The role of universal detection in modern liquid chromatography

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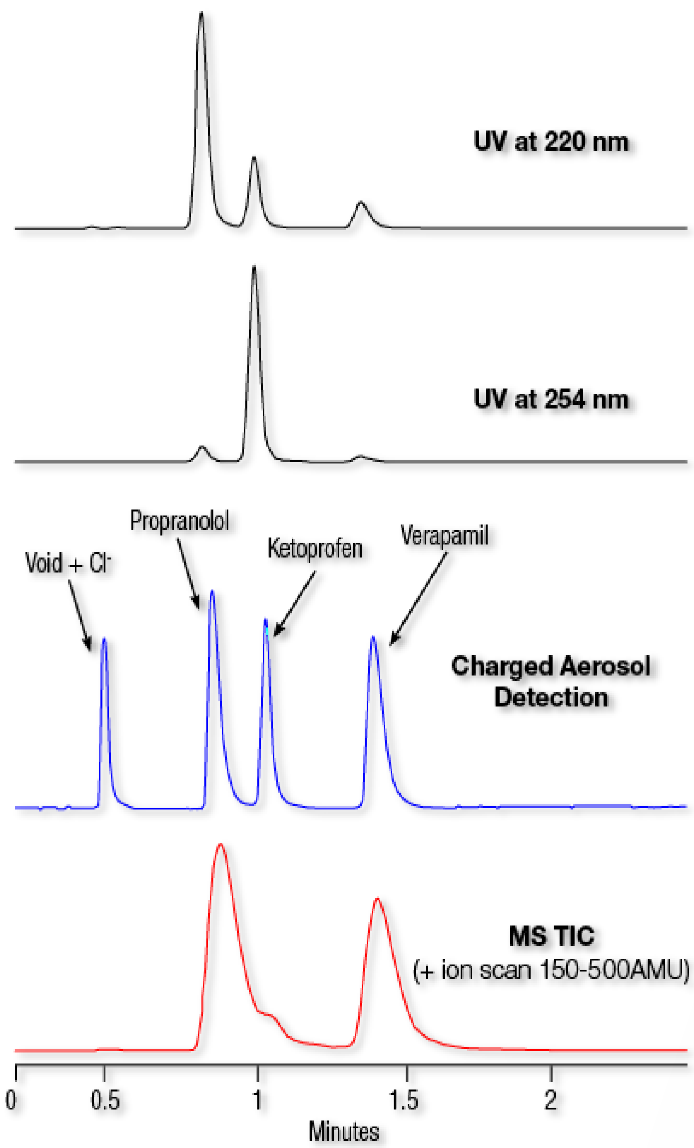
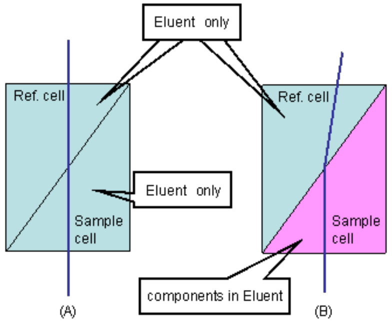
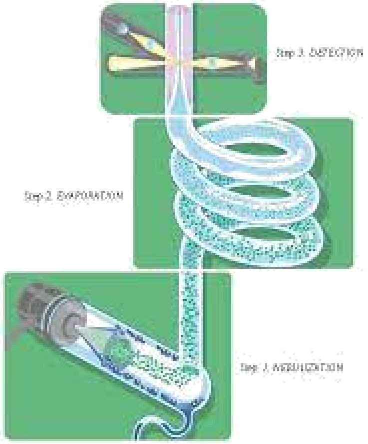
What is universal detection?

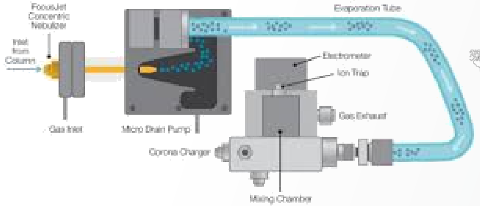


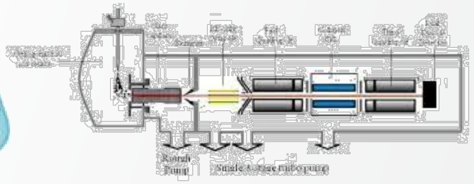
- A universal detector is characterized by providing a response for every component within eluent except mobile phase
- Contrary to selective detectors they do not rely on specific molecule properties (e.g., chromophore)

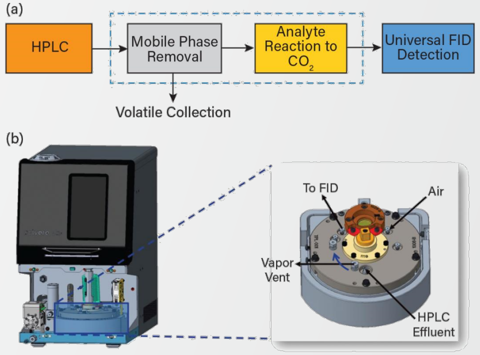
Comparison of universal detectors











Refractive Index Detector (RID)

Evaporative Light-Scattering Detector (ELSD)

Charged Aerosol Detector (CAD)

Mass Spectrometer (MS)

Flame Ionization Detector (FID)

Comparison of universal detectors

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	RID	ELSD	CAD	MS	FID
Costs	$	$$	$$	$$$$	$$$
Sensitivity	Low	Medium	Medium-high	Very high	Very low
Linearity	High	Low	Medium	High	High
Ease-of-use	High	Medium	High	Low	Low
Scope of detection	All analytes	Nonvolatiles, some semivolatiles	Nonvolatiles, many semivolatiles	Ionizable compounds	Nonvolatiles, few semivolatiles
Advantages	True universal detector for all analytes	No need for LC-MS grade solvents with assay	Uniformity of response, wide dynamic range	Specific analyte information	Potential uniformity of response
Limitations	No gradients, temperature sensitivity, assay only	Needs volatile additives, no combined impurity & assay	Needs LC-MS grade solvents, needs volatile additives	Complexity of use	Lack of robustness, assay only

Use of universal detectors

- Universal detectors inherently have lower performance compared to selective detectors
- Best use cases:
  - Complementary detector for higher data confidentiality
  - When selective detectors cannot be applied

Common HPLC detection methods

Detector	Detection limit	Destructive?
UV-Vis	ng	No
Fluorescence	fg	No
Refractive Index	µg	No
Evaporative Light Scattering	ng	Yes
Charged Aerosol	pg	Yes
Mass Spectrometry	pg	Yes

Learn more at thermofisher.com/howhplcdetectorswork

Impurity analysis with multi-detector set-up

CAD
Uniform response quantification

UV
Additional information, LC control, transfer to routine

MS
Analyte confirmation

Thermo Scientific™ Vanquish™ Duo UHPLC systems for Inverse Gradient

Thermo Scientific™ Vanquish™ ISQ EM Single Quadrupole Mass Spectrometer

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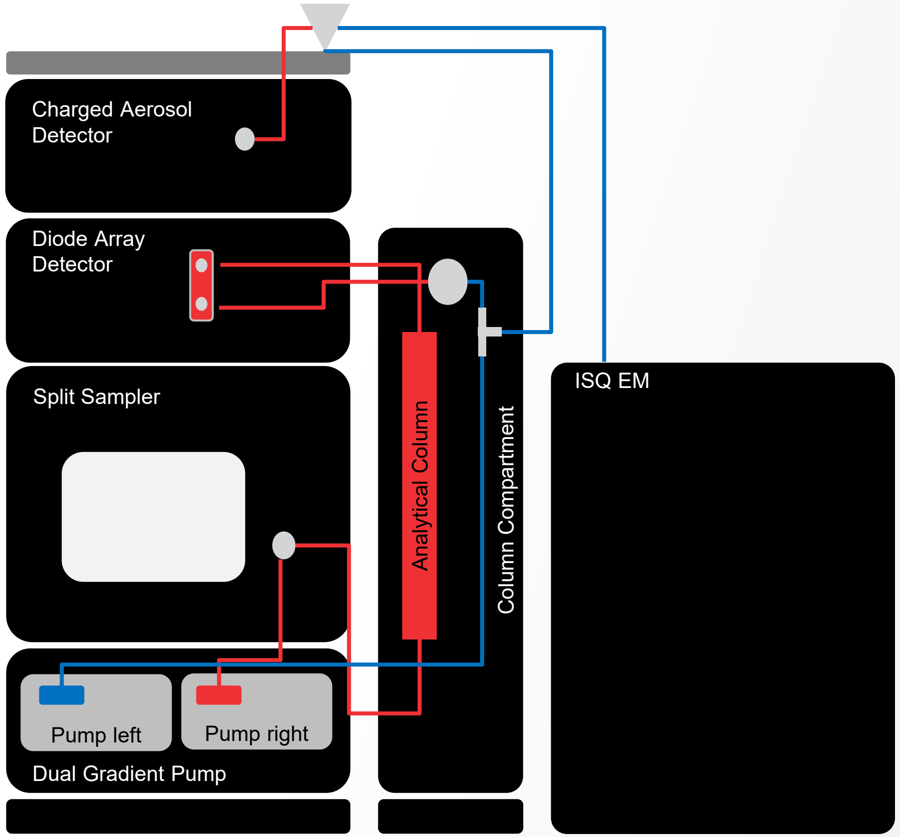

Vanquish Duo UHPLC systems for Inverse Gradient

Vanquish ISQ EM Single Quadrupole Mass Spectrometer

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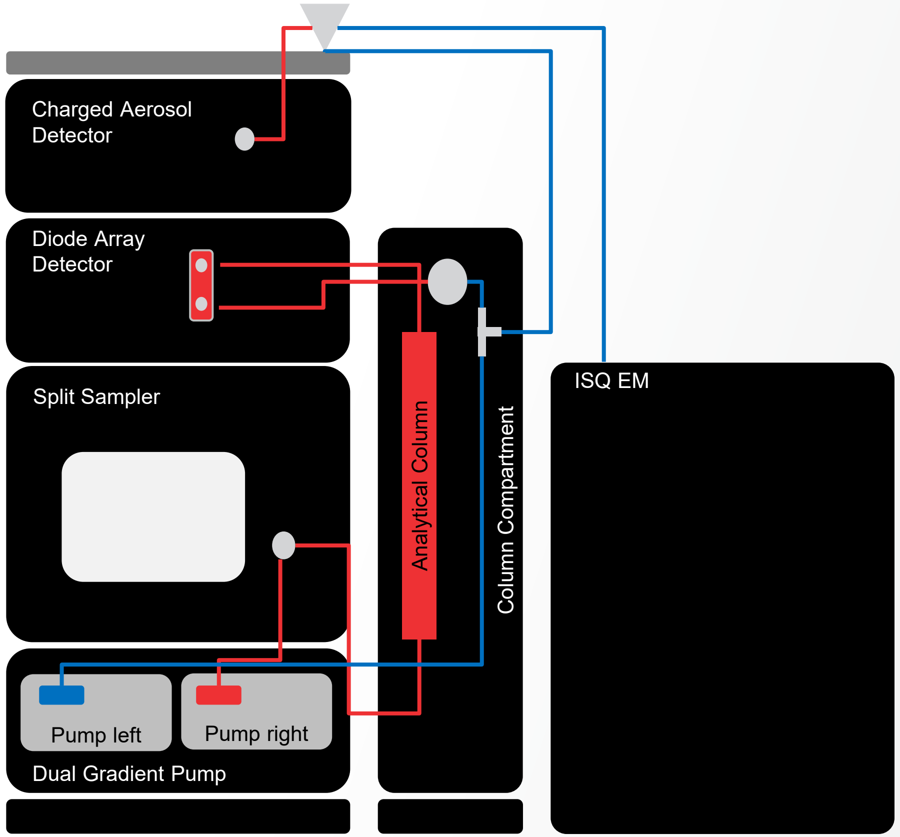

Vanquish Duo UHPLC systems for Inverse Gradient

Vanquish ISQ EM Single Quadrupole Mass Spectrometer

Analysis of single-use cell culture bag extracts

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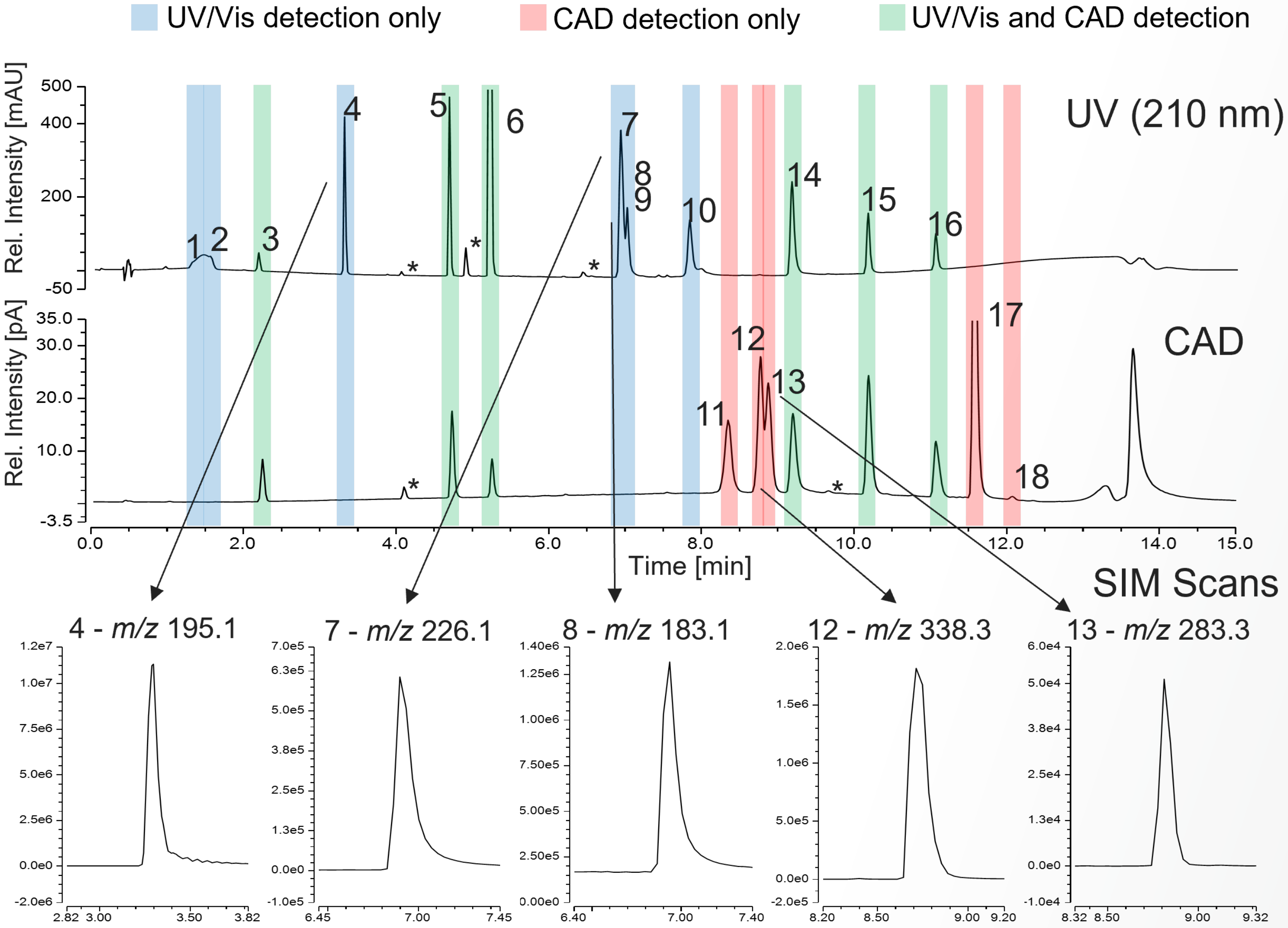
Comprehensive analysis with complementary detectors

18 extractables analyzed with standard multi-detector setup



#	Analyte	UV	CAD	MS	Mass Found	LOQ (µg/mL)
1	Phthalide	✓		✓	135.1	5 (UV)
2	Phthaldialdehyde	✓		✓	135.1	5 (UV)
3	BHET	✓	✓	✓	255.1	1
4	Dimethyl phthalate	✓		✓	195.1	1 (UV)
5	Bisphenol A	✓	✓	✓*	227.2	1
6	Butylparaben	✓	✓	✓	195.1	50
7	Tinuvin P	✓		✓	226.1	1 (UV)
8	Azobenzene	✓		✓	183.1	1 (UV)
9	2,4-di-t-Butylphenol	✓				1 (UV)
10	BHT	✓		✓	219.2	1 (UV)
11	Palmitic acid		✓	✓	255.2	1
12	Erucamide		✓	✓	338.3	1
13	Stearic acid		✓	✓	283.3	1
14	Tinuvin 234	✓	✓	✓	448.2	1
15	Irganox 1010	✓	✓	✓	1193.8	1
16	Irgafos 168	✓	✓	✓	645.4	1
17	Eicosane		✓			10
18	Tetracosane		✓			10

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Lipid Nanoparticles (LNPs)

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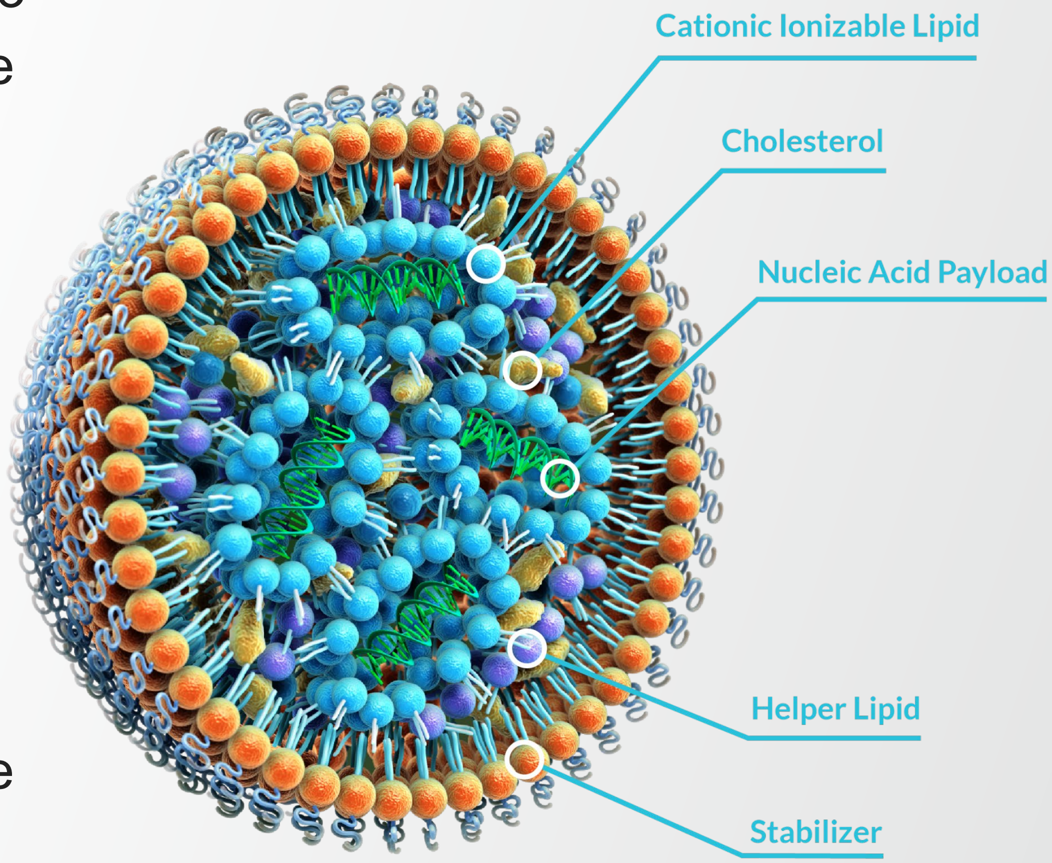
Therapeutic RNA

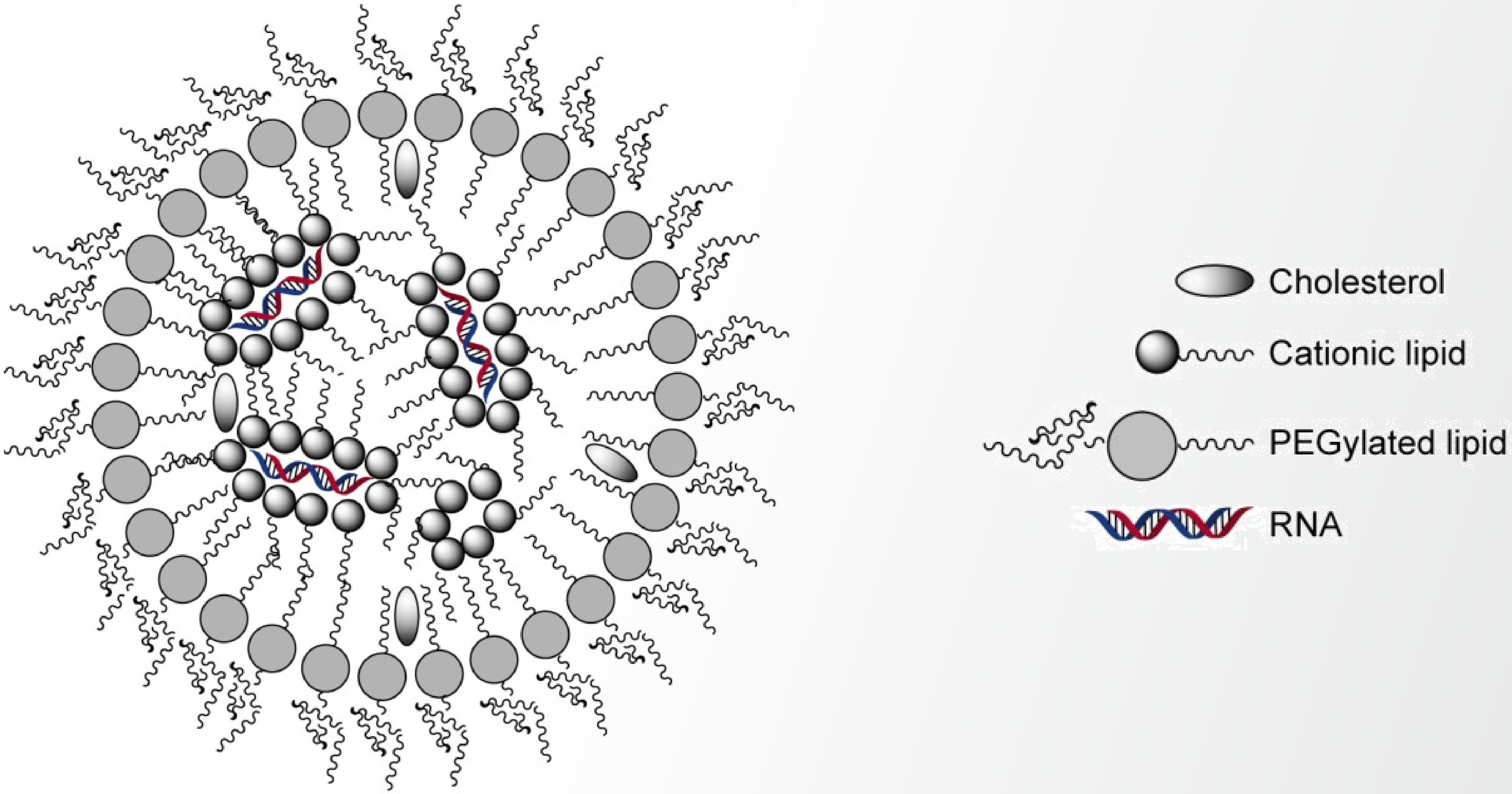
- In 2018, the FDA approved Alnylam's siRNA drug Onpattro (patisiran) for rare genetic neuropathy, the first drug to use LNPs as the drug delivery system
- Lipids intended for short siRNA strands did not work well for much longer mRNA strands, which led to extensive research during the mid-2010s into the creation of novel ironable cationic lipids appropriate for mRNA
- As of late 2020, several mRNA vaccines for SARS-CoV-2 use LNPs as their drug delivery system, including both the Moderna and Pfizer-BioNTech COVID-19 vaccines



https://www.precisionnanosystems.com/workflows/formulations/lipid-nanoparticles
https://www.gowinglife.com/why-lipid-nanoparticles-are-the-future-of-gene-therapy-and-what-needs-to-happen-next/

Schematic structure of lipid nanoparticles

Lipid nanoparticles (LNPs) are the most clinically advanced non-viral drug delivery system



Timofei S Zatsepin et al, Lipid nanoparticles for targeted siRNA delivery-going from bench to bedside, Int J Nanomedicine, 2016 https://doi.org/10.2147/IJN.S106625

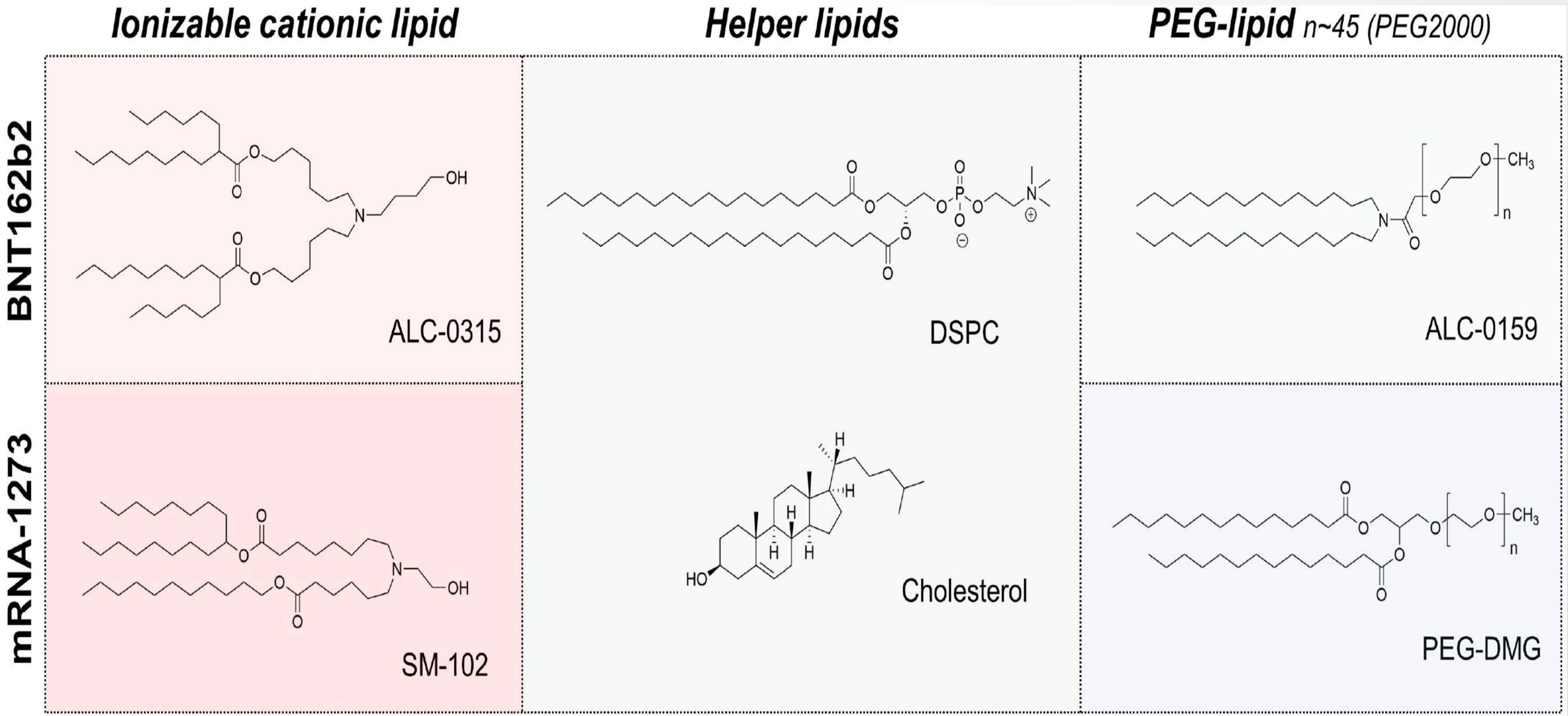
LNPs used in mRNA vaccines

LNPs used in mRNA vaccines for SARs-CoV-2 are made of four types of lipids:

	Moderna	**BioNTech / Pfizer**
An ionizable cationic lipid	SM-102 (its own proprietary)	ALC-0315 (from **Acuitas**)
A PEGylated lipid (for stability)	PEG-2000-DMG	ALC-0159
A phospholipid (for structure)	DSPC	DSPC
Cholesterol (for structure)	Same as BioNTech/Pfizer	Same as Moderna

L. Schoenmaker et al. mRNA-lipid nanoparticle COVID-19 vaccines: Structure and stability. International Journal of Pharmaceutics 601 (2021) 120586 https://doi.org/10.1016/j.ijpharm.2021.120586

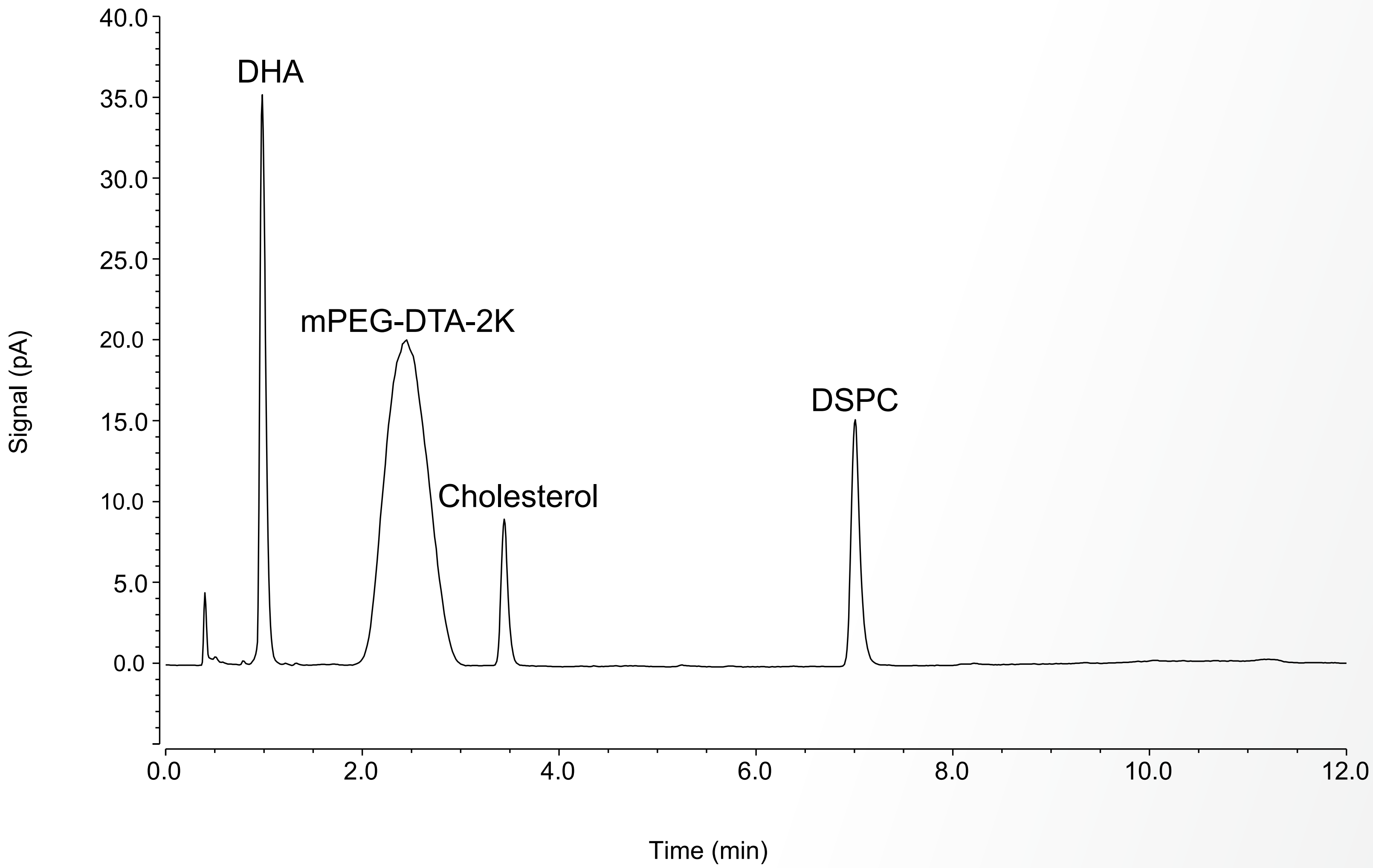
Lipids used in the mRNA-LNP COVID-19 vaccines



L. Schoenmaker et al. mRNA-lipid nanoparticle COVID-19 vaccines: Structure and stability. International Journal of Pharmaceutics 601 (2021) 120586 https://doi.org/10.1016/j.ijpharm.2021.120586

Characterization of LNP composition using UHPLC-CAD

Separation of LNP Standards



Column: Accucore C30, 2.6 µm
Format: 3.0 x 100 mm

Mobile phase A: 0.1% FA In Acetonitrile / Water (50:50)
Mobile phase B: 0.1% FA in Isopropanol / Acetonitrile / Water (60:30:10)

Needle wash: Mobile phase B

Gradient:

Time (min)	%A	%B
0.0	25	75
1.0	25	75
9.0	0	100
10.0	0	100
10.1	25	75
12.0	25	75

Flow rate: 0.9 mL/min
Inj. volume: 1 µL
Temperature: 50 ºC; Post-column cooler: 40 ºC
CAD setting: Power Function: 1.0; Gas resolution mode: Analytical; Evaporator Temperature: 35ºC; Filter: 3.6; Data rate: 2Hz.
System: Vanquish UHPLC

https://assets.thermofisher.com/TFS-Assets/CMD/Application-Notes/an-000465-uhplc-cad-lipid-nanoparticle-composition-an000465-na-en.pdf

Characterization of LNP composition using UHPLC-CAD

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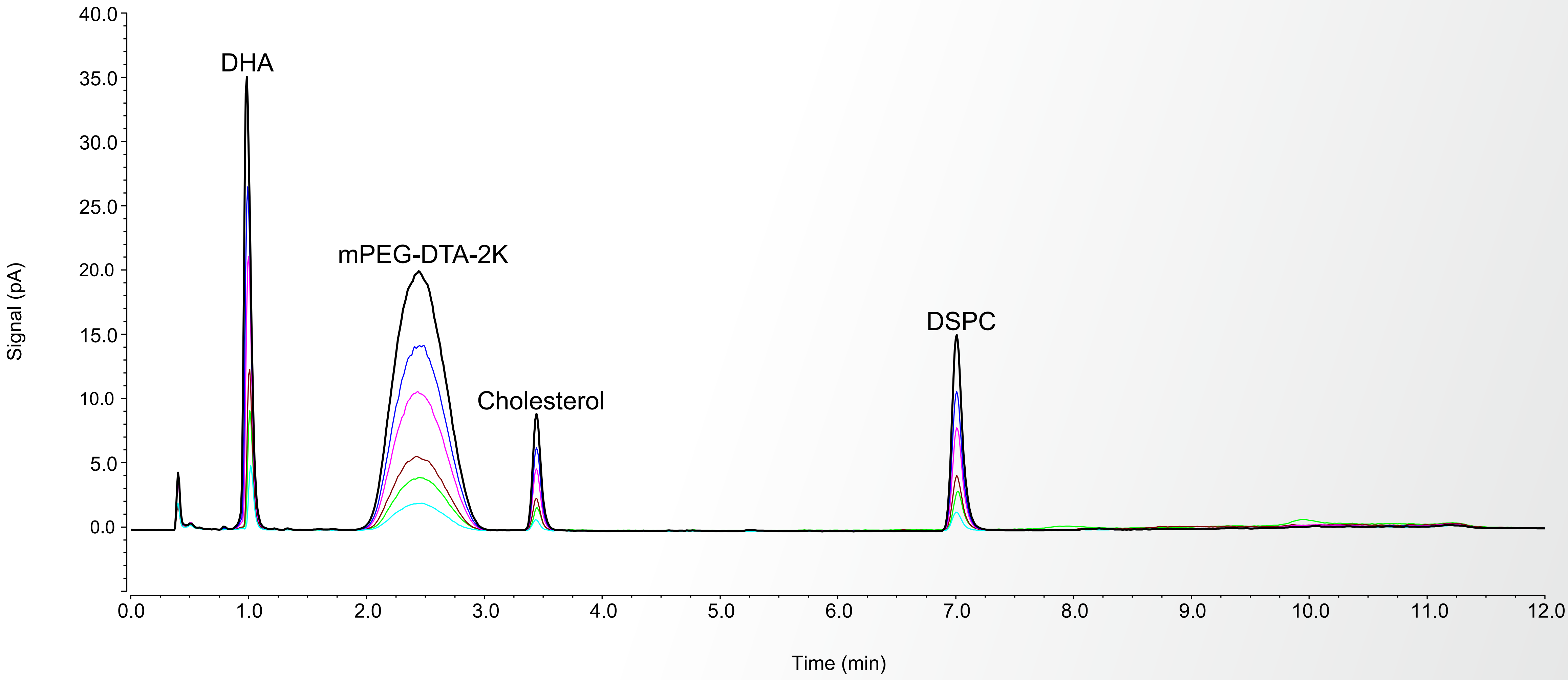
Overlaid chromatograms of calibration standards



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Characterization of LNP composition using UHPLC-CAD

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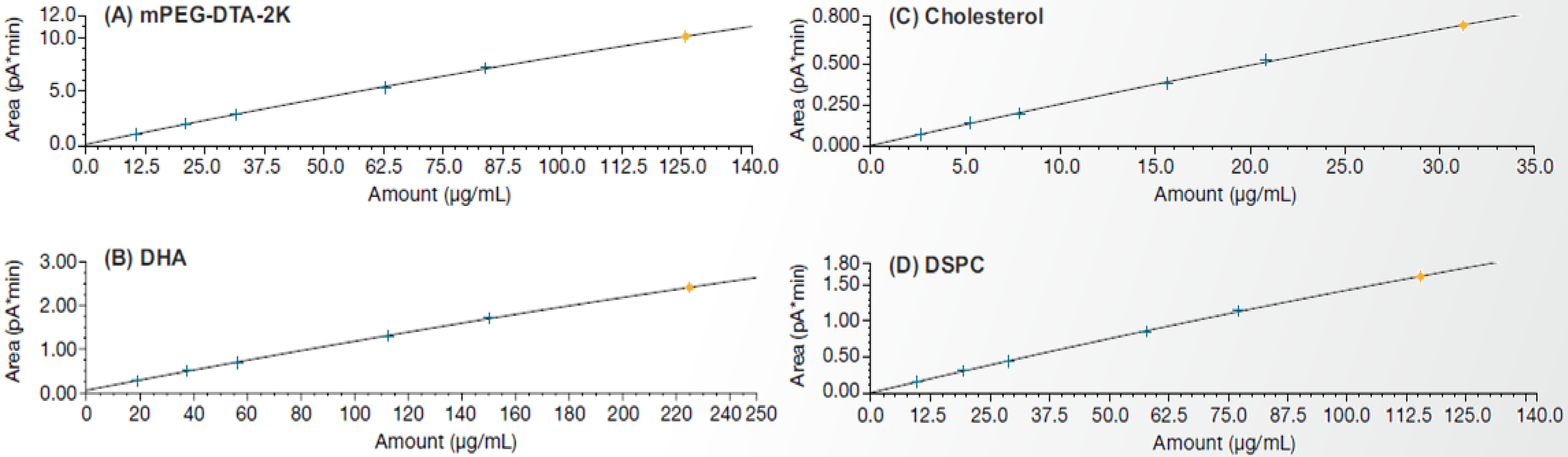
Calibration curves for the individual lipid components



Curve fit type: For CAD, it is possible to use either PFV optimization or nonlinear fits. Here a nonlinear fit (quadratic fit) was used.
Calibration coefficient >0.999

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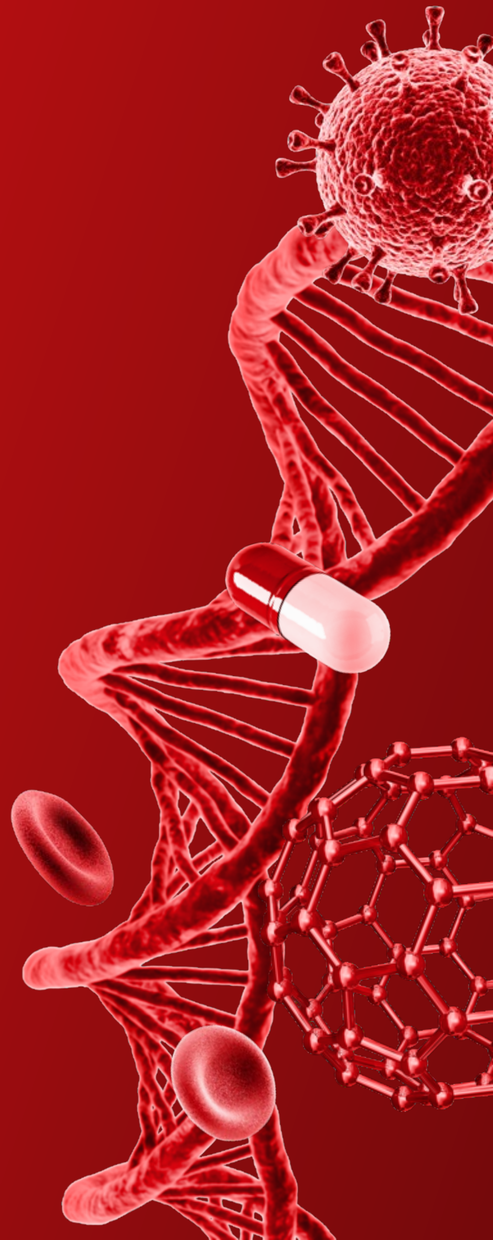

Thank you