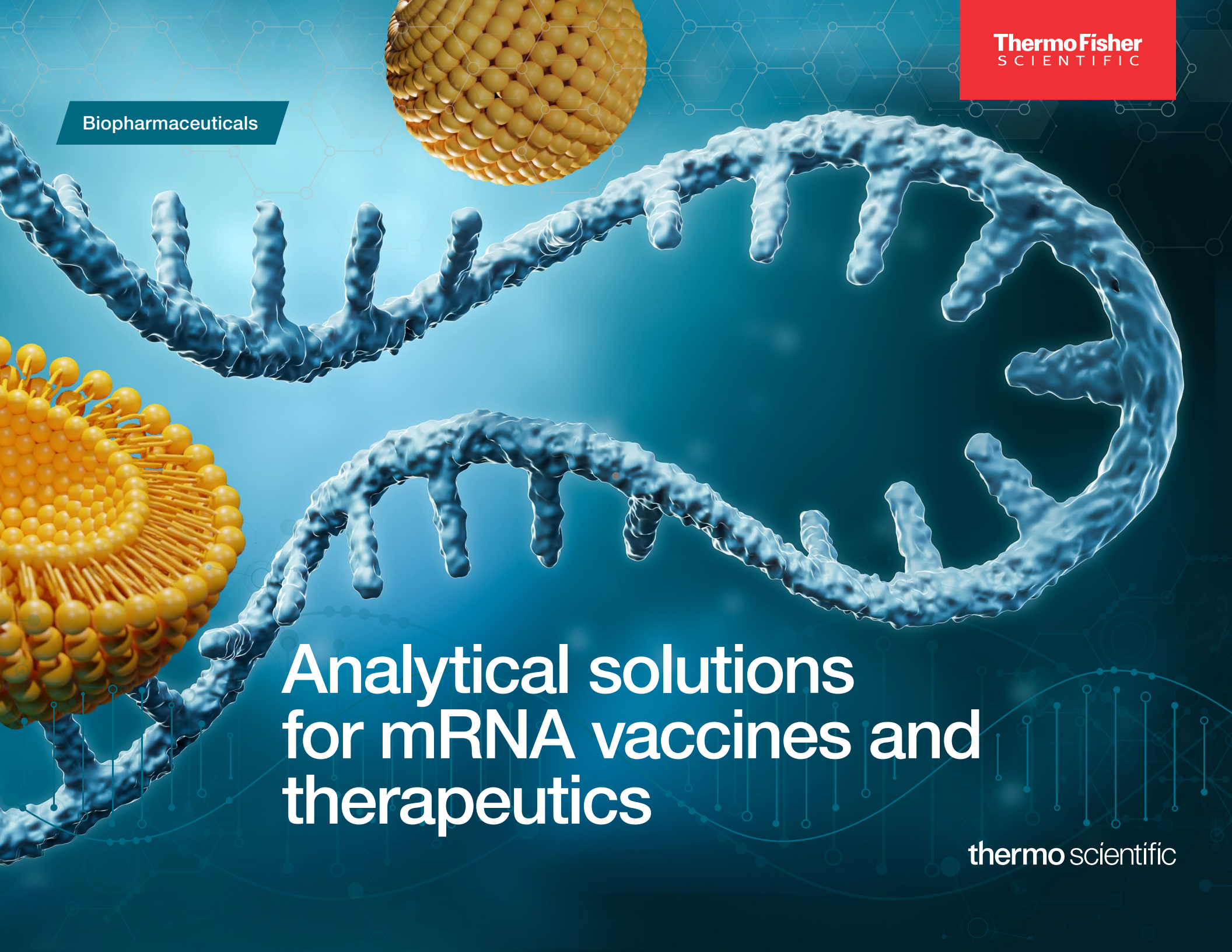
Thermo Fisher SCIENTIFIC

Biopharmaceuticals



Analytical solutions for mRNA vaccines and therapeutics

thermo scientific

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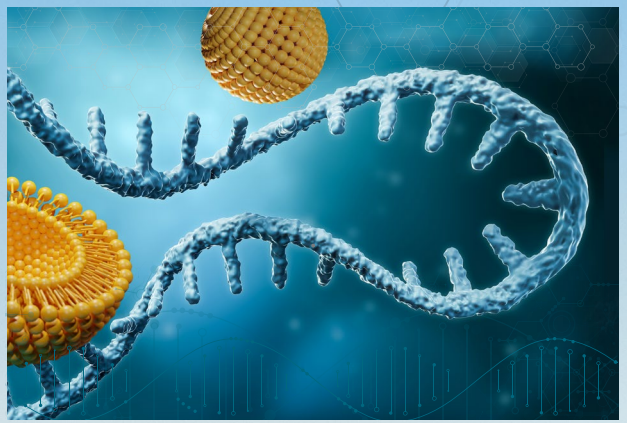

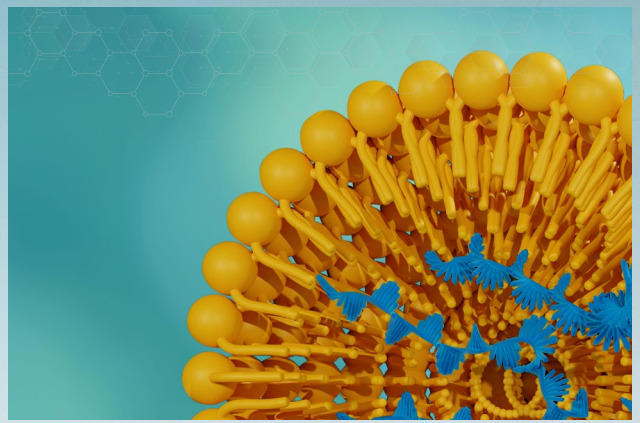

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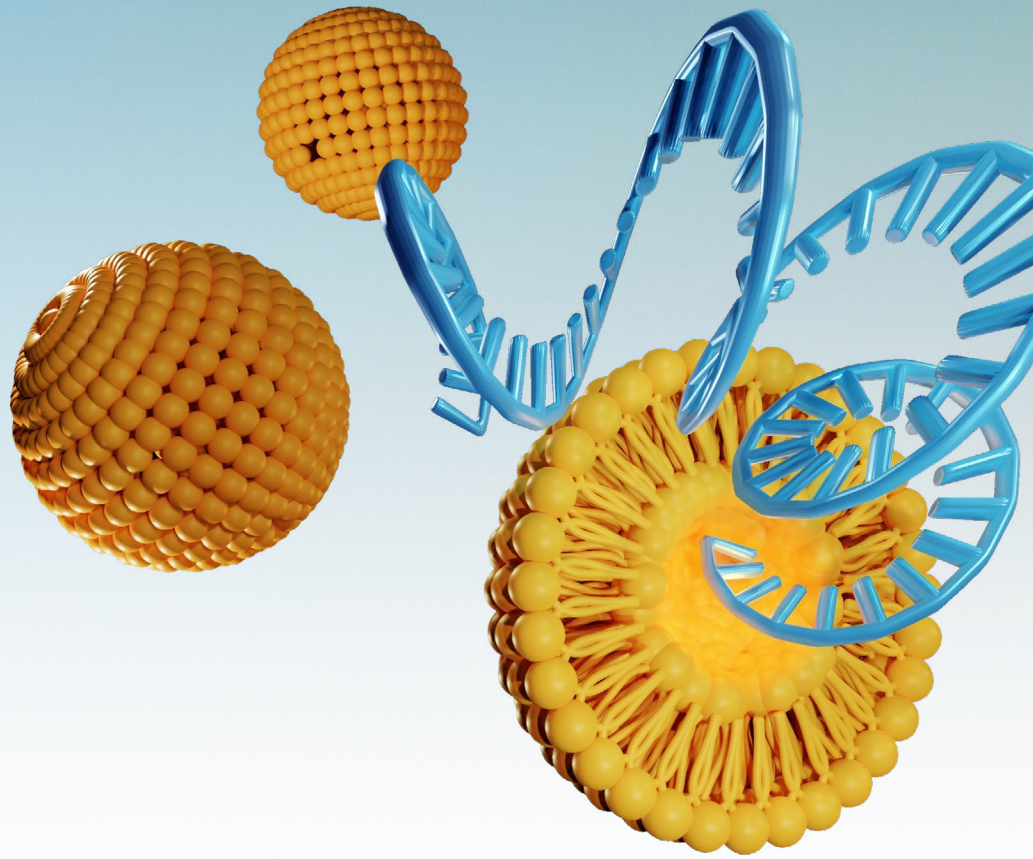
The complexity of mRNA vaccines and therapeutics

Messenger RNA (mRNA) therapy enables the body to make the proteins we need to prevent, treat, or cure diseases.

Unlike traditional biologics, mRNAs are large and delicate molecules that are produced using *in vitro* transcription (IVT), which need to be protected by lipid nanoparticles (LNPs) before they reach target cells.

Analytical characterization of mRNA therapeutics presents unique challenges that require new technologies and solutions.

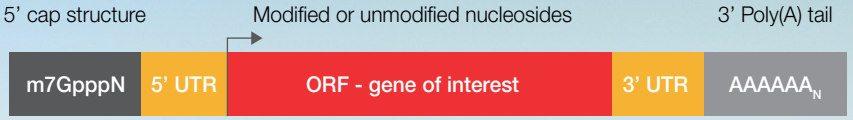


Critical quality attributes of mRNA therapeutics

mRNA

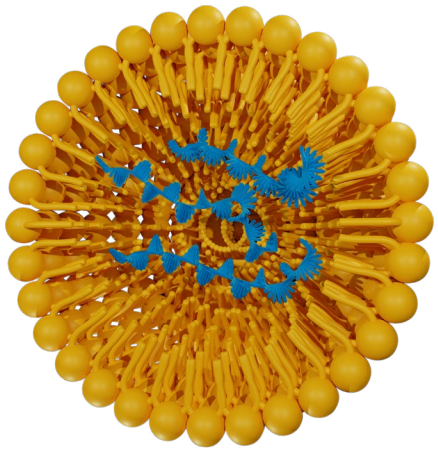
- Identify (sequence confirmation)*
- Purity (truncated forms, dsRNA, uncapped)*
- 5’ capping efficiency*
- 3’ poly(A) tail length*
- Process-related post translation modifications*

Schematic representation of *in vitro* transcribed (IVT) mRNA



Challenges:

- Release specifications have not been standardized
- Robust sequencing methods need to be developed



LNP

- Lipid purity*
- Lipid composition*
- Stability
- Size, polydispersity index (PDI), zeta potential
- Encapsulation
- Ionizable lipid pharmacokinetics and metabolism*

* LC, MS solutions are required or available

mRNA characterization

Direct mRNA sequence confirmation

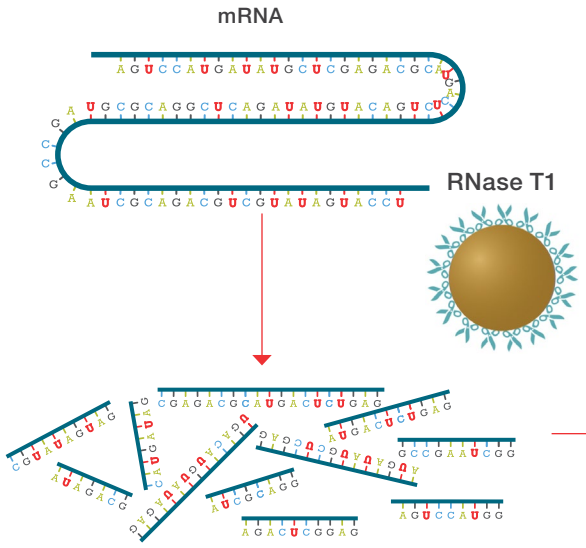
End-to-end LC-HRAM MS solution

RNA-Seq is the most common technology

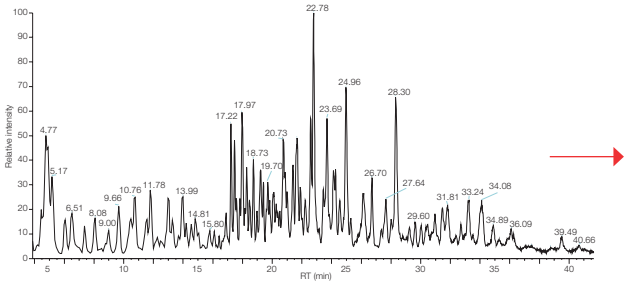
- Indirect. mRNA needs to be converted into DNA.
- Library/primer design is needed for each mRNA.
- Analysis takes >2 days.
- Multi-step, multi-instrument process.

An innovative LC-HRAM MS workflow

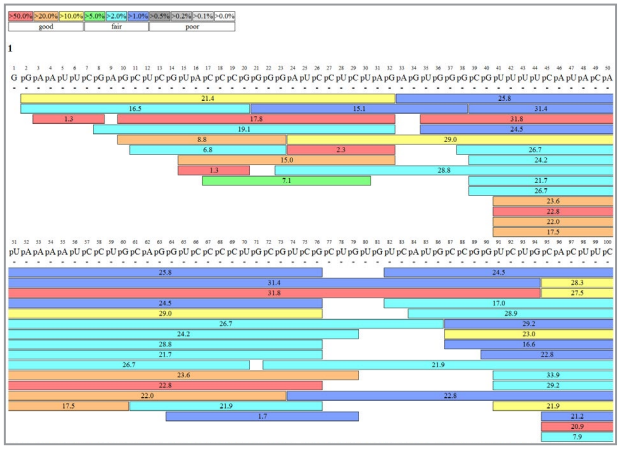
- **Direct** measurement of mRNA.
- **Universal**, no need for library/primer.
- **Fast** (~2 hr), reproducible.
- Accurate, **comprehensive sequence coverage (>85%)**.
- End-to-end solution with automation.



Reproducible, controlled partial digestion using immobilized RNAse magnetic beads.



High-resolution separation and high-quality MS, MS/MS data.

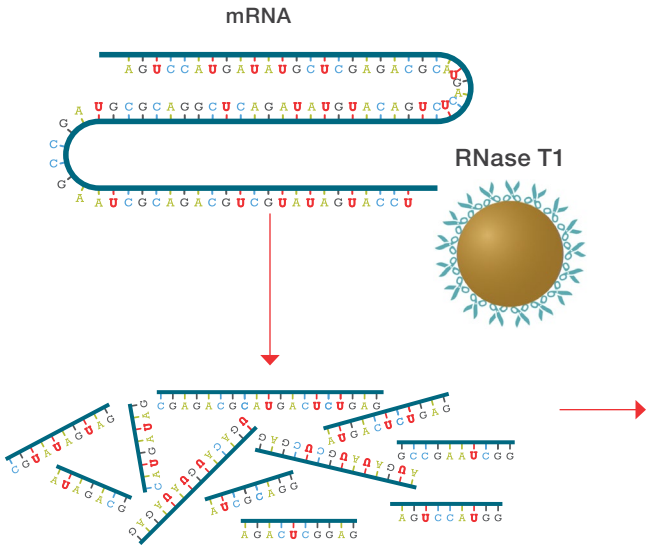


Confident, comprehensive sequence identification.

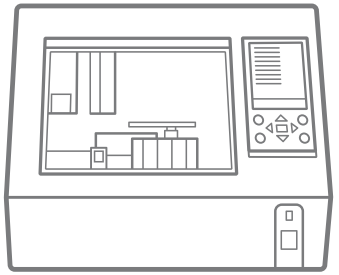
Direct mRNA sequence confirmation

Step 1: reproducible, controlled digestion

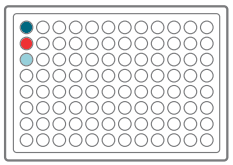
- **Controlled partial digestion** through immobilized RNAse avoids over-digestion, generates large fragments with unique sequences.
- Fast and complete removal of RNAse at the end of digestion **eliminates system contamination**.
- Digestion can be **automated** using the Thermo Scientific™ KingFisher™ Duo Prime purification system magnetic bead robot for **high reproducibility and throughput**.

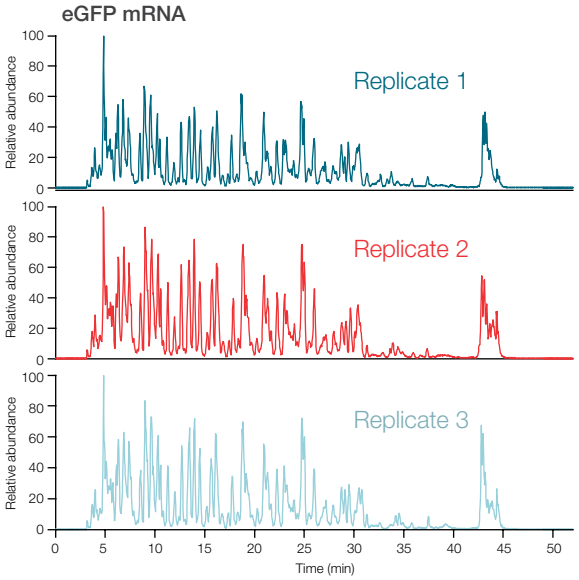


Reproducible, controlled partial digestion using immobilized RNAse magnetic beads.



KingFisher Duo Prime purification system



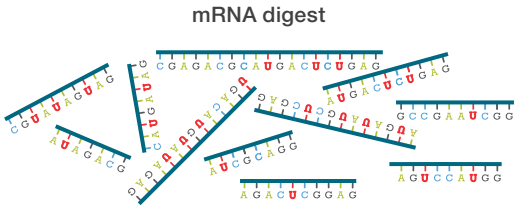


Chromatograms of three replicate eGFP mRNA digests show highly reproducible digestion.

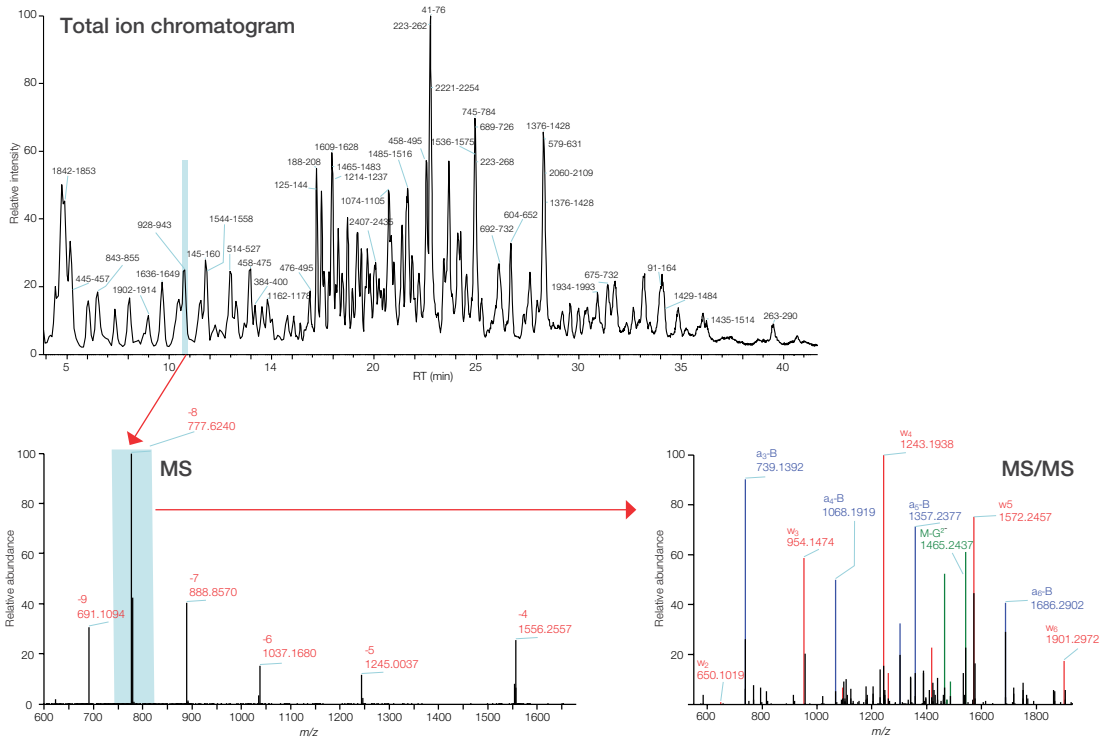
Direct mRNA sequence confirmation

Step 2: reproducible, high-quality LC-HRAM MS analysis

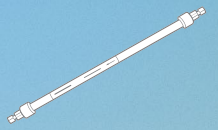
- **High-resolution separation** of RNA fragments is achieved at a high pH using Thermo Scientific™ DNAPac™ RP HPLC columns with unique polymeric structure.
- **Robust and reproducible separation** is ensured by using the biocompatible Thermo Scientific™ Vanquish™ UHPLC system.
- **Confident sequence identification** is ensured by high-quality MS, MS/MS spectra acquired on Thermo Scientific™ Orbitrap Exploris™ mass spectrometers.



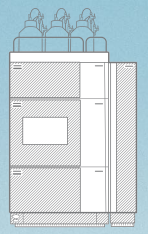
Reproducible, controlled partial digestion using immobilized RNAse magnetic beads.



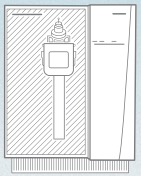
High-resolution separation and high-quality MS, MS/MS data.



Thermo Scientific™
DNAPac™ RP HPLC Columns



Thermo Scientific™
Vanquish™ Flex UHPLC System

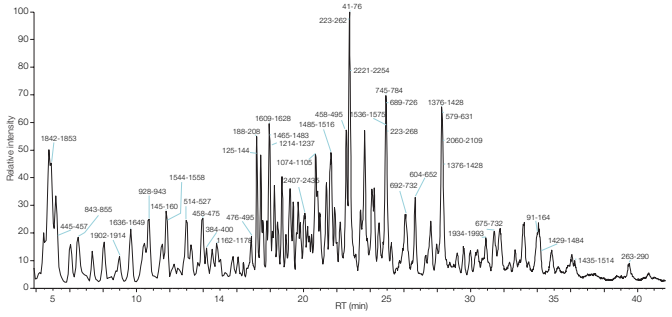


Thermo Scientific™
Orbitrap Exploris™ 240
Mass Spectrometer

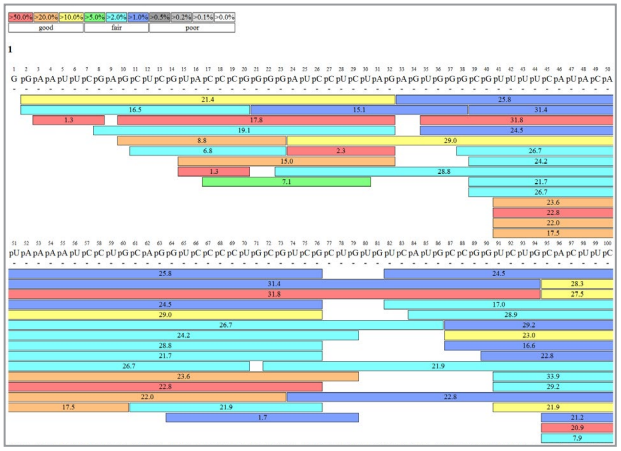
Direct mRNA sequence confirmation

Step 3: automated, streamlined data processing

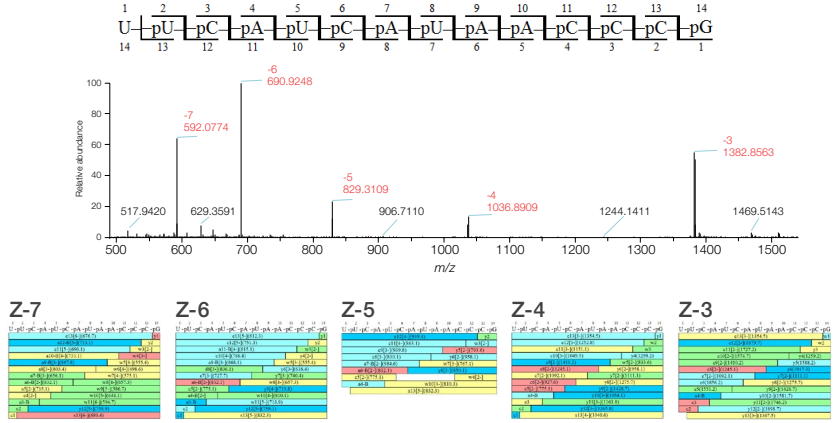
- **Confident oligo sequence identification** using MS/MS spectra of multiple charge states and innovative kinetic prediction algorithm.
- Intuitive user interface supports **customized building blocks and modifications**.
- Accurate identification and separation of **sequence isomers**.
- **Comprehensive sequence coverage at >85%.**



Thermo Scientific™ BioPharma Finder™ Software

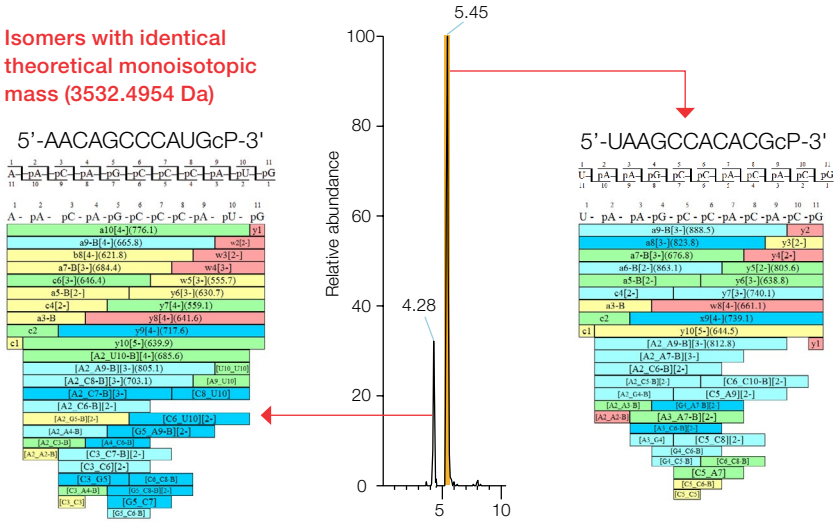


Confident sequence identification and comprehensive sequence coverage.



Improved sequence identification using HRAM MS/MS spectra of multiple charge states.

Isomers with identical theoretical monoisotopic mass (3532.4954 Da)

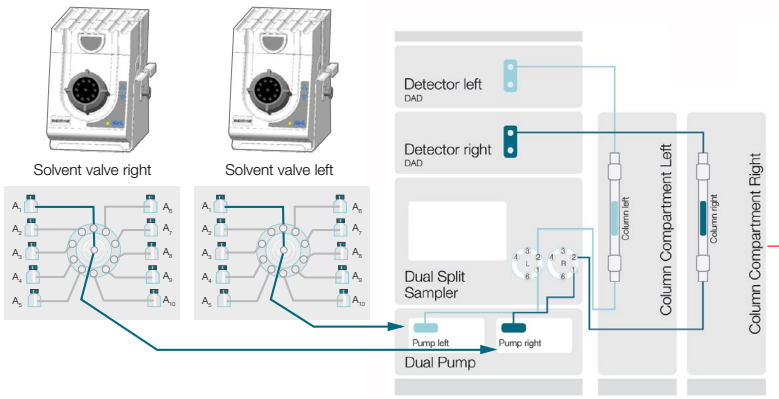


Sequence isomers are baseline separated and confidently identified using high-quality HRAM MS/MS spectra.

Optimize impurity analysis with ease

Determine the most suitable conditions for the detection of post-transcriptional impurities with a time-effective scouting approach

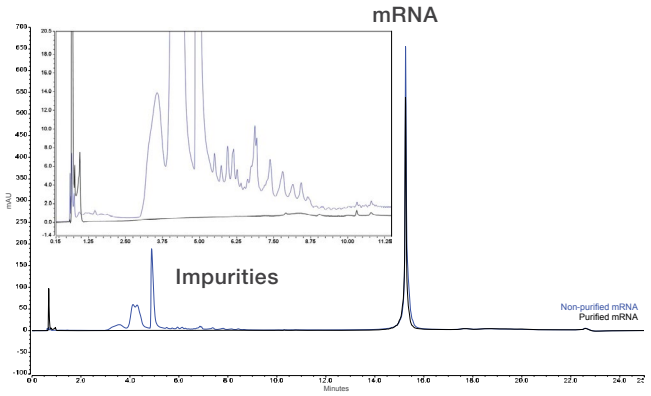
- **High selectivity and separation of mRNA impurities** using Thermo Scientific™ DNAPac™ RP HPLC columns and Thermo Scientific™ DNAPac™ PA 200RS HPLC columns.
- **Fast method optimization** through simultaneous scouting of columns with different chemistries on Thermo Scientific™ Vanquish™ Duo for Dual LC system.



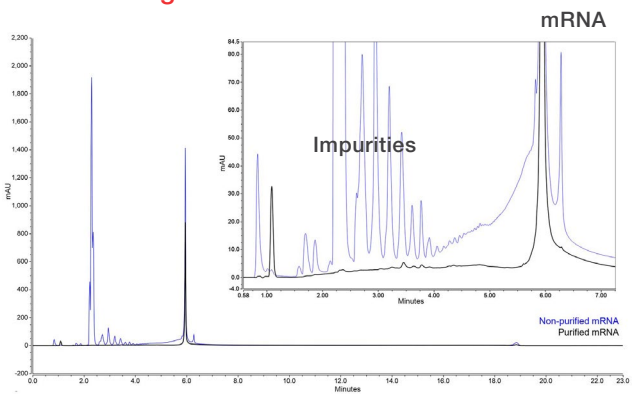
Flow scheme overview: Thermo Scientific Vanquish Duo for Dual LC with Solvent Extension Kits for automated method scouting. Dual pump and dual column compartment set-up.

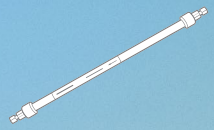
View complete application note

Reversed phase

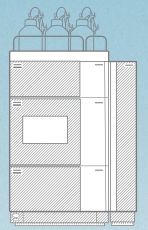


Ion exchange

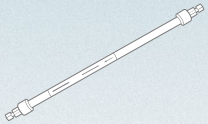




Thermo Scientific™ DNAPac™ RP HPLC Columns



Thermo Scientific™ Vanquish™ Duo HPLC System

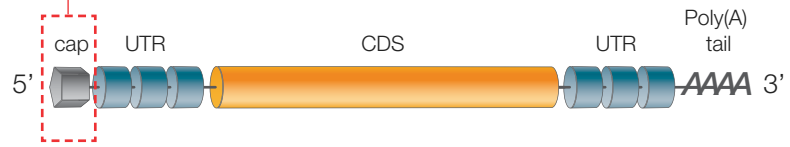


Thermo Scientific™ DNAPac™ PA200 Oligonucleotide HPLC Columns

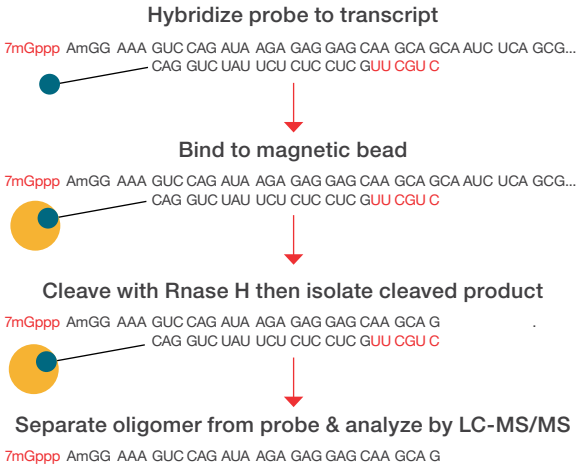
mRNA 5’ capping characterization

The 5’ cap features

- Prevents the degradation by exonucleases.
- Promotes translation.
- Incorporated *in vitro* via two methods: a two-step multi-enzymatic reaction or co-transcriptionally.

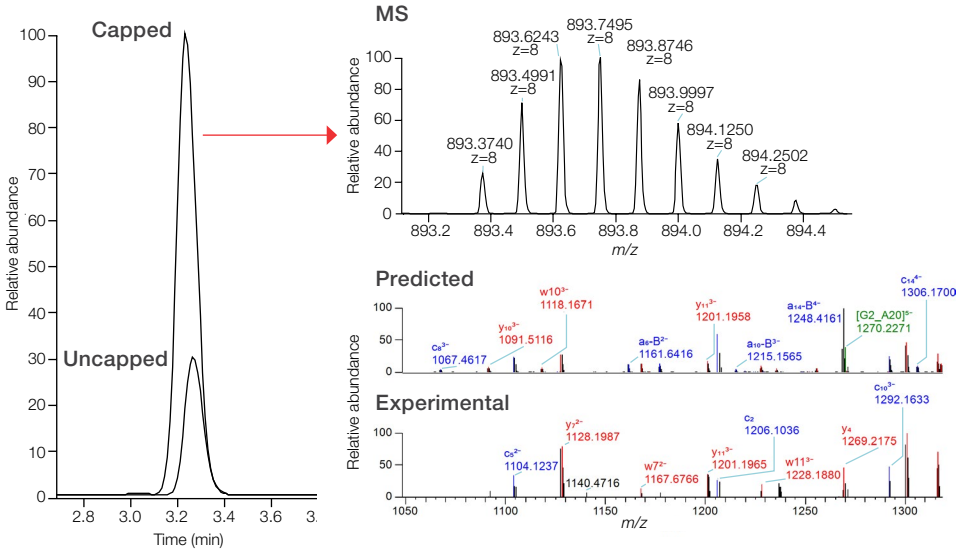


Expensive and labor intensive

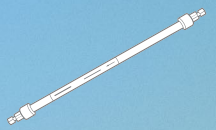


Accurate and sensitive characterization of capped and uncapped fragment with LC-HRAM MS

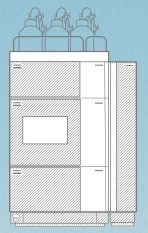
- 120 K resolution
- <1 ppm mass error



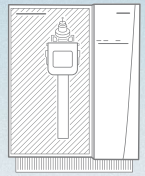
Extracted ion chromatograms of capped and uncapped fragments are used for accurate capping efficiency measurement. HRAM MS and MS/MS spectra ensure confident sequence confirmation.



Thermo Scientific™
DNAPac™ RP HPLC Columns



Thermo Scientific™
Vanquish™ Flex UHPLC System

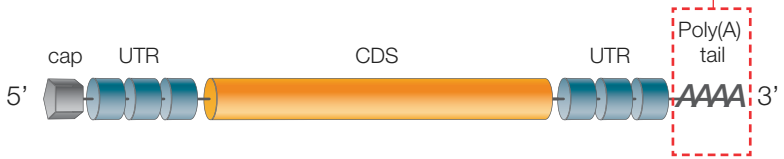


Thermo Scientific™
Orbitrap Exploris™ 240
Mass Spectrometer

Thermo Scientific™
BioPharma Finder
Software

mRNA 3’ Poly(A) characterization

Accurate, sensitive detection of poly(A) distribution

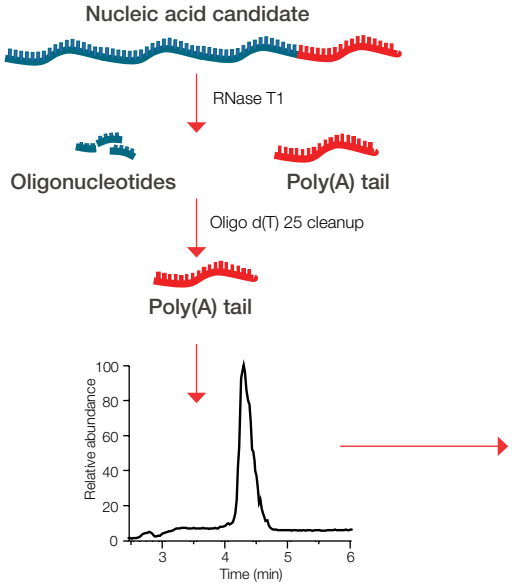


Poly(A) tails (117–134 As)

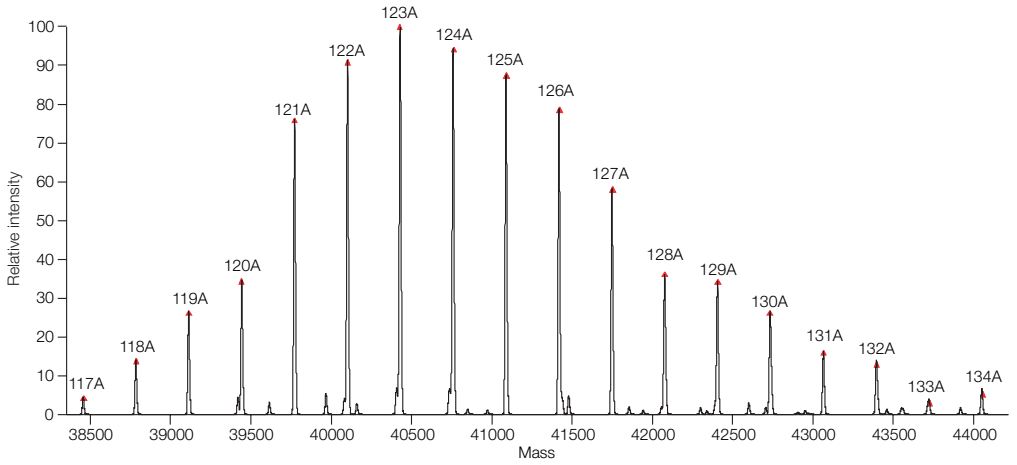
Poly(A) tail features

- Length is transcript dependent (100–250 nucleotide long).
- Protects against exonuclease degradation.
- Promotes translation.

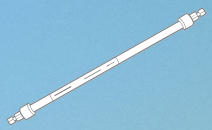
mRNA 3’ Poly(A) tail characterization



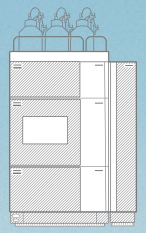
Poly(A) tails are baseline separated from the rest of mRNA digest.



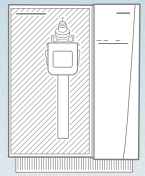
Poly(A) species with different lengths are characterized with high sensitivity and high mass accuracy (mass error <20 ppm) in the deconvoluted spectrum. Their relative abundance can be estimated using peak height.



Thermo Scientific™
DNAPac™ RP HPLC Columns



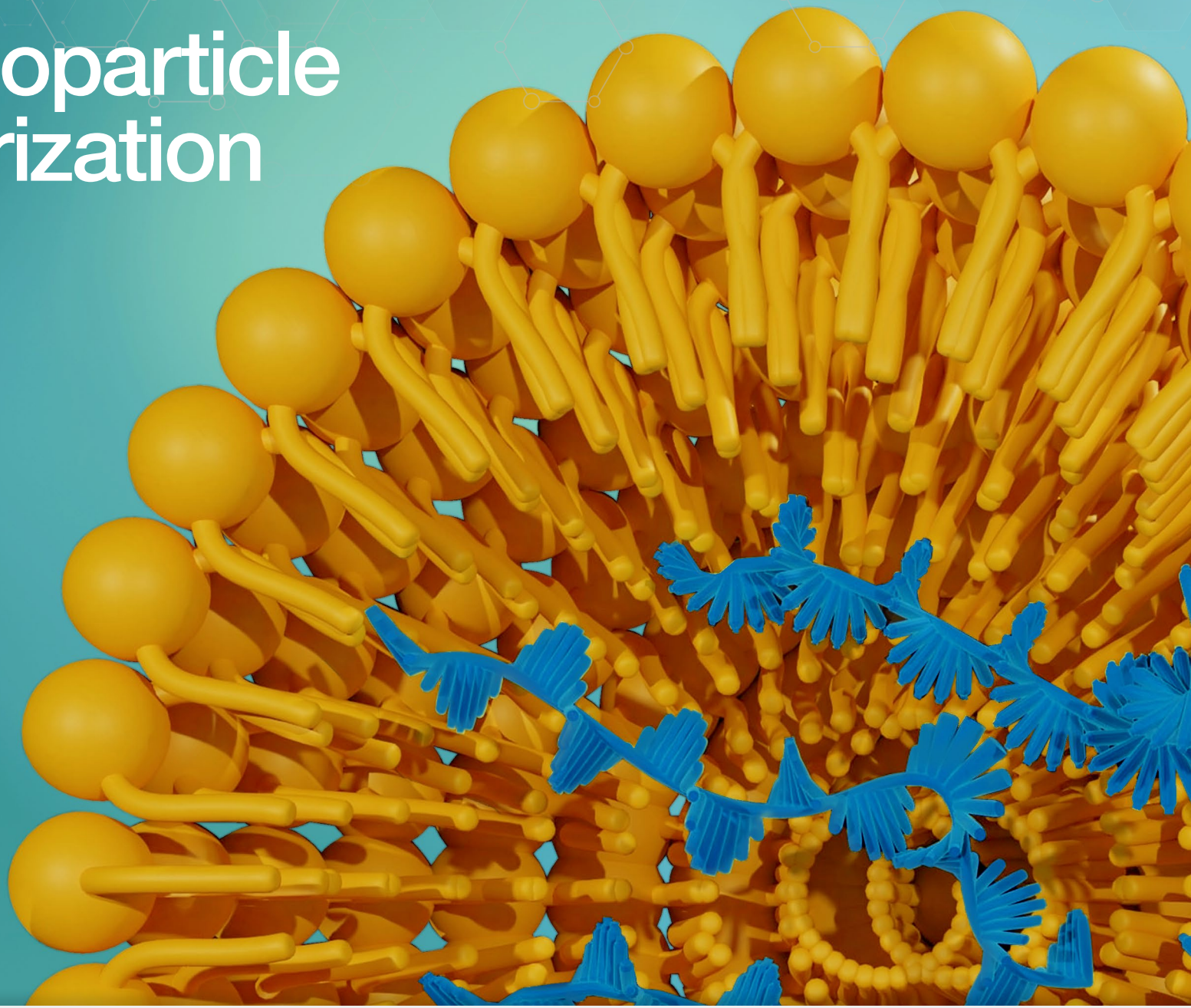
Thermo Scientific™
Vanquish™ Flex UHPLC System



Thermo Scientific™
Orbitrap Exploris™ 240
Mass Spectrometer

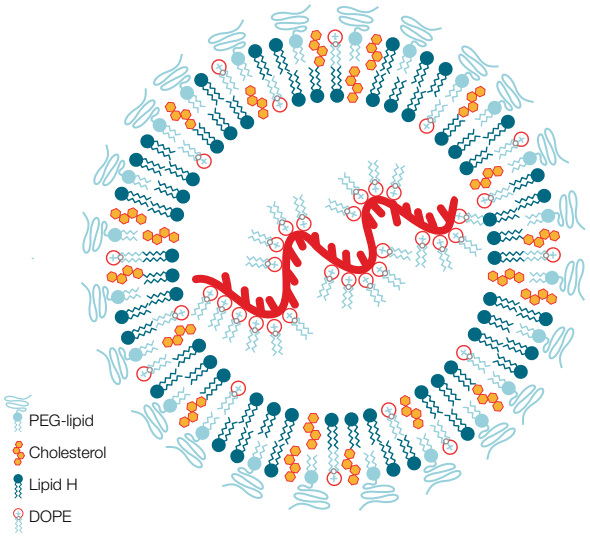
Thermo Scientific™
BioPharma Finder
Software

Lipid nanoparticle characterization

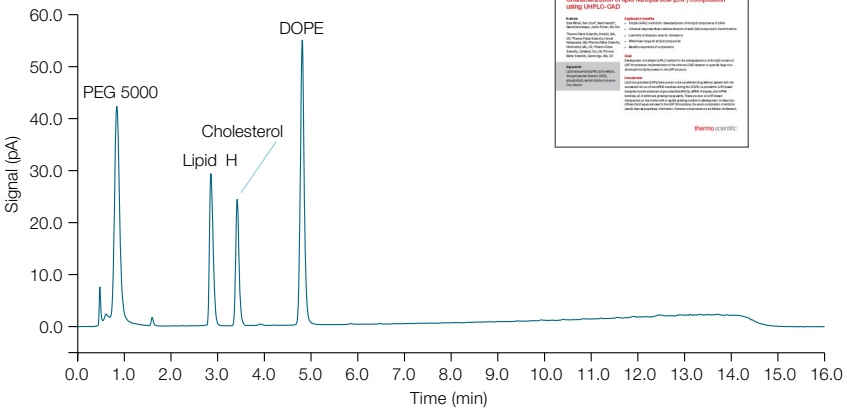


LNP composition analysis by LC-CAD

- **Fast high-resolution separation** of LNP components was achieved on the Thermo Scientific™ Accucore™ C30 column in 10 minutes.
- **Sensitive detection and accurate quantification** of all the components were obtained using Thermo Scientific™ Vanquish™ charged aerosol detector, the industry standard platform for lipid identity, lipid content and lipid impurity.
- Charged aerosol detector provides **universal detection with wide dynamic range** up to 10^5, allows quantification of low-level impurities and high-level API in a single run.

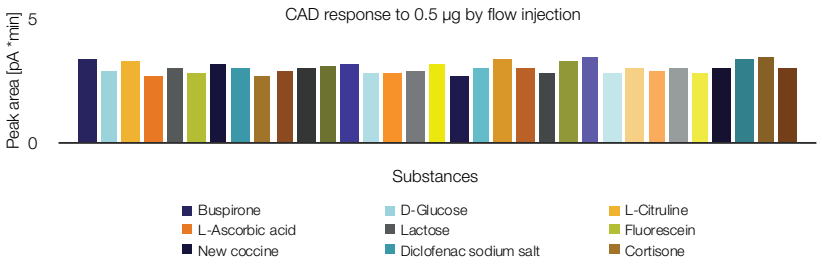


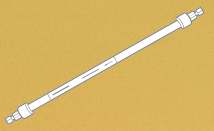
Base-line separation of lipid components and minor impurities



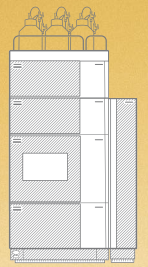
View complete application note

Universal response across broad range of molecules





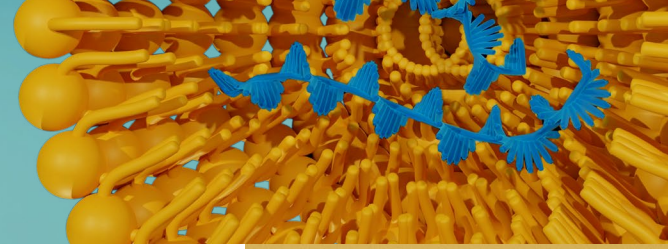
Thermo Scientific™ Accucore™ C30 HPLC Columns



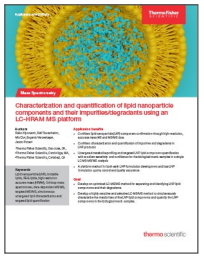
Thermo Scientific™ Vanquish™ System with Charged Aerosol Detector

LNP component analysis by LC-MS

Platform method for raw material and LNP formulation QC, suitable for different types of LNP formulations

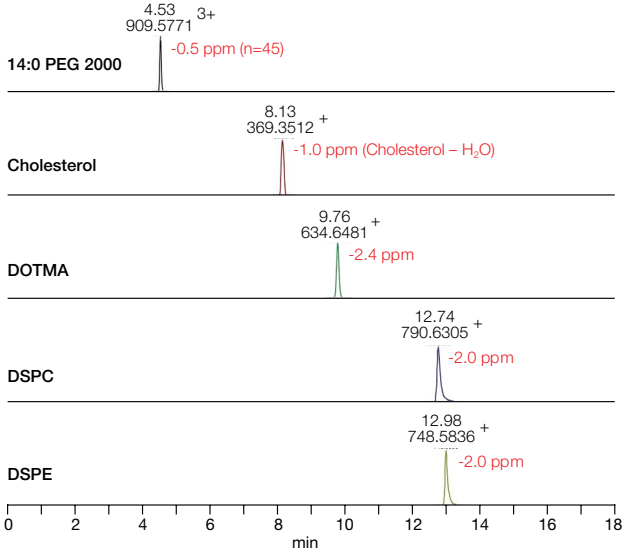


- **High-resolution separation of lipid isomers** using Thermo Scientific™ Accucore™ C30 UHPLC column coupled to Thermo Scientific™ Vanquish™ Horizon UHPLC system.
- **Confident confirmation of lipid components** using HRAM MS and MS/MS on Thermo Scientific™ Orbitrap Exploris™ 120 mass spectrometer.
- Sensitive detection and identification of impurities at **0.001%.**
- Simultaneous **lipid component quantification** and **metabolites characterization** in a single analysis.

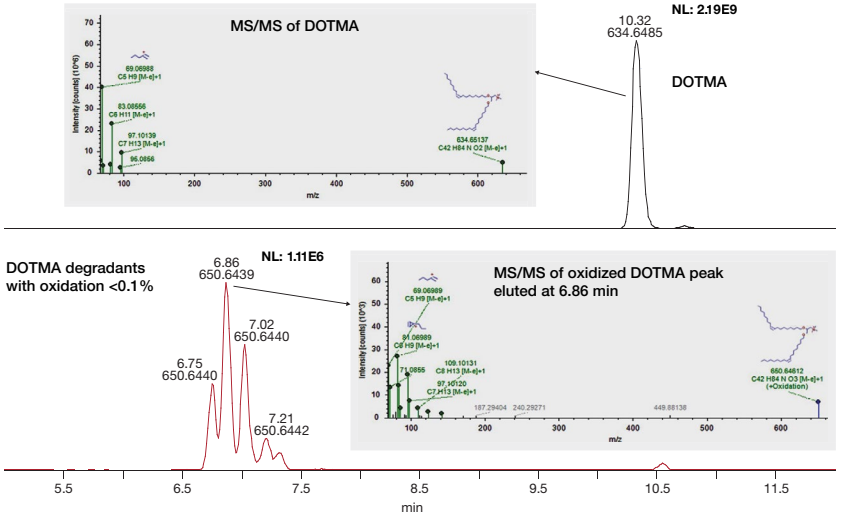


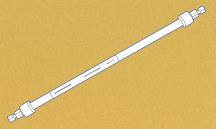
View complete application note

Excellent separation and accurate mass measurement (<3ppm) of lipid components

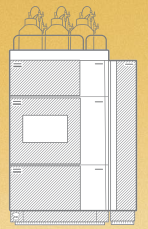


Confident detection and characterization of low abundant metabolites

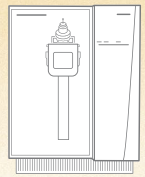




Thermo Scientific™ Accucore™ C30 HPLC Columns



Thermo Scientific™ Vanquish™ Horizon UHPLC System



Thermo Scientific™ Orbitrap Exploris™ 120 Mass Spectrometer

Featured products

Product name	Catalog number
Orbitrap Exploris 240 Mass Spectrometer	BRE725535
Orbitrap Exploris 120 Mass Spectrometer	BRE725531
Vanquish Horizon UHPLC System	IQLAAAGABHFAPUMZZZ
Vanquish Flex UHPLC System	IQLAAAGABHFAPUMBJC
Vanquish Duo UHPLC System	VQDUO-DUALLC
Vanquish Charged Aerosol Detector H	VH-D20-A
Extension Kit for Automated Method Scouting, Vanquish LC Systems	6036.0100
DNAPac RP HPLC Columns	088919
DNAPac PA200 Oligonucleotide HPLC Columns	082509
Accucore C30 HPLC Columns	27826-252130
BioPharma Finder Software	OPTON-30986
SMART Digest Bulk Magnetic RNase T1 Kit	60120-101
KingFisher Duo Prime Purification System	5400110
Dynabeads™ Oligo(dT)$_{25}$ mRNA isolation beads	61002

Resources

Application notes

- Characterization of mRNA 5' capping products using an LC-HRAM-MS/MS analytical platform and Thermo Scientific BioPharma Finder software solution
- Characterization of lipid nanoparticle (LNP) composition using UHPLC-CAD
- Characterization and quantification of lipid nanoparticle components and their impurities/degradants using LC-HRAM MS platform
- Simultaneous reversed-phase and anion-exchange method scouting with a dual system for mRNA impurity determination

Publications

- Characterization and sequence mapping of large RNA and mRNA therapeutics using mass spectrometry

Webinars

- On-demand: automated workflow for mRNA sequencing by high resolution LC-MS

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