PBR
Pharmaceutical & Biomedical Research

2026. Volume 12 Issue 3

Original Article

A Simple, Accurate, and Cost-effective HPLC-UV for Determination of Nanoliposome Components as Drug Carriers

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Article info:
Received: 05 Apr 2026
Accepted: 10 Aug 2026

Keywords:
Nanoliposomes, Drug carrier, Lipid analysis, High-performance liquid chromatography (HPLC), Method development

ABSTRACT

Background: Nanoliposomes are widely used as drug delivery systems owing to their excellent biocompatibility, biodegradability, and ability to enhance drug stability and targeted delivery. Accurate determination of lipid components in nanoliposomal formulations is essential for quality control, stability assessment, and regulatory compliance; however, simultaneous analysis of structurally similar lipids remains analytically challenging.

Objectives: This study aimed to develop and validate a simple, accurate, and cost-effective high-performance liquid chromatography coupled with ultraviolet detection (HPLC–UV) method for the simultaneous determination of α-(3-O-cholesteryloxy)-δ-(N-ethylmorpholine)-succineamide (MoChol), cholesteryl hemisuccinate, cholesterol, dioleoyl-phosphatidylethanolamine (DOPE) and palmitoyl-oleoylphosphatidylcholine (POPC) in nanoliposome formulations containing anti-BCL-2 DNA oligonucleotides.

Methods: Chromatographic separation was performed on a C18 column using a gradient elution system consisting of 0.1% trifluoroacetic acid (TFA) in water and 0.1% TFA in ethanol as mobile phases. Detection was performed at 202 nm with a flow rate of 1.0 mL min^{-1}. The method was validated according to International Council for Harmonization (ICH) guidelines for linearity, accuracy, precision, specificity, robustness, limit of detection (LOD), and limit of quantification (LOQ).

Results: Excellent linearity was obtained for all analytes over concentration ranges of 1–2134 mg L^{-1} with correlation coefficients (R^2) greater than 0.9997. The LOD and LOQ values ranged from 0.13–0.23 mg L^{-1} and 0.39–0.69 mg L^{-1}, respectively. Recovery values were within 98–102%, while precision expressed as relative standard deviation was below 1%. Resolution values exceeded 2.0 for all critical peak pairs, demonstrating adequate specificity. The method was successfully applied to evaluate lipid composition in nanoliposome samples and to monitor compositional changes after long-term storage

Conclusion: The developed HPLC–UV method provides a reliable, sensitive, and economical approach for the simultaneous determination of major lipid components in nanoliposomal drug delivery systems. Its satisfactory validation performance and applicability to stability studies make it suitable for routine quality control and pharmaceutical development of lipid-based formulations.

Citation Beheshti A, Tavakoli M, Karbasi V, Payeghadr M. A Simple, Accurate, and Cost-effective HPLC-UV for Determination of Nanoliposome Components as Drug Carriers. Pharmaceutical and Biomedical Research. 2026; 12(3):207-218. http://dx.doi.org/10.22034/PBR.12.3.1743.1

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Introduction

Due to unique characteristics and versatile applications, nanoliposomes known as lipid nanoparticles, have garnered significant attention in drug delivery [1]. With a lipid bilayer shell surrounding an aqueous core, these nanosized vesicles provide an ideal platform for nanoencapsulation and deliver various therapeutic (biologically active) compounds [2-4]. Composed primarily of naturally occurring phospholipids, nanoliposomes are biocompatible, biodegradable and mimic the lipid composition of cell membranes. This reduces the risk of immune responses or adverse reactions upon administration [3]. Furthermore, the nanoliposome components are metabolized and eliminated from the body, minimizing potential toxicity and long-term accumulation. The nanoliposomes' lipid bilayer structure provides a barrier against enzymatic degradation and environmental factors, preserving the integrity of encapsulated drugs, particularly sensitive or labile compounds, during storage and transportation [2, 4]. The hydrophobic drugs' solubility and bioavailability can be improved by facilitating their incorporation into nano carriers, thereby enhancing their therapeutic efficacy and reducing the required dosage [4]. In addition, nanocarriers can facilitate targeted drug delivery to specific cells or tissues through surface functionalization. Therefore, they can actively deliver to diseased sites or overexpressed receptors. This new strategy not only enhances drug accumulation and efficacy but also minimizes off-target effects. This targeted approach improves therapeutic outcomes while reducing systemic toxicity and adverse reactions [5-8]. Nanoliposomes can be engineered to exhibit sustained-release kinetics, prolonging the duration of drug action and minimizing the need for frequent dosing [4]. By modulating the size, surface properties, and lipid composition of nanoliposomes, it is possible to control the rate and extent of drug release, achieving desired pharmacokinetic profiles and therapeutic effects [9-12]. The composition and structure of spherical or multilayered spherical vesicles can be tailored with protective coatings or structural changes to optimize drug encapsulation, stability, and release kinetics. This modification makes them highly adaptable for a wide range of pharmaceutical formulations. Accurate determination of nanoliposomes components is crucial for ensuring product quality, efficacy, maintaining batch-to-batch consistency and meeting regulatory requirements [13, 14]. The analytical challenges associated with lipid determination become more pronounced. Labour-intensive and time-consuming traditional analytical methods, prone to variability result, in an unsuitable high-throughput process. Additionally, multiple lipid components with similar chemical properties pose challenges for selective quantification, further complicating nanocarrier analysis. Various analytical methods such as liquid chromatography with ultraviolet detection (HPLC-UV-Vis) [14-16] or gas chromatography [11, 17] as well as nuclear magnetic resonance spectroscopy [18, 19] and mass spectrometry [20] have been utilized before in nanoliposome component determination. While these methods offer high sensitivity and selectivity, they often require complex sample preparation and laborious instrumentation, limiting their suitability for routine high-throughput analysis. Despite progress in determining lipid content in nanoliposomes, there is a lack of simultaneous separation and analysis of multiple lipid components in nanoliposomes [21-23]. This study aimed to develop a robust, simple, accurate and cost-effective analytical method for the determination of nanoliposome components as a drug carrier. Leveraging the unique absorbance properties of lipid components, we optimized chromatographic conditions to achieve simultaneous separation and determination of α-(3-O-cholesteryloxy)-δ-(N-ethylmorpholine)-succineamide (MoChol, cationic), cholesteryl-hemisuccinate (Chems, anionic), cholesterol, dioleoyl-phosphatidylethanolamine (DOPE) and palmitoyl-oleoylphosphatidylcholine (POPC). The method development process focused on appropriate chromatographic conditions to maximize sensitivity, selectivity, and reproducibility. Validation studies were conducted in accordance with the International Council for Harmonization (ICH) Guidelines to demonstrate the reliability and robustness of the proposed method.

Experimental

Instrumentation and reagents

HPLC-based separation and analysis were performed on a Shimadzu Prominence system (Shimadzu Corporation, Kyoto, Japan) equipped with an LC-20AD pump, an online DGU-20A degassing system, a CTO-20A column oven, and an SPD-20A UV–visible detector. The separation was achieved using a Hector C18-M column (250×4.6 mm, 5 µm particle size). The system was controlled using Lab Solutions software, version 5.51 (Shimadzu), which was also applied for data processing. Column temperature was kept constant at 50 ˚C, and the flow rate was set at 1.0 mL/min with a mobile phase gradient (Table 1), where mobile phase A was 0.1% trifluoroacetic acid (TFA) in Water and mobile phase B was 0.1% TFA in ethanol. The injection was performed using a 20 µL injection loop and the detector wavelength was

Table 1. Gradient program used for HPLC separation of lipid components

Time in Minutes	Mobile Phase A (%)	Mobile Phase B (%)
0	32	68
30	15	85
30.1	32	68
40	32	68

adjusted to 202 nm. Ethanol was used as diluent. These chromatographic conditions were selected based on an optimization process, considering factors such as peak shape, retention time, and resolution between lipid components. MoChol, TFA, and ethanol (HPLC grade) were purchased from Merck (Germany). Cholesterol, DOPE, POPC and Chems were purchased from Sigma-Aldrich, Switzerland. In-house purified Water provided by a Milli-Q purification system (Millipore) was used. The anti-BCL-2 DNA oligonucleotide loaded nanoliposome was purchased from Polymon Company. Figure 1 shows the chemical structures of MoChol, Chems, DOPE, POPC, and cholesterol.

Preparation of standard and test solutions

The stock standard solution (2000 mg/L) of DOPE, POPC, MoChol, Chems, and cholesterol was prepared by weighing approximately 7.5 mg of each standard and transferring it into a 5 mL volumetric amber flask. The diluent was added, and the mixture was sonicated to dissolve. All diluted solutions were prepared by diluting a concentrated stock solution. The results from different concentration levels (1-2000 ppm) were used to create a calibration target curve for each component. The sample chromatogram showed high-resolution separations of these lipids (Figure 2). Additionally, 25 µL of synthesized nanoliposome was added to 1000 µL of Otyl β-D-glucopyanoside water solution (2% w/w) to prepare the nanoliposome test solution, which was vortexed for 30 s before injection.

Materials and Methods

HPLC with refractive index (RI) or evaporative light scattering (ELSD) detector is commonly utilized for lipid composition analysis. Either RI or ELSD detectors have drawbacks, including the laborious parameter optimization, sample decomposition, detector response fluctuations and the need to add internal standards. Furthermore, these costly techniques are limitedly accessible, which hinders their widespread application in lipid analysis. In addition, despite their versatility, RI detectors have limitations: Their response is dependent on temperature, pressure fluctuation, flow rate instability, and mobile phase composition, which make them inappropriate for gradient elution [24] because the lipids lack chromophores, their detection in the UV range is limited to approximately 200–210 nm [25] which restricts the solvents, buffers or additives choice in sampling procedure and HPLC analysis [26] as a mobile phase modifier, TFA forms ion pairs with basic analytes, suppress their interaction with column stationary phase and reduce peak tailing [27]. In the current study, TFA addition overcame the limitation in selecting an appropriate additive. Consequently, a gradient HPLC method was developed for cholesterol, MoChol, Chems, POPC and DOPE analysis in nanoliposomes. The standard peak response (average of three replicates) was plotted versus the lipid concentration in the range of 1 to 2000 mg L^{-1} (Table 2). The quantitative data obtained using the developed HPLC-UV method demonstrate the method's linearity, reliability, and accuracy. Parameters including the slope, intercept, calibration equation and linear range were established through linear regression analysis and are presented in Table 3. These data highlight the method's robustness, providing a reliable tool for lipid component quantification in nanoliposome formulations. The developed method ensures consistent product quality and is essential for large-scale manufacturing.

Results

Method validation

To ensure the results are trustworthy and precise, key parameters in validation according to ICH guidelines were assessed. This typically involves evaluating accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), specificity, and robustness [28].

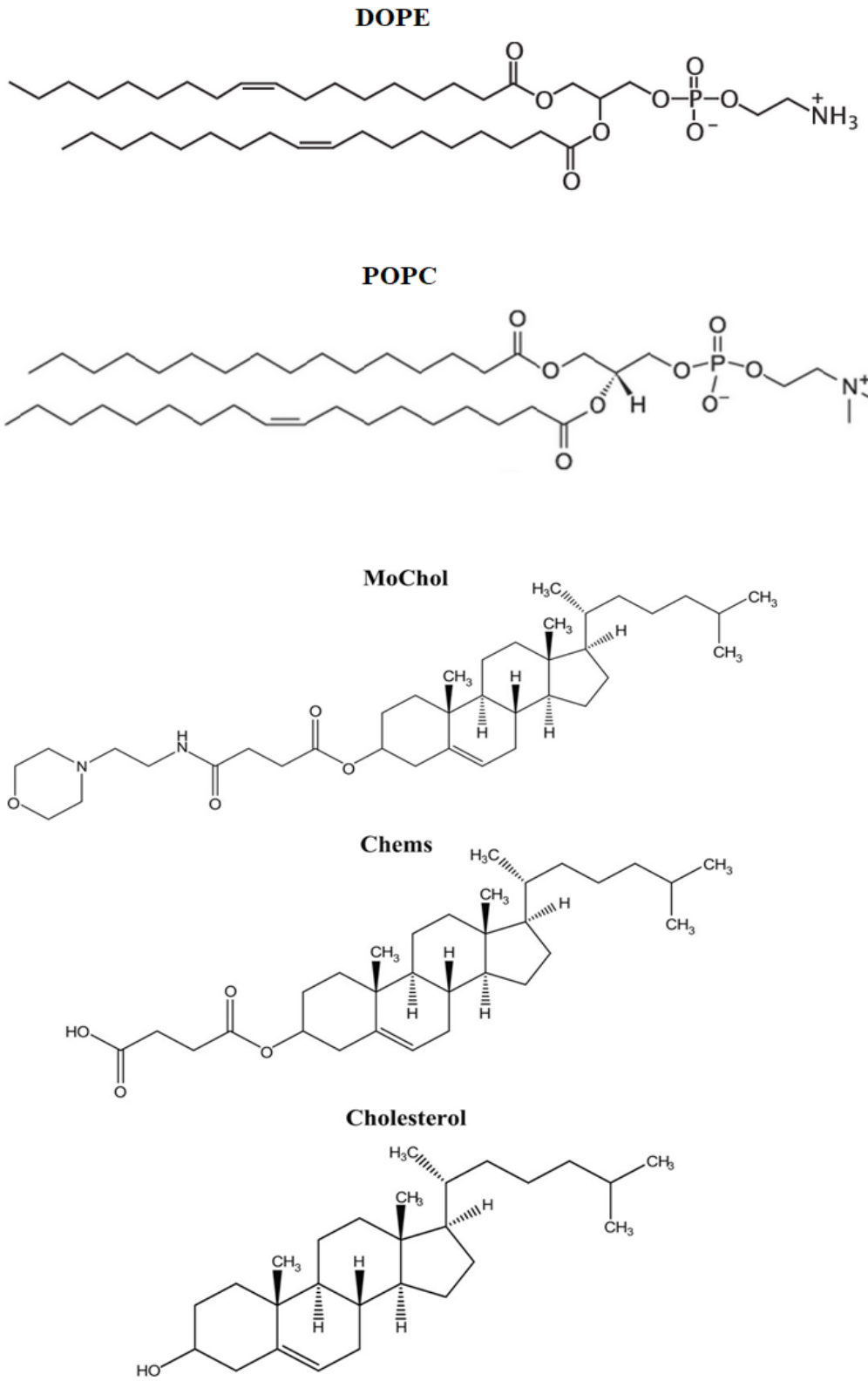


Figure 1. The chemical structures of DOPE, POPC, MoChol, Chems and Cholesterol

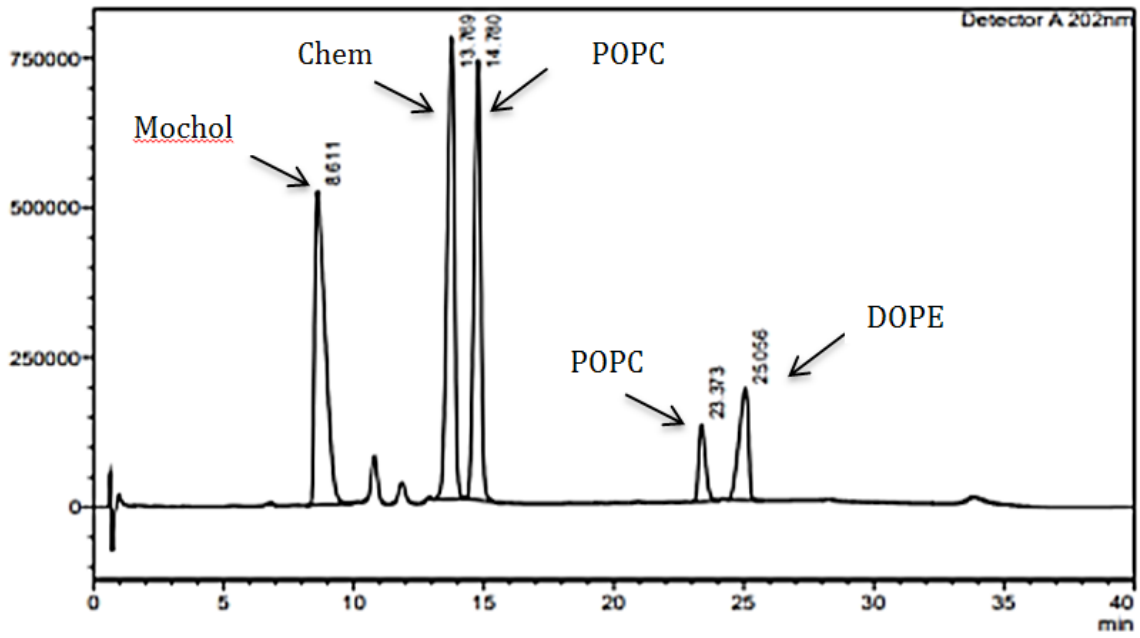


Figure 2. Chromatograms of DOPE, POPC, MoChol, Chems, and Cholesterol at 500 mg/L for each lipid

Table 2. Concentration of lipid components and corresponding peak area in HPLC-UV chromatograms

Mochol		Chems		Cholesterol		POPC		DOPE	
Conc. (ppm)	Ave. Area (n=3)	Conc. (ppm)	Ave.Area (n=3)	Conc. (ppm)	Ave.Area (n=3)	Conc. (ppm)	Ave.Area (n=3)	Conc. (ppm)	Ave. Area (n=3)
1.0	11965.0	1.0	10762.0	1.0	11800.0	1.0	1363.3	1.1	3635.0
1.9	23040.0	2.1	20591.0	2.1	23297.3	2.1	2977.0	2.2	7421.3
3.9	44343.0	4.0	40529.0	4.1	45280.7	4.2	5854.0	4.5	14102.3
7.8	87660.0	8.3	80118.0	8.2	90120.7	8.3	12235.0	8.9	27090.7
15.5	172150.0	16.6	158168.0	16.4	179788.0	16.7	25637.7	17.8	54517.0
31.0	341654.0	33.2	311920.0	32.8	357218.7	33.3	51058.3	35.7	107455.0
62.1	678217.0	66.3	621587.0	65.7	709577.0	66.7	102420.3	71.4	213526.0
124.1	1338935.0	132.6	1229839.0	131.4	1402286.0	133.4	202775.7	142.8	420095.0
248.3	2691191.0	265.3	2444848.0	262.8	2762997.7	266.8	408881.4	285.5	840831.2
496.5	5248027.0	530.5	4769047.0	539.5	5356538.2	533.5	807160.6	571.0	1652664.2
993.0	10082383.0	1061.0	9076080.0	1051.0	9939365.0	1067.0	1567506.4	1142.0	3198619.6
1986.0	19322007.0	2122.0	17635057.0	1982.0	18928147.3	2134.0	3046529.0	1713.0	4722104.8

Abbreviations: Chems: Cholesteryl hemisuccinate; DOPE: Dioleoyl-phosphatidylethanolamine; POPC: Palmitoyl-oleoylphosphatidylcholine; MoChol: α-(3-O-cholesteryloxy)-δ-(N-ethylmorpholine)-succineamide.

Note: The calibration curve was generated using triplicate injections of each solution, ensuring accuracy and consistency with the linear range

Table 3. Calibration data, linear range, LOD, and LOQ for the developed HPLC-UV method

Lipid	Slope	Intercept	r^2	Linear Range (mg/L)	LOD (mg/L)	LOQ (mg/L)
MoChol	100082.29	57864.07	0.9998	1-1986	0.13	0.39
Chems	8414.58	33063.69	0.9997	1-2122	0.16	0.49
Cholesterol	9517.34	67844.10	0.9997	1-1982	0.14	0.43
POPC	1434.30	9523.29	0.9997	1-2134	0.23	0.69
DOPE	2768.96	16467.92	0.9997	1.1-1713	0.20	0.59

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Abbreviations: Chems: Cholesteryl hemisuccinate; DOPE: Dioleoyl-phosphatidylethanolamine; POPC: Palmitoyl-oleoylphosphatidylcholine; MoChol: α-(3-O-cholesteryloxy)-δ-(N-ethylmorpholine)-succineamide; LOD: Limit of detection; LOQ: Limit of quantification.

Table 4. Accuracy and precision (n=5) of the target analytes in the developed method

Conc. (ppm)Real		Area	Found Conc. (ppm)	%		
				Relative Error	Recovery	RSD
Mochol	695.00	7078737	696.36	0.20	100.20	0.05
		7076358	696.12	0.16	100.16	
		7074541	695.94	0.14	100.14	
		7071146	695.60	0.09	100.09	
		7071508	695.64	0.09	100.09	
	1490.00	14826698	1464.83	-1.69	98.31	0.11
		14797767	1461.96	-1.88	98.12	
		14808139	1462.99	-1.81	98.19	
		14792542	1461.44	-1.92	98.08	
		14786202	1460.81	-1.96	98.04	
Chems	752.70	6470355	765.02	1.64	101.64	0.05
		6470909	765.08	1.64	101.64	
		6471321	765.13	1.65	101.65	
		6476289	765.72	1.73	101.73	
		6477043	765.81	1.74	101.74	
	1591.50	13293746	1575.92	-0.98	99.02	0.10
		13308620	1577.68	-0.87	99.13	
		13287472	1575.17	-1.03	98.97	
		13314937	1578.43	-0.82	99.18	
		13284176	1574.78	-1.05	98.95	

Conc. (ppm)Real		Area	Found Conc. (ppm)	%		
				Relative Error	Recovery	RSD
Cholesterol	760.70	7397835	770.17	1.25	101.25	0.35
		7346431	764.77	0.54	100.54	
		7349280	765.07	0.57	100.57	
		7393642	769.73	1.19	101.19	
		7391890	769.55	1.16	101.16	
	1576.50	15010095	1570.00	-0.41	99.59	0.40
		14942688	1562.92	-0.86	99.14	
		14987231	1567.60	-0.56	99.44	
		14866972	1554.96	-1.37	98.63	
		14901874	1558.63	-1.13	98.87	
POPC	746.90	1099474	759.92	1.74	101.74	0.08
		1099085	759.64	1.71		
		1098788	759.44	1.68	101.68	
		1099851	760.18	1.78	101.78	
		1101132	761.07	1.90	101.90	
	1600.50	2325101	1614.42	0.87	100.87	0.59
		2327527	1616.12	0.98	100.98	
		2299071	1596.28	-0.26	99.74	
		2303899	1599.64	-0.05	99.95	
		2302922	1598.96	-0.10	99.90	
DOPE	799.40	2246722	805.45	0.76	100.76	0.10
		2244785	804.75	0.67	100.67	
		2247331	805.67	0.78	100.78	
		2244419	804.62	0.65	100.65	
		2249924	806.60	0.90	100.90	
	1713.00	4749295	1709.24	-0.22	99.78	0.60
		4756235	1711.75	-0.07	99.93	
		4697933	1690.69	-1.30	98.70	
		4705426	1693.40	-1.14	98.86	
		4701635	1692.03	-1.22	98.78	

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Abbreviations: Chems: Cholesteryl hemisuccinate; DOPE, dioleoyl-phosphatidylethanolamine; POPC: Palmitoyl-oleoylphosphatidylcholine; MoChol: α-(3-O-cholesteryloxy)-δ-(N-ethylmorpholine)-succineamide; RSD: Relative standard deviation.

Table 5. Specificity parameters of the developed method

Lipid	TF[a]	Resolution	NTP[d]
Mochol	1.407	-	5149
Chems	1.033	10.127[b]	15249
Cholesterol	1.072	2.361[c]	17941
POPC	1.0182	19.780	44941
DOPE	0.942	2.927	49250

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Abbreviations: Chems: Cholesteryl hemisuccinate; DOPE: Dioleoyl-phosphatidylethanolamine; POPC: Palmitoyl-oleoylphosphatidylcholine; MoChol: α-(3-O-cholesteryloxy)-δ-(N-ethylmorpholine)-succineamide.

[a]Tailing factor, [b]resolution between mochol and chems, [c]resolution between chems and cholesterol, [d]number of theoretical plate.

Accuracy

Accuracy refers to how closely the results obtained from an analytical method match the true or accepted value. It measures the method's exactness and is assessed by comparing measured values to known reference values. In this study, accuracy was assessed by analyzing five replicate standard solutions at approximately 700 ppm and 1600 ppm. The recovery percentages shown in Table 4 were close to the range of 98–102%.

Precision

Precision refers to the consistency or reproducibility of the results and is monitored by the relative standard deviation percentage (RSD%) obtained from multiple standard or sample injections. As shown in Table 4, the proposed method precision was assessed by measuring the repeatability of peak areas for samples used in the accuracy test, meeting the criterion (RSD≤1%), indicating reproducible results.

Limit of detection and limit of quantification

LOD is the lowest concentration of an analyte that can be reliably detected but not necessarily quantified, while the LOQ is the lowest concentration that can be quantified accurately and precisely. These parameters were calculated using the y-intercept standard deviation value and the linearity plot slope. The calibration curve was created using samples with lower analyte content near the LOD. Table 3 presents the obtained LOD and LOQ values with the proposed method.

Specificity

As defined by the ICH guideline, specificity refers to the method's ability to measure the analyte in the presence of impurities, by-products, and matrix components accurately and selectively. Specificity of validation ensures that each peak response corresponds to a single component without overlap. Parameters commonly studied to evaluate specificity include resolution, tailing factor (TF) and number of theoretical plates (NTP). In the present study, it is crucial to achieve a suitable resolution between MoChol, Chems, and Cholesterol, as these are structurally cholesterol-related derivatives. The structural similarity between these lipids makes them difficult to distinguish during chromatographic separation. In addition, because their degradation can affect nanoliposome stability and efficacy, their specific separation is mandatory. Our method provides reliable and precise quantification by achieving a resolution >2.0. Table 5 presents the specificity parameters at 250 mg/L, demonstrating that the resolution between the five lipids exceeds 2.0, ensuring the integrity and performance of the proposed method.

Robustness

Method robustness refers to the ability to withstand minor but deliberate deviations in flow rate, mobile phase composition, and column temperature without significantly affecting results. It was evaluated by examining system suitability parameters, as shown in Table 6. The results indicate that the method remains stable, with NTP values consistently above 5,000, TF values below 1.5 for each lipid, and resolution exceeding 2. This indicates that the method remains unaffected by parameter variations and is reliable under normal usage.

Table 6. The results obtained for the robustness of the method

Parameter	Lipid	RSD of RT (%)	Average of TF	Resolution	Average of NTP
Flow rate (mL min^{-1})=0.9	Mochol	0.05	1.42		5870
	Chems	0.05	1.09	10.21	17689
	Cholesterol	0.03	1.11	2.42	19915
	POPC	0.06	1.09	19.92	45390
	DOPE	0.02	0.97	3.12	53190
Flow rate (mL min^{-1})=1.1	Mochol	0.07	1.38		5046
	Chems	0.06	1.03	10.08	15097
	Cholesterol	0.04	1.02	2.31	16865
	POPC	0.03	1.01	19.65	39548
	DOPE	0.03	0.92	2.87	47280
Column temperature (˚C)=47	Mochol	0.04	1.43		5515
	Chems	0.02	1.10	10.12	16774
	Cholesterol	0.03	1.12	2.41	17564
	POPC	0.04	1.10	19.72	44447
	DOPE	0.05	0.98	2.81	48708
Column temperature (˚C)=53	Mochol	0.07	1.37		5082
	Chems	0.03	1.02	10.08	15554
	Cholesterol	0.04	1.01	2.39	17582
	POPC	0.03	1.00	19.82	43323
	DOPE	0.04	0.91	3.01	51713
ACN in mobile phase (%)=28	Mochol	0.22	1.38		5870
	Chems	0.12	1.02	9.57	15401
	Cholesterol	0.15	1.08	2.10	177616
	POPC	0.18	1.05	18.85	448511
	DOPE	0.08	0.93	2.65	49743
ACN in mobile phase (%)=32	Mochol	0.16	1.43		5303
	Chems	0.14	1.04	10.86	15371
	Cholesterol	0.12	1.06	2.65	18443
	POPC	0.21	1.02	19.69	44132
	DOPE	0.19	0.92	2.93	48758

Abbreviations: RT: Retention time; TF: Tailing factor; NTP: Number of theoretical plate.

Table 7. Lipid content in the nanoliposome; the sample preparation was carried out by adding 25 µL of prepared nanoliposome to 1000 µL of Otyl β-D-glucopyanoside water solution (2% w/w) and vortexed for 30 s

No.	Lipid	Concentration of lipids (mg/L)[a]	Obtained Concentration of lipids (mg/L)[b]
1	Mochol	23340	22120
2	Chems	9450	9260
3	Cholesterol	900	2090
4	POPC	4030	4230
5	DOPE	15310	15950

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[a]Concentration of lipids immediately after preparation (initial lipid concentration), [b]obtained concentration of lipids after 8 months storage by the proposed method.

Discussion

The purchased nanoliposome real sample contained the following amounts (mg/L) of API: 1.53, MoChol: 23.34, Chems: 9.45, POPC: 4.03, DOPE: 15.31, and Cholesterol: 0.9. Also, it exhibits these characteristics: an average particle size of 124.4 nm, a polydispersity index of 0.13, a zeta potential of −32.4 mV, a pH of 7.56, and an endotoxin content of 3.44 EEU mL^{-1}. MoChol and Chems may undergo degradation through oxidation or hydrolysis under storage-related conditions. Oxidation produces peroxides that degrade MoChol and Chems into Cholesterol and other products. In addition, hydrolysis or enzymatic catalysis breaks ester bonds in the formulation or environment. MoChol and Chems' integrity in the nanoliposome is compromized by this degradation process, affecting its stability and efficacy. The nanoliposome compositions were determined after storage for 8 months at −20 ˚C to compare the stability of different batches. Preparation of the real sample involved adding nanoliposomes (25 µL) to Otyl β-D-glucopyanoside solution in water (1000 µL, 2% w/w) to achieve concentrations within the linear calibration ranges. The solution was vortexed for 30 s and injected into the HPLC. Table 7 presents the quantitative results. Slight degradation of MoChol and Chems over 8 months was revealed, while Cholesterol concentration significantly increased. The observed compositional changes underline the importance of this analytical approach for monitoring formulation stability.

Conclusion

In the current study, an HPLC-UV method for the simultaneous determination of lipid components in nanoliposome formulations was developed and validated. The method addressed significant technical challenges in lipid analysis. By optimizing chromatographic conditions, specifically the mobile phase composition, the method achieved high sensitivity, selectivity, and reproducibility. Key parameters, including linearity, precision, accuracy, and specificity, were thoroughly evaluated according to ICH guidelines. Linear regression analysis demonstrated strong correlation coefficients, and the method's precision met the predefined acceptance criterion of ≤1%. The accuracy, validated through recovery studies, was within the acceptable range of 98–102%. In addition to technical robustness, the method effectively detected minor degradation products, demonstrating its capability to monitor nanoliposome stability. The observed degradation product underlines the importance of this analytical approach in ensuring drug delivery systems' stability and efficacy. In addition, the method's ability to separate and quantify cholesterol derivatives and two phospholipids with sufficient resolution is crucial, due to their similarities. Specificity parameters, such as NTP, resolution, and TF, confirmed the method's adequacy in distinguishing these compounds without peak overlap. The developed HPLC-UV method offers a reliable, cost-effective way for routine analysis, facilitating quality control and regulatory compliance. Its robustness and sensitivity ensure consistent product quality, minimizing the risk of batch failure or deviations. This study's findings significantly contribute to the field of nanomedicine by providing a standardized analytical framework for future research and development in lipid-based drug delivery systems.

Ethical Considerations

Compliance with ethical guidelines

There were no ethical considerations to be considered in this research.

Funding

This research was financially supported by Tofigh Daru Research and Engineering Company, Tehran, Iran.

Authors' contributions

Supervision and conceptualization: Abolghasem Beheshti and Mahmood Payeghadr; Review and editing: Abolghasem Beheshti and Mehdi Tavakoli; Methodology: Abolghasem Beheshti, Mahmood Payeghadr and Vahid Karbasi; Resources, project administration and funding acquisition: Abolghasem Beheshti; Investigation, software, data curation, formal analysis, visualization: Vahid Karbasi; Writing the original draft, validation, formal analysis: Mehdi Tavakoli.

Conflict of interest

The authors declared no conflict of interest.

Acknowledgments

Authors thank Tofigh Daru Research and Engineering Company for the financial and technical support of this project.

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