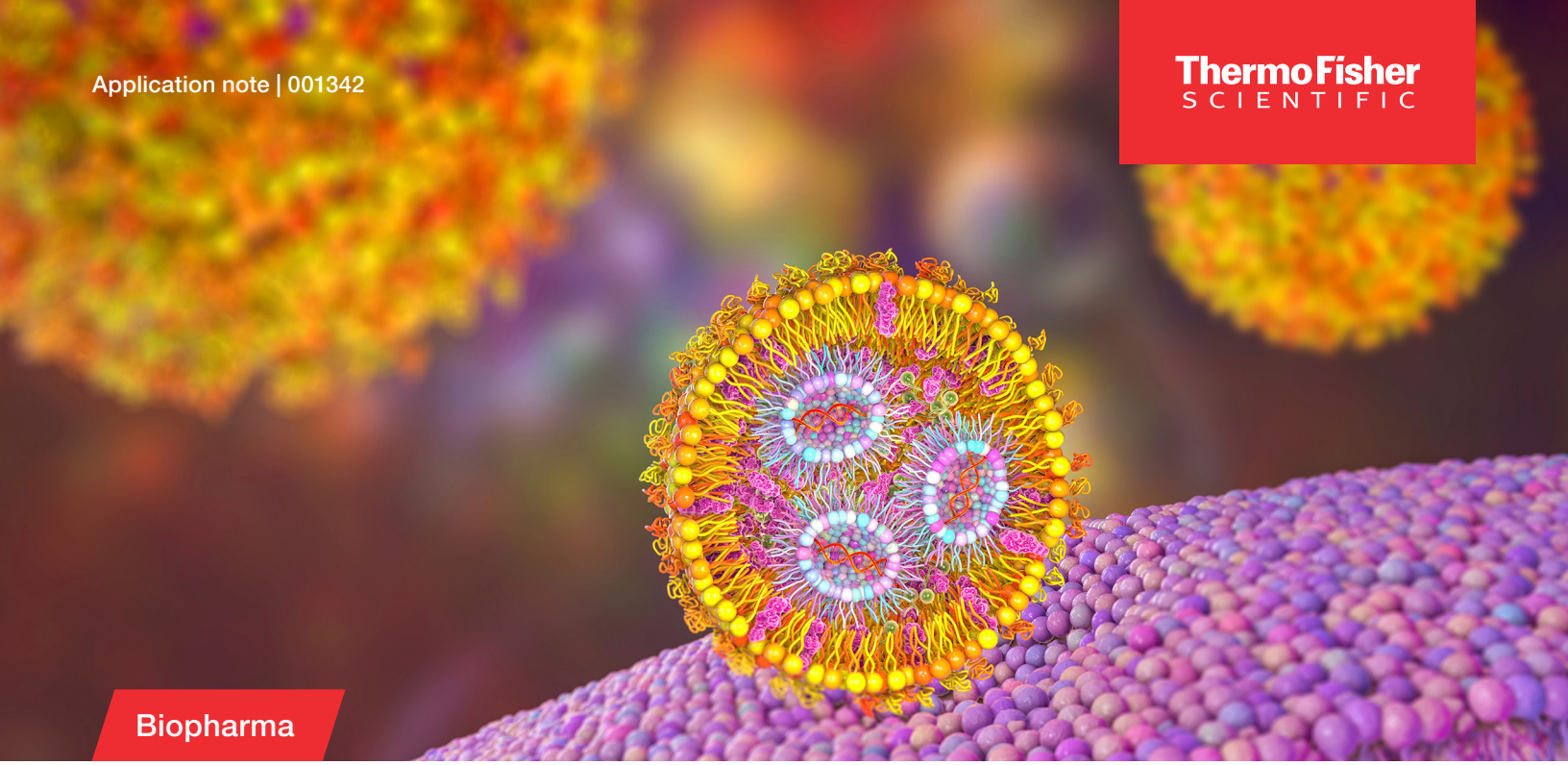
Application note | 001342



Biopharma

Profiling raw material impurities of the lipid nanoparticle (LNP) components

Authors

Sissi White[1], Junghyang Lee[2], Emily Hyekyung Choi[2], Min Du[3]

[1]Thermo Fisher Scientific, Franklin, MA, US

[2]Thermo Fisher Scientific, Seoul, South Korea

[3]Thermo Fisher Scientific, Cambridge, MA, US

Keywords

Lipid nanoparticle, LNP, lipid analysis, charged aerosol detection, CAD, UHPLC, cationic lipid, lipid excipient, Hypersil GOLD C8 HPLC column

Application benefits

- Simple UHPLC method represents an effective approach of the impurity profile of the lipid components of LNPs
- Uniform response shows relative amounts of each lipid compound along with impurities in the material
- Relative peak areas can be calculated and real-time reported by Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software

Goal

Development of a simple UHPLC method profiling the impurity of the raw materials from different vendors

Introduction

The identity, ratio, and purity of the lipid components in lipid nanoparticle (LNP) formulations are regarded as critical quality attributes that need to be well characterized and routinely monitored during development, manufacturing, and QC release testing to ensure safety and efficacy. The European Medicines Agency assessment report detailed the attributes have been included in the specification of mRNA -1273 LNP and in finished product release specification: lipid identity, lipid content and lipid impurities by UHPLC-CAD.[1] Charged aerosol detection (CAD) has become the preferred approach in lipid/lipidomic analysis due to its capabilities for measurement of any non- or semi-volatile compounds and its uniform response.

Besides the characterization of LNPs composition, it is also important for the associated impurities and degradants to be confirmed.[2] Lipid type, source, and quality/purity have a direct impact on the impurity profile and properties of final liposome and LNP formulations; using high-quality raw materials is important to synthesize lipids for reproducible results.[3] In this work, an efficient method is described for profiling the impurities of the raw materials from different suppliers. The column used is a Thermo Scientific™ Hypersil GOLD™ HPLC C8 column, which was specifically designed for outstanding selectivity and excellent peak shape in lipid chromatography. The Thermo Scientific™ Vanquish™ Flex and Horizon UHPLC systems are completely inert and have no iron that could potentially interact with the metal-chelating phosphate groups present on some of the lipids in the formulation.

Experimental

Chemicals

- Water Optima™ LC/MS grade, Fisher Scientific™ (P/N W64)
- Isopropanol (IPA), Optima™ LC/MS grade, Fisher Scientific™ (P/N A461-4)
- Methanol Optima™ LC/MS grade, Fisher Scientific™ (P/N A456-4)
- Ethyl alcohol (EtOH), 200 proof, 99.5+%, Thermo Scientific™ (P/N 61509-0010)
- Ammonium formate 10 M in H_2O BioUltra, Sigma-Aldrich (P/N 78314)
- Disposable controlled drop pipets, Fisherbrand™ (P/N 13-678-30)
- Pipets, Fisherbrand™ (P/N 13-678-25A)
- Autosampler inert vials and inserts, Thermo Scientific™ Chromacol™ GOLD-grade (P/N 13-622-351)

Instrumentation

Thermo Scientific™ Vanquish™ Flex System consisting of:

- Vanquish System Base (P/N VH-S01-A)
- Vanquish Dual Pump F (P/N VF-P32-A)
- Vanquish Split Sampler F (P/N VF-A10-A)
- Vanquish Column Compartment H (P/N VH-C10-A-02)
- Vanquish Charged Aerosol Detector H (P/N VH-D20-A)

Sample preparation

- It is recommended to use glass pipets to transfer lipids/LNPs in organic solvents.
- It is recommended to use glass inserts, vials, and bottles to store lipids and LNP samples.

Lipid vendor information is given in Table 1. Each lipid was individually dissolved in 100% ethanol and vortexed. The concentration of each component is shown in Table 2.

Table 1. Vendor information

	Vendor 1	Vendor 2	Vendor 3
DLIN-MC3_DMA		✓	✓
DSPE-MPEG(2000)	✓	✓	
DC-CHOL	✓	✓	
DMG-PEG(2000)	✓	✓	

Table 2. Sample composition for method development

Lipid	Formula weight	Concentration (mg/mL)
DLIN-MC3-DMA	642.1	10
DSPE-MPEG(2000)	2805.497 (average molecular weight)	4
DC-CHOL	537.26	1
DMG-PEG(2000)	2509.2 (average molecular weight)	1

LC conditions	
Column	Hypersil GOLD C8, 2.1 × 50 mm, 5 µm, P/N 25205-052130
Mobile phases	A: 5 mM ammonium formate in 100% water B: 5 mM ammonium formate in 70% IPA/30 methanol C: 100% IPA
Gradient	Table 3
Flow rate	0.5 mL/min
Column temperature	50 °C
Injection volume	1 µL
Injection wash solvent	Mobile phase B
Detector settings	Table 4

Table 3. LC gradient conditions

Time (min)	%A	%B	%C
0	60	10	30
4	20	30	50
6	10	40	50
7	10	40	50
7.1	60	10	30
12	60	10	30

Table 4. CAD settings

Parameter	Setting
Power function	1.0
Evaporator temperature	35 °C
Data rate	2 Hz
Filter	3.6

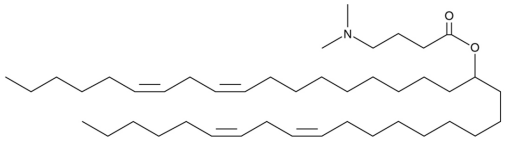
Chromatography Data System

Chromeleon CDS 7.2.10 ES was used for data acquisition and analysis.

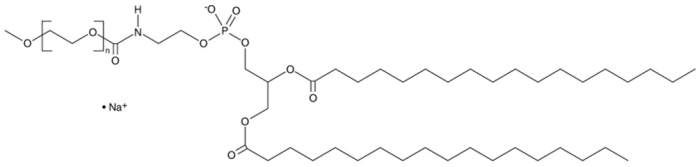
Results and discussion

Four lipids, representative of the starting materials used in liposomal and LNP formulations, were selected to demonstrate this analysis.

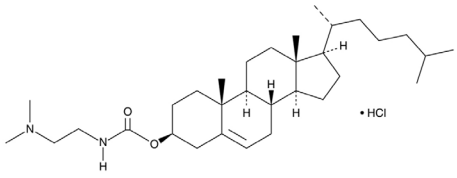
DLin-MC3-DMA is an ionizable amino lipid that has been used in combination with other lipids in the formation of LNPs for the delivery of nucleic acids.[4,5]



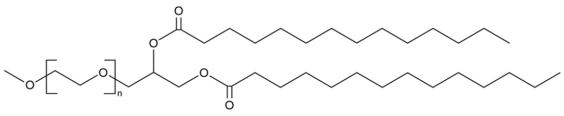
DSPE-MPEG(2000) is a PEGylated derivative of 1,2-distearoyl-sn-glycero-3-PE (DSPE). Formulations containing DSPE-MPEG(2000) have been used in the synthesis of liposomes for the delivery of anticancer and antimalarial agents. [4,5]



DC-cholesterol (DC-CHOL) is a cationic derivative of cholesterol with a quaternary amine salt attached via the 3-C position. DC-cholesterol has been shown to have potential as a nanocarrier for nucleic acids and drug therapies.[4,5]



DMG-PEG(2000) is a lipid excipient that has been used in combination with other lipids in the formation of LNPs.[4,5]



A key advantage of CAD is its ability to provide uniform response for analytes that behave as nonvolatiles. First, the comparison experiments were conducted with evaporation temperature settings of 35 °C and 50 °C to show the above lipid components and their impurities behave as non-volatiles. There was negligible difference between two chromatograms to approve the lipids and all impurities as nonvolatiles (Figures 1–4).

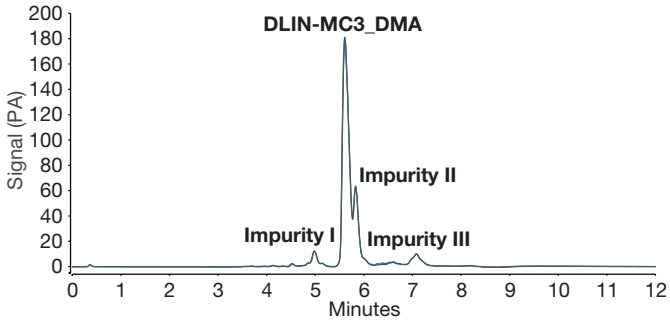


Figure 1. Overlaid chromatograms of DLIN-MC3_DMA

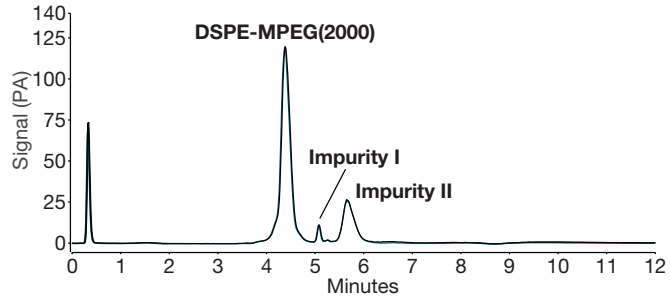


Figure 2. Overlaid chromatograms of DSPE-MPEG(2000)

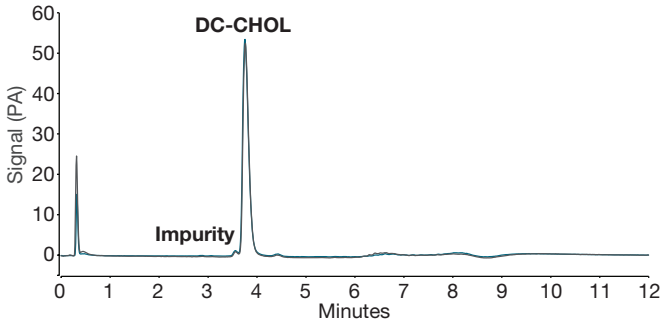


Figure 3. Overlaid chromatograms of DC-CHOL

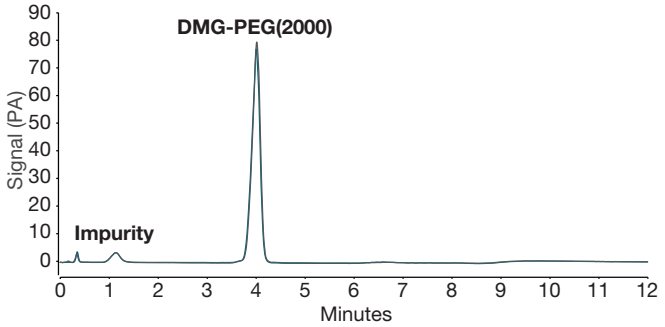


Figure 4. Overlaid chromatograms of DMG-PEG(2000)

The design of this experiment was that two suppliers were chosen according to different impurity levels. Three consecutive runs were performed for each lipid component, and the average of area percentage was calculated by impurity peak area/total peak area. Comparison chromatograms are demonstrated in Figures 5-9 and indicate comparable impurity peaks.

For analyte DLIN-MC3_DMA from vendor 3, the total impurity level determined by area percentage was 14.2%, while that for vendor 2 was 24.9%. The main difference is impurity II between different vendors.

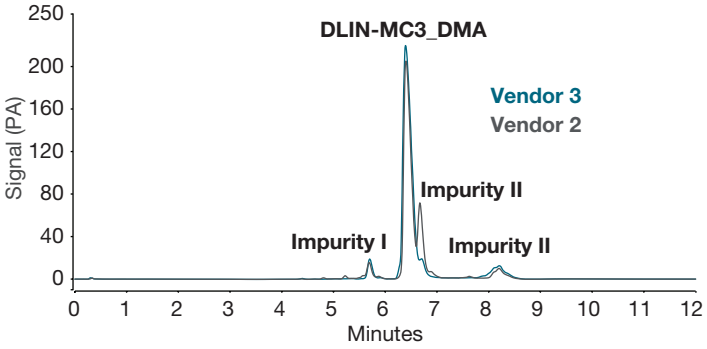


Figure 5. DLIN-MC3_DMA impurity comparison from different suppliers

In Figure 6, in the newer lot of DLIN-MC3-DMA from the same supplier, impurity I was below the detection limit before the main peak. Area percentage of this impurity peak was calculated to be 4.95% in the older lot. The main difference is impurity I between different lots with the same vendor.

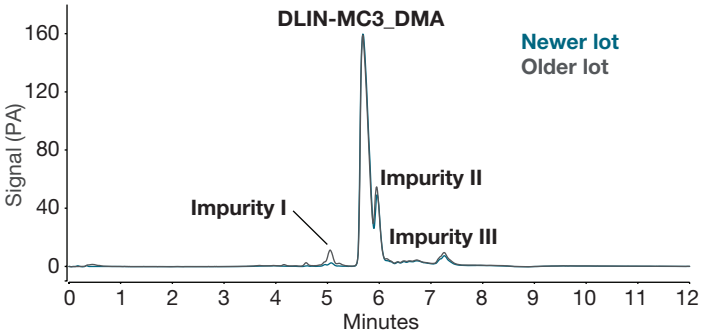


Figure 6. DLIN-MC3_DMA impurity comparison with different lots from the same supplier

For analyte DSPE-MPEG(2000) from vendor 1, the total impurity level determined by area percentage was 2.44%, while that for vendor 2 was 26.17% (Figure 7).

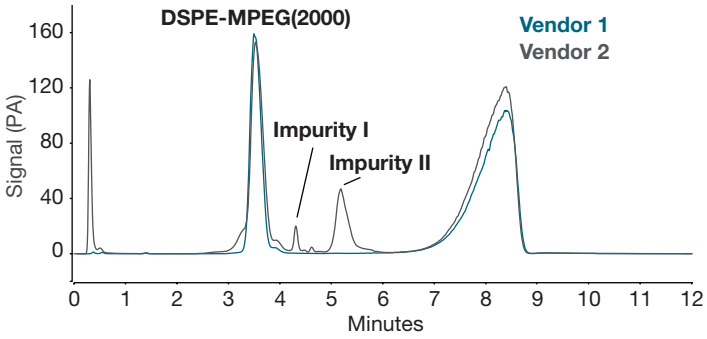


Figure 7. DSPE-MPEG(2000) impurity comparison from different suppliers

For analyte DC-CHOL from vendor 1, there was no impurity detected. For analyte DC-CHOL from vendor 2, the total impurity level determined by area percentage was 1.56% (Figure 8).

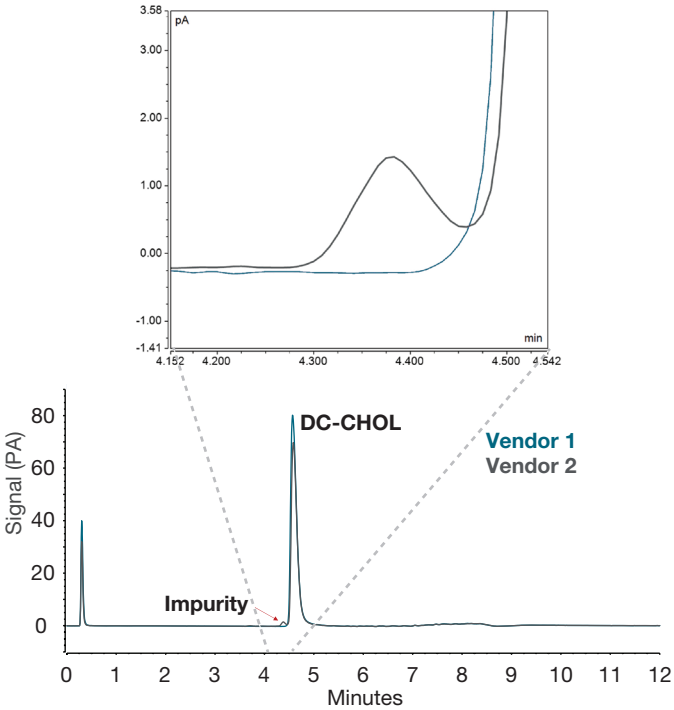


Figure 8. DC-CHOL impurity comparison from different suppliers

For analyte DMG-PEG(2000) from vendor 1, the total impurity level determined by area percentage was 1.58%, while that for vendor 2 was 0.23% (Figure 9).

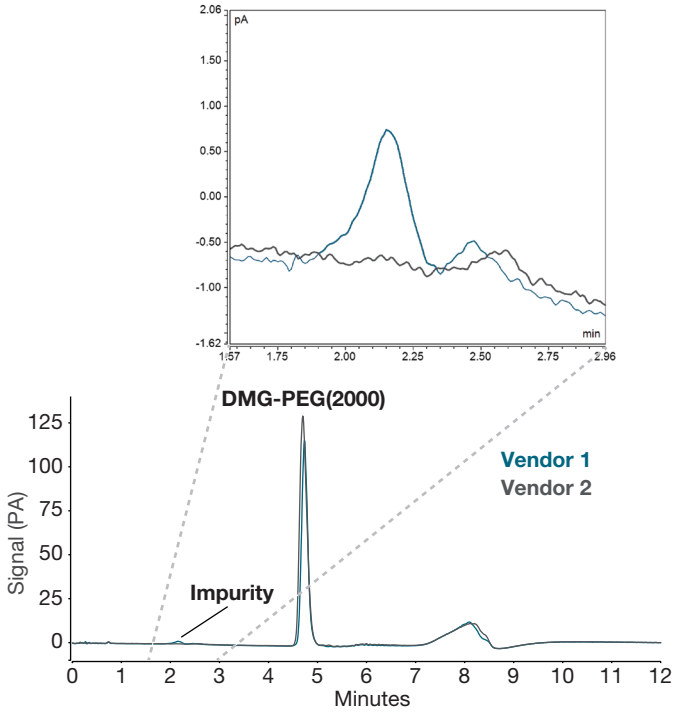


Figure 9. DMG-PEG(2000) impurity comparison from different suppliers

Figure 10 shows the separation of DC-CHOL, DSPE-MPEG(2000) and DLIN-MC3_DMA using higher purity standards. A binary eluent gradient had been used in this separation, however a ternary gradient (in method section) had better separation for detailed impurity analysis. Baseline separation of the main peaks can be well resolved and achieved within six minutes.

From the summary in Table 5, the high sensitivity of the CAD allows any trace impurities to be monitored. DSPE-MPEG(2000) had the most significant amount of impurities and DLin-MC3_DMA had less in comparison, while DC-CHOL and DMG-PEG(2000) had very small amounts of impurity.

Column:	Hypersil GOLD C8, 5.0 µm
Format:	2.1 × 50 mm
Mobile phase A:	5 mM AF in Water
Mobile phase B:	5 mM AF in 70IPA/30MeOH
Needle wash:	Mobile phase B
Gradient:	Time (min) %A %B
	0.0 70 30
	2.0 50 50
	4.0 0 95
	6.0 0 95
	7.0 70 30
	12.0 70 30
Flow rate:	0.5 mL/min
Inj. volume:	1 µL
Temperature:	50 °C
CAD setting:	Power function: 1.0
	Gas resolution mode: Analytical
	Evaporator temperature: 35 °C
	Filter: 3.6
	Data rate: 2 Hz
System:	Vanquish UHPLC

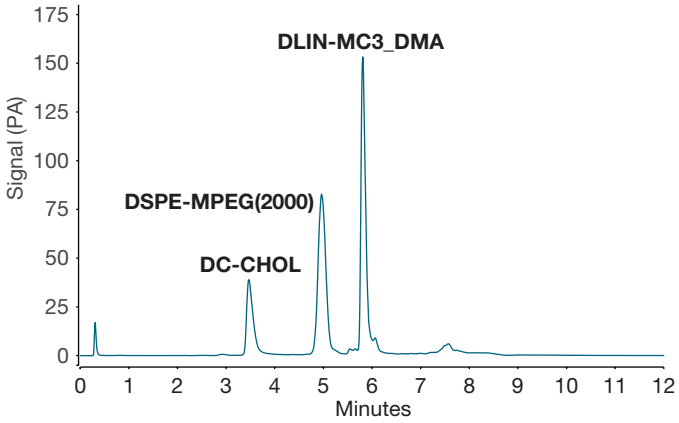


Figure 10. Separation of DC-CHOL, DSPE-MPEG(2000), and DLIN-MC3_DMA

Table 5. Summary of % area impurities

	Vendor 1 % area of impurities	Vendor 2 % area of impurities	Vendor 3 % area of impurities
DLIN-MC3_DMA	NA	29.9	14.2
DLIN-MC3_DMA (Newer Lot)	NA	24.9	NA
DSPE-MPEG2000	2.44	26.17	NA
DC-CHOL	0	1.56	NA
DMG-PEG2000	1.58	0.23	NA

NA: Not available

A reversed-phase gradient has been used in this method to demonstrate the impurity profile and the area percentage of the impurity peaks calculated in Chromeleon software was used as a simple means to estimate impurity levels. This approach is an estimated assessment of raw materials impurity analysis from different lots and vendor and as a precursor to more detailed quantitative analysis.

Conclusion

- The use of a Hypersil GOLD C8 column for DLIN-MC3_DMA, DSPE-MPEG(2000), DMG-PEG(2000), and DC-CHOL provides narrow peaks for outstanding efficiency.
- Peak purities have been assessed between different vendors or different lots and relative peak areas have been used to calculate purity level.
- The described method represents a simple and effective approach to profile comparable raw material impurity between different suppliers or different lots.

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