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Application News

LabSolutions™ MD : Software for Efficient Method Development based on Analytical Quality by Design

Efficient Method Development of Lipid Nanoparticle Components Using ELSD

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User Benefits

- LabSolutions MD enables efficient optimization of separation conditions for lipid nanoparticle components by streamlining the entire method development workflow.
- By utilizing switching valves, automated screening of multiple mobile phase and column combinations can be performed.
- For lipid nanoparticle components lacking UV absorption, an evaporative light scattering detector (ELSD) allows for reliable detection and quantification.

■ Introduction

In recent years, medical technologies utilizing messenger RNA (mRNA) have advanced rapidly, gaining attention not only for vaccines against infectious diseases but also in emerging applications such as cancer vaccines and therapies for genetic disorders. Due to the poor membrane permeability of mRNA, effective intracellular delivery requires specialized drug delivery technologies. Currently, lipid nanoparticles (LNPs) are the primary drug delivery system (DDS) used for mRNA-based therapeutics. LNPs typically consist of four lipid components: cholesterol, ionizable lipids, PEG-modified lipids, and neutral phospholipids. Accurate separation and quantification of each lipid component are essential to ensure the efficacy and safety of LNP-mRNA formulations. In LC analysis, the choice of mobile phase and column significantly influences the retention behavior of analytes. Therefore, screening across a variety of mobile phases and columns is desirable to achieve optimal separation. However, this process is often time-consuming. This article introduces a case study in which LabSolutions MD, a dedicated software for supporting method development, was used to streamline the search for optimal separation conditions during both the screening and optimization phases for the four lipid components of LNPs. Additionally, the optimized conditions were applied to the analysis of a real lipid nanoparticle sample. Detection was performed using an ELSD, as the lipid components lack UV absorbance.

■ Screening of Mobile Phases and Columns

In the screening phase (analytical conditions: Table 1), the optimal combination of mobile phase and column was investigated as key parameters significantly affecting retention and separation. For the mobile phase, 0.1% formic acid aqueous solution was used as the aqueous solvent, while the organic solvent consisted of acetonitrile and methanol mixtures with the methanol ratio varied from 0% to 90% in 10% increments (ten levels). Three types of columns with different stationary phases and pore sizes were evaluated. A comprehensive analytical schedule comprising 30 (10 × 3) conditions combining these mobile phases and columns was generated to identify the optimal combination. LabSolutions MD enables automated and error-free generation of analytical schedules with various LC parameters (steps (1) to (5) in Fig. 1). Furthermore, switching valves were employed to automatically alternate the selected mobile phase ((1) in Fig. 1) and column ((2) in Fig. 1). The adjustment of organic solvent composition in the mobile phase was performed automatically using the mobile phase blending function. By simply selecting the desired mobile phase, the system automatically prepared the mobile phase with the specified organic solvent ratio, significantly reducing manual preparation labor and eliminating preparation errors.

Table 1 Analytical Conditions for Screening

System	: Nexera™ X3 (Method Scouting System)
Sample	: Cholesterol*1, SM-102*2, DMG-PEG*3, DSPC*4
Column 1	: Shim-pack Scepter™ Phenyl-120*5
Column 2	: Shim-pack Scepter C8-120*6
Column 3	: Shim-pack Scepter C4-300*7
	(100 mm × 3.0 mm I.D., 1.9 µm : column 1~3)
Temperature	: 40 °C
Injection volume	: 0.5 µL (250 mg/L for each sample)
Sample solvent	: Ethanol
Mobile phases	
Pump A	: 0.1% formic acid in water
Pump B – Line A	: Acetonitrile
– Line B	: Methanol
– Line C	: Isopropanol (IPA)
Flow rate	: 0.6 mL/min
Time program (%B)	: 60% (0 min) →95% (5-15 min) →60% (15.01-21 min)
Detection	: ELSD-LTIII
Gain	: Wide
Filter	: 1 sec
Drift tube Temp.	: 40 °C
Nebulizer gas	: N_2
Gas pressure	: 350 KPa

*1 CAS RN：57-88-5
*2 CAS RN：2089251-47-6
*3 CAS RN：160743-62-4
*4 CAS RN：816-94-4
*5 P/N：227-31064-03
*6 P/N：227-31034-03
*7 P/N：227-31176-03

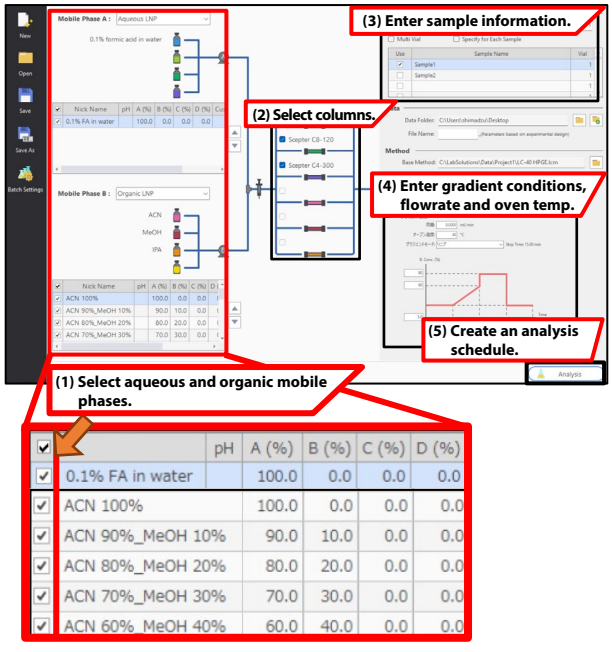

Fig. 1 Steps for Creating Analysis Schedule

■ Results of Screening

The results of the mobile phase and column screening are presented in Fig. 2–4. The retention behavior and peak shapes of the individual lipid components were found to vary significantly depending on the column type and the composition of the organic solvent in the mobile phase. For the Scepter Phenyl-120 column (Fig. 2), increasing the methanol ratio in acetonitrile led to an improvement in the peak shapes (increased peak heights) of SM-102 and DMG-PEG. In particular, chromatograms (7)–(9) in Fig. 2, corresponding to methanol ratios of 60%, 70%, and 80%, showed relatively good separation among the lipid components. In contrast, for the Scepter C8-120 column (Fig. 3), the peak heights of DMG-PEG remained low across all tested methanol ratios, and satisfactory peak shapes could not be achieved. With the Scepter C4-300 column (Fig. 4), an increase in methanol ratio similarly resulted in improved peak shapes for DMG-PEG and SM-102. However, tailing was consistently observed in the SM-102 peaks under all conditions. Among the screening results, the Scepter Phenyl-120 column under the three methanol conditions (60%, 70%, and 80%) demonstrated relatively favorable lipid separation, suggesting the potential for further optimization. Accordingly, additional experiments were conducted in which IPA was added to the organic solvent at different ratios to further improve resolution.

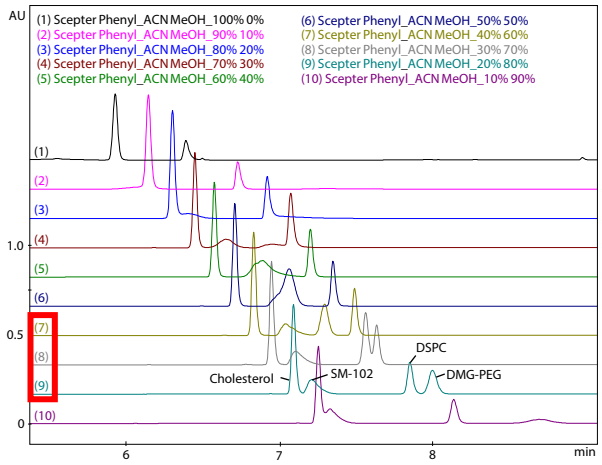

Fig. 2 Screening Results of Scepter Phenyl-120

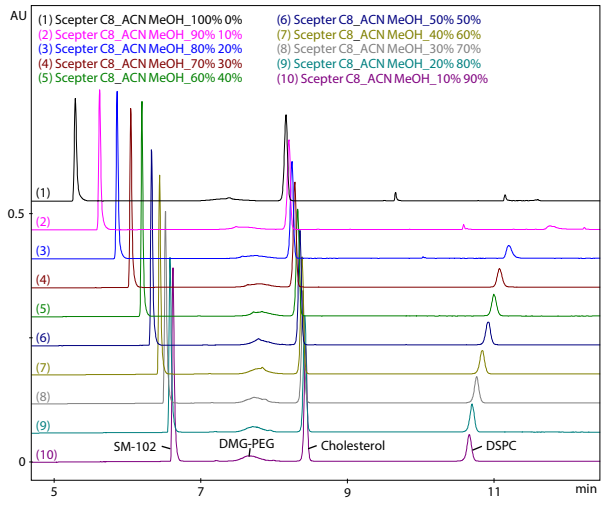

Fig. 3 Screening Results of Scepter C8-120

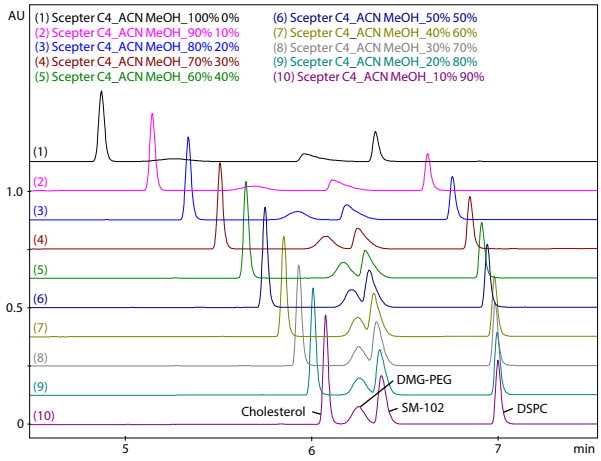

Fig. 4 Screening Results of Scepter C4-300

■ Investigation of IPA Addition Ratio to the Organic Solvent

Fig. 5 presents representative chromatograms obtained using Scepter Phenyl-120 column under the three mobile phase conditions (methanol-to-acetonitrile ratios of 60%, 70%, and 80%) that provided favorable separation in the screening. In these experiments, the acetonitrile-to-methanol ratio was held constant, while the proportion of IPA was varied from 0% to 50% in 10% increments, as summarized in Table 2.

Table 2 Conditions for IPA Addition

Acetonitrile : Methanol : IPA

(40 : 60) : 0	(30 : 70) : 0	(20 : 80) : 0
(36 : 54) : 10	(27 : 63) : 10	(18 : 72) : 10
(32 : 48) : 20	(24 : 56) : 20	(16 : 64) : 20
(28 : 42) : 30	(21 : 49) : 30	(14 : 56) : 30
(24 : 36) : 40	(18 : 42) : 40	(12 : 48) : 40
(20 : 30) : 50	(15 : 35) : 50	(10 : 40) : 50

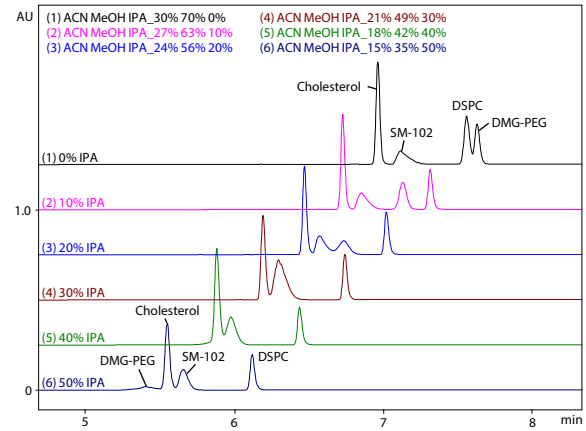

Fig. 5 Chromatograms at Different IPA Addition Ratios

It was confirmed that varying the addition ratio of IPA had a significant effect on the retention behavior and separation of the individual lipid components. As the proportion of IPA increased, the peak height of DMG-PEG tended to decrease, while that of SM-102 tended to increase. In total, 18 chromatograms corresponding to the mixing patterns listed in Table 2 were obtained during the investigation of IPA addition ratios. Evaluating which conditions achieved the desired separation requires substantial chromatographic expertise and effort. However, LabSolutions MD enables quantitative evaluation and ranking of the separation performance under each condition using Equation (Eq. 1), allowing rapid and straightforward identification of optimal conditions without relying on the experience in chromatography.

$$(\text{Evaluation Value}) = P \times (Rs_1 + Rs_2 + \ldots + Rs_{P-1}) \quad \text{(Eq. 1)}$$

Evaluation value is calculated as the number of peaks detected (P) multiplied by the sum of resolution factor (Rs) for all peaks. Fig. 6 shows the evaluation values obtained from the investigation of IPA addition ratios, arranged in descending order. The two conditions with the highest evaluation value were acetonitrile / methanol / IPA ratios of "27 : 63 : 10" and "40 : 60 : 0". Among these, the condition "27 : 63 : 10" (chromatogram (1) in Fig. 7) was selected as the optimal condition due to the higher peak height of SM-102.

MPB Nick Name	Evaluation Value
ACN MeOH IPA_27% 63% 10%	15.600
ACN MeOH IPA_40% 60% 0%	15.600
ACN MeOH IPA_24% 56% 20%	15.015
ACN MeOH IPA_20% 80% 0%	14.877
ACN MeOH IPA_20% 30% 50%	14.812
ACN MeOH IPA_15% 35% 50%	14.603
ACN MeOH IPA_30% 70% 0%	14.580
ACN MeOH IPA_36% 54% 10%	14.506
ACN MeOH IPA_16% 64% 20%	13.853
ACN MeOH IPA_10% 40% 50%	13.399

Fig. 6 Ranking of Each Condition by Evaluation Value
(top 10 chromatograms listed from the highest to the lowest)

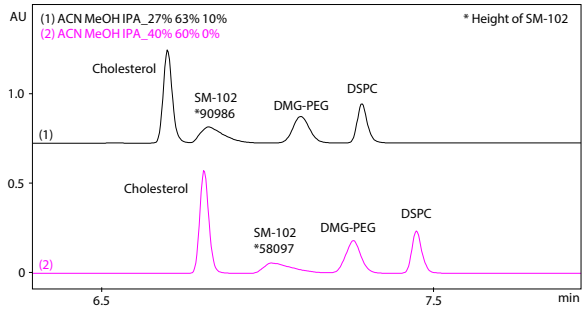

Fig. 7 Chromatograms with the Highest Evaluation Value

In the optimization phase, further improvements in resolution and the search for conditions with high robustness were conducted by examining the LC parameters, such as the gradient conditions and column oven temperature.

■ Optimization of LC Parameters

Based on condition (1) in Fig. 7, which provided the optimal separation in the screening, the separation of each lipid component was further optimized by varying the initial gradient concentration (50%, 60%, and 70%: three levels, Fig. 8), gradient time (4, 5, and 6 min: three levels, Fig. 8), and column oven temperature (30, 40, and 50 °C: three levels). The resulting chromatograms are shown in Fig. 9-11. Higher initial gradient concentrations and longer gradient times tended to improve the resolution of the peaks, whereas the column oven temperature showed a smaller effect on resolution. Additionally, higher column oven temperatures tended to reduce the peak widths. Subsequently, the resolution of each lipid component was visualized within the design space to facilitate the search for optimal conditions that provide both robustness and better resolution.

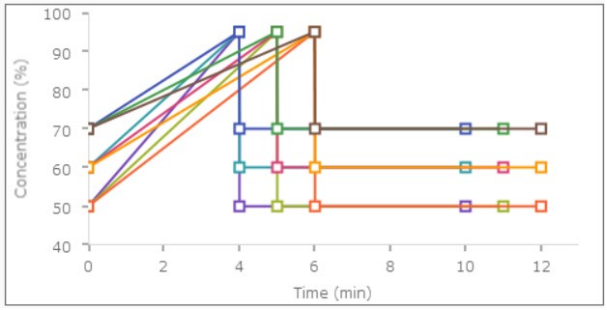

Fig. 8 Optimization of Gradient Conditions

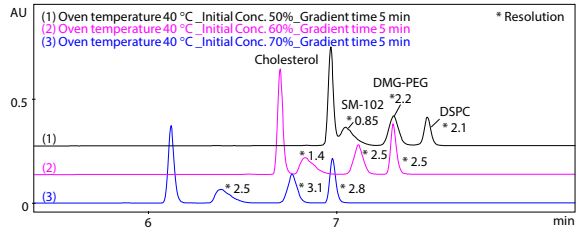

Fig. 9 Chromatograms with Different Initial Concentration
50% (1) , 60% (2) , 70% (3)

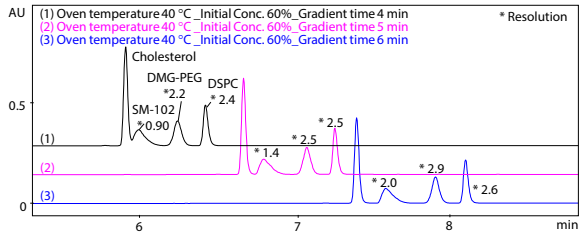

Fig. 10 Chromatograms with Different Gradient Time
4 min (1) , 5 min (2) , 6 min (3)

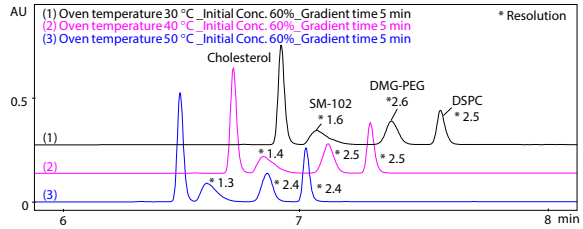

Fig. 11 Chromatograms with Different Column Oven Temperature
30 °C (1) , 40 °C (2) , 50 °C (3)

■ Exploration of Optimal Conditions by Design Space

The design spaces for the resolutions of each lipid component are shown in Fig. 12, where the vertical and horizontal axes represent the gradient time and initial gradient concentration, respectively. The red regions indicate areas of high resolution, while the blue regions represent areas of low resolution. The design space visualization reveals that higher initial gradient concentrations and longer gradient times lead to improved resolution.

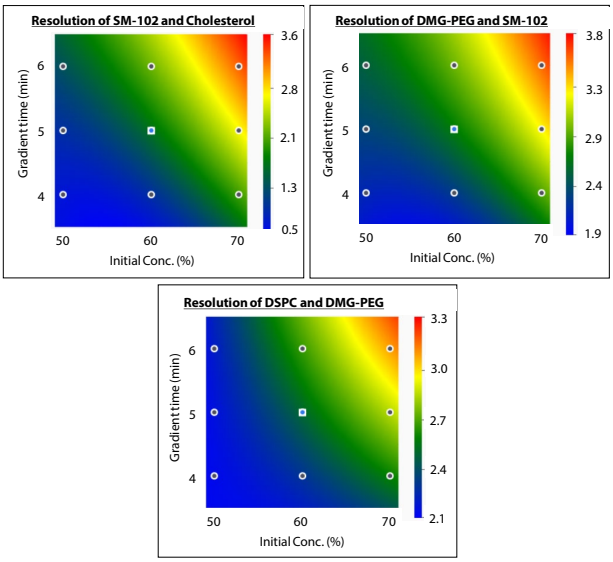

Fig. 12 Design Space for Resolution of Each Lipid Component
(column oven temperature: 40 °C)

LabSolutions MD can automatically explore the optimal conditions that satisfy multiple criteria by overlaying multiple design spaces. For example, the criteria applied in this study were: (1) a resolution of 1.5 or greater between cholesterol and SM-102, which is the most difficult pair to separate; (2) a peak height of 80,000 or greater for SM-102, which exhibits the lowest signal intensity; and (3) an elution time of 6 minutes or less for the final peak (DSPC) to reduce overall analysis time. The design spaces were overlaid to identify the region meeting all these criteria (Fig. 13).

In Fig. 13, the pink area represents regions where the resolution between cholesterol and SM-102 is below 1.5, the green area indicates regions where the peak height of SM-102 is below 80,000, and the yellow area corresponds to regions where the elution time of DSPC exceeds 6 minutes. The remaining area (hatched with black lines), including a point marked as A, was automatically identified as the optimal condition: initial gradient concentration of 70%, gradient time of 4 minutes, and column oven temperature of 50 °C. This approach demonstrates that by overlaying design spaces, LabSolutions MD allows for a straightforward and efficient search for conditions that simultaneously meet multiple user-defined criteria.

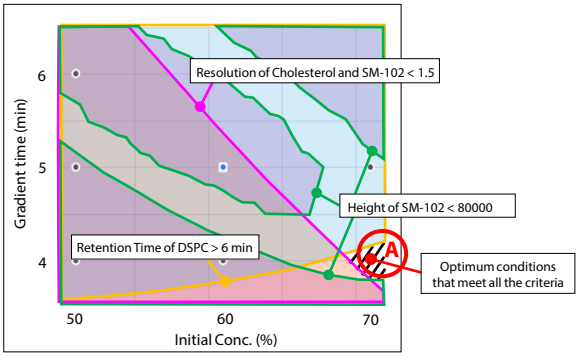

Fig. 13 Overlay of Design Spaces
(column oven temperature: 50 °C)

■ Chromatogram Under Optimal Conditions

A chromatogram obtained under the optimal conditions (point A) identified through design space evaluation is shown in Fig. 14. Under these conditions, the resolution between cholesterol and SM-102 was 1.5 or greater, the peak height of SM-102 exceeded 80,000, and the elution time of the final peak, DSPC, was within 6 minutes. These results demonstrate that the separation of the four lipid components was successfully optimized while also considering a reduction in analysis time. Visualization by design spaces enables systematic optimization without relying on the experience in chromatography.

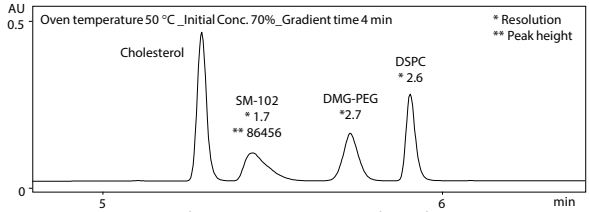

Fig. 14 Chromatogram at Optimal Conditions
(0.1% Formic acid in water, acetonitrile / methanol / IPA = 27 : 63 : 10, Scepter Phenyl-120)

■ Analysis and Quantification of Real Sample

The real lipid nanoparticle sample was analyzed under the optimized conditions (Table 3) to quantify each lipid component. Table 4 summarizes the calibration ranges and coefficient of determination (r^2) of the calibration curves, which were prepared using standard solutions of each lipid. Since the concentrations of individual lipids in the real sample varied widely, calibration curves were constructed over different concentration ranges. Good linearity was confirmed for all lipids, with r^2 values exceeding 0.998. The repeatability (%RSD) of retention times and peak areas, as well as the quantified concentrations for each component obtained from repeated analyses of the real sample, are listed in Table 5. The corresponding chromatogram of the sample is shown in Fig. 15.

Table 3 Optimized Analytical Conditions

System	: Nexera X3 (Method Scouting System)
Sample	: mRNA-LNP (1:10 dilution in ethanol, then analyzed)
Column	: Shim-pack Scepter Phenyl-120 (100 mm × 3.0 mm I.D., 1.9 µm)
Temperature	: 50 °C
Injection volume	: 1 µL
Sample solvent	: Ethanol
Mobile phases	
Pump A	: 0.1% formic acid in water
Pump B	: Acetonitrile/Methanol/IPA = 27:63:10
Flow rate	: 0.6 mL/min
Time program (%B)	: 70% (0 min) →95% (4-7 min) →70% (7.01-13 min)
Detection	: ELSD-LTIII
Gain	: Wide
Filter	: 1 sec
Drift tube Temp.	: 40 °C
Nebulizer gas	: N_2
Gas pressure	: 350 KPa

Table 4 Linearity of Each Lipid Component

	Calib. range (mg/L)	Coefficient of determination (r^2)
Cholesterol	6~30	>0.999
SM-102	20~100	>0.998
DMG-PEG	9~45	>0.998
DSPC	7~35	>0.998

Table 5 Concentration and Repeatability (%RSD) of Each Lipid Component in Seven Replicate Analyses

	Retention time (%RSD)	Peak area (%RSD)	Conc. (mg/L)
Cholesterol	0.060	2.35	24.3
SM-102	0.13	2.49	54.6
DMG-PEG	0.067	2.68	16.6
DSPC	0.060	3.82	17.5

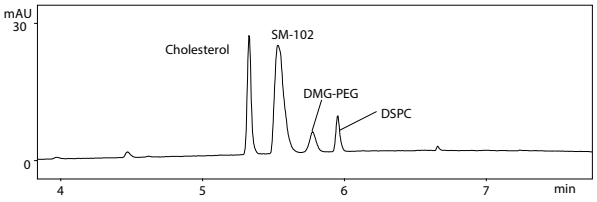

Fig. 15 Chromatogram of the Real Sample

■ Conclusion

LabSolutions MD enables automated switching of mobile phases and columns, as well as automated preparation of mobile phases, allowing for efficient exploration of optimal separation conditions for lipid nanoparticle components. Moreover, the analysis of a large number of data can be streamlined by utilizing evaluation value and design spaces. This approach allows for the exploration of optimal conditions without relying on the experience in chromatography. Additionally, the use of an evaporative light scattering detector (ELSD) makes it possible to detect and quantify lipid nanoparticle components that lack UV absorbance.

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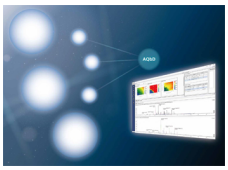

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