Analytical Approaches for Assessing Curcumin and Nicotinamide Co-Encapsulated in Liposomal Formulation: UV Spectrophotometry and HPLC Validation

Ali Fahdawi[1], Naeem Shalan[1], Zainab Lafi[1*], Omar Markab[1]

[1] Pharmacological and Diagnostic Research Center, Faculty of Pharmacy, Al-Ahliyya Amman University, Amman, Jordan.

ABSTRACT

Background: The study presents two distinct analytical methods tailored for the precise determination of curcumin (CUR) and nicotinamide (NIC) within liposomal formulations, addressing the needs of researchers and analysts in the biomedical and food supplement sectors.

Method: UV spectrophotometry provides a swift and cost-effective solution for quantification, while High-Performance Liquid Chromatography (HPLC) offers enhanced specificity and sensitivity, particularly in complex matrices. Method validation, especially for HPLC, ensures reliability and suitability for rigorous analysis, advancing the field of Analytical Chemistry and strengthening development and quality assurance processes in the pharmaceutical and biotechnology industries.

Results: The encapsulation efficiencies of CUR and NIC into liposomes, primarily composed of DPPC and CHO, were found to be 30% ± 6% and 80% ± 5%, respectively. The developed analytical methods using UV spectrophotometry and reverse-phase HPLC demonstrated robustness and efficiency, allowing for the simultaneous analysis of CUR and NIC with high specificity, accuracy, and precision. Validation according to ICH Q2 guidelines revealed excellent system suitability, linearity, and robustness, with relative standard deviation consistently below 2%. Stability studies over three weeks at 4°C showed minimal changes in liposomal characteristics, indicating good stability. Furthermore, release studies at 37°C demonstrated enhanced solubility and increased release of curcumin, suggesting the potential of the liposomal formulation for drug delivery applications.

Conclusion: This study developed straightforward, time-efficient, and cost-effective analytical methods using UV spectrophotometry and reverse-phase HPLC to quantify CUR and NIC encapsulated in liposomal formulations.

Keywords: Liposomes, Analytical Methods, UV Spectrophotometric, HPLC.

1. INTRODUCTION

Natural compounds offer numerous advantages in the quest for novel antioxidants, anti-inflammatory agents, anticancer agents, and antimicrobials (1-4). They often exhibit unique mechanisms of action that can selectively target specific pathways involved in oxidation, cell growth, and immunomodulation. Moreover, natural compounds hold promise for overcoming drug resistance, a common challenge encountered in many diseases (5, 6).

Curcumin (Figure 1A), a polyphenol present in turmeric, has demonstrated promising effects across various biological systems, modulating multiple signaling pathways implicated in general health (7). On the other hand, CUR has shown antibacterial activity against both Gram-negative and Gram-positive bacteria in many studies. CUR disrupts bacterial membranes and inhibits bacterial biofilm formation, which leads to oxidative stress (8, 9).

[*]***Corresponding author: Zainab Lafi***
z.lafi@ammanu.edu.jo
Received: 09/02/2024 Accepted: 10/03/2024.
DOI: https://doi.org/10.35516/jjps.v17i3.2359

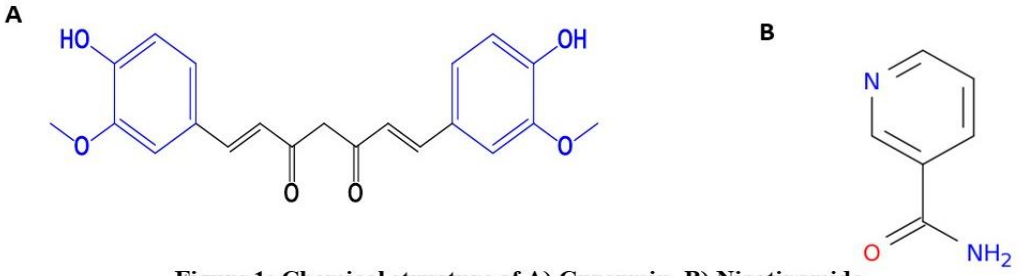


Figure 1: Chemical structure of A) Curcumin, B) Nicotinamide

Nicotinamide (Figure 1B), a derivative of Vitamin B3, has displayed notable potential in treating infectious diseases such as acne. NIC has shown promise as an adjuvant therapy when combined with other agents, enhancing their effectiveness while mitigating adverse effects (10). Its role in cellular metabolism is crucial, as it is involved in maintaining genomic stability and facilitating DNA repair processes (11-14). Nicotinamide has also demonstrated the ability to enhance the effectiveness of conventional cancer therapies, including radiation and chemotherapy. By sensitizing cancer cells to the damaging effects of these treatments, it makes them more vulnerable to therapy, potentially allowing for reduced doses (15). Additionally, NIC has the capacity to modulate the energy metabolism of cancer cells, targeting their altered metabolic pathways. NIC disrupts these metabolic adaptations, leading to energy depletion and increased susceptibility to treatment (16). Consequently, in this study, these two bioactive compounds (CUR and NIC) were combined in a liposomal nanoparticle formulation as immune-modulating supplements.

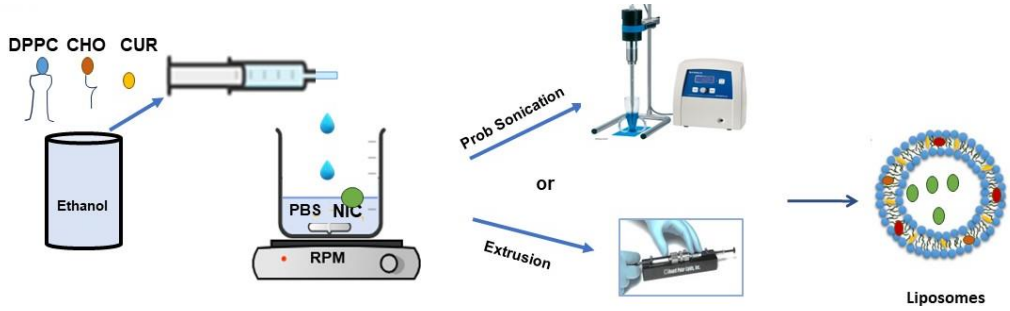


Figure 2: Schematic representation of CUR-NIC preparation by ethanol injection method

Liposomes are lipid-based vesicles that serve as carriers for both hydrophilic and hydrophobic drugs, enabling targeted delivery of therapeutic agents to specific anatomical sites. Their unique structure allows for the encapsulation of both water-soluble and lipid-soluble drugs, thereby enhancing solubility and stability (17, 18). Moreover, liposomes can protect drugs from premature degradation and metabolism, minimizing systemic toxicity and optimizing the therapeutic index (Joy et al., 2022) (19). Additionally, liposomal formulations offer the potential for incorporating targeting ligands on their surface, enabling active recognition of cancer cells. These

ligands can specifically bind to receptors overexpressed on cancer cells, promoting the internalization of liposomes and improving drug delivery to the tumor site (20).

This study aims to develop and validate fast and consistent UV-spectrophotometry and HPLC methods for the simultaneous quantification of CUR and NIC in liposome suspensions, in accordance with current official ICH guidelines. This is the first study to encapsulate a combination of the natural compounds NIC and CUR inside a liposomal delivery system to enhance their promising antioxidant and anti-inflammatory therapeutic activities. Accurate and validated analytical methods are essential for the consistent and precise quantification of encapsulated drugs in liposomal formulations. To date, no method has been reported that simultaneously quantifies these two bioactive compounds in supplement formulations.

2. MATERIALS AND METHODS

2.1. Materials:

CUR was sourced from ICT (Japan), while NIC was acquired from Sigma-Aldrich (USA). 1,2-Dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) was obtained from Avanti Polar Lipids, Inc. (Alabaster, Alabama, USA), and cholesterol (CHO) was procured from Carbosynth (UK). Phosphate Buffer Saline (PBS) was purchased from LONZA® (USA). HPLC-grade methanol was obtained from Sigma-Aldrich (USA), whereas HPLC-grade ethanol and methanol were sourced from the Carbon Group (England). All other chemicals and solvents used were of high analytical purity, and no further treatment was conducted on any of the reagents or chemicals.

2.2. Liposomes Preparation

Liposomes were prepared using the conventional ethanol injection method. Specifically, 4 mg of DPPC, 1.0 mg of CHO, and 1.0 mg of CUR were dissolved in 0.3 mL of ethanol, which was then warmed to 40°C in a water bath. Meanwhile, NIC was dissolved in phosphate-buffered saline (PBS) and heated on a hot plate at approximately 50°C for 14 minutes with continuous stirring at 700 rpm. Subsequently, the warm ethanol solution containing lipids and CUR was swiftly injected into the NIC-containing PBS under continuous stirring and heating conditions.

The resulting liposomal suspension underwent sequential extrusion, if necessary, through a polycarbonate membrane to achieve the desired size of 100 nm, using a Mini-Extruder (Avanti Polar Lipids, Inc., USA) at a temperature of 50°C. Following extrusion, free drug molecules were removed by washing the liposomes twice with PBS solution using a dialysis bag. Finally, the liposomes were stored at 4°C to maintain stability (19).

2.2. Liposomes Characterization

2.2.1. Hydrodynamic diameter, zeta potential and polydispersity Index of liposomes.

The average particle size, zeta potential (charge), and polydispersity index (PDI) of the liposomes were determined through dynamic light scattering (DLS) experiments using a Zetasizer Nano-ZS (Malvern Instruments Ltd., Malvern, UK). Liposomal samples were appropriately diluted with deionized water (10:990 µL, v/v) to achieve a suitable counting rate. Before each measurement, all samples were equilibrated at room temperature for 30 seconds in the specimen holder of the Zetasizer. This ensured consistent and accurate measurements of particle size, zeta potential, and PDI, providing valuable insights into the characteristics and stability of the liposomal formulations (21).

2.2.2. *In vitro* Release and Stability of prepared liposomes:

The stability of the liposomes was evaluated over a storage period of 4 weeks at 4°C. At regular intervals, liposome samples (50 µL) were collected, and the mean hydrodynamic diameter and zeta potential of the loaded liposomes were determined using the previously described DLS method. All liposome samples were appropriately diluted in deionized water before measurements to ensure accurate results.

To assess the release profile of encapsulated CUR and NIC, liposome suspensions were placed in dialysis bags (molecular weight cutoff 10 kDa, Thermo Fisher Scientific) containing 1 mL PBS. These dialysis bags were then suspended in a release volume of 20 mL PBS at pH 7.4 and maintained at 37°C. At regular intervals, 200 µL of the release medium was collected for HPLC analysis, with an equivalent volume of fresh PBS buffer at the same temperature immediately added to maintain a constant release volume. Consistency in the length of the dialysis tubing was maintained across all experiments to ensure a uniform surface area for dialysis. This comprehensive approach allowed for the monitoring of liposomal stability and the assessment of drug release kinetics over the specified storage period.

2.2. Measuring of liposomal Encapsulation Efficiencies:

To assess the encapsulation efficiencies (EE%) of CUR and NIC in the liposomal formulations, 200 µL of the liposome suspension was mixed with 800 µL of methanol to disrupt the liposomes. The resulting solution was vortexed for 3 minutes and then centrifuged at 15,300 rpm for 15 minutes. This process was conducted in triplicate, and the results were recorded as the mean ± standard deviation (SD).

$$Encapsulation\ Efficiency\ (EE\%) = \frac{\text{Actual Ammount of Drug Loaded}}{\text{Total Theoritical Amount of Drug Used}}\ X\ 100\ \% \quad (1)$$

$$Loading\ efficeiency\ (EE\%) = \frac{\text{Actual ammount of drug loaded}}{\text{Total lipid and drug used}}\ X\ 100\ \% \quad (2)$$

Where:

The total amount of drug in liposomes refers to the amount of drug encapsulated within the liposomes, while the amount of free drug is the drug remaining in the supernatant after centrifugation. The total amount of drug added is the initial amount of drug introduced during liposome preparation. This methodology ensured accurate quantification of drug encapsulation within the liposomal formulations, providing insights into their efficacy and performance.

2.3. Standard solution UV system and HPLC:

Two stock solutions were prepared for CUR and NIC. The first stock standard solution of CUR (2.0 mg/mL) was prepared by dissolving 2.0 mg of CUR in 2.0 mL of methanol. The second stock standard solution of NIC (1.0 mg/mL) was prepared by dissolving 1.0 mg of NIC in 1.0 mL of either ethanol or methanol. Both solutions were subjected to ultrasonication in a water bath for 10 minutes to ensure complete dissolution. Subsequently, calibration curves were established using eight standard solutions with increasing concentrations of CUR and NIC for the linearity assay. Prior to analysis, all solutions were filtered through a 0.45 µm cellulose membrane and then injected into the HPLC system, with each measurement performed in triplicate (n=3).

This rigorous preparation and analysis protocol ensured the accuracy and reliability of the HPLC method for quantifying CUR and NIC concentrations, facilitating the establishment of robust calibration curves for subsequent sample analysis.

2.4. HPLC Conditions and parameters:

The analysis was conducted using a Shimadzu LC-2030 HPLC system equipped with a UV detector (Shimadzu Corporation, Kyoto, Japan). A Thermo Scientific™ Hypersil™ C18 HPLC column, with dimensions of 4.6 × 150 mm and a particle size of 5 µm, featuring a 100 Å pore size, was employed. The mobile phase consisted of methanol and water (80:20, v/v) with a pH of 3. Chromatographic data acquisition and integration were performed using Laboratory Solutions version 5.92. Prior to use, the mobile phase was filtered through a nylon Millipore membrane filter with a pore size of 0.2 µm and degassed to remove any trapped air. The mobile phase was pumped at a flow rate of 1.0 mL/min, and the injection

volume was set to 20 µL. The analytical column temperature was maintained at 40°C throughout the analysis. The chromatographic run time was set to 10 minutes, during which CUR and NIC were detected at a wavelength of 262 nm using the UV detector. This comprehensive setup ensured precise separation and quantification of CUR and NIC in the analyzed samples, providing accurate and reliable results for further data interpretation and analysis.

2.5. Validation of the method

The method was validated according to the International Conference on Harmonization (ICH) guidelines regarding the following parameters: specificity, linearity, detection and quantification limits, precision, and accuracy. System suitability parameters, such as the theoretical plate number, tailing factor, and resolution between CUR and NIC peaks, were evaluated.

2.5.1. Selectivity and Specificity

Analytical method specificity was determined by evaluating the blank and unloaded liposomes compared to CUR and NIC-loaded liposomes at the concentrations used in the working standard. The samples were analyzed under the same chromatographic conditions to verify the absence of interference or overlaps from excipients, phospholipids, and sucrose. The method was validated according to ICH guidelines.

2.5.2. Calibration curve and linearity range:

Six standard solutions of CUR and NIC were prepared as detailed in Table 1. Three independent calibration curves were established, and linearity was evaluated by least-squares regression analysis. The lower limit of detection (LOD) and the lower limit of quantification (LOQ) were determined from the calibration plot based on the following equations:

$$LOD = \frac{3.3\,\sigma}{S} \quad (3)$$

$$LOQ = \frac{10\sigma}{S} \quad (4)$$

where σ is the Standard Deviation of the Intercept and S is the Slope of the Calibration Plot (22) .

Table 1: System suitability results for CUR and NIC.

Parameters	Value		Limits
	CUR	NIC	
Number of theoretical plates	2200	2300	> 2000
Tailing factor	0.938	0.907	< 2

2.5.3. Assessment of method Precision

Intermediate precision, also known as inter-day precision, was evaluated by measuring samples with the same concentrations—250 µg/mL for CUR and 250 µg/mL for NIC—on three different days by two different analysts. The results were expressed as the relative standard deviation (RSD%). Repeatability, also referred to as intra-day precision, was assessed by measuring six sample solutions, each in triplicate, at the same concentrations on a single day under identical experimental conditions. The results were also expressed as RSD%. These precision studies were essential for evaluating the consistency and reliability of the analytical method across different days and analysts, as well as within the same day, providing valuable insights into the robustness and reproducibility of the HPLC method for quantifying CUR and NIC concentrations.

2.5.4. Assessment of method Accuracy

Accuracy was assessed by triplicate assays of samples with known liposome concentrations spiked with three different concentrations of CUR and NIC, as well as standard solutions at four different levels (50%, 100%, and 150%). Recovery (%) was calculated based on the differences between the measured concentration of the spiked solutions

and the expected concentration, with results expressed as the RSD% of the triplicate measurements. This accuracy evaluation was crucial for determining the reliability and correctness of the analytical method in quantifying CUR and NIC concentrations in liposomal formulations across a range of spiked concentrations.

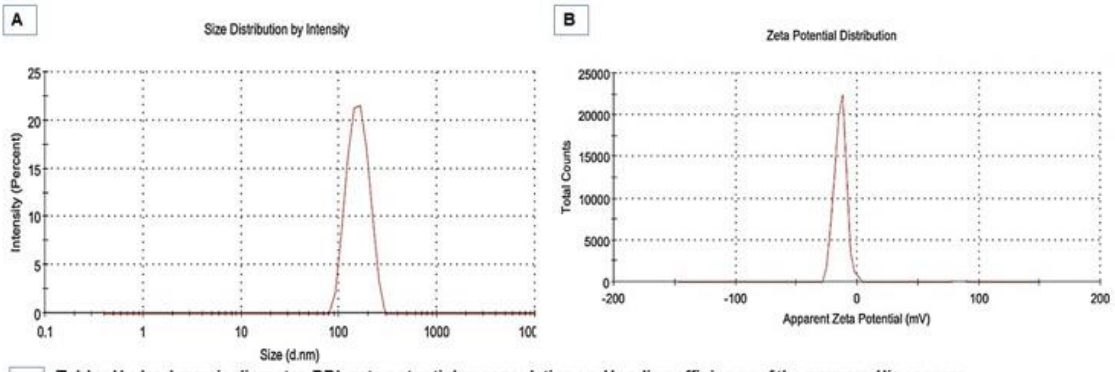
2.5.5. Assessment of method Robustness

Method Robustness was examined by measuring the sample under various method conditions to assess the impact of each variation.

3. RESULT AND DISCUSSIONS

3.1. Liposomes Characterization

In the current study, CUR and NIC were encapsulated into a liposomal formulation composed mainly of DPPC and CHO. The CUR and NIC liposomes exhibited an average particle size of 158.5 ± 6.1 nm and a polydispersity index (PDI) of less than 0.2. The zeta potential of the prepared liposomes was −16.6 ± 0.74 mV. The encapsulation efficiencies were found to be 30 ± 0.82% for CUR and 80.10 ± 0.39% for NIC, which are considered promising and high, especially for hydrophobic drugs encapsulated into the liposomal bilayer simultaneously. The experiments were conducted to develop a quick and effective method to simultaneously analyze CUR and NIC using both UV-spectrophotometry and HPLC with a UV detector (Figure 3A, 3B, 3C).



C Table : Hydrodynamic diameter, PDI, zeta potential encapsulation and loading efficiency of the prepared liposomes.

Liposomes (n=3)	Hydrodynamic diameter (nm ± SD)	Polydispersity index (PDI ± SD)	ζ-potential (mV ± SD)	Encapsulation efficiency %	Loading efficiency %
Lipo-NC-CUR	155.6±10.3	0.086±0.5	-22±5	NC: 80% CUR: 30%	NC:16% CUR: 5%

Figure 3: liposomes DLS characterization of CUR-NIC mixture (A) Size distribution by intensity (B) Apparent zeta potential (C) DLS measurements and encapsulation and loading efficiencies.

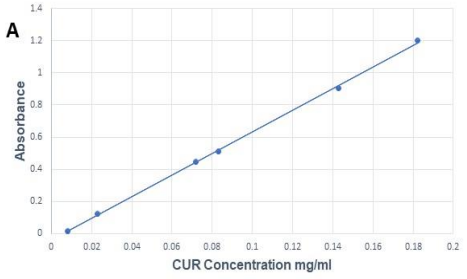
The chromatographic method employed utilizes a mobile phase consisting of methanol and water (pH 3) in an 80:20 v/v ratio, yielding symmetric peaks with retention times of 2.796 and 4.644 minutes for CUR and NIC, respectively (see Figure). These conditions facilitate swift and routine analyses. The selected chromatographic parameters include a column temperature of 40 ± 2°C, a 20 µL sample injection volume, an isocratic flow rate of 1.0 mL/min, a detection wavelength of 262 nm, and a 10-minute run time. These conditions have been deemed suitable for further procedures, including method validation.

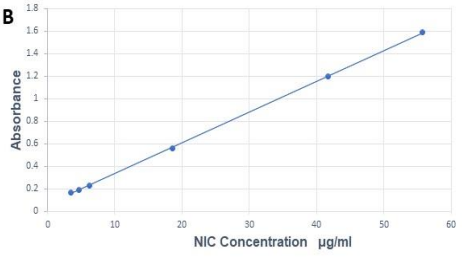
Furthermore, the chromatogram of blank liposomes shows no peaks that might interfere with the retention times of CUR and NIC, indicating that the liposome constituents do not interfere with the quantification of these drugs. This confirms the method's reliability (Figure 4A, 4B, 4C, 4D).

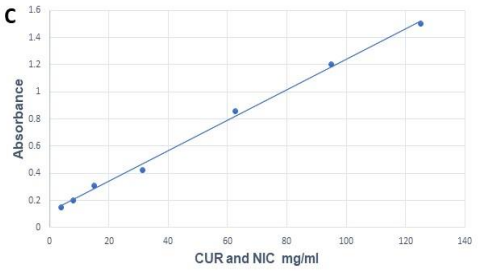
3.1.1. Validation of HPLC Method/ Limit Of Detection and Limit of Quantification:

Validation was performed following ICH Q2 guidelines. The tailing factors for both CUR and NIC peaks remained below 2 in all instances, with the number of theoretical plates exceeding 2,000, indicating the column's efficacy. The validation process included assessments of system suitability, specificity, linearity range, limit of detection (LOD), limit of quantification (LOQ), precision, accuracy, and robustness. Repeated analyses consistently placed CUR and NIC retention times at approximately 2.8 and 4.6 minutes, respectively, demonstrating commendable resolution between the two peaks. The relative standard deviation (%RSD) of recorded retention times was consistently below 2%, indicating excellent precision on the HPLC system. Overall, these results reflect the method's robustness and reliability for the simultaneous quantification of CUR and NIC (Table 1).

Linearity was assessed using calibration curves for both CUR and NIC, with both analytes showing an R^2 value of 0.999, indicating a strong correlation between concentration and peak area (Figure 4D). The LOD and LOQ for CUR were 0.0152 µg/mL and 0.0171 µg/mL, respectively. The measured LOD and LOQ values for CUR and NIC were 0.02 µg/mL and 0.025 µg/mL (LOD), and 0.06 µg/mL and 0.075 µg/mL (LOQ), respectively. Notably, no significant differences were observed when comparing the calculated and measured LOQ values on the linear calibration curve ($p < 0.05$) (Figure 4D).







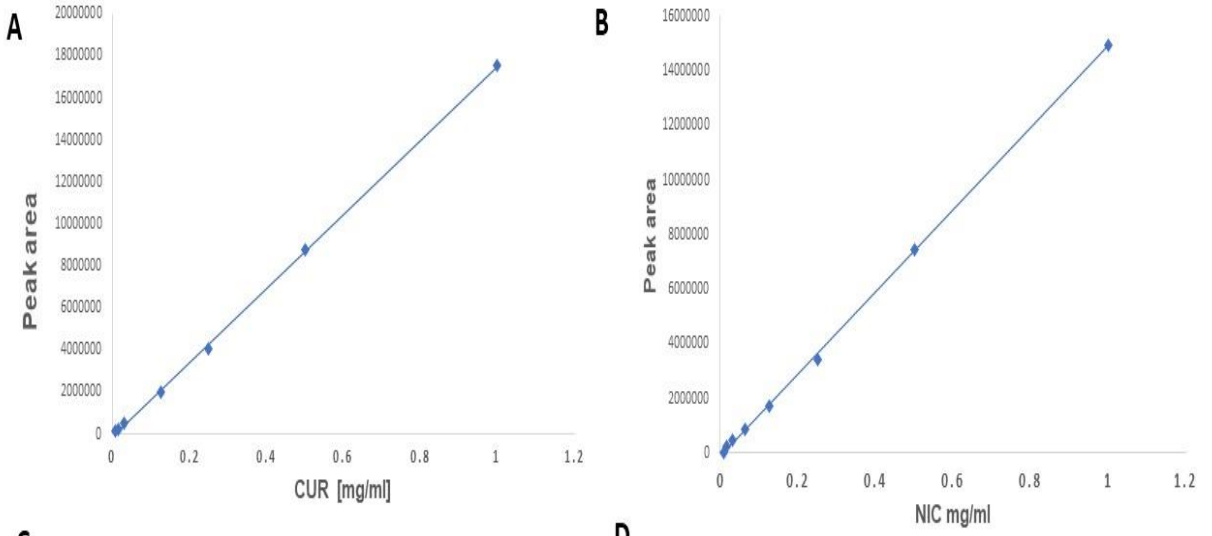
D

Parameter	CUR	NIC	CUR-NIC Ratio1:1
Calibration equation	y = 6.7166x - 0.0384	y = 0.0274x + 0.0599	y = 0.0113x + 0.1153
Determination coefficient (R^2)	R^2 = 0.9993	R^2 = 0.9998	R^2 = 0.997
Linearity range (µg/mL)	4-125 µg/ mL	3.5- 50 µg/ mL	4-125 µg/ mL
Wavelength	262	262	262
Number of points	6	6	6

Figure 4: Calibration curve of A) CUR B) NIC and C) CUR-NIC mixture (1:1) using UV spectrophotometry D) Table of the measuring parameters

3.1.2. Selectivity and Specificity:

In adherence to guidelines, specificity in the developed method is defined as the ability to distinguish the analytes without any interference. The peaks corresponding to CUR and NIC exhibited well-defined separation at distinct retention times, with no interference from additives in the liposomal formulation. This clear separation of peaks indicates the method's specificity and selectivity, underscoring its ability to differentiate CUR and NIC from each other and the lipids in the sample (Figures 5 and 6).



C

Instrument	Optimized chromatographic conditions
Detector	UV Detector (Shimadzu Corporation, Kyoto, Japan)
Column	C18 HPLC Column, 100Å, 5µm, 4.6 x 150mm
Mobile phase	Methanol: water 80:20; v/v (pH= 3)
Flow rate	1.0 mL/ min
Wavelength	262 nm
Temperature	40°C
Retention time	NIC ≈ 2.8 min
	CUR ≈ 4.7 min

D

Parameter	CUR	NIC
Calibration equation	$y = 2*10^7x - 103600$	$y = 2*10^7x - 92189$
Determination coefficient (R^2)	$R^2 = 0.9996$	$R^2 = 0.9996$
Linearity range (µg/mL)	7.813-1000	7.813-1000
Number of points	6	6
LOD (µg/mL)	0.0171	0.0152
LOQ (µg/mL)	0.052	0.046

Figure 5: A) Calibration curve of CUR B) Calibration curve of NIC C) Instrument optimized condition (1:1) using HPLC D) Table of the measuring parameters

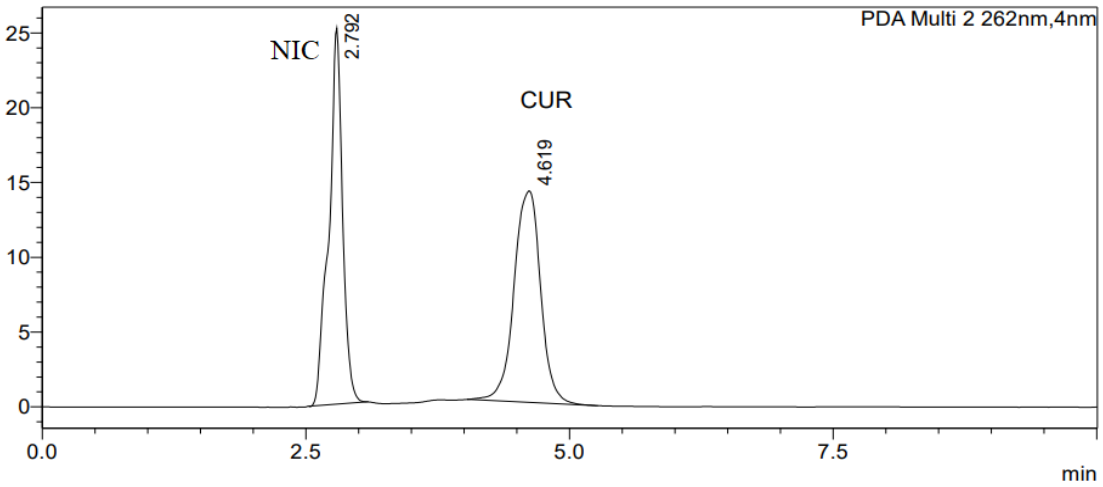


Figure 6: Representative HPLC chromatograms obtained from A) disrupted CUR and NIC loaded liposomes.

3.1.3. Accuracy and Precision:

The accuracy of the developed method was verified by measuring the recovery of standard samples. The recovery rates were acceptable, with %RSD values below 2%, indicating good accuracy. Specifically, the method achieved a recovery of 100.9% for CUR and 99.7% for NIC. These results, along with %RSD values consistently below 2% (Tables 2 and 3), confirm the method's accuracy.

Table 2: Precision study results of CUR and NIC

Sample	Injected concentration (mg/mL)	Intra- day (n=6)		Inter- day (n=6)	
		Measured concentration (mg/mL)	RSD%	Measured concentration (mg/mL)	RSD%
CUR	0.5	0.51	0.70%	0.491	0.7%
NIC	0.5	0.473	0.47%	0.509	0.6%

Table 3: Accuracy study results of CUR and NIC

Injected Sample (µg/mL)		Theoretical Concentration (µg/mL)	Measured concentration (µg/mL)	Recovery %	RSD%
CUR encapsulated into liposomes EE % = 30 %	**Standard**	**30**	**31**	**103%**	**0.24%**
	50%	15	14.06	93.73%	1.05%
	100%	30	28.83	96%	0.24%
	150%	45	40. 85	90.8%	1.5%
NIC encapsulated into liposomes EE % =80 %	**Standard**	80	80.9	101.13%	0.10%
	50%	40	39.2	98%	1.21%
	100%	80	76 ± 3.1	95%	0.11%
	150%	120	118 ± 1.9	92.5%	0.9%

3.1.4. Robustness of the method:

In accordance with recommended guidelines, the robustness of the developed HPLC analytical method was evaluated by assessing its ability to withstand minor variations in the UV wavelength (262 ± 2 nm). The method demonstrated robustness, with recovery results falling within the specified guideline range of 80–120%. Additionally, the relative standard deviation (%RSD) values remained below 2%. These findings indicate that the method is robust, even when subjected to slight variations in analytical conditions (Table 4).

Table 4: Robustness study results of CUR and NIC

Sample concentration	Conditions	Measured concentration (mg/mL)	Recovery %	RSD%
CURC (mg/mL)	**Reference**	0.50	100.%	0.27%
0.5	**260 nm**	0.486	97.2%	1.34%
	262 nm	0.5.07	101.28%	0.27%
	264 nm	0.543	108.68%	1.2%
NIC (µg/mL)	**Reference**	0.505	101%	0.6%
0.5	260 nm	0.4825	96.50%	1.29%
	262 nm	0.5064	101.28%	0.27%
	264 nm	0.529	105.8%	1.7%

3.2. Liposomes *in vitro* Stability

The stability of the prepared liposomes was investigated by measuring changes in the average particle size and zeta potential over a period of three weeks at a storage temperature of 4°C. The results demonstrated good stability of the CUR and NIC liposomes, as indicated by minimal changes in both average particle size and zeta potential at the tested temperature (Figures 7A, 7B, 7C). A release study conducted at 37°C revealed that the liposomal formulation enhanced the release of curcumin due to improved solubility (Figure 7D).

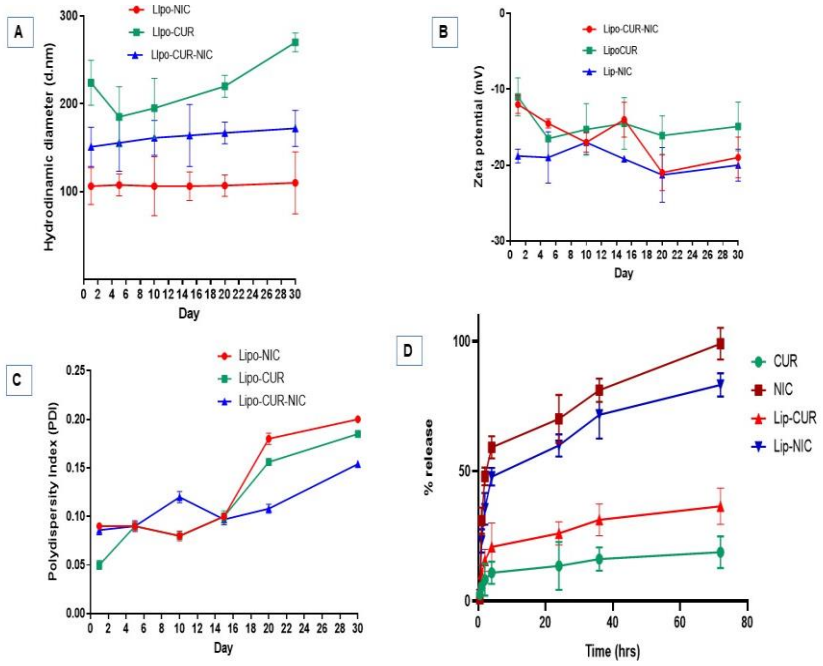


Figure 7: Colloidal stability of CUR and NIC liposomes in terms of average particle size and zeta potential changes over 4 weeks at 4 oC A) Hydrodynamic diameter B) Zeta potential C) Polydispersity index, D) In vitro release curve of a CUR and NIC loaded liposomes and free drug in phosphate buffer at 37 °C (mean ± SD, n = 3)

5. CONCLUSIONS

In this study, straightforward, time-efficient, and cost-effective analytical methods were developed using UV spectrophotometry and reverse-phase HPLC to quantify CUR and NIC encapsulated in liposomal formulations. Liposomes, widely recognized as a versatile lipid drug delivery system, provide an effective platform for encapsulating both hydrophilic and hydrophobic drugs. This approach helps to mask undesirable drug properties, improve release profiles, enhance pharmacokinetics, and serve as a promising drug carrier. The validated analytical methods were employed for the simultaneous estimation of CUR and NIC in liposome formulations containing DPPC, prepared using the ethanol injection technique, with a specific focus on accurately determining encapsulation efficiencies.

REFERENCES

1. Hussain Y, Alam W, Ullah H, Dacrema M, Daglia M, Khan H, et al. Antimicrobial Potential of Curcumin: Therapeutic Potential and Challenges to Clinical Applications. *Antibiotics (Basel).* 2022;11(3).
2. Homayoonfal M, Asemi Z, Yousefi B. Potential anticancer properties and mechanisms of thymoquinone in osteosarcoma and bone metastasis. *Cellular & Molecular Biology Letters.* 2022;27(1):21.
3. Lafi Z, Aboalhaija N, Afifi F. Ethnopharmacological importance of local flora in the traditional medicine of Jordan:(A mini review). *Jordan Journal of Pharmaceutical Sciences*. 2022;15(1):132-44.
4. Lafi Z, Alshaer W, Ma'mon MH, Zihlif MA, Asha NY, Abdelnabi H, et al. A review Echinomycin: A Journey of Challenges. *Jordan Journal of Pharmaceutical Sciences*. 2023;16(3):640-54.
5. Zargar A, Chang S, Kothari A, Snijders AM, Mao JH, Wang J, et al. Overcoming the challenges of cancer drug resistance through bacterial-mediated therapy. *Chronic Dis Transl Med*. 2019;5(4):258-66.
6. Hashem S, Ali TA, Akhtar S, Nisar S, Sageena G, Ali S, et al. Targeting cancer signaling pathways by natural products: Exploring promising anti-cancer agents. *Biomedicine & Pharmacotherapy*. 2022;150:113054.
7. Giordano A, Tommonaro G. Curcumin and Cancer. *Nutrients*. 2019;11(10).
8. Dai C, Lin J, Li H, Shen Z, Wang Y, Velkov T, et al. The Natural Product Curcumin as an Antibacterial Agent: Current Achievements and Problems. *Antioxidants*. 2022;11(3):459.
9. Hammad HM, Imraish A, Al-Hussaini M, Zihlif M, Harb AA, Abu Thiab TM, et al. Ethanol extract of Achillea fragrantissima enhances angiogenesis through stimulation of VEGF production. *Endocrine, Metabolic & Immune Disorders-Drug Targets (Formerly Current Drug Targets-Immune, Endocrine & Metabolic Disorders).* 2021;21(11):2035-42.
10. Nikas IP, Paschou SA, Ryu HS. The Role of Nicotinamide in Cancer Chemoprevention and Therapy. *Biomolecules*. 2020;10(3).
11. Peng M, Shi L, Ke S. Nicotinamide-based diamides derivatives as potential cytotoxic agents: synthesis and biological evaluation. *Chemistry Central Journal*. 2017;11(1):109.
12. Biniecka P, Matsumoto S, Belotti A, Joussot J, Bai JF, Majjigapu SR, et al. Anticancer Activities of Novel Nicotinamide Phosphoribosyltransferase Inhibitors in Hematological Malignancies. *Molecules*. 2023;28(4).
13. Nsairat H, Alshaer W, Lafi Z, Ahmad S, Al-Sanabrah A, El-Tanani M. Development and Validation of Reversed-Phase-HPLC Method for Simultaneous Quantification of Fulvestrant and Disulfiram in Liposomes. *Bioanalysis*. 2023;15(23):1393-405.

14. Teggar N, Bakchiche B, Abdel-Aziz ME-S, Bardaweel SK, Ghareeb MA. Chemical composition and biological evaluation of Algerian propolis from six different regions. *Jordan Journal of Pharmaceutical Sciences*. 2023:184-97.
15. Yan Y, Liao Z, Shen J, Zhu Z, Cao Y. Nicotinamide potentiates amphotericin B activity against Candida albicans. *Virulence*. 2022;13(1):1533-42.
16. Sharma A, Chabloz S, Lapides RA, Roider E, Ewald CY. Potential Synergistic Supplementation of NAD+ Promoting Compounds as a Strategy for Increasing Healthspan. *Nutrients*. 2023;15(2):445.
17. Liu P, Chen G, Zhang J. A Review of Liposomes as a Drug Delivery System: Current Status of Approved Products, Regulatory Environments, and Future Perspectives. *Molecules*. 2022;27(4):1372.
18. Lafi Z, Alshaer W, Hatmal MM, Zihlif M, Alqudah DA, Nsairat H, et al. Aptamer-functionalized pH-sensitive liposomes for a selective delivery of echinomycin into cancer cells. *RSC Adv*. 2021;11(47):29164-77.
19. Alshaer W, Zraikat M, Amer A, Nsairat H, Lafi Z, Alqudah DA, et al. Encapsulation of echinomycin in cyclodextrin inclusion complexes into liposomes: in vitro anti-proliferative and anti-invasive activity in glioblastoma. *RSC advances*. 2019;9(53):30976-88.
20. Nsairat H, Lafi Z, Al-Sulaibi M, Gharaibeh L, Alshaer W. Impact of nanotechnology on the oral delivery of phyto-bioactive compounds. *Food Chemistry*. 2023; 424: 136438.
21. Alshaer W, Zraikat M, Amer A, Nsairat H, Lafi Z, Alqudah DA, et al. Encapsulation of echinomycin in cyclodextrin inclusion complexes into liposomes: in vitro anti-proliferative and anti-invasive activity in glioblastoma. *RSC Advances*. 2019;9(53):30976-88.
22. Pharmaceutical development. ICH Q8 (R2). 2009.

طرق تحليلية لتقييم الكركمين والنيكوتيناميد في تركيبة الجسيمات الشحمية،اليبوسومات: القياس باسنخدام جهاز اللأشعة فوق البنفسجية و جهاز.HPLC

علي الفهداوي[1]، نعيم شعلان[1]، زينب لافي[1*]، عمر مركب[1]

[1] مركز الأبحاث الدوائية والتشخيصية، كلية الصيدلة، جامعة عمان الأهلية، عمان، الأردن.

ملخص

المقدمة: تقدم الدراسة طريقتين تحليليتين متميزتين مصممتين لتحديد دقيق للكركمين والنيكوتيناميد ضمن تركيبات الجسيمات الشحمية (الليبوسومات)، لتلبية احتياجات الباحثين والمحللين في قطاعات الطب الحيوي والمكملات الغذائية .

الطريقة: يوفر قياس الطيف الضوئي للأشعة فوق البنفسجية حلاً سريعًا وفعالاً من حيث التكلفة للقياس الكمي، بينما يوفر التحليل اللوني السائل عالي الأداء خصوصية وحساسية. يضمن التحقق من صحة الطريق، الموثوقية والملاءمة للتحليل الدقيق، وتطوير مجال الكيمياء التحليلية ودعم عمليات التطوير وضمان الجودة في الصناعات الدوائية والتكنولوجيا الحيوية باستخدام جهازي ، خاصة بالنسبة لجهاز. (HPLC & UV spectrophotometer))

النتائج: تم العثور على كفاءة تغليف الكركمين وفيتامين ب و في الجسيمات الشحمية، المكونة أساسًا من DPPC وCHO، لتكون 30% ± 6% و80% ± 5%، على التوالي. أظهرت الطريقة التحليلية المطورة باستخدام القياس الطيفي للأشعة فوق البنفسجية و HPLCللمرحلة العكسية المتانة والكفاءة، مما يسمح بالتحليل المتزامن لـ CUR و NICبدرجة عالية من الخصوصية والدقة والدقة. كشفت عملية التحقق من الصحة وفقًا لإرشادات ICH Q2 عن ملاءمة النظام الممتازة، والخطية، والمتانة، مع انحراف معياري نسبي أقل باستمرار من 2%. أظهرت دراسات الثبات على مدار ثلاثة أسابيع عند 4 درجات مئوية تغيرات طفيفة في خصائص الجسيمات الشحمية، مما يشير إلى ثبات جيد. علاوة على ذلك، أظهرت دراسات الإطلاق عند 37 درجة مئوية زيادة في قابلية الذوبان وزيادة إطلاق الكركمين، مما يشير إلى إمكانية استخدام تركيبة الجسيمات الشحمية في تطبيقات توصيل الأدوية.

الاستنتاج: في هذه الدراسة، تم تطوير طرق تحليلية واضحة وموفرة للوقت وفعالة من حيث التكلفة باستخدام القياس الطيفي للأشعة فوق البنفسجية و ذو الطور العكسي لتحديد كمية المغلفة في تركيبات الجسيمات الشحمية.

الكلمات الدالة: الجسيمات الشحمية، الطرق التحليلية، قياس الطيف الضوئي للأشعة فوق البنفسجية، HPLC.

[*] ***المؤلف المراسل: زينب لافي***

z.lafi@ammanu.edu.jo

تاريخ استلام البحث 2024/02/09 وتاريخ قبوله للنشر 2024/03/10.