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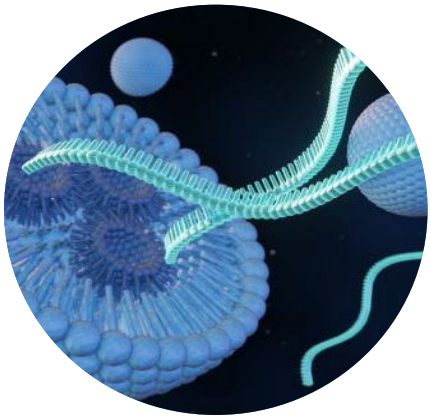
Lipid Nanoparticle Characterization by RP-HPLC-CAD on a C6-Phenyl Column

Jun Lu[1], Yidi Zhang[1], Liuliu Wu[1], Shilin Cheung[2] and Kevin Zhao[2]
[1]Bonna-Agela Technologies Co., Ltd, 179 South Street, West Zone, Tianjin Economic-Technological Development Area (TEDA), Tianjin, China, 300462
[2]Phenomenex Inc., 411 Madrid Ave., Torrance, CA 90501 USA



Introduction

Lipid Nanoparticles (LNPs) are effective delivery systems for gene therapies such as mRNA vaccines. LNPs contain four components (cholesterol, helper lipids, ionizable lipids, and PEGylated lipids) and the identity and content of each component are critical attributes of the LNP product. To determine these attributes, a reversed-phase liquid chromatographic method coupled with charged aerosol detection (RP-UHPLC-CAD) is often used with a C18 chromatographic column.[1] Herein, a 20 min RP-UHPLC-CAD method was developed, utilizing the Gemini(R) C6 Phenyl column, to improve chromatographic efficiencies.

Acknowledgement

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VectorBuilder

KeyWords

Lipid Nanoparticles (LNPs); Gemini C6-phenyl; HPLC; CAD; SM-102; DMG-PEG 2000; Cholesterol; DSPC

Table 1. Lipid Components

Component	CAS Number	Molecular Formula	Molecular Weight (g/mol)
SM-102	2089251-47-6	C44H87NO5	710.17
DMG-PEG 2000	160743-62-4	(C2H4O)nC32H62O5	2526.00
Cholesterol	57-88-5	C27H46O	386.65
DSPC	287399-26-2	C44H88NO8P	790.14

LC Conditions

Column: Gemini® 3 µm C6-Phenyl
Dimensions: 150 x 4.6 mm, 110Å
Part No.: 00F-4443-E0
Mobile Phase: A: 0.1% Trifluoroacetic Acid (TFA) in Water
B: 0.1% Trifluoroacetic Acid (TFA) in Methanol:Acetonitrile (3:2)

Gradient:

Time (min)	% B
0	85
15	100
15.1	85
20.0	85

Flow Rate: 1.0mL/min
Injection Volume: 10uL
Temperature: 30°C
LC System: Thermo Scientific™ Ultimate 3000
Detection: Charged Aerosol Detector (CAD)
Detector: Thermo Scientific™ Corona™ CAD

CAD Conditions

Data collection frequency: 10
Filtering: 5.0
Source Temperature: 35°C
Peak width: 0.02 min

Reagents & Materials

Water, trifluoroacetic acid, methanol, acetonitrile, and anhydrous ethanol were of chromatographic grade.

Samples

Purified lipid controls, shown in **Table 1**, were serially diluted with anhydrous ethanol into a range of concentrations. The LNP samples were demulsified by diluting with anhydrous ethanol (added at three times the volume of the LNP solution) and vortexed. Centrifugation was used to separate the the lipids into the supernatant which were extracted for chromatographic analysis.

Results and Discussion

Development of the Separation Method

The separation gradient and mobile phase were developed using the lipid standards. A high percentage of organic was needed for the elution of the lipids, i.e., from 85% to 100% of mobile phase B, see **Figure 1**. The elution order were SM-102, Cholesterol, DSPC and DMG-PEG 2000. All the analytes and their impurities were well resolved, with symmetrical peak shapes, see **Figure 2**. This indicated that the method may also be used for the quality control testing of the lipid starting materials.

Figure 1. The separation of SM-102 (7.472 min), Cholesterol (10.535 min), DSPC (13.545), DMG-PEG 2000 (12.896 min) and their impurities.

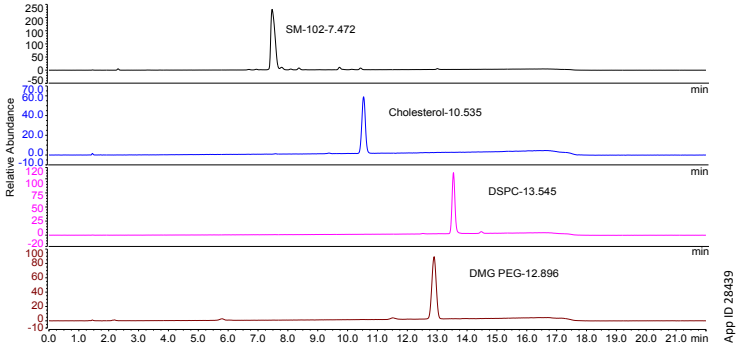
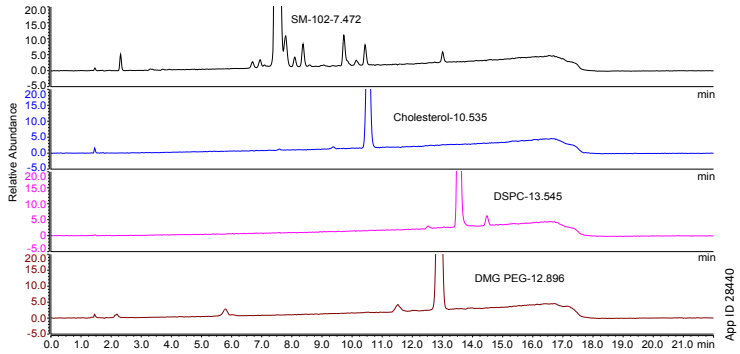


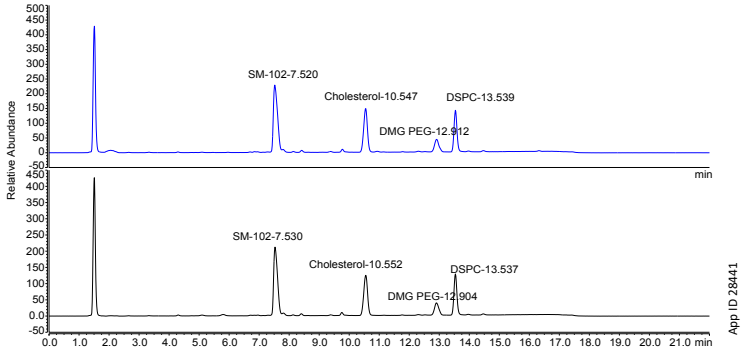
Figure 2 . Zoomed in chromatograms of lipid standards in **Figure 1** to show the separation of impurities within each standard.



Applicability to LNP Samples

Two LNP samples manufactured from two separate batches were tested. To demulsify the LNP samples, a 0.25x dilution into anhydrous ethanol and rapid vortex were performed. The lipids were then separated from the other impurities by centrifugation and the supernatant was injected into the LC system for chromatographic analysis. The chromatograms of the two LNP samples are shown in **Figure 3**. All four lipid components (SM-102, Cholesterol, DSPC and DMG-PEG 2000) were observed in both samples. The lipids were well resolved from each other and the relative amounts of the lipids in the samples were similar.

Figure 3 . The separation of two LNP samples from batch 1 (top) and batch 2 (bottom).



Analytical performance

A preliminary study of on-column recovery was performed by plotting the peak area response versus a range of lipid concentrations, see **Figures 4, 5, 6, and 7**. A good correlation was observed for the four lipid standards, where the R^2 were greater than 0.9999 and thus, siuggested good on column recoveries for the ranges tested. For SM-102 and DMG-PEG 2000 the samples lied within the standard curve. For Cholesterol, one of the samples was outside the bracketed range and for DSPC, both samples lied outside the bracketed range. As such, further lipid ratio calculations were not performed. The retention times (RT) for the lipid standards and samples were highly reproducible (all %RSD of RT < 0.5% for n = 3) and it allowed confident identification of the four lipid analytes in the samples.

Figure 4 . Standard curve of SM-102 and the determination of two LNP samples.

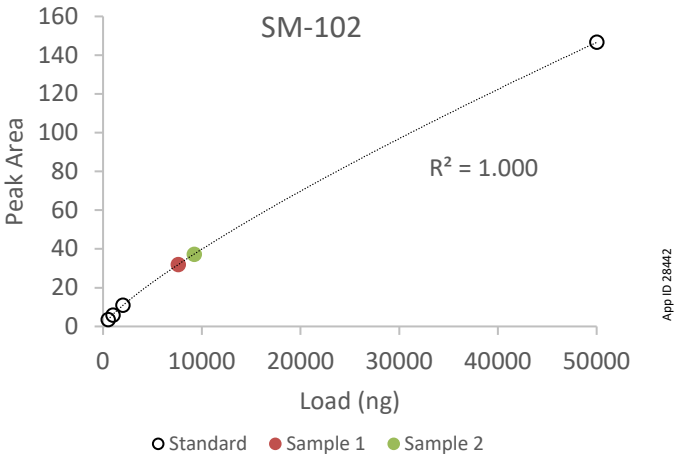


Figure 5 . Standard curve of Cholesterol and the determination of two LNP samples.

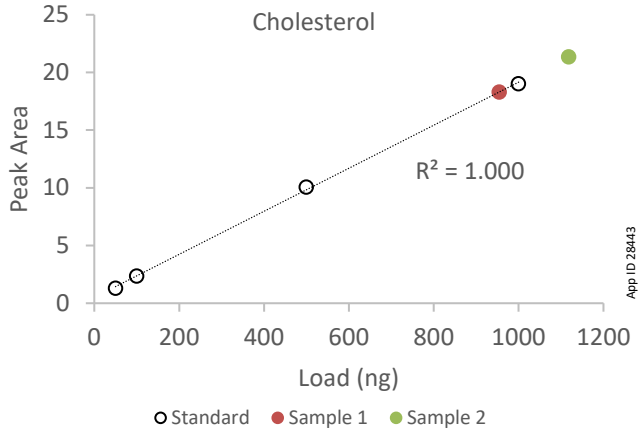


Figure 6 . Standard curve of DSPC and the determination of two LNP samples.

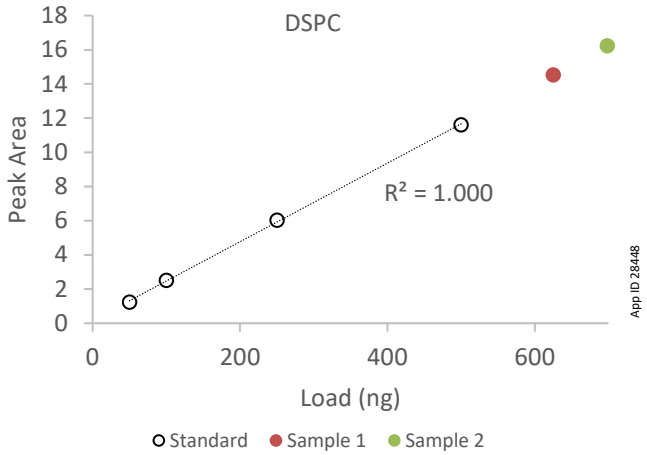
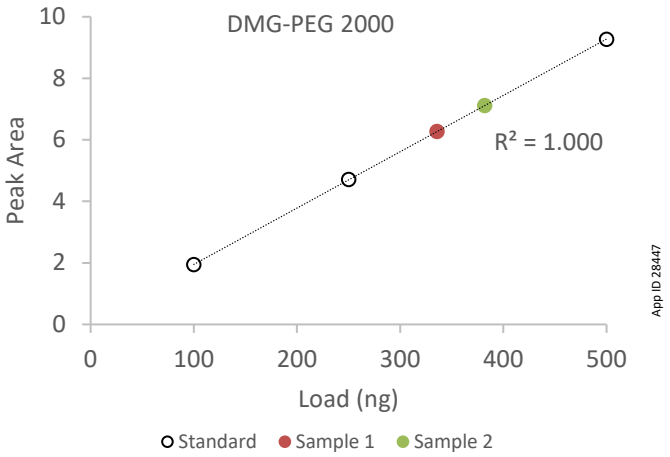


Figure 7 . Standard curve of DMG-PEG 2000 and the determination of two LNP samples.



Conclusions

A 20 min RP-UHPLC-CAD method was developed utilizing a™ Phenomenex Gemini® C6-Phenyl column. The method was applied to determine the lipid identity and preliminary content assessment of two LNP samples. All four lipid components were well resolved with symmetrical peak shapes and good retention time reproducibility due to the orthogonal chemistry of C6-Phenyl compared to traditional C18. Future studies will include the determination of the lipid mole ratios in different LNP samples.

References

1. USP Analytical Procedures for mRNA Vaccine Quality – Draft Guidelines: 3rd Edition.

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t: +32 (0)2 503 4015 (French)
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beinfo@phenomenex.com

Canada
t: +1 (800) 543-3681
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China
t: +86 400-606-8099
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Czech Republic
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Denmark
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Finland
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France
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Germany
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Hong Kong
t: +852 6012 8162
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India
t: +91 (0)40-3012 2400
indiainfo@phenomenex.com

Indonesia
t: +62 21 3952 5747
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Ireland
t: +353 (0)1 247 5405
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Italy
t: +39 051 6327511
italiainfo@phenomenex.com

Japan
t: +81 (0) 120-149-262
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Luxembourg
t: +31 (0)30-2418700
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Mexico
t: 01-800-844-5226
tecnicomx@phenomenex.com

The Netherlands
t: +31 (0)30-2418700
nlinfo@phenomenex.com

New Zealand
t: +64 (0)9-4780951
nzinfo@phenomenex.com

Norway
t: +47 810 02 005
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Poland
t: +48 22 51 02 180
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Portugal
t: +351 221 450 488
ptinfo@phenomenex.com

Singapore
t: 800-852-3944
sginfo@phenomenex.com

Slovakia
t: +420 272 017 077
sk-info@phenomenex.com

Spain
t: +34 91-413-8613
espinfo@phenomenex.com

Sweden
t: +46 (0)8 611 6950
nordicinfo@phenomenex.com

Switzerland
t: +41 (0)61 692 20 20
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Taiwan
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Thailand
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