Thermo Fisher
SCIENTIFIC

Biopharmaceuticals

Analytical solutions for mRNA vaccines and therapeutics

thermo scientific

Table of contents

mRNA vaccines and therapeutics

mRNA characterization

Lipid nanoparticle characterization

Click on titles to jump to page.

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.
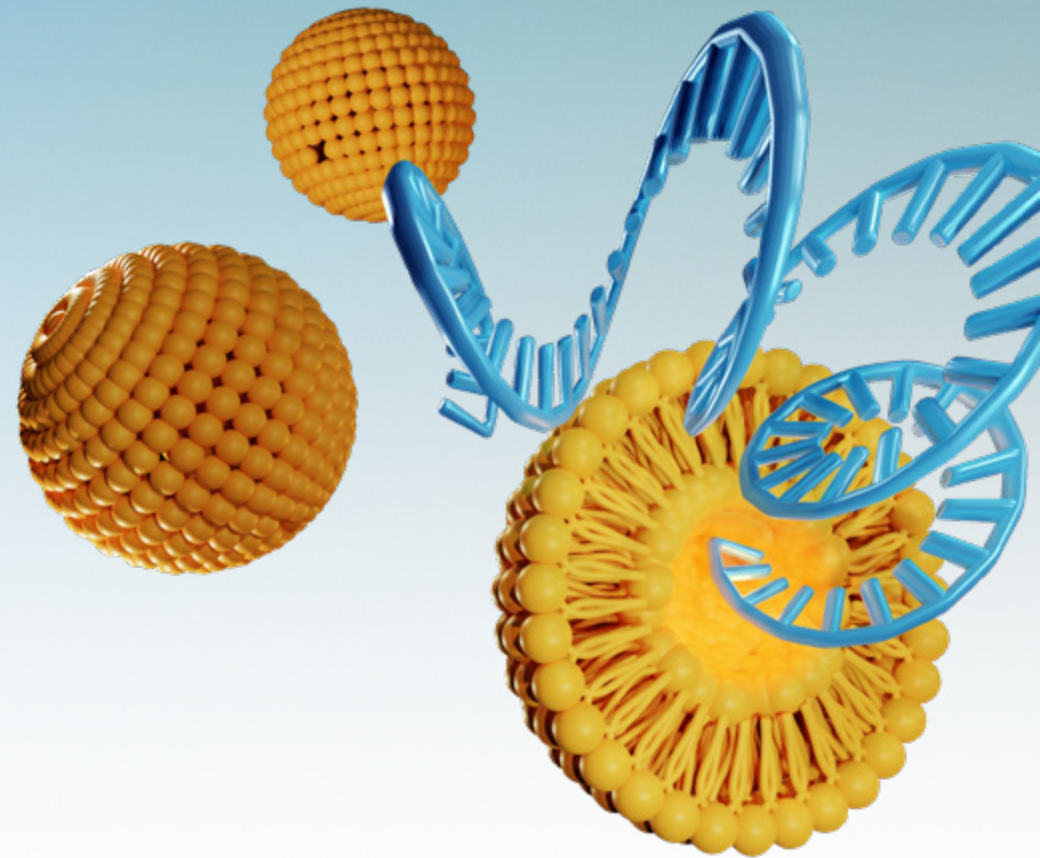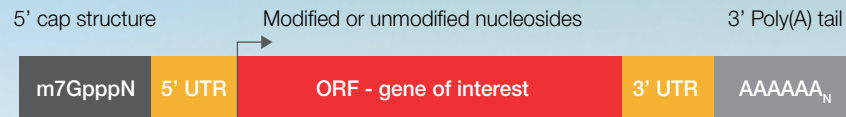
# Critical quality attributes of mRNA therapeutics

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

5' cap structure		Modified or unmodified nucleosides		3' Poly(A) tail
m7GpppN	5' UTR	ORF - gene of interest	3' UTR	AAAAAA$_N$

Challenges:

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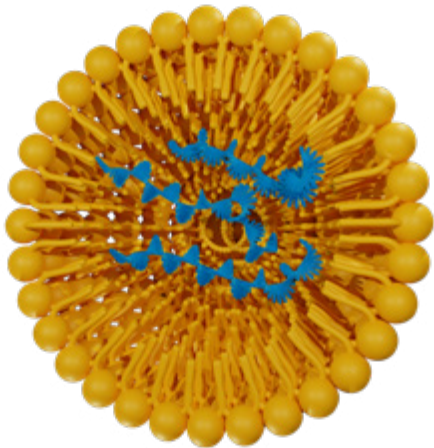
LNP

- 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

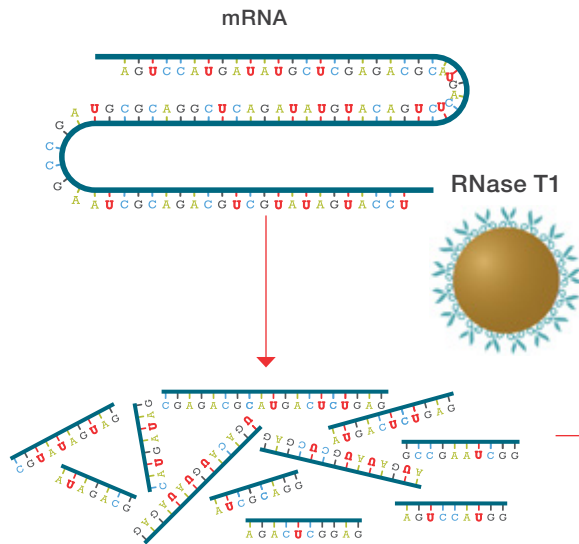# Direct mRNA sequence confirmation
End-to-end LC-HRAM MS solution

## 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.

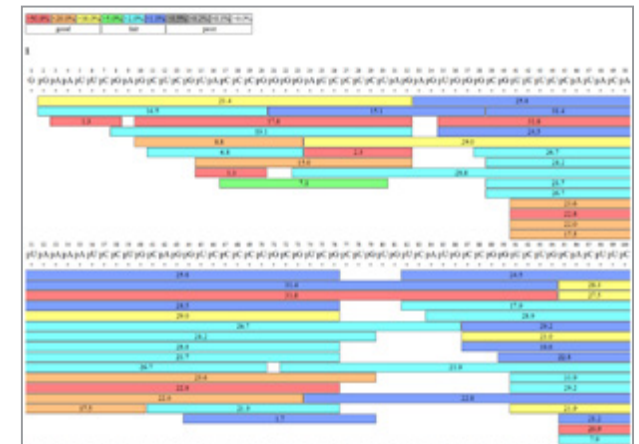## An innovative LC-HRAM MS workflow

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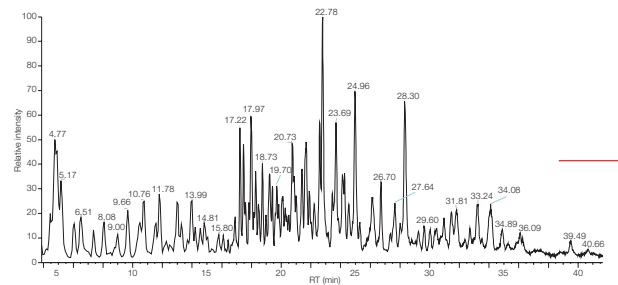
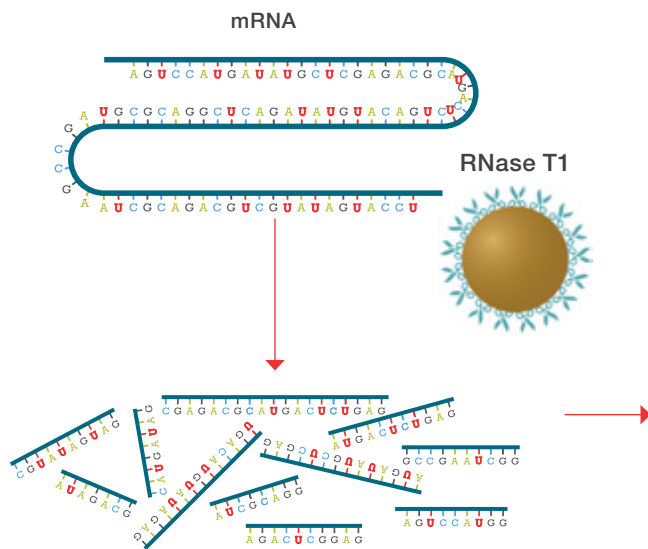High-resolution separation and high-quality MS, MS/MS data.

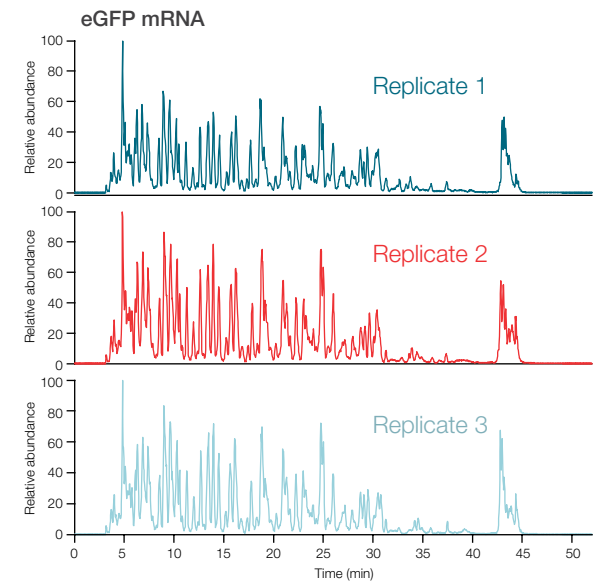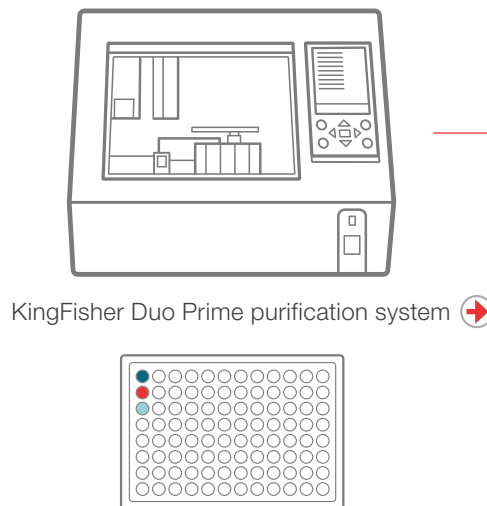Confident, comprehensive sequence identification.

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.
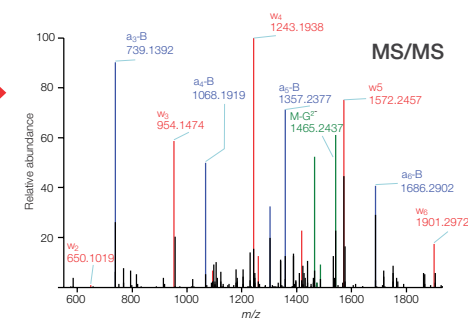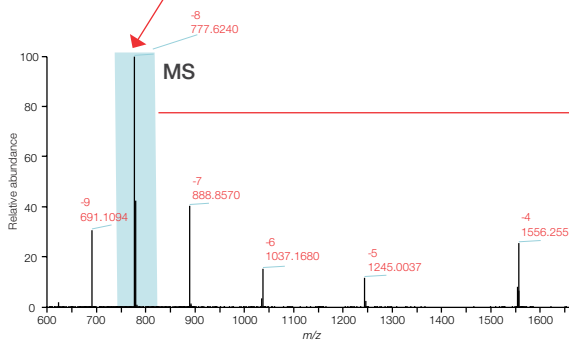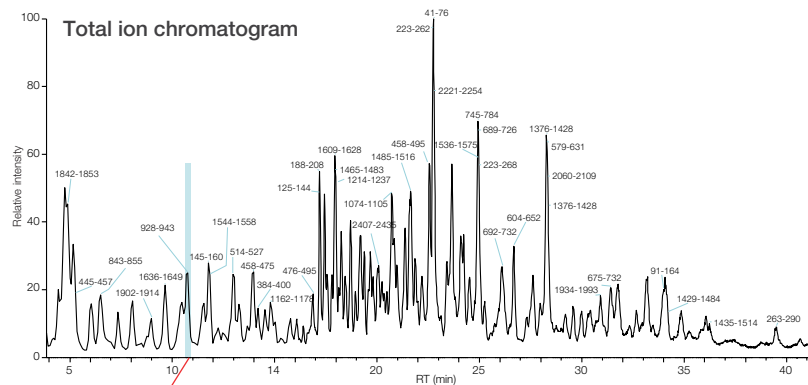
Direct mRNA sequence confirmation
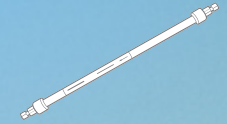## Step 2: reproducible, high-quality LC-HRAM MS analysis

- **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.
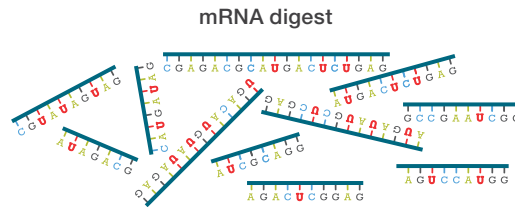
mRNA digest

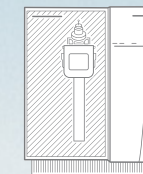Reproducible, controlled partial digestion using immobilized RNAse magnetic beads.

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

mRNA vaccines | **mRNA characterization** | Lipid nanoparticle characterization

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%.

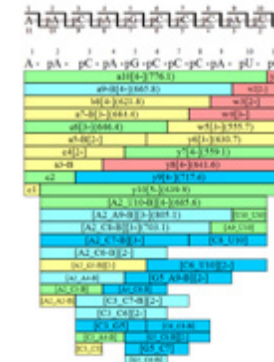Thermo Scientific™ BioPharma Finder™ Software
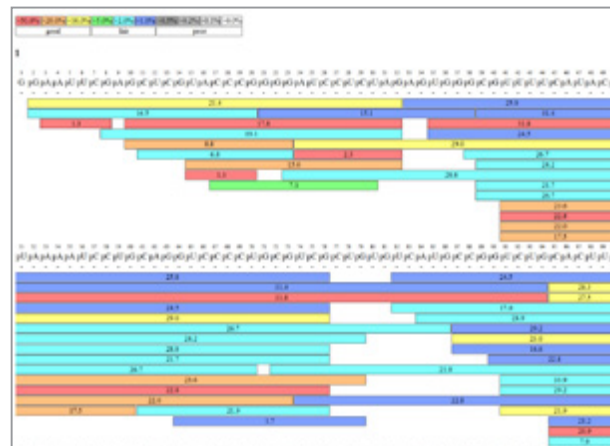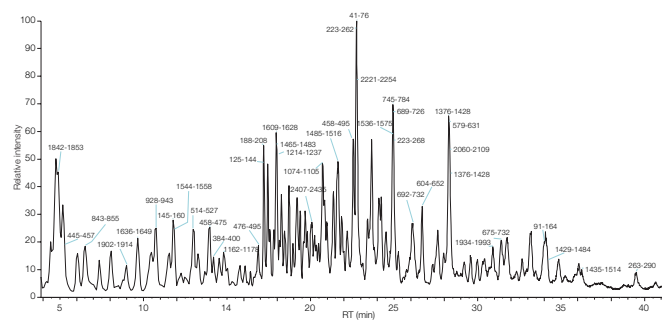
Confident sequence identification and comprehensive sequence coverage.
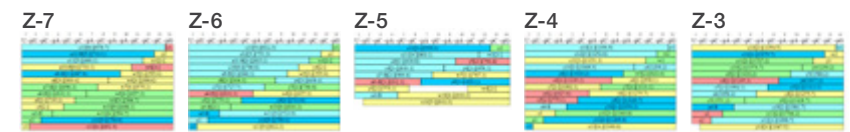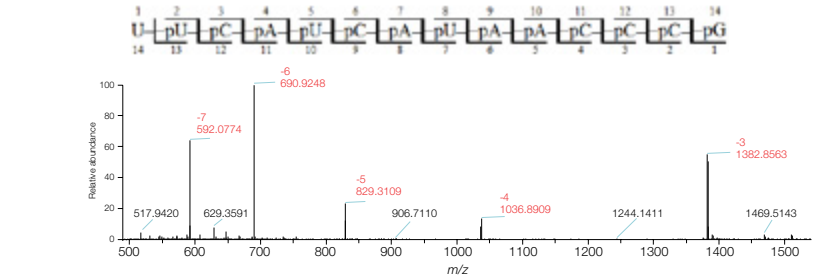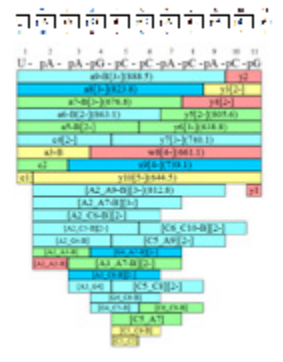

Improved sequence identification using HRAM MS/MS spectra of multiple charge states.

Isomers with identical theoretical monoisotopic mass (3532.4954 Da)

5'-AACAGCCCAUGcP-3'

5'-UAAGCCACACGcP-3'

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

mRNA vaccines | mRNA characterization | Lipid nanoparticle characterization

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.

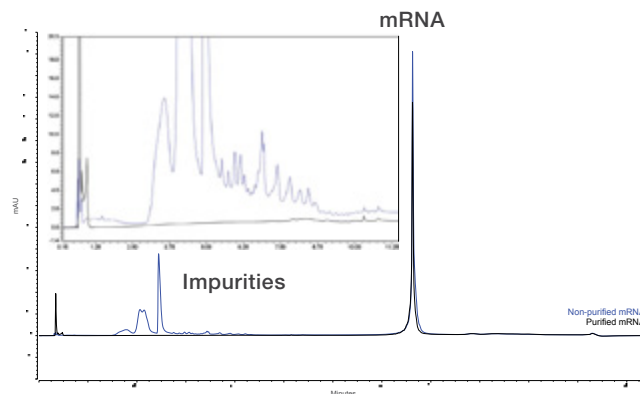Solvent valve right   Solvent valve left
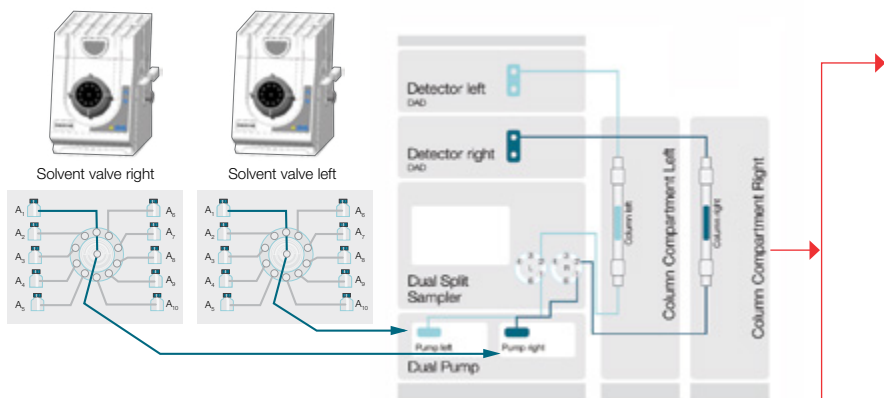
Flow scheme overview: Thermo Scientific Vanquish Duo for Dual LC with Solvent Extension Kits for automated method scouting. Dual pump and dual column compartment set-up.
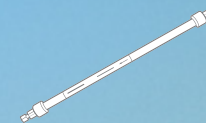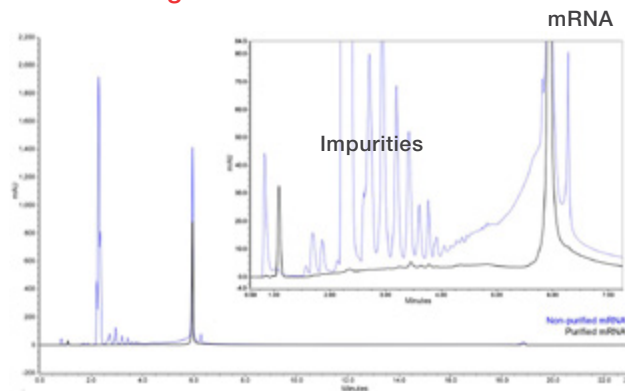
View complete application note

Reversed phase

mRNA

Impurities

Non-purified mRNA
Purified mRNA

Ion exchange

mRNA

Impurities

Non-purified mRNA
Purified mRNA
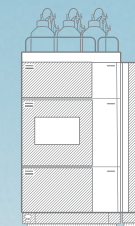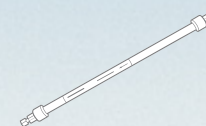
Thermo Scientific™ DNAPac™ RP HPLC Columns

Thermo Scientific™ Vanquish™ Duo HPLC System

Thermo Scientific™ DNAPac™ PA200 Oligonucleotide HPLC Columns

---

mRNA vaccines | mRNA characterization | Lipid nanoparticle characterization

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.

5' cap UTR CDS UTR Poly(A) tail AAAA 3'
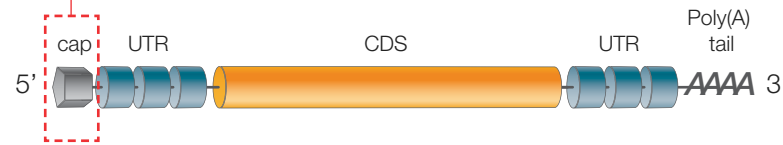
Expensive and labor intensive

Hybridize probe to transcript

7mGppp AmGG AAA GUC CAG AUA AGA GAG GAG CAA GCA GCA AUC UCA GCG...
CAG GUC UAU UCU CUC CUC GUU CGU C

Bind to magnetic bead

7mGppp AmGG AAA GUC CAG AUA AGA GAG GAG CAA GCA GCA AUC UCA GCG...
CAG GUC UAU UCU CUC CUC GUU CGU C

Cleave with Rnase H then isolate cleaved product

7mGppp AmGG AAA GUC CAG AUA AGA GAG GAG CAA GCA G
CAG GUC UAU UCU CUC CUC GUU CGU C

Separate oligomer from probe & analyze by LC-MS/MS
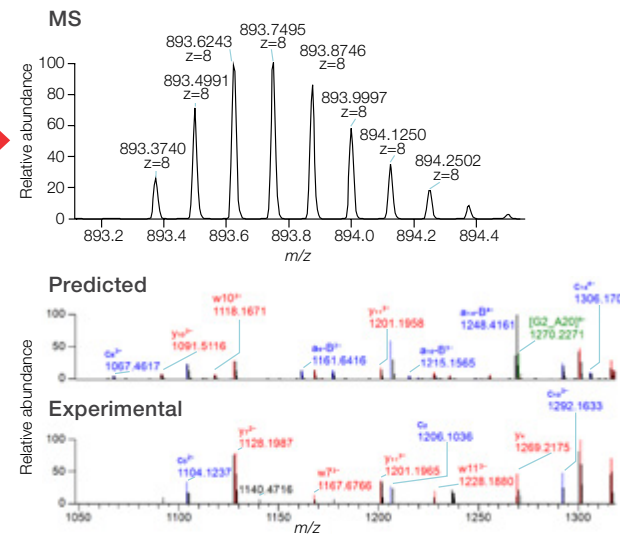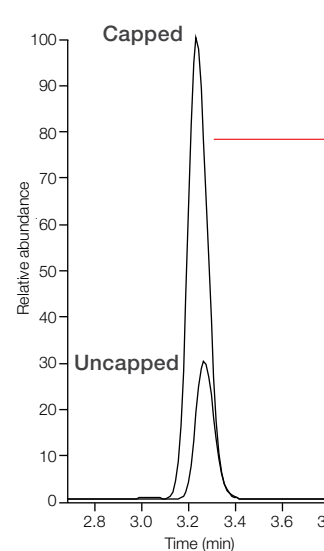
7mGppp AmGG AAA GUC CAG AUA AGA GAG GAG CAA GCA G

View complete application note

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

- 120 K resolution
- <1 ppm mass error

MS

Capped

Uncapped

893.3740 z=8
893.4991 z=8
893.6243 z=8
893.7495 z=8
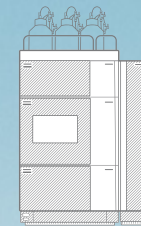893.8746 z=8
893.9997 z=8
894.1250 z=8
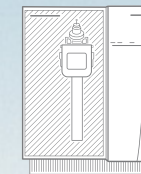894.2502 z=8

Predicted

Experimental

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.
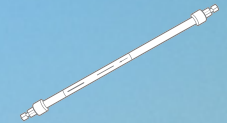
Thermo Scientific™ DNAPac™ RP HPLC Columns

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

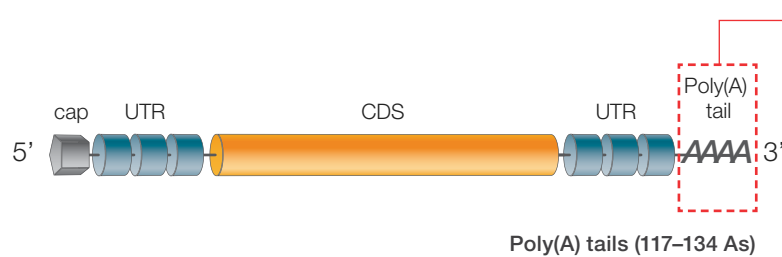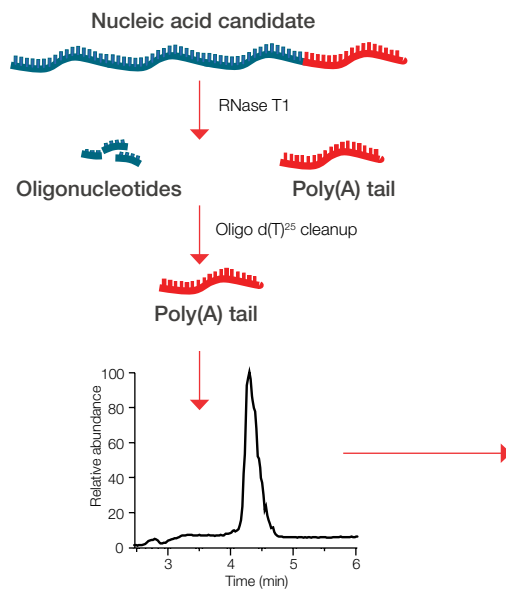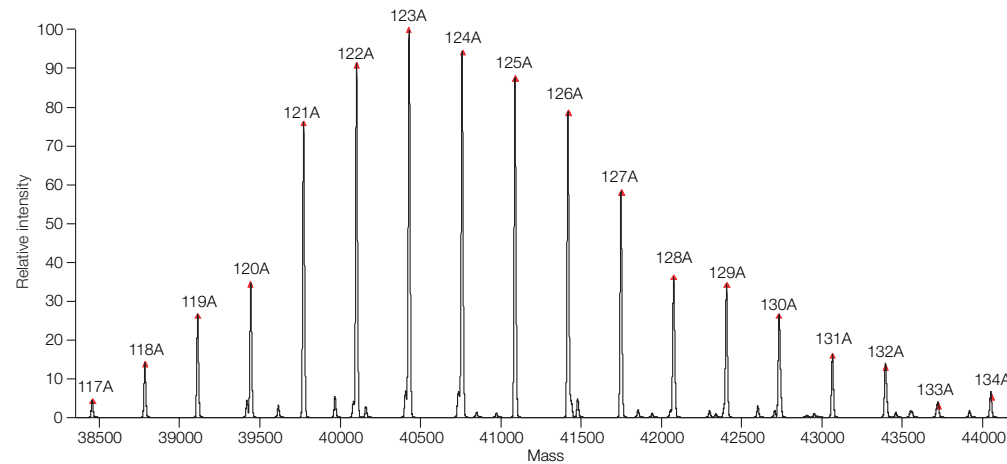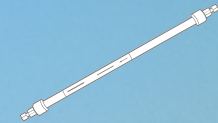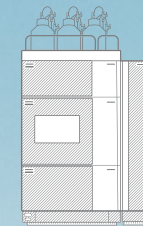Thermo Scientific™ Orbitrap Exploris™ 240 Mass Spectrometer

Bf Thermo Scientific™ BioPharma Finder™ Software

mRNA vaccines | mRNA characterization | Lipid nanoparticle characterization

mRNA 3' Poly(A) characterization

Characterization of *in vitro*-transcribed (IVT) mRNA poly(A) tail by LC-HRAM-MS and BioPharma Finder 5.0 software
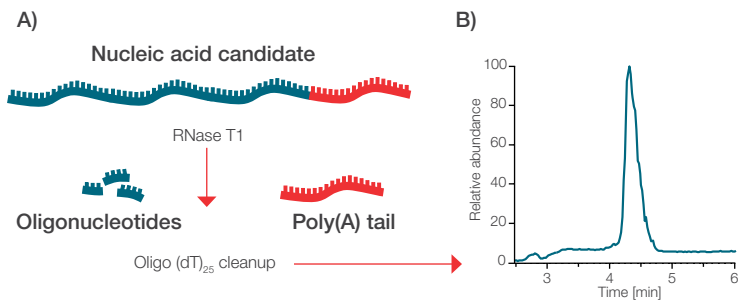
- Confident identification and sequence confirmation of polyadenylated tails in synthetic mRNA transcripts using high-resolution, accurate mass spectrometry (HRAM)
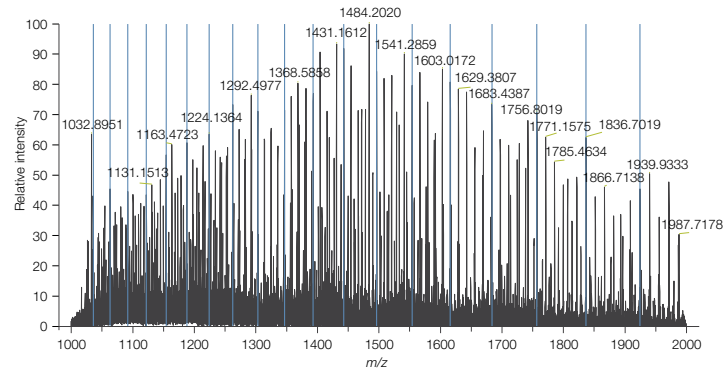
- Streamlined workflow for the characterization of the polyadenylated tail using Thermo Scientific™ BioPharma Finder™ 5.0 software

RNase T1 digestion

A) Representation of the digestion workflow.
B) Chromatographic peak of purified poly(A) tails.

Mass spectrum of purified poly(A) pool

Mass spectrum of a purified poly(A) pool. Blue lines indicate charge states used for Xtract algorithm deconvolution of the mass of a singular length of poly(A) in the sample.
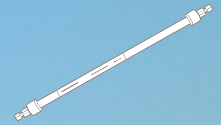
Isotopically resolved 30mer
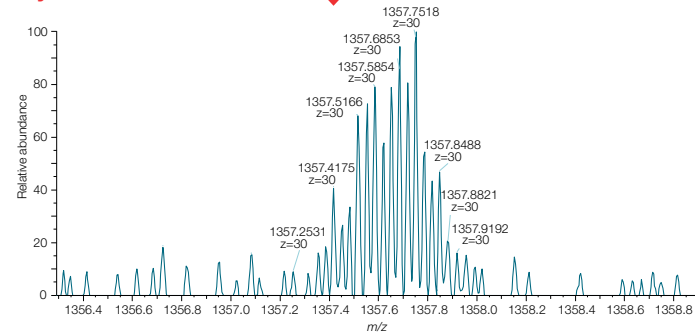
Zoomed image of an isotopically resolved 30mer. Isotopic resolved peaks are used with the Xtract deconvolution algorithm for determination of monoisotopic mass.

View complete application note
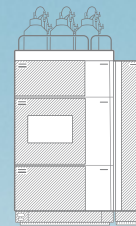
Thermo Scientific™ DNAPac™ RP HPLC Columns

Thermo Scientific™ Vanquish™ Horizon UHPLC System

Thermo Scientific™ Orbitrap Exploris™ 240 Mass Spectrometer

Thermo Scientific™ BioPharma Finder™ Software

---

mRNA vaccines | **mRNA characterization** | Lipid nanoparticle characterization

mRNA direct sequence mapping

Using automated partial digestion with magnetic nuclease and LC-HRMS

- Direct sequence analysis of large mRNAs by LC-HRMS

- Reproducible and comprehensive sequence coverage at >85%

- Simple automated workflow suitable for unmodified and modified mRNA

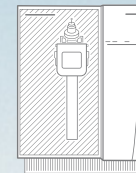- Automatic data annotation and mapping for simpler data analysis

View complete customer application note

Thermo Scientific™ DNAPac™ RP HPLC Columns

Thermo Scientific™ Vanquish™ Horizon UHPLC System

Thermo Scientific™ Orbitrap Exploris™ 240 Mass Spectrometer

Thermo Scientific™ BioPharma Finder™ Software

mRNA sequence mapping workflow

Data processing

LC-MS/MS analysis

Partial RNase T1 digest
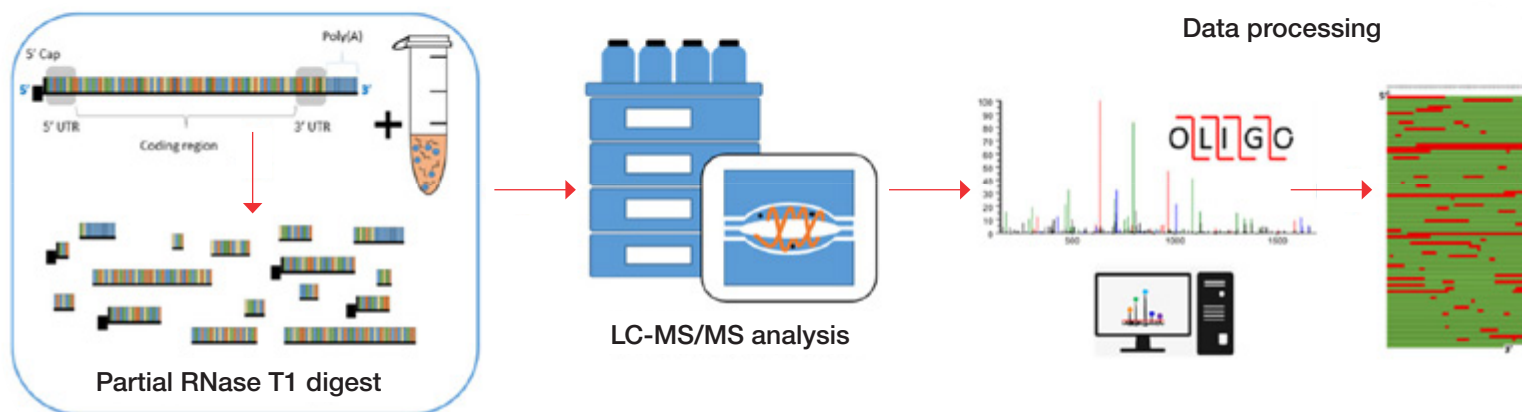
Schematic representation of direct mRNA sequencing workflow.
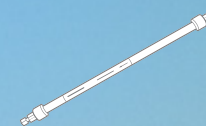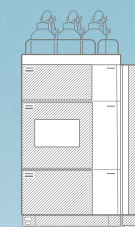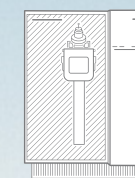
Lipid nanoparticle characterization
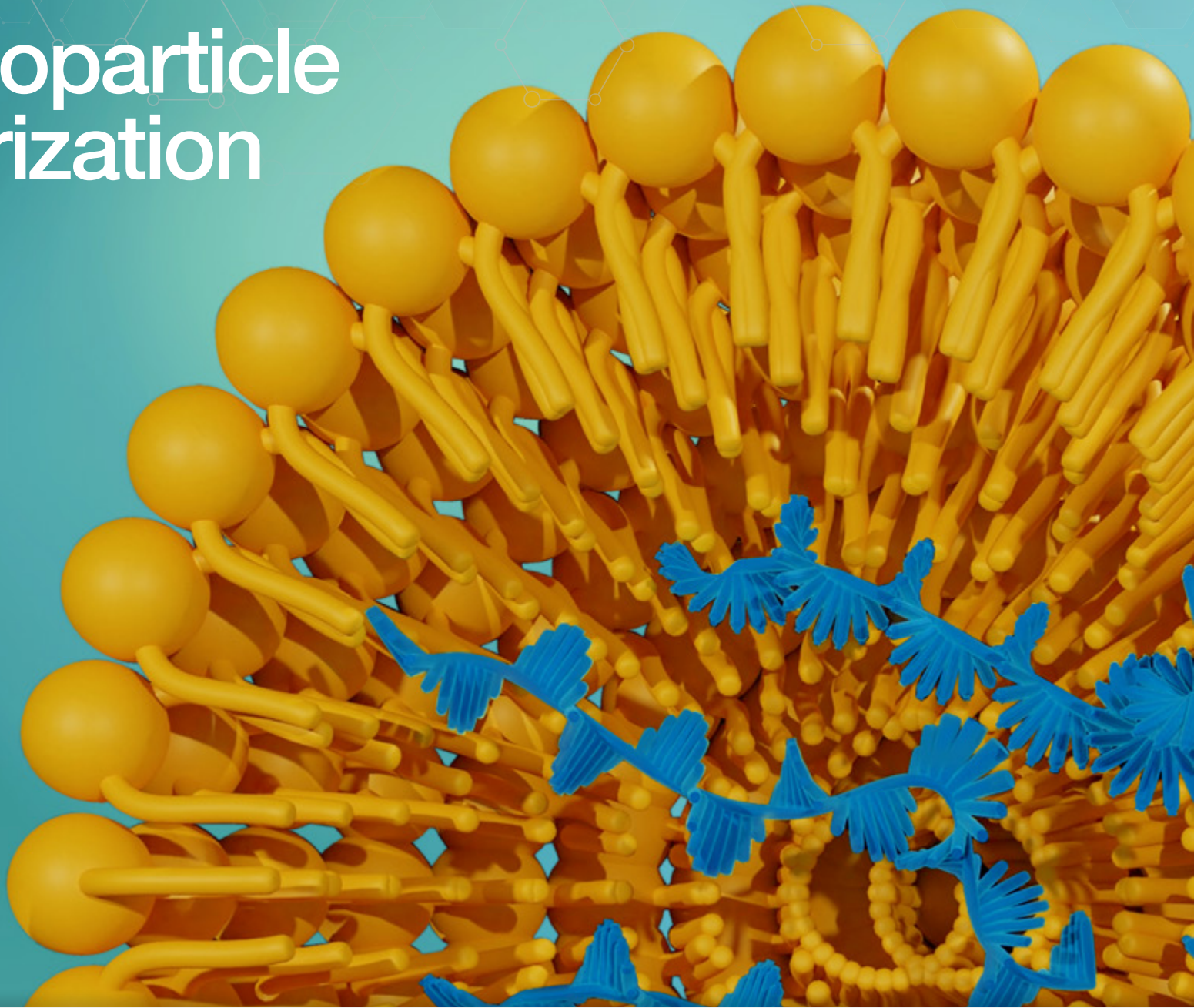
LNP composition analysis by LC-CAD

- **Fast high-resolution separation** of LNP components was achieved on the Thermo Scientific™ Accucore™ C30 column in 10 minutes.

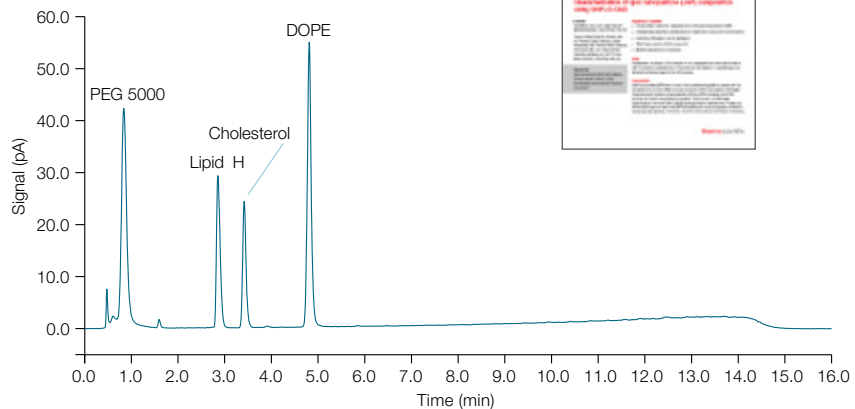- **Sensitive detection and accurate quantification** of all the components were obtained using Thermo Scientific™ Vanquish™ charged aerosol detector, the industry standard platform for lipid identity, lipid content and lipid impurity.

- 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

CAD response to 0.5 µg by flow injection

- Buspirone
- L-Ascorbic acid
- New coccine
- D-Glucose
- Lactose
- Diclofenac sodium salt
- L-Citruline
- Fluorescein
- Cortisone
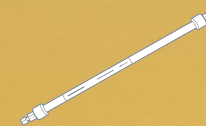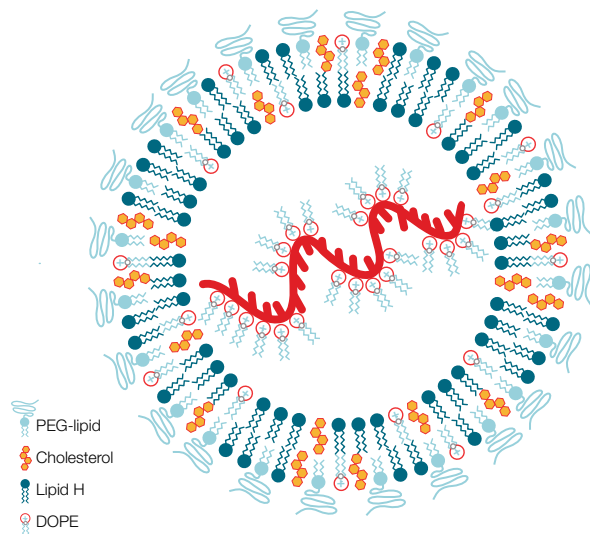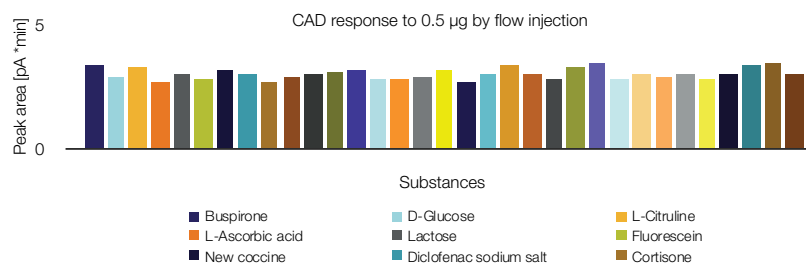
Thermo Scientific™ Accucore™ C30 HPLC Columns
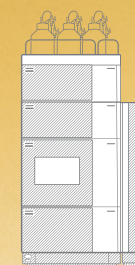
Thermo Scientific™ Vanquish™ System with Charged Aerosol Detector

PEG-lipid
Cholesterol
Lipid H
DOPE

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.

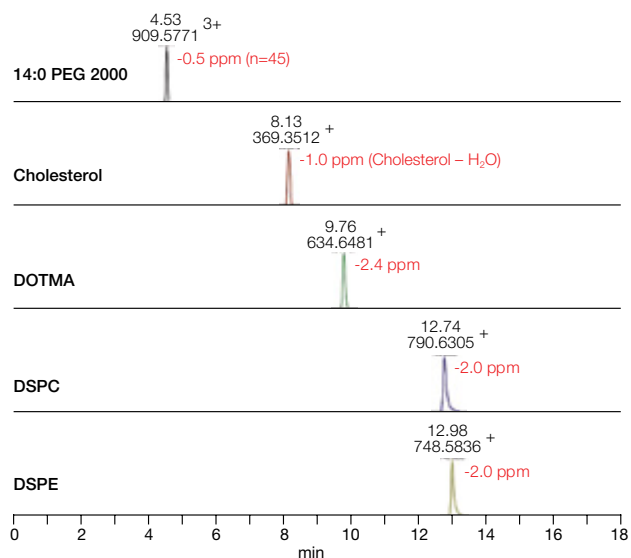- Sensitive detection and identification of impurities at **0.001%.**

- Simultaneous **lipid component quantification** and **metabolites characterization** in a single analysis.
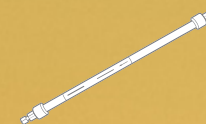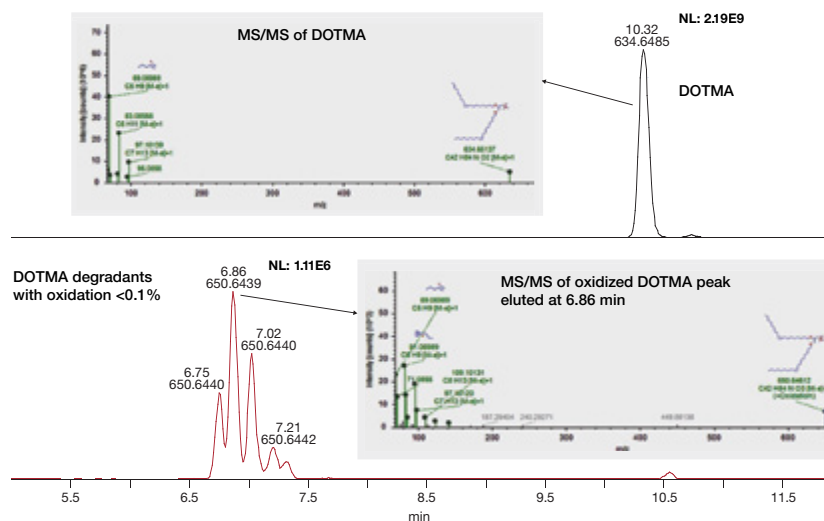
View complete application note

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

14:0 PEG 2000
4.53
909.5771 3+
-0.5 ppm (n=45)

Cholesterol
8.13
369.3512 +
-1.0 ppm (Cholesterol – H_2O)

DOTMA
9.76
634.6481 +
-2.4 ppm

DSPC
12.74
790.6305 +
-2.0 ppm

DSPE
12.98
748.5836 +
-2.0 ppm

Confident detection and characterization of low abundant metabolites

MS/MS of DOTMA

10.32
634.6485
NL: 2.19E9

DOTMA

DOTMA degradants with oxidation <0.1%

6.86
650.6439
NL: 1.11E6

7.02
650.6440

6.75
650.6440

7.21
650.6442

MS/MS of oxidized DOTMA peak eluted at 6.86 min

Thermo Scientific™ Accucore™ C30 HPLC Columns

Thermo Scientific™ Vanquish™ Horizon UHPLC System

Thermo Scientific™ Orbitrap Exploris™ 120 Mass Spectrometer

LNP component analysis by LC-MS
Applying UHPLC-HRAM MS technology to characterize and quantify lipid components in vivo to support new LNP development

- A sensitive and robust LC-MS based platform method to support efficient development and preclinical studies of novel lipid nanoparticles (LNPs)

- A single HRAM method for both quantification and characterization of LNP lipid components and their metabolites

Thermo Scientific™ Accucore™ C30 HPLC Columns

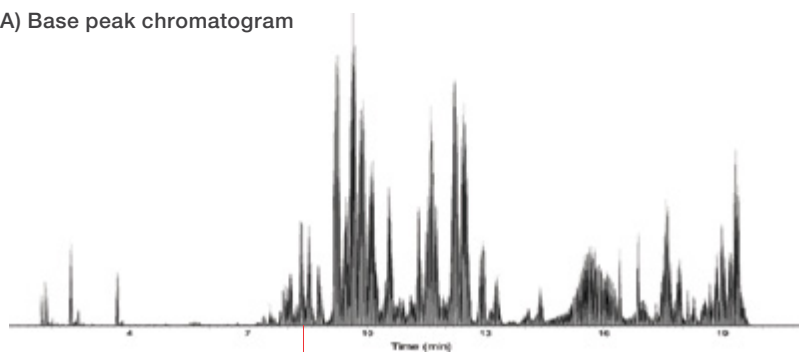### High quality MS/MS spectra for metabolite ID at 1 pg OC with 2 ppm mass accuracy

SM-102 quantification using targeted MS/MS.
A) The base peak chromatogram of control liver lipid extract with spiked-in SM-102 standard at 10 pg/µL.

B) Full scan MS spectrum observed at the retention time of 8.8 min where the SM-102 was eluted with other co-eluting lipid species. The relative intensity signal ratio of SM-102 (shown in the insert) vs. the strongest lipid species (precursor ion: m/z 703.5737) was less than 0.2%.

C) MS/MS spectrum of the SM-102 observed with the targeted MS/MS experiment of m/z 710.6642. The fragment ions highlighted with red circles were used for SM-102 quantification.
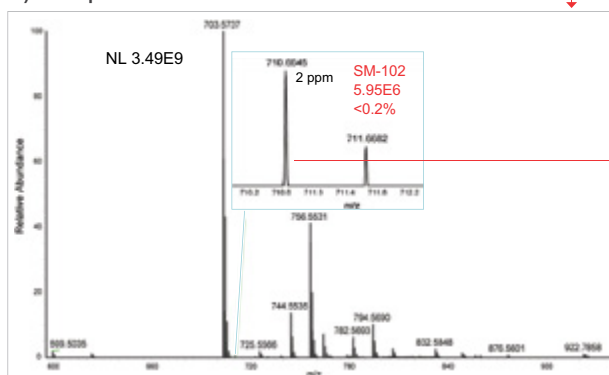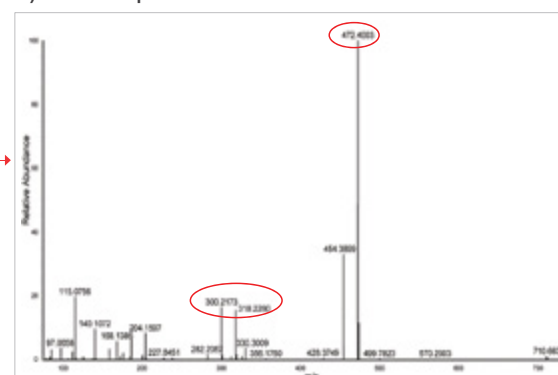
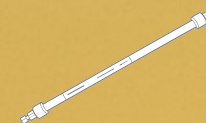Thermo Scientific™ Vanquish™ Flex UHPLC System

Thermo Scientific™ Vanquish™ Horizon UHPLC System

Thermo Scientific™ Orbitrap Exploris™ 480 Mass Spectrometer

A) Base peak chromatogram

View complete application note

B) MS spectrum at RT 8.8 min

C) MS/MS spectrum of SM-102
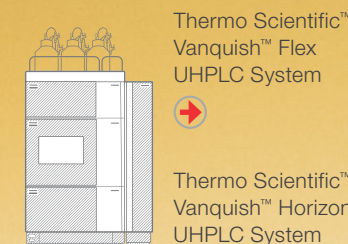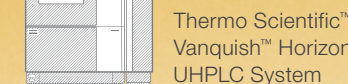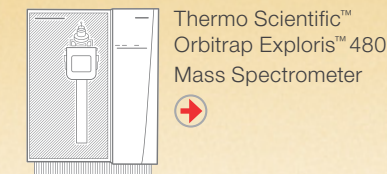
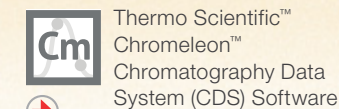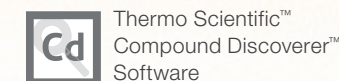Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) Software

Thermo Scientific™ Compound Discoverer™ Software

Featured products

Product name	Catalog number	
Orbitrap Exploris 480 Mass Spectrometer	BRE725533	→
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	→
Compound Discoverer Software	OPTON-31061	→
Chromeleon CDS	CHROMELEON7	→
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

Simultaneous reversed-phase and anion-exchange method scouting with a dual system for mRNA impurity determination →

Characterization of mRNA 5' capping products using an LC-HRAM-MS/MS analytical platform and Thermo Scientific BioPharma Finder software solution →

Characterization of *in vitro*-transcribed (IVT) mRNA poly(A) tail by LC-HRAM-MS and BioPharma Finder 5.0 software →

mRNA direct sequence mapping using automated partial digestion with magnetic nuclease and LC-HRMS →

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 →

Applying UHPLC-HRAM MS technology to characterize and quantify lipid components *in vivo* to support new LNP development →

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 →

Insights into direct mRNA sequencing LC-MS →

← Back to contents

Learn more at **thermofisher.com/vaccineanalysis**

thermo scientific