Atlantic Journal of Life Sciences
Volume 2025,Article ID 11, pages 8
https://doi.org/10.71005/a0ht4r94

Original Research Article

Development and Validation of RP-HPLC Method for Quantification of Clindamycin Phosphate and Tretinoin Drug-Loaded Microgel

Muhammad Ibrahim[1], Yasir Mehmood[2], Hafiz Muhammad Bilal Khalid[1], Muhammad Muaz ul Islam[3], Maida Minahil Mushtaq[4]

[1]Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, Government College University, Faisalabad 38000, Pakistan|[2] Riphah Institute of Pharmaceutical Sciences (RIPS), Riphah International University, Faisalabad, PO.Box 38000, Pakistan.|[3]Nuclear Institute for Agriculture and Biology, Faisalabad Department of Pharmacy|[4] The University of Lahore, Lahore, Pakistan

Correspondence should be addressed to Yasir Mehmood; Yasirmehmoodamjad@gmail.com

Received: 25 February 2025 ; Revised: 18 April 2025; Accepted: 20 May 2025 ; Published: 31 May 2025

KEYWORDS

Clindamycin phosphate
Tretinoin
Microgel
RP-HPLC
Method validation
Entrapment efficiency

ABSTRACT

The current study aimed to develop and validate a reverse-phase high-performance liquid chromatographic (RP-HPLC) method that is quick, easy, and sensitive for the simultaneous measurement of tretinoin and clindamycin phosphate in lipid-based semisolid pharmaceutical formulations, particularly microgel. Due to the difficulties involved in co-analysing a hydrophilic and a lipophilic drug inside the same matrix, a strong analytical technique was necessary for precise and repeatable quantification. Chromatographic separation was accomplished using a Phenomenex C-18 column (150 mm × 4.6 mm, 5 µm) and an isocratic mobile phase consisting of 0.01 N phosphate buffer and acetonitrile (20:80, v/v) at a flow rate of 1.0 mL/min. Clindamycin phosphate and tretinoin were detected at wavelengths of 210 and 353 nm, respectively. Standard calibration curves were created across a concentration range of 2–50 µg/mL for both analytes. The limits of detection (LOD) and quantification (LOQ) for clindamycin phosphate and tretinoin were 6.78 µg/mL and 20.56 µg/mL, respectively, and 0.14 µg/mL and 0.42 µg/mL, demonstrating the method's good linearity ($R^2 = 0.99$). With %RSD values constantly below 2%, recovery trials at three concentration levels (70%, 100%, and 120%) showed mean accuracy ranging from 98.18% to 102.93% for clindamycin phosphate and 116.80% to 123.36% for tretinoin. These results validated the accuracy, precision, and repeatability of the approach. This method effectively verified the entrapment efficiency of the drug-loaded microgel, meeting ICH requirements, and is suitable for regular quality control of combination formulations containing tretinoin and clindamycin.

1. Introduction

Acne is a chronic condition of the pilosebaceous unit that commonly affects teenagers and adults, presenting with inflammatory lesions such as papules, pustules, comedones (both open and closed), cysts, and nodules (Vargas, Martinez, & Gomez, 2024). The pathophysiology of acne is complex, involving excessive sebum secretion, abnormal keratinisation within hair follicles, overgrowth of Propionibacterium acnes, and subsequent inflammatory responses. Acne is prevalent across all racial and ethnic groups, making it one of the most frequently encountered dermatological disorders (Hazarika, 2021). Post-inflammatory hyperpigmentation (PIH) is a common issue for individuals with darker skin who suffer from acne (Vargas et al., 2024). Melanocytes produce more melanin when inflammatory acne lesions damage the basal layer of the epidermis. Patients with darker skin tones are frequently affected by PIH, which can persist for months or even years. The best course of action is to avoid or minimize the incidence of PIH, even if it may react to therapies such as chemical peels and laser therapy (Seck, Hamad, Schalka, & Lim, 2023).

Reliable analytical techniques are becoming increasingly necessary to support the development and assessment of topical formulations, such as microgels comprising drugs like clindamycin phosphate (CLD) and tretinoin (TRN), given the clinical challenges associated with acne and its consequences, including post-inflammatory hyperpigmentation (PIH). Our study presents a simplified isocratic RP-HPLC method using a shorter phenomenex C-18 column (150 × 4.6 mm) with a mobile phase of 0.01N phosphate buffer and acetonitrile (20:80, v/v) in comparison to the reported gradient method using a longer C-18 column (250 × 4.6 mm) with orthophosphoric acid and methanol (Sarfraz et al., 2022). Following an initial trial, the mobile phase ratio was chosen based on the best resolution, peak symmetry, and retention times of both analytes. At the same time, the phosphate buffer at low ionic strength maintained pH stability and reduced peak tailing, particularly due to tretinoin's acidic and hydrophobic nature. The high acetonitrile concentration enabled sharper peaks and shorter run durations. The isocratic elution technique further guaranteed reproducibility and simplicity. The previous method requires complex gradient programming and a more extended analysis time, whereas our method guarantees faster run time, lower solvent consumption, and ease of execution. Moreover, both drugs were successfully detected at 210 and 353 nm, providing a reproducible and economical alternative for routine analysis in quality control facilities.

The simultaneous extraction, separation, and quantification of active pharmaceutical ingredients (APIs), such as clindamycin and tretinoin, presents a significant analytical challenge in semisolid formulations, including creams, ointments, and gels. This intricacy arises from the two distinct physicochemical characteristics and polarity differences of APIs, specifically that tretinoin is relatively lipophilic, whereas clindamycin phosphate is highly hydrophilic. Due to their varied solubility characteristics, a meticulously designed chromatographic

system is necessary to ensure sufficient resolution without sacrificing sensitivity. Consequently, choosing the right mobile phase becomes crucial. In addition to resolving the polarity mismatch, our method's 20:80 (v/v) ratio of 0.01 N phosphate buffer (aqueous) and acetonitrile (organic) enables effective isocratic elution, which is necessary to produce distinct peaks and achieve repeatable quantification in a single chromatographic run (Sarfraz et al., 2022). Due to their bilayer vesicular structure, as presented in Figure 1, which enhances skin penetration and drug retention, microgels are well known for improving topical medication administration (Matos, 2023). Phospholipids self-assemble into vesicles when dispersed in an aqueous phase, encasing hydrophilic medicines in the aqueous core and hydrophobic drugs in the bilayer. Due to their dual compatibility, microgels are ideal for co-delivery systems, such as tretinoin and clindamycin phosphate. Microgels greatly enhance penetration across epidermal membranes compared to traditional techniques (Peng, Shi, Zhu, & Wang, 2022).

Entrapment efficiency (EE), which affects the formulation's release profile and therapeutic efficacy, is a crucial metric for assessing microgel performance. Regulatory rules for quality control also require the quantification of energy efficiency (EE). Therefore, a strong analytical method is needed to calculate EE in intricate semisolid matrices precisely (Ramadon et al., 2021), Jahanfar. There is currently no recognised compendial approach for the simultaneous measurement of clindamycin phosphate and tretinoin in lipid-based microgel formulations, despite the availability of several analytical methods (Sarfraz et al., 2022). Therefore, there is an immediate need for an analytical technique that can be applied to determine the EE of the drug (Banala, Mukherjee, Mahajan, & Singh, 2022). The objective of the present work is to develop and validate a sensitive, rapid, and accurate HPLC analytical method for clindamycin phosphate and tretinoin-loaded microgel, and it also aims to estimate and assess the entrapment efficiency of the drugs in pharmaceutical lipid-based formulations.

2. Materials and methods

2.1. Chemicals and Reagents

Clindamycin and tretinoin were obtained from Aladdin Biochemical Technology Co., Ltd., Shanghai. Soft paraffin and lipids were obtained from Lipoid GmbH, Nattermannallee, Switzerland. Cholesterol was obtained as a gift from Diamond Sangon Biotech® (Shanghai) Co., Ltd., China. Potassium dihydrogen phosphate (KH_2PO_4), methanol, chloroform, and HPLC-grade acetonitrile were purchased from HmbG® Chemicals Sdn Bhd, Malaysia.

2.2. Instrumentation

The HPLC analyses were performed using a Shimadzu LC-20AD system (Malaysia), equipped with a variable-wavelength UV/Vis detector, autosampler, prominence pump, and column oven. OpenLab operating software was employed for HPLC analysis. A reverse-phase Phenomenex C-18 column (150 x 4.6 mm) with a pore size of 5 µm (Agilent Technologies, USA) was used for the chromatographic operation (Y. Mehmood et al., 2023).

2.3. Method of preparing the sample solution

Approximately one gram of the microgel formulation was mixed with 25 mL, which comprised 12 mg of clindamycin phosphate and 0.25 mg of tretinoin. Next, 90% methanol was added to reach a final concentration of 0.48 mg/mL of clindamycin phosphate and 0.012 mg/mL of tretinoin. Thereafter, the mixture was sonicated for 30 minutes before being filtered thoroughly using a 0.45 µm syringe filter. Next, the filtrate was injected into the HPLC system for real-time quantification of clindamycin phosphate and tretinoin.

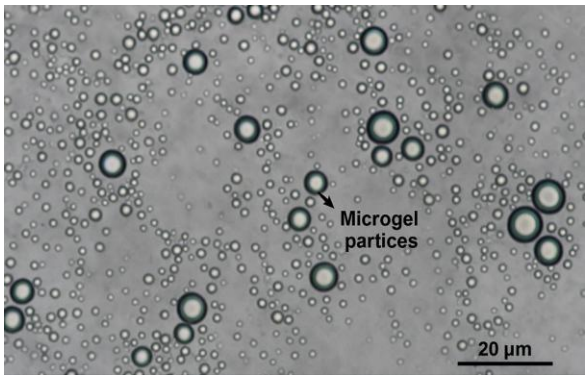

Figure 1: Microgel properties

2.4. Preparation of standard solutions

Approximately 24 mg of clindamycin phosphate, serving as a standard, was dissolved in 30 mL of 90% methanol and then sonicated to ensure complete dissolution (designated as Solution A). Separately, 30 mg of tretinoin was dissolved in methanol and sonicated to ensure homogeneity (Solution B). To prepare the final concentration,1.0 mL of Solution B and 1.0 mL of Solution A were combined in a 90% methanol solution. This yields a standard solution with final concentrations of 0.48 mg/mL for clindamycin phosphate and 0.012 mg/mL for tretinoin.

2.5. HPLC method validation

The critical parameters for analytical method validation, such as specificity, selectivity, linearity, accuracy, precision, robustness, limit of detection (LOD), and limit of quantification (LOQ), were followed in the validation of the developed RP-HPLC method, as described in the ICH Q2 (R1) guidelines (2005) (Mehmood, Khan, Shahzad, Khalid, Asghar, et al., 2019; Mehmood & Shahid, 2024). These globally agreed-upon standards provide a generally recognised foundation for ensuring the accuracy of drug analysis in pharmaceutical laboratories. At UV wavelengths of 210 and 353 nm, the drug content was quantitatively measured using a standard calibration curve in the 2-50 µg/mL range (T. Mehmood et al., 2023; Mehmood, Khan, Shahzad, Khalid, Irfan, et al., 2019; Shabir, 2003).

2.6. System Suitability

Approximately 24 mg of clindamycin phosphate was accurately weighed out in a 50 mL volumetric flask, and 30 mL of 90% methanol was added. The mixture was then sonicated for 30 minutes (Solution A). Separately, 30 mg of tretinoin was dissolved in methanol and sonicated to ensure homogeneity (Solution B). One millilitre of solution B was added to solution A. Then the volume was adjusted using 90% methanol to achieve a final concentration of 0.48 mg/mL of clindamycin phosphate and 0.012 mg/mL of tretinoin (Sravya & Kuber, 2023). Six replicate injections of the prepared standard solution were injected to assess system suitability parameters for the developed RP-HPLC method based on the following criteria:

The relative standard deviation (RSD) of peak areas for six replicate injections should be $\leq 2\%$,

ii. Tailing factor should be ≤ 2.0, and

iii. Theoretical plates should be ≥ 2000 for both analytes.

All results fell within the specified acceptance limits, confirming the method's precision, peak symmetry, and adequate column performance. The method was deemed suitable for quantitative analysis of clindamycin phosphate and tretinoin.

2.7. Specificity and selectivity

Specificity, an essential HPLC validation parameter, was assessed to confirm that the excipients used in the microgel formulations did not

interfere with the detection and quantification of the active ingredients. The specificity of the developed method for Safderm microgel was evaluated by analyzing potential interference from the diluent, placebo, and excipients. Individual and combined chromatograms of the diluent, placebo, excipients, clindamycin phosphate, tretinoin (active pharmaceutical ingredient, API), and Safderm microgel samples were analyzed. Figure 2 shows the combined chromatogram where clindamycin and tretinoin are well eluted.

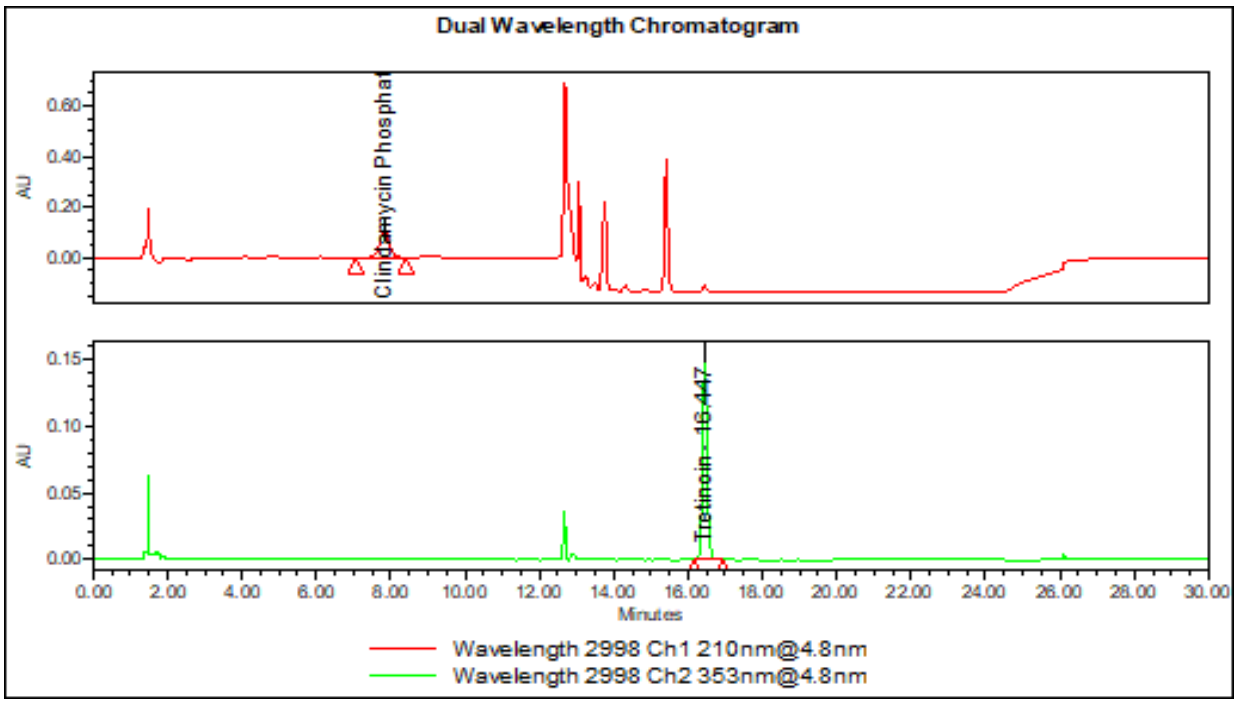

Figure 2: Chromatogram of Clindamycin Phosphate and Tretinoin

2.8. Limit of detection and limit of quantification

The limit of detection (LOD) and limit of quantification (LOQ) were calculated by using the standard formulas:

$$LOD = 3.3\ \sigma/m \text{ and } LOQ = 10\ \sigma/m$$

Where σ is the standard deviation of the response and m is the slope of the calibration curve. Both slope (m) and σ were derived from the regression line of the calibration curve for clindamycin phosphate and tretinoin. The residual standard deviation of a regression line was used as σ (Jaicharoensub, Sakpakdeejaroen, & Panthong, 2023). The LOD and LOQ values were quantitatively computed for both analytes based on these parameters.

2.9. Linearity and Range

A stock solution of tretinoin was prepared by dissolving 40.3 mg in a 100 mL volumetric flask using methanol as a diluent (solution C). Five linear dilutions of clindamycin phosphate and tretinoin were prepared as follows:

Standard A: 23.9 mg of clindamycin phosphate + 1 mL of solution C → diluted to 100 mL

Standard B: 36.1 mg + 2 mL of solution C → diluted to 100 mL

Standard C: 48.1 mg + 3 mL of solution C → diluted to 100 mL

Standard D: 72 mg + 4 mL of solution C → diluted to 100 mL

Standard E: 96.4 mg + 4 mL of solution C→ diluted to 100 mL-

Each solution was brought to volume with the appropriate diluent. This resulted in the following final concentrations:

Clindamycin phosphate: 0.240, 0.360, 0.480, 0.720, 0.960 mg/mL, while, Tretinoin: 0.00403, 0.00806, 0.01209, 0.01612, and 0.02015 mg/mL.

A calibration curve was plotted by graphing peak area versus concentration for both analytes. % RSD values and the correlation coefficient (r^2) were determined through regression analysis to assess linearity and reproducibility of the method.

2.10. Precision and Accuracy of the Method

To assess the precision of the established RP-HPLC method, One gram of microgel (equivalent to 12 mg clindamycin phosphate and 0.25 mg tretinoin) was transferred to a 25 mL volumetric flask and diluted with 90% methanol to achieve final concentrations of 0.480 mg/mL for clindamycin phosphate and 0.0120 mg/mL for tretinoin. The solution was vortexed, sonicated for 30 minutes, and then filtered through a 0.45 µm syringe filter (Kadam, Yadav, Bhingare, & Patil, 2018). Six replicate samples were made, and the relative standard deviation (RSD) of the results was calculated using the formula:

$$\%RSD = \frac{\text{SD (Standard deviation)}}{\text{Mean value of six sample}} \times 100$$

To determine accuracy, recovery studies were performed at three concentration levels: 2 µg/mL, 20 µg/mL, and 60 µg/mL for both clindamycin phosphate and tretinoin. The % recovery was calculated by comparing the measured concentrations with the known theoretical concentrations. A mean recovery within the 98% to 102% range was considered acceptable per analytical method validation standards.

2.11. Force degradation study

A stress study was performed to ensure specificity and make the method stability-indicating. Samples were stored under relevant stress conditions, including light, heat, acid/base hydrolysis, and oxidation, to determine whether any stress degradation factor interfered with the drug. Batch no. T-02 was used in this study.

6.11.1 Acid Hydrolysis

Approximately 1 g of the microgel formulation was transferred into a 25 mL volumetric flask. About 30% of the final volume of the diluent was added and shaken vigorously. The mixture was then sonicated for 30 minutes to ensure complete dissolution of the particles. 5 mL of 0.15 N HCl was added to the same flask, and the solution was left at room temperature for 8 hours to facilitate acid hydrolysis. After this incubation, 5 mL of 0.15 N NaOH was added to neutralise the solution. Then, the volume was adjusted with the diluent to reach the final concentrations of 0.480 mg/mL for clindamycin phosphate and 0.0120 mg/mL for tretinoin. After filtration, the final solution was injected into the HPLC through a 0.45 µm syringe filter. As previously mentioned, a fresh reference and sample solution were prepared and run in parallel to stress the sample under optimised chromatographic conditions to evaluate % recovery and degradation.

6.11.2 Base Hydrolysis

Using a volumetric flask, one gram of microgel was mixed with 25 mL of 30% diluent and vigorously shaken before being sonicated for 30 minutes. Next, 5 mL of 0.15 N NaOH was added to neutralise the solution. Notably, the solution was kept at room temperature for 8 hours. Subsequently, 5 mL of 0.15 M HCl was added to this flask to neutralise the sample and achieve a final concentration of 0.480 mg/mL for clindamycin phosphate and 0.0120 mg/mL for tretinoin, before vortexing. Next, the sample was filtered through a 0.45 µm syringe filter before being injected into the HPLC apparatus. A fresh reference and sample solution were also prepared under the heading "8.4" and run in parallel to stress the sample under optimised chromatographic conditions to evaluate the percentage recovery and degradation.

2.11.3. Oxidation Stress

One gram of the microgel formulation was transferred into a 25 mL volumetric flask. About 30% of the final volume of the diluent was added and shaken vigorously. The mixture was then sonicated for 30 minutes to ensure complete dissolution of the particles. Next, 5 mL of 3% H_2O_2 was added to the same flask, and the solution was then kept for 8 hours at room temperature. Afterwards, the volume was adjusted using the diluent to reach a final concentration of 0.480 mg/mL for clindamycin phosphate and 0.0120 mg/mL for tretinoin before vortexing. The final solution was injected into the HPLC after filtration through a 0.45 µm syringe filter, and then injected into the HPLC apparatus.

A fresh reference solution and sample solution were prepared as previously described and run in parallel to stress the sample under optimised chromatographic conditions to evaluate % recovery and degradation.

2.11.4. Thermal Stress

Using a volumetric flask, about one gram of microgel was mixed with 25 mL of 90% methanol and vigorously shaken before being sonicated for 30 minutes. After sonication, 90% methanol was used to adjust the final concentrations to 0.480 mg/mL for clindamycin phosphate and 0.0120 mg/mL for tretinoin. Next, samples were filtered using a 0.45 µm syringe filter before being injected into the HPLC. Thereafter, the samples were placed under thermal stress (60 °C in an oven). A fresh reference and sample solution were also prepared under the heading "8.4" and run in parallel to stress samples under optimised chromatographic conditions to evaluate the percentage recovery and degradation.

2.11.5. Photolytic Stress

The photo effect was studied in two segments: primary and secondary. The samples were kept in a photostability chamber to receive illumination for 1.2 × 10^6 lux hours. For this purpose, the 25,000 lux light was used for 48 hours. After completing illumination, the samples were removed from the photostability chamber and treated as previously described.

2.12. Statistical Analysis

Each measurement was done three times. The data were statistically evaluated and displayed as mean ± standard deviation using Microsoft Excel sheets. A p-value of less than 0.05 for the developed HPLC method was considered significant.

3. Results and discussion

3.1. System Suitability

The system suitability parameters for clindamycin phosphate and tretinoin complied with the established criteria. The %RSD of the peak areas was within the acceptable limit of <2%, with values of 0.861% for clindamycin phosphate and 0.377% for tretinoin. The tailing factors were also within the specified range of <2, observed as 1.137 and 1.332 for clindamycin phosphate and tretinoin, respectively. Theoretical plates, indicative of column efficiency, exceeded the minimum requirement of 2000, with values of 5185 for clindamycin phosphate and 117,939 for tretinoin. All obtained parameters are summarised in Table 1 and demonstrate full compliance with the system suitability criteria, ensuring the robustness, reproducibility, and consistency of the developed analytical method.

Table 1: System suitability results of clindamycin phosphate and tretinoin

Material	Area		Tailing factor		Theoretical plates	
Sr. No	(CLD)	(TRN)	(CLD)	(TRN)	(CLD)	(TRN)
1	1,817,041	1,341,058	1.130	1.33	5,118	117,899
2	1,839,694	1,353,398	1.140	1.33	5,178	118,284
3	1,846,712	1,353,268	1.130	1.33	5,213	117,942
4	1,864,848	1,355,116	1.150	1.33	5,180	117,858
5	1,856,459	1,355,281	1.140	1.33	5,200	117,918
6	1,859,698	1,355,545	1.130	1.34	5,222	117,731
Average	1,847,408	1,352,278	1.137	1.332	5,185	117,939
%RSD	0.861	0.377	0.656	0.280	0.720	0.150

3.2. Specificity and selectivity

The specificity of the method was assessed by examining the diluent, excipients, placebo, standard solutions of clindamycin phosphate and tretinoin, and a microgel sample to detect any potential interferences. The method was confirmed to be specific, as no interfering peaks were observed at the retention times of the two APIs, indicating complete resolution from formulation components. Repeatability was evaluated using six replicate measurements. For clindamycin phosphate, the mean recovery was 102.03%, with a coefficient of variation (CV) of 0.543% and a mean absolute error (MAE) of 0.222 mg/mL. For tretinoin, the mean recovery was 116.34%, with a coefficient of variation (CV) of 1.753% and mean absolute error (MAE) of 0.716 mg/mL. According to typical analytical validation criteria, a CV ≤ 2.0% and MAE < 2% are acceptable for assay precision and accuracy. The 95% confidence limits were 102.03 ± 0.46% for clindamycin phosphate and 116.34 ± 1.68% for tretinoin, as shown in Table 2, indicating good repeatability and accuracy.

Table 2: Evaluation of repeatability of clindamycin phosphate and tretinoin in microgel

Material	Clindamycin sulphate			Tretinoin		
Parameters	Added amount	recovered amount	Recovery	Added amount	recovered amount	Recovery
Unit	mg/mL	mg/mL	%	mg/mL	mg/mL	%
1	0.4765	0.4866	102.13	0.0099	0.0118	118.37
2	0.4770	0.4845	101.57	0.0099	0.0117	117.78
3	0.4783	0.4847	101.33	0.0100	0.0117	117.39
4	0.4799	0.4920	102.53	0.0100	0.0113	112.62
5	0.4812	0.4953	102.92	0.0100	0.0118	117.27
6	0.4789	0.4871	101.71	0.0100	0.0114	114.63
Mean	0.4786	0.4884	102.03	0.0100	0.0116	116.34
Statistical Data of precision (repeatability) of microgel clindamycin phosphate and tretinoin						
Standard deviation			0.554		2.039	
Coefficient of variation			0.543		1.753	
Mean absolute error			0.222		0.716	
Confidence Limit			102.03 ± 0.46		116.34 ± 1.68	

3.3. Determination of limit of detection and limit of quantification

The limit of detection (LOD) is the lowest concentration of an analyte that can be reliably distinguished from background noise, though not necessarily quantified with precision. The LOD was determined using the response's standard deviation and the calibration curve's slope of 6.78 µg/mL for clindamycin phosphate. The limit of quantification (LOQ), defined as the lowest concentration that can be quantitatively determined with acceptable precision and accuracy, was calculated as 20.56 µg/mL (Table 3). For tretinoin, the limit of detection (LOD) was found to be 0.14 µg/mL, and the limit of quantification (LOQ) was 0.42 µg/mL, indicating the method's high sensitivity for detecting even trace amounts of tretinoin (Adib, Mandal, Mohamed, & Chatterjee, 2017). These values meet the ICH acceptance criteria, where the method is considered valid if typically verified by %RSD ≤ 2% at the LOQ level (Branch, 2005).

Table 3: Summary of LOD and LOQ for Clindamycin phosphate and Tretinoin

Parameter	Clindamycin Phosphate	Tretinoin
Concentration Range (µg/mL)	239.00 – 964.00	4.03 – 20.15
Regression Equation	Y = 4008.48X – 49269	Y = 115818X + 11605
Correlation Coefficient (R^2)	0.99998	0.99998
Residual Standard Deviation (σ)	8240.21	4830.96
Slope (m)	4008.48	115818
Limit of Detection (LOD)	6.78 µg/mL	0.14 µg/mL
Limit of Quantification (LOQ)	20.56 µg/mL	0.42 µg/mL

3.4. Linearity and range

The correlation values (R^2) of 0.99998 for both clindamycin phosphate and tretinoin verified the method's linearity, showing good linearity across the tested concentration range. These results are comparable to or better than those found in earlier research, where, under similar chromatographic conditions, the correlation coefficient between tretinoin and clindamycin phosphate was ≥ 0.990. Furthermore, our study's linear regression equations (y = 4008.5x – 49269) for clindamycin phosphate and tretinoin (y = 115818x + 11604.5) show a strong linear association between peak area and concentration as well as sound sensitivity (Figure 3 and Table 4). This indicates that the linearity requirements specified in regulatory recommendations, such as ICH Q2 (R1), are satisfied and exceeded by our approach, which is highly repeatable and dependable (Walfish, 2006).

.

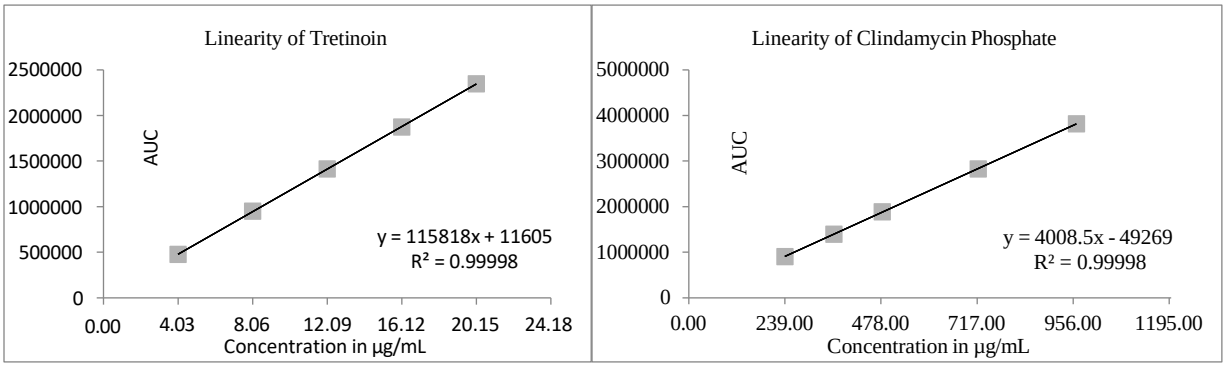

Figure 3: Linearity curve of clindamycin phosphate and tretinoin

Table 4: Linearity parameters for Clindamycin phosphate and Tretinoin

Clindamycin Phosphate			Tretinoin		
Concentration (µg/mL)	Mean Area ± S.E	RSS $(Y_i-\hat{Y}_i)^2$	Concentration (µg/mL)	Mean Area ± S.E	RSS $(Y_i-\hat{Y}_i)^2$
239.00	903,834 ± 2.979	908,758	4.03	476,546 ± 0.393	478,350
361.00	1,398,228 ± 0.144	1,397,792	8.06	948,969 ± 0.192	945,096
481.00	1,889,593 ± 0.088	1,878,810	12.09	1,412,804 ± 0.117	1,411,842
720.00	2,829,043 ± 0.049	2,836,836	16.12	1,872,265 ± 0.034	1,878,588
964.00	3,816,403 ± 0.158	3,814,905	20.15	2,348,627 ± 0.203	2,345,334
Statistical Data of Calibration Curve					
Parameter		Clindamycin Phosphate		Tretinoin	
Regression Equation		Ŷ = 4008.5X – 49268.8		Ŷ = 115818X + 11605	
Correlation Coefficient (R^2)		0.99998		0.99998	
Number of points (n)		5		5	

3.5. Precision and Accuracy

The calculated F-value for clindamycin phosphate was 4.4844 and 1.4708 for different analysts and days, respectively. The F value for different instruments is 1.2588 (Tables 5 and 6). The F-value for tretinoin was 1.2346 and 3.2078 for different analysts and days, respectively. The F value for different instruments is 2.5879. Results were found below the tabulated F-value of 5.05 at a 95% confidence level, indicating good precision (Atila Karaca & Yeniceli Uğur, 2018; Nugrahani & Dillen, 2018). The amount of analyte in the matrix may impact the percentage of recovery findings (Marson et al., 2020). The accuracy of the validated method was confirmed through a recovery study at three different concentration levels (70%, 100%, and 120%) by triplicate analysis. The accuracy results are reported in Table 5. The recovery range was found to be 98.18% to 102.93% for clindamycin phosphate and 116.80% to 123.36% for tretinoin; thus, the method is accurate for quantitative estimation, as the statistical results are within the acceptance range, i.e., 95-105% for clindamycin phosphate and 114-126% for tretinoin.

Table 5: Summary of recovery analysis on day 1 and day 2 (n = 6**)** of microgel on different days by the same analyst using the same instrument (CLD**)**

Parameter	Day 1	Day 2
Added amount (mg/mL)	0.4751 ± 0.0010	0.5042 ± 0.0082
Found amount (mg/mL)	0.4752 ± 0.0031	0.5042 ± 0.0082
Mean recovery (%)	100.03 ± 0.57	100.00 ± 0.47
Coefficient of variation	0.566	0.467
%RSD of recovery	0.57%	0.47%
Mean absolute error	0.231	0.191
Confidence Limits (p = 0.95)	100.03 ± 0.47	100.00 ± 0.38

Table 6: Summary of recovery analysis on day 1 and day 2 (n = 6) of microgel on different days by the same analyst using the same instrument (TRN)

Parameter	Day 1	Day 2
Added amount (mg/mL)	0.0099 ± 0.00	0.0105 ± 0.0002
Found amount (mg/mL)	0.0116 ± 0.0002	0.0125 ± 0.0002
Mean Recovery (%)	117.59 ± 1.20	119.15 ± 0.67
Coefficient of variation	1.02%	0.56%
% RSD of recovery	1.02%	0.56%
Mean absolute error	0.42	0.23
Confidence limits (p = 0.95)	117.59 ± 0.98	119.15 ± 0.55

3.6. Force degradation study

Forced degradation conditions were used in the current investigation to assess the method's capacity to indicate stability. Besides oxidative degradation, which occurred when 3% hydrogen peroxide was present and resulted in a notable 51.17% degradation, our results demonstrated that clindamycin phosphate remained stable under all stress conditions. In primary packaging, on the other hand, tretinoin showed significant instability under basic (10.07%), thermal (5.90%), and photolytic stress (13.23%), although it remained stable under oxidative circumstances. These findings align with earlier research that identified tretinoin as the component most prone to deterioration, particularly when exposed to heat and photolysis. According to the published study, tretinoin degraded by 87.2% following temperature exposure and 65.2% in sunshine, suggesting even more severe deterioration under longer-term or more demanding circumstances. In contrast, the same study found that clindamycin phosphate was reasonably stable (RSD = 99%) in thermal and photolytic environments. The stability-indicating capability of the present RP-HPLC technology, particularly its ability to distinguish APIs from their degradation products, is enhanced by the consistency of degradation patterns.

4. Conclusion

This work successfully developed and validated a reliable, sensitive, and stability-indicating RP-HPLC technique for the simultaneous measurement of tretinoin and clindamycin phosphate in lipid-based microgel formulations. According to ICH Q2 (R1) criteria, the technique demonstrated outstanding linearity, accuracy, precision, and specificity. The accuracy of the approach was confirmed by recovery values falling within acceptable ranges, and repeatability among analysts, equipment, and days was ensured by %RSD and F-values staying well below critical limits. Importantly, the validated method was successfully applied to determine the drug entrapment efficiency in microgel formulations, supporting its utility in formulation development and routine quality control. Thanks to its precision, reliability, and sensitivity, this method offers a valuable tool for pharmaceutical analysis. It can be confidently adopted for the standardisation and regulatory compliance of commercial clindamycin phosphate and tretinoin-based topical products. The method also demonstrated robustness under various analytical conditions and was able to differentiate APIs from their degradation products under stress.

Declarations

Ethics approval and consent to participate
Not applicable.
Ethical consideration
Not applicable.
Consent for publication
Not applicable.
Competing interests
The authors declare that they have no competing interests.

Acknowledgements
The authors express their heartfelt gratitude to Saffron Pharmaceuticals (Pvt.) Ltd., Pakistan, for granting them access to advanced analytical equipment, lab space, and technical support necessary for the successful completion of this study. We especially appreciate the HPLC equipment, reagents, and other resources that made it possible to design, validate, and use the approach in a real-world pharmaceutical scenario. We also like the Quality Control Department's facilitation and collaborative atmosphere

Author's contribution
Conceptualisation, writing the original draft**:** Muhammad Ibrahim, Yasir Mehmood. Formal analysis, investigations: Hafiz Muhammad Bilal Khalid, Muhammad Muaz ul Islam. Funding acquisition, resources, project administration, reviewing and editing, data validation, and data curation: Maida Minahil Mushtaq

Funding
This work received no external funding

Data Availability
Data are available from the corresponding author upon reasonable request

References

Adib, N. A. M., Mandal, U. K., Mohamed, F., & Chatterje(2017). Fast and simple gas chromatographic method for simultaneous estimation of camphor, menthol and methyl salicylate in analgesic ointment: application in stability study. *Journal of pharmaceutical Investigation, 47*(3), 275-285. doi: 10.1007/s40005-017-0305-0

Atila Karaca, S., & Yeniceli Uğur, D. (2018). Chemometrically assisted optimization and validation of a new HPLC method for the determination of paliperidone in pharmaceuticals. *Journal of Liquid Chromatography & Related Technologies*, *41*(3), 129-134. doi: 10.1080/10826076.2018.1426599

Banala, V. T., Mukherjee, D., Mahajan, S., & Singh, P. K. (2022). Chapter 20 - Current status of FDA-approved marketed nano drug products: regulatory considerations. In N. K. Mehra, S. Srivastava, J. Madan, & P. k. Singh (Eds.), *Multifunctional Nanocarriers* (pp. 501-521): Elsevier.

Branch, S. K. (2005). Guidelines from the international conference on harmonisation (ICH). *Journal of Pharmaceutical and Biomedical Analysis, 38*(5), 798-805. https://doi.org/10.1016/j.jpba.2005.02.037

Hazarika, N. (2021). Acne vulgaris: new evidence in pathogenesis and future modalities of treatment. *Journal of dermatological treatment*, *32*(3), 277-285. https://doi.org/10.1080/09546634.2019.1654075

Jaicharoensub, J., Sakpakdeejaroen, I., & Panthong, S. (2023). Validation of HPLC method for quantitative determination of active compounds in Thai traditional herbal medicine to treat gastrointestinal disease. *Talanta Open*, *7*, 100227. doi: https://doi.org/10.1016/j.talo.2023.100227

Kadam, P. V., Yadav, K. N., Bhingare, C. L., & Patil, M. J. (2018). Standardization and quantification of curcumin from Curcuma longa extract using UV visible spectroscopy and HPLC. *Journal of Pharmacognosy and Phytochemistry*, *7*(5), 1913-1918.

Marson, B. M., Concentino, V., Junkert, A. M., Fachi, M. M., Vilhena, R. O., & Pontarolo, R. (2020). Validation of analytical methods in a pharmaceutical quality system: An overview focused on HPLC methods. *Química Nova, 43*, 1190-1203.

Matos, C. M. (2023). Liposomes: The Brave Old World. *Int J Mol Sci, 24*(5). doi: 10.3390/ijms24054343

Mehmood, T., Hanif, S., Azhar, F., Ali, I., Alafnan, A., Hussain, T., Syed, M. A. (2023). HPLC Method Validation for the Estimation of Lignocaine HCl, Ketoprofen and Hydrocortisone: Greenness Analysis Using AGREE Score. *International Journal of Molecular Sciences*, *24*(1), 440. https://doi.org/10.3390/ijms24010440

Mehmood, Y., Khan, I. U., Shahzad, Y., Khalid, S. H., Asghar, S., Yousaf, A. M., . . . Khalid, I. (2019). A New, Rapid, Cost-Effective, Easy and Validated RP-HPLC Method for Determination of Antiviral (Sofosbuvir) in Bulk Forms. *Pakistan Journal of Analytical & Environmental Chemistry*, *20*(1), 11. http://doi.org/10.21743/pjaec/2019.06.02

Mehmood, Y., Khan, I. U., Shahzad, Y., Khalid, S. H., Irfan, M., Asghar, S., . . . Khalid, I. (2019). Development and validation of a stability-Indicating RP-HPLC method for simultaneous estimation of sofosbuvir and velpatasvir in fixed dose combination tablets and plasma. *Pak. J. Pharm. Sci*, *32*(4), 1835-1842.

Mehmood, Y., & Shahid, H. (2024). Development and Validation of RP-HPLC Method for Roflumilast Nano Particles in Rat Plasma for Pharmacokinetic Study Analysis. *Current Pharmaceutical Analysis*, *20*(9), 1083-1091. https://doi.org/10.2174/0115734129348436241209110149

Mehmood, Y., Shahid, H., Yousaf, H., Riaz, H., Farooq, U., Arshad, R. K. M., & Ahmad, W. (2023). RP-HPLC method to assay ethinylestradiol and drospirenone with 3D chromatograph model in fixed dose in rat plasma for pharmacokinetic study analysis. *Pakistan journal of pharmaceutical sciences*.

Nugrahani, I., & Dillen, N. (2018). Rapid assay development of diclofenac sodium COATED TABLET ASSAY USING FTIR COMPARED TO HPLC METHOD. *International Journal of Applied Pharmaceutics*, *10*(4), 43-50. doi: 10.22159/ijap.2018v10i4.25682

Peng, M., Shi, X., Zhu, L., & Wang, Z. (2022). Follow-up management service and health outcomes of hypertensive patients in China: a cross-sectional analysis from the National Health Service survey in Jiangsu province. *Frontiers in Public Health*, *10*. https://doi.org/10.3389/fpubh.2022.956711

Sarfraz, S., Hussain, S., Javed, M., Raza, A., Iqbal, S., Alrbyawi, H., Pashameah, R. A. (2022). Simultaneous HPLC determination of clindamycin phosphate, tretinoin, and preservatives in gel dosage form using a novel stability-indicating method. *Inorganics*, *10*(10), 168. https://doi.org/10.3390/inorganics10100168

Seck, S., Hamad, J., Schalka, S., & Lim, H. W. (2023). Photoprotection in skin of color. *Photochemical & Photobiological Sciences*, *22*(2), 441-456.

Shabir, G. A. (2003). Validation of high-performance liquid chromatography methods for pharmaceutical analysis: Understanding the differences and similarities between validation requirements of the US Food and Drug Administration, the US Pharmacopeia and the International Conference on Harmonization. *Journal of chromatography A*, *987*(1-2), 57-66. https://doi.org/10.1016/S0021-9673(02)01536-4

Sravya, D., & Kuber, B. R. (2023). A stability-indicating reverse phase-HPLC method development and validation for newly approved drug, Belzutifan in bulk and pharmaceutical dosage forms. *Journal of Applied Pharmaceutical Science*, *13*(7), 233-240. DOI: 10.7324/JAPS.2023.114351

Vargas, E. J., Martinez, J. C., & Gomez, L. A. (2024). Use of Plasma-Rich in Growth Factors (PRGF) in the Treatment of Acne. *OBM Transplantation*, *8*(4), 1-5. doi:10.21926/obm.transplant.2404225

Walfish, S. (2006). Analytical methods: a statistical perspective on the ICH Q2A and Q2B guidelines for validation of analytical methods. *BioPharm International*, *19*(12), 1-6.