WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.453
Volume 14, Issue 14, 1000-1013. Research Article ISSN 2277–7105

# DEVELOPMENT AND VALIDATION OF NEW RP-HPLC METHOD FOR ESTIMATION OF NADIFLOXACIN AND ADAPALENE IN GEL FORMULATION

**Prashanta Basnet**[1*]

[1*]Quality Assurance Department, Aadee Remedies Pvt. Ltd., Lalitpur, Nepal.

Article Received on
30 May 2025,

Revised on 20 June 2025,
Accepted on 10 July 2025,

DOI: 10.20959/wjpr202514-37622

***Corresponding Author**
**Prashanta Basnet**
Quality Assurance Department, Aadee Remedies Pvt. Ltd., Lalitpur, Nepal.

## ABSTRACT

This paper describes a validated high-performance liquid chromatographic (HPLC) method for estimation of Adapalene and Nadifloxacin in gel formulation. For estimation of Nadifloxacin, the HPLC separation was achieved on a stainless steel column 15cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (5µm) using a mobile phase of 650 volumes of water, 350 volumes of acetonitrile and 10 volumes of triethylamine adjusted to pH 4.0 with orthophosphoric acid, with a flow rate of 1.0 ml per minute, with an injection volume of 10µl and 295nm spectrophotometer. Similarly, for estimation of Adapalene, the separation was achieved on an Octadecylsilane ($L_1$), 4.6mm x 25cm column using Acetonitrile, Trifluroactetic acid and water in the ratio of 43:36:0.02:21, with a flow rate of 1.0ml per minute, with an injection volume of 20µl and 235nm spectrophotometer. The calibration curves showed good linear relationship with $r^2 = 0.9907$ for Adapalene and $r^2 = 0.9995$ for Nadifloxacin in the concentration range of 25, 50, 75, 100, and 150% of target concentration. The method was validated in terms of linearity, accuracy, precision (repeatability), precision (intermediate), specificity, solution stability, and robustness based on ICH guidelines ($Q_2$ $R_1$). The method was successfully applied for routine analysis of Adapalene and Nadifloxacin in gel formulation.

**KEYWORDS:** *Adapalene, Nadifloxacin, HPLC, validation, gel formulation.*

## 1. INTRODUCTION

Acne is a chronic inflammatory disease of pilosebaceous skin units, with multifactorial aetiology,[1,2] and characterized by presence of comedones, papules, pustules, nodules, cysts, which might result in permanent scars.[3]

There are several main etiological factors implicated in acne pathogenesis: the increased sebum production (frequently induced by testosterone secretion), irregular follicular desquamation (abnormal desquamated corneocytes are gathered in the sebaceous follicle along with other lipids), bacterial proliferation (Propionibacterium acnes determining local skin inflammation by producing pro-inflammatory mediators) and inflammation of the affected area.[6] Acne treatment includes systemic administration of retinoids, antibiotics or oral contraceptives, physical treatment, diet and nevertheless topical treatment. Topical treatment of *acne vulgaris* has the advantage of targeting directly the affected area and decrease of systemic absorption; literature data confirm that the combination therapy proved to be more effective than monotherapy with antimicrobial agents or retinoids.[4,5,6]

Nadifloxacin is a topical antibiotic that treats bacterial skin infections and acne. It's a second-generation fluoroquinolone that's effective against aerobic and anaerobic bacteria, including Gram-negative bacteria, Gram-positive bacteria, Propionibacterium species, Streptococcus species, and Staphylococcus species. Nadifloxacin works by preventing the synthesis of essential proteins and inhibiting the activity of bacterial enzymes. Nadifloxacin is intended for external use only. Some side effects that may occur during treatment include burning and itching, contact dermatitis, dryness, and skin irritation.[7]

Adapalene is a third generation topical retinoid primarily used in the treatment of mild-moderate acne, and is also used off-label to treat keratosis pilaris as well as other skin conditions. Studies have found adapalene is as effective as other retinoids, while causing less irritation. It also has several advantages over other retinoids.[2] The adapalene molecule is more stable compared to tretinoin and tazarotene, which leads to less concern for photodegradation.

**Nadifloxacin**[8,9]

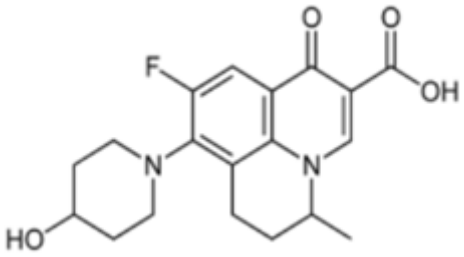


**Fig. 1: structure of nadifloxacin.**

It is also chemically more stable compared to the other two retinoids, allowing it to be used in combination with benzoyl peroxide. Due to its effects on keratinocyte proliferation and differentiation, adapalene is superior to tretinoin for the treatment of comedonal acne and is often used as a first-line agent.

**Chemical Name:** 9-Fluoro – 8 - (4 – hydroxyl - 1-piperidinyl) – 5 – methyl – 1 – oxo - 6, 7-dihydro-1H, 5 Hpyrido [3, 2, 1-ij] quinoline-2-carboxylic acid.

**Molecular Formula:** C19H21FN2O4 Molecular weight 360.379 g/mol.

**Drug Category:** Antibacterial Mechanism of action: Inhibits enzyme DNA gyrase that is involved in bacterial DNA synthesis and replication, thus inhibiting the bacterial multiplication. **Indication:** Used in treatment of bacterial skin infection *i.e.* acne vulgaris.

**Adapalene**[10,11]

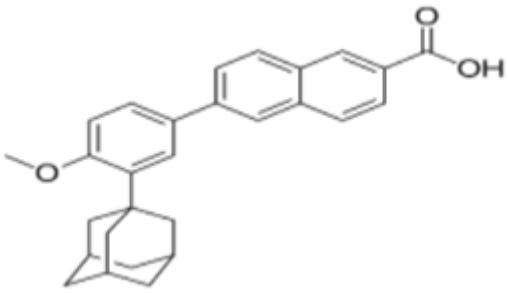


**Fig. 2: structure of adapalene.**

**Chemical Name:** 6 - [3 - (adamantan – 1 - yl) - 4- methoxyphenyl] naphthalene -2-carboxylic acid.

**Molecular Formula:** C28H28O3

**Molecular Weight:** 412.52 g/mol

**Drug Category:** Topical retinoid

**Mechanism of Action:** It acts on retinoid receptor. It is modulator of cell differentiation, keratinization and inflammatory processes which is pathology of acne vulgaris. Indication Used in treatment of *acne vulgaris*.

Literature review shows that many methods have been developed for Nadifloxacin but with other drugs like Mometasone furoate, Terbinafine hydrochloride, Clobetasol Propionate and Miconazole nitrate and also for Adapalene in combination with other drugs like Benzoyl peroxide, Clindamycin Phosphate. But very few methods have been reported for estimation of Nadifloxacin and Adapalene in a combined semi-solid dosage form. Therefore, this study is aimed at developing and validating a new method for estimation of both molecules in a combined semi-solid dosage form.

## 2. MATERIALS AND METHODS

### 2.1 MATERIALS

The pure Adapalene and Nadifloxacin raw materials were received from Sinobright Pharma Pvt. Ltd, Hongkong, China, and Sudarshan Trading, India, respectively. The working standard of Adapalene used was WS/ADP-05/166 (Potency 101.32 % as is basis) and that of Nadifloxacin was WS/NDF-01/168 (Potency 99.45% as is basis. Similarly, HPLC grade Acetonitrile, Tetrahydrofuran, Trifluoroacetic acid, Triethanolamine and Orthophosphoric acid were received from Himedia, India.

### 2.2 APPARATUS

The method was developed using a Shimadzu LC-2030 (Prominence-I).

For Nadifloxacin: A stainless steel column 15cm x 4.6mm, packed with octadecylsilane bonded to porous silica (5µm) was used with a flow rate of 1.0ml per minute. The elution was monitored at 295nm and injection loop volume was 10µl.

For Adapalene: an Octadecylsilane (L1), 4.6mm x 25cm column using Acetonitrile, Trifluroactetic acid and water in the ratio of 43:36:0.02:21, with a flow rate of 1.0ml per minute, with an injection volume of 20µl and 235nm spectrophotometer.

Standard and sample solutions ware filtered through a 0.45µm nylon membrane prior to HPLC injection.

## 2.3 PREPARATION OF REFERENCE SOLUTION

### 2.3.1 Preparation of Adapalene reference Stock solution

Accurately weighed 25 mg of Adapalene Reference Standard was transferred to 100ml volumetric flask, and 2 ml of Tetrahydrofuran was added and sonicated to dissolve. The volume was made to the mark with mobile phase. Again, 10 ml of solution was taken and diluted to 25ml with mobile phase to obtain the concentration of 0.1ml/ml.

### 2.3.2 Preparation of Nadifloxacin Stock Reference solution

A 0.001%w/v solution of Nadifloxacin RS in the solvent mixture.

## 2.4 PREPARATION OF TEST SOLUTION

### 2.4.1 Preparation of Adapalene test solution

5.0g of Gel was transferred to a 50ml volumetric flask and 12.5ml of Tetrahydrofuran was added, sonicated to dissolve. 12.5ml of acetronitrile was added and sonicated for 20minutes. It was then colled to room temperature and the volume was made up to the mark with mobile phase.

### 2.4.2 Preparation of Nadifloxacin test solution

A quantity of the gel containing about 10mg of nadifloxacin was dispersed in 70ml of the solvent mixture, heated on the water bath at 60°C for 10 minutes with shaking to obtain a uniform dispersion. It was then mixed with the aid of ultrasound for 5minutes with intermittent swirling and diluted to 100ml with the solvent mixture and then filtrated, discarding the first few ml of the filtrate. Finally, 5ml was taken and then diluted to 50ml with solvent mixture.

## 2.5 METHODS

### 2.5.1 Analytical Method Validation for Adapalene

#### 2.5.1.1 Linearity

A series of standard solution of five concentrations: 25%, 50%, 75%, 100%, and 150% of target concentration were prepared, three replicates at each concentration were analyzed. Regression was plotted against graph and found to be significant**.**

#### 2.5.1.2 Accuracy

Samples were prepared at three concentrations over the range of 50%, 100%, and 150% of the target concentration.

#### 2.5.1.3 Precision

**Repeatability:** Six replicate injections of standard solution were performed at 100% of expected concentration.

**Intermediate Precision:** Samples were prepared in duplicate of target concentration (100%) by two different analysts on two different days.

**Solution Stability**

Sample was prepared and stored at 2-8°C for 24hours to demonstrate the solution stability.

#### 2.5.1.4 Specificity

Specificity was investigated by injecting the blank solution and placebo solution to demonstrate the absence of interference with the elution of analyte.

#### 2.5.1.5 Robustness

**Robustness test for Adapalene**

The investigation of robustness was done by altering the mobile phase ratio from Acetonitrile: Tetrahydrofuran: Trifluoroacetic Acid: Water (43:36:0.02:21) to (45:40:0.03:15), changing the flow rate from 1.0ml/min to 1.5ml/min, changing column temperature from 35°C to 30°C, and changing column from C18a Shim Pack to C18b Perkin.

**Robustness test for Nadifloxacin**

The investigation of robustness was done by altering the mobile phase ratio from Water: Acetonitrile: Triethylamine (65:35:1) to (70:30:0.8), changing the flow rate from 1.0ml to 1.2ml /minute, changing column temperature from 35°C to 40°C, and changing column from C18-17 to C18-18.

#### 2.5.1.6 System Suitability parameters

During the whole analysis, the column efficiency should be not less than (NLT) 1200 theoretical plates, the tailing factor should be less than 2.0, and the relative standard deviation of replicate injections should not be more than 2.0% to comply with the system suitability test.

## 3. RESULTS AND DISCUSSION

Calibration Curve

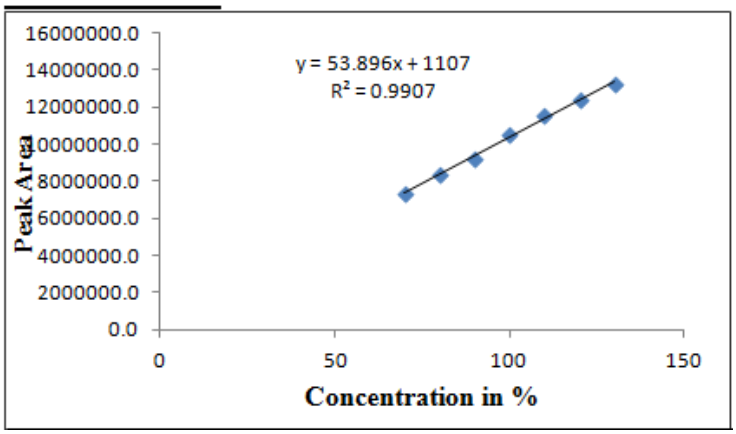


**Fig. 3: Linearity curve for %concentration vs. peak area for Adapalene.**

Calibration Curve

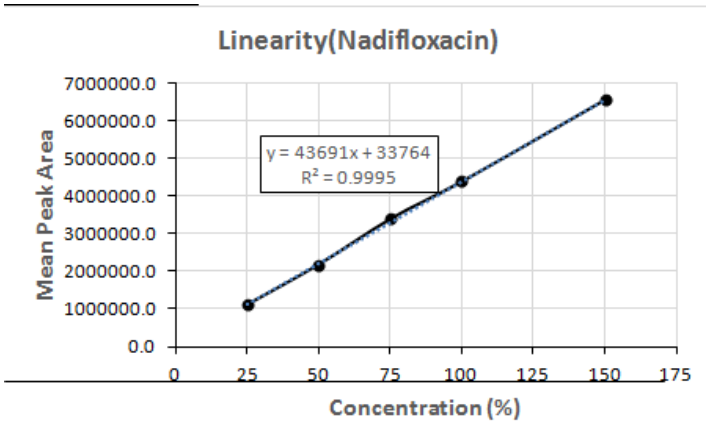


**Fig. 4: Linearity curve for %concentration vs. peak area for Nadifloxacin.**

The calibration curves showed good linear relationship with $r^2$ = 0.9907 for Adapalene and $r^2$ = 0.9995 for Nadifloxacin in the concentration range of 25, 50, 75, 100, and 150% of target concentration.

## ACCURACY

**Table 1: Accuracy datasheet for standard (Adapalene).**

| S. No. | Replicate | Area | Mean | Std. Dev. | RSD % |
|---|---|---|---|---|---|
| 1. | Std 1 | 14334123 | 14336122.67 | 11643.933 | 0.081 |
| 2. | Std 2 | 14337442 | | | |
| 3. | Std 3 | 14321482 | | | |
| 4. | Std 4 | 14326170 | | | |
| 5. | Std 5 | 14344322 | | | |
| 6. | Std 6 | 14353197 | | | |

**Table 2: Accuracy datasheet for test (Adapalene).**

| Parameters | Replicate | Level | | |
|---|---|---|---|---|
| | | 50% | 100% | 150% |
| Area | Test 1a | 7360105 | 14617897 | 20996404 |
| | Test 1b | 7360457 | 14624345 | 21171337 |
| | Test 1c | 7362499 | 14636546 | 20932185 |
| Recovery | Test | 50.84 | 101.74 | 150.83 |
| Recovery % | | 101.68 | 101.74 | 100.56 |

**Table 3: Accuracy datasheet for standard (Nadifloxacin).**

| S.No. | % Conc. of target analyte | Peak Area | | | Mean of Peak area | Std. Dev. | RSD |
|---|---|---|---|---|---|---|---|
| | | I | II | III | | | |
| 1. | 25 | 1109354 | 1110239 | 1106421 | 1108671.3 | 1998.45 | 0.1803 |
| 2. | 50 | 2180834 | 2184657 | 2179629 | 2181706.7 | 2625.14 | 0.1203 |
| 3. | 75 | 3393826 | 3395307 | 3393939 | 3394357.3 | 824.37 | 0.0243 |
| 4. | 100 | 4391013 | 4392742 | 4397843 | 4393866.0 | 3551.02 | 0.0808 |
| 5. | 150 | 6568308 | 6566766 | 6564846 | 6566640.0 | 1734.44 | 0.0264 |

**Table 4: Accuracy datasheet for test (Nadifloxacin).**

| Parameters | Replicate | Level | | |
|---|---|---|---|---|
| | | 50% | 100% | 150% |
| Area | Test 1a | 2088136 | 4442158 | 6158159 |
| | Test 1b | 2087595 | 4439370 | 6140984 |
| | Test 1c | 2087447 | 4440609 | 6128071 |
| Recovery | Test | 49.07 | 99.44 | 147.37 |
| Recovery (%) | | 98.14 | 99.44 | 98.25 |

The accuracy was found to be in the range of 100.56% to 101.74%, for Adapalene and 98.14% to 99.44% for Nadifloxacin, which are within the specified limit of 98% to 102%.

**PRECISION**

**Repeatability (Adapalene)**

**Table 5: Datasheet for Precision.**

| Inj. No. | Retention time | Peak Area | Peak Height |
|---|---|---|---|
| 1 | 8.590 | 13926.57813 | 1016.56409 |
| 2 | 8.535 | 13926.04590 | 1025.05933 |
| 3 | 8.504 | 13959.94629 | 1034.04382 |
| 4 | 8.485 | 13961.81152 | 1035.53857 |
| 5 | 8.496 | 13968.53320 | 1035.67688 |
| 6 | 8.519 | 13976.29102 | 1035.60608 |
| Mean | 8.5215 | 13953.20101 | 1030.414795 |
| Std. Dev. | 0.038 | 21.60575 | 7.93202 |
| RSD (%) | 0.444 | 0.155 | 0.770 |

**Table 6: Interpretation of Results.**

| Parameter | Result | Specification |
|---|---|---|
| RSD (%) Retention time | 0.444 | Not more than 2.0% |
| RSD (%) Peak Area | 0.155 | Not more than 2.0% |
| RSD (%) Peak Height | 0.770 | Not more than 2.0% |

**Repeatability (Nadifloxacin)**

**Table 7: Datasheet for Precision Repeatability.**

| Inj. No. | Retention time | Peak Area | Peak Height |
|---|---|---|---|
| 1 | 6.298 | 4345144 | 340597 |
| 2 | 6.298 | 4343267 | 340657 |
| 3 | 6.299 | 4343073 | 340697 |
| 4 | 6.299 | 4342924 | 340797 |
| 5 | 6.299 | 4344121 | 340917 |
| 6 | 6.300 | 4344249 | 340987 |
| **Mean** | 6.299 | 4343796.33 | 340775.33 |
| **Std. Dev.** | 0.001 | 859.173 | 153.15569 |
| **RSD (%)** | 0.012 | 0.020 | 0.045 |

**Table 8: Interpretation of Results (Nadifloxacin).**

| Parameter | Result | Specification |
|---|---|---|
| RSD (%) Retention time | 0.012 | Not more than 2.0% |
| RSD (%) Peak Area | 0.020 | Not more than 2.0% |
| RSD (%) Peak Height | 0.045 | Not more than 2.0% |

The relative standard deviation for retention time, peak area, and peak height were not more than 2%. Hence, the method complies for repeatability.

**Intermediate Precision (Adapalene)**

**Table 9: Intermediate Precision Datasheet (Adapalene)**

| Parameters | Replicate | Analyst 1(Day1) | | Analyst 2(Day2) | |
|---|---|---|---|---|---|
| | | Std(Avg) | Spl(Avg) | Std(Avg) | Spl(Avg) |
| **Area** | Test 1 | 13769237.8 | 14431164 | 13858827.8 | 14041769 |
| | Test 2 | | 14337748 | | 14310346 |
| **% Assay** | Test 1 | | 104.20 | | 105.73 |
| | Test 2 | | 104.73 | | 104.75 |
| **%Mean Assay** | | | 104.47 | | 105.24 |

**Table 10: RSD data across the two analysts (Adapalene).**

| | % Assay | Mean | Std. Dev. | RSD (%) |
|---|---|---|---|---|
| **Analyst 1** | 104.47 | 104.86 | 0.544 | 0.519 |
| **Analyst 2** | 105.24 | | | |

**Intermediate Precision (Nadifloxacin)**

**Table 11: Intermediate Precision datasheet (Nadifloxacin).**

| Parameters | Replicate | Analyst 1(Day1) | | Analyst 2(Day2) | |
|---|---|---|---|---|---|
| | | Std(Avg) | Spl(Avg) | Std(Avg) | Spl(Avg) |
| Area | Test 1 | 4420317 | 4552181 | 4362526.4 | 4451373 |
| | Test 2 | | 4526591 | | 4354169 |
| % Assay | Test 1 | | 98.47 | | 97.77 |
| | Test 2 | | 99.35 | | 97.45 |
| %Mean Assay | | | 98.91 | | 97.61 |

**Table 12: RSD data across two analysts (Nadifloxacin).**

| | % Assay | Mean | Std. Dev. | RSD(%) |
|---|---|---|---|---|
| Analyst 1 | 98.91 | 98.26 | 0.919 | 0.933 |
| Analyst 2 | 97.61 | | | |

The relative standard deviation of data across two analysts was RSD 0.519% for Adapalene and 0.933% for Nadifloxacin (less than 2.0%). Hence, the method complies for intermediate precision.

**SOLUTION STABILITY**

**Table 13: Datasheet for solution stability (Adapalene).**

| | Assay (%) | Solution Stability (%) |
|---|---|---|
| Fresh Sample | 106.24 | 101.04 |
| Stored sample | 107.34 | |

**Table 14: Datasheet for solution stability (Nadifloxacin).**

| | Assay (%) | Solution Stability (%) |
|---|---|---|
| Fresh Sample | 100.10 | 101.42 |
| Stored sample | 101.52 | |

The solution stability of Adapalene was 101.04% in comparision to freshly prepared solution, and that of Nadifloxacin was found to be 101.42% which both are within the specified limit of 97.5% to 102.5%. Hence, the method passed solution stability testing.

**SPECIFICITY**

**Table 15: Datasheet for specificity (Adapalene).**

| Chromatograph Results | | | |
|---|---|---|---|
| S.No. | Sample | Retention Time | Area |
| 1 | Blank Solvent | N/A | N/A |
| 2 | Placebo | N/A | N/A |
| 4 | Standard | 8.378 | 13893.23340 |
| 3 | Sample | 8.390 | 14307.82910 |

**Table 16: Datasheet for specificity (Nadifloxacin).**

| Chromatograph Results | | | |
|---|---|---|---|
| **S.No.** | **Sample** | **Retention Time** | **Area** |
| **1** | Blank Solvent | N/A | N/A |
| **2** | Placebo | N/A | N/A |
| **3** | Standard | 6.294 | 4283741 |
| **4** | Sample | 6.295 | 4550173 |

As blank and placebo solutions did not show peak response at the same time as sample and standard solution, the method is considered to be specific for the given sample.

**ROBUSTNESS (ADAPALENE)**

**Table 17: Change in mobile phase ratio.**

| **Parameters** | **Replicate** | **ACN:THF:TFA:H2O(43:36:0.02:21)** | | **ACN:THF:TFA:H2O(45:40:0.03:15)** | |
|---|---|---|---|---|---|
| | | **Std(Avg)** | **Spl(Avg)** | **Std(Avg)** | **Spl(Avg)** |
| **Area** | Test 1 | 13911734.6 | 14470319 | 13493.08262 | 14461.85792 |
| | Test 2 | | 14685215 | | 14410.31005 |
| **%Assay** | Test 1 | | 105.52 | | 106.95 |
| | Test 2 | | 106.64 | | 104.98 |
| **Mean (%Assay)** | | | 106.08 | | 105.96 |

**Table 18: Change in flow rate.**

| **Parameters** | **Replicate** | **Flow rate(1ml/min)** | | **Flow rate(1.5 ml/min)** | |
|---|---|---|---|---|---|
| | | **Std(Avg)** | **Spl(Avg)** | **Std(Avg)** | **Spl(Avg)** |
| **Area** | Test 1 | 13911734.6 | 14470319 | 9249.807032 | 9550.61328 |
| | Test 2 | | 14685215 | | 9719.90088 |
| **%Assay** | Test 1 | | 105.52 | | 106.65 |
| | Test 2 | | 106.64 | | 107.85 |
| **Mean (%Assay)** | | | 106.08 | | 107.25 |

**Table 19: Change in column temperature.**

| **Parameters** | **Replicate** | **Column Temp.(35°C)** | | **Column Temp.(30°C)** | |
|---|---|---|---|---|---|
| | | **Std(Avg)** | **Spl(Avg)** | **Std(Avg)** | **Spl(Avg)** |
| **Area** | Test 1 | 13911734.6 | 14470319 | 13708.75469 | 14137.32227 |
| | Test 2 | | 14685215 | | 14340.90820 |
| **%Assay** | Test 1 | | 105.52 | | 106.52 |
| | Test 2 | | 106.64 | | 107.37 |
| **Mean (%Assay)** | | | 106.08 | | 106.95 |

**Table 20: Change in column.**

| Parameters | Replicate | Column C18a Shim pack | | Column C18b Perkin | |
|---|---|---|---|---|---|
| | | Std(Avg) | Spl(Avg) | Std(Avg) | Spl(Avg) |
| **Area** | Test 1 | 13911734.6 | 14470319 | 13339.45391 | 14686.81983 |
| | Test 2 | | 14685215 | | 14710.93311 |
| **%Assay** | Test 1 | | 105.52 | | 107.63 |
| | Test 2 | | 106.64 | | 108.20 |
| **Mean (%Assay)** | | | 106.08 | | 108.02 |

**ROBUSTNESS (NADIFLOXACIN)**

**Table 21: Change in mobile phase ratio.**

| Parameters | Replicate | Water:ACN:TEA(65:35:1) | | Water:ACN:TEA(70:30:0.8) | |
|---|---|---|---|---|---|
| | | Std(Avg) | Spl(Avg) | Std(Avg) | Spl(Avg) |
| **Area** | Test 1 | 4383335.8 | 5000418 | 4412205.2 | 5049880 |
| | Test 2 | | 5234246 | | 5271911 |
| **%Assay** | Test 1 | | 97.56 | | 97.88 |
| | Test 2 | | 97.29 | | 97.35 |
| **Mean (%Assay)** | | | 97.42 | | 97.61 |

**Table 22: Change in flow rate.**

| Parameters | Replicate | Flow rate(1ml/min) | | Flow rate(1.2 ml/min) | |
|---|---|---|---|---|---|
| | | Std(Avg) | Spl(Avg) | Std(Avg) | Spl(Avg) |
| **Area** | Test 1 | 4383335.8 | 5000418 | 3653222.4 | 4167046 |
| | Test 2 | | 5234246 | | 4351228 |
| **%Assay** | Test 1 | | 97.56 | | 97.54 |
| | Test 2 | | 97.29 | | 97.04 |
| **Mean (%Assay)** | | | 97.42 | | 97.29 |

**Table 23: Change in column temperature.**

| Parameters | Replicate | Column Temp.(35°C) | | Column Temp.(40°C) | |
|---|---|---|---|---|---|
| | | Std(Avg) | Spl(Avg) | Std(Avg) | Spl(Avg) |
| **Area** | Test 1 | 4383335.8 | 5000418 | 4393430.4 | 5022039 |
| | Test 2 | | 5000782 | | 5246769 |
| **%Assay** | Test 1 | | 97.56 | | 97.75 |
| | Test 2 | | 97.29 | | 97.30 |
| **Mean (%Assay)** | | | 97.42 | | 97.52 |

**Table 24: Change in column.**

| Parameters | Replicate | Column C18-17 Shim pack | | Column C18-18 Shim pack | |
|---|---|---|---|---|---|
| | | Std(Avg) | Spl(Avg) | Std(Avg) | Spl(Avg) |
| **Area** | Test 1 | 4383335.8 | 5000418 | 4505180.2 | 4893485 |
| | Test 2 | | 5000782 | | 5003295 |
| **%Assay** | Test 1 | | 97.56 | | 97.87 |
| | Test 2 | | 97.29 | | 97.91 |
| **Mean (%Assay)** | | | 97.42 | | 97.89 |

The results as shown in the tables after changing several parameters like solvent ratio, change in flow rate, change in column temperature and change in column itself demonstrate that the method is robust enough for the routine analysis. While performing whole analysis the column efficiency was found to be more than 1200 theoretical plates, the tailing factor was less than 2.0, and the relative standard deviation of replicate injections was found to be below 2.0%. So, the analytical method followed complies with the system suitability test.

## 4. CONCLUSION

This study is a typical example of the development of an assay method following ICH guidelines. A new HPLC method has been developed and validated for determination of Adapalene and Nadifloxacin in the gel formulation. The results of the validated studies showed that HPLC method possesses significant linearity, precision, accuracy, specificity, sensitivity, high efficiency and resolution, and no interference from the excipients, as were demonstrated. The proposed method was successfully applied and is suggested for the quantitative analysis of Adapalene and Nadifloxacin in combined pharmaceutical formulations for QC, where economy and time are essential and to assure therapeutic efficacy.

## 5. ACKNOWLEDGEMENT

I would like to express my deepest gratitude to Aadee Remedies Pvt. Ltd, Quality Control Department for providing continuous support during this study. I would also like to thank Sinobright Pharma Pvt. Ltd, Hongkong, China, and SudarshanTrading, India, for providing Nadifloxacin and Adapalene active raw materials for gel preparation, respectively.

## 6. Conflict of Interest

The author declares no conflict of interest and no third party funding in this study.

## 7. REFERENCES

1. Gieler U, Gieler T, Kupfer JP. Acne and quality of life- impact and management. J Eur Acad Dermatol, 2015; 29(4): 12-14.
2. Al-Talib H, Al-Khateeb A, Hameed A, Murugaiah C. Efficacy and safety of superficial chemical peeling in treatment of active *acne vulgaris*. An Bras Dermatol, 2017; 92(2): 212-216.
3. Fox L, Csongradi C, Aucamp M, du Plessis J, Gerber M. Treatment modalities for acne. Molecules, 2016; 21(8) pii: E1063: 1-20.

4. AI Hussein SM. Evaluation of new drug formulation for topical use in acne pathology. Dermato-cosmetic procedures and dietary factors as adjivant non-pharmacological methods. Ph thesis, University of Medicine and Pharmacy from Targu, Romania, 2017 (available in Romania).
5. Kobayashi M, Nakagawa T, Fukamachi K, Nakamura M, Tokura Y. Efficacy of combined topical treatment of *acne vulgaris* with adapalene and nadifloxacin: A randomized study. J Dermatol, 2011; 38: 1163-1166.
6. Le Cleach L, Lebrun- Vignes B, Bachelot A, Beer F, Berger P, Brugere S, Chasting M, et al. French Acne Guidelines working Group and Centre of Evidence of Dermatology Guidelines for the management of acne: recommendations from a French multidisciplinary group. Br J Dermatol, 2017; 177(4): 908-913.
7. Jacobs M and Appelbaum P. Nadifloxacin: a quinolone for topical treatment of skin infections and potential for systemic use of its active isomer, WCK 771, Expert Opin Pharmacother, 2006; 7(14): 1957-1966.
8. Drugbank, "Nadifloxacin Drug profile", https://go.drugbank.com/drugs/DB12447 [Accessed 4 June 2025].
9. Nadifloxacin - PubChem https://pubchem.ncbi.nlm.nih.gov/compound/Nadifloxacin [Accessed 4 June 2025].
10. Drugbank, "Adapalene Drug profile", https://go.drugbank.com/drugs/DB00210 [Accessed 4 June 2025].
11. Adapalene - PubChem https://pubchem.ncbi.nlm.nih.gov/compound/Adapalene [Accessed 4 June 2025].