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Simultaneous HPLC Determination of Clindamycin Phosphate, Tretinoin, and Preservatives in Gel Dosage Form Using a Novel Stability-Indicating Method

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Abstract: The most well-known, effective medicines for acne therapy are clindamycin phosphate and tretinoin. For the first time, we have developed and validated a reversed-phase HPLC stability-indicating technique for the detection of clindamycin phosphate (CLP), tretinoin (TRN), and two preservatives, methylparaben (MP) and imidazolidinyl urea (IU), simultaneously in this work. Most of the chromatographic conditions in the present study were optimized to achieve better separation. The best separation results were obtained using gradient elution on a C-18 (250 × 4.6 mm), 5 µm column, with a mobile phase consisting of solution A (1 mL/L ortho-phosphoric acid in water) and solution B (methanol), at a flow rate of 1.0 mL/min, with UV detection at wavelengths of 200 nm and 353 nm. Standard parameters such as system suitability, precision, accuracy, specificity, robustness, linearity, range, detection limit, quantification limit, and reagent stability were used to validate the developed technique. According to the standards of the International Council for Harmonization, all of the experimental parameters were found to be within allowable bounds (ICH). The simultaneous concentrations of clindamycin phosphate, tretinoin, methylparaben, and imidazolidinyl urea in pharmaceutical formulations were successfully determined using the suggested approach. The proposed RP-HPLC method detected no interfering peaks in the chromatogram. We may conclude from the data that the new RP-HPLC method can be utilized in pharmaceutical laboratories to simultaneously assess clindamycin phosphate, tretinoin, and two preservatives, methylparaben and imidazolidinyl urea, for both qualitative and quantitative analyses.

Keywords: RP-HPLC; clindamycin phosphate; tretinoin; imidazolidinyl urea; methylparaben; method development and validation

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1. Introduction

One of the most common inflammatory infections found more frequently in women than men is acne vulgaris [1]. It is a type of recurring infection, and its effects are persistent. Acne vulgaris affects nearly everyone at one point or another in their lives [2]. Clindamycin phosphate and tretinoin are the best-known, proficient prescriptions for acne treatment among various antibiotics. Both of these are frequently used as topical treatments on their own. However, according to some of the most recent research findings, combining two or more topical medicines with different action mechanisms produces more significant results than employing solo treatments [3]. These findings imply that combining clindamycin phosphate and tretinoin can produce better acne treatment results than using them alone. Furthermore, clindamycin phosphate aids in reducing tretinoin's inflammatory effects [4,5]. Since adverse effects of combined usage of clindamycin phosphate and tretinoin are underreported, the safety of clindamycin phosphate together with tretinoin in pregnancy has not yet been established [6]. In addition, in a 5-year retrospective investigation of patch test reactions in six Spanish hospitals, the most prevalent allergens found were quaternium-15 (0.88%), formaldehyde (1.72%), imidazolidinyl urea (1.05%), and diazolidinyl urea (0.79%) [7].

A common antibiotic used to treat a variety of bacterial diseases is clindamycin phosphate. Both Gram-positive and Gram-negative bacteria are resistant to it [8]. Common bacterial infections include skin inflammation problems, respiratory tract infections, and gynecological, abdominal, and subcutaneous tissue infections [9]. Clindamycin's primary action method is its bacteriostatic effect: binding to the 50 S ribosomal subunit prevents bacterial protein synthesis by hampering ribosomal translocation. Several methods for estimating clindamycin phosphate have been published, each employing a different approach [10–12].

Tretinoin is mostly sold with the trading name "all-trans-retinoic acid", and it is a member of the retinoids group. Retinoids are a group of natural metabolites of retinol (vitamin A), including retinoic acid with the all-trans isomer, 13-cis isomer, and 9-cis isomer [13]. It has remarkable properties in treating various types of inflammatory skin diseases. Commercially, tretinoin is marketed as a cream and a gel because of its capacity to regulate epithelial cell proliferation, differentiation, sebum production, and collagen formation [14]. In addition, tretinoin works by interfering with gene expression by attaching to specific nuclear receptors. As a result, it influences immunological processes and may even prevent skin cancer from spreading [15]. Since tretinoin is susceptible to heat, light, and oxidation while in storage, precise and accurate quantification of tretinoin is crucial for maintaining the quality of completed goods [16]. Most pharmaceutical preparations necessitate sufficient safety precautions to avoid contamination jeopardizing the product's stability. This is accomplished by adding an antimicrobial ingredient to the product, which destroys and inhibits microbial growth that contaminates it during manufacturing or use. Parabens (alkyl esters of p-hydroxybenzoic acid), which are frequently coupled with imidazolidinyl urea, have a wide range of preservation properties and are widely employed as antimicrobial agents in food and pharmaceutical preparations [17]. Paraben is used as a preservative in a wide range of therapeutic goods due to its biodegradability, chemical stability, and antibacterial properties [18]. Paraben's most common side effects include its vulnerability to the endocrine system and reproductive system, and it is also thought to be a risk factor for breast cancer [19–21]. Imidazolidinyl urea is a formaldehyde releaser that can be used alone or in conjunction with other preservatives as an antibacterial preservative [18,22].

It may also induce contact dermatitis due to its potent antimicrobial properties. Contact sensitivity to imidazolidinyl urea and other formaldehyde releasers has been reported in several studies [23–27]. Given the perils of preservatives, it is necessary to undertake a comprehensive test of preserved materials to determine the presence of antimicrobial agents, as recommended by the ICH [28]. One of the most often used tests in this regard is stability testing. It aids in the determination of pharmaceutical products' shelf life

by supplying data on degradability under various environmental circumstances such as temperature, light, and moisture. The initial stress testing results are used to assess the product's stability in terms of product degradation, then evaluate the analytical approach's resistance. As a result of conducting product stability testing, pharmacists can prescribe storage conditions for extending the shelf life of items. Accurate and precise assessment of API (active pharmaceutical ingredients) quantitative testing of interfering chemicals, degradation products, excipients, or any other potential contaminants is among the remarkable outcomes of stability-indicating methods.

Several studies have been published in which clindamycin phosphate and tretinoin, either individually or in combination with other medications, were used for method development and validation, including HPLC and spectrophotometric approaches [8,29–33]. However, there have been no reports on establishing a method that includes a preservative. Due to this knowledge gap, the current work seeks to develop and verify a straightforward, sensitive, and stability-indicating analytical approach for the simultaneous determination of clindamycin phosphate, tretinoin, and two preservatives in dermatological and gel dosage forms. The present work is the first publication developing a method for concurrently determining clindamycin phosphate and tretinoin using two preservatives in a single HPLC run.

2. Materials and Method

2.1. Chemicals and Reagents

Merck Chemicals, Germany, provided methanol (99.9%), acetonitrile (99.8%), deionized water for chromatography (HPLC grade), and ortho-phosphoric acid (85%-AR grade). Clindamycin phosphate (Fengchen Group, Qingdao, China), tretinoin (Olson, PA, Italy), methylparaben (UENO Fine Chemical, Itami, Japan), and imidazolidinyl urea (Haihang Industry, Jinan, China) were used as received. We then used the suggested method to calculate the percentage assay of clindamycin phosphate, tretinoin, and preservatives in four topical gels named Clinician T-Gel, Acdermin Gel, Cleret Gel, and Clinda-T Gel randomly collected from the local market.

2.2. Instrumentation

This study used Shimadzu HPLC-20 and Agilent 1260 Infinity Series II paired with a variable wavelength detector. The dual-wavelength mode allows for the acquisition of even more sample data. The system also had an autosampler aligned with the Agilent HPLC column and an integrated solvent degasser and gradient elution capability. Agilent C18 (250 × 4.6 mm) 5 µm, ACE C18 (250 × 4.6 mm) 5 µm, and Merck C18 (250 × 4.6 mm) 5 µm were the columns utilized. This study additionally made use of an analytical balance (PB210S, Sartorius, Gottingen, Germany), a pH meter (S220, Mettler Toledo, Columbus, OH, USA), and an ultrasonic water bath (8510, Branson, Brookfield, CT, USA).

2.3. Preparation of Solutions

2.3.1. Mobile Phase Preparation

One milliliter of ortho-phosphoric acid in 1000 milliliters of water (solution A) and different *v/v* percentage compositions of methanol were used as the mobile phase (solution B). Gradient elution was carried out with solutions A and B in a 50:50 (*v/v* percent) ratio for 0.00 to 10.0 min, 06:94 (*v/v* percent) for 10.01 to 23 min, and 50:50 (*v/v* percent) for 23.01 to 35 min. The diluent was an 85:15 (*v/v* percent) mixture of acetonitrile and solution A.

2.3.2. Standard Stock Solution I (Tretinoin)

Accurately weighing 50 mg of tretinoin, a 100 mL volumetric flask was filled with the medication. By sonicating the flask for 10 to 15 min, it was dissolved into 70 mL of diluent. To acquire the actual concentration of 0.5 mg/mL of tretinoin, the volume was raised to the mark (100 mL) with diluent.

2.3.3. Standard Stock Solution II (Methyl Paraben and Imidazolidinyl Urea)

In a 100 mL volumetric flask, accurately weighed amounts of methylparaben (52 mg) and imidazolidinyl urea (86 mg) were added. Almost 70 mL of diluent was added to this flask, followed by sonication to obtain a homogeneous solution. The volume was made up to the mark, i.e., 100 mL with the diluent, thus getting the actual concentration, which is equal to 0.52 mg/mL and 0.86 mg/mL of methylparaben and imidazolidinyl urea, respectively.

2.3.4. Preparation of Standard Solution

Clindamycin, at 24 milligrams, was precisely weighed and added to a volumetric flask with a capacity of 100 mL. Then, 1 mL of stock solution I and 10 mL of stock solution II were added to the flask. After adding 70 mL of diluent, the mixture was sonicated for around 20 min to create a homogenous solution. By dilution with the diluent, the solution's final volume was brought up to the required level. The solution was diluted, put through a 0.2 m glass nylon filter, and kept at room temperature after that. The resultant solution included final concentrations of 0.24, 0.052, 0.086, and 0.005 mg/mL for clindamycin phosphate, methylparaben, imidazolidinyl urea, and tretinoin, respectively. The filtered solution was then fed into the HPLC apparatus to produce the reference chromatogram.

2.3.5. Preparation of Sample Solution

In this investigation, a clinical T gel (Pilot Batch) manufactured by Seatle Pvt. Ltd. (Lahore, Pakistan) was used as a sample. Clindamycin phosphate 1.2% and tretinoin 0.025% were present in a clinical T gel sample (methylparaben and imidazolidinyl urea amounts not declared). A 2.0 gm gel sample was placed in a 100 mL volumetric flask and 80 mL of diluent. After that, the solution was sonicated for around 30 min to achieve homogeneity. The final volume of the solution was raised to the mark (100 mL) using diluent after sonication. After that, a 0.2 μm glass nylon filter was used to filter it. To obtain the sample chromatograms, the filtered solution was added to an HPLC machine.

2.3.6. Stability/Stress Testing Studies

In order to determine the generation of likely degradation products, stress/stability tests were carried out on the experimental gel preparation and working standards of clindamycin phosphate, tretinoin, and preservatives. These tests served as a count of specificity stability-indicating testing of the proposed analytical method. Following the decision trees of study for different types of stressful situations, the initial stress testing included degradation tests for each active pharmaceutical ingredient (API), preservatives, and operating standards under various stress settings. To limit the likelihood of subsequent degradation, a degradation rate of 20% to 30% was targeted in the current investigation [1]. These peaks do not co-elute with any other peaks or with any of the APIs or preservatives, as shown by the data collected, which also enabled us to calculate the relative retention times of each API/degradation product. Each of the degraded products and APIs/preservatives was further validated using the peak purity test. The deterioration of the gel and placebo was evaluated using the suggested approach. The acquired results allowed us to rule out the possibility of deteriorated products interfering with detecting active substances and preservatives in the trial gel. In addition, the peak purity of all active compounds and preservatives in the gel was also examined.

All of the samples were kept in dark-colored glass bottles in a "stream of nitrogen" and were carefully sealed with a plastic cover. All clindamycin phosphate, tretinoin, and preservative samples were evaluated, and pH and density were measured before conducting stress measurements. Thermal stress tests were conducted on the experimental gel formulation, a placebo, and a placebo with each API/preservative, a placebo with two, a placebo with three, and so forth, until all potential combinations had been identified. This thermal test was conducted in a climate room at a temperature of 40 °C and a relative humidity of 75%. The temperature of the environment room where the control samples

were stored was 20 °C. Thermal analysis was done over a three-month period. In order to conduct the light stress trials, the experimental gel formulation, a placebo, and the regular working solution of clindamycin phosphate, tretinoin, and preservatives were all exposed to direct sunlight for 10 h at a temperature of 25 to 30 °C. Each sample was maintained in a quartz cell with a tight closure. In quartz cells, control samples were maintained concurrently at room temperature and shaded from the sun.

3. Results and Discussion

3.1. Method Development

In order to assure regulatory compliance and ICH guidelines Q2 compliance (R1), the majority of analytical parameters were examined in this research to assess system appropriateness in terms of measuring standard deviation, relative standard deviation (RSD), tailing factor, and accuracy (Table 1). Furthermore, method validation was based on accuracy, specificity, robustness, correlation coefficient (R) in linearity, the limit of detection (LOD), and the limit of quantification (LOQ). The mentioned chromatographic conditions were optimized to build a simple HPLC technique. Under the chromatographic conditions described, three distinct HPLC columns were used: Agilent C-18 (250 × 4.6 mm) 5 µm, ACE C-18 (250 × 4.6 mm) 5 µm, and Merck C-18 (250 × 4.6 mm) 5 µm. The Agilent C-18 (250 × 4.6 mm) 5 µm column performed well. To resolve all four peaks for the desired components, different *v/v*% compositions of the mobile phase were used.

Table 1. System suitability studies of the pharmaceutical drugs.

API/Preservative	tR (min)	Parameters and Acceptance Criteria			
		Instrument Precision (RSD ≤ 2%)	**Theoretical Plates (≥2000)**	**Tailing Factor (<2.0)**	**Resolution (>2)**
Imidazolidinyl urea	2.2	0.32%	21,482.500	1.128	-
Methyl paraben	7.3	0.64%	21,247.500	1.205	4.7
Clindamycin Phosphate	8.9	0.78%	23,318.500	1.105	2.4
Tretinoin	24.8	0.73%	40,487.000	1.083	-

a. temperature selection

Samples analysis was carried out at different temperature i.e., 30, 35, and 40 °C. In the present study, 40 °C temperature was found to be good for the best elution of components.

b. mobile phase composition

Different concentrations, i.e., 1%, 0.5%, and 0.1% of glacial acetic acid (named Solution A) were tried. Of all the concentrations used, 0.1% (1 mL/1000 mL) glacial acetic acid in water gave good results.

c. elution rate

Samples were analyzed on different elution rates, i.e., 0.5, 1.0,and 1.5 mL/min. as demonstrated by the data values given in Table 1, the elution rate of 1.0 mL/min was found satisfactory because the elution rate 0.5 mL/min shifted the analysis to a very long run time and 1.5 mL/min resulted in unresolved peaks in the chromatogram.

d. wavelength selection

Following a review of the literature [8], we individually UV scanned each API and preservative. Clindamycin phosphate, methyl paraben, and imidurea all exhibit absorption at 200 nm in their respective spectra. Therefore, 200 nm was chosen as the intermediate wavelength for all three components, with tretinoin's spectra showing its peak absorption at 353 nm. As a result, wavelength values of 353 nm was selected for tretinoin analysis. Figure 1a–d represent absorption spectra of all four APIs and preservatives.

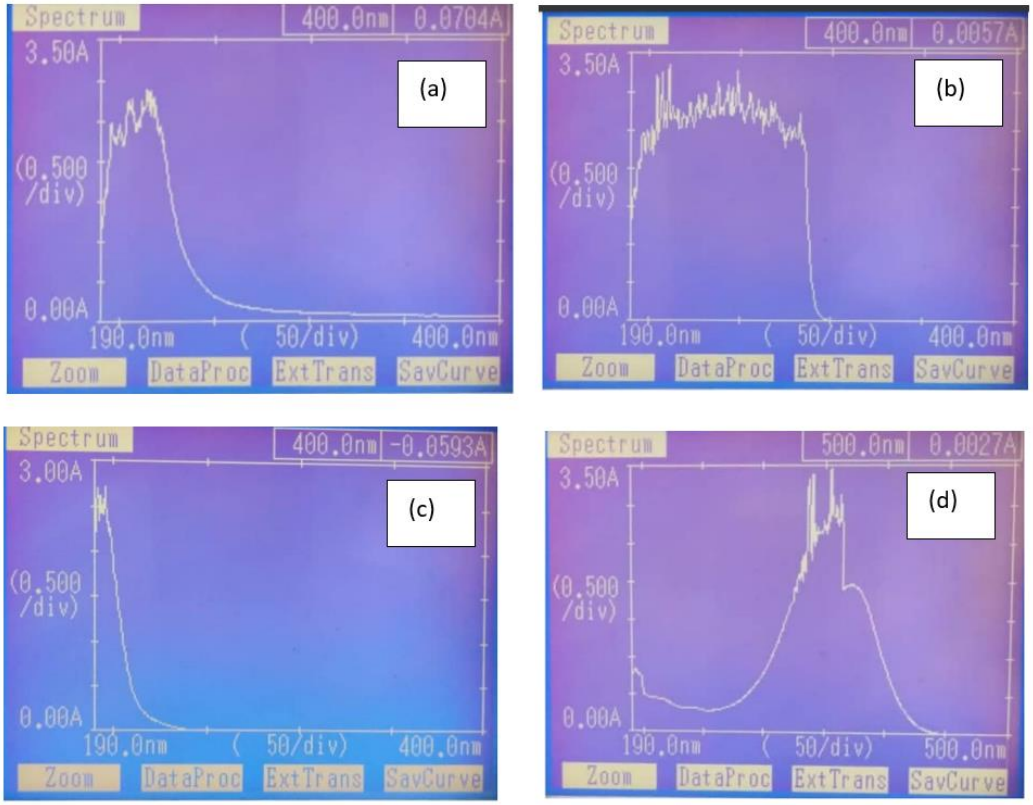

Figure 1. UV-Vis spectra of (**a**) clindamycin phosphate, (**b**) imidazolidinyl urea (**c**) methyl paraben, (**d**) tretinoin.

3.1.1. Optimized Chromatographic Conditions

The following chromatographic conditions produced the best results: C-18 (octyl-decyl silane) column 250 × 4.6 mm, 5 µm at 40 °C in a gradient mode (the technique of varying the composition of the mobile phase during the chromatographic run) with a mobile phase of solution A (1 mL ortho-phosphoric acid in 100 mL water) and Solution B (methanol). Gradient elution was carried out at a flow rate of 1.0 mL.min-1 with 50:50 *v/v* percent (A:B) for 0.00 to 10.0 min, 06:94 *v/v* percent (A:B) for 10.01 to 23 min, and 50:50 *v/v* percent (A:B) for 23.01 to 35 min at UV-Vis detection levels of 200 nm and 353 nm [34,35]. The system was equilibrated for 30 min before injecting the solution into the column. In all HPLC runs, the injection volume was 20 µL. HPLC was operated in gradient mode to decrease run times, improve peak responses, and meet suitability standards, thus enhancing the accuracy and precision of the procedure.

3.1.2. Formulation Analysis

A constant baseline was acquired using the above-mentioned optimized chromatographic settings. Standard and sample solutions were introduced into the HPLC apparatus once the baseline had stabilized. Four unique peaks were observed and recorded. The amount of drug and the assay % were computed (Figures 2–4). It is worth mentioning that since analysis was carried out using a dual-wavelength mode selector, each chromatogram has been presented in two parts (section), one part at 200 nm (for clindamycin phosphate, peaks: imidazolidinyl urea, methyl paraben) and the second part at 353 nm for tretinoin.

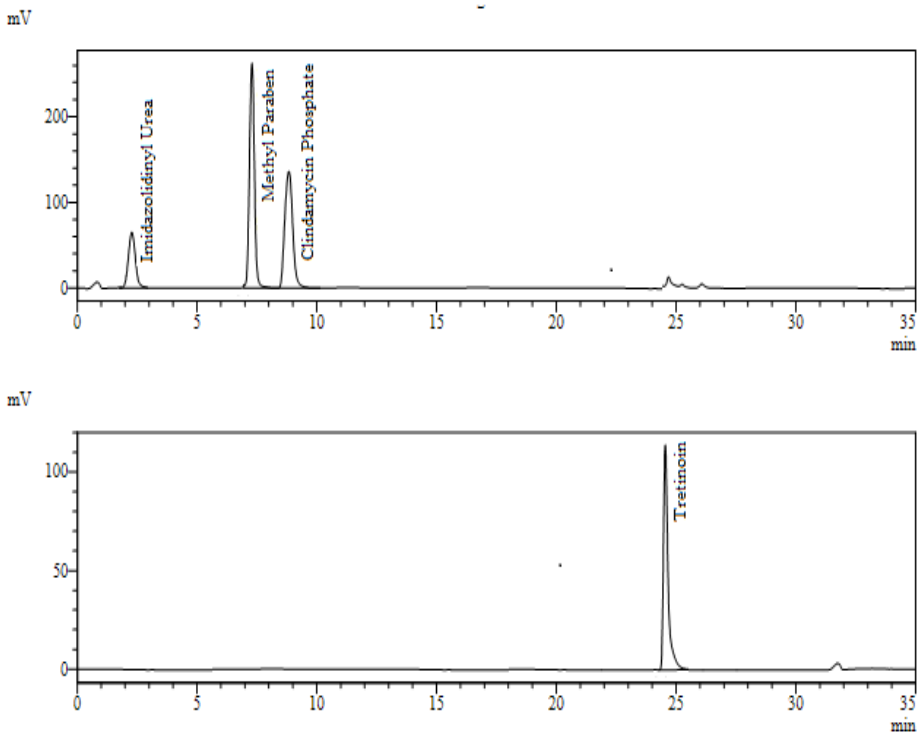

Figure 2. HPLC chromatogram of standard solution. Peaks: imidazolidinyl urea, t_R∼2.2 min; methyl paraben, t_R∼7.3 min; clindamycin phosphate, t_R∼8.9 min; tretinoin, t_R∼24.8 min.

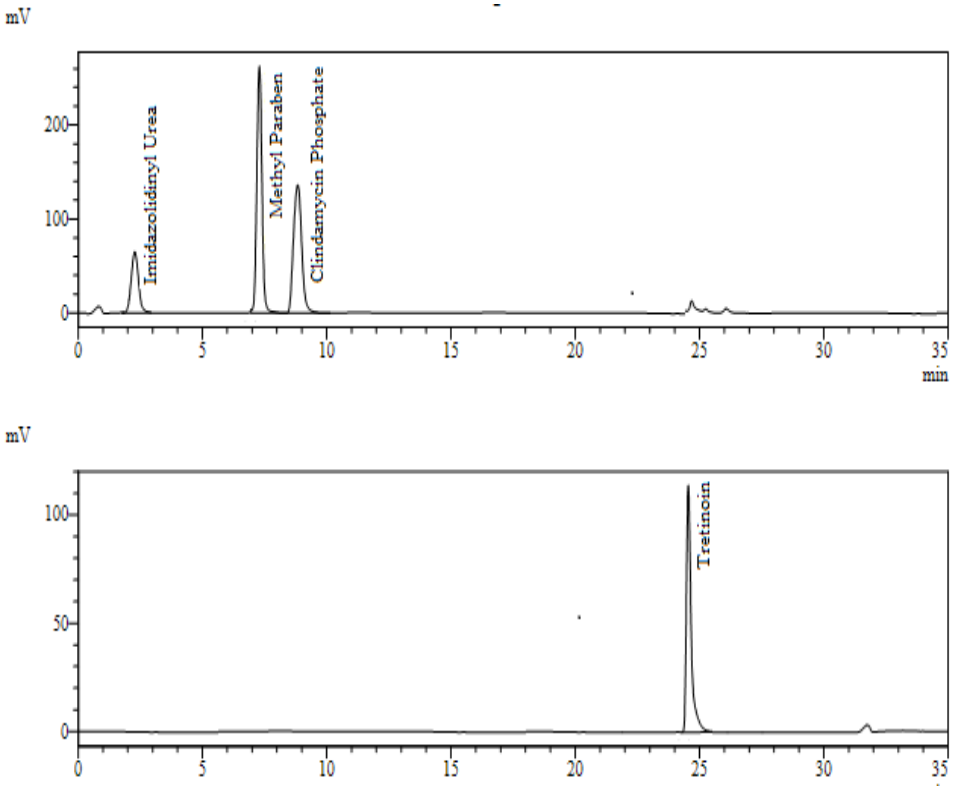

Figure 3. HPLC chromatogram of experimental gel preparation initial analysis. Peaks: imidazolidinyl urea, t_R∼2.2 min; methyl paraben, t_R∼7.3 min; clindamycin phosphate, t_R∼8.9 min; tretinoin, t_R∼24.8 min.

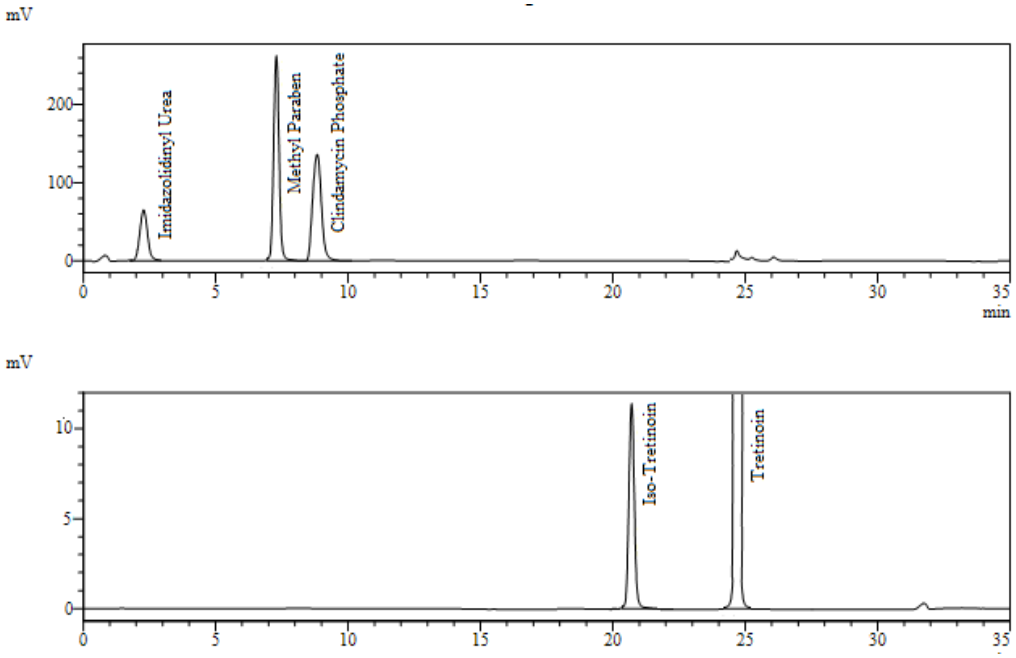

Figure 4. HPLC chromatogram of experimental gel preparation after 3 months of stress testing at 40 °C/75%RH. Peaks: imidazolidinyl urea, t_R∼2.2 min; methyl paraben, t_R∼7.3 min; clindamycin phosphate, t_R∼8.9 min; iso-tretinoin, t_R∼20.8 min; tretinoin, t_R∼24.8 min.

3.2. System Suitability

A system suitability study was conducted to validate the correctness of the proposed approach. For the clindamycin phosphate, tretinoin, methylparaben, and imidazolidinyl urea system suitability test, six replicated injections of the reference solution were administered at 100% each. The RSD values for retention time (≤2.0%), tailing factor (≤2.0), and theoretical plates (≥2000) were found to be within the standard acceptability requirements for the system suitability parameters [36]. Table 1 summarizes the findings.

3.3. Method Validation

According to the criteria of the International Conference on Harmonization [36], the suggested technique was validated for parameters such as linearity, range, precision, specificity, accuracy, the limit of detection, robustness, and the limit of quantitation.

3.3.1. Specificity

To test the specificity of the proposed analytical technique, an individual solution of the placebo, each API/preservative standard, and a blank were injected into the HPLC system. Although the placebo included several extra and minor peaks, these were acceptable due to their spacing from the needed peaks.

3.3.2. Linearity

Clindamycin phosphate, tretinoin, methylparaben, and imidazolidinyl urea were injected into the HPLC system at concentrations of 144–336 µg/mL, 3.0–7.0 µg/mL, 31.2–72.8 µg/mL, and 51.6–120.4 µg/mL, respectively, under defined chromatographic conditions. The correlation coefficient value was established after plotting a graph between various concentrations and their peak responses to assess linearity (R). Table 2 shows the obtained values of the correlation coefficient and regression equation. According to established guidelines, the correlation coefficient should be ≥0.990 [37].

Table 2. Analytical performance data for the determination of the studied drugs by the proposed method.

API/Preservative	Validation Parameters							
	Linearity		Repeatability	Range (µg/mL)	Accuracy		LOD (Limit of Detection) (µg/mL)	LOQ (Limit of Quantitation) (µg/mL)
	Regression Equation	R			Mean Recovery (%)	RSD		
Clindamycin Phosphate	y = 8543.2x + 238.4	1.00	0.52%	144–336	100.35	0.50	0.351	1.063
Methyl Paraben	y = 6952.6x − 504.55	1.00	0.26%	31.2–72.8	99.79	0.27	0.047	0.141
Imidazolidinyl Urea	y = 1175.6x + 2563.7	0.999	0.31%	51.6–120.4	100.15	0.17	1.101	3.335
Tretinoin	y = 252086.4x − 40937.5	0.999	0.33%	3.0–7.0	100.48	0.57	0.144	0.436

3.3.3. Range

The lower and upper concentrations of analyte in a sample for which the proposed analytical technique has an appropriate precision, accuracy, and linearity are referred to as a method's range. As shown in Table 2, all of these essential parameters have been examined for the validity of the devised analytical approach, and the acquired findings fall within the standard acceptance standards.

3.3.4. Precision

Repeatability and intermediate precision were used to measure accuracy.

3.3.5. Repeatability

Six separate samples were produced from homogenous blends of marketed samples of clindamycin phosphate, tretinoin, methylparaben, and imidazolidinyl urea with concentrations of 240 µg/mL, 5.0 µg/mL, 52.0 µg/mL, and 86.0 µg/mL, respectively, to test the repeatability of the proposed approach. The relative standard deviation (RSD) and standard deviation (SD) values for these samples are shown in Table 2. The relative standard deviation value for each of the samples prepared was found to be <2.0% based on the data provided. The obtained RSD value is within the standard allowed range, indicating that the proposed approach is precise [37].

3.3.6. Intermediate Precision

The HPLC system tested composite samples for several days to validate the suggested method's intermediate precision while operating circumstances remained the same, as mentioned earlier. On two different days, two operators evaluated six samples of clindamycin phosphate, tretinoin, methylparaben, and imidazolidinyl urea using the HPLC equipment. As shown in Table 3, the relative standard deviation (RSD) on both days was found to be $\leq$2%. Since the RSD for the APIs/preservatives assay for intermediate precision was within acceptable limits, the findings demonstrated that the proposed method's intermediate precision limit was excellent.

Table 3. Intermediate precision data for the determination of the studied drugs.

Parameters	Clindamycin Phosphate	Tretinoin	Methyl Paraben	Imidazolidinyl Urea
Analyst 1	99.88	100.95	100.29	100.05
Analyst 2	99.98	100.39	100.12	100.07
RSD $\leq$ 2%	**0.07%**	**0.39%**	**0.12%**	**0.01%**
Day 1	100.13	100.31	100.24	100.30
Day 2	100.06	100.77	100.28	100.06
RSD $\leq$ 2%	**0.05%**	**0.32%**	**0.03%**	**0.17%**

3.3.7. Accuracy

The accuracy (or trueness) describes how near the findings are to the accepted or real/true standard value. Clindamycin phosphate, tretinoin, methylparaben, and imidazo-

lidinyl urea were spiked in triplicate at 80%, 100%, and 120% concentrations, respectively. Under certain chromatographic conditions, these solutions were added to the HPLC system, and a three-level mean recovery was computed. Table 3 displays the results collected. Based on these data, the average recovery values found are within 98–102% of the acceptance criterion [37].

3.3.8. Robustness

The method was also validated by a slight variation in its operating conditions, such as the detection wavelength, flow rate, and column temperature, which was carried out to check the consistency and reliability of the test method during routine analysis. These parameters may vary during routine analysis, and we need to ensure that a minor variation will not affect the test method's reliability. Six replicates of each clindamycin, tretinoin, methylene paraben, and imidazolidinyl sample were injected into the HPLC system under chromatographic conditions by altering the (i) flow rate (0.1 mL/min), (ii) wavelength (200 ± 2 nm, 353 ± 2 nm), and (iii) column temperature maintained (35 °C and 45 °C). The peak responses were recorded to evaluate the suggested method's robustness in terms of standard deviation and RSD. Clindamycin phosphate, tretinoin, methylparaben, and imidazolidinyl urea had relative standard deviation values of 0.56%, 0.80%, 0.69%, and 0.45%, respectively, for various parameters. The observed findings vary within the prescribed parameters, demonstrating that deliberately changing the flow rate, wavelength, and column temperature did not affect the analytical procedure. As a result, the proposed system's robustness was established under various experimental settings.

3.3.9. Limit of Detection

The limit of detection (LOD) is the least quantity of analyte that can be detected but not necessarily measured under the specified experimental circumstances. The following formula was used to evaluate this parameter under linearity, and the estimated value (1.40 ppm) is shown in Table 2.

$$\text{LOD} = 3.3 \times \text{Standard deviation of response/Slope of calibration curve} \quad (1)$$

3.3.10. Limit of Quantification

The least quantity of analyte in a sample that can be precisely and correctly quantified under the specific experimental conditions is referred to as the limit of quantification (LOQ). The following method was used to evaluate this parameter under linearity, and the results for clindamycin phosphate, tretinoin, methylparaben, and imidazolidinyl are listed in Table 2.

$$\text{LOQ} = 10 \times \text{Standard deviation of response/Slope of calibration curve} \quad (2)$$

From the data given in Table 4, a comparison of assay results with the previously reported studies demonstrates that the percentage recovery attained in the current study is higher than the other studies reported on simultaneous determination of clindamycin phosphate in the presence of preservatives [8,38], hence making our findings valuable and giving a notable advantage to the present study.

Table 4. A comparative assay of results of the present study with the reported studies.

APIs	% Recovery (%)	RSD (%)	LOD (µg/mL)	LOQ (µg/mL)	Reference
PHE	98.8	1.72	0.3	1.0	[38]
MP	101.7	0.91	0.2	0.6	
CP	99.0	1.67	7.0	20.0	
ADA	101.3	0.49	0.2	0.5	
CP	99.2	0.6	-	-	[39]
Tretinoin	100.3	0.8	-	-	

PHE = phenoxyethanol, MP = methyl paraben, CP = clindamycin phosphate, ADA = adapelene.

3.4. Stress Testing Analysis

3.4.1. Temperature Stress Analysis

Thermal analysis is commonly used to determine a pharmaceutical product's stability. In this study, we evaluated the sample after it had been kept at a temperature of up to 40 °C for around three months. All analytes, including clindamycin phosphate, methylparaben, and imidazolidinyl urea, were relatively stable (RSD = 99%), except for tretinoin, which was shown to be degraded to roughly 87.2% (Figure 4). The impurity peak of iso-tretinoin was identified by injecting its reference standard on HPLC and then comparing its retention time with that obtained with the sample under study (Figure 4). Table 5 shows the results of the API/preservative assays at the temperature settings mentioned previously.

Table 5. Observed data for forced degradation study of the studied drugs.

API/Preservative	Temperature 40 °C, 3 Months	Sunlight, 10 h (25–30 °C)	Remarks
Clindamycin Phosphate	99.6%	99.7%	No major degradation products formed
Methyl Paraben	99.2%	99.4%	No major degradation products formed
Imidazolidinyl Urea	98.6%	99.8%	No major degradation products formed
Tretinoin	87.2%	65.2%	One major degradation product was formed

3.4.2. Sunlight Stress Analysis

Photo-stability refers to measuring a product's stability when exposed to sunlight or UV rays, which provides information on the active ingredients' stability. The sample was exposed to sunlight for roughly 10 h before being put into the HPLC apparatus under the prescribed conditions. Table 4 summarizes the results in percentage terms. According to the results, tretinoin was significantly damaged by sunlight exposure, with 65.2% lost. Still, clindamycin phosphate, methylparaben, and imidazolidinyl urea were found to be reasonably stable (RSD = 99% each). In addition, the chromatograms showed that all investigated APIs/preservatives were well-separated from the peaks of degraded products created after photo-stress studies (Figure 5). It is worth mentioning that in the forced degradation studies carried in this work, no major degradation products were formed except in the case of tretinoin, which was degraded by higher temperatures and sunlight exposure (Table 4).

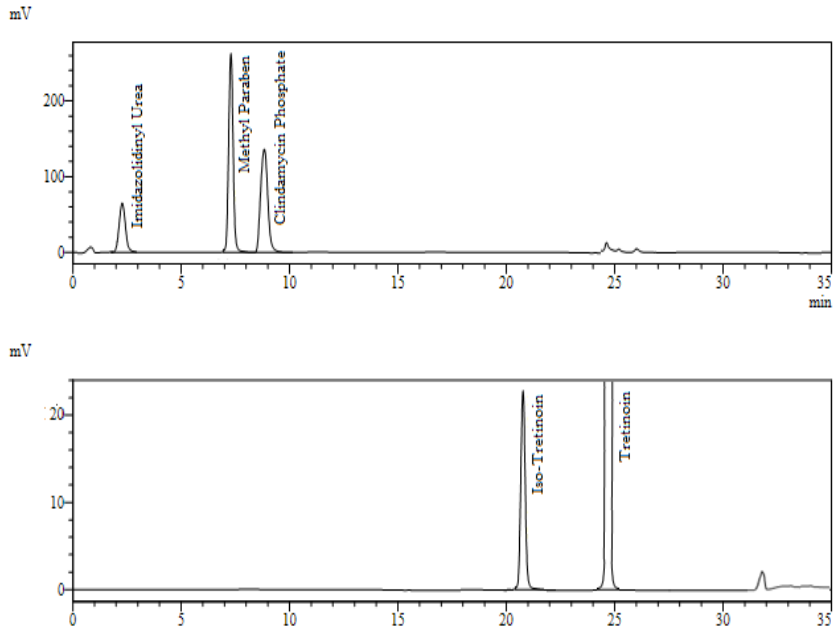

Figure 5. HPLC chromatogram of experimental gel preparation after 10 h stress testing in sunlight (25–30 °C). Peaks: imidazolidinyl urea, t_R~2.2 min; methyl paraben, t_R~7.3 min; clindamycin phosphate, t_R~8.9 min; iso-tretinoin, t_R~20.8 min; tretinoin, t_R~24.8 min.

3.5. Evaluation of Suggested Analytical Approach in Clindamycin Phosphate/Tretinoin Assay in Commercially Available Topical Gel Products

According to one of the published studies, 10% of moisturizing creams evaluated on the Swedish market had preservatives listed on the label that were not actually present in the products [39]. Additionally, 17% of the goods included one or more of the nine target preservatives that were not identified on the ingredient list [40]. To support this finding, the proposed stability-indicated approach was applied to a clindamycin phosphate/tretinoin assay in four topical gels randomly collected from the local market. Clinician-T Gel, Acdermin Gel, Cleret Gel, and Clinda-T Gel are brand names for the same product. The gels' clindamycin phosphate/tretinoin content was labeled, but other components, including the preservative, were only stated in the packaging list. These gels are comparable in clindamycin phosphate/tretinoin composition, but they differ in their excipient composition and appearance. The results of injecting these gel samples onto the HPLC column under identical conditions as indicated before are shown in Table 6. It is clear from the data that a decent separation of APIs and preservatives was accomplished (Figures 6–8).

Table 6. Determination of the studied drugs in commercial products by the proposed method.

API/Preservative	Clinacin-T Gel		Acdermin Gel		Cleret Gel		Clinda-T Gel	
	Labeled (mg/gm)	**Found (mg/gm)**	**Labeled (mg/gm)**	**Found (mg/gm)**	**Labeled (mg/gm)**	**Found (mg/gm)**	**Labeled (mg/gm)**	**Found (mg/gm)**
Clindamycin Phosphate	12	12.10	12	12.05	12	12.04	12	12.05
Methyl Paraben	Not declared	2.28	Not declared	2.14	Not declared	2.00	Not declared	Not found
Imidazolidinyl Urea	Not declared	4.32	Not declared	Not found	Not declared	Not found	Not declared	Not found
Tretinoin	0.25	0.29	0.25	0.26	0.25	0.26	0.25	0.27

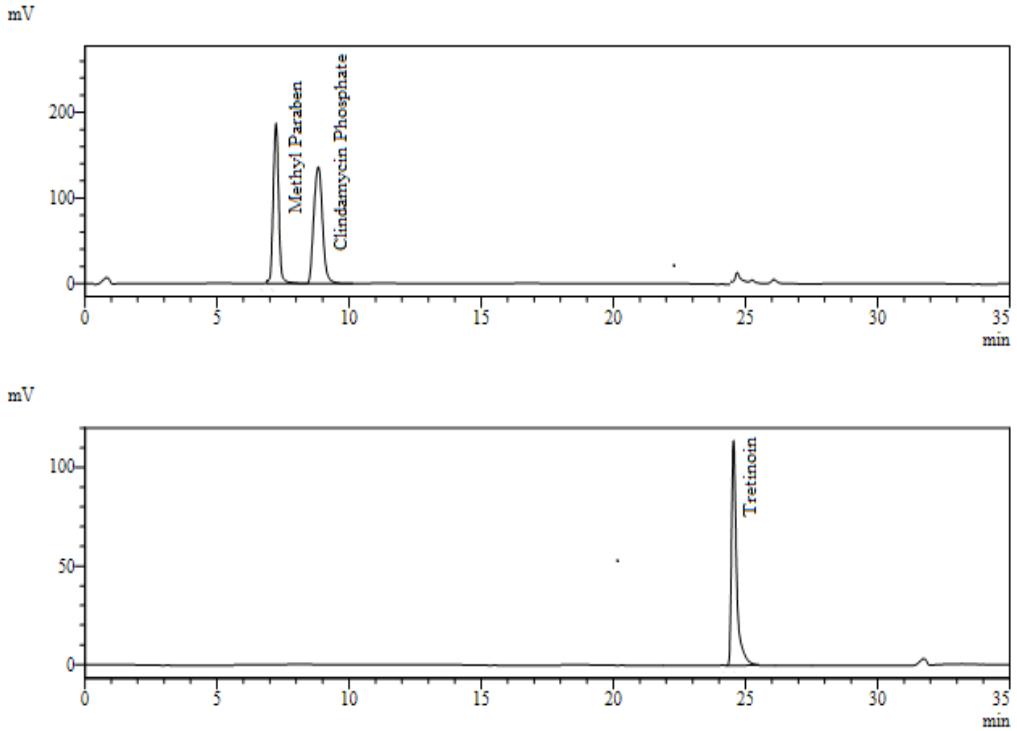

Figure 6. HPLC chromatogram of Acdermin Gel analysis. Peaks: methyl paraben, t_R~7.3 min; clindamycin phosphate, t_R~8.9 min; tretinoin, t_R~24.8 min.

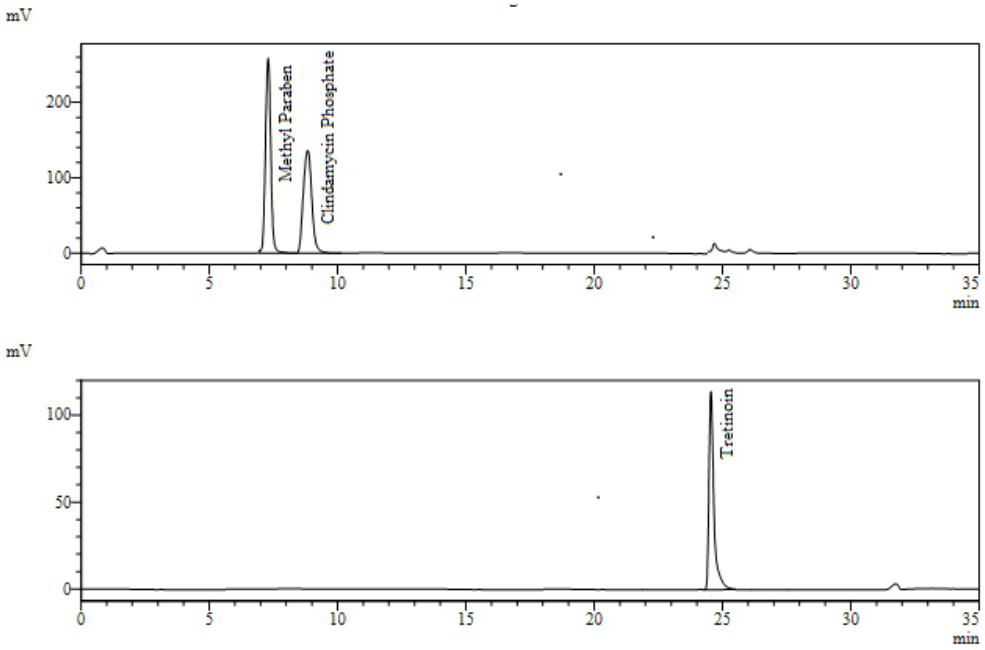

Figure 7. HPLC chromatogram of Cleret Gel analysis. Peaks: methyl paraben, t_R~7.3 min; clindamycin phosphate, t_R~8.9 min; tretinoin, t_R~24.8 min.

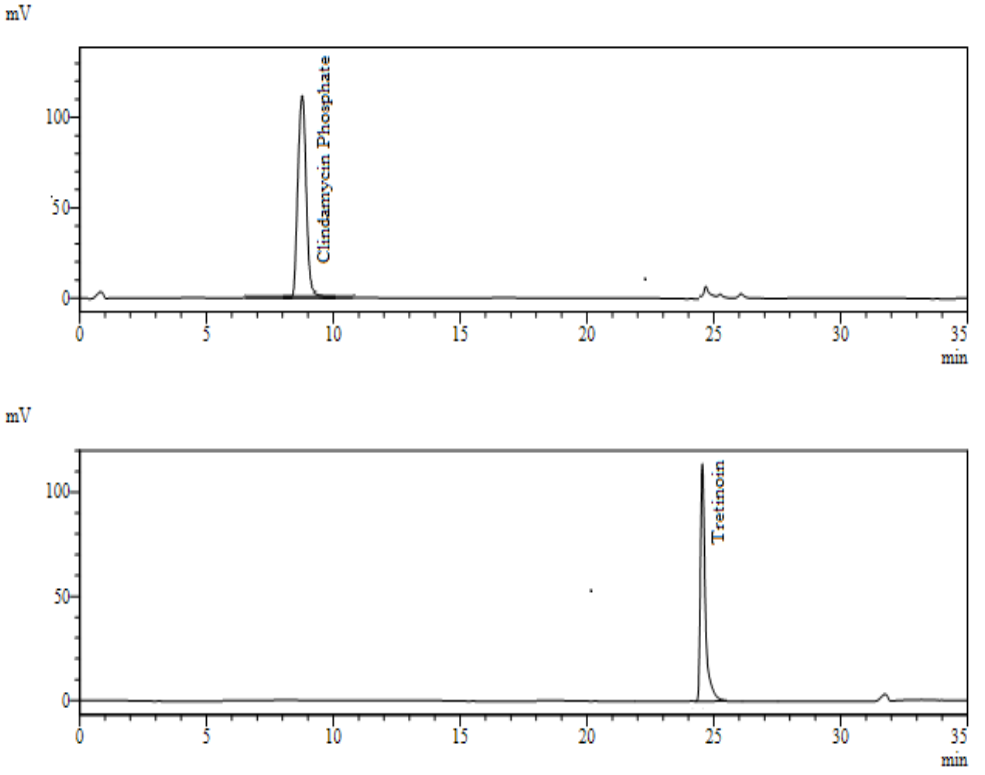

Figure 8. HPLC chromatogram of Clinda-T Gel analysis. Peaks: clindamycin phosphate, t_R~8.9 min; tretinoin, t_R~24.8 min.

4. Conclusions

According to the results, a rapid, easy, affordable, and reliable RP-HPLC technique has been created and validated for testing acne-treating drugs such as tretinoin and clindamycin phosphate in combination when preservatives are present. The devised approach is selective, precise, accurate, linear, and durable according to ICH criteria. The parameters were

statistically analyzed using standard deviation and RSD, and the findings were determined to be well within the specified ranges. In comparison to previous analytical techniques described in the literature, the new RP-HPLC approach is appropriate for the simultaneous measurement of clindamycin phosphate, tretinoin, and two preservatives with their degradation products in dermatological and gel dosage forms. The proposed method's reliability was demonstrated by temperature and photo-stress tests. Based on the findings, we propose that this RP-HPLC method can be used in pharmaceutical laboratories for routine quality control assessments of clindamycin phosphate and tretinoin in dermatological and gel dosage forms rapidly via simultaneous qualitative-quantitative analysis.

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