*REDVET - Revista electrónica de Veterinaria* - ISSN 1695-7504
Vol 25, No. 1S (2024)
http://www.veterinaria.org
Article Received: Revised: Accepted:

# Method Development & Validation Of Stability Indicating Assay Method Of Clindamycin In Adapalene And Clindamycin Gel Formulation By RP-HPLC

**K. Ranjith[1], Durga Panikumar Anumolu[2], Veera Shakar Pulusu[3], Raj K. Keservani[4], Ramanlal N Kachave[5], Navneet Tiwari[6], Himanshu Lohani[7], Anand Mundada[8*]**

[1]General Manager-Quality Assurance, Sionc Pharmaceuticals Pvt. Ltd, JN Pharmacity, Parawada, Anakapalli, Andhra Pradesh, 531021, India
[2]Associate Professor, Department of Pharmaceutical Analysis, Gokaraju Rangaraju College of Pharmacy, Osmania University, Hyderabad, Telangana, 500090, India
[3]Ohio University, Department of Chemistry & Biochemistry, Athens, OH, 45701, USA
[4]Faculty of B. Pharmacy, CSM Group of Institutions, Prayagraj, Uttar Pradesh, 212111, India
[5]GES's Sir Dr. M. S. Gosavi College of Pharmaceutical Education and Research Nashik, Affiliated Savitribai Phule Pune University, Pune, Nashik, Maharashtra, 422005, India
[6]Assistant Professor, Graphic Era Hill University Bhimtal, Nainital, Uttarakhand, 263136
[7]Associate Professor, Samrat Prithviraj Chauhan College of Pharmacy Kashipur, Udham Singh Nagar, Uttarakhand, 244713, India
[8]Associate Professor, R. C. Patel Institute of Pharmaceutical Education and Research, Shirpur, Dhule, Maharashtra, 425405, India

***Corresponding author:** Anand Mundada,
*Associate Professor, R. C. Patel Institute of Pharmaceutical Education and Research, Shirpur, Dhule, Maharashtra, 425405, India; Email: anandmundadarcp@gmail.com

**Abstract:**
**Background:** It is applied to the skin to treat bacterial vaginosis, which usually stops Propionibacterium acnes and acne vulgaris from growing on the skin.
**Objectives:** It was the goal of this study to create and test a new RP-HPLC method that can show stability while measuring stabilizers, CP, and ADA in topical gel formulations at the same time.
**Material and Methods:** The best way to separate analytes and stress-induced degradant peaks on the XBridge C18 (50 x 4.6 mm, 3.5 µm) column was to use a mobile phase that was a variable mix of acetonitrile, tetrahydrofuran, and ammonium hydrogen phosphate buffer (pH 2.50), along with gradient elution. At 210 nm, phenoloxyethanol, methylparaben, and clindamycin phosphate were found. At 321 nm, adapalene was found.
**Results and Conclusion:** The developed RP-HPLC method was validated for determination of Clindamycin using linearity, accuracy, precision, precision Day-2, Ruggedness, robustness, Specificity, Solution Stability Study. All parameters % RSDs were below two, demonstrating the method's validity and stability, and indicating that the assay results obtained using this procedure are highly consistent.

**Keywords:** Validation, Clindamycin Phosphate, Adapalene Stability Study

## Introduction:

Clindamycin is the name for the 2-phosphate form of clindamycin. Clindamycin is a chemical that is partly manufactured and partly natural. It works as an antibiotic against Gram-negative and Gram-positive bacteria that don't need oxygen, as well as Gram-positive aerobes [1, 2]. It is applied to the skin to treat bacterial vaginosis, which usually stops Propionibacterium acnes and acne vulgaris from growing on the skin. When Propionibacterium acnes is stopped from working, the amount of free fatty acids on the skin's surface goes down [1-3].

When clindamycin phosphate is applied to the skin, it deeply penetrates open comedones, causing a large number of them to show up. Adapalene (ADA) is a synthetic retinoid from the third generation that is used to treat acne. It comes from naphthoic acid and is a chemical that likes fats a lot. Combination therapy with a topical retinoid and an antibacterial drug is the best way to treat acne for most people because it gets rid of most of the problems that cause acne [2-4].

Adapalene has been shown to help clindamycin get deeper into hair follicles. This combination treatment works well and is well tolerated. Topical treatments need to have a way to keep them fresh so that microbes don't grow while they're being stored [3-5]. Because they kill germs and fungi, preservatives are an important part of many products. Phenoxyethanol, methylparaben, and propylparaben are common preservatives found in topical treatments that are water-based. Preservatives in medicines need to be checked often to see how well they work during the shelf life [4-6].

The aim of this study was to create and test a new RPHPLC method that can show how stable something is while also measuring the amounts of preservatives, CP, and ADA in dermal gels. In line with ICH guidelines, the procedure's precision, accuracy, linearity, specificity, solution stability, and durability were all carefully checked.

*REDVET - Revista electrónica de Veterinaria* - ISSN 1695-7504
Vol 25, No. 1S (2024)
http://www.veterinaria.org
Article Received: Revised: Accepted:

## MATERIAL AND METHODS:

### Materials:

Waters Alliance HPLC system having PDA detector. Separation was achieved on LiChroCart-Lichrospher RP-Select B (250 mm × 4.6 mm, 5.0 µ) [COL/ADL/14-020]. The peaks were checked with the PDA detector. All weighing were done on electronic balance (Model Shimadzu AUW-220D)[BAL/NO.-004,005].pH meter used for adjusting pH was Digital pH Meter. All samples and Mobile phase sonicated by using Ultrasonicator model Ultrasonic Cleaner.

### Method Development:

Various chromatographic settings were employed to facilitate the elution of the component. Clearly defined symmetrical peaks were achieved by monitoring the eluent response under optimal conditions following comprehensive experimental trials that can be described [5-7].

### Selection of Mobile Phase:

At first, various ratios of mobile phases were tested in order to estimate Clindamycin simultaneously. Considering system suitability parameters such as RT, tailing factor, number of theoretical plates, and HETP, the optimal mobile phase for the analysis was determined to be buffer [6-8].

### Preparation of Mobile phase and Buffer:

Prepare a mixture of 225:775 acetonitrile and buffer, then pass it through a 0.45 µ nylon membrane filter and degas it. Measure out 1000 milliliters of water, dissolve 13.68 grams of potassium dihydrogen phosphate in it, and then use orthophosphoric acid to bring the pH level up to 2.5+0.1 [7-9].

### Preparation of Standard Solution:

Precisely transfer 23.74 milligrams of Clindamycin by weighing it. Put 20 milligrams of Clindamycin (the phosphate working standard) into a 100 milliliter volumetric flask, add 70 milliliters of mobile phase, and sonicate until dissolved. Add enough mobile phase to make up the volume, then mix [8-10].

### Preparation of Placebo Solution:

Transfer around 2 grams of the placebo to a 100 milliliter volumetric flask after carefully weighing it. Add enough mobile phase to reach the desired volume, stir, shake vigorously to evenly distribute the gel, and sonicate for 5 minutes while whirling occasionally to include the mobile phase. When cooled, return to a room temperature state. Separate the liquid sample by centrifuging it. This liquid is called the supernatant [9-11].

### Preparation of Sample Solution:

Precisely measure out 2 grams of the sample and put it to a 100 milliliter volumetric flask. Add enough mobile phase to reach the desired volume, stir, shake vigorously to evenly distribute the gel, and sonicate for 5 minutes while whirling occasionally to include the mobile phase. When cooled, return to a room temperature state. Separate the liquid sample by centrifuging it. This liquid is called the supernatant [10-12].

### Method Validation:

In terms of specificity, linearity, accuracy, precision, and robustness, the recently created method was validated in accordance with the ICH standards. Injecting a standard solution determined that the system was suitable. We looked for any additional peaks in the chromatograms. There was no evidence of chromatographic interference caused by the excipients. The precision of the method was assessed by utilizing six separate test solutions [11-13].

### Specificity:

Even when excipients were present, Clindamycin remained completely separated. The retention time of the Clindamycin chromatogram of the formulation solution was also unaffected by any interference [12-14].

### Linearity:

Five calibration standard solutions were created over the concentration range in order to construct the calibration curves. Clindamycin was shown to have linearity between 80 and 120 µg mL-1. A value of 0.994 was recorded for the correlation coefficient ('r2') [13-15].

### Precision:

The repeatability of the results was checked after six sets of standard solutions of Clindamycin (Conc. 20 µg/ml) were prepared. Six sets of standard solutions were prepared for Precision Day-2 [14-16].

*REDVET - Revista electrónica de Veterinaria* - ISSN 1695-7504
Vol 25, No. 1S (2024)
http://www.veterinaria.org
Article Received: Revised: Accepted:

**Accuracy:**
In order to conduct recovery investigations, the pre-analysis samples were supplemented with 21, 26, and 31 µg of Clindamycin, respectively. Chromatograms were recorded after injecting the resulting sample solutions. Results were obtained after three determinations were conducted at each level [15-17].

**Robustness:**
Modifying the chromatographic settings slightly in accordance with ICH criteria allowed us to assess the method's robustness. The chromatograms showed no significant changes, indicating that the established RP-HPLC technique and System suitability parameters were within acceptable bounds. The table shows that the test procedure worked well under all of the different scenarios. So, the approach could withstand the usual fluctuations in chromatographic conditions [16-18].

**Ruggedness:**
Ruggedness was performing by preparing six set of standard solution (concentration 20 µg/ml) of Clindamycin and check out reproducibility of result [17-19].

**Force Degradation study:**
**Acid Degradation:**
Weigh accurately and transfer about 2gm gel sample into 100 ml volumetric flask. Add 10 ml of 0.1N methanolic Hydrochloric acid solution, and sonicate for 10 min, keep at room temp for 24 hours. Neutralized with 0.1N methanolic NaOH solution make up the volume with mobile phase and mix. Centrifuge the sample solution to get the clear supernatant liquid [18-20].

**Base Degradation:**
Weigh accurately and transfer about 2gm gel sample into 100 ml volumetric flask. Add 10 ml of 0.1N methanolic NaOH solution, and sonicate for 10 min, keep at room temp for 24 hours. Neutralized with 0.1N methanolic Hydrochloric acid solution make up the volume with mobile phase and mix. Centrifuge the sample solution to get the clear supernatant liquid [19-21].

**Peroxide Degradation:**
Weigh accurately and transfer about 2gm gel sample into 100 ml volumetric flask. Add 10 ml of 3Percentage v/v methanolic H2O2 solution, and sonicate for 10 min, keep at room temp for 24 hours make up the volume with mobile phase and mix. Centrifuge the sample solution to get the clear supernatant liquid [20-22].

**Thermal Degradation:**
Sample to be exposed at $105^0$C for at least 24 hours and made up sample as per previous procedure, and analyze by using a Photodiode Array Detector [21-23].

**Humidity Degradation:**
Sample to be exposed at $25^0$C/92Percentage RH condition for at least 24 hours and made up sample as per previous procedure, and analyze by using a Photodiode Array Detector [22-24].

**Photolytic Degradation:**
Sample to be exposed at 1.2 million Lux hours for at least 24 hours and made up sample as per previous procedure, and analyze by using a Photodiode Array Detector [23-25].

**Solution Stability Study:**
Freshly prepared Standard solution and Sample solution was taken as Initial Stability Standard and Sample solutions. Initial Stability Standard and Sample Solutions was taken as 23 Hours Stability Standard and Sample solutions resp. Initial Stability Standard and Sample Solutions was taken as 33 Hours Stability Standard and Sample solutions resp. [24-26].

**HPLC Method Development:**
The drug's solubility and spectral characteristics informed the selection of Acetonitrile: Buffer as the diluents, and following extensive testing, a mixture of 25:75 buffer and Acetonitrile was chosen as the mobile phase. Continuous chromatographic conditions were maintained throughout the technique to determine the concentrations of these medicines using high-performance liquid chromatography [26, 27].

*REDVET - Revista electrónica de Veterinaria* - ISSN 1695-7504
Vol 25, No. 1S (2024)
http://www.veterinaria.org
Article Received: Revised: Accepted:

**RESULTS AND DISCUSSION:**

**Method Development:**

To get the component out, different chromatographic settings were tried. After many tries at experiments, clearly defined symmetrical peaks were found when the eluent's response was tested in perfect conditions. The best clarity can be seen at a flow rate of 1 milliliter per minute. The PDA detector's reaction was studied, and it was found that 210 nm is the best wavelength for getting the best results.

**Selection of Mobile Phase:**

To figure out how much Clindamycin was present at the same time, different combinations of mobile phases were first tried, as shown below. The best mobile phase for the study was found to be buffer, taking into account factors like retention time, tailing factor, amount of theoretical plates, and height equivalent to a theoretical plate (HETP). A 0.45 µm nylon membrane filter was used to remove particle waste from the mobile phase. The mixture was then degassed using sonication. For the test, a flow rate of 1 ml/min was used. The table 1 shows the results that have been added together (figure 1).

**Table 1: Selection of Mobile Phase**

| Sr. No. | Mobile Phase | Observation | Result |
|---|---|---|---|
| 01 | Methanol: Water (50:50) | Only one peak | Method Rejected |
| 02 | (1.368 gm $KH_2PO_4$ in 100ml water (pH-3) and ACN (50:50) | Poor Resolution | Method Rejected |
| 03 | 1.368 gm $KH_2PO_4$ in 100ml water (pH-2) and ACN (30:70) | Poor Resolution | Method Rejected |
| 04 | 1.368 gm $KH_2PO_4$ in 100ml water (pH-2.5) and ACN (25:75) | Good resolution with acceptable System suitability parameters | Method accepted |

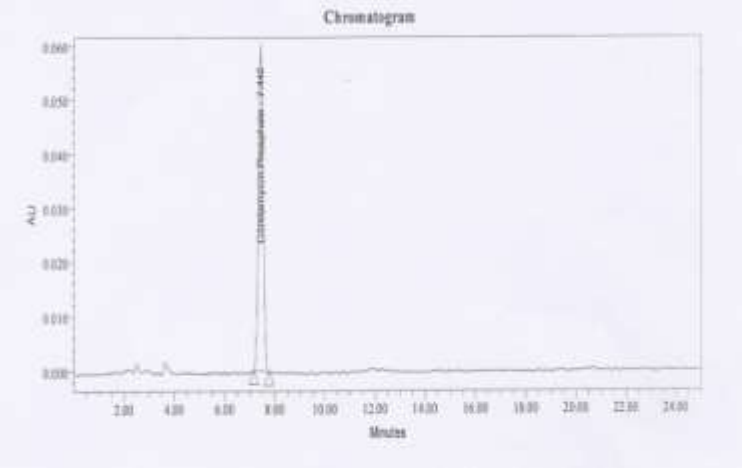


**Figure 1: Chromatogram for method accepted**

**Preparation of Mobile phase and Buffer:**

Mix acetonitrile and buffer in a 225:775 ratio. Then, filter the mixture through a 0.45 µ nylon membrane filter and remove any air bubbles. Weigh 13.68 grams of potassium dihydrogen phosphate, dissolve it, and then use orthophosphoric acid to bring the pH level back to 2.5 ± 0.1.

**Preparation of Standard Solution:**

Accurately measure and move about 23.74 milligrams of clindamycin. Put 20 mg of Clindamycin (the phosphate working standard) into a 100 ml volumetric jar. Then add 70 ml of mobile phase and sonicate to break up the Clindamycin. To get the amount you want, mix the mixture and the mobile phase together.

**Preparation of Placebo Solution:**

Very carefully weigh out two grams and put them in a 100-milliliter volumetric flask. After adding the mobile phase to get the right amount and mixing well, stir the mixture very well to make sure the gel is spread out evenly, and sonicate it with the mobile phase for five minutes, swirling it every now and then. Let it cool down to room temperature. To get the clear liquid supernatant out of the sample solution, centrifuge it.

*REDVET - Revista electrónica de Veterinaria* - ISSN 1695-7504
Vol 25, No. 1S (2024)
http://www.veterinaria.org
Article Received: Revised: Accepted:

**Preparation of Sample Solution:**
Weigh the sample carefully and move about 2 grams of it to a 100-milliliter volumetric flask. After adding the mobile phase to get the right amount and mixing well, stir the mixture very well to make sure the gel is spread out evenly, and sonicate it with the mobile phase for five minutes, swirling it every now and then. Let it cool down to room temperature. To get the clear liquid supernatant, centrifuge the sample solution. The table 2 shows an overview of the best chromatographic settings.

**Table 2: Chromatographic Condition**

| Parameter | Value |
|---|---|
| Column | LiChroCart-Lichrospher RP-Select B (250 mm × 4.6 mm, 5.0 µ) [COL/ADL/14-020]. |
| Mobile Phase | Acetonitrile : Buffer (225:775v/v) |
| Flow rate | 1.0 ml $min^{-1}$ |
| Run time | 25 min |
| Retention Time | 7 min |
| Column Temperature | $25^0$C |
| Injection volume | 20 µL |
| Detection wavelength | 210 nm |
| Diluents | Mobile phase |

**Method Validation:**
The method of getting the standards from the excipients was used to check that it was right. Three different amounts of the real standards were added to the samples that were used for the pre-analysis. The created HPLC method was used to separate and study the mixtures. There were solutions made for the linearity measurement. To test how resilient the method was, changes were made on purpose to the end experimental conditions, and the outcomes were studied. The flow rate was changed by ±0.05 ml $min^{-2}$, the pH was changed by ±0.05 pH units, and the mobile phase percentage was changed by ±2%.

**Specificity:**
It was seen that clindamycin was completely separated when it was mixed with other substances. Also, there was no interference seen during the Clindamycin chromatogram's retention time in the preparation solution (figure 2).

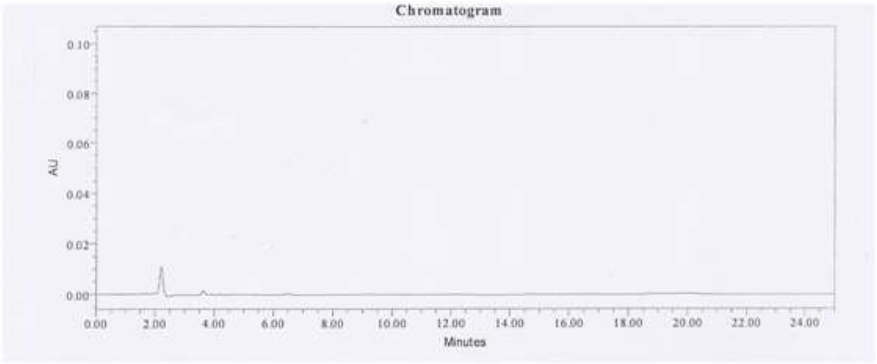


**Figure 2: Chromatogram -placebo**

**Linearity:**
To make the calibration graphs, five standard solutions for calibration were made across the concentration range. Clindamycin was found to be linear between 80 and 120 µg mL-1. The result of the correlation coefficient, or "$r^2$," was 0.994. The effects of linearity can be seen in Table 3 and figure 3.

**Table 3: Linearity of Clindamycin**

| Sr. No | Clindamycin | | | | |
|---|---|---|---|---|---|
| | Conc. (µg/ml) | Conc. of std (PPM) | Area | SD | % RSD |
| 1 | 50 % | 91.80 | 382174 | 2371.64 | 0.621 |
| 2 | 80 % | 146.88 | 614193 | 8452.05 | 1.376 |
| 3 | 90 % | 165.24 | 679048 | 3160.06 | 0.465 |
| 4 | 100 % | 183.59 | 764052 | 13490.89 | 1.766 |
| 5 | 110 % | 201.95 | 851098 | 163.34 | 0.019 |

| | | | | | |
|---|---|---|---|---|---|
| 6 | 120 % | 220.31 | 918093 | 1567.66 | 0.171 |
| 7 | 150 % | **275.39** | **1156088** | 9130.87 | 0.790 |
| Correlation Coefficient ($r^2$) | | | | | 0.99974 |
| Slope (m) | | | | | 4221 |
| Y-Intercept | | | | | -8543 |

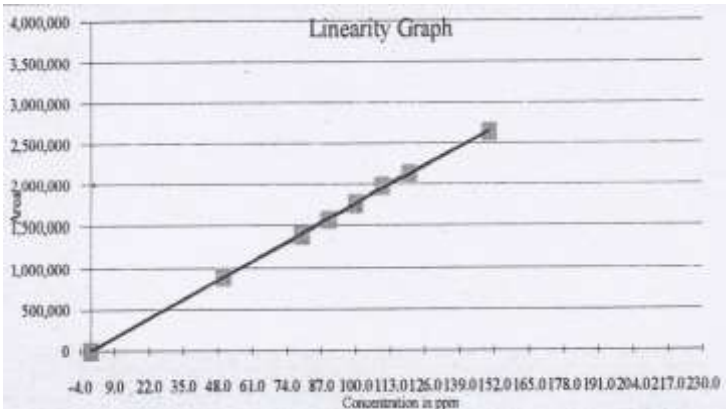


**Figure 3: Clindamycin Linearity Graph**

**Precision:**
A precision test was conducted by creating six sets of a Clindamycin standard solution with a concentration of 20 µg/ml and then checking the repeatability of the results (figure 4 and table 4).

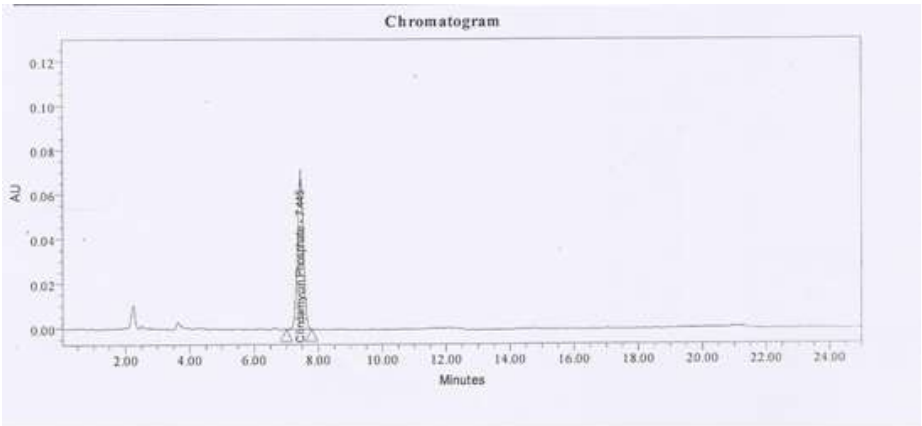


**Figure 4: Chromatogram of Clindamycin – Precision**

**Table 4: Precision of Clindamycin**

| **Sr. No** | **Clindamycin** | | |
|---|---|---|---|
| | **Conc. (µg/ml)** | **Area (mV)** | **Conc. found (µg/ml)** |
| 1 | 20 | 882753 | 19.67 |
| 2 | 20 | 887174 | 19.34 |
| 3 | 20 | 887814 | 20.27 |
| 4 | 20 | 885469 | 20.34 |
| 5 | 20 | 873402 | 19.73 |
| 6 | 20 | 884540 | 20.12 |
| | | **Mean** | 19.91 |
| | | **S.D** | 0.593 |
| | | **Percentage RSD** | 0.568 |

**Precision Day-2:**
To see if the data could be used again and again, on Precision Day 2, six sets of standard solutions containing 20 µg/ml of clindamycin were made (figure 5 and table 5).

*REDVET - Revista electrónica de Veterinaria* - ISSN 1695-7504
Vol 25, No. 1S (2024)
http://www.veterinaria.org
Article Received: Revised: Accepted:

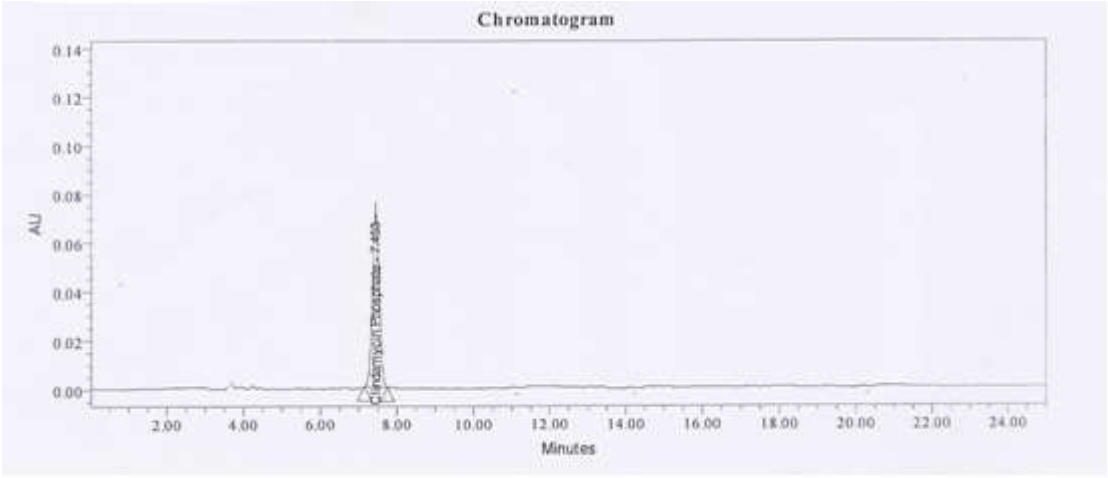


**Figure 5: Chromatogram of Clindamycin – Precision Day-2**

**Table 5: precision of Clindamycin (Precision Day-2)**

| Sr. No. | Clindamycin | | |
|---|---|---|---|
| | **Conc. (µg/ml)** | **Area (mV)** | **%Conc. found** |
| 1 | 20 | 888679 | 103.5 |
| 2 | 20 | 898316 | 103.8 |
| 3 | 20 | 897627 | 104.1 |
| 4 | 20 | 898937 | 105.7 |
| 5 | 20 | 873402 | 105.4 |
| 6 | 20 | 884540 | 105.0 |
| | | **Mean** | 104.6 |
| | | **S.D** | 0.906 |
| | | **Percentage RSD** | 0.866 |

**Accuracy:**

To do recovery tests, 21 εg, 26 µg, and 31 µg of Clindamycin were added to the sample that had already been analyzed. The chromatograms were made after the sample liquids were injected. There were three decisions, and results at each level. The results are shown in tables (table 6 and figure 6).

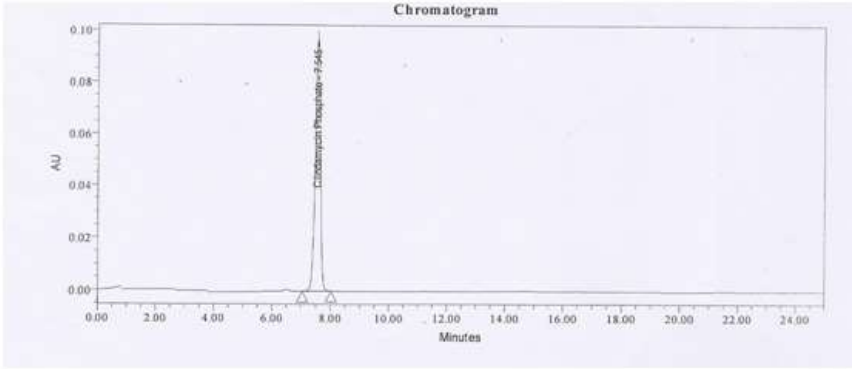


**Figure 6: Chromatogram of Clindamycin – Accuracy**

**Table 6: Accuracy of Clindamycin**

| **Recovery Level** | | **Amount added** (µg/ml) | **Amount Recovered** (µg/ml) | **Percentage Recovery** |
|---|---|---|---|---|
| 80% | Set-1 | 16.2539 | 16.4186 | 101.0 |
| | Set-2 | 16.2539 | 16.5618 | 101.9 |
| | Set-3 | 16.2539 | 16.3390 | 100.5 |
| 100% | Set-1 | 20.3174 | 20.2875 | 99.9 |
| | Set-2 | 20.3174 | 20.2197 | 99.5 |
| | Set-3 | 20.3174 | 20.2762 | 99.8 |

| | | | | |
|---|---|---|---|---|
| 120% | Set-1 | 24.3809 | 24.7113 | 101.4 |
| | Set-2 | 24.3809 | 24.7396 | 101.5 |
| | Set-3 | 24.3809 | 24.4812 | 101.5 |
| | | | **Mean** | 101.0 |
| | | | **SD** | 0.841 |
| | | | **Percentage RSD** | 0.833 |

**Robustness:**
ICH guidelines said that small changes should be made to the chromatographic settings in order to test how reliable the method was. There were no noticeable changes in the chromatograms, which means that the RP-HPLC method worked as expected and the system suitability parameters were found to be within acceptable ranges. The data in the table show that the testing method works reliably in all possible situations. In the end, the method showed enough resilience to handle expected changes in chromatographic conditions (table 7).

**Table 7: Robustness Study of Clindamycin**

| **Parameter** | **Conditions and sets** | **Variation** | **Parameters** | | | |
|---|---|---|---|---|---|---|
| | | | **Area** | **% Conc. found** | **SD** | **% RSD** |
| Flow rate ( mL min $^{-1}$) (± 0.1 mL) | Low Flow set-1 | 0.9 | 1003178 | 107.1 | | |
| | Set-2 | 0.9 | 1014939 | 108.2 | | |
| | Set-3 | 0.9 | 1014939 | 104.1 | | |
| | | | **Mean** | **105.6** | **1.641** | **1.554** |
| | High Flow | 1.1 | 799221 | 104.4 | | |
| | Set-2 | 1.1 | 829616 | 108.1 | | |
| | Set-3 | 1.1 | 803340 | 104.8 | | |
| | | | **Mean** | **105.3** | **1.405** | **1.334** |
| M.P. Composition (ACN: Buffer) (±2%) | Low comp. Set-1 | 23:77 | 1006667 | 101.3 | | |
| | Set-2 | 23:77 | 1012296 | 102.1 | | |
| | Set-3 | 23:77 | 1009672 | 101.7 | | |
| | | | **Mean** | **101.9** | **0.327** | **0.321** |
| | High comp. Set-1 | 27:73 | 1005162 | 102.6 | | |
| | Set-2 | 27:73 | 1012903 | 103.6 | | |
| | Set-3 | 27:73 | 1007806 | 102.9 | | |
| | | | **Mean** | **102.5** | **0.665** | **0.649** |

**Ruggedness:**
Ruggedness was performing by preparing six set of standard solution (concentration 20 µg/ml) of Clindamycin and check out reproducibility of result (figure 7 and table 8).

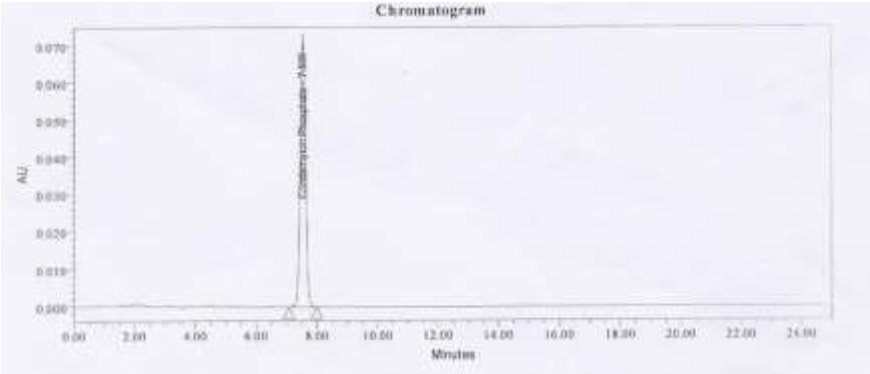


**Figure 7: Chromatogram of Clindamycin – Ruggedness**

**Table 8: Ruggedness Study of Clindamycin**

| **Sr. No.** | **Clindamycin** | | |
|---|---|---|---|
| | **Sample taken (mg)** | **Area (mV)** | **% Conc. Found** |

| | | | |
|---|---|---|---|
| 1 | 2051.04 | 999949 | 101.7 |
| 2 | 2046.04 | 1000063 | 102.0 |
| 3 | 2049.41 | 997881 | 101.6 |
| 4 | 2042.28 | 993556 | 101.5 |
| 5 | 2047.01 | 998380 | 101.8 |
| 6 | 2050.06 | 993792 | 101.1 |
| | | **Mean** | 101.6 |
| | | **S.D** | 0.306 |
| | | **Percentage RSD** | 0.301 |

**Force Degradation study:**

**Acid Degradation:**

Weigh a 2-gram sample of the gel carefully and carefully move it into a 100-ml volumetric flask. As soon as the 10 milliliters of 0.1N methanolic hydrochloric acid solution is done being sonicated, leave it at room temperature for 24 hours. Change the amount of the mobile phase and mix it after neutralizing it with a 0.1N methanolic NaOH solution. By centrifuging the sample solution, you can separate the clear liquid supernatant (figure 8).

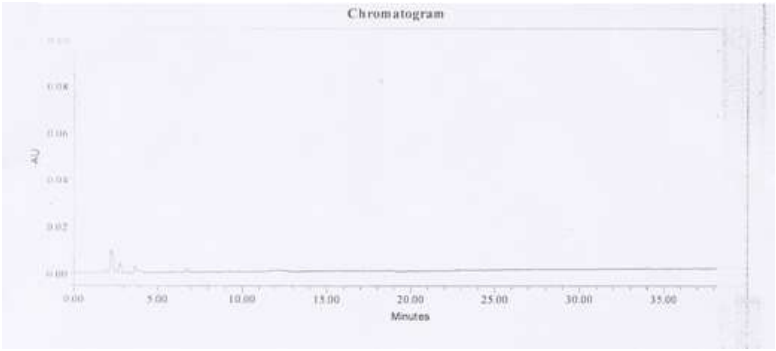


**Figure 8: Chromatogram of Clindamycin – Acid Degradation**

**Base Degradation:**

Put approximately 2 grams of gel sample into a 100 milliliter volumetric flask after carefully weighing it. Sonicate for 10 minutes after adding 10 milliliters of 0.1N methanolic NaOH solution; then, let stand at room temperature for 24 hours. Combine the mobile phase with the neutralized 0.1N methanolic hydrochloric acid solution to achieve the desired volume. Separate the liquid sample by centrifuging it. This liquid is called the supernatant (figure 9).

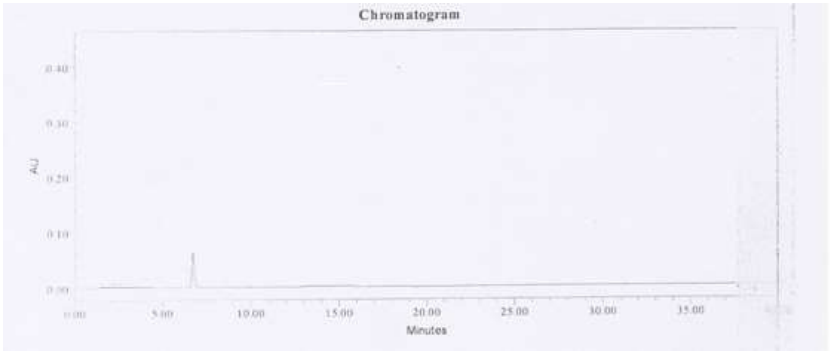


**Figure 9: Chromatogram of Clindamycin – Base Degradation**

**Peroxide Degradation:**

Accurately weigh and transfer a 2 gram sample of the gel into a 100 ml volumetric flask. To make up the volume, add 10 ml of the percentage v/v methanolic H2O2 solution, sonicate for 10 minutes, and then leave at room temperature for 24 hours. Then, combine. Extract the clear liquid supernatant by centrifuging the sample solution (figure 10).

*REDVET - Revista electrónica de Veterinaria* - ISSN 1695-7504
Vol 25, No. 1S (2024)
http://www.veterinaria.org
Article Received: Revised: Accepted:

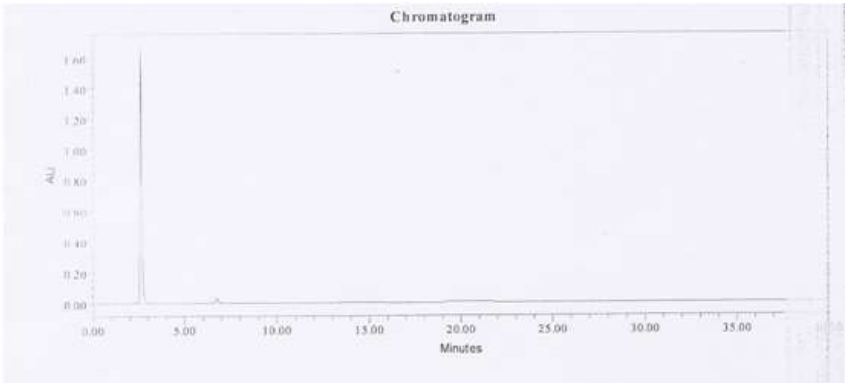


**Figure 10: Chromatogram of Clindamycin – Peroxide Degradation**

**Thermal Degradation:**
Sample to be exposed at $105^0$C for at least 24 hours and made up sample as per previous procedure, and analyze by using a Photodiode Array Detector (figure 11).

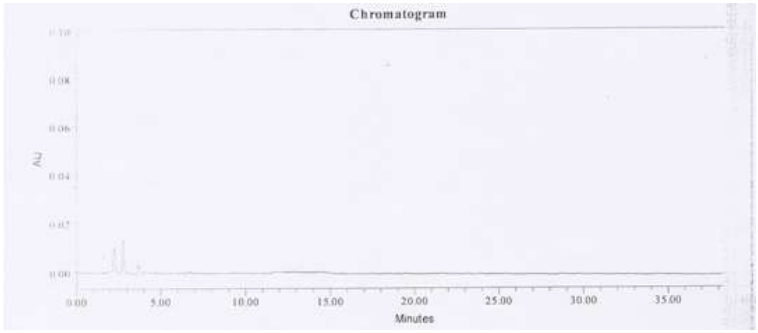


**Figure 11: Chromatogram of Clindamycin – Thermal Degradation**

**Humidity Degradation:**
Sample to be exposed at $25^0$C/92Percentage RH condition for at least 24 hours and made up sample as per previous procedure, and analyze by using a Photodiode Array Detector (figure 12).

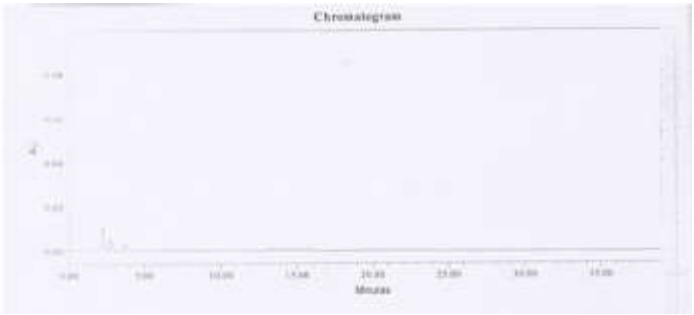


**Figure 12: Chromatogram of Clindamycin – Humidity Degradation**

**Photolytic Degradation:**
The sample must be prepared in accordance with the preceding protocol, exposed at 1.2 million Lux hours for at least 24 hours, and then examined using a photodiode array detector (figure 13).

*REDVET - Revista electrónica de Veterinaria* - ISSN 1695-7504
Vol 25, No. 1S (2024)
http://www.veterinaria.org
Article Received: Revised: Accepted:

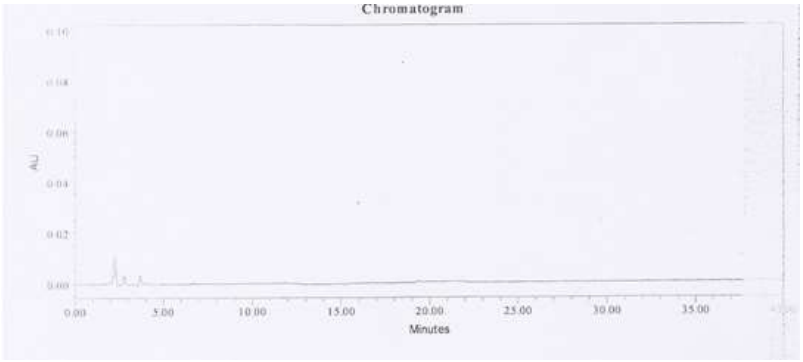


**Figure 13: Chromatogram of Clindamycin – Photo Degradation**

**Solution Stability Study:**
Newly made Standard and Sample solutions were used to make preliminary Stability Standard and Sample solutions. Both the basic stability standard and the sample solutions were set up after 23 hours. Thirty hours were set aside for the sample solutions and the basic stability standard (table 9).

**Table 9: Solution Stability Study of Clindamycin**

| Sr. No. | Sample Identity | Clindamycin | | |
|---|---|---|---|---|
| | | Sample taken (mg) | Area (mV) | Co-relation |
| 1 | Initial Sample Solution Stability | ---- | ---- | ---- |
| 2 | 23-Hours Sample Solution Stability | 2051.04 | 997755 | 99.7 |
| 3 | 33-Hours Sample Solution Stability | 2051.04 | 1012053 | 101.2 |
| 4 | Initial Standard Solution Stability | 23.28 | 854667 | ---- |
| 5 | 23-Hours Standard Solution Stability | 23.28 | 850852 | 99.5 |
| 6 | 33-Hours Standard Solution Stability | 23.28 | 993792 | 99.2 |

It was found that the suggested ways of measuring Clindamycin in gel form were exact, easy to use, and effective. The methods described can be used to do routine drug screening of gel formulations. The tests on gel recovery got results between 98% and 102%. The formulation's excipients don't get in the way of anything. It is possible to do routine quality control research quickly and easily. All three processes have been checked to make sure they meet ICH standards for being correct, easy to use, quick, precise, reliable, sensitive, repeatable, and cost-effective. A correlation value that is close to 1 in clindamycin's working range shows that it is linear. The biggest relative standard deviation that was found was less than 2, which shows that the data is reliable, precise, and accurate. As a result, all of the validation values are within the acceptable range. Because of this, the method can be used to regularly test for Clindamycin.

**Conclusion:**
Its main purpose was to discover Clindamycin in gel using HPLC. Trial and error and a literature study gave rise to the statistical principle of sampling. The method's simplicity, efficacy, repeatability, and low cost fulfill this study's goal. This study employed an HPLC Waters-Alliance with a PDA Detector to analyze a medication formulation using RP-HPLC. Clindamycin was measured using the novel RP-HPLC technology. Testing linearity, accuracy, precision, Day-2 precision, durability, sensitivity, and solution stability proved it worked. The procedure was valid, stable, and generated consistent assay findings because all factors had %RSD values less than two.

**Funding:**
None

**Conflict of Interest:**
None

**References:**
1. Modi PB, Shah NJ. Novel stability-indicating RP-HPLC method for the simultaneous estimation of clindamycin phosphate and adapalene along with preservatives in topical gel formulations. Scientia Pharmaceutica. 2014 Oct;82(4):799.
2. Khatri RH, Patel RB, Patel MR. A new RP-HPLC method for estimation of Clindamycin and Adapalene in gel formulation: Development and validation consideration. TJPS. 2014;38(1):44-8.

3. Sarfraz S, Hussain S, Javed M, Raza A, Iqbal S, Alrbyawi H, Aljazzar SO, Elkaeed EB, Somaily HH, Pashameah RA, Alzahrani E. Simultaneous HPLC determination of clindamycin phosphate, tretinoin, and preservatives in gel dosage form using a novel stability-indicating method. Inorganics. 2022 Oct 10;10(10):168.
4. Singh N, Singh Y, Bhadauria RS, Govindasamy J. A review on analytical method development, optimization and validation of combination of Azithromycin and benzoyl peroxide by RP-HPLC using design of experiment as per ICH guideline. Indian Journal of Pharmaceutical and Biological Research. 2018 Jun 30;6(02):53-63.
5. Korany MA, Youssef RM, Ragab MA, Amine MT, Afify MA. Development and validation of stability indicating HPLC-DAD assay of adapalene, methylparaben and propylparaben in a cosmeceutical gel matrix with blueness, greenness and whiteness assessment via relative ranking: An ab initio-sub finem juxtaposed non-traditional approaches. Microchemical Journal. 2024 Oct 1;205:111342.
6. Roy C, Panigrahi L, Chakrabarty J. Validated stability-indicating RP-HPLC method for the estimation of degradation behaviour of organic peroxide and third-generation synthetic retinoids in topical pharmaceutical dosage formulation. Scientia Pharmaceutica. 2015 Apr;83(2):321.
7. Chaudhary AM, Modi JI, Shaikh MA. RP-HPLC method development and validation for simultaneous estimation of clindamycin phosphate and nicotinamide in pharmaceutical dosage form. Int Bull Drug Res. 2014;4:160e74.
8. Navkhare MS, Gaidhane HK, Chaple DR, Ingale PL, Ghodekar SV. Validated stability indicating analytical method for the determination of clindamycin phosphate and adapalene in topical formulation. Anal Chem. 2013 Mar;13(210):e5.
9. Ibrahim F, El-Deen AK, El Abass SA, Shimizu K. An ecofriendly green liquid chromatographic method for simultaneous determination of nicotinamide and clindamycin phosphate in pharmaceutical gel for acne treatment. journal of food and drug analysis. 2017 Jul 1;25(3):741-7.
10. Raj V, Prava K, Seru G. RP-HPLC method development and validation for the simultaneous determination of clindamycin and miconazole in pharmaceutical dosage forms. Methods. 2014 Jul 1;5:56-60.
11. Shravya N, Mary P. Development And Validation Of A HPLC Method For Adapalene And Benzoyl Peroxide In Bulk And Gel. Nveo-Natural Volatiles & Essential Oils Journal| Nveo. 2021 Dec 18:6536-43.
12. Pereira MN, Matos BN, Gratieri T, Cunha-Filho M, Gelfuso GM. Development and validation of a simple chromatographic method for simultaneous determination of clindamycin phosphate and rifampicin in skin permeation studies. Journal of Pharmaceutical and Biomedical Analysis. 2018 Sep 10;159:331-40.
13. MuthuKumar S, Kathram S, Navanethan J, Selvakumar D, Banji D. Method development and validation of rp-hplc method for simultaneous determination of clindamycinphosphate and clotrimazole in soft gelatinvaginal suppositories. International Journal of Pharmacy & Therapeutics. 2013;4(4):270-5.
14. Usman M, Abbas T. Development and validation of stability indicating HPLC–PDA method for the simultaneous determination of benzoyl peroxide and tretinoin in topical dosage form. Future Journal of Pharmaceutical Sciences. 2023 Mar 31;9(1):26.
15. Roy C, Chakrabarty J, Rao S, Modi BP, Vairale A. Residue determination of clindamycin phosphate and tretinoin on the surface of manufacturing equipment by RP-HPLC. J. Pharm. Res. 2012 Jul;5:3665-9.
16. Khairy MA, Hamad A, Hamed M, Locatelli M, Mansour FR. A stability indicating RP-HPLC-UV assay method for the simultaneous determination of hydroquinone, tretinoin, hydrocortisone, butylated hydroxytoluene and parabens in pharmaceutical creams. Journal of Pharmaceutical and Biomedical Analysis. 2024 May 15;242:116021.
17. Aher P, Surana K, Ahire E, Patil D, Sonawane D, Mahajan S. Development and validation of RP-HPLC method for quantitative determination of 4-amino benzene sulphonamide in sulphonamide hydrochloride. Trends in Sciences. 2023 Mar 15;20(6):5209-.
18. Prashanthi Y. A New High-performance Liquid Chromatography Method Development for Cleaning Validation of Adapalene Active Pharma Ingredient. Asian Journal of Pharmaceutics (AJP). 2017 Apr 13;11(01).
19. Rao TN, Apparao K, Rao R. A new high-performance liquid chromatography method development for cleaning validation of adapalene active pharma ingredient. Asian J Pharm. 2017 Jan 1;11:37-46.
20. Mandour AA, Nabil N, Zaazaa HE, Ibrahim MM, Ibrahim MA. Two stability indicating chromatographic methods: TLC densitometric versus HPLC method for the simultaneous determination of brinzolamide and timolol maleate in ophthalmic formulation in the presence of probable carcinogenic oxidative degradation product of timolol maleate. Separations. 2023 Jan 6;10(1):37.
21. Sarkis N, Sawan A. Development and validation of derivative UV spectroscopic method for simultaneous estimation of nicotinamide and tretinoin in their binary mixtures and pharmaceutical preparations. BMC chemistry. 2022 Mar 18;16(1):15.
22. Alghamdi AF. Development and validation of anodic stripping voltammetry method for the determination of tretinoin in human urine and plasma using glassy carbon electrode. Journal of King Saud University-Science. 2020 Jul 1;32(5):2635-40.
23. Kotak V, Tanna N, Patel M, Patel R. Determination of Asenapine Maleate in Pharmaceutical and Biological Matrices: A Critical Review of Analytical Techniques over the Past Decade. Critical Reviews in Analytical Chemistry. 2022 Nov 7;52(8):1755-71.

24. Júnior JV, dos Santos JA, Ferreira GL, Porto DL, de Santana Oliveira A, Nogueira FH, de Souza FS, Aragão CF. Validation of HPLC and UHPLC methods for the simultaneous quantification of ferulic acid and nicotinamide in the presence of their degradation products. Analytical Methods. 2019;11(36):4644-50.
25. Gackowski M, Koba M, Mądra-Gackowska K, Kośliński P, Kruszewski S. Recent Applications of High Performance Thin Layer Chromatography and Derivative Spectrophotometry in Pharmaceutical Analysis. Current Pharmaceutical Analysis. 2020 Sep 1;16(6):671-89.
26. Wu GK, Gupta A, Khan MA, Faustino PJ. Development and application of a validated HPLC method for the determination of clindamycin palmitate hydrochloride in marketed drug products: an optimization of the current USP methodology for assay. Journal of Analytical Sciences, Methods and Instrumentation. 2013 Oct 8;3(4):202-11.
27. Cho SH, Im HT, Park WS, Ha YH, Choi YW, Lee KT. Simple method for the assay of clindamycin in human plasma by reversed-phase high-performance liquid chromatography with UV detector. Biomedical Chromatography. 2005 Dec;19(10):783-7.