Agilent

Trusted Answers

Composition Analysis of Lipid Nanoparticle Components with Agilent 1290 Infinity II ELSD

Authors

Ryu, Chae-Young
Korea Agilent Technologies
Co., Ltd.

Brian Liau
Agilent Technologies, Inc.

Abstract

Lipid nanoparticles (LNPs) used in mRNA vaccines typically consist of an ionizable lipid, neutral helper lipid, PEG-lipid, and cholesterol. As these components lack strong chromophores, they cannot be detected by UV absorbance.

This application note demonstrates quantitative analysis of LNP components using an Agilent 1290 Infinity II evaporative light scattering detector (ELSD). The developed method has a linear range of 5 to 1,000 μM and an interday RSD of ≤2%. The method is capable of sensitive and specific detection of forced degradation products.

Introduction

mRNA vaccines, which were recently developed in response to SARS-CoV-19, typically use LNPs as a delivery vector to muscle or antigen-presenting cells. LNPs prevent rapid degradation of mRNA *in vivo* and greatly enhance their uptake and expression in tissues.

LNPs generally consist of an ionizable lipid, neutral helper lipid, PEG-lipid, and cholesterol. The identities and relative abundance of these components are important quality attributes of the formulated product and can influence both stability and biological efficacy.[1,2] Ionizable lipids enhance the sequestration of mRNA within nanoparticles by electrostatic interactions, and PEG-lipids reduce mRNA cleavage by RNase enzymes. Targeting moieties may also be included on modified lipids to enhance receptor-mediated uptake.[1,2]
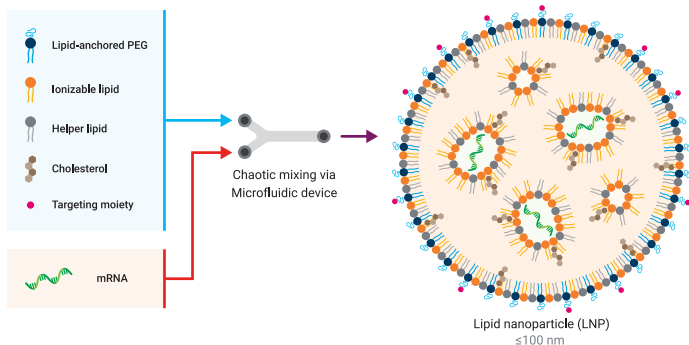
Figure 1. Structure of the [LNP1]

Lipid degradation products, such as N-oxides and aldehydes, are known to inhibit mRNA expression, so the quantification of such products is also of great importance.[2]

This application note demonstrates quantitative analysis of LNP components using HPLC separation and a 1290 Infinity II ELSD.

Experimental
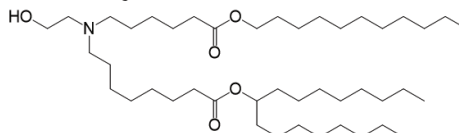
Standards and reagents

Lipids standards SM-102 (BroadPharm), DMG-PEG2000, DSPC, and cholesterol (Sigma-Aldrich) were acquired and used at ratios designed to mimic the mRNA vaccine developed by Moderna corporation (50:1.5:10:38.5).[3]

- ALC-0315: (4-Hydroxybutylbutyl)azanedyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate)
- ALC-0159: 2-[(Polyethylene glycol)-2000]-N,N ditetradecylacetamide
- DSPC: 1,2-Disteroyl-sn-glycero-3-phosphocholine
- SM-102: (Heptadecan-9-yl 8-((2-hydroxyethyl)(6-oxo-6-(undecyloyl)hexyl)amino)octanoate
- DMG-PEG2000: 1-monomethoxypolyethyleneglycol-2,3-dimyristylglycerol with polyethylene glycol of average molecular weight 2,000

The structures and molecular weights of the LNP components are shown in Figure 2.
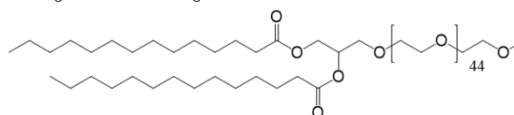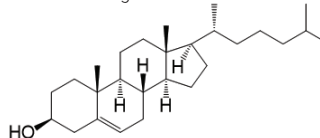

Figure 2. Chemical structure of LNP composition.
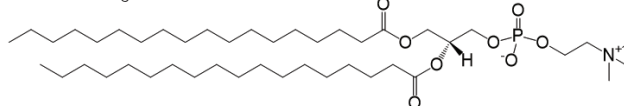
HPLC grade acetonitrile and methanol were purchased from B&J. Ethanol was purchased from Fisher, and ammonium formate was purchased from Sigma-Aldrich.

Preparation of LNP component standards

1. SM-102, DMG-PEG2000, DSPC, and cholesterol standards were dissolved in ethanol as 10 mM working solutions, then mixed further to achieve the final concentrations as shown in Table 1.

Table 1. Standard solution concentration.

Compound Name	Molecular Weight (g/mol)	Molecular Ratio (%)	Concentration (mM)	Concentration (mg/L)
SM-102	710.2	50	5	3,551.0
DMG-PEG2000	2,509.2	1.5	0.15	376.4
DSPC	790.1	10	1	790.1
Cholesterol	386.7	38.5	3.85	1,488.8
Sum	–	100	10	–

2. Two milliliters of the standard solution (Table 1) was then diluted to 10 mL in ethanol to yield a final concentration of 2 mM total lipids. This solution was then serially diluted to 1, 5, 10, 20, 50, 100, 200, 500, and 1,000 µM in ethanol.

3. For forced degradation experiments under acidic or basic conditions, 100 µL of 0.1 M HCl or NaOH was added to 100 µL of each lipid component and heated to 60 °C for 24 hours, then cooled to room temperature and neutralized with 100 µL of 0.1 M HCl or NaOH as appropriate, then made up to 1 mL with ethanol.

For forced oxidation experiments, 100 µL of 30% hydrogen peroxide was added to 100 µL of each lipid component and heated to 60 °C for 24 hours, then cooled to room temperature and made up to 1 mL with ethanol.

HPLC-ELSD instrumentation and settings

Table 2. Agilent HPLC analysis conditions.

| Parameters	Value
Instrument	Agilent 1260 Infinity II HPLC, Agilent 1290 Infinity II ELSD (cooled)
Column	Agilent Poroshell CS-C18, 2.1 × 100 mm, 2.7 µm
Flow Rate	0.6 mL/min
Column Temperature	40 °C
Injection Volume	5 µL
Sampler Temperature	4 °C
ELSD Conditions	Evaporator temperature 80 °C Nebulizer temperature 80 °C Gas flow rate 1.6 SLM
Mobile Phase	A: 5 mM ammonium formate in D.W./acetonitrile/methanol = 25/35/40 B: 5 mM ammonium formate in methanol/ethanol = 60/40
Gradient	Time (min) %A %B 0 85 15 1 85 15 30 0 100 35 0 100 35.1 85 15 42 85 15

Results and discussion

Chromatographic separation, quantitative range, and repeatability

Figure 3 shows baseline separation of the components of a standard solution consisting of 1 mM total lipid concentration. Strong ELSD signals were observed for all compounds despite the most abundant component (SM-102) being 33-fold more concentrated than the least abundant component (DMG-PEG2000).

To determine the quantitative range for each component, standard solutions ranging from 5 to 1,000 µM total lipid concentration were analyzed using the developed method (Figure 4). Calibration curves were constructed for each component and fitted with a quadratic function with R^2 ≥0.99 (Figure 5).
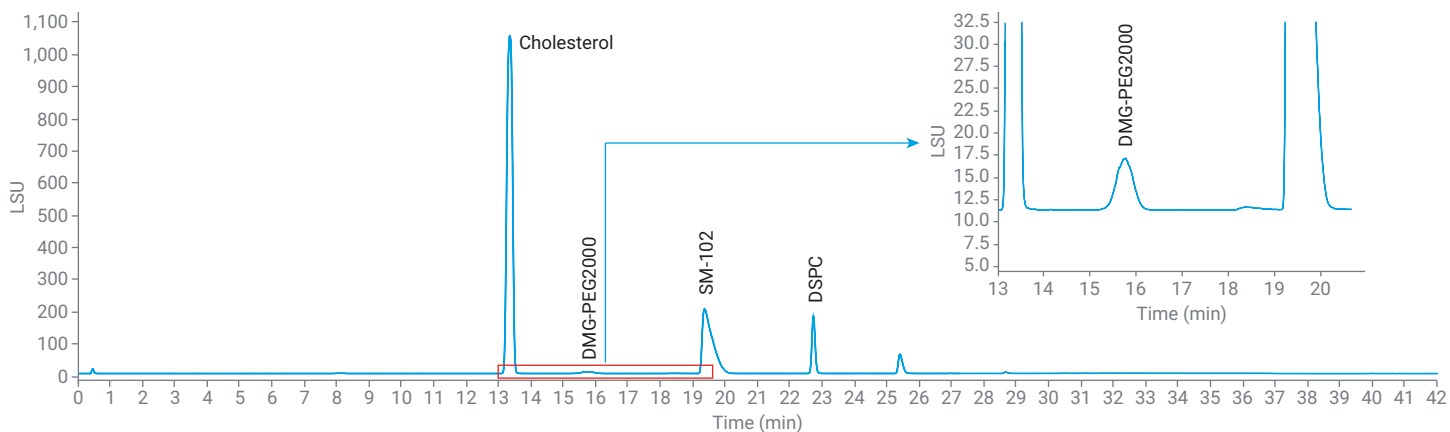
Figure 3. Chromatography of SM-102, DMG-PEG2000, DSPC, and Cholesterol.
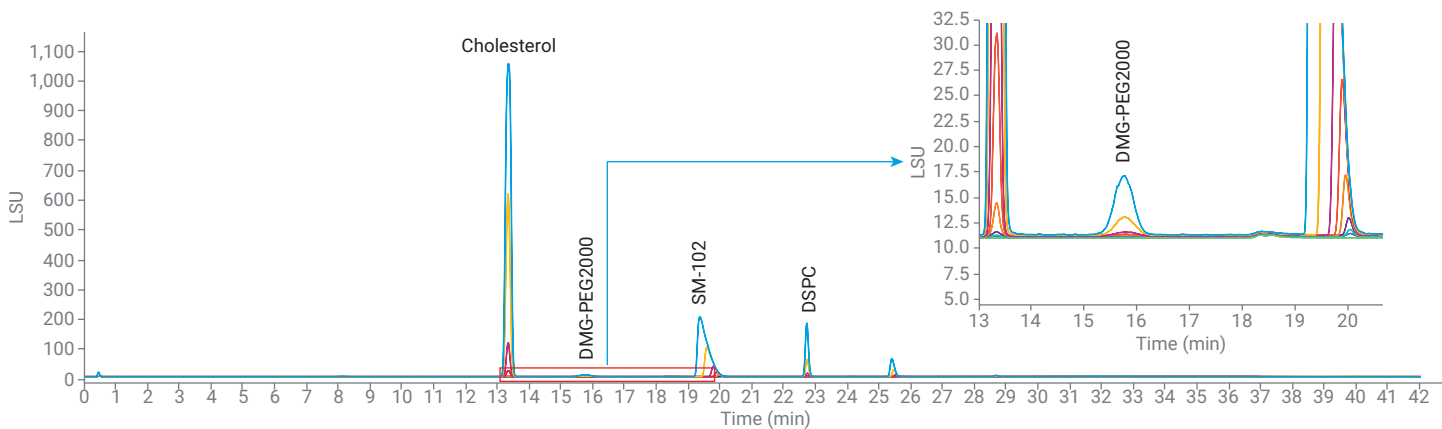
Figure 4. Overlaid chromatograms of standard solutions ranging from 5 to 1,000 µM total lipid concentration.

Cholesterol, 13.314 min
Formula:
$y = 0.0040 x^2 + 9.2867x - 440.7033$
r = 0.99634
R^2 = 0.99268

Residual standard deviation:
453.99784
Origin: Ignore
Weighting method: None

DMG-PEG2000, 15.743 min
Formula:
$y = 0.0001 x^2 + 0.0439x - 1.0637$
r = 0.99998
R^2 = 0.99996

Residual standard deviation:
0.43499
Origin: Ignore
Weighting method: None

SM-102, 19.229 min
Formula:
$y = 0.0023 x^2 + 2.1552 x - 33.2317$
r = 0.99967
R^2 = 0.99934

Residual standard deviation:
40.68915
Origin: Ignore
Weighting method: None

DSPC, 22.682 min
Formula:
$y = 0.0009 x^2 + 0.3217x - 8.8112$
r = 0.99987
R^2 = 0.99974

Residual standard deviation:
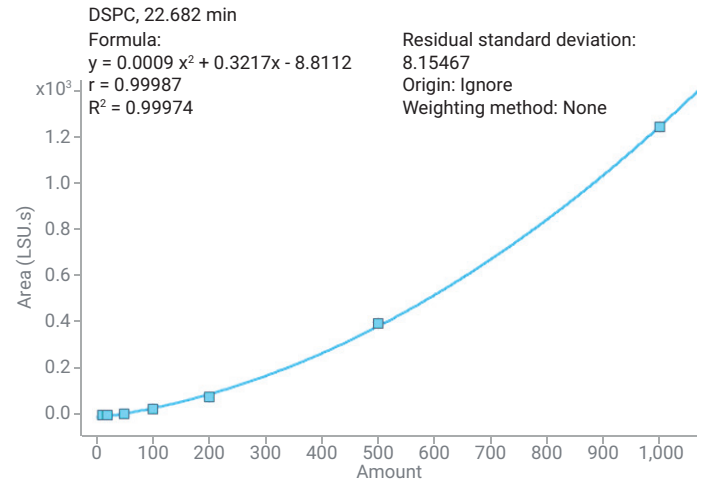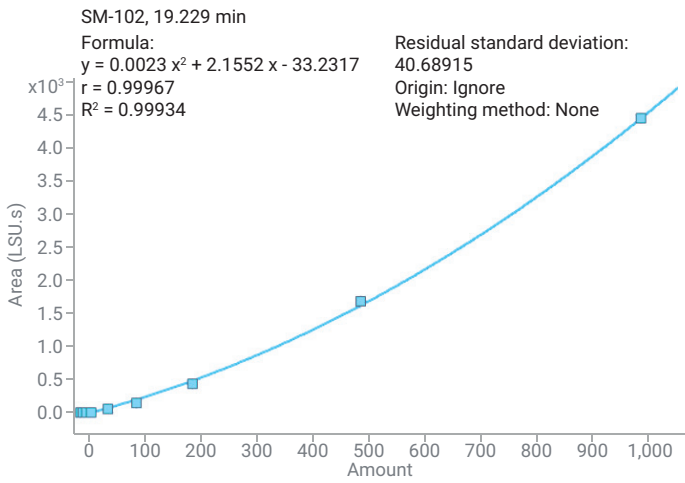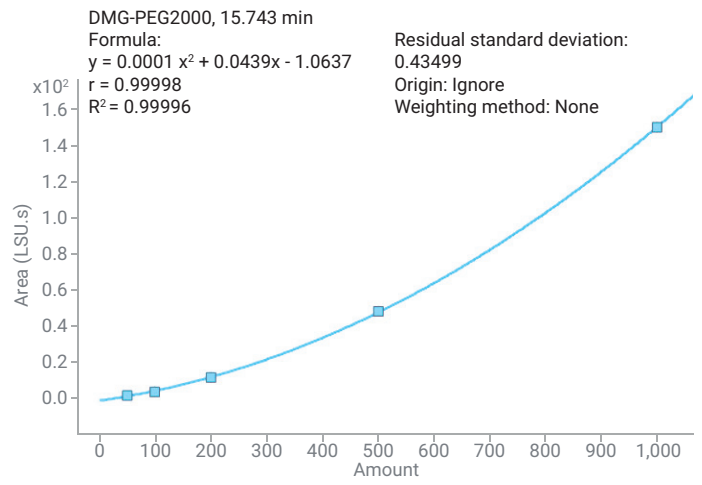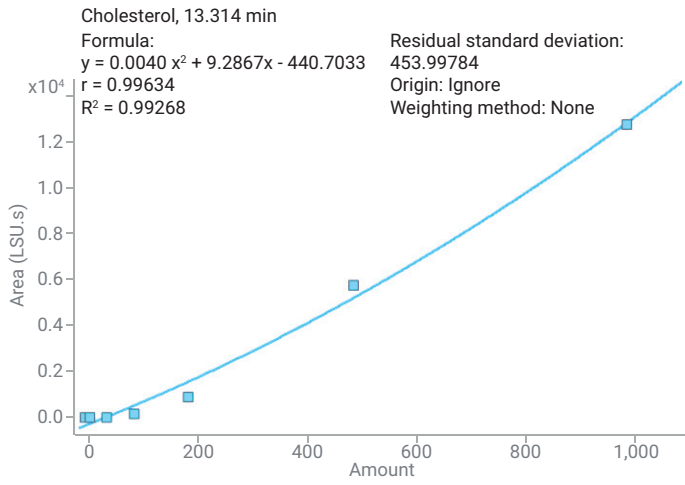8.15467
Origin: Ignore
Weighting method: None

Figure 5. Calibration curves of cholesterol, DMG-PEG2000, SM-102, and DSPC.

To determine the limit of quantitation (LOQ), the signal-to-noise ratio of each component was determined over the range of tested concentrations. The LOQ was defined by a signal-to-noise ratio of ~10. As shown in Table 3, the LOQ for cholesterol and DSPC were 10 µM, SM-102 was below 5 µM, and DMG-PEG2000 was 100 µM, underscoring the high sensitivity of the method.

The formula used to calculate the signal-to-noise ratio is as follows:

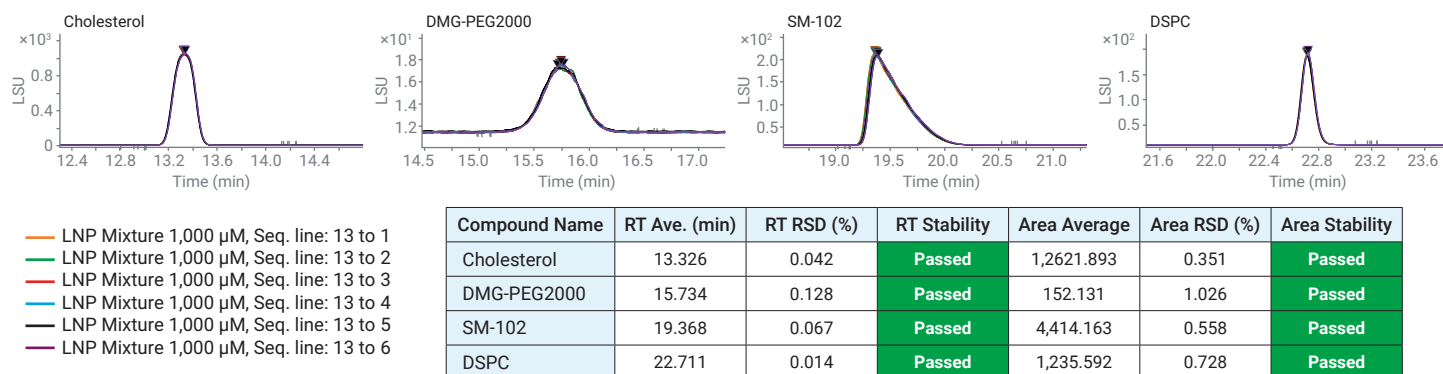$$\text{Signal-to-noise} = \frac{2H}{h}$$

H: Peak height

h: Height of noise

To establish repeatability, six repeated measurements of the 1 mM total lipid concentration standard solution confirmed that all four substances were within 2% relative standard deviation (Figure 6).

Table 3. Signal-to-noise ratio results for each LNP component.

Compound Name	Parameter	1 µM	5 µM	10 µM	20 µM	50 µM	100 µM	200 µM	500 µM	1,000 µM
Cholesterol	Concentration (mg/L)	0.15	0.74	1.49	2.98	7.44	14.89	29.78	74.44	148.88
	Signal-to-noise	–	–	12.7	27.0	213.8	1,554.5	10,451.9	35,973.3	84,680.2
DMG-PEG2000	Concentration (mg/L)	0.04	0.19	0.38	0.75	1.88	3.76	7.53	18.82	37.64
	Signal-to-noise	–	–	–	–	4.4	12.0	37.3	106.3	467.2
SM-102	Concentration (mg/L)	0.36	1.78	3.55	7.10	17.76	35.51	71.02	177.55	335.10
	Signal-to-noise	–	25.6	57.3	111.4	386.8	1,200.5	3,151.2	5,801.3	16,125.7
DSPC	Concentration (mg/L)	0.08	0.40	0.79	1.58	3.95	7.90	15.80	39.51	79.01
	Signal-to-noise	–	–	9.8	19.7	77.5	285.9	1,044.9	3,386.0	14,406.3

Blue highlight = concentration and signal-to-noise ratio results when the signal-to-noise ratio is approximately 10.

— LNP Mixture 1,000 µM, Seq. line: 13 to 1
— LNP Mixture 1,000 µM, Seq. line: 13 to 2
— LNP Mixture 1,000 µM, Seq. line: 13 to 3
— LNP Mixture 1,000 µM, Seq. line: 13 to 4
— LNP Mixture 1,000 µM, Seq. line: 13 to 5
— LNP Mixture 1,000 µM, Seq. line: 13 to 6

Compound Name	RT Ave. (min)	RT RSD (%)	RT Stability	Area Average	Area RSD (%)	Area Stability
Cholesterol	13.326	0.042	**Passed**	1,2621.893	0.351	**Passed**
DMG-PEG2000	15.734	0.128	**Passed**	152.131	1.026	**Passed**
SM-102	19.368	0.067	**Passed**	4,414.163	0.558	**Passed**
DSPC	22.711	0.014	**Passed**	1,235.592	0.728	**Passed**

Figure 6. LNP 1,000 µM (n = 6 replication results of standard solution).

Detection of impurities

Material that had been subjected to forced degradation under acidic, basic, or oxidative conditions were analyzed with the developed method to determine if degradation products could be observed. Several decomposition products were observed in SM-102 and DSPC as shown in Figure 7.
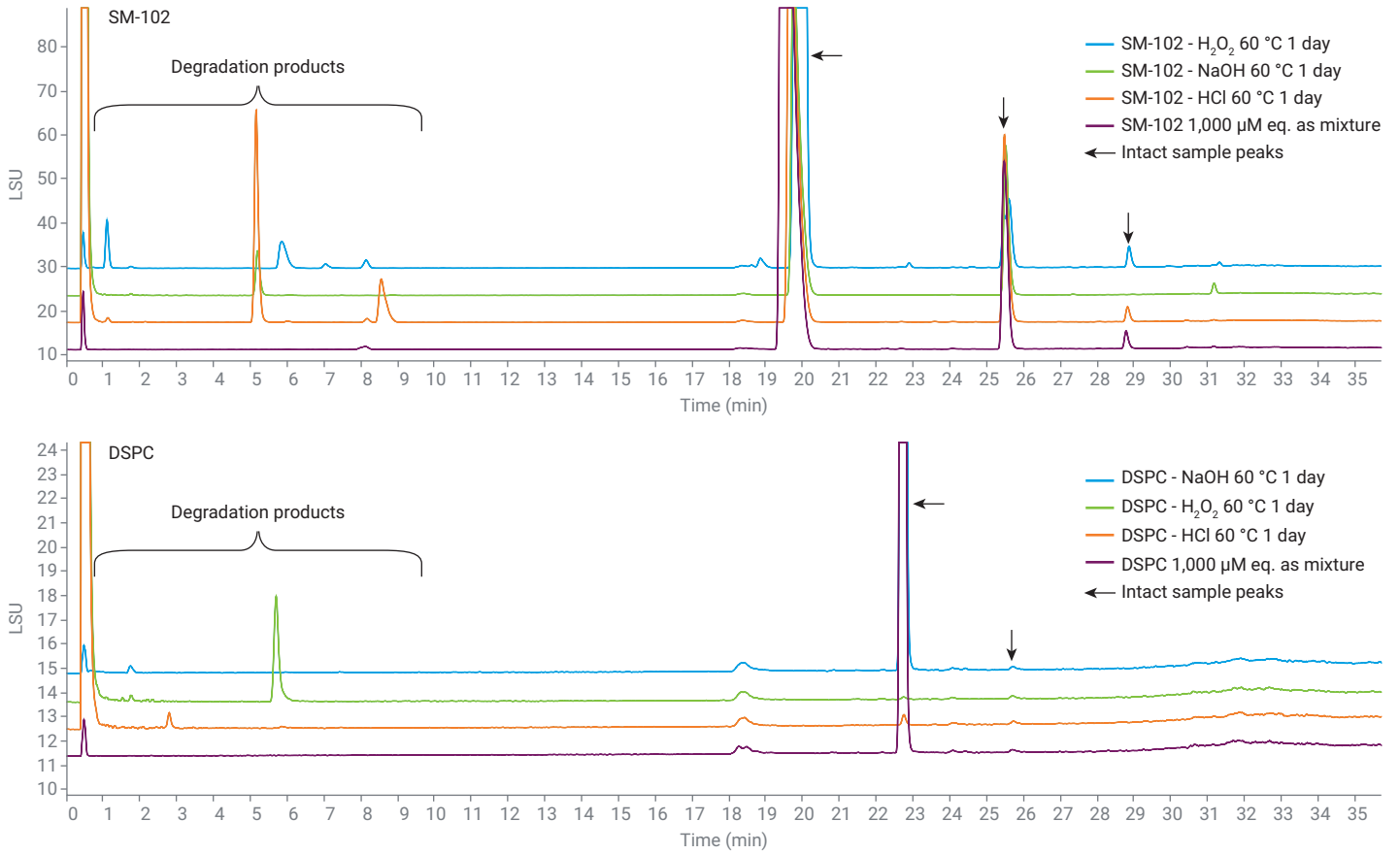

Figure 7. SM-102 and DSPC 1,000 µM. Results of the equivalent of forced degradation conditions.

Conclusion

LNP component standard solutions ranging from 5 to 1,000 µM were successfully analyzed using an Agilent 1260 Infinity II HPLC and an Agilent 1290 Infinity II ELSD with good quantitative range, linearity, sensitivity, and repeatability. SM-102 and DSPC were labile under acidic, basic, and oxidative conditions, and degradation products could be observed using the developed method. This work may be useful in studying the stability of LNP formulations.

Table 4. Summary of analysis results.

	Linearity (R^2)	Quantitative Limit (mg/L)	Repeatability (%RSD)
Cholesterol	0.99268	1.49	0.351
DMG-PEG2000	0.99996	3.76	1.026
SM-102	0.99934	1.78	0.558
DSPC	0.99974	0.79	0.728

References

1. Kelsey, L. *et al.* Lipid Nanoparticle-Mediated Delivery of mRNA Therapeutics and vVaccines, *Trends in Molecular Medicine*, June **2021**, *27(6)*.

2. Schoenmaker, L. *et al.* mRNA-lipid Nanoparticle COVID-19 Vaccines: Structure and Stability. *International Journal of Pharmaceutics*, *601*, **2021**

3. Meredith, P. *et al.* A Novel Mechanism for the Loss of mRNA Activity in Lipid Nanoparticle Delivery Systems. *Nature Communications*, **2021**, *12*, 6777.

www.agilent.com

DE14392059

Agilent

Trusted Answers